



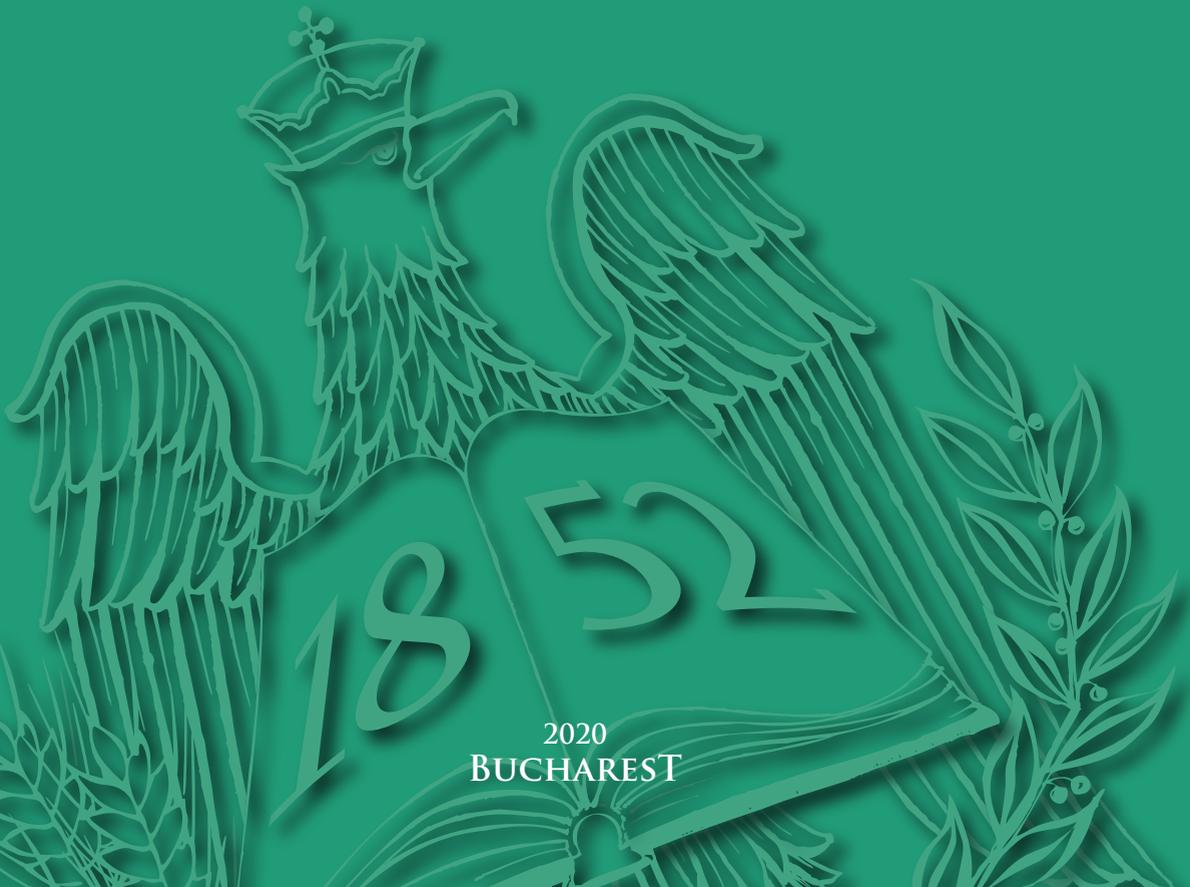
UNIVERSITY OF AGRONOMIC SCIENCES
AND VETERINARY MEDICINE OF BUCHAREST
FACULTY OF AGRICULTURE



SCIENTIFIC PAPERS

SERIES A. AGRONOMY

VOLUME LXIII, No. 1



2020
BUCHAREST

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SOIL SCIENCES

EFFECTS AND CONSEQUENCES OF SEWAGE SLUDGE FROM URBAN WASTEWATER AND THEIR COMBINATIONS WITH ZEOLITE ON SOIL FERTILITY AND PRODUCTIVITY OF GRAIN CROPS

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Abstract

The intensive development of agricultural production in the forest-steppe regions requires solving the problem of preserving the soils and their effective fertility. In this regard, the development and implementation of technological methods for preventing soil degradation in agricultural areas using local cheaper raw materials and agronomic ores (fertilizing ores) are the urgent areas of modern agronomic science. The purpose of the research was to study the effects and consequences of sewage sludge from urban waste waters and their combinations with zeolite on soil fertility and productivity of grain crops. It was found that the reclamation rates of urban wastewater sludge in combination with zeolite-containing agronomic ores increased the humus content in the arable layer of the soil by 0.23-0.36%, provided transformation soils from unsatisfactory structural state into a class with good soil and reduced the bulk soil density of the arable layer by 0.12-0.21 g/cm³. The urban sewage sludge (160 and 180 t/ha) and the urban sewage sludge (140-180 t/ha) in combination with zeolite increased the productivity of grain crops by 57.8-70.0%.

Key words: zeolite, humus, structure, density, acidity.

INTRODUCTION

Intensive use of arable land with a low level of application of organic and mineral fertilizers has led to the development of physical, chemical, biological degradation of soils and reduced productivity of crops.

Among degradation processes the most widespread are dehumification, destructuring, acidification, re-compaction and soil slitization (Arefyev et al., 2017).

Improvement and stabilization of soil fertility is impossible without the development and implementation of methods of biological and chemical land reclamation.

At present, there is significant foreign experience in using local raw materials and agronomic ores to prevent soil degradation and increase crop productivity (Alvarenga et al., 2019; Mitri et al., 2019; Melece and Shena, 2019).

In Russian agriculture the use of local raw materials and agronomic ores is limited. In this regard, there is a need for scientific justification, development and implementation of technological methods for biological and chemical land reclamation of soils of the forest-

steppe Volga region based on them (Proskoryakova, 2005; Korolev, 2007).

MATERIALS AND METHODS

To achieve this goal in the first agricultural soil allotment in the Penza region, field experience was laid down according to the following scheme: 1. Without Urban Sewage Sludge (USS) and zeolite (control); 2. Zeolite 10 t/ha; 3. USS 100 t/ha; 4. USS 120 t/ha; 5. USS 140 t/ha; 6. USS 160 t/ha; 7. USS 180 t/ha; 8. USS 100 t/ha + zeolite 10 t/ha; 9. USS 120 t/ha + zeolite 10 t/ha; 10. USS 140 t/ha + zeolite 10 t/ha; 11. USS 160 t/ha + zeolite 10 t/ha; 12. USS 180 t/ha + zeolite 10 t/ha.

The experiment was conducted three times, the variants in the experiment were placed by the method of randomized repetitions, the accounting area of the first allotment was 4 m². The studies were carried out in arable crop rotation. The soil of the experimental allotment is represented by meadow-chnozem leached low humus medium thick medium loamy soil. The humus content in the arable layer was 5.09-5.12%, alkaline hydrolyzable nitrogen - 119.9-120.6 mg/kg of soil, mobile phosphorus -

101.7-102.1 mg/kg of soil, mobile potassium - 151.8 -152.1 mg/kg of soil.

In the experiment the sewage sludge from the city of Penza (Russia) was used. It was mechanically treated and dehydrated (after four-years storage on the silt site), and could be characterized by the following indicators: pH (KCl) - 6 units and hydrolytic acidity - 2.4 meq/100 g of the urban sewage sludge, and with content of the nutrients: nitrogen - 291.0, phosphorus - 116 and potassium - 120 meq/100 g of the urban sewage sludge; carbon organic matter - 21.2%.

In our research the chemical composition of the urban sewage sludge was determined in laboratory conditions by the following methods: pH (KCl) extraction - according to the method for determination of exchangeable acidity (State Standard 26484-85); hydrolytic acidity - according to the Determination of hydrolytic acidity by Kappen method modified by CINAO (State Standard 26212-91); gross nitrogen - according to the Determination of mobile compounds of phosphorus and potassium by Chiricov method modified by

CINAO (State Standard 26204-91), the organic matter content - according to the Methods for determination of organic matter (State Standard 26213-91).

In the experiment, zeolite (zeolite-containing agronomic ore) was used as a chemical ameliorant. The clinoptilolite content in the zeolite-containing agronomic ore was 41%. The rate of chemical ameliorant was calculated by the content of clinoptilolite in it.

Urban sewage sludge and chemical reclamation was introduced into the steam field according to the experimental scheme in 2014 for the main tillage.

RESULTS AND DISCUSSIONS

This research has established that the one-sided effect and consequences of reclamation norms for sewage sludge from urban wastewater and their effect and consequences in combination with zeolite-containing agronomic ore had a positive effect on the humus content in the arable layer of meadow-chernozem soils (Table 1).

Table 1. Humus content in the arable layer of meadow-chernozem soils, %

Variant	Pure vapour 2014	Winter wheat		Corn		Spring wheat		Oat		Pea	
		2015	± from 2014	2016	± from 2014	2017	± from 2014	2018	± from 2014	2019	± from 2014
1. Without USS and zeolite (control)	5.12	5.10	-0.02	5.08	-0.04	5.09	-0.03	5.10	-0.02	5.09	-0.03
2. Zeolite 10 t/ha	5.10	5.12	0.2	5.12	0.02	5.13	0.03	5.15	0.05	5.14	0.04
3. USS 100 t/ha	5.09	5.23	0.14	5.26	0.17	5.29	0.20	5.28	0.19	5.24	0.15
4. USS 120 t/ha	5.10	5.26	0.16	5.31	0.21	5.35	0.25	5.35	0.25	5.32	0.22
5. USS 140 t/ha	5.11	5.30	0.19	5.35	0.24	5.38	0.27	5.37	0.26	5.35	0.24
6. USS 160 t/ha	5.10	5.34	0.24	5.39	0.29	5.44	0.34	5.42	0.32	5.39	0.29
7. USS 180 t/ha	5.10	5.37	0.27	5.44	0.34	5.46	0.36	5.45	0.35	5.42	0.32
8. USS 100 t/ha + zeolite 10 t/ha	5.10	5.25	0.15	5.30	0.20	5.34	0.24	5.35	0.25	5.33	0.23
9. USS 120 t/ha + zeolite 10 t/ha	5.10	5.27	0.17	5.34	0.24	5.38	0.28	5.40	0.30	5.37	0.27
10. USS 140 t/ha + zeolite 10 t/ha	5.10	5.32	0.22	5.38	0.28	5.42	0.32	5.42	0.32	5.40	0.30
11. USS 160 t/ha + zeolite 10 t/ha	5.09	5.35	0.26	5.40	0.31	5.45	0.36	5.46	0.37	5.43	0.34
12. USS 180 t/ha + zeolite 10 t/ha	5.09	5.39	0.30	5.45	0.36	5.48	0.39	5.47	0.38	5.45	0.36
Least significant difference (LSD) p = 0.05		0.11		0.12		0.14		0.13		0.12	

Before introducing the sewage sludge from urban wastewater and zeolite, the humus content in the arable layer of meadow-chernozem soils was 5.09-5.12%.

The unilateral action and consequences of a zeolite-containing agronomic ore had an insignificant effect on the accumulation of humus in the arable layer. The humus content

in this experiment varied over the years of research in the range from 5.12 to 5.15%, exceeding the initial values by 0.02-0.05%.

The sewage sludge from urban wastewater with their unilateral action and consequences, increased the humus content in the arable layer in winter wheat crops by 0.14 (USS 100 t/ha) - 0.27% (USS 180 t/ha), in corn crops - by 0.17-0.34%, in crops of spring wheat - by 0.20-0.36%, in crops of oats - by 0.19-0.35% and in crops of peas - by 0.15-0.32%

The humus content in the arable layer against the one-sided effect and consequences of the urban sewage sludge varied from 5.23 to 5.46%, depending on the sludge rate, according to years of research.

Against the one-sided effect and consequences of the urban sewage sludge in combination with zeolite-containing agronomic ore, the humus content in the arable layer slightly

exceeded its content in similar variants using urban sewage sludge without zeolite and varied in 2015 in the range from 5.25 (USS 100 t/ha + zeolite 10 t/ha) up to 5.39% (USS 180 t/ha + zeolite 10 t/ha), in 2016 - from 5.30 to 5.45%, in 2017 - from 5.34 to 5.48%, in 2018 - from 5.35 to 5.47%, in 2019 - from 5.33 to 5.45%, exceeding the initial values by 0.15-0.39%.

In the arable layer of meadow-chernozem soils without spreading urban sewage sludge and zeolite-containing agronomic ore, within the years of the current research the soil acidity varied from 5.33 to 5.38 units pH (Arefiev A.N. et al., 2019). Zeolite-containing agronomic ore against the one-sided effect and consequences reduced the value of metabolic acidity in the arable layer by 0.63-0.94 pH units. After pea harvesting in 2019, the pH (KCl) value in this experiment was 6.27 units (Table 2).

Table 2. Soil acidity of the arable horizon of meadow-chernozem soils, pH (KCl)

Variant	Pure vapour 2014	Winter wheat		Corn		Spring wheat		Oat		Pea	
		2015	± from 2014	2016	± from 2014	2017	± from 2014	2018	± from 2014	2019	± from 2014
1. Without USS and zeolite (control)	5.38	5.37	-0.01	5.35	-0.03	5.36	-0.02	5.35	-0.03	5.33	-0.05
2. Zeolite 10 t/ha	5.37	6.00	0.63	6.22	0.85	6.31	0.94	6.29	0.92	6.27	0.90
3. USS 100 t/ha	5.38	5.65	0.27	5.69	0.31	5.71	0.33	5.69	0.31	5.67	0.29
4. USS 120 t/ha	5.38	5.71	0.33	5.75	0.37	5.78	0.40	5.77	0.39	5.74	0.36
5. USS 140 t/ha	5.38	5.76	0.38	5.81	0.43	5.87	0.49	5.86	0.48	5.84	0.46
6. USS 160 t/ha	5.37	5.83	0.46	5.87	0.50	5.90	0.53	5.90	0.53	5.87	0.50
7. USS 180 t/ha	5.36	5.89	0.53	5.94	0.58	5.96	0.60	5.94	0.58	5.92	0.56
8. USS 100 t/ha + zeolite 10 t/ha	5.36	6.28	0.92	6.53	1.17	6.60	1.24	6.62	1.26	6.58	1.22
9. USS 120 t/ha + zeolite 10 t/ha	5.35	6.34	0.99	6.58	1.23	6.65	1.30	6.68	1.33	6.64	1.29
10. USS 140 t/ha + zeolite 10 t/ha	5.36	6.40	1.04	6.64	1.28	6.75	1.39	6.77	1.41	6.72	1.36
11. USS 160 t/ha + zeolite 10 t/ha	5.36	6.46	1.10	6.72	1.36	6.80	1.44	6.82	1.46	6.78	1.42
12. USS 180 t/ha + zeolite 10 t/ha	5.37	6.53	1.16	6.82	1.45	6.87	1.50	6.88	1.51	6.84	1.47
Least significant difference (LSD) p = 0.05		0.16		0.18		0.17		0.19		0.17	

The action and effect of urban sewage sludge reduced the value of soil acidity, depending on the sludge dose, by 0.27-0.60 pH units. The pH (KCl) value before pea harvesting in 2019 varied as the sediment rate increased from 5.67 to 5.92 units.

Despite the complex effect and consequences of urban wastewater and zeolite, the pH (KCl) value at the end of the research varied from

6.58 to 6.84 units, exceeding the initial values by 1.22-1.47 units.

As shown by the data presented in Table 3, the structural state of the arable layer in the meadow-chernozem soils before laying the experience was characterized as unsatisfactory. The content of water-resistant aggregates in the arable layer before the introduction of urban sewage sludge and zeolite was 38.3-38.9%.

Under grain crops without introducing the urban sewage sludge and zeolite, there was a tendency to decrease the content of water-resistant aggregates in the arable layer of meadow-chernozem soils (Table 4). After

harvesting peas in 2019 the content of water-resistant aggregates in the arable layer in this embodiment was 37.7%, which was 1.2% lower than the initial one.

Table 3. The content of water-resistant aggregates in the arable horizon of meadow-chernozem soils, %

Variant	Pure vapour 2014	Winter wheat		Corn		Spring wheat		Oat		Pea	
		2015	± from 2014	2016	± from 2014	2017	± from 2014	2018	± from 2014	2019	± from 2014
1. Without USS and zeolite (control)	38.9	38.5	-0.4	37.4	-1.5	37.7	-1.2	38.0	-0.9	37.7	-1.2
2. Zeolite 10 t/ha	38.5	40.6	2.1	41.5	3.0	42.1	3.6	43.2	4.7	43.1	4.6
3. USS 100 t/ha	38.6	46.2	7.6	47.3	8.7	48.4	9.8	48.5	9.9	47.9	9.3
4. USS 120 t/ha	38.4	48.0	9.6	49.3	10.9	50.5	12.1	50.7	12.3	50.4	12.0
5. USS 140 t/ha	38.5	50.0	11.5	51.3	12.8	52.8	14.3	53.4	14.9	53.1	14.6
6. USS 160 t/ha	38.8	51.9	13.1	53.3	14.5	55.2	16.4	56.0	17.2	55.8	17.0
7. USS 180 t/ha	38.7	54.0	15.3	55.6	16.9	57.2	18.5	57.8	19.1	57.7	19.0
8. USS 100 t/ha + zeolite 10 t/ha	38.3	48.9	10.6	50.4	12.1	52.3	14.0	53.1	14.8	52.5	14.2
9. USS 120 t/ha + zeolite 10 t/ha	38.6	51.0	12.4	53.1	14.5	55.2	16.6	56.0	17.4	55.6	17.0
10. USS 140 t/ha + zeolite 10 t/ha	38.4	53.1	14.7	55.1	16.7	57.4	19.0	58.1	19.1	58.0	19.0
11. USS 160 t/ha + zeolite 10 t/ha	38.6	55.0	16.4	57.5	18.9	59.7	21.1	60.8	22.2	60.8	22.2
12. USS 180 t/ha + zeolite 10 t/ha	38.5	57.2	18.7	59.8	21.3	61.5	23.0	62.0	23.5	61.6	23.1
Least significant difference (LSD) p = 0.05		1.9		2.4		2.8		2.6		2.5	

Table 4. Bulk density of the arable layer of meadow-chernozem soils, g/cm³

Variant	Winter wheat		Corn		Spring wheat		Oat		Pea	
	2015	*	2016	*	2017	*	2018	*	2019	*
1. Without USS and zeolite (control)	1.33	–	1.35	–	1.34	–	1.35	–	1.37	–
2. Zeolite 10 t/ha	1.29	0,04	1.31	0,04	1.29	0,05	1.30	0,05	1.33	0,04
3. USS 100 t/ha	1.26	0,07	1.27	0,08	1.26	0,08	1.27	0,07	1.30	0,07
4. USS 120 t/ha	1.24	0,09	1.26	0,09	1.24	0,10	1.25	0,10	1.29	0,08
5. USS 140 t/ha	1.22	0,11	1.23	0,12	1.21	0,13	1.23	0,12	1.27	0,10
6. USS 160 t/ha	1.21	0,12	1.21	0,14	1.19	0,15	1.20	0,15	1.24	0,13
7. USS 180 t/ha	1.19	0,14	1.20	0,15	1.18	0,16	1.20	0,15	1.23	0,14
8. USS 100 t/ha + zeolite 10 t/ha	1.23	0,10	1.23	0,12	1.20	0,14	1.22	0,13	1.25	0,12
9. USS 120 t/ha + zeolite 10 t/ha	1.22	0,11	1.21	0,14	1.19	0,15	1.20	0,15	1.24	0,13
10. USS 140 t/ha + zeolite 10 t/ha	1.19	0,14	1.19	0,16	1.17	0,17	1.19	0,16	1.22	0,15
11. USS 160 t/ha + zeolite 10 t/ha	1.16	0,17	1.17	0,18	1.14	0,20	1.17	0,18	1.19	0,18
12. USS 180 t/ha + zeolite 10 t/ha	1,14	0,19	1.16	0,19	1.13	0,21	1.15	0,20	1.18	0,19
Least significant difference (LSD) p = 0.05		0,03		0,02		0,04		0,04		0,03

*Deviation from control

Zeolite-containing agronomic ore increased the content of water-resistant aggregates by 4.6%.

The number of water-resistant aggregates in this variant upon completion of research (2019)

was 43.1%, and the structural state of the arable layer was assessed as satisfactory.

The urban sewage sludge with rates from 100 to 140 t/ha, as well as the unilateral action of zeolite-containing agronomic ore, provided a satisfactory structural state of the arable layer. The content of water-resistant aggregates in the arable layer against the one-sided consequences of urban sewage sludge varied in 2019 from 47.9 to 53.1%, exceeding the initial values by 9.3-14.6%.

A satisfactory structural state of the arable layer was also noted after introduction of 100 t/ha of urban sewage sludge in combination with zeolite-containing agronomic ore. The number of water-resistant aggregates in the arable layer in this embodiment exceeded the initial value by 14.2% and increased to 52.5%.

A good structural condition in the arable layer was ensured by the introduction of the urban sewage sludge with dose of 160-180 t/ha and the introduction of the urban sewage sludge with dose of 120 to 180 t/ha in combination with zeolite-containing agronomic ore. The number of water-resistant units at the same time exceeded the initial value by 17.0-23.1% and amounted to 55.6-61.6% after pea harvesting in 2019.

In the arable layer of meadow-chernozem soils without urban sewage sludge and zeolite-containing agronomic ore, the bulk density after winter wheat harvesting was 1.33 g/cm³, after corn harvesting - 1.35 g/cm³, after spring wheat harvesting - 1.34 g/cm³, after oats harvesting - 1.35 g/cm³, after peas harvesting - 1.37 g/cm³. The drift from the optimal density according to the gradation of A.G. Bondarev was 0.03-0.07 g/cm³ (Korchagin et al., 2011). The action and aftereffect of zeolite-containing agro-ore significantly reduced the bulk density in the arable layer by 0.04-0.05 g/cm³.

In case of unilateral action and aftereffect of urban wastewater sludge, the bulk density in the arable layer after winter wheat harvesting was 1.19-1.26 g/cm³, after corn harvesting - 1.20-1.27 g/cm³, after spring wheat harvesting - 1.18-1.26 g/cm³, after oats harvesting - 1.20-1.27 g/cm³, after peas harvesting - 1.23-1.30 g/cm³. Deviations from the control option were significant and varied from 0.07 to 0.14 g/cm³ in 2015, from 0.08 to 0.15 g/cm³ in 2016, from 0.08 to 0.16 g/cm³ in 2017, in 2018

- from 0.07 to 0.15 g/cm³, and in 2019 - from 0.07 to 0.14 g/cm³.

The most significant decrease in the equilibrium density in the arable layer was observed against the background of the action and aftereffect of urban wastewater precipitation in combination with zeolite. The value of the equilibrium density against the background of their combined action and aftereffect significantly decreased in relation to the control in 2015 by 0.10-0.19 g/cm³, in 2016 - by 0.12-0.19 g/cm³, in 2017 - by 0.14-0.21 g/cm³, in 2018 - by 0.13-0.20 g/cm³, and in 2019 - by 0.12-0.19 g/cm³.

An analysis of the total crop productivity by the method of conversion the crop into grain units made it possible to express the quantitative side of the resulting production (Arefyev et al., 2019). Studies conducted in the period from 2014 to 2019 showed the high efficiency of unilateral action and aftereffect of the urban wastewater sludge, as well as in combination with zeolite in the cultivation of grain crops.

Without the use of urban wastewater sludge and zeolite-containing agronomic ore, the total productivity of grain crops increased to 14.49 t/ha in standard units (s.u.). Despite the one-sided effect and consequences of a zeolite-containing agronomic ore, the total crop productivity was 16.23 t/ha s.u., exceeding the control by 1.74 t/ha s.u. or 12.0% (Table 5).

Table 5. The productivity of grain crops, t/ha

Variant	Total grain crops productivity	Deviation from control	
		t/ha s.u.	%
1. Without USS and zeolite (control)	14.49	–	–
2. Zeolite 10 t/ha	16.23	1.74	12.0
3. USS 100 t/ha	19.84	5.35	36.9
4. USS 120 t/ha	20.81	6.32	43.6
5. USS 140 t/ha	21.74	7.25	50.0
6. USS 160 t/ha	22.87	8.38	57.8
7. USS 180 t/ha	23.01	8.52	58.8
8. USS 100 t/ha + zeolite 10 t/ha	21.41	6.92	47.8
9. USS 120 t/ha + zeolite 10 t/ha	22.37	7.88	54.4
10. USS 140 t/ha + zeolite 10 t/ha	23.18	8.69	60.0
11. USS 160 t/ha + zeolite 10 t/ha	24.44	9.95	68.7
12. USS 180 t/ha + zeolite 10 t/ha	24.63	10.14	70.0

The effect and aftereffect of urban wastewater sludge without a zeolite-containing agronomic ore increased the crop productivity, depending on the sludge dose, by 5.35 (USS 100 t/ha) - 8.52 t/ha s.u. (USS 180 t/ha), or by 36.9-58.8%. The complex effect and consequences of the urban sewage sludge with zeolite-containing agronomic ore increased the productivity of grain crops by 6.92-10.14 t/ha s.u. or 47.8-70.0%.

CONCLUSIONS

The unilateral action and consequences of the urban sewage sludge with rates from 100 to 180 t/ha and their action and consequences in combination with zeolite-containing agronomic ore provided the maximum accumulation of humus in the arable layer of meadow-chnozem soils. The humus content during the unilateral action of urban wastewater sludge and in combination with zeolite-containing agronomic ore exceeded the initial value at the end of the research by 0.16 (USS 100 t/ha) - 0.36% (USS 180 t/ha + zeolite 10 t/ha).

The most significant effect on the acidity of the soil was exerted by the complex effect and consequences of urban sewage sludge and zeolite-containing agronomic ore. The pH value at the end of the studies varied from 6.58 to 6.84 units, exceeding the initial values by 1.22-1.47 units.

The action and consequences of the urban sewage sludge with dose of 160 and 180 t/ha and the urban sewage sludge with dose of 120 to 180 t/ha in combination with zeolite-containing agronomic ore ensured the transfer of soils from a class with an unsatisfactory structural state to a class with a good one. The number of water-resistant aggregates at the same time exceeded the initial value by 17.0-23.1% and amounted to 55.6-61.6% after pea harvesting in 2019.

REFERENCES

- Alvarenga, P., Rodrigues, D., Mourinha, C., Tarelho, L. A.C., Rodrigues, S. (2019). Use of wastes from the pulp and paper industry for the remediation of soils degraded by mining activities. Chemical, biochemical and ecotoxicological effects. *Science of the Total Environment*, 1152–1163.
- Arefyev, A.N., Kuzina, E.E., Kuzin, E.N. (2017) Changes in the fertility of leached chernozem soils depending on the nature of anthropogenic impact on the soil. *Volga Region Farmland*, 3(44), 9–16.
- Arefyev, A.N., Kuzin, E.N., Kuzina, E.E., Stehlmakh K.N. (2019). Action and consequences of urban sewage sludge in combination with zeolite-containing agro-ore on water availability and productivity of grain-pasture crop rotation. *Volga Region Farmland*, 1(1), 6–10.
- Arefyev, A.N., Kuzin, E.N., Kuzina, E.E. (2019). Effect and aftereffect of urban sewage sludge (USS) and their combinations with zeolite-containing agro-ore on the acid-basic properties of meadow-black soil. *Volga Region Farmland*, 3(52), 62–67.
- Korolev, A.A. (2007). Changes in the agro-reclamation state of leached chernozem under the influence of zeolite-bearing rocks, defecate and organic fertilizers in the conditions of the forest-steppe Volga region. *Scientific Papers on PHD in agriculture*. Penza.
- Korchagin, A.A., Mazirov, M.A., Shushkevich, N.I. (2011). *Soil physics: lab-workshop*. Vladimir: Publishing House of Vladimir State University.
- Mitri, G., Nasrallah, G., Gebrael, K., Masri, N., Choueiter, D. (2019). Ssessing land degradation and identifying potential sustainable land management practices at the subnational level in Lebanon. *Environmental Monitoring and Assessment*, 191(9), 567–572.
- Melece, L., Shena, I. (2019). Soil degradation and possible mitigation measures in Latvia. *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*, 19(3.2), 395–402.
- Proskoryakova, M.V. (2005). Agricultural reclamation techniques for maintaining soil fertility in the forest-steppe of the Middle Volga. *Scientific Papers on PHD in agriculture*. Penza.

CHANGE OF CHERNOZEMS SALT REGIME IN IRRIGATED AND POST-IRRIGATED PERIODS

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Abstract

The results of studies of chernozems salt regimes in the forest-steppe and steppe of Ukraine during irrigation, in post-irrigated period and their non-irrigated analogues are presented. In irrigated soils accumulation of salts up to the level of classification-significant changes when using "suitable" and "limited suitable" waters (national classification of the irrigation water quality) not observed. With long-term use of "unsuitable" water for irrigation, the degree of soil salinization is low. Salt regimes of post-irrigated chernozems are characterized by a tendency to restore their parameters to values characteristic of non-irrigated analogues. The intensity of transformations depends of the degree of soil changes during irrigation, the climatic features, soil properties and duration of post-irrigated period. In post-irrigated period after long-term irrigation by "suitable" and "limited suitable" waters, forest-steppe and steppe chernozems restore the salt regime to the level of non-irrigated analogues in 4-12 years. The ending of irrigation with "unsuitable" waters of the steppe chernozems also make for their desalinization, however, it is needed a longer period of time. The predictive model of the soil processes direction in post-irrigated period is developed.

Key words: desalinization, irrigated soils, irrigation water, salinization, salt regime.

INTRODUCTION

An analysis of natural conditions of Ukraine (Baliuk et al., 2018) and current trends of climate transformation on different spatial scales (e.g. global, continental, regional, watershed-scale) (Fischer et al., 2007; Gondim et al., 2012; Save et al., 2012) gives reason to believe that high-productivity agriculture in the steppe and forest-steppe zones of Ukraine is possible only with irrigation. There are three climatic zones: excessively moistened Forest (25% of the territory), insufficiently moist Forest-steppe (35%) and arid Steppe (40%) in Ukraine. Crops are grown in conditions of insufficient natural moisture on 75% territory of the country. Moisture deficiency is a major factor limiting productivity in these regions.

The country's food and resource supply depends to a large extent on the availability, condition and efficiency of the use of the irrigated land (Bezugliy et al., 2012; Stashuk et al., 2009; Gadzalo, 2018; EU Water Framework Directive, 2000; United Nations Convention, 1994)). The irrigated lands occupied the greatest area (2.6 mln. ha) at the beginning of the 90's of past century, which is

8% of area of plowed land. In recent years in the Ukraine the areas of the irrigated lands has decreased (predominantly spontaneously, without control). Currently, irrigation sector is facing a period of unprecedented challenge. Today, only 500-600 thousand hectares are irrigated in Ukraine. Large areas of previously irrigated lands are being used for rainfed farming. After years of reduced irrigation the need for rehabilitation and modernization is becoming ever more pressing.

At present, it has to be admitted that under the influence of the existing system of agriculture in Ukraine, there is often a deterioration of the soil-reclamation state of the land, loss of soil fertility and the imbalance of natural systems in general (Baliuk et al., 2018). The Sustainable Development Goals identify the need to restore degraded soils and improve soil health. At the 39th session of the FAO Conference, members unanimously approved a new edition of the World Soil Charter as an instrument for promoting and institutionalizing the sustainable use of soil resources at all levels (FAO, 2015). According Revised World Soil Charter (2015): "Soil management is sustainable if the supporting, provisioning, regulating, and

cultural services provided by soil are maintained or enhanced without significantly impairing either the soil functions that enable those services or biodiversity". Voluntary Guidelines for Sustainable Soil Management (FAO, 2017) were developed on account of concerns about the state of soils resulted. In a broader context, the 2030 Agenda for Sustainable Development adopted a number of related targets in 2015, i.a. those aimed at restoring degraded soil, striving to achieve a land degradation-neutral world and implementing resilient agricultural practices that progressively improve soil quality and minimize soil contamination. Sustainable soil management strongly contributes to collective efforts towards climate change adaptation and mitigation, combating desertification and promoting biodiversity, and therefore has specific relevance to the United Nations Framework Convention on Climate Change (UNFCCC) (1992) and United Nations Convention to Combat Desertification (UNCCD) (1994).

Irrigation restoration is a key tool development of the agrarian sector of economy and increase of export potential of Ukraine. It will minimize the impact of climate change on the processes of socio-economic development. The Cabinet of Ministers of Ukraine approved Irrigation and drainage strategy of Ukraine until 2030 (Strategy) in 2019. It is a nationwide cross-sectoral policy document. The scope of the Strategy is to ensuring sustainable eco-balanced agriculture development in Ukraine. The authors participated in the development of the Strategy. Our research focused on the necessary to clarify the soil aspects of the implementation of the Strategy in Ukraine given the current state of irrigated lands, the need to restore and increase the area of irrigated lands, the significant prevalence of degradation phenomena.

The adoption of the Strategy makes possible to use post-irrigated soil in irrigated agricultural again.

In this context, special studies of the modern status of irrigated and post-irrigated soils are necessary.

Studies of salt regimes in these soils are especially relevant to evaluate possibility and preventing the development of degradation

processes (Aidarov, 1978; 2012; Aidarov & Zavalin, 2015; Baliuk, 2016; 2017; EU Association, 2017; Vargas, 2018).

The aim of our study was to reveal the character of salt regime changes in the irrigated and post-irrigated soils in the forest-steppe and steppe of Ukraine (Kharkiv and Donetsk regions). The influence of irrigated and non-irrigated periods on the soil properties was studied.

MATERIALS AND METHODS

About the direction of the salinization-desalinization processes we judged from the analysis of long-term data on the contents of soluble salts, the composition of exchangeable bases and pH in the soil profile. In order to reveal the character of changes in the soils, we compared the properties of the post-irrigation soils with the soil properties during the irrigation period and non-irrigated areas.

Each studied stationar was characterized by a pit, soil samples were taken from the genetic horizons. In addition, auger samples were taken on experimental sections from boreholes.

The soluble salt content in water extracts, the composition of exchangeable bases and pH of water suspensions (with the soil-to-solution ratio of 1: 5) were determined in the laboratory. The average values of total soluble salt, exchangeable bases and pH were calculated from 10-15 point values.

The data on the contents of soluble salts, the composition of exchangeable bases, pH of water suspensions in the irrigated soils were taken from authors archived materials.

Grakovo stationar (Chuguevsky district of Kharkiv region) is located in the southern part of the Left-Bank Forest-Steppe of Ukraine with chernozem typical (Chernozems Chernic, WRB). The studies were conducted in long-term stationary field experience. Experience was founded in 1967-1968. Irrigation continued from 1970 to 1990. Since 1991, no irrigation was carried out.

Mineralization of irrigation water during irrigation was 0.4-0.6 g/l. Options: non-irrigated areas without fertilizers (control), excluded from irrigation without fertilizers and with aftereffect of mineral fertilizers.

Irrigation was carried out by DDA-100 M sprinkling machines with using "suitable" waters (national classification of the irrigation

water quality). Irrigation norms in experiment were: 380-2380 m³/ha. Groundwater depth was 9.4-9.9 m.

Pervomaysk stationar (Pervomaysky district of Kharkov region) is located within the northern part of the Left-Bank Steppe with ordinary chernozem (Chernozems Chernic, WRB). The experimental sections are located on non-irrigated and irrigated arable land under automorphic conditions. Irrigation continued from 1977 to 1995. Irrigation has not been carried out since 1996.

Mineralization of irrigation water during irrigation was 1.1-1.2 g/l. Water is limitedly suitable for irrigation because of the danger of soil alkalization and, at certain times, water sampling - because of the risk of soils salinization. Irrigation during irrigation was carried out by DDA-100 M sprinkling machines. The irrigation rate ranged from 300-500 to 1000-1500 m³/ha. Groundwater depth was from 1.5 to 7.0 m.

Maryinka stationar (Maryinsky district of Donetsk region) is located within the northern part of the Left-Bank Steppe of Ukraine with ordinary chernozem (Chernozems Chernic, WRB). The experimental sections are located on non-irrigated and irrigated arable land under automorphic conditions. Irrigation continued from 1965 to 1995. Irrigation has not been carried out since 1996.

Mineralization of irrigation water during irrigation was 2.9-3.1 g/l. Water is unsuitable or limited suitable for irrigation due to the risk of alkalization and salinization of the soil. Irrigation during irrigation was carried out by DDA-100 M sprinkling machines. The irrigation rate ranged 1500-3000 m³/ha. Groundwater depth was more than 10 m.

In present irrigated lands of studied stationars are being used for rainfed farming.

RESULTS AND DISCUSSIONS

The content of soluble salts in irrigated soils depends on the quality of irrigation water, groundwater depth and lithological composition of parent materials (Lyubimova & Novikova, 2016). Researches by S.A. Balyuk on Grakovo stationar (Stashuk et al., 2009) during the irrigation period established that, using fresh water, salt reserves form a stable salt regime in a long-term cycle, seasonal invertible

seasonality with insignificant (0.01-0.05%) dynamics. At the same time, irrigation causes a noticeable transformation of the qualitative composition of salts. The total toxic alkalinity (HCO₃-Ca) increases slightly (by 0.01-0.14 meq/100 g). The content of water-soluble sodium increases, and the calcium content tends to decrease. As a result of this, the ratio of calcium to sodium (Ca:Na) narrows (Table 1).

Table 1. Change of salt regime in chernozems of Grakovo stationar

Option	Depth, cm	Total soluble salt, %	Na ⁺ , mmol (equiv.) /100 g of soil	Ca:Na
Non-irrigation	0-30	0.06	0.11	6.2
	30-50	0.08	0.10	11.6
	50-100	0.09	0.07	17.6
Irrigation, 20 years	0-30	0.07	0.24	2.4
	30-50	0.09	0.21	4.4
	50-100	0.09	0.20	4.5
Post-irrigation, 10 years	0-30	0.04	0.12	3.8
	30-50	0.08	0.15	6.2
	50-100	0.09	0.16	6.1

In the post-irrigation period, the total amount of water soluble salts decreased. The decrease was due to sodium bicarbonates and sulfates. In 10 year after irrigation, the studied soils of Grakovo stationar according to salt characteristics corresponded to non-irrigated analogues. In this time the Ca:Na ratio in the soil of the studied variants reached the level of rainfed control.

In post-irrigation soils, a gradual decrease in sodium content is observed, which is a natural consequence of the influence of fresh atmospheric precipitation, and after 10 years, the studied options reached the level of rainfed. Research by S.A. Balyuk on Pervomaysk stationar (Stashuk et al., 2009) during the irrigation period established that using by irrigation with weakly mineralized water (1.1-1.2 g/l) caused a slight increase in salt content due to sodium chlorides and sulfates, but to a depth of 2 m the salt content did not exceed the toxicity threshold. The concentration of chlorine ion in the soil profile reaches the toxicity threshold of 0.3 mmol (equiv.)/100 g of soil. The author noted an increase in the salt content from spring to autumn, which were washed out by precipitation during the cold period. Moreover, along with an increase in the

content of water-soluble sodium, there is a noticeable decrease in the calcium content and, as a consequence, a narrowing of the ratio of Ca to Na (Table 2).

In the period after irrigation, the total amount of water-soluble salts decreased. In 10 years after irrigation, the studied soils of Pervomaysk stationar of total soluble salts content corresponded to non-irrigated analogues. The Ca:Na ratio in the studied soil reached the level of rainfed control. In the soils after irrigation, the content of water-soluble sodium was a gradual decrease. After 10 years, its content in the soil layer of 0-50 cm reached the rainfed level. In deeper layers, the sodium content remains somewhat elevated.

Table 2. Change of salt regime in chernozems of Pervomaysk stationar

Option	Depth, cm	Total soluble salt, %	Na ⁺ , mmol (equiv.)/100 g of soil	Ca:Na
Non-irrigation	0-30	0.09	0.06	8.2
	30-50	0.10	0.09	8.0
	50-100	0.11	0.08	12.6
Irrigation, 18 years	0-30	0.08	0.29	1.0
	30-50	0.11	0.36	2.7
	50-100	0.14	0.32	4.7
Post-irrigation, 10 years	0-30	0.08	0.06	8.6
	30-50	0.10	0.08	8.7
	50-100	0.12	0.16	8.6

Researches by L.I. Vorotyntseva on Maryinka stationar during the irrigation period established that using by irrigation with mineralized water (2.9-3.1 g/l) caused an increase in salt content due to magnesium and sodium chlorides and sulfates in the 2 m soil layer (Vorotyntseva, 2016; 2017). Significant qualitative and quantitative changes in the composition of water-soluble salts are noted: the content of water-soluble sodium increases significantly, the calcium content decreases markedly, the ratio of Ca to Na sharply narrows to values characteristic of highly and medium-degraded soils (0.2-0.5) (Table 3). The horizon of salt accumulations is noted at a depth of 100-150 cm.

In the post-irrigation period, halochemical processes gradually fade and desalination processes developing the soil layer of 0-25 cm. In 16 years after irrigation, the studied soils of Maryinka stationar of total soluble salts content corresponded to non-irrigated analogues in the soil layer 0-25 cm only. The Ca:Na ratio in the

in the upper half-meter layer of studied soil increased, but it didn't reach the level of rainfed control. The content of water-soluble sodium in the lower horizons remained high due to prolonged irrigation with mineralized water and aridity. The Ca:Na ratio in deeper layers of the studied soil did not increase significantly.

Table 3. Change of salt regime in chernozems of Maryinka stationar

Option	Depth, cm	Total soluble salt, %	Na ⁺ , mmol (equiv.)/100 g of soil	Ca:Na
Non-irrigation	0-30	0.09	0.12	6.8
	30-50	0.09	0.22	4.0
	50-100	0.07	0.08	7.0
Irrigation, 30 years	0-30	0.13	1.20	0.2
	30-50	0.13	0.96	0.4
	50-100	0.16	1.20	0.5
Post-irrigation, 16 years	0-30	0.10	0.19	4.2
	30-50	0.12	0.38	2.1
	50-100	0.11	0.56	1.1

The content and composition of exchange bases allows us to evaluate changes in soil quality. Changes in the composition of the soil-absorption complex are caused by irrigation water of any composition, however, the severity of these changes may vary depending on the quality of irrigation water, the initial soil properties and ecology-ameliorative condition of irrigated land.

In the soil of the Grakovo stationar, the content of exchangeable calcium tends to a gradual decrease during irrigation with fresh water. At the same time, the content of exchangeable sodium and potassium increases from 0.6-1.0% to 1.4-1.9% (Figure 1). An increase in the content of absorbed sodium takes place to a depth of 2 to 3 m.

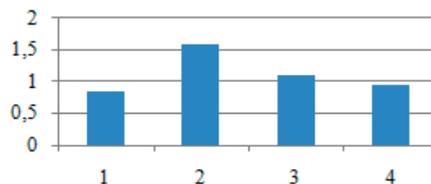


Figure 1. Exchangeable sodium and potassium in soil surface layer (0-30 cm) of the Grakovo stationar, % (1 - non-irrigation; 2- irrigation, 20 years; 3 - post-irrigation, 10 years; 4 - post-irrigation, 12 years)

In the post-irrigation period, the composition of the soil absorbing complex undergoes a reverse

transformation and approaches its initial state under the influence of fresh atmospheric precipitation. This is expressed in a noticeable decrease in the content of exchangeable sodium, the content of exchangeable calcium almost does not change.

The contents of all exchangeable cations in post-irrigation soils return to non-irrigated control for about 12 years.

In the soil of the Pervomaysk stationar, the content of exchangeable calcium also tends to a gradual decrease during irrigation with weakly mineralized water. At the same time, the content of exchangeable sodium and potassium increases from 1.2-1.8% to 2.4-4.6% (Figure 2), and the content of exchangeable potassium increases too. Soil chemical properties have been altered by long-term irrigation with weakly mineralized water - irrigation caused a slight degree of soils secondary salinization.

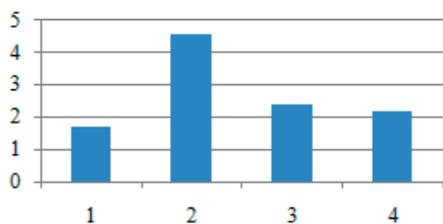


Figure 2. Exchangeable sodium and potassium in soil surface layer (0-30 cm) of the Pervomaysk stationar, % (1 - non-irrigation; 2 - irrigation, 18 years; 3 - post-irrigation, 5 years; 4 - post-irrigation, 10 years)

In the post-irrigation period, we observe the process of soil desalinization: the content of exchangeable sodium is significantly reduced. The content of exchangeable calcium is practically unchanged.

The total content of exchangeable cations in post-irrigation soils does not return to non-irrigated control over 10 years of observation.

Long-term irrigation with mineralized waters induces the development of degradation processes (secondary salinization and alkalization) in soil of Maryinka stationar. The process of soil alkalization reached an average degree, and the amount of exchangeable sodium and potassium was 6.1-7.1% of the amount of exchangeable cations (Figure 3). The content of exchangeable calcium decreases during irrigation with mineralized water.

In the post-irrigation period, the process of soil dealkalinization begins: the content of exchangeable sodium is significantly reduced and corresponds to slight degree of soils secondary alkalization. The content of exchangeable calcium is practically unchanged.

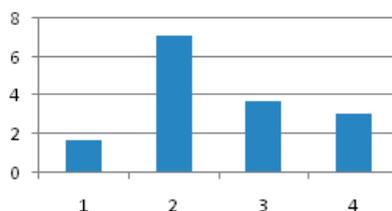


Figure 3. Exchangeable sodium and potassium in soil surface layer (0-25 cm) of the Maryinka stationar, % (1 - non-irrigation; 2 - irrigation, 30 years; 3 - post-irrigation, 6 years; 4 - post-irrigation, 16 years)

The total content of exchangeable cations in post-irrigation soils does not return to non-irrigated control over 16 years of observation.

To predict the development of soil processes in Maryinka stationar, the method of exponential smoothing was used.

$S_t = S_0 + at$; S_0 - the value of the indicator at the time of the last observation from which the calculation is made; a - coefficient calculated from experimental data; t - the period for which the forecast is developed, year.

Soil evolution in post-irrigated period is directed toward approaching the parameters of the non-irrigated analog. According to forecast model, processes desalinization and dealkalinization will continue depending on time and weather conditions. The composition of the soil absorption complex, which underwent changes during irrigation by mineralized water, is restored more slowly than the salt composition. Ordinary chernozem will reach the level of non-irrigated analogue on the content of exchangeable sodium and potassium for 37 years, on the content of toxic salts - 28 years (0-25 cm).

Changes in pH are one of the main indicators of salinity. The variation of pH in the surface layer was from 0.1-0.2 (Grakovo stationar) to 0.3-0.5 (Pervomaysk and Maryinka stationars).

The rise in pH was also observed in the lower part of the profile of studied soils. In the post-irrigation soils pH in the surface layer is reduced. It does not return to non-irrigated

control in soils of Maryinka stationar. The variation of pH was not significant in the lower part of the profile of post-irrigation soils.

CONCLUSIONS

Long-term irrigation with «suitable» and «limited suitable» waters did not induces accumulation of salts in soil. However, it caused an increase in the content of water-soluble sodium, a decrease in the calcium content, and the pH changed. Long-term irrigation with «unsuitable» waters also induces salt accumulation in soil.

Irrigation induced increase of exchangeable sodium and potassium in soil and decrease of exchangeable calcium.

Salt regimes of post-irrigated chernozems are characterized by a tendency to restore their parameters to values characteristic of non-irrigated analogues. The intensity of transformations depend of the degree of soil changes during irrigation, the climatic features, soil properties and duration of post-irrigated period. In post-irrigated period after long-term irrigation by «suitable» and «limited suitable» waters, forest-steppe and steppe chernozems restore the salt regime to the level of non-irrigated analogues in 4-12 years. The ending of irrigation with “unsuitable” waters of the steppe chernozems also make for their desalinization, however, it is needed a longer period of time. The predictive model of the soil processes direction in post-irrigated period is developed - ordinary chernozem will reach the non-irrigated analogue on the content of exchangeable sodium and potassium for 37 years.

REFERENCES

- Aidarov, I.P. (2012). *Ecological basis for land amelioration*. Moscow. 163 pp.
- Aidarov, I.P., Zavalin, A.A. (2015). *The basis for complex land amelioration (theory and practice)*. Moscow. 128 pp.
- Averianov, S.F. (1978). *Salinity control within irrigated lands*. Moscow. 276 pp.
- Baliuk, S., Nosonenko, A., Zakharova, M., Drozd, E., Vorotyntseva, L., Afanasyev, Y. (2017). Criteria and Parameters for Forecasting the Direction of Irrigated Soil Evolution, in: D. Dent and Y. Dmytruk (eds.), *Soil Science Working for a Living*, Spr. Int. Pub., 149–159.
- Baliuk, S., Drozd, E., Zakharova, M. (2016). Scientific approaches to the rational use and management of saline soils fertility in Ukraine. *Arid Lands Studies (JALS)*, 25(3), 69–72.
- Baliuk, S.A., Medvedev, V.V., Nosko, B.S. and Eds. (2018). *Adaption of agrotechnologies to climate change: soil-agrochemical aspects*. Kharkiv, 364.
- Bezugliy, M.D., Baliuk, S.A., Truskavetskiy, R.C. (2012). Soils and Their Fertility in Legal Field of Land Market Relations. *News of agrarian science*, 5, 5–10.
- EU Water Framework Directive. Official Journal of the European Communities (2000). L 327, 1–72.
- EU Association Agreement between the European Union and its Member States, of the one part, and Ukraine, of the other part (2017). Retrieved January 5, 2020, from www.kmu.gov.ua/storage/app/media/uploaded-files.pdf.
- FAO (2015). Revised World Soil Charter. Rome, Italy, 10.
- FAO (2017). Voluntary Guidelines for Sustainable Soil Management. Rome, Italy, 26.
- Fischer, G., Tubiello, F.N., van Velthuizen, H., Gondim, R.S., de Castro, M.A.H., Maia, A.D.N., Evangelista, S.R.M., Fuck, S.C.D. (2012). Climate change impacts on irrigation water needs in the Jaguaribe River Basin. *Journal of the American Water Resources Association*, 48(2), 355–365.
- Gadzalo, Y., Romashchenko, M., and Yatsiuk, M. (2018). Conceptual framework to ensure water security in Ukraine. *Proc. IAHS*, 376, 63–68.
- Irrigation and drainage strategy of Ukraine until 2030. *Official Bulletin of Ukraine* of 13.09.2019-2019, No 70, Article 2473, 194, act code 95918/2019. Retrieved January 5, 2020, from <https://zakon.rada.gov.ua>
- Lyubimova, I.N., Novikova, A.F. (2016). Changes in the Properties of Solonetzic Soil Complexes in the Dry Steppe Zone under Anthropogenic Impacts. *Eurasian Soil Science*, 49(5), 581–590.
- Save, R., de Herralde, F., Aranda, X., Pla, E., Pascual, D., Funes, I., Biel, C. (2012). Potential changes in irrigation requirements and phenology of maize, apple trees and alfalfa under global change conditions in Fluvia watershed during XXIst Century: results from a modeling approximation to watershed-level water balance. *Agricultural Water Management*, 114, 78–87.
- Stashuk, V., Baliuk, S., Romashchenko M. (2009). The scientific basis for the protection and management of irrigated land in Ukraine. Kiev, *Agricultural Science*, 619.
- United Nations (1992). United Nations Framework Convention On Climate Change, Retrieved 2016, 33.
- United Nations (1994). Convention to Combat Desertification In those Countries Experiencing Serious Drought And/Or Desertification, Particularly In Africa, A/AC.241/27, 58. Retrieved January 5, 2020, from www.unccd.int/convention/text/pdf/conv-eng.pdf
- United Nations (2015). Transforming our world: the 2030 Agenda for Sustainable Development, A/RES/70/1, 41. Retrieved January 5, 2020, from www.sustainabledevelopment.un.org/post2015/transformingourworld/publication.

- Vargas, R., Pankova, E.I., Baliuk, S.A., Krasilnikov, P.V. and Khasankhanova G.M. (2018). Handbook for saline soil management. FAO and Lomonosov Moscow State University, 144.
- Vorotyntseva, L.I. (2017). Irrigated soils of Donetsk region: ecology-ameliorative condition, complex of measures for the protection and rational use. Kharkiv, 207.
- Vorotyntseva, L.I. (2016) Transformation of properties of chernozem ordinary under irrigation by waters of different quality. *Bulletin of Agricultural Science*, 1, 56–60.

RECYCLED PAPER CRUMBLE; BENEFITS AND LIMITATIONS AS A LIVESTOCK BEDDING AND A SOIL ENHANCER

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Abstract

*Soil productivity and quality is influenced by the application of different fertilisers and soil structure enhancers to provide optimal crop growth and protect ecosystems and the environment. Commonly, straw is used as a livestock bedding material and is applied to land once it has been discarded. The use of straw is becoming less sustainable due to competition from the bioenergy market and adverse weather patterns affecting costs. This means an effective alternative is needed. This study presents an insight into Recycled Paper Crumble (RPC) applied to soil, once discarded from livestock enclosures in the form of farmyard manure (FYM). Analysis of soil nutrients and ecosystem studies were undertaken, and additionally, method of application, contrasting digging in (ploughing) and applying to surface (mulch) to provide knowledge for agriculturalists, horticulturalists and ecologists. Ecosystem studies were undertaken at week nine using the mustard extraction method. Few distinguishable differences were found between RPC and FYM applications with both adding to the nutritive values of the soil and being effective for earthworm (*Lumbricus terrestris*) activity. Significant increases in pH ($P < 0.001$) were found in RPC FYM applications suggesting potential as a lime replacement. In addition, no significant differences ($P > 0.05$) were found in organic matter, between applications and its depletion, seen over time. Ecosystems analysis concluded that RPC show significant difference compared to straw in the number of earthworm's present ($P < 0.01$). The findings in this study indicate RPC is a suitable alternative to straw as an all-round dual-purpose livestock bedding material and soil enhancer.*

Key words: bioenergy, earth worms, ecosystem, livestock bedding material, recycled paper crumble, soil structure enhancer.

INTRODUCTION

The aim of this research was to consider alternatives to straw for use as a dual-purpose product (livestock bedding/FYM), and to develop an understanding of new varieties of organic fertilisers with the intention of enhancing the nutritive value and structure of soil whilst being beneficial from an ecological aspect. The objective of this study was to prove whether Recycled Paper Crumble (RPC) provided a viable alternative to commonly used straw in terms of analysing breakdown and nutritive content of RPC Farmyard Manure (FYM) in comparison with straw FYM, to observe the effects on soil health and ecosystem habitats. Understanding the implication of fertiliser application to soil and how it can influence nutrient quality, fertility, outputs, soil structure, ecosystems and the environment is of vital importance for sustainable agriculture (Holland et al., 2018).

Research by Khan et al. (2013) refers to product uniformity to gain consistent soils. A level of inconsistency is shown in various fertilisers when adding $\text{NO}_3\text{-N}$. Ploughing in retains 90% of available N (Masvaya et al., 2017). A study undertaken by Luebbe et al. (2011) found fresh FYM (stored less than three months) applied to surface on medium/heavy soils will only supply 15% of total N. Ploughing is an effective method to boost $\text{NO}_3\text{-N}$. Research to magnify the differences between applications to surface and ploughing in is needed to limit environmental impacts and enhance value of N in FYM which is often lost to volatilisation leading to eutrophication (Wu et al., 2019).

Soil minerals account for half the soil volume and serve as sources and sinks of essential plant nutrients (Sanz et al., 2018). The type, proportion and concentration determine properties such as texture, structure and cation exchange capacity (CEC). Potassium (K) availability in plants is highly dependent on its release from the weathering of primary soil minerals (Behera et

al., 2015). K, an abundant essential element, varies from 0.5-2.5% of soil mass providing plant life with various functions (Hillel, 2008). Huang et al. (2005) states 98% of total available K is bound in mineral form, the remaining 2% is in soil solution and exchangeable phase affecting availability. K deficiency is often caused by extreme pH levels, liming, lack of oxygen or true soil deficiency (Potash Development Association, 2011). Research by Quan et al. (2005) suggests no visible off-site environmental problems are present after K leaves the soil system being non-toxic and not causing eutrophication in aquatic systems. Phosphorus (P), a macro-element, required for plant nutrition, is found in organic compounds and minerals (Bernardo et al., 2019). UK surface soils have a P content averaging 0.6% (Stuart and Lapworth., 2016). Studies have highlighted concerns regarding application of FYM with abundant P carrying the risk of runoff responsible for eutrophication. A review by Smith et al. (1998) concluded restricting extractable P levels to 70 mg/l minimizes the risk of unnecessary P enrichment and subsequent leaching. This means that land managers may need to reassess their fertiliser applications. Rupp et al. (2018) identified that whilst FYM is an important organic fertiliser, excessive utilization may cause P accumulation and eutrophication of surface waste. Similarly, a pollution survey by Chen et al. (2019) concluded 55% of P pollution from agriculture is coming from FYM. These concerns suggest effective control measures, FYM types and application methods, need consideration to limit negative impacts.

Magnesium (Mg) is mainly present in inorganic compounds although sufficient amounts appear in organic material (OM) (Smith et al.,1998). Effective crop production requires enough Mg for plant metabolic processes and reactions which are adversely affected by Mg deficiencies. Decreased Mg directly correlates with low pH, cold temperatures, desiccation and predominant competing elements such as K and calcium (Ca). Synthetic fertilisers supply Mg but are often insoluble and contain chlorides (Tried and Tested, 2014). This negatively impact on crop quality.

Rothamsted Research (2019) draws on an extensive range of sources to assess earthworm biodiversity in grasslands. Their studies suggest average topsoil has nine earthworms for every five-inch² (2790/m²) of soil compared to high quality soils which are three times higher. Their research concluded 42% of soils have poor earthworm biodiversity with few or no surface dwelling and deep burrowing worms. The absence of deep burrowing worms on 16% of soils significantly affects water infiltration due to lack of vertical burrows (Onrust and Piersma, 2019). Several FYM application methods can be used such as a mulch or ploughing in. A mulch provides OM whilst creating a cool, moist climate which is effective for earthworms (Bertrand et al., 2015). FYM's high in N have potential to create unfavourable conditions for earthworms. N content is at its highest in fresh FYM's so takes several weeks to age to be effective for ecosystems. The use of synthetic fertilisers creates hostile ecosystems as they increase acidity (Onrust and Piersma, 2019). Many nutrients eaten by livestock are excreted increasing the value of FYM as fertiliser (Chadwick et al., 2015). Equal amounts of FYM from different species have different effects on soil composition and nutrient uptake (Larney and Olsen, 2006). A proportion of nutrients obtained from the soil can be lost atmospherically before they can be made available for crop uptake as FYM's decompose during storage and moisture contents reduce. Several studies investigating N losses during storage of cattle FYM show a mass loss range of 7-39% (Larney and Olsen, 2006; Larney et al., 2008; Luebbe et al., 2011). Greater loss estimates reported with a range of 32-54% at 180 days (Rotz and Leytem, 2015) can be related to relatively low crop available nutrients (Table 1).

Table 1. Nutrient availability of different FYM types (Adapted from Tried & Tested, 2014)

Type of FYM and DM content (%)	Total Nutrients (kg/t)			Crop Available Nutrients(kg/t)		
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O
Cattle FYM (25%)	6.0	3.2	8.0	1.2	1.9	7.2
Sheep FYM (25%)	7.0	3.2	8.0	1.4	1.9	7.2
Pig FYM (25%)	7.0	6.0	8.0	1.8	3.6	7.2
Layer FYM (35%)	19.0	14.0	9.5	9.5	8.4	8.6

An investigation by Duruogbo et al. (2007) reported poultry FYM increased soil pH, OM and K, several micronutrients, and decreased salinity. A similar study by Whalen et al. (2000) indicated cattle (*Bos taurus*) and sheep (*Ovis aries*) FYM stored over a short period reduces soil acidity and increases the quantity of P and K and crop available nutrients in each FYM type. This research indicates that species influence FYM quality as differing species can harbour different nutrient qualities. This is dependent on soil type, material type, previous soil management and method of application. Straw is the most used FYM due to its traditional use as a livestock bedding material. Straw is a key source of OM, P, K, Mg and benefit organisms which break down cellulose (Copcea et al., 2017).

Challenges in the straw market including ploughing crop residues in and competition from the biofuel industry. These factors result in an increase in sparseness and cost, specifically in certain areas of the UK where few crops are grown (Venturini et al., 2019). The use of by products, including shavings and woodchip, are viable alternatives, however, these products have never been able to fully compete with straw as an all-round product (Teixeira et al., 2015). This is partly due to the time taken for them to break down especially if treated (Copcea et al., 2017). Overall, there is a requirement for an alternative, dual purpose livestock bedding material and FYM source. There is very little research around types of wastepaper to meet the demand for a dual-purpose material. Paper-based materials are often used as organic amendments rather than fertilisers due to their low mineral contents (Bellamy et al., 1995). Pulp and paper industries generate a mass of solid waste (Azevedo et al., 2019) creating a market for use in agriculture.

According to Royer-Tardif et al. (2019), paper-based material provides a latent alkalinity, controlling pathogens in the soil and increasing pH when applied, reducing the need for lime application (Quaye et al., 2011). The market value of paper-based materials is £40-85/Tonne (Azevedo et al., 2019). Price varies depending on whether the de-inking process is needed to remove toxic compounds (Villagra et al., 2011). Research by Quaye et al. (2011) into the impacts

of paper sludge FYM on soil suggests a major drawback is its relatively high C (carbon): N ratio being detrimental by immobilising N. Applications of FYM with higher N have the potential to relieve deficiencies whilst maintaining organic residues from paper. A pilot study, requested by the product manufacturer Cows & Co Group (2018), was carried out in February 2018, comparing RPC to straw as a material in sheep enclosures. Product effectiveness was proven, with statistical analysis showing lower material temperatures than straw and no significant differences ($P > 0.05$) in the instances of lameness or health issues. Analysis showed no metals were traceable in RPC from the paper making process. This is important as they can be toxic to animals and the environment (Carolin et al., 2017). As part of the pilot study, analysis was undertaken on fresh weight samples of the 2 material types (Table 2) and a further analysis on RPC as to the quality it provides when spread as FYM on land (Table 3). Pilot study findings led to a research project into the use of RPC as a fertiliser and as a soil structure enhancer. It is vital that materials prove effective when applied to soil, to provide required outputs, optimize soil structure and enhance nutrient values.

Table 2. Analysis of RPC in comparison to straw on a fresh weight basis (Authors own, 2018)

Sample type	Dry Matter (%)	pH	Total Nitrogen (kg/t)	Ammonia Nitrogen (kg/t)	Total Phosphate		Total Potassium	
					as P (kg/t)	As P ₂ O ₅ (kg/t)	as K (kg/t)	as K ₂ O (kg/t)
Straw	38.70	8.60	8.91	2.04	1.89	4.33	6.87	8.28
RPC	37.22	8.40	6.90	1.49	1.20	2.74	5.14	6.20

Table 3. RPC FYM value when added to a field based at Lee Farm; March 2018 (Authors own, 2018)

pH	Potassium		Phosphorus		Magnesium		Organic Nitrogen (N storage) mg/litre
	mg/l	Index	mg/l	Index	mg/l	Index	
5.60	250	3.0	38	3.6	180	4.0	4245

MATERIALS AND METHODS

Research carried out at Myerscough College/University of Central Lancashire. Research commenced 21st November 2018, until completion on 23rd February 2019. Soil analysis carried out in the Myerscough College laboratories. All chemicals and consumables were supplied by Fisher Scientific (2019).



Figure 1: Map of Myerscough and Bilsborrow, UK (Red + mark trial plot area) (Google Earth, 2019)

applied on the soil surface/RPC dug, refers to RPC based FYM which had been dug in.

Area A and B were divided into nine trial plots, measuring 183 cm x 122 cm and had an application rate of 6 kg, according to RB209 guidelines (AHBD, 2019). All dug in plots (including control) had the soil turned prior to the investigation. Each plot had a soil sample and moisture content taken prior to treatment (week 0) and then on a three-week basis (weeks 3, 6 and 9) thereafter, allowing the FYM time to degrade into the soil. After initial application of treatments, trial plots were left untouched (other than soil sampling every third week). Soil samples were taken using an auger in a W shape. A Delta-T ML2x Theta Probe was used, inserting the probe into the soil in several places in a W shape. After 30 seconds of recording, the moisture content shown as % vol. on the HH2 meter and the average of each plot was calculated.



Figure 2: Kitchen Garden Trial plot

The trial plots were set up; bedding materials applied were used in sheep enclosures, prior to use in this study. The soil type of the trial plot area was analysed pre-trial. It consisted of 54% sand, 32% silt, 14% clay. The area was split into two sections, A and B. Section A had used material dug in (15 cm depth) and B had used material left on the surface. The plots were set up using a randomized complete block design. Plot numbers were selected for ease of statistical analysis. Plot names: for example, RPC surface, refers to RPC based FYM which had been

After soil sample collection of each plot in a W shape (15 cm depth), the soils were dried in a 30^oC oven for a week before they were ground up using a pestle and mortar and retained until all samples had been collected. All 72 samples were tested together to reduce testing variability. Methods of soil/nutrient content analysis were adapted from the work of Bailey (1985). A 20 ml volume of air-dried soil, ground to pass a 2 mm mesh sieve, was transferred into a 175 ml bottle and 50 ml of calcium sulphate solution added. Bottles were capped and shook on the shaking machine for 15 minutes. Next, they were filtered through 125 mm Whatman No. 2 filter paper into a 60 ml bottle and the filtrate

retained for determination of nitrate-nitrogen. The VWR 2100 L Meter and Orion ISE nitrate electrode were set according to manufactures instructions. Exactly 10 ml of each working standard was added to 10 ml of ISA Buffer. Starting with the lowest concentration, the electrodes were immersed into 1 mg/l nitrate-nitrogen working standard solution ensuring the solution was stirred. The mV readings were recorded when a steady value was obtained. The electrodes were then removed and rinsed. The mV readings for corresponding nitrate working standard solutions (4, 10, 20, 50, 100) were recorded. The temperatures at which the measurements were made were recorded. The standards were constructed on a graph on semi logarithmic paper. The temperature of the extracts was brought to that of the nitrate working standard solutions. The electrodes were immersed into the extract keeping the extract stirred a reading was taken when a steady value was obtained. The electrodes were removed and rinsed after each reading was taken.

A 10 ml sample of air-dried soil, ground to pass a 2 mm mesh sieve, was transferred into a 175 ml bottle and 50 ml of M ammonium nitrate added. The bottle was capped and placed on the shaking machine for 30 minutes. The solution was filtered through a 125 mm Whatman No. 2 filter paper and the filtered extract then retained for the determination of potassium.

The potassium working standard solutions containing 0 and 50 mg/l of potassium were nebulised into natural gas flame. The controls on the Corning 410 Flame Photometer were adjusted until steady at zero and maximum readings were then obtained. The intermediate standard working solutions were nebulised and a graph constructed relating noted meter readings to mg/l of potassium in all the standard solutions.

The content of potassium, phosphorus, magnesium, organic material and pH measurement were calculated using standard laboratory procedures. After the completion of soil sampling, earthworm extraction took place in mid-February to allow the weather to be warmer increasing the chances of worm presence and limiting the chances of frost in the process.

RESULTS AND DISCUSSIONS

Data sets were compiled on Microsoft Excel (2017) and imported onto Minitab (2018) for statistical analysis with the inclusion of descriptive statistics to determine standard deviations (\pm SD). Individual data sets were tested for normality using the Kolmogorov-Smirnov test. Parametric data was analysed using a General Linear Model, One-Way ANOVA's and Tukey Pairwise Comparison's. Where appropriate, data was transformed onto a logarithmic scale for detailed analysis. For all tests conducted, statistical significance was set to $P \leq 0.05$. Letters indicated heterogeneity by Tukey ($P < 0.05$).

Data was analysed for all the parameters that were measured in the laboratory. Data that showed no significant difference or relevance to the hypothesis was not included in the following section. General linear models were used to check for the effect of time, treatment and method of application on each of the analysis undertaken. The space allocated for research was within a working garden and resulted in variation within the soil from week 0, allowing some significant differences between plots.

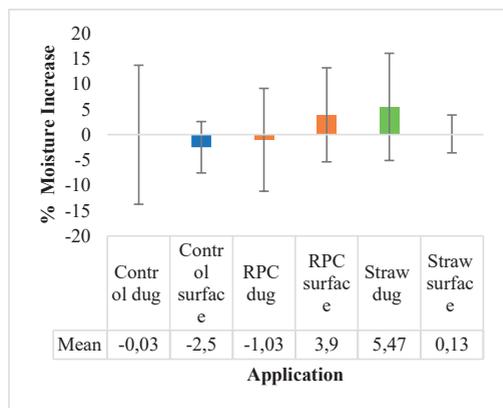


Figure 3. Mean (\pm SD) increase in % moisture of all application types by week nine

No significant difference ($P > 0.05$) could be seen between application types.

ST Dev: 13.72, 5.07, 10.15, 9.28, 10.58, 3.74 ($P = 0.893$, $F = 0.32$, $DF = 5$, $n = 3$).

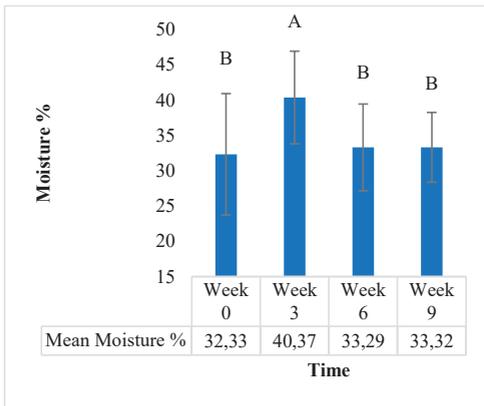


Figure 4. Mean (\pm SD) moisture of 18 plots over the duration of the trial

A significant increase ($P < 0.01$) in moisture content was present in week 3 (A) compared to other weeks (B). ST Dev: 8.59, 6.56, 6.15, 4.94 ($P = 0.005$, $F = 4.78$, $DF = 3$, $n = 18$).

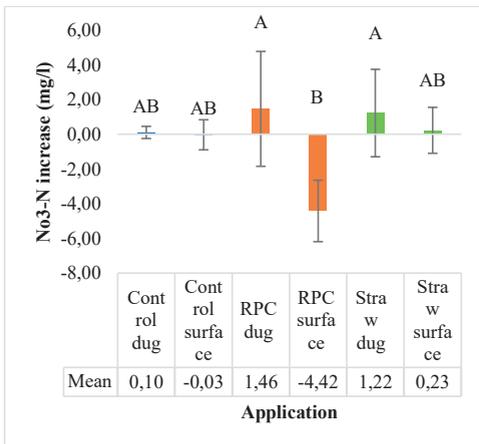


Figure 5. Mean (\pm SD) increase in $\text{NO}_3\text{-N}$ of all application types by week nine

There is a significant difference ($P < 0.05$) in RPC dug in (A) and straw dug in (A) when compared to RPC surface (B). No significant differences ($P > 0.05$) were found between control dug, control surface and straw surface (AB) in comparison to other applications. ST Dev: 0.35, 0.87, 3.31, 1.77, 2.52, 1.32 ($P = 0.033$, $F = 3.56$, $DF = 5$, $n = 3$).

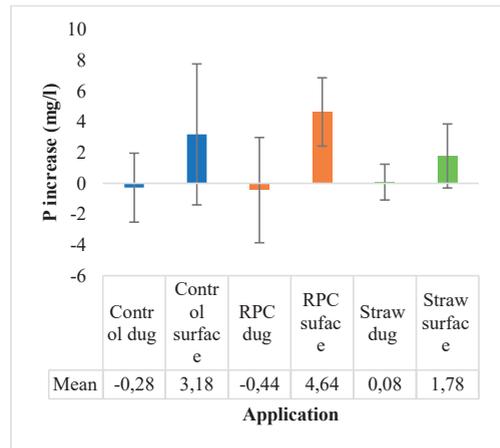


Figure 6. Mean (\pm SD) increase in P of all application types by week nine

There was no significant difference ($P > 0.05$) in any types of application. ST Dev: 2.24, 4.58, 3.42, 2.22, 1.16, 2.08 ($P = 0.229$, $F = 1.62$, $DF = 5$, $n = 3$).

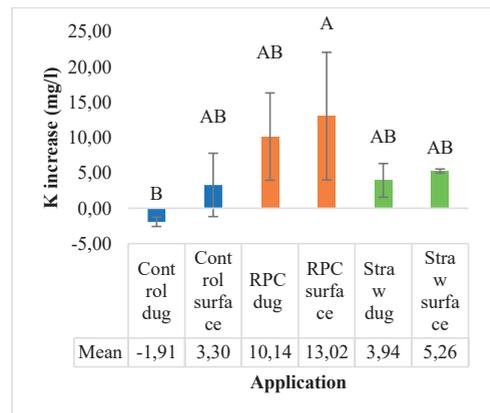


Figure 7. Mean (\pm SD) increase in K of all application types by week nine

There is a significant difference ($P < 0.05$) in RPC on surface (A) in comparison to control dug in (B) No significant differences ($P > 0.05$) were found between control surface, RPC dug straw dug and straw surface (AB) comparison to other applications. ST Dev: 0.66, 4.48, 6.17, 9.03, 2.39, 0.29 ($P = 0.036$, $F = 3.46$, $DF = 5$, $n = 3$).

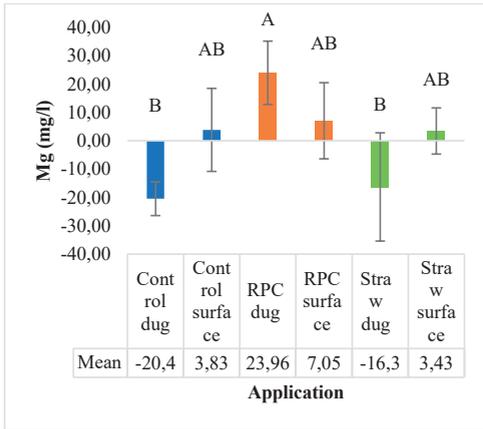


Figure 8. Mean (\pm SD) increase in Mg of all application types by week nine

There is a significant difference ($P < 0.05$) in RPC dug in (A) in comparison to control dug in and straw dug in (B). No significant differences ($P > 0.05$) were found between control surface, RPC surface and straw surface (AB) comparison to other applications. ST Dev: 5.95, 14.65, 11.19, 13.43, 19.10, 8.15 ($P = 0.012$, $F = 4.87$, $DF = 5$, $n = 3$).

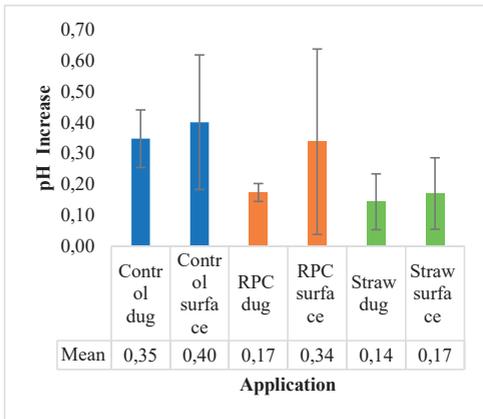


Figure 9. Mean (\pm SD) increase in pH of all application types by week nine

No significant difference was found in application types ($P > 0.05$). ST Dev: 0.09, 0.22, 0.03, 0.30, 0.09, 0.12 ($P = 0.314$, $F = 1.34$, $DF = 5$, $n = 3$).

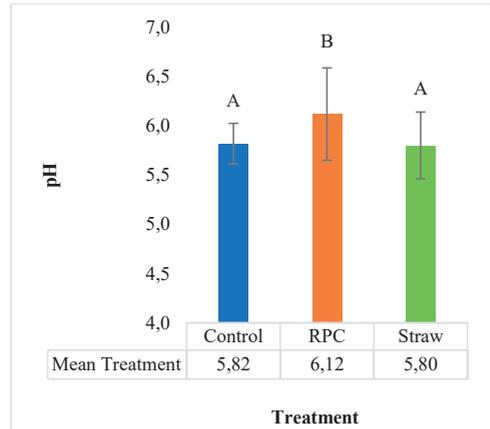


Figure 10. Mean (\pm SD) pH of 18 trial plots throughout the trial considering treatment

There was no significant difference ($P < 0.001$) in the mean pH amongst control (A) and straw (A) treatments, however there was a significant increase ($P < 0.05$) in RPC plots (B). ST Dev 0.21, 0.47, 0.34 consecutively ($P < 0.001$, $F = 18.50$, $DF = 2$, $n = 24$).

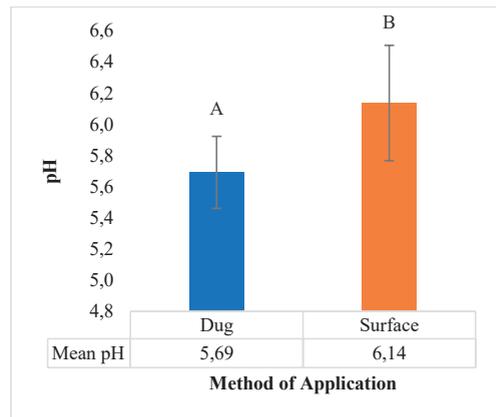


Figure 11. Mean (\pm SD) pH of 18 trial plots throughout the trial considering method of application

There was a significant difference ($P \leq 0.001$) in the pH of surface plots (B) in relation to dug in plots (A). ST Dev: 0.23, 0.37 consecutively ($P < 0.001$, $F = 86.02$, $DF = 1$, $n = 36$).

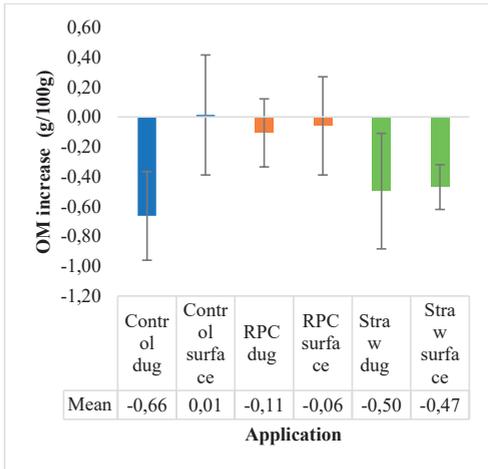


Figure 12. Mean (\pm SD) increase inorganic matter of all application types by week nine

No significant difference was found in applications ($P > 0.05$). ST Dev: 0.30, 0.40, 0.23, 0.33, 0.39, 0.15 ($P = 0.097$, $F = 2.43$, $DF = 5$, $n = 3$).

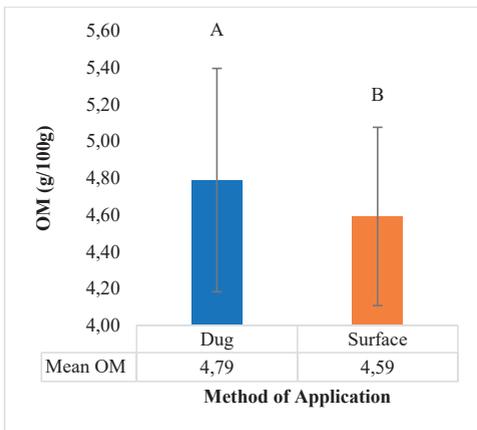


Figure 13. Mean (\pm SD) organic matter throughout the trial in relation to method of application

There was a significant difference ($P < 0.05$) in OM contents in dug in plots (A) in relation to surface plots (B) ST Dev: 0.61, 0.48 ($P = 0.048$, $F = 4.13$, $DF = 1$, $n = 36$).

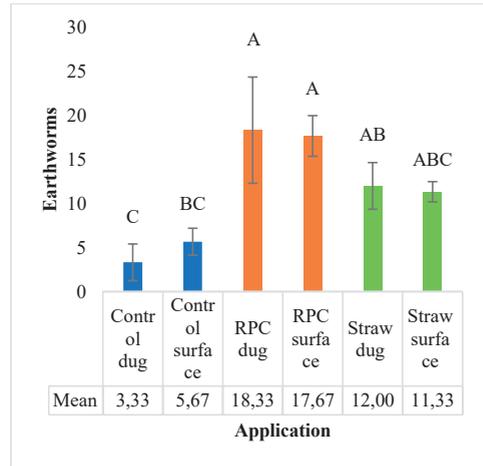


Figure 14. Mean (\pm SD) earthworm content in trial plots in relation to application at week 9

There was a significant difference ($P < 0.001$) in the number earthworms in RPC dug (A) and RPC surface (A) than control dug (C) and control surface (BC). Control dug (C) also had significantly lower ($P < 0.05$) earthworm presence than straw dug (AB) ST Dev: 2.08, 15.3, 6.03, 2.31, 2.65, 1.16 ($P < 0.001$, $F = 11.79$, $DF = 5$, $n = 3$).

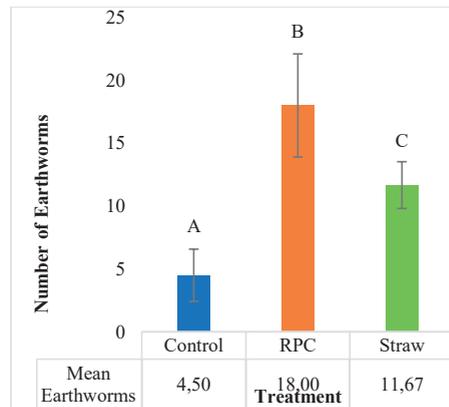


Figure 15. Mean (\pm SD) number of earthworms in plots of different treatments at week 9

Control plots (A) had a significantly lower ($P < 0.001$) worm count than RPC plots (B) and straw (C). Straw plots (C) were significantly lower than RPC plots ($P < 0.001$). RPC plots (B) had significantly higher ($P < 0.001$) worm counts than other treatments ST Dev 2.07, 4.10, 1.86 ($P < 0.001$, $F = 28.98$, $DF = 2$, $n = 6$).

CONCLUSIONS

Nitrate-Nitrogen contents differed pre-application at week zero. Potentially, this is due to previous crops or chemicals that have been applied; this is not ideal but makes the study more realistic to what happens in industry. The development of a precision agriculture technique that could apply FYM rates in differing depending on soil N requirements would be an effective technological

The phosphorus content of applications showed no significant increase over the period of the study for any application types This may show that a longer period is required for P to break down in the soil. Lack of significant difference and very minor increases (sometimes decreases) imply alternative applications or a longer time frame may be required to boost P nutrient content. Both RPC and straw plots showed an increase in potassium suggesting the means for aiding growth and reproduction in plants. The gains demonstrated in both RPC and straw plots show equal potential for supplying soils with K; RPC plots showed the greatest increase with straw plots showing a lesser increase; the K increase demonstrates that the liming effect of RPC is not creating K deficiencies as stated by Potash Development Association (2011).

The average pH of RPC plots was 6.12 (mildly acidic), in comparison to straw at 5.80 implying that RPC is more appropriate for providing an acceptable pH for soil nutrient bioavailability and plant productivity. Surface plots had a significantly higher pH than dug in plots

Dug in plots have a higher OM than when applied on the surface. Both methods of application showed a moderate concentration of OM, this could be due to OM being in the process of breaking down, assisted by the help of earthworm thus having a greater effect on plots with treatments further into the soil

Overall, analysis shows that neither treatment is effective for the addition of OM. However, if necessary, to apply, digging in (ploughing) is the effective method. OM result in this study greatly differs from most research in the agricultural industry.

Earthworms presence was significantly higher in RPC dug in and on surface plots than in control plots. The elevated number of

earthworms in RPC plots implies RPC soils fit the requirements of earthworm quantity specified by Rothamsted Research (2019). This suggests that RPC is effective at allowing earthworms to convert digestible C into a form that stays in the soil. Earthworms convert C emitting microbes into a form of organic matter (stabilisation) which decreases the amount of emissions entering into the atmosphere in the form of carbon dioxide correlates with earthworms thriving in a more neutral soil (between 6.0 and 7.0) as demonstrated in RPC plots.

Both straw and paper are effective at increasing the number of earthworms in a soil. However, as RPC had the greatest number of earthworms present this is the preferred treatment with application acceptable through either method.

With the correct marketing RPC could prove a viable product to horticulturalists because of ease of soil application present due to particle size in comparison to other products on the market. Market gardeners may find the product viable due to the size of RPC particles and the 25 kg bales available providing ease of use.

It is necessary to find a material that can replace straw due to lack of sustainability, this study provides a basis for progression within the agricultural industry to supply high quality, dual purpose materials that prove effective for both housing livestock and applying to soil to all in all optimize output across all aspects of modern-day agriculture.

There is little proof within this research to indicate that RPC would be effective as a mulch to conserve soil moisture, reduce overland flow and detach and transport sediments (Das et al., 2019). Crop cover (fleece) use has the potential to specify the performance of treatments with increased accuracy in relation to moisture associated factors. Positive results could promote the use of RPC on a global scale as a mulch to retain moisture in dry climates. An example of its use could be on vineyards and for cereals grown in Mediterranean climates (Prosdocimi).

Heavy rainfall in week three showed all application types can be considered free draining and not a cause of water logging. This contradicts literature suggesting the addition of material to the soils surface retains large amounts of water although it has been suggested

that materials left on the soil surface formed a layer between the soil and the atmosphere thus at times both sunlight and moisture may have been prevented from reaching the soil surface of the trial plots.

Further testing is required to provide evidence on its long-term effectiveness.

REFERENCES

- Azevedo, A.R.G., Marvil, T.M., Junior, W. (2019). Assessing the potential of sludge generated by the pulp and paper industry in assembling locking blocks. *Journal of Building Engineering*, 23, 334–340.
- Behera, S.K. and Shukla, A.K. (2015). Spatial distribution of surface soil acidity, electrical conductivity, soil organic carbon content and exchangeable potassium, calcium and magnesium in some cropped acid soils of India. *Land Degradation & Development*, 26(1), 71–79.
- Bellamy, K.L., Chong, C., Cline, R.A. (1995). Paper sludge utilization in agriculture and container nursery culture. *Journal of Environmental Quality*, 24(6), 1074–1082.
- Bernardo, L., Carletti, P., Badeck, F.W., Rizza, F., Morcia, C., Ghizzoni, R., Roupael, Y., Colla, G., Terzi, V. and Lucini, L. (2019). Metabolomics responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. *Plant Physiology and Biochemistry*, 137, 203–212.
- Bertrand, M., Barot, S., Blouin, M., Whalen, J., De Oliveira, T. and Roger-Estrade, J. (2015). Earthworm services for cropping systems. A review. *Agronomy for Sustainable Development*, 35(2), 553–567.
- Carolin, C.F., Kumar, P.S., Saravanan, A., Joshiba, G.J. and Naushad, M. (2017). Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of Environmental Chemical Engineering*, 5(3), 2782–2799.
- Chadwick, D., Wei, J., Yan'an, T., Guanghui, Y., Qirong, S. and Qing, C. (2015). Improving manure nutrient management towards sustainable agricultural intensification in China. *Agriculture, Ecosystems & Environment*, 209, 34–46.
- Chen, X., Gao, M., Li, Y., Zhang, X., Zhang, F., Hu, B. (2019). Effects of freeze-thaw cycles on the physiochemical characteristics of animal manure and its phosphorus forms. *Waste Management*, 88, 160–169.
- Copcea, A.D., Ilea, R. and Crîsta, I. (2017). Studies regarding grain loss in straw cereals. *Research Journal of Agricultural Science*, 49(2), 43–45.
- Cows&Co Group (2018). *Livestock Bedding*. [Online]. Available from: <https://envirosystems.co.uk/products/envirobed-original/> [Accessed 17 April 2018].
- Duruogbo, C.I., Obiefuna, J.C., Onweremadu, E.U. (2007). Effect of poultry manure rates on soil acidity in an ultisol. *International Journal of Soil Science*, 2, 154–158.
- Hillel, D. (2008). Soil fertility and plant nutrition. *Soil in the Environment*, 18, 151–162.
- Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A. and Hayes, R.C. (2018). Liming impacts on soils, crops and biodiversity in the UK: a review. *Science of the Total Environment*, 610, 316–332.
- Huang, P.M., Zhou, J.M., Xie, J.C., Wang, M.K. (2005). Potassium in soils. *Soils and the Environment*, 3, 303–314.
- Khan, N., Shah, Z., Ahmed, N., Ahmad, S., Mehmood, N., Junaid, M. (2013). Use of biochar, FYM and nitrogen fertilizer on soil organic fertility. *Pure Applied Biology*, 2(2), 42–47.
- Larney, F.J., Olson, A.F., DeMaere, P.R., Handerek, B.P., Tovell, B.C. (2008). Nutrient and trace element changes during manure composting at four southern Alberta feedlots. *Canadian Journal of Soil Science*, 88(1), 45–59.
- Luebbe, M.K., Erickson, G.E., Klopfenstein, J., Greenquist, M.A., Benton, J.R. (2011). Composting or stockpiling of feedlot manure in Nebraska: Nutrient concentration and mass balance. *The Professional Animal Scientist*, 27(2), 83–91.
- Onrust, J. and Piersma, T. (2019). How dairy farmers manage the interactions between organic fertilizers and earthworm ecotypes and their predators. *Agriculture, Ecosystems & Environment*, 273, 80–85.
- Quan, P., Schoenau, J.J., King, T., Japp, M. (2005). Effects of repeated manure application on potassium, calcium and magnesium in soils and cereal crops in Saskatchewan. *Soil Science*, 5, 1–9.
- Quaye, A.K., Volk, T.A., Hafner, S., Leopold, D.J., Schirmer, C. (2011). Impacts of paper sludge and manure on soil and biomass production of willow. *Biomass and Bioenergy*, 35, 2796–2806.
- Rothamsted Research (2019). *Earthworm research spurs farmers to act* [online]. <https://www.rothamsted.ac.uk/news/earthworm-research-spurs-farmers-act> [Accessed 12 March 2019].
- Rotz, C.A and Leytem, A.B. (2015). Reactive nitrogen emissions from agricultural operations. *Pasture Systems and Watershed Management Research*, 12, 16–24.
- Royer-Tardif, S., Whalen, J., Rivest, D. (2019). Can alkaline residues from the pulp and paper industry neutralize acidity in forest soils without increasing greenhouse gas emissions? *Science of the Total Environment*, 663, 537–547.
- Rupp, H., Meissner, R., Leinweber, P. (2018). Plant available phosphorus in soil as predictor for the leaching potential insight from long-term lysimeter studies. *Ambio*, 47, 103–113.

- Sanz, J., Asenjo, I., Santiagi-Martin, A., Ramon, J., Concepcion, N., Lafuente, L., Dieguez, A. (2018). Enzyme activity indicates soil functionality affectation with low levels of trace elements. *Environmental Pollution*, 243(B), 1861–1866.
- Smith, K.A., Chalmers, A.G., Chambers, B.J., Christie, P., 1998. Organic manure phosphorus accumulation, mobility and management. *Soil Use and Management*, 14, 154–159.
- Stuart, M.E. and Lapworth, D.J., 2016. Macronutrient status of UK groundwater: Nitrogen, phosphorus and organic carbon. *Science of the Total Environment*, 572, 1543–1560.
- Teixeira, D.L., Miranda-de la Lama, G., Villarroel, M., Olleta, J.L., García-Belenguer, S., Escós, J. and María, G.A. (2015). Effects of alternative bedding substrates on lamb welfare, productive performance, and meat quality during the finishing phase of fattening. *Journal of Veterinary Behaviour*, 10(2), 171–178.
- Tried & Tested, 2014. *Nutrient Management* [online]. Available from <http://www.nutrientmanagement.org/assets/12029> [Accessed 1 November 2018].
- Venturini, G., Pizarro-Alonso, A. and Münster, M. (2019). How to maximise the value of residual biomass resources: The case of straw in Denmark. *Applied Energy*, 250, 369–388.
- Whalen, J.K., Chang, C., Clayton, G.W., Carefoot, J.P., 2000. Cattle manure amendments can increase the pH of acid soils. *Soil Fertility and Plant Nutrition*, 64(3), 962–966.
- Wu, H., Du, S., Zhang, Y., An, J., Zou, H., Zhang, Y., Ya, N. (2019). Effects of irrigation and nitrogen fertilization on greenhouse soil organic nitrogen fractions and soil-soluble nitrogen pools. *Agricultural Water Management*, 216, 415–424.

THE STUDY ON THE USE OF THE TOPOGRAPHIC METHOD COMBINED GPS-TOTAL STATION IN THE WORKS OF FORESTRY CADASTRE

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Abstract

The paper aims to present a modern method of topo-cadastral lifting of land surfaces in the forest area, which involves the use of the combined technology of the type of global GPS positioning systems and total stations, to ensure accuracy, efficiency and very high efficiency of the works. Following the study, it was found that the method used is fully applicable and useful to forestry surveys, as relevant and significant results were obtained regarding the accuracy, correctness and speed of execution of the field and office operations (data processing and preparation plan). The obtained documentation fits perfectly with the requirements of the norms and regulations imposed by the legislation in force, in the field of forest cadastre. The precision obtained by the combined method in terms of distances and surfaces is of the order of millimetres, and in terms of angles it is below 5cc. Finally, it was found that the documentation obtained is very good for the proposed purpose, ie the first registration in the Land Book because, subsequently, a verification of the obtained results was carried out, by consulting the cadastral documentation registered with OCPI Dolj with no. 77401/2007 and it was found that there is no overlap, and the surfaces obtained were those registered in the deed of ownership.

Key words: cadastral survey, forest area, GPS systems, precision, total stations.

INTRODUCTION

The purpose of the procedure is to implement the provisions of art. 121 of the Law no. 46/2008 - The Forest Code, with the subsequent modifications and completions (Bădescu et al., 2009).

(1) The public property right of the state or of the administrative-territorial units on the forest fund is tabulated in the integrated cadastre and land book system at the request of the forest fund administrators, public property of the state, respectively of the owner, in the case of public property of the administrative territorial units, based on the property documents and the cadastral documentation drawn up according to the legal provisions (Law no. 46/2008).

(2) By exception from the provisions of par. (1), in the absence of the ownership documents, the provisional registration is made, based on the valid forestry arrangement, not updated. In this situation, the coordinates of the points on the limit of the respective property will be determined, in Stereographic System 1970, by vectorization, at the level of administrative-

territorial unit. Any disputes regarding possible overlaps are settled amicably or through the court according to the regulations provided by the Law on cadastre and real estate advertising no. 7/1996, republished, with subsequent amendments and completions (Burghilă et al., 2016).

(3) Justification of the provisional registration for the situations provided in par. (2) is made on the basis of the valid updated forest management of the forest fund public property of the state or of the administrative-territorial units, which confirms the surface and the identity between the building from the cadastral documentation and the one highlighted in the updated forest management. The procedure is applied within the subordinate institutions A.N.C.P.I.

MATERIALS AND METHODS

The cadastral documentation for the first registration in the integrated system of cadastre and land book for the buildings that are the object of art. 121, para. (2) of Law no. 46/2008 - The Forest Code contains the pieces provided in

art. 83, para. (1) of the Regulations, except those provided in letter. e), h), i), p), as well as the copy of the plan with the certified forestry arrangement, "in accordance with the original" and the document by which the administrator/owner of the forestry fund, as the case may be, confirms the location, surface and identity between the building from the cadastral documentation and the one highlighted in the forest management plan (Law no. 46/2008). The coordinates of the points on the boundary of the building are determined in the national reference system, by vectorization, at UAT level. The location and delimitation plan will bear the mention "Real estate registered in the cadastral plan with graphically determined coordinates, according to art. 121, para. (2) of Law no. 46/2008 - "The Forest Code". This mention is also made in the digital documentation, under the "comments" section, in the section "Textual data of the land" (Bădescu et al., 2018).

The administrator / owner of the forestry fund, as the case may be, signs the declaration regarding the consequences of the mention "Building registered in the cadastral plan with graphically determined coordinates, according to article 121, paragraph (2) of Law no. 46/2008 - Forest Code", respectively the possibility to change the geometry, the dimensions of the sides and the surface resulting from vectorization. The mention is deleted based on the report drawn up by the inspector and approved by the chief engineer, when updating the related cadastral documentation, by carrying out field measurements (Law no. 46/2008). Verification of the geometry of the building - forest fund, according to art. 46, para. (1), lit. g) from the Regulation, is made including on the basis of the digital graphic support for Law no. 165/2013 regarding the measures for completing the process of restitution, in kind or by equivalent, of the buildings taken abusively during the communist regime in Romania (Călina and Călina, 2019), with the subsequent modifications and completions, respectively annex no. 9, to the Norms of application of Law no. 165/2013.

At this work for the first registration in the Land Book it was necessary to verify the geometry of the building by measurements made on the ground. For this purpose, for lifting the forested area, a support network was established using

GPS measurements, using the static method, so that the collected data is as accurate as possible (Bergerman et al., 2016; Braun et al., 2018). The surface studied being large, for determining its geometry, several closed traverse were used, supported on the starting point, following and precisely determining all the contour points that make up the boundary of the surface. The works of topographic measurements by the traverse method were performed with total stations SOKKIA SET 610 and SOKKIA SET 630 R, of very good accuracy (Sălăgean et al., 2011; Rodriguez-Moreno et al., 2017).

The planimetric details were collected by specific methods such as polar coordinates method, obtaining all the data necessary to draw up a complete topographic plan, which corresponds technically, to all the precision requirements and the norms imposed for such work (Călinovici and Călina, 2008; Gonzalez-de-Santos et al., 2017). In order to record as accurately as possible and the possibility of easily recovering the border points, the topographic specialists made a detailed description of the geodesic points, a very important aspect especially in the case of forested lands (Geipel et al., 2016; Radu et al., 2017).

RESULTS AND DISCUSSIONS

At the request of the owner it was decided to survey the immovable property, which is a forested area located on the territory of Ghercești - Dolj commune, with a view to its first inscription in the Land Book. The topo-geodesic works were carried out in such a way that the provisions of the Cadastre Law and the real estate advertising no. 7/1996, republished, with the subsequent modifications and completions, Law 46/2008 - the Forest Code, with the subsequent modifications and completions and the Government Decision no. 1288/2012 for the approval of the Regulation for the organization and functioning of the National Agency for Cadastre and Real Estate Advertising.

In order to be able to perform the topo-geodesic survey in the best conditions, with high accuracy and maximum efficiency, the surveyors used to raise the support network and the detail points a new topographic method such as the Global

Positioning Systems (GPS) combined with total stations. The topographic apparatus used for the support and thickening network is modern and high performance GPS-type from Leica (Sui, 2014; Sala et al., 2020). GPS measurements were performed using the static method using records from the permanent

stations CRAI (CRAIOVA), SLAT (SLATINA), as well as the recording at the triangulation point of the first order Cârcea, obtaining the following GPS points: 1, 2, 3, 4, 5, 6 (Figure 1).

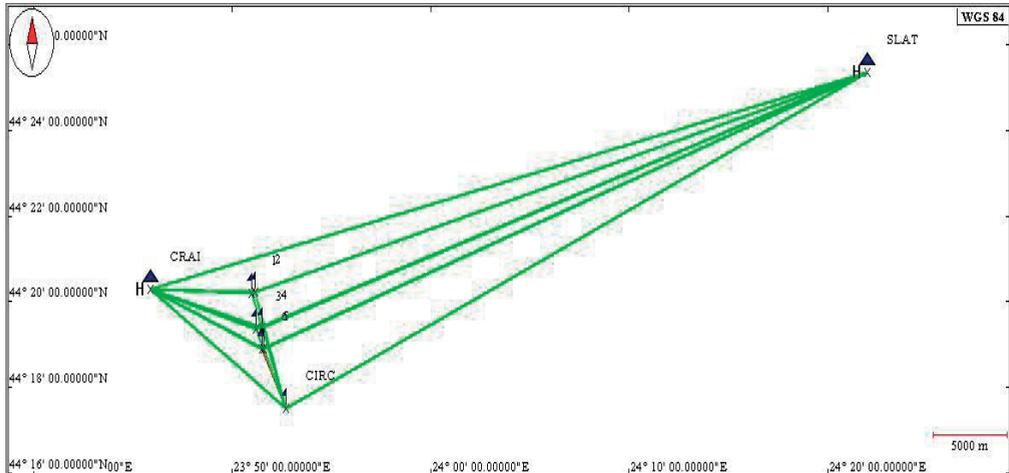


Figure 1. GPS support network

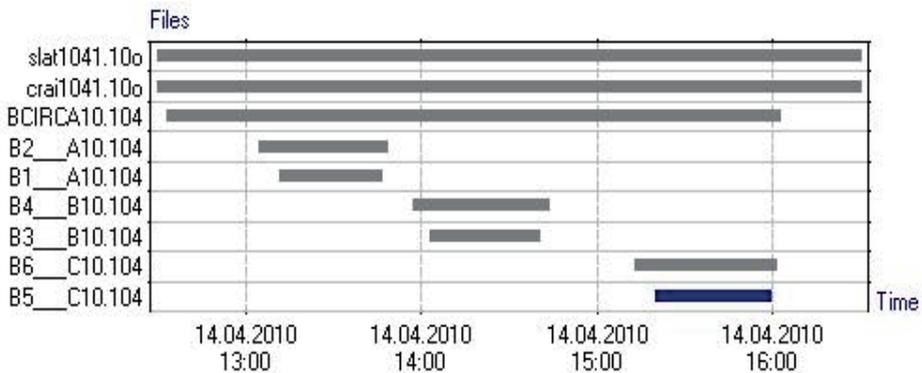


Figure 2. Stationary times in the GPS support network

Using these old points determinations of new support points were made near the detail points from where detailed measurements had to be made, for the elaboration of the plan of location and delimitation on a large scale. Measurements for the determination of new support points were carried out using GNSS (Global Navigation Satellite Systems) methods for determining the autonomous geo-spatial position (Mihai et al., 2015). The equipment used comprised 4 Leica SR530 satellite receivers, on 24 channels with

two working frequencies, the measurement method - static. The processing of the GNSS bases was done with specialized software (Leica Geo Office), in the ETRS 89 coordinate system, starting from the permanent station of Craiova and Băilești. Taking into account their lengths, the short bases (less than 20 km) were processed separately from the long bases, the bases with unresolved ambiguities in the processing were not taken into account when compensating (Călina et al., 2018).

First we obtained - geocentric cartesian coordinates - ellipsoid WGS 84/GRS 80 - XW, YW, ZW, for points 1, 2, 3, 4, 5, 6, and ellipsoidal coordinates - ellipsoid WGS 84/GRS 80 - (BW, LW, HW) (Table 1), then using the 7

parameters of a 3D Helmert transformation (dX, dY, dZ, m, rx, ry, rz), the coordinates of points 1, 2, 3, 4, 5, 6, were transformed into Stereographic system 1970 and Black Sea 1975 quota system (Table 2).

Table 1. Calculation of GPS support network

Name	Long	Lat	Ellips height	Status	Constraints	Surv_ Horz_ Conf	Surv_ Height_ Conf	Type
1	23° 50' 59"E	44° 20' 13"N	246.272	Adjusted	No constraints	0.003	0.01	Logged Point
3	23° 51' 11"E	44° 19' 22"N	206.101	Adjusted	No constraints	0.003	0.01	Logged Point
6	23° 51' 30"E	44° 18' 55"N	205.694	Adjusted	No constraints	0.003	0.01	Logged Point
2	23° 51' 09"E	44° 20' 14"N	246.663	Adjusted	No constraints	0.004	0.01	Logged Point
4	23° 51' 29"E	44° 19' 23"N	227.157	Adjusted	No constraints	0.003	0.01	Logged Point
5	23° 51' 35"E	44° 18' 54"N	207.321	Adjusted	No constraints	0.003	0.01	Logged Point
CIRC	23° 52' 42"E	44° 17' 30"N	246.56	Adjusted	No constraints	0.004	0.01	Logged Point
CRAI	23° 45' 52"E	44° 20' 17"N	150.16	Adjusted	Horiz. Fixed (2D)	0	0.01	Control Point
SLAT	24° 22' 01"E	44° 25' 21"N	235.972	Adjusted	Horiz. Fixed (2D)	0	0	Control Point

Table 2. Stereo 70 coordinates of the support points

PUNCT	X	Y
1	315883.617	408409.431
2	315927.458	408609.398
3	314324.347	408627.900
4	314335.323	409037.578
5	313450.775	409147.079
6	313465.358	409055.788
CIRC	310844.716	410600.274

Next, to determine all the bending and contour points, the total stations of the same precision were used SOKKIA SET 610 and SOKKIA SET 630 R, on the directions the accuracy was 1.9 mgon and on the distances the accuracy was 3 + 2 ppm, with data processing on the computer. (Călina et al., 2018).

By analyzing and interpreting very correctly and realistically the situation on the ground, it was concluded that four contour traverses supported on known GPS coordinate points must be made, these points being oriented and verified on the points of the state triangulation network (Figure 3).

Traverse pd - supported on Landmark 1 (GPS determined point) oriented on T110 and T109 with closure on Landmark 4 (GPS determined point), with orientation on Landmark 3 (GPS determined point). By this route were determined the stations: 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, and from the station 106 was given a station thrown on point 121 (Figure 3).

Traverse pd1 - supported on Landmark 2 (GPS determined point) oriented on T5, T109, T110, Teisi Hill Pyramid and Teišani Pyramid with closing on Landmark 4 (GPS determined point),

with orientation on Landmark 3, T33, T114 and T116. Through this process were determined the points: 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 (Figure 3).

Traverse pd2 - supported on Landmark 4 (GPS determined point) oriented on Landmark 3 (GPS determined point), T33, T114, T116 with closure on Landmark 5 (GPS determined point) with orientation on Landmark 6 (GPS determined point) and T114. Through this process were determined the points: 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211 (Figure 3).

Traverse PD3 - supported on the Landmark 3 (point determined GPS) oriented on Landmark 4 (determined GPS point), T33, T109, T110, closing on the terminal Landmark 6 (point determined GPS) oriented on the Landmark 5 (determined GPS point). By this route were determined the stations: 101, 102, 103, and from Landmark 6 was given a thrown station 121, from Landmark 5 (GPS determined point), was given to the thrown station 122 (Figure 3).

For a precise and rigorous verification of the measurements made, a series of old geodesic

points of known coordinates, from the state geodetic network, taken over by OJCPI Dolj were used: T109 - COS FUM ENERG; T110 - COS FUM TERMOFICARE; T14 - DEALUL TEIS; T5 - FABRICA DE AVIOANE; T33-BLOC A1 ROVINE; T114 - CA FRIGORIFER; T116 - SFERA METEO, TEIȘANI (Figure 3). At these points the stability and the degree of confidence had to be checked, finding that their condition was good.

Based on the measurements made on the field (distances and orientations), the relative and absolute rectangular coordinates of the points

were calculated and entered in Table 3. In order to be used in other later topographic surveys, it was necessary to compensate and verify them. Only then can they be safely used in new topographic elevations. According to the sketch in Figure 3, it is found that for the determination of all contour and detail points, the method of polar coordinates was applied to the field. From the support points, rays were taken to all the detail points and based on the measured data on the field, the absolute coordinates of the points were obtained, which were passed in Table 4.

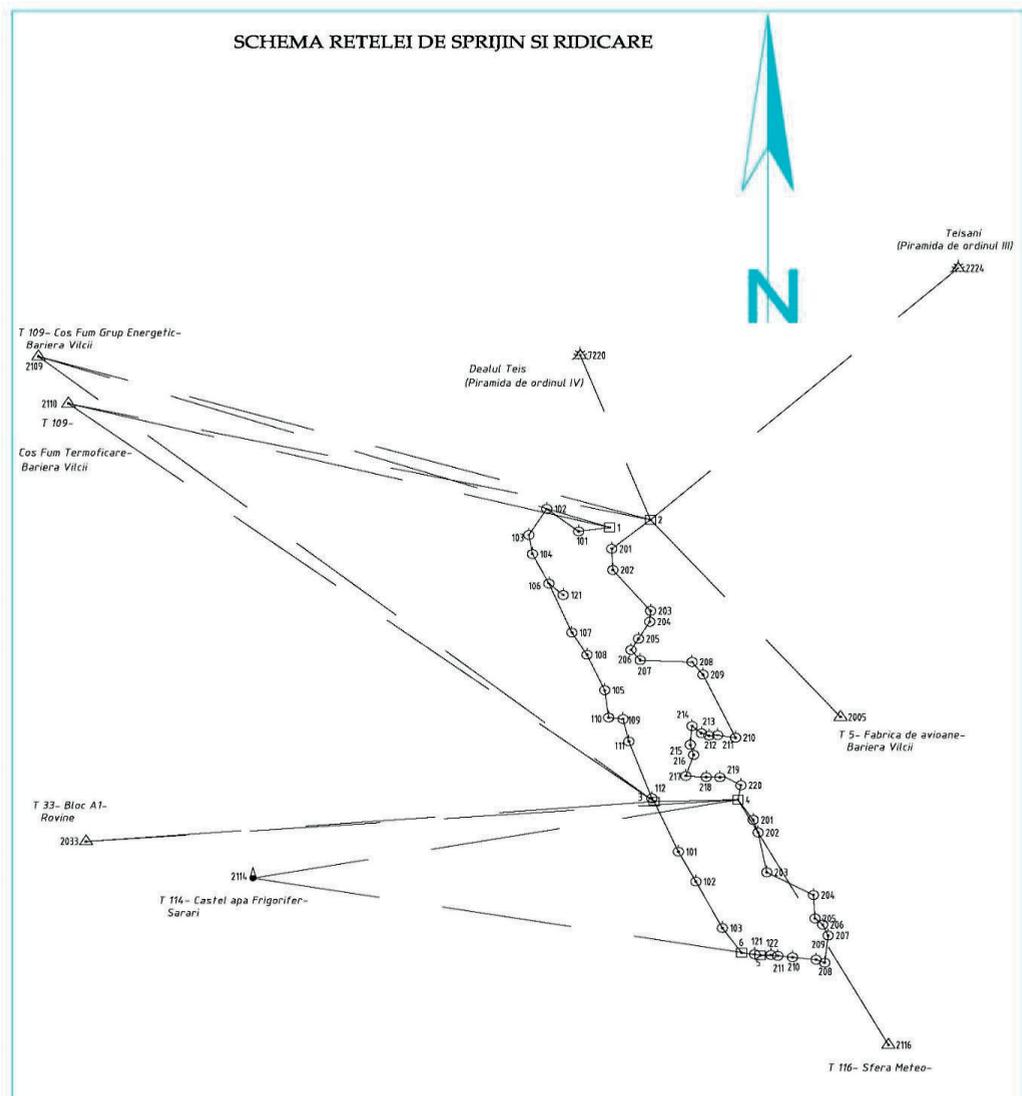


Figure 3. Outline of the support network and lifting of the studied surface

Table 3. Calculation of the supported traverses based on known coordinate points

Station point	Target point	Visa	Reduced distance	DX	DY	X	Y
2	1	286.260				315927.458	408609.398
2	201	254.478	251.377	-164.813	-189.808	315762.632	408419.572
		0.002		-0.013	-0.018		
		254.480		-164.826	-189.826		
201	202	197.091	121.009	-120.883	5.520	315641.743	408425.083
		0.004		-0.006	-0.009		
		197.095		-120.889	5.511		
202	203	157.171	296.793	-232.140	184.924	315409.588	408609.986
		0.006		-0.015	-0.021		
		157.177		-232.155	184.903		
203	204	204.443	61.935	-61.784	-4.327	315347.800	408605.655
		0.008		-0.003	-0.004		
		204.451		-61.787	-4.331		
204	205	232.773	111.650	-97.171	-54.987	315250.624	408550.660
		0.010		-0.006	-0.008		
		232.783		-97.177	-54.995		
205	206	233.356	73.354	-63.506	-36.712	315187.114	408513.942
		0.012		-0.004	-0.005		
		233.368		-63.510	-36.717		
206	207	157.989	73.522	-58.097	45.059	315129.013	408558.996
		0.015		-0.004	-0.005		
		158.004		-58.101	45.054		
207	208	102.532	254.124	-10.169	253.920	315118.831	408812.898
		0.017		-0.013	-0.018		
		102.548		-10.182	253.902		
208	209	159.283	89.836	-72.095	53.598	315046.731	408866.489
		0.019		-0.005	-0.006		
		159.302		-72.100	53.592		
209	210	173.298	392.399	-358.437	159.686	314688.274	409026.147
		0.021		-0.020	-0.028		
		173.319		-358.457	159.658		
210	211	310.446	89.288	14.617	-88.083	314702.887	408938.058
		0.023		-0.005	-0.006		
		310.469		14.612	-88.089		
211	212	295.304	42.083	-3.085	-41.970	314699.799	408896.085
		0.025		-0.002	-0.003		
		295.328		-3.087	-41.973		
212	213	322.290	39.463	13.552	-37.063	314713.349	408859.019
		0.027		-0.002	-0.003		
		322.317		13.550	-37.066		
213	214	346.419	62.108	41.400	-46.298	314754.746	408812.717
		0.029		-0.003	-0.004		
		346.448		41.397	-46.302		
214	215	205.023	107.029	-106.692	-8.487	314648.049	408804.222
		0.031		-0.006	-0.008		
		205.054		-106.698	-8.495		
215	216	182.471	60.380	-58.114	16.386	314589.932	408820.604
		0.033		-0.003	-0.004		
		182.504		-58.117	16.382		
216	217	219.599	125.666	-119.737	-38.146	314470.188	408782.449
		0.035		-0.006	-0.009		
		219.634		-119.743	-38.155		
217	218	104.023	100.207	-6.386	100.003	314463.797	408882.444
		0.037		-0.005	-0.007		
		104.060		-6.391	99.996		
218	219	101.357	66.886	-1.467	66.870	314462.327	408949.310
		0.039		-0.003	-0.005		
		101.397		-1.470	66.865		
219	220	126.088	112.189	-44.765	102.871	314417.556	409052.172
		0.041		-0.006	-0.008		
		126.129		-44.771	102.863		
220	4	211.136	83.512	-82.228	-14.590	314335.323	409037.576
		0.044		-0.004	-0.006		
		211.179		-82.232	-14.596		
4	3	298.249					
		0.046					
		298.295					

Stations no. 22.000 dif x: -0.135
 Azimuthal error: 0.0456 dif y: -0.188
 Orientation tolerance: 0.0469 Coord er: 0.231
 kx: -0.00005100 Coord tol: 0.676
 ky: -0.00007200

Table 4. Calculation of the detail points

Point no.	Coordinates		Point no.	Coordinates	
	X (m)	Y (m)		X (m)	Y (m)
782	314914.857	408849.229	816	314680.671	409017.117
783	314892.761	408844.100	817	314669.785	409046.329
784	314824.685	408839.362	1121	314911.260	408848.394
785	314799.595	408836.905	1123	314903.181	408852.717
786	314766.083	408834.185	1124	314881.215	408870.431
787	314710.639	408826.726	1126	314854.565	408894.006
788	314670.716	408824.161	1128	314828.186	408917.813
789	314740.992	408830.675	1131	314789.426	408951.748
807	314760.771	408977.957	1135	314754.100	409001.342
808	314739.058	408998.361	1136	314756.928	409007.892
809	314699.386	409014.297	1137	314760.018	409020.590
810	314682.152	408888.311	1139	314756.342	409021.856
811	314678.147	408931.981	1141	314741.738	408997.980
812	314674.203	409002.150	1143	314704.650	409013.626
813	314678.679	409004.974	1221	314670.871	408839.595
814	314681.817	409009.284	1222	314688.451	408840.848
815	314680.114	409014.968	1223	314684.367	409007.119

After accurately determining all the contour points, based on their absolute coordinates, the

total area of the building was calculated, this being 24925 sqm (Table 5).

Table 5. Surface calculation

Point no.	Coordinates		Distance (m)	Point no.	Coordinates		Distance (m)
	X (m)	Y (m)			X (m)	Y (m)	
1121	314911.260	408848.394		812	314674.203	409002.150	5.29
1123	314903.181	408852.717	9.16	811	314678.147	408931.981	70.28
1124	314881.215	408870.431	28.22	810	314682.152	408888.311	43.85
1126	314854.565	408894.006	35.58	1222	314688.451	408840.848	47.88
1128	314828.186	408917.813	35.53	1221	314670.871	408839.595	17.62
1131	314789.426	408951.748	51.52	788	314670.716	408824.161	15.43
807	314760.771	408977.957	38.83	787	314710.639	408826.726	40.01
1141	314741.738	408997.980	27.63	789	314740.992	408830.675	30.61
808	314739.058	408998.361	2.71	786	314766.083	408834.185	25.34
1143	314704.650	409013.626	37.64	785	314799.595	408836.905	33.62
809	314699.386	409014.297	5.31	784	314824.685	408839.362	25.21
1223	314684.367	409007.119	16.65	783	314892.761	408844.100	68.24
813	314678.679	409004.974	6.08	1121	314911.260	408848.394	18.99
Surface 24925 sqm							

For the first registration in the Land Book the team of specialists had to draw up the large scale location and delimitation plan 1:2000 (Figure 4). Due to the fact that a new, combined technology was used, such as global positioning systems (GPS) and total stations, as well as state-of-the-art topographic equipment and specialized processing programs, it was possible to draw up a very easy plan with a high precision, which made it possible to strictly comply with all the norms and regulations imposed by the laws and

government decisions in force, in the field of forest cadastre.

Also due to this method of measurement and the experience in the field of the working team it was found that the area taken into study was correctly and precisely determined because subsequently a verification of the obtained results was carried out, by consulting the cadastral documentation registered with OJCPI Dolj with no. 77401/2007 and it was found that there was no overlap.

PLAN DE AMPLASAMENT SI DELIMITARE A IMOBILULUI
SCARA:1:2000
(extravilan)

Nr. cadastral	Suprafata masurata [mp]	Adresa imobilului
	24925	Extravilan Comuna Ghercesti, tarla 41, parcela 478, jud. Dolj Ocolul Silvic Amaradia, Amenajamentul U.P. IV Viisoara, u.a. 58B
Carte Funciara nr.	UAT	GHERCESTI

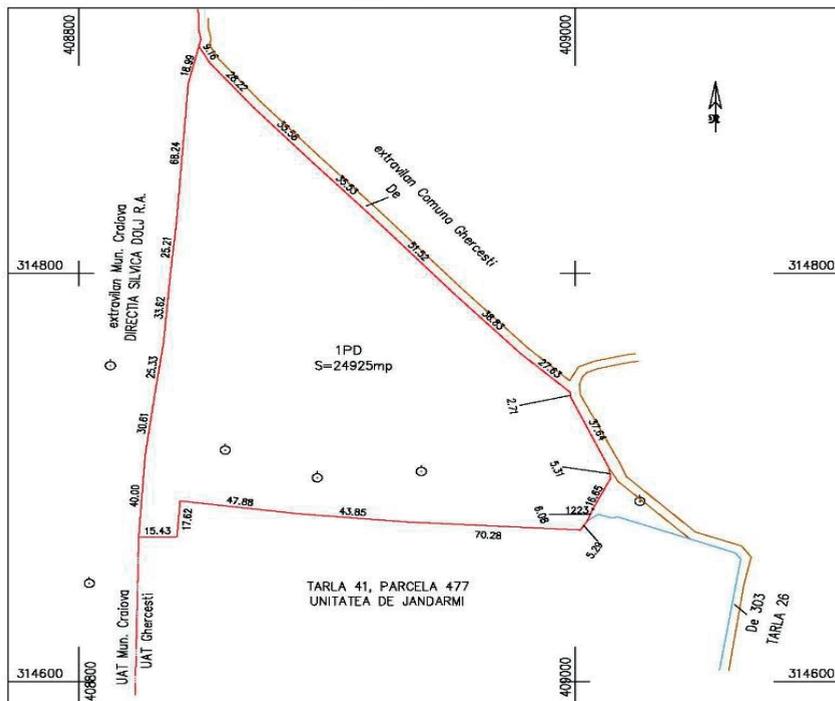


Figure 4. The plan of location and delimitation on scale 1:2000 of the studied surface

CONCLUSIONS

First of all, it was found that the combined topocadastral method used by the team of surveyors was very well adapted to the situation existing on the ground, to the type of work that had to be performed, namely the first registration in the land book of a forestry property, as and the precision required for such a work.

Using GPS technology, a network of new support points could be built very quickly and precisely, which formed the backbone of subsequent lifting, with the help of total stations. With the support network built precisely and with maximum efficiency, it was possible to carry out its thickening, four traverses supported on the GPS points, previously determined.

All the support points were located near the details that were to be picked up by other

methods, which allowed their visibility and accessibility to be raised, an aspect that essentially contributed to the increase of the precision and efficiency for topographic work performed with the total stations.

The processing of the measured data was carried out automatically with the help of the Toposys program, which allowed the export of all the results obtained in Autocad, a program with which the plan of location and delimitation of the studied real estate was drawn up and correct (also noted by Călina and Călina, 2019).

The remarkable results obtained by applying this combined method, namely high precision, correctness, accuracy and high efficiency, makes this method one that is required as a representative and relevant method for the execution of the cadastral measurements in the forest cadastre.

REFERENCES

- Bădescu, G., Ștefan, O., Bădescu, R., Badea, G., Badea, A.C. & Didulescu, C. (2009). Air-borne photogrammetric system used in topographic and cadastral works in Romania. In *Recent Advances in Remote Sensing*, Proceedings of the 5th WSEAS International Conference on Remote Sensing, Genova, Italy, 2769. 22–27.
- Bădescu, G., Călina, J., Călina, A., Miluț, M., Stan, I. (2018). Use of modern methods of terrestrial laser scanning on preservation of the religious patrimony in Romania. *European Journal of Science and Theology*, 14(5), 201–207.
- Bergerman, M., Billingsley, J., Reid, J., van Henten, E. (2016). *Robotics in Agriculture and Forestry*. In: Siciliano B., Khatib O. (eds) Springer Handbook of Robotics. Springer, Cham, Springer-Verlag Berlin Heidelberg, doi.org/10.1007/978-3-319-32552-1_56.
- Braun, J., Kremen, T. and Pruska, J. (2018). *Micronetwork for Shift Determinations of the New Type Point Stabilization*. In 2018 Baltic Geodetic Congress (BGC Geomatics) (265–269), IEEE.
- Burghilă, C., Bordun, C., Cîmpeanu, S.M., Burghilă, D. & Badea, A. (2016). Why mapping ecosystems services is a must in EU biodiversity strategy for 2020. *AgroLife Scientific Journal*, 5(2), 28–37.
- Călina, A. and Călina, J. (2019). Research regarding the agriproductive properties of the typical reddish preluvisol between Jiu and Olt rivers and its evolution from 1997-2017 in farms and agritouristic households. *Romanian Agricultural Research*, 36, 251–261.
- Călina, J., Călina, A., Bădescu, G., Vangu, G.M. and Ionică, C.E. (2018). Research on the use of aerial scanning for completing a GIS database. *AgroLife Scientific Journal*, 7(1), 25–32.
- Călina, J. and Călina, A. (2019). Evolution of the mollic reddish preluvisol in a romanian riverine region and the assessment of its agro-productive properties in farms and agro-touristic households. *Environmental Engineering and Management Journal*, 18(12), 2729–2738.
- Călinovici, I. and Călina, J. (2008). *Topography*. Ed. Mirton, Timișoara, 45–75.
- Gonzalez-de-Santos, P., Ribeiro, A., Fernandez- Quintanilla, C., Brandstoetter, M., Peruzzi, A., Kaplanis, G., Valero, C., Marco Vieri, M., Deblilde, B. (2017). Fleets of robots for environmentally-safe pest control in agriculture. *Precision Agriculture*, 18, 574–614.
- Geipel, J., Link, J., Wirwahn, J.A., Claupein, W. (2016). A programmable aerial multispectral camera system for in season crop biomass and nitrogen content estimation. *Agriculture*, 6(1), 4.
- Mihai, D., Teodorescu, R.I., Burghilă, D., Mudura, R. (2015). A modern approach in data updating for a vineyard agro-system modernization. *Conference SGEM*, 2, 651–656.
- Radu, O., Cîmpeanu, S.M., Teodorescu, R.I. and Bucur, D. (2017). Technical efficiency of the subsurface drainage on agricultural lands in the Moldova river meadow. Kulshreshtha S. *Current Perspective on Irrigation and Drainage*, London: IntechOpen, 69–81.
- Rodriguez-Moreno, F., Kren, J., Zemek, F. (2017). Advantage of multispectral imaging with sub-centimeter resolution in precision agriculture: generalization of training for supervised classification. *Precision Agric.*, 18, 615.
- Sala, F., Popescu, C.A., Herbei, M.V. & Rujescu, C. (2020). Model of Color Parameters Variation and Correction in Relation to “Time-View” Image Acquisition Effects in Wheat Crop. *Sustainability*, 12(6), 2470.
- Sălăgean, T., Dirja, M., Ortelecan, M., Pop, N. & Deak, J. (2011). 3D Modeling of the USAMV Cluj-Napoca campus using integrated system google earth-sketchup and 3D warehouse. *Agricultura*, 79(3-4), 146–150.
- Söderström, M., Sohlenius, G., Rodhe, L. Piikki, K. (2016). Adaptation of regional digital soil mapping for precision agriculture. *Precision Agric.*, 17, 588.
- ***Law on cadastre and real estate advertising no. 7/1996, republished, with subsequent amendments and completions.
- ***Law no. 46/2008. The Forest Code.
- ***Law no. 165/2013.
- ***Government Decision no. 1288/2012 for the approval of the Regulation for the organization and functioning of the National Agency for Cadastre and Real Estate Advertising.

METHOD OF REMEDIATION AND SUSTAINABLE USE OF SOILS TESTED IN THE REPUBLIC OF MOLDOVA

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Abstract

The system of conservative agriculture is not suitable for compacted soils of the Republic of Moldova, which need first loosening, a fact confirmed by recent researches. In the drought years, due to the high resistance to the penetration of the dry arable layer, the penetration of water and roots into the depths is impossible, which leads to the decrease of the agricultural crops yields. The use of green fertilizers (autumn and spring vetch, sown in an agricultural year as a 'busy field' of 5-folds crop rotation), whose green mass is introduced into the soil as organic fertilizer, leads to the positive remediation of the quality status of this layer. As a result of applying the green fertilizers through disking for a period of 5 years showed that the balance of carbon and nitrogen in the soil became positive for future 3-4 agricultural years; the physical quality state of the arable layer became favorable for agricultural crops; the crop yield increase with 1.0-0.5 t/ha/year. In order to maintain the long-term quality status of the Mini-till cultivated soils, it is necessary that, starting with the third year of cultivation, the field should be sown again with vetch as an intermediate crop, used at the end of April or the beginning of May for next year as a green manure. This process must be repeated permanently once in three years, which will ensure that the soil quality condition is maintained in a favorable state and that the nitrogen fertilizer needs to be reduced to 60-70%.

Key words: carbon balance, mini-till, remediation, soil, vetch.

INTRODUCTION

Conservative agriculture, based on the No-till system of soil tillage, is not suitable for dehumidified, destroyed, compacted soils, which require first loosening in the Republic of Moldova (Cerbari, 2011; Wiesmeier et al., 2015). The basic indicators used to determine the variants used in the conservative agriculture system are (Руководство..., 1991):

- elimination of the plowing with the return of the furrows, totally or for a number of years;
- maintaining at the soil surface of vegetal debris, total or at least 30% of its total;
- reducing the number of soil tillage and other measures that ensure soil conservation.

The requirements of the conservative agriculture largely correspond to the No-till or Mini-till soil tillage variants that are recommended for implementation on the agricultural lands of the Republic of Moldova, together with other compartments thereof (weed control, disease and pests, organic and mineral fertilization etc.). The No-till variant of soil tillage provides for sowing directly in the stubble or on the ground

with vegetal debris of the previous plant. The main working unit is the drill. The main working unit is the seed drill. The main element of the No-till drill is the couler. It is stated that as a result of the implementation of the No-till working system the top layer of soil gradually becomes more structured, more loosed; an aeryhydric and food regime favorable to plants is formed; soil resistance to erosion is increased (Berca, 2011; Lal, 2011; Блэк, 1993; Agricultura..., 2009).

In the first 5-6 years, and if is necessary in the following years (once in 3 years), the subsoil is carried out at the depth of the soil postarable layer (10-30 cm) with the subsoiler aggregate to accelerate the process of loosening of this layer by the roots of the crop plants.

The *general objective* of the researches was to assess the quality status and production capacity of the degraded ordinary chernozems from southern Moldova by incorporating in the soil as organic fertilizer the green mass of 2 crop yields of vetch on a field used as a “busy field” (1 year) in - a 5-field crop rotation with the following crop alternation per year: “busy field” (2 crop of

vetch applied in the soil as a green organic fertilizer) → autumn barley → rapeseed → autumn wheat → sunflower.

The research aimed at the preventive restoration of the degraded properties of the former arable layer 0-30 cm of the soil, currently compacted, by the systematic use of green fertilizers in conjunction with the agrotechnical procedures for the implementation of the conservative agriculture, based on the No-till system.

MATERIALS AND METHODS

In order to assess the impact of the No-till implementation on the quality status of the ordinary chernozems of Southern Moldova, on the territory of the Larga Nouă commune, the Cahul rayon were studied by comparison the soils worked according to the variant with conventional technology and variant with conservative technology - No-till. The land selected for experimental plot organization was investigated pedologically (Canarache, 1990; Florea et al., 1987) in order to evaluate the initial state of soil quality after its work 2 consecutive years in according to the No-till technology (Figure 1).



Figure 1. Experimental polygon, used in the agricultural year 2013-2014 under autumn wheat sown with No-till seed drill

The land is located on the quasi-horizontal surface of the last (highest) terrace of the Prut River. The absolute altitude of the site of the soil semi-profiles is about 120-121 m. The last terrace of the Prut River on the territory of the Larga Nouă commune is practically not affected by the erosion processes. The agricultural lands are suitable for vineyards, orchards, cereals, vegetables for irrigation etc.

The soil cover of the terrace is made up of clayey ordinary chernozem with a moderately

humiferous profile. The parent rock of soil genesis on the high terrace of the Prut River is made up of loess deposits of wind origin. The soils are typical for southern Moldova formed in arid conditions than the usual chernozems of Central Moldova (Leah, 2017).

After being placed in the field at the end of September 2014, the experimental plot was sown with a mixture of autumn vetch (80 kg/ha) and winter wheat (50 kg/ha). The total area sowed with vetch was 1.15 ha (Figure 2).



Figure 2. The experimental plot sown with autumn vetch

One hectare of autumn vetch has been sown for seeds. The experimental plot (strip) occupied 0.15 ha. The autumn vetch from the experimental plot was incorporated into the soil at the beginning of May through disking. On the same day, spring vetch were sown again and incorporated into the soil as a green fertilizer by disking and plowing at the end of September (Figure 3).



Figure 3. The first green mass yield of autumn vetch incorporated into the soil through disking

At the end of September, the second crop mass of spring vetch from the experimental plot was incorporated into the soil by disking and plowing (Figure 4).



Figure 4. The second green mass yield of spring vetch incorporated into the soil as a green fertilizer at the end of September through disking and plowing

RESULTS AND DISCUSSIONS

The intensive conventional agriculture of the period 1950-1990 led to the deterioration of the physical, chemical and biological quality of the soils. The plowing of the soil with the cormana plow in Moldova is carried out at a depth of 30-35 cm. The intensive agrotechnical works have contributed to intensifying the processes of dehumidification and deterioration of the natural soil structure. As a result, the arable layer has lost resistance to compaction.

At the same time, the existence after the land privatization period (1990 - present) of a practically unbalanced correlation between the used volume of the chemical and organic fertilizers does not ensure a major increase of the soil production capacity (Cerbari, 2010).

Chernozems of Moldova are characterized by fine textures and a not always favorable correlation of the granulometric fractions. The high clay content in the arable layer of soils in conditions of compaction resistance lack of its layers, leads to strong settling of the lower part of this layer for 1-2 years after passing to the minimum basic soil work. As a result of the compaction, when implementing conservative soil tillage systems, the lower part of the degraded arable layer is not penetrated by the roots of the crop plants, which leads to the decrease of the volume of physiologically active soil and crops yields (Leah, 2018).

The partial reduction of the negative influence of the secondary compaction of the arable soil layer during the first 5-7 years of implementation of the conservative agriculture system, based on

the No-till or Mini-till soil tillage procedures, can be carried out by using the phyto-ameliorative and agro-technical procedures (Leah, 2018a; 2018b). These procedures, by increasing the flow of organic substance and periodically performing the subsoil, can contribute to the restoration of the structure and the gradual loosening of the compacted postarable layer.

At the moment, based on the situation existing in the republic, the restoration of the quality status of the arable layer is possible only by applied in the soil the green fertilizers and the secondary production of agricultural crops, at the same time with the mechanical loosening of the former arable layer 0-35 cm once at 3 years by subsoiling with chisel, "pinocchio" or other type of subsoiler (Leah, 2016a; 2016b).

The situation regarding the implementation in Moldova of different basic tillage systems that protect the soil depends on the initial characteristics of the soil (Canarache, 1990), the provision of the territory with precipitation and the technical possibilities of the farmers.

The program of conservative agriculture provides for both the preventive restoration of the initial quality status of the properties of the degraded arable soil layer, as well as the monitoring of the properties changes in time and space.

The preventive research of the experimental plots soil gave the possibility to make the following conclusions:

- The agricultural soils located on the high terraces of the river Prut are characterized by a dusty-sandy clay texture, excellent in terms of their tillage and implementation of No-till or Mini-till technologies.

- As a result of the implementation during 2 consecutive favorable climatic years (2013 and 2014) on the territory of Larga Nouă commune, Cahul rayon of the system of conservative agriculture, based on the No-till technology of soil tillage, the agricultural crops were increased using more efficient soil moisture, conserved due to the layer of mulch formed on soil surface and the balanced fertilization of the crop plants (Figure 5).

The years 2013 and 2014 were favorable in terms of atmospheric precipitation and soil moisture during the vegetation period of agricultural crops.



Figure 5. Mulches on the soil surface after harvesting the seed of vetch (consisting from mixture of straw and grains vetch that shook)

After the preventive research the following conclusions were made:

- The soils investigated are poorly supplied with mobile phosphorus, moderately provided with mobile potassium and require the balanced application of the respective chemical fertilizers every year. The balanced application of chemical fertilizers complex has led to a slow change in soil fertility in a positive direction.

- The increase of the mobile phosphorus content in the arable layer 0-20 cm of the soil up to 2-3 mg/100 g soil should be done by autumn plowing until the implementation of the conservative agriculture system.

- The physical quality state of the soils worked No-till for 2 consecutive years has worsened: the soil has become strongly compacted and with high penetration resistance in dry state starting from 5 cm depth from the soil surface. The only 0-5 cm layer of soil left loose, consisting of a mixture of organic debris from the mulch layer and soil granules.

The incorporation into the 0-10 cm soil layer of the green mass of a vetch crop led to the modification of the physical quality state of this soil layer from unfavorable to very favorable, the penetration resistance of this soil layer from the large became very small. There was little change in the positive direction and the quality condition of the upper part of the layer 10-20 cm as a result of the disking and incorporation in the soil of the green mass of vetch up to the depth of 10-12 cm (Tables 1-3).

The physical quality state of the layer 0-20 cm radically has also been modified as a result of the incorporation into the soil of the green mass of two crop of vetch. The bulk density of this unfavorable one became very favorable, and the penetration resistance from large to very large became extremely low and low, which

contributed to the easy penetration of the roots of autumn barley.

The structure (dry sifting) in the soil layers where the green mass was introduced (0-10 cm, one crop and 0-20 cm, two crops), as a result of this action and the proper tillage of the soil, became agronomically favorable. However, the hydrostability of the structure of soil layers 0-10 cm and 0-20 cm, in which the green mass of vetch was introduced, did not change radically and from very small became only small - with a more favorable step.

The content of organic matter in the soil layers where the green mass of vetch was introduced increased by about 0.16-0.26%. It is necessary to note that this organic mass with high nitrogen content, as a result of the microbiological processes in the soil, partially becomes gradually labile humus. The labile humus is not closely related to the mineral part of the soil and gradually compares easily to mineralize as a result of microbiology processes. However, the mineralization process of this labile humus occurs slowly, over several years, which ensures for a period of 4-5 years a normal activity of the microbiological processes in the soil and avoids the emergence of the "nitrogen starvation" that is influential, by usually, due to the low content of mobile nitrogen in the soil, necessary for microbes to mineralize the organic residues of previous crops (Sencovscaia and Cerbari, 2018). The changes of the properties parameters of the ordinary chernozem in result of the incorporation in the soil of the green fertilizers in the 2016-2019 are presented in Tables 1-4. The average statistical ecopedological values of the quality status of ordinary chernozem were assessed according to the methodology developed by N. Florea et al. (1987) and A. Canarache (1990).

The No-tillage of soil together with the introduction of green fertilizers improves the vital activity and diversity of the edaphic fauna of the ordinary chernozem under the conditions of the Southern Moldova. In average, the number of invertebrates increases 6.0 times, Fam. *Lumbricidae* - 3.0 times, biomass - 2.3 and 2.2 times compared to plowing at depth 25-27 cm. The diversity of invertebrates has improved significantly, the number of families increasing from 2-5 to 4-12. Saprophages prevailed in the composition of the edaphic fauna.

Application of the No-tillage and introduction of green fertilizers in conditions of the Southern area of Moldova has positive effects on the microorganisms of the ordinary chernozem. The largest changes are in the 0-10 cm layer. There is an increase in microbial biomass by 2.0-2.1 times, the share of microbial carbon in the total content - by 1.8 times, the reserves of microbial biomass by 2.3 times compared to the plowing system. In total, the biomass of microorganisms in the 0-30 cm of postarable layer increases on

average by 13.9% compared to the plowing (Sencovscaia and Cerbari, 2018).

The research results confirm that the preventive restoration of the quality state of the dehumified, destroyed and compacted arable layer is absolutely necessary to be carried out until the implementation of the conservative agriculture system, based on the No-till soil tillage technology (Сйорд et al., 2002).

Table 1. Content of agronomic structural aggregates in soil (2016-2019)

Horizon and depth, cm	Initial data, No-till - 2 years		Variant - the application in soil 1 yield of vetch				Variant - the application in soil 2 yields of vetch			
	2015, autumn		2016	2017	2018	2019	2016	2017	2018	2019
	value assessing		value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing
Content of agronomically favorable structural aggregates 10-0.25 mm (dry sieving, %)										
Ahp1 0-5	75.0 good	65.0 good	73.9 good	72.2 good	77.4 good	75.9 good	74.5 good	75.1 good	77.6 good	75.1 good
Ahp1 5-10	55.0 moderate		57.5 moderate	44.0 moderate	40.8 moderate	42.5 moderate	77.7 good	77.7 good	71.0 good	70.4 good
Ahp1 10-20	48.8 moderate		49.0 moderate	47.8 moderate	48.2 moderate	50.8 moderate	45.1 moderate	45.1 moderate	44.3 moderate	40.0 moderate
Ahp2 20-30	42.7 moderate									
Content of favorable hydrostabile agronomic structural aggregates (wet sieving, %)										
Ahp1 0-5	31.9 small	24.3 small	31.6 small	21.6 small	23.2 small	25.6 small	31.4 small	27.3 small	29.8 small	29.2 small
Ahp1 5-10	16.7 very small		23.9 small	19.4 very small	26.4 small	26.2 small	23.6 small	25.5 small	28.0 small	24.2 small
Ahp1 10-20	14.7 very small		14.1 very small	18.2 very small	19.4 very small	17.0 very small	13.4 very small	14.6 very small	15.0 very small	14.4 very small
Ahp2 20-30	13.3 very small									

Table 2. The value of bulk density and total porosity in soil (2016-2019)

Horizon and depth, cm	Initial data, No-till - 2 years		Variant - the application in soil 1 yield of vetch				Variant - the application in soil 2 yields of vetch			
	2015, autumn		2016	2017	2018	2019	2016	2017	2018	2019
	value assessing		value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing
The mean values of bulk density, g cm ⁻³										
Ahp1 0-5	1.01 very small	1.24 small	1.12 very small	1.21 small	1.22 small	1.24 small	1.18 very small	1.24 small	1.22 small	1.23 small
Ahp1 5-10	1.47 high		1.44 high	1.48 high	1.46 high	1.47 high	1.21 small	1.26 small	1.28 small	1.37 moderate
Ahp1 10-20	1.48 high		1.49 high	1.49 high	1.45 mare	1.48 mare	1.50 mare	1.44 mare	1.45 mare	1.47 mare
Ahp2 20-30	1.49 high									
The average values of total porosity, % v/v										
Ahp1 0-5	61.2 extrem-high	52.9 high	56.9 very high	54.0 high	55.1 high	53.2 high	54.6 high	52.9 high	53.2 high	53.6 high
Ahp1 5-10	44.5 moderate		45.7 moderate	44.6 small	44.9 small	45.4 moderate	54.3 mare	52.8 mare	51.7 mare	48.7 moderate
Ahp1 10-20	44.5 small		44.2 small	44.4 small	45.7 moderate	44.8 small	43.8 small	46.3 moderate	45.3 moderate	45.2 moderate
Ahp2 20-30	44.4 small									

Table 3. The values of resistance to penetration and humus content in soil (2016-2019)

Horizon and depth, cm	Initial data, No-till - two years		Variant - the application in soil 1 yield of vetch				Variant - the application in soil 2 yields of vetch			
	2015, autumn		2016	2017	2018	2019	2016	2017	2018	2019
	value assessing		value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing
Average values of resistance to penetration, kgf cm ⁻²										
Ahp1 0-5	5 very low	13 low	7 very low	8 very low	6 very low	6 very low	6 very low	11 low	6 low	5 very low
Ahp1 5-10	20 high		17 moderate	19 moderate	19 moderate	19 moderate	7 very low	12 low	15 low	10 low
Ahp1 10-20	20 high		20 high	20 high	23 high	22 high	21 high	16 moderate	22 high	21 high
Ahp2 20-30	20 high									
Average values of humus content, % v/v										
Ahp1 0-5	2.70 moderate	2.63 moderate	2.79 moderate	2.85 moderate	2.78 moderate	2.82 moderate	2.89 moderate	2.85 moderate	2.85 moderate	2.86 moderate
Ahp1 5-10	2.57 moderate		2.67 moderate	2.71 moderate	2.65 moderate	2.69 moderate	2.84 moderate	2.83 moderate	2.81 moderate	2.82 moderate
Ahp1 10-20	2.52 moderate		2.47 moderate	2.49 moderate	2.47 moderate	2.47 moderate	2.49 moderate	2.52 moderate	2.50 moderate	2.51 moderate
Ahp2 20-30	2.45 moderate									

Table 4. The average values of mobile phosphorus and potassium content in soil (2016-2019)

Horizon and depth, cm	Initial data, No-till - two years		Variant - the application in soil 1 yield of vetch				Variant - the application in soil 2 yields of vetch			
	2015, autumn		2016	2017	2018	2019	2016	2017	2018	2019
	value assessing		value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing	value assessing
Average values of mobile phosphorus content, mg kg ⁻¹ of soil										
Ahp1 0-5	2.2 moderate	1.9 moderate	1.8 moderate	2.0 moderate	2.4 moderate	2.6 moderate	1.8 moderate	1.8 moderate	2.3 moderate	2.9 moderate
Ahp1 5-10	1.7 moderate		1.2 low	1.3 low	1.2 low	1.0 low	1.8 moderate	1.5 moderate	1.4 low	1.8 low
Ahp1 10-20	1.5 low		1.2 low	1.2 low	0.9 very low	0.8 very low	1.1 low	0.9 low	1.2 low	1.3 low
Ahp2 20-30	1.3 low									
Average values of exchangeable potassium content, mg kg ⁻¹ of soil										
Ahp1 0-5	31 high	27 optimal	20 optimal	21 optimal	32 high	22 optimal	25 optimal	24 optimal	33 high	24 optimal
Ahp1 5-10	22 optimal		20 optimal	20 optimal	20 optimal	19 moderate	25 optimal	21 optimal	21 optimal	21 optimal
Ahp1 10-20	20 optimal		19 moderate	15 moderate	20 moderate	19 moderate	17 moderate	17 moderate		
Ahp2 20-30	18 moderate									

This can be achieved only by intensifying the organic matter flow in the arable layer of soils from any possible source, increasing the humus content, restoring the structure and stabilizing it loosening.

The most effective method to regenerate the degraded soils for Moldova at present is the systematic use of green fertilizers - autumn and spring vetch or peas consistently sown in an agricultural year on a "busy field", whose green mass is introduced into the soil as an organic green fertilizer concomitantly with the subsoiling being carried out once in 3 years at a depth of 30-35 cm of the former arable layer (Cerbari and Leah, 2016).

In the climatic conditions of Southern Moldova it is possible to use the autumn peas or vetch as an intermediate crop, sown in the middle of September and incorporated into the soil as an organic fertilizer through disking at the end of April or at the beginning of May.

After the implementation of the conservative agriculture system, based on the No-till technology of soil tillage, it is necessary to establish a permanent pedological monitoring on the fields tillage according to this technology in order to detect in time some negative phenomena and to carry out the measures to prevent and eliminate their consequences.

The research conducted in 2016-2019 showed that the introduction into the soil by disking in the agricultural year 2014-2015 of two green masses of autumn vetch, as intermediate crop, led to the restoration in the positive direction of the physical, chemical and biological properties of the arable layer 0-10 and 10-20 cm: the soil layer 0-20 cm became biogenic, the balance of humus in the soil used for 4 years under the basic crops became positive, the quality and quantity of agricultural production increased.

The economic efficiency of the tested method is presented by the crop yield every year:

The harvest of autumn barley in 2016 reached the size of 7.1 t ha^{-1} , the harvest increase by 2.2 t/ha/year , the cash value of the harvest increase: $2.2 \text{ t barley} \times 2200 \text{ lei} = 4840 \text{ lei (MDL)}$.

Rapeseed harvest in 2017 reached the size of 4.1 t ha^{-1} , the harvest increase - 1.0 t/ha/year , the cash value of the harvest increase: $1 \text{ t rapeseed} \times 7100 \text{ lei} = 7100 \text{ lei (MDL)}$.

The winter wheat harvest in 2018 reached the size of 4.6 t ha^{-1} , the harvest increase was equal to 0.8 t/ha/year : income was $0.8 \text{ t wheat} \times 3300 \text{ lei} = 2640 \text{ lei (MDL)}$.

The sunflower harvest in 2019 was 3.3 t/ha/year , the cash value of harvest increase by 0.5 t ha^{-1} : $0.5 \text{ t sunflower} \times 7000 \text{ lei} = 3500 \text{ lei}$.

The total monetary value for 4 years of the basic crop yields increase was: $4840 \text{ lei} + 7100 \text{ lei} + 2640 \text{ lei} + 3500 \text{ lei} = 18580 \text{ lei/ha}$.

Total expenses for experimental field organization, seed procurement, sowing, incorporation into the soil the green mass of 2 crop of vetch = 6000 lei .

The net income for 4 years from the basic crops yields constituted: $18080 \text{ lei} - 6000 \text{ lei} = 12080 \text{ lei (MDL)}$.

CONCLUSIONS

The implementation on the Southern area of Moldova the conservative agriculture system, based on the No-till technology of soil tillage, has led to the increase of agricultural crops production in the first two years as a result of more efficient use of soil moisture, due to the layer of mulch formed on the soil surface and the balanced application of complex fertilizers.

The 5-years experimental researches confirmed that the preventive restoration of the quality status of the dehumidified, destructured and

compacted arable layer of ordinary chernozem is absolutely necessary to be carried out until the implementation of the conservative agriculture system, based on the No-till soil technology.

The recommended method of preventive restoration of the quality status of the arable soil layer, based on the use of leguminous crops as green organic fertilizer, has led to the remediation of the physical, chemical and biological quality of this layer, to the increase of the soil production capacity and created prerequisites for the successful implementation of the conservative agriculture system - No-till.

The implementation in the Republic of Moldova the conservative agriculture system, based on the use of green fertilizers in conjunction with the No-till or Mini-till technologies of soil tillage, contributes:

- to the establishment of the permanent organic matter flow and the gradual positive restoration of the physical, chemical and biological properties of the soils;

- at the increase of the agricultural crop yields by 20-30%;

- at the reduction of the nitrogen fertilizer requirement by at least 50%;

- to the reduce of chemical pollution of agricultural production and the environment.

Soil as a means of production is a poorly renewable resource, spatially limited and cannot be multiplied. The regeneration of the soil can only take place if it is properly worked, it is not over exploited until it is exhausted, the conditions of protection and conservation are respected and it is ensured in a permanent flow of qualitative organic substance in its arable layer. Otherwise, the soil, being vulnerable to numerous degradation processes, can be damaged or even destroyed.

At the same time, we must recognize that the restoration of the damaged soil covering is very difficult to achieve and requires an extremely long time, very high costs and cannot be executed during the life of a people generation.

In this context, permanent monitoring of changes in soil quality status is absolutely necessary for timely implementation of measures to combat or mitigate the degradation processes of this important means of agricultural production.

In order to successfully implement this method of remediating the degraded arable soil layer, it is necessary to organize the system of non-polluting green fertilizers use in the agricultural sector of the Republic of Moldova and to create the seed base of legumes (autumn and spring vetch or peas).

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REFERENCES

- Agricultura conservativă. Fișă informativă nr. 5. *Agricultura durabilă și conservarea solului*. Metode și sisteme agricole nepericuloase pentru sol. Disponibil: <http://agrilife.jrc.ec.europa.eu/documents/ROFactSheet-05.pdf>; [accessed 21.01.2020].
- Berca, M. (2011). *Agrotehnica*. București: Ceres. ISBN 978-973-40-0899-5, 124–178.
- Canarache, A. (1990). *Fizica solurilor agricole*. București: Ceres. 268.
- Cerbari, V. (2011). No-Till – sistem de agricultură care protejează solul. *Agricultura Moldovei*, 8-9, 9–14.
- Cerbari, V. (coordonator), (2010). Monitoringul calității solurilor Republicii Moldova. Ch., Pontos, 476.
- Cerbari, V., Leah, C. (2016). Green Manure - only possibility to save Moldova's arable soils from degradation. *Scientific Papers. Agronomy*, 59(2).
- Florea, N., Bălăceanu, V., Canarache, A. et al. (1987). *Metodologia elaborării studiilor pedologice*. Partea a III-a. București, 226.
- Leah, T. (2016a). Improving conservation practices to protect soil and water quality in agriculture of Moldova. *Scientific Papers. Series Management, Economic Engineering in Agriculture and Rural Development*, 16(1), 253–258.
- Leah, T. (2016b). The influence of soil tillage on the some physical indicators of chernozem cambic from Moldova. *Scientific Paper. Series Agronomy*, 59(1), 197–200.
- Leah, T. (2017). *Modificarea stării de calitate a stratului arabil al cernoziomurilor cambice din Moldova Centrală sub influența îngrășămintelor verzi*. În: Cercetarea și gestionarea resurselor de sol: Materialele conferinței științifice cu participare internațională a SNMSS (8-9 septembrie 2017). Chișinău/red. resp.: Gh. Jigău, An. Tărăță. - Chișinău: SNMSS (CEP, USM). ISBN 978-9975-71-931-5, 250–259.
- Leah, N., Leah, T. (2018). The importance of crop rotation in the agriculture of the Republic of Moldova. *Proceedings & Abstracts, 1st International Congress on Agricultural Structures and Irrigation (26-28 September, Antalya, Turkey)*, 655.
- Leah, T. (2018a). Phytoremediation measures for restoring quality state of Cambic chernozem from Moldova. *Abstracts of the Joint Conference EBC-VII & ISEB-2018 275, Chania, Greece, June 25-28*, 6–9.
- Leah, T. (2018b). The importance of crop rotation and the role of legumes. *Researches on the field crops in the Republic of Moldova (National Conference with international partners, Bălți, June 21-22, 2018)*. *Tipografia Indigou Color, Chișinău*. 66–71.
- Leah, T., Leah, N. (2018). Organic farming in Moldova - solution for rebalancing the soil resources and environment. *Proceedings of International Scientific Agricultural Symposium "AgroSym 2018" (Jahorina, October 04-07, 2018)*, 1335–1340.
- Senicovscaia, I., Cerbari, V. (2019). Influence of no-tillage practices and green manure treatment on soil biota of the ordinary chernozem in the southern zone of the Republic of Moldova. *Scientific papers. Series Agronomy, LXII(1)*, 173–178.
- Wiesmeier, M., Lungu, M., Hübner, R., and Cerbari, V. (2015). Remediation of degraded arable steppe soils in Moldova using vetch as green manure. *Solid Earth*, 6, 609–620.
- Блэк, К.А. (1993). *Растение и почва*. М.: Колос, 503.
- Лал, Р. (2011). *10 законов самовосстанавливающегося управления почвой*. *Зерно*, № 9, 68–70.
- Руководство по производству. Нулевая обработка почвы. США – 1991, 42 с.
- Сйорд, В., Дейкер и Джоал, С. Майерс. (2002). *Почвы лучше при системе No-Till*, 20.

APPLICATION OF ANHYDROUS AMMONIA AS NITROGEN FERTILIZER, ITS INFLUENCE ON SOIL PROPERTIES AND YIELD OF AGRICULTURAL CROPS

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Abstract

The article presents the scientific results of anhydrous ammonia on the acid-base properties of leached chernozem carried out in the training and production center of Penza State Agrarian University (Russia). After adding anhydrous ammonia, an increase in pH values is observed. During the growing season, an increase in acidity is observed as nitrification of ammonium nitrogen into nitrate. This is especially manifested when ammonia is introduced in doses of 150 and 200 kg/ha. In variants using anhydrous ammonia in doses of 150 and 200 kg/ha, the amount of absorbed bases decreased by 1.4-2.2 meq/100 g of soil. The greatest decrease was observed in variants with doses of 200 kg/ha in physical weight. The introduction of anhydrous ammonia in doses of 100 to 200 kg/ha in physical weight increased the content of nitrate nitrogen in the soil from the beginning of the growing season to the time of harvesting and provided spring wheat plants with available nitrogen. When conducting plant diagnostics on options using anhydrous ammonia, signs of nitrogen starvation of spring wheat plants were not observed.

Key words: anhydrous ammonia, acidity, nitrate nitrogen, grain yield, spring wheat.

INTRODUCTION

One of the most effective ways of intensifying crop production is to improve the supply of plants with nitrogen, since it is this element that most often limits yield. Currently, one of the indispensable attributes of highly profitable farming using intensive technologies is the use of anhydrous ammonia.

At the same time, its limited use in a number of countries indicates that the use of this liquid nitrogen fertilizer not only has advantages, but is also accompanied by certain difficulties (Smiciklas et al., 2008; Kovács et al., 2015).

Anhydrous ammonia (NH₃) - the most concentrated ballastless fertilizer, contains 82% nitrogen. Get it by liquefying gaseous ammonia under high pressure. This is a colorless liquid with a characteristic pungent odor, one of the most dangerous chemicals used in agriculture.

The agronomic advantage of anhydrous ammonia over solid nitrogen fertilizers is the fact that the diffusion of nitrogen from the granule depends on soil conditions and most often occurs in the vertical direction, while ammonia turns into gas at atmospheric pressure, diffusing in the soil over a greater distance in all directions, which allows one to more evenly

distribute nitrogen in the soil layer and, accordingly, increase the coefficient of its assimilation by plants (Bundy et al., 2011; Zhang et al., 2015).

MATERIALS AND METHODS

In order to study the effect of anhydrous ammonia on the properties of leached chernozem and grain yield of spring wheat, studies were conducted on the experimental field of the training and production center of Penza state Agrarian University (Mokshansky district, Penza, Russia) in 2017-2019 according to the following scheme:

1. Without fertilizers (control);
2. Anhydrous ammonia (NH₃) - 100 kg/ha in physical weight (F.W.) when applied to a depth of 10 cm;
3. NH₃ - 100 kg/ha F.W. to a depth of 15 cm;
4. NH₃ - 150 kg/ha F.W. to a depth of 15 cm;
5. NH₃ - 200 kg/ha F.W. to a depth of 15 cm;
6. NH₃ - 100 kg/ha F.W. to a depth of 20 cm;
7. NH₃ - 150 kg/ha F.W. to a depth of 20 cm;
8. NH₃ - 200 kg/ha F.W. to a depth of 200 cm.

Experiments were carried out on leached chernozem medium humus loamy.

The plot area is 1230 m². The plot allocation is randomized. Three iterations. The total area of

the experiment is 29,520 m² (2,952 ha). The predecessor is the winter wheat.

Before the introduction of anhydrous ammonia, disking was carried out to a depth of 12-14 cm and soil samples were taken for the initial values. Anhydrous ammonia was introduced according to the research scheme. For application, the integrated Case IH 5300 aggregate with a working width of 8.4 m was used.

When conducting soil and plant analyzes, the following research methods were used:

- nitrate nitrogen in the soil by potentiometric method;
- pH of saline suspension by potentiometric method;
- hydrolytic acidity of the soil according to the Kappen;
- the amount of absorbed bases according to the Kappen-Gilkowitz method.

- crop accounting by the weighing method according to the experimental options in 3-fold repetition from 1.0 m².

- crudeprotein content in wheat grain by the Kjeldahl method;
- wetgluten content in wheat grain - by manual method;
- gluten quality on the IDK-3M device.

RESULTS AND DISCUSSIONS

The experiments have shown, before sowing spring wheat, the pH of the soil in the experimental plots varied between 5.01-5.85 units and was characterized as weakly acidic and close to neutral, while an increase in pH was observed in variants with the introduction of anhydrous ammonia (Table 1).

Table 1. Soil pH (KCl) (units) in a layer of 0-25 cm, depending upon the dose of anhydrous ammonia (average for 2018-2019)

Variant	Before implementation (October)	Sample Duration				Deviations from the initial values
		Before sowing (first decade of May)	Exit to the handset (third decade of June)	At the time of harvesting (second decade of August)	One month after harvesting (second decade of September)	
1. Without fertilizers (control)	5.02	5.01	4.99	4.95	4.92	-0.10
2. NH ₃ 100 kg/ha 10 cm	5.02	5.22	5.15	5.05	5.00	-0.02
3. NH ₃ 100 kg/ha 15 cm	5.07	5.48	5.10	5.03	5.03	-0.03
4. NH ₃ 150 kg/ha 15 cm	5.22	5.62	5.52	5.38	4.98	-0.23
5. NH ₃ 200 kg/ha 15 cm	5.20	5.64	5.54	5.42	4.92	-0.28
6. NH ₃ 100 kg/ha 20 cm	5.07	5.22	5.13	5.07	4.87	-0.19
7. NH ₃ 150 kg/ha 20 cm	5.13	5.49	5.22	5.15	4.95	-0.18
8. NH ₃ 200 kg/ha 20 cm	5.30	5.85	5.35	5.32	4.92	-0.38

The increase in pH depending on the dose of anhydrous ammonia was 0.2-0.55 units pH. The dynamics of increasing the pH of the soil is associated with the use of anhydrous ammonia and the alkalization of the soil solution and soil with the formed ammonium hydroxide.

The greatest changes were in the case where 200 kg/ha of ammonia was added to a depth of 20 cm. In the subsequent determination, the pH of the soil decreased by 0.07-0.5 units for all variants of the experiment pH.

At the time of harvesting, there was a further trend towards a decrease in pH both on options without fertilizers (control) and with the use of

different doses of anhydrous ammonia (Melbourne et al., 2014).

The largest decrease in pH was observed in the variants using anhydrous ammonia at doses of 150 and 200 kg/ha, deviations from the initial values range from 0.18 to 0.38. This tendency to lower soil pH is associated with mineralization and nitrification processes. Ammonium forms of nitrogen gradually turn into nitrate forms with acidification of the soil solution and soil.

Hydrolytic acidity prior to the introduction of anhydrous ammonia according to the test variants was in the range of 2.65-5.03 meq/100 g of soil. During the growing season, minor

changes occurred depending on the options of the experiment. In variants without fertilizers and with the use of anhydrous ammonia to a

depth of 10 cm, an increase in hydrolytic acidity by 0.19-0.22 meq/100 g of soil is observed (Table 2).

Table 2. Hydrolytic acidity in the soil layer 0-25 cm, depending upon the application dose of anhydrous ammonia, meq/100 g of soil (average values for 2018-2019)

Variant	Before implementation (October)	At the time of harvesting (second decade of August)	Deviations from the initial values
1. Without fertilizers (control)	4.55	4.77	0.22
2. NH ₃ 100 kg/ha 10 cm	4.41	4.60	0.19
3. NH ₃ 100 kg/ha 15 cm	4.76	4.45	-0.31
4. NH ₃ 150 kg/ha 15 cm	5.03	4.68	-0.35
5. NH ₃ 200 kg/ha 15 cm	2.65	2.49	-0.17
6. NH ₃ 100 kg/ha 20 cm	4.22	4.17	-0.05
7. NH ₃ 150 kg/ha 20 cm	3.59	3.38	-0.21
8. NH ₃ 200 kg/ha 20 cm	3.16	2.76	-0.40

In the variants with the introduction of anhydrous ammonia to a depth of 15 and 20 cm at the end of spring wheat vegetation, a decrease in the hydrolytic acidity index by 0.05-0.4 meq/100 g of soil was observed. This can be explained by the fact that when there is a lack of moisture in the soil, the nitrification process is slow and all the ammonia introduced has not yet completely converted to nitrate nitrogen.

The amount of absorbed bases before applying anhydrous ammonia was in the range of 34.3-38.9 meq/100 g of soil. During the growing season of cultivated crops, insignificant changes in the amount of absorbed bases occurred. On options for the experiment with the use of anhydrous ammonia, there was a tendency to decrease this indicator by 0.8-2.2 meq/100 g of soil. In the variant without fertilizers, the amount of absorbed bases decreased by 0.8 meq/100 g of soil (Table 3).

The greatest decrease in the amount of absorbed bases was observed for variants with doses of 150 and 200 kg/ha with values of 1.4-2.2 meq/100 g of soil. This tendency to reduce the amount of absorbed bases is due to the fact that when anhydrous ammonia is introduced from the soil absorption complex, calcium and magnesium are displaced into the soil solution and used by plants during the growing season.

Reserves of nitrate nitrogen in the soil layer 0-40 cm before the introduction of ammonia were in the range of 21.0-27.2 kg/ha. In the spring, when determining the reserves of nitrate nitrogen in the soil according to the experimental options, its content varied depending on the application dose and ranged from 46.0 to 113.5 kg/ha (Table 4).

Table 3. The amount of absorbed bases in the soil layer 0-25 cm depending on anhydrous ammonia application dose, meq/100 g of soil (average indicators for 2018-2019)

Variant	Before implementation (October)	At the time of harvesting (second decade of August)	Deviations from the initial values
1. Without fertilizers (control)	35.1	34.3	-0.8
2. NH ₃ 100 kg/ha 10 cm	34.3	33.1	-1.2
3. NH ₃ 100 kg/ha 15 cm	36.2	34.6	-1.6
4. NH ₃ 150 kg/ha 15 cm	37.6	36.3	-1.4
5. NH ₃ 200 kg/ha 15 cm	36.7	34.5	-2.2
6. NH ₃ 100 kg/ha 20 cm	35.0	34.3	-0.7
7. NH ₃ 150 kg/ha 20 cm	36.8	34.8	-2.0
8. NH ₃ 200 kg/ha 20 cm	38.9	36.7	-2.2

Table 4. The content of nitrate nitrogen in the soil layer of 0-40 cm, depending on the dose of anhydrous ammonia, kg/ha (average for 2018-2019)

Variant	Before implementation (October)	Sample duration			Deviations from the initial values
		Before sowing (first decade of May)	Exit to the handset (third decade of June)	At the time of harvesting (second decade of August)	
1. Without fertilizers (control)	26.6	21.5	14.1	11.6	-
2. NH ₃ 100 kg/ha 10 cm	23.8	46.0	24.2	15.5	3.9
3. NH ₃ 100 kg/ha 15 cm	22.0	55.8	34.4	27.1	15.5
4. NH ₃ 150 kg/ha 15 cm	24.4	88.6	35.0	38.1	26.5
5. NH ₃ 200 kg/ha 15 cm	21.0	113.5	77.5	73.0	61.4
6. NH ₃ 100 kg/ha 20 cm	24.4	58.4	24.8	18.8	7.2
7. NH ₃ 150 kg/ha 20 cm	23.2	86.6	31.5	30.6	19.0
8. NH ₃ 200 kg/ha 20 cm	27.2	113.0	45.8	54.6	43.0

Compared with the control variant, the nitrate content in the soil layer of 0-40 cm increased by 23.5-92.0 kg/ha. An increase in the content of nitrate nitrogen is associated with nitrification and the transition of ammonium forms to nitrate ones. The nitrification process under these conditions is faster than on the version without fertilizers (Zhou et al., 2014).

In the middle of the growing season, the same trend is observed. The highest nitrate nitrogen content was observed in variants with doses of 200 kg/ha in F.W. 45.8 kg/ha when applied to a depth of 20 cm and 77.5 kg/ha when applied to a depth of 15 cm. In the variant without fertilizers, the content of nitrate nitrogen decreased to 14.1 kg/ha, which is associated with an increase in plant nutrition with this element in the middle of the growing season (Diane et al., 2011). When comparing the data on the content of nitrate nitrogen in the soil with the previous determination period, it is seen that there was a decrease in the stock of nitrate nitrogen for all variants of the experiment.

At the time of harvesting, the content of nitrate nitrogen in all experiment variants decreased. The highest nitrate nitrogen content was in the variants with 200 kg/ha of ammonia and amounted to 73.0 kg/ha when applied to a depth of 15 cm. In the variants with different doses of ammonia, the nitrate content in the soil at the time of harvesting was higher compared to the control on 3.9-61.4 kg/ha. In the control variant, the nitrate nitrogen content decreased to 11.6 kg/ha. The largest deviation of the

nitrate nitrogen content in the soil in the 0-40 cm layer from the control at the time of harvesting was found in the options for using anhydrous ammonia at a dose of 200 kg/ha to a depth of 15 and 20 cm, the deviations were 43.0 and 61.4 kg/ha, relatively.

The spring wheat grain yield was experimentally influenced by the weather conditions of the vegetation periods of 2018 and 2019. Weather conditions during the summer growing season of 2018 were characterized as arid. During the growing season of spring wheat from May to August, 116 mm of precipitation fell. When comparing with the data of perennial values, in the growing season of 2018, 54% of precipitation fell from the average long-term values, which subsequently affected the size of the crop yield. The weather conditions in 2019 were characterized, as in 2018, as arid. During the growing season of spring wheat, 153 mm of precipitation fell, which was lower than the long-term average by 62 mm or 29%. The month of June was the most arid. The air temperature in June ranged from +5.8°C to +33.4°C, and the amount of precipitation was 22 mm. During this month, 8 days with precipitation were observed, and only once 11 mm of precipitation fell, the remaining precipitation was less than 5 mm and was unproductive.

The results of studies to determine the yield of spring wheat grain showed that the autumn use of anhydrous ammonia increased the yield of spring wheat grain by 0.12-2.28 t/ha. The use of anhydrous in a dose of 100 kg/ha in F.W. when

applied to a depth of 10 cm, the grain yield increased by 0.12 t/ha (6.3%). The greatest increase in spring wheat grain yield in the experiment was obtained with the option of introducing a dose of ammonia of 200 kg/ha to a depth of 20 cm. When applying doses of ammonia from 100 to 200 kg/ha in F.W. wheat grain productivity increased by 0.48-1.72 t/ha or

by 24.4-86.3% compared to the version without fertilizers.

The weight of 1000 grains in the variants ranged from 35.8-38.9 g. The lowest weight of 1000 seeds was obtained in the variant without fertilizers, and the highest in the variant using anhydrous ammonia at a dose of 200 kg/ha to a depth of 20 cm (Table 5).

Table 5. The yield of grain of spring wheat, depending on the dose of anhydrous ammonia (average values for 2018-2019)

Variant	Grain yield			Mass of 1000 grains, g
	t/ha	deviations from control, t/ha	deviations from control, t/ha	
1. Without fertilizers (control)	1.99	-	-	35.8
2. NH ₃ 100 kg/ha 10 cm	2.11	0.12	6.3	37.2
3. NH ₃ 100 kg/ha 15 cm	2.47	0.48	24.4	37.6
4. NH ₃ 150 kg/ha 15 cm	2.82	0.83	41.9	36.8
5. NH ₃ 200 kg/ha 15 cm	3.71	1.72	86.3	38.2
6. NH ₃ 100 kg/ha 20 cm	2.37	0.38	19.1	37.1
7. NH ₃ 150 kg/ha 20 cm	3.50	1.51	75.7	37.5
8. NH ₃ 200 kg/ha 20 cm	4.27	2.28	114.7	38.9
HCP ₀₅		0.11 ± 0.03		0.95 ± 0.06

Important quality indicators of spring wheat grain in the Russian Federation are the protein and gluten content.

Studies show that without the use of fertilizers, the protein and gluten content in

wheat grain is reduced (Table 6). The protein content in the variant without the use of fertilizers on the Granny variety was 12.57%, and the gluten content is 17.5%, this grain is rated as grade 5.

Table 6. Grain quality of spring wheat depending on the doses of anhydrous ammonia

Variant	Crude protein, %	Gluten			
		%	Grade	IDK	Quality group
1. Without fertilizers (control)	12.57	17.5	5	80	2
2. NH ₃ 100 kg/ha 10 cm	13.71	23.0	4	77	1
3. NH ₃ 100 kg/ha 15 cm	14.05	23.0	4	79	1
4. NH ₃ 150 kg/ha 15 cm	14.32	25.5	3	72	1
5. NH ₃ 200 kg/ha 15 cm	14.45	29.0	2	87	2
6. NH ₃ 100 kg/ha 20 cm	13.37	24.0	3	81	2
7. NH ₃ 150 kg/ha 20 cm	13.54	27.0	3	72	1
8. NH ₃ 200 kg/ha 20 cm	13.71	27.5	3	77	1

The use of anhydrous ammonia in different doses increased the protein content to 13.37-14.45%, and gluten to 23.0-29.0%. When using anhydrous ammonia at a dose of 100 kg/ha to a depth of 10 and 15 cm in conditions of 2018, they received fourth-grade spring wheat grain of the first quality group. The introduction of ammonia in a dose of 150 kg/ha to a depth of 15 cm and from

100 to 200 kg/ha to a depth of 20 cm made it possible to obtain grain of the third class.

The most productive was the introduction of a dose of 200 kg/ha of anhydrous ammonia on leached chernozem to a depth of 15 cm, this made it possible to obtain grain of the second class of the second quality group with a content of: 14.45% protein, high quality gluten 29%.

CONCLUSIONS

The introduction of anhydrous ammonia in doses from 100 to 200 kg/ha in F.W. increased the content of nitrate nitrogen in the soil from the beginning of the growing season to the time of harvesting and provided spring wheat plants with available nitrogen.

When conducting plant diagnostics on variants using anhydrous ammonia, signs of nitrogen starvation of spring wheat plants were not observed.

In variants with a dose of ammonia of 200 kg/ha, an increased nitrate content is observed during the entire growing season of spring wheat, while nitrate nitrogen in the soil accumulated faster than was used by plants during nutrition.

The use of anhydrous ammonia in doses of 100, 150 and 200 kg/ha at different depths in spring wheat cultivation technologies increases grain yield depending on application doses by 24.4-114.7% and improves grain quality, increasing protein and gluten content.

REFERENCES

- Bundy, L.G., Andraski, T.W., Ruark, M.D., Peterson, A.E. (2011). Long-term continuous corn and nitrogen fertilizer effects on productivity and soil properties. *Agronomy Journal*, 103(5), 1346–1351. doi: 10.2134/agronj2011.0094.
- Diane, E.A., Bhupinder, P.S., Ram, C.D. (2011.) Soil Health indicators under climate change: A Review of Current Knowledge. *Soil Biology*, 29, 25–45.
- Kovács, P., Van Scoyoc, G.E., Doerge, T.A., Camberato, J.J., Vyn, T.J. (2015). Anhydrous ammonia timing and rate effects on maize nitrogen use efficiencies. *Agronomy Journal*, 107(4), 1205–1214. doi: 10.2134/agronj14.0350
- Melbourne, L.J. (2014). Soil Chemical Analysis. *Environmental Science*.
- Smiciklas, K.D., Moore, A.S. (2008). Tile drainage nitrate concentrations in response to fertilizer nitrogen application. *Journal of Agronomy*, 7(2), 163–169.
- Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature*, 528(7580), 51–59. doi: 10.1038/nature15743
- Zhou, W., Lv, T., Chen, Y., Westby, A.P., Ren, V.J. (2014). Soil Physicochemical and Biological Properties of Paddy-Upland Rotation: A Review. *The Scientific World Journal*, Article ID 856352, 8 p.

PRINCIPLES OF ECOLOGICAL MANAGEMENT OF SOIL RESOURCES IN AGRICULTURE IN THE REPUBLIC OF MOLDOVA

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Abstract

In this article we tried to present both the results of scientific research and observations from the Republic of Moldova, as well as the experience of different countries of the world, who were convinced of the need for economic, ecological and social modernization of the modern agricultural system, in order to transition to a sustainable system of agriculture. We hope that the farmers will enrich this experience which it will be a good help at the beginning of the path towards organic farming in our countries. Soil resource management is a major social issue. The increase of agricultural production can only be achieved through the rational use of soil resources. Effective sustainable agriculture, based on conservative technologies, can be designed as part of a system of long-term protection and preservation of soil quality and production capacity. According to the directive no. 2 of 25.01.2011, the Ministry of Agriculture and Food Industry requested the elaboration of the Program for the development and implementation of conservative technologies in agriculture. As of January 1, 2011, the total area of the Republic of Moldova constituted 3384.6 thousand ha, including: 2498.28 thousand ha (73.8%) agricultural land. Of which: 1812.73 thousand ha (72.6%) arable land, 298.78 thousand ha (12.0%) perennial plantations, 352.55 thousand ha (14.1%) meadows and pastures, 34.21 thousand ha (1.4%) land not worked.

Key words: ecological management, erosion protection, soil, sustainable agriculture, fertility.

INTRODUCTION

Interest in organic farming is growing worldwide, due to the fact that the industrial intensification model of agriculture has not ensured a sustainable development of the agricultural sector (Dokuchaev, 1949).

Moreover, the negative consequences intensified: economic, ecological and social extensions of the concept of the "green revolution", based on increasing the use of non-renewable energy sources and their derivatives (mineral fertilizers, especially nitrogen, pesticides for the management of diseases, pests and weeds, irrigation, plowing with plow with cormane etc.). Limited sources of non-renewable energy and continuous growth of prices for them, as well as their industrial derivatives (nitrogenous fertilizers, pesticides etc.) dictate the need for continuous increase in prices for energy sources and industrial products used in agriculture. Thus, the competitiveness of farmers decreases, especially in countries that do not have their own renewable energy resources. In addition, the negative effects of narrow specialization and concentration of agricultural production became evident under the influence of intensive use of chemicals in

agriculture (Oades, 1991; Бойнчан, 1999), as well as heavy equipment for agricultural work (water and wind erosion, increased loss of soil organic matter compensated, soil compaction, loss of biodiversity in the aerial and underground part of the soil etc.).

The abrupt reduction of soil fertility has become one of the main limiting factors in the subsequent increase of crops. Stabilizing and reducing production levels is becoming a well-established trend for all countries of the world, including European countries, and, of course, for the Republic of Moldova. The market economy underestimates or totally ignores both the negative consequences on the environment and on society. For example, the worsening of people's health, the ruin of rural communities with the danger of their disappearance and so on. In this regard, the company has become increasingly aware that food prices do not reflect the real costs of production, because the negative consequences on the environment and human health are not taken into account. People want to know the origin of the consumed products, as well as the influence of their cultivation methods on the environment and their health. Agriculture needs a different

approach to its intensification process (Barber, 1988; Reid et al., 1981; Tansley, 1935; Vernadsky, 1965). For the transition to a more sustainable system of agriculture, including an ecological system of agriculture, it is very important that the soil be conceived as a living ecosystem, a living organism in which the biophilic elements and the energy in the form of synthesis - decomposition of matter - flow continuously soil organic matter (Vernadsky, 1965). Unfortunately, in modern agriculture the soil is often conceived as a substrate in which it is necessary to add water and nutrients, as well as to protect the plants against diseases, pests and weeds in order to maximize the level of production obtained. There is also a simplistic approach to assessing the impact of drought consequences and the design of fertile soil capacity. Both are a consequence of the lack of soil surface of live or dead mulch, as well as due to deterioration of soil structure, because of insufficient restitution of organic residues in the soil to increase the biological activity of the soil and, as a result, better supply of plants with nutrients. Traditional agriculture, based on the use of industrial inputs, is usually geared towards “fighting” the consequences, rather than eliminating the causes that caused problems. For example, the efficiency of mineral fertilizers is higher on more compacted soils than on poorly compacted soils (Krupenikov et al., 2011). A similar situation arises when using mineral fertilizers on poorer soils or more unfavorable predecessors for different crops. It is obvious that it would be preferable to reduce the dose of mineral fertilizers by enriching the soil with organic substance, which will also contribute to soil relaxation under the conditions of sunny conditions, which implies simultaneously respecting the location of crops after pre-fertilizers (Sheptukhov, 1993).

MATERIALS AND METHODS

After the renowned academic T.S. Malițev, the efforts of the scientists in Moldova have been oriented towards increasing the level of production, without taking into account the need to restore soil fertility. As a result, soil fertility became one of the limiting factors for the subsequent increase in the level of production. The data obtained from long-term field

experiments on permanent crops and cultivations of the researches from Republic of Moldova with a duration of more than 50 years testify that, despite improving the composition of crop varieties and improving cultivation technologies of them, in the mid 80's of the last century, the stabilization of autumn wheat and sugar beet crops began to stabilize with their subsequent decline in the last 20-25 years. A similar situation is marked for other cultures in the long-term experiences of the institute. In the European countries, the stabilization of the production started a little later: in Switzerland in 1990; in the Netherlands in 1993; in France and the United Kingdom in 1996 etc. There is a misconception that organic farming means giving up the country to use chemicals for plant nutrition and their protection against pests, diseases and weeds. This is important, but obviously not enough, because in the absence of systemic management capable of compensating for the renunciation of the use of chemicals, the situation can change dramatically, and the idea itself may be compromised. Modern research allows a deeper understanding of the polyfunctional role of the soil and the importance of the trophic chain in the soil. We must recognize that the biodiversity in the underground part of the soil is much higher than in the aerial part and remains little explored. Without adequate measures for the correct selection of predecessors for all crops in the wild, and without adequate measures to restore soil fertility, it is impossible to achieve the expected results in organic farming (Figure 1).

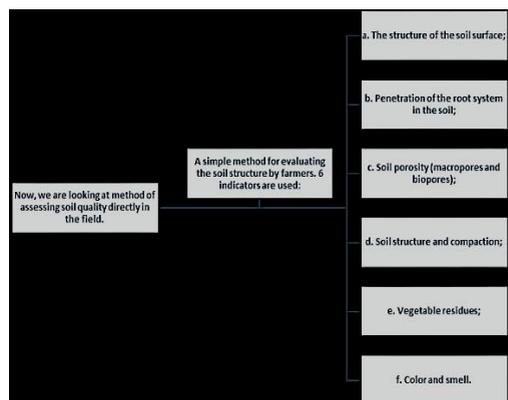


Figure 1. Basic indicators in the assessment of soil quality in the field

The situation is aggravated by ignorance and misunderstanding, especially the enormous role in soil of different groups of microorganisms in the transformation of soil organic matter.

In 10 grams of soil contains 1.5 times more microbes than the population of the earth. Satisfying their needs in nutrients and living conditions (air, water) is no less important than creating satisfactory living conditions for plant growth and development. The use of heavy equipment, the excessive consumption of mineral fertilizers and plant protection products in the control of diseases, pests and weeds will surely have a negative impact on the living conditions of the soil biota, in the formation of the soil structure (Berezin et al., 1985).

RESULTS AND DISCUSSIONS

The ecological management of the soil and of the crops implies the observance of a coherent

management system, oriented not only to obtaining the expected production, but also to restoring the fertility of the soil.

This goal can be achieved over a longer period. The accumulation of organic matter in the soil allows at the same time the accumulation of a larger quantity of water in the soil, which determines the success of the agricultural system in steppe conditions (French et al., 1979; Hadas, 1990).

Soil is a relatively non-renewable natural resource, by comparison with the duration of human life. Agriculture based on depletion of natural resources cannot achieve sustainable development. Moreover, deteriorating soil fertility leads not only to reducing crop productivity but also to other undesirable consequences (Bakhtin, 1969).

This can only be done if the order is established in the three basic pillars on which any agricultural system is based (Figure 2):

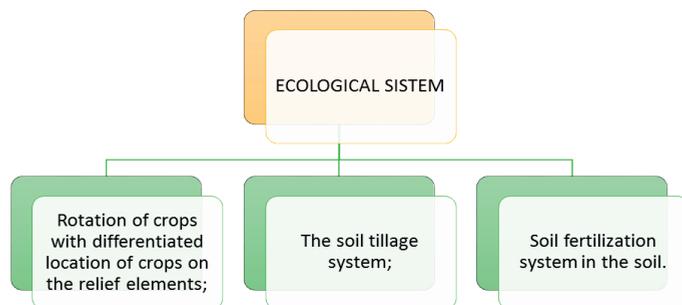


Figure 2. Basic pillars in the organic farming system

Drought and erosion are two sides of the same coin. Reduction the negative impact of drought and erosion cannot be achieved only in the context of desolation, although its role is extremely important (Bulygin, 1993; Milanovsky

et al., 2002; Snakin et al., 1995; Vasilievskaya, 1994).

To effectively achieve soil erosion protection, it is necessary to consider the following facts (Figure 3):

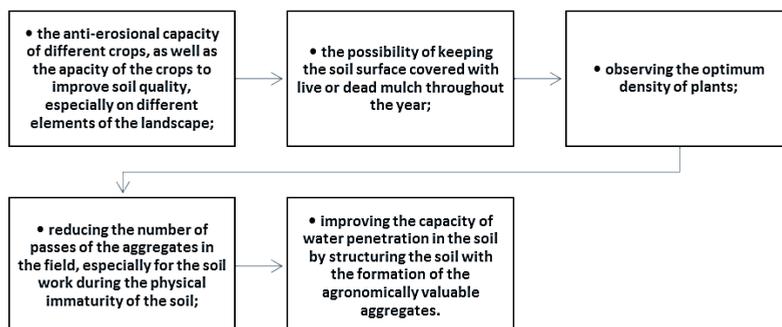


Figure 3. Analysis of the main facts in soil erosion protection

For the correct management of the organic matter of the soil are important: 1) permanent return of fresh organic matter in soil from different sources and of a diverse chemical composition; 2) use of different sources of organic matter; 3) keeping the soil covered with plants or mulch for as long as possible during the year, including during the vegetation period; 4) the minimum disturbance of the soil with mechanical works; 5) optimization of the fertilization system in isolation; 6) creating favorable conditions (habitat) for the life of organisms of the soil along the trophic chain.

The general principle for the slopes located on the slope is the need to reduce the weight of the crop and the size of the fields. As the slope increases, the share of compact seed crops, including perennial grasses, increases. The latter have the amazing property not only to better withstand erosion processes, but also to enrich the soil eroded on slopes with organic matter with an ideal distribution in the soil layers.

Kařtanov A.N., referring to the works of Prof. Zaslavski M.N., brings an ideal scheme for antierosion establishment of soils with the relationship between different cultures for the conditions of the Republic of Moldova (Бончан, 1999). So the relationship between the sowing crops and the compact sowing crops, including the perennial grasses for the arable lands located on slopes of different inclination, constitute (Krivenkov, 1967): - up to 1 degree with the ratio 3:1 (sowing crops: compact sowing crops); - 1-5 degrees with a ratio of 1:1; - 5-8 degree 1:3 ratios (including perennial crops - 1); - more than 8 degrees (including 2 and 3 under perennial grass).

It is important to choose the right species of trees and shrubs for steppe, drought tolerant and scorching conditions. The strips of forests need to be placed perpendicular to the prevailing wind direction.

The rotation of the crops allows the prevention, but not the "fight" against pests, diseases and weeds (Dokuchaev, 1949; Vernadsky, 1965).

In the Republic of Moldova, the legal framework corresponding to the European directives for the production of organic products is elaborated.

In addition, in the Republic of Moldova, a code of good agricultural practices was developed,

which are a firm step towards sustainable agriculture, including organic ones.

The soil used to produce organic produce must be homogeneous, fertile and uncontaminated. In the household is ensured the observance of the isolation during the last 5-6 years (Viter, 1969). For the ecological farming ideal would be to provide a closed circuit of nutrients and energy within each household, by combining the phytotechnics and zootechnics branch, in order to restore a maximum amount of nutrients and energy in the soil. In this way, the certification of the household is also facilitated, for the simple reason that the sources of soil fertility restoration become known (Sheptukhov, 1993). Manure is often used as waste for animal production. In fact, manure is an irreplaceable source for restoring soil fertility. It serves not only as a source of enrichment with soil nutrients, but also provides a guaranteed source of soil enrichment with organic matter and enhancement of soil biological activity.

At the time of sowing the grapefruit with the last cultivation between rows of maize, the grapefruit forms a carpet with a mass of 0.5-2.5 t/ha. Thus, the sowing of the ray between the rows allows not only to reduce the degree of thickening of corn with coarse pears, but also to obtain a vegetable mass for grazing animals and an underground biomass for enriching the soil with organic matter.

Often we do not appreciate the role played by the greater diversity of weed species in breeding and breeding animals. Farmers in organic farms appreciate this fact. Qualified farmers can diagnose their fields based on native weed species.

It is beneficial to crop rotation include high biological capacity to compete with the weeds (for example, buckwheat) and successive crops (rape, mustard, phacelia, vetch etc.). Incorporating them into the soil in the spring is more effective than the fall, but requires caution regarding the water regime for the next crop.

Soil management on an ecological basis implies respect for the entire (new) agricultural system, which differs essentially from the way of approaching soil and crop management. This is more than just observing crop cultivation technologies. Cultivation technologies need to be respected, but not necessarily in the context

of farms and in the systems of management, including agriculture.

Recently, areas planted with autumn cereal crops are being grown using the No-Till method. The efficiency of this process is determined by observing crop rotation and soil enrichment measures with fresh plant residues.

In the Register of varieties of the Republic of Moldova, there is a sufficient range of varieties suitable for cultivation under different climatic and soil conditions, as well as on different agrofunds, including varieties created in the Republic of Moldova. Each farmer must use 2-3 varieties of each crop.

The annual legumes have the ability to accumulate nitrogen from the atmosphere, due to the knots on the roots of the plants, but at the same time, the aerial biomass is also enriched in nitrogen.

Leguminous crops can enrich the soil with nitrogen only in case of incorporation into the soil of the aerial biomass, because at maturation most of the nitrogen is concentrated in seeds, which are extracted from the field with the harvest.

The amount of nitrogen extracted with primary and secondary production is equal to or even exceeds the amount of nitrogen returned back to the soil with the stubble and the underside of plants.

Soybeans and peas are a reliable source of protein for human and animal feed. Beans are the favorite food of the local population, but not only.

Maize is a traditional crop for agriculture of the Republic of Moldova. The successful cultivation of this crop is predetermined by the lack of droughts, which can totally compromise the production of maize. Compliance with the entire set of measures to increase productivity and restore soil fertility within the sustainable agriculture system allows to reduce the negative effects of droughts. When wheat grains are used as predecessors for maize, it is recommended to use successive crops as green manure. Sunflower is less suitable as a predecessor to maize, but is excellent when followed by corn at silo. This is explained by the fact that the green maize-silage table is harvested together with the sunflower, improving the nutritional qualities of the green mass.

In connection with the advancement of advanced technologies, used in recent years in agriculture, the minimum method of conservative

soil tillage - up to a depth of up to 15 cm, is increasingly applied.

Landowners and farmers could thrive on such land: no state in Europe has 80% of the black soil in its soil cover. However, the chernozem region, such as Moldova is, is currently experiencing environmental, economic, and social difficulties. One of the reasons is the improper use of soil cover. In an agricultural country, there is no more valuable natural wealth than soil. The crop yield, food security of the country depends on its condition. The need to double grain production in the world until 2030 is even more discouraging due to a decrease in cultivated land per capita and a decrease in fresh water reserves, the threat of a decrease in the efficiency of use of agricultural resources due to projected climate change. Thus, the need to identify processes, methods, and policies that support sustainable soil management is now even more relevant than ever. The goal of Sustainable Development is to reduce the risk associated with soil degradation by increasing its sustainability and improving the functioning of such a fragile and depleted resource as soil (Krupenikov et al., 2011).

CONCLUSIONS

A healthy soil provides a healthy root system and thus enhances the competitive capacity of crops in relation to weeds in the use of water and nutrients.

Regardless of the way of transition to ecological (organic) farming, the basic purpose remains to comply with the requirements regarding the growth of organic products in accordance with the applicable standards.

The key to success in the transition period depends on the health of the soil. Smooth and compacted soils need time for improvement. The desired results can be achieved only if the sun, the compost, the green fertilizers and other organic fertilizers are respected.

The rotation of the basic and successive crops must ensure the maximum coverage of the soil surface with vegetation and vegetal debris, not only during the growing season, but throughout the year. Thus, the development of erosion processes, nitrate leaching and other negative processes can be avoided during the most critical periods of the year.

A good improvement of the soils is due to the inclusion of perennial legumes in the settlement; it is possible to respect a closed circuit of nutrients and energy in each household. The plants are used for animal feed, and the manure is returned to the ground.

In order to increase the volume of agricultural production, at the same time with the long-term preservation of soil quality, it is recommended to implement conservative technologies, which include a complex of organizational, pedo-ameliorative and agro-technical measures.

REFERENCES

- Bakhtin, P.U. (1969). Studies of physico-mechanical and technological properties of the main types of soils of the USSR. M. Kolos, 271.
- Barber, S.A. (1988). The bioavailability of nutrients in the soil. *Mechanical approach*. M.: Agropromizdat, 376.
- Berezin, P.N., Voronin, A.D., Shein, E.V. (1985). The main parameters and methods for quantitative assessment of the soil structure. *Pedology*, 10, 58–68.
- Bulygin, S.Yu. (1993). Quantitative assessment of the erosion hazard of soils. *Pedology*, 3, 74–79.
- Dokuchaev, V.V. (1949). Selected Works. Moscow. Farm, Tom. 2, 426.
- Kachinsky, N.A. (1965). Soil physics. Moscow. Science, Higher School, 265.
- Krivenkov, N.P. (1967). The effect of soil density on the flow of nutrients into plants. *Abstract of dissertation Ph.D. Agricultural Institute*, Leningrad, 18.
- Krupenikov, I.A., Boincean, B.P., Dent, D. (2011). The Black Earth, Springer Dordrecht Heidelberg London New York, ISBN 978-94-007-0158-8, 11.
- French, N., Ryshkovsky, L., Titlyanova, A.A. et al. (1979). Comparison of the structure of organic matter in different ecosystems. Tom. 10, 2, 8–21.
- Friedland, V.M. (1972). The structure of the soil cover. Moscow, Think, 423.
- Hadas, A. (1990). Directional strength in aggregates as affected by aggregate volume and by a wet/dry cycle. Vol. 41(1), 85–93.
- Lee, K.T., Foster, R.C. (1991). Soil fauna and soil structure. *Austral Y. Soil Res.*, 29(6), 745–775.
- Milanovsky, E.Yu., Shein, E.V. (2002). Mechanisms of formation and stability of the soil structure. Soil resistance to natural and anthropogenic impacts. Moscow, Science, 83.
- Mirtskhulava, T.E. (1990). Reliability of the functioning of agroecological systems. *Bulletin of Science*, 12, 80–83.
- Mirtskhulava, T.E. (2001). Forecast soil washout. Reports of the RAAS, 4, 4–27.
- Oades, J.M. (1991). Waters, Aggregate hierarchy in soils. *Austral. J. Soil. Res.*, 29(6), 815–828.
- Reid, J.B., Goss, M.I. (1981). Effect of living roots of different plant species on the aggregate stability of two arable Soils, 32, 521–547.
- Snakin, V.V. et al. (1995). Ecological assessment of soil resistance to human impact, 5, 50–57.
- Sudakov, A.V., Petrushenko, S.E. (1982). The effect of soil compaction on the growth of root systems and the accumulation of above-ground corn mass. *Effect of fertilizers and tillage on their physical properties and crop yield*, Lenidrad, 10–15.
- Sheptukhov, V.N. (1993). Scientific and experimental substantiation of optimization of agrophysical properties and structure of the root zone of the soil in order to increase the sustainability of crop yields. Nemchinovka, 37.
- Tansley, A.G. (1935). The use and abuse of vegetational concepts and terms. *Ecology*, 16, 248–307.
- Taranu, L., Scorpan, V., Toderas, V., Mironova, T. (2009). Agroclimatic evaluation of the impact of possible changes in the thermal regime on the area of cultivation of later mature hybrids of corn in the territory of the Republic of Moldova, 400–409.
- Vasilievskaya, V.D. (1994). Soil resistance to anthropogenic impacts. // Soil-ecological monitoring and soil protection. Moscow, 61–79.
- Vernadsky, V.I. (1965). The chemical structure of the Earth's biosphere and its environment. Moscow, Science, 352.
- Viter, A.F. (1969). Effect of varying soil density on fertilizer efficiency and yield. *Scientific notes of the Belotserkovsky Agricultural Institute*, 15(2), 117–1149.
- Боинчан, Б.П. (1999). Экологическое земледелие в Республике Молдова (севооборот и органическое вещество почвы). Chişinău: Ştiinţa, 269 с.

MACRO AND MICRONUTRIENTS DISTRIBUTION IN CALCARIC ALUVIOSOIL

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Abstract

In times when healthy diets and personalized nutrition is one of the EU top-priority, sustainable production systems that both preserve and improve soil qualities and grant the agricultural products nutritional value, must be used at most. In this context, the content of macro and micro-elements both in soil and crop plants plays high importance and should be continuously monitored and used for the benefit of food production chains. Microbial inoculants are one of the many ways to enhance the plant's nutrient uptake capacity and increase crop yield and quality. They participate in the geochemical cycling of micronutrients and increase the available form of primary/major nutrients near plants rhizosphere. Soil organic matter also contributes to the nutrients cycle and maintains soil structure. The present study presents the soil nutrients content (N, K, Ca, Mg, Na, F, Cu, Zn, Mo, Se) in 2019, for an experimental field cultivated with organic tomatoes in Buzău county, Romania and attempts to establish some correlation between these elements. The high throughput ICP-MS analysis gives the total content of those elements, of which a part became available throughout the year and the presence and activity of soil microorganisms may accelerate this availability, which can be further exploited inside the organic or sustainable production systems by farmers.

Key words: ICP-MS, macronutrients, micronutrients, total content, soil fertility.

INTRODUCTION

Macro and micronutrients are very important for physiological and metabolic processes in plants. Nitrogen, phosphorus, and potassium are the primary nutrients for plants and are often deficient in cropped soils. Reduced concentrations of nutrients are found in sandy soils, as alluvial soils, (prone to leaching, micronutrient deficiencies of iron, manganese, copper, zinc, and boron), soils with high pH (affect the availability of iron and manganese) or soils intensively cropped. Crop rotation, the addition of bio-products, as organic amendments, organic fertilizers or microbial inoculants, environment protection and good agricultural practices etc are used to balance the nutrients soil content and availability (Mikula et al., 2020; Dhaliwal et al., 2019; Ionescu et al., 2016; Rashid et al., 2016; Madjar et al., 2014; Vlahova et al., 2014; Nagacevschi, 2013). Nitrogen (N) is a vital element needed for the survival of all livings. For plants is essential

both for growth and development and for crops, it significantly increases and enhances yields and their quality. N has a vital role in biochemical and physiological functions in plants. Potassium (K) is considered the second most important nutrient for plants, often termed as "the quality nutrient", a key parameter of soil fertility and plant growth (Rashid et al., 2016). K is essential for photosynthesis, photosynthates translocation, maintenance of turgescence, activation of enzymes, and reducing excess uptake of ions such as Na and Fe in saline and flooded soils (Cakmak, 2005). High-yielding crops can remove a large amount of potassium from the soil. Phosphorus (P) is essential for proper development of plants roots and hastening plants maturity. It plays an important role in photosynthesis, storage and transfer of energy, respiration etc. Without enough supply of phosphorus, plants are unable to complete their production cycle as expected (Wakeel, 2013).

In addition to NPK, calcium (Ca), magnesium (Mg) and sodium (Na) are considered the secondary nutrients, with essential role in plants growth, development and health. Ca is the predominant nutrient in the soil clay and organic matter particles. Parent material with higher content in calcium or magnesium enrich soils content in these nutrients, depending on the soil evolution pathways (Kowalska et al., 2019; Kelling et al., 2013). Ca is involved in the mechanism of controlling P bioavailability in soils and ensuring a good aeration and improving soil structure by displacing sodium (Norton, 2013). It can also influence the abundance of genes in soil microbial communities (Neal et al., 2019), a very important factor for microbial inoculants use in crop technologies. Magnesium (Mg) is a key element of the chlorophyll molecule, so important for photosynthesis and for activation of most plant enzymes that are needed for growth while contributing to protein synthesis. The uptake of K, Ca, and Mg by the plants from the soil depends by the concentration and the ratios of these nutrients (Bonomelli et al., 2019). The micronutrients presence and availability to plants, even in trace amounts, is essential to plants growth.

Micronutrients, such as iron (Fe), copper (Cu), zinc (Zn), molybdenum (Mo), selenium (Se) are essential elements that plants need in smaller quantities. Are involved in activity of various enzymes responsible for the carbohydrate's metabolism, nucleic acids, proteins and lipids. Their deficiency can severely affects crop yield. Iron (Fe) is essential for plant growth, because it influence gene regulation, metabolic activity and elements distribution within cells and within plants. Limited iron availability in soils is one of the main limiting factors of yield and quality of agricultural productions worldwide, particularly in alkaline and calcareous soils (Mimmo et al., 2020; Chen et al., 2019).

Micronutrient deficiency can occur in intensively cropped soils fertilized only with macronutrients. Alluvial soil requires more frequent organo-mineral fertilisers applications (Stănilă et al., 2015).

The soil health and its structure is influenced by physical, biological and chemical processes (Cojocaru, 2019; Ku et al., 2019; Singh et al., 2016) and the availability of macro and

micronutrients in soil for plants is the result of a perpetual translocation of mineral nutrients from inaccessible forms in easy absorbable forms (Abanda et al., 2011; Pallardy, 2008).

In this study we used ICP-MS technique to quantify few of the macro and micronutrients in a calcareous aluviosoil soil profile in 2019, as this method has higher sensitivity, selectivity and detection limits than other elemental analysis techniques. The results will be used in the further years to identify correlations between the dynamic of these total contents at different soil depths and the soil microbiota, including the added microbial inoculants used in tomato crop technology.

MATERIALS AND METHODS

The experiment was conducted in the organic research plot from Vegetable Research and Development Station Buzău, Romania, in 2019. The macro and microelements content analysis were made in the Research Centre for Study of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The soil samples were taken at six depth intervals (0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, 80-100 cm and 100-120 cm).

Soil samples collected in plastic zip bags were than air-dried in the laboratory and milled. Subsamples were homogenized and sieved with 250 µm sieve. All used reagents were analytical grade and suprapure (nitric acid 65% and hydrochloric acid 37%, Merck). The water used for samples preparation and calibration curve was purified by the Milli-Q system. The microwave extraction method was used after EPA Method 200.2 and Method 3050B using both solvents nitric acid and hydrochloric acid. Microwave-assisted digestion procedure was used for soil samples preparation and quantification of macro and microelements at Agilent ICP-MS (with MassHunter Workstation software). The multi-element ICP-MS calibration standard was used for the calibration curve.

The total N content was determined with Kjeldahl method, using 0.1 N hydrochloric acid indicator for titration.

Statistical analyses were performed using Microsoft Excel 2016 from Windows 10.

RESULTS AND DISCUSSIONS

Among the primary nutrients, nitrogen and potassium were analysed.

The total nitrogen content (Figure 1) decreases with depths, from 1700 mg/kg in the arable layer till 500 mg/kg at 1.2 m depth. According to Madjar, 2008, a supply of total N between 0.141 and 0.22 % in soils indicate a medium content of this macronutrient and due to high requirements of vegetables in N, as tomatoes, supplementary fertilization is needed. This recommendation is enforced by the sandy soil structure, which makes N easily leachable.

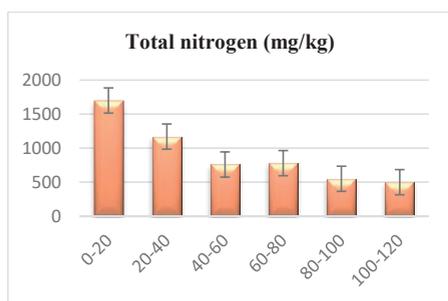


Figure 1. The total nitrogen content of the soil profiles

The concentration for K varied between 7,308 mg/kg to 4,221 mg/kg in soil profiles. The results are in concordance with Voica et al., 2012 who also find K concentration between 1,317.3 mg/kg to 7,767.4 mg/kg in Sălaj and Cluj soils. We also find a very high positive correlation ($r = 0.966$), $R^2 = 0.9337$ between K and Na content in soil profiles (Table 1). According to Madjar, 2008, a supply of total K below 0.8% in soils indicate a low content of this macronutrient.

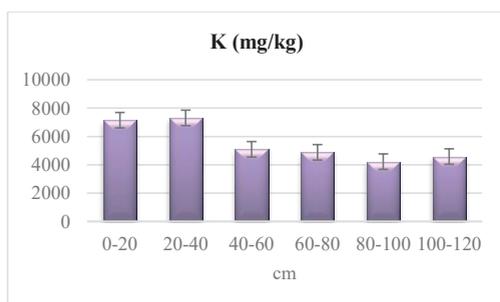


Figure 2. The total potassium content of the soil profiles

Considering the three forms of K - unavailable, slowly available and exchangeable, our analysis indicates the need of potassium based fertilisers.

Among the secondary nutrients, calcium, magnesium, and sodium were analysed.

The highest content of calcium (24,568 mg/kg) was found in 20-40 cm soil profile (Figure 3). Jodral-Segado et al. (2006) also found the results of calcium content of 18,790-49,470 mg/kg. We also find a high positive correlation with potassium ($r = 0.732$) and iron ($r = 0.796$). According to Madjar (2008) a Ca content of 2% indicate a medium content, a surprising result for a calcaric aluviosoil.

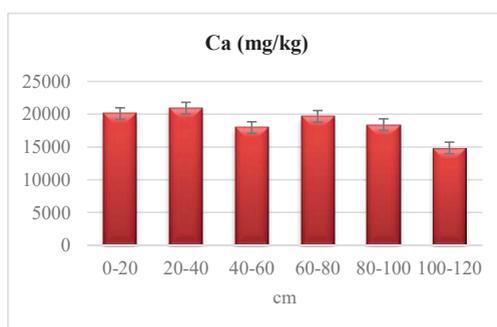


Figure 3. The total calcium content of the soil profiles

In the case of magnesium (Mg), it was observed a high positive correlation with potassium ($r = 0.865$) and calcium ($r = 0.746$) (Table 1). According to Madjar, 2008, a Mg content around 0.6% is more specific for a clay soil, than a sandy soil, which indicate high reserves for this element.

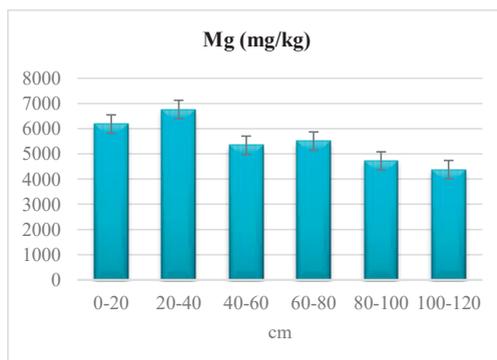


Figure 4. The total magnesium content of the soil profiles

Of the total exchangeable bases, Mg represents 18%, a confirmation of high content of this macronutrient in our soil.

Na content varied between 403 mg/kg in 0-20 cm soil profile to 235 mg/kg in 80-100 cm soil profile (Figure 5).

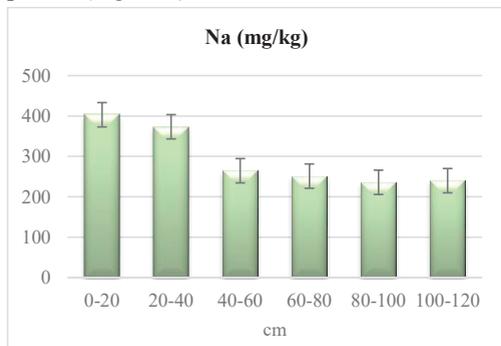


Figure 5. The total sodium content of the soil profiles

Although ICP-MS gives the total content of certain elements in soils, we could still analyse the results as a potential resource for the “total exchangeable bases”, which refers to the sum of the bases (calcium, magnesium, potassium, and sodium) from the soil, in exchangeable form (Table 1).

Table 1. Correlation between Ca, K, Mg and Na content in soil profiles in 2019

	Ca	K	Mg	Na
Ca	1			
K	0.732	1		
Mg	0.746	0.865	1	
Na	0.643	0.966	0.742	1

Of the total exchangeable bases, Ca represents 59.5%, K 21.1%, Mg 18.3% and Na 1.2%.

Among the micronutrients, iron, copper, zinc, molybdenum and selenium were analysed.

The total iron content, between 14.4 and 22.9% (Figure 6) is medium for Romanian soils, according to Madjar (2008), with highest values in the first 40 cm of depth, with a maximum of 2.29% in the second horizon. Still, if the total content may look somehow high, it must be remembered that only a very small amount of this content is soluble and available for plants.

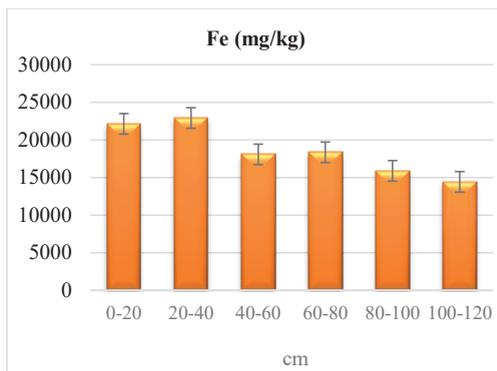


Figure 6. The total iron content of the soil profiles

The total copper content, between 15.74 and 36.4 mg/kg (Figure 7) is low for the deeper layers and high for the arable layer (Madjar, 2008). Usually, only 20-30% of this total content may be soluble and available for plants.

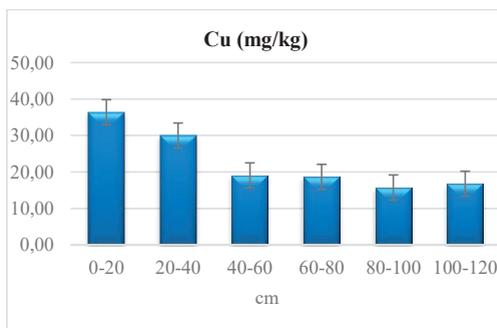


Figure 7. The total copper content of the soil profiles

The total zinc content, between 28.59 and 66.51 mg/kg (Figure 8) is under the know limits for Romanian soils (Madjar, 2008). Usually, only 10-30% of this total content may be soluble and available for plants.

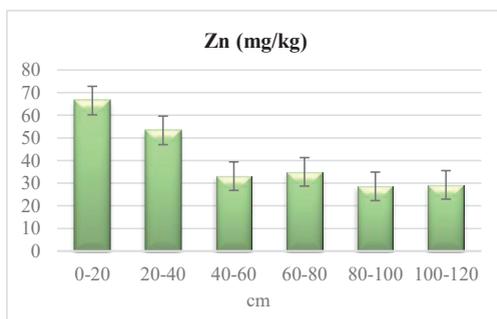


Figure 8. The total zinc content of the soil profiles

The total molybdenum content, between 0.75 and 1.29 mg/kg (Figure 9) falls under the known limits for Romanian soils, of 0.6-3.5 mg/kg (Madjar, 2008). Usually, only 0.05-0.15 mg/kg represents the accessible forms for plants.

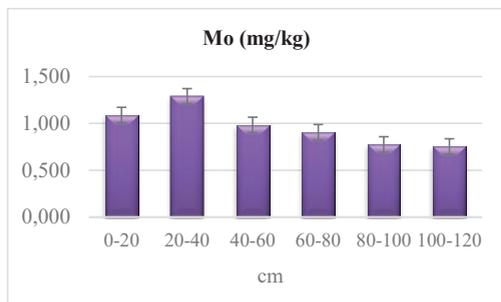


Figure 9. The total molybdenum content of the soil profiles

According to El-Ramady, 2015, non-seleniferous soils contain less than 1 mg/kg, whereas seleniferous soils can range between 2 and 100 mg/kg Se. Our analysis indicates a relatively low content of selenium in our soils, but a special attention should be given to the content from the arable layer.

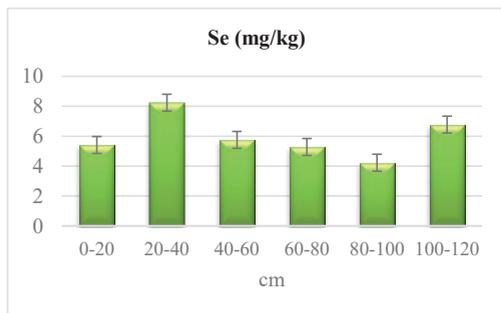


Figure 10. The total selenium content of the soil profiles

As a general remark, the soil total content in micronutrients reflects a good supply for the calcareous soil from Buzau county (Table 2).

Table 2. Correlation between analysed microelements content in soil profiles in 2019

	Zn	Cu	Fe	Se	Mo
Zn	1				
Cu	0.998	1			
Fe	0.895	0.896	1		
Se	0.296	0.340	0.407	1	
Mo	0.781	0.799	0.954	0.614	1

CONCLUSIONS

Soil fertility is one of the most important issues for modern agriculture today and the way we use the technological resources to maintain its health and prevent environmental pollution will deeply impact the generations of tomorrow. The fertilizations must be realized only considering the existing content of nutrients in soil, the physical and chemical processes that may lead to variations of available and unavailable forms and the requirements of the cultivated crops. Our analysis revealed that ICP-MS is a high throughput and reliable technique that allows fast analysis of the total content of nutrients in soils, providing results comparable with those obtained by other laborious techniques and may provide fast answers to farmers and valuable information about the nutrients dynamic in the plants rhizosphere through the cropping season.

ACKNOWLEDGEMENTS

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REFERENCES

- Abanda, A.P., Compton, J.S., Hannigan, R.E. (2011). Soil nutrient content, above-ground biomass and litter in a semi-arid shrubland, South Africa. *Geoderma*, 164(3-4), 128–137. doi.org/10.1016/j.geoderma.2011.05.015.
- Bonomelli, C., Gil, P.M., Schaffer, B. (2019). Effect of Soil Type on Calcium Absorption and Partitioning in Young Avocado (*Persea Americana* Mill.) Trees. *Agronomy*, 9(837), 1–11.
- Cakmak, I. (2005). The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science*, 168(4), 521–530. doi:10.1002/jpln.200420485
- Chen, X., Wei, X., Hao, M., Zhao, J. (2019). Changes in Soil Iron Fractions and Availability in the Loess Belt of Northern China After 28 Years of Continuous Cultivation and Fertilization. *Pedosphere*, 29(1), 123–131. doi.org/10.1016/S1002-0160(17)60331-X. doi:10.3390/agronomy9120837
- Cojocaru, O. (2019). Ecosystem - Reproduction of sustainable structure in agriculture as a factor of soil fertility. *AgroLife Scientific Journal*, 8(1), 71–76.
- Dhaliwal, S.S., Naresh, R.K., Mandal, A., Singh, R., Dhaliwal, M.K. (2019). Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review.

- Environmental and Sustainability Indicators*, 1-2, 100007. doi:10.1016/j.indic.2019.100007.
- El-Ramady, H., Abdalla, N., Alshaal, T., El-Henawy, A., Faizy, S. E.-D. A., Shams, M. S., Domokos-Szabolcsy, É. (2015). Selenium and its Role in Higher Plants. *Environmental Chemistry for a Sustainable World*, 235–296. doi:10.1007/978-3-319-19276-5_6.
- Ionescu, N., Popa, C.B., Chirilă, R., Drăgoi, S. (2016). New bio-products efficiency in sustainable agriculture. *AgroLife Scientific Journal*, 5(2), 79–84.
- Jodral-Segado, A.M., Navarro-Alarcon, M., Lopez-G De La Serrana, H., Lopez-Martinez, M. C. (2006). Calcium and Magnesium Levels in Agricultural Soil and Sewage Sludge in an Industrial Area from Southeastern Spain: Relationship with Plant (*Saccharum officinarum*) Disposition. *Soil & Sediment Contamination*, 15. 367–377, DOI: 10.1080/15320380600751736.
- Kelling, K.A, Schulte, E.E. (2013). Soil and Applied Calcium, A2523 Understanding Plant Nutrients, <http://corn.agronomy.wisc.edu/Management/pdfs/a2523.pdf>.
- Kowalska, J.B., Zaleski, T., Józefowska, A., Mazurek, R. (2019). Soil formation on calcium carbonate-rich parent material in the outer Carpathian Mountains – A case study. *CATENA*, 174, 436–451. doi:10.1016/j.catena.2018.11.025.
- Ku, Y.S., Rehman H.M., Lam H.M. (2019). Review Possible Roles of Rhizospheric and Endophytic Microbes to Provide a Safe and Affordable Means of Crop Biofortification; *Agronomy*, 9, 764. doi:10.3390/agronomy9110764.
- Madjar, R. (2008). *Agrochimie. Planta și solul*, Ed. INVEL Multimedia.
- Madjar, R., Scătețeanu, V.G., Peticilă, A., Tudor, M.S. (2014). Evaluation of nutrients availability by applying fertilizer at different doses in soil column. *Scientific Papers. Series A. Agronomy, LVII*, 46–53.
- Mikula, K., Izydorczyk, G., Skrzypczak, D., Mironiuk, M., Moustakas, K., Witek-Krowiak, A., Chojnacka, K. (2020). Controlled release micronutrient fertilizers for precision agriculture – A review, *Science of The Total Environment*, 712, 136365. <https://doi.org/10.1016/j.scitotenv.2019.136365>.
- Mimmo T., Terzano R., Viganì G. (2020). Special Issue Information "Role of Iron in Plant Nutrition, Growth and Metabolism", *Agronomy*.
- Neal, A.L., Glendining, M.J. (2019) Calcium exerts a strong influence upon phosphohydrolase gene abundance and phylogenetic diversity in soil. *Soil Biology and Biochemistry*, 139, 107613. <https://doi.org/10.1016/j.soilbio.2019.107613>.
- Norton, R. (2013) Focus on calcium: Its role in crop production. 1-10, <https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2013/02/focus-on-calcium-its-role-in-crop-production>.
- Pallardy, S.G. (2008). CHAPTER 10 - Mineral Nutrition. *Physiology of Woody Plants (Third Edition)*, Academic Press, 255-285, ISBN 9780120887651, <https://doi.org/10.1016/B978-012088765-1.50011-7>.
- Rashid, M.I., Mujawar, L.H., Shahzad, T., Almeelbi, T., Ismail, I.M.I., Oves, M. (2016). Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiological Research*, 183, 26–41. <https://doi.org/10.1016/j.micres.2015.11.007>.
- Singh, R, Arora, N.K. (2016). Bacterial formulations and delivery systems against pests in sustainable agro-food production. *Reference Module in Food Science*, Elsevier. ISBN: 978-0-08-100596-5.
- Stănilă, A.L., Dumitru, M., Mușat, M. (2015), Contributions to the knowledge of soils from the Pitești plain for sustainable use. *Scientific Papers. Series A. Agronomy, LVIII*, 105–112.
- Vlahova, V., Popov, V. (2014). Impact of biofertilisers on vegetative growth and leaf gas-exchange of pepper seedlings (*Capsicum annum* L.) in organic farming. *AgroLife Scientific Journal*, 3(1), 156–162.
- Voica, C., Dehelean, A., Iordache, A., Geana I. (2012). Method validation for determination of metals in soils by ICP-MS. *Romanian Reports in Physics*, 64(1), 221–231.
- Wakeel, A. (2013). Potassium-sodium interaction in soil and plant under saline-sodic conditions. *Journal of Plant Nutrition and Soil Science*, 176, 10.1002/jpln.201200417.
- EPA Method 3050B. Acid digestion of sediments, sludges, and soils, Revision 2, December 1996, <https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf>.
- EPA Method 200.2. Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements, Revision 2.8, EMMC Version, https://www.epa.gov/sites/production/files/2015-08/documents/method_200-2_rev_2-8_1994.pdf.

ALLOCHTHONOUS TREE SPECIES USED FOR AFFORESTATION OF SALT-AFFECTED SOILS IN ROMANIA

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Abstract

In Romania, in the last years, an increasing attention is given to the afforestation of degraded lands. According to recent statistics, across the country there are more than two million hectares of degraded terrains. In most of the cases, the afforestation works are carried out by specialized units and the projects are monitored by the territorial branches of the ministry responsible for forestry (i.e. Forest Guards). The afforestation projects are done based on the technical norms issued by the ministry responsible for forestry (i.e. Ministry of Environment, Waters and Forests). For each type of degraded land, situated in a certain region, one, two or even more alternatives regarding the usage of specific tree and shrub species are proposed. The aim of this study was to highlight the best afforestation solution (i.e. the most suitable allochthonous tree species) of the salt-affected terrains as they are described in the Technical Norms regarding the compositions, schemes and technologies for forest regeneration and afforestation of degraded lands. The best alternative resulted by using an Analytic Hierarchy Process (AHP), that took into account five allochthonous species and ten criteria.

Key words: afforestation, AHP, allochthonous tree species, degraded lands, salt-affected soils.

INTRODUCTION

In the last decade, in Romania, several afforestation projects of degraded lands were implemented. In most of the cases, the financial support was given by the Romanian Government, through specialized structures and programs. For example, between 2010 and 2017, more than 130 afforestation projects were financed by the Administration of the Environment Fund (AFM, 2018). Currently, the Agricultural Payments and Intervention Agency is financing afforestation projects, by the dedicated sub-measure 8.1 (APIA, 2019). In addition, the Ministry of Environment, Waters and Forests is providing funds for afforestation of degraded lands through its territorial units specialized in forestry (i.e. Forest Guards), following the procedure described by the Government Decision no. 1257/2011 and the Law no. 100/2010.

Across the country, several categories of degraded lands exist, the total area being around 2 million hectares (Șerbănescu, 2007). Among them, the terrains affected by salinity account for 614,000 hectares, of which the highest share is located in Romanian Plain (200,600 hectares), followed by the ones from Western Plain

(175,000 hectares), Moldova (114,000 hectares), Dobrogea (104,000 hectares) and Transylvania (20,400 hectares), respectively (Coteț and Eftene, 2009; Bălțeanu and Popovici, 2010).

Worldwide, the situation is even worse. It is estimated that the sodicity (or alkalinity) development in the soil is affecting an area of 434 million hectares (Bhardwaj et al., 2019), and more than 40% of the World's land surface may have potential salinity problems (Lal and Khanna, 1994). For example, in India, the salt-affected soils account for 7-10 million hectares, of which 3.6 million hectares are alkali soils (Mishra et al., 2006; Arya and Lohara, 2016). Other countries with large areas of salt-affected soils are Ethiopia and Pakistan, the total area accounting for more than 11 and 6.2 million hectares, respectively (Zaka et al., 2003; Qureshi et al., 2018). In most of the cases, the salt-affected soils frequently appear in arid and semi-arid regions, characterized by a low rainfall and very high temperatures (Abdel-Fattah et al., 2015).

The salt-affected soils are divided into two categories, namely saline soils and sodic (alkali) soils (Gill and Abrol, 1991), the salinization

taking place in different ways, such as: on bench-leveled rice plots through the redistribution of salts from a higher plot to a lower one, soil alkalization through microbiological reduction of sulfates in waterlogged, accumulation of salts originating from irrigation water, redistribution of salts from the subsoil to the surface of irrigated lands, rise of saline groundwater in irrigated plots and in nonirrigated adjoining plots (Maianu, 1984; Zhang et al., 2004).

Both primary salinization process (*i.e.* the salt is accumulating through natural processes due to the high salt content of the groundwater or the parent material) and secondary salinization processes (generated by human activities) represent major threats to both agricultural and forest management (Mihalache et al., 2015; Bhardwaj et al., 2019; Cuevas et al., 2019; Safdar et al., 2019; Zhang et al., 2019). For example, agricultural mismanagement of irrigated dry terrains or the vegetation change that influence the salt exchange between the groundwater and the ecosystems represent ones of the main factors of soil salinization (Nosetto et al., 2008; Hbirkou et al., 2011).

Soil salinization represents one of the most severe abiotic stresses affecting plant growth or survival (Jolly et al., 1993; Du et al., 2013; Sugadev et al., 2018; Sugai et al., 2019), seed germination percentages (El Nour et al., 2006; Gu et al., 2012) or different metabolic processes (Yue et al., 2019).

Worldwide, different planting techniques (Tomar 1997; Tomar et al., 1998) and shrub and tree species were tested. Good results were recorded in the case of *Elaeagnus angustifolia* L. in USA (Zalesny et al., 2019), *Prunus armeniaca* L., *Populus nigra* var. *pyramidalis* (Rozan) Spach, *Salix nigra* Marshall, *Thuja orientalis* (L.) Franco, *Populus euphratica* Olivier, *Ulmus pumila* L., *Fraxinus pennsylvanica* Marshall and *Morus alba* L. in Uzbekistan (Khamzina et al., 2006), *Tamarix smyrnensis* Bunge, *Elaeagnus angustifolia* L. and *Populus alba* L. in Turkey (Yildiz et al., 2017), *Eucalyptus* spp. L'Hér in Israel (Ohlde et al., 2019), *Atriplex nummularia* Lindl., *Pinus halepensis* Miller and *Eucalyptus gomphocephala* DC. in Tunisia (Zouari et al., 2019) and *Acer tataricum* L., *Elaeagnus angustifolia* L., *Ulmus pumila* L. in Romania (Enescu, 2015; Enescu, 2018a), respectively.

The aim of this study was to highlight the most suitable allochthonous tree species used in Romania for afforestation of the salt-affected soils.

MATERIALS AND METHODS

Five of the most common allochthonous tree species, namely black locust (*Robinia pseudoacacia* L.), Japanese pagoda tree (*Sophora japonica* L.), honey locust (*Gleditsia triacanthos* L.), Russian olive (*Elaeagnus angustifolia* L.) and Tree of Heaven [*Ailanthus altissima* (Mill.) Swingle] were taken into consideration. These species are among the ones recommended for afforestation of salt-affected lands by the Technical Norms regarding the compositions and technologies for forest regeneration and afforestation of degraded lands.

In order to highlight the most suitable allochthonous species an Analytic Hierarchy Process (AHP) was performed. Within AHP, the decision problem (*i.e.* the goal of this study) was decomposed into a hierarchy sub-problems (*i.e.* the ten criteria used), each of which can be independently analysed (Enescu, 2018b). A scale ranging from 1 to 5 was used for each criterion, namely:

- **criterion 1** - growth rate (from 1 - very slow growing rate to 5 - very fast growing rate),
- **criterion 2** - vegetative propagation (from 1 - no vegetative propagation to 5 - very intense vegetative propagation),
- **criterion 3** - seed dispersal (from 1 - the smallest to 5 - the highest),
- **criterion 4** - height (from 1 - the smallest to 5 - the highest),
- **criterion 5** - crown density (from 1 - rare crown to 5 - very dense crown),
- **criterion 6** - root system (from 1 - very less developed in depth and sidewise to 5 - very developed in depth and sidewise),
- **criterion 7** - demand for light (from 1 - very shade tolerant to 5 - very high demand for light),
- **criterion 8** - soil requirements (from 1 - extremely low requirements to 5 - very high requirements),
- **criterion 9** - temperature requirements (from 1 - resistant to low temperatures to 5 - resistant to high temperatures) and
- **criterion 10** - ornamental value (from 1 - very low value to 5 - very high value), respectively.

This methodology was used in a similar study aimed at highlighting the shrub species that should be used for establishment of the field shelterbelts in Romania (Enescu, 2018b). For analysing each criterion Expert Choice Desktop software (version 11.5.1683) was used. Two scenarios were taken into consideration, namely scenario 1 (all criteria received an equal share) and scenario 2 (the first two criteria received highest shares, namely 35.1% and 23.3%, respectively).

RESULTS AND DISCUSSIONS

By summarizing the information from specialized manuals and studies, a brief description of the five species was done in accordance with the ten considered criteria.

Black locust is a fast-growing, a very shade intolerant and a thermophilous tree species and it has a very good vegetative propagation system, due to its well-developed roots (Clinovschi, 2005; Şofletea and Curtu, 2008; Rédei et al., 2012; Du et al., 2013; Enescu and Dănescu, 2013).

Japanese pagoda tree has several economic, ornamental and medicinal values. It can reach up to 15-20 m in height, it has a well-developed root system and its seed propagation is very rarely used, the seed pods reaching up to 5 cm in length (Clinovschi, 2005; Kollár, 2012; Sajdak and Velazquez-Marti, 2012; He et al., 2016; Shu et al., 2019).

Honey locust can grow in different degraded terrains, including the salt-affected ones. It prefers the direct exposure to sunlight and a mild climate. It typically reproduces through the production of abundant seeds (Clinovschi, 2005; Vilches et al., 2019).

Russian olive has a rapid juvenile growth rate (Khamzina et al., 2009) and it can be propagated in both vegetative and generative ways (Busso et al., 2013). It has a well-developed root system (Şofletea and Curtu, 2008; Enescu, 2015), it is resistant to drought and frost (Stratu et al., 2016) and it can grow in almost any type of soil (Aksoy and Şahin, 1999), even in salt-affected soils (Katz and Shafroth, 2003).

Tree of Heaven is a shade intolerant species, preferring open spaces and it demands a warm climate, but is resistant to drought as well. This species has a very fast growing rate and the capability to reproduce itself at very early ages, both vegetative and by seeds (Enescu, 2014; Enescu et al., 2016).

The results of the AHP ranking are given in Table 1.

Table 1. AHP alternative ranking

Criterion	<i>R. pseudoacacia</i>	<i>S. japonica</i>	<i>G. triacanthos</i>	<i>E. angustifolia</i>	<i>A. altissima</i>
1	4	1	3	2	5
2	4	1	2	3	5
3	2	3	1	4	5
4	4	2	5	1	3
5	4	5	3	2	1
6	4	2	3	1	5
7	3	1	2	4	5
8	4	3	5	2	1
9	3	4	2	1	5
10	3	5	2	4	1

According to the results in scenario 1, the most suitable tree species among the five selected was the Tree of Heaven (Figure 1). Its placing in the top is mainly explained by the fact that it is a fast growing species that is easily propagating both on vegetative and generative ways.

In the second scenario, in comparison with the first one, no significant differences were recorded regarding the top two species (*i.e.* Tree of Heaven and black locust). The last three species (*i.e.* Honey locus, Russian olive and Japanese pagoda tree) recorded similar results (Figure 2).

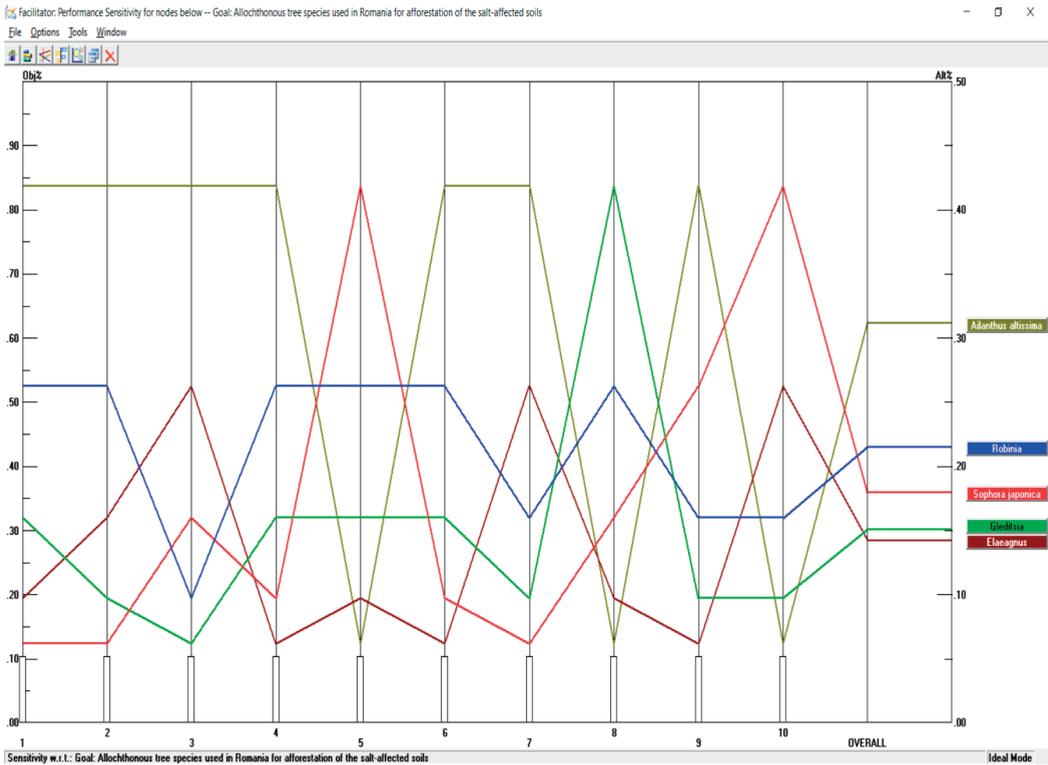


Figure 1. The ranking of the five tree species in the first scenario

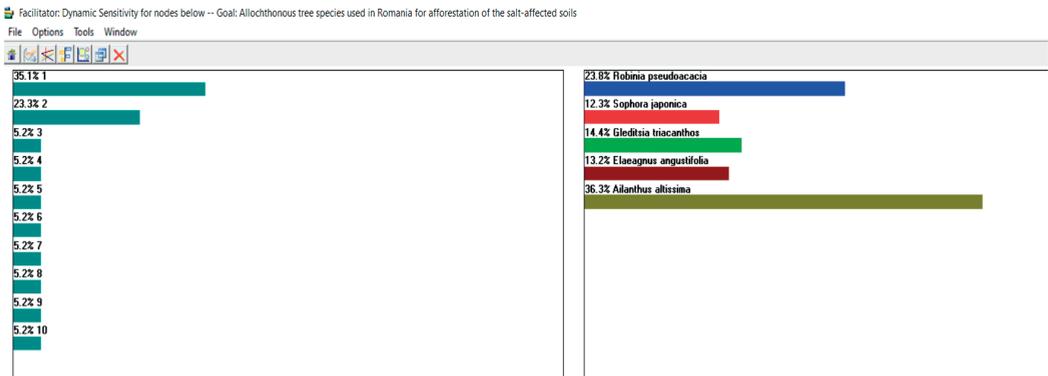


Figure 2. The ranking of the five tree species in the second scenario

CONCLUSIONS

Soil salinity is one of the major threats both in Romania and worldwide. In most of the cases, the salt-affected soils represent the consequence of an improper agricultural management, resulting large areas of degraded soils that could be economically exploited through afforestation.

Romania has great experience in the field of afforestation of degraded lands, by using several shrub and tree species, including the allochthonous ones.

This study that was based on a multi-decision analysis should be regarded as a first step in selecting the most suitable species for afforestation of salt-affected lands. Future research may consider additional criteria in accordance with the targeted scenarios.

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REFERENCES

- Abdel-Fattah, M.K., Fouda, S., Schmidhalter, U. (2015). Effects of Gypsum Particle Size on Reclaiming Saline-Sodic Soils in Egypt. *Communications in Soil Science and Plant Analysis*, 46(9), 1112–1122.
- Aksoy, A., Şahin, U. (1999). *Elaeagnus angustifolia* L. as a Biomonitor of Heavy Metal Pollution. *Turkish Journal of Botany*, 23, 83–87.
- Arya, R., Lohara, R.R. (2016). Enhancing forest productivity through afforestation on arid salt affected sandy soils in Rajasthan. *Journal of Agriculture and Ecology*, 1, 59–70.
- Bălteanu, D., Popovici, E.A. (2010). Land use changes and land degradation in post-socialist Romania. *Romanian Journal of Geography*, 54(2), 95–105.
- Bhardwaj, A.K., Mishra, V.K., Singh, A.K., Arora, S., Srivastava, S., Singh, Y.P., Sharma, D.K. (2019). Soil salinity and land use-land cover interactions with soil carbon in a salt-affected irrigation canal command of Indo-Gangetic plain. *CATENA*, 180, 392–400.
- Busso, C.A., Bentivegna, D.J., Fernández, O.A. (2013). A review on invasive plants in rangelands of Argentina. *Interciencia*, 38(2), 95–103.
- Clinovschi, F. (2005). *Dendrologie*. Universităţii Suceava Publishing House.
- Coteţ, V., Eftene, M. (2009). Some aspects of genesis, distribution and amelioration of saline soils from the Brăila Plain. *Scientific Papers, Series A, LII*, 124–129.
- Cuevas, J., Daliakopoulos, I.N., del Moral, F., Hueso, J.J., Tsanis, I.K. (2019). A Review of Soil-Improving Cropping Systems for Soil Salinization. *Agronomy*, 9, 295, doi:10.3390/agronomy9060295.
- Du, Z.Y., Wang, Q.H., Xing, S.J., Liu, F.C., Ma, B.Y., Ma, H.L., Liu, D.X. (2013). Fine root distribution, characteristics and rhizosphere soil properties in a mixed stand of *Robinia pseudoacacia* and *Fraxinus velutina* in a saline soil. *Silva Fennica*, 47(3), 1–13.
- El Nour, M., Khalil, A.A.M., Abdelmajid, E. (2006). Effect of Salinity on Seed Germination Characteristics of Five Arid Zone Tree Species. *U. of K. J. Agric. Sci.*, 14(1), 23–31.
- Enescu, C.M., Dănescu, A. (2013). Black locust (*Robinia pseudoacacia* L.) - an invasive neophyte in the conventional land reclamation flora in Romania. *Bulletin of the Transilvania University of Braşov, Series II: Forestry • Wood Industry • Agricultural Food Engineering*, 55(20), 23–30.
- Enescu, C.M. (2014). The role of Tree-of-Heaven in Forest Land Reclamation: A Brief Review. *Journal of Horticulture, Forestry and Biotechnology*, 18(2), 66–69.
- Enescu, C.M. (2015). Shrub and tree species used for improvement by afforestation of degraded lands in Romania. *Forestry Ideas*, 21(1), 3–15.
- Enescu, C.M., Houston Durrant, T., Caudullo, G. (2016). *Ailanthus altissima* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (Eds.), *European Atlas of Forest Tree Species*. Publ. Off. EU, Luxembourg.
- Enescu, C.M. (2018a). Russian olive (*Elaeagnus angustifolia* L.): a multipurpose species with an important role in land reclamation. *Current Trends in Natural Sciences*, 7(13), 54–60.
- Enescu, C.M. (2018b). Which shrub species should be used for the establishment of field shelterbelts in Romania? *Scientific Papers. Series A. Agronomy*, LXI(1), 464–469.
- Gill, H.S., Abrol, I.P. (1991). Salt affected soils, their afforestation and its ameliorating influence. *The International Tree Crops Journal*, 6, 239–260.
- Gu, J., Weina, L., Akinagbe, A., Wang, J., Jia, L., Yang, M. (2012). Effect of salt stress on genetic diversity of *Robinia pseudoacacia* seedlings. *African Journal of Biotechnology*, 11(8), 1838–1847.
- Hbirkou, C., Martius, C., Khamzina, A., Lamers, J.P.A., Welp, G., Amelung, W. (2011). Reducing topsoil salinity and raising carbon stocks through afforestation in Khorezm, Uzbekistan. *Journal of Arid Environments*, 75(2), 146–155.
- He, X., Bai, Y., Zhao, Z., Wang, X., Fang, J., Huang, L., Zeng, M., Zhang, Q., Zhang, Y., Zheng, X. (2016). Local and traditional uses, phytochemistry, and pharmacology of *Sophora japonica* L.: A review. *Journal of Ethnopharmacology*, 187, 160–182.
- Jolly, I.D., Walker, G.R., Thorburn, P.J. (1993). Salt accumulation in semi-arid floodplain soils with implications for forest health. *Journal of Hydrology*, 150(2-4), 589–614.
- Katz, G.L., Shafroth, P.B. (2003). Biology, ecology and management of *Elaeagnus angustifolia* L. (Russian olive) in Western North America. *WETLANDS*, 23(4), 763–777.
- Khamzina, A., Lamers, J.P.A., Worbes, M., Botman, E., Vlek, P.L.G. (2006). Assessing the potential of trees for afforestation of degraded landscapes in the Aral Sea Basin of Uzbekistan. *Agroforestry Systems*, 66, 129–141.
- Khamzina, A., Lamers, J.P.A., Vlek, P.L.G. (2009). Nitrogen fixation by *Elaeagnus angustifolia* in the reclamation of degraded croplands of Central Asia. *Tree Physiology*, 1–10.
- Kollár, J., 2012. The Pagoda Tree (*Sophora japonica* L.) seed damage by pest *Bruchophagus sophorae* Cr. et Cr. in Nitra City (Slovakia). *Acta entomologica serbica*, 17(1/2), 73–81.
- Lal, B., Khanna, S. (1994). Selection of salt-tolerant *Rhizobium* isolates of *Acacia nilotica*. *World Journal of Microbiology & Biotechnology*, 10, 637–639.
- Maiianu, A. (1984). Twenty years of research on reclamation of salt-affected soils in Romanian rice fields. *Agricultural Water Management*, 9(3), 245–256.

- Mihalache, M., Ilie, L., Marin, D.I. (2015). Romanian soil resources - "Healthy soils for a healthy life". *AgroLife Scientific Journal*, 4(1), 101–110.
- Mishra, A., Sharma, S.D., Pandey, R., Mishra, L. (2006). Amelioration of a highly alkaline soil by trees in northern India. *Soil Use and Management*, 20(3), 325–332.
- Nosetto, M.D., Jobbágy, E.G., Tóth, T., Jackson, R.B. (2008). Regional patterns and controls of ecosystem salinization with grassland afforestation along a rainfall gradient. *Global Biogeochemical Cycles*, 22(2), 1–12.
- Ohlde, G.W., Stadlander, T., Becker, K. (2019). Biomass Production and Carbon Sequestration by Cultivation of Trees under Hyperarid Conditions using Desalinated Seawater (Sewage Water). *Journal of Agriculture Food and Development*, 5, 33–42.
- Qureshi, A.S., Ertebo, T., Mehansiwala, M. (2018). Prospects of alternative cropping systems for salt-affected soils in Ethiopia. *Journal of Soil Science and Environmental Management*, 9(7), 98–107
- Rédei K., Csiha I., Keseru, Z., Végh, A.K., Gyori, J. (2012). The Silviculture of Black Locust (*Robinia pseudoacacia* L.) in Hungary: a Review. *South-East European Forestry*, 2, 101–107.
- Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., Ul Hussan, M., Sarwar, M.I. (2019). A review: Impact of salinity on plant growth. *Nature and Science*, 17(1), 34–40.
- Sajdak, M., Velazquez-Marti, B. (2012). Estimation of pruned biomass form dendrometric parameters on urban forests: Case study of *Sophora japonica*. *Renewable Energy*, 47, 188–193.
- Shu, W.J., Tang, J., Chen, Z.Y., Jian, Y.S., Wang, Z.F., Wei, X. (2019). Analysis of Genetic Diversity and Population Structure in *Sophora japonica* Linn. in China with Newly Developed SSR Markers. *Plant Molecular Biology Reporter*, 37, 87–97.
- Stratu, A., Costică, N., Costică, M. (2016). Wooden species in the urban green areas and their role in improving the quality of the environment. *PESD*, 10(2), 173–184.
- Sugadev, N., Arasu, P.A., Beaulah, A., Saravanapandian, P., Amutha, R. (2018). Study on effect of different salinity on growth and morphological traits of nerium cultivars (*Nerium oleander* L.). *Journal of Agriculture and Ecology*, 6, 22–30.
- Sugai, T., Yannan, W., Watanabe, T., Satoh, F., Qu, L., Koike, T. (2019). Salt Stress Reduced the Seedling Growth of Two Larch Species Under Elevated Ozone. *Frontiers in Forests and Global Change*, 2, 53, doi: 10.3389/ffgc.2019.00053.
- Șerbănescu, R. (2007). Considerații generale privind etapele proiectelor de reconstrucție ecologică forestieră a terenurilor degradate din țara noastră. *Agricultura - Știință și practică*, 3-4(63-64), 52–57.
- Șofletea, N., Curtu, L. (2008). Dendrologie. Pentru Viață Publishing House, Brașov.
- Tomar, O.S. (1997). Technologies of afforestation of salt-affected soils. *International Tree Crops Journal*, 9(2), 131–158.
- Tomar, O.S., Gupta, R.K., Dagar, J.C. (1998). Afforestation Techniques and Evaluation of Different Tree Species for Waterlogged Saline Soils in Semi-arid Tropics. *Arid Soils Research and Rehabilitation*, 12(4), 301–316.
- Vilches, C., Torremorell, A.M., Rodriguez Castro, M.C., Giorgi, A. (2019). Effects of the Invasion of Honey Locust (*Gleditsia triacanthos* L.) on Macrophytes and Algae of Pampean Streams (Argentina). *Wetlands*, 1–11.
- Yildiz, O., Altundag, E., Cetin, B., Güner, S.T., Sarginci, M., Toprak, B. (2017). Afforestation restoration of saline-sodic soil in the Central Anatolian Region of Turkey using gypsum and sulfur. *Silva Fennica*, 51(1B), 1–17.
- Yue, J., You, Y., Zhang, L., Fu, Z., Wang, J., Zhang, J., Guy, R.D. (2019). Exogenous 24-Epibrassinolide Alleviates Effects of Salt Stress on Chloroplasts and Photosynthesis in *Robinia pseudoacacia* L. Seedlings. *Journal of Plant Growth Regulation*, 38, 669–682.
- Zaka, M.A., Mujeeb, F., Sarwar, G., Hassan, N.M., Hassan, G. (2003). Agromelioration of Saline Sodic Soils. *OnLine Journal of Biological Sciences*, 3(3), 329–334.
- Zalesny, R.S., Stange, C.M., Birr, B.A. (2019). Survival, Height Growth, and Phytoextraction Potential of Hybrid Poplar and Russian Olive (*Elaeagnus angustifolia* L.) Established on Soils Varying in Salinity in North Dakota, USA. *Forests*, 10, 672, doi:10.3390/f10080672.
- Zhang, J., Xing, S., Li, J., F, M., Song, Y. (2004). Agroforestry and its Application in Amelioration of Saline Soils in Eastern China Coastal Region. *Forestry Studies in China*, 6(2), 27–33.
- Zhang, X., Liu, L., Chen, B., Qin, Z., Xiao, Y., Zhang, Y., Yao, R., Liu, H., Yang, H. (2019). Progress in Understanding the Physiological and Molecular Responses of *Populus* to Salt Stress. *International Journal of Molecular Sciences*, 20, 1312, doi:10.3390/ijms20061312.
- Zouari, M., Souguir, D., Bloem, E., Schnug, E., Hanchi, B., Hachicha, M. (2019). Saline soil reclamation by agroforestry species under Kalaât Landelous conditions and irrigation with treated wastewater in Tunisia. *Environmental Science and Pollution Research*, 26, 28829–28841.
- ***Administrația Fondului de Mediu (AFM) (2018). Împăduriri. Available at: <https://www.afm.ro/impaduriri.php>.
- ***Agenția de Plăți și Intervenție pentru Agricultură (APIA) (2019). Schema de ajutor de stat "Sprijin pentru prima împădurire și crearea de suprafețe împădurite" aferentă Măsurii 8 „Investiții în dezvoltarea zonelor împădurite și îmbunătățirea viabilității pădurilor”, Submăsura 8.1 „Împăduriri și crearea de suprafețe împădurite” din cadrul PNDR 2014-2020 - sesiunea 4, anul 2019. Available at: http://www.apia.org.ro/files/pages_files/prelungire_perioada_depuneri_m8.pdf.

SCIENTIFIC RESULTS ON JUSTIFICATION THE PARAMETERS OF A COMBINE U-SHAPED FURROW-OPENER

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Abstract

A combine U-shaped furrow-opener for laying and seeding crops and granules of mineral fertilizers with their multi-level application is presented. The research technique for substantiating the optimal parameters is presented. The results are determined on the basis of laboratory and laboratory field studies. The optimal design parameters of the combined opener with a U-shaped furrow were revealed: the width of the bed compactor is 13.1 mm, the distance between the planes of the outlet holes of the needle guide and the vas deferens is 35.4 mm, the distance from the base of the exit hole of the needle guide to the U-shaped furrow is 48.7 mm. The average traction resistance of the seeder was 13,325 N when the tractor moved in the VI gear. The distribution of seeds along the length of the row with the dual-level opener is 74.2%. The depth of seeding crops with an experimental seeder and a serial corresponding one was 49 ± 3 mm and 51 ± 5 mm. The soil layer between the seeds and fertilizers was 25 ± 3 mm.

Key words: combined opener, soil layer, multi-level application.

INTRODUCTION

Grain crops are the main source of food. Confirmation of this fact is the ever-increasing sown area of grain crops. So, in Russia in 2018, grain and leguminous crops occupied an area of 44.8 million ha, of which 26.7 million ha were sown with wheat. Ensuring stable and high yields of grain crops largely depends on the quality of the sowing, including the final operation - laying and planting seeds and fertilizers.

Estimated indicators of the quality of laying and sowing seeds of grain crops and granular mineral fertilizers when they are applied at different levels include: distribution of seeds in a row, depth of sowing seeds and fertilizers, soil layer between seeds and fertilizers. Improvement of these indicators could be achieved by using combined openers in grain seeders for laying and sowing seeds of grain crops and granules of mineral fertilizers when they are applied at different levels. However, seeders with serial openers have disadvantages, which include: the formation of a non-horizontal furrow bottom relative to the soil surface; shedding of the walls of the furrow; uneven soil layer between seeds and fertilizers. At the same time, the laying of seeds of grain crops is carried out on an unconsolidated bed. From this, the yield of agricultural culture is

declining. Therefore, it is necessary to develop a combined opener, the design of which ensures optimal placement and sowing seeds of grain crops and granules of mineral fertilizers when they are applied at different levels.

In this regard, studies devoted to improving the quality of laying and sowing grains and granular mineral fertilizers with the development of a combined opener with U-shaped furrow are relevant and have important economic and economic importance for the agricultural sector.

MATERIALS AND METHODS

The theoretical laws are based on the basic laws of classical mechanics, mathematics, and the working processes of sowing and planting machines. Experimental studies were carried out on the basis of comparative laboratory and field studies of estimated indicators for laying and planting seeds of grain crops and granular mineral fertilizers when they were applied at a different level with a seeder equipped with combined openers with a U-shaped furrow.

Experimental studies were performed using standard methods (GOST 31345–2007, STO AIST 5.6–2010). Analysis and processing of research results were carried out using the programs Statistica 6.0 RUS, Microsoft Office etc.

To determine the distribution of seeds along the length of a row of a combined U-shaped furrow-opener for laying and sowing seeds of grain crops and granules of mineral fertilizers with their multilevel application, experiments were carried out with the opener and with the serial opener. As a result, the frequencies of appearance of squares with the number of seeds (seedlings) were obtained. Based on the obtained experimental values, graphs were plotted reflecting the uneven distribution of seeds along the row length by the serial and studied openers.

RESULTS AND DISCUSSIONS

A multi-level method of sowing seeds with the simultaneous application of fertilizers allows to apply part of the fertilizer up to 30% together with the seeds, providing them with nutrients for a powerful start, and part of the fertilizer (the main dose - 70%) is applied under the seed bed with a soil layer to avoid damage seed material. Thus, providing plants with nutrients for the entire time of growth and development, and also allows to reduce the number of technological operations, reduce soil compaction.

In Penza State Agrarian University, a structural and technological scheme and model of a combined U-shaped furrow-opener shorting bar for laying and planting seeds of grain crops and granules of mineral fertilizers at their multi-level application was developed (Patented in the Russian Federation for invention No. 2671704) (Figure 1).

Opener multi-level fertilizer and sowing seeds works as follows. When the opener moves, fertilizers from the hopper (19) through the fertilizer meter (23) enter the fertilizer flow divider (22), where they are divided into the main and starting dose. The main dose of fertilizer through the neck (2) with the funnel (3) of the rack falls into the guide (5). Due to the use of a pipe ellipse-shaped in cross section of the guide 5 and bending its lower part back to the side opposite to the movement of the combined opener along an arc of a circle with a radius of $R = 0.3$ m, will even out the fertilizer flow of the main and starting doses and seeds, which will significantly improve the uniformity of their distribution along the length and depth of the sowing furrow.

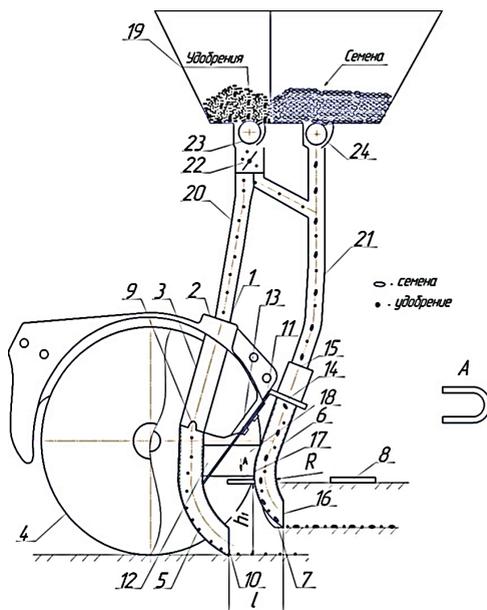


Figure 1. Structural-technological scheme of a combined U-shaped furrow-opener for laying and sowing seeds of grain crops and granules of mineral fertilizers when they are applied at different levels:

1 - stand; 2 - neck; 3 - funnel; 4 - disks; 5 - fertilizer guide; 6, 21 - seeds tube; 7 - bed seal; 8 - closing the working body; 9 - ear; 10 - outlet; 11 - an arm; 12 - stiffener; 13 - scrapers; 14 - shelf; 15 - neck; 16 - outlet; 17 - U-shaped furrow; 18 - level; 19 - hopper; 20 - piping; 22 - fertilizer flow divider; 23 - fertilizer metering device; 24 - sowing apparatus; l is the distance between the planes of the outlet openings of the guideway and the vas deferens; h/l is the distance from the base of the outlet of the guide to the U-shaped furrow; R is the bending radius of the lower part of the guide and the vas deferens

Fertilizers from the outlet (10) are evenly distributed along the bottom of the furrow formed by two disks (4) installed at an angle of 10-12°, while not mixing with the soil, due to the location of the holes (10) in the transverse vertical plane directed relative to the longitudinally vertical plane of symmetry the opener, while fertilizer, when leaving the hole (10), are distributed strictly at a given depth, since the base of the output hole (10) is located on a horizontal plane coinciding with the lower part of the cutting edges of the two combination opener discs. To exclude fertilizer spillage into the soil through the cracks, the upper part of the guide (5) has two ears (9), while the end surface of the ears (9) is made parabolic and identical to the end surface of the lower part of the funnel

(3) of the neck (2) of the rack (1), while their end surfaces fit snugly to friend. To increase the reliability of the opener, the guide (5) in the upper part is additionally fixed motionless with the aid of an arm (11) reinforced with a stiffener (12) at the attachment point of the internal coulters (13) of the coulters. The starting dose of fertilizers together with the seeds sown by the sowing apparatus (24) through the seeds tube (21) enters the neck (15) of the tubular seeds tube (6). To equalize the flow of seeds and fertilizers in the tubular seeds tube and for their uniform distribution along the length of the sowing furrow, the tubular seeds tube (6) is made of a transverse ellipsoidal tube sections, while the lower part of the tubular seeds tube (6) is bent backward, in the direction opposite to the movement of the combined opener, along a circular arc of radius $R = 0.3$ m to exclude mixing of fertilizers and seeds with soil at their exit from the tubular vas deferens (6), which contributes to the seeding of fertilizers and seeds in accordance with the requirements, as well as evenly distribute seeds and fertilizers along the length of the sowing furrow, at the bottom of the tubular seeds tube there is an outlet (16) located in transverse vertical plane directed relative to the longitudinally vertical plane of the opener. In order to lay seeds and fertilizers on a compacted bed, to obtain friendly seedlings of plants and better digestibility of fertilizers by the roots of plants in the initial period of their vegetation, under the outlet (16) on the tubular seeds tube (6) a seed bed (7) sealer (7) is installed, made in the form of a curved wedge

with two side the working faces made with an inclination equal to the inclination of the walls of the furrow cut by the opener discs (4) and the supporting working face ab made with a width equal to the width of the bottom of the furrow 14 mm, while the supporting working face ab curved wedge is located horizontally. For the final embedment of the furrow behind the opener, followed by the tubular seeds tube (6), at the level of the U-shaped furrow (8), a sealing body (17) U-shaped is made of a round bar with a diameter of 0.012-0.014 m, while the furrow (8) has a working width of 0.15 m (Kalabushev et al., 2018).

For laboratory and field studies, the SZ-5.4-0.6 seeder was used, equipped with combined U-shaped furrow-opener. The seeder consists of a frame, grain box, openers, seeds tubes. The seeder is available in three versions. The technical and economic characteristics of various modifications of the seeder are shown (Table 1). The studies had been conducted during 2017-2018 on the farm "ANTONOVO" of the Penza region, Russia (Figures 2 and 3).

When conducting research to create natural conditions is almost impossible. Therefore, an approximate technological scheme of the experimental opener was used. When conducting field trials of the SZ-5.4-0.6 seeder with combined U-shaped furrow-opener, a flat area was selected. The soil was ordinary chernozem of medium loamy granulometric composition. During field studies, Arkhat spring wheat seeds with a seeding rate of 200 kg/ha were taken.



Figure 2. A seeder with combined coulters on the fields of Antonovo farm



Figure 3. Combined opener with U-shaped clipper

Moisture and soil hardness were determined according to Interstate Standard (IS) 28168-89, IS 28268-89 on the day of the experiments at a depth of 0-15 cm along the diagonal of the plot with a tenfold repetition. To determine the humidity, we chose the thermal weight method. When determining the influence of the design parameters of a combined opener with a U-shaped furrow-opener on the quality of placement and sowing of grain seeds and granular mineral fertilizers when they are applied at different levels, the root-mean-square deviation of the soil layer between seeds and fertilizers was taken as an optimization criterion and a multivariate experiment of the D-optimal design was carried out.

According to the results of the sifting experiment, the most significant factors were determined: the distance from the base of the outlet of the guide to the U-shaped furrow ($h1$); the distance between the planes of the outlet openings of the guide and the seeds tubes (l); bed seal width (b) (Kalabushev, 2019).

In accordance with the methodology of the multifactor experiment, the parameters of the combined U-shaped furrow-opener are refined. After processing the experimental data, graphs were built and correlation relationships between the soil layer between the seeds and fertilizers and the studied parameters were determined (Larushin et. al, 2018).

The optimal value for the bed seal width indicator can be considered as 13.1 mm, while

the coefficient of variation of the soil layer between seeds and fertilizers would be 27.0%. The test results are presented in graph form Figure 4.

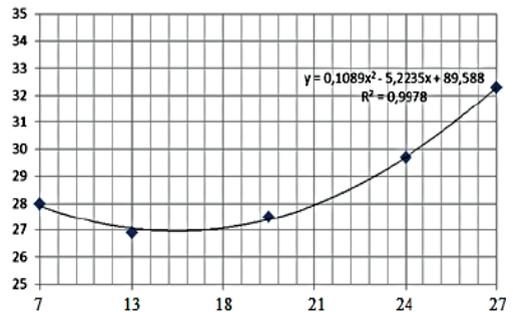


Figure 4. The effect of the width of the bed compactor on the coefficient of variation of the soil layer between seeds and fertilizers

According to the research data, the value of 35.4 mm could be considered the optimal value of the distance between the planes of the outlet openings of the needle guide and the seeds tubes. The coefficient of variation of the soil layer between seeds and fertilizers is 28.9%. The test results are presented in graph form Figure 5.

The optimal value of the distance from the base of the outlet of the guide to the U-shaped furrow could be considered a value of 48.7 mm. The coefficient of variation of the soil layer between seeds and fertilizers was 28.7%. The test results are presented in graph form Figure 6.

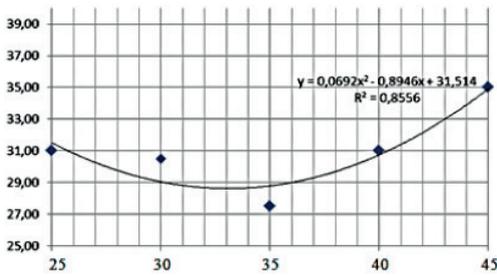


Figure 5. The effect of the distance between the planes of the outlets of the fertilizer guide and the vas deferens on the coefficient of variation of the soil layer between the seeds and fertilizers

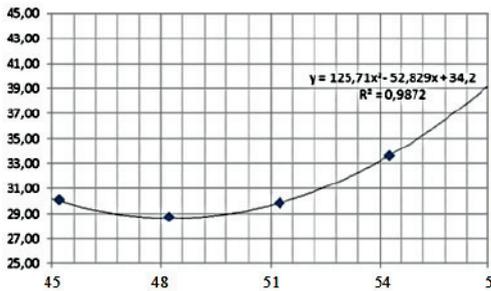


Figure 6. The effect of the distance from the base of the outlet of the fertilizer guide to the U-shaped shortener on the coefficient of variation of the soil layer between the seeds and fertilizers

Next, the results were compared. As it could be seen from the graph of dependencies, the seeder with experimental openers placed the seeds at a given depth better than the serial seeder.

As a result of the analysis of the obtained values, it was found that the number of seeds planted in a given layer (84.5-84.9%) is higher than the serial seeder (65.7-68.4%).

The number of spring wheat seeds planted to a predetermined depth, as well as the hardness and moisture of the soil, affects the dynamics of the appearance of wheat seedlings and field germination.

As a result of observations of spring crops of the Arkhat variety carried out by a seeder with experimental openers and a seeder with serial openers, we note that the seedling emergence rate in the first case is somewhat better.

After the passage of the experimental seeder, seedlings appeared one day earlier due to the multi-level fertilizer application than after the passage of the serial seeder. On the ninth day, the maximum difference in the number of

seedlings per day was noted - 9%. This is due to the fact that with a more uniform distribution of seeds along the depth of planting and row length, the plants are in more favorable conditions for growth and development than seeds that are planted with serial openers of the SZ-5.4-0.6 seeder.

The soil layer between spring wheat seeds and fertilizer granules during sowing was determined for each individual opener. After studying the data obtained, it follows that the number of seeds out of contact with the granules of the fertilizer (interval of more than 10 mm) is 95.5% and this amount is within acceptable limits.

During laboratory and field studies, the distribution of seeds along the length of the row with a combined U-shaped furrow-opener is 74.2% (serial - 61.5%). The depth of sowing of seeds of grain crops with an experimental seeder and a serial corresponding one was 49 ± 3 mm and 51 ± 5 mm. The distance of the soil layer between seeds and fertilizers was stable 25 ± 3 mm.

To determine the traction resistance of the SZ-5.4-0.6 seeder, equipped with combined U-shaped furrow-opener, a dynamometer of the VISKHOM design was used, electrically connected to the IP 238 MR information-measuring system. As a result of processing the experimental data, the average value of the traction resistance of the seeder was 13,325 N when moving in the VI gear.

To change the depth of embedding during the transition to different crops, it is recommended to use track rollers of a combined U-shaped furrow-opener, which allows to change the position of the fertilizer guide and the seeds tube in the soil, thereby setting the necessary depth for laying and seeding the seeds of grain crops and granular fertilizers.

CONCLUSIONS

As a result of laboratory and field studies of the seeder equipped with combined U-shaped furrow-opener, the design parameters obtained as a result of theoretical and laboratory tests are refined. The data obtained fully comply with the requirements for grain seeders with openers.

To improve the quality of sowing seeds of grain crops with seeders of the SZ-5.4-0.6 type, it is recommended to use combined U-shaped

furrow-opener for laying and seeding grain seeds and granules of mineral fertilizers when they are applied at different levels.

REFERENCES

- Kalabushev, A.N., Larushin, N.P., Shumaev, V.V. (2018). Laboratory studies of a combined opener for multilevel sowing seeds of grain crops and fertilizing. *Science in the Central Russia*, 3(33), 21–28.
- Kalabushev, A.N. (2019). Theoretical calculation of certain parameters of a combined coulter. *Volga Region Farmland*, 1(1), 94–97.
- Larushin, N.P., Kalabushev, A.N., Shumaev, V.V. (2018). Combined opener design for sowing crops and multi-level fertilizer. *Volga Region Farmland*, 2(47), 123–127.

EFFECT OF IRRIGATION AND FERTILIZATION ON THE CONTENT AND COMPOSITION OF HUMUS OF CHERNOZEM IN THE VEGETABLE-FODDER CROP ROTATION

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Abstract

Study was conducted in long-term field experiment (47 years) in irrigated vegetables cropping system on chernozem heavy loamy in Forest-Steppe zone of Ukraine. Different types of fertilization regimes were compared: mineral (NPK); organic (manure) and organo-mineral (manure + NPK). Humus content decreased by 10-12% after two 9-crops rotations compared to initial data (before irrigation) on all variants of experiment. Since the end of third 9 years cropping system humus has been increased up to 4.35% at the end of fifth crop rotation in organo-mineral fertilization regime. Mineral fertilization systems did not increase humus content compared to the control (without any fertilization). The highest humic compounds content in chernozem was observed on organic fertilization system. Long-term regular manure application led to significant increase of the content of humic complexes with calcium in comparison with mineral and organo-mineral fertilization regimes. Fulvic acids content was higher on variant with NPK application.

Key words: chernozem, composition of humus, different types of fertilization.

INTRODUCTION

Climate change adaptation, mitigation and food security may be addressed at the same time by enhancing soil organic carbon sequestration through environmentally sound land management practices (Rumpel et al., 2020). Soils are the largest terrestrial reservoir of organic carbon (C) and thus play a significant role in the global C cycle. The loss of organic matter from agricultural lands constrains our ability to sustainably feed a growing population and mitigate the impacts of climate change (Machmuller et al., 2015). Also, soil organic matter (SOM) has traditionally been viewed as the main yield determining factor in agriculture. Addressing these challenges development and implementation of practices that accumulate soil C are extremely important. Increase in agricultural production and productivity depends, to a large extent, on the availability of water. In arid and semi-arid regions, irrigation improves economic returns, but on the other hand irrigation can produce unwanted environmental consequences (Cirelli et al., 2009). A meta-analysis (Zhou et al., 2016) showed that, on average across all biomes, drought and irrigation similarly induced minor increases in soil C pool by 1.45% and 1.27%, respectively.

However, drought and irrigation oppositely affected both C fluxes and plant C pools as well as in agroecosystems (e.g., croplands and grasslands). Active management of agricultural soils may reduce the losses of soil organic matter, but full life cycle analyses for fertilized and irrigated soils seldom show net carbon sequestration (McGill et al., 2018). Positive effects of irrigation on soil organic carbon become less pronounced at higher initial soil organic carbon contents and higher precipitation (Troost et al., 2013). Soils with higher initial carbon contents and higher soil moisture offer better living conditions for microorganisms. Inputs of carbon by the cultivation of crops exceed the microbial decomposition. In addition to increased input of carbon by improved plant growth, irrigation shows direct effects on soil aggregate building and thus on the ability of soil to fix organic carbon long-term (Troost et al., 2013). Many researchers, as a general trend, note a slight decrease in humus stocks in the initial periods of irrigation and their gradual restoration over time, as well as a decrease of its content in the arable layer and an increase with depth (Komissarov, 2015).

The reduced soil organic carbon (SOC) content in irrigation treatment was attributed to drainage associated with greater stocking, together with accelerated decomposition of organic C resulting from elevated soil moisture maintained throughout the growing season (Condrón et al., 2014).

During the growing season, there was a fluctuation in the humus content in the layer of 0-40 cm with an increase at the end of the irrigation season from 7 to 23% and a subsequent decrease of 9-10% compared to the initial values at the beginning of the next irrigation season (Voevodina, 2017).

Compared to non-irrigated areas, by irrigation with fresh water, the humus content is less than by irrigation with weakly mineralized water (Shedrin et al., 2017).

The combination of irrigation with other agronomic management factors also influences the development of soil organic carbon content. Authors (Stoner et al., 2019) observed that frequent irrigation decreases the amount of long-term stable C in pastures. Despite no difference in soil C accumulation, fertilized pastures store C longer than unfertilized pastures.

The application of organic fertilizers has an important influence on the carbon exchange of agro-ecosystems, especially in irrigated agriculture. The joint application of organic fertilizers in irrigated areas can maintain a high crop yield and increase the soil organic carbon content and CO₂ net absorption of paddy soil ecosystems (Shihong et al., 2018).

MATERIALS AND METHODS

Long-term field experiment was conducted on chernozem typical at Institute of Vegetables and Melons NAAS of Ukraine (Kharkiv oblast, Ukraine). Experimental field is located in Forest-Steppe zone of Ukraine (49° 45' N, 35° 51' E and 110 m above mean sea level). The territory of experimental fields is characterized by a temperate continental climate. The sum of positive temperatures is about 2850°C. The vegetation period (days with an average daily temperature above 5°C) 195-220 days. The average annual precipitation is 560 mm.

Soil - chernozem typical heavy loamy with pH = 5.7, bulk density = 1.30 g cm⁻³, the amount of absorbed bases = 26.0 meq per 100 g of soil,

hydrolytic acidity = 2.8 meq per 100 g of soil, humus content = 4.3%. At the beginning of field experiment soil contained 15.2 mg kg⁻¹ available nitrogen (NH₄-N + NO₃-N), 106-119 mg kg⁻¹ available phosphorus (P₂O₅) and 173 mg kg⁻¹ potassium (K₂O).

Experimental plot were irrigated by sprinkler irrigation system during 47 years (2-4 times per year with the norm of 350-500 m³ ha⁻¹). An each experimental plot was 58.3 m² with 4 replicates. In all variant of experiment plow tillage was applied.

The chernozem samples were taken from the depth of 0-25 cm. Sampling locations varied in terms of fertilization: 1) Without fertilizer (control); 2) Mineral fertilization system (N₆₀P₅₇K₅₀); 3) Organic fertilization system (manure 21 t ha⁻¹); 4) Organo-mineral fertilization system (14 t ha⁻¹ of manure + N₆₀P₅₇K₅₀).

Crop rotation: barley - cucumber - winter wheat - onion - tomato - cabbage - beetroot. N₅₄₀P₅₁₀K₄₅₀ (mineral fertilization system), manure 189 t ha⁻¹ (organic fertilization system), 126 t ha⁻¹ of manure + N₃₃₀P₃₃₀K₄₅₀ (organo-mineral fertilization system) were applied for rotation.

Organic carbon content was determined by Tyurin method based on dichromate oxidation. Organic carbon content was re-calculated into humus using the mean coefficient (1.724). Humus composition was determined by Tyurin method according to Ponomareva-Plotnikova procedure (Ponomareva & Plotnikova, 1980). Different organic matter fractions were isolated: humic acid (HA), fulvic acid (FA), and humin. For humus fractional composition, the solutions of different NaOH concentrations were used for extraction: 0.1 M NaOH (room temperature); 0.02 M NaOH (hot extraction) also 0.05 M H₂SO₄ (for decalcitation, room temperature) at a soil solution ratio at 1:20. The extracted humic substances were then separated into humic and fulvic acid fractions by acidifying the extract to pH = 1.3-1.5 using 0.5 M H₂SO₄ at 68-70°C and humic acids were separated by filtering. Separated humic acids were re-dissolved in 0.1 M NaOH solution. Some humic and fulvic acid solutions of each fraction were evaporated and oxidized. Carbon content in the fractions of humic and a fulvic acid was determined by the dichromate oxidation procedure.

HA extracts were transferred to cuvettes. A solution of 0.02 M of NaOH was used as the blank. The absorbance of solutions at wavelengths of 465 and 665 nm was measured. The color indexes (E4:E6) were calculated as the ratio of E465:E665 nm. Spectral properties

of solutions were measured using an UV-Vis spectrometer (SF-26).

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

Initial soil test information collected in 1967 from 0-25 cm (Table 1).

Table 1. Fractional composition of humus in chernozem typical before the experiment

Corg, %	HA-1, %	HA-2, %	HA-3, %	FA-1, %	FA-2, %	FA-3, %	% to Corg			HA:FA
							HA	FA	Humin	
2.85	0.18	0.58	0.73	0.09	0.32	0.21	52.3	21.8	25.9	2.41

RESULTS AND DISCUSSIONS

Continuous irrigation (47 years) resulted in considerable changes of organic C in the topsoil (0-25 cm) in chernozem typical. Soil organic carbon content was by 10% less than in initial soil before irrigation.

An improved water supply leads, on the one hand, to an increased yield therefore to a higher input of carbon into the soil in the form of roots and plant material. On the other hand, consequently higher soil moisture results in an increased microbial decomposition of soil organic matter (Trost et al., 2013).

Analysis by Condron with coauthors revealed that amounts of SOC were significantly greater between the dry land (125.5 Mg ha⁻¹) and irrigation treatment (93.0 Mg ha⁻¹).

Nitrogen fertilisation promotes plant growth but may lead to a change in the Carbon:Nitrogen

ratio and hence to a higher decomposability of SOC.

In present study fertilization promoted to less carbon loss and in organo-mineral fertilization system to C accumulation (by 9%) compared to plots without fertilization.

An increase in organic carbon stocks in response to organic fertilization by irrigation was not limited to the surface soil, but it continued down the soil profile to a depth of 160 cm (Bugchio et al., 2016).

The fractional composition of humus of chernozem heavy loamy after long agricultural use (47 years) was determined that under the conditions of irrigation and application of mineral and organic fertilizers. A tendency to increase the content of mobile fulvic acids of fraction I from 3.5 to 5.1% of Corg (Table 2) was observed. The content HA-1 remained at 1.2% of Corg.

Table 2. Fractional composition of humus in chernozem typical under irrigation and fertilization

Treatment	Corg, %	HA-1, %	HA-2, %	HA-3, %	FA-1, %	FA-2, %	FA-3, %	% to Corg			HA:FA
								HA	FA	Humin	
Without fertilizer (control)	2.57	0.03	0.86	0.41	0.16	0.09	0.26	50.6	19.8	29.6	2.6
Mineral fertilization system	2.44	0.03	0.86	0.38	0.20	0.11	0.40	52.0	29.1	18.9	1.8
Organic fertilization system	2.41	0.03	1.23	0.41	0.17	0.07	0.26	69.3	20.7	10.0	3.3
Organo-mineral fertilization system	2.82	0.03	0.98	0.35	0.17	0.1	0.25	48.2	18.4	33.3	2.6
<i>LSD</i> _{0.05} ¹	0.41	0.01	0.11	0.10	0.03	0.03	0.08	-	-	-	-

¹LSD 0.05 - Least Significant Difference at p=0.05

The results of long-term investigations show that during irrigation the humus state of chernozems of the Steppe zone is met with significant transformation in the direction of decreasing the total humus content and its fulvization (Shedrin et al., 2017).

The systematic application of mineral fertilizer separately and in combination with manure under the organo-mineral fertilization system increased the content of the most mobile groups of humic substances. The use of manure twice per rotation resulted in a reduction of the content of the active components in the soil organic matter by 11% of C_{org} compared to the control. This indicates that mobile organic matter is rapidly mineralized and is a source of nutrients for crops.

The content HA-2 bound with calcium under the effect of mineral and organo-mineral fertilization systems was not changed significantly. By the organic fertilizer system it was observed an accumulation HA bound with calcium of up to 51% of C_{org}. With the increasing HA the content FA-2 decreases accordingly.

It was found that under the conditions of application of only organic fertilizers the content HA-3 increased from 16% of C_{org} in the control variant to 17% of C_{org} by the organic fertilization system.

The mineral fertilization system tends to increase the content FA bound with the mineral part of the soil.

With a slight variation in the relative content of mobile HA the influence of an agrogenic factor of different intensity on the indicator HA-1:FA-1 which reflects the direction of the first stage of the humification process was observed (Ovchinnikova, 2019). By the indicator of the ratio HA-1:FA-1 which according to the variants of the experiment was from 0.24 to 0.35 the low intensity of the process of humification at the stage of formation HA was observed.

The ratio of HA-1:FA-1 which characterized the polymerization of humus structures in the second stage of humification. Under organo-mineral fertilization system increased from 9.6 (on control) to 9.9 under the mineral fertilization system decreased to 7.8 which indicated a decrease in the intensity of formation of high-molecular compounds from low molecular ones.

The application of single organic fertilizers increased the expansion of the HA-2:FA-2 ratio to 17.6 due to the supply of fresh organic matter which is a source for the synthesis of young HA. Soil before the setting of the experiment in the arable layer of soil was characterized by a ratio of the amount of HA:FA at the level of 2.4 despite the anthropogenic influence (application of organo-mineral and organic fertilization systems in irrigated vegetable-fodder crop rotation) type of humus remained humate.

With long-term use of mineral fertilizers there was a narrowing of the HA:FA ratio to 1.8 in which the type of humus changed to fulvate-humate which was a sign of the negative orientation of the humification process.

Under conditions of long-term agricultural use of chernozem heavy loamy in irrigated vegetable-fodder crop rotation (9 crops) under different agrochemical loading signs of degradation transformation of humus with unequal degree of expression at different levels of its organization were revealed.

Although the humus content remained at a high level (at least 4%) in the arable soil layer. The losses were recorded ranging from 1.2 to 15.7% of C_{org} of the baseline to the experiment (Figure 1). Such losses are estimated to be relatively low.

Differences of humus state of chernozem heavy loamy in the layer of 0-20 cm under different agrochemical load were diagnosed by the complex of characteristics. Changes in the variants of the experiment in the form of a trend were observed at the maximum values of the fertility parameters. The degree of humification of organic matter was characterized as very high by the investigated variants among which was notable the organic fertilization system. With the high degree of humification of organic matter a very low content of "free" HA was observed. During the long agricultural use of chernozem heavy loamy the content of "free" HA in the arable layer of soil decreased by 5 times compared to 1967.

The content HA bound with calcium from low level (soil before setting of the experiment) was changed to high according to the investigated variants among which were notable organic and organo-mineral fertilization systems.

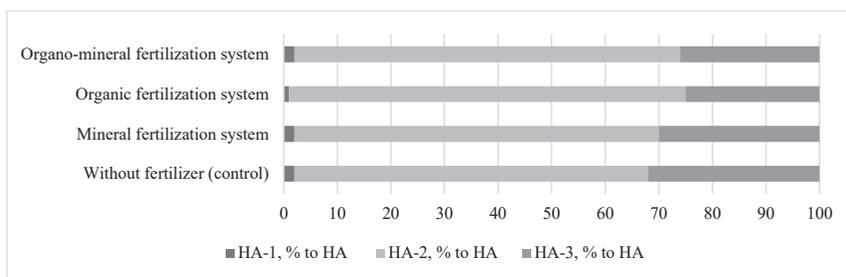


Figure 1. Content of different fractions of humic acids in chernozem typical under irrigation and fertilization

The content of strongly bound HA in the organic matter of chernozem heavy loamy remained high although changes in the direction of the conditions of humification were detected.

The primary information on the elements of humic substances was given by the values of E4:E6 ratio (ratio of the absorbances at 465 nm and at 665 nm). The E4:E6 ratio is considered to be inversely related to the degree of condensation and aromaticity of the humic substances and to their degree of humification (Senesi et al., 2003). The electronic absorption spectra of mobile fraction HA-1 at 465 nm and 650 nm are preferably lower than those of the other humus fraction Ca-bound fraction of humic acids (HA-2) and the fraction strongly bound with soil clay minerals (HA-3). Long-term agricultural use of chernozem heavy loamy without application of fertilizers resulted in a decrease in the E4:E6 ratio in labile humus compounds compared with soil before the experiment setting indicating that the aromatic nucleus with a condensed carbon atom network is predominant in the structure of HA-1 molecules.

The obtained data show that the organic matter of chernozem heavy loamy by different agrochemical loads has different degree of humification (Figure 2).

The decrease in molecular weight and carbon content the increase in the number of acidic functional groups and the oxidation of humic acids are observed under mineral and organo-mineral fertilization systems as evidenced by an increase of E4:E6 ratio by 38 and 67% compared to the control.

The E4:E6 ratio was higher in organo-mineral fertilization system compared with the control and initial soil test mainly due to the higher proportion of fresh SOM. Mineral fertilization increases the content of aliphatic compounds in

the humic substances while in organic fertilization system the E4:E6 ratio is lower which means a predomination of molecules with a high degree of aromaticity and condensation.

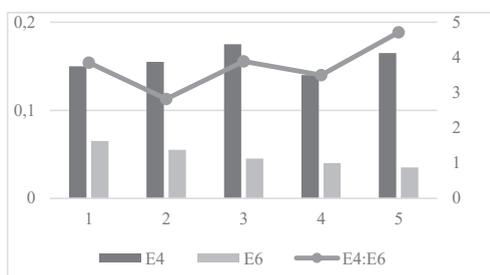


Figure 2. The ratio of the absorbances at 465 nm and 665 nm of humic acids in chernozem typical under irrigation and fertilization:

- 1 - initial soil (before the experiment);
- 2 - without fertilizer (control);
- 3 - mineral fertilization system;
- 4 - organic fertilization system;
- 5 - organo-mineral fertilization system

CONCLUSIONS

During 47 years of irrigation ($\approx 2000 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$) without fertilization a content of organic C in 0-25 cm of chernozem typical heavy loamy in a vegetable-fodder crop rotation have been decreased by 9.8% compared to initial data. There were no statistically significant differences in soil organic C from mineral and organic fertilization systems and control (without fertilization). Only organo-mineral system (126 t ha^{-1} of manure + $\text{N}_{330}\text{P}_{330}\text{K}_{450}$ per 9-course rotation) contributed to stabilization organic C content in chernozem typical under irrigation.

The topsoil highest humic acids (HA) content was observed under organic fertilization. Application of mineral fertilizers only led to increasing of the content of fulvic acids (FA) by 45% compared to other fertilization systems.

Fractional composition of humus showed that 47 years of irrigation led to decrease of content of mobile humic acids (HA-1) in 6 times due to leaching while fraction of HA-2 increased by 50-112% compared to soil before irrigation that could be explain by intensive bounding with calcium from irrigation waters. The intensity of the process of formation of humic acids and the process of polymerization of humus structures was evaluated according to the ratios HA-1:FA-1 and HA-2:FA-2, respectively. It was found that in organic fertilization humus structure is more compensated that in other fertilization systems. On comparing E4:E6 ratios among different fertilization systems the lower ratio was in organic fertilization system which correspond with higher degree of aromaticity of organic substances in soil. Mineral fertilization increased the content of aliphatic compounds in the humic substances.

REFERENCES

- Bughio, M.A., Wang, P., Meng, F., Qing, C., Kuzyakov, Y., Wang, X., Junejo, S.A. (2016). Neof ormation of pedogenic carbonates by irrigation and fertilization and their contribution to carbon sequestration in soil. *Geoderma*, 262, 12–19.
- Cirelli, A.F., Arumi, J.L., Rivera D., Boochs, P.W. (2009) Environmental Effects of Irrigation in Arid and Semi-Arid Regions. *Chilean Journal of Agricultural Research*, 69, 27–40.
- Condron, L.M., Hopkins, D.W., Gregorich, E.G., Black A., Wakelin, S.A. (2014). Long-term irrigation effects on soil organic matter under temperate grazed pasture. *European Journal of Soil Science*, 29, 815–823.
- Komissarov, A.V. (2015). Influence of prolonged irrigation on properties of chernozem leached in the Southern Cis-Urals. *Agriculture Journal*, 2, 5–9.
- Machmuller, M., Kramer, M., Cyle, T., Hill, N., Hancock, D. & Thompson, A. (2015). Emerging land use practices rapidly increase soil organic matter. *Nat Commun*, 6:6995, 64–72.
- McGill, B.M., Hamilton, S.K., Millar, N., Robertson, G.P. (2018). The greenhouse gas cost of agricultural intensification with groundwater irrigation in a Midwest U.S. row cropping system. *Global Change Biology*, 8, 5948–5960.
- Ovchinnikova, M.F. (2019). Features of the transformation of humic substances of sod-podzolic soil disturbed by the construction of the main pipeline route. *Vestn. Mosk. University of Ser. 17. Soil Science*, 1, 35–41.
- Ponomareva, V.V. & Plotnikova, T.A. (1980). *Humus and soil formation*. Leningrad: Nauka Publishing, 222.
- Rumpel, C., Amiraslani, F., Chenu, C., Cardenas, M., Kaonga, M., Koutika, L., Ladha, J., Madari, B., Shirato, Y., Smith, P., Soudi, B., Soussana, J., Whitehead, D., Wollenberg, E. (2020). The 4p1000 initiative: Opportunities, limitations and challenges for implementing soil organic carbon sequestration as a sustainable development strategy. *Ambio. A Journal of the Human Environment*, 49, 350–360.
- Senesi, N., D’Orazio, V., Ricca, G. (2003). Humic acids in the first generation of EUROSOILS. *Geoderma*, 116, 325–344.
- Shedrin, V.N., Dokuchaev, L.M., Yurkova, R.E. (2017). The humus state of various soil types during prolonged irrigation. *Scientific Journal of the Russian Research Institute of Land Reclamation*, 4(28), 1–19.
- Shihong, Y., Ya’nan, Xiao, Y. & Junzeng, Xu. (2018). Organic fertilizer application increases the soil respiration and net ecosystem carbon dioxide absorption of paddy fields under water-saving irrigation. *Environ Sci Pollut Res.*, 25, 9958–9968.
- Stoner, S., Trumbore, S., Baisden, T., Schipper, L., Sierra, C. (2019). Fertilization and irrigation effects on the time scale of carbon cycling in New Zealand Pastures. *Geophysical Research Abstracts*, 21, 11.
- Trost, B., Prochnow, A., Drastig, K., Meyer-Aurich, A., Ellmer, F. & Baumecker, M. (2013). Irrigation, soil organic carbon and N₂O emissions. A review. *Agronomy for Sustainable Development*, 33, 733–749.
- Voevodina, L.A. (2017). Drip irrigation impact on humus state of ordinary chernozem. Innovations in crop cultivation technologies. In: *Materials of the All-Russian Scientific and Practical Conference*, Don State Agrarian University, February 9th, pos. Persianovsky, Rostov Region, 11–17.
- Zhou, X., Zhou, L., Nie, Y., Fu, Y., Du, Z., Shao, J., Zheng, Z., Wang, X. (2016). Similar responses of soil carbon storage to drought and irrigation in terrestrial ecosystems but with contrasting mechanisms: A meta-analysis. *Agriculture, Ecosystems & Environment*, 228, 70–81.

OBSERVATIONS REGARDING THE EVOLUTION OF THE AGRICULTURAL LAND FUND IN ROMANIA ON CATEGORIES OF USE AFTER 1990

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Abstract

The paper presents the evolution of the areas of agricultural land in Romania, by categories of use, after the '90s. According to the Law of the land fund 18/1991 "the lands of any kind, regardless of destination, title on the basis of which they are owned or by the public or private domain of which they are part, constitute the land fund of Romania". If before 1989 there was a tendency to increase the areas destined for agriculture, especially of arable land, after the revolution, with the restoration of land rights, there was an oscillating evolution of agricultural areas, with a decreasing tendency. The study is based on data provided by the National Institute of Statistics. The year 1990 is taken as a reference, and for an overview, are presented the values of the surfaces for each category of agricultural use from 5 to 5 years. The surface of the Romanian land fund is 23,839,100 ha, being made up of agricultural and non-agricultural areas. These surfaces had certain variations throughout the period studied. The area of arable land had a maximum in 1990 when there were 9.45 million ha and a minimum in 1995 with only 9.33 million ha. The value closest to the maximum was recorded in 2005 (9.42 million ha).

Key words: agricultural land fund, category of use.

INTRODUCTION

The land fund of Romania is divided into 10 categories of use, between which we distinguish 5 categories of agricultural land and 5 non-agricultural categories, each having in turn subcategories of use (Călița, 2010).

The up-to-date maintenance of the areas by categories of use is done on the basis of the information collected from the agricultural register and the data provided by the county offices of cadastre and real estate advertising. The agricultural register is the official document of unitary primary evidence, which includes data on households and agricultural companies/associations, as well as any other individuals and/or legal entities that own land/use land and/or animals. The agricultural register is a source of data for the elaboration on a local level of some policies in the fields: fiscal, agrarian, social protection, cadastre, urbanistic, sanitary, school, public services of local interest and others. Therefore, the agricultural register is an administrative source of data for the

statistical information system, respectively: official statistics, preparation and organization of agricultural censuses, buildings and population, pilot surveys, organization of a system of statistical observations by survey, etc. The agricultural register provides the database for the realization with the help of electronic calculation systems of cross-checks between the data from the agricultural register and the data entered in specific registers kept by other institutions: Integrated Administration and Control System (IACS) from the Payments and Intervention Agency Agriculture (APIA); National Register of Farms (RNE) but also others (Order No. 734/2015 on the approval of the Technical Norms for completing the agricultural register).

If before 1989 there was a tendency to increase the areas destined for agriculture, especially of arable land, after the revolution, with the restoration of land rights, there was an oscillating evolution of agricultural areas, with a decreasing tendency. This evolution is closely related to the current needs of today's society.

MATERIALS AND METHODS

To carry out this study we used the statistical data provided by the National Institute of Statistics of Romania. The areas of agricultural categories registered after 1990 are compared, until 2014 because after this year the series of data were blocked until the completion of the country cadastre register by the National Agency for Cadastre and Land Registration.

According to the methodology of the National Institute of Statistics, land fund represents all lands no matter of destination, of the title based on which they are owned or of public or private sector to which they belong. Also, the agricultural area includes, by use, the lands with agricultural destination, owned by natural or legal persons, classified as follows: arable land, natural pastures and hayfields, vineyards and vine nurseries, orchards and tree nurseries.

The lands with agricultural destination include five categories of use, with the following main characteristics:

- The arable land includes land that is plowing every year or several years (2-6) and which are cultivated with annual or perennial plants: cereals, legumes for grains, technical and industrial plants, medicinal and aromatic plants, vegetables, fodder plants.

- The pastures are grassed, naturally or artificially by periodic re-seeding, at 15-20 years old used for grazing animals.

- The hayfields are grassed, naturally or artificially, through the sowing at 15-20 years, from which the grass is mowed for hay.

- The vineyards include the fields planted with "hybrid vineyards" (direct producers) or "noble", hops crops with a similar agrotechnics and vineyards.

- The orchards are plantations with fruit trees and shrubs, having as sub-categories classical orchards (with interleaved, grassy, pure crops), intensive and superintensive (high density, with directed crowns and mechanization of maintenance and harvesting works), plantations of fruit trees (raspberries, currants) (Miluț, 2018).

RESULTS AND DISCUSSIONS

According to the Law of the land fund 18/1991 "the lands of any kind, regardless of destination, title on the basis of which they are owned or by the public or private domain of which they are part, constitute the land fund of Romania".

As it is known, the surface of the Romanian land fund is 23,839,100 ha, being made up of agricultural and non-agricultural areas. These surfaces had certain variations throughout the period studied.

If we compare the year 1990 with 2014, the following breakdown by categories of agricultural use is shown by the data provided by the National Institute of Statistics:

Table 1. Total agricultural land fund, by use, in the year 1990 and 2014

Category of use	Area in the year 1990 (ha)	Area in the year 2014 (ha)
Arable	9450400	9395300
Pastures	3262500	3272200
Hayfields	1465300	1556300
Vineyards and nurseries	277400	209400
Orchards and nurseries	313400	196900
Total agricultural area	14769000	14630100

With reference to the year 1990, we observe a decrease in the area of arable land, vineyards and orchards and an increase of the areas of pastures and hayfields, against a decrease of 138900 ha of the total agricultural areas (Table 1).

Figures 1 and 2 shows the proportion of each category of agricultural land use in Romania in 1990, respectively 2014.

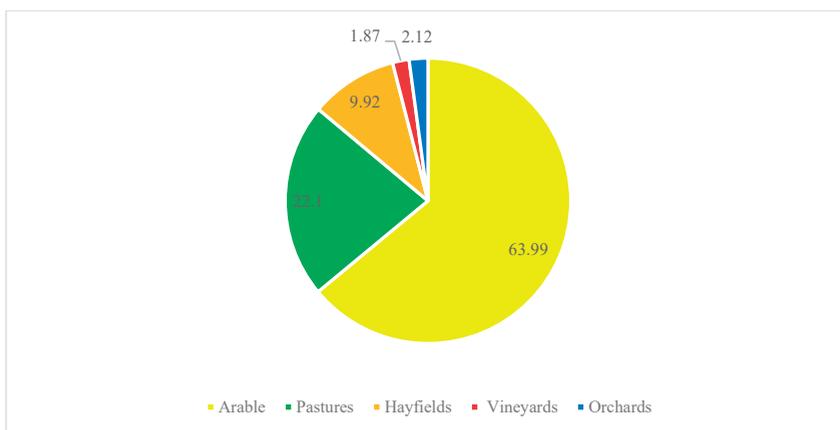


Figure 1. Agricultural land fund by use in Romania (%), 1990)

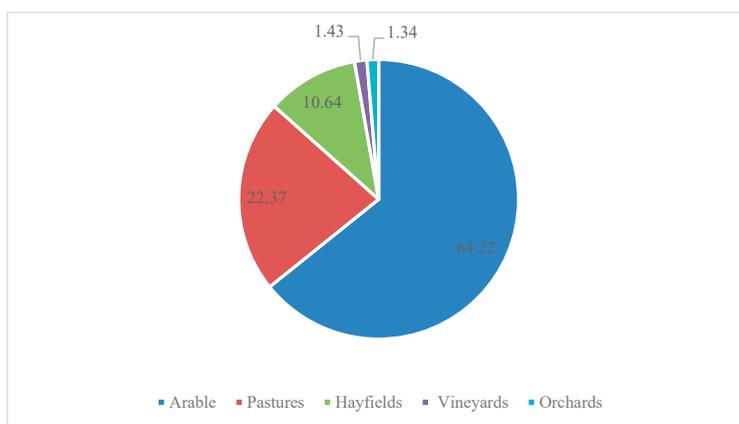


Figure 2. Agricultural land fund by use in Romania (%), 2014)

In order to have an image of the evolution after 1990 of the agricultural areas, in Table 2 the situation is presented for each category of agricultural use from 5 to 5 years, until 2014.

Table 2. Surfaces by agricultural category of use and by year (ha)

Category of use	Year					
	1990	1995	2000	2005	2010	2014
Arable	9450400	9337100	9381100	9420200	9404000	9395300
Pastures	3262500	3392400	3441700	3364000	3288700	3272200
Hayfields	1465300	1497700	1507100	1514700	1529600	1556300
Vineyards and vine nurseries	277400	292400	272300	224100	213600	209400
Orchards and tree nurseries	313400	277600	254600	218200	198600	196900

Figure 3 shows that the area of arable land had a maximum in 1990 when there were 9.45 million ha and a minimum in 1995 with only 9.33 million hectares.

The value closest to the maximum was recorded in 2005 (9.42 million ha).

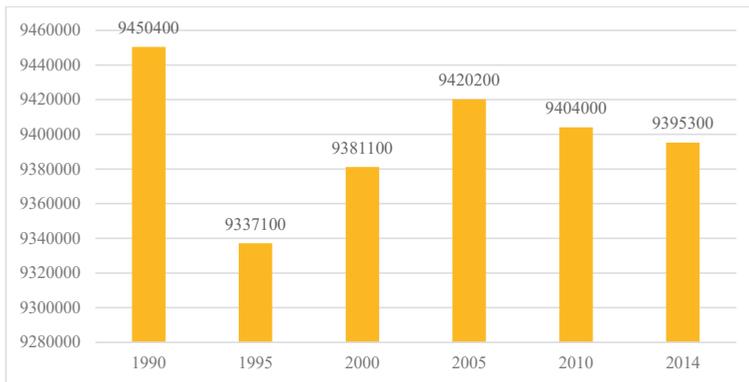


Figure 3. Variation of arable land surfaces by year (ha)

Regarding the pasture category, a rather oscillating evolution is observed during the analyzed period. As Figure 4 shows, there was an increase in the area of pasture until around

2000, from 3.26 million ha to 3.44 million ha, followed by a continuous decrease of them to 3.27 million ha in 2014.

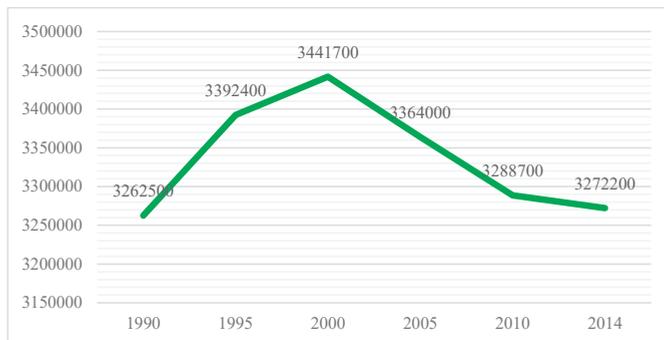


Figure 4. Variation of pasture areas in Romania, by years (ha)

If we refer to the hayfields category, they had a different evolution compared to pastures. Figure 5 shows a constant increase of them

from year to year, starting from 1.46 million ha in 1990 and reaching over 1.55 million ha in 2014.

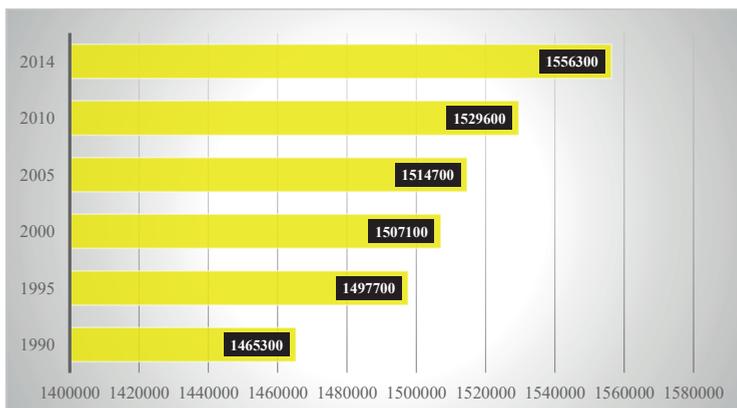


Figure 5. Variation of hayfields surfaces in Romania, by years (ha)

In the case of vineyards (Figure 6), there was a variation almost identical to that of pastures, in the sense that it had a positive evolution until around 1995, when there were 292400 ha, after which the areas decreased every year, reaching - it has an area of less than 210000 ha. This can be accounted for by the growth of the areas of hybrid vine after the revolution, when the

inhabitants replanted their surfaces at the time of collectivization. With the aging of the population in the rural area, with the migration of young people to cities or abroad, important areas of vines remained unused and then transformed into arable land, at least in the southern part of Romania.

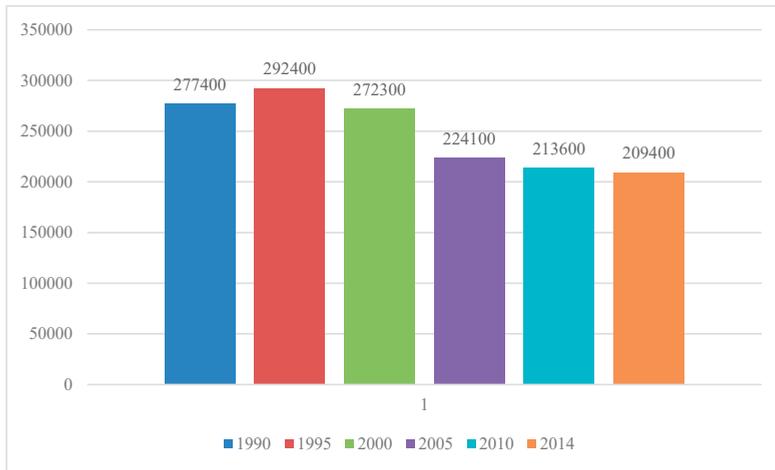


Figure 6. Variation of vineyards surfaces in Romania, by years (ha)

The category of orchards and orchards, showed a sharp decrease during the whole period analyzed, from 313400 ha in 1990 to 196900 ha in 2014 (Figure 7). This may be due to the fact that old plantations, without economic efficiency, have not been replanted.

However, it seems that in recent years there are signs that accessing programs on European funds, the areas planted with orchards have begun to grow.

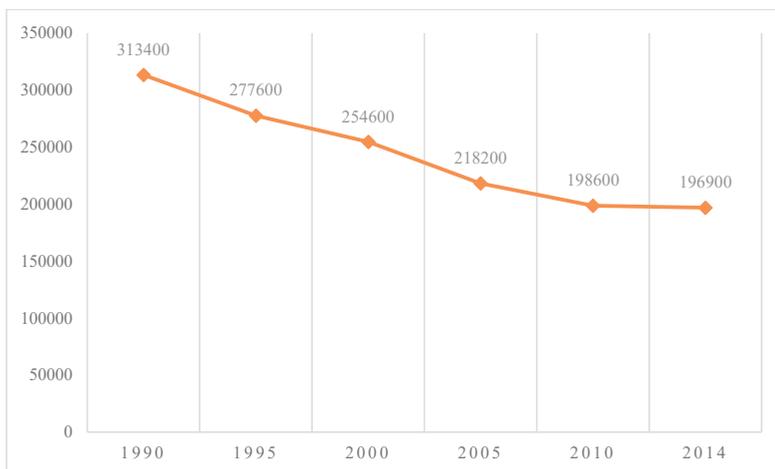


Figure 7. Variation of orchards areas in Romania, by years (ha)

CONCLUSIONS

The agricultural area of Romania registered a decrease of almost 140000 ha compared to 1990, with a share of 61.37% of the total land fund.

The surfaces by categories of agricultural use had positive or negative oscillations during the studied period, their evolution being determined by the current needs of the present society.

The surface of arable land decreased by about 55000 ha, the pastures increasing after 2000 to the detriment of the hayfields.

The vineyards and orchards showed a tendency of decreasing surfaces after 1990, the areas being reduced by about 1/3.

It is necessary to update the information regarding the categories of use, together with the completion of the country cadastre register.

REFERENCES

- Călina, J., Călina, A., Bădescu, G., Vangu, G.M. and Ionică, C.E. (2018). Research on the use of aerial scanning for completing a GIS database. *AgroLife Scientific Journal*, 7(1), 25–32.
- Călina, A., Călina, J. and Miluț, M. (2014). Study on topographic survey of a forest area using combined technology GPS and total station. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, 43(2), 45–53.
- Mihai, D., Teodorescu, R.I., Burghilă, D., Mudura, R. (2015). A modern approach in data updating for a vineyard agro-system modernization. *Conference SGEM*, 2, ISBN 978-619-7105-35-3, 651–656.
- Miluț, M. et al. (2015). The evolution of surfaces in Romania, by category of use during 2000-2012. *Annals of the University of Craiova, Series Agriculture, Montanology, Cadastre, XLV(2)*, 114–119.
- Miluț, M. et al. (2014). Elaborating documentations for changing the category of use to construct a field of photovoltaic panels. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, 27–131.
- Miluț, M. et al. (2018). *Cadastru - Note de curs*. Universitaria Publishing House, Craiova.
- Rodriguez-Moreno, F., Kren, J., Zemek, F. (2017). Advantage of multispectral imaging with sub-centimeter resolution in precision agriculture: generalization of training for supervised classification. *Precision Agric.*, 18, 615.
- Sala, F., Popescu, C.A., Herbei, M.V., Rujescu, C. (2020). Model of Color Parameters Variation and Correction in Relation to “Time-View” Image Acquisition Effects in Wheat Crop. *Sustainability*, 12(6), 2470.
- Sălăgean, T., Dirja, M., Ortelecan, M., Pop, N. & Deak, J. (2011). 3D Modeling of the USAMV Cluj-Napoca campus using integrated system google earth-sketchup and 3D warehouse. *Agricultura*, 79(3-4), 146–150.
- ***2014. Statistical Yearbook of Romania.
- ***1991. Law 18/1991.
- ***2015. Order No. 734/480/1003/3727 from 29 april 2015 on the approval of the Technical Norms for completing the agricultural register.
- ***<http://www.insse.ro/>.

ASSESSMENT OF HEAVY METALS CONTENT IN CALCARIC ALLUVIAL SOIL FROM BUZAU COUNTY UNDER RESTRICTION OF ORGANIC FARMING

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Abstract

One of the most important aspects regarding food safety is the heavy metal content of food and agricultural products. Due to the rapid development of different industries and other anthropogenic activities, the environmental contamination suffered an exponential increase. The assessment of the soils and crops heavy metal content is unavoidable nowadays, even mandatory for specific products. Also, it provides important information regarding the characterization of a specific area, of the soils properties variations and allows correlations between different elements and agricultural practices. Our study aims to evaluate some of the most common heavy metals (Pb, Cd, Ni, Co, Zn, Cu, Mo, As and Cr) found in one calcaric alluvial soil from Buzau county used for tomato cultivation in organic farming system. We aimed both to establish the compliance with national and European legislation and determine a correlation between these elements, in the context of using microbial inoculants for crop protections. The soil samples were taken only from the surface layers (0-20 cm depth), from plots planted with Florina 44 tomato variety, microwave digested and the elements were quantified using ICP-MS method. The obtained values showed that the soil content is within the legislation limits and allowed us to illustrate some correlations between different heavy metals.

Key words: heavy metals, organic farming, Buzau county, tomato.

INTRODUCTION

Over the past decades, heavy metal pollution has grown steadily, exposing both human health and the environment to increasing risks (Tchounwou et al., 2012). Heavy metals form a separate group of pollutants, especially due to properties such as long residual and half-life, soil residence time (> 1000 years), as well as bioaccumulation and bioamplification in food chains. However, some of these metals, such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are essential micronutrients for organisms, being necessary for different physiological and biochemical mechanisms (WHO, 1996). However, excessive amounts of these metals can cause adverse reactions in plant and animal organisms. Other metals such as lead (Pb) and cadmium (Cd) have no established biological functions and are considered non-essential (Hazrat et al., 2019). These are considered by EPA (The

Environmental Protection Agency) as hazardous contaminants.

Although heavy metals are naturally part of the earth's crust, anthropogenic activities play the biggest role in environmental contamination, the most notable being waste disposal & treatment, mining, chemical industries, agricultural and pharmaceutical activities, transportation (Raja Rajeswari et al., 2014). Soil is one of the most affected components of the environment. EEA (European Environmental Agency) estimates that in 2019, in Europe exists 2.5 million potentially contaminated sites, but only 45% of them have been identified. The existence of a significant number of unidentified sites demands that for the correct characterization of an area, a series of analyses must be carried out to determine heavy metal abundance. Since most heavy metal inputs act on the surface layer (0-20 cm), an analysis of this layer is sufficient for soil characterization (Hou et al., 2014). Also, mineral uptake is prevalent in this range for most crop plants (Kismányoky et al., 2010). Heavy metals are found in soils in different forms, and

they are influenced by several physical and chemical properties of the soil. Thereby, pH, conductivity or amount and type of other metals can influence solubility, mobility, bioavailability and biotoxicity of heavy metals (Shahid, 2017). Although the dissolved form fraction of heavy metals is the most important regarding availability for plants, the international and national authorities use the total content of heavy metal as reference values. (European Commission, 2001). As the European Union has not established general heavy metal limits for soils, each country has its own legislation. However, FOMA (Organic and Organo-mineral Fertiliser Manufacturers' Spanish Association) presents in a report published in 2014 (Revision of the Fertilisers Regulation) some limits of the European legislation, which appear in Directive 86/278/EEC (Table 1). In Romania, heavy metal limits are established in Order 756/1997 (Table 1). Because there is no specific legislation for organic farming, the same values are applied, as long as the Inspection and Certification Bodies for organic farming do not impose other restrictions.

Table 1. Maximum permissible levels of studied heavy metals in current legislation (mg/kg)

Metal	Directive 86/278/EEC (EU)	Order 756/1997 (Romania)		
	Limit values	Reference value in soils	Alert threshold *sensitive soils	Intervention threshold *sensitive soils
Cadmium	1-3	1	3	5
Copper	50-140	20	100	200
Nickel	30-75	20	75	150
Lead	50-300	20	50	100
Zinc	150-300	100	300	600
Mercury	1-1.5	0.1	1	2
Total Chromium	-	30	100	300
Cobalt	-	15	30	50
Molybdenum	-	2	5	10
Arsenic	-	5	15	25

*soils used for residential and recreational areas, for agricultural purposes, as protected areas or restrained sanitary areas, as well as the areas provided for such future uses

The present study evaluates the content of mineral elements on a field under conversion to organic farming, treated with microbial inoculants. Soil microorganisms can have different effect on each element. Certain elements can be solubilized, in this way becoming bioavailable, while others can be immobilized in the soil. These bio-

transformations have been studied for different heavy metals such as As (Khalid et al., 2017; Rahman et al., 2014), Hg (Leveque et al., 2014), Pb (Ahmad et al., 2016; Leveque et al., 2014) or Zn (Cavagnaro et al., 2010).

As the applied microbial treatment can change the soil composition, the analysis of the heavy metals was made as a safety measure, decreasing or increasing the concentration of them being a possible side effect. For this reason, we must ensure that the site remains within the limits required by law.

MATERIALS AND METHODS

The present study follows the evolution of the heavy metal content in a calcareous alluvial soil from Buzau county - Romania, which was treated with microbial inoculants. The field is in conversion to organic farming, and it was planted with Florina 44 tomato variety in 2019. Other studies were carried out in 2018 for the characterisation of the same soil, and the results confirmed that the soil composition respects the legislation limits (Dobrin et al., 2018).

Soil samples were taken from topsoil (0-20 cm), from several points of tomato field, in two stages: in summer (July - month 7) and in late autumn (November - month 11) - denoted as Exp3 and Exp4. We used as control samples from corn and pea crops (V_{mp} and V_{mm}) and two replicates from soils treated with microbial inoculants (V_1 and V_2). Samples preparation and physicochemical analyses have been done in the Research Center for Studies of Food and Agricultural Products Quality, USAMV Bucharest. The samples were dried at room temperature, ground with a laboratory soil grinder and sifted through a 250-micron sieve, in order to prepare for elemental analysis. Heavy metal analysis was performed using ICP-MS technique (Agilent 7700 system). To perform this analysis, the samples were subjected to acid digestion with aqua regia (65% HNO_3 and 37% HCl in 3 to 1 proportion). An amount of 0,1000 g of each sample was placed in Teflon tubes, adding 6 ml of HNO_3 and 2 ml HCl . The digestion was accomplished using a microwave system (Ethos Up), at 180°C for 15 minutes. The following heavy metals were included in this study: Pb, Cd, Ni, Co, Zn, Cu, Mo, As and Cr.

RESULTS AND DISCUSSIONS

This study aims to determine if the microbial treatment applied to the soil produces significant changes on heavy metals content, and if these changes break the law in force. Furthermore, depending on the determined values, some correlations between these values are shown. The results are presented in figures below (Figures 1-9). Each value represents the mean of three replicates. It was preferred to represent each element on different figure because every metal concentration has a different range of values, that cannot be represented clearly together.

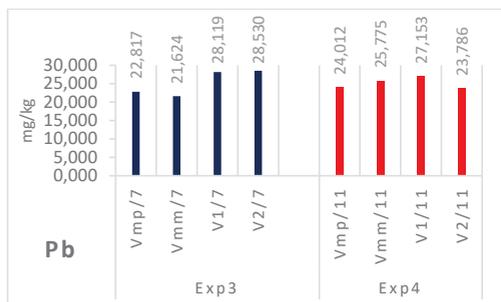


Figure 1. Pb content for the two stages

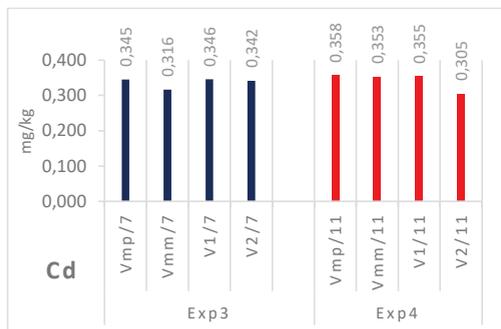


Figure 2. Cd content for the two stages

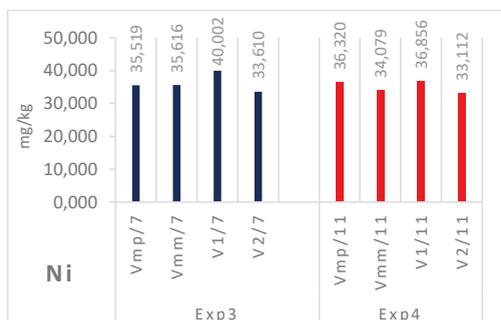


Figure 3. Ni content for the two stages



Figure 4. Co content for the two stages

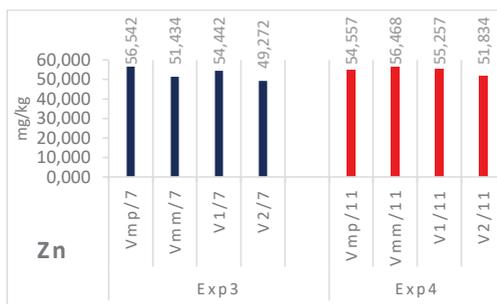


Figure 5. Zn content for the two stages



Figure 6. Cu content for the two stages

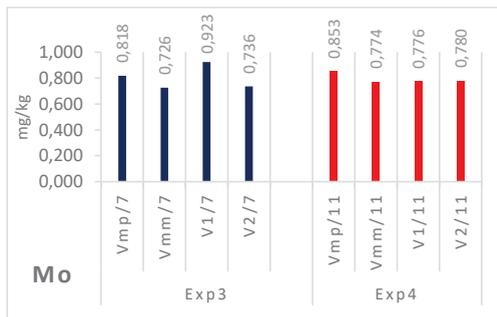


Figure 7. Mo content for the two stages

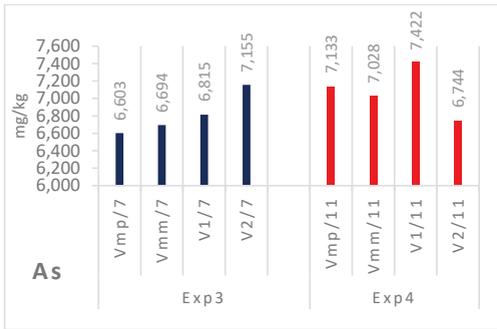


Figure 8. As content for the two stages

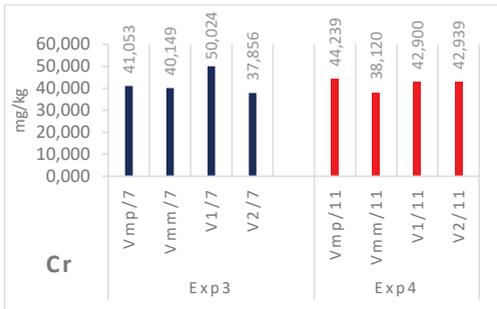


Figure 9. Cr content for the two stages

Applying T-test for the two groups of data (Exp3 and Exp4) ($\alpha = 0.05$) for each metal separately, it can be observed that there are no significant differences between the two sets of samples (Table 2).

Table 2. T-test value comparing the set of samples taken in summer with the set of samples taken in late autumn

Metal	p-value	Significance
Pb	0.9643	No significant diff
Cd	0.7272	No significant diff
Ni	0.5244	No significant diff
Co	0.8769	No significant diff
Zn	0.4261	No significant diff
Cu	0.3393	No significant diff
Mo	0.9190	No significant diff
As	0.2015	No significant diff
Cr	0.9435	No significant diff

Although the table looks flat, it is important to note that the heavy metal concentrations varied insignificantly. This result was rather expected, beside the fact that none of the values exceeded the maximum limit according to law. Considering the values of all 12 determinations (three replicates of four samples), some correlations can be found. Regarding the content of Zn and Cr in summer, it can be determined a weak positive correlation between them (Pearson correlation coefficient $\rho = 0.4805$). On

the same elements, in autumn there was a weak negative correlation ($\rho = -0.4178$) (Figure 10). That may be due to microbial treatment that can influence the solubility of one element over another.

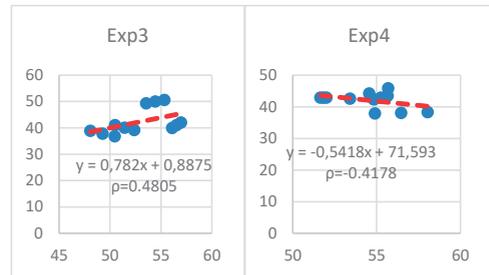


Figure 10. Change of direction between Exp3 and Exp4 on Zn and Cr correlation

Regarding the correlation between Co and As, can be observed that on first stage, there was no correlation ($\rho = -0.0469$) between them and at the second stage this relation was replaced by a strong positive correlation ($\rho = 0.9000$) (Figure 11).

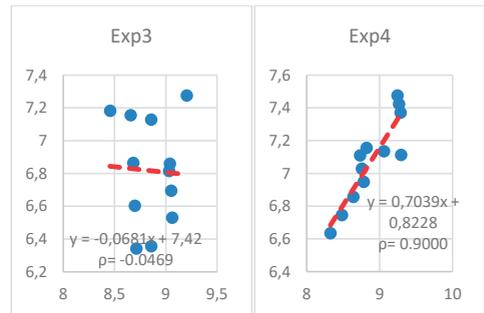


Figure 11. Occurrence of new correlation between Co and As on the second stage

On the other hand, some of the correlations were maintained in the same direction. It's the case of Cu and Ni whose values were in strong positive correlation ($\rho = 0.7283$) and kept a moderate to strong positive correlation ($\rho = 0.6270$) (Figure 12).

The same way, Pb and Cu were in weak positive correlation on the first stage ($\rho = 0.3182$) and became a strong positive correlation on the second stage ($\rho = 0.7898$) (Figure 13).

Since these groups of values followed the same trend, the microbial inoculants treatment didn't influence the behaviour of these metals in the soil enough to be observed.

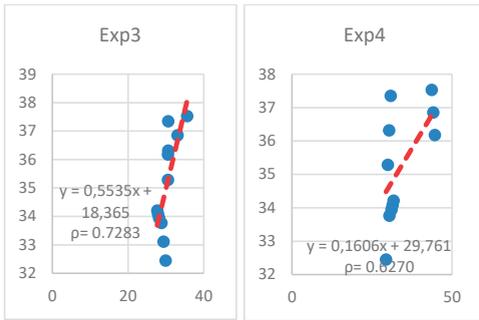


Figure 12. Maintenance of the same direction between Exp3 and Exp4 on Cu and Ni correlation

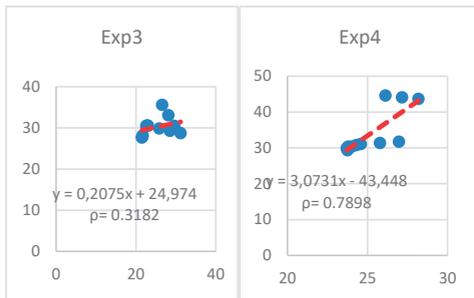


Figure 13. Maintenance of the same direction between Exp3 and Exp4 on Pb and Cu correlation

CONCLUSIONS

Regarding the heavy metal content of the soil, between the two sampling periods, no significant difference was observed in any of the elements analysed in this study. Also, none of the elements exceeds the maximum legal limit after the microbial treatment.

There was observed some changes, meaning that after the treatment, the positive correlation between Zn and Cr content has been reversed to negative correlation. Also, a new strong positive correlation emerged between Co and As after the microbial treatment.

The Cu and Ni content kept the same positive correlation for both stages of sampling, but at a lower level for the second stage.

The values for Pb and Cu kept also a positive correlation for both stages of sampling, but much stronger for the last taken samples.

Could not be established any relation for the rest of the heavy metals.

ACKNOWLEDGEMENTS

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REFERENCES

- Ahmad, I., Javed, M., Asghar, H.N., Shahid, M. (2016). Differential effects of plant growth-promoting rhizobacteria on maize growth and cadmium uptake. *J. Plant Growth Regul.*, 35, 303–315.
- Ali, H., Khan, E., Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*, 1–14.
- Cavagnaro, T.R., Dickson, S., Smith, F.A. (2010). Arbuscular mycorrhizas modify plant responses to soil zinc addition. *Plant Soil*, 329, 307–313.
- Dobrin, A., Moț, A., Mușat, M., Ciceoi, R., Madjar, R. (2018). Heavy Metals and Isotopes Content of an Organic Experimental Field for Vegetable Crops, Buzau Region, Romania. *Vision 2020: sustainable economic development and application of innovation management*, 5745–5752.
- Hou, Q., Yang, Z., Ji, J., Yu, T., Chen, G., Li, J., Xia, X., Zhang, M., Yuan, X. (2014). Annual net input fluxes of heavy metals of the agro-ecosystem in the Yangtze River delta. *China J. Geochem. Explor.*, 139, 68–84.
- Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I., Dumat, C. (2017). A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration*, 182(B), 247–268.
- Kismányoky, T., Tóth, Z. (2010). Effect of mineral and organic fertilization on soil fertility as well as on the biomass production and N utilization of winter wheat (*Triticum aestivum* L.). *Arch. Agron. Soil Sci.*, 56(4), 473–479.
- Leveque, T., Capowiez, Y., Schreck, E., Xiong, T., Foucault, Y., Dumat, C. (2014). Earthworm bioturbation influences the phytoavailability of metals released by particles in cultivated soils. *Environ Pollut*, 191, 199–206.
- Rahman, M.A., Rahman, M.M., Reichman, S.M., Lim, R.P., Naidu, R. (2014). Heavy metals in Australian grown and imported rice and vegetables on sale in Australia: Health hazard. *Ecotoxicol Environ. Saf.*, 100, 53–60.

- Raja Rajeswari, T., Sailaja, N. (2014). Impact of heavy metals on environmental pollution. *Journal of Chemical and Pharmaceutical Sciences*, 3, 175–181.
- Shahid, M. (2017). *Biogeochemical behaviour of heavy metals in soil-plant system*. Published: Higher Education Commission - Pakistan.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J. (2012). Heavy metal toxicity and the environment. *Experientia supplementum*, 101, 133–164.
- ***Directive 86/278/EEC, Official Journal of the European Communities.
- ***European Commission, APPLYING COMPOST BENEFITS AND NEEDS. Seminar Proceedings Brussels, 2001.
- ***European Environmental Agency. (2019). Progress in management of contaminated sites. Indicator Assessment.
- ***FOMA (2014). Revision of the Fertilisers Regulation: Feedback of the FWG members on essential safety requirements, Spain.
- ***Order 756/1997, pentru aprobarea Reglementării privind evaluarea poluării mediului.
- ***World Health Organization (1996). Trace elements in human nutrition and health, Geneva, ISBN 92 4 156173 4.

MACRO AND MICROELEMENTS CONTENT OF URBAN SOILS FROM PLOVDIV (BULGARIA)

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Abstract

The accumulation of some macro- (N, P, K) and microelements (Co, Cu, Mn, Zn) in urban soils has been studied by the analysis of topsoil horizon. Soil samples from the big park areas and along the main boulevards in the city of Plovdiv (Bulgaria) were collected. The chemical elements content was determined using standardized analytical methods. Data obtained showed significant differences, from a low up to excessive pollution level. The majority of investigated roadside soils recorded heavy metal loading when compared to the park soils from the same area. Generally, the K and P content was high in all soil samples and varied in the range 291.9-825.4 mg/kg for potassium and 410.5-1216.0 mg/kg for phosphorus, respectively. Nitrogen content was not so abundant and varied from low/insufficient (14.17-19.4 mg/kg) in some park soils to medium (20.85-31.36 mg/kg) in the rest samples.

Key words: traffic pollution, heavy metals, park soils, roadside soils.

INTRODUCTION

Urbanization is the main driver of rapid land-use change around the world with important consequences for soil quantity and quality (Zhiyanski et al., 2017). Urban soils are formed under the combined effect of both natural soil formation factors and anthropogenic factors (Stroganova & Prokofieva, 2001). Soil degradation is often observed in urban areas and is expressed as a complete or partial loss of individual soil functions.

Accumulation of heavy metals and toxic elements into surface layers is one of the main characteristics of urban soils. A wide range of substances being produced through combustion of fuels, abrasion of vehicle exploitation materials (mainly tyres), road de-icing, and industrial processes are emitted to the urban environment. These are mainly toxic gases and dust enriched with heavy metals. Such pollution undergoes dry and wet atmospheric deposition

and penetrates into urban soils (Mcbride et al., 2014; Shang et al., 2012). Transformed structure and chemistry of soils may lead to serious disturbances in their physio-chemical properties, biological activity and functioning, as well to the impoverishment of vegetation cover (Hu et al., 2013; Mao et al., 2014; Nikolov et al., 2019; Swiercz & Zajecka, 2018; Wu, 2014).

Urban soils derive from natural soils to the anthropogenic influence, caused by human settlement construction and their development. The transformation level is directly proportional to the intensity of urbanization process, human activities and traffic volume, as well as the type of land use and land cover changes (Zheng et al., 2002). Based on the above-mentioned reasons, the soil cover of the settlements could be divided to: *i*) soils consisting of a mixture of materials other than those in adjacent agricultural or forest areas that form a surface layer with a power of > 50 cm, greatly altered by human activity; *ii*)

soils in parks and gardens that differ from natural forest or agricultural soils by composition, type of land use and management; *iii*) soils that are the result of construction activities in urban areas and are often sealed (Morel et al., 2005).

Soil, as the component of urban green spaces (Setälä et al., 2013), plays a pivotal role in maintaining urban ecosystem services, such as biodiversity maintenance, water resources protection, microclimate regulation, carbon sequestration, food production, and fulfilment of cultural or recreational needs (Lovell & Taylor, 2013; Neil et al., 2014; Wu, 2014). Urban soils are well known as large pools of carbon, nitrogen, and other elements, serving as the foundation of urban ecosystems by supporting plant growth and sustaining biogeochemical cycles (Pouyat et al., 2002). However, the protection of urban soils is still poorly considered in the planning and development of urban areas, and there is a lack of profound knowledge regarding the potential of different types of vegetation cover and plant species to moderate the degradation or to improve the state of urban soils (Zhiyanski et al., 2017).

Quantifying soil properties and understanding soil conditions are essential for assessing ecosystem services provided by urban green spaces and detecting pollution in them (Mao et al., 2014). Having in mind that the urban soils going into different use, including vegetable and/or trees cultivation in the small gardens around the houses (Lăcătușu et al., 2008), the nutritive chemical elements level knowledge is of a great importance for the sustainable management of the urban green infrastructure. In this context, the aim of the present study concerns the urban soil chemistry, especially about macro- and microelements contents as premise for urban green spaces supporting and urban agriculture developing.

MATERIALS AND METHODS

Study area

The city of Plovdiv is located at 24°45' east longitude and 42°09' north latitude, at an altitude of 160 m a.s.l. It is the second largest city in Bulgaria after Sofia, and is also one of the most densely populated cities in the country with almost 350,000 inhabitants per 102 km² (NSI,

2018). Inside the city proper are six syenite hills, several industrial zones, densely populated central area, some moderately populated areas around it, wide network of busy streets and train tracks, big parks and other green yards.

Sample collection

Soil samples were taken in Mart 2019 according to the administrative regulation of the city of Plovdiv, urban gradient and the type of land use (Figure 1). Sampling plots have been chosen in permanent grassland areas along the main boulevards in the city, as well as in permanent grassland areas of park zones (as away from the traffic as possible). Soil samples were collected on the depth of 0-20 cm and each sample was formed by 5 subsamples (Petrova et al., 2013).

Laboratory analyses

Soil pH was measured using pHotoFlex Set, 2512000, WTW-Germany, and the soil organic matter content (SOM) was analysed using the weight loss method (Nikolov et al., 2019).

Analyses of the mobile forms of studied macroelements (N, P and K) followed ISO/TS 14256-1:2003 and GOST 26209:1991, respectively.

Total content of studied microelements (Co, Cu, Mn, and Zn) in air dried soil samples was estimated after MW digestion in closed PTFE vessels (ETHOS ONE, Milestone) by using a mixture of HNO₃ + H₂O₂. All reagents used are high purity analytical grade. For a control of possible contamination due to the reagents or sample preparation procedure, blank samples are prepared and analysed for every sample batch.

The total content of Cu (324.754 nm), Mn (348.291 nm) and Zn (206.200 nm) were determined by ICP-OES (iCAP 6300 Duo,S, Thermo Scientific) at the appointed spectral lines by axial plasma observation with an exception of Mn (for which radial plasma view mode was used). For quantification of low Co content the method ICP-MS (Agilent 7700) was used. The following isotopes were monitored: ⁵⁴Mn, ⁵⁹Co, ⁶³Cu, ⁶⁵Cu, ⁶⁴Zn and, ⁶⁶Zn.

The calibration solutions were prepared after appropriate dilution of Multi VI (30 elements in HNO₃, Merck, Darmstadt, Germany), traceable to NIST. For on-line correction of non-spectral matrix effect in ICP-MS the method of internal standard (¹⁰³Rh) was applied. Both instrumental methods have been validated (for the total content of elements) via analysis of certified reference material (CRM) Loam Soil ERM -

CC141. The same material was used for quality control purposes. A portion of CRM was digested and analysed together with samples. The measured concentrations of all tested elements were statistically comparable with the declared certified values (for extraction with Aqua Regia).

Statistical analyses

Descriptive analysis was obtained using the following variables: arithmetic mean, median,

range, standard deviation (SD), and coefficient of variation (CV). The cluster analysis was carried out, based on Ward's method in which the similarity criterion is the squared Euclidean distance. Euclidean distance provides a measure of the similarities between samples. The distance coefficients express the degree of similarity as distance in multidimensional space and thus, as the distance value decreases, the similarity increases (Melegy & El-Agami, 2004).

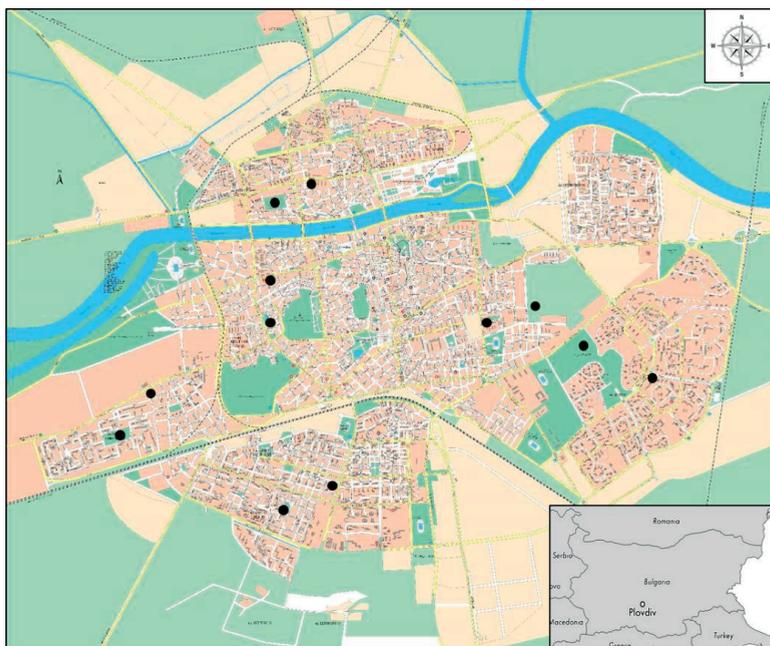


Figure 1. Map of Plovdiv and locations of sampling plots

RESULTS AND DISCUSSIONS

The influence of the anthropogenic factor on the contemporary soil formation process is well known and soils with the distinct influence of this factor are diagnosed as "Anthropogenic soils" (Anthrosols) (Ivanov et al., 2010). Depending on the main directions of anthropogenic activity, at the next taxonomic level Gencheva (2000) divides three soil types: agrogenic, urbogenic and technogenic.

According to their genesis, the soils in the Plovdiv area are classified as Fluvisols (FAO, World Reference Base for Soil Resources, 2014). Our previous studies on some park soils from the city of Plovdiv have shown that they exhibit urban changes not only in the topsoil

horizon but in deeper soil layers too, so they could be referred to Urbic Anthrosols (WRB, 2006). When regarding the soils of small green belts, especially roadside, we found that soil properties are significantly influenced and it is more appropriate to discuss them as Technosols (WRB, 2006) (Petrova et al., 2018).

The pH values of soils samples from the surface layer (0-20 cm) varied from 5.57 (medium acidic) up to 6.70 (close to neutral) (Table 1). The prevailing part of studied urban soils had a light acidic reaction with a median value of 6.37 in both park and roadside samples. Our findings well correspond with data about the pH of some urban garden soils from the Ferneziu area (NW Romania) - medium acid-neutral (5.7-7.5) (Mihali et al., 2013). Similar results have been

also obtained for soil pH of some industrial areas (median 6.46) in the city of Skarżysko-Kamienna (Poland) (Swierz & Zajecka, 2018), while the pH values of urban green areas (median 5.64) and allotment gardens (5.57) in this city are significantly lower from our data. Physio-chemical properties of urban soils are dependent on the characteristics of geological substrate as well as the conditions and structure of the urban environment (Greinert, 2015). As noted by Park et al. (2010), physio-chemical properties of soils are derivatives of the city age and spatial structure. It is caused by the factors influencing soils in various stages of urban development and concerns both industry and transportation growth, as well as the intensity of urbanisation. Moreover, soils are subject to numerous land-use treatments and thus unequivocally transform their structure and physio-chemical properties. Soil organic matter (SOM) in the present study varied in the range of 3.18% to 7.21% (park) and between 2.36-4.37% (roadside) (Table 1). As a whole, SOM in Plovdiv was quite elevated, more expressed in park samples. SOM is necessary for all soil functions, and it is the most important indicator of soil health. It consists of varying proportions of small plant residue (fresh), small living soil organisms, decomposing (active) organic matter, and stable organic matter (humus) in varying stages. SOM is a mineralizable source of nutrients for plants. It increases the availability of most nutrients, buffers the effects of high acidity, increases the available water capacity and moisture retention of the soil, helps to minimize compaction and surface crusting, increases water infiltration, provides food for micro-organisms that facilitate the availability of nutrients, holds soil aggregates together, decomposes pesticides, and acts as a carbon sink. Soil organic carbon (SOC) is a function of SOM, so we found the same tendency. All studied urban soils samples are above the recommended range of soil SOC (> 10 g/kg) for healthy plant growing (Whitcomb, 1987). It could be assumed that these soils possess significant potential for plant growing and provide a good media for urban greening. Data from Plovdiv are significantly higher than the organic carbon content in some urban soils from Romania with medium values about 1.7% (Lăcătușu et al., 2008).

Table 1. General characteristics of studied urban soils

Parameter	pH		Organic C, %		SOM, %	
	Park soil	Road-side soil	Park soil	Road-side soil	Park soil	Road-side soil
Mean	6.25	6.40	2.61	1.99	4.50	3.43
Median	6.37	6.37	2.42	1.91	4.17	3.29
Min	5.57	6.23	1.84	1.37	3.18	2.36
Max	6.63	6.70	4.18	2.71	7.21	4.67
SD	0.39	0.17	0.89	0.55	1.54	0.95
CV, %	7	3	34	28	34	28

Data concerning the content of main macro-elements (N, P and K) as mobile forms are presented on Table 2. Although the studied urban soils are rich in total nitrogen (data not shown), the level of mobile nitrogen is very low. The values range between 14.17 and 31.36 mg/kg with a median of 20.13 mg/kg. These values meaning a low level of nitric azote supplying, are specific for the soils that have not received any organic or mineral fertilizers. Therefore, the urban soils are poorly supplied with mobile nitrogen forms, that representing one of the specific chemical property of these soils (Lăcătușu, 2005).

Median values of mobile phosphorus in urban soils from all studied locations are 525 mg/kg (park) and 540 mg/kg (roadside), respectively, values that, without exception, defining a very high content domain.

Regularly, the potassium is in large quantities in urban soils. Medium total potassium values, rounding about 2.5%, are revealing a very good soil supplying with this macro-element. In our study, the mobile potassium content is very high, reaching a maximum value about 826 mg/kg in park soils. Median values of mobile potassium from all studied samples are 660 mg/kg (park) and 722 mg/kg (roadside), making point very high mobile potassium content in urban soils from Plovdiv.

Therefore, the analysed urban soils are containing low quantities of mobile nitrogen, but are highly supplied with mobile phosphorus and potassium. These findings are in agreement with data from Ferneziu area (NW Romania), reported by Mihali et al. (2013). An exception was found only for nitrogen supply - it's content in Romanians urban soil is medium due to natural content and anthropogenic inputs (as consequence of the fertilization of the soils in the vegetable gardens).

Table 2. Agrochemical characteristics of urban soils

Parameter	N, mg/kg		P, mg/kg		K, mg/kg	
	Park soil	Road-side soil	Park soil	Road-side soil	Park soil	Road-side soil
Mean	20.69	23.44	557.8	613.5	667.5	623.2
Median	20.13	24.03	525	540	660	722
Min	14.17	15.57	77	354	463	292
Max	31.36	28.17	1216	1117	826	745
SD	6.05	4.18	394.1	280.9	146.9	183.9
CV, %	29	18	71	46	22	30

The C/N ratio is very low for urban soils in Plovdiv (< 10). This shows that in these soils with more anthropogenic influence, the organic matter is subjected to a higher degree of mineralization and carbon loss in the atmosphere, while the humus accumulation process is weaker. We aimed to analyse also the content of some microelements with biogenic role - Co, Mn, Cu, Zn, and the results are shown in Table 3. According to content level or their abundance, these chemical elements could produce negative effects on environmental factors. In fact, from environmental sciences point of view, all these chemical elements are included in heavy metals generic terms.

Analytical dates, statistically calculated, have been reveal differences between both chemical elements and locations ($p < 0.05$). The content of Co varied between 5.6 mg/kg and 15.4 mg/kg in the park soils and between 6.1 mg/kg and 8.8 mg/kg in the roadside soils. It was found to be the microelement with the most significant differences in CV according to the type of land use - 38% (park) and 12% (roadside) ($p < 0.05$). Our data are 3 fold lower than the median values for Co content (24 mg/kg) in Romanian urban soils, reported by Lăcătușu et al. (2008) and Mihali et al. (2013). However, there is no Co deficiency in studied urban soils from Plovdiv (Bulgaria).

The content of Cu varied in the range 23-56 mg/kg, Mn content was between 421 and 891 mg/kg, while Zn ranged from 74 mg/kg to 193 mg/kg. The median content of Cu, Mn and Zn in our study was also quite the median values of these element, revealed by Lăcătușu et al. (2008) and Mihali et al. (2013) in Romanian urban soils. This fact could be due mainly to the genesis of microelements in the urbanized areas. Han et al. (2006) reported that coefficients of variations (CV) of heavy metals dominated by natural sources are relatively low, while those of heavy metals affected by anthropogenic sources are quite high. Thus, based on the CV values which

all are below 0.40, the microelements in studied urban soils from Plovdiv seem to be associated mainly with natural sources, although some anthropogenic inputs to Co, Cu and Zn should be considered.

Table 3. Content of microelements in studied urban soils

Parameter	Co, mg/kg		Cu, mg/kg		Mn, mg/kg		Zn, mg/kg	
	Park soil	Road side soil						
Mean	9.3	7.6	38	46	640	540	111	113
Median	8.85	7.5	38	49.5	564	516	96	113
Min	5.6	6.1	23	32	494	421	81	74
Max	15.4	8.8	56	56	891	691	193	160
SD	3.5	0.9	12.6	9.9	157	93	42	35
CV, %	38	12	33	22	25	17	38	31

To differentiate distinct groups of macro- and microelements as tracers of natural or anthropogenic sources, an explorative hierarchical cluster analysis has been performed (Figure 2), which maximises the variance between groups and minimizes the variance between members of the same group (Lee et al., 2006).

As can be noticed from Figure 2, the elements were grouped in two clusters: cluster I (N, Co, Cu, Zn) and cluster II (K, P, Mn). The similarity axes represent the degree of association between the elements, as follows: the higher their value, the higher the degree of association between the elements. The first cluster may be influenced by the anthropogenic pollution by means of the activities in the area (urban greening and road traffic), while cluster II seem to be associated with the geogenic and pedogenic sources.

Heavy metal content in street dust and upper soil horizons in cities is an index of pollution originating from various forms of human activity - operation of industrial plants, fuel consumption, exhaust emissions and the wear of various vehicle parts (Al-Khashman, 2007; Bilos et al., 2001). As Zglobicki et al. (2019) revealed, Zn was the element with the greatest enrichment in street dust in relation to the geochemical background for both fractions (63-200 μm and $< 63 \mu\text{m}$) for the whole 6-years period of survey in Lublin, Poland. The concentrations of Zn and Cu in the urban soils of Plovdiv could be influenced by the road traffic, while N and Co concentrations could be related with the mineral fertilisers used in landscaping activities (Maas et al., 2010). Cu abundance may be also influenced by the fertilisers and pesticides used in park areas (Nikolov et al., 2019).

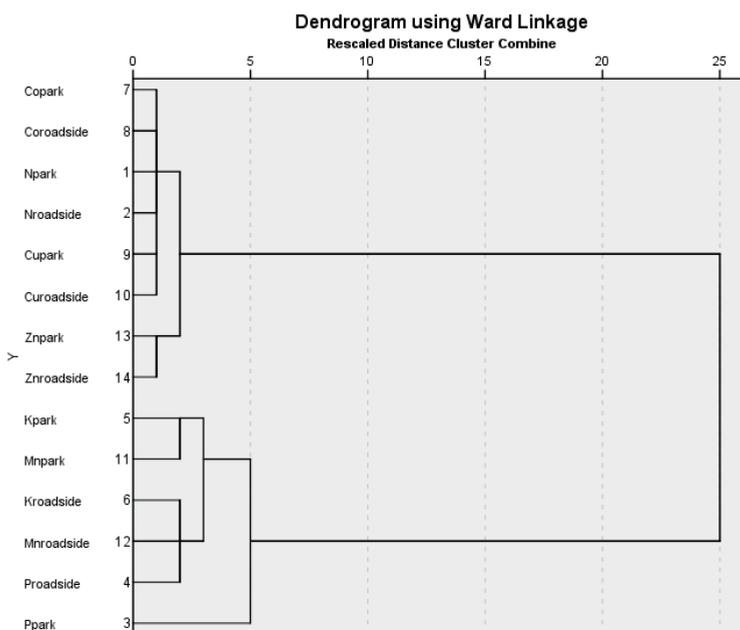


Figure 2. Hierarchical cluster analysis of macro- and microelements content of studied urban soils

CONCLUSIONS

As a whole, SOM in Plovdiv was quite elevated, more expressed in park samples. All studied urban soils are above the recommended range of soil SOC (> 10 g/kg) for healthy plant growing. It could be assumed that these soils possess significant potential for plant growing and provide a good media for urban greening. Generally, the K and P content was high in all soil samples and varied in the range 291.9-825.4 mg/kg for potassium and 410.5-1216.0 mg/kg for phosphorus, respectively. Nitrogen content was not so abundant and varied from low/insufficient (14.17-19.4 mg/kg) in some park soils to medium (20.85-31.36 mg/kg) in the rest samples. The C/N ratio is very low (< 10) which means that the organic matter is subjected to a higher degree of mineralization and carbon loss in the atmosphere, while the humus accumulation process is weaker.

Urban soils of Plovdiv are well supplied with microelements. Based on the CV values, which all are below 0.40, these microelements seem to be associated mainly with natural sources, although some anthropogenic inputs to Co, Cu and Zn should be considered.

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REFERENCES

- Al-Khashman, O.A. (2007). Determination of metal accumulation in deposited street dusts in Amman, Jordan. *Environmental Geochemistry and Health*, 29, 1–10.
- Bilos, C., Colombo, J.C., Skorupka, C.N. & Presa, M.J.R. (2001). Sources, distribution and variability of airborne trace metals in La Plata City area, Argentina. *Environmental Pollution*, 111, 149–158.
- Gencheva, S. (2000). *Updated classification and diagnosis of anthropogenic soils. II. Diagnosis of Anthropogenic Soils*. Scientific reports of the National conference “75 years of forest education in Bulgaria”, Forestry Section, Sofia, 196–204.
- Greinert, A. (2015). The Heterogeneity of Urban Soils in the Light of their Properties. *Journal of Soils and Sediments*, 15(8), 17–25.
- Han, Y.M., Du, P.X., Cao, J.J. & Posmentier, E.S. (2006). Multivariate analysis of heavy metal contamination in urban dusts of Xi’an, Central China. *Science of the Total Environment*, 355, 176–186.

- Hu, Y., Liu, X., Bai, J., Shih, Y., Zeng, E.Y. & Cheng, H. (2013). Assessing Heavy Metal Pollution in the Surface Soils of a Region that Had Undergone Three Decades of Intense Industrialization and Urbanization. *Environmental Science and Pollution Research*, 20(9), 6150–6159.
- Ivanov, P., Zhiyanski, M. & Doychinova, V. (2010). An overview of some classifications concerning the systematic nature of urban forest park soils. *Forest Science*, 2, 59–66.
- Lăcătușu, R. (2005). Consideration on urban soils. Factori și procese pedogenetice în zona temperata. *Serie noua*, 4, 69–78.
- Lăcătușu, R., Lăcătușu, A.R., Lungu, M. & Breaban, I.G. (2008). Macro- and microelements abundance in some urban soils from Romania. *Carpathian Journal of Earth and Environmental Sciences*, 3(1), 75–83.
- Lee, C.S.L., Li, X., Shi, W., Cheung, S.C.N. & Thornton, I. (2006). Metal Contamination in Urban, Suburban, and Country Park Soil of Hong Kong: A Study Based on GIS and Multivariate Statistics. *Science of the Total Environment*, 356(1-3), 45–61.
- Lovell, S.T. & Taylor, J.R. (2013). Supplying urban ecosystem services through multifunctional green infrastructure in the United States. *Landscape Ecology*, 28, 1447–1463.
- Maas, S., Scheifler, R., Benslama, M., Crini, N., Lucot, E., Brahmia, Z., Benyacoub, S. & Giraudoux, P. (2010). Spatial distribution of heavy metal concentrations in urban, suburban and agricultural soils in a Mediterranean city of Algeria. *Environmental Pollution*, 158, 2294–2301.
- Mao, Q., Huang, G., Buyantuev, A., Jianguo Wu J., Shanghua Luo, S. & Keming Ma, K. (2014). Spatial Heterogeneity of Urban Soils: The Case of the Beijing Metropolitan Region, China. *Ecological Processes*, 3(23), 23–28.
- Mcbride, M.B., Shayler, H.A., Spliethoff, H.M., Mitchell, R.G., Marquez, L.G., Ferenz, G.S., Russel, J.M., A.L. & Bachman, S. (2014). Concentrations of Lead, Cadmium and Barium in Urban Garden Grown Vegetables: The Impact of Soil Variables. *Environmental Pollution*, 194, 254–261.
- Melegy, A.A. & El-Agami, N.L. (2004). Factors controlling the chemistry and mineralogy of selected soil types of the Czech Republic and Egypt. *Bulletin of Geosciences*, 79(1), 71–79.
- Mihali, C., Oprea, G., Michnea, A., Jelea, S.G., Jelea, M., Man, C., Senila, M. & Grigor, L. (2013). Assessment of heavy metals content and pollution level in soil and plants in Baia Mare Area, NW Romania. *Carpathian journal of earth and environmental sciences*, 8(2), 143–152.
- Morel, J., Schwartz, C., Florentin, L. & De Kimpe, C. (2005). Urban soils. In: Hillel, D. (Ed), *Encyclopedia of soils in the environment*, Vol. 4. Oxford: Elsevier, 202–208.
- Neil, K., Wu, J., Bang, C. & Faeth, S. (2014). Urbanization affects plant flowering phenology and pollinator community: effects of water availability and land cover. *Ecological Processes*, 3, 17–23.
- Nikolov, B., Velcheva, I., Zheleva, E., Zaprianova, P., Valcheva, E., Bileva, T. & Petrova, S. (2019). Accumulation of Heavy Metals in the Urban Soils of Natural Monument “Bunardzhik” (Plovdiv, Bulgaria). *Ecologia Balkanica*, 11(2), 215–228.
- Park, S.J., Zhiqiang, C., Hanbae, Y., Morris, E.E., Sutherland, M., Mcspadden Gardener, B.B. & Grewal, P.S. (2010). Differences in Soil Chemical Properties with Distance to Roads and Age of Development in Urban Areas. *Urban Ecosystems*, 13(4), 483–497.
- Petrova, S., Yurukova, L., Velcheva, I. (2013). *Taraxacum officinale* as a biomonitor of metals and toxic elements (Plovdiv, Bulgaria). *Bulgarian Journal of Agricultural Science*, 18(5), 742–748.
- Petrova, S., Nikolov, B., Velcheva, I., Yankova, M., Kogan, E., Zheleva, E., Valcheva, E., Marhova, M., Tsankova, M. & Iliev, I. (2018). Dynamics of soil properties under a pollution gradient in urban areas (Plovdiv, Bulgaria). *Scientific Papers, Series A. Agronomy*, LXI(1), 109–116.
- Pouyat, R., Groffman, P., Yesilonis, I. & Hernandez, L. (2002). Soil carbon pools and fluxes in urban ecosystems. *Environmental Pollution*, 116(1), 107–118
- Setälä, H., Bardgett, R., Birkhofer, K., Brady, M., Byrne, L., de Ruiter, P., de Vries, F., Gardi, C., Hedlund, K., Hemerik, L., Hotes, S., Liiri, M., Mortimer, S.R., Pavao-Zuckerman, M., Pouyat, R., Tsiafouli, M. & van der Putten, W.H. (2013). Urban and agricultural soils: conflicts and trade-offs in the optimization of ecosystem services. *Urban Ecosystems*, 17, 239–253.
- Shang, L.Y., Sun, R.H., Wang, Z.M., Ji, Y.H. & Chen, L.D. (2012). Assessment of Heavy Metal Pollution in Surface Sediments of Rivers in Northern Area of Haihe River Basin. *China Journal of Environmental Sciences*, 33, 606–611.
- Stroganova, M. & Prokofieva, T. (2001). Urban soil classification for Russian cities of the taiga zone. In: Micheli, E., Nachtergaele, F.O., Jones, RJA et al. (Eds.). *Soil classification*. European Soil Bureau research report #7, EUR 20398 EN. Office for Official Publications of the European Communities, Luxembourg, 248, 153–156.
- Swiercz, A. & Zajecka, E. (2018). Accumulation of heavy metals in the urban soils of the city of Skarżysko-Kamienna (Poland) with regard to land use. *Carpathian Journal of Earth and Environmental Sciences*, 13(1), 249–266.
- Whitcomb, C.E. (1987). *Establishment and maintenance of landscape plants*. Lacebark.
- Wu, J. (2014). Urban Ecology and Sustainability: The State-of-the-Science and Future Directions. *Landscape and Urban Planning*, 125, 209–221.
- Zheng, Y.M., Yu, K., Wu, H.T., Huang, Z.C., Chen, H., Wu, X., Tian, Q.Z., Fan, K.K. & Chen, T.B. (2002). Lead concentrations of soils in Beijing urban parks and their pollution assessment. *Geographical Research*, 21, 418–424.

- Zhiyanski, M., Sokolovska, M., Glushkova, M., Vilhar, U. & Lozanova, L. (2017). Soil quality. In: Pearlmutter, D., Blaustein, J., Samson, R. et al. (Eds.). *The urban forest: Cultivating Green Infrastructure for People and the Environment*. Springer International Publishing AG.
- Zgłobicki, W., Telecka, M. & Skupiński, S. (2019). Assessment of short-term changes in street dust pollution with heavy metals in Lublin (E Poland) - levels, sources and risks. *Environmental Science and Pollution Research*, 26, 35049–35060.
- ***FAO (2014). *World Reference Base for Soil Resources*. Retrieved from <http://www.fao.org>.
- ***National Statistics Institute (NSI) (2018). Retrieved from <http://www.nsi.bg>.
- ***WRB (2006). IUSS Working Group, World Reference Base for Soil Resources. *World soil resources reports No. 103*, FAO, Rome.

THE EVALUATING METHOD OF THE BIOLOGICAL ACTIVITY AND RELATIVE PRODUCTIVITY FOR MIXED AND COMBINED THREE- COMPONENT CROPS

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Abstract

The production of cheap energy-rich and high-protein feed is possible due to the introduction of new types of feed crops. The use of new perennial low-growing crops based on the agrocenoses will make it possible to obtain cheap energy-saturated and high-protein feeds. In addition to the selection and introduction of new species of forage grasses, it is also necessary to evaluate their biological effectiveness in joint and mixed crops. A technique is proposed for assessing the biological activity and relative productivity of mixed and combined three-component crops. Formulas for calculating the land equivalents ratio (LER) and competitiveness coefficients (CR) in three-component mixtures are given. The proposed methodology is based on methods for assessing two-component mixed crops. According to the proposed method, the calculations of LER and CR of three-component joint and mixed crops of legumes, cereals and cruciferous herbs were carried out.

Key words: *three-component crops, high-protein feed, mixed agrocenoses, perennial herbs.*

INTRODUCTION

Currently, a very limited number of plant species are used in the feed production system. Expanding the assortment of feed crops through the introduction of new species may become a source of increasing production of cheap energy-rich and high-protein feeds. In this regard, the organization of adaptive fodder production based on the mixed agrocenoses through the selection of crops and the introduction of new species of forage grasses is of great importance (Ovtova et al., 2005).

One of the important aims in the fodder production is to find out, on the basis of resource-saving technologies, economically viable long-term agrocenoses with using new and perennial rare crops in order to obtain various types of feed.

The feasibility of widespread use of mixed crops was pointed out by K.A. Timiryazev, who noted that a plot of land sown with several varieties of grass gives a greater yield of hay than an area of equal size sown with the same species of grass. The relationship of plants in mixed crops depends on the biological nature of different plant species and on environmental conditions. Given these parameters, it is possible to identify and select crops and varieties that most fully use

the areas of cohabitation and vegetation time (Timoshkin et al., 2011).

Until recently, it was believed that high-quality herbage is produced only from a grass mixture consisting of 5-6 or more types of herbs with different biology (loose bush, rhizome) and a different formation of the shoot and of the foliage (top, half-top and bottom). However, under conditions of intensification and agricultural specialization, with a significant amount of work on the radical improvement of meadows, an increase in crop rotation area under grasses, it is difficult to seed so many herbs.

The purpose of the current article is to describe the new method for assessing the biological activity and relative productivity of mixed and combined three-component crops.

MATERIALS AND METHODS

Multivariate field experiments were carried out. In the first experiment, the comparative productivity of perennial herbs was studied. These perennial herbs were not mixed and we examined the productivity of every mentioned below herbs separately.

1. *Bunias orientalis* L.
2. *Galega orientalis* L.

3. *Medicago sativa* L.
4. *Trifolium pratense* L.
5. *Bromopsis inermis* Holub
6. *Dactylis glomerata* L.

In the second experiment, we examined *Bunias orientalis* L. in mixed crops. The second experiment was also bifactorial: Factor A (grass mixture) and Factor B (sowing method).

Factor A (grass mixture)

- Bunias orientalis* L. + *Galega orientalis* L.
- Bunias orientalis* L. + *Medicago sativa* L.
- Bunias orientalis* L. + *Trifolium pratense* L.
- Bunias orientalis* L. + *Dactylis glomerata* L.
- Bunias orientalis* L. + *Bromopsis inermis* Holub
- Bunias orientalis* L. + *Galega orientalis* L. + *Bromopsis inermis* Holub
- Bunias orientalis* L. + *Galega orientalis* L. + *Dactylis glomerata* L.
- Bunias orientalis* L. + *Medicago sativa* L. + *Bromopsis inermis* Holub
- Bunias orientalis* L. + *Medicago sativa* L. + *Dactylis glomerata* L.
- Bunias orientalis* L. + *Trifolium pratense* L. + *Bromopsis inermis* Holub
- Bunias orientalis* L. + *Trifolium pratense* L. + *Dactylis glomerata* L.

Factor B (sowing method)

1. Even spacing between individual seeds in the row.
2. Cross-sowing.

Pure crop seeding rates: *Bunias orientalis* L. - 40 kg/ha, *Galega orientalis* L. - 20 kg/ha, *Medicago sativa* L. - 12 kg/ha, *Trifolium pratense* L. - 10 kg/ha, *Bromopsis inermis* Holub - 20 kg/ha, *Dactylis glomerata* L. - 18 kg/ha. In grass mixtures, the sowing rate of legumes and *Bunias orientalis* L. was reduced by 20%, bluegrass - 2 times. One of the most common criteria for assessing the biological activity of mixed crops is the land equivalents ratio (LER) (Willey et al., 1980). In this case we used a well-known method for assessing biological activity for two-component mixtures, where LER is calculated by the formula given below:

$$LER = \frac{Y_{AB}}{Y_{AA}} + \frac{Y_{BA}}{Y_{BB}} = \frac{Y_{AB} \cdot Y_{BB} + Y_{BA} \cdot Y_{AA}}{Y_{AA} \cdot Y_{BB}}$$

where:

Y_{AA} - crop yield (component A) in clean sowing, t/ha;

Y_{BB} - crop yield (component B) in clean sowing, kg/ha;

Y_{AB} - crop yield (component A) in mixed sowing, t/ha;

Y_{BA} - crop yield (component B) in mixed, kg/ha.

To evaluate the relative productivity of crops in mixed crops, a competitive coefficient (CR) is used.

According to a known method for two-component mixtures, CR is calculated by the formula:

$$CR_{AB} = \frac{Y_{AB}}{Y_{AA}} \cdot \frac{Y_{BA}}{Y_{BB}} \cdot \frac{z_{BA}}{z_{AB}} = \frac{LER_A \cdot z_{BA}}{LER_B \cdot z_{AB}}$$

where:

LER_A - the ratio of the land equivalent of the crop (component A) in clean sowing;

LER_B - ratio of land equivalent of crop (component B) in clean sowing;

z_{AB} - proportion of culture (component A) in the mixed crop;

z_{BA} - proportion of culture (component B) in the mixed crop.

Based on the evaluation methodology for LER of two-component mixtures, a methodology is proposed for assessing the Land Equivalents ratio (LER) in three-component mixtures.

The land equivalents ratio (LER) is determined by the formula:

$$LER = \frac{Y_{ABC}}{Y_{AA}} + \frac{Y_{BAC}}{Y_{BB}} + \frac{Y_{CAB}}{Y_{CC}} = \frac{Y_{ABC}Y_{BB}Y_{CC} + Y_{BAC}Y_{AA}Y_{CC} + Y_{CAB}Y_{AA}Y_{BB}}{Y_{AA} \cdot Y_{BB} \cdot Y_{CC}}$$

where:

Y_{AA} - crop yield (component A) in clean sowing, t/ha;

Y_{BB} - crop yield (component B) in clean sowing, kg/ha;

Y_{CC} - crop yield (component C) in clean sowing, t/ha;

Y_{ABC} - crop yield (component A) in mixed sowing, t/ha;

Y_{BAC} - crop yield (component B) in mixed sowing, t/ha;

Y_{CAB} - crop yield (component C) in mixed sowing, t/ha.

Then the land equivalents ratio (LER) of the components of the mixture is determined as follows:

$$LER_A = \frac{Y_{ABC}}{Y_{AA}}; LER_B = \frac{Y_{BAC}}{Y_{BB}}; LER_C = \frac{Y_{CAB}}{Y_{CC}},$$

$$LER_{AB} = \frac{Y_{BAC}}{Y_{BB}} + \frac{Y_{ABC}}{Y_{AA}} \\ = \frac{Y_{BAC} \cdot Y_{AA} + Y_{ABC} \cdot Y_{BB}}{Y_{BB} \cdot Y_{AA}};$$

$$LER_{AC} = \frac{Y_{CAB}}{Y_{CC}} + \frac{Y_{ABC}}{Y_{AA}} \\ = \frac{Y_{CAB} \cdot Y_{AA} + Y_{ABC} \cdot Y_{CC}}{Y_{CC} \cdot Y_{AA}};$$

$$LER_{BC} = \frac{Y_{BAC}}{Y_{BB}} + \frac{Y_{CAB}}{Y_{CC}} \\ = \frac{Y_{BAC} \cdot Y_{CC} + Y_{CAB} \cdot Y_{BB}}{Y_{BB} \cdot Y_{CC}}.$$

Using the above method for determining the competitiveness coefficient in two-component mixtures, we propose a method for determining (CR) in three-component mixtures. Thus, the competitiveness coefficient in ternary mixtures is determined by the formulas:

$$CR_A = \frac{LER_A \cdot z_{BC}}{LER_{BC} \cdot z_A}; \quad CR_B = \frac{LER_B \cdot z_{AC}}{LER_{AC} \cdot z_B}; \\ CR_C = \frac{LER_C \cdot z_{AB}}{LER_{AB} \cdot z_C},$$

where:

LER_A - ratio of the land equivalent of the crop (component A) in clean sowing;

LER_B - ratio of land equivalent of crop (component B) in clean sowing;

LER_C - ratio of land equivalent of crop (component C) in clean sowing;

LER_{BC} - the ratio of the land equivalent of crops (component B and C) in a mixed three-component sowing;

LER_{AC} - ratio of the land equivalent of crops (component A and C) in a mixed three-component crop;

LER_{AB} - ratio of the land equivalent of crops (component A and B) in a mixed three-component crop;

z_{BC} - the proportion of crops (components B and C) in mixed sowing;

z_{AC} - the proportion of crops (components A and C) in mixed sowing;

z_{AB} - the proportion of crops (components A and B) in mixed sowing;

z_A - the proportion of culture (components A) in the mixed crop;

z_B - the proportion of culture (components B) in the mixed crop;

z_C - the proportion of culture (components C) in mixed sowing.

Statistical evaluation for the calculated results influence of the components in the current research and the new sowing method on the green mass yield in perennial grass mixtures (t/ha) was carried out using analysis of variance (ANOVA). The assessment was carried out by comparing the variances of the variants with the variance of the error according to the Fisher test F with a 95% probability. Thus, the average square of the random variance, which determines the random error of the experiment, was taken as the base - unit of comparison. In this case, the null hypothesis being tested was the assumption that all sample averages are estimates of the same general average, and, therefore, the differences between them are insignificant. In addition, the significance of particular differences in terms of the smallest significant difference (NDS) was additionally assessed and between what averages there were significant differences. When processing data from multivariate experience, analysis of variance allowed us to identify not only the main effects, but also to assess the significance of their interaction.

RESULTS AND DISCUSSIONS

The assessment showed that the greatest coefficient of biological efficiency is observed with the ordinary method of sowing, various variants of three-component agrocenoses. At the same time, on average for 1 year of vegetation of herbs, this indicator was higher for ordinary than for cross-sowing by 17.8%. In two-component crops, the LER value was less than unity - with ordinary sowing 0.28-0.92 and 0.18-0.90 with cross-sowing (Ovtova et al., 2005). It should also be noted that in double mixtures the highest LER value was observed in mixtures with cereals, regardless of the method of sowing.

In the three-component mixtures of the considered variants, the highest biological efficiency coefficient was found in the agroecosystems of *Bunias orientalis* L. + *Galega orientalis* L. + *Bromopsis inermis* Holub, 1.22-1.34, and *Bunias orientalis* L. + *Galega orientalis* L. + *Dactylis glomerata* L., 1.09-1.25.

So, in the 2nd year of vegetation, the LER value of ternary mixtures was 49.7% higher in row crops and 27.5% in cross crops than in binary crops. In ternary mixtures, the highest biological efficiency coefficient was observed in the agroecosystems of *Bunias orientalis* L. + *Galega orientalis* L. + *Bromopsis inermis* Holub and *Bunias orientalis* L. + *Galega orientalis* L. + *Dactylis glomerata* L. - 1.97 and 1.89. Analysis of the LER of binary mixtures showed that the highest LER was obtained in the agroecosystem of *Bunias orientalis* L. + *Bromopsis inermis* Holub, 1.15, and *Bunias orientalis* L. + *Bromopsis inermis* Holub, 1.06, the remaining mixtures were biologically less effective (Ovtova et al., 2005).

The competitiveness factor of the *Bunias orientalis* L. is mainly determined by the number of components in the agroecosystem and the method of sowing. The number of components in the mixture and the method of sowing had a significant impact on the competitiveness factor of the *Bunias orientalis* L. An increase in the components from two to three contributed to a decrease in the CR value of the *Bunias orientalis* L. by an average of 1.7 times, and the ordinary method of sowing increased the competitiveness of the *Bunias orientalis* L. by an average of 40.4%.

In the first year of vegetation, the highest CR (0.73) of *Bunias orientalis* L. was observed in the binary mixture of *Bunias orientalis* L. + *Galega orientalis* L. broom during row crops, and the highest coefficient of competitiveness was obtained from the *Dactylis glomerata* L. of the national team during cross-sowing (5.00) in the binary mixture with *Bunias orientalis* L.

In the second year of vegetation, the *Galega orientalis* L. is the most optimal component for *Bunias orientalis* L., so in a grass mixture with its participation, the *Bunias orientalis* L. CR is 0.99 for ordinary sowing. Among ternary mixtures, the highest *Bunias orientalis* L. CR was noted in the agroecosystem of *Bunias orientalis* L. + *Galega orientalis* L. + *Bromopsis inermis* Holub, while it can be noted that the

coefficient of competitiveness of the bean component of the mixture during cross-sowing increases by 28.3%.

By the third year of vegetation, in two-component and three-component mixtures, an increase in CR of *Bunias orientalis* L. is noted. In the double agroecosystems of the *Bunias orientalis* L. and the *Bromopsis inermis* Holub, the competitiveness coefficient increases to 1.19, and the *Bunias orientalis* L. and the *Galega orientalis* L. grow to 1.02, more than in the second year of vegetation. At the same time, cereal grasses reduce the coefficient of competitiveness of *Bunias orientalis* L., so rump - by 1.7 times, and hedgehog - by 23.6%. An analysis of three-component mixtures of *Bunias orientalis* L. showed that its competitiveness coefficient is lower than in binary mixtures by an average of 2.2 times.

On average, in the first year (Table 1), significant differences were obtained for factor A (set of components); for factor B (sowing method), the same yield (5.4 t/ha) was obtained in the *Bunias orientalis* L. + clover + *Bromopsis inermis* Holub mix. The maximum yield was obtained in the mixture of *Bunias orientalis* L. + clover + *Dactylis glomerata* L. (6.4 t/ha) for row crops, and the minimum (2.2 t/ha) in the variant *Bunias orientalis* L. + *Dactylis glomerata* L. for cross-sowing. On average, the yield of ordinary crops in the first year of vegetation was higher by 17.1%. At the same time, ordinary three-component crops (5.2 t/ha) were the most productive, with cross-sowing, these mixtures yielded 15.6% less green mass. Two-component row crops yielded 23.8% less than the three-component row crops, and with cross-sowing, the difference was 25% compared to the cross three-component crops and 44.4% in comparison with the three-component row crops. In the second and third years of vegetation, a significant influence on factor A was found, with the exception of mixtures of *Bunias orientalis* L. + *Medicago sativa* L. + *Dactylis glomerata* L. and *Bunias orientalis* L. + clover + *Bromopsis inermis* Holub in the third year of vegetation in ordinary crops of the main mowing (Table 1). According to factor B, the yield increase was also seen in the main mowing in all cases except:

Bunias orientalis L. + *Bromopsis inermis* Holub, *Bunias orientalis* L. + *Dactylis*

glomerata L., *Bunias orientalis* L. + *Medicago sativa* L. + *Bromopsis inermis* Holub in the second year of vegetation in the main mowing

and *Bunias orientalis* L. + clover + *Dactylis glomerata* L. - in the third year of the main mowing.

Table 1. Effect of a set of components and the sowing method on the crop of green mass of perennial grass mixtures, t/ha

Variety	Years of vegetation				
	1st	2nd		3rd	
		Main mowing	Aftergrass	Main mowing	Aftergrass
Ordinary crops					
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L.	4.2	32.5	6.6	35.8	15.8
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L.	5.3	27.6	4.6	35.6	12.8
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L.	5.9	26.8	2.7	35.0	13.2
<i>Bunias orientalis</i> L. + <i>Dactylis glomerata</i> L.	3.3	20.3	4.3	24.0	12.0
<i>Bunias orientalis</i> L. + <i>Bromopsis inermis</i> Holub	2.3	19.1	3.6	20.4	12.8
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Bromopsis inermis</i> Holub	4.6	36.0	5.7	43.0	19.2
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Dactylis glomerata</i> L.	3.6	34.7	5.9	41.2	20.6
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Bromopsis inermis</i> Holub	5.2	26.0	5.3	32.8	20.2
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Dactylis glomerata</i> L.	6.2	27.3	5.1	32.0	20.4
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Bromopsis inermis</i> Holub	5.4	26.4	5.9	32.0	19.0
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Dactylis glomerata</i> L.	6.4	28.0	5.1	30.0	18.8
Cross-sowing					
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L.	3.1	33.6	6.1	35.6	14.6
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L.	4.6	26.2	7.2	34.0	15.0
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L.	5.6	31.4	3.3	33.2	14.4
<i>Bunias orientalis</i> L. + <i>Dactylis glomerata</i> L.	2.3	20.5	4.6	25.6	12.6
<i>Bunias orientalis</i> L. + <i>Bromopsis inermis</i> Holub	2.2	19.1	3.9	24.8	11.7
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Bromopsis inermis</i> Holub	2.9	33.7	5.9	40.5	18.6
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Dactylis glomerata</i> L.	3.4	32.7	6.1	39.6	17.9
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Bromopsis inermis</i> Holub	4.5	25.9	5.0	31.2	15.8
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Dactylis glomerata</i> L.	5.2	28.1	5.0	31.5	16.0
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Bromopsis inermis</i> Holub	5.4	26.8	5.7	30.6	13.6
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Dactylis glomerata</i> L.	5.5	29.8	5.1	30.0	13.8
HCR ₀₅	0.15	0.48	0.08	0.39	0.12
HCR _{05A}	0.04	0.15	0.02	0.12	0.04
HCR _{05B}	0.10	0.34	0.05	0.28	0.09

In 1st, 2nd, 3rd years, in the main mowing, the maximum yields were given by three-component mixtures of *Bunias orientalis* L. with *Galega orientalis* L. and bluegrass crops. In the second year, in ordinary crops, the yield of the *Bunias orientalis* L. + *Galega orientalis* L. + *Bromopsis inermis* Holub variant exceeded

the average yield for factor A by 30% and by 29% by experience. In a mixture with a hedgehog - 25.3% and 24.4%, respectively. When cross-sowing, these mixtures yielded lower than in ordinary crops by 2.3 and 2.0 t/ha. The third year as a whole was more favorable and the yield for all options was higher on

average by 30.7% in ordinary crops and by 15.7 in cross crops. In the third year, in ordinary crops, the yield of the *Bunias orientalis* L. + *Galega orientalis* L. + *Bromopsis inermis* Holub variant exceeded the average for factor A by 18.8%, according to experience by 25.4%, the mixture with a hedgehog, respectively -13.8% and 20.1%. In cross-crops, the first mixture had this indicator at the level of 25% and 18.1%, the second - 22.2% and 15.5%. The remaining mixtures were less productive, although they gave a quite stable crop.

The nutritional value of the feed is estimated by the content of the main nutrients in the dry mass in mixtures, % (Table 2).

Based on the studies, we can conclude that the methods of sowing herbs have a significant impact on the chemical composition of the feed. So, on average, over three years of grass life, the highest protein content was noted in ordinary crops (Table 2), with the exception of mixtures of *Bunias orientalis* L. with *Galega orientalis* L.

and clover. The highest protein content was in the mix of *Bunias orientalis* L. and *Galega orientalis* L. (18.25%) with cross-sowing and floor; the mixture was the best with ordinary sowing (17.08%). In the first year of grass vegetation, the mixture of *Bunias orientalis* L. and *Galega orientalis* L. was less productive in comparison with the average by 0.18% in both cases. In the second year of grass vegetation, the mixture of *Bunias orientalis* L. and *Galega orientalis* L. at cross-sowing was 0.25% higher, and at ordinary lower by 0.18%. This year, cross sowing was generally more productive. In the third year of vegetation, these mixtures were the best and the method of sowing did not affect the nutritional value of the feed.

When comparing two-component crops with the content of one legume culture and three-component crops, we see a pattern: for row crops, the combined team is most favorable as the third component of the hedgehog, and for cross-sowing, the boneless beef (Table 2).

Table 2. Dynamics of the content of basic nutrients in the dry mass in mixtures, %

Variety	Protein	Fat	Fiber	Ash
Ordinary crops				
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L.	17.08	2.11	25.08	8.22
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L.	17.00	2.84	25.08	8.26
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L.	17.08	2.87	24.23	7.50
<i>Bunias orientalis</i> L. + <i>Dactylis glomerata</i> L.	13.23	3.64	27.65	7.96
<i>Bunias orientalis</i> L. + <i>Bromopsis inermis</i> Holub	15.05	2.85	27.72	8.30
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Bromopsis inermis</i> Holub	16.20	2.60	27.12	8.05
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Dactylis glomerata</i> L.	16.40	2.01	26.53	7.21
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Bromopsis inermis</i> Holub	15.38	1.85	27.22	9.48
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Dactylis glomerata</i> L.	15.65	2.21	26.92	7.51
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Bromopsis inermis</i> Holub	15.10	2.09	25.55	9.40
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Dactylis glomerata</i> L.	15.63	2.28	26.23	9.53
Cross-sowing				
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L.	18.25	2.25	24.87	7.98
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L.	16.92	2.91	25.68	8.34
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L.	17.22	2.05	24.50	7.79
<i>Bunias orientalis</i> L. + <i>Dactylis glomerata</i> L.	12.58	3.33	24.07	11.57
<i>Bunias orientalis</i> L. + <i>Bromopsis inermis</i> Holub	11.88	2.97	27.30	7.94
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Bromopsis inermis</i> Holub	15.87	2.66	27.08	8.84
<i>Bunias orientalis</i> L. + <i>Galega orientalis</i> L. + <i>Dactylis glomerata</i> L.	15.80	2.16	26.48	8.07
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Bromopsis inermis</i> Holub	14.74	2.15	27.13	8.43
<i>Bunias orientalis</i> L. + <i>Medicago sativa</i> L. + <i>Dactylis glomerata</i> L.	14.67	2.21	26.52	8.32
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Bromopsis inermis</i> Holub	15.04	2.16	25.60	8.69
<i>Bunias orientalis</i> L. + <i>Trifolium pratense</i> L. + <i>Dactylis glomerata</i> L.	14.79	2.16	26.00	8.76

The percentage of fat in the mixtures ranges from 1.85% (*Bunias orientalis* L. + *Medicago sativa* L. + *Bromopsis inermis* Holub in the ordinary way of sowing) to 3.64% (*Bunias orientalis* L. + *Bromopsis inermis* Holub in the ordinary way of sowing). At the same time, a high fat content on average is observed in cross crops. The same pattern was preserved in the first year of the vegetation of the grass, here there is a pattern: more protein - less fat.

The percentage of fiber ranged from 24.25% to 27.72% in ordinary crops and from 24.07% to 27.30% in cross crops. In both cases, these were two-component crops. In the three-component crops in the first case, fiber contained from 25.55% to 27.22% in the second from 25.60% to 27.13%. At the same time, according to factor B (sowing method), ordinary crops were distinguished.

CONCLUSIONS

Thus, the proposed methodology for calculating the coefficient of biological activity and the coefficient of competitiveness (CR) allows to evaluate three-component agrocenoses with different methods of sowing.

REFERENCES

- Ovtova, O.G. (2005). The formation of mixed agrocenoses of the *Bunias orientalis* L. in the forest-steppe zone. *Scientific paper on the PhD research*, Penza, Russia, 159.
- Timoshkin, O.A., Potehin, S.A. (2011). Competitive ability and biological effectiveness of mixed crops with fodder beans. *Achievements of Science and Technology of AIC*, 1, 53–55.
- Willey R.W., Rao M.R. (1980). A competitive ratio for quantifying competition between intercrops. *Experimental Agriculture*, 16(2), 117–125.

THE INFLUENCE OF BIOCARBON ON THE AVAILABILITY OF SOIL NUTRIENTS DURING ZUCCHINI CULTIVATION

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Abstract

The purpose of the experiment is to study the influence of incorporated carbonised plant residues on the nutrient storage in soil during the zucchini vegetation. The experiment was carried out in the experimental field on University of Forestry - Sofia (42° 7' N, 23° 43' E), on fluvisols. Six variants have been developed: 1) V1 - pure soil; 2) V2 - with manure (4 t/da); 3) V3 - with biochar (500 kg/da); 4) V4 - manure (4 t/da) + biochar (250 kg/da); 5) V5 - manure (4 t/da) + biochar (500 kg/da); 6) V6 - manure (4 t/da) + biochar (750 kg/da). The highest N content in the experimental variants was reported in V6 - (manure 4t/da + BCh 750 kg/da), which was found to be optimal for the development of zucchini. The lowest NPK values were reported in V1 and V3. Positive effects on soil nutrients, organic matter and zucchini yield indicates that biochar could partly replace chemical fertilizers.

Key words: biochar, manure, N, soil nutrients.

INTRODUCTION

Maintaining soil productivity and improving soil quality are one of the key elements for sustainable agriculture. In recent years, in the context of climate change, there is a great interest in studying biochar (BC) use in agriculture (Lehmann, 2007). Several studies have reported that biochar can adsorb nitrogen (as NH_4^+ -N, NO_3 -N, and Urea), phosphorus (as orthophosphate), potassium, and organic matter (Kasozi et al, 2010; Takaya, 2016; Kizito et al., 2019). As a soil amendment, biochar has been reported to offer several benefits, which include increased soil texture, soil carbon, nutrient retention, and cation exchange capacity, beside support to microbial diversity that increases mineralization and availability of nutrients in amended soils (Kizito et al., 2019). The incorporation of carbonized plant residues into the soil increases the efficiency of nitrogen uptake from plants grown on sandy soil (Zwieten et al., 2010). Thereby, seed germination, growth, development and yield of some plants are significantly increased by the application of biochar (Glaser et al., 2002).

The increasing interest in recent years on the fresh zucchini taste has leads to necessitated of study its growth and development.

Zucchini is an annual herbaceous plant of the family *Cucurbitaceae*, of the genus *Cucurbita*,

to which the zucchini (*Cucurbita pepo* var. *Giromontia Dutch*) belongs.

A number of researches have considered the zucchini yield obtained on different soil types after biochar application. According to some authors, application of biochar increases the yield of zucchini over the control variant by 2.2 t/ha (Zwieten et al., 2009). Other authors report a *Cucurbita pepo* crop increase ranging from 20% to 140% over control variants. There are also data that do not show a significant difference in zucchini yield (Joseph SD. et al., 2008), and those that report a decline in yield compared to control variants in a one-year experiment (Gaskin et al., 2010).

A very few studies about BC application and its effect on soil properties and nutrition uptake have been conducted in Bulgaria.

The aim of the experiment is to study the influence of incorporated carbonised plant residues on the nutrient storage in soil during the zucchini vegetation.

MATERIALS AND METHODS

The experiment was carried out on the experimental field of the University of Forestry - Sofia (42° 7' N, 23° 43' E). The soil is fluvisol, slightly stony, slightly acidic. This area came under a continental climatic sub region, in a mountain climatic region.

The experiment was set with two ameliorants - biochar and manure (used as a background). During the spring cultivation, the two ameliorants were incorporated into the soil at 15 cm depth. Six variants were developed: 1) V1 - pure soil; 2) V2 - with manure (4 t/da); 3) V3 - with biochar (500 kg/da); 4) V4 - manure (4 t/da) + biochar (250 kg/da); 5) V5 - manure (4 t/da) + biochar (500 kg/da); 6) V6 - manure (4 t/da) + biochar (750 kg/da). The experiment was carried out by randomized complete block design with four replications and protection zones.

To test the impact of BC, a field experiment with the zucchini variety Izobilna was carry out, this variety was chosen as the standard variety in the country. The sowing was done at the end of April. Three beds were formed on which the sowing of zucchini was carried out according to the standard scheme for growing two-row tape. The sowing is in nests, with a row spacing of 60 cm and 50 cm inside the row (two row row diagram: 100 + 60/50).

Plants are irrigated by a drip irrigation system; the tape drip hose used has the following characteristics: I-Tape 8 mil/distance between drippers 20 cm/5.3 lh. The irrigation rate is 40 mm.

Samples of used ameliorants (biochar and well-decomposed manure) were taken before conducting the experiment. Laboratory analyzes in beginning and in the end of the experiment was carried out. The content of basic nutrients, heavy metals and pH was obtained from soil and plants.

RESULTS AND DISCUSSIONS

Samples of used ameliorants were taken prior to the laying of the field experiment.

In the experiment well decomposed cow manure was used.

The pH reaction is slightly alkaline. The manure contains a high amount of organic carbon, well stored with total N and medium stored with P and K, the values of the mobile forms being approximately equal to the reported total amounts of P-0.89 and K-1.31. The ratio of

ammonium to nitrate forms indicates that the mineralization process is not fully complete (NH_4 - 79.9 and NO_3 - 0.9).

Table 1. Nutrient content of manure

Indicator	Unit	Method	Value
pH		BS EN 15933	7.51
Organic C	%	BS EN 13137	16.92
N Kjeldahle	%	BS EN 13342	2.06
K	%	BS EN 16170	1.40
P	%	BS EN 16170	1.39
K mobile	%	BS EN 16170	1.31
P mobile	%	BS EN 16170	0.89
NH_3	%	BS EN 16177	0.90
NH_4	mg/kg	BS EN 16177	79.9

The biochar used to conduct the experiment was made from wood chips. Table 2 presents the chemical analysis of used BC.

Table 2. Chemical characteristics of BC obtained from wood chips

pH	ELCD mS/m	C%	N%	P%	K%	Ca%	Mg%	CaCO ₃
10.8	45	61.8	0.39	0.22	0.85	2.18	0.23	5.4

The pH reaction in the analysed sample from BC is highly alkaline. It contains a large amount of carbon, which confirms the ability of BC to deposit carbon into the soil, reducing its release into the atmosphere. The mineral content of NPK is minimal. The presence of CaCO_3 is one of the causes of the highly alkaline reaction of the substrate.

Agrometeorological and phenological observations are a valuable source of information on the relationship between climate and plant development during the growing season.

During the experimental period (April-July), the weather conditions are favourable for the growth and development of zucchini (Figure 1). With average seasonal temperatures close to normal, no temperature anomalies are observed.

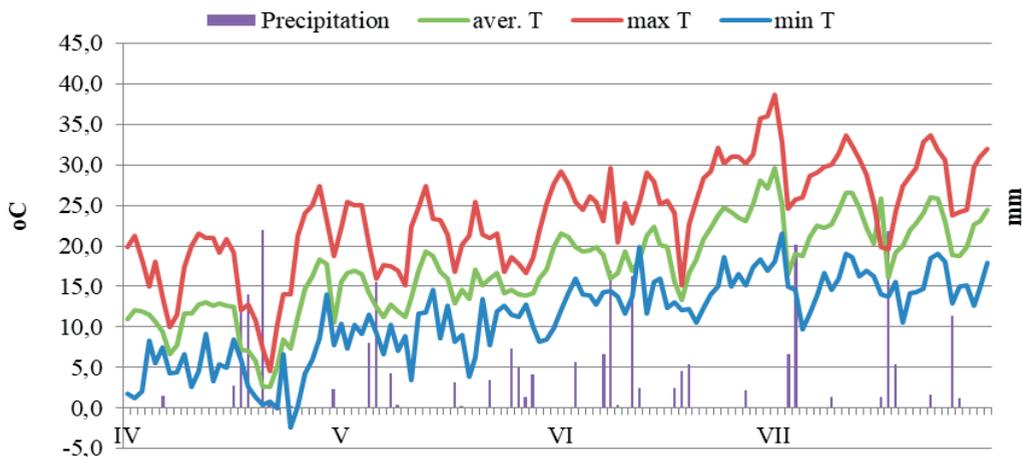


Figure 1. Temperatures and precipitation during the growing season of the zucchini

Only at the beginning and at the end of the second week of May lower positive temperatures (3.5°C and 3.8°C) were recorded, but average daily temperatures during this period did not fall below 12°C, which did not lead to a negative effect on the vegetative plant growth.

There is an even distribution of rainfall during the zucchini vegetation, ranging from 0.2 mm to 21.9 mm. The total rainfall is 63.1, with more than two-thirds falling in the first half of the month.

The agrochemical characteristics of the Fluvisols from experimental field (Vrajdna) are presented in Table 3. The sampling depth is consistent with the main root zone of the zucchini.

Table 3. Agrochemical characteristics of the soil before conducting the experience with zucchini

Depth cm	Humus %	pH H ₂ O	N, %	P ₂ O ₅ mg/100 g	K ₂ O mg/100 g
0-20	1.73	7.3	0.160	79.2	18.1
20-40	1.64	7.2	0.154	94.81	19.9

Soil data obtained before ameliorants incorporation, show a good degree of storage of K₂O, a very high content of P₂O₅, which increases in depth. The soil reaction is slightly alkaline, approaching a depth-neutral layer.

The soil is poorly humus with humus content varying between 1.73% for the arable horizon and 1.64% for the sub arable horizon. The obtained data are common for this kind of soil type.

After the end of zucchini vegetation, soil samples were taken again and give for agrochemical analysis. The obtained data are presented in Table 4.

Table 4. Soil analysis after zucchini vegetation

Variants	Humus (%) BBM	pH (H ₂ O) ISO 10390	N (%) Kjeldal	P ₂ O ₅ (mg/100g) BBM	K ₂ O (mg/100g) BBM
Var.1	1.36	7.2	0.165	53.60	10.0
Var.2	1.33	7.3	0.173	68.45	10.8
Var.3	1.30	7.3	0.143	51.05	11.0
Var.4	1.82	7.1	0.189	81.60	14.1
Var.5	1.91	7.3	0.194	84.32	17.8
Var.6	1.90	7.3	0.203	81.84	19.3

The soil samples were taken from a depth of 0-30 cm. It is obvious that the humus content increases in variants with higher BC content in combination with manure. The reduced content of total N in Var. 3 with biochar probably is due to the adsorption of NH₃ or organic N onto its surface by cations or anion exchange reactions and the increased immobilization of N, as a consequence of the additional C incorporate in soil with BC.

A decrease in P₂O₅ content in variant 3 is observed with BC alone, this is most likely due to the ability of BC to absorb phosphorus and nitrogen anions on its surface. The combined introduction of BC and manure has a positive effect on the potassium content of the soil as the soil storage of K increases from medium to very good. This is due to the ability of BC to increase

cation exchange capacity. Many authors report for increasing levels of K in soil after BC application (Cheng et al., 2008; Lentz and Ippolito, 2012). It is considered that available K in the BC composition can be rapidly absorbed by plants (Karer et al., 2013). However, some researchers have suggested that the high availability of K for plants may not continue beyond the year of application (Steiner et al. 2007).

It is noteworthy that despite the highly alkaline reaction of BC (pH = 10.8), when is combined with manure, it has no effect on soil acidity.

Mineral plant nutrition, along with photosynthesis, is a piece of plant-specific autotrophy. Mineral nutrition is closely related to soil fertility. Regulation of mineral nutrition is one of the most powerful factors for managing the physiological processes and productivity of agricultural plants.

The nutrient content of zucchini fruits is shown in Figure 2.

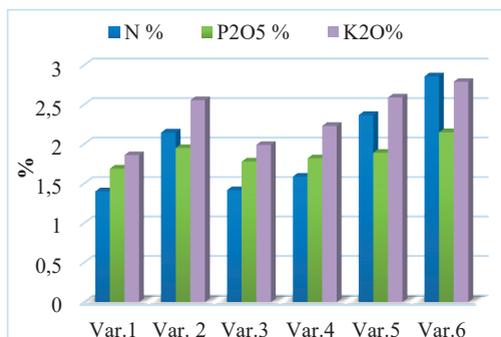


Figure 2. Percentage of nutrients in zucchini fruits

The lowest NPK values were again reported in Var. 1 clean soil and Var. 3 only with added BC. For BC variants, the N and K content increases with the increase the rate of imported carbonated plant residues. The highest nitrogen content in the experimental variants was reported in variant 6 - (manure 4 t/da + BC 750 kg/da), which is found to be optimal for the development of zucchini.

Nitrogen substances are found in the form of proteins, amino acids, amides, ammonia compounds and more. Their quantity in fruits is low and ranges from 1.41% to 2.86%.

The increase in nitrogen content in the variants with imported BC can be explained by biological nitrogen fixation due to the favourable development of microorganisms in the soil.

According to (Xu et al., 2014), the use of biochar for fertilization increases plant growth, soil pH, total carbon and nitrogen forms, C/N ratio and cation exchange capacity. Their results show that the application of biochar significantly increases the diversity and alters the relative abundance of some microbes that are associated with the carbon and nitrogen cycle. Overall nitrification and denitrification processes are stimulated while reducing N₂O emissions.

Despite the high values of P₂O₅ in soil samples, the content in zucchini fruits range between 1.69 to 2.15%. This is probably due to the fact that the majority of phosphorus compounds are poorly soluble in soil solution, which is one of the major difficulties in supplying plants with phosphorus. Highest values were reported for variant 2 (manure only) and variant 6, no significant difference was observed between the other variants.

Phosphorus, like nitrogen, is easily redistributed between the organs of the plant, moving from the old leaves to other parts of the plant (Kimenov, 1994). Phosphorus uptake by plants occurs mainly in two periods when the seeds germinate and when the fruit ripens, when a large number of organophosphorus compounds are formed, therefore the content of P₂O₅ in the fruits is higher than that of N.

Of all nutrient content in plants, potassium generally reaches the highest content. A large amount of potassium is associated with carbohydrate metabolism and plants water regime. The highest percentage of potassium was again reported in variant 6 - 2.79%, followed by variant 2 by 2.55%. It is noteworthy that as the rate of imported BC increases, the content of K₂O increases as well, due to the ability of the BC to capture positively charged cations through its negative charge, which develops on its surfaces, and this negative charge can buffer acidity in soil, as well as organic matter in general fertility. The low content in variant 3, which is almost equal to that in the control variant can be explained by the fact mentioned above, as well as by the minimum nutrients contained in biochar.

Nine harvests were made from the zucchini. They were obtained by variants and replicates. Data are averaged and equated to kilograms per decare at a planting density of 2,500 plants per hectare. The results obtained are shown in Figure 3.

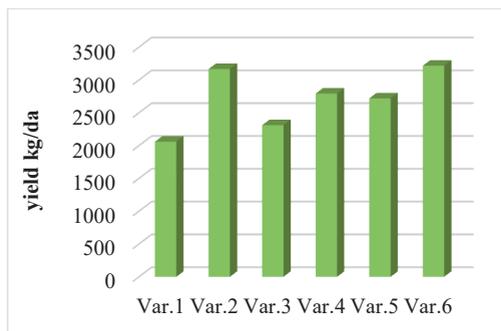


Figure 3. Average zucchini yield obtained by variants

The highest yield was reported in variant 6 - 3219 kg/da, followed by that in variant 2 with manure-3168 kg/da. The yield of zucchini fruits improves with the addition of BC, increasing with increasing of bio carbon application level. The increase in yield is probably due to the accumulation of nutrients and the improved microbiological activity of the soil that is associated with nitrogen fixation.

CONCLUSIONS

Based on the experiment the results demonstrate the positive effect of BC on soil organic matter content, water-physical properties and zucchini yield.

The soil and climatic conditions of the experimental year are favorable for the cultivation of zucchini. Uniform rainfall combined with a moderate irrigation rate is an indicator of optimum moisture content for their development.

For variants with higher BC content in combination with manure, humus content increases. The reduced content of total N in variant 3 probably is due to the adsorption of NH_3 or organic-N onto its surface by cationic or anion exchange.

The lowest NPK values were reported in variant 1 clear soil and variant 3 with only BC added. In the variants of BC, as the rate of imported carbonated plant residues increases, the NPK content also increases. The highest N content in the experimental variants is reported in variant 6 (manure 4 t/da + BV 750 kg/da), which is found to be optimal for the development of zucchini.

The obtained yield in the experiment exceeds the national average values, with the reported yield even in the control variant 1- 2031. 45 kg/da within the maximum yields reported

worldwide. The highest yield was reported for variant 6- 3219 kg/da, followed by variant 2 - 3168 kg/da.

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REFERENCES

- Cheng, C.H., Lehmann, J., & Engelhard, M.H. (2008). Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochimica et Cosmochimica Acta*, 72. 1598–1610.
- Gaskin G., Julia W., R.A. Speir, K. Harris, K.C. Das, R. D. Lee, A. Lawrence, S. Dwight, D. Fisher (2010). "Effect of Peanut Hull and Pine Chip Biochar on Soil Nutrients, Corn Nutrient Status, and Yield. *Agronomy Journal*, 102(2), 623–633.
- Glaser, B., J. Lehmann, W. Zech, (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal/VA review. *Biol. Fertil. Soils* 35:219Y230.
- Joseph, M., Lehmann, J., Solomon, O., Ngoze, D., Mugendi, N., James, M., Kinyangi, M., Riha, S., Verchot, L. John, W. (2008). Recha, and Alice N. Pell. "Reversibility of Soil Productivity Decline with Organic Matter of Differing Quality Along a Degradation Gradient. *Ecosystems*, 11.5, 726–39.
- Kasozi, G.N., Zimmerman, A.R., Nkedi-Kizza, P., Gao, M.B. (2010). Catechol and humic acid sorption onto a range of laboratory-produced black carbons (biochars). *Environ. Sci. Technol.*, 44. 6189–6195.
- Kimenov G., (1994). *Plant Physiology*. Univ. St. Cl. Ohridski, Sofia, 567 p.
- Kizito, S.; Luo, H.; Lu, J.; Bah, H.; Dong, R.; Wu, S. (2019). Role of Nutrient-Enriched Biochar as a Soil Amendment during Maize Growth: Exploring Practical Alternatives to Recycle Agricultural Residuals and to Reduce Chemical Fertilizer Demand. *Sustainability*, 11, 3211.
- Lehmann, J. (2007) Bio-energy in the black. *Frontiers in Ecology and the Environment*, 5, 381–387.
- Lentz, R., & Ippolito, J. (2012). Biochar and manure affect calcareous soil and corn silage nutrient concentrations and uptake. *Journal of Environmental Quality*, 41(4), 1033–1043.
- Steiner, C., et al. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil*, 291(1-2), 275–290.
- Takaya, C.A., Fletcher, L.A., Singh, S., Anyikude, K.U., Ross, A.B. (2016) Phosphate and ammonium sorption capacity of biochar and hydrochar from different wastes. *Chemosphere*, 145, 518–527.

- Xu Hui J., X. Wang, H. Yao, J. Su, Y. Zhu, (2014). Biochar Impacts Soil Microbial Community Composition and Nitrogen Cycling in an Acidic Soil Planted with Rape. *Environ. Sci. Technol.*, 48(16), 9391–9399.
- Zwieten L., S. KimberS, A. Downie, S. Morris, S. Petty, J. Rust, K. Y. Chan, (2010). A glasshouse study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. *Australian Journal of Soil Research*, 48, 569–576.
- Zwieten L., S. KimberS, A. Downie, S. Morris, S. Petty, J. Rust, K. Y. Chan, (2010). A glasshouse study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. *Australian Journal of Soil Research*, 48, 569–576.

THEORETICAL AND APPLIED BASIS FOR CREATION OF SPATIALLY-DIFFERENTIATED SYSTEMS OF SOIL QUALITY MANAGEMENT BASED ON SPACE SCANNING DATA

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Abstract

Global trends in agricultural production and environmental management require the mandatory use of geoinformation technologies and Earth remote sensing data as a basis for informational support for effective management of land and soil resources. Therefore, it is important to develop a methodology for using space scanning data to create spatially-differentiated soil quality management systems, which take into account the quantitative estimates of spatial soil variation and the results of pedotransfer modeling to evaluate soil quality and create optimal agrotechnologies. Experimental studies in different regions of Ukraine showed the effectiveness of the developed technology for soil decoding by multispectral space data with high spatial resolution to determine soils, which are different in complex assessment of soil physical quality for the diagnosis of especially valuable, optimal and modal parameters of soils, their degraded and underproductive kinds. Coherent analysis of the results of space image classification and geostatistical processing of soil field surveys is effective for a reasonable extrapolation of evaluation results at large territories and the development of agrotechnologies, which provide optimal conditions for development of agricultural plants and increase their productivity.

Key words: *geoinformation technologies, geostatistical processing, soil quality, remote sensing.*

INTRODUCTION

The need to integrate our country into the European systems of environmental monitoring and ecological land use, as well as the need to implement the sustainable development of market relations in the agrarian sector of Ukraine, require a development of the modern information support system for soil cover studies, especially in agricultural lands.

Every facet of the landscape can be accurately characterized by conventional soil survey but this is time-consuming, some areas are hard to reach and it is not practicable to analyse long-term soil change using field data alone. Remote sensing (RS) and geographic information systems (GIS) allow us to meet the challenge thanks to continual instrumental improvement and computerised image interpretation in GIS, elaboration of theoretical foundations for assessing the structure of soil cover and spatial heterogeneity, and application of geo-statistics to provide testable estimates of reliability (Goldshleger et al., 2010). In particular, high-resolution multispectral satellite imagery offers

objective data with the accuracy (geometric and geographic) and detail that we need to soil diagnose and map soil degradation. The use of high-resolution multispectral satellite images, which typically have geographical compliance, continuity, and are regularly updated, seems to be the promising approach to ensuring compliance to high requirements for the accuracy and impartiality of the data on the national soil resources. Additionally, satellite images, as up-to-date digital materials, in conjunction with modern GIS, provide means of precise determining the soil heterogeneity, both in detailed and large-scale surveys, which defines the basics of precision farming (Medvedev, 2007; Godwin et al., 2003; Havrankova et al., 2006; Mouazen et al., 2006; Medvedev et al., 2009).

Backed by numerous publications in many countries we can make a conclusion that precision agriculture is a very promising application of remote sensing methodology, not only as a new agricultural technology, but also for the formation of sustainable environments. The concept of precision agriculture is intended

to achieve a stability state of agrarian landscapes, risk minimization of incorrect agricultural activities (Mouazen et al., 2007). Precision farming involves calculation of the optimal parameters of agricultural activities, including the doses of fertilizers and methods of cultivation, which affect the overall environmental condition of soil and vegetation cover. Most of the parameters required for the selection of certain agricultural technologies are diagnosed by means of remote sensing. The task of remote sensing for precision agriculture is to identify similar parts of a field that require specific methods of cultivation or fertilization. Because precision farming involves restriction of fertilizers and other means of chemicals, it can be regarded as an admission of ecologisation, i.e. removal or prevention of negative effects of excessive treatment, pollution and other degradation processes on such a non-renewable resource as soil. The use of remote sensing methods is most important on the initial stage of elaboration of precision farming technologies in order to measure soil indicators (the main environmental constants). Additionally, the measuring point indicators based on remote sensing data allow to track a crop's status, i.e., to see whether diseases are developing, if the crop is suffering from water stress, nitrogen stress and so on. Soil physical measurements combined with soil analysis make it possible to precisely map agro-pedological conditions (Truskavetsky et al., 2015). The results of our researches have shown that integrated use of high quality satellite images can provide a necessary amount of numerical information for the correct recognition of soil-contours that have differences over the content of soil parameters, such as humus, agro physical soil indicators (Byndych, 2017). Cartographic materials obtained this way are used to determine quantitative estimates of soil anisotropy, and to development of optimal agricultural technologies. In our opinion, the above necessitates the use of a systematic approach for a land use optimization on arable land, which actualizes the need to development the methodological bases for a creation of spatially differentiated of soil quality management systems. Nowadays, in Ukraine there is no spatially differentiated soil quality management system

that will objectivize the evaluation of the quality of arable soils on the basis of taking into account updated data on the basic properties of arable soils, the latest methods of forecasting and modeling, the implementation of differentiated agrotechnologies taking into account zonal and regional peculiarities of soil heterogeneity. Significant disadvantages of existing approaches in the world is a limited number of evaluation criteria (Agricultural land classification survey of England and Wales, 1974), actually ignoring the most important factors of land productivity - moisture and heat supply, stocks of available nutrients (Petrasovits, 1978), the artificial nature of the division of criteria into basic and modification, unreasonableness of most correction coefficients, incorrect description of complex soil-crop system only with paired correlation analysis (Polupan et al., 2008), absence of production testing techniques (Methods of soil evaluation in Ukraine, 1992), as well as disregarding the heterogeneity of the soil cover (Glover et al., 2000; Granatstein, 2016).

MATERIALS AND METHODS

An practicing of a create a spatial-differentiated soil quality management system based on space scanning data was carried out on the example of the Korotych polygon. This polygon occupies 30 ha in Kharkiv Region. It lies within the Zolochiv-Chuguev physiogeography region of the Left-Bank Dnieper forest-steppe province of the Ukraine (Popov et al., 1968), which characterized by erosion-tree combinations of typical chernozems of deep middle and low humus and podzolized chernozem (Chernozem Chernic in WRB) and leachate chernozem (Chernozem Luvic in WRB).

We have tested modern technologies for creation of spatially-differentiated systems of soil quality management using Landsat 8 satellite data that provides digital images of the Earth's surface in the panchromatic (with a resolution of 15 m) and multispectral bands (with a resolution of 30 m) (<https://www.usgs.gov/land-resources/nli/landsat/landsat-8>). This imagery may be interpreted to monitor land use and crop rotation compliance, calculate the total area of crops, identify stages of crop development, detect soil erosion and salinity,

study natural conditions that affect agricultural activities (waterlogging, sharp changes in relief) and identify agricultural land that has suffered losses due to adverse natural conditions.

Imagery was acquired from the bare, dry soil surface (11.05.18). Research activities included: statistical analysis of the image, creation of a provisional soil map and system of soil sampling, field investigation of the soil pattern and laboratory analysis of soil samples, expert assessment of image complexity and analytical results as the basis for image classification and soil-cover models, derivation of mathematical models describing the relationship between optical characteristics of soil and other soil attributes, parameterization and geo-statistical analysis of the spatial variation of soil indicators, and extrapolation procedures based on interpretation of spectral signatures. A generalization of experimental data and calculations of soil, climate and land assessment, the fundamental and current value of arable soils were important parts of research.

With the aid of a GPS, a regular grid of elementary sites was established (one per 1 ha) for 35 soil sampling were collected from the 0-10 cm layer, and 3 soil pits were dug to characterise the soils (morphological structure of the soil profile, depth of humus profile, spatial configuration of plow layer) in the field. Samples were collected according to Soil Survey Standards of Ukraine (ISO 10694-1995, DSTU 4287:2004, DSTU 4728-2007, DSTU 4730-2007). Also in the field it was investigated the physical soil properties (density of structure, hardness - according to DSTU 5096:2008, specific resistance during plowing), the crop yield was recorded.

At the laboratory-analytical stage of the research, it was determined: granulometric composition of the soil (DSTU 4730:2007); total humus content (DSTU 4289: 2004); the content of mobile forms of phosphorus and potassium (DSTU 4115-2002; DSTU 4114-2001); pH (DSTU ISO 10390:2007) and structural-aggregate composition (DSTU 4744:2007).

Analytical data were compiled in a regional database. Statistical and data processing methods used GIS TNT-lite for geo-referencing of space image, NDVI calculation, primary image processing, transformation, general statistical analysis and image classification; and STATISTICA 10 for variance, correlation and regression analysis.

RESULTS AND DISCUSSIONS

Numerous studies have shown that an important feature of the soil cover is its heterogeneity, which is due to the heterogeneity of the terrain, parent rock, uneven fertilization and meliorants, tillage etc. (Dmitriev, 2001; Medvedev, 2007; Friedland, 1978; Wei Xue et al., 2019). As a result, the heterogeneity of soil physical, biological and agrochemical properties is observed within one field (Tittonell et al.). Therefore, taking into account the spatial heterogeneity of the soil cover is a necessary component for the optimal organization and use of agricultural land and the implementation of innovative agro-technologies for growing crops, which contribute to the conservation of soil from degradation and pollution, saving costs and obtaining competitive products.

Many works are devoted to the use of geoinformation technologies, which provide for mandatory use of space scanning data, as an objective information basis for determining the state of the Earth's surface, as well as geoinformation systems and to assess the spatial heterogeneity of arable soil properties.

Comprehensive analysis of theoretical bases for determining the soil cover heterogeneity and taking into account the specificity of space scanning data allows to group theoretical and methodological bases of the use of space imagery for spatial differentiation of a soil cover as a basic issue in the development of modern soil quality management systems (Figure 1).

The first group of methods consists generally scientific and classical research methods, which allow to process quantitative characteristics of a large number of studied objects and phenomena and to evaluate the reliability of the conclusions obtained. When using space survey data, researchers collide with an additional set of uncertainties of all kinds, which is related both to the multifactorial formation of the optical characteristics of the soil surface during survey and to the lack of knowledge of the nature of the relationship between soil properties and its spectral characteristics. In this regard, the fundamentals of probability theory, a statistical theory of pattern recognition and information theory are of particular importance.

A particular group of methods constitutes of multivariate statistical procedures (factor, discriminant and cluster analyzes) for interpreting

and classifying images during a thematic decryption, since space images are sensing in several bands of the spectrum. This group includes a geostatistica, which proves the regular nature of the variation and covariance of spatially distributed data.

The soil science foundations and methods are a separate group. For example, theories of soil cover organization and qualitative assessment of soil properties. For example, this group includes the complex of soil science techniques for using of space scanning data to determination of soil properties (total humus content in the arable layer, the content of fractions of physical clay, iron compounds), which determine the optical characteristics of the soil surface.

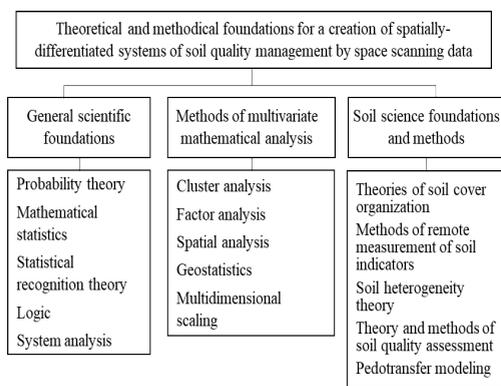


Figure 1. Scientific foundations for a creation of spatially-differentiated systems of soil quality management by space scanning data

A systematic approach to optimizing land use on arable land, which actualizes the need to develop methodological foundations for creating spatially-differentiated systems of soil quality management (Figure 2).

As a result of the research, a system of quality management of arable soils in Ukraine was developed, which represented a complex of interrelated methods, principles, and methods of purposeful impact on arable soils for their rational use, protection and increase of their fertility on the basis of detailed consideration of the regularities of spatial variation of soil properties, that was defined and evaluated based on thematic decryption of satellite imagery data. *The information unit* of the developed system is a set of information about the properties of arable soils from different sources, the use of

which allows to evaluate the quality of the soil. In this case, the main source of information for the spatial differentiation of the soil cover on arable agricultural lands is data of multispectral space scanning of high spatial resolution. The results of contour decryption of space images are supplemented by data on soil sample surveys, determination of the main climatic indicators (by administrative areas), yield data of major crops and the results of scientific research using pedotransfer modeling, that allows to obtain the required amount of data on the main agro-physical indicators.

The analytical evaluation unit includes the assessment of the quality of arable soils by updated data to establish the parameters of particularly valuable soils, determining the area of degraded and unproductive soils. At this stage there are estimates by the improved soil evaluation method and the highly cultivated and low fertile soils are identified, their ecological functions are evaluated and their evaluation changes as a result of increasing the intensity of cultivation or availability of land degradation are predicted. Based on the results of the evaluation, zonal and regional features are established to justify the transition from zonal to spatially differentiated agro-technologies by the set of physical, chemical and agrochemical properties of soils.

The management unit provides the implementation of space-differentiated agro-technologies taking into account the local heterogeneity of the soil cover, as well as the determination of such economic estimates as the value of arable soils and their agro-investment attractiveness, providing land users with sufficient information to reduce the risk from attracting investment in the development of the country's agricultural sector. This unit also provides for the legal and regulatory support of the functioning of the developed system for the possibility of its implementation in the field of land tenure and the development of measures to increase the level of fertility, rational use and protection of arable soils of the country. In addition, organizational and coordination activities require mandatory background (reference) and production monitoring of soils by a broad program of indicator parameters to control the quality of arable land in the country.

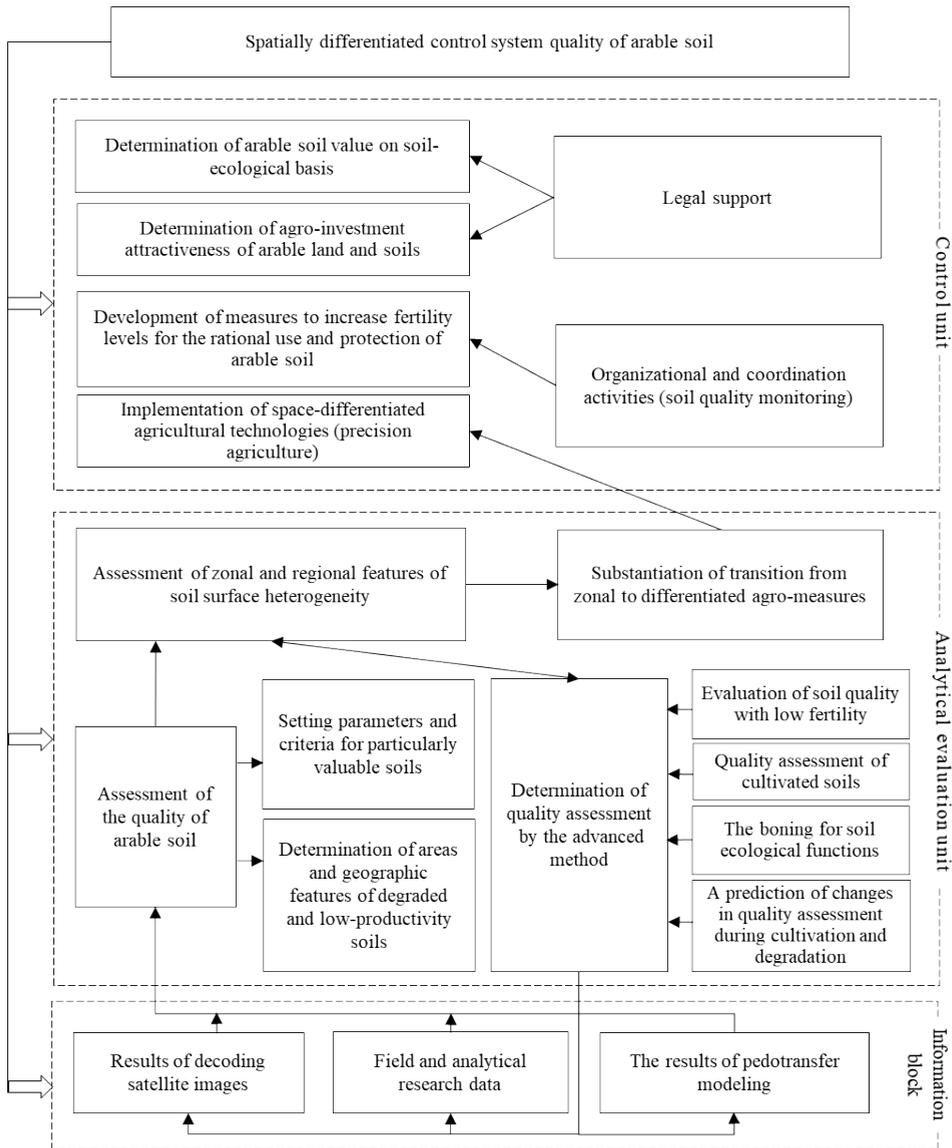


Figure 2. Scheme of spatial-differentiated quality management system of arable soil

The proposed system was elaborated taking into account the heterogeneity of the basic properties of arable soils in different natural and climatic zones of Ukraine. Here is an example of its use for Korotych test site. According to the results of contour decryption of Landsat-8 satellite data, three soil contours were identified within the test site, which presented varieties of dark gray podzolized soil according to the national classification of soils, which corresponds to the Haplic phaeozem according to the WRB classification (Figure 3).

The provisional soil map was created in the final stage of image processing by the K-means method of cluster analysis. This map was the basis for soil sampling and positioning of soil pits for field observations. Further, we observed correspondence between soil delineations and individual elements of the micro-relief so the map reflects a certain orderliness of the soil cover; its boundaries separate soil bodies with specific internal structure and variability characteristics.

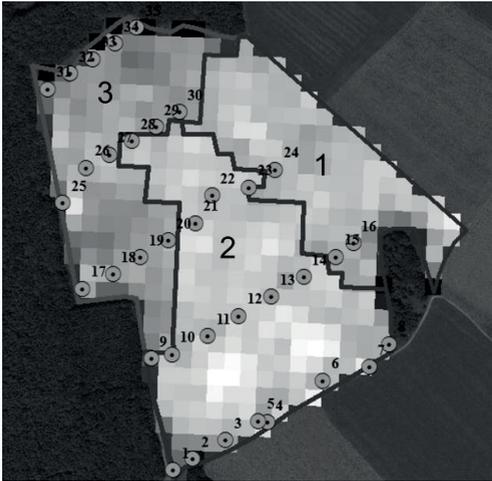


Figure 3. Soil map of the Korotych polygon, which derived from classification of Landsat-8 satellite data:



- the soil sampling location;

- 1 - conformed with dark gray podzolized soil on loess-like parent material (Haplic Phaeozems in WRB);
- 2 - conformed with dark gray podzolized soil on loess-like parent material, lightly eroded (Phaeozems Turbik in WRB);
- 3 - conformed with dark gray podzolized soil on loess-like parent material (Phaeozems Huperhumic Lamellic in WRB)

Following the field examination, analytical determinations of soil properties were conducted. The results, compiled in the regional database, revealed correlation between the brightness of multispectral image in different bands and various soil attributes including humus content and soil texture, for the infra-red range, correlation coefficients (r^2) between the brightness of the soil surface and total humus content and clay content were -0.78 and -0.69, respectively.

The taxonomic units represented by the mapping units are listed below, according to the Ukrainian soil nomenclature and the World Reference Base (WRB), using the scheme for harmonisation drawn up by Medvedev and others (2003).

The digital map was then used to analyse the structure of soil indicators (granulometric

composition of the soil, total humus content, the content of mobile forms of phosphorus and potassium, pH and structural-aggregate composition): visual field analysis and testing stationarity of the expected value; smoothing functions, determination of the nature of a trend and random function approximation; finding an analytical expression of regionally-correlated component and removing the trend; correlation analysis and identification of significant periodic components; analysis of the spectral density of the dispersion; verification of the allocation and estimates of the indicator distribution parameters.

Research has shown that certain soil contours are different in quality characteristics of basic physical properties. For each part of the test site, there were developed recommendations for the use of different intensities of pre-sowing and basic tillage, namely: a - no-till (the parameters are close to the requirements of the crops); b - with moderate tillage of zonal type (parameters close to modal values); c - with high intensity tillage (the parameters are unsatisfactory and more intensive pre-sowing of the soil is required).

According to the results of geostatistical analysis of data of ground-based tests of soils, which proves the presence of a regular component in the spatial variation of the investigated soil properties, the conclusion was made about the correctness of the map model created by the data of the space scanning of the local structure of the soil cover of the test site and the expediency of its implementation within precision tillage.

Statistical and geostatistical parameters of the investigated soil properties are given in Table 1. It was established that by 27% from the total area of the test site the density of the structure exceeded the value of 1.30 g/cm^3 , which was an obstruction for quality soil tillage and germination of agricultural crops. Localization of these parts of the test site is the basis for differentiated tillage in the form of additional tilling, the rest of the area does not require additional tillage.

Table 1. Statistical and geostatistical indicators of some properties of dark gray podzolized heavy loamy soil (Korotych test site)

Indicator	The bulk density in the sowing layer of soil, g/cm ³	Hardness in the layer of 0-10 cm, kgf/cm ²	Content of mobile forms of nutrients in the soil		
			N	P ₂ O ₅	K ₂ O
Swing range	0.47	9	2.2	62.4	55.6
Average value	1.31	27	1.1	15.6	20.0
Median	1.31	27	1.1	11.1	16.0
The coefficient of variation	0.08	0.09	0.42	0.81	0.59
Dispersion	0.01	5.9	0.21	158.6	140.4
Asymmetry coefficient	0.43	0.2	0.54	2.74	2.11
Nougat effect	0.0004	0	0	0	0
The dispersion threshold	0.004	-	0.23	73	41
Correlation radius, m	300	-	300	280	230

Most of the test site area has a hardness in the sowing layer of more than 20 kgf/cm², which prevents the germination of most cereals. Hardness in the plow sole reaches quite high values (30-40 kg/cm² and above). Due to the fact that these indicators do not have a solid configuration, deep tillage also requires differentiation.

It was established that the range of observed agrochemical parameters was quite clearly divided, in particular, had three classes and, accordingly, contours with three levels of supply of mobile nutrients: 1 - close to optimal, 2 - above the optimum level; 3 - level with a very low content. It was established that 2/3 of the test site can be classified as the 1st class, 15% - as the 2nd class, about 23% - to the 3rd class, which substantiates practical proposals for differentiation of doses of phosphate fertilizers. Moreover, it is possible to achieve regulatory returns on fertilizers and at the same time reduce their total application rate by saving fertilizers on sites of the area with the high availability.

The heterogeneity of the investigated soil properties indirectly testifies to the prospect of implementation of precision agriculture elements at the investigated test site. It is established that the higher the variability of soil properties, the more it is necessary to study it and identify spatial patterns, which allows to justify the boundaries between sites with different levels of fertility and to obtain economically justified results in the implementation of spatially differentiated agro-measures. In the course of the research, the evaluation of the qualitative state of the investigated soils of the test site was carried out according to an improved method of assessing the quality of

arable soil, which was developed by NSC ISSAR (Medvedev and Plisko, 2006).

Figure 4 shows the geostatistical indicators of spatial heterogeneity of the structural composition (for example, a fraction of 0.25-10 mm in size) of the Korotych test site.

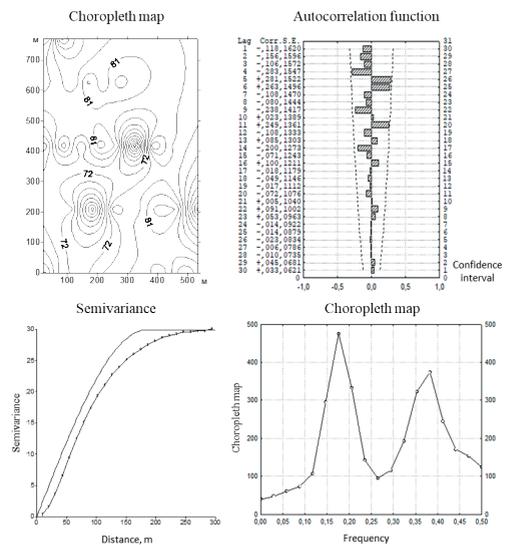


Figure 4. Spatial heterogeneity of the content of an agronomically valuable fraction of a structure in size 0.25-10 mm (Korotych test site)

The methodology allows to evaluate the productive and ecological functions of soils and has advantages over existing analogues due to: the use of an extended number of soil, climatic criteria and technological characteristics of the land site (field); distribution of soil variety as elementary spatial unit instead of soil agro-group; a unified 100-point country-wide rating

scale instead of country-specific assessment scales; a new way of calculating points - as the mean geometric ratio of real and optimal parameters. It was established that the soil quality points of the studied soils ranged from 39 to 51. At the same time, the highest points had dark gray podzolized soil, the score points of lightly washed kind were lower by 7 points compared to its full-profile counterpart.

The agroinvestment attractiveness of arable land of the test site for growing crops on the basis of an integrated assessment of soil quality, climatic and agricultural production of the land (test site) was determined. It was established that soils of the test site in terms of soil and climate properties can be considered attractive for investing and growing of agricultural crops.

CONCLUSIONS

In the course of the research, a spatially differentiated system of arable soil quality management was developed, which represents a complex of interrelated methods and techniques, principles, processes of purposeful influence on arable soils for their rational use, protection and increasing of their fertility level on the basis of taking into account the heterogeneity of the soil cover.

A coherent analysis of satellite images decoding results and data obtained from field surveys in the Forest-Steppe region of Ukraine proved the soil decoding technology for multispectral satellite imagery data in the optical range, developed herein, to be highly effective for determining the elements of soil cover heterogeneity, which vary in the vertical soil structure;

According to the results of regional studies, the coherent analysis of a satellite image and its classification results, as well as the autocorrelation analysis of the soil indicators of land survey data, is proven to be informative enough to identify soil variations to the development of spatially - differentiated soil quality management systems.

Directions for further research include:

- Systematization of equations which explain the relationship of optical properties of the soil surface with fundamental physical and chemical properties, so as to make real and useful differentiation of soil quality from satellite imagery.

- Methods, techniques and rules for the use of remote sensing data for diagnosis and quantitative assessment and mapping of the soil cover in our region.

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REFERENCES

- Byndych, T. (2017). Using Multispectral Satellite Imagery for Parameterisation of Eroded Chernozem. *Soil Science Working for a Living: Applications of soil science to present-day problems*, Part II, 57–65.
- Dmitriev, E.A. (2001). A chapter from the unfinished book "Soil heterogeneity". *Large-scale effects in soil research*, Moscow: Moscow State University, 8–39.
- Fridland, V.M. (1972). Soil cover structure. Moscow: Thought, 423 s.
- Glover, J., Reganold, J., Andrews, P. (2000). Systematic method for rating soil quality of conventional, organic and integrated apple orchards in Washington State. *Agriculture, Ecosystems, and Environ.*, 80, 29–45.
- Godwin, R.J., Wood, G.A., Taylor, J.C., Knight, S.M., Welsh, J.P. (2003). Precision Farming of Cereal Crops: a Review of a Six Year Experiment to develop Management Guidelines. *Biosystems Engineering*, 84(4), 375–391.
- Goldshleger, N., Ben-Dor, E., Lugassi, R., Eshel, G. (2010). Soil degradation monitoring by remote sensing: examples with three degradation processes. *Soil Science Society of America Journal*, 74(5), 1433–1445.
- Granatstein, D. (2016). Measuring soil quality: indicators of soil quality-developing a quantitative index. *Tilth Producers Quarterly*, URL: https://www.researchgate.net/publication/237611194_Measuring_Soil_Quality_Indicators_of_Soil_Quality_Developing_a_Quantitative_Index (date of the application: 05.03.2017).
- Havrankova, J., Godwin, R.J., Wood, G.A. (2006). Ground Remote Sensing Systems for Determining Canopy Nitrogen in Winter Wheat. *International Soil Tillage Research 17th Triennial Conference*, Kiel, Germany, 910–915.
- Medvedev, V.V., Plisko, I.V. (2006). *Valuation and qualitative assessment of arable land in Ukraine*. Kharkov: 13th Printing House, 386.

- Medvedev, V.V., Laktionova, T.M., Kanash, O.P. (2003). *Soils of Ukraine (genesis and agronomic characteristics)*. Kharkiv, 68 s.
- Mouazen, A.M., Malaki, M.R., Merckx, R., De Baerdemaeker, J., Ramon, H. (2006). Primary results on on-line measurement of some selected soil properties using a VIS-NIS sensor. International Soil Tillage Research Organization, 17th Triennial Conference, Kiel, Germany, 839–844.
- Mouazen, A.M., Maleki, M.R., De Baerdemaeker, J., Ramon, H. (2007). On-line measurement of some selected soil properties using a VIS-NIR sensor. *Soil & Tillage Research*, 93(1), 13–27.
- Petrasovits, I. (1978). A foldhasznalat es a melioracio osszefugeseirol. *Gazdalkodas. Evf.* 22, Sz. 8, 9–16.
- Polupan, M.I., Velychko, V.A., Solovey, V.B. (2008). Fertility of soils and soil cover of the Steppe of the Southern and Dry according to agricultural potentials of agricultural crops and bonus criteria. *Bulletin of Agrarian Science*, 2, 18–25.
- Popov, V.P., Marynich, A.M., Lan'ko, A.I. (1968). *Physical geographic regions of the Ukrainian SSR*. Kiev University Publishing House, Kiev.
- Tittonell, P., Muriuki, A., Klapwijk, C.J., Shepherd, K.D., Vanlauwe, B., Coe, R.D. (2020). Soil heterogeneity and soil fertility gradients in smallholder farms of the East African highlands. <http://worldagroforestry.org/publication/soil-heterogeneity-and-soil-fertility> (date of the application 05.02.2020).
- Truskavetsky, S., Byndych, T., Sherstyuk, A., Viatkin, K. (2015). Studying the condition of soil protection Agrolandscape in Ukraine using Remote Sensing Methods. *Journal of Agricultural Science and Technology A.*, 5(4), 235–240.
- Wei Xue, T., Martijn Bezemer & Frank Berendse (2019). Soil heterogeneity and plant species diversity in experimental grassland communities: contrasting effects of soil nutrients and pH at different spatial scales. *Plant and Soil*, 442, 497–509.
- ***Agricultural land classification survey of England and Wales (1974). Ministry of Agr., Fisheries and Food. *Press Notice*, 195(25), 1–3.
- ***Methods of appraisal of Ukrainian soils (1992). L.Ya. Novakovsky, Kiev, 102 s.
- ***Soil heterogeneity and precision farming. Part 2 (2009). Research results/Medvedev, V.V., Melnik, A.I., Plisko, I.V., Shevchuk, M.Y., Zinchuk, N.I., Pashchenko, V.F., Dorozhko, I.N., Onishko, M.I., Dontsova, L.V., Zavadsky, A.M.; under the editorship of Medvedev, V.V., Kharkov: KP "City Printing House", 260 s.
- ***<https://www.usgs.gov/land-resources/nli/landsat/landsat-8> (date of the application: 16.04.2018).

EFFECT OF LOW RATES OF MINERAL FERTILIZERS ON THE PRODUCTIVITY OF DURUM WHEAT (*Triticum durum* Desf.)

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Abstract

The aim of the study was to determine the effect of nitrogen and phosphorus fertilization applied at low rates on grain yield and some physical and chemical parameters for the durum wheat variety Progress. Nitrogen and phosphorus fertilizers were applied given in doses of 0, 40 and 80 kg/ha alone and in various combinations. A stationary trial with fertilization has been carried out since 1966 in the experimental field of Field Crops Institute in town of Chirpan, Bulgaria. The study included 2016 and 2017 crop years. It was found that the fertilizer rate increase did not necessarily lead to an increase in the studied parameters. The highest values were reported as follows: grain yield at $N_{80}P_{80}$; thousand-kernel weight at N_{40} ; test weight at $N_{40}P_{40}$; vitreous of grain at N_{80} ; protein content at N_{80} and gluten at $N_{80}P_{40}$. Correlation analysis showed the strongest and most significant correlations between protein content and gluten (0.906**).

Key words: fertilization, grain yield, physical and chemical parameters, *Triticum durum*.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. *durum*) is the second most important wheat species grown in the world next to bread wheat (*Triticum aestivum* L.) (Gerba et al., 2013). In Bulgaria durum wheat is a traditional culture. The two top producing countries are Turkey and Canada with estimated 2 million ha each (USDA, 2015; Statistics Canada, 2017), followed by Algeria, Italy and India, each cultivating over 1.5 million ha (Nagarajan, 2006; Le Lamer & Rousellin, 2011).

Considering that world population is increasing day by day on one hand, and that the fields used for agriculture have reached its limits on the other hand, it is coming to light that increase of yield is required to continue also in the future (Yildiz & Beyaz, 2019). Low soil fertility is one of the constraints in durum wheat production (Teklu & Hailemariam, 2009). In order to increase soil fertility in the short run, nutrient have to be added to the soil (Getinet & Wassie, 2019). The use of nitrogen is normally considered a key factor in cereal crops and numerous studies on the best N fertilization rates (Rossini et al., 2018). Current research efforts, however, are trying to reduce the use of N fertilizer to avoid unfavorable environmental consequence due to its losses without affecting

crop productivity (Ali et al., 2019). Moreover, the soil is nature resource whose exploitation should be seen through a conservative wise-use approach limiting all forms of degradation (Neffar et al., 2014). According to FAO data, global demand for mineral fertilizers increases every year. Chemical fertilizers, however, are expensive to purchase and for most small-scale farmers this is a problem (Gete et al., 2010; Getachew & Tilahum, 2017). On the other hand a high quality standard could be guaranteed with an increase in N input at rates often double those required to maximize grain yield (Gariddo-Lestache et al., 2005), but with a risk for the environment (i.e., nitrate pollution) (Ercoli et al., 2013).

Contrary to N, P is reasonable abundant in the Earth's crust (1.2 g kg⁻¹ on average) (Hinsinger et al., 2011). However, with the formation of soil and weathering, the total P content vents with time and the content of organic P accumulates at the expense of inorganic (Richardson et al., 2004). However that Otiz-Monasterio et al. (2002) reported that phosphorus is the second most widely occurring nutrient deficiency in cereal system around the world, and Kizilgoz & Sakin (2010) defined phosphorus as a third most abundant macronutrients in plants after nitrogen and potassium, P is undoubtedly one of the main nutrients. Therefore, it is expected high-grade

phosphate rocks are definitely expected to be exhausted within the next decades (Cordell et al., 2009), which calls in to question the sustainability of current P fertilizer use in developed and emerging countries (Hinsinger et al., 2011).

It is well known, that the conciliation of performance improvement of wheat crops depends on maintaining the stock of nutrients in soil, which is essential for plant growth (Haung et al., 2005; Casado-Vela et al., 2006; Chennafi et al., 2011). Determining the optimum rate of N and P fertilizer rate is the key to maximize the economic yields (Dugassa et al., 2019). This requires detailed research on the impact of mineral fertilizers. The aim of our study was to determine the effect of nitrogen and phosphorous fertilizers applied alone and in various combinations at low rates on grain yield and some physical and chemical parameters.

MATERIALS AND METHODS

The experiment was performed of the Field Crops Institute in Chirpan, Bulgaria (42°11'58"N, 25°19'27"E). The experiment was a stationary fertilizer trial set up in 1966. The

study included 2016 and 2017 crop years. The trial was conducted by the randomized complete block design in four replications in two-field crop system rotation of durum wheat and cotton in the experimental field of the Institute in Chirpan on soil type Pelic Vertisols (Kirchev et al., 2017).

Three N and P (alone) rates were applied - 0, 40 and 80 kg/ha and four NP fertilization combinations: N₄₀P₄₀; N₄₀P₈₀; N₈₀P₄₀ and N₈₀P₈₀ (kg/ha). N₀P₀ was taken as a control variant. The phosphorus fertilizer was incorporated during autumn with the deep plowing, the nitrogen in early spring at tillering stage of durum wheat.

The following traits were examined: grain yield (kg/ha); test weight (kg/hl); thousand-kernel weight (g); vitreous of grain (%), protein content (%) and gluten (%) of durum wheat.

According to Figure 1 precipitations were very unevenly distributed. In the harvest 2015/2016, the amount of precipitation in May was higher than in 2016/2017 and the multi-year period, and in June 2016 it was significantly less. In terms of temperature, the first harvest year was characterized by a mild winter and a warm spring. In the spring of 2017 the temperature curve was close to that of the climate norm.

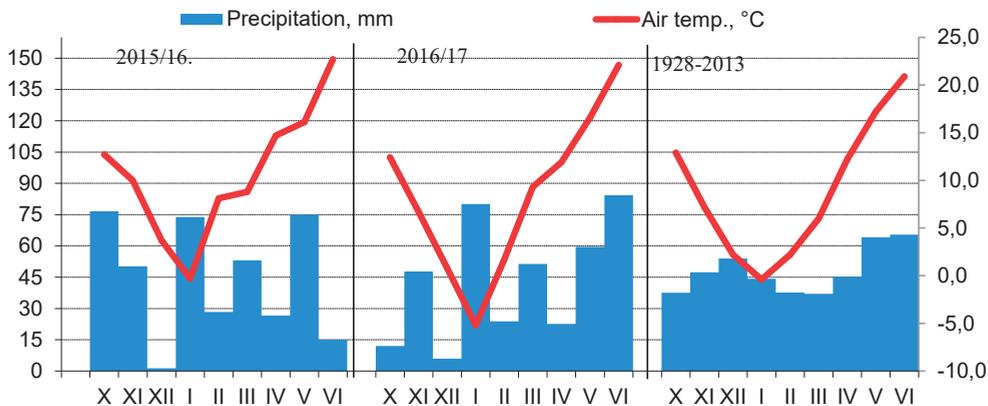


Figure 1. Precipitation and temperature security during the durum wheat vegetation period

Variance (ANOVA) analysis was used to determine the significance of differences between the studied variants. Correlation relationships were established by applying the Statistics 13.0 software (TIBCO, Software, 2018).

Abbreviations: GY - grain yield; TW - test weight; TKW - thousand-kernel weight; GV - vitreous of grain; CP - protein content; G - gluten.

RESULTS AND DISCUSSION

Data in Table 1 showed that in 2016 GY was lower than in 2017. The lowest value in 2016 was observed for the variant without fertilization - 935.0 kg/ha. Nitrogen fertilization significantly

increased the GY. With increasing the fertilizer rates GY values also increased. Of N₄₀ was reported GY 1,737.5 kg/ha. When raised to N₈₀, GY increased by 162.6% compared to the untreated plot, reaching 2,455.0 kg/ha. The results of P fertilization showed little effect.

Table 1. Grain yield, kg/ha - 2016, 2017 and average

Fertilization rates, kg/ha	2016		2017		Average	
	kg/ha	% St	kg/ha	% St	kg/ha	% St
N ₀ P ₀	935.0	100.0	3,255.3	100.0	2,095.2	100.0
N ₄₀	1,737.5***	185.9	3,813.8**	117.2	2,775.7	132.5
N ₈₀	2,455.0***	262.6	4,624.0***	142.1	3,539.5	168.9
P ₄₀	1,005.0 ^{ns}	107.5	2,320.3 ^{ns}	71.3	1,662.7	79.4
P ₈₀	1,067.5 ^{ns}	114.2	2,130.0 ^{ns}	65.4	1,598.8	76.3
N ₄₀ P ₄₀	1,845.0***	197.3	4,258.8***	130.8	3,051.9	145.7
N ₄₀ P ₈₀	2,045.0***	218.7	3,783.8**	116.2	2,914.4	139.1
N ₈₀ P ₄₀	2,485.0***	265.8	5,046.3***	155.0	3,765.7	179.7
N ₈₀ P ₈₀	2,607.5***	278.9	5,083.8***	156.2	3,845.7	183.6
<i>LSD</i>	5%	337.4	36.1	371.7	11.4	-
	1%	455.6	48.7	501.9	15.4	-
	0.1%	606.8	64.9	668.3	20.5	-

ns - no significant; *, **, ***significant at P = 5%, P = 1% and P = 0.1%

Attached low rate P₄₀ exceeded the control plot by 7.5%. The P₈₀ rate showed a slight increase in the value of the studied trait - by 14.2% above the control variant. Following these results, the differences were not be proved statistically.

Although self-P fertilization had no demonstrated effect in combination with N showed a good effect. The impact of N₄₀P₄₀ and N₄₀P₈₀ on GY statistically was confirmed in the highest degree. At fertilization with N₄₀P₄₀ GY was 1,845.0 kg/ha, and with an increase in the P rate (N₄₀P₈₀) GY increased by 200 kg/ha compared to the previous variant - 2,045.0 kg/ha.

At N₈₀P₄₀ GY was 2,485.0 kg/ha (165.8% over the control plot). It should be noted that the difference with N₈₀ was only (30 kg/ha) i.e. 3.2%. This slight difference could not be taken as an argument for the imported P for the conditions of this year. At fertilization with N₈₀P₈₀ GY was 2,607.5 kg/ha, which was the highest GY reported in 2016. High values were statistically confirmed with high confidence.

Large amount of rainfall during the flowering (Figure 1) led to abnormal pollination of the flowers, which might explain the low GY in 2016. Kolev & Tahsin (2010) observed the same situation in a study with another cereal crop – triticale.

GY in 2017 without fertilizers was 3,255.3 kg/ha. Alone-application of N fertilization led to an increased in values. Of N₄₀ GY of 3,813.8

kg/ha was reported. The GY was superior to control plot by 17.2%. The analysis of variance showed a high confidence for the effect of the N₈₀ rate by raising the GY by 42.1% (4,624 kg/ha) over the non-fertilizing variant.

The lowest values were reported from P fertilization. At P₄₀ GY was 2,320.3 kg/ha and when the rate increased to P₈₀ the value of the trait decreased to 2,130.0 kg/ha. Both variants had a lower GY compared to the control plot, by 28.7% and 34.6%, respectively.

The combined fertilization N₄₀P₄₀ showed a higher GY of 4,258.8 kg/ha compared to N₄₀P₈₀ - 3,783.8 kg/ha. As a consequence, the N₄₀P₄₀ variant has a significantly higher statistical impact than the N₄₀P₈₀.

From the combined fertilization N₈₀P₄₀ and N₈₀P₈₀ similar results were reported for the GY achieving 5,046.3 kg/ha (155.0%) and 5,083.8 (156.2%) kg/ha. Maximum GY in 2017 was reported at N₈₀P₈₀. However, the 1.2% difference from the N₈₀P₄₀ variant did not justify its application.

The insufficient effect of P fertilization was due to the weather conditions in 2017. In October, when the P fertilizer was incorporated, the amount of rainfall was low, which prevented its absorption. As reported by Fricke et al. (1997) under soil moisture deficiency, nitrogen uptake from the roots was limited. It could be said that

this effect was also valid for the other mineral fertilizers. Due to the large differences in GY during the two years of study statistical data processing was not carried out. However, the average data showed that the incorporated P had low effect. The P₄₀ showed lower GY by 20.6% than the control. At the P₈₀ rate GY was by 23.7% less than the variant without fertilizer. In contrast to our results, François et al. (2009) found higher but statistically unreliable values of GY under the impact of P. Grant and Bailey (1998) also reported an increase in GY under the impact of P, although in small quantities, even when the rate was increased.

The averaged data showed that N fertilization increased GY. With the application of N₄₀ by 32.5% more grain was obtained compared to control plot. Higher than the N₈₀ rate had a greater impact raising GY by 68.9% compared to without fertilizing. López-Bellido & López-Bellido (2001) confirmed an increase in GY due

to an increase in the N rate (N₅₀ - 2,548 kg/ha and N₁₀₀ - 2,929 kg/ha). These results, however, contradicted the data of Ali et al. (2019), who find that the rate increased in the range 0, 30, 60 kg N/ha, grain yield decreased (4.4, 4.5, 4.1 t/ha - 4,400, 4,500, 4,100 kg/ha).

From the combined fertilization with N₄₀P₄₀ and N₄₀P₈₀ GY was lower than the single N fertilization at a dose of 80 kg/ha. The difference between N₈₀ and N₄₀P₄₀ was 23.2% and the difference between N₈₀ and N₄₀P₈₀ was 29.8%. Fertilization with N₈₀P₄₀ GY was 3,765.7 kg/ha. This value exceeded the control plot by 79.7%. The highest GY reported for the average of the study was fertilization with N₈₀P₈₀ - 3,845.7 kg/ha, which was 83.6% above the non-fertilization variant. The effect of this fertilizer combination was confirmed in the study performed by Panayotova et al. (2018).

Table 2 presents the averaged values of the TW, TKW and GV grain properties.

Table 2. Test weight (kg/hl), thousand-kernel weight (g) and vitreous of grain (%) average for the period

Fertilization rates, kg/ha	TKW, g	% St	TW, kg/hl	% St	GV, %	% St
N ₀ P ₀	58.70	100.00	77.28	100.00	60.4	100.0
N ₄₀	62.08***	105.76	80.75***	104.49	68.4*	113.3
N ₈₀	60.60*	103.24	80.50***	104.17	72.5**	120.0
P ₄₀	59.30 ^{ns}	101.02	80.43***	104.08	67.4*	111.6
P ₈₀	58.38 ^{ns}	99.46	80.35***	103.97	68.2*	112.9
N ₄₀ P ₄₀	61.28**	104.40	80.95***	104.75	69.9**	115.7
N ₄₀ P ₈₀	60.75**	103.49	80.88***	104.66	70.4**	116.6
N ₈₀ P ₄₀	61.00**	103.92	80.73***	104.46	71.1**	117.7
N ₈₀ P ₈₀	60.23*	102.61	80.63***	104.34	72.4**	119.9
LSD	5%	1.35	0.60	0.78	6.8	11.3
	1%	1.94	0.86	1.11	9.4	15.6
	0.1%	2.85	1.27	1.64	12.7	21.0

ns - no significant; *, **, ***significant at P = 5%, P = 1% and P = 0.1%

Average for the two years of the study lowest TKW was under the control variant - 58.70 g. Nitrogen fertilization had a positive effect. However, the lower rate of N₄₀ (62.08 g) showed a better result than N₈₀ (60.60 g). The increasing of 5.76% over the control plot indicated that the N₄₀ variant had the highest TKW and statistically significant effect of the highest degree. This result was at odds with that found by Woyema et al. (2012) and Iancu et al. (2019), and in both studies it was reported that when the N rate increased, TKW values also increased. The incorporation of P reduced the values of TKW. Increasing the rate had an adverse effect. At P₄₀ fertilization values of 59.30 g were

reported. Although the variant was superior in value this without fertilization by 1.02%, the difference was not statistically significant. At increase to P₈₀ TKW was inferior to the control. The combination with low rates of fertilization (N₄₀P₄₀) showed TKW of 61.28 g, which was 4.40% more than without fertilization. As P, increased the value of the trait decreased (N₄₀P₈₀ - 60.75 g). Analysis of variance showed an average degree of impact of the factor in both variants.

The combination of a higher N rate and a low P (N₈₀P₄₀) increased the TKW value by 3.92% of the control plot. But with the increasing of P again negative effect was observed. Fertilization with N₈₀P₈₀ had a value of TKW 60.23 g, which

exceeded the variant without fertilization by 2.61%.

The average data from the two years of study of Progress durum wheat showed high TW values. Without fertilization 77.28 kg/hl was reported. Application of N₄₀ lead to an increase of 4.49% (80.75 kg/hl) compared to the control plot. However, when the rate was raised to N₈₀ the value of the trait decreased to 80.50 kg/hl which was 4.17% compared to the control plot. Woyema et al. (2012) observed the same trend. Unlike our study, Ali et al. (2019) reported the same TW for the non-fertilizing variant and N₃₀, and when the norm increased to N₆₀ the value decreased.

The lowest TW values for all variants tested were observed for P fertilization. At a rate of P₄₀ TW was 80.43 kg/hl or 4.08% above the control plot. Again, when the rate was raised, the value of the trait decreased - P₈₀ - 80.35 kg/hl.

The combined fertilization showed the same trend. The highest TW value over the study period was reported at N₄₀P₄₀ - 80.95 kg/hl. As the rates of combined fertilization increased, TW decreased. Thus, the lowest value for combined fertilization was observed at the highest rates - N₈₀P₈₀ - 80.63 kg/hl. Contrary to our results was the report of Makowska et al. (2008), where as the fertilizer rate increased, the test weight was increased.

The results for GV showed that the lowest value was reported from the version without fertilizer -60.4%. The application of 40 kg N/ha increased GV by 13.3% and N₈₀ by 20.0%, respectively, against the control plot. The resulting value of 72.5% GV from the N₈₀ was the highest of the variants considered. However, the difference was not enough for a statistically significant influence of the highest rank. The increase of GV with the increase of N was confirmed by the study of Campiglia et al. (2014).

P fertilization showed a slight increase in GV. At P₄₀, GV was reported 67.4% and at 80 kg P/ha 68.2% or 12.9% above the control plot.

Fertilization with N and P led to a consistent increase with increasing fertilizer rate. Therefore, the lowest combination was the lowest value - N₄₀P₄₀ - 69.9%. The highest GV was observed the N₈₀P₈₀ variant - 72.4%. Although this was the highest GV at combination fertilization, it should be noted that GV had a similar value when applied N at a rate

of 80 kg N/ha, although with a minimum difference of 0.1%. Compared to our study, Garrido-Lestache et al. (2005) also reported higher values of this property under the impact of self-fertilizing N in comparison with the combined NP fertilization.

Protein content and gluten strength are considered the most important features grain qualities needed for use in pasta (Rossini et al., 2018). In our study without fertilization and with fertilization P₈₀ the lowest PC - 12.0% were accounted (Table 3).

Table 3. Protein content (%) and gluten (%) average for the test period

Fertilization rates, kg/ha	Average		Average		
	PC, %	% St	G, %	% St	
N ₀ P ₀	12.0	100.00	22.1	100.00	
N ₄₀	13.2*	110.0	25.8***	116.7	
N ₈₀	15.1***	125.8	30.5***	138.0	
P ₄₀	13.3**	110.8	25.5***	115.4	
P ₈₀	12.0 ^{ns}	100.0	23.5*	106.3	
N ₄₀ P ₄₀	13.4**	111.7	25.2***	114.0	
N ₄₀ P ₈₀	13.4**	111.7	24.7***	111.8	
N ₈₀ P ₄₀	14.6***	121.7	30.7***	138.9	
N ₈₀ P ₈₀	14.1***	117.5	28.0***	126.7	
GV	5%	1.0	8.4	1.3	5.9
	1%	1.3	10.8	1.8	8.2
	0.1%	1.8	15.0	2.5	11.3

ns - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%

N fertilization had a positive effect on PC. Similar results were reported by Ames et al. (2003). Several studies showed that application a significant increase in protein content (Lerner et al., 2006; Makowska et al., 2008). The highest content of 15.1% PC was observed when fertilizing with N₈₀. This variant showed high statistical significance of influence. Ierna et al. (2015) confirmed the positive effect of N fertilizers when the rate was raising. The combinations N₈₀P₄₀ (14.6%) and N₈₀P₈₀ (14.1%) had the same effect. Average for the years of studying 22.1% G was reported from the variant without fertilization. A minimal increase was observed at P₈₀, by 6.3% above the control. This rate remained statistically the least reliable for influence. Fertilization N₈₀P₄₀ showed the largest G - 30.7%. However, the difference with the application of N₈₀ was minimal G - 0.2%. The increase at the low rates of combined fertilization was also small - N₄₀P₄₀ - 25.2% G. Gerba et al. (2013) confirmed that with nitrogen increasing the content of gluten increased.

Of the correlation analysis performed (Table 4), the strongest significant relationship was between

CP and G (0.906**). A number of studies, such as those by Brites and Carrillo (2001), Bilgin et al. (2010), Sieber et al. (2015) and Fu et al. (2018) confirmed the positive relationship between CP and G. From the established interdependence between chemical and physical parameters, with the exception of TW and G (0.263^{ns}), good evidence of the yield values under the influence of applied mineral fertilization (Table 4) might suggest that the improved nutrition of durum wheat was from crucial for improving quality.

Table 4. Correlation coefficients between the studied traits

	TKW	TW	GV	CP	G
TKW	1				
TW	0.4713*	1			
GV	0.412*	0.598**	1		
CP	0.577**	0.391*	0.718**	1	
G	0.482*	0.263 ^{ns}	0.645**	0.906**	1

*, **Significant at 5% and 1% level of probability

The presented data give reason to summarize that the low rates of alone fertilization with nitrogen and phosphorus, as well as their combined application, showed a favorable effect on TKW and TW, while an increase in the fertilizer rate positively affected GV. The impact of increasing rates of mineral fertilization was unidirectional on PC and G.

CONCLUSIONS

The highest GY of 3,845.7 kg/ha was found at fertilizing rate of N₈₀P₈₀ which was 83.6% over the control variant.

The highest TKW 62.08 g was found when fertilizing with N₄₀. All higher rates of self and combined fertilization had a weaker effect.

Fertilization had a statistically significant effect on TW at all variants. Although the differences were small the biggest effect was found at combined fertilization N₄₀P₄₀ – by 4.75% over the control.

The effect of the N₈₀ was strongest on the GV trait. This rate was superior to all variants and was 20.0% more than the control.

The N₈₀ rate increased the most CP by 25.8% compared to the non-fertilized control variant.

The best result on G was obtained at N₈₀P₄₀ - 30.7% G. However, the difference with the N₈₀ was negligible - 0.2% G.

The highest and significant correlation was found between CP and G (0.906**).

Increasing rates of mineral fertilizers (N and P) did not necessarily increase the values of the studied traits. In some cases, the lower rates had a more favorable effect than the higher rates.

REFERENCES

- Ali, S.A., Tedone, L., Verdini, L., Cazzato, E., Mastro, G. De (2019). Wheat Response to No-Tillage and Nitrogen Fertilization in a Long-Term Faba Bean-Based Rotation. *Agronomy*, 9, 50.
- Ames, N.P., Dexter, J.E., Woods, S. M., Selles, F., Marchylo, B. (2003). Effect on nitrogen fertilizer on protein quality and gluten strength parameters in durum wheat (*Triticum turgidum* L. var. *durum*) cultivars of variable gluten strength. *Cereal Chem.*, 80, 203–211.
- Bilgin, O., Korkut, K. Z., Baser, I., Daglioglu, O., Öztürk, I., Balkan, A., Kahraman, T. (2010). Variation and heritability for some semolina characteristics and grain yield and their relations in durum wheat (*Triticum durum* Desf.). *World J. Agric. Sci.*, 6(3), 301–308.
- Brites, C. and Carrillo, J.M. (2001). Influence of High Molecular Weight (HMW) and Low Molecular Weight (LMW) Glutenin Subunits by *Glu-1* and *Glu-3* Loci on Durum Wheat Quality. *Cereal Chem.*, 78(1), 59–63.
- Campiglia, E., Mancinelli, R., Radicetti, E., Baresel, J. P. (2014). Evaluating spatial arrangement for durum wheat (*Triticum durum* Desf.) and subclover (*Trifolium subterraneum* L.) intercropping systems. *Field Crops Research*, 169, 49–57.
- Casado-Vela, J., Selle's, S., Navarro, J., Bustamante, M. A., Mataix, J., Guerrero, C. Gomez, I. (2006). Evolution of composted sewage sludge as nutritional source for horticultural soils. *Waste Manag.*, 26(9), 946–952.
- Chennafi, H., Hannachi, A., Touahria, O., Fellahi, Z. E. A., Makhlof, M. & Bouzerzour, H. (2011). Tillage and residue management effect on durum wheat [(*Triticum turgidum* (L.) Thell spp. *turgidum* conv. *durum* (Desf.) Mac Key] growth and yield under semi-arid climate. *Adv. Environ Biol.*, 5, 3231–3240.
- Cordell, D., Drangert, J.-O., White, S. (2009). The story of phosphorus: global food security and food for thought. *Glob Environ Change*, 19, 292–305.
- Dugassa, A., Belete, K., Shimbir, T. (2019). Response of Wheat (*Triticum aestivum* L.) to Different Rate of Nitrogen and Phosphorus at Fiche-Salale, Hihglands of Ethiopia. *International Journal of Plant Breeding and Crop Science*, 6(1), 474–480.
- Ercoli, L., Masoni, A., Pampana, S., Mariotti, M., Arduini, I. (2013). A durum wheat productivity is affected by nitrogen fertilization management in

- Central Italy. *European Journal of Agronomy*, 44, 38–45.
- FAO <http://www.fao.org/3/a-i4691e.pdf>
- François, M., Grant, C., Lambert, R., Sanvé, S. (2009). Prediction of cadmium and zink concentration in wheat grain from soil affected by the application of phosphate fertilizers varying in Cd concentration. *Nut Cycl Agroecosyst*, 83, 125–133.
- Fricke, W., McDonald, A. J. S. & Mattson-Djos, L. (1997). Why do leave and cell of N-limited barley elongate at reduced rates? *Plata*, 202, 522–530.
- Fu, B.X., Wang, K., Dupuis, B., Taylor, D., Nam, S. (2018). Kernel vitreousness and protein content: Relationship, interaction and synergistic effect on durum wheat quality. *J. Cereal Sci.*, 79, 210–217.
- Garrido-Lestache, E., López-Bellido, R.J., López-Bellido, L. (2005). Durum wheat quality under Mediterranean conditions as affected by N rate, timing and splitting, N form and S fertilization. *European Journal of Agronomy*, 23(3), 265–278.
- Gerba, L., Getachew, B., Walelign, W. (2013). Nitrogen fertilization effect on grain quality of durum wheat (*Triticum turgidum* L. var. durum) varieties in central Ethiopia. *Agricultural Science*, 4, 123–130.
- Getachew, A. & Tilahun, A. (2017). Integrated Soil Fertility and Plant Nutrient Management in Tropical Agroecosystems: A review. *Pedosphere*, 27(4), 662–680.
- Gete, Z., Getachew, A. & Shahid, R. (2010). A Report on Fertilizer and Soil Fertility Potential in Ethiopia: Constraints and opportunities for enhancing the system. IFPRI.
- Getinet, A. & Wassie, H. (2019). Yield and Nitrogen Uptake of Wheat as Affected by Nitrogen Fertilizer and Compost in the Central Rift Valley of Ethiopia. *Etiop. J. Agric. Sci.*, 29(1), 85–97.
- Grant, C.A. & Bailey, L. D. (1998). Nitrogen, phosphorus and zink management effects on grain yield and cadmium concentration in two cultivars of durum wheat. *Can. J. Plant Sci.*, 78, 63–70.
- Haug, Y., Chen, L., Fu, B., Haug, Z., Gong, J. (2005). The wheat yield and water use efficiency in the loss plateaus: Straw much and irrigation effects. *Agr. Water Manage.*, 72, 209–222.
- Hinsinger, P.H., Betencourt, E., Bernard, A., Plassard, C., Shen, J., Tang, X., Zhang, F. (2011). P for Two, Sharing a Scarce Resource: Soil Phosphorus Acquisition in the Rhizosphere of Intercropped Species. *Plant Physiology*, 156, 1078–1086.
- Iancu, P., Păniță, O., Soare, M. (2019). Response of some new wheat genotypes to nitrogen fertilization and prospects of yield breeding based on yield elements. *Romania Agricultural Research*, 36.
- Ierna, A., Lombardo, G.M., Mauromicales, G. (2015). Yield, nitrogen use efficiency and grain quality in durum wheat as affected by nitrogen fertilization under a Mediterranean environment. *Expl. Agric.*, 1–16. Doi: 10.1017/S0014479715000113
- Kirchev, H., Dobreva, S., Muhova, A. (2017). Productivity and quality of durum wheat (*Triticum durum* Desf.) at increasing rates of nitrogen fertilization under long-term accumulation of nutrients in pelic vertisols. XXII Savetovanje O Biotehnologij. *Zbornik radova, Knjiga, 1*, 165–169.
- Kizilgoz, I. & Sakin, E. (2010). The effects of increased phosphorus application on shoot dry matter, shoot P and Zn concentration in wheat (*Triticum durum* L.) and maize (*Zea mays* L.) grown in a calcareous soil. *African Journal of Biohehnology*, 9(36), 5893–5896.
- Kolev, T. & Tahsin, N. (2010). Productivity of triticale varieties under the ecological conditions of the Plovdiv region. *Journal of Mountain Agriculture on the Balkans*, 13(3), 688–696.
- Le Lamer, O. & Rousselin, X. (2011). The durum wheat market. In *Studies of FranceAgriMer*; Bova, F. Ed.; FranceAgriMer, Montreuil-sous-Bois cedex, France, 1–46.
- Lerner, S.E., Seghezzo, M.L., Molfese, E.R., Ponzio, N.R., Cogliatti, M., Rogers, W.J. (2006). N- and S-fertilizers on grain composition, industrial quality and end-use in durum wheat. *J. Cereal Sci.*, 44, 2–11.
- López-Bellido, R.J. & López-Bellido, L. (2001). Efficiency of nitrogen in wheat under Mediterranean conditions: effect of tillage, crop rotation and N fertilization. *Field Crops Research*, 71(1), 31–46.
- Makowska, A., Obuchowski, W., Sulewska, H., Koziara, A., Paschke, H. (2008). Effect of nitrogen fertilization of durum wheat varieties on some characteristics important for pasta production. *Acta. Sci. Pol., Technol. Aliment.*, 7(1), 29–39.
- Neffar, S., Chenchouni, H., Beddiar, A., Redjel, N. (2014). Rehabilitation of degraded rangeland in drylands by Prickly pear (*Opuntia ficus-indica* L.) plantations: effect on soil and spontaneous vegetation. *Ecol. Balkanika*, 5, 63–76.
- Nagarajan, S. (2006). Quality characteristics of Indian wheat. In *Future of Flour*; Popper, L., Schäfer, W., Freund, W., Eds; *AgriMedia GmbH, Germany*, 79–86.
- Otiz-Monasterio, J.I., Peña, R.J., Pfeiffer, W.H., Hede, A.H. (2002). Phosphorus use efficiency, grain yield, and quality of triticale and durum wheat under irrigated conditions. *Proceeding of the 5th International Triticale Symposium, Annex, June 30-July 5, 2002, Radzików, Poland*, 9–14.
- Panayotova, G., Kostadinova, S., Manolov, I. (2018). Mineral balances nitrogen and phosphorus in cotton-durum wheat crop rotation depends on the fertilization system. *53rd Croatian and 13th International Symposium of Agriculture, Zbornik Radova*, 333–337.
- Richardson, S.J., Peltzer, D.A., Allen, R.B., McGlone, M.S., Parfitt, R.L. (2004). Rapid development of phosphorus litation in temperate rainforest along the Franz Josef soil chronosequence. *Ecologia*, 139, 267–276.
- Rossini, F., Provenzano, M.E., Sestili, F., Ruggeri, R. (2018). Sinergistic Effect of Sulfur and Nitrogen in the Organic and Mineral Fertilization of Durum Wheat: Grain Yield and Quality Traits in the Mediterranean Environments. *Agronomy*, 189.

- Sieber, A.N., Würschum, C., Longin, C.F.H. (2015). Vitreosity, its stability and relationship to protein content in durum wheat. *J. Cereal Sci.*, 61, 71–77.
- Statistic Canada (2017). Canada: outlook for principal field crops. Available online: https://www.google.com/search?q=hppt://www.arg.gc/ca/eng/industry-markets-and-trade/statistics-and-market-information/by-product-sector/crops-industry/outlook-for-principal-field-crops-in-canada/Canada-outlook-for-principal-field-crops-february-16-2016/?id%3D145572069951&spell=1&sa=X&ved=0ahUKewj_y6Cz19biAhUt2aYKHYBSBHcQBQgsKAA&biw=1680&bih=939 (accessed on 7 April 2017).
- Teklu, E. & Hailemariam, T. (2009). Agronomic and economic efficiency of manure and urea fertilizers use on vertisols in Ethiopian Highlands. *Agricultural Sciences in China*, 8(3), 352–360.
- USDA (2015). Foreign Agricultural Service. Grain and Feed Annual. GAIN Report №TR5016, 30th March 2015, Ankara, Turkey.
- Woyema, A., Bultosa, G., Taa, A. (2012). Effect of different nitrogen fertilizer on yield and yield related traits for seven durum wheat (*Triticum turgidum* L. var. Durum) cultivars grown at Sinana, South Eastern Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, 12(3), 6079–6094.
- TIBCO, Software (2018).
- Yildiz, M. & Beyaz, R. (2019). The effect of gamma radiation on *Agrobacterium tumefaciens* – mediated gene transfer in durum wheat (*Triticum durum* Desf.). *Fresenius Environmental Bulletin*, 28(1), 488–494.

EFFECT OF NITROGEN RATES ON CONCENTRATION OF NITROGEN, PHOSPHORUS AND POTASSIUM IN SORGHUM

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Abstract

The response of nitrogen, phosphorus and potassium concentration of grain sorghum to nitrogen fertilization in rates 0, 60, 120, 180, 240 and 300 kg N·ha⁻¹ was studied in the experimental field of Agricultural University of Plovdiv, Bulgaria in 2017-2019 under non-irrigated conditions. It was established proven effect of nitrogen fertilization on the concentration of nitrogen, phosphorus and potassium in the sorghum straw. The average grain nitrogen concentration ranged from 1.96% N at N₀ to 2.35% at N₃₀₀. The applying of higher N₁₈₀₋₃₀₀ rates proven increased the concentration of grain nitrogen. The increase of grain phosphorus concentration was up to N₁₂₀ and higher nitrogen rates decreased its value. The hydro-thermal conditions during the sorghum vegetation slightly affected the concentration of nitrogen and phosphorus in plant parts, but the drought conditions in 2017 significantly decreased the grain potassium concentration.

Key words: nitrogen, phosphorus, potassium, concentration, sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth leading cereal grain produced worldwide after wheat, corn, rice, and barley (FAO, 2013). In Bulgaria the production of grain sorghum has increased in recent years and sorghum is one of the top ten grown crops in the country. It can be used as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production) and organic by-products (Fernandes et al., 2013). This crop has better ability to tolerate drought stress compared with other crops and is known as an index for drought resistance of agronomic crops (Kebede, 2001; Wenzel, 2001). Nutrient uptake of sorghum precedes dry matter accumulation because nutrients are required for growth and dry matter accumulation (Soleymani et al., 2011). Sorghum is mainly grown under non-irrigated fields where stressful conditions during grain filling can limit productivity and increase the dependence of the yield of spare assimilations (Kaye et al., 2007). It is a multipurpose crop belonging to the Poaceae family, which are C₄ carbon cycle plants with high photosynthetic efficiency and productivity (Tari et al., 2012). Nitrogen is the main nutrient for C₄ plant productivity (Hao et al., 2014). It plays a critical role in cell division during the plant growth (Stals and Inzé, 2001) and the deficit of soil

nitrogen leads to lower sorghum biomass due to reductions in leaf area, chlorophyll index, and photosynthetic rate (Zhao et al., 2005; Hirel et al., 2007; Mahama et al., 2014). Nitrogen fertilizer is known to boost the aboveground biomass yield (Amaducci et al., 2004; Anderson et al., 2013). Nitrogen fertilization had a significant impact on the concentration of protein in the grain and grain protein yield (Anfinrud et al., 2013). Application of nitrogen in rates 120-240 kg N·ha⁻¹ increase crude protein content to higher levels to support rapid weight gains and milk yields (Hoffman et al., 2001; Kaufman et al., 2013). The nitrogen doses 50-200 kg ha⁻¹ contributed to an increase in the crude protein together with an increase in dry matter and/or protein concentration and crude protein increased 59.5-312.9% (Melo et al., 2017). The phosphorus and potassium fertilization slightly affected grain protein yield of sorghum grown under good phosphorus and potassium soil availability (Franco et al., 2017). Improper nitrogen fertilization and excess nitrogen resulted in environmental impacts, such as the pollution by nitrate (NO₃-N) leaching and nitrous oxide emissions (Miller and Cramer, 2004; Ramu et al., 2012), as well as increasing production costs (Marsalis et al., 2010). Thus, coordination of sorghum N demand with N supply is critically important to maximize economic efficiency, optimize biomass quality,

and minimize loss of soil NO₃-N and environmental pollution (Schroder et al., 2000; Zhu et al., 2000; Rooney et al., 2007; Cui et al., 2008; Meki et al., 2017).

The objective of this study was to determine the effect of nitrogen fertilization rates on the concentration of protein in the grain and grain protein yield of sorghum grown under non-irrigated conditions.

MATERIALS AND METHODS

The investigation was carried out during the period 2017-2019 on the experimental field of Agricultural University of Plovdiv, Bulgaria, under non-irrigated conditions after wheat as predecessor. The effect of nitrogen fertilization in rates 0, 60, 120, 180, 240 and 300 kg N·ha⁻¹ on the concentration of nitrogen, phosphorus and potassium in the grain and stover was studied at grain sorghum hybrid EC Alize. The experimental design consisted of a randomized, complete block design with four replications. The size of individual trial plots was 20 m². Total nitrogen as NH₄NO₃ was applied as pre-sowing fertilization on the background P₅₀K₅₀ fertilization as triple superphosphate and potassium chloride, respectively. Standard farming practices for the region of Southern Bulgaria were applied.

Table 1. Content of available nitrogen, phosphorus and potassium in the soil

Year	Soil depth, cm	Nmin, mg·kg ⁻¹	P ₂ O ₅ , mg·100 g ⁻¹	K ₂ O, mg·100 g ⁻¹
2017	0-30	27.6	15.8	21.0
	30-60	22.1	13.9	24.0
2018	0-30	33.8	17.3	23.1
	30-60	20.4	14.1	22.9
2019	0-30	31.8	16.9	24.1
	30-60	24.2	12.8	24.4

The soil type of the experimental field is alluvial-meadow Mollic Fluvisols (FAO, 2006) with slightly alkaline reaction pH (H₂O) = 7.80. The content of available nutrients in the soil before sowing of the sorghum was determined in soil layers 0-30 and 30-60 cm and pointed out in Table 1. The soil had low content of mineral nitrogen and it was good supplied with available phosphorus (Egner-Ream method) and exchangeable potassium (extracted by 2N HCL). Meteorological conditions during vegetation period of sorghum were recorded daily in the

experimental area and are given in Table 2, together with the long-term average of temperature and precipitations.

Table 2. Hydro-thermal conditions during sorghum vegetation period

Year	April	May	June	July	August
Temperature (°C)					
2017	12.7	17.6	23.7	25.1	25.4
2018	16.4	19.2	28.8	30.5	24.2
2019	12.6	18.2	23.4	23.5	24.6
Long-term norm	12.2	17.2	20.9	23.2	22.7
Precipitation (L·m ⁻¹)					
2017	26.1	52.7	15.4	29.8	9.2
2018	25	112.3	118.9	94.7	35.1
2019	76.5	21.3	196.7	67.5	30.6
Long-term norm	45	65	63	49	31

The values of temperature and precipitations during the vegetation period of sorghum characterized hydro-thermal conditions of 2017 as warm and dry. In contrast, the months of May, June and July of 2018 were characterized as extremely humid. The amount of precipitation exceeded nearly twice the values of long-term norm for the region. The amount of rainfall during the sorghum vegetation period in 2019 differed sharply from the long-standing value. In May, when sorghum was sown, 3 times less rainfall or 43.7 mm less fell compared to the long-term value of the month.

The concentration of nitrogen, phosphorus and potassium were analyzed in sorghum grain and stover after wet digestion by H₂SO₄ and H₂O₂ as a catalyst (Tomov et al., 2009).

An overall analysis of variance (ANOVA) was performed to evaluate the effect of the experimental treatments on the referred variables. In order to establish the difference among the means Duncan's multiple range test at level of significance $p \leq 0.05$ was used.

RESULTS AND DISCUSSIONS

Nitrogen fertilization had a positive effect on the percentage of nitrogen in the grain (Table 3). The average nitrogen concentration in sorghum grains varied from 1.96 % N at N₀ and up to 2.35% at N₃₀₀. The increase in the average concentration of nitrogen in the grain was up to the N₁₈₀rate, differences between this norm and the increased fertilization variants N₂₄₀ and N₃₀₀ were insignificant. Plants grown without nitrogen fertilization contained least nitrogen in the range

of 1.95 and 1.98% N, except in 2017, where the difference between the control and N₆₀ was not proven.

Table 3. Grain nitrogen concentration (N, %) depending on nitrogen fertilization

N rate	2017	2018	2019	2017-2019
N ₀	1.98 ^d	1.96 ^c	1.95 ^c	1.96 ^d
N ₆₀	2.03 ^d	2.11 ^d	2.13 ^d	2.09 ^c
N ₁₂₀	2.16 ^c	2.21 ^c	2.25 ^c	2.21 ^b
N ₁₈₀	2.20 ^{ab}	2.29 ^b	2.31 ^b	2.27 ^{ab}
N ₂₄₀	2.22 ^{ab}	2.33 ^b	2.35 ^{ab}	2.30 ^{ab}
N ₃₀₀	2.28 ^a	2.39 ^a	2.39 ^a	2.35 ^a
Year	2.14^{ns}	2.22	2.23	

The conditions of the year did not affect the concentration of nitrogen in the grain and the mean values obtained by year were close to each other. During the drought 2017, the differences between nitrogen concentrations at moderate and higher (N₁₈₀, N₂₄₀, N₃₀₀) rates were unproven and their values did not differ significantly from one another. The results show that the application of the higher N₃₀₀ nitrogen in 2018 and 2019 experimental years resulted in a significantly higher grain nitrogen concentration as well as the highest obtained during the experimental period. Differences between the values of nitrogen concentration in sorghum grain at fertilizer rate N₁₈₀ and N₂₄₀ were not demonstrated on average over the period and separately over the three years of the study.

Nitrogen fertilization raised the average nitrogen content of sorghum stover from 0.67% N at N₀ to 1.17% at N₃₀₀ (Table 4). The effect of nitrogen fertilization had been demonstrated compared to the control over the three years. The lowest concentrations of nitrogen in sorghum stover during the three-year experimental period were obtained in plants that were not fertilized with nitrogen. The nitrogen concentration in the stover was the lowest at N₆₀ of the nitrogen fertilized variants. Effect of applied high nitrogen N₂₄₀ and N₃₀₀ rates proven to lead to the straw with the highest nitrogen content 1.16-1.17% N, but differences between the averages values were not significance. The nitrogen content of sorghum stover at fertilizer rates N₁₂₀ and N₁₈₀ was not demonstrated on average over the period and separately over the three years of the study. The highest concentrations of nitrogen were obtained during the humid 2018

and 2019 at fertilizer rates of N₃₀₀ and N₂₄₀. The conditions of the year did not have a significant effect on the nitrogen concentration in the sorghum stover and the obtained average annual values were close.

Table 4. Stover nitrogen concentration (N, %) depending on nitrogen fertilization

N rate	2017	2018	2019	2017-2019
N ₀	0.69 ^d	0.69 ^c	0.62 ^c	0.67 ^d
N ₆₀	0.76 ^c	0.81 ^d	0.74 ^d	0.77 ^c
N ₁₂₀	0.98 ^b	0.94 ^c	0.98 ^c	0.97 ^b
N ₁₈₀	1.03 ^b	0.95 ^c	0.93 ^c	0.97 ^b
N ₂₄₀	1.15 ^a	1.13 ^b	1.19 ^a	1.16 ^a
N ₃₀₀	1.16 ^a	1.26 ^a	1.10 ^b	1.17 ^a
Year	0.96^{ns}	0.96	0.93	

The average concentration of phosphorus in sorghum grain varied between 1.01% P₂O₅ at N₀ and 0.86% at N₃₀₀ (Table 5). Plants grown without nitrogen fertilization and under conditions of lower fertilizer norms contained the most phosphorus in the range 1.01 and 1.08% of P₂O₅. The increase in the average concentration of phosphorus in the grain was up to N₁₂₀, with insignificant differences between N₀, N₆₀ and N₁₂₀ both on average over the period and in 2019. Fertilizer norms higher than N₁₂₀ led to a decrease in the percentage of phosphorus in the grain of sorghum. Differences in the concentration of phosphorus in sorghum grain in 2019 and the average over the period at elevated fertilizer rates N₁₈₀, N₂₄₀ and N₃₀₀ were insignificant. The conditions of the year did not affect the concentration of phosphorus in the grain and the mean values obtained over the years were very close to each other. In 2017 and 2018, the concentration of phosphorus in the sorghum grain in the all studied variants was slightly influenced by nitrogen fertilization and the differences were insignificant.

Table 5. Grain phosphorus concentration (P₂O₅, %) depending on nitrogen fertilization

N rate	2017	2018	2019	2017-2019
N ₀	1.04 ns	0.97 ns	1.02 ^b	1.01 ^{ab}
N ₆₀	1.08	1.01	1.07 ^{ab}	1.05 ^a
N ₁₂₀	1.10	1.03	1.11 ^a	1.08 ^a
N ₁₈₀	0.93	0.86	0.97 ^c	0.92 ^{bc}
N ₂₄₀	0.85	1.00	0.93 ^c	0.93 ^{bc}
N ₃₀₀	0.89	0.83	0.85 ^d	0.86 ^c
Year	0.98^{ns}	0.95	0.99	

Nitrogen fertilization raised the average phosphorus content of sorghum stover from 0.33% P₂O₅ at N₀ to 0.49% P₂O₅ at N₃₀₀ (Table 6). The effect of nitrogen fertilization had been demonstrated compared to the control over the three experimental years. The lowest concentrations of phosphorus in sorghum stover during the three-year experimental period were obtained in non-fertilized plants.

Table 6. Stover phosphorus concentration (P₂O₅, %) depending on nitrogen fertilization

N rate	2017	2018	2019	2017-2019
N ₀	0.31 ^b	0.34 ^b	0.33 ^d	0.33 ^c
N ₆₀	0.39 ^a	0.43 ^a	0.42 ^c	0.41 ^b
N ₁₂₀	0.41 ^a	0.46 ^a	0.43 ^{bc}	0.43 ^b
N ₁₈₀	0.43 ^a	0.48 ^a	0.45 ^b	0.45 ^{ab}
N ₂₄₀	0.46 ^a	0.50 ^a	0.49 ^{ab}	0.48 ^a
N ₃₀₀	0.45 ^a	0.50 ^a	0.51 ^a	0.49 ^a
Year	0.41^{ns}	0.45	0.44	

Fertilization with moderate and elevated nitrogen levels N₁₈₀, N₂₄₀ and N₃₀₀ were proven to accumulate the stover with the highest phosphorus content, but differences between their values were not proven.

The concentration of phosphorus in stover in 2017 and 2018 among all fertilized variants were close to each other and the differences were not significant. In 2019, the phosphorus content of sorghum stover increased in parallel and was highest at the N₂₄₀ and N₃₀₀ fertilizer rates. The conditions of the year did not have a significant effect on the phosphorus concentration in the sorghum stover, with the average annual values obtained were close.

The obtained average values for the period 2017-2019 did not indicate a proven effect of nitrogen fertilization on the concentration of potassium in the sorghum grain, despite a tendency for higher values with increasing fertilization up to N₂₄₀ (Table 7). The effect of the more favorable rainfall in 2018 and 2019 on the concentration of potassium in sorghum grains on average from the nitrogen fertilizer rates tested was proven to the dried 2017.

The highest concentrations of potassium in the grain were obtained in variants N₁₂₀, N₁₈₀ and N₂₄₀. The differences between these three rates were unproven except for N₁₂₀ in 2018.

Table 7. Grain potassium concentration (K₂O, %) depending on nitrogen fertilization

N rate	2017	2018	2019	2017-2019
N ₀	0.43 ^c	0.52 ^d	0.49 ^c	0.48 ^{ns}
N ₆₀	0.44 ^b	0.58 ^c	0.53 ^b	0.52
N ₁₂₀	0.46 ^a	0.60 ^{bc}	0.58 ^a	0.55
N ₁₈₀	0.48 ^a	0.64 ^a	0.61 ^a	0.58
N ₂₄₀	0.47 ^a	0.63 ^{ab}	0.62 ^a	0.57
N ₃₀₀	0.42 ^c	0.50 ^d	0.53 ^b	0.48
Year	0.45^b	0.58^a	0.56^a	

The difference between the control and the nitrogen fertilized plants was only demonstrated in 2019. The results show that the application of the higher N₃₀₀ norm proven reduced the potassium concentration in the grain closed to values of the control plants.

In contrast to the concentration of potassium in the grain, nitrogen fertilization raised the average potassium content of sorghum stover from 1.06% P₂O₅ at N₀ to 1.32% P₂O₅ at N₁₈₀ (Table 8). The effect of nitrogen fertilization was demonstrated compared to the control over the three experimental years and averaged over the period. Without nitrogen fertilization, the sorghum stover contained 1.04-1.08% K₂O. Nitrogen fertilization raised the concentration of potassium in sorghum stover to N₁₈₀. Higher fertilization rates did not increase the concentration of potassium in stover. No differences between fertilizer rate N₂₄₀ and N₁₂₀ were obtained and the values were close to each other. Fertilizing with the high N₃₀₀ norm led to a concentration of potassium in stover of sorghum close to variant N₆₀. Only the average concentration for the period 2017-2019 was an exception. The conditions of the year did not have a significant effect on the concentration of potassium in the sorghum stover, with the obtained average annual values being close.

Table 8. Stover potassium concentration (K₂O, %) depending on nitrogen fertilization

N rate	2017	2018	2019	2017-2019
N ₀	1.08 ^d	1.04 ^d	1.06 ^d	1.06 ^c
N ₆₀	1.15 ^c	1.16 ^c	1.12 ^c	1.14 ^d
N ₁₂₀	1.22 ^b	1.22 ^b	1.25 ^b	1.23 ^b
N ₁₈₀	1.30 ^a	1.31 ^a	1.35 ^a	1.32 ^a
N ₂₄₀	1.22 ^b	1.23 ^b	1.26 ^b	1.24 ^b
N ₃₀₀	1.17 ^c	1.18 ^c	1.22 ^{bc}	1.19 ^c
Year	1.19^{ns}	1.19	1.21	

The obtained results indicated that the nitrogen concentration in the sorghum grain was 2.23-2.40 times higher than the concentration of nitrogen of the stover. Similar results were found for phosphorus, with its concentration in the grain exceeding 2.11-2.39 times the percentage of phosphorus in stover. In contrast to these two elements, the potassium content of stover was higher than that of sorghum grain 2.05-2.64 times on average per year.

CONCLUSIONS

Nitrogen fertilization increased the concentration of nitrogen in sorghum hybrid EC Alize from 1.96% N (at N₀) up to 2.35% (at N₃₀₀) in the grain, and from 0.67% N (at N₀) to 1.17% N (at N₃₀) in the sorghum stover. The applying of higher N₁₈₀₋₃₀₀ rates proven increased the concentration of grain nitrogen over the period 2017-2019. The increase of grain phosphorus concentration was up to N₁₂₀ and higher nitrogen rates decreased its value, but the higher nitrogen rates N₂₄₀ and N₃₀₀ significantly increased the phosphorus concentration of the stover.

The studied nitrogen rates 0-300 kg N·ha⁻¹ slightly affected the potassium concentration of the grain, but the percentage of potassium in sorghum stover proven increased with nitrogen rates up to N₁₈₀ with value of 1.32% K₂O. Nitrogen concentration in the sorghum grain was 2.23-2.40 times higher than the concentration of nitrogen of the stover. Similar results were found for phosphorus, with its concentration in the grain exceeding 2.11-2.39 times the percentage of phosphorus in stover. In contrast to these two elements, the potassium content of stover was higher than that of sorghum grain 2.05-2.64 times on average per year. The hydro-thermal conditions during the sorghum vegetation slightly affected the concentration of nitrogen and phosphorus in the plant parts, but the drought conditions in 2017 significantly decreased the average grain potassium concentration.

REFERENCES

Amaducci, S., Monti, A., Venturi, G. (2004). Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. *Ind. Crop Prod.*, 20, 111–118.

Anderson, E.K., Parrish, A.S., Voigt, T.B., Owens, V.N., Hong, C., Lee, D.K., (2013). Nitrogen fertility and harvest management of switchgrass for sustainable bioenergy feedstock production in Illinois. *Ind. Crop Prod.*, 48, 19–27.

Anfinrud, R., Cihacek, L., Johnson, B.L., Ji, Y., Berti, M.T., (2013). Sorghum and kenaf biomass yield and quality response to nitrogen fertilization in the Northern Great Plains of the USA. *Ind. Crop Prod.*, 50, 159–165.

Cui, Z., Zhang, F., Chen, X., Miao, Y., Li, J., Shi, L., Xu, J., Ye, Y., Liu, C., Yang, Z., Zhang, Q., Huang, S., Bao, D., (2008). On-farm evaluation of an in-season nitrogen management strategy based on soil Nmin test. *Field Crop Res.*, 105, 48–55.

FAO, (2013). FAOSTAT online database: <http://faostat3.fao.org/download/Q/QC/E>.

Fernandes, E., Pereira, W., Hackenhaar, L., Rodrigues, R. & Terra, R. (2013). The use of whole grain sorghum in broiler feeds. *Revista Brasileira de Ciência Avícola*, 15(3), 217–222.

Franco, A. (2011). Marcha de absorção e acúmulo de nutrientes na cultura do sorgo. Dissertação (Mestrado em Produção Vegetal), *Universidade Estadual de Montes Claros*, Beasil, 13–19.

Hao, B., Xue, Q., Bean, B.W., Rooney, W.L., Becker, J.D., (2014). Biomass production, water and nitrogen use efficiency in photoperiod-sensitive sorghum in the Texas High Plains. *Biomass Bioenergy*, 62, 108–116.

Hirel, B., Le Gouis, J., Ney, B., Gallais, A., (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.*, 58, 2369–2387.

Hoffman, P.C., Esser, N.M., Bauman, L.M., Denzine, S.L., Engstrom, M., Chester-Jones, H., (2001). Short communication: effect of dietary protein on growth and nitrogen balance of Holstein Heifers1. *J. Dairy Sci.*, 84, 843–847.

Kaye N.M., Mason S.C., Jackson D.S., Galusha T.D., (2007). Crop Rotation and Soil Amendment Alters Sorghum Grain Quality. *Crop Science*, 47, 722–729.

Kaufman, R.C., Wilson, J.D., Bean, S.R., Presley, D.R., Blanco-Canqui, H., Mikha, M. (2013). Effect of Nitrogen Fertilization and Cover Cropping Systems on Sorghum Grain Characteristics, *Journal of Agricultural and Food Chemistry*, 61, 5715–5719.

Kebede H., Subudhi P.K., Rosenow D.T., Nguyen H.T., (2001). Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). *Springer-Verla. TheorAppl Genet*, 103, 266–276.

Mahama, G.Y., Prasad, P.V.V., Mengel, D.B., Tesso, T.T., (2014). Influence of nitrogen fertilizer on growth and yield of grain sorghum hybrids and inbred lines. *Agron. J.*, 106, 1623–1630.

Mariotti, F., Tomé, D., Mirand, P. (2008). Converting Nitrogen into Protein - Beyond 6.25 and Jones' Factors. *Critical Reviews in Food Science and Nutrition*, 48(2), 177–184.

Marsalis, M.A., Angadi, S.V., Contreras-Govea, F.E., (2010). Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different

- plant populations and nitrogen rates. *Field Crop Res.*, 116, 52–57.
- Meki, M.N., Ogoshi, R.M., Kiniry, J.R., Crow, S.E., Youkhana, A.H., Nakahata, M.H., Littlejohn, K., (2017). Performance evaluation of biomass sorghum in Hawaii and Texas. *Ind. Crop Prod.*, 103, 257–266.
- Melo, D., Orlando Da Ros, C., Ferreira da Silva, R., José Coldebella, I. (2017). Effect of N, P or K doses on the dry matter and crude protein yield in maize and sorghum, *Pesquisa Agropecuária Tropical*, 47(1), 21–43.
- Miller, A.J., Cramer, M.D., (2004). Root nitrogen acquisition and assimilation. *Plant Soil*, 274, 1–36.
- Ramu, K., Watanabe, T., Uchino, H., Sahrawat, K.L., Wani, S.P., Ito, O., (2012). Fertilizer induced nitrous oxide emissions from Vertisols and Alfisols during sweet sorghum cultivation in the Indian semi-arid tropics. *Sci. Total Environ.*, 438, 9–14.
- Rooney, W.L., Blumenthal, J., Bean, B., Mullet, J.E., (2007). Designing sorghum as a dedicated bioenergy feedstock. *Biofuel Bioprod. Biorefin.*, 2, 147–157.
- Schroder, J., Neeteson, J., Oenema, O., Struik, P., (2000). Does the crop or the soil indicate how to save nitrogen in maize production? Reviewing the state of the art. *Field Crop Res.*, 68, 151–164.
- Soleymani, A., Shahrajabian, M. H., & Naranjani, L. (2011). The effect of plant density and nitrogen fertilization on yield, yield components and grain protein of grain sorghum. *Journal of Food, Agriculture and Environment*, 9(3-4), 244–246.
- Stals, H., Inzé, D., (2001). When plant cells decide to divide. *Trends Plant Sci.*, 6, 359–364.
- Tari, I., Laskay, G., Takács, Z. & Poor, P. (2012). Responses of Sorghum to Abiotic Stresses: A Review. *Journal of Agronomy Crop Science*, 199(4), 264–274.
- Tomov, T., Rachovski, G., Kostadinova, S. & Manolov, I. (2009). Handbook of Agrochemistry. *Academic publisher of Agricultural University of Plovdiv*, Bulgaria.
- Wenzel W.G., Van Rooyen P.J., (2001). Moisture Stress and Potential Sorghum Yield. *ISMN*, 42, 28–29.
- Zhao, D., Reddy, K.R., Kakani, V.G., Reddy, V.R., (2005). Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *Eur. J. Agron.*, 22, 391–403.
- Zhu, Z.L., Chen, D.L., (2002). Nitrogen fertilizer use in China—contributions to food production, impacts on the environment and best management strategies. *Nutr. Cycl. Agroecosyst.*, 63, 117–127.

INNOVATIVE WORKING BODIES OF OPENERS FOR SEEDING GRAIN CROPS

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Abstract

The working bodies of the openers are presented, which help to avoid the rolling of seeds along the length of the bottom of the furrow, as well as the displacement of seeds along the depth of their placement. Studies show that the depth of seed placement does not always coincide with the depth of the openers. This is explained by the fact that loose soil mass enters the internal space of the openers and is located on some inclined surface. To ensure an even predetermined placement depth, the seeds must be directed to the front of the openers where the scree does not fall and where the bottom of the furrow is horizontal. To do this, in the lower part of the openers set a plate inclined forward, hitting which, the seeds are discarded to the toe of the coulter. However, the seeds, having hit the inclined plate, randomly fall onto the prematurely sprinkled bottom of the furrow, and the uniform distribution of seeds along the row length and the depth of their placement is violated.

Key words: grain seeder opener, crops, working bodies, grain speed quencher.

INTRODUCTION

Currently, the functioning of agricultural production questions that reveal the problems of sowing grain crops are especially relevant. At the same time, the development of technological and technical systems for the cultivation of grain crops that most fully meet the agrotechnical requirements is a priority.

One of the prime areas for the development of agriculture in Russia is the improvement of the working bodies of agricultural machinery in order to increase the efficiency of the technological operations they perform (Larushin et al., 2018).

One of the most important operations in the cultivation of crops is sowing. The yield of the cultivated crop, the biological productivity of sowing, and the saving of seed material depend on the quality of sowing.

MATERIALS AND METHODS

In the review and comparative analysis of existing seeders and grain seeder openers, theoretical methods based on the principles of classical mechanics, mathematical analysis, modeling were used. The results of experimental studies of these seeders and grain seeder openers during laboratory and laboratory field studies were obtained using the theory of multivariate experiment, mathematical statistics and current

Interstate Standard (IS). Processing of experimental data from research on innovative grain seeder openers bodies were performed using the application programs “Statistica 6.0”, “MathCAD”, 3D modeling programs etc.

RESULTS AND DISCUSSIONS

The invention relates to agricultural machinery, in particular to close-up working bodies of grain seeder openers.

The grain seeder opener (USSR Author's Certificate No. 1273006 A1) contains a casing, two discs mounted at an angle to each other and secured to the casing with a hinge and secured with a cord-shaped knife, with the aim of improving the quality of seed placement and reliability of work on soils of any kind humidity, the disks are located between the cheeks of the snake-shaped knife, the latter being equipped with a furrow-forming tool with a keel-shaped mouth mounted under the disks and attached to its cheeks.

The disadvantages of this grain seeder opener include the use of a skid-shaped knife of a furrow former installed in front of the discs, which increases the resistance of this grain seeder opener when it moves in the soil. Also, the used runner does not exclude the sticking of soil to it and soil unloading in front of the opener, which leads to disruption of the opener working process and to a

change in the seed placement depth and uniform distribution along the furrow length and seeding depth. In addition, the seeds are fed into the furrow behind a skid-shaped knife, where the soil crumbles into the furrow, and the sowing uniformity along the furrow depth and length would also be violated. All this leads to a decrease in crop yields.

Another grain seeder opener (RF Patent No. 2435356 C1) comprises a housing, a leash for attaching to the frame, two flat pointed discs, a seed guide, a scraper, two ball bearings, the opener having a figured plate made of highly wear-resistant steel, which is rigidly fixed with an adapter to the opener body and the adapter, made of spring steel, in the upper part has longitudinal holes for fixing bolts that allow to adjust the height of the adapter and curly plate, changing the density of the soil mass around the seeds, located at the bottom of the furrow.

The disadvantages of that kind of the grain seeder opener include soil sticking to a curly plate, which results in poor compaction of the bottom of the furrow and shifting of seeds along the furrow, which disturbs the uniform distribution of seeds along the depth and length of the furrow. In addition, in the opener, the seed guide is made of an open type, which leads to the ingress of seeds not to the bottom of the furrow, but to its walls, thereby violating the uniform distribution of seeds along the depth and length of the furrow. All these shortcomings lead to a decrease in crop yields.

One more grain seeder opener (RF Patent No. 2427124 C1) contains a furrow-forming disk mounted on a frame with a leash, on which there is a push rod with a spring, a seed guide located behind the furrow-forming disk, a shut-off valve, a coulter-stopper depth limiter coulter, characterized in that the furrow-forming disk on the periphery has a wedge-shaped shape, the wedge of the disk being rounded, the seed guide in the lower front part is equipped with a two-sided scraper made integrally with the seed guide, a shut-off valve is installed angles on the shafts inside the guide seeds and has a center of gravity offset relative to this shafts, earthing devices stroke limiter depth, the grain seeder opener is mounted on the guide frame for seeds. One of the drawbacks of this grain seeder opener is the installation of the seed guide behind the furrow-forming disk above the soil level, which leads to the shedding of the soil into the furrow before the seeds reach its bottom. Therefore, the seeds are laid in violation of the uniform

distribution of them along the depth and length of the furrow. In addition, the presence of a shut-off valve in the seed guide complicates the design and leads to its enveloping with plant residues and clogging of the seed guide, interfering with the normal operation of the grain seeder opener. Because the opener-limiter of the depth of the opener stroke is made of a passive type, it unloads the soil during operation, which negatively affects the uniform distribution of seeds along the depth and length of the furrow. The installation of a disk scraper in the soil leads to its clogging with plant residues and soil, while the furrow-forming disk is slipping, or it stops completely and the coulter will heap the soil.

There the grain seeder opener of RF Patent No. 2427124 C1 should also be mentioned. It comprises a housing and two discs mounted at an angle of 18° to each other, while to increase the efficiency of seed distribution over the feeding area, a plate is installed between the discs to form a horizontal profile of the seed bed and a seed diffuser her.

The disadvantages of that grain seeder opener include the manufacture of a seed diffuser in the form of a plate, which, when the grain seeder opener is used, leads to seeds getting on the grain seeder opener discs and engaging them in rotation with the disc, while the uniformity of the supply and distribution of seeds in the furrow along the depth and length of the furrow is violated. In addition, the manufacture of the profiler also in the form of a plate leads to the falling of the furrow formed by it before the seeds get into it from the seed diffuser, which leads to a violation of the uniformity of the depth of seed placement and the uniform distribution along the length of the furrow.

It is necessary to pay attention to the opener and device for planting seeds (Certificate for utility model of the Russian Federation No. 37904 U1) and disk grain seeder opener (RF Patent No. 2237396 C2), including two discs, seed guide and device for planting seeds.

The disadvantages of highly mentioned grain seeder opener include the production of the seed guide tube vertical or curved and open type, and also due to the lack of a device for preliminary compaction of the walls and the bottom of the furrow, when sowing, the walls of the furrow are shed before the seeds get into it and the bottom of the furrow is not horizontal, and the seeds bounce off the bottom of the furrow and the

seeds are laid on an unconsolidated bed, which leads to a violation of the uniform distribution of seeds in depth and further worsens the germination of seedlings.

A grain seeder opener battery is known (RF Patent No. 2125359 C1), including a shaft, spherical disks and racks installed between them with the fertilizer and the seed guide, moreover, paired bows are fixed on each rack, the lower edges of which are placed at the level of the lower edge of the spherical disks, while the working surfaces narralniks (for drawing a furrow in the soil) are made in the form of convex parts of truncated spherical segments facing towards each other, and between them in front of the direction of placement of the pipelines.

The disadvantages include the fact that the opener battery, as well as the openers, are rigidly attached to the seeder frame on one shaft, without taking advantage of the opener suspension, which negatively affects the uniformity of the sowing depth. Also, spherical discs form pile ridges and an uneven bottom of the furrow, violating the uniformity of sowing of seeds and fertilizers in depth. Paired arrays on not sufficiently leveled soil form grooves of different depths, which is unacceptable by agrotechnical requirements when sowing.

The opener (USSR Author's Certificate No. 1688796 A1) comprises a casing, two discs mounted at an angle to each other on the casing, a seed guide and a cultivator in the form of a dihedral wedge located in front of the discs at an acute angle to the horizontal plane, while increase the uniformity of seed placement in depth, the cultivator is equipped with a stabilizer made in the form of wings fixed in its lower part. Moreover, the wings of the stabilizer are directed upward at an acute angle to the horizontal.

The opener's disadvantages include the manufacture of a seed guide in the form of a plate and a cultivator in the form of an open type chute, which leads to seeds getting onto the opener discs and engaging them in rotation together with the discs, while the uniformity of seed supply and distribution in the furrow along the depth and length is violated furrows. Also, the manufacture of a cultivator in the form of an open gutter leads to the rolling of seeds along the bottom of the furrow and falling asleep of the furrow formed by it until the seeds get from the

cultivator, which leads to a violation of the uniformity of the depth of seed placement and the uniform distribution along the length of the furrow. In addition, the installation of a cultivator with a stabilizer in the front of the opener does not allow to obtain an even furrow bottom, since the stabilizer made with wings creates vibrations of the coulter in the longitudinal-vertical plane, which will affect the depth of seed and fertilizer placement and their uniform distribution along the furrow length. Also, the installation of a cultivator with a stabilizer in the front of the coulter cannot be carried out for design reasons, since in this part of the opener discs there is no necessary gap between the coulter discs for installing a cultivator with a stabilizer between them. With an increase in the size of the gap between the opener discs of more than 1 mm, the discs become clogged with soil in the front part of the opener while they are working, and the openers deepen and the seeds are distributed in violation of the set depth. Also, the design of a cultivator with a stabilizer will lead to a violation of the stability of the opener in the soil in a longitudinally vertical plane, which would not allow the formation of the necessary furrow for laying out the seeds, while the formed furrow has loose sides and a bottom of the furrow, which eliminates the attraction of moisture to the seeds. All this leads to lower crop yields and higher production costs.

To solve the problem of improving the uniformity of seed distribution along the furrow length and the depth of their seeding, a coulter was developed and manufactured (RF Patent No. 2687368 C1) in Penza State Agrarian University (Vanin et al., 2017; Zubarev et al., 2019).

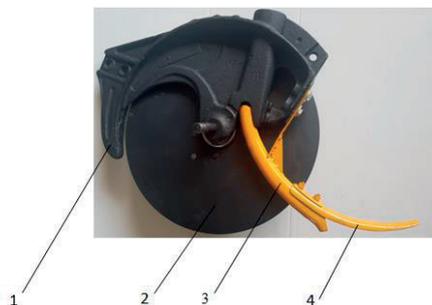


Figure 1. Grain seeder opener: 1 - housing; 2 - disk; 3 - seed guide; 4 - damper

The opener (Figure 1) contains a housing, two discs mounted at an angle to each other on the housing, a seed guide and a cultivator, characterized in that the seed guide and cultivator are made of an ellipsoidal tube as a whole, while the ellipsoid tube is bent to the side opposite to the motion of the opener, while the longitudinally vertical plane of symmetry of the ellipsoid pipe coincides with the longitudinally vertical plane of symmetry of the opener, while the shafts of symmetry of the pipe of the ellipsoid section is made with a radius $R = 250-300$ mm, at the same time, the ellipsoidal section pipe simultaneously serves as a seed guide and cultivator, while the upper part of the ellipsoidal section pipe is fixedly fixed with a funnel to the neck of the opener body, while inside the ellipsoidal section pipe, by gumming, a coating is applied, for example, from rubber, acting as a calming agent seed, while the coating inside the ellipsoidal tube has elastic-elastic and antifriction properties, while the ellipsoidal tube in the lower part has it an ellipsoidal outlet that is identical to its contour, while the major axis of the outlet of the ellipsoidal tube is 30-35 mm, while the minor shafts of the outlet of the ellipsoidal tube is 15-17 mm, and all-metal is made below the outlet of the ellipsoidal tube a device made of a wear-resistant material in the form of a curved wedge, while the heel width of the curved wedge is $e = 12-14$ mm, while the curved wedge is attached to the ellipse-shaped pipe with the help of welding, while the middle part of the ellipsoid pipe is fixed to the coulter body using a bracket installed at the attachment point of the internal opener scrapers, and a seed speed damper is installed over the outlet of the ellipsoid pipe with a screw connection, while the seed speed damper has the mounting part, made in the form of a thickening at the place of its attachment to the ellipsoidal pipe and the working part, while the mounting part has a longitudinal groove intended for installation fixing screws in it, while the longitudinal axis of symmetry of the seed velocity damper coincides with the longitudinally vertical plane of symmetry of the ellipsoidal tube, while the seed velocity damper is directed backward relative to the outlet of the ellipsoid tube toward the bottom of the furrow, with a clearance h between the seed velocity damper and the furrow bottom, measured between its rear part and the furrow

bottom, is equal to the smaller thickness of the seeds, while the contour of the working part of the seed velocity damper is made in the form the isosceles trapezoid with a slope of its lateral sides identical to the slope of the furrow walls, while the working part of the seed velocity damper is made concave relative to the longitudinal shafts of symmetry of the bottom of the furrow, while the profile of the concave working part of the seed velocity damper has the shape of a circular arc made with a radius $r = 25-30$ mm, while the longitudinal axis of symmetry of the working part of the seed speed quencher is made convex along a circular arc of radius $r_1 = 95-100$ mm, relative to the shafts of symmetry of the bottom of the furrow, while the a width of the working part is grain speed quencher, in its front part, located at a height equal to the major shafts of the ellipse of the outlet of the ellipsoid tube, is 30-33 mm, while the width b of the rear working part of the seed velocity damper, located at the bottom of the furrow formed by the heel of the curved wedge, equal to 12-14 mm, while the thickness from the profile of the working part of the seed velocity damper is 2.5-3.0 mm, while the length l of the working part of the seed velocity damper is 120-150 mm, while the seed velocity damper is made of nylon. The opener (RF patent No. 2692622 C1) contains a casing (Figure 2), two discs mounted at an angle to each other on the casing, a seed guide and a cultivator, characterized in that the seed guide and a cultivator are made as a single

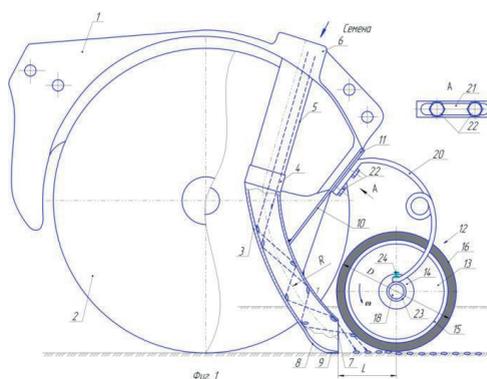


Figure 2. Opener: 1 - body, 2 - disk, 3 - guide, 4 - socket, 5 - gate, 6 - neck, 7 - hole, 8 - wedge, 9 - heel, 10 - bracket, 11 - scrapers, 12 - roller, 13 - disk, 14 - hub, 15 - rim, 16 - tire, 17 - bearing, 18 - axis, 19 - head, 20 - strut, 21 - groove, 22 - screws, 23 - sleeve, 24 - lock screw

unit of a rectangular pipe, while the pipe of rectangular cross section is bent to the side opposite to the movement of the opener, while the axis of symmetry of the pipe of rectangular section is made with a radius $R = 150-160$ mm, while the longitudinally-vertical plane of symmetry of the rectangular pipe coincides with the longitudinally vertical plane of symmetry of the opener, while the width of the pipe of rectangular section is 14 mm and the height is 25 mm, while the pipe of rectangular section simultaneously serves as a seed guide and cultivator, while the upper part pipes of rectangular cross section is fixedly fixed by a bell to the funnel of the neck of the opener body, while the pipe of rectangular cross section in the lower part has a rectangular outlet, the width of the outlet of the rectangular pipe is 11 mm and the height is 30 mm, while the gaps between the opener discs and the side surfaces of the rectangular pipe, measured near the cutting edges of the rear of the opener discs, are at least 12 mm on each side of the side surface of the pipe a rectangular section, while below the outlet of the pipe of rectangular section, a curved wedge is installed by welding, while the width of the heel of the curved wedge is equal to the width of the pipe of rectangular section 14 mm, while the middle part of a rectangular pipe is fixed to the opener body using a bracket installed at the attachment point of the internal opener disc scrapers, while a furrowed roller is installed behind the outlet of a rectangular pipe, and the longitudinally vertical plane of symmetry of the furrowed roller coincides with the longitudinally vertical the plane of symmetry of the pipe of rectangular cross section, while the furrowed packer roller consists of a disk with a hub and welded a rim on which is firmly attached, for example, with glue, to a tire made of rubber massif, while a furrowed packer roller is supported by a tire made of rubber massif to the bottom of the furrow formed by the opener discs, while the width of the tire made of rubber massif is equal to the width of the bottom furrows in = 12-14 mm, while the diameter of the furrow packer roller is $D = 100-120$ mm, while the distance between the transverse vertical plane of the outlet of the rectangular pipe and the transverse vertical plane of symmetry of a solid packer roller is $L = 60-65$ mm, while in the hub

of the furrow packer roller a sliding bearing is installed, made in the form of a sleeve of antifriction material, while the hub of the furrow packer roller is supported through the bearings on the shafts, while the axis of the hub of the furrow packer roller has a head on one side, while the furrow packer roller has a spring strut, while the spring strut is made of alloy spring steel, while the upper part of the spring strut has a fastening part with a longitudinal groove for accommodating the screw connection necessary for fastening the spring strut to the opener body at the place of attachment of the internal opener disc scrapers, while the bush is fixedly attached to the bottom of the spring strut, while the bush is dressed on the shafts of the furrow-seaming hub roller and fixed motionlessly with a locking screw, while the shafts of the hub of the furrow packer roller from axial displacement relative to the hub of the furrow packer roller is held bare shafts and the end of the sleeve on the axle hub clad furrow packer roller, while on both ends of the hub, set of antifriction material washer.

CONCLUSIONS

Designed, innovative openers made, mounted on the seeder SZ-5.4 and tested in the field of the Penza region (Russia). The results of processing the experimental data showed an improvement in the uniformity of the distribution of seeds along the length of the row and the depth of their seeding, which led to an increase in the yield of grain crops by 12%.

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REFERENCES

- Larushin N.P., Vanin, D.V., Shumaev, V.V. (2018). Ларюшин, Н.П. Grain seeder opener design for sowing crops. *Volga Region Farmland*, 4(49), 137–143.
- Vanin, D.V., Larushin, N.P., Shumaev, V.V. (2017). Ванин, Д.В. Results of search experiments on choosing the optimal opener design. *Scientific papers: Energy-efficient and resource-saving technologies and systems*. Saransk: MSU Publishing, 617–622.
- Zubarev, A.G., Laryshin, N.P., Shukon, A.V. (2019). Development of working bodies for a grain seeder for sowing using resource-saving technologies. *Science in the Central Russia*, 3(39), 78–83.

CROP SCIENCES

YIELD AND QUALITY OF CONFECTIONERY SUNFLOWER SEEDS AS AFFECTED BY FOLIAR FERTILIZERS AND PLANT GROWTH REGULATORS IN THE LEFT-BANK FOREST-STEPPE OF UKRAINE

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Abstract

A three years (2016-2018) field experiment was conducted in the Left-Bank Forest-Steppe of Ukraine to assess the influence of 7 different treatments with foliar fertilizers and plant growth regulators (control; Sol Bor + Basfoliar 6-12-6; Basfoliar 6-12-6; Wuxal Bio Aminoplant + Wuxal Boron; Wuxal Boron; Spectrum Askorist + Spectrum B + Mo; Spectrum B + Mo) on seed yield and quality of 3 new confectionery sunflower genotypes (Confeta F1, Lakomka, Oniks). Foliar applications largely had a favourable effect on all parameters studied (plant height, leaf surface area, number of seeds per head, seed weight per head, 1000-seed weight, seed yield, and protein content) compared to the control. Significantly ($P < 0.05$) higher average seed yield occurred in the variety Lakomka (2.42 t/ha). Again, the greatest average protein content was obtained from Lakomka (23.0%). Sequential foliar application of Sol Bor + Basfoliar 6-12-6 thrice gave significantly ($P < 0.05$) greater average seed yield (2.37 t/ha) for all three genotypes combined compared to the other treatments. Based on foliar sprays, the increase in protein content ranged from 0.1-0.5% compared to the control.

Key words: confectionery sunflower, foliar fertilizer, plant growth regulator, seed quality, seed yield.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is among the four key oilseed plants worldwide (along with palm, soy, and rapeseed) and recognized as one of the two most indispensable oil crops in Europe, together with rapeseed (Jocić et al., 2015). Ukraine has maintained its spot as leading producer of sunflower seeds globally for the past 10 years. It is recently reported in 2018 that, Ukraine currently (2017-2018) ranks first in sunflower production in the world with an output of 13.70 million metric tons (MMT), representing 28.9% of the total global sunflower output of 47.41 MMT [United States Department of Agriculture (USDA), 2018]. Breeding for protein content and enhancement of amino acid level of sunflower seeds received huge interest, especially in areas where soybean and rapeseed were not the key crops (Fick & Miller, 1997). Apparently, the principal production standards for confectionery hybrids which increase their market value are: seed yield, seed protein content, mass of 1000 seeds, hull/kernel ratio and dehullability of the seed

(Hladni & Miladinović, 2019). Also, studies on confectionery sunflower breeding is directed at increasing genetic potential for yield, yield stability, health safety, and nutritional value, besides increasing production efficiency (Hladni et al., 2011). Indeed, Jocić et al. (2015) advised that, newly developed confectionery hybrids should exhibit higher yield potential, higher self-fertility rate, and larger seeds with high oleic acid and vitamin E (tocopherol) content to enrich their nutritional value and extend seed shelf life. Thus, specific aims in confectionery sunflower breeding include, increase of total protein content in seed beyond 25%, increase of essential amino acid content, 1000-seed mass, kernel content, and reduction of oil content in the seed to 40% with concomitant increase of oil stability, uniform seed size and color, and enhanced seed hulling. Relatedly, it is recommended that, confectionery sunflower seed must preferably exceed 80 g 1000-seed weight, oil content less than 30%, bigger seed size, lower cadmium rate, and higher protein, oleic acid, and vitamin E (tocopherol) content (Lofgren, 1997;

Jovanović et al., 1998). Yet, it is also stated that confectionery sunflower seeds have high 1000-seed weight that is often above 100 g (Dozet & Jovanović, 1997). Also, seeds of confectionery sunflower notably possess an enormous share of hull, normally 40–50% (Jovanović, 2001).

In spite of these unique qualities/characteristics, environmental factors seems to limit current sunflower yields to the production range of 1.5-3.0 t/ha (Kaya, 2015), for a high genetic potential for seed yield exceeding 5 t/ha (Jocić et al., 2015). Recently, Kaya (2015) recommended that Breeders should pay particular attention to eliminating or minimizing extreme environmental factors to guarantee a minimum of 4 t/ha sunflower yields. A vital reserve for the attainment of plant biological potential is the application of foliar fertilizers and plant growth regulators (PGRs). Foliar fertilization of crops offers a valuable supplement to the application of nutrients through the soil and in certain conditions, foliar fertilization is more economical and effective (Fageria et al., 2009). Of significance, this mode of fertilizer application guarantees instant uptake and translocation of nutrients to several plant organs via the leaf tissues and thus supports prompt correction of nutrient deficiencies (Fageria et al., 2009). Besides, foliar fertilization is endorsed for integrated plant production as it not only increases crop yield and quality, but is as well ecologically safe (Fageria et al., 2009; El-Aal et al., 2010; Zodape et al., 2011). This is because the nutrients are directly delivered to the plant in limited amounts, thereby assisting to reduce the environmental impact linked with soil fertilization (Fernández & Eichert, 2009).

PGRs according to Oosterhuis and Robertson (2000) involve a wide-ranging category of compounds that promote, inhibit, or otherwise alter plant physiological or morphological processes. It is also acknowledged that, the agricultural practice that is effectively employed to eliminate the negative impacts of stressful condition on crop productivity is the application of PGRs (Calvo et al., 2014). The positive effect of foliar fertilizers and plant growth regulators on yield and quality of sunflower is also reported (Vyakaranahal et al., 2001; Shaker & Mohammed, 2011;

Hassanlouee & Baghbani, 2013; Mátyás et al., 2014; Khan et al., 2015; Klimenko, 2015; Mekki, 2015; Ernst et al., 2016; Eremenko, 2018; Melnyk et al., 2019). However, recently in 2019, Melnyk and collaborators seems to be the first to have reported in the Left-Bank Forest-Steppe of Ukraine, the effect of foliar fertilizers and plant growth regulators on productivity and quality of high-oleic sunflower (Melnyk et al., 2019). The influence of these foliar applications on confectionery sunflower genotypes in the Left-Bank Forest-Steppe of Ukraine is yet to be documented. Besides, market demands and production area of confectionery sunflower indicate a steady increase globally and in Eastern Europe too, owing to its nutritional value and use in human nutrition (Hladni & Miladinović, 2019). In the year 2017, 740 sunflower genotypes were registered and approved for distribution in Ukraine, with 22 classified as confectionery types [Ukrainian Institute for Plant Variety Examination (UIPVE), 2017].

The present study therefore investigates the influence of foliar fertilizers and plant growth regulators on seed yield, yield components and quality of confectionery sunflower under the climatic condition of the Left-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

A three-year (2016-2018) field research was performed in Poltava region in the Left-Bank Forest-Steppe of Ukraine. The experimental site was located about 10 km SW from Poltava (Latitude: 49.6; Longitude: 34.9; 113 m above sea level) on black soil, typical for coarse-medium loam. Seeds of three confectionery sunflower genotypes (Confeta F1, Lakomka, Oniks) were sown in first decade of May and harvested in the third decade of September of the investigated years. The origins of the genotypes are as follows: Confeta F1 (May Agro Tohumculuk Sanayive Tisaret A.S., Turkey); Lakomka (State Scientific Institution All-Russian Research Institute of oil crops named after. V.S. Pustovoit, Russia); Oniks (Department of Crop Production of Sumy National Agrarian University, Ukraine).

Data on rainfall and air temperature during the growing period were acquired from Poltava

Regional Center for Hydrometeorology of Ukraine. Analysis of weather conditions, in particular Hydrothermal coefficient (HTC) as described by Selyaninov (1937), revealed that the growing season for the research years were characterized as follows: 2016 - sufficiently wet (normal moisture) year (HTC = 1.00); 2017 - extremely dry year (HTC = 0.45); 2018 - moderately dry year (HTC = 0.59). HTC were

calculated using the formula: $HTC = \frac{\Sigma p \times 10}{\Sigma t}$, where Σp is the amount of precipitation/rainfall (mm), for a period with an average daily air temperature above Σt is the sum of temperatures ($^{\circ}C$), for the period with average daily air temperature above $10^{\circ}C$. The key weather indexes for the studied periods are given in Table 1.

Table 1. Weather indexes for the period of field trials with confectionery sunflower

Year	2016		2017		2018		Long-term average	
	AT ($^{\circ}C$)	RA (mm)						
May	15.1	109.4	14.8	39.4	18.1	30.6	15.8	47.9
June	19.9	71.4	20.1	41.2	20	48.6	19.4	63.2
July	22.6	40.3	21.5	29.9	22.6	49.8	21.8	60.7
August	22.6	62.7	24	7.2	23	2.2	20.9	38.3
September	15.2	7	18	16.6	18.4	51.8	14.9	7
Total	95.4	290.8	98.4	134.3	102.1	183	92.8	217.1

Note: AT - Air Temperature; RA - Rainfall Amounts.

The trials were established in a Randomized Complete Block Design (RCBD) with 4 replications on a plot size of 25 m². The main plots had the sunflower genotypes while the sub plots were 7 different foliar applications (control; Sol Bor + Basfoliar 6-12-6; Basfoliar 6-12-6; Wuxal Bio Aminoplant + Wuxal Boron; Wuxal Boron; Spectrum Askorist + Spectrum B + Mo; Spectrum B + Mo). Seeds were sown for a plant density of 40,000 plants/ha with 4 rows in each plot. An inter row space of 70 cm was maintained. Application of background fertilizer to the soil was at the rate of N₉₄P₄₈K₄₈. Sowing was done using the New Holland tractor with 8 rows Baural Planter Maxima in the unit. Chemical and mechanical methods were employed to effectively control weeds in high debris field conditions. Soil herbicide Prim Extra TZ Gold 500 SC (4.5 l/ha), composing the active substances S-Metolachlor (290 g/l) + Atrazine (370 g/l), was also applied. All other cultural practices including pest and disease control were executed.

Foliar fertilizers and plant growth regulators were sprayed sequentially twice or thrice on sub plots at recommended rates as follows: Sol Bor (3 l/ha); Basfoliar 6-12-6 (6 l/ha); Wuxal

Bio Aminoplant (3 l/ha); Wuxal Boron (3 l/ha); Spectrum Askorist (3 l/ha); Spectrum B+Mo (2.5 l/ha). Foliar applications were undertaken at the following stages using the BBCH scale: BBCH 15-17; BBCH 27-37; and BBCH 47-57 (Meier, 2001). Accordingly, Sol Bor, Wuxal Bio Aminoplant and Spectrum Askorist were applied first, before respectively adding Basfoliar 6-12-6, Wuxal Boron and Spectrum B + Mo twice. These new set of foliar fertilizers (Basfoliar 6-12-6, Wuxal Boron and Spectrum B + Mo) were also applied in sequence twice on sub-plots lacking the first applications. Hence, there were double applications (Basfoliar 6-12-6, Wuxal Boron, Spectrum B + Mo) and triple applications (Sol Bor + Basfoliar 6-12-6; Wuxal Bio Aminoplant + Wuxal Boron; Spectrum Askorist + Spectrum B + Mo). Plots with no foliar applications served as control. Foliar preparations were obtained from popular companies including: ADOB (Sol Bor, Basfoliar 6-12-6); Unifer (Wuxal Bio Aminoplant, Wuxal Boron); and Spectrum-Agro (Spectrum Askorist, Spectrum B + Mo). Nutrient compositions of foliar fertilizers and plant growth regulators are presented (Table 2).

Table 2. Nutrient compositions of foliar applications

Foliar applications	N	P ₂ O ₅	K ₂ O	MgO	Mn	Cu	Fe	B	Zn	Mo	Amino acids	*EM B
Sol Bor (%)								15.0				
Basfoliar 6-12-6 (%)	7.2	14.4	7.2	0.012	0.012	0.012	0.012	0.012	0.06	0.005		
Wuxal Bio Aminoplant (g/l)	22.6	22.6	22.6								141.3	
Wuxal Boron (g/l)	110	137.0			0.69	0.69	1.37	95.9	0.69	0.014		
Spectrum Askorist (%)	3.7	1.76	3.0		0.02	0.003	0.01	0.014	0.01	0.001		20
Spectrum B+Mo (%)								15.0		0.75		

*Seaweed extract of *Ascophyllum nodosum*

Data on the following parameters were collected and/or determined from two inner rows of 5 representative tagged plants at reproductive stage (R-1 to R-4) prior to flowering (Schneider and Miller, 1981): plant height; width of 7th leaf and length of 7th leaf, before leaf surface area was calculated using the method described by Osipova and Litun (1988). Harvesting was done manually at physiological maturity by harvesting the 5 tagged plants from two inner rows per plot. Accounting, measurement, and related observations were undertaken according to the methods of field experience by Dospekhov (1985). Seed protein content was determined

using an infrared analyzer SupNir 2700. Data were subjected to statistical analysis of variance (ANOVA) followed by least significant difference (LSD) test at 5% level of probability ($p < 0.05$) using the software Statistica (version 8) (StatSoft. Inc.).

RESULTS AND DISCUSSIONS

Among the three confectionery sunflower genotypes investigated, Lakomka created a significantly ($P < 0.05$) taller average plant (192.6 cm) (Table 3). The difference in average plant height between Confeta F1 (139.3 cm) and Oniks (114.0 cm) is also significant.

Table 3. Effect of foliar applications on average plant height of confectionery sunflower genotypes for the investigated period (2016-2018) (cm)

Foliar applications (Factor B)	Genotypes (Factor A)			
	Confeta F1	Lakomka	Oniks	Average (Factor B)
Control	138.0	191.4	109.4	146.3
Sol Bor + Basfoliar 6-12-6	138.1	193.3	111.4	147.6
Basfoliar 6-12-6	138.6	192.7	111.3	147.5
Wuxal Bio Aminoplant + Wuxal Boron	140.9	193.0	118.3	150.7
Wuxal Boron	139.3	193.8	116.2	149.8
Spectrum Askorist + Spectrum B + Mo	140.2	192.2	116.1	149.5
Spectrum B + Mo	139.7	192.0	115.6	149.1
Average (Factor A)	139.3	192.6	114.0	

The least significant difference (LSD) at $p < 0.05$: A - 1.12; B - 1.71 cm

When Wuxal Bio Aminoplant was applied once before spraying Wuxal Boron twice (sequential triple application), it generated the tallest average plant (150.7 cm). However, this did not result in a significantly ($P < 0.05$) taller plant compared to foliar applications of Wuxal Boron (149.8 cm), Spectrum Askorist + Spectrum B + Mo (149.5 cm), and Spectrum B + Mo (149.1 cm). Except for Sol Bor + Basfoliar 6-12-6 (147.6 cm) and Basfoliar 6-

12-6 (147.5 cm), all other foliar applications produced a significantly higher plant height than the control (146.3 cm). Regarding all the individual genotypes, the control had the least average plant height compared to each foliar spray. The increase in average plant height due to foliar sprays ranged from 1.2-4.4 cm. Similar effects were recently reported for high oleic sunflower in that, the least plant height was recorded for the control (Melnyk et al., 2019).

The greatest influence on plant height was caused by the genotypes with a share of 93.5%. The second factor was the combination of genotypes and foliar applications (6%) while foliar applications was next with a share of 0.2%. Other factors had a 0.3% influence on plant height. In contrast, it is reported that, the greatest effect on plant height emanated from the combination of hybrids and foliar applications with a share of 75.6%, before hybrids (19.1%), foliar applications (3.1%) and other factors (2.2%). The difference in the level of influence of these factors could be due to differences in genetics since the previous study involved high oleic sunflower hybrids while the present research is based on the confectionery sunflower genotypes.

Leaf area growth determines light interception and is a chief parameter that determines plant productivity (Gifford et al., 1984; Koester et al., 2014). Also, Leaf area and Leaf area index are notably good surrogate measures of plant

and crop photosynthesis which is a central factor of growth rate and ultimate seed yield (Al-Amery et al., 2011). Lakomka formed significantly ($P<0.05$) larger mean leaf surface area ($0.76 \text{ m}^2/\text{plant}$) than Confeta F1 ($0.72 \text{ m}^2/\text{plant}$) and Oniks ($0.59 \text{ m}^2/\text{plant}$). Additionally, that of Confeta F1 was significantly larger than Oniks (Table 4). With respect to foliar applications, triple applied Spectrum Askorist + Spectrum B + Mo formed the largest average leaf surface area ($0.72 \text{ m}^2/\text{plant}$) but was only significantly ($P<0.05$) higher than foliar application of Basfoliar 6-12-6 ($0.68 \text{ m}^2/\text{plant}$), Wuxal Boron ($0.68 \text{ m}^2/\text{plant}$) and control ($0.65 \text{ m}^2/\text{plant}$). Also, all foliar applications generated a significantly larger leaf surface area than the control. In a related study with sunflower, similar results were recently reported (Al-Amery et al., 2011; Melnyk et al., 2019). There was a $0.03\text{-}0.07 \text{ m}^2/\text{plant}$ extension in leaf surface area based on foliar applications.

Table 4. Effect of foliar applications on mean leaf surface area of confectionery sunflower genotypes for the investigated period (2016-2018) (m^2/plant)

Foliar applications (Factor B)	Genotypes (Factor A)			
	Confeta F1	Lakomka	Oniks	Average (Factor B)
Control	0.67	0.71	0.57	0.65
Sol Bor + Basfoliar 6-12-6	0.70	0.82	0.62	0.71
Basfoliar 6-12-6	0.68	0.77	0.60	0.68
Wuxal Bio Aminoplant + Wuxal Boron	0.77	0.76	0.58	0.70
Wuxal Boron	0.75	0.72	0.57	0.68
Spectrum Askorist + Spectrum B + Mo	0.76	0.79	0.60	0.72
Spectrum B + Mo	0.74	0.76	0.59	0.70
Average (Factor A)	0.72	0.76	0.59	

The least significant difference (LSD) at $p<0.05$: A - 0.02; B - $0.03 \text{ m}^2/\text{plant}$

In all the genotypes, three-fold foliar spray formed a larger leaf surface area compared to their respective two-fold spray only. As well, all the varieties/hybrid formed a higher leaf surface area for the foliar sprays than control, except Oniks, which formed equal leaf surface area in the control and foliar applied Wuxal Boron ($0.57 \text{ m}^2/\text{plant}$). Together, genotypes and foliar applications influenced leaf surface area the greatest with a share of 58.2%. This was followed by only genotypes (33.1%) before foliar applications (2.9%). Other factors had an influence of 11.9%. Likewise, Melnyk et al. (2019) recently reported that, together, hybrids and foliar applications had the largest influence

on leaf surface area with a share of 47.5%. This was followed by only foliar applications (29.9%) before hybrids (10.7%), while other factors had an effect of 11.9%.

Lakomka produced a significantly ($P<0.05$) greater average number of seeds per head (789.3 pcs) among the three studied genotypes (Table 5). The next highest number of seeds per head was generated by Oniks (710.6 pcs), which was also significantly higher than Confeta F1 (388.2 pcs).

Regarding foliar sprays, double applied Basfoliar 6-12-6 and triple applied Sol Bor + Basfoliar 6-12-6 respectively caused a significantly ($P<0.05$) higher number of seeds

per head (643.6 and 640.7 pcs) compared to the control (621.0 pcs) but not for the other foliar applications. The others caused a lesser number of seeds per head in a decreasing sequence as follows: Spectrum B + Mo (629.3 pcs), Wuxal Boron (624.8 pcs), Spectrum Askorist + Spectrum B + Mo (624.3 pcs), and Wuxal Bio Aminoplant + Wuxal Boron (621.9 pcs). These other foliar applications also did not result in a significantly higher number of seeds per head

compared to control. Working with high-oleic sunflower in the same environmental condition, Melnyk et al. (2019) recently reported similar results. However, all of these same foliar applications caused a significant increase in this parameter. The inconsistencies could be due to differences in genetics of confectionery and high oleic sunflower. The increases in average number of seeds per head presently due to foliar sprays were in the range of 0.9-22.6 pcs.

Table 5. Effect of foliar applications on average number of seeds per head of confectionery sunflower genotypes for the studied period (2016-2018) (pcs)

Foliar applications (Factor B)	Genotypes (Factor A)			Average (Factor B)
	Confeta F1	Lakomka	Oniks	
Control	381.7	776.0	705.3	621.0
Sol Bor + Basfoliar 6-12-6	383.9	827.7	710.4	640.7
Basfoliar 6-12-6	395.8	821.3	713.6	643.6
Wuxal Bio Aminoplant + Wuxal Boron	386.9	771.5	707.4	621.9
Wuxal Boron	400.6	768.6	705.1	624.8
Spectrum Askorist + Spectrum B + Mo	379.9	779.0	713.9	624.3
Spectrum B + Mo	388.8	780.9	718.3	629.3
Average (Factor A)	388.2	789.3	710.6	

The least significant difference (LSD) at $p < 0.05$: A - 20.11; B - 13.22 pcs

Furthermore, triple foliar spray of Wuxal Bio Aminoplant + Wuxal Boron on Confeta F1 generated a little higher number of seeds per head (386.9 pcs) compared to only dual applied Wuxal Boron (400.6 pcs), but the reverse was obtained for Lakomka and Oniks. For all 3 genotypes, dual application of only Spectrum B + Mo produced a slightly greater number of seeds per head (Confeta F1, 388 pcs; Lakomka, 780.9 pcs; Oniks, 718.3 pcs) than triple applied Spectrum Askorist + Spectrum B + Mo (Confeta F1, 379.9 pcs; Lakomka, 779.0 pcs; Oniks, 713.9 pcs). Largely, all foliar sprays resulted in somewhat higher number of seeds per head than the control, except that, for Lakomka, foliar sprayed Wuxal Bio Aminoplant + Wuxal Boron produced a lesser number of seeds per head (771.5 pcs) compared to the control (776.0 pcs). Similar exception occurred for Oniks sprayed with Wuxal Boron, generating 705.1 pcs and control 705.3 pcs. For all 3 genotypes, Lakomka generated a significantly ($P < 0.05$) greater average seed weight per head (65.4 g) (Table 6). The difference between Oniks (55.9 g) and Confeta F1 (51.5 g) is also significant. Among the foliar sprays, spraying of Sol Bor + Basfoliar 6-12-6

in sequence thrice, caused the greatest average seed weight per head (59.6 g). In a descending order, the other foliar applications with their corresponding average seed weight per head were as follows: Basfoliar 6-12-6 (58.5 g), Wuxal Bio Aminoplant + Wuxal Boron (57.8 g), Spectrum Askorist + Spectrum B + Mo (57.6 g), Spectrum B + Mo (57.2 g), Wuxal Boron (57.0 g), and control (55.6 g). All foliar applications generated a significantly ($P < 0.05$) higher average seed weight per head than the control, except for Wuxal Boron and Spectrum B + Mo. Additionally, the differences among Sol Bor + Basfoliar 6-12-6, Basfoliar 6-12-6, and Wuxal Bio Aminoplant + Wuxal Boron are not significant ($P < 0.05$). A similar result was reported for the application of boron fertilizers (Vyakaranahal, 2001). In the present work, the seed weight per head had an increase of 1.4-4 g based on foliar sprays. The seed weights per head for the triple foliar applications were a bit greater than for dual applications in all 3 confectionery genotypes. As well, in all the genotypes, each foliar spray produced a little greater seed weight per head compared to their respective control.

Table 6. Effect of foliar applications on average seed weight per head of confectionery sunflower genotypes for the investigated period (2016-2018) (g)

Foliar applications (Factor B)	Genotypes (Factor A)			Average (Factor B)
	Confeta F1	Lakomka	Oniks	
Control	49.2	62.7	54.8	55.6
Sol Bor + Basfoliar 6-12-6	51.4	70.6	56.9	59.6
Basfoliar 6-12-6	51.1	68.0	56.3	58.5
Wuxal Bio Aminoplant + Wuxal Boron	53.9	63.8	55.6	57.8
Wuxal Boron	53.0	63.1	55.0	57.0
Spectrum Askorist + Spectrum B + Mo	51.1	65.2	56.4	57.6
Spectrum B + Mo	50.9	64.5	56.1	57.2
Average (Factor A)	51.5	65.4	55.9	

The least significant difference (LSD) at $p < 0.05$: A - 2.12; B - 1.85 g

The greatest average 1000-seed weight was created in Confeta F1 (132.1 g) and this was significantly ($P < 0.05$) greater than the other two genotypes (Lakomka and Oniks) (Table 7). As well, Lakomka had significantly greater 1000-seed weight (82.9 g) than Oniks (78.6 g).

However, it should be noted that the huskiness (husk content) of Confeta F1 was also the highest (52.2%). Respectively, Lakomka and Oniks had a lesser husk content of 32.4% and 30.2%.

Table 7. Effect of foliar applications on average 1000-seed weight of confectionery sunflower genotypes for the investigated period (2016-2018) (g)

Foliar applications (Factor B)	Genotypes (Factor A)			Average (Factor B)
	Confeta F1	Lakomka	Oniks	
Control	124.6	80.8	77.7	94.4
Sol Bor + Basfoliar 6-12-6	133.9	85.3	80.1	99.8
Basfoliar 6-12-6	129.1	82.8	78.9	96.9
Wuxal Bio Aminoplant + Wuxal Boron	139.3	82.7	78.6	100.2
Wuxal Boron	132.3	82.1	78.0	97.5
Spectrum Askorist + Spectrum B + Mo	134.5	83.7	79.0	99.1
Spectrum B + Mo	130.9	82.6	78.1	97.2
Average (Factor A)	132.1	82.9	78.6	

The least significant difference (LSD) at $p < 0.05$: A - 4.19; B - 6.40 (g)

Among the foliar sprays, Wuxal Bio Aminoplant + Wuxal Boron caused the greatest average 1000-seed weight (100.2 g). But, this was not significantly ($P < 0.05$) greater than the other treatments. The rest of the foliar sprays generated the following average 1000-seed weights in a reducing sequence: Sol Bor + Basfoliar 6-12-6 (99.8 g), Spectrum Askorist + Spectrum B + Mo (99.1 g), Wuxal Boron (97.5 g), Spectrum B + Mo (97.2 g), Basfoliar 6-12-6 (96.9 g), and control (94.4 g). Within the genotypes, sequential foliar sprays three-times generated a slightly greater mass of 1000 seeds than their corresponding double sprays. In addition, all foliar applications gave a slightly

higher 1000-seed weight than the control. Due to foliar applications, average 1000-seed weight increased by 2.5-5.8 g. The greatest influence was caused by genotypes (share 83.6%). This was followed by combination of genotypes and foliar applications (11.4%) before foliar applications only (0.5%). Other factors had a 4.5% influence.

Lakomka produced significantly ($P < 0.05$) higher average seed yield (2.42 t/ha) than Oniks (2.33 t/ha) and Confeta F1 (2.15 t/ha) (Table 8). Also, there was a significant difference between the seed yield of Oniks and Confeta F1.

Table 8. Effect of foliar applications on average seed yield of confectionery sunflower genotypes for the studied period (2016-2018) (t/ha)

Foliar applications (Factor B)	Genotypes (Factor A)			Average (Factor B)
	Confeta F1	Lakomka	Oniks	
Control	2.05	2.32	2.28	2.22
Sol Bor + Basfoliar 6-12-6	2.14	2.61	2.37	2.37
Basfoliar 6-12-6	2.13	2.52	2.34	2.33
Wuxal Bio Aminoplant + Wuxal Boron	2.24	2.36	2.31	2.30
Wuxal Boron	2.21	2.33	2.29	2.28
Spectrum Askorist + Spectrum B + Mo	2.13	2.41	2.35	2.30
Spectrum B + Mo	2.12	2.39	2.34	2.28
Average (Factor A)	2.15	2.42	2.33	

The least significant difference (LSD) at $p < 0.05$: A - 0.02; B - 0.03 t/ha

Foliar spray of Sol Bor + Basfoliar 6-12-6 produced significantly greater average seed yield (2.37 t/ha) than all other treatments as follows: Basfoliar 6-12-6 (2.33 t/ha); Wuxal Bio Aminoplant + Wuxal Boron or Spectrum Askorist + Spectrum B + Mo (2.30 t/ha); Wuxal Boron or Spectrum B + Mo (2.28 t/ha). As well, all foliar applications produced a significantly higher average seed yield than the control (2.22 t/ha). Similar results were recently reported (Al-Amery et al., 2011; Shaker & Mohammed, 2011; Khan et al., 2015; Ernst et al., 2016; Melnyk et al., 2019). Previously, Reddy et al. (2003) suggested that, increased achene (seed) yield of sunflower might be due to active role of boron in translocation of photosynthates particularly when applied at ray floret stage. Also, Al-Amery et al. (2011) reported that, the greatest effect of foliar applied boron is an increase in seed yield, and this partially might be due to reduction in seed sterility. Moreover, Brighenti and Castro (2008) demonstrated that, seed yield was increased by foliar application of boron on sunflower, and indicated that boron consumption increased the pollen fertility. Except for Wuxal Bio Aminoplant, all foliar applications contain boron in the present study.

Hence, similar reasons are attributable in the present study for the increased seed yield. The increase in average seed yield in the present study ranged from 0.06-0.15 t/ha because of foliar applications. However, Černý and Veverková (2012) found no statistically significant effect on sunflower seed yield after foliar application of two PGRs (Atonik and Pentakeep - V).

For all the genotypes, sequential foliar applications in threefold generated slightly higher seed yield than their corresponding applications in twofold. As well, all foliar applications for each genotype produced a little higher seed yield than the control. The greatest effect on average seed yield was triggered by the combined factors of genotypes and foliar applications (share is 74.7%). The second greatest influence was by genotypes (17.9%) and the third by foliar applications (2.7%). Other factors had a 4.7% effect on the average seed yield.

The main interest in confectionery sunflower is the seed protein content. Among the 3 genotypes investigated, Lakomka produced the highest average protein content (23.0%), followed by Confeta F1 (21.7%) and the least by Oniks (21.2%) (Figure 1).

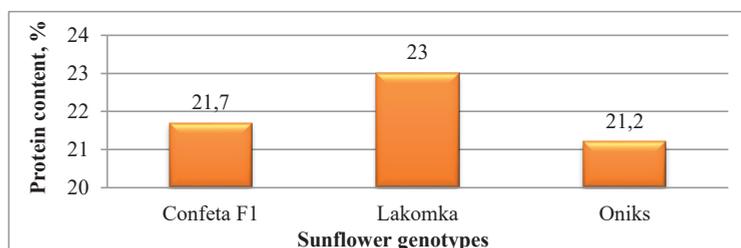


Figure 1. Effect of foliar applications on average protein content of confectionery sunflower genotypes

Sequential foliar application of either Wuxal Bio Aminoplant + Wuxal Boron thrice or Wuxal Boron twice, generated the highest

average protein contents (22.2%) and was followed by applying Spectrum B + Mo (22.1%) (Figure 2).

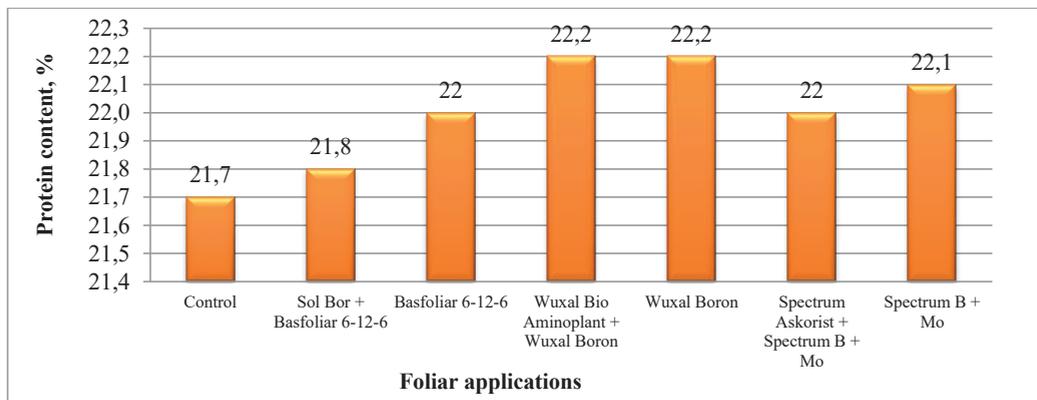


Figure 2. Average protein content of the combined sunflower genotypes as influenced by foliar applications

The next greatest average protein content (22.0%) was caused by either spraying Basfoliar 6-12-6 twice or Spectrum Askorist + Spectrum B + Mo thrice. The lowest average protein content occurred in the control (21.7%) after Sol Bor + Basfoliar 6-12-6 (21.8%).

There was an increase in average protein content from 0.1-0.5% as per the foliar sprays with average protein content for the various genotypes fluctuating between 20.7-23.2%. In a related study with high oleic sunflower hybrids using these same foliar applications, Melnyk et al. (2019) similarly reported increases in average oil content and oleic acid content by 0.6-1.6% and 1.8-4.1%, respectively.

CONCLUSIONS

Foliar applications generally had a favourable effect on all parameters studied (plant height, leaf surface area, number of seeds per head, seed weight per head, 1000-seed weight, seed yield, and protein content) compared to the control.

Significantly ($P < 0.05$) higher average seed yield occurred in the variety Lakomka (2.42 t/ha). Again, the greatest protein content was obtained from Lakomka (23.0%).

Sequential foliar application of Sol Bor + Basfoliar 6-12-6 thrice gave significantly ($P < 0.05$) greater average seed yield (2.37 t/ha) for the combined genotypes compared to all

other treatment. Based on foliar sprays, the increase in seed yield ranged from 0.06-0.15 t/ha when compared to control. Foliar sprays also caused increases in protein content between 0.1-0.5% compared to the control with average protein content for the various genotypes fluctuating between 20.7-23.2%.

In order to ensure stable high yield (2.42 t/ha) with high-quality confectionery sunflower seeds, there should be foliar application of Sol Bor (3.0 l/ha) at BBCH 15-17 and Basfoliar 6-12-6 (6.0 l/ha) at BBCH 27-37 and BBCH 47-57 on the Lakomka variety.

REFERENCES

- Al-Amery, M., Hamza, J. H., Fuller, M. P. (2011). Effect of boron foliar application on reproductive growth of sunflower (*Helianthus annuus* L.). *International Journal of Agronomy*, 5(6), 1–5. <https://doi.org/10.1155/2011/230712>
- Brighenti, A.M., Castro, C. (2008). Boron foliar application on sunflower (*Helianthus annuus* L.) associated with herbicides. *Helia*, 31(48), 127–136. <https://doi.org/10.2298/HEL0848127B>
- Calvo, P., Nelson, L., Kloepper, W.J. (2014). Agricultural uses of plant biostimulants. *Plant and Soil*, 383, 3–41. <https://doi.org/10.1007/s11104-014-2131-8>
- Černý, I., Veverková, A. (2012). Production parameters of sunflower (*Helianthus annuus* L.) influenced by weather conditions and foliar application of Pentakeep - v and Atonik. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(1), 887–896.

- Dospekhov, B.A. (1985). *Methods of field experience*, Moscow: Agropromizdat (in Russian).
- Dozet, B., Jovanović, D. (1997). Combination ability and mode of inheritance of seed size in F1 generation of confectionary sunflower [*Helianthus annuus* var. macrocarpus Dc (Ckll)]. *Selekcija i Semearstvo*, 3–4, 20–25.
- El-Aal, F.S.A., Shaheen, A.M., Ahmed, A.A., Mahmoud, A.R. (2010). Effect of foliar application of urea and amino acids mixtures as antioxidants on growth, yield and characteristics of squash. *Research Journal of Agriculture and Biological Sciences*, 6(5), 583–588.
- Eremenko, O.A. (2018). *Agrobiological bases of formation of oilseed crops productivity (Helianthus annuus L., Carthamus tinctorius L., Linum usitatissimum L.) in the Southern Steppe of Ukraine* (Doctoral dissertation). Kherson State Agrarian University, Kherson, Ukraine.
- Ernst, D., Kovár, M., Černý, I. (2016). Effect of two different plant growth regulators on production traits of sunflower. *Journal of Central European Agriculture*, 17(4), 998–1012. <https://doi.org/10.5513/JCEA01/17.4.1804>
- Fageria, N.K., Filho, M.P.B., Moreirab, A., Guimaresa, C.M. (2009). Foliar fertilization of crop plants. *Journal of Plant Nutrition*, 32(6), 1044–1064. <https://doi.org/10.1080/01904160902872826>
- Fernández, V., Eichert, T. (2009). Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Critical Reviews in Plant Sciences*, 28, 36–68. <https://doi.org/10.1080/07352680902743069>
- Fick, G.N., Miller, J.F. (1997). Sunflower Breeding. In: Schneiter, A. A., (Ed.), *Sunflower Technology and Production* (pp. 395–439). Madison, WI: American Society of Agronomy.
- Gifford, R.M., Thorne, J.H., Hitz, W.D., Giaquinta, R.T. (1984). Crop productivity and photoassimilate partitioning. *Science*, 24, 801–808. <https://doi.org/10.1126/science.225.4664.801>
- Hassanlouee, M., Baghban, F. (2013). Effects of stages and amount of nitrogen foliar application on yield and yield components in hybrid Alestar sunflower. *ARP Journal of Agricultural and Biological Science*, 8(3), 224–226.
- Hladni, N., Miladinović, D. (2019). Confectionery sunflower breeding and supply chain in Eastern Europe. *Oilseeds and fats Crops and Lipids*, 26(29), 1–9. <https://doi.org/10.1051/ocl/2019019>
- Hladni, N., Miklič, V., Jocić, S., Sakač, Z., Radić, V., Radeka, I. (2011). New confectionary sunflower hybrids in NS palette. *Field and Vegetable Crops Research*, 48, 49–56. <https://doi.org/10.5937/ratpov1101049H>
- Jocić, S., Miladinović, D., Kaya, Y. (2015). Breeding and genetics of sunflower. In: Martínez-Force, E., Dunford, N.T., Salas, J.J., (Eds.), *Sunflower: Chemistry, Production, Processing and Utilization* (1–26). Urbana, IL: AOCS Press.
- Jovanović, D. (2001). Possibilities of using sunflower and breeding for specific purposes. *Ratar Povrt*, 35, 209–221.
- Jovanović, D., Škorić, D., Dozet, B. (1998). Confectionery Sunflower Breeding. In: S.S. Sreten (Ed.), *Genetics and Breeding*. Proceedings of the 2nd Balkan Symposium on Field Crops (pp. 349–352). Novi Sad, Yugoslavia.
- Kaya, Y. (2015). Sunflower. In: Gupta, S.K., (Ed.), *Breeding oilseed crops for sustainable production: opportunities and constraints* (55–82). San Diego, CA: Elsevier.
- Khan, I., Anjum, S.A., Qardri, R.W.K., Ali, M., Chattha, M.U., Asif, M. (2015). Boosting achene yield and yield related traits of sunflower hybrids through boron application strategies. *American Journal of Plant Sciences*, 6, 1752–1759. <http://dx.doi.org/10.4236/ajps.2015.611175>
- Klimenko, I.I. (2015). Influence of plant growth regulators and micronutrient fertilizer on yield of seed of lines and hybrids of sunflower. *Selection and seed production*, 107, 183–188 (in Ukrainian).
- Koester, R.P., Skoneczka, J.A., Cary, T.R., Diers, B.W., Ainsworth, E.A. (2014). Historical gains in soybean (*Glycine max* Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. *Journal of Experimental Botany*, 65, 3311–3321. <https://doi.org/10.1093/jxb/eru187>
- Lofgren, J.R. (1997). Sunflower for confectionery food, bird food, and pet food. In: Schneiter, A.A. (Ed.), *Sunflower Technology and Production* (747–764). Madison, WI: American Society of Agronomy.
- Mátyás, M., Černý, I., Kovár, M. (2014). Sunflower (*Helianthus annuus* L.) yield-forming elements influenced by year, weather conditions and application of biological preparations Terra-Sorb and Unicum. *Journal of Microbiology, Biotechnology and Food Sciences*, 3, 131–133.
- Meier, U. (2001). Growth stages of mono- and dicotyledonous plants – BBCH Monograph. *German Federal Biological Research Centre for Agriculture and Forestry*. <http://dx.doi.org/10.5073/20180906-074619>. Retrieved from <http://www.jki.bund.de>
- Mekki, B.E.D. (2015). Effect of boron foliar application on yield and quality of some sunflower (*Helianthus annuus* L.) cultivars. *Journal of Agricultural Science and Technology B*, 5, 309–316. <http://dx.doi.org/10.17265/2161-6264/2015.05.002>
- Melnyk, A., Akuaku, J., Trotsenko, V., Melnyk, T., Makarchuk, A. (2019). Productivity and quality of high-oleic sunflower seeds as influenced by foliar fertilizers and plant growth regulators in the Left-Bank Forest-Steppe of Ukraine. *AgroLife Scientific Journal*, 8(1), 167–174.
- Oosterhuis, D., & Robertson, W.C. (2000). The use of plant growth regulators and other additives in cotton production. *Arkansas Agricultural Experiment Station Special Report 198*, Proceedings of the 2000 Cotton Research Meeting. Retrieved from

- https://www.researchgate.net/publication/242152959_The_use_plant_growth_regulators_and_other_additives_in_cotton_production/link/556deb0208aefcb861db94bf/download
- Osipova, L.S., Litun, P.P. (1988). Express method for determining the surface area of sunflower leaves. *Selection and seed production*, 64, 68–70 (in Ukrainian).
- Reddy, N.Y.A., Shaanker, R.U., Prasad, T.G., Kumar, M.U. (2003). Physiological approaches to improve harvest index and productivity in sunflower. *Helia*, 26, 81–90.
<http://dx.doi.org/10.2298/HEL0338081R>
- Schneider, A.A., Miller, J.F. (1981). Description of sunflower growth stages. *Crop Science*, 21(6), 901–903. <https://doi.org/10.2135/cropsci1981.0011183X002100060024x>
- Selyaninov, G.T. (1937). *Methods of Agricultural characteristics of climate* [World Agro-climatic reference book]. Leningrad, Moscow: Gidrometisdat. (in Russian).
- Shaker, A.T., Mohammed, S.A. (2011). Effect of different levels and timing of boron foliar application on growth, yield and quality of sunflower genotypes (*Helianthus annuus* L.). *Mesopotamia Journal of Agriculture*, 39(3), 16–26.
- Ukrainian Institute for Plant Variety Examination. (2017). State register of plant varieties suitable for dissemination in Ukraine in 2017. Retrieved from <http://sops.gov.ua/reiestr-sortiv-roslyn-ukrainy> [Retrieved July 8, 2017].
- United States Department of Agriculture. (2018). *Production, Supply, and Distribution (PSD) reports – Oilseeds*. Retrieved from <https://apps.fas.usda.gov/psdonline/app/index.html#/app/downloads> [Retrieved December 7, 2018].
- Vyakaranahal, B.S., Shekharagouda, M., Patil, S.A., Prabhakar, A.S., Giriraj, K. (2001). Effects of planting date and chemicals (TIBA and Boron) spray on seed yield, its attributes and quality of single and three-way cross sunflower hybrids during different seasons. *Seed Research*, 29(2), 127–135.
- Zodape, S.T., Gupta, A., Bhandari, S.C., Rawat, U.S., Chaudhry, D.R., Eswaran, K., Chikara, J. (2011). Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato (*Lycopersicon esculentum* Mill.). *Journal of Scientific and Industrial Research*, 70, 215–219.

AQUACROP WATERPRODUCTIVITY MODEL SIMULATION FOR MAIZE USING DIFFERENT METHODS OF CALCULATING EVAPOTRANSPIRATION

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Abstract

Maize is a drought-resistant plant, a feature ensured by low water consumption, the highly developed and deep root system, and has the ability of the plant to adapt to drought conditions by reducing the perspiration surface. In this paper, the water productivity model simulation Aquacrop was used for maize crop. AquaCrop is a crop water productivity model developed by the Land and Water Division of FAO to address food security and to assess the effect of environment and management on crop production. AquaCrop simulates yield response to water of herbaceous crops, and is particularly suited to address conditions where water is a key limiting factor in crop production. There were analyzed climatic, water, soil and crop parameters from April 1st to August 31 for the years 2014-2017 for maize crop. In the Aquacrop model we used two different methods for evaluate the evapotranspiration: simulated Evapotranspiration with ETo Calculator module and calculated Evapotranspiration using Penman-Monteith. Constantly monitoring and observation the risk/stress phenomena, lead to the most effective measures to prevent and mitigate the effects on the obtained agricultural productions.

Key words: agrometeorology, evapotranspiration, maize, productivity, water stress.

INTRODUCTION

There is an urgent need to increase the efficiency and productivity of agricultural water use in response to growing pressures on finite water resources worldwide (Schewe et al., 2014; Taylor, 2014; Richey et al., 2015). Critically, tackling this challenge will require a sound understanding of the biophysical response of crop yield to water (Steduto et al., 2012). The relationship between crop yield and water supply traditionally has been based on empirical production functions (Doorenbos and Kassam, 1979), which cannot be extrapolated reliably beyond the location for which they were developed.

Irrigations are measures of agricultural technique that consist in the directed supply of the soil with additional volumes of water, compared to those naturally received from rainfall and from the groundwater, with the purpose of intensifying agricultural production and stabilizing crops. Associated with the administration of fertilizers, with the use of varieties and hybrids with great productive

potential, with the use of different cultivation technologies, under the conditions of a good work organization, irrigation determines a substantial increase in the yield of agricultural crops.

Population growth, land use change, climate change, and increasing demand in non-agricultural sectors profoundly affect the availability and quality of water resources for irrigated agriculture. Amid increasing concerns that water scarcity and food security are among the main problems to be faced by many societies in the 21st century, a global challenge for the agricultural sector is to produce more food with less water (Greaves et al., 2016).

It is important to realize that several crop models are already available in literature to simulate yield response to water. They are used mostly by scientists, graduate students, and advanced users in highly commercial farming. However, it is also important to recognize that these models present substantial complexity and are rarely used by the majority of FAO target users, such as extension personnel, water user associations, consulting engineers,

irrigation and farm managers, planners and economists. Furthermore, these models require an extended number of variables and input parameters not easily available for the diverse range of crops and sites around the world. Some of these variables are much more familiar to scientists than to end users (e.g., leaf area index -LAI- or leaf water potential $-\psi_l$). Lastly, the insufficient transparency and simplicity of model structure for the end user were considered strong constraints for their adoption (Reference Manual, Chapter 1 - AquaCrop, 2011).

Maize is one of the most important crops known to humankind, accounting for nearly 30% of the total global grain production. The crop is cultivated on more than 197 million ha of land worldwide, producing over 1.134 million of grain (FAO, 2017).

The objective of this work was to analyze the maize productivity using soil, meteorological and groundwater as input parameters required for the AquaCrop water balance model, in order to obtain biomass and grain yield data of a rainfed-simulated corn crop, for 2014-2017 in Calarasi region.

AquaCrop is a crop water productivity model developed by the Land and Water Division of FAO to address food security and to assess the effect of environment and management on crop production.

AquaCrop simulates yield response to water of herbaceous crops, and is particularly suited to address conditions where water is a key limiting factor in crop production. AquaCrop uses only a relatively small number of explicit parameters and mostly-intuitive input-variables requiring simple methods for their determination. The calculation procedures are grounded on basic and often complex biophysical processes to guarantee an accurate simulation of the response of the crop in the plant-soil system. AquaCrop is designed to be widely applicable under different climate and soil conditions, without the need for local calibration, once it has been properly parameterized for a particular crop species. To this end the model is constructed with parameters falling into two groups. One group is considered conservative, in that the parameters should remain basically constant under different growing conditions and water

regimes. The other group encompasses parameters that are dependent on location, crop cultivar, and management practices, and must be specified by the user (Figure 1).

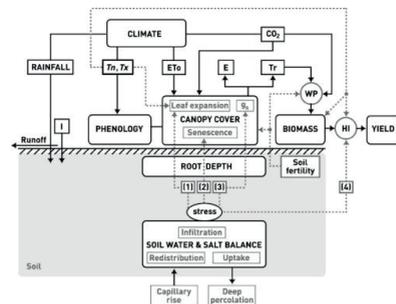


Figure 1. Chart of AquaCrop indicating the main components of the soil-plant-atmosphere continuum and the parameters driving phenology, canopy cover, transpiration, biomass production, and final yield (Reference Manual, Chapter 1 - AquaCrop, 2011)

A critical stipulation for many of the conservative parameters is that their values are based on data obtained from modern high-yielding cultivars grown with optimal soil fertility without limitation by any mineral nutrient, particularly nitrogen.

MATERIALS AND METHODS

The analysis of the thermal and water resources implies the identification of the parameters and the critical thresholds on specific calendar intervals that correspond to the process of growth and development of the maize plants during the vegetation period.

Real evapotranspiration (ETR) represents the amount of water actually lost depending on atmospheric parameters, soil and plant status. It is understood that the real evapotranspiration, in the case of a cultivated or uncultivated vegetable cover, can reach potential values only under the conditions of unlimited water supply from the soil. The intensity of water loss by evaporation from the soil or by evapotranspiration from the leaf surface, is largely determined by the vapor pressure gradient from the leaf or soil surface and from the atmosphere.

For the accomplishment of this work, the climatic data from April 1st to August 31st for the years 2014-2017 were processed in the analysis of the vegetation period of the maize

crop. Also, these data were compiled within the AquaCrop model in order to highlight the agricultural potential of the study area when using an irrigation method.

AquaCrop simulates in daily time steps because plant responses to water status are highly dynamic and cannot be easily represented as weekly or 10-day means. The model runs with 10-day or monthly mean temperature and evapotranspiration (ET_o) files, through interpolations. The results are, however, obviously approximations, and should not be used to calibrate or validate the model except as the last resort. ET_o is a key input for AquaCrop as the model calculates daily crop transpiration (Tr) and soil evaporation (E) using daily ET_o values. ET_o was calculated using the *FAO Penman-Monteith* equation from full daily weather data sets, as described by Allen et al. (1998). In this paper we also used a simulation program to do this calculation, named *ET_o Calculator* (FAO, 2009) available on the FAO website, Allen et al. (1998).

ET_o was obtained from the data recorded at the weather stations through the FAO Penman-Monteith equation and also through the ET_o Calculator application. In the ET_o Calculator application, data from a weather station can be specified in a wide variety of units, meteorological data can be imported, and procedures for estimating missing climate data are available. Climate files (*ET_o and *TMP) can be exported to AquaCrop.

ET_o represents the evapotranspiration rate on a reference surface, without water. The reference surface is considered worldwide as a large, flat surface covered with grass. The culture covers the whole soil, is well watered and develops under optimum vegetation conditions.

The rougher the approximation of ET_o, the less reliable would be the simulated results and derived AquaCrop parameters.

The climatic indicators used in the simulations performed by *ET_o Calculator* and the AquaCrop model were:

- The cumulative precipitation quantities during the April-September 2014, 2015, 2016 and 2017 periods;
- The average monthly temperatures recorded between April-September 2014, 2015, 2016 and 2017.

The ET_o Calculator application evaluates the potential reference evapotranspiration from the weather data through the FAO Penman-Monteith equation. This method was chosen by FAO because it accurately approximates the potential evapotranspiration in the analyzed perimeter, is physically based and explicitly incorporates both physiological and aerodynamic parameters.

The meteorological data needed for calculating the ET_o using the *FAO Penman-Monteith* equation: the actual duration of sunshine in a day - n (hour/day), the minimum and maximum air temperature (°C) and the rainfall (mm) between April 1st to August 31st for period 2014-2017.

The AquaCrop program can manage daily, decadal and monthly climate data. The data can be exported to several units of measurement and are data from commonly used climatic parameters. By selecting the minimum and maximum limits of the weather data, the program applies a quality check when specifying or importing data. The specified and derived climate data, including ET_o, can be exported to text files compatible with AquaCrop or graphically represented in different user-specified modes.

RESULTS AND DISCUSSIONS

In order to simulate AquaCrop response of crop production to water, were gathered daily meteorological parameters for one station, namely Călărași (NMA National Meteo Network), for the following period April 1st to August 31st for period 2014-2017.

AQUACROP model input includes different types of data: climate (meteorological), maize crop, soil, groundwater and irrigation data.

The weather data were needed to calculate the reference evapotranspiration (ET_o - calculated according to FAO standards) using the ET_o Calculator application (software developed by the FAO Department of Land and Water).

The meteorological data were uploaded: the actual duration of sunshine in a day - n (hour/day), the minimum and maximum air temperature (°C) and the rainfall (mm). In addition, there are the climatic station details to take into account, such as latitude, longitude, altitude and the location of the station, Figure 2.

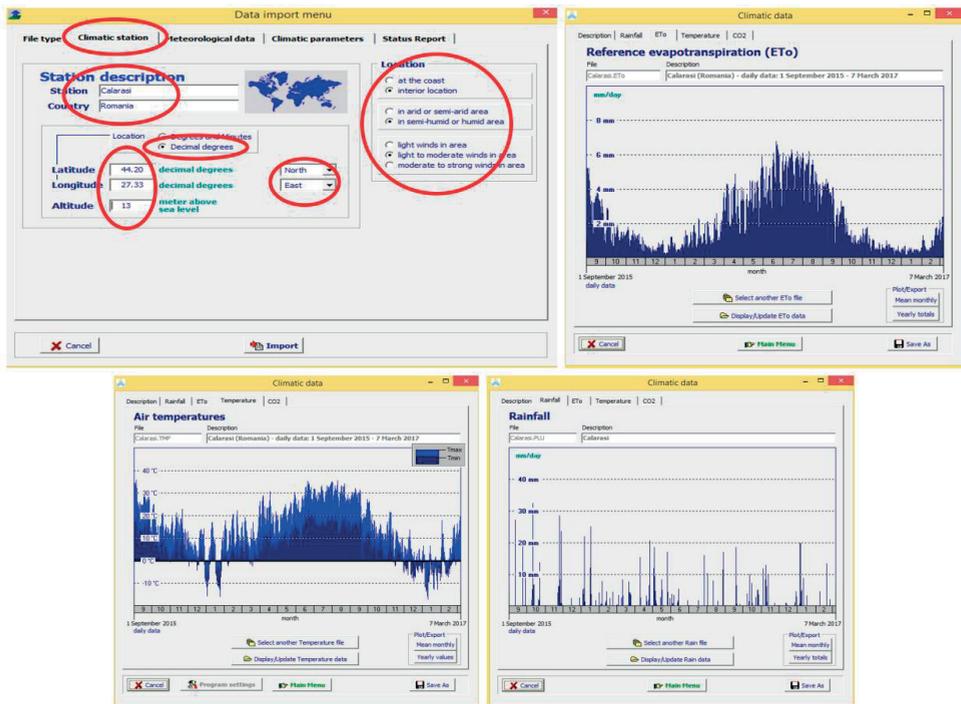


Figure 2. The climatic data for Călărași station

Maize crop data

FAO calibrated the agricultural parameters for the main crops and provided them as predefined values in this model. However, the user has the option to change these values so as to return the crop specificity according to area, climate, soil, etc. In this paper, for the characteristics of the crop were introduced data regarding the date of sowing, the method of sowing, average time intervals specific to the period of vegetation of maize, the thermal thresholds that can affect the development of the plants. For maize crop simulation, the following vegetative data were necessary (Figure 3):

- the sowing date;
- the initial canopy cover (%);
- the type of planting method, seedling (cm^2/plant);
- from day 1 after sowing to: emergence, maximum canopy cover, start of canopy senescence, maturity, flowering, maximum rooting depth (days);
- the length building up to harvest index (days);
- the duration of flowering (days);

- the threshold temperatures for crop development (minimum and maximum air temperature - $^{\circ}\text{C}$).

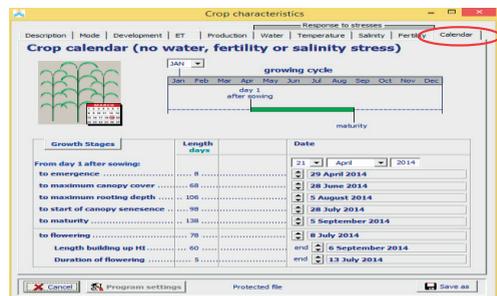


Figure 3. AquaCrop - crop data

Soil characteristics for AquaCrop simulation includes soil type, number of layers, thickness for each layer (m), curve number and readily evaporable water (mm); and the groundwater data: if the depth is constant or not, water quality, depth at which the groundwater is present (meters below the soil surface), salinity (dS/m). All the previously mention data are necessary in order to create the following AquaCrop files (Figure 4):

- 1 climate file;
- 2 crop files, for wheat and maize;
- 1 soil profile file;
- 1 groundwater file.

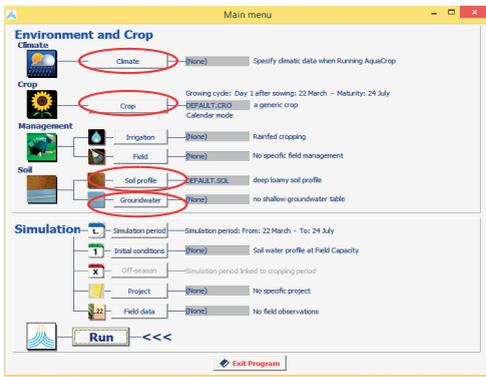


Figure 4. The AquaCrop menu and each file type created (NMA, 2018)

As for the irrigation file, the user has the option of determining whether the water needed to develop the crop comes strictly from rainfall or if a irrigation method is applied. If the land is irrigated, the irrigation method can be chosen, the watered surface, for each watering, the quality of the water is specified, the time at which it will be irrigated and the amount of water related. Also, it is possible to establish the net irrigation requirement and a program of application of the watering rules according to time and depth. Since the criteria may change during the vegetation period, the program may apply, depending on the phenological phase in progress, certain quantities of water. In this paper, the method of irrigation through furrows was chosen, ensuring an application on the whole cultivated area and requiring that the water supply available to the plants at the root level should not fall below 70% (Figure 5).

The characteristics of the groundwater considered by the model are its depth and salinity. The average depth of the groundwater in the study area is 5.8 meters, and the salinity is 1.5 dS/m (Figure 6).

For maize, precipitation registered in the summer months have a decisive influence on the final yield, and their uniform distribution is more important than the total amount of rainfall.

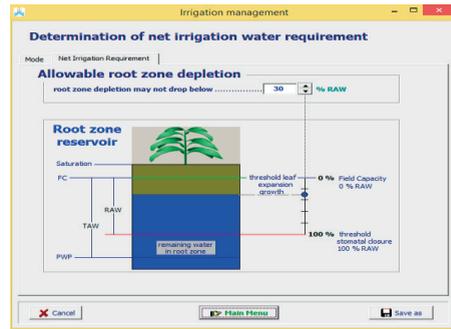


Figure 5. AquaCrop - irrigation data

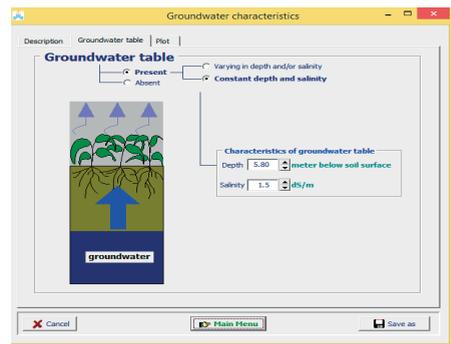


Figure 6. AquaCrop - groundwater data

It can be observed that **2014** is the **rainiest period** from those analyzed and that **2015** is the period in which the **precipitation deficit** was more pronounced (Figure 7).

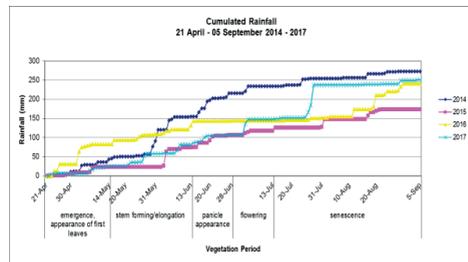


Figure 7. Cumulated rainfall, April-September, 2014-2017, Giurgiu meteorological station (NMA, 2019)

The *maximum air temperature* varies in the vegetation period, but more important are the values above 32°C registered in June 1- August 31, this being a biological threshold for resistance for the agricultural crops, affecting the growth and development processes and production in terms of quantity and quality. In accordance, in the 4 years analyzed, the number of days with the maximum air temperature which exceeds 32°C (June 1-August 31) ranges

between 25 (2014) and 46 (2015), out of a total of 92 days (Figure 8).

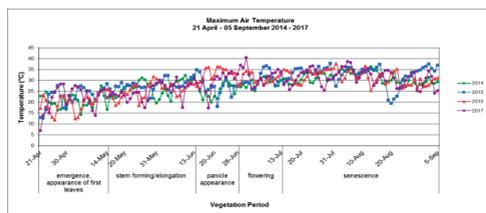


Figure 8. Maximum air temperature, April-September, 2014-2017, Giurgiu meteorological station (NMA, 2019)

In a second step, were calculated daily evapotranspiration database, based on the *Penman-Monteith equation (ETP)*.

The comparison between the two parameters of evapotranspiration, simulated with *ETo Calculator (ETo)* and calculated by *Penman-Monteith (ETP)*, is presented below.

The evapotranspiration were obtained by two different methods: simulated ETo Calculator module (FAO, 2017) and calculated Evapotranspiration using Penman-Monteith (Figure 9).

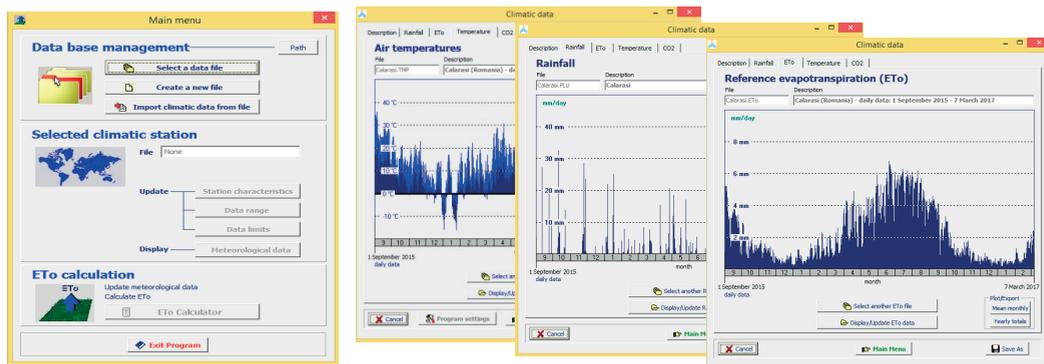


Figure 9. ETo Calculator: rainfall, reference evapotranspiration and temperature plots

Between April 21-September 5, 2014, which corresponds to the vegetation period in maize, the ETo values simulated using the ETo Calculator application are generally similar to the ETP data by the Penman-Monteith method. Deviations are between -1.4 mm/day, recorded on September 5th, in the maturity phase (milk/wax/full) and 1.0 mm on June 28, in the panicle phase, as well as on August 02, also in the maturity phase of the maize crop (Figure 10).

The estimation of the evolution of evapotranspiration (ETo) during the vegetation period active in maize cultivation in 2015 using the *ETo Calculator* application indicates values of this parameter between 1.8 mm and 7.0 mm. The potential evapotranspiration values calculated on the basis of the weather data using the *Penman-Monteith* equation for the same studied interval are between 1.6 mm and 5.8 mm/day.

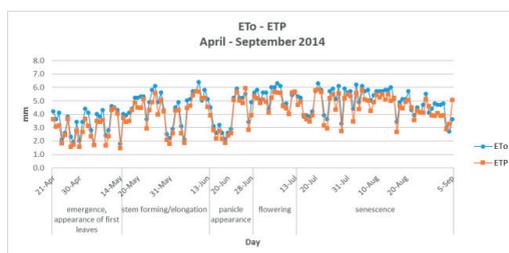


Figure 10. Dynamics of the potential evapotranspiration values between April 21 and September 5, 2014

It turns out that the differences between the two ways of estimation of the potential evapotranspiration analyzed during the whole season of maize vegetation are very small. The analysis of the values by vegetation phases indicates differences generally recorded in the canopy/first leaves (May 04) and maturity (July 7-August 10th) phases, ranging from -0.9 mm to 1.4 mm/day (Figure 11).

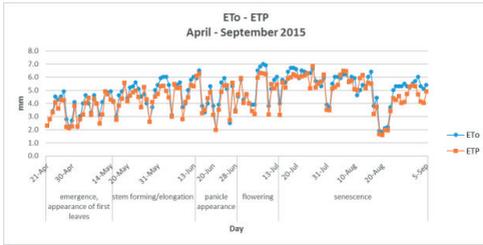


Figure 11. Comparisons of the potential and the reference evapotranspiration period April 21-September 05, 2015

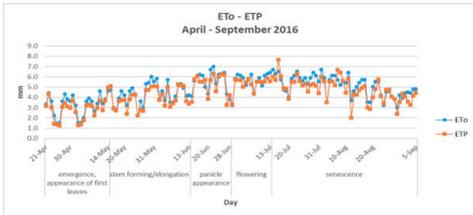


Figure 12. Comparisons of the values of potential evapotranspiration and of the reference evapotranspiration period April 21-September 05, 2016

In the agricultural year 2017, in the maize crop, the obtained values from the comparison between ETo and ETP indicate that during the whole vegetation season (April 21-September

05, 2017) the differences between the two methods of calculating the evapotranspiration they are small and have the range from -1.2 mm/day to 1.1 mm/day. The months of June, July and August are the months in which these maximum differences are recorded and correspond to the phases of panic emergence, panicle flowering and maturity in the maize crop (Figure 13).

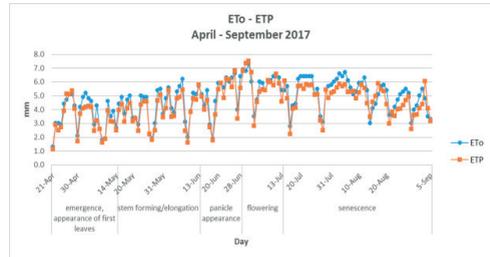


Figure 13. Comparisons of the values of potential evapotranspiration and of the reference evapotranspiration period April 21-September 05, 2017 (NMA Agromteteo)

AquaCrop run program simulates the soil water content (SWC), crop water use, crop growth, total biomass production (B) and yield (Y) based on the climatological and environmental conditions given in the files mentioned above (Figure 14).

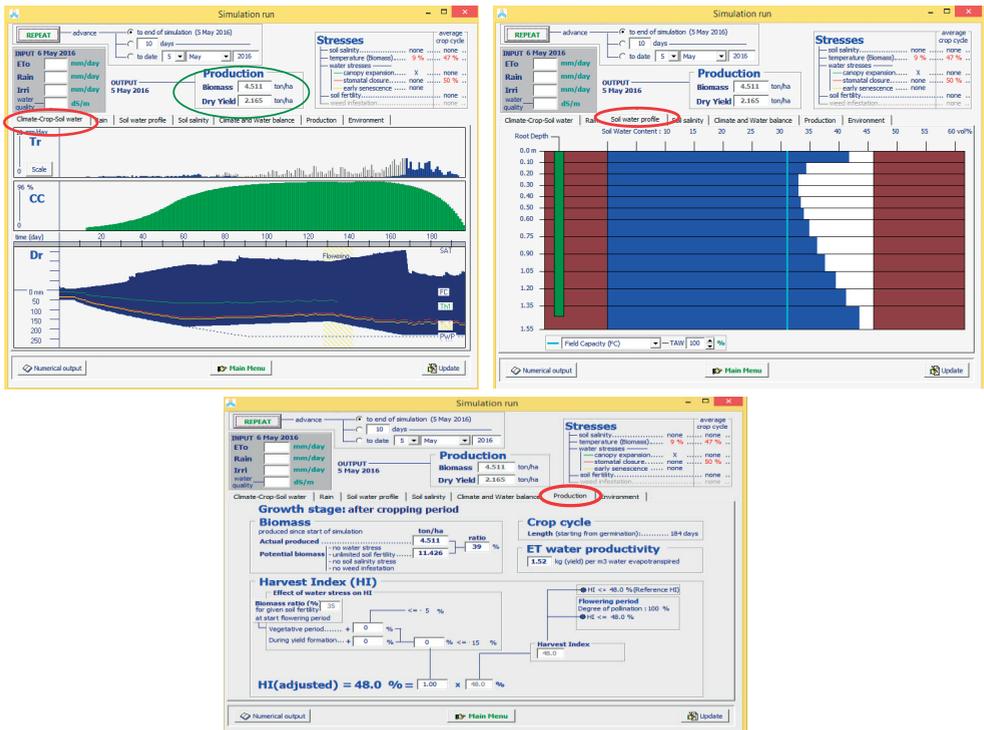


Figure 14. AquaCrop simulation products

The most productive year for the maize crop in the study area was 2014, the cumulated precipitation quantity was the highest and the number of days with the maximum air temperature which exceeds 32°C was the lowest (Figure 15).

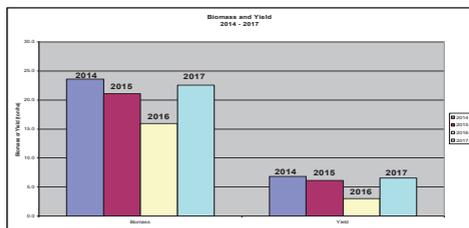


Figure 15. Biomass and Yield AquaCrop simulation

Agricultural production simulated with AquaCrop software (using calculated evapotranspiration (ETP) and simulated evapotranspiration (ETo) for maize crop in the period 2014-2017 were compared with INSSE (National Institute of Statistics data), thus, Figure 12 shows the differences between the simulated productions within the AquaCrop model by the two methods and the available statistical data, the differences being between 0 and 4 t/ha (Figure 16).

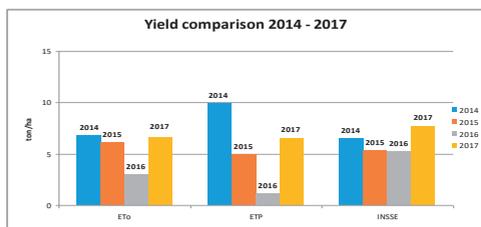


Figure 16. ETo-Calculator and FAO Penman-Monteith method comparing with the registered data of maize yield (2014-2017)

CONCLUSIONS

AquaCrop can be used as a planning or supplement / aid tool in decision making in both irrigated and non-irrigated agriculture. The model is particularly useful in: development of irrigation strategies under pedological drought conditions, studying the effect of geographical location, soil type, sowing date on agricultural production; analyzing the effect on agricultural production of different techniques of land management; comparing the possible production with the

actual production of an agricultural land, a farm or a region, better forecast of the impact of climate change on agricultural production.

AquaCrop is a water-driven simulation model that requires a relatively low number of parameters and input data to simulate the yield response to water of most major field and vegetable crops cultivated worldwide. When compared to other crop simulation models, its parameters are explicit and mostly intuitive and the model was built to achieve a balance between accuracy, simplicity, and robustness. The goal was to make the model as transparent as possible to users that generally do not belong to the research community and are not very familiar with the discipline of crop physiology.

Although the model is relatively simple, it gives particular attention to the fundamental processes involved in crop productivity and in its responses to water deficits, both from physiological and agronomic background perspectives. The fact that the simulated results agreed generally well with the measured data in the examples presented suggests that AquaCrop may be successful in achieving a good balance in simplicity, robustness, accuracy, and ease of use. These findings are particularly promising as they have been obtained with only limited calibration of the model.

The ETo values simulated using the ETo Calculator application are generally similar to the ETP data calculated from the weather data using the Penman-Monteith equation. It turns out that the differences between the two methods of determining the potential evapotranspiration analyzed over the entire growing season of maize crop are very small. The maximum differences are between -1.4 mm and 1.7 mm. These maximum deviations of the two compared parameters are generally recorded in July and August, these months corresponding to the flowering phases of the panicle and maturity (milk, wax and full) for the corn crop.

One important application of AquaCrop it is to compare the attainable with actual yields in a field, farm, or a region, to identify the constraints limiting crop production and the water productivity levels. It can also be used by economists, water agencies, and managers for scenario analysis and for planning purposes. It is suited for prospective studies such as those of

future climate change scenarios. Overall, it is particularly suited to develop agricultural water management strategies for a variety of objectives and applications.

REFERENCES

- Allen, R., Pereira, L., Raes, D. & Smith, M. (1998). Crop evapotranspiration (Guidelines for computing crop water requirements). *FAO Irrigation and Drainage Paper*, 56, Rome, 297 pp.
- Asseng, S. and Hsiao, T.C. (2000). Canopy CO₂ assimilation, energy balance, and water use efficiency of an alfalfa crop before and after cutting. *Field Crops Res.*, 67, 191–206.
- Ayers, R.S., Westcot, D.W. (1985). Water quality for agriculture. *FAO Irrigation and Drainage Paper*, 29, FAO, Rome.
- Belmans, C., Wesseling, J.G. and Feddes, R.A. (1983). Simulation of the water balance of a cropped soil: SWATRE. *J. of Hydrol.*, 63, 271–286.
- Bradford, K.J. & Hsiao, T.C. (1982). Physiological responses to moderate water stress. In: Lange, O.R., Nobel, P.S.
- Osmond, C.B., Ziegler, H., eds. Encyclopedia plant physiology new series. Physiological plant ecology II. Vol. 12B, Heidelberg, Springer, 264–324.
- Evans, L.T. (1993). *Crop evolution, adaptation and yield*. UK, Cambridge University Press, 500 pp.
- Evans, L.T. & Fischer, R.A. (1999). Yield potential: its definition, measurement, and significance. *Crop Sci.*, 39, 1544–1551.
- Hsiao, T.C., Heng, L., Steduto, P., Rojas-Lara, B., Raes, D. & Fereres, E. (2009). *AquaCrop*-the FAO crop model to simulate yield response to water: III. Model parameterization and testing for maize. *Agronomy Journal*, 101, 448–459.
- Mateescu, E., Alexandru, D. (2016). Guide for adapting agricultural technologies to climate change for the Center 7 Region. National Meteorological Administration Bucharest.
- McMaster, G.S. & Wilhelm, W.W. (1997). Growing degree-days: One equation, two interpretations. *Agricultural and Forestry Meteorology*, 87, 291–300.
- Raes, D., Steduto, P., Hsiao, T.C. & Fereres, E. (2009). *AquaCrop*-the FAO crop model to simulated yield response to water: II. Main algorithms and software description. *Agronomy Journal*, 101, 438–447.
- Raes, D., Steduto, P., Hsiao, T.C. & Fereres, E. (2011). *AquaCrop* Reference Manual. Rome, FAO (<http://www.fao.org/nr/water/aquacrop.html>).
- Steduto, P., Hsiao, T.C. & Fereres, E. (2007). On the conservative behavior of biomass water productivity. *Irrigation Science*, 25, 189–207.
- Steduto, P., Hsiao, T.C., Raes, D. & Fereres, E. (2009). *AquaCrop*-the FAO crop model to simulated yield response to water: Concepts and underlying principles. *Agronomy Journal*, 101, 426–437.
- Villalobos, F.J. & Fereres, E. (1990). Evaporation measurements beneath corn, cotton, and sunflower canopies. *Agronomy Journal*, 82, 1153–1159.
- Xu, L.K. & Hsiao, T.C. (2004). Predicted vs. measured photosynthetic water use efficiency of crops stands under dynamically changing field environments. *Journal of Experimental Botany*, 55, 2395–2411.
- ***FAO (2009). ETo calculator, land and water digital media Series, No. 36, Rome.
- ***Romanian statistical yearbook, National Institute of Statistics (2017). http://www.insse.ro/cms/sites/default/files/field/publicatii/anuar_statistic_al_romaniei_2017_format_carte.pdf.

INFLUENCES OF SOWING DATES ON THE GENERATIVE AND VEGETATIVE PROPERTIES OF DIFFERENT SWEET BASIL (*Ocimum basilicum* L.) VARIETIES

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Abstract

During the present study the effect of different sowing dates on five sweet basil varieties' ('Aromat de Buzău', 'Serafim', 'Busuioc Dulce', 'Genovese' and 'Grand Verte') parameters which are important from economic point of view (plant height, plant diameter and stem diameter, respectively average plant biomass) were compared between years (2016 and 2017). According to the results, it can be detected that in general, the IVth growth period (sowed on 11 May 2016) and the Vth and VIth growth periods (sowed on 18 May, respective 1 June 2017) had the most influential effects on sweet basil parameters except stem diameters in 2017. Plant biomass also varied between years and sowing dates. Plants of 'Genovese' and 'Serafim' varieties can be characterized with relatively low variation of the average plant weight, on the contrary 'Aromat de Buzău' had higher variations regarding average plant weight. Altogether we can conclude that the VIth period initiated on 1 June had the most positive effects on all plant parameters at all varieties, while the VIIIth period, sowed on 28 June had the most harmful effects, therefore we are not recommending this period as proper for sweet basil cultivation.

Key words: climate conditions, growth period, plant biomass, plant parameters.

INTRODUCTION

Ocimum basilicum L. (sweet basil) is an annual aromatic herb from the *Lamiaceae* Family. Native to Iran, Afghanistan and India (Asghari et al., 2012; Saha et al., 2010) it is none-the-less widely cultivated all around the world (Kiferle et al., 2011). Some species such as *Ocimum americanum* L., have insecticidal properties, while others have ornamental qualities with a particular leaf shape, size and colour, e.g. 'Purple Ruffles' (Kintzios et al., 2004; Phippen, 2000). The common sweet basil is of high economic importance because of the essential volatile oil derived from its leaves (Saha et al., 2010; Siddique & Anis, 2008; Sudhakaran & Sivasankari, 2002). These compounds also have valuable pharmaceutical, aromatic and culinary properties (Gopi & Ponmurugan, 2006; Sahoo et al., 1997). Basil is a source of rosmarinic acid but also contains caffeic acid and derivatives of lithospermic acid and lithospermic acid B, which help in

healing some renal diseases (Rady & Nazif, 2005). These compounds are also considered to be important due to their stomachic, antihelminthic, antipyretic, diaphoretic and diuretic effects, as well as in the treatment of purulent discharge of the ear and diseases of the heart and brain (Saha et al., 2010; Siddique & Anis, 2008; Singh & Sehgal, 1999). Volatiles are popular ingredients in dental and oral health care products, and the leaf extract is highly effective in inhibiting carcinogen-induced tumor development (Chandramohan & Sivakumari, 2009). Dried leaves of basil are used to flavor stew, sauces, salads, soups, meat and tea (Phippen, 2000; Siddique & Anis, 2008). Due to these high-value characteristics, sweet basil is cultivated and volatile compounds obtained cca 100 t/year (Begum et al., 2002; Daniel et al., 2010). Basil is usually propagated by sowing or by the use of seedlings (Bernáth, 2000). Under temperate climate conditions, the earlier seeding may cause substantial losses due to the negative

effect of low temperatures. While all the above parameters are highly important for growers, under temperate climate conditions, and especially when high variations in temperature and precipitations can be predicted year-by-year, one important issue, the effect of sowing date on sweet basil parameters have not been studied until now. This is crucially important for economic point of view to have clear information about the best variety that meant to be cultivated and because of maximization the bioactive compounds quantity and quality. Therefore, two main objectives were defined for this study: 1. to compare between years the effect of different sowing date on five different sweet basil varieties ('Aromat de Buzău', 'Serafim', 'Grand Verte', 'Genovese' and 'Busuioc Dulce') and 2. to test how different plant parameters (plant height, plant diameter and stem diameter, respectively average plant

biomass), that are important from economic point of view, vary with sowing period (early spring, late summer or between these periods), in two consecutive years.

MATERIALS AND METHODS

Experimental site

The field study took place in 2016 and 2017, in the Medicinal and Aromatic Plants Garden belonging to the Didactical and Research Field of the Faculty of Technical and Human Sciences, Târgu Mureş of Sapientia Hungarian University of Transylvania, situated in Târgu Mureş, Mureş County, Romania. The monthly average temperature values, respectively the sums of precipitation of 2016 and 2017 compared to the multiannual values (1971-2000 periods) are presented in Figure 1.

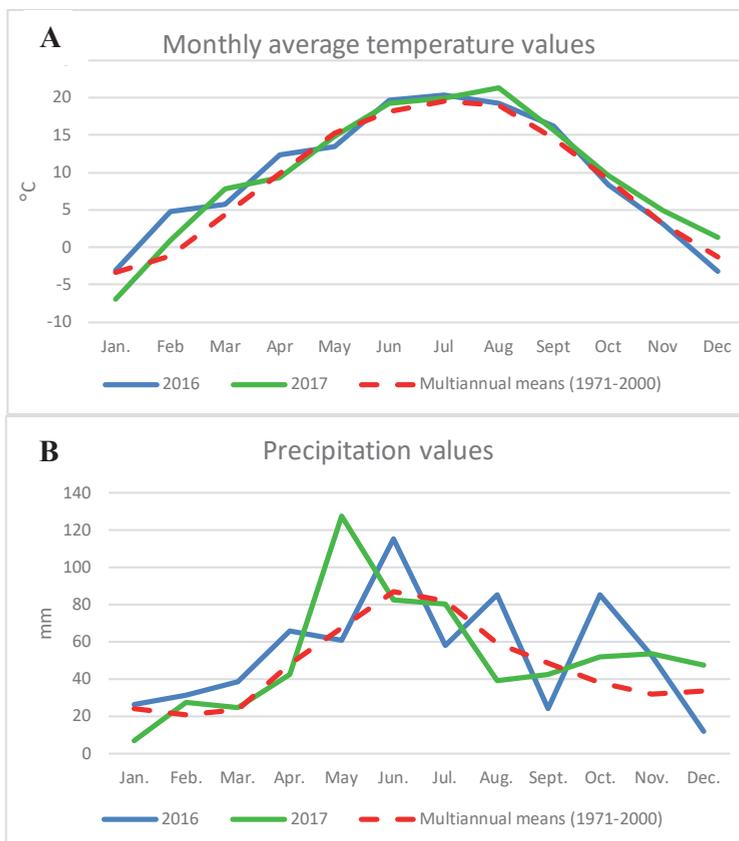


Figure 1. A: Mean daily temperature values and B: mean monthly precipitation sums of the experimental site in 2016 and 2017 compared to the multiannual (1971-2000) mean values of the region.

Considering the sums of precipitations of 2016 and 2017 it can be observed that they remained relatively similar to the multiannual values (1971-2000 periods) during the vegetation period (April-September), small differences being only in May and August. Data concerning the accumulated heat units (also called grower degree days - GDU) and the sum of precipitations during the experiment are presented also in Table 1. The accumulated heat units by each sweat basil variety were calculated by summarizing the mean daily

temperature values, starting from the sowing date of each growth period. This was also approximated with the number of days elapsed between two sampling dates. As example if one variety accumulated 9°C in 2016 by six consecutive days until 25 May while in 2017 accumulated approximately the same degree (8.93°C) in nine days until 25 May, we divided the cumulated temperature with elapsed days. Next, correlations between years were computed for each variety and each cumulated temperatures.

Table 1. Sowing and harvesting dates, length of the growth periods and, respectively grower degree-days (GDU's) and the sum of precipitations in the experimental years

Growth period	Experimental year	Sowing date	Date of harvest	Growth period (days)	GDU's (°C)	Sum of precipitations (mm)
I.	2016	4 May	-	-	-	-
	2017	4 April	-	-	-	-
II.	2016	11 May	-	-	-	-
	2017	11 April	-	-	-	-
III.	2016	19 May	29 Sept.	134	1148.1	303.6
	2017	27 April	1 Aug.	97	775.2	290.4
IV.	2016	27 May	29 Sept.	126	1116.5	287.2
	2017	11 May	8 Aug.	90	805.1	No data
V.	2016	10 June	29 Sept.	112	1015.4	247.6
	2017	18 May	22 Aug.	97	948.8	No data
VI.	2016	17 June	29 Sept.	105	967.2	211
	2017	1 June	22 Aug.	83	867.1	200.6
VII.	2016	30 June	28 Sept.	92	801.5	167.4
	2017	13 June	22 Aug.	71	760.5	No data
VIII.	2016	6 July	28 Sept.	86	732.4	164.7
	2017	28 June	27 Sept.	92	869.1	162

Plant material used in the experiment

The seed batches of 'Aromat de Buzău', 'Serafim', 'Busuioc Dulce' and 'Grand Verte' varieties were offered by the Buzău Research Station for Legumiculture (SCDL Buzău), whereas in the case of 'Genovese' variety they were purchased from a local merchant.

Preparation of the experimental field

In the autumn of 2016, a 25 cm deep plowing was performed and the same design used also in 2017. After the cold season has passed, the terrain was further worked using a roto tiller. The seedbeds were made by manual harrowing. Batches of seeds of each cultivar were sowed in eight different dates, constituting eight growth periods, which are denominated with roman numbers (I-VIII). The dates of sowing for both

years, respectively of harvesting and the lengths of the vegetation/growth periods (from sowing to harvesting) are presented in Table 1. Plants of the first two growth periods in each year marked as I and II in the Table 1 did not emerge properly and were not included in data analyses and are not presented in the results part.

Experimental design

The experimental field was divided into six equal parts (15.75 meters long and 5 meters wide), separated by one meter wide pathway, each part representing the parcel for the plants of a particular sowing date.

These parcels then were partitioned into seven plots (5 m long and 2.25 m wide), one for each basil cultivar. This way we gained a total number of 42 plots allocated for the treatments.

The seeds of a particular treatment were sowed in five rows (5 m long) with 45 cm distance in-the-row and 30 cm in-row spacing, the sowing depth being 1.5-2 cm. This way a total number of 85 plants were initiated (multiple seeds in each hole). On the date of 15.06.2016 and in 14.07.2017 the treatments were fertilized using mineral fertilizers. The applied doses were of 250 kg ha⁻¹ NPK 15:15:15 (37.5 kg ha⁻¹ for each nutrient) and 150 kg ha⁻¹ ammonium nitrate fertilizer with 33% (NH₄) (NO₃) content (50.25 kg ha⁻¹ N content).

Data collection

Data referring to the development of the plants were collected during each growing period on several times, differences occurring among the number of times of data collecting. Plant height was measured from ground level to the maximum height of the plant and expressed in centimeters. The diameter of the plant/bush was measured at its maximal value and expressed also in centimeters. The diameter of the stem was measured right above ground level and expressed in millimeters. At the end of each vegetation period plants were harvested one by one, the stems being severed at ground level using a pruning shear. The plants biomass harvested from a certain plot were weighted individually and the results were averaged constituting three repetitions. Harvest times were scheduled to fit as much as possible the flowering period of basil plants.

Data analyses

Data analyses were made using Principal Coordinates Analyses (PCoA) in PAST program. This was made because sowing was made on different dates, and plant parameters were collected according to the growing tendency. Therefore, parameters collected under different growth periods can be compared. The same method was used consecutively in 2016 and 2017. This method also allows adding parameters as heat units (GDD-s) and time elapsed between assessments. The presentations are made as bi-dimensional graphics, where different ellipses colors represent different growth periods, and the dimension and the tendency toward a

positive or negative direction of axis x or y represents negative or positive associations (growing tendency of the assessed plant parameter, i.e. ramifications) with the parameter tested (heat units in axis y and time elapsed in axis x). Altogether this representation presents graphically the tendency of each plant parameter do increase or decrease in time under different heat units. The highest and lowest values of each parameter assessed for each variety and sowing time are also represented near graphs in tables; lowest values are marked with yellow, highest values with green. Plant parameters were also compared using the similar accumulated GDDs in each year. These data were presented as bar-charts but because approximations between years were necessary because of different number of days elapsing between years to reach the same GDD, data were compared using correlation analyses between years. For these analyses linear correlations in plant parameters growth for each variety and for each GDDs were computed. Significant Rho values were presented in tables. Plants biomass was compared separately for each growth period. For 2016 data from each sowing time were normally distributed, therefore ANOVA and Tukey test were used to compare variables. For 2017 data from IIIrd to VIth sowing time were normally distributed; therefore, ANOVA and Tukey test were used to compare variables. Plant biomass data from VIIth and VIIIth growth periods were not distributed normally; therefore, Man-Whitney test was used to compare data. Results are presented in Tables 6 and 7.

RESULTS AND DISCUSSIONS

By comparing the sweet basil varieties diameter variations between years and under different sowing dates and heat units (also known as grower degree-days, GDD-s) high alterations between varieties can be detected. While the 'Aromat de Buzău', 'Serafim' and varieties have the highest diameter development when sowing was made on 18 May, respective 1 June 2016 (Vth and VIth growth period), and less at VIIIth growth period (sowed on 28 June 2016), 'Busuioc Dulce', 'Genovese' and 'Grand Verte' had a more

constant diameter growth when they were sowed on 11 May 2016 (IVth growth period) while the VIIIth growth period (sowing on 28 June 2016) has a relative harmful effect of plant diameters. No clear effect of sowing date on ‘Genovese’ can be detected, positive tendency toward VIIIth growth period (28 June 2016) however can be detected (Figure 2). Very different trends in 2017 were observed, for ‘Aromat de Buzău’ and ‘Grand Verte’ the IVth growth period (11 May 2017) was the most influential, for ‘Serafim’ and ‘Genovese’ the Vth and VIth growth periods (18 May,

respective 1 June 2017) had significant effect on diameter development, while for ‘Busuioc Dulce’ the VIth growth period (1 June 2017) was most important for plant diameter variations (Figure 2). While comparisons of plant diameters under cumulated GDD-s were tested between years, the same tendencies of variations were detected (Figure 2). When correlation analyses were made by comparing plant diameters using cumulated and approximated time periods between years, correlations were only detected for variety ‘Serafim’ (Table 2).

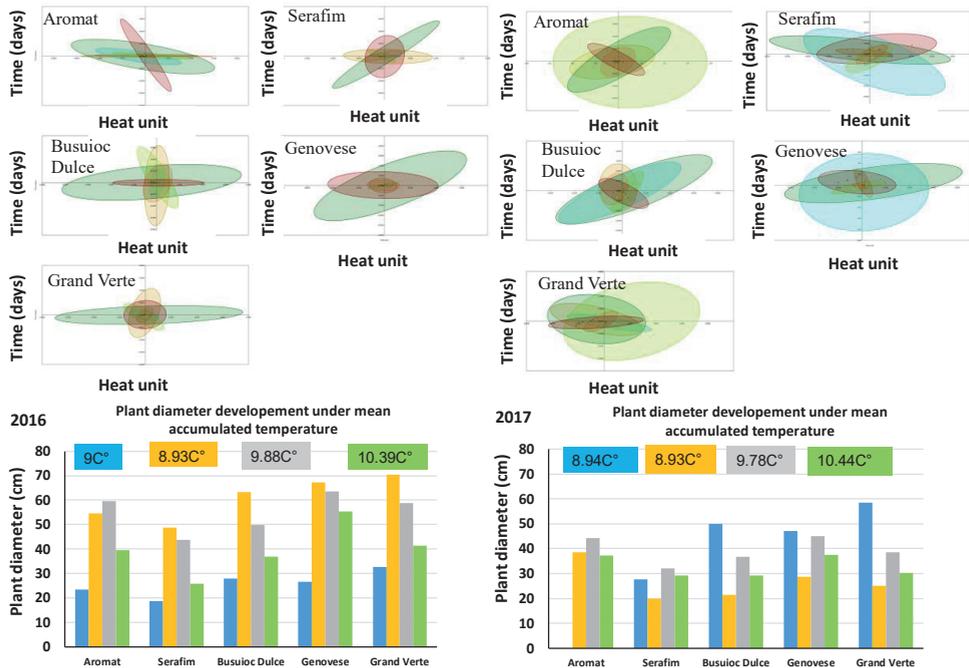


Figure 2. The effect of sowing time on sweet basil diameter variations under different heat units (air temperatures). Different colours refer to different growth periods as follows: Aqua IIIrd growth period, Chartreuse: IVth growth period, Coral Vth growth period, Darkgoldenrod: VIth growth period, Darkgreen: VIIth growth period, Darkred: VIIIth growth period

Table 2. Correlation analyses of sweet basil diameter variations between 2016 and 2017 under similar accumulated GDD-s. Before analyses the cumulated GDD-s were divided with days elapsed until the next sampling period. Bolded values represent significant positive correlations between values

2016	2017	Aromat	Serafim	Busuioc dulce	Genovese	Grand Verte
9°C	8.94°C	Rho=-	Rho=0.92**	Rho=0.32	Rho=0.34	Rho=0.17
8.93°C	8.93°C	Rho=0.09	Rho=0.90**	Rho=0.23	Rho=0.12	Rho=0.34
9.88°C	9.78°C	Rho=0.13	Rho=0.95**	Rho=0.12	Rho=0.41	Rho=0.40
10.39°C	10.44°C	Rho=0.24	Rho=0.87**	Rho=0.31	Rho=0.45	Rho=0.15

Clear positive effect of the VIIth growth period (sowed on 13 June 2016) on ‘Aromat de Buzău’, ‘Serafim’, ‘Busuioc Dulce’ and ‘Grand

Verte’ height can be detected and in all cases again temperature has harmful effect on plant height if sowing was made at 27 April. Again

no clear effect on ‘Genovese’ height can be detected at different growth periods, only a tendency of positive effect at VIIth (13 June 2016) and VIIIth growth period (28 June 2016) can be observed (Figure 3). Again very different tendencies in 2017 were detected. More influential effect on plant height variations were detected when plants were

sowed in Vth and VIth growing periods (Figure 3). Again, the plant height development under cumulated GDD-s presented a high variations between years (Figure 3) while positive correlations between plant height were detected at four three (‘Aromat de Buzău’, ‘Serafim’ and ‘Grand Verte’) (Table 3).

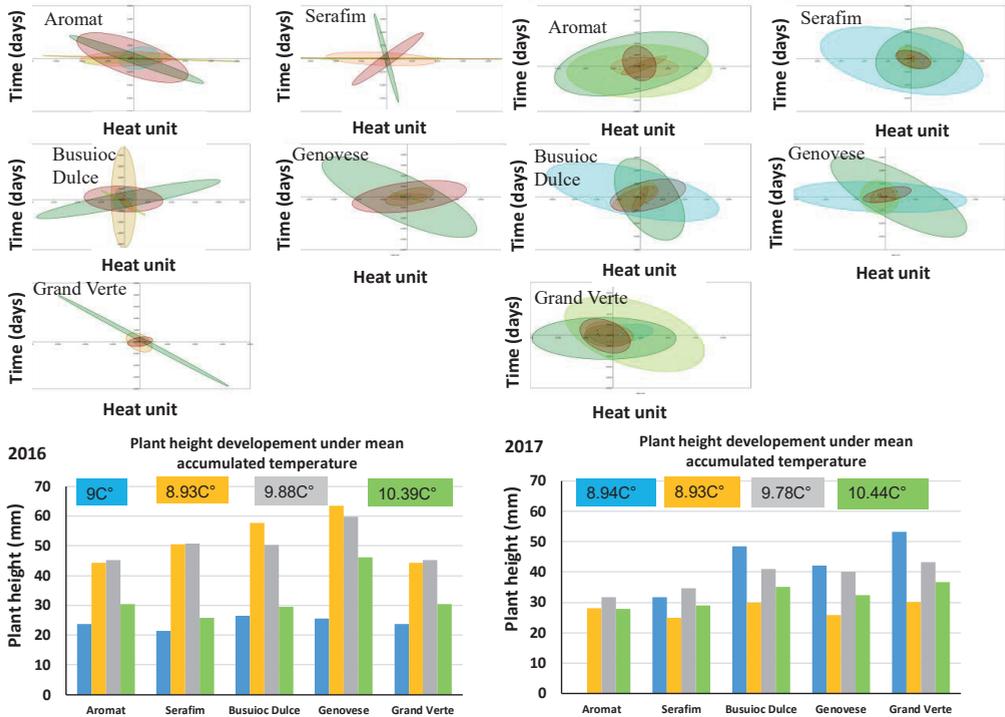


Figure 3. The effect of sowing time on sweet basil height variation under different heat units (air temperatures). Different colours refer to different growth periods as follows: Aqua IIIrd growth period, Chartreuse: IVth growth period, Coral Vth growth period, Darkgoldenrod: VIth growth period, Darkgreen: VIIth growth period, Darkred: VIIIth growth period

Table 3. Correlation analyses of sweet basil height variations between 2016 and 2017 under similar accumulated GDD-s. Before analyses the cumulated GDD-s were divided with days elapsed until the next sampling period. Bolded values represents significant positive correlations between values

2016	2017	Aromat	Serafim	Busuioc dulce	Genovese	Grand Verte
9°C	8.94°C	Rho=	Rho= 0.91**	Rho=0.33	Rho=0.32	Rho= 0.75*
8.93°C	8.93°C	Rho= 0.80**	Rho= 0.90**	Rho=0.24	Rho=0.24	Rho= 0.78*
9.88°C	9.78°C	Rho= 0.78*	Rho= 0.80**	Rho=0.31	Rho=0.21	Rho= 0.72*
10.39°C	10.44°C	Rho= 0.81**	Rho= 0.87*	Rho=0.30	Rho=0.38	Rho= 0.70*

Analyzing the stem diameter, similar tendency can be detected with plant height variations, positive effect of the VIIth growth period (13 June 2016) on stem diameters of ‘Aromat de Buzău’, ‘Serafim’, ‘Busuioc Dulce’, ‘Genovese’ and ‘Grand Verte’ were detected,

while also the IIIrd growth period (24 April 2016) has positive effect on, ‘Genovese’ and ‘Grand Verte’ stem diameter. ‘Genovese’ and ‘Grand Verte’ has relative stable and continuous stem diameter development both at IIIrd (24 April 2016), VIIth (13 June 2016) and

VIIIth growth periods (28 June) (Figure 4). For 2017 the most significant effect on stem diameters had the VIIth growth period. High variations between years can also be detected

considering by comparing the cumulated GDD-s (Figure 4). Correlations between stem diameter development were only observed at ‘Busuioc Dulce’ (Table 4).

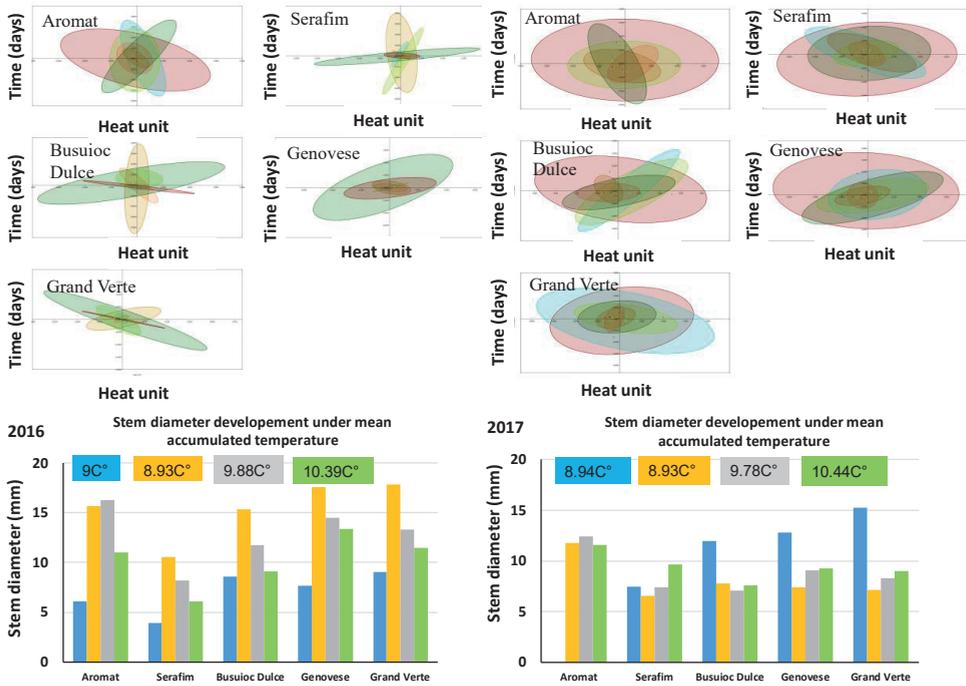


Figure 4. The effect of sowing time on sweet basil stem diameter variations under different heat units (air temperatures). Different colours refer to different growth periods as follows: Aqua IIIrd growth period, Chartreuse: IVth growth period, Coral Vth growth period, Darkgoldenrod: VIth growth period, Darkgreen: VIIth growth period, Darkred: VIIIth growth period

Table 4. Correlation analyses of sweet basil stem diameter variations between 2016 and 2017 under similar accumulated GDD-s. Before analyses the cumulated GDD-s were divided with days elapsed until the next sampling period. Bolded values represents significant positive correlations between values

2016	2017	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte
9°C	8.94°C	Rho=-	Rho=0.33	Rho= 0.74*	Rho=0.08	Rho=0.09
8.93°C	8.93°C	Rho=0.08	Rho=0.21	Rho= 0.71*	Rho=0.06	Rho=0.23
9.88°C	9.78°C	Rho=0.13	Rho=0.43	Rho= 0.77*	Rho=0.22	Rho=0.12
10.39°C	10.44°C	Rho=0.32	Rho=0.04	Rho= 0.68*	Rho=0.18	Rho=0.32

The average fresh aboveground plant weights are presented in Table 5.

Plant biomass analyses revealed that the majority of varieties had higher plant weight from the earlier sowing dates and lower values if sowed later during the years. ‘Genovese’ varieties had the highest values from the IIIrd growth period, ‘Busuioc Dulce’ and ‘Grand Verte’ from the IVth period, ‘Aromat de Buzău’ from the Vth, while ‘Serafim’ from the VIth growth period. Plants of ‘Genovese’ and

‘Serafim’ varieties can be characterized with relatively low variation of the average plant weight, on the contrary ‘Aromat de Buzău’ had higher variations regarding average plant weight (Tables 6 and 7). Differences can be observed between varieties only at IIIrd, IVth and Vth growth periods. At IIIrd growth period, significantly higher biomass at ‘Genovese’ variety was observed, followed by ‘Busuioc Dulce’ and ‘Grant Verte’ varieties. Very similar trend at IVth growth period were

detected, while at Vth growth period, the highest biomass at ‘Aromat’ were detected, followed by ‘Grant Verte’ and ‘Serafim’. No

other differences in plant biomass were detected at any other growth periods (Tables 6 and 7).

Table 5. The average fresh aboveground weight of sweet basil plants of the different growth periods of the experimental years expressed in g

Growth period	Experimental year	Sowing date	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte
III.	2016	19 May	259.60	121.77	247.25	519.31	355.66
	2017	27 April	-	109.6	235.01	325.4	224.4
IV.	2016	27 May	372.65	153.20	343.95	547.55	737.20
	2017	11 May	193	83.45	392.2	277.5	494
V.	2016	10 June	401.21	153.34	295.21	332.19	388.69
	2017	18 May	406.8	113.9	181.7	230.3	185.94
VI.	2016	17 June	270.33	176.11	348.68	551.33	334.00
	2017	1 June	272.7	171.7	122.7	223.6	169.7
VII.	2016	30 June	152.15	41.94	22.08	5.67	47.47
	2017	13 June	157.5	75.2	166.4	245.3	125.9
VIII.	2016	6 July	242.79	83.42	158.52	109.72	82.63
	2017	28 June	76.5	-	98.7	56.9	79.3

Table 6. Biomass comparison by sowing dates of sweet basil varieties in 2016, using ANOVA and Tukey and Mann-Whitney test

3 rd growth period						
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Aromat			0.44	0.99	0.04↓	0.74
Serafim	2.48			0.53	0.003↓	0.08
Busuioc Dulce	0.22	2.25			0.03↓	0.65
Genovese	4.67	7.15	4.89			0.29
Grand Verte	1.72	4.20	1.95	2.94		
4 th growth period						
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Aromat			0.04	0.99	0.13	0.001↓
Serafim	4.73			0.09	0.009↓	0.001↓
Busuioc Dulce	0.61	4.11			0.06	0.001↓
Genovese	3.77	8.50	4.38			0.09
Grand Verte	7.85	12.59	8.47	4.08		
5 th growth period						
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Aromat			0.001↑	0.14	0.47	0.99
Serafim	8.59			0.03	0.009↓	0.001↓
Busuioc Dulce	3.67	4.91			0.88	0.22
Genovese	2.39	6.19	1.28			0.64
Grand Verte	0.43	8.15	3.24	1.95		
6 th growing period						
	Serafim	Busuioc Dulce	Genovese	Grand Verte		
Serafim		0.11	0.002↓	0.15		
Busuioc Dulce	3.67		0.06	0.99		
Genovese	7.99	4.31		0.04↑		
Grand Verte	3.36	0.32	4.62			
7 th growth period						
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Aromat			0.001↑	0.001↑	0.001↑	0.001↑
Serafim	10.38			0.68	0.18	0.99
Busuioc Dulce	12.25	1.87			0.80	0.48
Genovese	13.79	3.41	1.54			0.10
Grand Verte	9.85	0.52	2.39	3.93		
8 th growth period						
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Aromat			0.001↑	0.06	0.003↑	0.001↑
Serafim	8.44			0.10	0.85	1
Busuioc Dulce	4.46	3.98			0.40	0.09
Genovese	7.05	1.39	2.58			0.84
Grand Verte	8.49	0.04	4.02	1.43		

Data above line represents P values, significant values are marked with grey. Data below line represents F values. ↑ represents highest significances at 0.01 confidential intervals.

Table 7. Biomass comparison by sowing dates of sweet basil varieties in 2017, using ANOVA and Tukey and Mann-Whitney tests

3rd growth period - Tukey test					
	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Serafim			0.16	0.01↓	0.18
Busuioc Dulce	3.34			0.34	0.99
Genovese	5.87	2.52			0.30
Grand Verte	3.21	0.13	2.65		
4th growth period					
	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Serafim			0.05	0.22	
Busuioc Dulce	4.24			0.53	
Genovese	2.65	1.59			
5th growth period					
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte
Aromat			0.001↑	0.002↑	0.001↑
Serafim	12.31		0.30	0.03↓	0.28
Busuioc Dulce	9.40	2.90		0.64	1
Genovese	7.42	4.88	1.97		0.68
Grand Verte	9.30	3.00	0.09	1.87	
6th growth period					
	Serafim	Busuioc Dulce	Genovese	Grand Verte	
Serafim			0.70	0.70	1
Busuioc Dulce	1.55			0.20	0.69
Genovese	1.55	3.10			0.71
Grand Verte	0.02	1.57	1.52		
7th growing period – Mann Whitney test					
	Aromat	Serafim	Busuioc Dulce	Genovese	Grand Verte
Aromat			0.38	0.19	0.66
Serafim	0.38		0.08	0.08	0.19
Busuioc Dulce	0	0.08		0.38	0.38
Genovese	0.19	0.08	0.38		0.08
Grand Verte	0.66	0.19	0.38	0.08	
8th growth period					
	Aromat	Busuioc Dulce	Genovese	Grand Verte	
Aromat			0.08	0.08	0.38
Busuioc Dulce	0.08			0.08	0.38
Genovese	0.08	0.08			0.08
Grand Verte	0.38	0.38	0.08		

Data above line represents P values, significant values are marked with grey. Data below line represents F values. ↑ represents highest significances at 0.01 confidential intervals.

CONCLUSIONS

Little research studies regarding the effect of sowing date in temperate climatic conditions of sweet basil on vegetative parameters are published (Kosecka et al., 2014; Sadeghi et al., 2009). More studies are made regarding the influence upon the bioactive compounds (micro and macro elements, phenolics and volatile oils) (Majkowska-Gadomska et al., 2014; Muráriková et al., 2017; Sims, 2014). For this reason, the data obtained in the present study are of great importance for the plant growers. According to the results, it can be concluded that in general the IVth growth period sowed on 11 May 2016 and the Vth and VIth growth periods (sowed on 18 May, respective 1 June 2017) had the most influential effects on sweet basil parameters except stem diameters in 2017, when the VIIIth growth period seems to

be more significant. Clear positive effect of the VIIth growth period (13 June 2016 and 2017) on ‘Aromat de Buzău’, ‘Serafim’, ‘Busuioc Dulce’ and ‘Grand Verte’ plant height can be detected and in all cases again temperature has harmful effect on plant height if sowing was made on 27 April. While analyzing the stem diameter, similar tendency can be detected with plant height variations, positive effect of the VIIth growth period (13 June) on stem diameters of ‘Aromat de Buzău’, ‘Serafim’, ‘Busuioc Dulce’, ‘Genovese’ and ‘Grand Verte’ were detected. Altogether we can conclude that the VIth period initiated on 1 June had the most positive effects on all plant parameters at all varieties, while the VIIIth period, sowed on 28 June had the most harmful effects, therefore we are not recommending this period as proper for sweet basil cultivation. Relative low performance and high sensitivity

to temperature at ‘Genovese’ and ‘Busuioac Dulce’ varieties were detected, while the most constant development and less sensitivity to temperature variations at ‘Aromat de Buzău’, ‘Serafim’ and ‘Grand Verte’ varieties were detected at VIth growth period (1 June).

Plant biomass also varied between years and the sowing time. Plants of ‘Genovese’ and ‘Serafim’ varieties can be characterized with relatively low variation of the average plant weight, on the contrary ‘Aromat de Buzău’ had higher variations regarding average plant weight. The mean precipitation variation may also be an important factor that can influence sweet basil development. In our cases the highest precipitation value in 2016 were detected in June, while in 2017 in May (Figure 1). Further analyses are carried out to detect the cumulative effect of precipitations, temperature and sowing data. Altogether it can be concluded that the present experiment is the first study that links the date of sowing of different varieties of basil, with the growth performances of the plants, following the same climatic conditions. Our experiment was also performed in 2018, but no analyses can be made on these data as a fungal disease compromised the crop during August. This can also be considered as important factor because it looks as several other parameters (pathogens, precipitations) may have significant influence on sweet basil cultivation. Further researches are necessary to detect the influence of these factors.

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REFERENCES

- Asghari, F., Hosseini, B., Hassani, A., & Shirzad, H. (2012). Effect of explants source and different hormonal combinations on direct regeneration of basil plants (*Ocimum basilicum* L.). *Australian Journal of Agricultural Engineering*, 3, 12–17.
- Begum, S., Amin, M.N., & Azad, M.A. (2002). In vitro Rapid Clonal Propagation of *Ocimum basilicum* L. *Plant Tissue Culture*, 12(1), 27–35.
- Bernáth, J. (Ed.). (2000). *Gyógy- és Aromanövények*. Mezőgazda Kiadó.
- Chandramohan, R., & Sivakumari, V. (2009). Micropropagation and preliminary phytochemical analysis of *Ocimum basilicum* L., 9, 19–21.
- Daniel, A., Kalidass, C., & Veerabahu, R. M. (2010). In vitro multiple shoot induction through axillary bud of *Ocimum basilicum* L. an important medicinal plant. *International Journal of Biological Technology*, 1, 24–28.
- Gopi, C., & Ponmurugan, P. (2006). Somatic embryogenesis and plant regeneration from leaf callus of *Ocimum basilicum* L. *Journal of Biotechnology*, 126(2), 260–264. <https://doi.org/10.1016/j.jbiotec.2006.04.033>
- Kiferle, C., Lucchesini, M., Mensuali-Sodi, A., Maggini, R., Raffaelli, A., & Pardossi, A. (2011). Rosmarinic acid content in basil plants grown *in vitro* and in hydroponics. *Open Life Sciences*, 6(6), <https://doi.org/10.2478/s11535-011-0057-1>
- Kintzios, S., Kollias, H., Straitouris, E., & Makri, O. (2004). Scale-up micropropagation of sweet basil (*Ocimum basilicum* L.) in an airlift bioreactor and accumulation of rosmarinic acid. *Biotechnology Letters*, 26(6), 521–523. <https://doi.org/10.1023/B:BILE.0000019561.89044.30>
- Kosecka, D., Jadcak, D., Grzeszczuk, M., & Berova, M. (2014). Effect of sowing date on the yielding of sweet basil cultivated for a bunch harvest in climatic conditions of western Pomeranian region of Poland. *Journal of International Scientific Publications: Agriculture and Food*, 2, 99–105.
- Majkowska-Gadomska, J., Wierzbička, B., & Dziedzic, A. (2014). The Effect of Seedling Planting Time on Macroelement and Microelement Concentrations in Basil (*Ocimum basilicum* L.) Leaves. *Polish Journal of Environmental Studies*, 23(1), 125.
- Muráriková, A., Ťažký, A., Neugebauerová, J., Planková, A., Jampílek, J., Mučaji, P., & Mikuš, P. (2017). Characterization of Essential Oil Composition in Different Basil Species and Pot Cultures by a GC-MS Method. *Molecules*, 22(7), 1221. <https://doi.org/10.3390/molecules22071221>
- Phippen, W.B. (2000). Anthocyanin inheritance and instability in purple basil (*Ocimum basilicum* L.). *Journal of Heredity*, 91(4), 289–296. <https://doi.org/10.1093/jhered/91.4.289>
- Rady, M.R., & Nazif, N.M. (2005). Rosmarinic acid content and RAPD analysis of *in vitro* regenerated basil (*Ocimum americanum*) plants. *Fitoterapia*,

- 76(6), 525–533. <https://doi.org/10.1016/j.fitote.2005.04.001>
- Sadeghi, S., Rahnavard, A., & Ashrafi, Y. (2009). The effect of plant-density and sowing-date on yield of Basil (*Ocimum basilicum*) in Iran. *Journal of Agricultural Technology*, 5(2), 413–422.
- Saha, S., Dey, T., & Ghosh, P. (2010). Micropropagation of *Ocimum kilimandscharicum* Guerke (*Labiatae*). *Acta Biologica Cracoviensia Series Botanica*, 52(2), <https://doi.org/10.2478/v10182-010-0023-7>
- Sahoo, Y., Remien, Y.N., & Yao, R.S. (1997). *In vitro* clonal propagation of an aromatic medicinal herb *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation. *In Vitro Cell. Devel. Biol. Plant Largo*, 33, 293–296.
- Siddique, I., & Anis, M. (2008). An improved plant regeneration system and *ex vitro* acclimatization of *Ocimum basilicum* L. *Acta Physiologiae Plantarum*, 30(4), 493–499. <https://doi.org/10.1007/s11738-008-0146-6>
- Sims, C. A. (2014). *Essential Oils in Holy Basil (Ocimum tenuiflorum L.) as Influenced by Planting Dates and Harvest Times in North Alabama*. <https://doi.org/10.7275/R5P26W1X>
- Singh, N.K., & Sehgal, C.B. (1999). Micropropagation of 'Holy Basil' (*Ocimum sanctum* Linn.) from young inflorescences of mature plants. *Plant Growth Regulation*, 29(3), 161–166. <https://doi.org/10.1023/A:1006201631824>
- Sudhakaran, S., & Sivasankari, V. (2002). *In vitro* flowering response of *Ocimum basilicum*. *Journal of Plant Biotechnology*, 4(4), 179–181.

STUDY REGARDING THE CELLULAR ACTIVITY IN GARLIC (*A. sativum*) BULBS AFFECTING BY *Sclerotium cepivorum*

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Abstract

The purpose of this study was to evaluate the cellular activity in meristematic roots of garlic bulbs (*Allium sativum*) affected by *Sclerotium cepivorum*, from the point of view of the mitotic index, the frequency of mitosis phases and the frequency and types of chromosomal and nuclear abnormalities. Results obtained indicated large differences between samples; thus, the mitotic index decreased from 14.64% for the control to 4.36-10.25% for samples affected by *S. cepivorum*. Also, it has been found the following main types of chromosome aberrations and nuclear abnormalities: laggards, stickiness, rings, micronucleus and nuclear dissolutions. From this point of view, the frequency of chromosomal aberrations and nuclear abnormalities was significantly higher in the case of variants affected by *S. cepivorum*, compared to the control variant (1.04% for control variant and 8.65-11.24% for variants affected by *S. cepivorum*). The results suggest that *S. cepivorum* has the ability to disturb the cellular activity in *A. sativum* meristematic roots, which affecting the growth and development of plants.

Key words: *A. sativum*, *Sclerotium cepivorum*, mitotic index, chromosomal anomalies.

INTRODUCTION

The production and the economic efficiency of the agricultural crops are in strong correlation with a good management of the respective vegetable genotype (Butnariu and Caunii, 2013; Dragomir and Partal, 2016; Roşculete and Roşculete, 2016; Roşculete and Roşculete, 2018, Pandia et al., 2019).

Garlic (*Allium sativum*) belongs to the genus *Allium* of the family *Alliaceae*. The generic name *Allium* is the Latin word for garlic, and the type species for the genus is *Allium sativum*, which means "cultivated garlic" (Block, 2009).

Some sources refer to Greek (*aleo* = to avoid) by reason of the smell of garlic (Block, 2009). Various *Allium* species have been cultivated from the earliest times, and about a dozen species are economically important as crops, or garden vegetables, and an increasing number of species are important as ornamental plants.

Garlic is grown around the world. It is related to onion, leeks, and chives. It is thought that garlic is native to Siberia, but spread to other parts of the world over 5000 years ago. Garlic is most commonly used for conditions related to the heart and blood system (Borrelli et al., 2007; Ahmadi et al., 2013). These conditions

include high blood pressure, high levels of cholesterol or other fats (lipids) in the blood (hyperlipidemia), and hardening of the arteries (atherosclerosis).

In foods and beverages, fresh garlic, garlic powder, and garlic oil are used to add flavor. Garlic produces a chemical called allicin. This is what seems to make garlic work for certain conditions. Allicin also makes garlic smell. Some products are made "odorless" by aging the garlic, but this process can also make the garlic less effective. It's a good idea to look for supplements that are coated (enteric coating) so they will dissolve in the intestine and not in the stomach (Garlic, Uses & Effectiveness, <https://www.webmd.com/vitamins/ai/ingredient-mono-300/garlic>).

Fungal pathogens may be present on plants before harvesting, in the time of harvest or after harvesting, in the time of storage, depending on storage conditions.

Sclerotium cepivorum is the causal agent of the disease commonly known as *Allium* root rot. It is a problem found all over the world on *Allium* spp. that can be very devastating since it can result in large crop losses. Once a field has *S. cepivorum* it is difficult and costly to continue growing *Allium* spp. there, if possible at all. The fungus is positively influenced mainly by

cool weather and survives in the soil as small, round structures known as sclerotia. These sclerotia can survive in the soil for decades (Sammour et al., 2012).

During cool weather there may be white, fluffy mycelia growth at the base of the stem plate when the leaves are yellowing. Mycelia growth is favoured by cool and moist soil, and in the right conditions the mycelia can cover the whole bulb. On these mats of mycelium black sclerotia will form that will be about the size of a poppy seed (Sammour et al., 2012).

Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants. For this purpose, the *A. cepa* and *A. sativum* are the most frequently used higher plant species (Bonciu et al., 2018). The vegetal meristematic tissues that are used for testing the effects of some mutagens on chromosomes should be easy to obtain and less expensive.

MATERIALS AND METHODS

For this study, bulbs of garlic (*A. sativum*, $2n = 16$) affected by *S. cepivorum* were selected, together with a control without traces of diseases or pests. After germination in distilled water, for fixing meristematic cells, Carnoy's fixator was used (ethanol and glacial acetic acid, 3:1 ratio). The biological material was then hydrolysed and stained with basic fuchsin 1%.

The mitotic activity was calculated as the percent ratio of number of cells in mitotic division and total numbers of cells.

The percent of the chromosomal aberrations and nuclear anomalies was determined as the percent ratio of number of cells with mitotic abnormalities and total number of cells in mitotic division.

For cytogenetic study it was used the Kruss Optronic microscope.

Statistical analysis was done using MS Excel 2007. The analysis of variance (ANOVA) was used to assess the significant differences between the control variant and each treatment.

RESULTS AND DISCUSSIONS

Species of the *Allium* genus are used very often in genetic researches, being easy to trace from a

cytogenetic point of view (Hao et al., 1994; Dolatyari et al., 2018; Rosculete et al., 2019). However, there are other species used as genetic models for research, such as: *V. faba* (Kosev and Georgieva, 2019); *C. libani* (Mercimek Takci et al., 2019); alfalfa (Nikolova and Georgieva, 2019) and mistletoe plants (Samfira et al., 2013). Regarding the present study, *S. cepivorum* fungus has ability to induce several effects to garlic (Table 1 and Figure 1). Analysis of the results showed a decrease of the MI between 14.64% (Control) and 10.25% (V4); 9.21% (V1); 8.23% (V5); 6.18 (V2) and 4.36% (V3).

Table 1. The mitotic activity to *A. sativum* bulbs affected by *S. cepivorum*

	Mitotic index (MI%±SE)	Frequency of mitosis phases (%)*			
		P	M	A	T
Ct	14.64±0.2	54.11±2.5	18.64±2.1	12.21±0.1	15.04±2.0
V1	9.21±0.2	40.21±4.5	30.21±2.1	13.16±1.2	16.42±2.0
V2	6.18±0.6*	43.31±2.5	26.42±0.4	15.05±2.1	15.22±3.1
V3	4.36±0.5*	40.25±3.0	31.15±2.4	14.51±2.1	14.09±1.5
V4	10.25±0.2	43.32±2.4	29.55±0.6	13.21±2.5	13.92±1.8
V5	8.23±0.2*	45.92±2.6	31.25±0.4	15.21±2.3	7.62±3.0

SE=standard error of the means; P=Prophase; M=Metaphase; A=Anaphase; T=Telophase *Significant at level 5% (p=0.05)

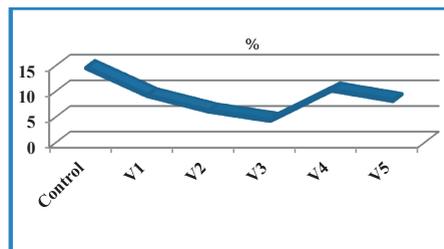


Figure 1. The depression of the mitotic index (MI%) in *A. sativum* cells exposed to *Sclerotium cepivorum*

The *S. cepivorum* can resist in the soil 4-5 years by sclerotia that are carried by irrigation water or by the maintenance works, ensuring the spread of the pathogen. The sclerotia can also be found on the bulbs intended for planting and can maintain their viability for 4-5 years. They can produce new root infections even one month after their formation. In crops there are two periods of maximum sensitivity of plants to this fungus. One in March-April and another after the formation of bulbs and until harvesting, the attack being favoured by the wounds that appear on the roots or bulbs. Plants in cold and moist soils are more sensitive, as the fungus can attack from 2°C to 35°C, when the mycelium is destroyed.

The mitotic index is important criteria used in the assessment of cells proliferation processes (Bonciu, 2012).

The analysis of the mitosis stages allowed finding some fluctuations in the experimental variants, compared to the control. Thus, they recorded the following values: prophase 54.11% (Ct) and 40.21-45.92% (V1-V5); metaphases 18.64% (Control) and 26.42-31.25% (V1-V5); anaphase 12.21% (Ct) and 13.16-15.21% (V1-V5); telophase 15.04% (Ct) and 7.62-16.42% (V1-V5).

Frequency of cellular abnormalities induced by *S. cepivorum* fungus to *A. sativum* is showed in Table 2.

Table 2. The genotoxicity effects induced by *S. cepivorum* to *A. sativum*

	Total CA and NA (%)	Specification (%)				
		L	S	R	MN	ND
Control	1.04	0.11	0.30	0.00	0.42	0.21
V1	8.94	3.15	1.02	0.89	1.74	2.14
V2	11.24	3.04	2.18	2.08	2.42	1.52
V3	9.45	3.11	1.20	1.09	2.96	1.09
V4	8.65	2.64	2.86	0.93	1.10	1.12
V5	10.12	2.81	3.23	0.99	2.02	1.07

CA = Chromosomal aberrations; NA=Nuclear abnormalities; L=Laggards; S=Stickiness; R=Rings; MN=Micronucleus; ND=Nuclear dissolutions

The cytological profile of the garlic meristematic roots analysed revealed a series of chromosomal and nuclear anomalies, represented by: laggards, stickiness, rings, micronucleus and nuclear dissolutions (Figure 3). The recorded values ranged between 8.65% (V4) and 11.24% (V2), while in the control variant, cellular abnormalities were identified only as a percentage of 1.04%. These results indicate the strong genotoxicity potential of *S. cepivorum* fungus in the garlic cells and suggest the possibility of impairing normal growth and development of the future plantlets. Chromosomal anomalies are changes in the number or structure of the chromosomes, while nuclear anomalies represent changes in the structure of the nucleus. In some cases, the structural anomalies of the plant cells dose not manifest at the plant in which they were identified, but the manifestations can occur at the descendants who inherited the respective modification (Bonciu, 2018; Bonciu et al., 2018).

Chromosomal abnormalities can be defined as chromosomes restructuring, after which whole groups are moved from one chromosome to another. They do not usually affect the structure of the genes but especially the links between them. Any change in the structure of the chromosomes can mean a change in the amount or arrangement of genetic information. These changes may induce some disturbances in plants development.

As can be seen in Figure 2, the limits of quantifiable values of the cellular anomalies identified were: 2.64-3.15% (laggards), 1.02-3.23% (stickiness), 0.89-2.08% (rings), 1.10-2.96% (micronuclei) and 1.07-2.14% (cells with nuclear dissolutions).

Some changes in chromosomes structure occur when the genetic material is broken and rearranged into a certain one way. For example, in the case of ring chromosomes, the terminations of a chromosome usually bind when the two endings of the chromosome are deleted. The ends that remain are "Sticky" and it is unite in the form of a ring. The effects that this chromosomal aberration will have it on the plant depend usually by the amount of genetic material that was lost before the ring is formed. Some previous studies in the literature explain the correlation of the diseases and pests attack depending on the environmental conditions or the culture management (Butnariu, 2012; Partal et al., 2013; Partal et al., 2014; Paraschivu et al. 2015; Paraschivu et al., 2017; Cotuna et al., 2018; Pandia et al., 2018). In order to limit the attack and the damages caused by *S. cepivorum* fungus, preventive and curative measures are required, such as: 5-6 years of crop rotation, deep ploughing preceded by burning of diseased plants, balanced fertilization, use of healthy bulbs for planting and their preventive treating, treatments with specific fungicides in the vegetation etc.

Many authors have described techniques for testing the viability of sclerotia and for measuring their germination in response to host plants (Coley-Smith, 1985; Banksler and Edgington, 1989; Davis et al., 2007; Esler and Coley-Smith, 2007; Amin et al., 2014).

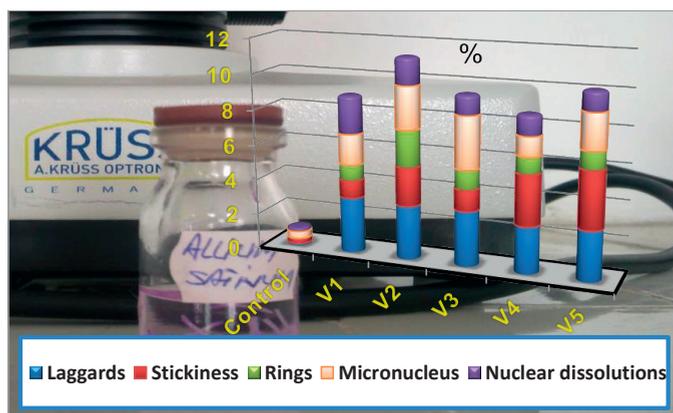


Figure 2. Frequency of the main types of cellular abnormalities identified in *A. sativum* meristematic roots under influence of *S. cepivorum*

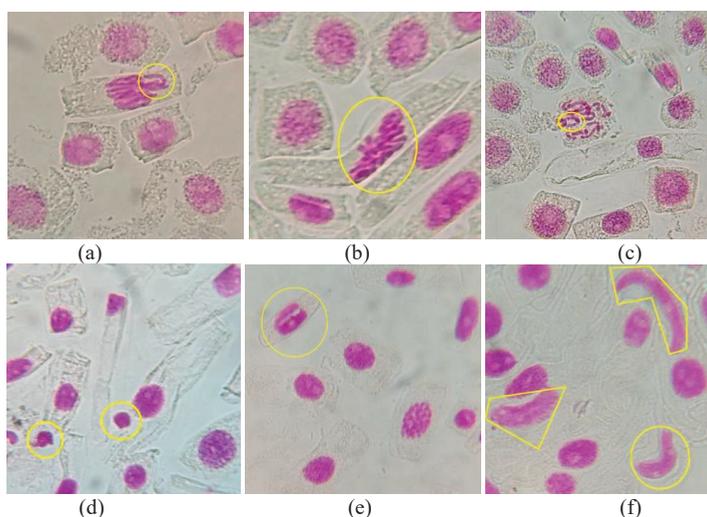


Figure 3. The aberrant appearance of *A. sativum* cells affected by *S. cepivorum*: laggards (a), stickiness (b), ring chromosome (c), micronuclei (d), nuclear dissolutions (e, f)

CONCLUSIONS

The evaluation of the influence of the *S. cepivorum* on the *A. sativum* cells suggests the depression of the mitotic index and the mitotic activity respectively. This can affect the growth and development of the garlic plants. The reduction of mitotic activity was correlated with the increase of the cellular abnormalities frequency.

Comparative cytological analysis in the garlic bulbs affected by the *S. cepivorum* and healthy bulbs, reveal specific features of inhibition of the mitotic activity process and phenotypic induction of the some types of

chromosomal and nuclear anomalies that indicate the strong cytotoxicity and genotoxicity of this fungus in *A. sativum* cells. In order to limit the attack and the damages caused by this fungus, preventive and curative measures are required, such as use of healthy bulbs for planting and treatments with specific fungicides in the vegetation.

REFERENCES

- Ahmadi, N., Nabavi, V., Hajsadeghi, F., Zeb, I., Flores, F., Ebrahimi, R., Budoff, M. (2013). Aged garlic extract with supplement is associated with increase in brown adipose, decrease in white adipose tissue and predict lack of progression in coronary

- atherosclerosis. *International Journal of Cardiology*, 168(3), 2310–2314.
- Amin, M., Tadele, S., Thangavel, S. (2014). White rot (*Sclerotium cepivorum*-Berk) - an aggressive pest of onion and garlic in Ethiopia: an overview. *J. Agric. Biotechnol. Sustain. Dev.*, 6(1), 6–15.
- Banks, E., Edgington, L.V. (1989). Effect of integrated control practices on the onion white rot pathogen in organic soil. *Can. J. Plant Pathol.*, 11(3), 268–272.
- Block, E. (2009). *Allium In: Index Nominum Genericorum*. Cambridge, UK: Royal Society of Chemistry.
- Bonciu E. (2012). Cytological effects induced by Agil herbicide to onion. *Journal of Horticulture, Forestry and Biotechnology*, 16(1), 68–72.
- Bonciu, E. (2018). Evaluation of cytotoxicity of the herbicide Galigan 240 EC to plants. *Scientific Papers. Series A. Agronomy, LXI*(1), 175–178.
- Bonciu, E., Firbas, P., Fontanetti, C.S., Wusheng, J., Karaismailoğlu, M.C., Liu, D., Menicucci, F., Pesnya, D.S., Popescu, A., Romanovsky, A.V., Schiff, S., Ślusarczyk, J., De Souza, C.P., Srivastava, A., Sutan, A., Papini, A. (2018). An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. *Caryologia*, 71(3), 191–209.
- Borrelli, F., Capasso, R., Izzo, A.A. (2007). Garlic (*Allium sativum* L.): adverse effects and drug interactions in humans. *Mol. Nutr. Food. Res.*, 51(11), 1386–1397.
- Butnariu, M. (2012). An analysis of *Sorghum halepense*'s behavior in presence of tropane alkaloids from *Datura stramonium* extracts. *Chemistry Central Journal*, 6, 75.
- Butnariu, M., Caunii, A. (2013). Design management of functional foods for quality of life improvement. *Annals of Agricultural and Environmental Medicine*, 20(4), 736–741.
- Coley-Smith, J.R. (1985). Methods for the production and use of sclerotia of *Sclerotium cepivorum* in field germination studies. *Plant Pathol.*, 34(3), 380–384.
- Cotuna, O., Paraschivu, M., Paraschivu, M., Olaru, L. (2018). Influence of crop management on the impact of *Zymoseptoria tritici* in winter wheat in the context of climate change: an overview. *Research Journal of Agricultural Science*, 50(3), 69–74.
- Davis, R.M., Hao, J.J., Romberg, M.K. (2007). Efficacy of germination stimulants of sclerotia of *Sclerotium cepivorum* for management of white rot of garlic. *Plant Dis.*, 91(2), 204–208.
- Dolatyari, A., Saeidi Mehrvarz, S., Shahzadeh Fazeli, S.A., Naghavi, M.R., Fritsch, R.M. (2018). Karyological studies of Iranian *Allium* L. (*Amaryllidaceae*) species with focus on sect. *Acanthoprason*. 1. Mitotic chromosomes. *Plant Syst Evol.*, 304, 583–606.
- Dragomir, C.L., Partal, E. (2016). Influence of different levels of water supply on production and economic efficiency under maize cultivated in Dobroea region of Romania. *Scientific Papers. Series A. Agronomy, LIX*, 289–293.
- Esler, G., Coley-Smith, J. (2007). Resistance to *Sclerotium cepivorum* in *Allium* and other genera. *Plant Pathology*, 33, 199–204.
- Garlic, *Uses & Effectiveness*. Retrieved from <https://www.webmd.com/vitamins/ai/ingredientmono-300/garlic>.
- Hao, S., Jiao, M.D., Zhao, J., Xing, M., Huang, B.C. (1994). Reorganization and condensation of chromatin in mitotic prophase nuclei of *Allium cepa*. *Chromosoma*, 103, 432–440.
- Kosev, V., Georgieva, N. (2019). Application of the Ecologo-genetic Model in Broad Bean (*Vicia faba* L.) Breeding. *Banat's Journal of Biotechnology*, X(20), 29–36.
- Mercimek Takci, H.A., Turkmen, F.U., Sari, M. (2019). In vitro mutagenic effect of cedar (*Cedrus libani* A. Rich) tar in the *Salmonella/Microsome* Assay System. *Banat's Journal of Biotechnology*, X(20), 13–18.
- Nikolova, I., Georgieva, N. (2019). Effect of biological products on the population of aphids and chemical components in alfalfa. *Banat's Journal of Biotechnology*, X(19), 51–57.
- Pandia, O., Sărăcin, I., Sărăcin, A.I. (2018). Management of agricultural culture establishment works. *Scientific Papers. Series Management, Economic Engineering in Agriculture and rural development*, 18(2), 315–318.
- Pandia, O., Sărăcin, I., Olaru, L. (2019). The importance of knowledge of foliage surface index (ISF) influence on production. *Scientific Papers. Series Management, Economic Engineering in Agriculture and rural development*, 19(2), 317–321.
- Pandia, O., Sărăcin, I., Olaru, L. (2019). The importance of knowledge concerning resistance to draught for some types of seeds cultivated on sandy soils. *Scientific Papers. Series Management, Economic Engineering in Agriculture and rural development*, 19(2), 311–315.
- Paraschivu, M., Cotuna, O., Paraschivu, M., Durau, C.C., Damianov, S. (2015). Assessment of *Drechslera tritici repentis* (Died.) Shoemaker attack on winter wheat in different soil and climate conditions in Romania. *European Biotechnology Congress, Journal of Biotechnology*, 208, S113.
- Paraschivu, M., Cotuna, O., Olaru, L., Paraschivu, M. (2017). Impact of climate change on wheat-pathogen interactions and concerning about food security. *Research Journal of Agricultural Science*, 49(3), 87–95.
- Partal, E., Sin, Gh., Alionte, E. (2013). The effect of management practices on the quality of wheat and maize harvest. *Annals of the Academy of Romanian Scientists. Series on Agriculture, Silviculture and Veterinary Medicine Sciences*, 2(1), 82–89.
- Partal, E., Paraschivu, M., Cotuna, O. (2014). Influence of seeds treatment on the cereales production.

- Research Journal of Agricultural Science*, 46(2), 270–276.
- Roșculete, C., Roșculete, E. (2016). The influence of foliar fertilizers on rape crops from SCDA Caracal. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, 46(1), 261–265.
- Roșculete, C.A., Roșculete, E. (2018). The use of chemical and organic fertilizers for sunflower culture on a sterile dump. *Scientific Papers. Series A. Agronomy*, LXI(1), 117–124.
- Roșculete, E., Roșculete, C.A. (2018). The influence of the interaction of some mineral fertilizers on the accumulation of some nutritive elements in wheat grains. *Scientific Papers. Series A. Agronomy*, LXI(1), 386–391.
- Rosculete, E., Olaru, A.L., Rosculete, C.A., Bonciu, E. (2019). Assessment of Cytological Effects of Food Preservative Potassium Metabisulphite to *Allium cepa*. *American Journal of Plant Sciences*, 11(1), 11–23.
- Samfira, I., Butnariu, M., Rodino, S., Butu, M. (2013). Structural investigation of mistletoe plants from various hosts exhibiting diverse lignin phenotypes. *Digest journal of nanomaterials and biostructures*, 8(4), 1679–1686.
- Sammour, R., Mahmoud, Y., Alhozeim, A.A. (2012). Biology, controlling and genetic variability in *Sclerotium cepivorum* Berk; the causal agent of *Allium* white rot disease. *Current trends in Microbiology*, 7, 101–111.

INVESTIGATION OF RELATIONSHIPS BETWEEN SEED YIELD AND AGRONOMIC TRAITS IN SUNFLOWER

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Abstract

Better understanding of the relationships between seed yield and agronomic traits could facilitate sunflower breeding programs. In this study, twenty sunflower hybrids were investigated to explore the relationships between yield and agronomic traits during two years (2017 and 2018), under Agricultural Research and Development Station (ARDS) - Simnic environmental conditions. The analyses of Pearson correlation coefficients showed that the seed yield was significantly and positively correlated only with the head diameter, suggesting a reliable selection criterion for improving of seed yield in sunflower. The plant height was significantly and positively correlated with thousand seed weight, while significantly and negatively correlated with hectolitre mass and days to physiological maturity. Also, hectolitre mass was significantly and negatively correlated with thousand seed weight. Days to flowering initiation were significantly and positively correlated with days to flowering completion.

Key words: correlation, head diameter, plant height, thousand seed weight, sunflower (*Helianthus annuus* L.).

INTRODUCTION

Sunflower (*Helianthus annuus* L.) belongs to the genus *Helianthus* of the family *Asteraceae*, is one of the most important oil crops worldwide with an annual production of about 51 Million tonnes. In 2018, Romania achieved 3.062 Million tons sunflower seeds (FAO, 2018).

Due to the increasing population, selection and cultivation of high yielding sunflower varieties is very important.

Seed yield in sunflower is a complex character which is products of interaction between a numbers of other traits (Arshad et al., 2010). According to Abad et al. (2013), the seed yield is a function of genetic potential of the genotype, external conditions in which the crop is grown, applied technology and the interaction of all these factors.

Understanding the nature of relationships between seed yield and associated traits is a prerequisite for screening programs in plant breeding. Direct selection for this trait has a lower efficiency due to low heritability, so indirect selection using yield traits may improve seed yield of sunflower (Ghaffari et al., 2019).

In breeding program for increasing yield, morphological and physiological characteristics that are easily measured and that demonstrate a causal relationship with seed yield, can be used as selection criteria (Hladni et al., 2007).

Correlation coefficients reveals the strength of relationship among the group of traits, thus is one of the important biometrical tools for formulating a selection index (Jayakumar et al., 2007). According to Yagdi and Sozen (2009), analysis of correlation coefficient is the most widely used statistical tool one among numerous methods.

Many researchers used correlation coefficient for displaying the relationship of sunflower traits. Darvishzadeh et al. (2011) reported that head diameter had the highest significant positive correlation with seed yield, in both well-watered and water-stress conditions. Zeinalzadeh-Tabrizi et al. (2019) reported positive correlations of plant height, head diameter and seed numbers with seed yield in drought stress condition.

Therefore, due to environmental effects, relationships among sunflower traits could be variable.

The objective of this study was to investigate the correlation coefficients of seed yield with agronomic traits in sunflower hybrids.

MATERIALS AND METHODS

Twenty sunflower hybrids coming from the NARDI Fundulea (noted conventionally HF1-HF20) were used in this study.

Experiments were conducted under rain fed conditions in the years 2017 and 2018.

The field experiments were located within the Agricultural Research and Development Station (ARDS) Simnic, Craiova (44⁰19' N, 23⁰48' E, and 182 m altitude).

Soil was a reddish preluvosol with a humus content of 2.68-2.23% and pH = 5.08-5.33 (Radu et al., 2019).



Figure 1. View from the experimental field (ARDS Simnic)

The experiment organized in a randomised block design with three replications (Figure 1). The row-to-row distance was 70 cm, and the plant-to-plant distance was 30 cm. Each 19.6 m² plot consisted of four 7.0 m long rows.

Sowing was performed on 12 April 2017 and 20 April 2018, respectively.

Fertilization was done with an application of 250 kg of complex fertiliser (N₂₀P₂₀K₀) prior to sowing.

Weeds were controlled by the help of herbicides, respectively Dual Gold 960 EC applied at a dose of 1.5 l/ha immediately after sowing. Also, one manual hoeing was performed.

The monthly precipitation, average temperatures and multiannual data for 2017 and 2018 are presented in Table 1.

Climatic conditions for the period 2017-2018, in general, were unfavourable for the growth and development of sunflower plants.

In 2017 growing season, the average annual temperature was 11.8°C, with lows of -5.1°C in January and highs of 25.4°C in August.

In 2018 the average annual temperature was 12.6°C, with minimal value of 0.8°C in February and maximum value of 24.1°C in August.

Compared to multiannual average, in both growing seasons, the average temperature was higher by 0.6°C and 1.4°C, respectively.

The total annual precipitation was 483.4 mm in 2017 and 905.4 mm in 2018, being with -81.7 mm lower and, respectively, with +340.3 mm higher than the multiannual average.

Although the June and the July precipitations in 2018 amounted to 182.3 mm and 177.3 mm, respectively, the higher average temperature during August caused the manifestation of diseases on the plants and so reduced production.

Data were recorded on the following parameters:

Days to flowering initiation were recorded from date of sowing till about ten percent of the buds opened flowers in each plot.

Days to flowering completion were recorded when about 75% of the buds opened flower in each plot.

Days to physiological maturity were recorded when back of the heads turned yellow and bracts started turning brownish in colour.

Plant height: a total of five plants randomly selected in each plot at the time of maturity and were measured from ground level to attachment of head with stem to record data on plant height.

Head diameter was measured from one edge of the disk to other at five plants.

Hectolitre mass was determined using the Perten AM 5200-A.

Thousand seed weight data was recorded by weighing thousand grains randomly taken, with the help of electronic balance, and then average weight was calculated for each hybrid.

Seed yield per hectare adjusted to 9% moisture.

The relationships between the yield and associated traits were established using Pearson correlation coefficients (r). The variability presence in the hybrids was estimated by coefficient of variations (CV) using the procedure suggested by Săulescu and Săulescu (1967).

RESULTS AND DISCUSSIONS

The analysis of some agronomical traits of 20 new sunflower hybrids in the experimental field during 2017 and 2018 are given in Table 2.

Table 1. Monthly and growing season precipitation and temperature at ARDS Simnic in 2017 and 2018

Months	Temperature (°C)			Precipitation (mm)		
	2017	2018	Multiannual average	2017	2018	Multiannual average
October	10.3	12.7	11.8	63.3	100.2	44.5
November	5.2	6.4	5.5	75.2	70.3	44.9
December	-0.3	3.2	0.4	5.0	62.0	45.1
January	-5.1	1.4	-1.4	11.1	36.3	32.7
February	1.6	0.8	1.0	31.2	72.5	30.6
March	9.8	3.9	5.6	32.1	95.0	33.7
April	11.1	16.6	11.8	71.1	11.1	46.0
May	16.7	19.2	16.9	74.2	60.2	66.9
June	23.4	21.6	20.4	0	182.3	67.9
July	24.2	22.3	22.6	89.2	177.3	61.5
August	25.4	24.1	22.1	5.0	19.2	48.9
September	19.4	19.2	17.5	26.0	19.0	42.4
Average/Total	11.8	12.6	11.2	483.4	905.4	565.1

The average value for seed yield during the period of study (two years) was 1452.08 kg/ha. Hybrid HF1 has the highest average value for seed yield (1995.5 kg/ha) and the minimal yield observed at hybrid HF17 (1184.0 kg/ha). The results are in agreement with values reported by Bonea et al. (2010) and Bonea et al. (2012) for sunflower hybrids cultivated at ARDS Simnic in drought conditions.

Higher values for this trait at 20 new sunflower hybrids were presented by Clapco et al. (2019), who observed that the seed yield in 2017 ranged between 2139.1-3152.9 kg/ha, with an average value of 2743.5 kg/ha, and in 2018 seed yield varied between 1474-3202 kg/ha, with average value of 2442.5 kg/ha.

Regarding the plant height, the average value was 119.98 cm, and the hybrids HF19 was the tallest among tested hybrids with the height of 138.0 cm.

The average values for head diameter was 18.08 cm. The highest head diameter (20.5 cm) was observed in hybrid HF3 and the shortest head diameter (15.5 cm) was observed in hybrids HF17 and HF18. The diameter values are similar with the values reported by Clapco et al. (2019), but are higher comparative to those reported by other authors (Khan et al., 2018; Sincik and Goksoy, 2014).

The average value for hectolitre mass was 45.17 kg/hl. The highest value in both analysed years was showed by the hybrid HF6 (47.7 kg/hl), while the lowest value was showed by the hybrid HF14 (43.2 kg/hl).

Obtained data has shown that the average value of thousand seed weight of all hybrids was 52.22 g. High value of thousand seed weight was noticed in HF10 (69.3 g), while the lowest value of thousand seed weight (35.4 g) was noticed in HF5.

The average value for days to flowering initiation was 58.68 days. Hybrid HF15 took maximum days (60.5) to flowering initiation whereas hybrid HF17 took minimum days (56.5) for flowering initiation.

Regarding the days to flowering completion, the average value was 75.5 days. Hybrid HF8 took maximum days (78.0) for their flowering completion while hybrid HF13 took minimum days (72.5) to complete their flowers.

The average value for days to physiological maturity was 129.83 days. HF7 took maximum days (134.5) to be matured whereas HF14 and HF15 took minimum days (124.5) to be matured.

The moderate coefficient of variation was obtained for seed yield and thousand seed weight of 13.27 % and 16.62 %, respectively.

Similar results was also observed for 20 sunflower varieties tested in Ovche Pole

locality, Republic of Macedonia by Gorgieva et al. (2015).

Table 2. Mean values for agronomic traits in sunflower hybrids during the period of study

Hybrids	SY	PH	HD	HM	TSW	DFI	DFC	DPM
HF1	1995.5	104.5	19.5	45.8	47.1	59.0	74.5	133.0
HF2	1528.0	110.5	19.5	44.9	48.1	60.0	76.5	133.0
HF3	1368.5	102.5	20.5	46.0	50.2	59.0	74.0	133.0
HF4	1196.0	111.5	16.5	46.1	37.9	60.0	76.0	134.0
HF5	1253.5	108.5	19.0	45.5	35.4	57.5	76.0	134.0
HF6	1420.0	112.0	18.5	47.7	42.1	57.5	75.0	131.0
HF7	1646.0	118.0	18.5	45.1	52.0	57.5	74.5	134.5
HF8	1567.5	112.0	18.0	45.1	50.7	59.5	78.0	132.0
HF9	1529.0	121.0	18.5	45.4	63.9	59.5	77.5	129.5
HF10	1589.5	122.0	18.5	45.9	69.3	58.5	76.0	129.5
HF11	1388.0	118.0	19.0	43.4	59.4	58.5	75.0	129.5
HF12	1510.5	137.5	18.0	44.2	59.3	59.5	75.5	130.5
HF13	1469.5	124.0	16.5	44.6	55.9	59.0	72.5	130.0
HF14	1655.5	131.0	19.0	43.2	55.8	57.5	73.0	124.5
HF15	1364.5	127.5	16.5	47.0	49.9	60.5	77.0	124.5
HF16	1435.5	124.5	19.5	44.7	51.3	57.5	75.5	125.0
HF17	1184.0	122.5	15.5	45.3	44.8	56.5	73.5	128.0
HF18	1203.5	121.5	15.5	45.7	49.1	57.5	75.5	128.0
HF19	1443.0	138.0	17.0	44.1	59.5	59.5	77.0	128.0
HF20	1294.0	132.5	18.0	43.6	62.7	59.5	77.5	125.0
Average	1452.08	119.98	18.08	45.17	52.22	58.68	75.50	129.83
Minim	1184.0	102.5	15.5	43.2	35.4	56.5	72.5	124.5
Maxim	1995.5	138.0	20.5	47.7	69.3	60.5	78.0	134.5
CV%	13.27	8.53	7.74	2.52	16.62	1.9	2.02	2.54

SY= Seed yield; PH = Plant height; HD = Head diameter; HM = Hectolitre mass; TSW = Thousand seed weight; DFI = Days to flowering initiation; DFC = Days to flowering completion; DPM = Days to physiological maturity

The correlation analyses of the combined two years of data revealed that seed yield was significant and positively correlated only with the head diameter ($r = 0.511^*$; $p = 0.05$), but non-significantly positive correlated with thousand seed weight, days to flowering initiation and days to physiological maturity (Table 3).

Previous studies have similarly reported that the association between seed yield and the head diameter is positive and significant (Bonea et al., 2008; Tahir et al., 2002; Sincik and Goksoy, 2014; Zeinalzadeh-Tabrizi et al., 2019)

Many researchers observed a positive but low correlation between seed yield and head diameter (Bonea et al., 2012; Bonea et al., 2013; Clapco et al., 2019).

In contrast to our results, Manivannan et al. (2007) reported that the correlation between seed yield and the head diameter was negative and non-significantly for most studied varieties and hybrids.

According to Sincik and Goksoy (2014), this discordance among results for correlations is most likely associated with differences in the genetic material and environmental conditions used in studies.

Plant height exhibited positive and significant association with thousand seed weight ($r = 0.587^*$; $p = 0.05$), and a negative significantly association with hectolitre mass ($r = -0.526^0$; $p = 0.05$) and with days to physiological maturity ($r = -0.721^{00}$; $p = 0.01$).

Tahir et al. (2002) found that plant height revealed significant and positive correlation with seed yield, head diameter and 1000-seed weight.

On the other hand, Khan et al. (2018) observed that the correlation of plant height with days to physiological maturity was non-significant.

Arshad et al. (2010), Sujatha and Nadaf (2013) concluded that plant height correlated positively and significantly with days to physiological maturity.

Table 3. Pearson correlation coefficients between seed yield and other agronomic yield traits

Traits	PH	HD	HM	TSW	DFI	DFC	DPM
SY	-0.117	0.511*	-0.101	0.297	0.125	-0.123	0.174
PH		-0.415	-0.526 ⁰	0.587*	0.065	0.104	-0.721 ⁰⁰
HD			-0.109	0.091	0.016	-0.018	0.271
HM				-0.494 ⁰	0.023	0.099	0.321
TSW					0.236	0.164	-0.433
DFI						0.555*	0.073
DFC							-0.052
DPM							

⁰, significant at p = 0.05 probability level; ⁰⁰, significant at p = 0.01 probability level;

SY = Seed yield; PH = Plant height; HD = Head diameter; HM = Hectolitre mass; TSW = Thousand seed weight; DFI = Days to flowering initiation; DFC = Days to flowering completion; DPM = Days to physiological maturity

Head diameter and thousand seed weight had insignificant correlative associations with all of the other observed traits.

In addition, hectolitre mass was negatively and significantly associated with thousand seed weight ($r = -0.494^0$; $p = 0.05$) and days to flowering initiation were positively and significantly associated with days to flowering completion ($r = 0.555^*$; $p = 0.05$).

The our findings are in agreement with those of Khan et al., (2018) and Zeinalzadeh-Tabrizi et al. (2019), who reported that the association between days to flowering initiation and days to flowering completion is positive and significant.

CONCLUSIONS

The results from this study indicated that seed yield was positively and significantly correlated with head diameter.

It can be conclude that head diameter can be good selection criteria in breeding program to obtain the maximum seed yield in sunflower hybrids.

REFERENCES

- Abad, A., Khajehpour, M.R., Mahloji, M., Soleymani, A. (2013). Evaluation of phenological, morphological and physiological traits in different lines of barley in Esfahan region. *International Journal of Farming and Applied Science*, 2(18), 670–674.
- Arshad, M., Khan, M.A., Jadoon, S.A., Mohmand, A.S. (2010). Factor analysis in sunflower (*Helianthus annuus* L.) to investigate desirable hybrids. *Pakistan Journal of Botany*, 42(6), 4393–402.
- Bonea, D., Urechean, V., Constantinescu, E., Pandia, O. (2008). The interrelations between the capacity of production and the component elements at the sunflower. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca - Agriculture*, 65(1), 355.
- Bonea, D., Borleanu, C.I., Urechean, V., Constantinescu, E. (2010). Agronomic value of some sunflower hibryds cultivated in Oltenia area. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, XL(2), 46–51.
- Bonea, D., Urechean, V., Constantinescu, E., Iancu, D. (2012). The behaviour of some sunflower (*Helianthus annuus*) hybrids from abroad under water and heat stress at ARDS Simnic. *Scientific Papers. Series A. Agronomy*, LV, 129–132.
- Bonea, D., Urechean, V., Mindrilă, G., Iancu, D. (2013). Results on the yield and correlations between characters in some sunflower (*Helianthus annuus* L.) hybrids grown in Oltenia. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, XLIII(1), 73–77.
- Clapco, S., Gisca, I., Cucereavii, A., Duca, M. (2019). Analysis of yield and yield related traits in some sunflower (*Helianthus annuus* L.) hybrids under conditions of the Republic of Moldova. *Scientific Papers. Series A. Agronomy*, LXII(1), 248–257.
- Darvishzadeh, R., Hatami-Maleki H., Sarrafi, A. (2011). Path analysis of the relationships between yield and some related traits in diallel population of sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. *Australian journal of Crop Science*, 5(6), 674–680.
- FAO. (2018). <http://www.fao.org/faostat/en/#data/QC> Accessed on February. 06, 2020.
- Ghaffari, M., Davaji, A.M.N.R., Ghadimi, F.N. (2019). Oil yield determinant of sunflower in climatically different regions of Iran. *Bulgarian Journal of Agricultural Science*, 25(1), 67–71.
- Gorgieva, B., Ilija, K., Sasa, M., Ruzdik, M., Emilija, K., Biljana, K. (2015). Correlation and path analysis in sunflower (*Helianthus annuus* L.). *Helia*, 38(63), 201–210.
- Hladni, N., Škorić, D., Kraljević-Balalić, M., Sakač, Z., Miklič, V. (2007). Heterosis for eagronomically important traits in sunflower (*Helianthus annuus* L.). *Helia*, 30(47), 191–198.
- Jayakumar, J., Sundaram, T., Prabu, D.A., Rajan, A.R.R. (2007). Correlation studies in maize (*Zea mays* L.) evaluated for grain yield and other yield attributes. *International Journal of Agriculture Sciences*, 3(2), 57–60.

- Khan, H., Ali, S., Ahmad, I., Khan, I., Hussain, S., Khan B.A., Suhaib, M. (2018). Agronomic and qualitative evaluation of different local sunflower hybrids. *Pakistan Journal of Agricultural Research*, 31(1), 69–78.
- Manivannan, N., Karthika, R., Punitha, B., Vindhiyavarman, P., Muralidharan, V. (2007). Association pattern among the yield attributes in varieties and hybrids of sunflower (*Helianthus annuus* L.). *Madras Agricultural Journal*, 94(1-6), 84–88.
- Radu, V., Popa, I., Dodocioiu, A.M., Buzatu, G.D. (2019). The quality status of the reddish preluvosol from ARDS Simnic. *Analele Universității din Craiova, seria Biologie, Horticultură, Tehnologia Prelucrării Produselor Agricole, Ingineria Mediului*, 24, 213–217.
- Săulescu, N.A., Săulescu, N.N. (1967). *Câmpul de experiență*. Editura Agro-Silvică, București.
- Sincik, M., Goksoy, A.T. (2014). Investigation of correlation between traits and path analysis of confectionary sunflower genotypes. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 42(1), 227–231.
- Sujatha, K., Nadaf, H.L. (2013). Correlation for yield and yield related trait in mutant and segregating genotypes in sunflower (*Helianthus annuus* L.). *Molecular Plant Breeding*, 4(32), 265–266.
- Tahir, M.H.N., Imran, M., Hussain, M.K. (2002). Evaluation of sunflower (*Helianthus annuus* L.) inbred lines for drought tolerance. *International Journal of Agriculture and Biology*, 4(3), 398–400.
- Yagdi, K., Sozen, E. (2009). Heritability, variance components and correlations of yield and quality traits in durum wheat (*Triticum durum* Desf.). *Pakistan Journal of Botany*, 41(2), 753–759.
- Zeinalzadeh-Tabrizi, H., Ghaffari, M., Hosseinpour, A. (2019). Correlation and path analysis of yield and related traits in sunflower (*Helianthus annuus* L.) under normal and drought stress conditions. *Bioscience Research*, 16(1), 658–666.

IMPACT OF THE UNIVERSAL LIQUID FERTILIZER LACTOFOL ON SEED PRODUCTIVITY OF SOYBEAN (*Glycine max* (L.) Merrill.)

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Abstract

A two-year field experiment was conducted at the Experimental Station on Soybean in order to study the effect of foliar feeding by the universal fertilizer Lactofol on the elements that structure soybean seed production. The experiment was carried out as a two-factor by the method of fractional plots. Factorial combinations were made with two initial phases of application of foliar fertilizing (R₃ and R₅) and with three formulations of leaf fertilizer Lactofol, each administered in two doses (500 and 750 ml/da). According to the results, the characteristics studied were more influenced by the formulation and the dose of foliar fertilizing than the treatment phase. Foliar fertilizing by Lactofol O applied in the onset of flowering at dose of 0.500 ml/da increased the seed yield per plant significantly in both experimental years. In a humid and cooler year, this formulation of leaf fertilizer increases the number of seeds per plant. In conditions of optimal to low soil and air humidity, Lactofol K/Ca (0.750 ml/da) applied in the phenophase of flowering-pod formation (R₅) also increases the number and yield of plant seeds, as well as the absolute seed weight.

Key words: *Glycine max*, fertilizer, seed yield.

INTRODUCTION

The development and implementation of innovative technologies in agriculture is related to the preservation of natural resources and the provision of good health status for both animals and humans. Contemporary agriculture incorporates green farming best practices for organic production (Lee and Song 2007; Wasule et al., 2007; Son et al., 2006; Argaw 2012; Marinova et al., 2019; Marinov-Serafimov and Golubinova, 2019; Rosculete et al., 2019). The introduction of natural substances through foliar nutrition, improves metabolism and balances the nutrition of plants (Churkova, 2013; Churkova, 2014; Vasileva, 2015; Marinova and Ivanova, 2018).

Soy is an important and widespread protein and oilseed crop all over the world. According to FAOSTAT (2017), 65% of crop exports come from the USA and Brazil. Countries such as Argentina, China and India are third, fourth and fifth, respectively.

The seeds of *Glycine max* (L.) Merrill., are widely used in the medical, food and cosmetic industries (Akpapobi, 2009). They are a rich source of fat (from 18% to 22-25%) and crude

protein (from 30% to 42-50%). The protein fraction comprises the eight essential amino acids required for protein building in animals and humans (Raei et al., 2008; Ali, 2010; Argaw, 2012). Being also called 'Golden Bean', soybean is a good source of calcium, iron, glycine and isoflavones (Kumar, 2007). Its seed productivity requires an optimal nutritional regime of the above ground mass, often associated with additional nutrient input through soil or leaf nutrition (Shrivastava et al., 2000; Singh et al., 2003; Devi et al., 2012). The fertilizers containing sulfur and boron increase the seed number per pods and have a positive effect on nitrogenase activity and nitrogen fixation, which in turn increases the quality of soybean (Devi et al., 2012). The foliar application of micro and macronutrients (Fe + Zn + Mn + B) in combination, significantly increases the values of the indicators, such as: plant height, number of branches per plant, number of pods per plant, weight per 100 seeds, seed yield, content and fat and protein yield (El-Haggan, 2014).

The treatment of leaf mass with biostimulants allows the direct contact and immediate reaction of the crop to the compositions of the

liquid fraction (Zayed et al., 2011). The effect of biologically active substances depends on the concentration of the fertilizer that is applied and the phenophase of plants. If the bio fertilizers are applied in the vegetation period, they are absorbed entirely by plants.

They also have an impact on the metabolism and content of unsaturated fatty acid composition (Bellaloui et al., 2010). Foliar nutrition of *Glycine max* (L.) Merrill., increased the protein concentration in the grain and enhances the growth processes of the above ground parts (Mannan, 2014).

The agrotechnic overcomes the physiological stress, increases the resistance to diseases and pests and improves the values of the structural elements of production in that legume crop.

The aim of the present study was to determine the impact of foliar feeding with Lactofol on the structural elements of seed production, the development of cultivar agrotechnics and technology for seed production of modern Bulgarian soybean cultivars.

MATERIALS AND METHODS

The study was conducted at the Experimental Station on Soybean for two consecutive years (2014-2015) under non irrigated conditions. The Bulgarian cultivar Richie was used, which is from the group with middle-early ripening period.

The soil is leached chernozem, characterized by average storage of movable phosphorus and nitrogen, a good storage of potassium and a neutral reaction. The altitude is 144 m. The precipitation amounts for the period of April-October were 485 mm (2014) and 369 mm (2015) at an average air temperature of 19.8°C and 19.3°C for the first and second experimental year, respectively.

June and July correspond to the phases of treatment. In 2014, the amount of precipitation during the summer months was higher (June - 91 mm; July - 106 mm) and the air temperature lower (June - 21.6°C; July - 23.4°C) compared to 2015 (June - 86 mm; July - 20 mm and June - 21.0°C; July - 25.6°C).

The experiment was conducted as a two-factor using the methods of fractional plots. The number of repetitions was four, the size of the harvest plot was 5m².

The factorial combinations were made with two initial phases of application of foliar fertilizing and with three formulations of the leaf fertilizer Lactofol, each applied in two doses.

The large plots were occupied by the different phases of foliar fertilization and the small plots were occupied by the different formulations and doses of the leaf fertilizer.

The initial phenophases of the application are: R₃ - flowering onset and R₅ - onset of flowering. From each initial phase, 2 treatments with a 14 day interval were made.

The following leaf fertilizer formulations were studied: Lactofol B, Lactofol K/Ca and Lactofol Basic (O), administered in the following two doses - 500 and 750 ml/da.

Composition of foliar fertilizers

Lactofol B: Macroelements (v/w) - Total nitrogen (N) 20.0; Nitrate nitrogen - 10.0; Ammonium nitrogen - 5.7; Amide nitrogen - 4.3 and Potassium oxide (K₂O) - 20.0. Microelements (v/w) - Boron (B) - 1.07; Iron (Fe) - 0.04; Manganese (Mn) - 0.02; Zinc (Zn) - 0.01; Copper (Cu) - 0.02; Magnesium oxide (MgO) - 2.21; Molybdenum (Mo) - 0.001 and Physiologically active substances.

Lactofol K/Ca: Macroelements (v/w) - Total nitrogen (N) - 20.0; Nitrate nitrogen - 10.0; Ammonium nitrogen - 3.3; Amide nitrogen - 6.7 and Potassium oxide (K₂O) - 12.21. Microelements (v/w) - Boron (B) - 0.02; Manganese (Mn) - 0.02; Zinc (Zn) - 0.01; Copper (Cu) - 0.02; Calcium oxide (CaO) - 10.0; Molybdenum (Mo) - 0.001 and Physiologically active substances.

Lactofol Basic (Lactofol O): Macroelements (v/w) - Total nitrogen (N) - 30.0; Nitrate nitrogen - 10.0; Ammonium nitrogen - 5.7; Amide nitrogen - 14.3; Phosphorus pentoxide (P₂O₅) - 7.5 and Potassium oxide (K₂O) - 15.0. Microelements (v/w) - Boron (B) - 0.30; Iron (Fe) - 0.38; Manganese (Mn) - 0.03; Zinc (Zn) - 0.02; Copper (Cu) - 0.02; Molybdenum (Mo) - 0.002 and Physiologically active substances.

Observed indicators and data analysis

When the crop ripened, 5 plants of each replicate, respectively 20 of each variant, were subjected to biometric analysis. The following indicators are taken into account: plant height (cm); number of branches per plant; number of

Pods per plant; number of seeds per plant; seed yield per plant (g), absolute seed weight - weight of 100 seeds - M100 (g) and harvest index - HI (%) - ratio of grain yield to above-ground biological yield.

Experimental data were processed by ANOVA and mean separations were performed through the Duncan multiple range test, with reference to 0.05; 0.01 and 0.001 probability level, using Microsoft Office Excel 2007 and Stat graphics Plus for Windows software package.

RESULTS AND DISCUSSIONS

Changes in the values of the structural elements of yield under the influence of the factors tested in the first experimental year

The factors studied have a reliable effect ($P < 0.05$ - $P < 0.001$) on the values of all studied parameters, except for the number of branches and pods formed by the plant - Table 1.

Table 1. Extent and significance of factorial influences on the structural elements of seed yield in the first experimental year (2014)

Sources of variation	Phenophase of Application		Formulation and dose of Lactofol		Interaction of studied factors	
	η^2 (%)	P	η^2 (%)	P	η^2 (%) and P	P
Height of plant (cm)	2.6	$P < 0.05$	28.1	$P < 0.001$	9.5	$P < 0.05$
Branches, number	0.3	ns	8.7	ns	6.6	ns
Number of pods per plant	0.1	ns	3.1	ns	13.8	ns
Number of seeds per plant	0.1	ns	3.1	ns	13.8	$P < 0.05$
Seed yield per plant (g)	3.8	$P < 0.05$	16.1	$P < 0.05$	5.2	ns
M100 (g)	0.1	$P < 0.001$	4.3	$P < 0.001$	12.0	$P < 0.001$
HI (%)	0.4	ns	11.4	$P < 0.05$	8.5	ns

The formulation and application dosage of the tested leaf fertilizer had a greater effect (η^2 was in the range 3.1 to 28.1%) on the values of the indications that structure seed yield in the soybean genotype used, compared to the phase of treatment (η^2 - 0.1 to 3.8%).

The interaction between the studied factors causes a significant part of the factorial dispersion on the indications of plant height ($\eta^2 = 9.5\%$), as well as in number and absolute weight of seeds per plant ($\eta^2 = 13.8$ and $\eta^2 = 12.0\%$, respectively).

According to the results of the first experimental year, the harvest index was significantly influenced by the composition and dose of application of Lactofol O. The increase was by 2.6 and 4.1 percentage units compared to the control in the variants treated with

Lactofol O (0.750 ml/da) - Table 2. In wetter and cooler years, foliar fertilizing adversely affected the plant height, except for the variant with Lactofol B (0.750 ml/da) and applied in the beginning of flowering phenophase. The findings suggest that the combination of increased soil moisture and treatment with boron formulation will positively affect the number of nodes and seeds on the central stem and branches of the plant. Foliar fertilizing in the later phase leads to a greater reduction in plant height.

The application of Lactofol O (0.500 ml/da) significantly ($P < 0.05$) increased the number of seeds per a plant in both phenophases studied (R_3 and R_5). The application of a higher (0.750 ml/da) dose of Lactofol O had the same effect, but for earlier treatment (R_3) variants.

Table 2. Changes in the values of the structural elements of production in *Glycine max* (L.) Merrill., treated with the universal liquid fertilizer Lactofol in the first experimental year (2014)

Phenophase of application	Control	Lactofol B 0.500	Lactofol B 0.750	Lactofol K/Ca 0.500	Lactofol K/Ca 0.750	Lactofol O 0.500	Lactofol O 0.750
	K	1	2	3	4	5	6
Height of plants (cm)							
Beginning of flowering	108.8	103.4	110.0	106.4	99.8	99.0	103.2
Flowering – pod formation	108.8	105.4	102.8	98.6	102.8	96.2	101.8
		LSD _{0.05} = 4.4					
		LSD _{0.01} = 5.8					
		LSD _{0.001} = 7.5					
Branches (number)							
Beginning of flowering	2.2	3.0	3.2	2.4	2.2	2.8	2.8
Flowering – pod formation	2.2	2.6	3.4	3.4	3.0	2.4	2.4
		LSD _{0.05} = 1.3					
		LSD _{0.01} = 1.5					
		LSD _{0.001} = 1.8					
Number of pods per plant							
Beginning of flowering	82.0	91.2	76.4	58.8	76.6	65.8	95.8
Flowering – pod formation	82.0	67.6	80.6	78.8	74.2	115.6	59.8
		LSD _{0.05} = 28.5					
		LSD _{0.01} = 37.8					
		LSD _{0.001} = 49.1					
Number of seeds per plant							
Beginning of flowering	178.4	213.4	172.8	136.0	173.8	257.3	230.4
Flowering – pod formation	178.4	156.6	185.2	162.2	156.8	252.6	141.0
		LSD _{0.05} = 44.4					
		LSD _{0.01} = 65.5					
		LSD _{0.001} = 91.9					
Seed yield per plant (g)							
Beginning of flowering	27.5	29.5	22.3	20.8	24.7	40.6	33.1
Flowering – pod formation	27.5	20.5	24.2	21.7	20.2	32.3	19.9
		LSD _{0.05} = 9.1					
		LSD _{0.01} = 12.0					
		LSD _{0.001} = 15.6					
M100 (g)							
Beginning of flowering	15.3	13.8	13.1	15.4	13.9	15.7	14.3
Flowering – pod formation	15.3	13.1	13.0	14.1	13.0	12.2	13.9
		LSD _{0.05} = 1.3					
		LSD _{0.01} = 1.7					
		LSD _{0.001} = 2.2					
HI (%)							
Beginning of flowering	46.3	46.7	45.6	48.4	45.8	42.4	48.9
Flowering – pod formation	46.3	46.4	46.2	44.0	43.2	47.0	50.2
		LSD _{0.05} = 2.4					
		LSD _{0.01} = 3.8					
		LSD _{0.001} = 5.3					

Individual plant productivity (seed yield per plant) was significantly affected ($P < 0.05$) only by leaf fertilization with Lactofol O (0.500 ml/da) in the onset of flowering. The increase was by 47.6% compared to the control nontreated variant. Lactofol B, regardless of its

phase and dose, resulted in a significant ($P < 0.05$) decrease in absolute seed weight.

The same results were observed for Lactofol K/Ca applied at a higher dose (0.750 ml/da) and for Lactofol O, which was applied into a later phenophase (R_5).

Changes in the values of the structural elements of yield under the influence of the factors tested in the second experimental year
 In the second experimental year, the factorial impacts were significant ($P < 0.001$) for plant

height (Table 3). The formulation and the dose of the leaf fertilizer ($\eta^2 = 45.7\%$) exceeded degree of influence the factor phase treatment ($\eta^2 = 9.3\%$).

Table 3. Degree and significance of factorial impacts on the structural elements of seed yield in the second experimental year (2015)

Sources of variation	Phenophase of application		Formulation and dose of Lactofol		Interaction of studied factors	
	η^2 (%)	P	η^2 (%)	P	η^2 (%)	P
Height of plants (cm)	9.3	$P < 0.001$	45.7	$P < 0.001$	10.5	$P < 0.05$
Number of branches	4.0	$P < 0.05$	3.7	ns	2.4	ns
Number of pods per a plant	0.8	ns	6.2	ns	11.4	ns
Seeds per a plant	0.4	ns	6.3	ns	10.8	$P < 0.05$
Yield of seeds per a plant (g)	0.1	ns	8.4	ns	12.6	$P < 0.05$
M100 (g)	0.1	ns	17.7	$P < 0.05$	3.6	ns
HI (%)	1.9	ns	9.2	ns	1.7	ns

The values exceeded the control by 5.3% (Lactofol O - 0.500 ml/da) to 16.9% (Lactofol K/Ca - 0.500 ml/da) compared to the control in variants with earlier fertilizing phase (R_3) - Table 4. Treatment of plants in the phenophase of flowering-pod formation (at lower soil and air humidity) had a less pronounced effect on the height of the crop. The excess was from 3.2% (Lactofol O - 0.750 ml/da) to 15.7% (Lactofol K/Ca - 0.750 ml/da) compared to the nontreated variant. An exception is observed for the crops of the second variant, where the plant height treated by Lactofol B (0.500 ml/da) were 0.4 cm lower than the control.

The analysis data clearly show the effect of fertilizer applied (in both test phases - R_3 and R_5) on the number of branches of *Glycine max* (L.) Merrill. The values were significantly affected ($P < 0.05$) in the leaf treatment variants with Lactofol O (0.750 ml/da) in the beginning of flowering phenophase. The excess over the control was 31.3%. The interaction between the studied factors had a significant effect on the values of number and yield of seeds. The treatment with Lactofol K/Ca (0.750 ml/da) in phenophase of flowering-pod formation increased the values by 57.5% and ($P < 0.05$) and 85.3% ($P < 0.01$), respectively. In the same phase, the variant had the highest number of

pods per plant. The excess over the nontreated control was 40.6% ($P < 0.05$). The yield of seeds per plant was significantly affected ($P < 0.05$) in the variants with foliar fertilizing of Lactofol O in beginning of flowering, regardless of the administered dose.

In contrast to the first experimental year, the data from the second year indicate a significant increase in the absolute weight of seeds in a large number of the variants studied. The highest values were found in the variants with a higher dose (0.750 ml/da) Lactofol O and Lactofol K/Ca applied in the beginning of flowering phenophase. The excess in comparison with the control was 27.0% and 24.3%, respectively.

In case of severe soil drought, the formulations: Lactofol O (0.750 ml/da), Lactofol K/Ca (0.500 ml/da) and Lactofol K/Ca (0.750 ml/da), introduced into the phenophase of flowering-pod formation, showed a high degree of positive influence ($P < 0.01$ and $P < 0.05$) on the absolute weight of seeds.

In the second experimental year, changes in harvest index values were not significantly affected by the treatment phase alone and in interaction with the formulation and dose of the tested foliar fertilizer.

Table 4. Changes in the values of the structural elements of production in *Glycine max* (L.) Merrill., treated with the universal liquid fertilizer Lactofol in the second experimental year (2015)

Phenophase of application	Control	Lactofol B 0.500	Lactofol B 0.750	Lactofol K/Ca 0.500	Lactofol K/Ca 0.750	Lactofol O 0.500	Lactofol O 0.750
	K	1	2	3	4	5	6
Height of plants(cm)							
Beginning of flowering	86.4	92.4	100.0	101.0	96.8	91.0	95.2
Flowering – pod formation	86.4	86.0	92.0	93.4	100.0	89.4	89.2
	LSD _{0.05} = 3.3 LSD _{0.01} = 4.4 LSD _{0.001} = 5.7						
Branches (number)							
Beginning of flowering	3.2	3.6	3.6	3.6	3.4	3.4	4.2
Flowering – pod formation	3.2	2.6	3.2	3.2	3.2	3.2	3.4
	LSD _{0.05} = 0.8 LSD _{0.01} = 1.1 LSD _{0.001} = 1.4						
Number of pods per plant							
Beginning of flowering	46.8	57.8	52.0	50.2	45.6	61.4	55.0
Flowering – pod formation	46.8	41.4	56.0	46.8	65.8	54.8	33.8
	LSD _{0.05} = 15.0 LSD _{0.01} = 19.5 LSD _{0.001} = 25.0						
Number of seeds per plant							
Beginning of flowering	84.8	111.0	95.4	93.6	93.0	114.8	111.8
Flowering – pod formation	84.8	84.4	105.6	94.0	133.6	106.0	63.0
	LSD _{0.05} = 39.5 LSD _{0.01} = 51.3 LSD _{0.001} = 63.6						
Seed yield per plant (g)							
Beginning of flowering	9.5	13.5	10.8	11.6	11.6	14.7	15.7
Flowering – pod formation	9.5	10.5	13.0	12.6	17.6	13.4	8.9
	LSD _{0.05} = 5.2 LSD _{0.01} = 6.8 LSD _{0.001} = 8.2						
M100 (g)							
Beginning of flowering	11.1	12.1	11.2	12.4	13.8	12.7	14.1
Flowering – pod formation	11.1	12.2	12.2	12.9	12.6	12.4	13.1
	LSD _{0.05} = 1.5 LSD _{0.01} = 2.0 LSD _{0.001} = 2.5						
HI (%)							
Beginning of flowering	40.7	41.6	39.2	40.3	40.4	41.0	43.1
Flowering – pod formation	40.7	43.6	40.6	40.8	42.7	41.6	42.9
	LSD _{0.05} = 2.9 LSD _{0.01} = 3.8 LSD _{0.001} = 4.9						

The application effectiveness of a technology is evaluated on the basis of changes in the values of the basic structural components concerning the yield and quality of the legume crop (El-Shairy and Hegazi, 2009; Hristozkova et al., 2011; Bozhanska et al., 2017; Bozhanska, 2018). According to Mandić et al. (2015), the treatment of soybeans under conditions of even

rainfall distribution during the vegetation season results in a significant increase of the average values of the following: height (108.8 cm), number seed (121.0) and seed yield (20.76 g) per plant. According to the results of the present study, under conditions of lower humidity, foliar fertilizing positively affected the height, number and absolute weight of

seeds, as well as the seed yield per a single plant. Under conditions of greater amount of vegetative precipitation, other structural elements of seed productivity, such as number of beans and branches per a plant, were positively affected. Some authors (Barger, 2001; Oko et al., 2003; Mallarino, 2005) have determined that the phase of fertilizer application is essential for the results of foliar fertilizing, such as treatment in earlier phases (R₂-R₃), increases seed yield by 15-30% to 68%. According to the results of the present study in the second experimental year, fertilization in the phenophase of beginning of flowering (R₃) led to a greater increase in the height of the crop compared to the later treatment. The same applies to the absolute weight of seeds (weight per 100 seeds). In the first experimental year, when foliar fertilizing lowers the height of the plants, treatment in phenophase of flowering-pod formation led to a greater reduction in the values. According to the results obtained, foliar fertilizing in later phenophase (R₅) had a greater impact on other characteristics, such as maximum increase in the number of pods, number and yield of seeds per plant.

Differences in the result of foliar fertilizing of soy depending on the composition and formulation of leaf fertilizer have been identified in a number of studies (Milev and

Todorova, 2014; Jarecki et al., 2016; Moreira et al., 2017; Kahraman, 2017). Mallarino et al. (2001) define the soybean foliar fertilizing with macronutrients as multifaceted in effect. According to Moreira et al. (2017) foliar N generally increased seed yield, irrespective of N source and analysis pooled over three growing seasons showed average seed yield increase of 5.0% (211 kg ha⁻¹) and 6.1% (259 kg ha⁻¹) for the 5 and 10 kg N ha⁻¹ over control, respectively. In the present study, Lactofol O containing compounds with increased N and P concentration had a clearly positive effect on the absolute weight of seed, number of branches and number of pods per soybean plants.

Potassium imported by foliar fertilizing increases the number of pods, yield and the amount of protein in the composition of soybean (Anuradha and Sharma, 1995; Tiwari

et al., 2001). This is in support of the results that we got in the variants with Lactofol K/Ca. The formulation enriched with the K and Ca macronutrients influences to the maximum extent the height, yield and number of seeds in the soybean genotype used.

Fertilizing soybean by foliar application of boron is considered effective when soils are poorly stocked and are not recommended as a permanent agricultural practice (Bruns, 2017). Hamurcu et al. (2019) observed a positive effect of foliar fertilizing with this microelement in terms of the quality composition of the grain. According to Sabev and Todorova (2015), the treatment of soybean with Lactofol O + 2% B contributes the least to improving its productivity. Our findings confirm that foliar fertilizing with Lactofol enriched with boron has the least effect on the structural elements of soybean seed production, regardless of the conditions of the experimental year. As a probable cause, we can point to the sufficient presence of this element in the leached black soil. At the same time, according to Pawlowski et al. (2019), the physiological response of soybeans to the deficiency and excess of this element is variety dependent. It may be considered that 'Ritchie' cultivar used for the study does not respond to foliar boron nutrition.

CONCLUSIONS

In the wetter and cooler year, Lactofol O (0.500 ml/da) introduced into the two phenophases tested increased significantly (P<0.01) the number of seeds per plant by 41.6 to 44.2%. At a higher dose (0.750 ml/da) of Lactofol O and in the earlier phase (R₃), the values increased by 29.1% (P<0.05). Seed production was significantly increased by 47.6% (P<0.05) only from the formulation Lactofol O (0.500 ml/da) introduced at the beginning of flowering phase. In conditions of optimal to low soil and air humidity, foliar fertilizing significantly increases the height of the plants. The values of the variants fertilized in the earlier phase (R₃) exceeded the control by 5.3% Lactofol O (0.500 ml/da) to 16.9% Lactofol K/Ca (0.500 ml/da). The number and yield of plant seeds exceeded the control by 57.5% (P<0.05) and 85.3% (P<0.01), respectively, in the variants

with Lactofol K/Ca (0.750 ml/da) applied in the flowering-bean-forming phenophase (R₅). The amount of seeds per plant was also significantly affected (P<0.05) by Lactofol O, introduced into the phenophase beginning of flowering, regardless of dose. The application of Lactofol O (0.750 ml/da) and Lactofol K/Ca (0.750 ml/da) in phenophase R₃ increased the absolute seed weight by 27.0% and 24.3%, respectively (P<0.001).

The harvest index was increased by 2.6 and 4.1 percentage points compared to the control in Lactofol O variants (0.750 ml/da).

REFERENCES

- Akparobi, S.O. (2009). Evaluation of six cultivars of soybean under the soil of rainforest agro-ecological zones of Nigeria. *Middle-East Journal of Scientific Research*, 4(1), 6–9.
- Ali, N. (2010). Soybean processing and utilization. The Soybean Botany, Production and Uses, (Ed.): Singh, G. CABI International, UK, 345–374.
- Anuradha, K., Sharma, P.S. (1995). Effect of moisture stress and applied potassium on yield and biochemical parameters of soybean in vertisols. *Journal of Oilseeds Research*, 12, 275–278.
- Argaw, A. (2012). Evaluation of co-inoculation of *Bradyrhizobium japonicum* and phosphate solubilizing *Pseudomonas* spp. effect on soybean (*Glycine max* L. Merr.) in Assossa area. *Agricultural Science and Technology*, 14(1), 213–224.
- Barge, G.L. (2001). Foliar fertilizer applications for soybean production. *Special circular*, 197. 71–73.
- Bellaloui, N., Gillen, A. M., Reddy, K. N., Abel, C. A. (2010). Nitrogen metabolism and seed composition as influenced by foliar boron application in soybean. *Plant Soil*, 336, 143–155. DOI: 10.1007/s11104-010-0455-6
- Bozhanska, T. (2018). Botanical and morphological composition of artificial grassland of bird's-foot-trefoil (*Lotus corniculatus* L.) treated with Lumbrical and Lumbrex. *Banat's Journal of Biotechnology*, 9(19), 12–19. DOI: 10.7904/2068-4738-IX(19)-12
- Bozhanska, T., Churkova, B., Mihovski, T. (2017). Biological, morphological and qualitative characteristics of perennial legume forage grasses treated with growth regulators and biofertilizers. *Journal of Mountain Agriculture on the Balkans*, 20(2), 100–113.
- Bruns, H.A. (2017). Effects of boron foliar fertilization on irrigated soybean (*Glycine max* L. Merr.) in the Mississippi River Valley Delta of the midsouth, USA. *Archives of Agriculture and Environmental Science*, 2(3), 167–169.
- Churkova, B. (2013). Influence of treatment with biofertilizers over the chemical composition and the energy nutritional value of forage from birdsfoot trefoil. *Banat's Journal of Biotechnology*, 4(8), 20–25. DOI: 10.7904/2068-4738-IV(8)-20
- Churkova, B. (2014). Influence of Some Organic Mineral Fertilizers on the Seed Productivity of Birdsfoot trefoil. *American Journal of Agricultural Science and Technology*, 2(1), 42–48. DOI:10.7726/ajast.2014.1005
- Devi, K.N., Singh, L.N.K., Singh, M.S., Singh, S.B., Singh, K.K. (2012). Influence of Sulphur and Boron Fertilization on Yield, Quality, Nutrient Uptake and Economics of Soybean (*Glycine max*) under Upland Conditions. *Journal of Agricultural Science*, 4(4), 1–10.
- El-Haggan, E.A.L.M.A. (2014). Effect of micronutrients foliar application on yield and quality traits of soybean cultivars. *International Journal of Agriculture and Crop Sciences*, 7(11), 908–914.
- El-Shraiy, A.M., Hegazi, A.M. (2009). Effect of acetylsalicylic acid, indole – 3 – bytric acid and glbberelic acid on plant growth and yield of pea (*Pisum sativum* L.). *Australian Journal of Basic and Applied Sciences*, 3(4), 3514–3523.
- FAOSTAT (2017). Food and Agriculture Organization of the United Nations. <http://www.fao.org/faostat/en/#data>
- Hamurcu, M., Arslan, D., Hakki, E.E., Ozcan, M.M., Pandey, A., Khan, M.K., Gezgin, S. (2019). Boron application affecting the yield and fatty acid composition of soybean genotypes. *Plant, Soil and Environment*, 65(5), 238–243, DOI: 10.17221/679/2018-PSE
- Hristozkova, M., Geneva, M., Stancheva, I. (2011). Regulation of Nitrogen Assimilation in foliar Fed Legume Plants at Insufficient Molibdenum Supply. *Plant Growth and Promrting Bacteria, Microbiology Monographs*, 18, 417–431. DOI: 10.1007/978-3-642-13612-2_18
- Jarecki, W., Buczek, J., Bobrecka-Jamro, D. (2016). Response of soybean (*Glycine max* L. Merr.) to bacterial soil inoculants and foliar fertilization. *Plant, Soil and Environment*, 62(9), 422–427. DOI: 10.17221/292/2016-PSE
- Kahraman, A. (2017). Nutritional value and foliar fertilization in soybean. *Journal of Elementology*, 22(1), 55–66, DOI: 10.5601/jelem.2016.21.1.1106
- Kumar, A. (2007). A study of consumer attitudes and acceptability of soy food in Ludhiana. MBA research project report, Department of Business Management, Punjab Agricultural University, Ludhiana. <https://krishikosh.egranth.ac.in/display/bitstream?handle=1/5810125005>
- Lee, J., Song, S.H. (2007). Evaluation of Groundwater Quality in Coastal Areas: Implications for Sustainable Agriculture. *Environmental Geology*, 52, 1231–1242. <http://DOI.ORG/10.1007/S00254-006-0560-2>
- Mallarino, A.P. (2005). Foliar fertilization of soybean: Is it useful to supplement primary fertilization? *Integrated Crop Manag.*, IC-494, 15, 125–126.
- Mallarino, A.P., Haq, M.U., Witty, D., Bermudez, M. (2001). Variation in soybean response to early season

- foliar fertilization among and within fields. *Agronomy Journal*, 93(6), 1220–1226. DOI:10.2134/AGRONJ2001.1220
- Mandić, V., Simić, A., Krnjaja, V., Bijelić, Z., Tomić, Z., Stanojković, A., Ruzić Muslić, D. (2015). Effect of foliar fertilization on soybean grain yield. *Biotechnology in Animal Husbandry*, 31(1), 133–143. <http://DOI.ORG/10.2298/BAH1501133M>
- Mannan, M.A. (2014). Foliar and soil fertilization effect on seed yield and protein content of soybean. *Bangladesh Agronomy Journal*, 17(1), 67–72. DOI: <https://doi.org/10.3329/baj.v17i1.23678>
- Marinova, D., Ivanova, I. (2018). Effect of foliar fertilization with total care on some morphological traits and forage productivity in Prista 5 alfalfa (*Medicago sativa* L.) variety. *Journal of Mountain Agriculture on the Balkans*, 21(4), 118–131.
- Marinova, D., Soivanova, S., Petrova, I. (2019). Study of the effect of biostimulants application on green mass and dry matter yield in alfalfa (*Medicago sativa* L.) Prista 4 variety. *Journal of Mountain Agriculture on the Balkans*, 22(3), 64–80.
- Marinov-Serafimov, P., Golubina, I. (2019). Effect of growth regulator on *Agropyron desertorum* (Fisch.) Schultes. *Journal of Mountain Agriculture on the Balkans*, 22(4), 88–102.
- Milev, G., Todorova, R. (2014). Effect of the complex suspension foliar fertilizers Lactofol and Amalgerol premium on grain yield from soybean (*Glycine max* L. Merr.) under the conditions of Dobrudzha region. *Agricultural Science and Technology*, 6(4), 445–450.
- Moreira, A., Moraes, L.A.C., Schroth, G., Becker, F.J., Mandarino, J.M.G. (2017). Soybean yield and nutritional status response to nitrogen sources and rates of foliar fertilization. *Agronomy Journal*, 109(2), 629–635. DOI:10.2134/AGRONJ2016.04.0199
- Oko, B.F.D., Eneji A.E., Binang, W., Irshad, M., Yamamoto, S., Honna, T., Endo, T. (2003). Effect of foliar application of urea on reproductive abscission and grain yield of soybean. *Journal of Plant Nutrition*, 26, 1223–1234. <https://doi.org/10.1081/PLN-120020366>
- Pawlowski, M.L., Helfenstein, J., Frossard, E., Hartman, G.L. (2019). Boron and zinc deficiencies and toxicities and their interactions with other nutrients in soybean roots, leaves, and seeds. *Journal of Plant Nutrition*, 42(6), 634–649. <https://doi.org/10.1080/01904167.2019.1567782>
- Raei, E., Sedghi, M., Sayed Sharifi, R. (2008). Effect of Bradirizobium inoculation, application of nitrogen and weeding on growth and seed filling rate in soybean. *Journal of Science and Technology of Agriculture and Natural Resources*, 12(43), 91–81.
- Rosculete, C.A., Bonciu, E., Rosculete, E., Olaru, L.A. (2019). Determination of the Environmental Pollution Potential of Some Herbicides by the Assessment of Cytotoxic and Genotoxic Effects on *Allium cepa*. *International Journal of Environmental Research and Public Health*, 16(1), 75. DOI:10.3390/IJERPH16010075
- Sabev, V., Todorova, R. (2015). Determining the soybean productivity and efficiency under the effect of biostimulants and Folia fertilizers. *Anniversary collection – 90 years since the creation of Experimental station on soybean in Pavlikeni*, 135–143.
- Shrivastava, U.K., Rajput, R.L., Devedi, M.L. (2000). Response of soybean-mustard cropping system to sulphur and biofertilizers on farmer's field. *Legume Research- An International Journal*, 23, 277–78.
- Singh, M.V., Patel, K.P., Ramani, V.P. (2003). Crop responses to secondary and micronutrients in swell-shrink soils. *Fertilizer News*, 48(4), 63–66.
- Son, T.T.N., Diep, C.N., Giang, T.T.M. (2006). Effect of Bradyrhizobia and Phosphate Solubilizing Bacteria Application on Soybean in Rotational system in the Mekong Delta. *Omonrice*, 14, 48–57.
- Tiwari, S.P., Joshi, O.P., Vyas, A.K., Billore, S.D. (2001). Potassium Nutrition in Yield and Quality. 307–320.
- Vasileva, V. (2015). Root biomass accumulation in vetch (*Vicia sativa* L.) after treatment with organic fertilizer. *Banat's Journal of Biotechnology*, 6(11), 100–105. DOI: 10.7904/2068–4738–VI(11)–100
- Wasule, D.L., Wadyalkar, S.R., Buldeo, A.N. (2007). Effect of Phosphate Solubilizing Bacteria on Role of *Rhizobium* on Nodulation by Soybean. *First International Meeting on Microbial Phosphate Solubilization*, 139–142.
- Zayed, B.A., Salem, A.K.M., Sharkawy, H.M.E. (2011). Effect of different micronutrient treatments on rice (*Oryza sativa* L.) growth and yield under saline soil conditions. *World Journal of Agricultural Sciences*, 7(2), 179–184.

VARIATION OF THE GROWTH PERIOD AT SUNFLOWER HYBRIDS ACCORDING TO GROWING CONDITIONS

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Abstract

For a given sunflower hybrid, even the length of the growth period is a specific characteristic, this being determined by the genetics of the hybrid, this is influenced between some limits by the growing conditions. These limits of variation are important to be known by the farmers as they could predict the time when the plants reach a certain growing stage or the maturity.

The aim of the present paper is to identify the variation limits of the growth period of an assortment of 18 sunflower hybrids cultivated in 2018 and 2019 in five locations covering the most important regions for growing sunflower in Romania. Sunflower hybrids behave differently regarding the duration of the growth period, according to the specific growing conditions of the location and the year. The variation limits of the growth period at the sunflower hybrids studied in the five location from the most important regions for growing sunflower in Romania and in the years 2018 and 2019 were of 52-74 days for the period from emergence to beginning of flowering process (10% flowered heads) and of 101-135 days for the period from emergence to maturity.

Key words: growth period, hybrid, limits of variation, sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) adapted during the time to various growing areas, which are characterised through different environmental conditions. This adaptation process has involved several mechanisms among which the length of the growth period is one of the most important.

Sunflower is a temperate zone crop which can perform well under various climatic and soil conditions (Qadir et al, 2007). This is a versatile crop, this being reflected in the fact that it can grow on a wide range of soils in many latitudes and are tolerant of dry conditions (Myers, 2017).

The total time required for development of a sunflower plant and the time between the various stages of development depends on the genetic background of the plant and the growing environment (Schneiter & Miller, 1981). So, the length of the growth period is given by the genetics of the hybrid and it is influenced by the environmental conditions

which characterise a certain geographical location.

Early maturing varieties are ready for harvesting 90 to 120 days after planting, and late maturing varieties 120 to 160 days after planting (Fernández-Luqueño et al., 2014).

Practically, due to its adaptation to a large production area, with different climate features in light intensity, temperature and altitude, the crop cycle may vary from 80 to 130 days, depending on the cultivar and sowing date (Castro and Leite, 2018).

The time taken for a sunflower plant to develop through the growth stages from planting to maturity is affected by planting time, temperature, day length, nutrition and soil moisture; thus, temperature may vary the time taken to reach physiological maturity by as much as 25-30 days (GRDC, 2017).

Climatic variability is a major cause of inability of agricultural crops to achieve potential yield (Agele, 2003). This is an important source for the variability of the length of the growth period of the crops. Among the climatic factors,

temperature has a major influence on the growth and development processes of the plants, as well as on the length of the growth period.

Temperature affects the duration of all phenological stages (Alberio et al., 2015; GRDC, 2017; Ştefan et al., 2008). Having wide adaptability, different sunflower hybrids require different total number of cumulative degree-days or growing degree days for growth, development and maturity (Qadir et al., 2007). The increase in the average annual temperature will decrease the duration of the growth period till flowering with 4-6 days/°C and the duration of the growth period till maturity with 7-12 days/°C (Guilioni et al., 2010).

Another important factor affecting the length of the growth period is the day length, respectively the photoperiod. The flowering time, for example can be increased by up to 15 days due to day length (GRDC, 2017).

Generally, a longer growth period of a given sunflower hybrid is associated with a higher yielding capacity, this being due to the fact that the plants can produce more through photosynthetic process during a longer period of growth. But, increasing the vegetation period itself do not lead to a high productivity and in each groups of earliness there are hybrids with high and low productivity (Gontcharov & Zaharova, 2008). The longer growth period has to be put into values through optimal values of the environmental factors.

For a given sunflower hybrid, even the length of the growth period is a specific characteristic, this being determined by the genetics of the hybrid, this is influenced between some limits by the growing conditions. These limits of variation are important to be known by farmers as they could predict the time when the plants reach a certain growing stage or the maturity.

The aim of the present paper is to identify the variation limits of the growth period of an assortment of 18 sunflower hybrids cultivated in 2018 and 2019 in five locations covering the most important regions for growing sunflower in Romania.

MATERIALS AND METHODS

An assortment of 18 commercial sunflower hybrids was studied under rainfed conditions in

two years (2018 and 2019) and in five field experiments located in the most important regions for growing sunflower in Romania.

The studied hybrids were the following: P63LL06, SY Flamenco, NK Neoma, LG55.42 CL, FD15E27, FD15CL44, LG 55.55 CLP, Paraiso 1000 CL Plus, ES Arcadia SU, P64LE25, P64LE99, MAS 85.SU, Rustica 172, Rustica 170, MAS 83.HO, Suntec HO CL, ES Poetic HO, P64HE118.

The five locations of the field experiments were the following:

- Negreşti (Vaslui district), which is representative for eastern Romania; the area is characterised by haplic chernozems with neutral pH, well supplied with potassium and mid supplied with phosphorus and nitrogen.
- Cogealac (Constanţa district), which is representative for south-eastern Romania; the area is characterised by chernozems with basic pH, well supplied with potassium and mid supplied with nitrogen and phosphorus.
- Mircea Vodă (Brăila district), which is representative for south-eastern Romanian Plain; the area is characterised by chernozems with neutral-basic pH, well supplied with potassium and mid supplied with phosphorus and nitrogen.
- Peciu Noi (Timiş district), which is representative for western of Romania; the area is characterised by haplic chernozems with neutral-basic pH, well supplied with potassium, mid supplied with phosphorus and poorly supplied with nitrogen.
- Şimleu Silvaniei (Sălaj district), which is representative for north-western of Romania; the area is characterised by haplic luvisols with neutral-basic pH, well supplied with potassium, mid supplied with phosphorus and poorly supplied with nitrogen.

For the five locations, the year 2018 is characterised as being warmer and dryer than 2019 (Table 1). The average temperature registered in the period April-August, which is associated with the growing period of the sunflower in Romania, in the two experimental years varied between 19.2 and 22.4°C, while the sum of rainfall varied between 164.2 and 367.7 mm, according to location.

Table 1. Temperatures and rainfall registered in the period April-August, which is associated with the growing period of the sunflower in Romania

Location	Average temperatures (°C)		Sum of rainfall (mm)	
	2018	2019	2018	2019
Negrești	21.3	22.3	221.9	367.7
Cogealac	20.5	19.9	213.0	181.0
Mircea Vodă	20.7	19.8	164.2	216.0
Peciu Nou	22.4	20.1	292.4	296.9
Șimleu Silvaniei	21.0	19.2	265.7	327.5
Average values for the five locations	21.2	20.3	231.4	277.8

The field experiments were organised in split plots with three replications. The sowing was performed in April at 70 cm row spacing. The plant density was of 51,000 plants/hectare.

The weeds were controlled by the help of herbicides, which were applied pre-emergence (Dual Gold 960EC herbicide with 960 g/l of S-metolaclof as active substances, applied in a rate of 1.5 l/ha for controlling annual grasses and some annual broad-leaved weeds) and in vegetation in the 6 leaves stage (Select Super 120 EC herbicide with 120 g/l of Clethodim as active substances, applied in a rate of 1.5 l/ha for controlling annual and perennial monocotyledonous weeds).

Fertilization was performed with complex fertilizers containing nitrogen and phosphorus, which were of type 18:46:0, applied before seed bed preparation in a rate of 300 kg/ha. In vegetation, there was applied 150 kg/ha of ammonium nitrate.

RESULTS AND DISCUSSIONS

Duration of the period from emergence to beginning of the flowering process (10% flowered heads) at the studied sunflower hybrids in the five different locations in Romania was in average of 59 days, with limits of variations from 52 to 68 days in 2018, and it was in average of 66 days, with limits of variations from 54 to 74 days in 2019 (Table 2). These figures are according to those obtained by previous studies performed in South Romania, when the number of days from emergence to the beginning of flowering (10% flowered heads) varied between 64 and 70 days (Ion et al., 2006), even this time the minimum values are smaller.

The differences between maximum and minimum values registered by the studied hybrids according to the specific growing conditions of the location was in average of 10 days, with limits of variation from 7 to 14 days in 2018, and it was of 14 days, with limits of variation from 11 to 19 days in 2019. The higher variation registered in 2019 is confirmed by the value of the coefficient of variation (CV) which was in average of 7.64 in 2019 compared to 5.92 in 2018 (Table 2).

Duration of the period from emergence to maturity at the studied sunflower hybrids in the five different locations in Romania was in average of 116 days, with limits of variations from 101 to 130 days in 2018, and it was in average of 122 days, with limits of variations from 110 to 135 days in 2019 (Table 3). These figures are according to those mentioned by Vrânceanu (2000), respectively the growth period at the sunflower hybrids is of 115-136 days.

The differences between maximum and minimum values registered by the studied hybrids according to the specific growing conditions of the location was in average of 21 days, with limits of variation from 16 to 27 days in 2018, and it was of 22 days, with limits of variation from 18 to 25 days in 2019. Differences between maximum and minimum values registered by the studied hybrids in 2018 and 2019 are quite similar, this being shown also by the coefficient of variation (CV) which was in average of 6.59 in 2018 and 6.31 in 2019 (Table 3).

It has to be notices that the differences between maximum and minimum values registered by the studied hybrids according to the specific growing conditions of the location are much higher, up to double for the duration of the period from emergence to maturity compared to the duration of the period from emergence to beginning of the flowering process. This means that the differences between maximum and minimum values registered for the duration of the period from emergence to beginning of the flowering process are amplified as time goes on to the maturity leading to higher differences for the duration of the period from emergence to maturity.

Table 2. Duration of the period from emergence to beginning of the flowering process at different sunflower hybrids cultivated in five different locations in Romania in 2018 and 2019

Sunflower hybrid	Period from emergence to beginning of flowering (days)									
	2018					2019				
	Minimum	Average	Maximum	Difference (Max.-Min.)	CV* (%)	Minimum	Average	Maximum	Difference (Max.-Min.)	CV* (%)
P63LL06	53	57	64	11	6.76	56	65	73	17	9.31
SY Flamenco	55	58	62	7	4.40	58	66	73	15	7.55
NK Neoma	54	58	62	8	6.03	56	64	71	15	8.09
LG55.42 CL	54	58	62	8	5.14	57	64	70	13	6.86
FD15E27	53	59	64	11	6.58	56	65	72	16	8.57
FD15CL44	54	59	65	11	6.35	60	67	74	14	8.11
LG 55.55 CLP	52	56	60	8	5.42	54	62	68	14	8.35
Paraiso 1000 CL Plus	55	60	64	9	4.89	60	67	71	11	6.27
ES Arcadia SU	53	56	61	8	5.44	54	63	73	19	9.80
P64LE25	54	58	63	9	6.63	57	66	72	15	7.93
P64LE99	55	59	68	13	8.15	58	67	72	14	7.57
MAS 85.SU	55	59	64	9	5.42	60	67	73	13	6.88
Rustica172	54	60	65	11	6.11	60	68	73	13	7.50
Rustica170	54	62	68	14	7.52	60	67	72	12	6.87
MAS 83.HO	55	60	64	9	5.48	61	68	73	12	6.58
Suntec HO CL	56	60	63	7	4.28	60	67	73	13	6.74
ES Poetic HO	55	59	64	9	5.39	57	64	70	13	6.99
P64HE118	52	57	61	9	6.56	56	64	69	13	7.51
Minimum	52	56	60	7	4.28	54	61	68	11	6.27
Average	54	59	64	10	5.92	58	66	72	14	7.64
Maximum	56	62	68	14	8.15	61	68	74	19	9.80

*CV = coefficient of variation.

Table 3. Duration of the period from emergence to maturity at different sunflower hybrids cultivated in five different locations in Romania in 2018 and 2019

Sunflower hybrid	Period from emergence to maturity (days)									
	2018					2019				
	Minimum	Average	Maximum	Difference (Max.-Min.)	CV* (%)	Minimum	Average	Maximum	Difference (Max.-Min.)	CV* (%)
P63LL06	104	115	129	25	7.56	110	122	130	20	5.90
SY Flamenco	101	115	128	27	8.16	111	120	131	20	6.03
NK Neoma	106	116	127	21	6.49	110	122	133	23	6.53
LG55.42 CL	109	115	125	16	5.07	110	120	132	22	6.00
FD15E27	109	118	128	19	5.35	111	122	132	21	6.30
FD15CL44	104	116	130	26	7.92	114	124	132	18	5.75
LG 55.55 CLP	104	115	123	19	6.11	110	121	135	25	7.01
Paraiso 1000 CL Plus	109	117	128	19	5.92	114	125	135	21	6.10
ES Arcadia SU	105	114	125	20	6.25	110	122	132	22	6.41
P64LE25	103	116	129	26	7.83	110	121	132	22	6.02
P64LE99	107	117	130	23	7.17	111	123	133	22	6.93
MAS 85.SU	105	116	127	22	6.85	111	122	133	22	6.33
Rustica172	109	117	126	17	5.16	110	121	131	21	6.09
Rustica170	106	116	126	20	6.49	111	124	133	22	7.03
MAS 83.HO	105	117	130	25	7.70	113	124	135	22	6.03
Suntec HO CL	108	117	126	18	5.30	110	123	133	23	7.29
ES Poetic HO	104	114	125	21	6.35	110	121	133	23	6.35
P64HE118	107	116	129	22	6.96	111	121	131	20	5.53
Minimum	101	114	123	16	5.07	110	120	130	18	5.53
Average	106	116	127	21	6.59	111	122	133	22	6.31
Maximum	109	118	130	27	8.16	114	125	135	25	7.29

*CV = coefficient of variation.

The duration of the period from emergence to beginning of the flowering process and the duration of the period from emergence to maturity, as well as the variation of these periods according to the specific growing conditions are important to be known by sunflower growers. In this respect, Gontcharov & Zaharova (2008) concluded that seed yield is mainly determined by duration of the period from emergence to flowering (VE-R5.1), and oil content is mainly determined by duration of the period from beginning of flowering to maturity (R5.1-R8).

CONCLUSIONS

Sunflower hybrids behave differently regarding the duration of the growth period, according to specific growing conditions of the location and the year.

The variation limits of the growth period at the sunflower hybrids studied in the five location from the most important regions for growing sunflower in Romania and in the years 2018 and 2019 were of 52-74 days for the period from emergence to beginning of flowering process (10% flowered heads) and of 101-135 days for the period from emergence to maturity.

The differences between maximum and minimum values of the duration of the growth period registered by the studied hybrids according to the specific growing conditions of the study location and year was of 7-19 days for the period from emergence to beginning of flowering process and of 16-27 for the period from emergence to maturity.

The variation of the duration of the period from emergence to maturity is much more important than the variation of the duration of the period from emergence to beginning of flowering process.

For all the studied sunflower hybrids no matter the location and year the coefficient of variation for the growth period was less than 10%.

REFERENCES

- Agele, S.O. (2003). Response of sunflower to weather variations in a tropical rainforest zone. *African Crop Science Conference Proceedings*, 6, 1–8.
- Alberio, C., Izquierdo, N.G., Aguirrezábal, L.A.N. (2015). Sunflower Crop Physiology and Agronomy. Chapter 3 in: *Sunflower Chemistry, Production, Processing, and Utilization*. Editors: Martínez-Force, E., Dunford, N. T., Salas, J. J., AOCS Press, pp. 53–91.
- Castro, C., Leite, R.M.V.B.C. (2018). Main aspects of sunflower production in Brazil. *Oilseeds & Fats Crops and Lipids - OCL*, 25(1), D104.
- Fernández-Luqueño, F., López-Valdez, F., Miranda-Arámbula, M., Rosas-Morales, M., Pariona, N., Espinoza-Zapata, R. (2014). An Introduction to the Sunflower Crop. Chapter 1 in: *Sunflowers*, p. 1–18. Editor: Juan Ignacio Arribas. Nova Science Publishers, Inc.
- Gontcharov, S., Zaharova, M. (2008). Vegetation period and hybrid sunflower productivity in breeding for earliness. *Proc. 17th International Sunflower Conference, Córdoba, Spain*, 531–533.
- GRDC, 2017. Sunflowers GrowNotes. Grains Research and Development Corporation, Australia.
- Guilioni, L., Brisson, N., Levrault, F. (2010). C6 - Tournesol. Éléments sur le changement climatique et la culture du tournesol. En : Brisson, N., Levrault, F., éditeurs. *Changement climatique, agriculture et forêt en France : simulations d'impacts sur les principales espèces*. Le Livre Vert du projet CLIMATOR (2007-2010). ADEME, p. 201–212.
- Ion, V., Ștefan, V., Ion, N., Roman, G.V., Dumbravă, M. (2006). Date privind înflorirea la un sortiment de hibrizi străini de floarea-soarelui admiși pentru cultivare în România. *Lucrări științifice, Simpozionul Internațional de Apicultură "Managementul durabil al exploatațiilor apicole în concordanță cu cerințele UE"*, Tulcea, pp. 9.
- Qadir, G., Fayyaz-Ul-Hassan, Malik, M. A. (2007). Growing Degree Days and Yield Relationship in Sunflower (*Helianthus annuus* L.). *International Journal of Agriculture & Biology*, 9(4), 564–568.
- Myers, R. (2017). Sunflowers: A Versatile Native Crop. g4701, University of Missouri Extension, 7 p.
- Schneiter, A.A., Miller, J.F. (1981). Description of Sunflower Growth Stages. *Crop Sci.*, 21, 901–903.
- Ștefan, V., Ion, V., Ion, N., Dumbravă, M., Vlad, V. (2008). *Floarea-soarelui*. Editura Alpha MDN.
- Vrânceanu, A.V. (2000). *Floarea-soarelui hibridă*. Editura Ceres, București.

ASSESSMENT OF THE ADAPTIVE CAPACITY OF SELECTION POPULATIONS OF BIRDS FOOT TREFOIL IN AGROECOLOGICAL CONDITIONS OF THE CENTRAL BALKAN MOUNTAIN

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Abstract

In the period 2016-2019, the following selection populations of birds foot trefoil were tested in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture (RIMSA)-Troyan using the block method in four replications, with a size of the harvest plot of 5 m², compared to the Bulgarian variety 'Targovishte 1': Syn₁, Syn₂, Syn₃, Syn₄, Syn₅. It was found that for the specific environmental conditions of Troyan, the Syn₃ population achieved the highest productivity. Birds foot trefoil retained a high presence in the grassland during the second and third years of the experimental period in all tested populations, and the amount of stems predominated the leaves. Plant height ($r = 0.7185$) had the greatest influence on the formation of dry mass yield. A strong correlation dependence ($r = 0.7891$) was found between the height of plants and the amount of stems.

Key words: *Lotus corniculatus L., yield, morphological composition, height.*

INTRODUCTION

Birds foot trefoil (*Lotus corniculatus* L.) refers to legume forage plants with good adaptability to different soil and climatic conditions, high productivity and quality of forage. It is grown in pure crop or as a major legume component in mixtures (Vasileva, 2015; Vasileva and Ilieva, 2017) for combined use (Bozhanska, 2017; Bozhanska and Churkova, 2019) in Bulgaria. Soils with acid reaction are suitable for its growing (Churkova, 2013a). Different cultivars and populations of bird's foot trefoil originating in different regions are characterized by specific biological and morphological properties, such as growth and development, number and length of stems, productivity (Chourkova, 2010; Vasileva, 2017). The botanical composition of grassland provides information on the sustainability and stability of the yield of meadow grasses imported into Bulgaria. Specific adaptation and purposeful use may be the main determinants of species and crop selection (Parsons et al., 2011; Vasileva and Vasilev, 2012).

Studies on cultivars and populations of bird's-foot-trefoil, grown under the foothill conditions of Bulgaria, have been made, both with

selection and practical orientation (Churkova, 2013b; 2013c; Bozhanska, 2018). The effect of adaptive variability of local populations and foreign cultivars on the yield of fresh and dry mass (Golubinova, 2018), persistence (Naydenova and Mitev, 2015), use, reproductive capacity and quality of feed (Churkova, 2011) has been registered.

Geographic location, biodiversity in structure and composition have an impact on the effective use of grasslands. The production of high quality feed organic production requires an optimal combination of environmental conditions and agrotechnical events (Allen et al., 2013; Semmartin et al., 2010) for the maximum development of grasslands. Appropriate adaptation of meadow grass species and cultivars is essential for realizing their genetic potential and hence their productive potential (DeBoer et al., 2017).

Recently, global environmental studies shown that the earth's climate is gradually warming. Modern agricultural science develops and implements innovative approaches to the selection of species and cultivars of fodder grasses for study in order to cultivate and use them in the changing environmental conditions (Golubinova and Marinov-Serafimov, 2019;

McDonagh et al., 2016; O'Donovan et al., 2016).

The aim of the present study is to evaluate the adaptive capacity of birds foot trefoil selection populations under the agroecological conditions of the Central Balkan Mountains.

MATERIALS AND METHODS

The experiment was conducted in the period 2016-2019 in the experimental field of RIMSA-Troyan using the block method in four replications, with a size of the harvest plot of 5 m². The experimental variants were: Syn₁ (restricted free pollination of genotypes of 'Bright' and 'Georgia 1' cultivars), Syn₂ (pre-pollinated plants from the local population originating from the village of Staro selo and the Hungarian variety 'Ergechi'), Syn₃ (synthetic population from local genetic types originating in the Central Northern Bulgaria), Syn₄ (synthetic population from the Hungarian cultivars 'Pecoli' and 'Gjiki'), Syn₅ (synthetic population of genotypes originating from the towns of Shumen, Nessebar and Kiten) compared to the Bulgarian variety 'Targovishte 1' (control). The sowing was done manually, in a dispersed manner at a sowing rate of 12.0 kg ha⁻¹.

During the four years of the experimental period, two regrowths were harvested in hay harvesting stage in the phase of bud-formation period - beginning of flowering of birds foot trefoil.

The following indicators were registered: Dry vegetative matter yield (t ha⁻¹) - determined by drying average samples (200 g) in laboratory conditions until they reached constant weight at a temperature of 105°C, hence it was calculated by regrowths, years and average over the experimental period based on green matter yield and dry matter content. The height (cm) of different mowings of the grassland was monitored at the time of harvesting. Plants (4 spots) were measured from the soil surface to the top of the tallest stems along both diagonals of each plot. The height of 40 birds foot trefoil plants was registered in each variant by years and regrowths. The average values were calculated from the data obtained.

Morphological composition of the grassland (%) was determined by the weight percentage of 40 plants, taken from each variant and each replication during the grassland harvesting. The quantity of stems and leaves was determined by the weighting method and on its basis their percentage share was determined. The dry mass yields were processed by dispersion analysis method using the Analysis Toolpak software for Microsoft Excel 2010 and Statgraphics Plus v.2.1. The height and morphological composition of the grassland are represented by: limit values (min and max), average value (x), control deviation (SD) and coefficient of variation (CV, %). The degree of variability is considered to be very low, low, medium, high or very high with CV values, respectively: up to 7%; from 7.1 to 12%, from 12.1 to 20%, from 20.1 to 40% and over 40% (Lidanski, 1988).

RESULTS AND DISCUSSIONS

The meteorological characteristics of the vegetation period (March-October) during the survey years are quite different in terms of precipitation, which specifically affected the development and productivity of the grassland. The highest values are found in 2017 for the overall vegetation period and over the months and the lowest in 2019 (555.2 mm). The average temperature during the vegetation period had similar values over the years, respectively 15.2; 15.0; 15.3 and 15.6°C. The impact of climatic factors is a key indicator of the normal sprouting, growth, development and productivity of birds foot trefoil.

Dry mass yield (Table 1) in selection populations in the first year was relatively low compared to the control. Syn₂ population was distinguished with a slightly higher productivity than the control with a yield of 3.72 t ha⁻¹ (+ 5.50% in comparison with the control). This is the only population with a yield above the control. Population Syn₅ had a similar value to control, and all other populations were significantly less productive. The lower productivity is determined by lower values of the main characteristics: plant height, leaf weight and stems.

Table 1. Dry mass yield (t ha⁻¹) over the years and average for the period 2016-2019

Populations	2016		2017		2018		2019		average for the period	
	t ha ⁻¹	compared to control %	t ha ⁻¹	compared to control %	t ha ⁻¹	compared to control %	t ha ⁻¹	compared to control %	t ha ⁻¹	% compared to control %
Targovishte 1	3.53	100.00	5,65	100.00	13.07	100.00	8.16	100.00	7.60	100,00
Syn ₁	3.21	91.00	5.48	97.00	13.13	100.44	7.78	95.33	7.40	97.34
Syn ₂	3.72	105.50	5.56	98.39	12.89	98.63	9.55	117.07	7.93	104.33
Syn ₃	3.42	96.95	6.34	112.34	13.35	102.13	10.01	122.68	8.28	108.93
Syn ₄	2.88	81.79	6.06	107.23	12.90	98.70	10.29	126.07	6.46	84.98
Syn ₅	3.49	98.95	5.93	104.96	1.57	111.44	8.60	105.34	8.15	107.15
LSD 5%	0.97	27.67	0.11	19.84	2.40	18.39	1.96	15.85	0.71	8.24
LSD 1%	1.35	38.32	1.54	27.48	3.33	25.47	2.72	21.96	0.98	11.41
LSD 0.1%	1.86	52.87	2.13	37.91	4.59	35.14	3.75	30.29	1.36	15.75

LSD – mathematical proof of differences

In the second year of the experimental period, Syn₁ and Syn₂ populations had lower yields than the control. Population Syn₃ showed the highest productivity. It exceeded the standard by 12.34%. Yields exceeding the control were also reported in Syn₄ and Syn₅ populations. The high productivity in the second year of the experimental period was due to favourable climatic conditions. The combination of higher soil and air humidity with intense birds foot trefoil growth and development during this period explains the high productivity of its populations.

In the third year, Syn₃ and Syn₅ populations were more productive than the control. The highest value was found in Syn₅ population with a yield of 14.57 t ha⁻¹ and an increase of 11.44%. The yield of dry mass of plants from populations that are more productive than the control is statistically unproven (Table 1).

In the fourth experimental year, dry mass yield varied from 4.23 to 8.77 t ha⁻¹. All populations exceeded the yield of the control, but Syn₄ population occupies the first position (10.29 t ha⁻¹) and exceeds the statistically significant control (26.07%). The excess was from 5.34% to 22.68% for the other populations. Syn₁

population is an exception and it is below the control. The high productivity in the fourth experimental year is due to the moisture, especially in April - 106.9 mm, June - 234.6 mm and July - 106.7 mm. That created excellent conditions for the growth and development of the plants and the formation of the two regrowths. The high yield prove the good persistence and survival of the plants until the end of the fourth vegetation in a great share of the studied populations, which are equal or have higher persistence than the control variety.

Over the study period, with the exception of Syn₁ and Syn₄ populations, all other populations were more productive than the control. Syn₃ population achieved the highest productivity. Syn₅ population ranks second in productivity with almost similar dry matter yield (8.15 t ha⁻¹), exceeding the control by 7.15%. The difference in the dry mass yield of the studied populations for each year of the experimental period can be explained by genetic differences, since the test was conducted under the same agro ecological conditions.

Table 2. Morphological analysis (%) by years and average for the period 2016-2019

Populations	2016		2017		2018		2019		average for the period	
	leaves	stems	leaves	stems	leaves	stems	leaves	stems	leaves	stems
Targovishte 1	50.0	50.0	50.0	50.0	50.0	50.0	41.7	58.3	47.9	52.1
Syn ₁	60.0	40.0	45.5	54.5	54.5	45.5	41.4	58.6	50.3	49.6
Syn ₂	50.0	50.0	46.7	53.3	53.8	46.2	41.7	58.3	48.1	51.9
Syn ₃	55.6	44.4	43.8	56.2	54.5	45.5	40.0	60.0	48.5	51.5
Syn ₄	57.1	42.9	37.5	62.5	53.8	46.2	36.1	63.9	46.1	53.9
Syn ₅	44.4	55.6	50.0	50.0	53.3	46.7	42.9	57.1	4.6	52.4
X	52.8	47.1	45.6	54.4	53.3	46.7	40.6	59.4	48.1	51.9
SD	5.73	5.7	4.7	4.7	1.7	1.7	2.4	2.4	1.4	1.4
VC	10.8	12.2	10.2	8.6	3.2	3.6	5.9	4.0	2.8	2.6
Min	44.4	40.0	37.5	50.0	50.0	45.5	36.1	57.1	46.1	49.6
Max	60.0	55.6	50.0	62.5	54.5	50.0	42.9	63.9	50.3	53.9

The morphological composition of the grassland (Table 2) is an important indicator that affects especially the quality of the feed. The morphological composition of the grassland in the first year showed that Syn₄ population had the leaf mass of 57.1%. The average value of the stems was 47.1%, with a variation factor of 10.8%. The maximum value was registered for Syn₅ population - 55.6% and the minimum value for Syn₁ population - 40.0%.

In the second year, the number of stems exceeded the number leaves. The same percentage of leaves and stems was registered for the control and Syn₅ population. In the third year, the number of leaves was predominant and they ranged from 50.0% to 54.5%. Their degree of

variability was very low and almost similar both in terms of stems (VC - 3.6%) and in leaves (VC - 3.2%), according to the values of the variation coefficient.

The same trend as in the third year was observed in the fourth year, ie. The maximum values of the stalks were 63.9% and those the leaves 42.9%.

The number of stems in the majority of the population is average over the study period, with no significant variance found. This is also evident from the values of the variation coefficient for the stems and leaves, which are almost identical (2.8% and 2.6%). According to the results obtained for the percentage of leaves and stems, the populations do not differ from each other. The number of leaves varies within a very narrow range (46.1 to 50.3%). The same trend was observed in the percentage of stems (from 49.6 to 53.9%). On average for the experimental period, the lowest values were observed for the genotypes of Syn₄ population (46.1%) and the highest values for Syn₁ population (50.3%). Low values of control deviation indicate less scattering of the quantity of stems and leaves.

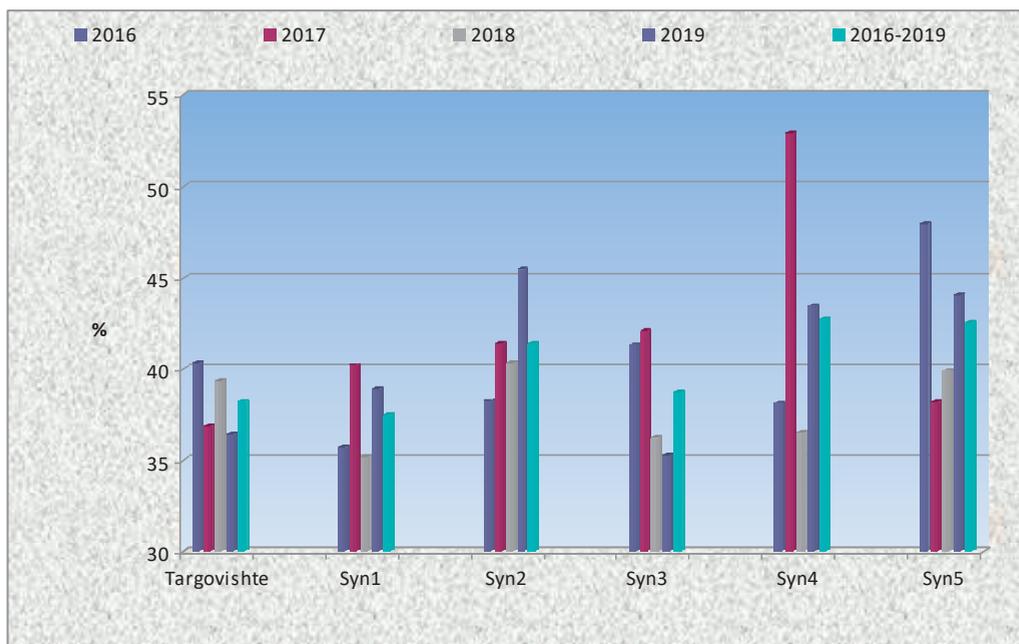


Figure 1. Height (cm) of stems of selection populations of birds foot trefoil over the years and average for the period 2016-2019

Table 3. Statistical processing of stem height (cm) of birds foot trefoil selection populations over the years and average for the period 2016-2019

Statistical values	2016	2017	2018	2019	Average for the period
X	40.24	41.91	37.87	40.55	40.14
SD	4.25	5.72	2.20	4.29	2.31
VC	10.56	13.66	5.80	10.57	5.76
Min	35.63	36.80	35.13	35.25	37.44
Max	47.93	52.88	40.28	45.40	42.72

The plants of Syn₅ population of the first regrowth in 2016 formed higher stems than 'Targovishte 1' (47.94 cm) (Figure 1). All tested populations except Syn₃ remained below the control. Syn₂ (38.20 cm) and Syn₄ (38.10 cm) populations had similar values of stem height. Syn₄ population (52.88 cm) had the highest stems in the second experimental year. All populations had statistically proven higher stems than the control. There is a tendency of a parallel increase in the values of dry mass production with an increase in plant height. In the third year of 2018, Syn₂ population (40.28 cm) had the highest values of stem height. The relatively similar height values of stems in the tested populations are noticeable.

The stem height in population Syn₂ (45.40 cm) exceeded 'Targovishte 1' in the first regrowth in 2019. The average stem height was 40.55 cm at a low degree of variability according to the value of the variation coefficient (CV-10.57%).

The average stem height (Table 3) for the study period from all populations was 40.14 cm, with a very low degree of variability (CV - 5.76%), determined by the maximum (42.72 cm) and minimum values (37.44 cm). Variability of plant height in selection populations can also be used as an indicator of the adaptability of plants of selection populations to the agroecological conditions of the area. According to the values of variation coefficient

(CV), the degree of variability was low for the three experimental years and average for 2017. These values in the individual populations and

the values of the coefficient of variation reflect the poorly manifested effect of environmental conditions on the height of the grassland.

Table 4. Correlation dependences among yield, percentage of stems, leaves and heights for birds foot trefoil populations (2016-2019)

Indicators	yield	leaves	stems	heights
Yield	1			
Leaves	-0.5210	1		
Stems	0.4582	-0.7484	1	
Height	0.7185	-0.7147	0.7891	1

Table 4 represents the linear correlation coefficients as plant height ($r = 0.7185$) is more influenced by the formation of dry mass yield. These results are consistent with Golubinova (2018), according to which the stem height has a greater impact on yield than their number regardless of the year of study ($r = 0.703$). The formation of dry mass yield in populations is mainly determined by the relative proportion of stems, which is confirmed by the positive relationship between these two indicators ($r = 0.4582$). A high level of correlation dependence ($r = 0.7891$) is characterized by the height to stem ratio.

Soil and climate conditions change the extent and nature of the relationship between productivity and quantitative characteristics of birds foot trefoil populations (Table 3). Debely et al. (2015) said that it is essential for plant selection to evaluate the individual elements determining yield, depending on environmental conditions. The same authors argue that the height of vetch determines productivity to the greatest extent, as this feature is characterized by high inheritance. These results are explained by the fact that the height of plants, as well as the percentage of leaves and stems in the grassland, are strongly influenced by the environmental conditions. In the presence of favourable soil and climatic conditions, the value of the correlation coefficient expressing the dependence of the characteristics with the productivity is positive and high.

CONCLUSIONS

Syn₃ had the highest dry mass productivity and yield stability from the tested populations. Its plants can be included as parental components to create a synthetic population.

Birds foot trefoil retained a high presence in the grassland during the second and third years of the experimental period in all populations, and the number of stems predominated the leaves. Plant height ($r = 0.7185$) had the greatest influence on the formation of dry matter yield. A strong correlation ($r = 0.7891$) was found between the height of plants and the quantity of stems.

REFERENCES

- Allen, E., Sheaffer, C., Martinson, K. (2013). Forage Nutritive Value and Preference of Cool-Season Grasses under Horse Grazing. *Agronomy Journal Abstract - Crop Economics, Production & Management*, 105(3), 679–684.
- Bozhanska T. (2017). Study on perennial legume-grass mixtures in the conditions of the Central Balkan mountain. *Banat's Journal of Biotechnology*, VIII(15), 34–42.
- Bozhanska T. (2018). Botanical and morphological composition of artificial grassland of bird's-foot-trefoil (*Lotus corniculatus* L.) treated with Lumbrical and Lumbrax. *Banat's Journal of Biotechnology*, IX(19), 12–19.
- Bozhanska, T., Churkova, B. (2019). Growth and of Legume and Grass Components in Mixed Grasslands Grown in the Central Balkan Mountain. *Trakia Journal of Sciences*, 1, 19–27.
- Churkova, B. (2010). Productive capacities, botanical composition and morphological characteristics of varieties and populations of birdsfoot trefoil. *Journal of Mountain Agriculture on the Balkans*, 5(2), 829–838.
- Churkova, B. (2011). Biochemical traits and correlation associations between chemical composition and some parameters yield in birdsfoot trefoil. *Banat's Journal of Biotechnology*, II(3), 25–28.
- Churkova, B. (2013a). Agro-ecological aspects of the weed infestation control in birdsfoot trefoil grown for forage. *Banat's Journal of Biotechnology*, IV(7), 29–34.
- Churkova, B. (2013b). Biological potential and adaptive capacity of varieties birds foot trefoil in the

- agroecological conditions of Troyan. *Banat's Journal of Biotechnology*, IV(7), 69–73.
- Churkova, B. (2013c). Content of crude protein, crude fiber and crude ash in dry mass of birdsfoot trefoil varieties and populations. *American Journal of Agricultural Science and Technology*, I(3), 77–83.
- Debelyj, G. A., Kalinina, L. V., Mednov A. V., Goncharov, A. V. (2015). New generation of varieties of leguminous crops released by the Moscow Research Institute of Agriculture. *Scientific and production journal Grain-leguminous crops and cereals*, I(13), 1–14.
- DeBoer, M. L., Sheaffer, C. C., Grev, A. M., Catalano, D. N., Wells, M. S., Hathaway, M. S., Martinson, K. L. (2017). Yield, Nutritive Value, and Preference of Annual Warm-Season Grasses Grazed by Horses. *Agronomy Journal Abstract - Crop Economics, Production & Management*, 109(5), 2136–2148.
- Golubinova, I. (2018). Comparative testing of varieties and local populations birdsfoot trefoil (*Lotus corniculatus* L.) *Journal of Mountain Agriculture on the Balkans*, 21(6), 44–56.
- Golubinova, I., Marinov-Serafimov, P. (2019). Influence of Meteorological Factors on the Productivity of Accessions Birdsfoot Trefoil (*Lotus corniculatus* L.). *Journal of Mountain Agriculture on the Balkans*, 22(2), 82–93.
- Lidanski, T. (1988). Statistical methods in biology and agriculture, Zemizdat, Sofia, BG.
- McDonagh, J., Donovan, M. O., McEvoy, M., Gilliland, T. J. (2016). Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013. *Euphytica*, 212(2), 187–199.
- Naydenova, G., Mitev, D. (2015). Study of Breeding Populations of Birdsfoot Trefoil and Red Clover in Mixtures. *Plant Science*, LII(5), 30–36.
- O'Donovan, M., McHugh, N., McEvoy, M., Grogan, D., Shalloo, L. (2016). Combining seasonal yield, silage dry matter yield, quality and persistency in an economic index to assist perennial ryegrass variety selection. *Journal of Agricultural Science*, 155, 556–568.
- Parsons, A. J., Edward, G. R., Newton, P. C., Chapman, J. D. F., Caradus, R., Rasmussen, S., Rowarth, J. S. (2011). Past lessons and future prospects: plant breeding for yield and persistence in cool-temperate pastures. *Grass and Forage Science*, 66, 153–172.
- Semmartin, M., Bella, C. D., Salamone, I. G. (2010). Grazing-induced changes in plant species composition affect plant and soil properties of grassland mesocosms. *Plant and Soil*, 328, 471–481.
- Vasileva V., Vasilev, E. (2012). Study on Productivity of Some Legume Crops in Pure Cultivation and Mixtures. *Agriculturae Conspectus Scientificus*, 77(2), 91–94.
- Vasileva, V. (2015). Morphological parameters and ratios in some mixtures with subclover. *Science International*, 3(4), 107–112.
- Vasileva, V. (2017). Parameters Related to Nodulating Ability of Some Legumes. *International Journal of Agriculture Food Science & Technology*, 3(1), 005–008.
- Vasileva, V., Ilieva, A. (2017). Some physiological parameters in mixtures of cocksfoot and tall fescue with subterranean clover. *Bulgarian Journal of Agricultural Science*, 23(1), 71–75.

INFLUENCE OF DIFFERENTIATED FERTILIZATION ON YIELD AND QUALITY OF WINTER WHEAT CULTIVATED ON THE ȘIMNIC LUVOSOIL

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Abstract

Between 2016-2018, 15 varieties of winter wheat were tested in 3 variants of fertilization ($V1 =$ unfertilized, $V2 = N_{40}P_{40}$ administered in autumn and $V3 = N_{100}P_{40}$ administered in autumn + spring) on Simnic luvosoil. The following were analyzed: yield, thousand grains weight, test weight, protein content and sedimentation index. Wheat yield was significantly higher when the nitrogen dose increased from N_0 to N_{40} and from N_0 to N_{100} . All tested varieties, without exception, registered a statistically assured protein increase when the nitrogen dose was increased to N_{100} . The correlation between the protein content obtained in the unfertilized variant and the protein content when fertilized with $N_{40}P_{40}$ is extremely strong ($r = 0.912$). For the studied interval, the coefficient of determination suggests that approximately 83% of the variability of the protein content to $N_{40}P_{40}$ is associated with the protein content from the unfertilized variant. Also, the correlation between the protein content obtained in the unfertilized variant and the protein content when fertilized with $N_{100}P_{40}$ is positive and extremely strong ($r = 0.877$). Approximately 77% of the variability of the protein content at $N_{100}P_{40}$ is associated with the protein content in the unfertilized variant.

Key words: wheat, quality, fertilization levels, yield, protein.

INTRODUCTION

Nitrogen is the main factor in growth and development of plants, with positive influence on rooting, tillering, leaf system development and photosynthesis process, the elements of productivity and also quality (Matei, 2014). It is understood that in addition to nitrogen, the other nutrients play a role in the balanced nutrition of wheat, in which phosphorus is an important role. In fact, the highest yields are obtained when fertilizing using these two types of fertilizers (Naidin & Păunescu, 2003).

Under the climatic conditions specific to Câmpia Română, the wheat satisfies the nitrogen needs necessary for growth and development, especially by absorbing large quantities of nitrates, as nitrogen in the form of ammonium ions is converted quickly into nitrates, and their dynamics in soil is dependent on the weather (Nedelciuc et al., 1995).

Therefore, the application of nitrogen fertilizers becomes a necessary and obligatory measure for the continuous supply of plants with this essential nutrient and especially under irrigation conditions (Nicolescu et al., 1994).

The harvest results obtained on the cambic chernozem from Câmpia Banato-Crișană showed that even on this type of soil with good fertility, doubling the dose of nitrogen fertilizers from N_{70} to N_{140} , on the basis provided by P80K80 is motivated, the average harvest increase per 3 genotypes studied being 12% (+565 kg/ha), statistically assured as very significant. Wheat varieties of the species *Triticum aestivum* ssp. *vulgare* (Vill.), Ciprian and Kristina registered 5997 kg/ha and 6499 kg/ha, respectively, average yields on the two agrofondos (Andronache, 2010).

The highest positive correlations were between yield and grain weight/spike, yield and HW and HW and grain weight/spike. For those characters there were also identified a very positive correlation with the N doses (Iancu et al., 2019).

Seventeen winter wheat cultivars were tested with recommended and reduced N fertilization during 4 years in three locations. Relative grain yield, protein concentration and protein yield per hectare under reduced fertilization, as percentage from the performance with recommended N fertilization, showed very

large variation due to environment, the effect of cultivars being much smaller, but still significant. The effect of reduced N fertilization was larger for grain yield than for protein concentration. For grain yield and protein yield per hectare the highest reduction was found in cultivars originating from the Fundulea breeding program, while the smallest reduction was found in cultivar Adelina (bred at Șimnic), suggesting a possible relationship with the natural soil fertility at the breeding site (Marinciu et al., 2018).

MATERIALS AND METHODS

The experiment was placed in the experimental field of the Breeding Laboratory at ARDS Simnic. Between 2016 and 2018, 15 varieties of winter wheat (A factor) were tested in 3 fertilization levels (B factor): V1 = unfertilized, V2 = N₄₀P₄₀ administered in autumn and V3 = N₁₀₀P₄₀ administered in autumn + spring, on Simnic luvisol.

The following were analyzed: yield, thousand grains weight, test weight, protein content and sedimentation index.

Each variant was placed in 3 repetitions on a plot with 10.5 m surface which 9 m were harvested.

Calculated and interpreted: influence of A factor (variety), influence of B factor (fertilization level), interaction variety x fertilization level (A x B - between fertilization levels at the same variety) and interaction fertilization level x variety (B x A - between varieties at the same fertilization level).

The protein content was determined with the infrared apparatus INFRATEC, test weight with GRANOMAT, TGW with CONTADOR (grains count) and the sedimentation index according to the Zeleny method.

The grain yield was calculated at STAS humidity (14%).

The climatic conditions, except for 2016, provided the necessary precipitation for the normal development of wheat crops.

RESULTS AND DISCUSSIONS

Yield. Among the management technologies, nitrogen fertilization is the most important for increasing grain yield in cereals (Flores et al., 2012; Arenhardt et al., 2015). In wheat, the nutrient is responsible for the formation of biological molecules and determinant of productivity and grain quality (Fageria et al., 2006; Silva et al., 2015).

Important results regarding wheat yield depending on nitrogen fractionation during crop fertilization presented Brezolin et al. in 2017.

In our experiment, the yield limits at the dose of N₁₀₀P₄₀ were between 5644 kg/ha in the Ursita variety and 4014 kg/ha in Bezostaia, on average for 3 years. At the dose of N₄₀P₄₀ the highest yield was recorded by Uнитар variety (5002 kg/ha) and the smallest of the same variety Bezostaia (3667 kg/ha). In the unfertilized, yield limit was 3274 kg/ha in the Alex variety (Ursita is very close with 3242 kg/ha) and 2707 kg/ha in Bezostaia.

Over 5500 kg/ha was reached only at the increased dose of nitrogen by the Ursita and Uнитар varieties. Very close was the variety Adelina.

All the varieties studied had very significant yield increases when the nitrogen dose increased either to N₄₀ or to N₁₀₀ on the basis of P₄₀ (Table 1).

Overall, yield of wheat was significantly higher when the nitrogen dose was increased from N₀ to N₄₀ and from N₀ to N₁₀₀ (+1313 kg/ha, respectively 2087 kg/ha).

Regardless of the level of fertilization, the yield of the tested varieties was at the control level - the Glosa variety, with the exception of the Bezostaia variety (a landmark for genetic progress over time) whose yield was distinctly significantly diminished.

At the N₀P₀ fertilization level, the Bezostaia variety recorded a yield significantly lower than the control variety, which indicates that it does not support the absence of nitrogen as well.

Table 1. Differentiated fertilization influence on yield at tested varieties (2016-2018 average)

A factor (variety)	B factor (fertilizationlevel)					A factor infl ence
	Yield at N0P0 (kg/ha) -b ₁ (ct)-	Yield at N40P40 (kg/ha) -b ₂ -	Yield at N100P40 (kg/ha) -b ₃ -	A x B dif b ₂ -b ₁	A x B dif b ₃ -b ₁	
B x A						
-a ₁ -GLOSA(ct)	3181	4193	5156	1012 ***	1975 ***	4177
-a ₂ -BOEMA 1	2981	4277	5035	1296 ***	2054 ***	4098
-a ₃ -LITERA	3198	4219	4851	1021 ***	1652 ***	4089
-a ₄ -MIRANDA	3099	4225	5462	1126 ***	2363 ***	4262
-a ₅ -IZVOR	3029	3839	4545 ^{oo}	810 ***	1517 ***	3804
-a ₆ -OTILIA	3041	4415	5251	1374 ***	2210 ***	4236
-a ₇ -PITAR	3011	4228	5071	1217 ***	2060 ***	4103
-a ₈ -PAJURA	3108	4230	5406	1122 ***	2298 ***	4248
-a ₉ -URBITA	3242	4734**	5664**	1492 ***	2422 ***	4547
-a ₁₀ -UNITAR	3131	5002 ***	5616*	1871 ***	2485 ***	4583
-a ₁₁ -PROFUND	3060	4763**	5449	1703 ***	2390 ***	4424
-a ₁₂ -ADELINA	2872	4665*	5492	179 ***	2621 ***	4343
-a ₁₃ -S 60	3109	4626*	5221	151 ***	2111 ***	4319
-a ₁₄ -ALEX	3274	4647*	5118	1373 ***	1845 ***	4346
-a ₁₅ - BEZOSTAIA	2707 ^o	3677 ^{oo}	4014 ^{ooo}	969 ***	1307 ***	3466 ^{oo}
Average	3070	4383	5157			
B factor influence	ct	1313***	2087***			

A factor: DL 5% = 470 kg/ha; DL 1% = 620 kg/ha; DL 0.1% = 810 kg/ha
 B factor: DL 5% = 200 kg/ha; DL 1% = 300 kg/ha; DL 0.1% = 430 kg/ha
 A x B interaction: DL 5% = 350 kg/ha; DL 1% = 460 kg/ha; DL 0.1% = 600 kg/ha
 B x A interaction: DL 5% = 370 kg/ha; DL 1% = 500 kg/ha; DL 0.1% = 650 kg/ha

At the N₄₀P₄₀ level of fertilization, recent creations of the breeding process were highlighted as superior to the Glosa variety: Ursita, Unitar, Profund, Adelina, Şimnic 60 and the Alex variety from the old varieties.

Increasing the nitrogen dose to N₁₀₀ on P₄₀ base favored the Ursita and Unitar varieties, which obtained higher yields than the Glosa variety, with statistical assurance. Izvor variety had a significantly reduced yield compared to the same control variety.

The Bezostaia variety was inferior to the Glosa variety, with statistical assurance at each of the studied fertilization levels.

There is a positive close correlation between the yield obtained when fertilized with N₄₀P₄₀ and the yield obtained when not fertilized (r = 0.453). For the interval studied, the correlation suggests that when an increase of yield by 100 kg/ha occurs in the unfertilized, at N₄₀P₄₀ the increase is 118 kg/ha (Figure 1).

About 23% of yield variability at N₄₀P₄₀ is associated with yield at N₀P₀. As the latter grows, grow yield in nitrogen addition plots. The varieties Ursita and Alex were noted that had very high yields in both fertilization variants.

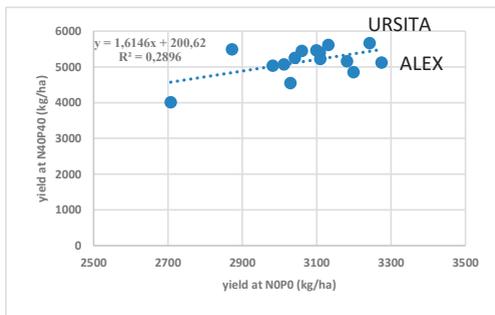


Figure 1. Correlation between N₀P₀ yield and N₄₀P₄₀ yield (r = 0.475)

The correlation between the yield obtained when fertilized with N₁₀₀P₄₀ and the yield obtained when not fertilized (r = 0.538) is stronger. For the studied interval, the correlation suggests that when an increase of yield by 100 kg/ha occurs in the unfertilized, at N₁₀₀P₄₀ the increase is 161 kg/ha (Figure 2). About 29% of yield variability at N₁₀₀P₄₀ is associated with yield at N₀P₀. And here, as the latter grows, grows yield in the variant with the higher dose of nitrogen.

The varieties Unitar, Alex and Ursita were noted, which had very high yields in both variants (unfertilized and N₁₀₀P₄₀).

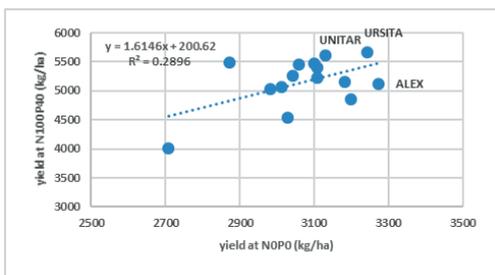


Figure 2. Correlation between N₀P₀ yield and N₁₀₀P₄₀ yield (r = 0.538)

Test weight (TW). Under the experimentation conditions, not all the varieties tested obtained TW above limit of 76 kg/hl (Table 2). There are several varieties where the test weight decreased

with statistical assurance when the nitrogen dose was increased: Miranda (-1.8/-2.0 kg/hl), Izvor (-1.4/-1.3 kg/hl), Unitary (-1.2 kg/hl), Adelina (-1.3 kg/hl), S60 (-1.4/-2.1 kg/hl) and Alex (-1.2/-1.3 kg/hl).

Table 2. Differentiated fertilization influence on test weight at tested varieties (2016-2018 average)

A factor (variety)	B factor (fertilizationlevel)			A x B dif b ₂ -b ₁	A x B dif b ₃ -b ₁	A factor infl ence
	TW at N0P0 (kg/hl) -b ₁ - (ct)	TW at N40P40 (kg/hl) -b ₂ -	TW at N100P40 (kg/hl) -b ₃ -			
B x A						
-a ₁ -GLOSA (ct)	75.9	75.8	76.2	-0.2	0.2	76.0
-a ₂ -BOEMA 1	76.1	75.8	76.9	-0.3	0.8	76.3
-a ₃ -LITERA	76.0	75.4	75.9	-0.7	-0.1	75.8
-a ₄ -MIRANDA	75.9*	74.1*	73.9 ⁰⁰⁰	-1.8 ⁰⁰	-2.0 ⁰⁰	74.6*
-a ₅ -IZVOR	77.5*	76.1	76.2	-1.4 ⁰⁰	-1.3*	76.6
-a ₆ -OTILIA	77.2	76.2	76.7	-0.9	-0.4	76.7
-a ₇ -PITAR	76.8	75.7	76.5	-1.1	-0.3	76.3
-a ₈ -PAJURA	75.2	75.9	76.8	0.7	1.6**	76.0
-a ₉ -URSITA	74.2*	77.4*	77.7*	3.2***	3.5 ***	76.4
-a ₁₀ -UNITAR	75.3	74.1*	74.8*	-1.2*	-0.5	74.8*
-a ₁₁ -PROFUND	76.9	76.0	76.2	-0.9	-0.7	76.4
-a ₁₂ -ADELINA	77.4*	76.1	76.8	-1.3*	-0.6	76.7
-a ₁₃ -S 60	76.9	75.6	74.8*	-1.4*	-2.1 ⁰⁰⁰	75.8
-a ₁₄ -ALEX	75.9	74.7	74.5*	-1.2*	-1.3*	75.0
-a ₁₅ - BEZOSTAIA	76.4	77.2*	77.4	0.8	1.0	77.0
Average	76.2	75.7	76.1			
B factor influence	ct	-0,5*	-0,1			

A factor: DL 5% = 1.2 kg/hl; DL 1% = 1.6 kg/hl; DL 0.1% = 2.1 kg/hl
 B factor: DL 5% = 0.5 kg/hl; DL 1% = 0.7 kg/hl; DL 0.1% = 1.1 kg/hl
 A x B interaction: DL 5% = 1.2 kg/hl; DL 1% = 1.6 kg/hl; DL 0.1% = 2.1 kg/hl
 B x A interaction: DL 5% = 1.3 kg/hl; DL 1% = 1.8 kg/hl; DL 0.1% = 2.3 kg/hl

On 3 years average, the lowest value was obtained by the Miranda variety (73.9 kg/hl) at N₁₀₀P₄₀.

The study of the influence of the variety, regardless of the level of fertilization, revealed that the variety Glosa was not significantly exceeded and the varieties Miranda and Unitar were significantly lower.

Surprisingly, increasing the nitrogen dose significantly decreased the test weight in variants on the basis of N₄₀P₄₀ and kept it approximately equal to the dose of N₁₀₀P₄₀.

At the N₀P₀ fertilization level, the Miranda, Izvor, Adelina varieties were higher than the control and the Ursita variety was significantly lower than this indicator.

At the N₄₀P₄₀ fertilization level, the varieties Ursita and Bezostaia were highlighted as superior on Glosa.

Increasing the nitrogen dose to N₁₀₀ on the P₄₀ base favored only the Ursita variety. And the Pajura variety presented the upper TW but only

at N₁₀₀P₄₀. The varieties Miranda, Unitar, Şimnic 60 and Alex were inferior with statistical assurance in relation to the same control variety.

The Ursita variety was the only variety superior to the Glosa variety in point of view of the values of the test weight, with statistical assurance at the nitrogen fertilization levels. In contrast, the varieties Miranda, Izvor, Şimnic 60 and Alex registered decreases with statistical assurance in the conditions of increasing either 40 or 100 kg/ha of the nitrogen dose. In conclusion, the test weight is more influenced by the interaction variety x fertilization level than unique contribution of nitrogen.

The 1000 grains weight (TGW). The 1000 grains weight had very high values during the three years of experimentation, at all three levels of fertilization (over 39 g/1000 grains). The values of this element of productivity were between 39.3 g at the Otilia variety and 47.3 g at the Unitar in variant without nitrogen; between 42.4 g at the Otilia variety and 51.3 g at Profund line in the N₄₀P₄₀ variant; between 39.9 g at Alex and 50.4 g at Profund in the N₁₀₀P₄₀ variant (Table 3).

Table 3. Differentiated fertilization influence on 1000 grains weight at tested varieties (2016-2018 average)

A factor (variety)	B factor (fertilizationlevel)			A x B dif b ₂ -b ₁	A x B dif b ₃ -b ₁	A factor infl ence
	TGW at N0P0 (g) -b ₁ - (ct)	TGW at N40P40 (g) -b ₂ -	TGW at N100P40 (g) -b ₃ -			
B x A						
-a ₁ -GLOSA (ct)	46.0	49.2	46.8	3.2*	0.8	47.3
-a ₂ -BOEMA 1	41.8 ⁰⁰⁰	43.3 ⁰⁰⁰	42.4 ⁰⁰⁰	1.5	0.6	42.5 ⁰⁰
-a ₃ -LITERA	42.0 ⁰⁰	44.7 ⁰⁰⁰	43.9 ⁰	2.7*	2.0	43.5 ⁰
-a ₄ -MIRANDA	45.1	48.5	46.2	3.4**	1.0	46.6
-a ₅ -IZVOR	42.3 ⁰⁰	45.9 ⁰⁰	42.6 ⁰⁰⁰	3.5**	0.3	43.6*
-a ₆ -OTILIA	39.3 ⁰⁰⁰	42.4 ⁰⁰⁰	40.5 ⁰⁰⁰	3.1*	1.2	40.7 ⁰⁰⁰
-a ₇ -PITAR	44.3	45.4 ^{0*}	45.1	1.0	0.7	44.9
-a ₈ -PAJURA	44.0	47.4	44.8	3.4**	0.8	45.4
-a ₉ -URSITA	44.4	44.8 ⁰⁰⁰	45.9	0.4	1.5	45.1
-a ₁₀ -UNITAR	47.3	46.5*	45.2	-0.8	-2.0	46.3
-a ₁₁ -PROFUND	46.5	51.3*	50.4**	4.8***	3.9 ***	49.4
-a ₁₂ -ADELINA	41.9 ⁰⁰⁰	45.4 ^{0*}	43.7 ⁰⁰⁰	3.5**	1.8	43.6*
-a ₁₃ -S 60	46.6	47.2	47.1	0.6	0.6	47.0
-a ₁₄ -ALEX	39.4 ⁰⁰⁰	47.6	39.9 ⁰⁰⁰	8.2***	0.5	42.3 ⁰⁰
-a ₁₅ - BEZOSTAIA	42.2 ⁰⁰	44.8 ⁰⁰⁰	43.6 ^{0*}	2.6*	1.4	43.5 ⁰
Average	43.5	46.3	44.5			
B factor influence	ct	2.8	1.0			

A factor: DL 5% = 3.5 g; DL 1% = 4.7 g; DL 0.1% = 6.1 g
 B factor: DL 5% = 3.3 g; DL 1% = 4.4 g; DL 0.1% = 5.7 g
 A x B interaction: DL 5% = 2.5 g; DL 1% = 3.3 g; DL 0.1% = 4.3 g
 B x A interaction: DL 5% = 2.4 g; DL 1% = 3.1 g; DL 0.1% = 4.1 g

The increase of the nitrogen dose had a very significant influence only at the Profund line (+3.9 g at N₁₀₀P₄₀ and +4.8 g at N₄₀P₄₀). At the N₄₀P₄₀ dose, increases of TGW with statistical assurance had the majority of varieties.

The results suggest that the nitrogen dose does not influence the 1000 grains weight, although there are increases, they do not have statistical assurance.

The influence of the variety, regardless the fertilization level, exists and is reflected in the fact that five of the varieties tested are inferior to the variety Glosa: Boema, Litera, Izvor, Otilia, Adelina, Alex and Bezostaia.

Also, the interaction fertilization level x variety revealed that the varieties Boema, Litera, Izvor, Otilia, Adelina and Bezostaia have a 1000 grains weight inferior with statistical assurance at all fertilization levels. In contrast, in the presence of nitrogen, Profund wheat line is over Glosa with statistical assurance, on a 3 years average. In conclusion, the 1000 grains weight is not influenced by the fertilization level but is influenced by the variety because it is a genetic impregnated character.

Protein content (PC). Under experimentation conditions, only two of the varieties had a protein content above the 10.5% limit at the N₁₀₀P₄₀ variant (Profund - 10.9% and Bezostaia - 10.7%). In the unfertilized variant, the protein content was between 8.3% (Miranda) and 10% in Bezostaia; in the fertilized variant with N₄₀P₄₀, the values of the protein content were between 8.9% at several varieties and 10.4% at Profund line (its name comes from the combination “protein (pro) Fundulea (fund)”, which represents the variety with the largest protein content created at NARDI Fundulea) and for the variant fertilized with N₁₀₀P₈₀ the limits were 9.4% (Unitar) and 10.9% (Profund), on 3 years average (Table 4).

All varieties, without exception, registered a statistically assured increase in protein when the nitrogen dose was increased to N₁₀₀.

The obtained results suggest that the high quantity of nitrogen influences the protein content, with increases between 0.7-1.5%. At two levels of fertilization, the varieties Profund and Bezostaia were superior to the control variety Glosa. The fact that the Bezostaia variety was superior in the absence

of nitrogen indicates that it is genetically impregnated with a high protein content.

Table 4. Differentiated fertilization influence on protein content at tested varieties (2016-2018 average)

A factor (variety)	B factor (fertilization level)					
	PC at N0P0 (%) -b ₁ - (ct)	PC at N40P40 (%) -b ₂ -	PC at N100P40 (%) -b ₃ -	A x B dif b ₂ -b ₁	A x B dif b ₃ -b ₁	A factor influence
B x A						
-a ₁ -GLOSA (ct)	9.3	9.5	10.2	0.2	0.8**	9.7
-a ₂ -BOEMA 1	9.3	9.3	10.1	0.0	0.8**	9.6
-a ₃ -LITERA	9.0	9.4	10.0	0.4	1.0**	9.4
-a ₄ -MIRANDA	8.3 ^{oo}	8.9	9.8	0.6*	1.5** *	9.0
-a ₅ -IZVOR	9.1	9.6	10.3	0.5	1.3** *	9.7
-a ₆ -OTILIA	8.7	9.1	9.7	0.4	1.0**	9.2
-a ₇ -PITAR	9.4	9.6	10.0	0.2	0.7*	9.7
-a ₈ -PAJURA	9.1	9.2	10.0	0.1	1.0**	9.4
-a ₉ -URSITA	8.6 ^o	9.0	9.6	0.4	1.0**	9.1
-a ₁₀ -UNITAR	8.6 ^o	8.8 ^o	9.4 ^o	0.2	0.7*	8.9 ^o
-a ₁₁ -PROFUND	9.8	10.4**	10.9*	0.5	1.1 ***	10.4
-a ₁₂ -ADELINA	9.0	9.0	9.7	0.0	0.7*	9.2
-a ₁₃ -S60	8.8	8.9	9.6	0.1	0.7*	9.1
-a ₁₄ -ALEX	8.7	8.9	9.7	0.3	1.0**	9.1
-a ₁₅ -BEZOSTAIA	10.0*	10.2*	10.7	0.2	0.7*	10.3
Average	9.0	9.3	10.0			
B factor influence	ct	0.3	1.0***			

A factor: DL 5% = 0.8%; DL 1% = 1.1%; DL 0.1% = 1.4%
 B factor: DL 5% = 0.5%; DL 1% = 0.6%; DL 0.1% = 0.8%
 A x B interaction : DL 5% = 0.6%; DL 1% = 0.8%; DL 0.1% = 1.1%
 B x A interaction : DL 5% = 0.7%; DL 1% = 0.9%; DL 0.1% = 1.1%

Miranda variety much better utilized a reduced amount of nitrogen in relation to all its varieties.

The correlation between the protein content obtained in the unfertilized variant and the protein content when fertilized with N₄₀P₄₀ is extremely strong (r = 0.912) (Figure 3).

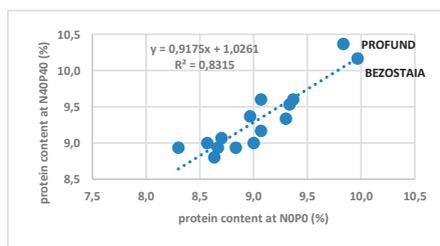


Figure 3. Correlation between protein content at N0P0 and protein content at N₄₀P₄₀ (r = 0.912)

For the studied interval, the coefficient of determination suggests that approximately 83% of the variability of the protein content to N₄₀P₄₀ is associated with the protein content from the unfertilized variant Profund and Bezostaia varieties with high protein content were noted in both variants.

Also, the correlation between the protein content obtained in the unfertilized variant and the protein content when fertilized with N₁₀₀P₄₀ is positive and extremely strong ($r = 0.877$). Approximately 77 % of the variability of the protein content at N₁₀₀P₄₀ is associated with the protein content in the unfertilized variant (Figure 4).

The same two varieties were noticed: Profund and Bezostaia.

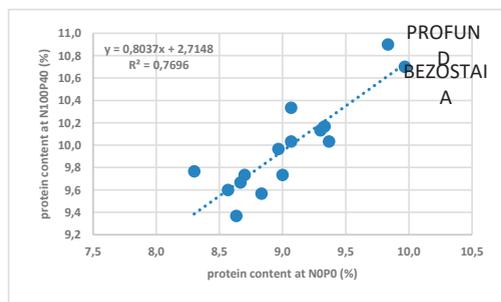


Figure 4. Correlation between protein content at N₀P₀ and protein content at N₁₀₀P₄₀ ($r = 0.877$)

Sedimentation index. With three exceptions (Miranda, Şimnic 60 and Alex at N₀P₀) in all the fertilization variants, the varieties tested had the sedimentation index over 60 ml, the limit for a good flour for baking.

The sedimentation index values at the nitrogen dose N₁₀₀ on the P₄₀ basis were within 70 ml at Miranda and 84 ml at Otilia variety, while at the nitrogen dose N₄₀ on the P₄₀ basis, the interval was 61 ml (Miranda) - 81 ml (Profund). In the unfertilized, the limits were 53 ml at Miranda and 79 ml at Profund (Table 5).

Pitar and Profund varieties were superior to the Glosa control variety at all fertilization levels, while Miranda, Izvor and Şimnic 60 were lower - in both cases, with statistical assurance. Exist an influence of the variety but also an influence of the fertilization level on the sedimentation index. The varieties Otilia, Pitar, Profund and Bezostaia presented a higher

sedimentation index with statistical assurance compared to the Glosa variety, while the Litera, Miranda, Izvor, Pajura, Alex and Şimnic 60 varieties had diminished values with statistical assurance.

The obtained results show that during the three years of experimentation, the quality of baking reflected through the sedimentation index was very good and the presence of nitrogen in high dose brings very significant increases of this indicator (+4 ml at N₄₀P₄₀ and 10 ml at N₁₀₀P₄₀).

Table 5. Differentiated fertilization influence on sedimentation index at tested varieties (2016-2018 average)

A factor (variety)	B factor (fertilization level)			A x B dif	A x B dif	A factor influence
	SI at N ₀ P ₀ (%)	SI at N ₄₀ P ₄₀ (%)	SI at N ₁₀₀ P ₄₀ (%)			
	-b ₁ - (ct)	-b ₂ - B x A	-b ₃ -	b ₂ -b ₁	b ₃ -b ₁	
-a ₁ -GLOSA (ct)	68	71	77	4*	10***	72
-a ₂ -BOEMA 1	61 ⁰⁰⁰	65 ⁰⁰⁰	75	4*	14***	67 ^o
-a ₃ -LITERA	61 ⁰⁰⁰	68	73 ⁰⁰⁰	8**	12***	67 ^o
-a ₄ -MIRANDA	53 ⁰⁰⁰	61 ⁰⁰⁰	70 ⁰⁰⁰	8***	17***	61 ⁰⁰⁰
-a ₅ -IZVOR	60 ⁰⁰⁰	66 ^{oo}	68 ⁰⁰⁰	7***	8***	64 ^{oo}
-a ₆ -OTILIA	77***	72	84***	-5 ^{oo}	7***	77*
-a ₇ -PITAR	74***	77***	82**	4*	8***	77*
-a ₈ -PAJURA	62 ⁰⁰⁰	65 ⁰⁰⁰	74	4*	13***	67 ^o
-a ₉ -URSITA	65	67 ^o	75	2	10***	69
-a ₁₀ -UNITAR	67	66 ^{oo}	71 ⁰⁰⁰	-1	4*	68
-a ₁₁ -PROFUND	79***	81***	81*	3	2	80***
-a ₁₂ -ADELINA	63 ^{oo}	68	75	5**	12***	69
-a ₁₃ -SIMNIC 60	57 ⁰⁰⁰	67 ^o	72 ^{oo}	10****	15***	65 ^o
-a ₁₄ -ALEX	58 ⁰⁰⁰	64 ⁰⁰⁰	76	7***	19***	66 ^o
-a ₁₅ -BEZOSTAIA	77***	73	81*	-4 ^o	4*	77*
Average	65	69	75			
B factor influence	ct	4***	10***			

A factor: DL 5% = 5 ml; DL 1% = 6 ml; DL 0.1% = 8 ml

B factor: DL 5% = 2 ml; DL 1% = 3 ml; DL 0.1% = 4 ml

A x B interaction: DL 5% = 4 ml; DL 1% = 5 ml; DL 0.1% = 6 ml

B x A interaction: DL 5% = 4 ml; DL 1% = 5 ml; DL 0.1% = 6 ml

CONCLUSIONS

All the varieties studied had very significant yield increases when the nitrogen dose increased either to N₄₀ or to N₁₀₀ on the basis of P₄₀. About 23% of yield variability at N₄₀P₄₀ is associated with yield at N₀P₀. As the latter grows, grow yield in nitrogen addition plots. The varieties Ursita and Alex were noted that had very high yields in both fertilization variants. About 29% of yield variability at N₁₀₀P₄₀ is associated with yield at N₀P₀. And here, as the latter grows, grows yield in the variant with the higher dose of nitrogen. The varieties Unitar, Alex and Ursita were noted,

which had very high yields in both variants (unfertilized and N₁₀₀P₄₀).

The test weight is more influenced by the interaction variety x fertilization level than unique contribution of nitrogen

The 1000 grains weight is not influenced by the fertilization level but is influenced by the variety because it is a genetic impregnated character.

All varieties, without exception, registered a statistically assured increase in protein when the nitrogen dose was increased to N₁₀₀. The obtained results suggest that the high quantity of nitrogen influences the protein content, with increases between 0.7-1.5%. The correlation between the protein content obtained in the unfertilized variant and the protein content when fertilized with N₄₀P₄₀ is extremely strong (r = 0.912). For the studied interval, the coefficient of determination suggests that approximately 83% of the variability of the protein content to N₄₀P₄₀ is associated with the protein content from the unfertilized variant. Also, the correlation between the protein content obtained in the unfertilized variant and the protein content when fertilized with N₁₀₀P₄₀ is positive and extremely strong (r = 0.877). Approximately 77% of the variability of the protein content at N₁₀₀P₄₀ is associated with the protein content in the unfertilized variant.

The obtained results show that during the three years of experimentation, the quality of baking reflected through the sedimentation index was very good and the presence of nitrogen in high dose brings very significant increases of this indicator (+4 ml at N₄₀P₄₀ and 10 ml at N₁₀P₄₀).

REFERENCES

- Andronache, A.M. (2010). *Study on the production capacity and quality of an assortment of species, subspecies and varieties of the genus Triticum*. PhD Thesis USAMV Timișoara.
- Arenhardt, E.G., Silva, J., Gewehr, E., Oliveira, A.C., Binelo, M.O., Valdiero, A.C., Gzergorczyk, M.E., Lima, A. (2015). The nitrogen supply in wheat cultivation dependent on weather conditions and succession system in southern Brazil. *African Journal of Agricultural Research*, 10(48), 4322–4330.
- Brezolin, A.P., Gonzalez D.S., Roos-Frantz, F., Binelo, M.O., Menegassi, C.A., Krüger, B., Ghisleni, E., Anderson, M.R., Mantai, D., BrunelauScremin, O., Dornelles, E.F. (2017). Wheat yield obtained from nitrogen dose and fractionation. *African Journal of Agricultural Research*, 12(8), 566–576.
- Fageria, C.K., Baligar, V.C., Clarck, R.B. (2006). *Physiology of crop production*. I. Publishing House New York: Incorporated, 335 p.
- Flores, R.A., Urquiaga, S.S., Alves, B.J.R., Collier, L.S., Morais, R.F., Prado, R.M. (2012). Effect of nitrogen fertilizer and cutting age on the dry matter production of elephant grass in Savana. *Rev. Bras. E. Agr. Amb.*, 16(12), 1282–1288.
- Iancu, P., Păniță, O., Soare, M. (2019). Response of some new wheat genotypes to nitrogen fertilization and prospects of yield breeding based on yield elements. *Romanian Agricultural Research*, 34, 41–49.
- Marinciu, C.M., Șerban, G., Ittu, Gh., Mustățea, P., Manda, V., Păunescu, G., Voica, M., Săulescu, N.N. (2018). Response of several winter wheat cultivars to reduced nitrogen fertilization. *Romanian Agricultural Research*, 35, 177–182.
- Matei, GH. (2014). *Fitotehnie*. Craiova, RO: Sitech Publishing House.
- Naidin, C., Păunescu, G. (2003). Impact of precipitation and N P fertilization on wheat yield. *Scientific Papers I.C.D.A. Fundulea*, București, 26 mai.
- Nedelciuc, M., Nedelciuc, C., Văduva, I., Iagăru, Gh., Groza, N. (1995). Economic efficiency of some crop rotation and nitrogen fertilization for irrigation conditions in the South-West Romanian Plain. *Romanian Agricultural Research*, 4.
- Nicolescu, M., Croitoru, I., Mititelu, C. (1994). The influence of chemical fertilizers with nitrogen and phosphorus applied for a long time on some physical and chemical properties of the brown soil - luvic red, under irrigation conditions. *Scientific Papers Agricultural Management*, IX, 170–189.
- Silva, J.A.G., Arenhardt, E.G., Krügers C., Lucchese A.O., Metz M., Marolli, A. (2015). The expression of the components of wheat yield by technological class and nitrogen use. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 19(1), 27–33.

EFFICACY OF SOME HERBICIDES AND HERBICIDE TANK MIXTURES AGAINST WEEDS AND SELF-SOWN PLANTS IN DURUM WHEAT (*Triticum durum* Desf.)

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Abstract

The research was conducted during 2015-2017 on pellicvertisol soil type. Under investigation was Bulgarian durum wheat cultivar Elbrus (*Triticum durum* Desf.). Factor A included untreated control and 4 antigraminaceous herbicides - Axial 050 EC (pinoxaden) - 900 ml/ha, Topic 080 EC (clodinafop) - 450 ml/ha, Traxos 045 EC (pinoxaden + clodinafop) - 1.20 l/ha and Scorpio super 7.5 EB (fenoxaprop-ethyl) - 1 l/ha. Factor B included untreated control and 4 antibroadleaved herbicides - Biathlon 4 D (tritosulfuron + florasulam) - 55 g/ha, Lintur 70 WG (triasulfuron + dicamba) - 150 g/ha, Granstar super 50 SG (tribenuron-methyl + tiphensulfuron-methyl) - 40 g/ha and Secator OD (amidosulfuron + iodosulfuron) - 100 ml/ha. All of antigraminaceous herbicides, antibroadleaved herbicides and their tank mixtures were treated in tillering stage of the durum wheat. Self-sown plants of Clearfield canola in durum wheat crops are successfully controlled by herbicide Biathlon only. Self-sown plants of Clearfield and ExpressSun sunflower are controlled by herbicides Biathlon, Lintour and Secator. Self-sown plants of coriander are controlled by herbicides Lintur, Granstar super and Secator. Self-sown plants of milk thistle are controlled by herbicides Lintur and Secator. There is synergism in herbicide Traxos and very good efficacy against *Bromus arvensis* L. There is antagonism in herbicide tank mixture Scorpio super + Lintur and decreasing of the efficacy against *Galium aparine* L., *Chamomilla recutita* Rauchert, *Myagrum perfoliatum* L., *Cirsium arvense* Scop. and *Apera spica-venti* P.B. For complete control of all weeds and self-sown plants in durum wheat crops, two herbicides should be combined - both antigraminaceous and antibroadleaved.

Key words: durum wheat, weeds, self-sown plants, herbicides, herbicide tank mixtures, efficacy, selectivity.

INTRODUCTION

In Bulgaria, durum wheat is grown mainly in crop rotations with sunflower or canola. The most part of winter oilseed canola is grown by Clearfield technology - about 60%. Oil-bearing sunflower is grown mainly by Clearfield and ExpressSun technologies - between 93% and 95%. Hybrids of Clearfield canola, Clearfield sunflower and ExpressSun sunflower are characterized by resistance to herbicides different than that of conventional canola and sunflower hybrids. This resistance is transmitted to hybrid generation (Covarelli and Stagnari, 2002; Smajlagić and Đikić, 2011; Lobkov et al., 2012).

In the latest years the coriander significantly increased the areas and became the sixth culture in Bulgaria, deferring only to wheat, sunflower, maize, canola and barley. Currently, about 200.000 hectares in the Bulgaria already "are weed infested" with self-sown plants of coriander (Delchev, 2018a). These self-sown

plants are destroyed difficult in wheat crops (Vaculík, 2007).

Self-sown plants of milk thistle are an unpleasant weed for next crops, especially for winter cereals (Delchev, 2018b). The seeds maintain their germination ability for a long time and easily spread by wind at harvest. Uneven maturation time of individual anthodia is typical and the achenes tend to fall out from ripe anthodia, so optimal time of harvest must be selected (Spitzová, 1997; Moudrý, 2001).

Seeds from self-sown plants of Clearfield canola, Clearfield sunflower, ExpressSun sunflower, coriander and milk thistle are becoming a big problem in the durum wheat crops (Nakayama et al., 2010; Gupta et al., 2011; Tsyuganov and Potarenko, 2011). Effective weed control is one the important conditions for full realization of the biological potential of the durum wheat (Delchev, 2018).

The purpose of the investigation was to establish the efficacy and selectivity of some antigraminaceous herbicides, antibroadleaved

herbicides and their tank mixtures on durum wheat.

MATERIALS AND METHODS

The research was conducted during 2015-2017 on pellicvertisol soil type. A field experiment was carried out with Bulgarian durum wheat cultivar Elbrus (*Triticum durum* Desf.). Two factors experiment was conducted under the

block method, in 4 repetitions; the size of the crop plot was 15 m². Factor A included untreated control and 4 antigraminaceous herbicides - Axial 050 EC, Topic 080 EC, Traxos 045 EC and Scorpio super 7.5 EB. Factor B included untreated control and 4 antibroadleaved herbicides - Biathlon 4 D, Lintur 70 WG, Granstar super 50 SG and Secator OD. The active substances and doses of the investigated herbicides are given in Table 1.

Table 1. Investigated variants

№	Herbicide	Active substance	Dose
Antigraminaceous herbicides			
1	Control	-	-
2	Axial 050 EC	pinoxaden	900 ml/ha
3	Topic 080 EC	clodinafop	450 ml/ha
4	Traxos 045 EC	pinoxaden + clodinafop	1.20 l/ha
5	Scorpio super 7.5 EB	fenoxaprop-ethyl	1 l/ha
Antibroadleaved herbicides			
1	Control	-	-
2	Biathlon 4 D	tritosulfuron + florasulam	55 g/ha
3	Lintur 70 WG	triasulfuron + dicamba	150 g/ha
4	Granstar super 50 SG	tribenuron-methyl + tiphensulfuron-methyl	40 g/ha
5	Secator OD	amidosulfuron + iodosulfuron	100 ml/ha

Herbicide Biathlon was used in addition with adjuvant Dash HC - 500 ml/ha and herbicide Granstar super - with adjuvant Trend 90 - 0.1%.

All of antigraminaceous herbicides, antibroadleaved herbicides and their tank mixtures were treated in tillering stage of the durum wheat, with working solution 200 l/ha. Mixing was done in the tank on the sprayer. Due to of low adhesion of the herbicide Biathlon it was used in addition with adjuvant Dash HC - 500 ml/ha and herbicide Granstar super - with adjuvant Trend 90-0.1 %.

It was investigated efficacy and selectivity of herbicides and their tank mixtures. Efficacy of herbicides against weeds and self-sown plants was appointed according to 100% scale of EWRS (European Weed Research Society). Selectivity of herbicides to durum wheat plants was followed according to the 9 - rate scale of EWRS (rating 1 - without damages, rating 9 - crop is completely destroyed).

RESULTS AND DISCUSSIONS

Annual broadleaved weeds in the experience are represented by *Anthemis arvensis* L., *Chamomilla recutita* Rauchert, *Galium aparine* L., *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Capsella bursa-pastoris* L., *Falopia convolvulus* Leve, *Lithospermum arvense* L., *Papaver rhoes* L., *Consolida regalis* Gray, *Viola tricolor* L., *Myagrum perfoliatum* L., *Lamium purpureum* L., *Veronica hederifolia* L., *Stellaria media* Cyr. Perennial broadleaf weeds are established *Cirsium arvense* Scop., *Convolvulus arvensis* L., *Cardaria draba* L., *Sonchus arvensis* L. Graminaceous weeds are reported *Avena fatua* L., *Avena ludoviciana* Durien., *Alopecurus myosuroides* L., *Apera spica-venti* P.B., *Lolium*

temulentum L., *Lolium multiflorum* L., *Bromus arvensis* L.

Self-sown plants in experience are from Clearfield canola (*Brassica napus* L.), Clearfield and ExpressSun sunflower (*Helianthus annuus* L.), coriander (*Coriandrum sativum* L.) and milk thistle (*Silybum marianum* Gaertn.). They are sown manually when sowing durum wheat.

Antibroadleaved herbicides Biathlon and Secator have high efficacy against represented in the experience annual broadleaved weeds (Table 2). Herbicide Lintur is less effective only against *Consolida regalis* Gray - 45%. It destroys mainly less developed plants. Herbicide Granstar super has satisfactory efficacy against *Falopia convolvulus* Leve, but the efficacy against *Galium aparine* L. is unsatisfactory.

Table 2. Efficacy of some herbicides and herbicide tank mixtures against annual broadleaved weeds at durum wheat according to the 100% visual scale of EWRS (mean 2015-2017)

Herbicides		Weeds								
Antigraminaceous	Antibroadleaved	<i>Galium aparine</i>	<i>Chamomillare cutita</i>	<i>Papaver rhoes</i>	<i>Consolida regalis</i>	<i>Sinapis arvensis</i>	<i>Raphanus raphanistrum</i>	<i>Anthemis arvensis</i>	<i>Myagrumperf olatum</i>	<i>Falopia convolvulus</i>
	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	100	100	98	100	100	100	100	100
	Lintur	100	100	100	45	100	95	100	100	100
	Granstar super	75	100	100	100	100	100	100	100	92
	Secator	100	100	98	98	100	100	100	100	100
	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	100	100	98	100	100	100	100	100
	Lintur	100	100	100	45	100	95	100	100	100
	Granstar super	75	100	100	100	100	100	100	100	92
	Secator	100	100	98	98	100	100	100	100	100
	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	100	100	98	100	100	100	100	100
	Lintur	100	100	100	45	100	95	100	100	100
	Granstar super	75	100	100	100	100	100	100	100	92
	Secator	100	100	98	98	100	100	100	100	100
	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	100	100	98	100	100	100	100	100
	Lintur	100	100	100	45	100	95	100	100	100
	Granstar super	75	100	100	100	100	100	100	100	92
	Secator	100	100	98	98	100	100	100	100	100
	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	100	100	98	100	100	100	100	100
	Lintur	98	95	100	45	100	95	100	95	100
	Granstar super	75	100	100	100	100	100	100	100	92
	Secator	100	100	98	98	100	100	100	100	100

Antibroadleaved herbicides Biathlon, Lintur and Secator have very high efficacy against perennial broadleaved weeds *Cirsium arvense* Scop., *Cardaria draba* L. and *Sonchus arvensis* L. (Table 3). Herbicide Granstar super has fewer efficacies against *Cirsium arvense* Scop. compared to herbicide Granstar (Delchev, 2013; 2015). Granstar super controls it at 90%,

versus Granstar controls it at 100%. Obviously, the reduction in the amount of tribenuron-methyl at the expense of the addition of thifensulfuron-methyl at Granstar super increases the efficacy of this herbicide against some annual broadleaved weeds but reduces its efficacy against *Galium aparine* L.

Table 3. Efficacy of some herbicides and herbicide tank mixtures against perennial broadleaved weeds and self-sown plants at durum wheat according to the 100% visual scale of EWRS (mean 2015-2017)

Herbicides		Weeds and self-sown plants								
Antigraminaceous	Antibroadleaved	<i>Cirsium arvense</i>	<i>Convolvulus arvensis</i>	<i>Cardaria draba</i>	<i>Sonchus arvensis</i>	<i>Brassica napus</i> ¹	<i>Helianthus annuus</i> ²	<i>Helianthus annuus</i> ³	<i>Coriandrum aivum</i> ⁴	<i>Silybummaria num</i> ⁵
-	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	0	100	100	100	100	100	70	80
	Lintur	100	86	100	100	65	100	100	100	100
	Granstar super	90	0	90	100	10	15	10	100	0
	Secator	100	0	90	90	82	100	100	100	100
Axial	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	0	100	100	100	100	100	70	80
	Lintur	100	86	100	100	65	100	100	100	100
	Granstar super	90	0	90	100	10	15	10	100	0
	Secator	100	0	90	90	82	100	100	100	100
Topic	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	0	100	100	100	100	100	70	80
	Lintur	100	86	100	100	65	100	100	100	100
	Granstar super	90	0	90	100	10	15	10	100	0
	Secator	100	0	90	90	82	100	100	100	100
Traxos	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	0	100	100	100	100	100	70	80
	Lintur	100	86	100	100	65	100	100	100	100
	Granstar super	90	0	90	100	10	15	10	100	0
	Secator	100	0	90	90	82	100	100	100	100
Scorpio super	-	0	0	0	0	0	0	0	0	0
	Biathlon	100	0	100	100	100	100	100	70	80
	Lintur	95	86	100	100	65	100	100	100	100
	Granstar super	90	0	90	100	10	15	10	100	0
	Secator	100	0	90	90	82	100	100	100	100

¹ - self-sown plants of Clearfield canola; ² - self-sown plants of Clearfield sunflower; ³ - self-sown plants of ExpressSun sunflower; ⁴ - self-sown plants of coriander; ⁵ - self-sown plants of milk thistle

Antibroadleaved herbicides Biathlon, Granstar super and Secator are inefficacy against

Convolvulus arvensis L., although they have high efficacy against *Cirsium arvense* Scop.,

Cardaria draba L. and *Sonchus arvensis* L. This is due to the fact that the massive emergence of the bum is late and occurs after the time of herbicide treatment through the tillering stage of the durum wheat.

From the antibroadleaved herbicides only Lintur has satisfactory efficacy against *Convolvulus arvensis* L. - 86%. This herbicide, besides foliar action, also has soil action. Thus, he can control *Convolvulus arvensis* L. when germinating its shoots through stem elongation stage of the durum wheat.

Herbicide Biathlon successfully controls self-sown plants of Clearfield canola (*Brassica napus* L.) and Clearfield and ExpressSun sunflower (*Helianthus annuus* L.), but has insufficient efficacy against self-sown plants of coriander (*Coriandrum sativum* L.) and of milk thistle (*Silybum marianum* Gaertn.) - respectively 70% and 80%.

Herbicide Lintur has satisfactory efficacy against self-sown plants of Clearfield canola - 65%. This herbicide is efficacy against self-sown plants of Clearfield and ExpressSun sunflower, of coriander and of milk thistle.

Herbicide Granstar super has high efficacy against self-sown plants of coriander, but is inefficacy against self-sown plants of Clearfield canola, of Clearfield and ExpressSun sunflower and of milk thistle.

Herbicide Secator successfully controls self-sown plants of Clearfield and ExpressSun sunflower, of coriander and of milk thistle, but has fewer efficacies against self-sown plants of Clearfield canola - 82%.

Antigraminaceous herbicide Axial is effective against almost all annual graminaceous weeds - *Avena fatua* L., *Avena ludoviciana* Durien., *Alopecurus myosuroides* L., *Apera spica-venti* P.B., *Lolium temulentum* L., *Lolium multiflorum* L. (Table 4). This herbicide is inefficacy against *Bromus arvensis* L. only.

Antigraminaceous herbicides Topicand Traxos have very high efficacy against *Avena*

fatua L., *Avena ludoviciana* Durien., *Alopecurus myosuroides* L., *Apera spica-venti* P.B., *Lolium temulentum* L., *Lolium multiflorum* L. Herbicide Topic can not control *Bromus arvensis* L. only.

The combination of the active substances of herbicides Axial and Topic - respectively pinoxaden and clodinafop - in herbicide Traxos, results in synergism and very good herbicide efficacy against *Bromus arvensis* L. - 96%.

Herbicide Scorpio super has very high efficacy against *Alopecurus myosuroides* L., *Avena fatua* L., *Avena ludoviciana* Durien. and good efficacy against *Apera spica-venti* P.B. This herbicide is inefficacy against *Lolium temulentum* L., *Lolium multiflorum* L. and *Bromus arvensis* L.

There is antagonism in herbicide tank mixture Scorpio super + Lintur. There is reduction in the efficacy of antibroad leaved herbicide Lintur against *Galium aparine* L., *Chamomilla recutita* Rauchert, *Myagrum perfoliatum* L. and *Cirsium arvense* Scop. There is also reduction in the efficacy of antigraminaceous herbicide Scorpio super against *Apera spica-venti* P.B. There is no evidence of antagonism against other graminaceous and broadleaved weeds.

Herbicide tank mixtures of antigraminaceous herbicide Scorpio super with antibroadleaved herbicides Biathlon, Granstar super and Secator, as well as of antibroadleaved herbicide Lintur with antigraminaceous herbicides Axial, Topic and Traxos do not exhibit antagonism in their herbicide action.

Antigraminaceous herbicides Axial, Topic and Traksos exhibit excellent miscibility with antibroadleaved herbicides Biatlon, Granstar super and Secator. The herbicides exhibit an additive effect in their herbicide action when they treated as tank mixtures.

The investigated antigraminaceous herbicides - Axial, Topic, Traksos and Scorpio super, antibroadleaved herbicides Biatlon, Lintur, Granstar super and Secator, as well as their tank mixtures exhibit very high selectivity to durum wheat - rating 1 by the scale of EWRS (Table 4).

Visible signs of phytotoxicity to durum wheat were observed only in the herbicide tank mixture Scorpio super + Lintur - rating 2 by the scale of EWRS. It leads to short developmental disorders, resulting in a slight

Table 4. Efficacy of some herbicides and herbicide tank mixtures against annual graminaceous weeds at durum wheat according to the 100% visual scale of EWRS and selectivity according to the 9-rate scale of EWRS (mean 2015-2017)

Herbicides		Weeds							Selectivity
Antigraminaceous	Antibroadleaved	<i>Avena fatua</i>	<i>Avena ludoviciana</i>	<i>Lolium ulitiform</i>	<i>Lolium temulentum</i>	<i>Alopecurus myosoides</i>	<i>Apera spica-venti</i>	<i>Bromus arvensis</i>	
-	-	0	0	0	0	0	0	0	1
	Biathlon	0	0	0	0	0	0	0	1
	Lintur	0	0	0	0	0	0	0	1
	Granstar super	0	0	0	0	0	0	0	1
	Secator	0	0	0	0	0	0	0	1
Axial	-	100	100	100	100	100	100	0	1
	Biathlon	100	100	100	100	100	100	0	1
	Lintur	100	100	100	100	100	100	0	1
	Granstar super	100	100	100	100	100	100	0	1
	Secator	100	100	100	100	100	100	0	1
Topic	-	100	100	98	99	100	100	0	1
	Biathlon	100	100	98	99	100	100	0	1
	Lintur	100	100	98	99	100	100	0	1
	Granstar super	100	100	98	99	100	100	0	1
	Secator	100	100	98	99	100	100	0	1
Traxos	-	100	100	100	100	100	100	96	1
	Biathlon	100	100	100	100	100	100	96	1
	Lintur	100	100	100	100	100	100	96	1
	Granstar super	100	100	100	100	100	100	96	1
	Secator	100	100	100	100	100	100	96	1
Scorpio super	-	100	100	0	0	100	93	0	1
	Biathlon	100	100	0	0	100	93	0	1
	Lintur	100	100	0	0	100	90	0	2
	Granstar super	100	100	0	0	100	93	0	1
	Secator	100	100	0	0	100	93	0	1

chlorosis on the leaves. Signs of phytotoxicity are overcome by durum wheat for about 4-5 days after treatment.

CONCLUSIONS

Self-sown plants of Clearfield canola in durum wheat crops are successfully controlled by herbicide Biathlon only.

Self-sown plants of Clearfield and ExpressSun sunflower are controlled by herbicides Biathlon, Lintour and Secator.

Self-sown plants of coriander are controlled by herbicides Lintur, Granstar super and Secator. Self-sown plants of milk thistle are controlled by herbicides Lintur and Secator.

There is synergism in herbicide Traxos and very good efficacy against *Bromus arvensis* L. There is antagonism in herbicide tank mixture Scorpio super + Lintur and decreasing of the efficacy against *Galium aparine* L., *Chamomilla recutita* Rauchert, *Myagrurn perfoliatum* L., *Cirsium arvense* Scop. and *Apera spica-venti* P.B.

For complete control of all weeds and self-sown plants in durum wheat crops, two herbicides should be combined - both antigraminaceous and antibroadleaved.

REFERENCES

- Covarelli, G., Stagnari, F. (2002). Carfentrazone-ethyl for post-emergence weed control in wheat (*Triticum aestivum* L.). *Attidelle Giornate Fitopatologiche*, 1, 183–188.
- Delchev, Gr. (2018). *Mixability of herbicides with growth regulators and foliar fertilizers*. Monograph, ISBN: 978-613-6-64820-0, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 329.
- Delchev, Gr. (2018). *Chemical control of weeds and self-sown plants in eight field crops*. Monograph, ISBN: 978-613-7-43367-6, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 397.
- Delchev, Gr. (2018). *Late use of herbicides in durum wheat crop (Triticum durum Desf.)*. Monograph, ISBN: 978-613-8-26945-8, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 141.
- Gupta, A., Aggarwal, A., Chhavi, M., Kumar, A., Tanwar, A. (2011). Effect of herbicides fenoxaprop-ethyl and 2, 4-d ethyl-ester on soil mycoflora including vam fungi in wheat crop. *Indian Journal of Weed Science*, 43(1-2), 32–40.
- Lobkov, V., Plygin, C., Abakumov, N., Bobkov, J. (2012). The role of tillage and herbicide application "Trizlak" when growing winter wheat grain quality. *Russian Journal of Agricultural and Socio-Economic Sciences*, 4(4), 32–37.
- Moudrý J. (2001). *Alternativní plodiny*, ISBN 978-80-86726-40-3, Profi Press, Praha, pp. 142.
- Nakayama, S., Nakatani, K., Hamaguchi, H. (2010). Control effects of soil-applied herbicides in soybeans sprayed over wheat-residue mulch. *Weed research*, 55(2), 62–68.
- Smajlagić, A., Đikić, M. (2011). Karakteristik ekoroške zajednice pšenice nastale primjenom različitih agrotehničkih mjera. *Works of the Faculty of Agricultural and Food Sciences University of Sarajevo, LVI*(61/2), 7–20.
- Spitzová I. (1997). Ostropestřec mariánský - staronová léčivá rostlina. *Úroda*, 45(8), 28–29.
- Tsyuganov, A., Potarenko, M. (2011). Comparative efficacy of herbicides in winter wheat under conditions of 'Raia Gro Service' Senno district. *Agriculture plant protection: Scientific journal*, 1, 24–26.
- Vaculík, A. (2007). *The influence of the herbicides treatment on the yield and essential oil content of spice plants growth in the Czech Republic*. Dissertation thesis. Department of Crop Science, Breeding and Plant Medicine, Brno, Czech Republic.

EFFECT OF SOWING PERIOD ON SEED YIELD AND ESSENTIAL OIL COMPOSITION OF CORIANDER (*Coriandrum sativum* L.) IN SOUTH-EAST BULGARIA CONDITION

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Abstract

Coriander (Coriandrum sativum L.) is one of the most important spice and aromatic plants grown worldwide. It is an annual plant, belonging to the Apiaceae family, which is cultivated mainly for its fruits (seeds). The productivity of coriander is influenced by weather conditions, agronomic and genetic factors. In this paper the influence of six sowing periods (October, November, December, February, March and April) on yield and essential oil quality of the coriander cultivar 'Aleksievski' is discussed. The study was carried out during 2015-2018 in south-east Bulgaria on vertisols leached soil type. Field experiments were performed by means of a block method with four replications; experimental field area was 15 m². The pre crop of coriander was winter wheat. All the stages of the established technology for coriander growing were followed. The indicators seeds yield (kg ha⁻¹), essential oil content (%), essential oil yield (kg ha⁻¹) and essential oil composition of coriander were determined. The obtained data was statistically processed by the dispersion and correlation analyses methods. The obtained results showed that sowing period in combination with meteorological conditions during the years of the study had a significant influence on productivity of coriander, grown in the study. The highest seeds and essential oil yields (2302 kg ha⁻¹ and 26.8 kg ha⁻¹, respectively) in the October sowing period were reported, and the lowest ones (1582 kg ha⁻¹ and 11.8 kg ha⁻¹) in the April sowing was recorded. The essential oil content in coriander fruits was lower by the later sowing period. The year of the experiment and sowing period affected essential oil composition.

Key words: coriander, sowing period, seed yield, essential oil, essential oil composition.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is one of the most important spice and aromatic plants grown in world. It is an annual plant, belonging to the *Apiaceae* family, which is cultivated mainly for its seeds (Delibaltova et al., 2012). The productivity of coriander is determined by genetic traits of cultivars, the environmental factors as well as by the agro-technical practices (Marinov et. al., 2017; Nowak and Szemplinski, 2014). The maximum fruit and essential oil yields are attained only when an appropriate combination of these factors are provided for the plant (Gil et al., 2002; Kassu et al., 2018). Sowing period is some of the major agronomic factors for the vegetative growth and ultimate yield expressions. Any early or lateness in sowing may hamper the growth, yield as well as quality of the coriander (Sharangi and Roychowdhury, 2014). According to the investigations of Bhadkariya et al. (2007) the sowing period have the effect on plant height, number of branches per plant,

number of umbel per plant, number of the seeds per umbel and seeds yield. The sowing period affected yield and essential oil quality of coriander as well as on the seeds yield (Zheljzakov et al., 2008). Gujar et al. (2005) reported that the maximum values were recorded for all the characters when the seeds were sown on 10th of October. Yildirim and Gok (2012) found that the highest essential oil ratio was obtained as 0.5% from May 20th sowing period. The essential oil average yields changed from 0.09-0.116 l ha⁻¹ and Linalool which essential oil component ratio was changed from 68.3-74.8%. Gamma terpinen ratio was changed from 7-8%. The highest gamma terpinen ratio was obtained as 8.8% from April the 20th sowing period. Because of obtaining the highest fruit yield, Moosavi (2012) recommended to sow the coriander on March the 30th. A field trail performed in Iran (Ghobadi and Ghobadi, 2012) showed that at late sowing period the coriander produced satisfactory seed, essential and oil yields. Carrubba et al. (2006) reported that the most

productive sowing period was December, and sowing after this period resulted in lower yields. Tanchev (2011) investigated the effect of sowing period on seed yield of coriander of Strandhja region (Bulgaria) and reported those highest yields were formed by sowing in autumn. The obtained yield was 84% higher than those of springs sowing. In this regard, for coriander, comprehensive information is very much scanty and very little research is conducted in Bulgaria to study the effect of sowing period on seed yield and essential oil of this crop.

The aim of the study is to determine the effect of specific climatic factors of year and sowing period as well as their interaction on yield, the seed oil content and the oil chemical composition in coriander seeds.

MATERIALS AND METHODS

Field trials were carried out in the period of 2015-2018 in the region of Elhovo town, South-East Bulgaria. The study was performed by means of a block method with four replications; experimental field area - 15 m². The soil type is vertisols leached. The pre crop was winter wheat. The studied coriander cultivar was 'Alekseevski'. The crop was sown in six different periods - October, November, December, February, March and April. The study was carried out by the following practices: soil cultivation - ploughing of the stubble in July and August at a depth of 20-22 cm, twice pre-sowing cultivation with harrowing, the last being at a depth of 5-6 cm. The fertilizer was introduced at the time of sowing of di-ammonium phosphate (P-N 48-18) at a rate of 200 kg ha⁻¹. The sowing between row distances were 12-15 cm and a seed rate was 250 germinating seeds per m² at a depth of 3-4 cm. Weeds were controlled by treatment with the herbicide Linurex 45 SC – 2.00 l ha⁻¹, applied after sowing, before germination of the crop. At "rosette" stage dressing with 100 kg ha⁻¹ N was applied. Harvesting was done at full crop maturity. The seed yield is determined with standard grain moisture of 9%. The indicators seeds yield (kg ha⁻¹), essential oil content (%), essential oil yield (kg ha⁻¹) and essential oil composition of coriander were determined.

The essential oil from coriander dried seeds was extracted by distillation using a Clevenger collector apparatus. Compositions of the essential oil were determined by Gas Chromatography-Mass Spectrometry (GC/MS) analysis.

The obtained data for the values of the all indices were statistically processed by the method of dispersion and correlation analyses.

The major climatic factors determining the productivity and quality of coriander are temperature and rainfall, their combination and distribution throughout the vegetation season (Dyulgerov and Dyulgerova, 2016). Comparing the values of the average monthly temperatures during the years of the study showed that they were very close in all the three years and they were higher than those measured for a long period of time, with no significant deviations from the coriander requirements (Figure 1). The years of the study (2015-2018) differed significantly in the amount and distribution of rainfall during vegetation (Figure 2). Its amount in 2015-2016 experimental year was 433.2 mm and those values were very close to the values reported for a long period of time (414 mm), which determined the first year of the experiment as good for the plants. The lowest amount of precipitation was reported in 2017 economic year - 328.9 mm i.e. 85 mm little than the amount measured for a long period of time. That year was characterized by uneven distribution of rainfall, which was not enough to meet the plant requirements for water at the critical stages. In April-June at the stages of buttoning, flowering and fruit setting, the amount of rainfall was 108.6 mm versus 138 mm for a multiple-year period, i.e. about 30 mm less, that determined the second experimental year as less favorable for the productivity of coriander. The last year of the study (2017-2018) was characterized by the greatest amount of rainfall during the vegetation season - 538.0 mm and exceeded the values for the period 1961-1991 by 124.0 mm. Precipitation was evenly distributed during vegetation and in a combination with the reported temperature values, it was considered to be the most favorable for coriander cultivation of the experimental years.

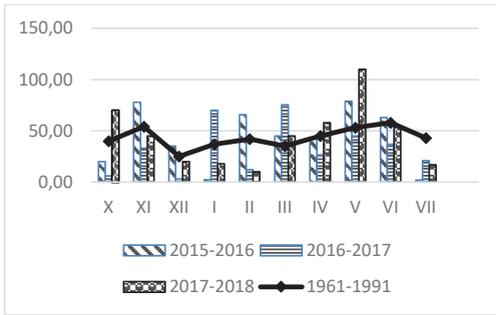


Figure 1. Rainfall, mm

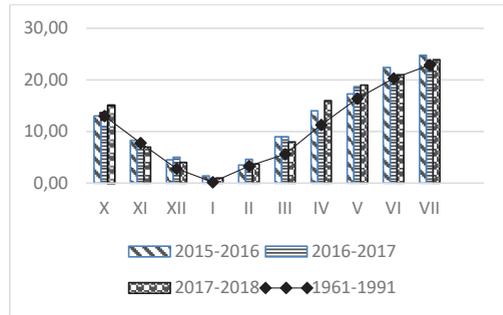


Figure 2. Average monthly air temperature, °C

RESULTS AND DISCUSSIONS

The yields of coriander seeds during the experiment varied depending on the climatic conditions throughout the years and the sowing period (Table 1). In years with the rainfalls reaching 433.2 (2015-2016) and 538.0 mm (2017-2018) the seed yields were 2041 and 2183 kg ha⁻¹, respectively, which was 30.0 and 38.9 % higher than in the period of 2016-2017. During the first experimental year, the highest statistically significant yield was obtained from October sowing - 2384 kg ha⁻¹, followed by November (2142 kg ha⁻¹), and the lowest - from April sowing (1690 kg ha⁻¹).

Table 1. Seeds yield, kg ha⁻¹

Sowing time	Years of study			Average for the period kg ha ⁻¹
	2015-2016	2016-2017	2017-2018	
October	2384 ^c	1963 ^f	2560 ^f	2302
November	2142 ^d	1840 ^c	2345 ^c	2109
December	2100 ^c	1726 ^d	2230 ^d	2017
February	2065 ^c	1400 ^c	2110 ^c	1858
March	1863 ^b	1325 ^b	1980 ^b	1723
April	1690 ^a	1180 ^a	1875 ^a	1582
Mean for year	2041	1572	2183	

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test.

The insufficient rainfall during the vegetation season of 2016-2017, especially during the stages of coriander buttoning, flowering and fruit setting, was the reason for the low seed yields, which varied from 1180 to 1963 kg ha⁻¹

for the studied sowing period. In that year, coriander sown in November surpassed in yields - December, February and March sowing by 6.6, 23.4 and 30.2%, respectively, but fall behind October sowing by 6.7%, the values are being significant. The more favorable climatic factors in 2017-2018 preconditioned the formation of a higher seed yield than in the other two experimental years. During the three experimental years, the highest yield of seeds formed by October sowing - 2560 kg ha⁻¹ and it exceeded the November, December, February and March sowing by 215, 330, 450 and 580 kg ha⁻¹, respectively, the differences were statistically significant.

The lowest seed yield was formed after April sowing, the values being 36.5% lower than the yield from October, 25.1% lower than November, 18.9% lower than December, 12.5% lower than February and 5.6% lower than March sowings, which was statistically proved. This result corresponds with Bhadkariya et al. (2007), Ghobadi and Gobadi (2012), Moosavi (2012) and Tanchev (2011), who reported that earlier sowing of coriander led to the highest seed yields. The dispersion analysis about the effect of the factors Sowing time and Year, as well as their interaction, on the seed yield, showed a significant influence of the factors on the changes of the characteristic and statistically insignificant effect of the interaction between them (Table 2).

Table 2. Analysis of variance ANOVA

Source of Variation	Sum of Square	Df	Mean Square	F	P-value	F crit
Year**	4904087	2	2452044	23695.48	0.00	3.168246
Sowing time**	4175610	5	835122	8070.255	0.00	2.38607
Interaction**	190198.2	10	19019.82	183.7993	0.00	2.011181
Within	5588	54	103.4815			

*F-test significant at $P<0.05$; **F-test significant at $P<0.01$; ns non-significant

The content of essential oil in coriander seeds was affected by both the year and the sowing period (Figure 3). In 2016, when the average air temperature during the period of fruit maturation was 24.8°C and the rainfall equalled

2 mm., depending on sowing period, the coriander seeds had oil content from 0.87 to 1.23%, which was higher than in 2017, when the average daily temperature was 22.0°C and the rainfall was 21 mm.

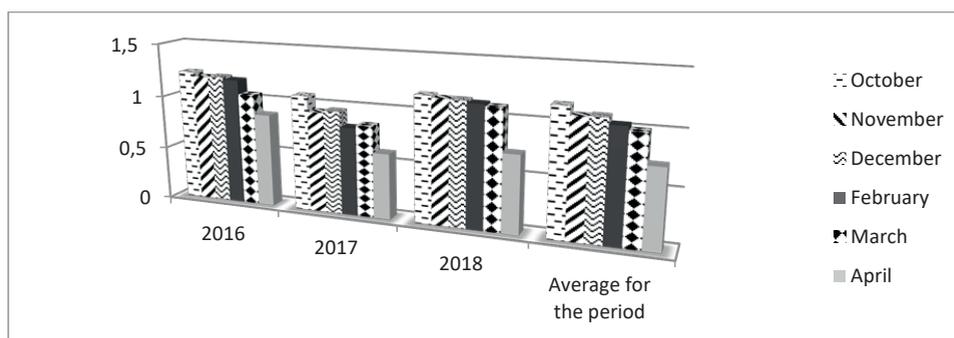


Figure 3. Effect of sowing time on essential oil content, %

The content of oil in coriander seeds ranged from 0.61 to 1.08%. In the other years of the experiment (2018), that characteristic was from 0.72 to 1.17%. This is also confirmed by an experiment conducted by Zawislak (2011) in Poland, where the concentration of oil in coriander fruits was distinctly different in two consecutive years (1.87% in 2007 and 2.33% in 2006). On average for the period of 2015-2018, the highest essential oil content of 1.17% was obtained when coriander was sown in October. Sowing after that time the values of this indicator decreased and ranged from 0.73 to 1.09%. The sowing period in this experiment,

similarly to the obtained data from trials conducted by Moosavi et al. (2012) as well as Zheljzakov et al. (2008) induce differences in the content of essential oil in coriander seeds. In Iran and Canada, seeds from an early date of sowing contained more essential oil than seeds from plant sown latter.

The results of the dispersion analysis about the effect of the factors Sowing time and Year, as well as their interaction on the indicator seed yield are presented in Table 3. The results showed a statistically significant effect of the studied factors and insignificance of their interaction.

Table 3. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Year*	0.847136	2	0.423568	40.7277	0.00	3.168246
Sowing time*	1.373294	5	0.274659	26.40951	0.00	2.38607
Interactions ^{ns}	0.108564	10	0.010856	1.043884	0.42	2.011181
Within	0.5616	54	0.0104			

*F-test significant at $P<0.05$; **F-test significant at $P<0.01$; ns non-significant

The essential oil yield of coriander was affected significantly by different sowing periods (Figure 4). The results confirm that the values of that indicator depended to a sowing period, ranging from 14.7 to 29.3 kg ha⁻¹; 7.2-21.2 kg ha⁻¹ and 13.5-29.9 kg ha⁻¹ in 2016, 2017 and 2018, respectively. The highest oil yield, on average for the period of the study, was obtained from October sowing period

(26.8 kg ha⁻¹) surpassing by 15.5%, 19.1%, 34.7 and 52.3% the November sowing period, December, February and March, and the lowest yield was obtained from April sowing period - 11.5 kg ha⁻¹. The fact that coriander tends to essential lower oil yield when sown later has also been reported by other researchers (Zheljzakov et al., 2008; Özel et al., 2009; Moosavi et al., 2012).

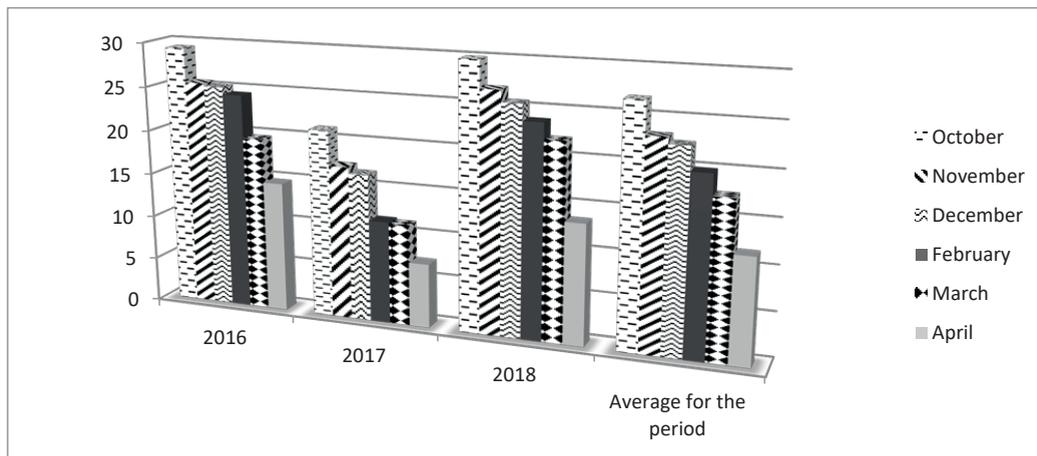


Figure 4. Effect of sowing period on essential oil yield, kg ha⁻¹

The results of the dispersion analysis about the influence of the factors and their interaction on the essential oil yield showed clear statistically

significant variations, and the interaction between the two factors was statistically insignificant (Table 4).

Table 4. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Year*	1230.163	2	615.0817	69.94606	0.00	3.168246
Sowing time*	1346.758	5	269.3516	30.63021	0.00	2.38607
Interactions ^{ns}	147.53	10	14.753	1.677686	0.11	2.011181
Within	474.8575	54	8.793657			

*F-test significant at P<0.05; **F-test significant at P<0.01; ns - non-significant

The composition of coriander oil, in which a profile of 12 chemical compounds were determined, was dominated by linalool (Table 5). This is the major constituent of essential oil in coriander seeds, responsible for their typical aroma (Carrubba et al., 2006). In the present experiment the concentration of linalool depending on the sowing time range from 61.0 to 67.0% in 2016, 58.0-65.0 in 2017 and 63.0-68.0% in 2018. Many authors report similar percentages of linalool in coriander essential oil. For example, Nowak and Szemplinski (2014) determined the concentration of linalool

in coriander oil at 65.3 and 67.1% from fruits in Olsztyn in two years. In Poland, the percentage determined by Zawislak (2011) ranged from 69.9 to 72.5%, while in Canada the results obtained by Zheljzakov et al. (2008) revealed from 64.0 to 84.6% of linalool in coriander essential oil. Delayed sowing period decreased the content of linalool in coriander oil (Zheljzakov et al., 2008). Other dominant component of coriander essential oil were camphor, α -pinene, γ -terpinene, ρ -cymene, limonene, α -terpineol, geranyl acetate, geraniol, camphene, sabinene and myrcene. The

concentration of camphor, α -pinene, ρ -cymene, α -terpineol, geranyl acetate and myrcene was greater in seed oil from the October sowing compared to the other sowing periods in the experiment. The sowing time influences the essential oil composition of the coriander seeds

with the exception of the limonene, sabinene γ -terpinene and camphene. The year with specific climatic conditions has a strong influence on the content of this composition, with the exception of sabinene. The strongest is the impact of the year on the content of camphor.

Table 5. Essential oil composition of coriander, %

Constituents	Years of study	Sowing time						Significance	
		October	November	December	February	March	April	Year	Sowing time
α -pinene	2015-2016	6.3	5.3	5.5	5.5	4.5	4.2	*	*
	2016-2017	7.0	6.2	6.0	6.1	5.3	4.5		
	2017-2018	6.0	5.6	5.4	4.7	4.1	3.2		
ρ -cymen	2015-2016	1.4	1.4	1.1	1.3	1.0	-	*	*
	2016-2017	1.6	1.6	1.3	1.2	1.1	-		
	2017-2018	1.3	1.1	1.0	1.0	0.8	-		
α terpineol	2015-2016	0.6	0.6	0.5	0.5	-	-	*	*
	2016-2017	0.4	0.4	0.3	-	-	-		
	2017-2018	0.7	0.6	0.4	0.5	0.48	-		
Limonene	2015-2016	1.5	1.4	1.4	1.5	1.3	1.0	*	ns
	2016-2017	2.0	2.5	2.8	2.8	2.8	3.4		
	2017-2018	1.6	1.5	1.5	1.6	1.6	2.1		
Myrcene	2015-2016	1.1	1.0	0.9	0.8	0.5	0.5	*	*
	2016-2017	0.8	0.8	0.5	0.3	0.2	-		
	2017-2018	1.2	1.1	1.2	0.9	0.9	0.5		
Geranyl acetate	2015-2016	2.6	2.4	1.9	1.7	1.6	1.6	*	*
	2016-2017	1.7	1.3	1.1	-	-	-		
	2017-2018	2.6	2.5	2.0	2.1	1.3	1.2		
Sabinene	2015-2016	0.5	0.5	0.3	0.18	0.15	0.15	ns	ns
	2016-2017	0.4	0.2	0.4	0.45	0.48	0.48		
	2017-2018	0.6	0.6	0.4	0.2	0.1	0.16		
γ - terpinene	2015-2016	6.5	6.5	6.3	6.0	6.2	6.0	*	ns
	2016-2017	5.8	5.9	5.3	5.2	5.0	5.0		
	2017-2018	7.1	7.2	7.0	7.5	6.1	5.3		
Linalool	2015-2016	67.0	65.0	63.0	63.0	62.0	61.0	*	*
	2016-2017	65.0	63.0	61.0	60.0	59.0	58.0		
	2017-2018	68.0	66.0	64.0	64.0	64.0	63.0		
Camphor	2015-2016	6.9	6.6	5.8	5.1	4.5	4.1	**	*
	2016-2017	8.4	8.0	7.6	6.7	6.5	6.3		
	2017-2018	6.0	5.6	5.1	4.2	4.0	4.0		
Geraniol	2015-2016	2.0	2.1	1.9	1.7	1.5	1.4	*	*
	2016-2017	1.3	2.0	1.9	1.15	1.30	1.35		
	2017-2018	2.5	2.3	2.0	2.1	1.9	1.7		
Camphene	2015-2016	0.61	0.61	0.65	0.60	0.50	0.45	*	ns
	2016-2017	0.87	0.91	1.20	1.15	1.30	1.32		
	2017-2018	0.52	0.50	0.48	0.47	0.42	0.40		

*F-test significant at $P < 0.05$; **F-test significant at $P < 0.01$; ns - non-significant

The results of correlation analysis between the seed yield, the essential oil content and the oil yield as well as the major components of coriander essential oils, are presented in Table 7. The results show that the highest correlation

coefficients obtained from the correlation between essential oil yield and seed yield (0.963), essential oil yield and essential oil content (0.954), myrcene and seed yield (0.958), geranyl acetate and seed yield (0.953),

linalool and seed yield (0.957), essential oil yield and myrcene (0.952), essential oil yield and linalool (0.907), essential oil yield and geranyl acetate (0.926), geranyl acetate and

myrcene (0.904), linalool and myrcene (0.923) as well as between geranyl acetate and linalool (0.902).

Table 7. Values of the coefficient of correlation

	seed yield	essential oil	essent.oil yield	α -pinene	ρ -cymen	α -terpineol	Limonene	Myrcene	Geranyl acetate	Sabinene	γ -terpinene	Linalool	Camphor	Geraniol	Camphene
seed yield	1														
essential oil	0,855*	1													
essent.oil yield	0,963**	0,954**	1												
α -pinene	0,231	0,398	0,336	1											
ρ -cymen	0,415	0,649*	0,536	0,841*	1										
α -terpineol	0,810*	0,778*	0,844*	0,570	0,631	1									
Limonene	-0,737	-0,726	-0,735	0,144	-0,076	-0,404	1								
Myrcene	0,959**	0,880*	0,952**	0,312	0,494	0,814*	-0,696	1							
Geranyl acetate	0,953**	0,832*	0,926**	0,184	0,340	0,800*	-0,812	0,904**	1						
Sabinene	0,146	0,107	0,193	0,557	0,319	0,435	0,265	0,152	0,106	1					
γ -terpinene	0,850*	0,777*	0,851*	0,106	0,284	0,713*	-0,729	0,856*	0,864*	0,109	1				
Linalool	0,957**	0,784*	0,907**	0,312	0,460	0,780*	-0,637	0,923**	0,902**	0,223	0,771*	1			
Camphor	-0,130	-0,011	-0,060	0,840*	0,631*	0,299	0,510	-0,050	-0,136	0,541	-0,296	0,008	1		
Geraniol	0,819*	0,631*	0,768*	0,105	0,304	0,764*	-0,419	0,807*	0,766*	0,236	0,767*	0,757*	-0,087	1	
Camphene	-0,744	-0,556	-0,652	0,372	0,119	-0,304	0,897	-0,682	-0,760	0,379	-0,717	-0,667	0,672	-0,510	1

*significant at 5% probability level; **significant at 1% probability level

High positive correlation coefficients were obtained from the correlation between essential oil content and seed yield (0.855), α -terpineol and seed yield (0.810), γ -terpinene and seed yield (0.850), geraniol and seed yield (0.819), essential oil content and myrcene (0.879), essential oil content and geranyl acetate (0.832), γ -terpinene and essential oil yield (0.844), α -pinene and ρ -cymen (0.841), α -pinene and camphor (0.841), α -terpineol and myrcene (0.814), geranyl acetate and γ -terpinene (0.864).

CONCLUSIONS

In general, the obtained results showed that the sowing period in combination with meteorological conditions during the years of the study had a significant influence on coriander seed yield, essential oil content and oil yield, grown in the region of the town Elhovo, South-East Bulgaria.

The highest seed and essential oil yields (of 2302 kg ha⁻¹ and 26,8 kg ha⁻¹) were reported in the October sowing and the lowest one - of 1582 kg ha⁻¹ and 11.8 kg ha⁻¹ in the April sowing. The content of essential oil was lower for the later sowing periods.

The years of the experiment and sowing period affected essential oil composition such as linalool, camphor, α -pinene, ρ -cymene, α -terpineol, geranyl acetate, geraniol and myrcene.

REFERENCES

- Bhadkariya, K., Gupta, A., Bobade, A., Kasana, B., Tomar, S. (2007). Effect of different times of sowing on growth, yield and seed quality of coriander (*Coriandrum sativum*). *Indian Journals*, 22(3), 268–275.
- Carrubba, A., Torre, R., Saiano, F., Alonzo, G. (2006). Effect of sowing time on coriander performance in a semiarid Mediterranean environment. *Crop Sci.*, 46, 437–447.
- Ghobadi, M. E., & Ghobadi, M. (2012). Effects of late sowing on quality of coriander (*Coriandrum sativum*). *World Acad. Sci. Eng. Tech.*, 67, 432–435.
- Gujar, S., Warade, A., Mohariya, A., Paithankar, D. (2005). Effect of dates of sowing and nitrogen levels on growth, seed yield and quality of coriander. *Crop Res. Hisar.*, 29(2), 288–291.
- Gil, A., de la Fuente, E. B., Lenardis, A. E., López Pereira, M., Suárez, S. A., Bandoni, A., Ghersa, C. M. (2002). Coriander essential oil composition from two genotypes grown in different environmental conditions. *Journal of Agricultural and Food Chemistry*, 50(10), 2870–2877.
- Delibaltova, V., Kirchev, H., Zheliazkov, I., Yanchev, I. (2012). Influence of predecessor and sowing rate oh

- seed yield and yield components of coriander (*Coriandrum sativum*) in South-East Bulgaria. *J. Agric. Sci.*, 18, 315–319.
- Dyulgerov, N., & Dyulgerova, B. (2016). Effect of some meteorological factors on main breeding traits in coriander. *Rasteniev'dni Nauki/Bulgarian Journal of Crop Science*, 53(5/6), 60–66.
- Kassu, K., Dawit, H., Wubengeda, A., Almaz, A., Asrat, M. (2018). Yield and yield components of coriander under different sowing dates and seed rates in tropical environment. *Advances in Horticultural Science*, 32(2), 193–203.
- Marinov-Serafimov, P., I. Golubinova, Sht. Kalinova, M. Yanev, A. Ilijeva, (2017). Allelopathic activity of some parasitic weeds. *Bulgarian Journal of Agricultural Science*, 23(2), 219–226.
- Moosavi, S. (2012). Yield and yield components of *Coriandrum sativum* L. as affected sowing date and plant density. *Technical Journal of Engineering and Applied Sciences*, 2(4), 88–92.
- Moosavi, S.G.R., Seghatoleslami, M.J., & Zareie, M.H. (2012). The effect of planting date and plant density on morphological traits and essential oil yield of coriander (*Coriandrum sativum* L.). *International Journal of Agriculture and Crop Sciences (IJACS)*, 4(8), 496–501.
- Nowak, J., Szemplinski, W. (2014). Influence of sowing date on yield and fruit quality of coriander (*Coriandrum sativum* L.). *Acta Sci. Pol., Hortorum Cultus*, 13(2), 83–96.
- Özel, A., Güler, İ., & Erden, K. (2009). Effect of different sowing time on yield and some plant characters of Coriander (*Coriandrum sativum* L.) under the Harran Plain condition. *Journal of the Faculty of Agriculture of Harran University (Turkey)*.
- Tanchev, D. (2011). Investigation on the sowing date and sowing norm of the coriander in condition Strandhza region. *Rasteniev'dni Nauki*, 48(4), 351–354.
- Sharangi, A. B., Roychowdhury, A. (2014). Phenology and yield of coriander as influenced by sowing dates and irrigation. *The Bioscan*, 9(4), 1513–1520.
- Zheljazkov, V., Pickett, K., Caldwell, C. (2008). Cultivar and sowing date effects on seed yield and oil composition of coriander in Atlantic Canada. *Industrial crops and products*, 28, 88–94
- Zawiślak, G. (2011). The chemical composition of essential oil from the fruit of coriander (*Coriandrum sativum* L.). *Annal Univers Mariae Curie-Skłodowska Sect DDD: Pharm*, 24, 169–175.
- Yildirim, B., Gok, N. (2012). Effect of sowing date and varieties on essential oil ratio and essential oil components of coriander (*Coriandrum sativum* L.) in Van ecological condition. *Journal of Animal and Veterinary Advances*, 11(11), 1925–1929.

BIOLOGICAL EFFICACY OF HERBICIDES AND HERBICIDE COMBINATIONS APPLIED TO CORN

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Abstract

*In the period 2018-2019, the biological efficacy of new herbicides and herbicide combinations for maize was studied in the experimental field of Agricultural University, Plovdiv. The experiment was performed using the block method with DuPont Pioneer hybrid P0216 and included preparations applied in phase 5-6 leaf of the crop. In both experimental years, annual late-spring dicotyledonous species predominate - *Amaranthus retroflexus* L., *Chenopodium album* L., *Solanum nigrum* L., *Xanthium strumarium* L., *Datura stramonium* L., *Abutilon theophrasti* Medic., *Portulaca oleracea* L. From the perennial weeds 2 species are common - *Sorghum halepense* (L.) Pers and *Convolvulus arvensis* L. The best control of weeding was observed when using the combination of *Victus OD* - 1250 ml/ha + *Cabadox Extra* - 300 ml/ha. These results correlate both with the yields obtained from the crop and the physical characteristics of the grain.*

Key words: weeds, efficacy, herbicides, *Zea mays* L, yield.

INTRODUCTION

Corn (*Zea mays* L.) is one of the most important food and feed crops in the world. Over the last few years, there has been a steady tendency to an increase of the planted areas in Bulgaria, expanding from 328 000 ha in 2010 to 550000 ha in 2019 (Agricultural Reports 2010-2019, website of the Ministry of Agriculture and Food in the Republic of Bulgaria).

The main harmful factor for obtaining a high and quality yield from the crops are the weeds. (Tonev et al., 2019; Dimitrova et al., 2018; Zhelyazkov I., 2007). Agricultural practice has shown that the grain yield could decline between 70 and 90% in highly weeding maize fields (Mahana et al., 2014; Troyer, 2009). It has been proven that weeds not only reduce the yield but also adversely affect the quality of the grain, which makes combating them effective and economically viable (Koprivlenski et al., 2015; Dimitrova, et al., 2013a; Dimitrova, et al., 2013b; Haall et al., 1992).

The aim of the present study was to follow out the biological efficacy of new herbicides and herbicide combinations in maize and the

effect of herbicides on physical characteristics of the grain.

MATERIALS AND METHODS

In 2018 and 2019 in the Training-and-Experimental Fields of the Agricultural University in Plovdiv, precise field trials were carried out with maize hybrids P0216. The plants were grown at planting densities 70000 plants per hectare. The experiments were set by the split-plot design method with a perpendicular location of the factors. Drip irrigation was provided in the experimental field during the trial years.

The soil in the experimental field of the Agricultural University - Plovdiv has been determined as alluvium, which based on the international classification of FAO belongs to the category of Mollic Fluvisols. It is characterized by average sandy-clay mechanical composition, not high humus content of 1.01-1.32%, a weak alkaline reaction of the soil (pH 7.6-7.9) (Popova et al., 2012).

Variants of the trial:

1. Untreated control - (K₁);

2. Industrial control – untreated area with 1-2 hoeing (K₂);
3. Victus OD - 1250 ml/ha + Derby Super - 330 g/ha;
4. Victus OD - 1250 ml/ha + Starane Gold - 1200 ml/ha;
5. Victus OD - 1250 ml/ha + Kabadex Extra - 300 ml/ha;
6. Arigo WG - 330 g/ha + 0.1% Trend;
7. Principal Plus - 440 g/ha + 0.1% Trend;
8. Elumis OD - 1600 ml/ha.

Plant Material

Seeds of the maize hybrid for forage production of Pioneer Company were used.

According to the length of their vegetation period the hybrid P 0216 - according to FAO 480. Stay green type with open leaves wrapping the cob. It is recommended for the production of biogas. It can also be used for silage. It has an excellent yield potential.

(www.pioneer.com)

Agro-technical practices

The experimental field was fertilized with a nitrogen fertilizer at the rate of 240 kg/ha NH₄NO₃, applied at the stage of 5th-6th leaf of the crop. During the vegetation, treatment was carried out at the 4th-6th leaf stage of the crop with different herbicides.

Selectivity of the herbicide preparations to the crop was reported following EWRS (*European Weed Research Society*) scale: from score 1 - no damage to the crop plants, to score 9 - the crop is completely destroyed.

Foliar herbicides were applied by a back-sack sprayer with spray solution 300 l/ha.

The agro-technical measures were carried out according to the generally accepted technology for maize growing (soil cultivation, fertilization, sowing, rolling).

Data analysis

The data were processed statistically by method ANOVA to determine the significance of the differences between the tested variants.

RESULTS AND DISCUSSIONS

In both experimental years, the growing season was warm, with temperatures around and above average over a long period of time (Figures 1 and 2). In terms of temperature, the year 2018 was warmer compared to the second year of the

experiment. There is a substantial difference in the average monthly temperatures in years during the initial period of the growth of the crop in April and May, in 2018 the temperatures were higher than those registered in 2019. Regarding the rest of the vegetation period, the values of this indicator in the different months are quite similar and exceed the average values for the period of many years (Figure 1).

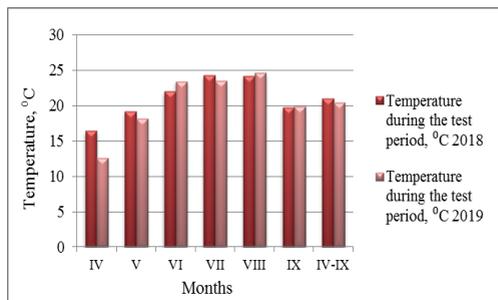


Figure 1. Temperature during the test period, °C

Despite the large quantity of the rainfall in May and June 2018 (112.3 and 118.9 mm, respectively), it is extremely unevenly distributed, which necessitated conducting the first irrigation of the crop with a norm of 40 m³. The year 2019 was characterized by the high values of the rainfall, which during the vegetation period of the crop exceeded the threefold average values for the period of many years (Figure 2).

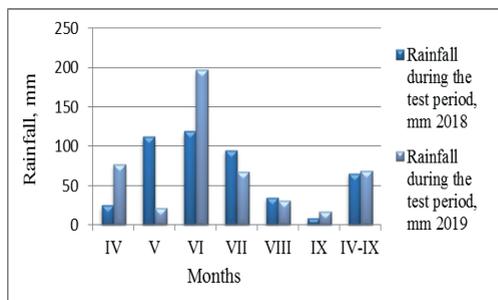


Figure 2. Rainfall during the test period, mm

In the period 2018-2019, the experimental areas were heavily infested by weeds from the group of the annual late-spring species. Major representatives of that group were: *Solanum nigrum* L., *Xanthium strumarium* L., *Chenopodium album* L., *Amaranthus retroflexus* L., *Portulaca oleraceae* L., *Datura stramonium* L., *Setaria* spp., *Echinochloa crus-*

galli L. The perennial weed species found were: *Sorghum halepense* Scop. and *Convolvulus arvensis* L. Those weed species are typical for the maize crop in the region (Mitkov et al., 2018; Dimitrova et al., 2013; Zhelyazkov, 2007).

The obtained results on the efficacy of the tested herbicides during the years of the experiment are shown in Tables 1 and 2.

On the 20th day of the first year of the experiment, after applying the respective

treatment (05.06.2018), the number of the weeds in the untreated control sample reached 108.0 weeds/m², of which 84.3 weeds are annual and 23.7 weeds are perennial (*Sorghum halepense* Scop. and *Convolvulus arvensis* L.). The highest was the density of *Solanum nigrum* L. - 39.6 weeds/m², *Xantium strumarium* L. - 14.2 weeds/m², *Chenopodium album* L. - 8.4 weeds/m², *Amaranthus retroflexus* L. and *Setaria* spp. - 6.4 weeds/m².

Table 1. Weed density on the 20th days after treatment, 2018

Variants	Weed density on the 20 th days after treatment (05.06.2018), number/m ²												
	Annual									Total annual	Perennial		Total perennial
	<i>Amaranthus retroflexus</i>	<i>Solanum nigrum</i>	<i>Datura stramonium</i>	<i>Setaria</i> spp.	<i>Abutilon theophrasti</i>	<i>Xantium strumarium</i>	<i>Chenopodium album</i>	<i>Portulaca oleracea</i>	<i>Sorghum halepense</i>		<i>Convolvulus arvensis</i>		
1. Untreated control	6.4	39.6	2.7	6.5	2.9	14.2	8.4	3.6	84.3	18.3	5.4	23.7	
2. Industrial control – untreated area with 1-2 hoeing (K ₂)	1.2	11.3	0.7	1.2	0.6	2.8	1.7	1.5	21.0	4.1	1.8	5.9	
3. Victus OD – 1250 ml/ha + Derby Super – 330 g/ha	0.2	0.4	0.2	0.2	0.6	0.8	0	0.4	2.8	0.5	0.8	1.3	
4. Victus OD – 1250 ml/ha + Starane Gold – 1200 ml/ha	0.1	0.3	0.2	0.2	0.2	0.5	0.4	0.3	2.2	0.5	0	0.5	
5. Victus OD – 1250 ml/ha + Kabadex Extra–300 ml/ha	0.1	0.3	0	0	0	0.2	0	0.4	1.0	0.4	0.2	0.6	
6. Arigo WG – 330 g/ha + 0,1% Trend	0	0.4	0	0.1	0	0.9	0	2.3	3.7	0.7	1.8	2.5	
7. Principal Plus – 440 g/ha + 0,1% Trend	0	2.5	0.2	0	0	0.8	0	0.4	3.9	0.2	0.4	0.6	
8. Elumis OD – 1600 ml/ha	0.1	0.4	0	0.6	0.8	1.1	0	1.7	4.7	0.2	4.0	4.2	

The data on Table 1 show the high efficacy of the applied herbicides and combinations, which compared to the overall weed coverage ranges from 98.5% (var. 5. Victus OD - 1250 ml/ha + Kabadex Extra - 300 ml/ha) to 91.7% (var. 8 Elumis OD - 1600 ml/ha). The weaker effect of the combination in variant 8 is primarily due to the weaker control of the type *Xantium strumarium* L. - 89% compared to the untreated control sample and *Convolvulus arvensis* L. - 74%. Table 1 shows the effect of the preparations on the separate weed types. These are foliar herbicides and this allows us to apply the herbicides having the highest level of efficacy in a differentiated manner, after we have determined the type composition to be found among the crops. On the 40th day after spraying (25.06.2018), the number of the weeds in the untreated control sample reached 139.6

weeds/m², as the density of the annual types is 108.0 weeds/m².

The farm control sample was dug only once, which led to a reduction of almost 2.5 times in the level of weed coverage.

The governing laws that we found during the first reporting on the efficacy of the applied herbicides and combinations were preserved. Considering the overall level of weed coverage, it is the highest in variant 5 (Victus OD - 1250 ml/ha + Kabadex Extra - 300 ml/ha - 97.2% and variant 4 (Victus OD - 1250 ml/ha + Starane Gold - 1200 ml/ha) - 95%.

The large quantity of the rainfall in June and July 2018, 118.9 and 94.7 mm, respectively, which exceeded the norm during this period 2.7 times, was the reason for the secondary weed coverage, which emerged after the 40th day of the treatment using herbicides.

During the second year of the experiment, there is lower density of the weeds compared to the first year but the type composition is the same as in the year 2018.

On the 20th day after treatment (06.06.2019), the number of the weeds in the untreated control sample reached 104.2 weeds/m², of which 80.7 weeds are annual and 23.5 weeds are perennial (*Sorghum halepense* Scop. and *Convolvulus arvensis* L.).

The highest was the density of the European black nightshade *Solanum nigrum* L. - 45.0 weeds/m², *Xanthium strumarium* L. - 15.3, *Chenopodium album* L. and *Setaria* spp., 5.6-5.3 weeds/m² (table 2).

The data show the high efficacy of the applied herbicides and combinations like the previous year of the experiment.

Considering the overall weed coverage, it ranges from 97.7% in variant 5 to 92% in variant 8

(Elumis OD), which is due to its weaker effect on *Xanthium strumarium* L.

On the 40th day after spraying (26.06.2019), the number of the weeds in the untreated control sample reached 128.7 weeds/m² and the density of the annual types was 95.9 weeds/m².

The farm control sample was dug only once, which led to a decrease of the level of weed coverage by almost 2.6 times. The governing laws established during the first reporting on the efficacy of the applied herbicides and combinations are preserved.

The quantity of the rainfall in June and July 2019, 196.7 and 67.5 mm, respectively, as well as the high summer temperatures, are the reason for the substantial secondary weed coverage which emerged after the 40th day of the treatment using herbicides.

Table 2. Weed density on the 20th days after treatment, 2019

Variants	Weed density on the 20 th days after treatment (06.06.2019), number/m ²											
	Annual								Total annual	Perennial		Total perennial
	<i>Amaranthus retroflexus</i>	<i>Solanum nigrum</i>	<i>Datura stramonium</i>	<i>Setaria viridis</i>	<i>Abutilon theophrasti</i>	<i>Xanthium strumarium</i>	<i>Chenopodium album</i>	<i>Portulaca oleracea</i>		<i>Sorghum halepense</i>	<i>Convolvulus arvensis</i>	
1. Untreated control	4.0	45.0	1.4	5.3	1.9	15.3	5.6	2.2	80.7	19.3	4.2	23.5
2. Industrial control – untreated area with 1-2 hoeing (K ₂)	0.9	10.1	0.4	0.8	0.4	6.1	2.2	0.9	21.8	7.8	1.4	9.2
3. Victus OD – 1250 ml/ha + Derby Super – 330 g/ha	0.1	0.8	0	0.3	0.1	1.4	0	0.1	2.8	0.8	1.0	1.8
4. Victus OD – 1250 ml/ha + Starane Gold– 1200 ml/ha	0.1	0.5	0.1	0	0	1.5	0.1	0.1	2.4	0.9	0.6	1.5
5. Victus OD – 1250 ml/ha + Kabadex Extra– 300 ml/ha	0	0.4	0	0	0	0.5	0	0.1	1.0	0.9	0.5	1.4
6. Arigo WG – 330 g/ha + 0,1% Trend	0	0.5	0	0.2	0	0.9	0	0.6	2.2	1.0	1.4	2.4
7. Principal Plus – 440 g/ha + 0,1% Trend	0.1	3.0	0.1	0.2	0.3	1.0	0	0.2	4.9	0.3	0.5	0.9
8. Elumis OD – 1600 ml/ha	0.1	0.8	0.1	0.6	0.7	1.5	0.1	1.0	4.9	0.4	3.5	3.9

Tables 3, 4 and 5 show the results from the conducted dispersion analysis of the obtained data on the yield and the physical characteristics of the grain - hectoliter weight and the weight of 1000 grains, under the influence of the studied herbicides applied on maize hybrid P0216. The grain yield of maize plants is presented in Table 3, on average for the two years of the study. The

yield was harvested at 12% grain moisture content.

An assessment was made of the proven differences of the data during the two years of the experiment regarding the untreated control sample. The results related to the three studied signs show that the highest values were registered in variant 5 (Victus OD - 1250 ml/ha + Kabadex extra - 300 ml/ha) and variant 4

(Victus OD - 1250 ml/ha + Starane Gold - 1200 ml/ha. The gradation of the variants with reference to the 3 signs is relatively identical. The differences have been proven to be significant with levels of significance $P_{1\%}$ and $P_{0.1\%}$.

Regarding the yield, even in the farm control sample a higher value was registered (though on the lowest level $P_{5\%}$). With reference to the hectoliter weight and weight of 1000 grains, the values of the farm control sample are at the same level as those of the untreated control sample.

Table 3. Grain yield of corn (kg/ha)

Variants	\bar{X} kg/ha	(D)	Significance
5	15459.0	8692.0	+++
4	14908.0	8141.0	+++
3	14720.0	7953.0	+++
6	14511.0	7744.0	+++
7	14482.5	7715.5	+++
8	14179.0	7412.0	+++
2	8228.5	1461.5	+
1 K ₁	6767.0		

Table 4. Hectoliter weight (kg/hl)

Variants	\bar{X} (kg/hl)	(D)	Significance
5	77.20	3.80	+++
4	76.95	3.55	+++
7	76.60	3.20	+++
6	76.40	3.00	+++
3	76.40	3.00	+++
8	76.15	2.75	+++
2	73.90	0.50	Ns
1 K ₁	73.40		

Table 5. Weight of 1000 grains (g)

Variants	\bar{X} (g)	(D)	Significance
5	355.40	101.50	++
4	353.45	99.55	++
3	352.60	98.70	++
6	352.50	98.60	++
7	352.50	98.60	++
8	352.00	98.10	++
2	272.84	18.94	ns
1 K ₁	253.90		

CONCLUSIONS

In the period 2018-2019, the experimental areas were heavily infested by weeds from the group of the annual late-spring species. Major representatives of that group were: *Solanum nigrum* L., *Xanthium strumarium* L., *Chenopodium album* L., *Amaranthus retroflexus* L., *Portulaca oleraceae* L., *Datura stramonium* L., *Setaria* spp., *Echinochloa crus-galli* L. The perennial weed species found are: *Sorghum halepense* Scop. and *Convolvulus arvensis* L.

The highest density was registered among the types *Solanum nigrum* L., *Xanthium strumarium* L., *Chenopodium album* L., *Setaria* spp. and *Sorghum halepense* Scop.

The large quantity of the rainfall in June and July, which exceed 2-3 times the norm for this period, is the main reason for the emergence and the high density of the secondary weed coverage among the crops.

During the two years of the experiment, on the 20th and the 40th days after spraying, the highest density of overall weed coverage was registered in var. 5 - 97% (Victus OD - 1250 ml/ha + Kabadex extra – 300 ml/ha) and var. 4 - 95% (Victus OD - 1250 ml/ha + Starane Gold - 1200 ml/ha).

The yield in all variants treated with herbicides exceed the zero control sample (6767 kg/ha) 2.1 up to 2.3 times. Their average values for the period range from 14179.0 kg/ha (var. 8) to 15459.0 kg/ha (var. 5).

The physical indicators of the grain also manifest higher values compared to the untreated control sample. The weight of 1000 grains is 34-40% larger. The hectoliter weight ranges from 76.15 to 77.2 kg/hl, exceeding the control sample by 3.7% to 5.2%.

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REFERENCES

- Dimitrova M., Dimitrov Y., Palagacheva N., Vitanova M., Minev N., Yordanova, N. (2018). Maize – weeds, diseases and pests. Fertilization. *Videnov & son*, Sofia (Book in Bulgarian).

- Dimitrova, M., Dimova, D., Zhalnov, Iv., Zovorski, P., Zhelyazkov, I., Valcheva, E., Popova, R. (2013a). The influence of new herbicides on the growth and the some structural elements of the yield of fodder maize. *Scientific Papers. Series A. Agronomy, LVI*, 226–230.
- Dimitrova, M., Zhalnov I., Zhelyazkov, I., Stoychev, D. (2013b). Efficiency and selectivity of new herbicides on fodder maize. *Agrolife Scientific Journal*, 2(1), 47–50.
- Haall, M.R. et al., 1992. The critical period of weed control in grain corn (*Zea mays* L.). *Weed Science*, 40, 441–447.
- Koprivlenski, V., Dimitrova, M., Zhalnov (2015). Economic evaluation of new herbicides for weed control in maize grain. *Bulgarian Journal of Agricultural Science*, 21(2), 315–319.
- List of authorized plant protection products for sale and use, registered fertilizers, soil improvers and nutrient media. Ministry of Agriculture and Food (2018-2019). *Vidinov & son*, Sofia.
- Mahana, B., Seglar, B., Owens, F., Dennis, S., Newell, R. (2014). Silage Zone Manual. *Du Pont Pioneer. Johnston, IA* In: Johnston, I. A. (Ed.).
- Mitkov, A., Yanev, M., Neshev, N., Tonev, T. (2018). Biological efficacy of some soil herbicides at maize (*Zea mays* L.). *Scientific Papers. Series A. Agronomy, LXI*(1), 340–346.
- Popova, R., Zhalnov, I., Valcheva, E., Zorovski, P., Dimitrova, M. (2012). Estimates of environmental conditions of soils in Plovdiv Region in applying the new herbicides for Weed control in major field crops. *JCEA*, 13(3), 595–600.
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Zhelyazkov, I., Vasilev, A., Tityanov, M., Mitkov, A., Yanev, M. (2019). *Herbology. Vidinov & son*. ISBN: 978-954-8319-75-1 (Text book in Bulgarian).
- Troyer, A. (2009). Development of hybrid corn and the seed corn industry. in: Bennetzen J. L. and S. Hake, (Eds.), *Maize Handbook: Genetics and Genomics, Vol. II*. (87-95). Springer, New York.
- Zhelyazkov, I. (2007). Integration of new and classical methods against heavy weed infestation in some field crops. *PhD Thesis*, Plovdiv, Bulgaria.
- *** www.pioneer.com

INHERITANCE, HETEROSIS AND COMBINING ABILITY IN F₁ INTRASPECIFIC *G. hirsutum* L. COTTON CROSSES

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Abstract

Line × tester analysis of 5 Bulgarian cotton varieties used for mothers and 3 foreign ones as fathers was applied to study the inheritance of the most important economic traits and the breeding value of parental forms (G. hirsutum L.) and F₁ hybrids. The trial was conducted in 2013-2014 by block method in three replicates. Different types of inheritance were observed by the productivity, boll weight and length and lint percentage of the fibre. Exhibition of heterosis was most pronounced by productivity reaching 39.1%. For all studied traits the genetic variance was mainly of non-additive type and the prognosis for selection in early segregate generations could not be good. The varieties of Bulgarian selection, Boyana and IPK Nelina had high GCA for productivity and fibre lint percentage. Their high GCA was combined with low SCA variances making them suitable for the synthetic selection. Of the foreign varieties, the Turkish Nazilli 954 had high GCA for the fibre lint percentage, the Greek Corina - for the fibre length. Because of the high SCA variances they appeared to be more suitable for the heterosis selection.

Key words: *G. hirsutum* L., genetic control, productivity, fibre properties.

INTRODUCTION

The method of intraspecific hybridization in the selection of cotton in Bulgaria is basic and continues to be so. Local varieties, which are early and with reduced heat-loving, are crossed mainly with foreign ones which are later mature, but have high fiber quality and high fiber lint percentage, and some of them are resistant to the most dangerous and economically important disease in cotton - Verticillian wilt (*Verticillium dahliae* Kleb.).

The main varieties for cotton production in Bulgaria, characterized by high earliness and high yield potential, were obtained by intraspecific hybridization within *G. hirsutum* L. species (Bojinov and Dimitrova, 1981a; 1981b; 1989; Bojinov et al., 1996). The newer and latest varieties - Beli Iskar, Beli Lom, IPTP Veno, Boyana and Denitsa, obtained by this method, are a step forward in the selection for increasing the productivity and lint percentage of cotton fiber (Bozhinov and Bojinov, 2004; Bozhinov and Bojinov, 2008; Valkova and Bojinov, 2010; Valkova, 2014).

The success of each breeding program depends to a large extent on the right selection of the parental forms for crossing and their combining ability.

The aim of this research was to study the inheritance and breeding value of parental forms by economically most important traits in F₁ intraspecific (*G. hirsutum* L.) crosses of Bulgarian and foreign varieties, with a view to their effective usage in breeding programs with cotton.

MATERIALS AND METHODS

15 F₁ intraspecific crosses (*G. hirsutum* L.) of 5 Bulgarian varieties - Chirpan-539, Helius, Rumi, Boyana and IPK Nelina, used for mothers, with 3 foreign ones - Nazilli 954 (Turkish), Mytra and Corina (Greek), used as fathers, were studied. The trial was carried out in the experimental field of the Field Crops Institute in town of Chirpan, in 2013-2014, on leached soil type, set by block method design, in three replicates. Each F₁ hybrid combination and the parental varieties were sown in 2 rows, 3 m long, randomized, with a 60 × 20 × 1 sowing scheme. 10 plants of each replicate were observed. There were studied the following traits: productivity/plant; boll weight; fiber length determined by the "butterfly" method and fiber lint percentage. Productivity per plant and boll weight data are given for one year (2014), for fiber length and fiber lint

percentage - for two years (2013-2014) and are presented by years.

Crossing (mothers × fathers) and combining ability analysis (general combining ability - GCA and specific combining ability - SCA) are given by the method of Savchenko (1984). The sum of the squares of the crosses was divided into variation due to mothers, variation due to fathers and variation due to mothers × fathers interaction. The main effects of mothers and fathers are equivalent to the GCA, and of the mothers × fathers interaction represents SCA.

The years of the study were characterized as follows: in terms of temperature security 2013 was warm, 2014 was average; in terms of rainfall 2013 was moderately dry and 2014 was moderately wet.

RESULTS AND DISCUSSION

The inheritance of productivity/plant determined by the “d/a” indicator in 6 of the 15 crosses studied was positively over-dominance, which caused heterosis up to 39.1% and productivity/plant reached 40.3-50.2 g (Table 1). Only one cross showed negative over-dominance. At other six crosses the inheritance was completely and incompletely dominant to the parent with the higher or lower productivity and additively at one cross. The genotype of the parent varieties (mothers and fathers) and their combining ability were important for the inheritance of productivity/plant.

The crosses with the Turkish variety Nazilli 954 inherited the boll weight with positive

Table 1. Inheritance and heterosis by productivity/plant and boll weight in the F₁ hybrids obtained from the crosses of 5 Bulgarian cotton varieties with 1 Turkish and 2 Greek varieties in 2014

Hybrid combination	P ₁	P ₂	F ₁	d/a	HP %
Productivity/plant, g					
Chirpan-539×Nazilli 954	30.5	35.1	34.3	0.65	97.7
Chirpan-539 × Mytra	30.5	36.9	31.5	-0.69	85.4
Chirpan-539 × Corina	30.5	36.1	32.5	-0.29	90.0
Helius × Nazilli 954	29.9	35.1	35.6	1.19	101.4
Helius × Mytra	29.9	36.9	35.2	0.51	95.4
Helius × Corina	29.9	36.1	40.3	2.35	111.6
Rumi × Nazilli 954	31.5	35.1	22.9	-5.78	65.2
Rumi × Mytra	31.5	36.9	33.0	-0.44	89.4
Rumi × Corina	31.5	36.1	41.2	3.22	114.1
Boyana × Nazilli 954	33.0	35.1	34.0	-0.05	96.9
Boyana × Mytra	33.0	36.9	44.4	4.85	120.3
Boyana × Corina	33.0	36.1	39.7	3.32	110.0
IPK Nelina × Nazilli 954	30.2	35.1	31.4	-0.51	89.5
IPK Nelina × Mytra	30.2	36.9	45.1	3.45	122.2
IPK Nelina × Corina	30.2	36.1	50.2	5.78	139.1
GD 5.0%; 1.0%; 0.1%	3.6; 4.8; 6.3				
Boll weight, g					
Chirpan-539×Nazilli 954	5.3	5.7	5.9	2.00	103.5
Chirpan-539 × Mytra	5.3	5.8	5.7	0.60	98.3
Chirpan-539 × Corina	5.3	6.0	5.8	0.43	96.7
Helius × Nazilli 954	5.4	5.7	5.9	2.33	103.5
Helius × Mytra	5.4	5.8	5.7	0.50	98.3
Helius × Corina	5.4	6.0	5.6	0.33	93.3
Rumi × Nazilli 954	5.5	5.7	5.8	2.00	101.8
Rumi × Mytra	5.5	5.8	5.6	-0.33	96.6
Rumi × Corina	5.5	6.0	5.6	-0.60	93.3
Boyana × Nazilli 954	5.4	5.7	5.8	1.67	101.8
Boyana × Mytra	5.4	5.8	5.7	0.50	98.3
Boyana × Corina	5.4	6.0	5.9	0.67	98.3
IPK Nelina × Nazilli 954	5.2	5.7	5.9	1.80	103.5
IPK Nelina × Mytra	5.2	5.8	5.7	0.67	98.3
IPK Nelina × Corina	5.2	6.0	5.8	0.50	96.7
GD 5.0%; 1.0%; 0.1%	0.6; 0.7; 0.9				

over-dominance and slightly manifested heterosis effect of 1.8-3.5%. Eight crosses showed incomplete dominance of the parent with the higher value.

The crosses of Rumi variety with the Greek varieties Mytra and Corina showed incomplete dominance towards the parent with the lower value.

The inheritance of the fiber lint percentage at the crosses with the Turkish variety Nazilli 954 during the first year of the study was partially dominant towards the parent with the lower value to negative over-dominance (Table 2). An exception was the cross IPK Nelina × Nazilli 954 in which the inheritance of the trait was incompletely dominant to the parent with the higher value. At the crosses with the Greek varieties Mytra and Corina, the inheritance of this trait was positively over-dominance, except the crosses of Rumi, in which the inheritance

was negatively over-dominance. In the second year of the study, positive over-dominance was found only at the Chirpan-539 × Corina cross, negative over-dominance was observed in four crosses, and in the others the inheritance was incompletely dominant to the parent with the lower or higher value.

Averaged data for two years showed that three crosses (with Corina variety) inherited the fiber lint percentage with positive over-dominance, while other three crosses showed negative over-dominance. The inheritance of this trait was complete and incomplete dominant to the parent with the higher value in 4 crosses, to the parent with the lower value - in 2 crosses and additively - in 3 crosses. The genotype of paternal forms had great influence on the inheritance of the trait also relevant was the genotype of the maternal components and their combining ability.

Table 2. Inheritance and heterosis by fiber lint percentage in F₁ hybrids obtained from the crosses of 5 Bulgarian cotton varieties with 1 Turkish and 2 Greek varieties in 2013-2014

Hybrid combination	2013					2014					Average	
	P ₁	P ₂	F ₁	d/a	HP%	P ₁	P ₂	F ₁	d/a	HP%	F ₁	HP%
Chirpan-539×Nazilli954	40.1	42.2	39.8	-1.29	94.3	41.1	43.6	41.8	-0.46	95.7	41.3	96.3
Chirpan-539 × Mytra	40.1	39.3	41.1	3.50	102.5	41.1	43.7	42.7	0.28	97.9	41.5	100.0
Chirpan-539 × Corina	40.1	39.3	41.1	3.50	102.5	41.1	38.4	43.0	2.41	104.6	42.1	103.7
Helius × Nazilli 954	37.7	42.2	39.5	-0.20	93.6	39.9	43.6	37.6	-2.24	86.2	38.6	90.0
Helius × Mytra	40.1	39.3	41.1	3.50	102.5	39.9	43.7	40.9	-0.47	93.6	41.0	98.8
Helius × Corina	40.1	39.3	41.1	3.50	102.5	39.9	38.4	39.5	0.47	99.0	40.3	103.6
Rumi × Nazilli 954	37.7	42.2	39.5	-0.20	93.6	43.0	43.6	41.4	-6.33	95.0	40.5	94.4
Rumi × Mytra	38.3	39.3	37.2	-3.20	94.7	43.0	43.7	40.7	-7.57	93.1	39.0	94.0
Rumi × Corina	38.3	39.3	38.2	-1.20	97.2	43.0	38.4	40.1	-0.26	93.3	39.2	97.0
Boyana × Nazilli 954	39.6	42.2	40.8	-0.08	96.7	42.5	43.6	42.8	-0.45	99.1	42.0	97.9
Boyana × Mytra	39.6	39.3	39.3	-1.00	99.2	42.5	43.7	42.1	-1.73	94.1	40.2	96.9
Boyana × Corina	39.6	39.3	40.1	4.33	101.3	42.5	38.4	39.6	-0.41	93.2	39.9	97.1
IPK Nelina×Nazilli 954	38.6	42.2	41.2	0.44	97.6	41.9	43.6	43.5	0.88	99.8	42.4	98.8
IPK Nelina × Mytra	38.6	39.3	39.6	1.86	100.8	41.9	43.7	43.0	0.22	97.4	41.3	99.5
IPK Nelina × Corina	38.6	39.3	39.5	1.57	100.5	41.9	38.4	41.7	0.89	99.5	40.6	100.7
GD 5.0%; 1.0%; 0.1%	0.9; 1.2; 1.6					1.1; 1.4; 1.9						

There was also diversity in the type of inheritance of the fiber length (Table 3). At the crosses with the Turkish variety Nazilli 954 as father, the inheritance of this trait was from partial dominance of the longer fiber of this variety to positive over-dominance. An exception was the cross IPK Nelina × Nazilli 954, in which the inheritance of the fiber length was positively over-dominance in the first year and negatively over-dominance in the second

year of the study. At the crosses with fathers the Greek varieties Mytra and Corina, inheritance was from partial dominance of the longer fibers of these varieties to positive over-dominance, but negative over-dominance was also observed. The genotypes of mothers and fathers were important for inheritance of this trait. Environmental conditions (years of testing) also affected its inheritance.

Table 3. Inheritance and heterosis by fiber length in F₁ hybrids obtained from the crosses of 5 Bulgarian cotton varieties with 1 Turkish and 2 Greek cotton varieties in 2013-2014

Hybrid combination	2013					2014					Average	
	P ₁	P ₂	F ₁	d/a	*HP	P ₁	P ₂	F ₁	d/a	*HP	F ₁	*HP
Chirpan539×Nazilli954	27.0	27.4	27.5	1.50	100.4	26.1	27.0	26.6	0.11	98.5	27.1	99.6
Chirpan-539 × Mytra	27.0	27.5	27.3	0.20	99.3	26.1	27.8	27.1	0.18	97.5	27.2	98.2
Chirpan-539 × Corina	27.0	28.4	28.2	0.71	99.3	26.1	26.6	27.7	5.40	104.1	28.0	101.8
Helius × Nazilli 954	27.5	27.4	27.5	1.00	100.0	25.3	27.0	27.1	1.12	100.4	27.3	100.4
Helius × Mytra	27.5	27.5	28.1	-	102.2	25.3	27.8	26.3	-0.20	94.6	27.2	98.2
Helius × Corina	27.5	28.4	27.3	-1.44	96.1	25.3	26.6	26.0	0.08	97.7	26.7	97.1
Rumi × Nazilli 954	27.4	27.4	27.3	-	99.6	26.6	27.0	27.9	5.50	103.3	27.6	101.5
Rumi × Mytra	27.4	27.5	27.6	3.00	100.4	26.6	27.8	27.0	-0.33	97.1	27.3	98.6
Rumi × Corina	27.4	28.4	26.8	-2.20	94.4	26.6	26.6	26.8	-	100.8	26.7	97.1
Boyana × Nazilli 954	26.7	27.4	28.2	3.29	102.9	26.1	27.0	26.9	0.78	99.6	27.6	101.5
Boyana × Mytra	26.7	27.5	27.5	1.00	100.0	26.1	27.8	27.2	0.29	97.8	27.4	98.9
Boyana × Corina	26.7	28.4	28.5	1.12	100.4	26.1	26.6	27.0	2.60	101.5	27.8	101.1
IPK Nelina×Nazilli 954	27.5	27.4	28.2	15.00	102.5	27.1	27.0	25.8	-25.0	95.2	27.0	98.9
IPK Nelina × Mytra	27.5	27.5	28.3	-	102.9	27.1	27.8	26.2	-3.57	94.2	27.3	98.6
IPK Nelina × Corina	27.5	28.4	28.0	0.11	98.6	27.1	26.6	27.7	3.40	102.2	27.9	101.5
GD 5.0%; 1.0%; 0.1%	<i>1.1; 1.5; 1.9</i>					<i>0.8; 1.1; 1.4</i>						

There were significant GCA and SCA effects for productivity/plant (Table 4). The involvement of σ^2_{GCA} and σ^2_{SCA} in the genetic variance revealed that non-additive gene effects were more important for the inheritance of productivity/plant. The results obtained were similar to those reported by Senthilkumar et al. (2010), Karademir et al. (2016), Sajjad et al. (2016), Memon et al. (2017), Sivia et al. (2017), who also found predominance of non-additive gene effects for raw cotton yield using the line × tester method.

The GCA and SCA for the fiber lint percentage were significant in both years of the study and as for the fiber length the GCA effects only of mothers and SCA were significant only in 2014.

The sum of the squares of the SCA effects for the fiber lint percentage was 49.7% in 2013 and 44.7% in 2014 of the total variation indicating that additive and non-additive gene effects were equally important for the inheritance of this trait. The $\sigma^2_{GCA}/\sigma^2_{SCA}$ ratio revealed that non-additive gene effects were more important for the inheritance of the fiber lint percentage.

The sum of squares of the SCA effects for the fiber length was 71.1% in 2014, revealing that the main component of the genetic variance was of the non-additive type. The calculated components of the genetic variances and their ratio $\sigma^2_{GCA}/\sigma^2_{SCA}$ confirmed the greater importance of the non-additive gene effects in the inheritance of this trait.

Significant positive GCA for the productivity/plant was found, of the mothers for Boyana variety, with the highest productivity under the conditions of 2014, and IPK Nelina variety, and of the fathers for Mytra and Korina varieties, which had higher productivity than the Turkish variety Nazilli 954. Chirpan-539 and Rumi varieties of the mothers and Nazilli 954 variety of the fathers had negative GCA (Table 5). Helius variety had a positive but non-significant GCA for productivity/plant. Significant positive SCA effects were found for 6 of 15 crosses (Table 5), F₁ hybrids exceeded or equalized with the mean of the two parents. Some crosses (Rumi × Corina, Boyana × Mytra, IPK Nelina × Mytra and IPK Nelina × Corina) surpassed the parent with the higher value and showed heterosis from 14.1% to 32.1% (see Table 1).

Table 4. Analysis of the variance of combining ability by productivity/plant, lint percentage and length of the fiber in F1 hybrids of 5 Bulgarian cotton varieties with 1 Turkish and 2 Greek cotton varieties in 2013-2014

Source of variation	Degree of freedom	2013 g.			2014 g.		
		Sum of squares	Mean square	F-exp.	Sum of squares	Mean squares	F-exp.
<i>Productivity/plant, g</i>							
GCA - mothers	4	-	-	-	244.879	61.220	36.868 ⁺⁺⁺
GCA - fathers	2	-	-	-	211.311	105.655	63.627 ⁺⁺⁺
SCA	8	-	-	-	232.451	29.056	17.498 ⁺⁺⁺
Errors	15	-	-	-	-	1.661	-
Components of the variance		-	-	-	$\sigma^2_{GCA}=0.712; \sigma^2_{SCA}=9.132;$		
		-	-	-	$F=0, \sigma^2_A=2.848; \sigma^2_D=36.527$		
		-	-	-	$F=1, \sigma^2_A=1.424; \sigma^2_D=9.132$		
<i>Lint percentage, %</i>							
GCA - mothers	4	5.219	1.305	11.046 ⁺⁺	12.193	3.048	41.383 ⁺⁺
GCA - fathers	2	5.422	2.711	22.951 ⁺⁺	6.201	3.101	42.092 ⁺⁺
SCA	8	10.498	1.312	11.101 ⁺⁺	14.855	1.857	25.209 ⁺⁺
Errors	15	-	0.118	-	-	0.074	-
Components of the variance		$\sigma^2_{GCA}=0.007; \sigma^2_{SCA}=0.398$			$\sigma^2_{GCA}=0.201; \sigma^2_{SCA}=0.594$		
		$F=0, \sigma^2_A=0.028; \sigma^2_D=1.592$			$F=0, \sigma^2_A=0.804; \sigma^2_D=2.376$		
		$F=1, \sigma^2_A=0.014; \sigma^2_D=0.398$			$F=1, \sigma^2_A=0.402; \sigma^2_D=0.594$		
<i>Fiber length, mm</i>							
GCA - mothers	4	-	-	-	1.376	0.344	5.272 ⁺⁺
GCA - fathers	2	-	-	-	0.207	0.04	1.587
SCA	8	-	-	-	3.898	0.487	7.469 ⁺⁺
Errors	15	-	-	-	-	0.065	-
Components of the variance		-	-	-	$\sigma^2_{GCA}=-0.223; \sigma^2_{SCA}=0.141$		
		-	-	-	$F=0, \sigma^2_A=-0.892; \sigma^2_D=0.564$		
		-	-	-	$F=1, \sigma^2_A=-0.446; \sigma^2_D=0.141$		

Table 5. Evaluation of the GCA and SCA effects by productivity/plant in F1 hybrids of 5 Bulgarian varieties with 1 Turkish and 2 Greek cotton varieties in 2013-2014

Mothers	GCA	Fathers	SCA	Fathers/SCA effects			
				Nazilli	Mytra	Corina	σ^2_{Si}
Chirpan-539	-4.844	Nazilli 954	-4.895	7.451	-4.049	-3.402	40.858
Helius	0.500	Mytra	0.671	3.473	-2.493	-0.971	8.735
Rumi	-4.178	Corina	4.224	-4.549	-1.555	4.564	19.878
Boyana	2.822			-0.449	4.351	-3.902	16.295
IPK Nelina	5.700			-5.927	2.207	3.720	26.031
Stand. error	1.052		0.815				
σ^2_{Sj} -fathers				30.015	10.717	14.723	

The values obtained for the heterosis effect were much lower than those reported in the literature, Wankhade et al. (2009) reported a maximum heterosis effect for raw cotton yields of 231.99% and 115.14% in diallel crosses, but

they were commensurable or close to those reported by Karademir and Gencer (2010) - 39.39-56.11%

All parental forms with high GCA, of mothers Boyana and IPK Nelina varieties, of fathers

Mytra and Corina varieties, had low variances of SCA (Table 5). The low variances of SCA indicated that their high GCA effects were mainly determined by additive gene effects.

In four of the crosses with positive SCA, the parental forms had significant positive GCA, in the other two, only one of the two parents had a positive GCA.

Significant positive and constant by years GCA for the fiber lint percentage was found for Boyana and IPK Nelina varieties of the mothers and for Nazilli 954 variety of the fathers (Table 6). The other varieties, mothers and fathers, had constant, significant negative GCA for this trait. An exception was Chirpan-539 variety, which showed inconstant, negative in the first year of the study and positive in the second year, but non-significant in the two years GCA by this trait. Average for the two years, of the mothers IPK Nelina variety with high fiber lint percentage had the highest GCA,

and of the fathers Nazilli-954 variety with the highest fiber lint percentage was with positive and high GCA.

Positive SCA effects for the fiber lint percentage were found for 5 crosses in the first year and for 8 crosses in the second year of the study (Table 7). Two crosses Rumi × Corina and IPK Nelina × Nazilli 954 showed constant positive SCA in the two years. At the first cross, both parental forms had negative GCA for this trait, while at the second one they had positive and high GCA. Maternal forms with high GCA (Boyana and IPK Nelina varieties) had low variances of SCA indicating that their high GCA effects were mainly due to additive gene effects. Nazilli 954 variety (of fathers) with high GCA had high variance of SCA.

The high variance of the SCA indicates that its high GCA was due to the presence of both additive and non-additive gene actions and interactions.

Table 6. Evaluation of GCA and SCA effects by fibre lint percentage in F₁ hybrids of 5 Bulgarian cotton varieties with 1 Turkish and 2 Greek varieties during 2013-2014

2013				2014			
Mothers	GCA	Fathers	GCA	Mothers	GCA	Fathers	GCA
Chirpan-539	-0.091	Nazilli 954	0.842	Chirpan-539	0.100	Nazilli 954	0.807
Helius	-0.258	Mytra	-0.318	Helius	-1.589	Mytra	-0.031
Rumi	-0.924	Corina	-0.524	Rumi	-0.111	Corina	-0.077
Boyana	0.631			Boyana	0.455		
IPK Nelina	0.642			IPK Nelina	1.145		
Stand. error	0.281		0.217	Stand. error	0.222		0.172

Table 7. Evaluation of GCA and SCA effects by fibre lint percentage in F₁ hybrids of 5 Bulgarian cotton varieties with 1 Turkish and 2 Greek varieties in 2013-2014

Fathers Mothers	2013				2014			
	Nazilli 954	Mytra	Corina	σ^2_{Si}	Nazilli 954	Mytra	Corina	σ^2_{Si}
Chirpan-539	-0.409	2.018	-1.609	3.351	0.893	-0.193	-0.700	0.623
Helius	-0.575	-0.382	0.958	0.634	-2.484	1.495	0.989	4.654
Rumi	0.824	-0.982	0.158	0.772	-0.162	0.018	0.144	-0.015
Boyana	-0.098	-0.438	0.535	0.181	1.071	-0.149	-0.922	0.971
IPK Nelina	0.258	-0.215	-0.042	-0.006	0.682	-1.171	0.489	0.999
σ^2_{Sj}					2.113	0.878	0.605	

In terms of fiber length, of the mothers Chirpan-539 and Rumi varieties were with positive GCA, of the fathers Corina variety was with positive but non-significant GCA (Table 8). IPK Nelina variety, which had the longest fiber of the mothers, had a negative GCA for this property and Helius variety, with the shortest fiber, also had a negative GCA.

Boyana variety had positive but insignificant GCA. Nazilli 954 and Mytra varieties of the fathers had negative but insignificant GCA. The insignificant values of the fathers showed that they did not differ in GCA for the fiber length, which confirmed the results obtained from the analysis of the variance of the combining

ability of the parental forms by this trait (see Table 4).

Specific combining ability for the fiber length was found for 6 crosses (Table 8), which average values were equal to the average parental value or exceeded it. Some crosses IPK Nelina × Corina, Rumi × Nazilli 954 and Chirpan-539 × Corina surpassed the better parent and exhibited heterosis of 2.2 to 4.1%. At crosses with manifested SCA effects, both parental forms had positive GCA effects (Chirpan-539 × Corina), or negative GCA effects (Helius × Nazilli 954), or one of parental forms showed positive GCA and the other one - negative GCA. Chirpan-539 variety with positive GCA was with low variances of SCA, its high SCA was due mainly to additive

gene effects. Rumi variety showed higher GCA than Chirpan-539 had medium high variances of SCA, which means that its high GCA, except by additive genes, to a considerable extent it was due to non-additive gene actions and interactions. Corina variety, with positive but insignificant GCA, had high variances of SCA, indicating that its positive GCA was conditioned by additive and non-additive gene actions and interactions.

GCA effects for productivity/plant and fiber length were inconsistent by years as a result of the specific response of F₁ hybrids to environmental conditions. The SCA effects were also unstable for most of the crosses which was due to their interaction with the environmental conditions (year conditions).

Table 8. Evaluation of GCA and SCA effects by fibre length in F₁ hybrids of 5 Bulgarian varieties with 1 Turkish and 2 Greek cotton varieties in 2013-2014

Mothers	GCA	Fathers	GCA	Fathers SCA effects			
				Nazilli 954	Mytra	Corina	σ^2_{Si}
Chirpan-539	0.242	Nazilli 954	-0.011	-0.489	0.071	0.418	0.175
Helius	-0.424	Mytra	-0.138	0.678	-0.062	-0.615	0.386
Rumi	0.342	+Corina	0.149	0.711	-0.129	-0.582	0.396
Boyana	0.131			-0.144	0.349	-0.204	0.057
IPK Nelina	-0.291			-0.755	-0.229	0.984	0.761
St. error/	0.209		0.161				
σ^2_{Sj}				0.414	0.015	0.441	

CONCLUSIONS

At the studied 15 intraspecific crosses of 5 Bulgarian cotton varieties with three foreign ones, different types of inheritance were found for the productivity/plant, boll weight, fiber length and lint percentage.

Positive over-dominance, dominance of parent with the higher value and additive inheritance were observed by all traits studied.

The heterosis effect was most pronounced for the productivity/plant and reached 39.1%, which was a prerequisite for transgressive segregation in F₂ generation.

Additive and non-additive gene effects were of importance for the inheritance of the traits studied.

The genetic variance was mainly of non-additive type and the prognosis for the selection in the early segregating generations could not be good.

Of the cotton varieties Bulgarian selection, included in the crosses, Boyana and IPK Nelina emerged as good common combiners for the productivity and fiber lint percentage, differed with high performance for both traits and high GCA. Chirpan-539 and Rumi varieties appeared to be good common combiners for the fiber length.

Their high GCA was combined with low variances of the SCA, making them very suitable for the synthetic selection.

Of the foreign varieties, the Turkish Nazilli 954, possessing high fiber lint percentage, emerged as a good common combiner by this trait, the Greek variety Corina had high GCA for the fiber length.

Their high GCA was combined with high variances of the SCA and they appear to be more suitable for the heterosis selection.

REFERENCES

- Ahuja, S.L., Dhayal, L.S. (2007). Combining ability estimates for yield and fibre quality traits in 4×4 line×tester crosses of *Gossypium hirsutum* L. *Euphytica*, 153, 87–98.
- Basal, H., Unay, A., Canavar, O., Yavas, I. (2009). Combining ability for fiber quality parameters and within-boll yield components in intra- and inter-specific cotton populations. *Span J. Agric. Res.*, 7(2), 364–374.
- Bojinov, M., Dimitrova, L. (1981a). New cotton varieties (No 432 and No 996). Ed. *CNTI*, S., 8–14.
- Bojinov, M., Dimitrova, L. (1981b). Biological and economic qualities of the new cotton varieties No 26, No 432 and No 996. *Crop Science*, 6.
- Bojinov, M., Dimitrova, L. (1989). Ogosta (No 644). *Crop Science*, 26, 10, 31–35.
- Bojinov M., Dimitrova, L., Bozhinov, B. (1996). Chirpan-603 and Chirpan-539 - new cotton varieties. *Crop Science*, 33(2), 35–38.
- Bojinov M., Bozhinov, B. (2004). Beli Lom (393) and Beli Iskar (800) - new cotton varieties. *Plant Science*, 41, 380–383.
- Bojinov, M., Bozhinov, B. (2008). Veno - a new cotton variety. *Crop Science*, 45(3), 277–278.
- Karademir, E., Gencer, O. (2010). Combining ability and heterosis for yield and fiber quality properties in cotton (*G. hirsutum* L.) obtained by half diallel mating design. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 38(1), 222–227.
- Karademir, E., Karademir C., Basal, H. (2016). Combining Ability and Line × Tester Analysis on Heat Tolerance in Cotton (*Gossypium hirsutum* L.) *Indian Journal of Natural Sciences*, 6(34), 10515–10525.
- Memon, Sh., Jatoi, W.A., Khanzada, S., Kamboh, N., Rajput, L. (2017). Line × Tester Analysis for Earliness Yield and Yield Contributing Traits in *Gossypium hirsutum* L. *Journal of Basic & Applied Sciences*, 13, 287–292.
- Sajjad, M., Azhar, M.T., Malook, S. (2016). Line × tester analysis for different yield and its attributed traits in Upland Cotton (*Gossypium hirsutum* L.). *Agric. Biol. J. N. Am.*, 7(4), 163–172.
- Savchenko, B.K. (1984). *Genetic analysis in line x teter crossings*. Minsk, BLR “Science and Technology”.
- Senthilkumar, R., Ravikesavan, R., Punitha, D., Rajarathinam, S. (2010). Genetic analysis in cotton. *Electronic Journal of Plant Breeding, Coimbatore: Indian Society of Plant Breeders*, 1(4), 846–851.
- Sivia, S.S., Siwach, S.S., Sangwan, O., Lingaraja, L.R. Vekariya, D. (2017). Combining Ability Estimates for Yield Traits in Line×Tester Crosses of Upland Cotton (*Gossypium hirsutum* L.) *Int. J. Pure App. Biosci.*, 5(1), 464–474.
- Valkova, N., Bojinov, M. (2010). "Boyana" cotton variety. *Field Crops Studies*, VI(3), 395–398.
- Valkova, N. (2014). Denitsa - a new high quality cotton variety. *Field Crop Studies*, IX(1.2), 227–232.
- Wankhade, S.N., Patil, S.P., Burghate, S.K., Chikhale, N.J. (2009). Heterosis for seed cotton yield and its quantitative characters of *Gossypium hirsutum* L. *Journal of Cotton Research and Development, Hisar: Cotton Research and Development Association*, 23(1), 27–31.

INFLUENCE OF THE IRRIGATION METHOD ON THE SOYBEAN PRODUCTIVITY

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Abstract

The aim of this work is to determine the impact of irrigation methods on soybean productivity. The field experiment was conducted during the period 2008-2010 in Agricultural University, Plovdiv. Three irrigation techniques are tested – by gravity, microsprinklers and drip irrigation. A non-irrigation plot was the control. Irrigation for all three irrigation methods is given at 80% FC for the layer 0-0.60 m. Under non-irrigation conditions, the yield is between 104 and 202 kg/da. Irrigation by furrows increases yields with 66%. The average yield for this technique is 277 kg/da. Yield increases by over 75% and reaches 295 kg/da under sprinkler irrigation, the irrigation water use efficiency (IWUE) is 0.595 kg/m³. Using drip irrigation, the yield increases by an average of 50% and reaches 250 kg/da, and the IWUE is much lower - 0.377 kg/m³. From an economic point of view, the best results are by using sprinkler irrigation. It gives yield 18% higher than that of drip and 6% higher than that of furrow irrigation.

Key words: soybean, irrigation, irrigation water use efficiency.

INTRODUCTION

Global climate change is adversely affecting the geographical area in which our country is located. To enhance the efficiency of Bulgarian agriculture, it is of particular importance to create conditions for the full utilization of the productive potential of natural resources - soil and water. In the context of the contemporary economic development of our country and the associated increasing consumption of fresh water, those technologies that require less water are becoming increasingly important. The efficiency of drip irrigation and micro sprinkling is determined by the amount of investment and annual operating costs, additional production, saving of irrigation water, etc. indicators. Such studies have been done for some crops, but soybean is not available.

In today's conditions, the choice of irrigation equipment is relatively large, due to the availability of a wide variety of elements of high quality materials on the market. The correct choice of irrigation method and type of irrigation equipment is an important prerequisite for efficient use of water, provision of suitable soil moisture and high yields from crops. According to research conducted in Austria, irrigation by sprinklers is more

effective than irrigation by furrows, yield is 23% higher than that obtained under non-irrigation conditions (Klik A. & Cepuder P., 1991). In support of this are the results of a long experiment (20 years) exported by Paltineanu et al., 1994, according to which the yield and productivity of the total irrigation rate in sprinkler irrigation are higher than those in gravity irrigation. Riter & Scarborough (1988) attach more importance to the irrigation regime than to the irrigation technique. Shuravilin et al. (2015) found that irrigation of early soybean varieties provided yields of 250-270 kg/da, but when drip irrigation was applied it increased and was 100 kg/da higher. According to the same study, maximum efficiency of sprinkler irrigation is reached at the seasonal rate of 400 mm, which is less than 70% of that found in drip irrigation. Babayan (2018) reports that carrying out 1-2 irrigations through the sprinklers during the critical phenophases leads to a significant increase in the yield of soybeans (from 44 to 101%), but according to the author the best results are obtained in combination between drip irrigation and mineral fertilization, with yields increasing by more than 20% comparing with sprinkler. Balakay et al. (2019) recommend that, in the case of sprinkler and drip irrigation, a soil moisture level 80% of FC for the 0-0.60 m

layer. Significant water losses at soybean sprinkler irrigated have been reported by Zhelyazko & Vcherashnyi (2015). According to the authors the total coefficient of water loss during sprinkling is in the range 0.84-0.94, depending on the growing season.

The purpose of the research is to identify the impact of irrigation methods on soybean productivity and to find the most economically suitable of them.

MATERIALS AND METHODS

The experiment was conducted with soybean (variety Srebrina) in the period 2008-2010 on alluvial-meadow soil, in the experimental field of the Agricultural University, Plovdiv. Experience includes three variants with irrigation techniques - gravity (short closed furrows), micro sprinklers and drip irrigation. A non-irrigated variant was used for the control. Irrigations for all three variants were given at soil moisture 80% of field capacity (FC) for the layer 0-0.60 m. Irrigation time is determined by gravimetric method. The experiment is based on the method of the long plots in three repetitions, with the size of the experimental plots of 30 m² and the harvest plots - 10 m². The sowing was carried out mechanically in the last decade of April or early May, depending on the conditions of the year, at a density in the range of 25-30 plants per m². Soybean was grown after a corn maize precursor. All necessary agro-technical activities related to the cultivation of the crop have been carried out. Grain yield was determined at a humidity of 14%. By means of variance analysis (ANOVA1), the degree of proof of the differences between the variants, both in terms of yield and its main structural components, was established. On the basis of production data, output and costs associated with soybean production, an analysis was made to determine the economic performance of each of the irrigation techniques used.

RESULTS AND DISCUSSIONS

Water supplying by rainfall

The effect of irrigation and its intensity depend to a large extent on the amount and distribution of vegetation rainfall. As far as they are

concerned, the experimental year 2008 is averaged with a 45% security and a vegetation rainfall of 231.0 mm. During the reproductive period this experimental year is dry, with the sum of rainfall below 25 mm from the third ten days of June to the second of September. At the end of vegetation rainfall 83 mm have no effect on irrigation and its effect on soybean productivity. The second experimental year is average dry, with a security of 69% and a precipitation amount of 190.2 mm. As a distribution, the vegetation rainfall in this experimental year is more favorable in terms of water supply of rain-fed soybean. The fall of about 60 mm in May and June, together with the autumn-winter moisture reserves are completely sufficient to secure the plants until the end of the growing season. During the reproductive period (July and August), precipitation is a total of 96 mm, with $\frac{3}{4}$ of them falling during the first half of the period, when soybeans bloom and form beans, there is a very intense water consumption, and the stress of meteorological factors is greatest. The third experimental year is close in characteristics to the first, with a sum of rainfall for the period May-September of 234 mm and a security of 52%. Typical for this experimental year is that most of the vegetation rainfall has fallen during the grain filling period (99.2 mm), when water demand begins to gradually decrease. Precipitation in June (44 mm) is of great importance for the beginning of the reproductive period.

Elements of irrigation regime

The difference in natural humidification conditions during the experimental years is reflected in the irrigation regime elements, with yearly data presented in Table 1.

During the first experimental year, the optimum soil moisture is maintained naturally throughout the growing season, extending to the period of mass flowering and the onset of beans formation, when the first 60 mm irrigation was applied in all three techniques. The second irrigation by gravity irrigation was also carried out during the period of mass flowering of bean formation with the norm of 50mm, while in the case of sprinkler and drip irrigation, the second irrigation was carried out 10 days later (during the period of bean

formation and the beginning of grain filling). This difference in sprinkler is due to the slower depletion of water from the soil, which is why the irrigation rate is 50 mm. The delay of the second irrigation during drip irrigation is due to the daily and, at the same time, small rainfall, which hinder its realization. This creates a slight water deficit requiring a higher irrigation rate (75 mm). The third irrigation with all three techniques was performed during the period of grain filling, while maintaining the difference in the irrigation realization between the different variants of the experiment. Irrigation rates are consistent with the reported soil moisture. Under these conditions, the irrigation rate for sprinkler and gravity irrigation is practically the same (150 mm), while for drip irrigation it is 50 mm higher.

Table 1. Irrigation rates at three different techniques

№	period	Irrigation rates (mm)					
		Furrow		Sprinkler		Drip	
		m	M	m	M	m	M
2008							
1	II	50.1	148.7	60.0	151.1	60.0	201.4
2	II	50.1		50.0		74.8	
3	III	48.5		41.1		66.6	
2009							
1	II	55.1	232.6	55.1	236.8	55.1	216.2
2	II	56.7		56.7		44.4	
3	III	56.7		47.7		57.5	
4	III	64.1		77.3		59.2	
2010							
1	II	66.0	224.0	66.0	216.0	66.0	201.5
2	II	59.0		55.0		39.0	
3	III	50.0		45.0		49.0	
4	III	49.0		50.0		47.5	
m – irrigation rate, M – total irrigation rate							
II – flowering and pod development, III – pod filling							

The number of irrigations in the average dry year 2009 is the same for the three irrigation techniques. They are 4 and are distributed only within the reproductive period, since during this experimental year the vegetative period runs under conditions of optimal natural water supply. The first irrigation with all three techniques was carried out at the beginning of the mass flowering (July 20) with the standard 51mm. The second watering was carried out during the period of mass flowering and the beginning of bean formation. The third irrigation was carried out during the flowering and intensive beanning period, while in the drip and gravity irrigation, it was applied 5-6 days

later when the grain irrigation period began. The last (fourth) watering is during the period of intensive grain filling (end of July and beginning of August). The total irrigation rate M in this experimental year was higher than in 2008, but varied within narrower limits (up to 20 mm) between techniques.

In the third experimental year, the number of irrigations was again 4, the first being at the beginning of flowering, the second during the period of mass flowering and bean-forming, and the third fourth - during the grain filling period. The irrigation rate for drip irrigation is the smallest, followed by that for sprinkler irrigation and gravity irrigation, with differences being relatively small. Compared to the previous two years, the circumstances allow the watering of the various techniques to take place at the same time.

Grain yield, depending on irrigation method

The impact of irrigation on soybean yield is presented in Table 2. During the first experimental year, irrigation under extreme drought results in a significant and statistically proven increase in yield, most notably in irrigation by furrows - 75%. During this experimental year, the differences in production and between techniques are also statistically proven. For example, in sprinkler irrigation, the yield represents 94% of that in gravity irrigation, but has been proven. A more significant difference is reported for drip irrigation, with a difference of nearly 13%. In both cases, the number of beans per plant can be considered as the main cause, since the data show deviations in the same range (6 and 14%, respectively). This is supported by data on the number and weight of seeds per plant. In the case of rain, the number of seeds decreases by 4% and their weight by more than 10%. In the case of drip irrigation, the number of seeds is reduced by almost 9% and the mass - by more than 11%. However, with all three techniques used, the yield is over 300 kg/da, which is close to the maximum productive capacity of this soybean (380 kg/da). In the second year, which is characterized by medium dryness, the four irrigations increased yields by 2.2 times in furrow irrigation, 2.5 times in rainfall and 85% in drip irrigation, with statistically proven differences in each of the three cases (Table 2). During this

experimental year, the yield results were the best in sprinkler, and probably one of the reasons is that the first three of the total 4 irrigations were realized at different stages of the flowering period, with the third coinciding with the intensive growth of the beans. In the case of gravity and drip irrigation, it is applied 5-6 days later (at the beginning of the seed filling period). Thus, as in the previous year, the structural elements of yield in gravity irrigation and in sprinkler vary within relatively narrow limits, but in drip the variations are more significant, which affects productivity. The number of beans per plant is about 8% less than that of sprinkler and about 10% less than that of gravity irrigation. In addition, during

this experimental year, the mass of 1000 seeds for drip irrigation was 8% less than that for sprinkler and more than 10% compared to furrow irrigation. Under these conditions, the yield is below 200 kg/da (192.2 kg/da), behind the sprinkler by more than 25% and the furrow by more than 15%.

In the third experimental year, which is average and relatively favorable in meteorological conditions, soil moisture optimization increases yield by 27-28% in furrow and drip irrigation and by 48% in sprinkler. Relative to the non-irrigated soybean, these differences are statistically proven (Table 2). The yields of gravity and drip irrigation are almost the same, with a difference of less than 0.5%.

Table 2. Impact of irrigation method on grain yield in 2008, 2009 and 2010 years

Variant	2008				2009				2010			
	Y (kg/da)	differences			Y (kg/da)	differences			Y (kg/da)	differences		
		± kg/da	%	warranty		± kg/da	%	warranty		± kg/da	%	warranty
Relative to rain-fed												
rain-fed	202.1	St.	100.0	St.	103.7	St.	100.0	St.	195.8	St.	100.0	St.
furrow	353.8	+151.7	175.1	C	228.6	+124.9	220.4	C	248.6	52.8	127.0	B
sprinkler	332.1	+130.0	164.3	C	261.7	+158.0	252.4	C	289.8	94.0	148.0	C
drip	308.8	+106.7	152.8	C	192.2	+88.5	185.3	C	249.6	53.8	127.5	B
Relative to gravity irrigation												
rain-fed	202.1	-151.7	57.1	C	103.7	-124.9	45.4	C	195.8	-52.8	78.8	B
furrow	353.8	St.	100.0	St.	228.6	St.	100.0	St.	248.6	St.	100.0	St.
sprinkler	332.1	-21.7	93.9	A	261.7	+33.1	114.5	B	289.8	41.2	116.6	A
drip	308.8	-45.0	87.3	C	192.2	-36.4	84.1	B	249.6	1.0	100.4	n.s.
Relative to sprinkler irrigation												
rain-fed	202.1	-130.0	60.9	C	103.7	-158.0	39.6	C	195.8	-94.0	67.6	C
furrow	353.8	+21.7	106.5	A	228.6	-33.1	87.4	B	248.6	-41.2	85.8	A
sprinkler	332.1	St.	100.0	St.	261.7	St.	100.0	St.	289.8	St.	100.0	St.
drip	308.8	-23.3	93.0	A	192.2	-69.5	73.4	C	249.6	-40.2	86.1	A
Relative to drip irrigation												
rain-fed	202.1	-106.7	65.4	C	103.7	-88.5	54.0	C	195.8	-53.8	78.4	B
furrow	353.8	+45.0	114.6	C	228.6	+36.4	118.9	B	248.6	-1.0	99.6	n.s.
sprinkler	332.1	+23.3	107.5	A	261.7	+69.5	136.2	C	289.8	40.2	116.1	A
drip	308.8	St.	100.0	St.	192.2	St.	100.0	St.	249.6	St.	100.0	St.
	GD (kg/da) 5% = 16.9; 1% = 24.3; 0.1% = 35.7				GD (kg/da) 5% = 21.7; 1% = 30.9; 0.1% = 44.7				GD (kg/da) 5% = 28.2; 1% = 42.8; 0.1% = 68.7			

Drip irrigation provides the highest number of beans per plant (average 58), while in the sprinkler they average 52 (difference is 11%). In gravity irrigation, the number of beans per plant is 39, ie. significantly less than with drip irrigation. In addition to the number of beans, drip irrigation accounts for the highest number of seeds per plant (average 120), although the differences between the three techniques are not statistically proven. Despite these advantages with drip irrigation, the sprinkler

yield is 16% higher. The main reason is the higher yield by 4.4% by weight per 1000 seeds and by 17% higher yield per plant. The large number of beans in drip irrigation is largely offset by the small relative share of fully developed grains in them. Of the 148 seeds per plant planted, only 36.4% are fully developed, 44.7% are underdeveloped and 18.9% are missing. Compared to drip irrigation, in the case of rain and gravity irrigation, the seeds developed are more than 30% higher.

Figure 1 presents the average soybean yield data under non-irrigation (dry) conditions, as well as using the three irrigation techniques. Without irrigation, an average of 167 kg/da is obtained. Drip irrigation increases yield by 50% on average, furrow irrigation - by 66%. Sprinkling has the most favorable effect on yield, increasing it by an average of 76%. Compared to sprinkler irrigation, the yield for furrow irrigation is 94% and for the drip - 85%.

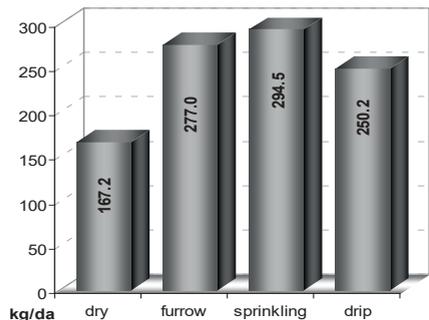


Figure 1. Soybean yield depending on irrigation method - average for three years

Irrigation water productivity (IWUE)

The productivity of the irrigation rate represents the additional yield of every 1 m³ of irrigation water, but it can also be expressed as the amount of irrigation water to produce a unit of additional yield. The year data are presented in Table 3.

Table 3. The productivity of the irrigation rate

variant	ΔY (kg/da)	M (mm)	IWUE kg/m ³	IWUE m ³ /kg
1	2	3	4	5
2008				
furrow	151.7	148.7	1.020	0.980
sprinkler	130.0	151.1	0.860	1.162
drip	106.7	201.4	0.530	1.888
2009				
furrow	124.9	232.6	0.537	1.862
sprinkler	158.0	236.8	0.667	1.499
drip	88.5	216.2	0.409	2.443
2010				
furrow	52.8	224.0	0.236	4.242
sprinkler	94.0	216.0	0.435	2.298
drip	53.8	201.5	0.267	3.745
average				
furrow	109.8	201.8	0.598	2.362
sprinkler	127.3	201.3	0.654	1.653
drip	83.0	206.4	0.402	2.692
ΔY - additional yield, M - total irrigation rate				
IWUE - irrigation rate productivity				

The use of irrigation water for drip irrigation is less effective. For the three experimental years the values are significantly lower than those for gravity irrigation and sprinkler (Table 3, column 4), with values ranging from 0.267 to 0.530 kg/m³. Significantly higher efficiency of irrigation rate is reported for gravity irrigation, and in the first year of the experiment it is highest compared to the other two techniques, exceeding 1 kg/m³.

Under the conditions of the experiment, the productivity of the irrigation rate of sprinkler irrigation varies within the narrowest range (between 0.435 and 0.860 kg/m³). In addition, in the first year it is slightly lower than that of gravity irrigation, and in the second and third years it is the highest. Column 5 of Table 3 lists the values of the irrigation rate productivity giving an idea of the amount of irrigation water needed to obtain 1 kg of additional yield. Here again, irrigation water is used most effectively at sprinkler, with an average of 1.653 m³ of water (between 1.162 and 2.298 m³) being consumed to obtain 1 kg of additional yield. The variation of these values in the gravity irrigation is the largest - between 0.980 and 4.242 m³. However, on average during the experimental period, to obtain 1 kg of additional yield, most water is required for drip irrigation (2.692 m³/kg). The average data of irrigation water productivity are illustrated in Figures 2 and 3.

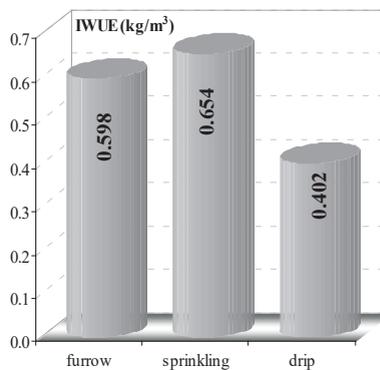


Figure 2. Irrigation rate productivity (kg/m³)

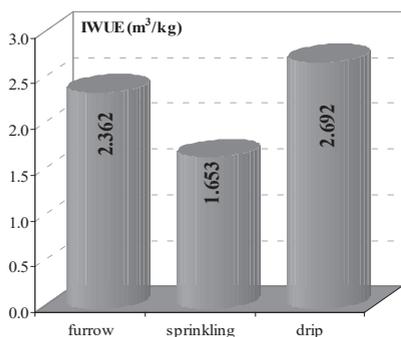


Figure 3. Necessary irrigation water for producing 1 kg soybean (m³/kg)

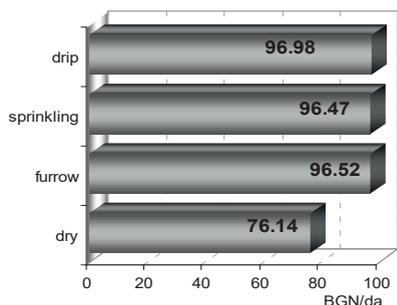


Figure 4. Producing cost in BGN/da

Economic Indicators

The parameters of the main economic indicators have been established and the costs have been determined on the basis of a technological map with specified prices for each agricultural practice (G. Georgiev et al., 2015). Four soil treatments are included, one being basic (autumn plowing), sowing costs, three herbicide treatments (soil, vegetative against wheat weeds and vegetative against deciduous weeds), as well as harvesting with a harvester combine and grain removal. Irrigation costs have also been added to the irrigation costs (excluding investment costs for the irrigation equipment). The soybean market price for 2019, which is 1 BGN/kg, is used to calculate revenue, meaning that the values of yield and total production are numerically the same.

• Costs of soybean producing

Under non-irrigation conditions, the costs are 76.14 BGN/da in the three experimental years. Irrigation increases costs, with gravity increasing by between 20 and 31% (average 27%) and reaching an average of 96.52 BGN/da (Figure 4), depending on the number of irrigations and the size of the irrigation rate vary between 91 and 100 BGN/da. In the sprinkler, costs vary in the same range, with an average of 96.47 BGN/da for the three years. The average costs at drip irrigation do not differ significantly (BGN 96.98/da), but unlike the other two techniques, there is practically no variation over the years (BGN 96.48-97.98/da). The data in the different variants of the experiment give reason to believe that the irrigation costs do not significantly depend on the method of irrigation.

• Cost price

The cost of production under non-irrigation conditions does not differ significantly from that under irrigation, but is still higher (on average 0.46 BGN/kg), being relatively low (0.38-0.39 BGN/kg) in the average 2008 and 2010 years, but over years with prolonged periods of drought and lower yields, the cost of production has increased to over 0.70 BGN/kg, which is still below the market sale price of the production. The irrigation method does not significantly affect this indicator (Figure 5).

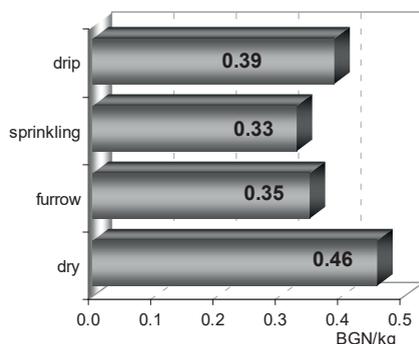


Figure 5. Cost price - average for three years

However, the lowest cost is recorded in the sprinkler, depending on the number of irrigations it varies between 0.28 and 0.38 BGN/kg (average 0.33 BGN/kg). In the case of gravity irrigation, the average increase is minimal (0.02 BGN/kg), but depending on the conditions of the year the variation is slightly wider - between 0.26 and 0.44 BGN/kg. The use of drip irrigation provides the highest cost of production, although it averages below 0.40 BGN/kg. However, in dry years, it may exceed 0.50 BGN/kg, while in more favorable years it ranges from 0.31-0.39 BGN/kg.

- Rate of profitability

This indicator represents the ratio between net income and production costs, with the highest average values being 205.3% for the conditions of sprinkler irrigation. Gravity irrigation (187%) and drip irrigation (158%) rank similarly to other economic indicators. Under rain-fed conditions, the rate of return is on average 120%.

- Net income

Under non-irrigation conditions, net income is relatively low, especially in dry years when yields are low and costs are commensurate with those in more favorable years. Therefore, in the average dry year 2009 it amounts to BGN 28/da, while for the other two, which are characterized as average, the profit is between 120 and 126 BGN/da. On average over the three years of non-irrigation experience, net income is 91 BGN/da (Figure 6).

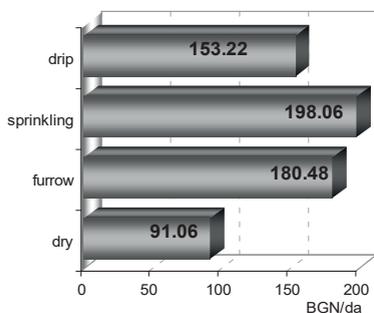


Figure 6. Net incom, in BGN/da

Irrigation increases net income significantly, especially in the drier years, although in such years its absolute values decrease due to higher irrigation costs. For furrow soybean irrigation, the average yearly profit increases from 25% to more than 2 times if there are periods of longer droughts during the year (as in 2008).

During average dry and dry years, furrow irrigation provides a net income increase of 4-5 times that under irrigation conditions. With this irrigation technique, the absolute values of this indicator range from 129 to 263 BGN/da, and on average over the three-year period it is 180 BGN/da. From an agronomic and profit standpoint, soybean sprinkling gives the best results, exceeding that in dry years by more than 20% gravity irrigation. However, in more climate-friendly years, the values for both techniques are commensurate. On average over

the three experimental years, sprinkling provides a net income of 198 BGN/da, which is 9% higher than reported for gravity irrigation and 2.2 times compared to that under non-irrigation conditions (up to 5-6 times on average dry and dry years). The use of soybean drip irrigation increases the profit by 68% on average against non-irrigated soybean, depending on the cost of irrigation and the absolute yield obtained, ranging from 94 to 212 BGN/da (average 153 BGN/da), ie. an increase in the range of 28% to more than 3 times. However, net income from drip irrigation is inferior to that obtained under the other two techniques, regardless of the conditions of the year. It represents on average 85% of that in furrow irrigation and 77% (58 to 88%) of that in sprinkler.

CONCLUSIONS

The irrigation method does not significantly affect the elements of the irrigation regime in soybean, in the average years 3-4 irrigations are needed, and in the middle of the dry season - four times with rate of 50-60 mm. The average total irrigation rate for gravity irrigation, rain and drip irrigation is practically the same (201-206 mm).

Without irrigation, an average of 167 kg/da is obtained. Drip irrigation increases yield by 50% on average, gravity irrigation - by 66%. Sprinkling has the most favorable effect on yield, increasing it by an average of 76%. Compared to sprinkler irrigation, the yield for gravity irrigation is 94% and for the drip - 85%. Drip irrigation creates prerequisites for strong vegetative growth and the laying of more beans and seeds per plant than rain and gravity irrigation. As a result, the relative share of undeveloped and not fully developed seeds in the total yield increases. The mass of 1000 seeds is also smaller. For the conditions of this experiment, this is the main reason for the significantly lower yields than the other two methods.

Irrigation water is used most effectively in the sprinkler, with an average of 1.653 m³ being consumed to obtain 1 kg of additional yield, and most of the water required for drip irrigation (2.692 m³/kg) to obtain 1 kg of additional yield. The irrigation rate of sprinkler

irrigation exceeds that of furrow irrigation by 9% and that of drip irrigation by 63%.

The best economic results are obtained by sprinkler irrigation with the lowest production cost of 0.33 BGN/kg and the highest net income - 198 BGN/da. Thus sprinkling is recommended as a method of soybean irrigation. If there are not suitable conditions for sprinkler irrigation, it is also recommended furrow irrigation. With this type of irrigation, the average net income is 180 BGN/da. Soybean drip irrigation is not recommended because of its significantly lower agronomic and economic results. This method of irrigation can only be used when sprinkler and gravity irrigation are not possible.

REFERENCES

- Babayan, B.R. (2018). Assessing the possibility of cultivating soybeans in Transnistria, taking into account the use of irrigation and fertilizers. Biodiversity and factors affecting the ecosystem of the Dniester basin, Tiraspol: Eco-TIRAS, Materials of the scientific-practical conference, 18–21.
- Balakai, G.T., Selitskiy, S.A. (2019). Soybean Varieties Yield by Sprinkling and Drip Irrigation in the Rostov Region. *Scientific Journal of the Russian Research Institute of Melioration*, 23(35), 80–97.
- Click, A., Cepuder, P. (1991). Irrigation of soybeans improves quality. *Forderungsdinst*, 39(11), (322–324).
- Georgiev, G, Sabev V., Todorova R., Alexieva, A., Naydenova G. (2015). Advanced Soybean (*Glycine max* (L) Merrill.) Cultivation. Soybean Research Station - Pavlikeny State-owned Enterprise, p. 34.
- Paltineanu, I.C., Negrila, C., Craciun, M., Craciun I. (1994). Long term trails on irrigated field crops in the semiarid area of Romania. *Romanian Agricultural Research*, 1, 85–92.
- Riter, W.F., Searborough, R.W. (1988); Irrigation of soybeans on the Delmarva Peninsula. *Appl. Eng. In Agr.*, 4(2), 136–141.
- Shuravilin, A.B., Borodychev, V.V., Lytov, M.N. (2015). Ways of Irrigation and Methods of Soy Cultivation in the Lower Volga Area. *AGRO XXI*, 7-9, 37–40.
- Zhelyazko, V.I., Vcherashniy, E.A. (2015). Water Losses of the Sprinkling Irrigated Soya Using the Irriland Raptor. *Bulletin of the Belarusian State Agricultural Academy*, 2, 120–123.

ECOLOGICAL AND ECONOMIC ASPECTS OF ORGANIZATION OF CROP ROTATIONS IN MARKET TYPE AGRICULTURAL ENTERPRISES

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Abstract

The ecological and economic problems arising from the organization of crop rotations in agricultural enterprises, formed during the period of land relations reform in rural territories, are analyzed. Large agroholdings neglect the crop rotation factor in an effort to maximize the economic impact by growing highly profitable market-oriented crops in large areas. Their attempt to replace crop rotation by increasing the use of synthetic mineral fertilizers and pesticides can lead to environmental and socio-economic problems in the future. Farmers cannot organize crop rotations to the full extent due to the short land lease term. It is shown that the scientifically substantiated crop rotation can harmonize the economic interests of agricultural producers (high and sustainable crop yields, accompanied by the minimum use of material and energy resources) and the environmental safety (preserving the quality of the soil cover and the environment). In order to organize crop rotations in a changing market and climatic environment, it is suggested to use methodological approaches. These approaches are based on short-term rotation of crops, on dynamics of their placement depending on ecological and economic factors, on formation of homogeneous (by quality indicators) lands (fields), on choice of crops on the basis of evaluation of land prior to its cultivation, on compulsory environmental assessment of projected crop rotations using balance calculations.

Key words: crop rotation, agricultural enterprises, land management, ecological and economic justification.

INTRODUCTION

Crop rotation is a determining factor for economically efficient and environmentally safe use of agricultural land, ensuring sustainable agricultural land use, as opposed to the constant cultivation of the same plants on the same land plot for a long period, resulting in a decrease in their yield, in a depletion of the soil nutrients and moisture, and in the intensity of the spread of diseases and pests (Saiko et al., 2002). Therefore, scientifically substantiated crop rotation prevents the irrational use of land by continuously growing the most profitable crops on the same fields (in particular, sunflower or rapeseed); and without additional capital expenditures, it contributes to the reproduction of the soil fertility, provides the normative phytosanitary state of crops and ecological state of the environment, which certainly has a positive impact on the results of economic activity of market-based agricultural enterprises.

Transformation of land relations in the agrarian sector of the economy, change of boundaries and sizes of land business entities, formation of personal farms on the basis of former collective and state farms were accompanied, among other things, by the destruction of existing crop rotations. Because of this, the problem of forming cost-effective crop rotations for various organizational and economic forms of land use becomes especially relevant.

The other side of the problem is the issue of harmonization of the economic interests of land users (maximizing profits through the cultivation and sale of highly profitable crops) with the requirements of environmental safety (compliance with environmental regulations, preservation and reproduction of soil fertility, etc.). Moreover, the economic and environmental aspects of crop rotation are closely interlinked, since scientifically substantiated crop rotation can ensure high productivity of agro-ecosystems (Shevchenko et al., 2017) and significantly reduce the amount of material and

energy resources used to control weeds, pests and plant diseases arising from monocultural cultivation.

The purpose of the case study is the analysis of ecological and economic aspects of organization of crop rotations in market-type agro-formations and substantiation of the possibility of introducing new methodological approaches to the formation of crop rotation arrays on the example of agricultural enterprises of the Kyiv region.

Literature review. The results of scientific studies, conducted in Ukraine and abroad, confirm the growing organizing role of crop rotation in solving the main tasks of modern farming systems: ensuring high productivity of agrocenoses in combination with the simultaneous protection of the environment from harmful anthropogenic influence. In particular, foreign scientists consider crop diversification with appropriate crop rotation and agro-biodiversity to be a mechanism to reduce market risks, to increase resource efficiency (Roest et al., 2018), to be an important factor in the sustainable provision of environmentally friendly food and in reducing the negative effects of climate change (Frison et al., 2011), to be a method of increasing the stability of agroecosystems (Erisman et al., 2016), to be the means of harmonizing (balancing) productivity, profitability and environmental friendliness of production (Davis et al., 2012) by reducing the use of chemical plant protection products and synthetic mineral fertilizers.

According to domestic scientists, the complete development of scientifically grounded crop rotations, developed for different soil and climatic zones of the country, in combination with other agrotechnical measures allows to increase the productivity of arable land by 40-50%, while ensuring the fertility of the surrounding environment (Kovalenko, 2007; Lebid et al., 1992).

The historical experience of agricultural development in the forest-steppe zone of the country convinces the existence of dependence of economic and industrial stability of a certain agricultural enterprise, in particular in market conditions, on the breadth of the range of highly profitable commodity products produced by the economy. In addition, weather and

climatic conditions during the growing season have a significant impact on the productivity of agrocenoses, as they can be favorable either for all crop rotations or only for individual crops (Saiko et al., 2002).

Therefore, the diversity of crops in the structure of the cultivated land is the means of offsetting the negative impact of adverse abiotic factors, of increasing the resistance of agrocenosis to the harmful effects of biocenotic factors. This is especially true in the conditions of insufficient provision of chemical protection of crops in the vast majority of farms (Brazhenko et al., 2008).

MATERIALS AND METHODS

For the analysis of ecological and economic aspects of crop rotation organization in market-type agro-formations, we used the data of state statistical reporting, provisions of legislative and regulatory acts, as well as the materials of scientific works relevant to the chosen direction of research.

Land management planning and surveying activity were carried out at the territory of the State Enterprise "Research Entity Skvyrske" of the Institute of Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine, where the technological aspects of production are studied. During the agrochemical examination of soils, we used methods, complying with the current national standard DSTU, GOST standard and methodical instructions. The humus content was determined according to DSTU 4289: 2004 by the Tiurin method (DSTU, 2004); the content of hydrolysable nitrogen – according to the DSTU 7863: 2015 by the Cornfield method (DSTU, 2015); the content of mobile compounds of phosphorus and potassium - according to DSTU 4115-2002 by the modified Chyrykov method (DSTU, 2002).

One of the main principles of short-term crop rotation should be the placement and rotation of crops in accordance with the laws of fruit rotation. This is important from the environmental (normative state of the environment) and the economic point of view (ensuring food security and expanding the range of quality crop products).

It is widely recognized that the only mechanism for practical implementation of scientific and

methodological know hows in this field is the development of land regulation projects that provide ecological and economic justification for crop rotation and land management. However, today, according to official statistics, a significant number of land users are evading the development and introduction of crop rotation projects and are in constant search for the ways to circumvent the rules of the law (Poleiko, 2013). Some scientists attribute frequent violation of crop rotation principle in agricultural enterprises to the predominant orientation of commodity producers on the formation of static crop rotations (Kazmir et al., 2014), the methods of which are sufficiently covered in special literature (Kazmir, 2009).

The static crop rotation design algorithm provides justification for the size (area) of rotation, for the designation of the uniform-size fields (or with a normative deviation from the average field size), for drawing up a scheme of crop rotation, and for the designation of forest strips and field roads (Kazmir, 2009). However, the designed fields are not always characterized by homogeneous quality indicators.

The organization of dynamic crop rotation begins with the very formation of homogeneous fields, which are understood not as equal sizes of the crop rotation fields, but as the most homogeneous (in terms of quality) areas with fixed boundaries. For each such field, depending on its geographical features, a unique scheme of crop rotation is adopted, as well as protective forest strips, field roads, etc. are designed. The boundaries of such fields remain stable, regardless of changes in the types of crop rotation or changes in the economic situation. The stability of the fields contributes to the monitoring of lands and to the accounting for material and energy resources used, unlike fields of static crop rotation, where these measures are impossible in a long run.

Therefore, the number of fields and their area in dynamic crop rotations is determined not by the structure of the cultivated areas and the period of crop rotations, but above all by the soil and climatic conditions of a certain area. Because of this, organizing such crop rotations requires more detailed information on the qualitative status of each field. In addition, the effectiveness of forming a dynamic crop

rotation system is related not only to the quality of the project design, but also to the annual operational work on these projects' introduction. Given the wide variety of organizational and economic forms of land tenure and land use, the diversity of soil and climatic conditions of the regions of the country, there is an urgent need to improve methodological approaches to the organization of crop rotation areas in market type agroformations and to their ecological and economic development.

The set of crops in short rotations is influenced by the specialization of the business entity, which, in turn, depends on the geographical zone's soil and climate, as well as the market conditions. Given that, in order to maximize the productivity of crops, it is necessary to achieve the highest possible level of compliance in the soil-plant system: the soil-climatic conditions must meet the agrobiological requirements of the crops grown. The practical implementation of this is to assess the suitability of arable land for a particular farm to grow crops.

From an economic point of view, an important criterion for the effectiveness of the projected crop rotation is crop yields and the level of profitability of crop production, which, in particular, amounted to 24.2% for cereals and legumes and 31,1% for sunflower in 2018. However, the ecological assessment of crop rotations requires considering the influence of the crop rotation on the basic parameters of soil fertility. Given the key role of humic substances in determining the level of soil fertility, it is advisable to carry out the balance calculations of these substances for environmental assessment of the projected crop rotation. In order to calculate the average annual balance of humic substances in the soil without taking into account the losses from erosion, the method of G. Chesniak was used (Batsula et al., 1987):

$$B_{a.a.} = \frac{(\sum N_1 + \sum N_2)}{t_r} - \frac{\sum M}{t_r},$$

where:

$B_{a.a.}$ - average annual balance of humic substances during a single crop rotation, t/ha;

N_1 - quantity of the new humic substances due to plant residues, t/ha;

N_2 - quantity of the new humic substances due to organic fertilizers, t/ha;

M - mineralization of humic substances under crops, t/ha;

t_r - rotation duration (time), years.

RESULTS AND DISCUSSIONS

Analysis of the process of organization of crop rotations in market-type agro formations showed that large agro holdings are focused on maximizing profits by expanding the cultivation areas of highly profitable crops. At the same time, the negative impact of the constant cultivation of such crops is overcome by the significant use of synthetic mineral fertilizers and pesticides, which will undoubtedly lead to socio-environmental problems in the near future (environmental pollution, deterioration of health of rural population, etc.). In their turn, farms, the vast majority of which use land plots of not more than 100 hectares, are unable to organize long-term crop rotations because of the short-term land lease. Therefore, for such farms, the optimal form of organization of their land use territory should be implemented through the introduction of highly specialized short-term crop rotations. Long-term crop rotations were justified in large collective farms, because they performed the function of providing maneuverability in the placement of crops (with respect to various soil and landscape factors), of providing more complete use of the bioclimatic potential of the territory, and contributed to the conservation and reproduction of fertility resources.

The result of the mapping and the land preparation work is the formation of a crop rotation area on the territory of the State Enterprise "Research Entity Skvyrske" of the Institute of Agroecology and Nature Management of the National Academy of Agrarian Sciences of Ukraine with a total area of 229.41 ha, where it is proposed to place a four-course arable crop rotation (average field size - 57.35 ha), which can be used as static or dynamic.

The territory of the studied entity is characterized by the soil and climatic conditions of the Skvyrske natural and agricultural district, which is located in the southwestern part of Kyiv region. The climate of the region is temperate continental, with an average temperature of -6.5°C in January and $+19.6^{\circ}\text{C}$ in July. In the summer, the air can warm up to $+30^{\circ}\text{C}$ and to cool down to -30°C in winter. Up to 550 mm of rainfall occur on average per year, and the rainfall is often stormy, which is a cause of increased erosion risk on sloping areas. The relief of the district is an undulating plain with a dense network of river valleys with numerous beams and ravines. Absolute heights of inter-river space vary within 230-250 m. The most common source rocks are water-glacial and glacial forest sediments. The northern part of the district is characterized by the dominance of the podzolic chernozem in the soil cover, and the low-humus chernozem is typical for the southern part. The valleys of beams and floodplains of rivers are covered with meadow and swamp soils. The upper root layer of chernozem soils has a humus content of 2.1-3.8%. Soils with high acidity occupy approximately a half of the arable areas of the district, which is why the average soil quality index of arable land is 55 points, perennial stands - 49, hayfields - 33, pastures - 30 points. In the soil cover of the studied entity, the light and medium loamy chernozem is rather typical, and 94.6% of it is untouched by the water erosion, 3.9% is lightly eroded, and 1.5% is medium-eroded. Accordingly, a qualitative assessment of these soil erosion differences is 56, 54 and 42 points.

The results of the agrochemical examination of the soil cover of the crop rotation area are shown in Table 1.

Table 1. Basic fertility parameters of the soil cover of the entity

№ of field	Humus, %			N			P ₂ O ₅			K ₂ O		
	mg/100 g of soil											
	1*	2*	Difference	1*	2*	Difference	1*	2*	Difference	1*	2*	Difference
I	2.90	3.14	+0.24	14.42	11.76	-2.66	15.71	11.23	-4.48	8.41	9.20	+0.79
II	2.92	3.14	+0.22	14.40	11.64	-2.76	15.87	11.35	-4.52	8.35	9.33	+0.98
III	2.94	3.16	+0.22	14.56	11.76	-2.80	15.65	11.47	-4.18	8.13	9.37	+1.24
IV	2.90	3.18	+0.28	14.42	11.39	-3.03	15.71	11.11	-4.60	8.47	9.25	+0.78
Average	2.91	3.15	+0.24	14.45	11.64	-2.81	15.73	11.29	-4.44	8.34	9.29	+0.95

*1 - data from the X round of the soil examination (2011-2015)

*2 - data from the XI round of the soil examination (2016-2020)

Source: developed by authors.

As shown in Table 1, the soil cover of the entity according to the results of the X round of agrochemical examination is characterized by an average content of humus, and of the XI round - increased, which is explained by an increase in the flow of organics to the soil and increase in its humification. A similar trend was observed for potassium metabolism, which increased by an average of 11.4%. At the same time, the content of mobile forms of nitrogen and phosphorus during the indicated period decreased by 19.4 and 28.2%, respectively, which is due to a significant decrease in the supply of these nutrients to the soil with synthetic mineral fertilizers.

These soil and climatic conditions are generally favorable for the cultivation of the majority of crops, in particular, winter wheat, barley, corn, sugar beet, sunflower, etc., which is confirmed by the data in Table 2. The data in Table 2 indicates the presence of high adaptive cultivation potential of the forest-steppe zones of the Kyiv region; the deep chernozem is suitable for such crops as basic cereals and industrial crops (I class of suitability), the meadow-chernozem soils for winter wheat, barley (I class of suitability), maize, sugar beet and sunflower (suitability class II).

Table 2. The suitability of the soil cover of the Kyiv forest-steppe zone for growing crops

Soil types	Net area		Class of suitability of arable land for crop cultivation:								
	in thousand hectares	%	winter wheat	winter rye	barley	maize	potato	sugar beets	sunflower	soy*	rapeseed*
Deep chernozem, 54 (light loamy soils, medium loamy soils)	621.3	33.1	I	I	I	I	-	I	I	I	I
Meadow and meadow-chernozem soils, 121 (light loamy soils), 124 (hard loamy soils)	125.0	6.6	I	II	I	II	-	II	II	III	III
Shallow chernozem, 52 (sandy loam soil), 53 (medium loamy soils)	89.7	4.8	II	I	II	II	-	II	II	II	II

*authors' own calculations
Source: Dobriak et al., 2009.

In order to improve the quality of the soil cover, it is necessary to plant perennial legumes (clover or alfalfa) which, due to the deep root system, can transfer calcium from the lower layers of soil and from the source rock, and accumulate it in the root layer. In addition, increasing the area under perennial legumes is an important measure to preserve and restore soil fertility, especially soil with an increased erosion risk on slopes of 3 to 5°.

The high agricultural potential of the above-mentioned crops in the Kyiv region is confirmed by the State Statistics Committee of Ukraine. Its 2018 data shows that wheat yield averaged 46.7 q/ha on the area of 190.1 thousand hectares (16.0% of the total cultivated area of the region), maize corn - 97.2 q/ha on an area of 290.9 thousand hectares (24.4%), soybeans - 25.8 q/ha on an area of 135.1 thousand hectares (11.3%), sunflower - 29.7 q/ha on an area of 191.7 thousand hectares (16.1%); sugar beet - 606,0 q/ha on the area of

23.1 thousand hectares (1.9% of the total cultivated area of the region) (State Statistics Service of Ukraine, 2019).

Given that, the following set of crops is suggested for the projected crop rotation field: field I - half a field - perennial grasses, another half - legumes; II - winter cereals; III - half a field - sugar beets, another half - maize corn; IV - half a field - spring cereals, another half - oilseeds. In this crop rotation, the direct use of arable land is one. In this crop rotation, the index of the direct use of arable land equals to one. At the same time, cereals and legumes occupy 62.5% of the crop rotation area, including winter crops - 25% and industrial crops - 25% (12.5% of them are the sugar beets and 12.5% - oilseeds). The main products of almost all of the listed crops can be sold outside the enterprise as commodities.

In this order, crop rotations are all placed after the best precursors: winter wheat - after perennial grasses and legumes, sugar beets and

maize corn - after winter cereals, spring corns - after sugar beets, oilseeds - after maize corn. The crop set of this crop rotation allows one to maintain the optimum periodicity of the return of a particular crop to its previous location. In particular, in order to return oilseeds (such as sunflowers) and sugar beets to their previous place of cultivation not after 4 years, but after 8 years, it is necessary to exchange perennial leguminose grasses and leguminous crops after every rotation in the first field (from which the rotation starts). In addition, it is possible to expand or reduce the acreage of a particular crop in such crop rotation (but without disruption of the accepted order of crop placement) in accordance with the market situation, weather peculiarities of the year, organizational and economic reasons. If necessary, such crop rotation has the opportunity to change the set of cultures. For example, the composition of the spring crops can be wheat, barley, buckwheat, millet, and

among the oilseeds - sunflower, rapeseed, soybeans.

For the ecological assessment of the projected crop rotation, we used indicators of the annual average balance of humic substances in the soil, the initial data for which is given in Table 3.

Given the indicators of new humic substances (ΣN_i) and mineralization of humic substances (ΣM) shown in Table 3, the humus balance in this rotation without organic fertilizers ($\Sigma N_2 = 0$) is:

$$B_{a.a.} = \frac{(3,38 + 0)}{4} - \frac{3,25}{4} = 0,03 \text{ t/ha}$$

Therefore, a crop set of this crop rotation can provide a non-deficit balance of humic substances in the soil even without the use of organic fertilizers.

The additional application of 10 tons of organic fertilizers per 1 ha of cultivated area will provide an annual increase in humus of 0.02%, and 20 t/ha respectively 0.03%.

Table 3. Humus balance in a projected four-year crop rotation

№ of field	Culture	Crop yield, q/ha	Coefficient of plant residues	Coefficient of humification	New humic substances (N_i), t/ha	Mineralization of humic substances (M), t/ha
1	½ clover	222	0.20	0.25	1.11	0.30
	½ pea	24	0.80	0.23	0.44	0.80
2	winter wheat	47	1.10	0.25	1.29	0.70
3	½ sugar beets	606	0.04	0.10	0.24	1.50
	½ maize corn	97	0.80	0.20	1.55	1.10
4	½ buckwheat	16	1.10	0.22	0.39	0.60
	½ soy	26	0.80	0.23	0.48	0.80
Σ					3.38	3.25

Source: developed by authors.

CONCLUSIONS

The basis for an effective organization of modern crop rotations is a harmonious combination of environmental and economic aspects. The environmental aspect lies within the possibility of reducing the level of use of synthetic mineral fertilizers and pesticides on the soil cover due to scientifically based crop rotation, resulting in a normative state of the environment. The economic aspect involves adapting a set of cultivated crops to the changing conditions of the market environment, because the ultimate goal of any producer is to maximize profits by growing highly profitable crops.

In its turn, the highly productive crop rotation contributes to ensuring the food security of the country, increases revenues to local budgets, and helps to maintain the health of rural population through the safe environment, as well as to maintain the health of the urban population through the high-quality agricultural products. The only mechanism for solving this problem is the development and implementation of land management projects for ecological and economic justification of land regulation and crop rotation. The use of crop rotations with short rotation period is promising in market conditions for small land-use areas. The main methodological approach to the organization of these areas is the

formation of homogeneous (by the soil cover) lands and a set of crops based on the assessment of the suitability of arable land for their cultivation.

The introduction of the short-term crop rotations will allow farmers to address issues related to the reduction of crop rotation development time, to provide a high level of specialization in cultivation of market-oriented crops, to reduce the set of tools and machines for crop production and costs for their maintenance and operation, to simplify the management system of the crop care processes, to use the land efficiently under short-term lease conditions through the correct combination of static and dynamic crop rotations. The latter one will allow to get out of strict frameworks (restrictions) of static crop rotations and to provide economically feasible (taking into account the changing market conditions of the crop production) and environmentally safe (preserving the quality of land through the selection of the best agro-technical variants of growing separate crops and prompting introduction of new technological developments in the practice of agricultural production) use of crop rotation arrays.

REFERENCES

- Batsula, O., Holovachov, Ye., Derevianko, R. (1987). Zabezpechennia bezdefitsytnoho balansu humusu v hruntii [Providing a non-deficit balance of humus in the soil]. Ukraine, Kyiv: Urozhai, 128.
- Brazhenko, I., Brazhenko, L. (2008). Optymalni sivozminy Lisostepu [Optimal crop rotations of the forest-steppe zone]. *Propozytsiia*. Retrieved March 24, 2020, from <https://propozitsiya.com/ua/optimalni-sivozmini-lisostepu-povna-versiya>
- Davis, A.S., Hill, J.D., Chase, C.A., Johanns, A.M., Liebman, M. (2012). Increasing Cropping System Diversity Balances Productivity, Profitability and Environmental Health. *PLOS ONE* 7(10), e47149. Retrieved March 24, 2020, from <https://doi.org/10.1371/journal.pone.0047149>
- Dobriak, D., Kanash, O., Babmindra, D., Rozumnyi, I. (2009). Klasyfikatsiia silskohospodarskykh zemel yak naukova peredumova yikh ekolohobezpechnoho vykorystannia [Classification of agricultural land as a scientific prerequisite for its environmentally friendly use]. Ukraine, Kyiv: Urozhai, 464.
- DSTU 4115: 2002 Hrunty. Vyznachennia rukhomykh spoluk fosforu I kaliu za modufikovanyim metodom Chyrykova [DSTU 4115: 2002 Soils. Determination of mobile phosphorus and potassium compounds by the modified Chirikov method] (2002). Retrieved March 23, 2020, from http://online.budstandart.com/ua/catalog/doc-page?id_doc=58863
- DSTU 4289: 2004 Yakist hruntu. Metody vyznachennia orhanichnoi rehovyny (method Tiurina) [DSTU 4289: 2004 Soil quality. Methods for Determining Organic Substance (Tiurin Method)] (2004). Retrieved March 23, 2020, from http://online.budstandart.com/ua/catalog/doc-page?id_doc=56400
- DSTU 7863: 2015 Yakist hruntu. Vyznachennia lehkohidralizovanoho azotu metodom Kornfilda [DSTU 7863: 2015 Soil quality. Determination of easily hydrolysed nitrogen by the Cornfield method] (2015). Retrieved March 23, 2020, from <https://dbn.co.ua/load/normativy/dstu/7863/5-1-0-1777#load>
- Erismán, J.W., van Eekeren, N., de Wit, J., Koopmans, C., Cuijpers, W., Oerlemans, N., Koks, B.J. (2016). Agriculture and biodiversity: a better balance benefits both. *Agriculture and Food*, 1, 157–174. Doi: 10.3934/agrfood.2016.2.157
- Frison, E.A., Cherfas, J., Hodgkin, T. (2011). Agricultural Biodiversity Is Essential for a Sustainable Improvement in Food and Nutrition Security. *Sustainability*, 3, 238–253. Retrieved March 25, 2020, from <https://doi.org/10.3390/su3010238>
- Kazmir, P. (2009). Orhanizatsiia silskohospodarskoho vykorystannia zemel na landshaftno-ekolohichnii osnovi: monohrafiia [Organization of agricultural land use on landscape-ecological basis: monograph]. Ukraine, Lviv: Spolom, 254.
- Kazmir, P., Chernetskyi, P., Kazmir, L. (2014). Metodychne zabezpechennia orhanizatsii systemy sivozmin v umovakh formuvannia dualistichnoi modeli silskohospodarskoho vyrobnytstva v Ukraini [Methodological support for the organization of crop rotation system in the conditions of formation of dualistic model of agricultural production in Ukraine]. *Teoretychni osnovy i praktychni aspekty vykorystannia resursooschadnykh tekhnolohii dlia pidvyshchennia efektyvnosti ahropromysloвого vyrobnytstva i rozvytku silskykh terytorii: materialy mizhnarodnogo naukovopraktychnoho forumu*. Ukraine, Lviv: LNAU, 180–185.
- Kovalenko, N. (2007). Optyimizatsiia struktury posivnykh ploshch i spetsializovanykh sivozmin metodom ekonomiko-matematichnogo modeliuvannia [Optimization of acreage structure and specialized crop rotation by economic and mathematical modeling]. *Zbirnyk naukovykh prats instytutu tsukrovyykh buriakiv NAAN*, 9, 245–251.
- Lebid, Ye., Andrusenko, I., Pabat, I. (1992) Sivozminy pry intensyvnomu zemlerobstvi [Crop rotations in intensive agriculture]. Ukraine, Kyiv: Urozhai, 224.
- Poleiko, T. (2013). Zemelkorystuvachi ne pospishaiut vprovadzhuvaty sivozminy, a shukaiut, yak obmynuty zakon [Land users are in no hurry to implement rotations but are looking for a way around the law]. *Zemlevporiadnyi visnyk*, 4, 10–11.
- Roest, K., Ferrari, P., Knickel, K. (2018). Specialization and economies of scale or diversification and

- economies of scope? Assessing different agricultural development pathways. *Journal of Rural Studies*, 59, 222–231. Retrieved March 25, 2020, from <https://doi.org/10.1016/j.jrurstud.2017.04.013>
- Saiko, V.F., Boiko, P.I. (2002). Sivozminy u zemlerobstvi Ukrainy [Crop rotation in Ukrainian agriculture]. Ukraine, Kyiv: Ahrarna nauka, 146.
- Shevchenko, O.V., Openko, I.A., Tsviakh, O.M. (2017). Ekonomichni peredumovy chervuvannia kultur yak sposib zapobihannia dehradatsii ahrolandshaftu [Economic background crop rotation as a way to prevent the degradation of agricultural landscapes]. *Land Management, Cadastre and Land Monitoring*, 2, 58–65. Retrieved March 24, 2020, from <http://dx.doi.org/10.31548/zemleustriy2017.02.058>
- State Statistics Service of Ukraine (2019). Statystychnyi shchorichnyk Ukrainy za 2018 rik [Statistical Yearbook of Ukraine for 2018]. Ukraine, Zhytomyr: TOV «BUK-DRUK», 482. Retrieved March 24, 2020, from http://www.ukrstat.gov.ua/druk/publicat/kat_u/2019/zb/11/zb_yearbook_2018.pdf

GENETIC ANALYSIS OF WET GLUTEN CONTENT IN GRAIN IN DIALLEL CROSS OF DURUM WHEAT

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Abstract

In the period 2014-2016, a diallel cross design was performed and harvested without the reciprocal crosses. The study includes five modern durum wheat varieties: Victoria (BG), Deni (BG), Superdur (AT), Progress (BG), Predel (BG). Parents and hybrids were sown by block method with three replications in the experimental field of the Field Crops Institute in Chirpan, Bulgaria. The trait studied was wet gluten content in grain. The results of the technological analysis were statistically processed by Hayman - Jinks methods. Both the additive and dominant parameters have influenced the inheritance of the studied character. The dominant parameters are higher and they have a greater role in inheritance. To increase the wet gluten content, recessive genes are accumulated in the genotype. Heritability coefficients indicate that efficient genotype selection is possible by phenotype, but it should start in the later segregated generations. The graphical analysis points out that the trait is under the control of an additive-dominant genetic system with manifestations of epistasis. In all cases the epistasis is of a complementary type with involved overdominance.

Key words: breeding, durum wheat, genetics, inheritance, wet gluten.

INTRODUCTION

Inheritance of quantitative traits is the subject of numerous scientific studies. Knowledge of genetic architecture and the inheritance of various traits is of particular importance for the development of varieties that meet modern requirements. The use of diallel crosses and the information received from them gives a clear idea of the manipulations that may be carried out on the trial objects. Studies are topical since the manifestation of various components in inheritance is essential to set the right strategy for breeding in durum wheat. Knowledge about the genetic capabilities of various genotypes is the basis of their effective use as donors in the development of new high-quality varieties. The prevailing content of dominant or recessive genes in parents is critical to their breeding value. Determining what kind of genes are accumulated to increase the magnitude of the trait allows for an effective estimate defining the rate of the expected result. The results of various studies exhibit a diversity of inheritance mechanisms for wet gluten content in grain. Rathod et al. (2008); Singh et al. (2009) report that additive and dominant parameters are significant in the inheritance of the wet gluten content in grain. According to

Akram et al. (2007) wet gluten content is influenced by dominant gene effects with preponderance of overdominance and manifestations of non-allelic interactions. They conclude that this will hinder effective selection in earlier generations. The correlation between the mean value of parents and their sum (V_r+W_r) indicated a tendency for high wet gluten content to be associated with accumulated of dominant genes. Sadeghi et al. (2013) determine that non-additive gene effects have a greater role in inheritance of the wet gluten content. According to them, the inheritance of the character exhibits a simple additive-dominant genetic system with overdominance and the trait values increase with the accumulation of recessive genes. The authors note that effective selection has to begin in later segregated generations. The calculation of the heritability coefficients determines the opportunities both for running efficient selection and the appropriate units to start it from. Aydogan and Yagdi (2004) examine a 6 x 6 diallel cross to establish the genetic nature of the wet gluten content in grain. They find significant D, H_1 , and H_2 parameters, noting that the dominant ones are higher. They establish medium heritability coefficients in narrow sense and high ones in

broad sense. From the graphical diallel analysis they report that partial dominance is fundamental to inheritance.

The results presented by the various authors show that in the inheritance of the character of great importance are the parents used and they determine the differences in the genetic architecture of the hybrids. This necessitates the study of this trait in durum wheat with modern genotypes. The aim of this study is to investigate the genetic components and their variances for wet gluten content in the grain. Receiving inheritance information for this trait will allow recommendations to be made to lead an effective selection and speed up the breeding process.

MATERIALS AND METHODS

The study was conducted under field conditions in the Field Crops Institute, Chirpan. The experiment included five durum wheat varieties: Victoria (BG), Deni (BG), Superdur (AT), Progress (BG) and Predel (BG) and 10 crosses with them. A diallel cross was made and tested for three years (2014, 2015 and 2016). This allowed the cultivation of three generations - F₁ and two F₂. The experiment was carried out by a block method in three replications by the approved technology for growing durum wheat. The length of the row was 2 m, the row spacing was 20 cm and in the row 5 cm. 20 plants from parents and F₁ generation were randomly plucked off and from F₂ - 30 plants. The grain from the replication was pooled and used for technological analysis under laboratory conditions. The studied trait was wet gluten content in grain from an average sample for each replication and the technological analysis was carried out under BDS EN ISO 21415-2: 2008. Half of the seeds of F₁ were used to sow F₂ generation.

The obtained results were processed with graphic and parametric diallel analysis by Jinks (1954); Hayman (1954; 1957); Mather (1967). The obtained results are processing using the publication of Aksel and Johnson (1962) for diallel analysis. Suitability of data for diallel analysis was assessed by the regression coefficient *b* and *t* (*W_r-V_r*) (Mather and Jinks, 1982; Singh and Chaudhary, 1985). Heritability

coefficients were calculated by the formulas of Mather and Jinks (1982). The calculated parameters are as follows: *D* - Component of genetic variance due to additive effect of genes, *H₁* - Components of genetic variance due to dominance effect, *H₂* - Proportion of dominance variance due to the positive and negative effects of the genes in the parents, *h₂* - Dominance effects, as algebraic sum over all the loci in heterozygous phase in all the crosses, *F* - Co-variance of additive and non-additive effects in a single array. It may be positive or negative. The parameters served for calculation of indicators: (*H₁/D*)^{1/2} - Mean degree of dominance, *H₂/4H₁* - Proportion of genes with positive and negative effects on the parents, *K_d/K_r* - Proportion of dominant and recessive genes in the parents, *h₂/H₂* - number of groups of genes with positive and negative effects on the parents, *r_{Y_r(W_r+V_r)}* - correlation coefficient between the value of the parent and their respective sum *W_r + V_r*, indicate the direction of action of the dominant or recessive factors.

RESULTS AND DISCUSSIONS

The variance analysis exhibits reliable differences between genotypes in the three generations F₁ and the two F₂ (See Table 1). This allows the data to be processed statistically and to obtain significant information from them. Table 2 lists the genetic parameters and indicators by Hayman (1954); Jinks (1954) from the diallel cross for all tested cases.

Table 1. Mean squares (MS) from ANOVA by years

Source of variation	F ₁ -2014 MS	F ₁ -2015 MS	F ₁ -2016 MS	F ₂ -2015 MS	F ₂ -2016 MS
Genotype	20.13***	8.93**	18.58***	9.79**	12.47**
Replication	21.88***	6.37*	12.52***	36.96**	18.57**
Error	0.44	3.45	1.57	2.37	3.55

*P >0.05; **P >0.01; ***P >0.001

Parameter *D* has significant values for the F₁ and F₂ generations (Table 2). These reliable values show the remarkable impact of the additive gene action in the inheritance of the character. That correlates to the results obtained by other authors (Aydogan and Yagdi, 2004; Rathod et al., 2008; Singh et al., 2009). The table presents the dominant parameters of *H₁*

and H_2 , which are also significant. They are greater than (D) and prove that dominance has a major role in the inheritance of the wet gluten content in grain (See Table 2). Other researchers also report proven dominant

parameters from experiments with diallel analysis for wet gluten content (Aydogan and Yagdi, 2004; Rathod et al., 2008; Singh et al., 2009).

Table 2. Genetic parameters and indicators for wet gluten content in grain of durum wheat from the diallel cross

Parameters and indicators	F ₁ - 2014 y.	F ₁ - 2015 y.	F ₁ - 2016 y.	F ₂ - 2015 y.	F ₂ - 2016 y.
D	6.54 ± 0.37	1.61 ± 0.59	5.14 ± 3.25	1.55 ± 0.73	4.42 ± 1.76
F	-2.25 ± 0.93	-2.02 ± 1.47	10.48 ± 8.13	-1.30 ± 1.84	9.72 ± 4.42
H ₁	13.04 ± 1.01	3.70 ± 1.59	30.87 ± 8.79	5.79 ± 1.99	20.83 ± 4.78
H ₂	12.70 ± 0.92	3.76 ± 1.44	23.20 ± 7.97	5.14 ± 1.80	14.99 ± 4.33
h ₂	20.27 ± 0.15	-0.10 ± 0.24	2.76 ± 1.32	2.05 ± 0.30	0.87 ± 0.72
(H ₁ /D) ^{1/2}	1.41	1.51	2.45	1.93	2.17
H ₂ /4H ₁	0.24	0.85	0.18	0.22	0.17
Kd/Kr	0.78	0.41	2.42	0.64	3.05
h ₂ / H ₂	1.59	0.02	0.11	0.39	0.05
r _{Yr_i(Wr+Vr)}	0.28	-0.82	0.78	0.26	0.78
H ²	0.94	0.69	0.91	0.7	0.74
h ²	0.55	0.45	0.15	0.40	0.05

D - Component of genetic variance due to additive effect of genes, H₁ - Components of genetic variance due to dominance effect, H₂ - Proportion of dominance variance due to the positive and negative effects of the genes in the parents, h₂ - Dominance effects, as algebraic sum over all the loci in heterozygous phase in all the crosses, F - Covariance of additive and non-additive effects in a single array. (H₁/D)^{1/2} - Mean degree of dominance, H₂/4H₁ - Proportion of genes with positive and negative effects on the parents, Kd/Kr - Proportion of dominant and recessive genes in the parents, h₂/H₂ - number of groups of genes with positive and negative effects on the parents, r_{Yr_i(Wr+Vr)} - correlation coefficient between the value of the parent and their respective sum Wr + Vr, indicate the direction of action of the dominant or recessive factors, H² - broad sense heritability coefficient and h² - narrow sense heritability coefficient.

A number of authors find major impact of the dominant parameters, expressed by greater dominant parameter than the additive one (Aydogan and Yagdi, 2004; Akram et al., 2007; Sadeghi et al., 2013). The mean degree of dominance presented by the parameter (H₁/D)^{0.5} exhibits that overdominance has a major role in the inheritance of the character. Other researchers also report the same results for this trait (Akram et al., 2007; Sadeghi et al., 2013). The values of the parameter F suggest that positive genes are not particularly balanced. In cases where the parameter F has a negative sign, it indicates that there are more recessive genes. The values of indicator (H₂/4H₁) (which differ by 0.25) confirm the uneven distribution of genes with positive and

negative effects on parents. The exception is only F₁-2014 where this indicator has values very close to 0.25. The ratio of the number of dominant to the number of recessive genes Kd/Kr shows that recessive genes dominate over the dominant genes in F₁-2014, F₁-2015, and F₂-2015, while in 2016 it is the opposite in the F₁ and F₂ generations. This is confirmed by other researchers such as Mandloi et al. (1974) and Akram et al. (2007). Throughout all years of study, the component H₁ is greater than H₂, indicating that positive and negative alleles in the loci exhibiting dominance in parents are not proportional. It should be borne in mind that H₁ and H₂ differ reliably with proven values. For the F₁-2015 case only, the H₂ parameter is greater than H₁. The indicator (h₂/H₂) refers to

the participation of 1 gene or group of genes exhibiting dominance in the control of the trait, except for F₁-2014 where the involvement of two genes or a group of genes is assumed. Other authors also report the involvement of a gene or a group of genes in inheritance of trait (Aydogan and Yagdi, 2004; Akram et al., 2007). The correlation coefficient ($r_{Yr:(Wr+Vr)}$) between the magnitude of the trait in parents and their sum (Wr+Vr) shows that the increase of the amount of wet gluten in the grain is connected to the accumulation of recessive genes in the genotype (Table 2). Authors Aydogan and Yagdi (2004) also reported that the increase in trait values has resulted from the accumulation of recessive genes. Only in F₁-2015 the correlation coefficient changed its sign, which points to the phenomenon of redefinition of the genetic formulas of the trait. This indicates that under different environmental conditions, the increase of the trait depends both on dominant and recessive genes. Akram et al. (2007) also report the accumulation of dominant genes would increase the values of the trait. The obtained heritability coefficients in broad sense H^2 are high in F₁-2014 and F₁-2016 and medium in F₁-2015, F₂-2015, and F₂-2016, which means that it is possible to conduct efficient genotype selection by phenotype. Others also report such heritability coefficients in broad sense (Aydogan and Yagdi, 2004; Khodadadi et al., 2012). Heritability coefficients in narrow sense h^2 are from low to medium. These coefficients denote that selection needs necessarily to start in later segregated generations. This conforms to the results obtained by a number of other authors (Vallega, 1985; Blanco et al., 2002; Aydogan and Yagdi, 2004; Khodadadi et al., 2012). Researchers Akram et al. (2007); Sadeghi et al. (2013) also reported that selection by wet gluten content in grain will be hindered in the early segregated generations and they recommend to start it in the later ones. The graphic diallel analysis gives the visual idea about the genetic nature of the trait in the

parents used. On Figures 1, 2, 3, 4 and 5, one can see a graphical analysis of individual environments by generation and year: F₁-2014, F₁-2015, F₁-2016, F₂-2015, F₂-2016. Figures 1, 2 and 5 (F₁-2014, F₁-2015, F₂-2016) show that the wet gluten content in durum wheat is determined by a simple additive-dominant genetic system since b differs reliably from 0 and does not differ from 1. The two cases shown on Figures 3 and 4 (F₁-2016 and F₂-2015) illustrate that complementary epistasis is involved in the inheritance of the character.

This becomes clear from the regression coefficient (b), which has values reliably different from 1 and inclines the regression line down to the abscissa (Vr). Akram et al. (2007); Sadeghi et al. (2013) also report that the trait is under the control of a simple additive-dominant genetic system with manifestations of epistasis. In F₁ regression line crosses Wr below the start of F₁-2014 (Figure 1) and around the start of F₁-2015 (Figure 2) and F₁-2016 (Figure 3). This exhibits that the inheritance in F₁ generation is dominated by complete dominance and only in F₁-2014 over dominance prevails. For F₂-2015 (Figure 4) and F₂-2016 (Figure 5) partial dominance and over dominance in the expression of the trait are respectively expressed in inheritance. Aydogan and Yagdi (2004) report about the manifestation of partial dominance. The figures present the location of the parental points through which it can be determined which varieties contain more dominant and which more recessive genes. The graphs show that parental points exchange their places in different years and generations, which is explained by the genotype-environment interaction. These parents, who are closer to the beginning of the coordinate system, contain more dominant ones, and vice versa, those who are closer to the interception point of the regression and the parabola contain more recessive ones.

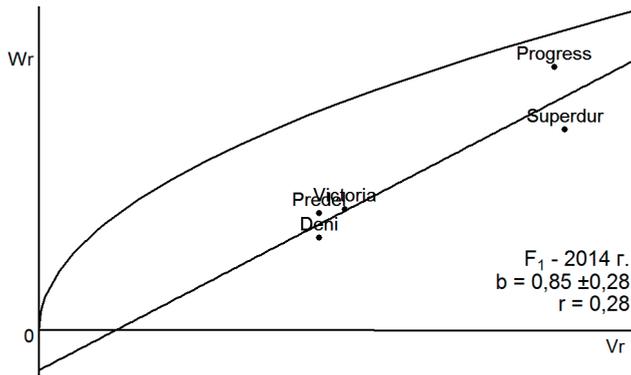


Figure 1. Graphical diallel analysis for wet gluten content in F₁-2014 year; Wr - Mean parent-offspring covariance of arrays; Vr - Mean variance of arrays; b – regression coefficient

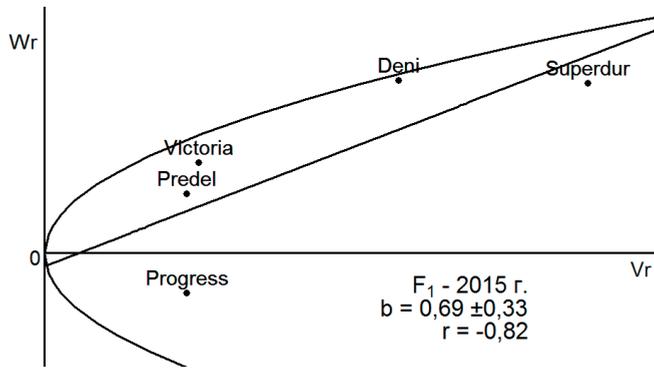


Figure 2. Graphical diallel analysis for wet gluten content in F₁-2015 year

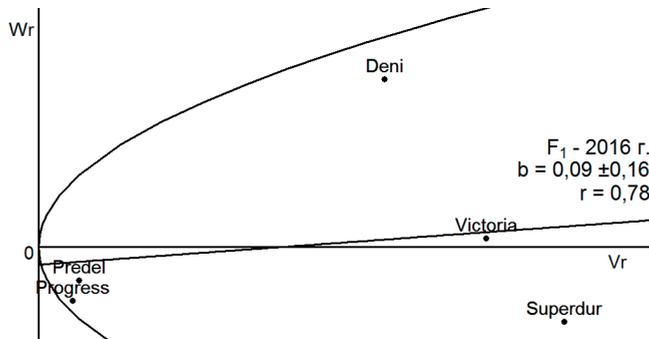


Figure 3. Graphical diallel analysis for wet gluten content in F₁-2016 year

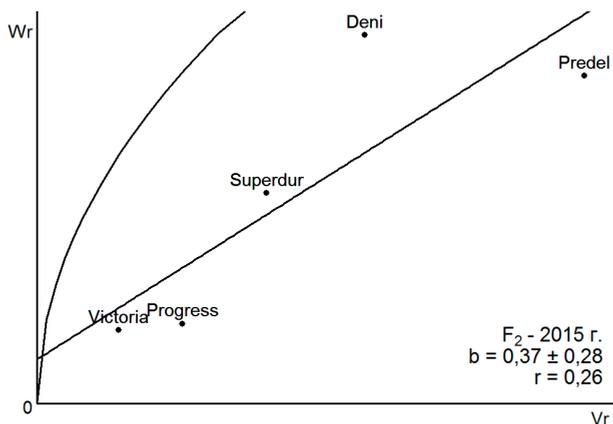


Figure 4. Graphical diallel analysis for wet gluten content in F₂-2015 year

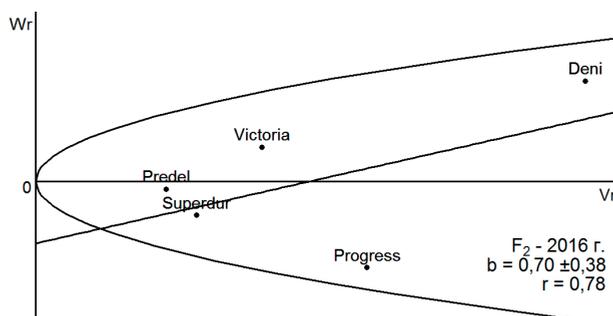


Figure 5. Graphical diallel analysis for wet gluten content in F₂-2016 year

In F₁-2014 (Figure 1), varieties Deni, Victoria and Predel had the greatest number of dominant genes, while the rest move away and occupy a more distant position from the beginning (or closer to the parabola and regression intersection point) and therefore contain more recessive genes. In the F₁-2015 (Figure 2) case, varieties Victoria, Predel and Progress have more dominant genes, while Superdur variety contains more recessive genes. In the third year F₁ (figure 3) the studied varieties are located close to the beginning of the coordinate system and contain more dominant genes. In the F₂ generations (Figures 4 and 5) varieties are located relatively close to the beginning of the coordinate system. The impression is that Superdur variety changes its place and in F₂ it contains more recessive genes. Victoria and Progress varieties retain their positions against the F₁ generation and therefore contain more dominant genes. Deni and Predel varieties are located in the farthest part, indicating that they contain more recessive genes for this trait.

CONCLUSIONS

The results obtained lead to the following conclusion. The trait wet gluten content in the grain is controlled by a simple additive-dominant genetic system.

In some cases, inheritance control is achieved by the involvement of non-allelic interactions, too, in the form of complementary epistasis. Partial to overdominance in the inheritance of the character wet gluten content in grain is observed. It has been found that increasing the values of the wet gluten content trait is associated with the accumulation of recessive genes in the genotype.

The heritability coefficients make it apparent that efficient genotype selection is possible by phenotype, but it should begin in later segregated generations.

REFERENCES

- Akram, Z., Ajmal, S. U., Kiani, A.A., Jamil, M. (2007). Genetic analysis of protein, lysine, gluten and flour yield in bread wheat (*Triticum aestivum* L.). *Pakistan Journal of Biological Science*, 10, 1990–1995.
- Aksel, R., Johnson, L. P. V. (1962). Analysis Of a diallel cross. A Worked out example. *Advances Frontier Plant Science*, 2, 37–64, Program software.
- Aydogan, E., Yagdi, K. (2004). Determination of inheritance of some yield and quality traits in winter wheat (*Triticum aestivum* L.), *Biotechnology & Biotechnological Equipment*, 18(2), 194–200.
- Blanco, A., Pasqualone, A., Troccoli, A., Di Fonzo, N., Simeone, R. (2002). Detection of grain protein content QTLs across environments in tetraploid wheats. *Plant Molecular Biology*, 48, 615–623.
- Hayman, B.I. (1954). The theory and analysis of diallel crosses. *Genetics*, 39, 789–809.
- Hayman, B.I. (1957). Inheritance, heterosis, and diallel crosses. *Genetics*, 42, 330–355.
- Jinks, J.L. (1954). The analysis of continuous variation in diallel cross of *Nicotiana rustica* varieties. *Genetics*, 39, 767–788.
- Khodadadi, E., Aharizad, S., Sabzi, M., Shahbazi, H., Khodadadi, E. (2012). Combining ability analysis of bread quality in wheat. *Annals of Biological Research*, 3(5), 2464–2468.
- Mandloi, K.C., Rao, M. V., Austin, A. (1974). Inheritance of two baking quality characteristics in common wheat. *Indian Journal of Genetics and Plant Breeding*, 34, 646:646.
- Mather, K. (1967). Complementary and duplicate gene interactions in biometrical genetics. *Heredity*, 22, 97–103.
- Mather, K., Jinks, J. L. (1982). Biometrical genetics, study of continuous variation, 3rd edition., London, New York, Chapman and Hall, pp 396–403.
- Rathod, S., Dobariya, L., Jivani, L., Ponkia, H.P. (2008). Analysis for test weight, protein content and grain yield in six crosses of bread wheat (*Triticum aestivum* L.). *Advances in Plant Science*, 21(1), 99–101.
- Sadeghi, F., Dehghani, H., Najafian, G., Aghae, M. (2013). Estimation of genetic structure traits attributes bread-making quality in bread wheat (*Triticum aestivum* L.) using GGEbiplot and diallel method. *Cereal Research Communication*, 2(4), 263–277.
- Singh, K., Gupta, P.K., Tewari, N. (2009). Combining ability analysis for yield and quality traits in spring wheat (*Triticum aestivum* Linn. Emend Thell). *Progressive Agriculture*, 9(2), 235–241.
- Singh, R.K., Chaudhary, B.D. (1985). Biometrical methods in quantitative genetic analysis. Kalyani Publishers Ludhiana, New Delhi.
- Vallega, V. (1985). Identification of a major protein gene compatible with high grain yields in semi-dwarf *Triticum durum* genotypes. *Cereal Research Communication*, 13, 201–207.

RESEARCH REGARDING CEREAL AUTHENTICATION BY USING EFFICIENT METHODS OF ANALYSIS. A REVIEW

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Abstract

Cereals are found in many foods. Testing their authenticity is necessary to comply with the rules of labeling and to avoid unfair competition. To protect consumers, European Union (EU) law requires labeling of ingredients that cause allergies or intolerances, especially for cereals containing gluten, such as wheat (including common wheat, durum wheat, spelled wheat), rye, barley, oats or their hybridized strains and products thereof (OJEU, 2011; OJEU, 2014). Thus, the identification of cereals in a product is of paramount importance, not only to prevent the risks related to food safety in sensitive or allergic persons but also to avoid economic fraud.

Key words: authenticity, *Triticum aestivum*, PCR methods, molecular characterisation, spectroscopy.

INTRODUCTION

Cereals are present in many food products. Authenticity testing it is necessary in order to comply to labelling norms and avoid unfair competition.

EU regulations impose mentions on products labels of ingredients that could induce allergies or intolerance reactions, especially for gluten presence in such as wheat (including common wheat, durum wheat, spelled wheat), rye, barley, oats or their hybridized strains and products thereof (OJEU, 2011; OJEU, 2014).

Thus, the identification of cereals in a product is of paramount importance, not only to prevent the risks related to food safety in sensitive or allergic persons but also to avoid economic fraud (James and Schmidt, 2004).

The authenticity of cereals has been based in recent years on high performance techniques analyzing either protein content or DNA (Hernandez et al., 2005; Tavoletti et al., 2009; Bottero and Dalmaso, 2011).

The use of Real time PCR with TaqMan samples is a good alternative when specific and sensitive detection of the smallest DNA fragments is required, such as in processed foods (Bottero and Dalmaso, 2011; Hernandez et al., 2005; Tavoletti et al., 2009).

The increased number of researches generated the need of systematic reviews (Simpkins and Harrison, 1995). However new results make necessary new reviews.

MATERIALS AND METHODS

The present article reviews some of the main researches in cereal authenticity, focused on wheat. There are considered genetic studies, different analysis technics, including spectrometry or PCR. The selection considered also a diachronic approach for PCR studies section, in order to suggest the evolution of researches focuses in the field.

RESULTS AND DISCUSSIONS

Luo et al. (2015), Li et al. (2016) used the method of element analyser-stable isotope ratio mass spectrometry, in order to discriminate the geographical origin of wheat, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Studies on magnetic field effect on cell differentiation on different wheat genotypes showed that differences between wheat genotypes and level of magnetic fields were significant (Kahrizi et al., 2013). Brescia et al. (2002) established that the isotopic signature can be used to develop reliable fingerprints for

regional determination. IR spectrometry was used in order to develop simple, rapid technology to determine the origin of products (González-Martín et al., 2014; Zhao et al., 2013), as well as to determine wheat species (Ziegler et al., 2016). Fuzzy chromatographic mass spectrometry proved to be efficient in discriminate between whole wheat and refined wheat flours (Geng et al., 2016). Koenig et al. (2015) used HPLC technics to classify spelt cultivars, from 'typical spelt' to 'similar to common wheat'.

Mass spectrometry, combined with polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis were used for a proteomic study to characterize serpin polymorphisms along 177 Australian and 19 foreign hexaploid as well as 6 tetraploid wheat varieties (Wu et al., 2012).

Konieczny et al. (2005), studied the extracellular matrix surface network transformation during plant regeneration for wheat anther culture. Microscopic observations revealed two distinct types of cells on the callus surface, arranged in multicellular clusters.

Determination of multi-element composition of wheat proved to be effective in developing a fingerprint of geographical origin (Zhao et al., 2013).

Popping (2002), Pauli et al. (2014), Zörb et al. (2009) used chemical methods to determine authenticity of wheat, or adulterations, including for *Triticum aestivum* L.

Voorhuijzen et al. (2011), developed a padlock probe ligation and detection method, a DNA-based multiplex detection tool to determine traceability and authenticity for crop plant materials, wheat included.

Mass spectrometry proves to be an effective method in contaminants and food adulteration detection (Gharechahi et al., 2016).

Escherichia coli expression, Western blotting and tandem mass spectrometry were used to identify and confirm authenticity of two novel x-type HMW-GS from wheat line CNU608, as possibly originated from one octapeptide deletion and two unequal cross-over events (Wang et al., 2016; Liang et al., 2015).

Genetic studies

Wang et al., 2000, contributed to the first comprehensive analysis made of restriction

fragment length ploimorphism of the mitochondrial (mt) DNA of *Triticum aestivum* L. This led to clarification of the nature of mtDNA variability.

Maat, 2001, offers an overall analysis on genetic researches and stakeholders cooperation in wheat breeding in Netherlands, as a best practice experience.

Genetic analysis of Russian wheat, the history of prebreeding studies and the genetic diversity evaluation is reviewed by Mitrifanova (2012), Novoselskaya-Dragovich et al. (2015).

Results of some new approaches, like "systems biology", "genome informatics" or "computational genome science" are concentrated on pre-mRNA splicing, organisation of transposable elements, identification of protein-coding genes and RNA genes were developed into tools by Brendel et al. (2004). Gremme et al. (2005) proposed a software predictive tool for gene structure in higher organisms.

The analysis of several storage protein loci, allow differentiating Asian and European *Triticum spelta* L. (Kozub et al., 2014).

Microsatellite markers and in situ hybridization are valuable techniques in molecular analysis of triticale lines with different *vrn* gene systems (Leonova et al., 2005), as well in confirming the authenticity of inter-varietal chromosome substitution lines of *Triticum aestivum* L. (Pestsova et al., 2000).

Schmidt et al. (2004), Kara et al. (2018), used microsatellite SSR markers in molecular characterization of *Triticum aestivum* L. genotype. The results demonstrate the utility of microsatellite markers for detecting polymorphism to estimate genetic diversity.

Similar researches were conducted by Ahmad et al. (2018), on studying molecular diversity of *Triticum aestivum* L. genotypes resistance to rice weevi (*Sitophilus oryzae* L.). Results indicated that microsatellite markers are able to acces genetic diversity among wheat genotypes for weevil resistance.

Genetic similarity studies with SSR markers were conducted on 43 wheat varieties to reveal genetic relationships in wheat varieties by Zhang et al. (2002). Studies revealed that genetic similarities should be based on data from all genomes, rather than any one genome. Assessing genetic modification impact on allergenicity of wheat species concluded that

the differences observed between GM wheats and their parents are within the range of cultivated wheats (Lupi et al., 2014)

Song et al. (2002), determined the abundance of nine different trinucleotide microsatellites in the wheat genome, the repeat length distributions of

each and the rates at which they could be developed into informative markers.

The stress tolerance traits in wheat revealed that 11 important quantitative trait loci clusters located on chromosomes 1 BL, 1D, 2A, 2B, 4A, 6B and 7B (Zhang et al., 2014). Disease resistance studies showed that a wheat - *L. mollis* double substitution line DM96 could induce high resistance to stripe rust and Fusarium head blight (Zhao et al., 2013).

Influence of regional origin, harvest year and genotypes are significant in the fingerprints of the wheat kernels (Liu et al., 2015).

Korzun et al. (1997) showed the role of microsatellites and their markers as a tool in determination of wheat authenticity.

The combining ability and authentication of F₁ hybrids in *Triticum aestivum* L. using SSR markers revealed that LU₂6S as best general combiner for plant height (Ahmet et al., 2012). New progress was reported on utilization of Golden Ball (GB) wheat cultivar and Langdon - GB lines for genetic and genomic studies in tetraploid wheat and for improvement of stem solidness in both durum and bread wheat (Xu et al., 2014).

Genetic studies on heat stress for *Triticum aestivum* L. observed significant differences among the 19 genotypes considered (Pankj et al., 2019).

Low-molecular-weight glutenin subunits encoded by Glu-3 complex loci in hexaploidy wheat, were found to impact the flour quality, as a comprehensive study revealed. Molecular characteristics and functional properties were conducted (Zhen et al., 2014).

Researches on chinese wheat *Triticum aestivum* L. landrace Banjiemang identified two novel HMW-GS genes, designated as 1Bx14* and 1Bx15*, novel allelic variations of HMW-GS at Glu-B1 locus, which were probably exploitable as new resources for quality improvement of *Triticum aestivum* L. (Shao et al., 2015).

Authentication of *Triticum aestivum* L. lines with specific rust resistance using molecular markers were done for yielding cultivars PBW343, UP2338 and WH542, used to incorporate multiple rust resistance genes from winter wheat or agronomical inferior wheat lines (Datta et al., 2008).

By using quantitative trait locus (QTL) detection techniques, Cui et al. (2012), found that though co-located QTL were universal, every trait owned its unique QTL and even two closely related traits were not excluded.

Chromosome sequencing techniques were used to reveal the partitioning correlated with meiotic recombination for 1-gigabase chromosome 3B of hexaploidy bread wheat. Comparative analyses indicated high wheat-specific inter and intrachromosomal gene duplication activities, source of variability, for increased adaptability (Choulet et al., 2014).

Kabir et al. (2015) also used two wheat populations in mapping QTL's associated with root traits. Root morphological parameters were measured for both populations. In total, 54 QTLs for roots traits were detected.

Bagherikia et al. (2014), studied translocation of chromosome arm 1RS (*Secale cereale*) to *Triticum aestivum* L. improvement. 1AL.1RS offering higher biotic and abiotic stress tolerance. Results 1AL.1RS confirmed "Sholeh" wheat cultivar as the only cultivar (1.5%) that carries 1AL.1RS, as a successful translocation process result.

The full-length cDNA sequence (1158 bp) encoding a ribosomal L5 protein, designated as TaL5, was firstly isolated from common wheat (*Triticum aestivum* L.) using the rapid amplification of cDNA ends method (RACE). Stress studies indicated that TaL5 gene was dramatically induced by salt, drought and freezing. These implied that TaL5 gene could preserve function in several stress conditions in wheat plants (Kang et al., 2012).

Molecular cloning techniques were applied to isolate the starch-branching enzymes isoform SBEIII cDNA sequence (3,780 bp) from common wheat (*Triticum aestivum* L.) using RACE. The SBE activity of the protein expressed in *Escherichia coli* (BL21) was measured and verified. During the wheat grain filling period, TASBEIII was constitutively expressed (Kang et al., 2013).

Molecular characterization using real-time PCR method

Adulteration studies on wheat variety content in traditional Italian pasta addressed identification of *Triticum aestivum* L. presence, as adulteration agent to *Triticum durum*. The PCR of some sequences of *T. aestivum* has been optimised using two sets of primers designed on *puroindoline b* gene. The analyses showed that this method works well also on high-temperature dried pasta (Arlorio et al., 2003). Similar interest showed the studies of Terzi et al. (2003). They proposed qualitative and quantitative PCR-based methods to detect hexaploid wheat adulteration in pasta.

PCR techniques were designed for phylogenetic analysis of 59 external transcribed spacers (ETS) region of the 18S ribosomal RNA genes for some species, including *Triticeae*. It was demonstrated that the complete ETS sequences of the *Triticeae* yield coherent phylogenetic information (Sallares and Brown, 2004).

Mafra et al. (2008), revue on main novelties on animal products food authentication based on PCR methods. They emphasized on the method effectiveness in species authentication or detection of allergens and GMOs.

PCR was revealed as most efficient method for a rapid and specific wheat virus diagnostic tool that also has the potential for investigating the epidemiology of viral diseases, like dwarf viruses or mosaic viruses (Deb and Anderson, 2008).

The method was extended in detecting also vector leafhopper (*Psammotettix alienus* Dahlb.), by Zhang et al. (2010).

A combination of STS markers and multiplex PCR techniques for Glu-A3 alleles in *Triticum aestivum* L. The markers and multiplex-PCR systems were validated on 141 CIMMYT wheat varieties and advanced lines with different Glu-A3 alleles, confirming that they can be efficiently used in marker-assisted breeding (Wang et al., 2010)

Specific detection and quantification of *Aspergillus flavus* and *Asperigillus parasiticus* in wheat flour was studied using two qPCR assays. Both assays could detect spore concentrations equal or higher than 106 spores/g in flour samples without prior incubation. The assays proved to be valuable tools to improve diagnosis at an early stage in

all critical control points of food chain (Patiño et al., 2011).

Real-time PCR was tested in quantification of wheat contamination in gluten-free for celiac patients. Values obtained were compared with those from R5 ELISA. They were similar for majority of tests; however real-time PCR showed a better sensitivity of the DNA for some samples. The method was proposed to be used also as a non-immunological tool to confirm the presence of wheat (Mujico et al., 2011).

One hundred and eighty-two bread wheat cultivars were characterised for low molecular weight glutenins using SDS-PAGE and allele-specific PCR. Data found greater consistency between SDS-PAGE and PCR amplification patterns for some of the alleles and less consistency for others. More studies are needed in order to achieve unambiguous identifications (Ram et al., 2011)

Amar et al. (2012) studied predictive and early detection of mycotoxigenic *Fusarium culmorum* in wheat. They used multiplex PCR to detect toxigenic agent with no need of prior DNA extraction. They concluded the method is a suitable strategy for high throughput screening of mycotoxigenic *Fusarium*.

PCR assay was used in confirming the presence of HMW-GS in the 29 genotypes of wheat. Differences between Arabian Australian and American varieties were identified (Ghazi et al., 2012).

Kutateladze et al. (2013), have developed methods of reliable and fast detection of maize (*Zea mays* L.) wheat (*Triticum aestivum* L.) and soybean (*Glycine max* L.). They used novel multiplex PCR techniques. New soybean and maize specific PCR-primers were developed, as well as a species-specific triplex PCR targeting maize invertase gene, soybean lectin gene and wheat low-molecular-weight glutenin subunit.

Authentication of wheat, barley, rye and oats in food and feed was studied using four TaqMan real-time PCR assays. Three specific primers were used. The system showed high specificity and sensitivity in experimental flour binary mixture. Method was further applied for 270 food and pet food products, proving to be an effective tool in authentication of foods with different labelling schemes, in which the presence of the targeted cereals was either

declared, not declared or declared as possible traces (Pegels et al., 2015).

Studies on Fusarium head blight caused by *Fusarium graminearum* were performed in order to discriminate quantitative resistance in barley and wheat genotypes. A pathogen inoculation and a quantitative PCR based protocol were reported.

The method proved to be effective and could be applied for medium to high throughput barley and wheat breeding programmes (Kumar et al., 2015).

Carlioni et al. (2017) developed new PCR-related techniques to detect common wheat adulteration of durum wheat for pasta production. They demonstrated the limits of the method based on gliadin gene. A new molecular method, based on DNA extraction from semolina and real-time PCR determination of *Triticum aestivum* L. in *Triticum* spp., was validated.

The variation of high-molecular-weight glutenin subunits in wheat was studied, based on a combination of two techniques, PCR amplification and digestion with endonucleases. Data allowed detection of allelic variations that were not clearly by one technique alone (Wang et al., 2018).

Silieti et al. (2019), studied untargeted DNA-based methods for authentication of ancient wheat species and other cereals, present in modern food products, particularly pasta, bread and cookies. They used DNA fingerprinting through tubulin-based species and tested a series of commercial food products. The assay has a sensitivity of 0.5-1% w/w in binary detect possible adulterations.

CONCLUSIONS

There are a large variety of analytical methods in determination and authentication of cereals. Infrared techniques, mass spectrometry, chromatography and chemical determinations are however less studied compared to genetic methods, PCR particularly. The last years the scientists are more focussed in refining old techniques, overcoming their limits by developing new assays or by using complex mixture of techniques.

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REFERENCES

- Ahmad, E., Jaiswa, J.P., Badoni, S., Akhtar, M. (2018). Molecular Diversity of Wheat (*Triticum aestivum* L.) Genotypes Resistance to Rice Weevil (*Sitophilus oryzae* L.) Revealed by SSR Markers. *International Journal of Current Microbiology and Applied Sciences*, 7(07): DOI: 10.20546/ijcmas.2018.707.093.
- Abdel-Mawgood, A.L. (2008). Molecular markers for predicting end-products quality of wheat (*Triticum aestivum* L.). *African Journal of Biotechnology*, 7(14), 2324–2327.
- Ahmed, M.S., Fhaliq, I., Farooq, J., Awan, S.I. (2011). Assessment of the combining ability and authentication of F1 hybrids using SSR markers in wheat (*Triticum aestivum* L.). *Frontiers of Agriculture in China*, 5(2), 135–140.
- Amar, A.B., Oueslati, S., Ghorbel, A., Mliki, A. (2012). Prediction and early detection of mycotoxigenic *Fusarium culmorum* in wheat by direct PCR-based procedure. *Food Control*, 23(2), 506–510.
- Arlorio, M., Coisson, J., Cereti, E., Travaglia, F., Capasso, M., Martelli, A. (2003). Polymerase chain reaction (PCR) of puroindoline b and ribosomal/puroindoline b multiplex PCR for the detection of common wheat (*Triticum aestivum*) in Italian pasta. *European Food Research and Technology*, 216(3), 253–258.
- Bagherikia, S., Karimzadeh, G., Naghavi, M.R. (2014). Distribution of 1AL.1RS and 1BL.1RS wheat-rye translocations in *Triticum aestivum* using specific PCR. *Bioch. Systematics and Ecology*, 55, 20–26.
- Bildanova, L., Salina, E., Pershina, L. (2003). Specific Features of the Nuclear Genome Recombination in Backcross Progenies of Barley-Wheat Hybrids *Hordeum vulgare* L. (2n = 14) × *Triticum aestivum* L. (2n = 42). *Russian J. of Genetics*, 39(12), 1420–1425.
- Bottero, M.T. & Dalmaso, A. (2011). Animal species identification in food products: evolution of biomolecular methods. *The Veterinary Journal*, 190, 34e38.
- Brendel, V., Xing, L., Zhu, W. (2004). Gene structure prediction from consensus spliced alignment of multiple ESTs matching the same genomic locus. *Bioinformatics*, 20(7), 1157–1169.
- Brescia, M.A., Di Martino, G., Guillou, C., Reniero, F. (2002). Combination of the ⁸⁷Sr/⁸⁶Sr ratio and light stable isotopic values (δ¹³C, δ¹⁵N and δD) for identifying the geographical origin of winter wheat in China. *Rapid Communication in Mass Spectrometry*, 16(24), 2286–2290.
- Carlioni, E., Amagliani, G., Omiccioli, E., Ceppetelli, V., Del Mastro, M., Rotundo, L., Brandi, G., Magnani,

- M. (2017). Validation and application of a quantitative real-time PCR assay to detect common wheat adulteration of durum wheat for pasta production. *Food Chemistry*, 224, 86–91.
- Choulet, F., Alberti, A., Theil, S., Glover, N., Barbe, V., Daron, J., Pingault, L., Sourdille, P., Couloux, A., Paux, E., Leroy, P., Mangenot, S., Guilhot, N., Le Gouis, J., Balfourier, F., Alaux, M., Jamilloux, V., Poulain, J., Durand, C., Bellec, A., Gaspin, C., Safar, J., Dolezel, J., Rogers, J., Vandepoele, K., Aury Jm Mayer, K., Berges, H., Quesneville, H., Wincker, P., Feuillet, C. (2014). Structural and functional partitioning of bread wheat chromosome 3B. *Science*, 345(6194):1249721. doi: 10.1126/science.1249721.
- Cui, F., Ding, A., Li, J., Zhao, C., Wang, L., Wang, X., Qi, X., Li, X., Li, G., Gao, J., Wang, H. (2012). QTL detection of seven spike-related traits and their genetic correlations in wheat using two related RIL populations. *Euphytica*, 186(1), 177–192.
- Datta, J.K., Bhardwaj, S.C., Prashar, M. (2008). Development of bread wheat (*Triticum aestivum* L.) lines with specific rust resistance genes and their authentication through molecular markers. *Indian Journal of Genetics and Plant Breeding*, 68(2), 113–123.
- Deb, M., Anderson, J.M. (2008). Development of a multiplexed PCR detection method for Barley and Cereal yellow dwarf viruses, Wheat spindle streak virus, Wheat streak mosaic virus and Soil-borne wheat mosaic virus. *Journal of Virological Methods*, 148(1-2), 17–24.
- Geng, P., Harnly, J., Chen, P. (2016). Differentiation of bread made with whole grain and refined wheat (*T. aestivum*) flour using LC/MS-based chromatographic. *Journal of Food Composition and Analysis*, 47. DOI: 10.1016/j.jfca.2015.12.010.
- Gharechahi, J., Zeinolabedini, M., Salekdeh, G.H. (2016). Proteomics in Detection of Contaminations and Adulterations in Agricultural Foodstuffs. *Agricultural Proteomics*, 1, 67–85.
- Ghazy, A.I., Zanouny, A.I., Moustafa, K.A., Al-Doss, A.A. (2012). Molecular screening of high molecular weight glutenin genes in spring bread wheat genotypes in Saudi Arabia. *Journal of Food, Agriculture & Environment*, 10(1), 157–161.
- González-Martín, M.I., Moncada, G.W., González-Pérez, C., Zapata San Martín, N.Z., López-González, F., Ortega, I.L., Hernández-Hierro, J.M. (2014). Chilean flour and wheat grain: Tracing their origin using near infrared spectroscopy and chemometrics. *Food Chemistry*, 145, 802–806.
- Gremme, G., Brendelb, V., Sparksc, M.E., Kurtz, S. (2005). Engineering a software tool for gene structure prediction in higher organisms. *Information and Software Technology*, 47(15), 965–978.
- Hernandez, M., Esteve, T., Pla, M. (2005). Real-time polymerase chain reaction based assays for quantitative detection of barley, rice, sunflower, and wheat. *Journal of Agricultural and Food Chemistry*, 53, 7003e7009.
- Huang, X.-Q., Brülé-Babel, A. (2011). Development of simple and co-dominant PCR markers to genotype puroindoline a and b alleles for grain hardness in bread wheat (*Triticum aestivum* L.). *Journal of Cereal Science*, 53(3), 277–284.
- Huber, I., Block, A., Sebah, D., Debode, F., Morisset, D., Grohmann, L. et al. (2013). Development and validation of duplex, triplex, and pentaplex real-time PCR screening assays for the detection of genetically modified organisms in food and feed. *Journal of Agricultural and Food Chemistry*, 61, 10293e10301.
- James, D. & Schmidt, A.-M. (2004). Use of an intron region of a chloroplast tRNA gene (trnL) as a target for PCR identification of specific food crops including sources of potential allergens. *Food Research International*, 37, 395e402.
- Kabir, M.R., Liu, G., Guan, P., Wang, F., Khan, A.A., Ni, Z., Yao, Y., Hu, Z., Xin, M., Peng, H. (2015). Sun q., Mapping QTLs associated with root traits using two different populations in wheat (*Triticum aestivum* L.). *Euphytica*, 206(1), 175–190.
- Kahrizi, D., Cheghamirza, K., Akbari, L., Rostami-Ahmadvandi, H. (2013). Effects of magnetic field on cell dedifferentiation and callus induction derived from embryo culture in bread wheat (*Triticum aestivum* L.) genotypes. *Molecular Biology Reports*, 40(2), 1651–1654.
- Kang, G., Li, S., Zhang, M., Peng, H. (2018). Molecular Cloning and Expression Analysis of the Starch-branching Enzyme III Gene from Common Wheat (*Triticum aestivum*). *Biochemical Genetics*, 51(5), DOI: 10.1007/s10528-013-9570-4.
- Kang, G.-Z., Peng, H.-F., Han, Q.-X., Wang, Y.-H., Guo, T.-C. (2012). Identification and expression pattern of ribosomal L5 gene in common wheat (*Triticum aestivum* L.). *Gene*, 493(1), 62–68.
- Kara, K., Rached-Kanouni, M., Mezghani, N., Mnasri, S., Ben Naceur, M. (2018). Molecular characterization of bread wheat genotypes *Triticum aestivum* L. through microsatellites *ssr* markers. *SGEM Scientific Papers DataBase*, <https://www.sgem.org/sgemlib/spip.php?article13341>.
- Koenig, A., Konitzer, K., Wieser, H., Koehler, P. (2015). Classification of spelt cultivars based on differences in storage protein compositions from wheat. *Food Chemistry*, 168, 176–182.
- Konieczny, R., Bohdaniwicz, J., Czaplicki, A., Przywara, L. (2005). Extracellular matrix surface network during plant regeneration in wheat anther culture. *Plant Cell Tissue and Organ Culture*, 83(2), 201–208.
- Korzun, V., Börner, A., Worland, A.J., Law, C.N., Röder, M.S. (1997). Application of microsatellite markers to distinguish inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.). *Euphytica* 95(2), 149–155.
- Kozub, N.A., Boguslavskii, R.L., Sozinov, I.A., Tverdokhle, Ye.V., Xynias, I.N., Blume, Ya.B., Sozinov, A.A. (2014). Alleles at Storage Protein Loci in *Triticum spelta* L. Accessions and Their

- Occurrence in Related Wheat., *Cytology and Genetics*, 48(1), 33–41.
- Kumar, A., Karre, S., Dhokane, D., Kage, U., Hukkeri, S., Kushalappa, A.C. (2015). Real-time quantitative PCR based method for the quantification of fungal biomass to discriminate quantitative resistance in barley and wheat genotypes to fusarium head blight. *Journal of Cereal Sciences*, 64, 16–22.
- Kutateladze, T., Gabriadze, I., Vishnepolsky, B., Karseladze, M., Datukishvili, N. (2013). Development of triplex PCR for simultaneous detection of maize, wheat and soybean. *Food Control*, 34(2), 698–702.
- Leonova, I.N., Dobrovolskaya, O.B., Kaminskaya, L.N., Adonina, I.G., Koren, L.V., Khotyljova, L.V., Salina, E.A. (2005). Molecular Analysis of the Triticale Lines with Different Vrn Gene Systems Using Microsatellite Markers and Hybridization In Situ. *Russian Journal of Genetics*, 41(9), 1014–1020.
- Li, C., Dong, H., Luo, D., Xiang, Y., Fu, X. (2016). Recent Developments in Application of Stable Isotope and Multi-element Analysis on Geographical Origin Traceability of Cereal Grains. *Food Analytical Methods*, 9(16), 1512–1519.
- Liang, X., Zhen, S., Han, C., Wang, C., Li, X., Ma, W., Yan, Y. (2015). Molecular characterization and marker development for hexaploid wheat-specific HMW glutenin subunit 1By18 gene. *Molecular Breeding*, 35, 221; DOI: 10.1007/s11032-015-0406-2.
- Liu, H., Guo, Z., Wei, Y., Wei, S., Ma, Y., Zhang, W. (2015). Effects of region, genotype, harvest year and their interactions on d13C, d15N and dD in wheat kernels. *Food Chemistry*, 171, 56–61.
- Luo, D., Dong, H., Luo, H., Xian, Y., Wan, J., Guo, X., Wu, Y. (2015). The application of stable isotope ratio analysis to determine the geographical origin of wheat. *Food Chemistry*, 174, 197–201.
- Lupi, R., Mascia, S., Rogniaux, H., Tranquet, O., Brossard, C., Lafiandra, D., Moneret-Vautrin, D.A., Denery-Papini, S., Larré, C. (2014). Assessment of the allergenicity of soluble fractions from GM and commercial genotypes of wheats. *Journal of Cereal Science*, 60(1), 179–186.
- Maat, H. (2001). Genetics and Plant Breeding: Wheat in the Netherlands. *The International Library of Environ., Agricultural and Food Ethics*, 117–142.
- Mafra, I., Ferreira, I.M.P.L.V.O., Beatriz, M., Oliveira, P.P., (2008). Food authentication by PCR-based methods. *European Food Research and Technology*, 227(3), 649–665.
- Mitrofanova, O.P. (2012). Wheat Genetic Resources in Russia: Current Status and Prebreeding Studies. *Russian Journal of Genetics Applied Research*, 2(4), 277–285.
- Mujico, J.R., Lombardía, M., Mena, M.C., Méndez, E., Albar, J.P. (2011). A highly sensitive real-time PCR system for quantification of wheat contamination in gluten-free food for celiac patients. *Food Chemistry*, 128(3), 795–801.
- Novoselskaya-Dragovich, A.Yu., Besselova, L.A., Shishkina, A.A., Melnik, V.A., Upelnik, V.P., Fisenko, A.V., Dedova, L.V., Kudryavtsev, A.M. (2015). Genetic Diversity of Common Wheat Varieties at the Gliadin Coding Loci. *Russian Journal of Genetics*, 51(3), 262–271.
- Pankaj, Y.K., Vasantro, M.J., Prakash, N., Jat, R.K., Kumar, R., Kumar, V., Kumar, P. (2019). Characterization of wheat (*Triticum aestivum* L.) genotypes unraveled by molecular markers considering heat stress. *Open Agriculture*, 4(1), DOI: <https://doi.org/10.1515/opag-2019-0037>.
- Patiño, B., González-Jaén, M., Vázquez, C., Gil-Serna, J. (2011). Specific detection and quantification of *Aspergillus flavus* and *Aspergillus parasiticus* in wheat flour by SYBR® Green quantitative PCR. *International Journal of Food Microbiology*, 145(1), 121–125.
- Pauli, E.D., Barbieri, F., Garcia, P.S., Madeira, T.B., Accquaro Junior, V.R., Scarmino, I.S., Da Camara, C.A.P., Nixdorf, S.L. (2014). Detection of ground roasted coffee adulteration with roasted soybean and wheat. *Food Research International*, 61, 112–119.
- Pegels, N., Alonso, I.G., Garcia, T., Martin R. (2015). Authenticity testing of wheat, barley, rye and oats in food and feed market samples by real-time PCR assays. *Lebensmittel-Wissenschaft + [i.e. und] Technologie*, 60(2), 867–875.
- Pereira, F., Carneiro, J. & Amorim, A. (2008). Identification of species with DNA based technology: current progress and challenges. *Recent Patents on DNA & Gene Sequences*, 2, 187e200.
- Pestsova, E., Salina, E., Börner, A., Korzun, V., Maystrenko, O.I., Röder, M.S. (2000). Microsatellites confirm the authenticity of inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 101(1-2), 95–99.
- Popping, B. (2002). The application of biotechnological methods in authenticity testing. *Journal of Biotechnology*, 98(1), 107–112.
- Ram, S., Sharma, S., Verma, A., Tyagi, B.S., Peña, R.J., (2011). Comparative analyses of LMW glutenin alleles in bread wheat using allele-specific PCR and SDS-PAGE. *Journal of Cereal Science*, 54(3), 488–493.
- Sallares, R., Brown, T.A. (2004). Phylogenetic analysis of complete 59 external transcribed spacers of the 18S ribosomal RNA genes of diploid Aegilops and related species (Triticeae, Poaceae). *Genetic Resources and Crop Evolution*, 51(7), 701–712.
- Schmidt, A.L., Gale, K.R., Ellis, M.H., Giffard, P.M. (2004). Sequence variation at a microsatellite locus (XGWM261) in hexaploid wheat (*Triticum aestivum*) varieties. *Euphytica*, 135, 239–246.
- Shao, H., Liu, T.-H., Ran, C.-F., Li, L.-Q., Yu, J., Gao, X., Li, X.-J. (2015). Isolation and molecular characterization of two novel HMW-GS genes from Chinese wheat (*Triticum aestivum* L.) landrace Banjiemang. *Genes & Genomes*, 37(1), 45–53.
- Silletti, S., Morello, L., Gavazzi, F., Giani, S., Braglia, L., Breviaro, D. (2019). Untargeted DNA-based

- methods for the authentication of wheat species and related cereals in food products. *Food Chemistry*, 271, 410–418.
- Simpkins, W., Harrison, M. (1995). The state of the art in authenticity testing. *Trends in Food Science & Technology*, 6(10), 321–328.
- Song, Q.J., Fickus, W., Cregan, P.B. (2002). Characterization of trinucleotide SSR motifs in wheat. *Theoretical and Applied Genetics*, 104(2-3), 286-293.
- Tavoletti, S., Iommarini, L. & Pasquini, M. (2009). A DNA method for qualitative identification of plant raw materials in feedstuff. *European Food Research and Technology*, 229, 475-484.
- Terzi, V., Malnati, M., Barbanera, M., Stanca, A.M., Faccioli, P. (2003). Development of analytical systems based on real-time PCR for Triticum species-specific detection and quantitation of bread wheat contamination in semolina and pasta. *Journal of Cereal Science*, 38(1), 87–94.
- Voorhuijzen, M.M., Van Dijk, J., Van Hoef, A. (2011). Development of a multiplex DNA-based traceability tool for crop plant materials. *Analytical and Bioanalytical Chemistry*, 402(2), 693–701.
- Wang, C., Shen, X., Wang, K., Yanlin, L. (2016). Molecular characterization and functional properties of two novel x-type HMW-GS from wheat line CNU608 derived from Chinese Spring Ae. caudata cross. *Journal of Cereal Science*, 68, 16–24.
- Wang, G.-Z., Matsuoka, Y., Tsunewaki, K. (2000). Evolutionary features of chondriome divergence in *Triticum* (wheat) and *Aegilops* shown by RFLP analysis of mitochondrial DNAs. *Theoretical and Applied Genetics*, 100(2), 221–231.
- Wang, L., Li, G., Peña, R.J., Xia, X., He, Z. (2010). Development of STS markers and establishment of multiplex PCR for Glu-A3 alleles in common wheat (*Triticum aestivum* L.). *Journal of Cereal Science*, 51(3), 305–312.
- Wang, Z., Huang, L., Wu, B., Hu, J., Jiang, Z., Qi, P., Zheng, Y., Liu, D. (2018). Identification and characterization by PCR-RFLP analysis of the genetic variation for the Glu-A1x and Glu-B1x genes in rivet wheat (*Triticum turgidum* L. ssp. *turgidum*). *International Journal of Molecular Sciences*, 19(4), 923, doi: 10.3390/ijms19040923.
- Wu, M.J., McKay, S., Howes, N., Chin, J., Hegedus, E. (2012). Identification of novel serpin isoforms and serpin polymorphisms among Australian wheat. *Journal of Cereal Science*, 55, 202–209.
- Xu, S.S., Chu, C., Chao, S., Klindworth, D. (2010). Marker-assisted characterization of durum wheat Langdon-Golden Ball disomic substitution lines. *Theoretical and Applied Genetics*, 120(8), 1575–1585.
- Zhang, H., Cui, F., Wang, H. (2014). Detection of quantitative trait loci (QTLs) for seedling traits and drought tolerance in wheat using three related recombinant inbred line (RIL) populations. *Euphytica*, 196(3), 313–330.
- Zhang, X., Zhou, G., Wang, X. (2010). Detection of wheat dwarf virus (WDV) in wheat and vector leafhopper (*Psammotettix alienus* Dahlb.) by real-time PCR. *Journal of Virological Methods*, 169(2), 416–419.
- Zhang, X., Li, C., Wang, L., Wang, H., You, G., Dong, Y. (2002). An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties. I. Information from large-scale planted varieties and cornerstone breeding parents in Chinese wheat improvement and production. *Theoretical and Applied Genetics*, 106(1), 112–117.
- Zhao, H., Guo, B., Wei, Y., Zhang, B. (2013). Multi-element composition of wheat grain and provenance soil and their potentialities as fingerprints of geographical origin. *Journal of Cereal Sciences*, 57(3), 391–397.
- Zhao, H., Guo, B., Wei, Y., Zhang, B. (2013). Near infrared reflectance spectroscopy for determination of the geographical origin of wheat. *Food Chemistry*, 138(2-3), 1902–1907.
- Zhao, J.-X., Du, W.-L., Wu, J., Cheng, X.-N. (2013). Development and identification of a wheat-Leymus mollis multiple alien substitution line. *Euphytica*, 190(1): DOI: 10.1007/s10681-012-0772-3.
- Zhen, S., Han, C., Ma, C., Gu, A. (2014). Deletion of the low-molecular-weight glutenin subunit allele Glu-A3a of wheat (*Triticum aestivum* L.) significantly reduces dough strength and breadmaking quality. *BMC Plant Biology*, 14, 367. doi: 10.1186/s12870-014-0367-3
- Zhen, S., Han, C., Ma, C., Gu, A., Zhang, M., Shen, X., Li, X., Yan, Y. (2014). Deletion of the low-molecular-weight glutenin subunit allele Glu-A3a of wheat (*Triticum aestivum* L.) significantly reduces dough strength and breadmaking quality. *BMC Plant Biology*, 367. DOI:10.1186/s12870-014-0367-3
- Ziegler, J.U., Leitenberger, M., Friedrich, C., Longin, H., Würschum, T., Carle, R., Schweiggert, R.M. (2016). Near-infrared reflectance spectroscopy for the rapid discrimination of kernels and flours of different wheat species. *Journal of Food Composition and Analysis*, 51, 30–36.
- Zörb, C., Betsche, T., Langenkämper, G. (2009). Search for Diagnostic Proteins to Prove Authenticity of Organic Wheat Grains (*Triticum aestivum* L.). *Journal of Agricultural Chemistry*, 57(7), 2932–2937.

***Official Journal of the European Union (OJEU) (2011). Commission Regulation 1169/ 2011/EC of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No. 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/ 496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. The Official Journal of the European Union, L304, 18e63.

URL <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF>
Accessed 23.01.14.

***Official Journal of the European Union (OJEU) (2014). Commission Regulation 78/ 2014/EC of 22 November 2013 amending annexes II and III to Regulation (EU) No. 1169/2011 of the European

Parliament and of the Council on the provision of food information to consumers, as regards certain cereals causing allergies or intolerances and foods with added phytosterols, phytosterol esters, phytostanols or phytostanol esters. The Official Journal of the European Union, L27, 7e8.

EFFECT OF HUMIDITY AND TEMPERATURE ON THE INCIDENCE OF ATTACK OF *Zymoseptoria tritici* IN WHEAT

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Abstract

Septoria tritici blotch, caused by *Zymoseptoria tritici*, is a major disease of wheat in many areas of the world. Optimal conditions for development of *Zymoseptoria tritici* take place during April and May. High risk of *Septoria tritici* blotch disease periods are defined as days with at least 10 mm rain, or else up to three consecutive days with 10 mm or more rain. The research was conducted during 2015-2019 (four agricultural years). The climatic conditions specific for these interval of time made it to be present in wheat fields *Zymoseptoria tritici* which causes *Septoria tritici* blotch disease of wheat. The aim of this paper is to describe the behaviour of eleven Romanian winter wheat varieties (Glosa, Izvor, Miranda FDL, Otilia, Pajura, Pitar, Semnal, Ursita, Andrada, Codru and Bezostaia 1) under natural infection conditions with *Zymoseptoria tritici*.

Key words: winter wheat, *Zymoseptoria tritici*, diseases, weather conditions.

INTRODUCTION

The filamentous fungus *Zymoseptoria tritici* infects wheat plants and causes the disease known as *Septoria tritici* blotch. Because of its negative impact on crop management and yield *Septoria tritici* blotch is considered, nowadays, the major diseases of wheat (Greiner et al., 2019; Arraiano et al., 2001). Until 1960s, economically significant losses caused by this disease were occasionally reported (Eyal et al., 1987) but from the 1980s *Zymoseptoria tritici* becomes an extremely important disease, being reported all over the world (Eyal, 1999; Halama, 1996), causing yield losses ranged between 10% and 50%, depending on the region and the pressure with which the disease manifests during the vegetation period (Heick et al., 2017). This situation was favoured by the replacement of the local wheat varieties with semi-dwarf cultivars, with early maturation which proved to be susceptible to this disease (Muckle, 2013; Eyal, 1987). Besides this, the intensification of the agriculture of that period, the application of high doses of synthetic fertilizers and, very important, the climatic conditions favourable to the development of the disease, represented by high precipitation and low temperatures (Eyal, 1999; Lovell et al.,

1997) made *Septoria tritici* blotch the most popular foliar disease of wheat with the greatest negative economic impact. Yield losses caused by this disease have large limits, from 10-20% when the infection pressure is moderate, reaching severe losses, up to 60% in the years when the pathogen produces epidemics (Fones et al., 2015; Torriani et al., 2015; Goodwin et al., 2004).

Even if beneficial measures are taken to control the disease, such as cultivation of resistant varieties and application of fungicides, yield losses of about 5-10% are frequently recorded (Fones et al., 2015). The production losses are mainly due to the reduction of the size and weight of wheat kernels and their number on spike (Shipton et al., 1971).

Zymoseptoria tritici is considered the most economically important fungal disease in Europe and the second, after black rust, in the United States of America (Ponomarenko et al., 2011; Palomar, 2014), the production losses are estimated to be around \$400 million in Europe and \$275 million in the USA.

Being a such important disease, in Europe, of \$2.4 billion, as it represents the market for fungicides used for cereals, \$1.7 billion represents fungicides applied to wheat cultivation, and of this value about 70% (\$1.2

billion) represents the value of the fungicides aimed at the management of the pathogen *Zymoseptoria tritici* (Torriani et al., 2015).

MATERIALS AND METHODS

To know the effect that the climatic conditions, represented by the air temperature and humidity, have on the development of the *Zymoseptoria tritici* and on the infection pressure of this pathogen on the wheat plants, in the period 2015-2019, within the Didactic Station Iasi - Ezareni Farm, a research was carried out that aimed at the analysis of eleven Romanian wheat varieties. The experience was placed in the experimental field of the Iasi Didactic Station, the Ezareni Farm, being organized according to the randomized block diagram, in three replicates, each wheat cultivar representing an experimental variant.

The crop technology applied in the experimental field was classical technology. Soil tillage consisted of the ploughing and the preparation of seedbed, the fertilization was realized by applying moderate doses of fertilizers to the preparation of the seedbed and in the spring. Regarding the protection of wheat crop, it should be noted that no phytosanitary treatments were applied, which is why the studied cultivars were analysed in terms of their behaviour of winter wheat cultivars under natural conditions of infection with the pathogens.

The observations made to identify the presence of pathogens were conducted between March-June each year. In order to determine Frequency (F%), Intensity (I%) and to calculate the Degree of Attack (DA%), were made observations with metric frame (50 x 50 cm) in each variant. The genotype reaction was estimated by F.A.O. scale rate, with 9 attack classes in which, 1 = very resistant; 9 = very sensitive (Roelfs et al., 1992; FAO, 2016).

For a real and objective estimation of the intensity of the attack (I%) of the identified pathogens, guides and scientific papers were studied, of which the most useful ones were published by Muhammad et al., 2017; Manandhar et al., 2016; FAO, 2016.

Data regarding air temperature, atmospheric precipitation, relative humidity for the studied period (2015-2019) were obtained from the

Meteorological Station 000019B located on the territory of the Ezareni Farm - where the observations of the present study were made - through the site www.fieldclimate.com.

Data on the multiannual values of air temperature, precipitation and relative humidity, as well as other climate information, were obtained considering studies published by the National Meteorological Administration (www.meteoromania.ro) and the National Institute of Statistics (www.insse.ro).

The data obtained during the four years of observations were statistically interpreted using the SPSS program (IBM SPSS Statistics 20), and the graphical representation was performed using the Excel program within the Microsoft Office 2016 package.

RESULTS AND DISCUSSIONS

Based on the economic importance of *Septoria tritici* blotch disease, a series of studies have been carried out to understand the effects of the weather on the incidence of the pathogen's attack. Some of the risk factors have been identified and described (e.g. air temperature, precipitation, air relative humidity), with varying degrees of validation (O'Driscoll et al., 2014; Fones et al., 2015; Simon et al., 2002; Gladders et al., 2001; Thomas et al., 1989).

Analysis of *Septoria tritici* blotch disease development under weather conditions records show that optimal conditions for development of *Zymoseptoria tritici* take place during April and May. High risk of *Septoria tritici* blotch disease periods are defined as days with at least 10 mm rain, or else up to three consecutive days with 10 mm or more rain (Gladders et al., 2001; Thomas et al., 1989).

Considering that the research was performed under field conditions, the results were influenced by weather. Table 1 presents the air temperatures, rainfall and relative humidity registered between 2015-2019 at Ezareni Farm - Iasi Didactic Station during the vegetation period of wheat. The weather data recorded were carefully analysed (Table 1).

During the research period the average monthly air temperature showed some differences compared to the multiannual value of temperature, the most obvious differences were noted at the level of 2018, when the deviations

from the multiannual monthly average were significant. The biggest difference was observed in April 2018, when the monthly air temperature was with 5.2°C higher compared to the multiannual average of air temperature. Also, the next two months (May and June),

when the wheat crop is in the phenophase of intense growth, the monthly air temperatures were higher than the multiannual average. For the other years, March was the month in which the temperatures shown the most obvious differences from the multiannual average.

Table 1. Climatic conditions at Ezareni Farm - Iasi Didactic Station, during 2016-2019

Month	March	April	May	June	July	
Air Temperature (°C)						
Multiannual average (°C) (last century)		3.1	10.2	16.0	19.5	21.2
2016	Month average (°C)	6.5	13.3	15.3	20.9	22.6
	Deviation (°C)	+3.4	+3.1	-0.7	+1.4	+1.4
2017	Month average (°C)	8.0	10.0	16.1	21.1	21.6
	Deviation (°C)	+4.9	-0.2	+0.1	+1.6	+0.04
2018	Month average (°C)	1.2	15.4	18.7	20.8	21.3
	Deviation (°C)	-1.9	+5.2	+2.7	+1.3	+0.1
2019	Month average (°C)	7.3	10.6	16.1	21.9	21.2
	Deviation (°C)	+4.2	+0.4	+0.1	+2.4	0.0
Rainfall (mm)						
Multiannual sum (mm) (last century)		28.4	43.9	55.9	82.6	69.3
2016	Month sum (mm)	33.8	76.2	70.4	97.0 ^a +45.4 ^b	24.0
	Deviation (mm)	+5.4	+32.3	+14.5	+59.8	-45.3
2017	Month sum (mm)	107.0	140.4	72.8	17.6 ^a +54.0 ^b	84.4
	Deviation (mm)	+78.6	+96.5	+16.9	-11.0	+15.1
2018	Month sum (mm)	56.8	18.0	16.8	57.0 ^a +159.0 ^b	136.6
	Deviation (mm)	+28.4	-25.9	-39.1	+133.4	+67.3
2019	Month sum (mm)	40.4	62.6	125.2	67.6 ^a +46.2 ^b	24.2
	Deviation (mm)	+12.0	+18.7	+69.3	+31.2	-45.1
HC Relative Humidity (%)						
Multiannual average (%) (last century)		80.0	72.0	70.0	72.0	72.0
2016	Month average (%)	72.9	69.0	70.7	76.0	60.0
	Deviation (%)	-7.1	-3.0	+0.7	+4.0	-12.0
2017	Month average (%)	80.1	65.7	65.9	65.6	69.0
	Deviation (%)	+0.1	-6.3	-4.1	-6.4	-3.0
2018	Month average (%)	85.3	60.0	59.5	73.1	80.1
	Deviation (%)	+5.3	-12.0	-10.5	+1.1	-8.1
2019	Month average (%)	61.5	65.4	80.9	78.2	70.8
	Deviation (%)	-18.5	-6.6	+10.9	+6.2	-1.2

^aRainfall recorded between 01-15 of June

^bRainfall recorded between 16-30 of June

Regarding the air relative humidity, the values of this climatic element data recorded during the studied period showed deviations from the multiannual monthly values, the most obvious differences being recorded during the wheat vegetation period of the last two years of research.

Of the three climatic elements analysed, the atmospheric precipitation represents the element that presented the most volatile values, being also the most important climatic element that influenced the frequency and intensity of

attack with which *Zymoseptoria tritici* manifested.

In the years when during the wheat vegetation period a significant amount of precipitation was recorded (2017 and 2019), the characteristic symptoms produced by this disease (Figure 1) were easy to notice.

Carefully analysing the distribution of atmospheric precipitation during these four years of research, it was observed that when the days with rainfall exceeding 10 mm, or two or three consecutive days in which precipitations

fell was more than 10 mm were more numerous, the intensity of the attack of the pathogen was higher. Considering this situation

during the research 12 cases were registered in 2016, 13 cases in 2017, 4 cases in 2018 and 14 in 2019.

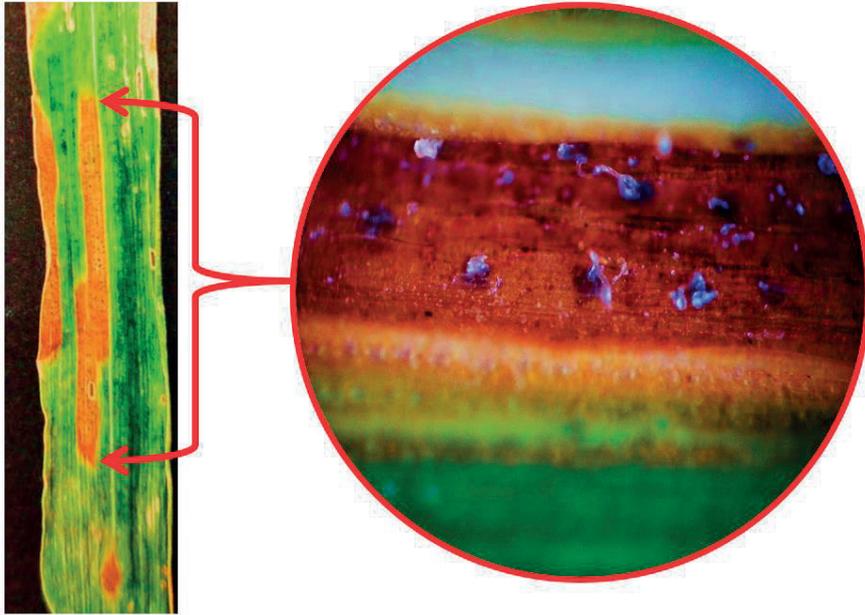


Figure 1. *Zymoseptoria tritici* disease symptoms on wheat plants (Photo: A.M. Gafencu)

The presence of these typical periods with precipitations witch favour the evolution of the disease can be observed very well by analysing the values of the degree of attack with which the pathogen was manifested during this period (Table 2). In the years when were recorded a

higher number of disease periods (2017, 2019) the evolution of the disease was favoured, the symptoms produced by it gradually covering the leaves of the wheat and extending its covered surface.

Table 2. *Septoria tritici* blotch caused by *Zymoseptoria tritici* - degree of attack (DA %), during 2015-2019

No.	Variety	<i>Zymoseptoria tritici</i> Degree of Attack (%)							
		2016		2017		2018		2019	
1.	BEZOSTAIA 1	0.84±0.12	Cv	3.46±0.59	Cv	0.66±0.22	Cv	3.22±0.38	Cv
2.	GLOSA	0.39±0.03	NS	2.68±0.62	NS	0.76±0.32	NS	3.19±0.19	NS
3.	IZVOR	0.45±0.18	NS	1.79±0.24	*	0.27±0.05	NS	2.29±0.36	NS
4.	MIRANDA FDL	0.45±0.12	NS	2.31±0.55	NS	0.84±0.29	NS	3.80±0.60	NS
5.	OTILIA	0.57±0.10	NS	2.17±0.46	NS	0.29±0.06	NS	1.47±0.36	**
6.	PAJURA	0.14±0.03	*	1.95±0.75	NS	0.46±0.19	NS	1.85±0.70	*
7.	PITAR	0.80±0.21	NS	3.35±0.37	NS	1.66±0.20	**	1.97±0.56	*
8.	SEMNAL	0.95±0.39	NS	2.91±0.24	NS	0.42±0.11	NS	1.36±0.23	**
9.	URSITA	0.98±0.36	NS	2.10±0.81	NS	0.39±0.06	NS	1.50±0.04	**
10.	ANDRADA	0.32±0.06	NS	2.26±0.52	NS	0.67±0.51	NS	3.38±0.38	NS
11.	CODRU	0.81±0.20	NS	1.97±0.14	NS	0.43±0.12	NS	2.05±0.33	NS

Ns

- Not Significant (P>0.05)

Cv – control variant

*

- Significant (P>0.01)

**

- Distinguish significant (P>0.001)

- Very significant (P<0.001)

Analyzing Figure 2, it is observed that the values of the degree of attack with which *Zymoseptoria tritici* manifested during this

period were influenced by the climatic conditions of the studied period.

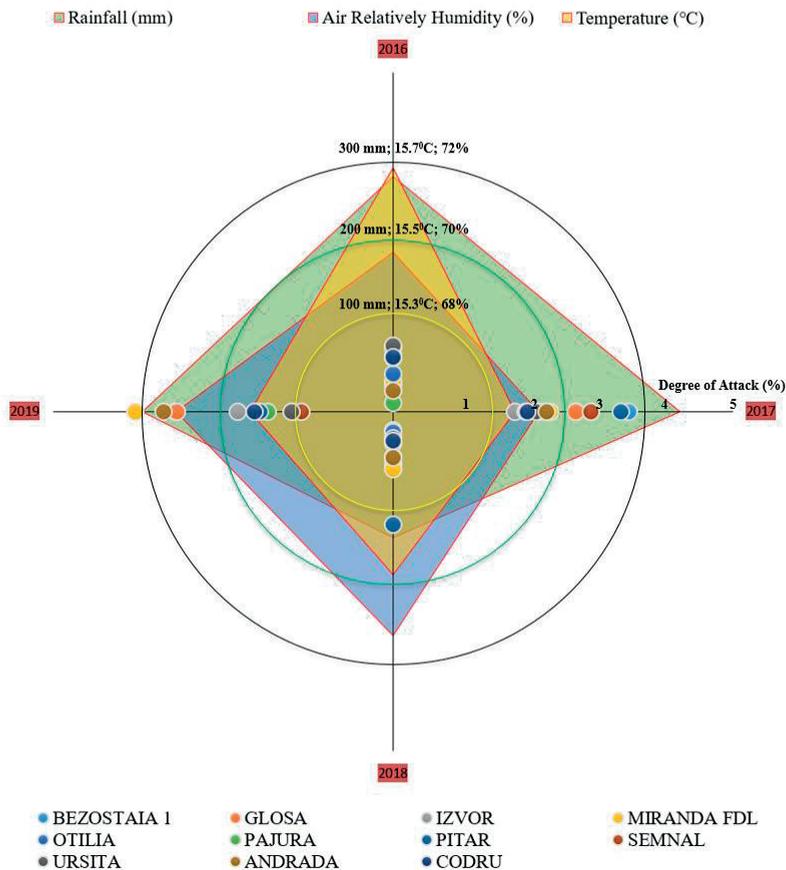


Figure 2. Evolution of the degree of attack of the *Zymoseptoria tritici* under the influence of precipitation, air temperature and air relative humidity, during 2015-2019

In 2017, when the amount of rainfall was higher and the air temperature was lower, the values of the degree of attack with which *Zymoseptoria tritici* manifested were the highest. A similar situation was encountered also at the level of 2019, when the precipitations were higher, but the average value of the temperatures was slightly higher, which is why the values of the attack degree of the pathogen were lower.

In the other two years studied, when the precipitations recorded were reduced, and the air temperature was higher it is observed that the pathogen manifested with low values of the degree of attack.

CONCLUSIONS

As a result of the research carried out, it can be observed that atmospheric precipitation, together with other climatic elements influence the occurrence and development of the disease produced by *Zymoseptoria tritici*.

The rains over 10 mm fallen within 24 hours, or the sum of the precipitations fallen during 3 consecutive days which exceed 10 mm of rain favours the evolution of disease.

Knowing the behaviour of wheat varieties in front of pathogens attack is important, because under the current situation of agriculture and plant protection, the most important measure

for limiting the pressure of pathogens in the field is the cultivation of genotypes that show resistance to them.

REFERENCES

- Arraiano, L.S., Brading, P.A., Brown, J.K.M. (2001). A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. *Plant. Pathol.*, 50, 339–346.
- Eyal, Z. (1999). The *Septoria tritici* and *Stagonospora nodorum* blotch diseases of wheat. *Eur. J. Plant Pathol.*, 105, 629–641.
- Eyal, Z., Scharen, A.L., Prescott, J.M., van Ginkel, M. (1987). *The Septoria Diseases of Wheat: Concepts and Methods of Disease Management*. CIMMYT, Mexico.
- FAO (2016). *Guidelines for monitoring diseases, pests and weeds in cereal crops*.
- Fones, H., Gurr, S. (2015). The impact of *Septoria tritici* Blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology*, 79, 3–7.
- Gladders, P., Paveley, N.D., Barrie, I.A., Hardwick, N., Hims, M.J., Langton, S., Taylor M.C. (2011). Agronomic and meteorological factors affecting the severity of leaf blotch caused by *Mycosphaerella graminicola* in commercial wheat crops in England. *Ann. Appl. Biol.*, 138, 301–311.
- Goodwin, S.B., Waalwijk, C., Kema, G.H.J. (2004). Genetics and Genomics of *Mycosphaerella graminicola*: A Model for the Dothideales. *Applied Mycology & Biotechnology*, 4, 315–330.
- Greiner, S.D., Racca, P., Jung, J., von Tiedemann, A. (2019). Determining and modelling the effective period of fungicides against *Septoria* leaf blotch in winter wheat. *Crop Protection*, 117, 45–51.
- Halama, P. (1996). The occurrence of *Mycosphaerella graminicola*, teleomorph of *Septoria tritici* in France. *Plant Pathol.*, 45(1), 135–138.
- Heick, T.M., Justensen, A.F., Jorgensen, L.N. (2017). Anti-resistance strategies for fungicides against wheat pathogen *Zymoseptoria tritici* with focus on DMI fungicides. *Crop Protection*, 99, 108–117.
- Lovell, D.J., Parker, S.R., Hunter, T., Royle, D.J., Coker, R.R. (1997). Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. *Plant Pathol.*, 46, 126–138.
- Manandhar, H.K., Timila, R.D., Sharma, S., Joshi, S., Manandhar, S., Gurung, S.B., Sthapit, S., Palikhey E., Pandey, A., Joshi, B.K., Manandhar, G., Gauchan, D., Jarvis, D.I., Sthapit, B.R. (2016). *A field guide for identification and scoring methods of diseases in the mountain crops of Nepal*. NARC, DoA, LI-BIRD and Bioersivity International, Nepal.
- Morais, D., Laval V., Sache I., Suffert F. (2015). Comparative pathogenicity of sexual and asexual spores of *Zymoseptoria tritici* (*Septoria tritici* blotch) on wheat leaves. *Plant Pathology*, 64(6), 1429–1439.
- Muckle, A.E. (2013). *Management of Fusarium Head Blight and Septoria tritici Blotch in Winter Wheat through the use of Host Resistance and Chemical Controls and the Investigation of Fusarium graminearum Chemotype Diversity, Aggressiveness and Toxicity*. MSc. Thesis, University of Guelph, Ontario, Canada.
- Muhammad, R., Yong, W. (2017). Estimation of Yield Losses due to Leaf Rust and Late Seeding on Wheat (*Triticum aestivum* L) Variety Seher-06 in District Faisalabad, Punjab, Pakistan. *Advances in Biotechnology and Microbiology*.
- O'Driscoll, A., Kildea, S., Doohan, F., Spink, J., & Mullins, E. (2014). The wheat - *Septoria* conflict: a new front opening up? *Trends in plant science*, 19(9), 602–610.
- Palomar, M.A.Q. (2014). *Impact of fungicide mode of action and application timing on the control of Mycosphaerella graminicola and the physiology and yield of wheat*. PhD Thesis. Institute of Phytopathology and Applied Zoology, JLU Giessen.
- Ponomarenko, A., Gooding, S.B., Kema, G.J. (2011). *Septoria Tritici Blotch (Stb)*. Plant Health Instructor (www.apsnet.org).
- Roelfs, A.P., Singh, R.P., Saari, E.E. (1992). *Rust Diseases of Wheat: Concepts and methods of disease management*. CIMMYT, Mexico.
- Shipton, W.A., Boyd, S.R.J., Rosielle, A.A., Shearer, B.I. (1971). Common *Septoria* Diseases of Wheat. *Botanical Review*, 37(2), 231–262.
- Simon, M.R., Perello, A.E., Cordo, C.A., Struik, P.C., (2002). Influence of *Septoria tritici* on yield, yield components, and test weight of wheat under two nitrogen fertilization conditions. *Crop Sci.*, 42, 1974–1981.
- Thomas, M.R., Cook, R.J., King, J.E., (1989). Factors affecting development of *Septoria tritici* in winter wheat and its effect on yield. *Plant Pathology*, 39, 246–257.
- Torriani, S. F., Melichar, J. P., Mills, C., Pain, N., Sierotzki, H., & Courbot, M. (2015). *Zymoseptoria tritici*: a major threat to wheat production, integrated approaches to control. *Fungal Genetics and Biology*, 79, 8–12.

YIELD OF SEEDS AND SOME STRUCTURAL ELEMENTS OF THE PSEUDANTHIUM IN TRIBENURON-METHYL RESISTANT SUNFLOWER HYBRIDS, GROWN UNDER DIFFERENT SOIL NUTRITION REGIME

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Abstract

In the experimental field of the Department of Crop Science at the Agricultural University - Plovdiv during the two harvest 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. The effect of two soil nutrition regimes - lower and higher has been investigated (main plots). Five sunflower hybrids, all from the Tribenuron-methyl resistant hybrids group have been studied: P64LE25 (standard); LG 59.580 SX; Subaru HTS; ES Arcadia SU and Magma SU. Yield of seeds has been calculated from the harvest plots. The following pseudanthium yield components have been investigated: pseudanthium (head) diameter; density of the head and head harvest index. It has been found out that in both years of the study, the factor soil nutrition had a statistically proven positive effect on the yield of seeds. Soil nutritional regime had a positive effect on the diameter of the head by all studied hybrids. By most of the studied hybrids, the higher nutrient contain in soil results in a higher density of the head, an exception is the Magma SU hybrid. The reason for this negative result is the excessively high effect of the higher soil nutritional regime on the diameter of the head by this hybrid, which cannot be compensated by the higher number of seeds.

Key words: sunflower, tribenuron-methyl resistance, yield, pseudanthium, structural elements.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the most widespread technical crop in Bulgaria. This is not a coincidence, given that sunflower oil is the traditional vegetable oil that has been consumed in Bulgaria over the last 90 years. Increased consumption of vegetable fats in our country is consistent with the global trend of displacement of animal fats, and recently the dietary value of vegetable oils is explained by their antioxidant role in the human body (Stoyanova et al., 1977).

The Plovdiv region falls into an area with unfavorable sunflower growing conditions. However, sunflower here has a significant place in the field of crop rotation due to the lack of alternative crops suitable for cultivation as well as for the predecessor (Tahsin and Yankov, 2015; Yankov and Tahsin, 2015). One of the methods in sunflower farming in recent years is to make it easier to control weeds by introducing herbicide-resistant hybrids (Dochev et al., 2016; Poienaru & Sarpe, 2006).

The use of herbicide resistance crops has been the dominant weed management technology for

the past 20 years (Green, 2011). Glyphosate resistant crops became available when growers desperately needed the technology to effectively control problem weeds (Green & Owen, 2011).

Most herbicide tolerance genes are transgenic and therefore must be approved by the appropriate government agencies prior to cultivation. The DuPont™ Express Sun™ trait is a non-transgenic trait and therefore did not require the same approval process as transgenic traits. The DuPont™ ExpressSun™ (Tribenuron-methyl resistant) technology has been a revolution in aiding sunflower growers internationally with a genetic herbicide trait system designed to maximize weed control in sunflower crops, thereby enhancing production and yield (Streit, 2012).

The main indicator that characterizes each sunflower hybrid is its productive potential. Apart from some basic morphological features of the plant (height and thickness of the stem, number of leaves and leaf area, etc.), some structural elements of the sunflower head (pseudanthium) - the diameter of the pseudanthium and its density are most

important parameters which effect on a yield in sunflower (Mizzotti et al., 2015; Fambrini and Pugliesi, 2017).

MATERIALS AND METHODS

On the experimental field of the Department of Crop Science at the Agricultural University - Plovdiv during the two harvest years - 2018 and 2019 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 has been studied: P64LE25 - Pioneer® (standard); LG 59.580 SX - Limagrain®; Subaro HTS - Syngenta®, ES Arcadia SU - Euralis®, Magma SU - Caussade semences®. Seed yield has been calculated from the harvest plots in four replications. The following pseudanthium yield components have been investigated:

- Head (pseudanthium) diameter (Hd), cm;
- Density of the head (Ph), number of seeds/cm² (1):

$$(1) \quad Ph = \frac{Ns}{\pi \cdot r^2}, \text{ where:}$$

Ns is number of seeds per head;
r² is head radius².

- Head harvest index (HI) (2):

$$(2) \quad HI = \frac{S}{H}, \text{ where:}$$

S is mass of the seeds per head;
H is mass of whole head, g.

RESULTS AND DISCUSSIONS

The first year of the study is generally characterized as warmer and more humid compared to the multi-annual data for the area (Figure 1). Compared to the climate norm, the differences in temperature are minimal, during the whole vegetation, except September where they are from 1 to 3 degrees higher.

The amount of rainfall during the first month of vegetation, which coincides with the sowing and emergence of sunflower, is 20 mm less than the climatic norm, but still sufficient for the crop development. In May, June and July, the amount of rainfall exceeds the average multi-year values of the area. Particularly impressive is July, when rainfall is 88.3 mm higher than the climate norm. These rainfall conditions reflect favorably on the growth and development of sunflower, because they coincide with critical periods of the culture. The second harvest year begins with favorable climatic conditions for sowing, characterized by an average monthly temperature of 10.6°C for the month of March, as sowing have been done at the end of the same month. Comparing the temperature sum by months, it is clear from the climatic norm that the second harvest year also stands out as warmer, as with these favorable conditions and higher temperatures in the months of June and July conducive the acceleration of flowering.

The amount of rainfall during the second harvest year is characterized with drastic changes. The first month of vegetation - March is characterized with a very low amount of rainfall (8.8 mm), which is a very low value, considering that for this period culture needs water to germinate. This is the reason why germination occurs in 16 days, contrasting to the first year, when germination occurred in 10 days. However, the month of April differs with 31.5 mm more rainfall compared to the multiannual period and this compensates for the lack of moisture at the beginning of the development of the crop. In the following months of vegetation, higher sums of rainfall, compared to the multiannual period, are of great importance to sunflower, as they coincide with the budding and beginning of flowering phases, when the crop needs more moisture.

In the first year of the study, factor soil nutritional regime had a statistically proven action on yield of seeds (Table 2). In all tested sunflower hybrids, higher soil fertility had a positive effect on the amount of seeds. The most significant effect on yield of seeds is in the ES Arcadia SU hybrid, in which the difference in yield in the higher soil nutrition regime than the lower is 137.2 kg/da (Table 1). The following are P64LE25 (standard) - 114.8

kg/da; Magma SU - 114.5 kg/da; LG 59.580 SX - 94.3 kg/da), and with the lowest effect on

the yield, the higher soil regime has by the Subaru HTS hybrid - 74.5 kg/da.

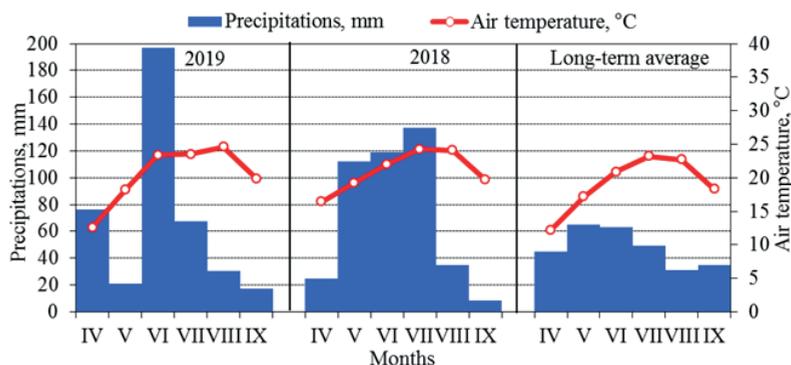


Figure 1. Climatogram of the year and the average for many years

In lower (NRL) soil nutrition regime the highest yield of seed have been reported in the LG 59.580 SX hybrid - 351.4 kg/da, followed by Subaru HTS (337.6 kg/da); P64LE25 (337.5 kg/da); Magma SU (296.9 kg/da) and lowest in the ES Arcadia SU hybrid - 280.3 kg/da.

Table 1. Yield of seeds, kg/da

Year	Soil nutrition regime	Hybrids				
		P64 LE25	LG 59.580 SX	Subaro HTS	ES Arcadia SU	Magma SU
2018	NRL	337.5	351.4	337.6	280.3	296.9
	NRH	452.4	445.7	412.1	417.5	411.4
	± D, cm ²	114.8	94.3	74.5	137.2	114.5
2019	NRL	194.9	271.3	203.8	186.5	174.0
	NRH	303.0	316.2	247.8	207.5	247.1
	± D, cm ²	108.1	44.9	44.0	21.1	73.1

In higher (NRH) soil nutrition regime the highest yield of seeds have been obtained from the P64LE25 (standard) - 452.4 kg/da, followed by LG 59.580 SX - 445.7 kg/da; ES Arcadia SU - 417.5 kg/da; Subaru HTS - 412.1 kg/da and lowest in the Magma SU hybrid - 411.4 kg/da.

Despite the described differences above, between the individual sunflower hybrids, from the implemented Anova Two-Factor analysis (Table 2), for the factor hybrid, the value of F is less than F crit., which defines this factor as having no proven effect on the yield of seeds. A similar result have been obtained when considering the interaction between the two factors, indicating that the availability of nutrients in the soil is not associated with a

dependent change in yield of seeds in the individual sunflower hybrids.

Table 2. Two-way ANOVA analysis of the yield of seeds

Year	Source variation	SS	df	MS	F	P-value	F crit
2018	Soil nutrition regime	114639.8	1	114639.8	70.868	0.00*	4.171
	Hybrids	16502.2	4	4125.6	2.550	0.06 ^{ns}	2.690
	Interaction	4494.5	4	1123.6	0.695	0.60 ^{ns}	2.690
2019	Soil nutrition regime	33868.9	1	33868.9	38.94	0.00*	4.17
	Hybrids	46178.8	4	11544.7	13.27	0.00*	2.69
	Interaction	8937.9	4	2234.5	2.57	0.06 ^{ns}	2.69

*Significance at P<0.05, ns - no significance.

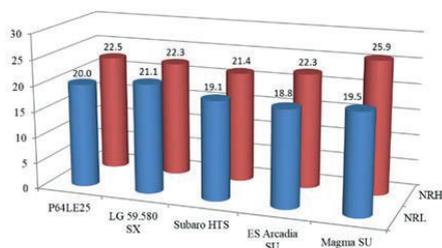
During the experienced 2019 year, the impact of soil nutrition regimes on yield of seeds is positive and again statistically proven (Table 2). Compared to the first harvest year, the impact of higher soil nutrition regime on yield of seeds is significantly lower. Higher levels of macronutrients, in all sunflower hybrids, result in a higher amount of seeds. The effect on yield of seeds is most pronounced in the P64LE25 hybrid where the difference in yield in the higher soil nutritional regime versus the lower stock is 108.1 kg/da, followed by the Magma SU hybrid - 73.1 kg/da; LG 59.580 SX - 44.9 kg/da; Subaru HTS - 44.0 kg/da, and the lowest effect on the yield, the higher soil nutrition regime has on the ES Arcadia SU hybrid - 21.1 kg/da. In lower (NRL) soil nutrition regime the highest yield of seeds is again as in the first year by the hybrid LG 59.580 SX - 271.3 kg/da, followed by Subaru HTS - 203.8 kg/da and P64LE25 - 194.9 kg/da; while the ES Arcadia SU hybrids - 186.5 kg/da and the

lowest value for the Magma SU hybrid - 174.0 kg/da, exchange their places.

In higher (NRH) soil nutrition regime the highest yield have been obtained in the LG 59.580 SX hybrid - 316.2 kg/da, followed by P64LE25 - 303.0 kg/da, Subaru HTS - 247.8 kg/da, Magma SU - 247.1 kg/da and the lowest for the ES Arcadia SU hybrid - 207.5 kg/da. From the two-factor analysis of variance (Table 2), the influence of the two factors is confirmed, and they independently confirm a proven influence on yield of seeds, but the interaction between them is unproven.

Soil nutritional regime had a positive effect on the diameter of the head by all studied hybrids in both years of the study (Figure 2).

2018



2019

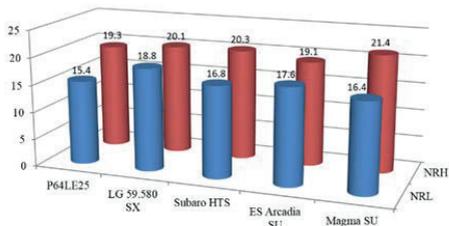


Figure 2. Head (pseudanthium) diameter (Hd), cm

The differences in the content of macronutrients in the soil has the strongest influence on the diameter of the head in the Magma SU hybrid - 6.38 cm larger head than the lower soil nutrition regime (NRL), followed by ES Arcadia SU - 3.15 cm; P64LE25 - 2.46 cm; Subaru HTS - 2.25 cm and LG 59.580 SX - 1.12 cm. In lower (NRL) soil nutritional regime the largest pseudanthium (head) forms LG 59.580 SX hybrid - 21.13 cm, followed by the standard P64LE25 - 20.00 cm; Magma SU - 19.50 cm; Subaru HTS - 19.13 cm and ES Arcadia SU - 18.75 cm.

In higher (NRH) soil nutrition regime the largest head is formed in the Magma SU hybrid - 25.88 cm, followed by the standard P64LE25 - 22.46 cm; ES Arcadia SU - 22.26 cm; LG 59.580 SX - 22.25 cm and Subaru HTS - 21.38 cm.

In the second year of the study (2019), soil nutrition regime again had a positive effect on the diameter of the pseudanthium in all studied hybrids (Figure 2).

Similarly to the first year of this study, soil nutrition regime had the strongest influence on the diameter of the head in the Magma SU hybrid - 21.38 cm in the NRH and 16.38 cm in the NRL; followed by Subaru HTS - 20.25 cm in NRH and 16.75 cm in NRL, as these two hybrids have the largest diameter of the head. The next LG 59.580 SX hybrid does not stand out from them, forming a head with a diameter of 20.13 cm for the NRH, and 18.75 cm for the NRL. The other two hybrids P64LE25 (standard) and ES Arcadia SU, form significantly smaller pseudanthium diameter, with the differences from the one with the highest head accordingly - 2.13 cm and 2.25 cm for the higher (NRH) soil nutrition regime.

Table 3. Two-way Anova analysis of the Head (pseudanthium) diameter

Year	Source of variation	SS	df	MS	F	P-value	F crit
2018	Soil nutrition regime	98.91	1.00	98.91	86.10	0.00*	4.17
	Hybrids	30.47	4.00	7.62	6.63	0.00*	2.69
	Interaction	31.83	4.00	7.96	6.93	0.00*	2.69
2019	Soil nutrition regime	93.03	1.00	93.03	70.43	0.00*	4.17
	Hybrids	19.56	4.00	4.89	3.70	0.01*	2.69
	Interaction	19.79	4.00	4.95	3.75	0.01*	2.69

*Significance at P < 0.05, ns - no significance.

From the performed two-factor analysis (Table 3), it is clear that the value of F for the soil nutrition regime is much greater than F crit, which clearly confirms the strong influence of the soil nutrition on the increase of the head diameter of sunflower. The influence of the hybrid factor has also been proven, which confirms the thesis that hybrids are different because of their distance in terms of their different origins.

In addition to the diameter of the pseudanthium and the quantitative content of seeds in it, basic

importance determining the productive potential of the hybrid is the quantitative ratio of seeds per unit area, determining the density of seeds in the head (Table 4).

Table 4. Density of the head (Ph), number of seeds/cm²

Year	Soil nutrition regime	Hybrids				
		P64LE25	LG 59.580 SX	Subaro HTS	ES Arcadia SU	Magma SU
2018	NRL	2.14	2.65	2.30	1.96	2.19
	NRH	2.35	2.98	2.40	2.05	2.00
	± D, cm ²	0.21	0.33	0.10	0.09	-0.19
2019	NRL	2.26	2.06	1.89	1.65	1.82
	NRH	2.34	2.31	1.98	1.67	1.46
	± D, cm ²	0.08	0.25	0.09	0.02	-0.36

In the first years of the study (2018), the highest density of the head at the first nutritional regime of the soil (NRL) has been reported by the LG 59.580 hybrid - 2.65 seeds per cm², followed by Subaro HTS - 2.30 seeds per cm². The other three hybrids have lower values ranging between 1.96 (for the ES Arcadia SU hybrid) and 2.19 seeds per cm² (for the Magma SU hybrid). Despite the described differences between the hybrids, they are not significantly proven due to the higher level of F-crit., compared to F, which exceeds the level of P several times, showing the unproven effect of the factor "hybrid" (Table 5).

The increased level of macronutrients in the soil leads to a proven effect of the factor on the density of the head (Table 5). In most of the studied hybrids, the higher nutritional regime resulted in a higher density of the pseudanthium, from 0.09 (in the ES Arcadia SU hybrid), to 0.33 seeds per cm² in the LG 59.580 SX hybrid. An exception is the Magma SU hybrid, where the higher soil regime leads to 0.19 less seeds in the head. The reason for this negative result is the excessively high effect of the higher soil nutritional regime on the diameter of the head by this hybrid (Figure 2), which cannot be offset by the higher number of seeds.

In the second year of this study (2019), higher soil nutritional regime lead to a proven effect of the factor on the density of the head, as well as the effect of the "hybrid" factor (Table 5). The highest density of the head at the NRL has been recorded in hybrid P64LE25 - 2.26 seeds per cm², followed by LG 59.580 SX - 2.06

seeds per cm². The other three hybrids have lower values ranging from 1.89 (in the Subaro HTS hybrid) to 1.65 seeds per cm² (in the ES Arcadia SU hybrid). Similar to the first nutritional regime of the soil, the second, (NRH) has the same sequence of the most densest head - in hybrid P64LE25 (2.34 seeds per cm²), followed by LG 59.580 SX (2.31 seeds per cm²), Subaro HTS (1.98 seeds per cm²), ES Arcadia SU (1.67 seeds per cm²) and the lowest density of pseudanthium in a Magma SU hybrid (1.46 seeds per cm²).

Table 5. Anova: Two-Factor analysis of head density (Ph)

Year	Source of Variation	SS	df	MS	F	P-value	F crit
2018	Soil nutrition regime	2.026	1	2.026	0.512	0.480 ^{ns}	4.171
	Hybrids	349.292	4	87.323	22.076	0.000*	2.690
	Interaction	57.543	4	14.386	3.637	0.016*	2.690
2019	Soil nutrition regime	30.695	1.000	30.695	8.366	0.007*	4.171
	Hybrids	354.771	4.000	88.693	24.174	0.000*	2.690
	Interaction	56.652	4.000	14.163	3.860	0.012*	2.690

*Significance at P < 0.05, ns - no significance.

Similarly to the first year of this study, the exception to the Magma hybrid is applies again, so higher soil nutritional regime lead to 0.36 seeds less sown in the head. The reason for this negative result is the excessively high effect of the higher soil nutritional regime on the diameter of the head in this hybrid (Figure 2), which cannot be offset by the higher number of seeds.

The different proportion of the organs of the plant is the main reason for the seed content of the total mass of the head, expressed by the harvest index (Table 6). The highest correlation of seeds in head is in the LG 59.580 SX hybrid - between 0.692 and 0.689 relative to the total weight of the head, followed by the P64LE25 (standard) - between 0.651 and 0.603; Magma SU - between 0.622 and 0.512; Subaro HTS - between 0.615 and 0.611 and the smallest harvest index have been reported by the ES Arcadia SU hybrid- between 0.579-0.580. Only in the last hybrids, the harvest index is higher in the NRH soil nutrition regime than the NRL. In all other hybrids, the higher soil stock has negative effects on this indicator.

Table 6. Head harvest index (HI)

harvest	Soil nutrition regime	Hybrids				
		P64LE25	LG 59.580 SX	Subaro HTS	ES Arcadia SU	Magma SU
2018	NRL	0.651	0.692	0.615	0.579	0.622
	NRH	0.603	0.689	0.611	0.580	0.512
2019	NRL	0.633	0.575	0.568	0.563	0.598
	NRH	0.625	0.583	0.488	0.523	0.528

In the second harvest year, the standard P64LE25 has the highest correlation of seeds in the pseudanthium - between 0.633 and 0.625 relative to the total weight of the head; followed by Magma SU - between 0.588 and 0.528; LG 59.580 SX - between 0.575 and 0.583; Subaro HTS - between 0.568 and 0.488 and the smallest harvest index again as in the first year by the ES Arcadia SU hybrid-between 0.563 and 0.523. Only in one of the hybrids (LG 59.580 SX), the harvest index is higher in the NRH soil nutrition regime than the lower. In the other four hybrids, better soil conservation of microelements, again has a negative effect on this indicator.

CONCLUSIONS

Yield of seeds in all studied sunflower hybrids was positively affected by higher soil fertility and by the more favorable climatic conditions of the year. The hybrid Arcadia differs with the highest yield in the first year and in the second-hybrid P64LE25. The structural elements of the yield (pseudanthium) of the studied sunflower hybrids are affected differently depending on the nutrient storage of the soil. The higher nutrition regime found out to have a positive effect on the indicator the diameter of the head. The density of the pseudanthium is also positively affected, with exception only of the Magma hybrid, where the higher soil fertility has a negative effect.

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REFERENCES

- Dochev, C., Mitkov, A., Yanev, M., Neshev, N., Tonev, T. (2016). Herbicide control of wild hemp (*Cannabis sativa* L.) at sunflower grown by "Express Sun" technology. *VII International Scientific Agriculture Symposium, "Agrosym 2016"*, 6-9 October 2016, Jahorina, Bosnia And Herzegovina. *Proceedings*, 1339–1344.
- Fambrini, M., Pugliesi, C. (2017). CYCLOIDEA 2 Clade Genes: Key Players in the Control of Floral Symmetry, Inflorescence Architecture, and Reproductive Organ Development. *Plant Molecular Biology Reporter*, 35(1), 20–36.
- Georgieva, R. (2019). Alteration of yield components of triticale depending on treatment with plant stimulants in the condition of different soil nutrition regime. *Journal of Mountain Agriculture on the Balkans*, 22(1), 130–138.
- Green, J.M. (2011). Outlook on weed management in herbicide-resistance crops: need for diversification. *Outlooks on Pest Management*, 22, 100–104.
- Green, J. M. and Owen, M.D.K. (2011). Herbicide-resistant crops: Utilities and limitations for herbicide-resistant weed management. *J. Agric. Food Chem.*, 59, 5819–5829.
- Mizzotti, S., Fambrini, M., Caporali, E., Masiero, S., Pugliesi, C. (2015). A CYCLOIDEA-like gene mutation in sunflower determines an unusual floret type able to produce filled achenes at the periphery of the pseudanthium. *Botany*, 93(3), 171–181.
- Poienaru, S., Sarpe, N. (2006). Selectivity, efficacy and yield of PR64E83 sunflower hybrid treated with Express 50 SG herbicide. *Lucrari Stiintifice, Universitatea De Stiinte Agricole Si Medicina Veterinara "Ion Ionescu De La Brad" Iasi, Seria Agronomie*, 49, 151–156.
- Stoyanova, J., Simeonov, B., Sabev, G., Petrov, D., Georgiev, I., Dimitrov, I., Georgieva-Todorova, J., Rangelov, L., Petrova, M., Ivanov, P., Palazov, P., Kontev, C. (1977). *Sunflower in Bulgaria*. BAS. Sofia (in BG).
- Streit, L. (2012). Du Pont™ Express Sun™ Herbicide Technology in Sunflower. In: *Proc. 18th Sunflower Conf., Mar del Plata-Balcarce, Argentina*, 143–149.
- Tahsin, N., Yankov, B. (2015). Performance of some sunflower's genotypes grown in South Bulgaria. *Journal of Central European Agriculture*, 16(3), 299–306.
- Yankov, B., Tahsin, N. (2015). Genetic variability and correlation studies in some genotypes of sunflower (*Helianthus annuus* L.). *Journal of Central European Agriculture*, 16(2), 212–220.

RESEARCHES CONCERNING EFFECTIVENESS OF THE SUNFLOWER SEEDS TREATMENT FOR CONTROLLING OF THE MAIZE LEAF WEEVIL (*Tanymecus dilaticollis* Gyll), IN SOUTH-EAST OF THE ROMANIA

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Abstract

Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is the main pest of the sunflower crops, mainly in south and south-east of the Romania. Each year, around one half million hectares cultivated with sunflower is attacked by this pest, with different level of the attack intensities. This paper presents some results of the researches concerning the effectiveness of the sunflower seeds treatments with imidacloprid, clothianidin and thiamethoxam active ingredients, for weevils control, in the climatic conditions from south-east of the Romania, at NARDI Fundulea experimental field. Both, in 2018 and 2019, there weren't registered significant statistical differences between weevils attack at treated plots. Highest statistical differences concerning weevils attack intensity it has registered between untreated and treated plots ($p < 0.05$). In 2018, at treated plots, saved plants percent ranged from 89.59 to 93.47% while in 2019, the percentage of saved plants, at treated plots, presented lower values comparative with previous year, ranged from 51.67 to 64.17%. A possible reason for this fact is because of higher attack of turnip moth larva (*Agrotis* spp.) at sunflower plants in 2019 comparative with 2018.

Key words: sunflower, pests, seed treatment, control.

INTRODUCTION

In last years, Romania has more than 1.0 million hectares cultivated with sunflower which represents the highest area within the EU28 (Eurostat, 2018; MADR data, 2018; Romanian Statistical Yearbook, 2018). According Chiriac et al. (2018), in 2016, sunflower cultivated area represented 12.37% of Romania's total cultivated area compared to 4.20% in 1990. Higher areas with this crop are located in south and south-east of the country (Maria et al., 2017; Kaya, 2019). Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is the main pest of both, maize and sunflower crops, mainly in south and south-east of the Romania (Paulian et al., 1969; 1974; Voinescu et al., 1985; Barbulescu et al., 1991; 1995; 2001a; Popov, 2002; Popov et al., 2004; 2005; 2006a). Each year, around one half million hectares cultivated with sunflower is attacked by this pest, with different level of the attack

intensities (Popov et al., 2007a). The pest is dangerous when sunflower crops are in early vegetation stages, from plants emergence until four leaves stage (Barbulescu et al., 1991; Rosca et Rada, 2009). Same authors mentioned that, after BBCH 14 stage, weevils have feeding only with leaf margins and damages are less economically important. In same time, weevils attack in early vegetation stages can be dangerous because insects can cut plants stem during feeding process and sunflower seedlings can be destroyed. However, in some cases, weevils attack can occur before plants emergence above soil surface, causing high yield losses, sometimes compromising not only maize crops, but also sunflower and sugar beet (Barbulescu et al., 2001b). Data from the literature make in evidence that in south-east of the Romania, yield losses ranged between 10 and 26 %, in case of moderate weevils attack at sunflower untreated plants (Barbulescu et al., 1993c; 1994; Barbulescu, 1995; 1997; 2001; Popov, 2003). Same authors mentioned that in

case of high weevils attack at sunflower untreated plants, yield losses ranged between 32 and 60%. Barbulescu et al. (1991; 1993a) make in evidence that in spring of the years 1990-1992, in south east of the Romania, pest density at sunflower crops ranged between 7.5 to 35.0 weevils/m², while at Tulcea County it has recorded 58 weevils/m². In same time, in Moldavia plateau and West Plane, pest density was low (0.5-1.5 weevils/m²). Researches made at NARDI Fundulea revealing that higher biological reserve of the maize leaf weevil (*T. dilaticollis*) it has registered in case of maize monoculture and sunflower cultivated after maize (Paulian, 1972; Barbulescu and Voinescu, 1998; Voinescu and Barbulescu, 1998). In some cases, even if the sunflower was sowed in plots with low pest reserve, the weevils can migrate from neighborhood plots that were sowed with maize in previous years (Barbulescu, 1996; Popov and Barbulescu, 2007). As result of both, increasing area cultivated with sunflower and decreasing number of economically effective crops, in favorable pest area from south and south-east of the Romania, farmers couldn't make a proper rotation, in many cases sunflower is sowed after maize (Dachim, 2016; Lup et al., 2017; Popescu et al., 2019). Maize leaf weevil is a thermo and xerophilous insect specie, being spreading especially in arid and semi-arid areas from Romania (Sisesti and Staicu, 1958, cited by Paulian, 1972). According Popov et al. (2006b) weevils are very active at high air temperatures and low humidity, registered in period when sunflower plants are in early vegetation stages while low air temperatures and high rainfall amount represent unfavorable conditions for weevils activity. Duffenbaugh et al. (2008) mentioned that climate changes will increase the prevalence of insect pests in maize and sunflower agro-ecosystems from Central and South-East of the Europe, including Romania. Other long term studies confirm this theory (Olesen, et al., 2011; Bebbler et al., 2014; Pietrapertosa et al., 2018; Choudhary et al., 2019). In the climatic conditions from south and south-east of the Romania, seeds treatment with systemic insecticides is the most effective method to control maize leaf weevil (*T. dilaticollis*) attack at sunflower crop (Barbulescu, 1995; 1997; 2001; Barbulescu et

al., 1993c; 1994; 2001; Popov, 2002; 2003; Popov et al., 2007b; Popov and Barbulescu, 2007; Georgescu et al., 2015; 2018; Trotus et al., 2018). After European Commission Regulations 218/783, 218/784 and 218/785, the use of imidacloprid, clothianidin and thiamethoxam active ingredients for all field crops, both like seeds treatment and foliar application will be total banned in UE, from 2019 (Official Journal of the European Union, 2018a; 2018b; 2018c). As result no insecticides will remain available for sunflower seed treatment against *T. dilaticollis* in Romania. According Ionel (2014), lack of the seeds treatment alternatives for spring crops, including sunflower, can have negative impact in Romanian agriculture in following years. Kathage (2018) mentioned that after ban of seeds treatment with neonicotinoids of the maize, sunflower and oilseed rape in EU, farmers use foliar and soil treatments that are more expensive comparative with seeds treatment. Same author mentioned that further studies is required to assess the effectiveness and sustainability of these alternatives compared with the restricted insecticides. In this paper it has presented some results of the researches concerning the effectiveness of the sunflower seeds treatments with imidacloprid, clothianidin and thiamethoxam active ingredients, for controlling of the maize leaf weevil (*T. dilaticollis*), in climatic conditions from south-east of the Romania, at NARDI Fundulea.

MATERIALS AND METHODS

The experiment were carried out at the experimental field of the Plants and Environment Collective, from National Agricultural Research Development Institute (NARDI) Fundulea, Calarasi County, Romania (latitude: 44° 46'; longitude: 26° 32'; altitude: 68 m), between 2018 and 2019.

Table 1. Active ingredients used in this research

Variant no.	Active ingredient (concentration)	Rate (l.c.p./t)*
1	control (untreated)	—
2	imidacloprid (600 g/l)	10.0
3	clothianidin (600 g/l)	9.0
4	thiamethoxam (350 g/l)	10.0

*litter commercial product per ton seeds

Active ingredients used in this study were listed on table 1. Experimental plots were arranged

according randomized blocks scheme. Each plot has 10 m length and 4.2 m wide as result plot area was of 42 m². In 2018 sunflower was sowed on 23 April and plants emergence occurred on 5 May while in 2019 sunflower was sowed on 12 May and plants emergence occurred on 18 May. For this experience it has used Performer sunflower hybrid. In both years, sunflower was sowed after maize. Distance between rows was 0.7 m. For this experience it has used lower plants density. On each plot it has sowed 180 sunflower seeds, that correspond on a density of 42857 seeds/ha. The purpose for using of lower plants density in this experience is to assure better conditions for weevils attack.

Pest density was rated two times, before sowing and when sunflower plants are in BBCH 14 stage. These assessments were made with metric branch, after noon, when air temperatures were higher, sky was without clouds or less clouds and wind was lower or absent. This weather conditions are favorable for weevils activity on soil surface. At each plot it has made 10 assessments with metric branch for determine weevils density per square meter.

Attack intensity of weevils was assessed when sunflower plants arrive in four leaves stage (BBCH 14). At each plot it has marked 20 plants, from four central rows (5 plant/row). Before assessment, the plants were marked with sticks, in stair system. Weevils attack was rated on a scale from 1 to 9, as follows:

- Note 1: plant not attacked;
- Note 2: plant with 2-3 simple bites on the leaf edge;
- Note 3: plants with bites or clips on all leaf edge;
- Note 4: plants with leafs chafed in proportion of 25%;
- Note 5: plants with leafs chafed in proportion of 50%;
- Note 6: plants with leafs chafed in proportion of 75%;
- Note 7: plants with leafs chafed almost at the level of the stem;
- Note 8: plants with leafs completely chafed and beginning of the stem destroyed;
- Note 9: plants destroyed, with stem chafed close to soil level.

Saved plant percent was rated at 30 days from sunflower emergence, by counting all the

emerged plants from a plot and comparing them with the number of sowed sunflower seeds/plot.

Plants height at 50 days from sunflower emergence was rated at same plants that, previously, it has assessed attack intensity.

Meteorological data were collected from automatic weather stations (iMethos), placed in the field at 100 meters from the experimental plots. It has registered average air temperature, soil temperature at 5 cm depth and daily rainfalls amount.

The data were **statistical analyzed** using Student - Newman - Keuls (SNK) test for multiple comparisons used to identify sample means that are significantly different from each other (Student, 1927; Neuman, 1939; Keuls, 1952).

RESULTS AND DISCUSSIONS

At experimental field from NARDI Fundulea, average air temperatures registered, both in April and May, 2018 were over multiyear average with a positive deviation of +4.7°C (April) and +2.5°C (May). Contrarily, average air temperatures registered, both, in April and May, 2019 were slightly over multiyear average, with a positive deviation of +0.1 °C in April and + 0.3°C in May (Figure 1).

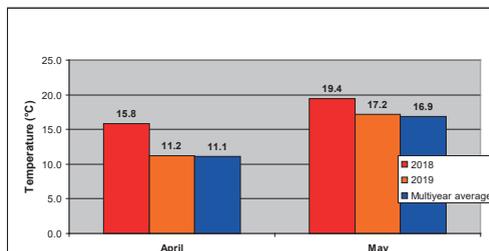


Figure 1. Average air temperature registered in April and May, 2018 and 2019, at NARDI Fundulea

In April, 2018, in south-east of the Romania, rainfalls amount level was close to zero. At experimental field from NARDI Fundulea, in April, 2018, it has registered only 3.0 mm of rains, while multiyear average for this month is 59.0 mm. In May, 2018, rainfalls amount was below multiyear average, with a negative deviation of 29.5 mm (Figure 2). In 2019, at NARDI Fundulea experimental field, rainfalls amount registered in April were higher then multiyear average with a positive deviation of

11.6 mm, while in May it has registered double rainfalls amount comparative with multiyear average, with a positive deviation of 125.1 mm. Overall weather conditions from spring period, at NARDI Fundulea, were favorable for *T. dilaticollis* attack at sunflower plants in 2018 and less favorable in 2019. However, weevils attack was higher in 2019 and lower in 2018.

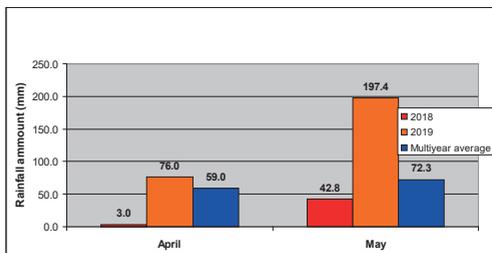


Figure 2. Rainfalls amount registered in April and May, 2018 and 2019, at NARDI Fundulea

Possible explication for lower weevils attack, at sunflower plants, in May 2018 is because of daily weather conditions evolution. Even if total rainfalls amount registered in this month was below multiyear average and average air temperature were higher, however between 8 and 18 May, when sunflower plants were in early vegetation stages (BBCH 10-14), the most susceptible period for pest attack, air and soil temperatures were lower while rainfalls amount where higher (Figure 3). In this period, daily weather conditions weren't favorable for weevils attack, because insects activity on soil surface was lower as result feeding process of the weevils were low and sunflower untreated plants presented low damages.

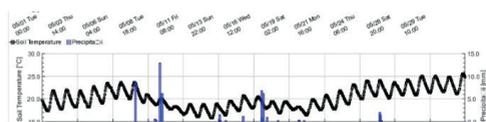


Figure 3. Daily soil temperatures and rainfalls amount recorded in May, 2018, at NARDI Fundulea, during early sunflower vegetation stages (BBCH 10-14)

In May, 2019, at experimental field of NARDI Fundulea, from sowing until emergence of sunflower plants it has registered 45.4 mm of rains. As result plants emergence it has occurred at 6 days after sowing, comparative with 12 days in 2018. In same time, daily average air temperatures were lower

comparative with multiyear average. From plants emergence (BBCH 10) until four leaves stage (BBCH 14) it has registered 20.2 mm of rains, while average air temperatures were higher then previous period. Weather conditions registered in early stages of sunflower plants were favorable for weevils activity on the soil surface. As result intensity of feeding process was high and damages at untreated sunflower plants was higher. On 31 May it has registered 72.0 mm of rains (Figure 4). Rainfalls amount registered in last day of May it was slight equal with multiyear average for all this month (72.3 mm). However rainfalls from 31 May didn't influence weevils attack, because sunflower was less sensitive for pest attack (BBCH 15-16).

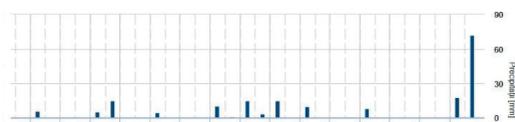


Figure 4. Daily rainfalls amount recorded in May, 2019, at NARDI Fundulea, during early sunflower vegetation stages (BBCH 10-14)

Bozo (2011) mentioned that precipitation evolution in Central and South-East of Europe show a decreasing trend, especially in spring period, but increasing precipitations is visible as a shorter term tendency. In last years, at NARDI Fundulea it has observed short periods with higher rainfalls amount, especially in May (Georgescu et al., 2014). This atypically evolution of weather conditions, especially when sunflower plants were in early vegetation stages (BBCH 10-14), can have effect on weevils activity (Georgescu et al., 2015). Further studies are necessary to evaluate daily weather conditions influence on both, *T. dilaticollis* activity and host plants reaction.

In 2018 and 2019, at experimental field from NARDI Fundulea, located in south-east of the Romania, before sowing of sunflower and when plants were in four leaves stage (BBCH 14) it has made assessments concerning weevils density. In last 10 days of the April, 2018, pest density has slight variability, ranged from 6.70 to 6.82 weevils/m². At second assessment, made in same day with those concerning attack intensity, it has noticed that at untreated variant pest density decreasing, at

5.35 weevils/m² while at treated variants, pest density decreasing below 3.50 weevils/m² (Figure 5). According Student - Newman - Keuls (SNK) test, there weren't registered significant statistical difference between pest densities, at treated variants. In same time there were significant statistical differences between treated and untreated variants ($p < 0.05$).

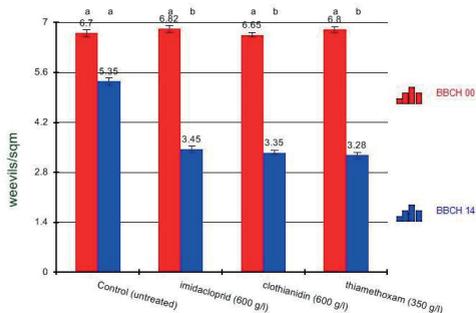


Figure 5. Weevils density, at experimental field from NARDI Fundulea, in 2018

Pest density was higher in 2019 comparative with 2018 (Figure 6). At first assessment, made at 12 May, before sowing of sunflower (BBCH 00), it has registered minimum 8.81 weevils/m² and maximum 9.43 weevils/m². At second assessment, made when sunflower was at BBCH 14 stage, pest density, at untreated variant was 8.65 weevils/m² while at treated variants ranged from 5.41 to 5.59 weevils/m².

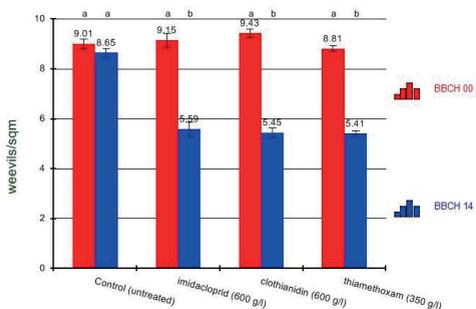


Figure 6. Weevils density, at experimental field from NARDI Fundulea, in 2019

In both years from this research, seeds treatment with imidacloprid, clothianidin and thiamethoxam active ingredients has result in decreasing of weevils density. Possible explication for higher pest reserve in 2019 comparative with 2018 is both, because of

increasing pest reserve from one year to another and migrations of weevils from neighbor plots. Also, daily weather evolution between 8 and 18 May, 2018, was less favorable for weevils feeding process.

Table 2. Attack intensity of *T. dilaticollis* at sunflower plants, in field conditions, at NARDI Fundulea

Active ingredient (concentration)	Rate (l.c.p./t)	Attack intensity (1-9)	
		2018	2019
control (untreated)	—	3.09	5.09
imidacloprid (600 g/l)	10.0	2.29	3.51
clothianidin (600 g/l)	9.0	2.18	3.58
thiamethoxam (350 g/l)	10.0	2.25	3.60
LSD P=0.05		0.399	0.560
Standard deviation (SD)		0.250	0.350
Variation coefficient (C.V.)		10.18	8.88

*Means followed by same letter or symbol do not significantly differ ($p < 0.05$, Student - Newman - Keuls test)

According Rosca et Rada (2009) in normal climatic conditions, economic threshold of *T. dilaticollis* is 5 weevils/m². In spring of 2018 and 2019, before sowing of the sunflower, pest density was higher then economic threshold. Also, in 2019, when sunflower plants were in BBCH 14 stage, even if the pest density were lower then at assessment made before sowing, however it was higher then 5 weevils/m². In same time, pest density registered at experimental field from NARDI Fundulea, both in 2018 and 2019 was lower then densities mentioned by Barbulescu (1991; 1993a).

Data from Table 2 ascertained that weevils attack intensity at sunflower plants, on a scale from 1 to 9, was lower in spring of 2018 and higher in spring of 2019. In both years from this study, at treated variants, attack intensity was lower comparative with untreated variant. According Student - Newman - Keuls (SNK) test, there weren't registered significant statistical differences between weevils attack at treated variants ($p < 0.05$). In same time, in both years, in the climatic conditions from experimental location, it has registered significant statistical difference between weevils attack at sunflower plants from treated variants comparative with untreated variant ($p < 0.05$). Also variation coefficient (CV) has a slight variation in the two years of this study.

Data from Table 3 make in evidence that, in 2018, at treated plots, saved plants percent, at 30 days from sunflower emergence, ranged from 89.59 to 93.47%.

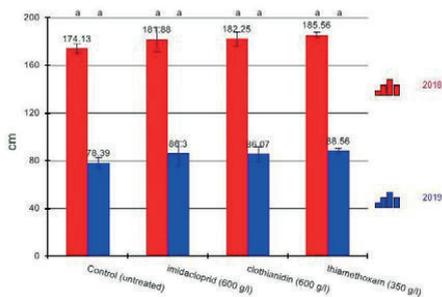


Figure 7. Plants height at 50 days after sunflower emergence, NARDI Fundulea (2018-2019)

Table 3. Influence of the seeds treatment, concerning saved plants percent, at 30 days from sunflower emergence (NARDI Fundulea)

Active ingredient (concentration)	Rate (l.c.p./t)	Attack intensity (1-9)	
		2018	2019
control (untreated)	—	84.59	51.25
imidacloprid (600 g/l)	10.0	89.59	51.67
clothianidin (600 g/l)	9.0	93.48	64.17
thiamethoxam (350 g/l)	10.0	89.86	57.50
LSD P=0.05		10.117	22.418
Standard deviation (SD)		6.325	14.016
Variation coefficient (C.V.)		7.08	24.96

*Means followed by same letter or symbol do not significantly differ (p<0.05, Student - Newman - Keuls test)

In 2019, the percentage of saved plants, at treated plots, presented lower values comparative with previous year, ranged from 51.67 to 64.17%. In both years there weren't registered significant statistical differences between experimental variants (p<0.05). However there were higher differences concerning saved plants percent in 2018 comparative with 2019. Also, variation coefficient (CV) was higher in 2019 comparative with 2018. Similar situation it has registered in case of plants height at 50 days from emergence (Figure 7). Possible explication for this is because of both, higher rainfalls amount from summer period of 2018 and high attack of turnip moth larva (*Agrotis* spp.) at sunflower plants from experimental field of NARDI Fundulea, in spring of 2019. In different climatic conditions from spring period, at experimental field of NARDI Fundulea, located in south-east of the Romania, sunflower seeds treatment with imidacloprid, clothianidin and thiamethoxam active ingredients provide effective protection of the sunflower plants against maize leaf weevils (*Tanymecus dilaticollis* Gyll) attack. Further researches are necessary for finding possible

alternatives of sunflower protection after permanent ban in EU of the neonicotinoid insecticides used both, for seed treatment and foliar application. Also, new researches are necessary for evaluate effect of the climate changes concerning attack of the *Agrotis* spp. and other similar pests at sunflower plants in Romania.

CONCLUSIONS

In the climatic conditions from south-east of the Romania, at experimental field from NARDI Fudulea, *T. dilaticollis* attack at sunflower plants, in early vegetation stages (BBCH 10-14) was lower in spring of the year 2018 and higher in spring of the year 2019.

Weevils density was lower in spring of the year 2018 and higher in spring of the year 2019.

Seeds treatment with imidacloprid, clothianidin and thiamethoxam active ingredients has effect in a reduction of both, weevils density and attack intensity at sunflower plants.

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REFERENCES

- Barbulescu, A. (1995). Results obtained in 1994 in the research on diseases and pests of cereals and some industrial and forage crops. *Problems of Plant Protection*, 23(2), 125–170.
- Barbulescu, A. (1996). Results obtained in 1995 in the research on diseases and pests of cereals and some industrial and forage crops. *Problems of Plant Protection*, 24(2), 87–142.
- Barbulescu, A. (1997). Results obtained in 1996 in the research on diseases and pests of cereals and some industrial and forage crops. *Problems of Plant Protection*, 25(2), 99–164.
- Barbulescu, A., Voinescu, I. (1998). Influence of the crop rotation on the evolution of *Tanymecus dilaticollis*. *Annals of I.C.C.P.T. Fundulea*, 65, 321–326.
- Barbulescu, A. (2001). Results obtained in 2000, regarding the diseases and pests of cereals and some

- industrial and forage crops. *Problems of Plant Protection*, 29(2), 123–178.
- Barbulescu, A., Ciurdarescu, G., Mateias, M.C., Popov, C., Gheorghe, M., Pelaug, A., Pelmus, V., Rugina, M., Voinescu, I., Bigiu, L., Costescu, P., Bratu, R., Vonica, I., Kozinschi, T. (1991). Evolution of some diseases and pests of grain industrial and forage crops in our country during 1990. *Problems of Plant Protection*, 19(1-2), 57–73.
- Barbulescu, A., Mateias, M.C., Popov, C., Rugina, M., Guran, M., Voinescu, I., Bratu, R., Vonica, I., Kozinschi, T. (1993a). Evolution of some diseases and pests of cereal, industrial and forage crops in our country during 1992. *Problems of Plant Protection*, 21(1), 47–68.
- Barbulescu, A., Popov, C., Voinescu, I., Mateias, M.C. (1993b). The impact of chemical control of pests and diseases in cereals and some industrial and forage plants, on the environment. *Problems of Plant Protection*, 21(1), 67–98.
- Barbulescu, A., Mustea, D., Sapunaru, T., Sendru, T., Burdea, T., Emilia, B., Ignatescu, I., Mateias, M. C., Popov, C., Rosca, I., Alister, C., Elena, B., Csep, N., Goga, N., Valeria, I., Dana, M., Felicia, M., Elena, N., Bratu, R., Lucica, P., Emilia, P., Marta, R., Stoica, V., Elena, T., Angela, U., Florina, V., Voicu, M., Voinescu, I., Emilia, L., Laura, M., Maria, O., Aurelia, S., Doina, C. (1993c). Results obtained in 1992 in the research on diseases and pests of cereals and some industrial and forage crops. *Problems of Plant Protection*, 21(2), 149–170.
- Barbulescu, A., Mustea, D., Sapunaru, T., Sendru, T., Burdea, T., Emilia, B., Ignatescu, I., Mateias, M. C., Popov, C., Rosca, I., Elena, B., Csep, N., Goga, N., Valeria, I., Dana, M., Felicia, M., Elena, N., Lucica, P., Emilia, P., Marta, R., Stoica, V., Elena, T., Angela, U., Florina, V., Voicu, M., Voinescu, I., Emilia, L., Laura, M., Maria, O. (1994). Results obtained in 1993 in the research on diseases and pests of cereals and some industrial and forage crops. *Problems of Plant Protection*, 22(2), 143–216.
- Barbulescu, A., Mateias, M.C., Popov, C., Guran, M., Voinescu, I., Stanciu, M., Raranciu, S. (1995). Evolution of some diseases and pests of grains, industrial and forage crops in our country during 1994. *Problems of Plant Protection*, 23(1), 75–92.
- Barbulescu, A., Popov, C., Mateias, M.C., Voinescu, I., Guran, M., Raranciu, S., Spiridon, C., Vasilescu, S., Valasn, D. (2001a). Evolution of some diseases and pests of grains, industrial and forage crops in our country during 2000. *Problems of Plant Protection*, 29(1), 1–16.
- Barbulescu, A., Voinescu, I., Sadagorschi, D., Penescu A., Popov, C., Vasilescu, S. (2001b). Cruieser 350 FS-A new product for maize and sunflower seed treatment against *Tanymecus dilaticollis* Gyll. *Romanian Agricultural Research*, 15, 77–87.
- Bebber, D.P., Holmes, T., Gurr, S.J. (2014). The global spread of crop pests and pathogens, *Global Ecology and Biogeography*, 23(12), 1398–1407.
- Bozo, L. (2011). Climate change in Central Europe: Observations and Model Scenarios. *Agrisafe final conference: Climate change: Challenges and Opportunities in Agriculture*, 9–11.
- Chiriac, A.R., Cristea, S., Popescu, M., Rasnoveanu, L. (2018). The evolution of sunflower crops in Romania in the context of the pre-and post-accession to the European Union, *In Agrarian Economy and Rural Development-Realities and Perspectives for Romania. 9th Edition of the International Symposium*, 156–162.
- Choudhary, J.S., Madhumita, K., Fand, B.B. (2019). Linking insect pest models with climate change scenarios to project against future risks of agricultural insect pests. *CAB Reviews*, 14(055), 1–13.
- Dachin, A. (2016). Structure of production costs for field crops in Romania—a regional analysis. *Competitiveness of Agro-Food and Environmental Economy*, 101–109.
- Diffenbaugh, N.S., Krupke, C.H., White, M.A., Alexander, C.A. (2008). Global warming presents new challenges for maize pest management. *Environmental Research Letters*, 3(4), 1–9.
- Georgescu E., Cana L., Popov C., Gargarita, R., Rasnoveanu, L., Voinea, L. (2014). Maize leaf weevil (*Tanymecus dilaticollis* Gyll) in the context of neonicotinoid seed treatment restriction. *Annals of N.A.R.D.I. Fundulea*, 82, 251–277.
- Georgescu, E., Cana, L., Gargarita, R., Voinea, L., Rasnoveanu, L. (2015). Atypically behavior of the maize leaf weevil (*Tanymecus dilaticollis* Gyll) on maize and sunflower crops, in climatic conditions of the Year 2014, in South-East of Romania. *Agriculture and Agricultural Science Procedia*, 6, 9–16.
- Georgescu, E., Toader, M., Ionescu, A.M., Cana, L., Rasnoveanu, L. (2018). Researches concerning sunflower seeds treatment against *Tanymecus dilaticollis*, in green house conditions, “Agriculture for Life, Life for Agriculture” Conference Proceedings, 1(1), 64–71.
- Ionel, I.I. (2014). Regulation implications on banning the treatment of seeds with neonicotinoid insecticides on the seed market in Romania. *Scientific Papers Agricultural Management*, 16(2), 223–228.
- Kathage, J., Pedro, C., José, L.A.P., Manuel, G.B., Emilio, R.C. (2018). The impact of restrictions on neonicotinoid and fipronil insecticides on pest management in maize, oilseed rape and sunflower in eight European Union regions. *Pest management science*, 74(1), 88–9.
- Kaya, Y. (2019). An overview of sunflower production in the Balkan region. *Oilseeds Focus*, 5(1), 40–41.
- Keuls, M. (1952). The use of the "studentized range" in connection with an analysis of variance. *Euphytica*, 1, 112–122.
- Lup, A., Miron, L., Alim, I.D. (2017). Studies and strategies regarding the evolution of crop yields per unit of land area in Romania, studies. *Scientific Papers Series Management, Economic Engineering*

- in *Agriculture and Rural Development*, 17(2), 227–233.
- Maria, M.C., Teodor, M., Nicoleta, A.A., Nicoleta, M.S. (2017). Evolution of cultivated areas and yield in the main crops in Romania. *Agricultural Management, Scientific Papers, Series I*, 19(3), 299–304.
- Newman, D. (1939). The distribution of range in samples from a normal population, expressed in terms of an independent estimate of standard deviation. *Biometrika*, 31(1), 20–30.
- Olesen, J.E., Tmka, M., Kersebaum, K.C., Skjelvåg, A. O., Seguine, B., Peltonen-Sainio, P., Rossig, F., Kozyrah, J., Micalei, F. (2011). Impacts and adaptation of European crop production systems to climate change. *European Journal of Agronomy*, 34(2), 96–112.
- Paulian, F., Popov, C., Marcela, D.P. (1969). The corn leaf weevil (*Tanymecus dilaticollis* Gyll.) in Romania and its control. *Contemporary Agriculture*, 5-6, 643–652.
- Paulian, F. (1972). Contribution at knowledge of the development, ecology and control of the *Tanymecus dilaticollis* specie (Contribuții la cunoașterea dezvoltării, ecologiei și combaterii speciei *Tanymecus dilaticollis*). *Doctoral thesis, I.A.N.B. Bucharest*, pp. 300.
- Paulian, F., Ciurdarescu, G., Mateias, M.C., Brudea, V., Caea, D., Ignatescu, I., Perju, T., Peteanu, S., Sapunaru, T., Sandru, I. (1974). Actual problems concerning diseases and pest of the forages. *Problems of Plant Protection*, 2(1), 76–109.
- Pietrapertosa, F., Valeriy, K., Salvia, M., Carmelina, C. (2018). Climate change adaptation policies and plans: A survey in 11 South-East European countries. *Renewable and Sustainable Energy Reviews*, 81, 3041–3050.
- Popescu, A., Stoian, E., Serban., V. (2019). Oil seeds crops cultivated area and production in the EU-28 - trends and correlations, 2008-2018. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 19(4), 265–272.
- Popov, C. (2002). Researches concerning the protection of the cereals, leguminous for grains, technical and forage plants against pathogen agents and pests, year 2001. *Problems of Plant Protection*, 30(2), 109–190.
- Popov, C. (2003). Researches regarding the protection of cereals, leguminous for grains, industrial and fodder crops to pathogens and pests performed in 2002 year. *Problems of Plant Protection*, 31(2), 7–84.
- Popov, C., Guran, M., Raranciuc, S., Rotarescu, M., Spiridon, C., Vasilescu, S., Gogu, F. (2004). Phytosanitary state of cereals, leguminous for grain, industrial and fodder crops in Romania in 2003. *Problems of Plant Protection*, 32(1), 1–23.
- Popov, C., Guran, M., Raranciuc, S., Rotarescu, M., Spiridon, C., Vasilescu, S., Gogu, F. (2005). Phytosanitary state of the cereals, leguminous for grains, forage and technical crops from Romania, year 2004. *Problems of Plant Protection*, 34(1-2), 15–37.
- Popov, C., Guran, M., Raranciuc, S., Rotarescu, M., Spiridon, C., Vasilescu, S., Gogu, F. (2006a). Phytosanitary state of the cereals, leguminous for grains, forage and technical crops from Romania, year 2005. *Problems of Plant Protection*, 33(1-2), 7–30.
- Popov, C., Trotus, E., Vasilescu, S., Barbulescu, A., Rasnoveanu, L. (2006b). Drought effect on pest attack in field crops. *Romanian Agricultural Research*, 23, 43–52.
- Popov, C., Raranciuc, S., Spiridon, C., Vasilescu, S., Cana, L. (2007a). Phytosanitary state of the cereals, leguminous for grains, forage and technical crops from Romania, year 2006. *Problems of Plant Protection*, 35(1), 1–24.
- Popov, C., Barbulescu, A., Raranciuc, S. (2007b). Seed treatment a modern, efficient and less pollutant method for field crops protection. *Annals of N.A.R.D.I. Fundulea*, 74, 133–139.
- Popov, C., Barbulescu, A. (2007). 50 years of scientific activity in field protection domain against diseases and pests. *Annals of N.A.R.D.I. Fundulea*, 75, 371–404.
- Rosca, I., Rada, I. (2009). *Entomology (Agriculture, Horticulture, Forest)*. Alpha Mdn Publishing House, 699 pp (pg. 119-123).
- Trotus, E., Mincea, C., Dudoiu, R., Pintilie, P.L., Georgescu, E. I. (2018). The preliminary results regarding the impact of the neonicotinoids insecticides, applied at rape, sunflower and maize seed treatment, on the harmful entomofauna and honey bees. *Annals of N.A.R.D.I. Fundulea*, 87, 251–260.
- Student (1927). Errors of Routine Analysis, *Biometrika*, 19(1/2), 151–164.
- Voinescu, I. (1985). Maize seed treatment with carbamic insecticides, effective method for controlling of the weevil *T. dilaticollis* Gyll. *Problems of Plant Protection*, 13(2), 151–156.
- Voinescu I., Barbulescu A. (1998). Evolution of maize leaf weevil (*Tanymecus dilaticollis* Gyll.) in various crops depending on the preceding crop. *Proceedings of International Symposium on Integrated Protection of Filed Crops*, Vrnja-ka Banja, 157–164.
- ***EUROSTAT. (2018). <http://ec.europa.eu/eurostat/data/database>
- ***MADR data (2018). <https://www.madr.ro/culturi-de-camp/plante-tehnice/floarea-soarelui.html>
- ***Official Journal of the European Union. (2018a). Commission implementing regulation (EU) 2018/783 of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance imidacloprid. 61(L132), 31-34, ISSN 1977-0677. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.132.01.00.31.01.ENG&toc=OJ:L:2018:132:FULL
- ***Official Journal of the European Union (2018b). Commission implementing regulation (EU) 2018/784

of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance clothianidin. 61(L132), 35-39, ISSN 1977-0677. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.132.01.0035.01.ENG&toc=OJ:L:2018:132:FULL

***Official Journal of the European Union (2018c). Commission implementing regulation (EU) 2018/785

of 29 May 2018 amending Implementing Regulation (EU) No 540/2011 as regards the conditions of approval of the active substance thiamethoxam. 61(L132), 40-44, ISSN 1977-0677. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2018.132.01.0040.01.ENG&toc=OJ:L:2018:132:FULL

***Romanian Statistical Yearbook (2018). Chapter "Agriculture and Silviculture", 489-528.

STUDY ON THE PRODUCTIVITY OF IRRIGATION WATER ON COTTON CROP

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Abstract

The analysis of the climate change in the country shows that during the summer months there is a decrease in soil moisture, as a result of the increase in temperature and less precipitation during the period. Agriculture affects both the quantity and quality of available water resources for other uses. The purpose of this study was to investigate the effect of irrigation and productivity of irrigation water in certain varieties of cotton grown in irrigated and non-irrigated conditions. Established productivity of three varieties of cotton. The estimated climate indices and coefficients and analyzes the productivity of irrigation water and the effect of 100 m³ irrigation water to produce a kg of cotton per hectare can be used in the design and operation of irrigation systems and in determining the economic impact of drip irrigation, taking into account security of rainfall for periods of committed research. When two waterings are made, the productivity ranges from 4.99-7.40 kg/mm. Productivity of the water, after the completion of four irrigations ranges of 2.66 to 3.26 kg/mm.

Key words: cotton, irrigation, varieties, yield, productivity.

INTRODUCTION

Global climate change towards warming and drought raises the question of more economical ways of using irrigation water. One area where new or updated technologies and policies can have a significant impact on more efficient use of water resources is crop irrigation (Ziad et al., 2010; Moteva et al., 2016; Kireva et al., 2018). Demand for water saving technologies in agriculture is a priority for many countries in the European Union and the world. Increasing the efficiency of irrigation water is related to water supply and absorption of water through the vegetation of the crops. Drip irrigation has proved its advantages and effectiveness in a number of crops. In this way localized on which the irrigation water can lead to a 30 ÷ 50% saving of irrigation water and 20 ÷ 40% increase in yield, considered Ravender et al. (2010). Efficiency of irrigation is studied in a wide range of crops (Saldzhiev et al., 2011; Stoyanova et al., 2018; Mahan et al., 2018; Stoyanova et al., 2019).

When cultivated agricultural crops, including and cotton in a market economy and private economy growing emphasis on agrotechnics of crops, productivity and quality, the optimal mineral nutrition and soil fertility in general,

the specific economic and environmental conditions.

In drip irrigation the irrigation rates may be regulated, restricted wetted surface and reduces evaporation from the soil surface. Possibility of applying fertilizers together with the irrigation water makes it possible to provide an appropriate diet of the plants in various stages of development and a better distribution of nutrients, reducing labor costs and reducing the amount of fertilizers (Petkov et al., 2013). Investigation of the influence of the feeding of nitrogen fertilizer deposited with irrigation water, has established that the reduction of the emission of 18.75% does not reduce the quality and quantity of the yields and increases the efficiency of utilization of the water supplied for irrigation (Zugui et al., 2003; Aujla et al., 2005; Li et al., 2017).

Yan et al. (2008) found that there was a significant correlation between root length density and cotton yield in the flowering stage and opening the box. There are a significant correlation between root length density and yields of cotton at flowering and boll opening stage.

The regression between irrigation amounts and yields of cotton are $y = -0.0026x^2 + 18.015x - 24845$ ($R^2 = 0.959$). The regression showed that

the irrigation amounts of 3464.4 m³/hm² can access to appropriate root length density and yields of 6360.8 kg/hm².

Study efficient use of irrigation water, in three varieties of cotton, it is the basis of this field study.

MATERIALS AND METHODS

The agricultural study was taken in the field trials of the Department of Plant Production at the Thracian University - Stara Zagora. During the period 2018-2019 on a soil type of typical meadow-cinnamon soil, in a fertilizer experiment under irrigation and non-irrigation conditions a field experiment with three varieties of cotton was laid. The subject of the study are cotton varieties and their productivity under the influence of different levels of fertilization and moisture supply. Agricultural experience has been set by the method of fractional plots in four replicates at the size of the harvest plot 15 m² (1.80 x 8.34 m). The varieties studied are Helius, Darmi and Isabell. Fertilization is carried out at four norms of nitrogen fertilizer (N₀; 8; 16; 24).

The varieties are Bulgarian selection approved by Executive Agency for Variety Testing, Testing and Seed Control. Helius was obtained by the method of experimental mutagenesis - irradiation with gamma rays (150 Gy) of seeds. Helius and Darmi were established and introduced into production as early as 2007-2008. Isabell variety is an achievement in the selection of cotton in our country and marks the beginning of a new generation of naturally colored fibers with high environmental and economic effect. The variety was obtained by intraspecific hybridization by crossing the breeding lines with brown and white fibers. The variety was recognized in 2009. High biological potential and high fiber quality are at the heart of the selection of varieties.

Due to the change of the climate elements and their influence on the growth, development and fruitfulness of the crops, the humidity coefficient and the hydrothermal coefficient

(HC) have been determined. The active temperature sums were used to determine climatic indicators and coefficients. The active temperature sums are determined by the equation:

$$\Sigma T^{\circ}\text{C} > 10^{\circ} = (t_1 + t_2 + t_3 + \dots + t_n),$$

where: $\Sigma T^{\circ}\text{C} > 10^{\circ}$ - sum of effective temperatures (for the period with average daytime temperature $> 10^{\circ}$) in $^{\circ}\text{C}$; $t_1, t_2, t_3, \dots, t_n$ - consecutive observations of daily average air temperatures in $^{\circ}\text{C}$; 1, 2, ... n - index for the daily number of days during the established period.

The wetting coefficient of Ivanov (1941) is determined by the following dependence:

$$E = 0.0018 * (t + 25) 2 * (100 - a),$$

where: t is the average monthly (decade) air temperature, $^{\circ}\text{C}$; a - average relative humidity, %. The values of the hydrothermal coefficient (by Selyaninov) are calculated by the formula:

$$K = P * 10 / \Sigma T^{\circ}$$

where P is the sum of precipitation (mm) over a period of time, Σ - this is the sum of average daily air temperatures ($^{\circ}\text{C}$) for the same period.

RESULTS AND DISCUSSIONS

Climatic conditions and coefficients

The elements of the climate that mainly influence the development, productivity and quality of cotton are temperature and rainfall. The air temperature in the ground of the test field is characterized by values close to the temperature norm (Figure 1). The fluctuations in the first trial year are larger. The sowing and the beginning of the growing season takes place at temperatures higher than normal. The average monthly values for April 2018 exceed by +3.68 $^{\circ}\text{C}$ in April compared to the annual norm set for a period of 89 years. Deviations were reported in May (+1.94 $^{\circ}\text{C}$) and August (+2.43 $^{\circ}\text{C}$). In the second year of the experiment, the measured temperature values show an excess in June (+2.27 $^{\circ}\text{C}$), August (+1.83 $^{\circ}\text{C}$) and September (+1.53 $^{\circ}\text{C}$). The total temperature is 10.8 $^{\circ}\text{C}$ higher in 2018 compared to the multiannual norm.

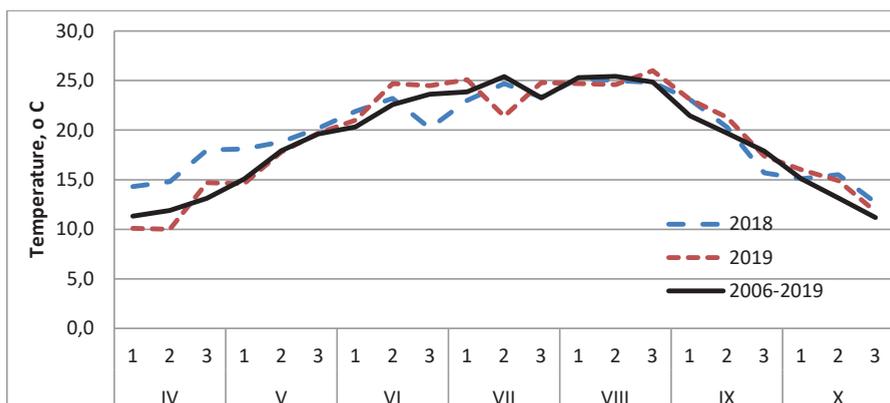


Figure 1. Dynamics of average air temperatures for the period growing season for cotton in the Stara Zagora region, 2018-2019

The data in Figure 2 show the amount and distribution of precipitation over ten days. The total rainfall over the entire growing season varies within a narrow range by year. At 346.0 mm (April-October), we can see how close the values are to the perennials. With regard to the moisture supply of cotton during the growing season, it is important to distribute the rainfall by ten days. The first year of the Field study is characterized by a distribution that guarantees

the availability of easily accessible moisture for the plants. Tensions are reported in July and the second and third ten days of August. A number of mathematical and statistical methods are used in the analyzes of climatic factors to calculate different climatic indicators and indices. Rainfall values and average monthly air temperature are the main parameters in calculating "evaporation".

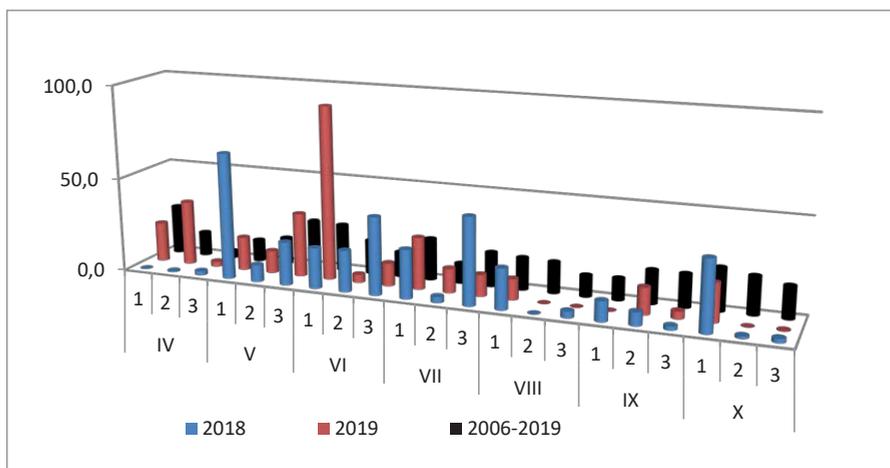


Figure 2. Dynamics of rainfalls during the growing season for cotton in the region of Stara Zagora, 2018-2019

The humidity coefficient and the moisture balance factor reflect the warmth and humidity of the area. The empirical formula for calculating the evaporation of Ivanov shows the degree of humidity over ten days for the period during which the plants need sufficient

moisture in the soil. In the last ten days of July, the humidity in the first year was 70% lower than the second. The water deficit during these periods necessitates watering to increase the water supply in the soil.

Table 1. Humidification and hydrothermal coefficients

Year	Humidity coefficient of Ivanov																	
	IV			V			VI			VII			VIII			IX		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
2018	1,14	0,91	1,36	1,00	1,07	1,40	1,23	1,59	1,14	1,37	1,87	1,01	1,86	2,21	2,01	1,79	1,33	1,25
2019	0,69	0,64	1,19	1,02	0,99	1,26	1,10	1,73	1,72	1,94	1,43	1,74	1,87	2,30	2,57	2,00	2,08	1,10
Hydrothermal coefficient																		
2018	0,01	0,04	0,12	3,71	0,48	1,14	0,99	0,95	2,03	1,13	0,13	1,97	0,86	0,00	0,14	0,46	0,35	0,13
2019	2,09	3,41	0,18	1,22	0,67	1,71	4,39	0,17	0,50	1,10	0,60	0,46	0,45	0,00	0,00	0,00	0,67	0,23

The humidity differs by a coefficient of variation, VC = 27.42%. The values of the hydrothermal coefficient range from 0.00 to 4.39 for 2019, which shows the nature of the year. High variability is characterized by CTC in the period VI-VII. The coefficient of variation of the moisture balance for the whole period at CTC is very high VC = 36.68%.

Irrigation water productivity

The productivity results of cotton grown under natural moisture conditions show a great variety over the two experimental years. Helios N16 fertilization reported an increase of 70.3% compared to zero fertilization in the first year, characterized by a more favorable rainfall distribution. In the second year, the increase was 114.6%. The water deficit during the period from flowering to fruiting influenced the flowering and retention of the boxes on the fruiting twigs. This has reduced the yield of

raw cotton. The results show that Darmi and Isabelle have the highest yields recorded in nitrogen fertilization with N24 under conditions of periodic water deficit in 2018. The excess ranges from 29.9% (Darmi) to 36.8% (Isabell).

In the second year, which is characterized by a higher humidity coefficient, the increase is in the narrower range from 1.4% to 3.3% for fertilization with N24. With better soil moisture, the results show higher yields of N16 fertilizer application. For all three varieties, productivity gains were measured within 6.8% to 14.6%. The distribution of moisture provided by rainfall creates the conditions for the development of more powerful stems, with larger boxes. On average for the study period, yields with nitrogen fertilizer at a dose of N16, under non-irrigation conditions, were highest for Helios (42.5%).

Table 2. Water productivity in three varieties of cotton grown at four levels of fertilization

Cotton varieties	Variants	2018		2019		Water produktivity kg/mm		Average		
		Yield, kg/ha	M	Yield, kg/ha	M	2018	2019	Yield, kg/ha	M	Water produktivity kg/mm
Helios	N ₀	1541,8	300	1668,7	600	5,14	2,78	1605,25	450	3,57
	N ₈	1496,7	300	1870,0	600	4,99	3,12	1683,35	450	3,74
	N ₁₆	1745,0	300	1817,0	600	5,82	3,03	1781,00	450	3,96
	N ₂₄	1533,4	300	1758,9	600	5,11	2,93	1646,15	450	3,66
	Average	1579,2	300	1778,6	600	5,26	2,96	1678,90	450	3,73
Darmi	N ₀	1891,8	300	1793,0	600	6,31	2,99	1842,40	450	4,09
	N ₈	1996,7	300	1840,4	600	6,66	3,07	1918,55	450	4,26
	N ₁₆	2220,1	300	1954,6	600	7,40	3,26	2087,35	450	4,64
	N ₂₄	1965,0	300	1808,9	600	6,55	3,01	1886,95	450	4,19
	Average	2018,4	300	1849,2	600	6,73	3,08	1933,80	450	4,30
Isabell	N ₀	1790,1	300	1597,6	600	5,97	2,66	1693,85	450	3,76
	N ₈	2171,7	300	1892,6	600	7,24	3,15	2032,15	450	4,52
	N ₁₆	1901,7	300	1869,4	600	6,34	3,12	1885,55	450	4,19
	N ₂₄	1795,0	300	1765,2	600	5,98	2,94	1780,10	450	3,96
	Average	1914,6	300	1781,2	600	6,38	2,97	1847,90	450	4,11

The productivity of irrigation water is an important point in modern conditions when water resources are scarce and of high value.

This study calculated the water productivity of the different fertilization and irrigation options (Table 2). The productivity of irrigation water

is defined as the ratio between the yield and the irrigation rate. An analysis of the productivity of irrigation water shows that the submission of a lower irrigation rate in 2018 leads to an increase in water productivity. When two waterings are made, the productivity ranges from 4.99 - 7.40 kg/mm. The highest productivity was found at Darmi (6.73 kg mm) and at all four fertilization levels. For Helius the values range from 4.99 to 5.82 kg/mm. For the Isabella colored natural fiber variety, the average for the four fertilizer variants is 6.38 kg/mm. The tendency to decrease in productivity values after the realization of four waterings in the second year is observed in the three varieties. The variation is very narrow, from 2.66 to 3.26 kg/mm. During the study average, irrigation water productivity was estimated to be lowest for Helius (3.57 kg/mm), zero fertilization, and 3.66 kg/mm for high nitrogen (N₂₄). The highest water productivity

is Darmi. The calculated average period average is 4.64 kg/mm for the N₁₆ fertilizer variant. Darmi exceeds water productivity by 15.3% compared to Helius. In terms of Isabell, data show 10.2% higher productivity.

The effect of irrigation is expressed by the resulting yield of raw cotton (Table 3). The higher yields resulting from the irrigation were the result of the second year. Increasing the water supply of soil moisture guarantees better plant development, more vegetative mass and fruit twigs. Helius has the largest vegetative mass, many boxes that cannot be opened and this reduces the yield. The rapid growth rate and the betting on more boxes, many of which remain open after irrigation, account for the lower first-year results. Four waterings were conducted in the second year. The irrigation effect is the reported additional yield for all varieties and variants of fertilization.

Table 3. Additional yield, effect of 100 m³ irrigated water and effect of 100 m³ irrigated water in kg in three varieties of cotton, Stara Zagora

Cotton varieties	Variants	Yield without irrigation, kg/ha		Yield under irrigation, kg/ha		Additional yield, kg/ha		Irrigation norm, mm		Effect of 100 m ³ irrigated water in kg cotton	
		2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Helios	N ₀	1408,3	1400,8	1541,8	1668,7	133,5	267,9	300,0	600,0	44,5	44,7
	N ₈	2121,7	1469,2	1496,7	1870,0	-	400,8	300,0	600,0	-	66,8
	N ₁₆	2398,4	1604,7	1745,0	1817,0	-	212,3	300,0	600,0	-	35,4
	N ₂₄	1991,7	1438,5	1533,4	1758,9	-	320,4	300,0	600,0	-	53,4
Darmi	N ₀	1758,4	1515,7	1891,8	1793,0	133,4	277,3	300,0	600,0	44,5	46,2
	N ₈	2061,7	1639,5	1996,7	1840,4	-	200,9	300,0	600,0	-	33,5
	N ₁₆	2108,4	1678,5	2220,1	1954,6	111,7	276,1	300,0	600,0	37,2	46,0
	N ₂₄	2283,4	1536,7	1965,0	1808,9	-	272,2	300,0	600,0	-	45,4
Isabell	N ₀	1476,7	1450,8	1790,1	1597,6	313,4	146,8	300,0	600,0	104,5	24,5
	N ₈	1906,7	1544,9	2171,7	1892,6	265,0	347,7	300,0	600,0	88,3	58,0
	N ₁₆	1845,0	1549,0	1901,7	1869,4	56,7	320,4	300,0	600,0	18,9	53,4
	N ₂₄	2019,8	1499,0	1795,0	1765,2	-	266,2	300,0	600,0	-	44,4

When calculating the effect of 100 m³ of irrigation water, the amount of additional yield and irrigation rate shall be taken into account. The effect ranges from 18.9 to 104.5 kilograms of cotton per hectare. Better humidity in 2018 forms a high yield in non-irrigation options. The additional yield obtained by Isabel shows the susceptibility of the variety to irrigation. The artificial irrigation water contributed to the realization of additional yields from 265.0 to 313.4 kg/ha at low fertilizer rates and at zero

fertilization. The irrigation effect is higher in the second year of the field experience, which is characterized by lower values of the CTC. The four waterings provided contribute to an additional yield of 15.2% to 28.6% for Helius, 13.3% to 18.2% for Darmi, and for Isabell it is in the range of 18.3% to 24.0%. As a result of this additional yield, an increase in the effect of 100 m³ of irrigation water is also observed. The effect of 100 m³ of irrigation water in 2019 is highest in Helius, with an average of 50.1 kg

of cotton per hectare for fertilizing options. For Isabell, the average for 2019 is 45.0 kg of cotton per hectare, and for Darmi it is 42.8 kg. Over the two years, it is estimated that Darmi and Isabell produce more than 100 m³ of irrigation water.

The calculated climate indicators and coefficients and analyzes of irrigation water productivity and the effect of 100 m³ of irrigation water for the production of kilograms of cotton per hectare can be used in the design and operation of irrigation systems and in determining the economic effect of drip irrigation, taking into account rainfall coverage for the periods for which the respective surveys were carried out.

CONCLUSIONS

Analysis of the irrigation water productivity data shows that the submission of a smaller irrigation rate leads to an increase in water productivity.

When two waterings are made, the productivity ranges from 4.99 - 7.40 kg/mm. Water productivity after the implementation of four irrigation ranges from 2.66 to 3.26 kg/mm.

The effect of 100 m³ of irrigation water varies by year, depending on the additional yield and the irrigation rate achieved. The study ranged from zero to 66.8 kg of cotton per hectare.

REFERENCES

Aujla, M.S., Thind, H.S., Buttar, G.S. (2005). Cotton yield and water use efficiency at various levels of water and N through drip irrigation under two methods of planting. *Agricultural Water Management*, 71(2), 167–179.

Yan, Y., Sheng, Y., Feng, S., Li Z.L., Jia H., Zhao C. (2008). Effects of Soil Moisture on the Distribution of Root Length Density and Yield of Cotton under Drip Irrigation. *Journal of Irrigation and Drainage*, 5.

Ziad, A.M. and Jamous, S.A. (2010). Climate change and agricultural water demand: Impacts and adaptations. *African Journal of Environmental Science and*

Technology, 4(4), 183–191.
<http://www.academicjournals.org/AJEST>

Yanmei, L., Yanxin, S., Shangqiang, L., Guoyuan, Z., Tongke, Z., Yanhua, C., Jungang, Y., Lin, Z. (2017). Effects of two slow-release nitrogen fertilizers and irrigation on yield, quality, and water-fertilizer productivity of greenhouse tomato. *Agricultural Water Management*, 186, 139–146.

Ravender, S., Kundu, D.K. and Bandyopadhyay, K.K. Enhancing. (2010). Agricultural Productivity through Enhanced Water Use Efficiency. *Journal of Agricultural Physics*, 10, 1–15.

Kireva, R., Petrova-Branicheva, V. (2018). Irrigation Mode off Fruit And Vegetables, Breded In Drop Irritation In The Area Of Sofia Field. Annual of The University Of Architecture. *Civil Engineering And Geodesy*, 51(6), 181–188.

Mahan, J., Payton, P. (2018). Design and Implementation of a Rainfed Matrix for Cotton. *Agriculture*, 8, 193.doi:10.3390/agriculture8120193

Mahan, J.R., Young, A.W., Payton, P.R., Bange, M.P., Stout, J. (2014). Effect of Differential Irrigation on Accumulation of Canopy Temperature-Based Heat Units in Cotton. *J. Cotton Sci.*, 18, 129–136.

Petkov, P., Varlev, I., Karaivanov, K. (2013). Determining the economically optimal sizing and economic effect of drip irrigation of vegetables. *Water business*, 5-6, 13_s4. pdf.

Saldzhiev, I., Raykov, S. (2011). Wide-row cultivation of cotton under irrigation. *Field Crops Studies*, VII(2), 293–298.

Stoyanova A, Valchev, N. (2018). Productivity of irrigation water of greenhouse tomatoes. *Lucrări Științifice*, 47, Horticultură, Viticultură și vinificație, Silvicultură și grădini publice, Protecția plantelor, Universitatea Agrară de Stat din Moldova, 171–177.

Stoyanova, A., Kuneva, V., Valchev, N. (2019). Survey Of The Influence Of Fertilization And Irrigation In The Tomatoes, Greenhouse Production. *Scientific Papers. Series B, Horticulture*, LXIII(1).

Selyaninov, G. (1958). On agricultural climate assessment. *Proceedings of agricultural meteorology*, 20.

Moteva, M., Trifonova, T., Georgieva, V., Kazandjiev, V. (2016). Anupdate of theirrigation depths according to the current climatic conditions in Bulgaria. *Journal of Mountain Agriculture on the Balkans*, 19(6), 226–242.

Zugui, L., Aiwang, D., Haiqing, W., Jiyang, Z., Guangxing, W. (2003). Impacts of Water-and-Fertilizer Allocation on Tomato Yield and Water Use Efficiency in Drip Irrigation Greenhouse. *China Rural Water and Hydropower*, 01.

RESEARCH REGARDING THE CONTROL OF DICOTYLEDONOUS WEEDS IN RAPESEED CROPS, IN CALARASI COUNTY

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Abstract

Rapeseed crop is one of the most profitable agricultural investments, very wide opened to development in Romania. In the latest years, rapeseed has been cultivated on larger surfaces, as this plant may produce quality oil. It is also known as a biodiesel plant, more and more requested for fuel, so that the total area cultivated with rapeseed was 632 thousand ha in 2018. Of this surface, 63,389 ha were grown in Calarasi County. New agricultural technologies and new hybrids have allowed it to grow successfully under our country's climatic conditions. Beside of this, this crop has its own particularities that must be carefully taken into consideration in order to obtain high yields. To give best results, rapeseed needs to be carefully protected, just because this plant can hardly bear weeds infestation, which is one of the limiting factor of yield. In recent years, due to weather evolution, with very mild winters, many weeds species (*Galium* sp., *Lamium* sp., *Matricaria* sp., *Thlaspi arvense*, *Veronica* sp., etc.) have grown, even propagated, so that they became a problem more and more often. On the contrary, under normal winter conditions, even during freezing winters, this fact would have never happened. These weeds, among which some invasive species (*Veronica persica*) become immune to the cold and enter the spring well-developed and compete with the crops for water, air, light and nutrients. In this context, the paper reveals aspects regarding the control of the annual and perennial dicotyledonous weeds in the rapeseed crop grown in two distinct locations in Calarasi County, where post-emergence treatments with clopyralid herbicides formulated in the form of a soluble concentrate (SL) and of water soluble granules (SG) were applied in spring. Herbicides were applied at different rates and at different crop and weeds stages. The assessments concerned the effectiveness in control, the safety of the crop as well as a comparative analysis between the two formulations SL vs. SG respectively, regarding the mode of action on weeds and the level of weeds control. The research results indicated that clopyralid-based herbicides had good efficacy in controlling dicotyledonous weeds depending on the rate applied, the time of application and the degree of weed infestation.

Key words: rapeseed, weeds, herbicides, efficacy, Calarasi.

INTRODUCTION

Canola (*Brassica napus* L.) is one of the most economically important oilseed crop worldwide which is grown mainly for edible vegetable oil and biodiesels production as well as animal feed (Mohamed, 2017). Canola seeds are a rich source of oil (about 40-45%) and protein (25%) and it is cultivated in more than 120 countries mostly in Asia, Europe, North America and Australia (Roshdy et al., 2008). Weeds are one of the most problematic pests of canola all over the world which cause considerable loss in quantity and quality of canola yield production (Khan et al., 2003, Berca, 2004, Singh et al., 2001, Mekki et al., 2010, Kaur et al., 2015, Grădilă, 2017). There are many various grass and broadleaved weeds species infesting canola fields in the world and resulting in yield loss of 20-50% (Kaur et al., 2015). Weed control was

relatively simple for monocotyledonous species, but was more challenging for some dicotyledonous species, especially *Brassicaceae* weeds related to canola (Blackshaw, 1989).

Integrated weed control in oilseed canola is a combination of preventative, mechanical and chemical methods to reduce environmental pollution. (Delchev & Georgiev, 2015). To be economically efficient, application of herbicides must be done in accordance with damage thresholds prevailing weeds (Lukacs & Halasz, 1987; O'Donovan, 1991; Klaus, 1992; O'Donovan & Newman, 1996). Chemical weed control is more effective than mechanical processing.

In the spring, after the weather warming up, there is an overwhelming development of winter rapeseed plants, but at the same time, of the weeds that survived through the winter, too.

As emerged, weeds compete with crops and deprive them of nutrients. Therefore, management control should be taken as soon as possible, when the weeds are still in low stages of vegetation.

One of the important aspects of the location technology and the maintenance of the autumn crops is the spring herbicide application.

One of the most used spring - applied herbicides in rapeseed crop is Lontrel (with clopyralid as active ingredient). Clopyralid does not exhibit phytotoxicity to the winter rape and provides better control of annual and perennial broadleaf weeds (Wei et al., 2010). Leyhe et al. (1994) reported a high herbicidal efficacy and selectivity of Lontrel in oilseed canola, too. In this context, the paper reveals aspects regarding the control of the annual and perennial dicotyledonous weeds in the rapeseed crop grown in two distinct locations in Călărași county, where post-emergence treatments with clopyralid herbicides formulated in the form of a soluble concentrate (SL) and of water soluble granules (SG) were applied.

MATERIALS AND METHODS

The trials have been carried out at S.C. Profarma Holding S.R.L. Tămădău and SC Ghinea Prod. S.R.L., Călărași county on experimental plots (Figure 1 and Figure 2). The experiments were conducted in 2018-2019, in 4 repetitions with plot area of 30 m² on loamy clay soil with a pH of 6.5 and an organic matter content of 2.5%. Each experimental block included an untreated plot and one standard reference. The herbicide Clopyralid 30 SL (300 g/l active ingredient) was applied in a dose of 0.2, 0.3 and 0.4 l/ha and Clopyralid 72 SG (720 g/kg active ingredient) at 0.083, 0.125 and 0.167 kg/ha. The hybrids taken into account were DK Exprit at Dâlga and Hybrirock at Tămădău. The planting density was 450000 plants per hectare. Sowing was performed on August 24 at Dâlga and September 21 at Tămădău. The previous crop was wheat. The following agro-technical measures have been applied: systematic crop rotation, rational choice of the preceding plant, deep plowing up to 30 cm depth in summer, seedbed tillage by 2 passes with disc harrow followed by milling, high quality hybrids, and sowing at the right

time at appropriate densities in accordance with crop technology. At the same time 200 kg/ha of complex fertilizer (40 N + 13 SO₃) was applied. The pathogens were controlled by two insecticides applications with cipermetrin 100g/l (Faster 10 CE 0.2 l/ha) and cipermetrin 25% (Cyperguard 25 EC 0.1 l/ha) at Dâlga, and by one fungicide application with tebuconazol 250g/l (Orius 25 EW 0.2 l/ha) and one insecticide application with alfa-cipermetrin 50 g/l (Fastac Active 0.2 l/ha) at Tămădău, respectively. All treatments were applied in the autumn, both at Dâlga and Tămădău.



Figure 1. Location of rapeseed plots at Dâlga



Figure 2. Location of rapeseed plots at Tămădău

The herbicides were applied in postemergence when rape was on the stage of 7 and 8 visibly extended internodes at BBCH 37-38 and weeds on the stage of two to four leaves at BBCH 12-14. Weeds density was assessed in ground % and in number of plants per square meter. Weed control (efficacy) was assessed at 10, 28, and 40 days after each application in % control in comparison with the untreated plots. Also, there were observations on the weeds found in the experimental plots before treatment, and selectivity - at each date of the efficacy

assessments. Determination of segetal flora was performed on one square meter using a metric frame. Statistical data - processing of the assessments was based on the analysis of ARM-9 software (P=.05, Student - Newman - Keuls).

RESULTS AND DISCUSSIONS

Generally, the important weeds to occur in oilseed rape can be ordered into several groups as follows: dicotyledonous species (excluding *Brassicaceae* family), dicotyledonous species belonging to the *Brassicaceae* synonym *Cruciferae*, annual grasses (including volunteer), and perennials.

As for the experimental plots the previous crop was wheat, the weed species spectrum on rapeseed crops looks like those on grain crops. Thus, in the experimental field at Dâlga the predominant weeds were annual dicotyledonous: *Papaver rhoeas* L., *Galium aparine* L., *Polygonum persicaria* Gray. and perennial

dicotyledonous *Cirsium arvense* (L.) Scop. and *Sonchus* species. The common poppy is a hard to control weed that became resistant to herbicides in recent years.

At Tămădău the predominant weeds were annual dicotyledonous: *Viola arvensis* Murray., *Galium aparine* L., *Euphorbia cyparissias* L., *Matricaria inodora* L., and perennial dicotyledonous *Cirsium arvense* (L.) Scop. and *Raphanus raphanistrum* L.

There were present also the species: *Fumaria officinalis*, *Lamium spp.*, *Descurainia sophia*, *Chenopodium album*, *Thlaspi arvense*, *Centaurea cyanus*, *Veronica persica* but in a low number. Canola plants during its initial growth stages are very sensitive to weeds interference (Kaur et al., 2015). The critical weed-free period for oilseed rape is from emergence to early flowering stages (Deligios et al., 2018). The growth stage of dominant weeds in experimental plots is presented in Table 1.

Table 1. Growth stage of dominant weeds in rape crops

Assessment	Dâlga			Tămădău		
	Weeds	BBCH ¹	Description	Weeds	BBCH	Description
1 st assessment	PAPRH (<i>Papaver rhoeas</i>)	16	6 true leaves unfolded	VIOAR (<i>Viola arvensis</i>)	16	6 true leaves unfolded
2 nd assessment		25	5 side shoots visible		30	Beginning of stem elongation
3 rd assessment		42	First young plant visible		51	Inflorescence or flower buds visible
4 th assessment		55	First individual flowers visible		63	30% of flowers open
1 st assessment	GALAP (<i>Galium aparine</i>)	14	4 true leaves unfolded	GALAP (<i>Galium aparine</i>)	14	4 true leaves unfolded
2 nd assessment		24	4 side shoots visible		32	2 visibly extended internode
3 rd assessment		36	6 visibly extended internode		51	2 side shoots visible
4 th assessment		51	Inflorescence or flower buds visible		60	First flowers open (sporadically)
1 st assessment	POLPE (<i>P. persicaria</i>)	18	8 true leaves unfolded	EPHCY (<i>Euphorbia cyparissias</i>)	18	8 true leaves unfolded
2 nd assessment		26	6 side shoots visible		42	First young plant visible
3 rd assessment		28	8 side shoots visible		59	5 true leaves unfolded
4 th assessment		59	First flower petals visible		65	Full flowering
1 st assessment	CIRAR (<i>Cirsium arvense</i>)	14	4 true leaves unfolded	MATIN Matricaria inodora	14	4 true leaves unfolded
2 nd assessment		24	4 side shoots visible		34	4 visibly extended internode
3 rd assessment		42	First young plant visible		42	First young plant visible
4 th assessment		59	First flower petals visible		65	Full flowering
1 st assessment	SONSS (<i>Sonchus</i> species)	14	4 true leaves unfolded	CIRAR (<i>Cirsium arvense</i>)	16	6 true leaves unfolded
2 nd assessment		24	4 side shoots visible		36	6 visibly extended internode
3 rd assessment		42	First young plant visible		42	First young plant visible
4 th assessment		60	First flowers open		65	First young plant visible
1 st assessment				RAPRA (<i>Raphanus raphanistrum</i>)	23	3 side shoots visible
2 nd assessment					42	First young plant visible
3 rd assessment					60	First flowers open (sporadically)
4 th assessment					71	Fruits begin to develop

¹BBCH scale= is a scale used to identify the phenological stages of a plant development

Coverage with weeds species in the experimental field was high: *P. rhoeas* 22.0%, *G. aparine* 17.5% at Dâlga and 15% at Tămădău, *P. persicaria* 12.2%, *C. arvense* 15.5% at Dâlga and 19% at Tămădău, *S. species* 19% and *R. raphanistrum* 10.3%, as a

ground % at 42 days after treatment application (Table 4).

In these infestation conditions, herbicides Clopyralid 30 SL and Clopyralid 72 SG provided a good efficacy control on annual and perennial dicotyledonous weeds in rape, at Dâlga and Tămădău. At 10 days after treatment

the herbicide had a good efficacy in control of weeds at all tested doses, both at Dâlga and Tămădău, except *R. raphanistrum* species from *Brassicaceae* family, whose efficacy was lower (Table 2). Clopyralid is a pyridinecarboxylic acid, absorbed in the leaves and roots, ceasing plant growth. This unique mode of action makes Clopyralid excellent for use in control strategies and resistance against broadleaved weeds (Leyhe et al., 1994). Once the herbicide is applied, it is quickly absorbed and translocated throughout the whole plant, including the roots, flowing to increased metabolic activity areas. This ability makes Clopyralid effective against weeds with deep

roots that are difficult to fight against, such as *Galium aparine*, *Sonchus* species and *Cirsium arvense* (Grădilă & Jalobă, 2018). Weeds do not die immediately, but their growth and development are stopped. When finally translocated throughout the whole weed, Clopyralid interrupts water absorption and nutrients included, plant metabolism being affected. The leaves dry out and lose their functional properties and at last plants die, even their deep roots. Besides, there are others trials or results that report high efficacy of herbicides Lontrel in oilseed canola crops (Tibets & Saskevich, 2006; Saskevich et al., 2009).

Table 2. The efficacy of herbicides in crop after 10 days of treatment

Treatment name	Dose l or kg/ha	Weeds										
		Efficacy - % control in comparison with the untreated plots										
		Dâlga					Tămădău					
	PAPRH	GALAP	POLPE	CIRAR	SONSS	VIOAR	GALAP	EPHICY	MATIN	CIRAR	RAPRA	
Untreated (ground %)		8.75	10	7	6.50	9.50	12.5	9	6	12	10	8
Untreated	-	0.0e	0.0c	0.0b	0.0b	0.0b	0.0c	0.0c	0.0b	0.0b	0.0b	0.0d
Clopyralid 30 SL	0.2	87.6d	95.5b	97.9a	98.1a	94.0ab	82.2b	96.1ab	99.3a	92.9a	89.7a	72.9c
	0.3	94.6bc	96.9ab	99.6a	99.6a	95.5ab	87.5ab	97.5ab	100a	97.7a	96.1a	76.6bc
	0.4	97.9b	99.4ab	100a	100a	99.3a	92.8a	98.8ab	100a	99.7a	97.5a	83.0a
Clopyralid 72 SG	0.083	87.2d	93.7b	99.3a	99.3a	89.3b	82.2b	91.1b	99.5a	93.4a	94.4a	71.5c
	0.125	89.5cd	95.5ab	100a	100a	95.1ab	91.0ab	96.9ab	100a	96.8a	94.1a	76.8bc
	0.167	100a	98.6ab	100a	100a	99.2ab	91.2ab	100a	100a	99.1a	98.7a	84.0a
Lontrel 300 SL	0.4	97.6b	100a	100a	100a	98.1a	95.4a	100a	100a	99.4a	99.0a	81.5ab
LSD (P=.05)		1.0-6.1	2.2-5.3	1.4-1.9	1.3-1.8	3.5-6.4	5.5-7.4	1.9-6.2	0.6-1.0	3.9-5.6	5.4-7.4	45-2.1
Standard Deviation		4.002t	5891t	4.679t	4.542t	4.873 t	4.21t	5.39t	4.04t	5.88t	6.13t	2.28t

Subsequent observations (28 and 40 days after treatment) confirmed the good results of the clopyralid herbicide in control of annual and perennial dicotyledonous weeds in rape (Tables 3 and 4). At the dose of 0.4 l/ha Clopyralid 30 SL and at the dose of 0.167 kg/ha Clopyralid 72 SG the herbicidal effect was preserved throughout the growing season of rape. At the dose of 0.2 l/ha Clopyralid 30 SL, the weeds species are not entirely controlled and control rate decreased. For example, in case of *V. arvense* from 70.7% at 28 days to 53.8% at 42 days after treatment and at the dose of 0.3 l/ha from 76.9% at 28 days to 61.3% at 42 days after treatment. The results were also similar to

those of Clopyralid 72 SG, applied at the doses of 0.083 and 0.125 kg/ha (Tables 3 and 4). Overall, the effectiveness of clopyralid in controlling annual and perennial dicotyledonous weeds was slightly lower at Tămădău, being compared to Dâlga trial efficacy, as the density of weeds on square meter and the ground cover of the weeds were higher. No phytotoxicity symptoms have been shown in the experimental plot. No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with clopyralid were seen (*, 2014).

Table 3. Efficacy of herbicides in rape crop after 28 days of treatment

Treatment name	Dose l or kg/ha	Weeds										
		Efficacy - % control in comparison with the untreated plots										
		Dălga					Tămădău					
		<i>PAPRH</i>	<i>GALAP</i>	<i>POLPE</i>	<i>CIRAR</i>	<i>SONSS</i>	<i>VIOAR</i>	<i>GALAP</i>	<i>EPHY</i>	<i>MATIN</i>	<i>CIRAR</i>	<i>RAPRA</i>
Untreated (ground %)		17.5	13.7	10	11.2	12.7	20.5	12	6.5	16.2	15	10
Untreated	-	0.0c	0.0d	0.0d	0.0c	0.0c	0.0c	0.0b	0.0b	0.0c	0.0e	0.0c
Clopyralid 30 SL	0.2	75.2b	86.9bc	89.3c	83.4b	82.6b	70.7b	86.0a	98.6a	79.8b	75.9d	62.5b
	0.3	83.1ab	90.4bc	94.0bc	90.9ab	87.3ab	76.9ab	89.8a	99.0a	85.4b	83.4bcd	68.3b
	0.4	93.3a	94.1abc	98.5ab	94.8a	94.5a	84.1a	94.8a	100a	94.5a	93.0a	75.8a
Clopyralid 72 SG	0.083	75.4b	82.8c	88.1c	83.6b	82.9b	70.5b	90.8a	98.8a	78.9b	75.4d	64.3b
	0.125	80.1b	87.1b	95.2bc	90.3ab	88.8ab	76.0ab	89.3a	99.4a	83.3b	81.3cd	69.05
	0.167	92.8a	98.1a	100a	95.3a	93.6a	82.3ab	96.3a	100a	93.4a	89.1abc	80.0a
Lontrel 300 SL	0.4	92.7ab	95.3ab	100a	97.2a	94.0a	83.0ab	96.9a	100a	92.3a	91.3.ab	79.2a
LSD (P=.05)		7.8-10.2	4.9-8.4	1.3-6.4	5.7-8.4	4.7-6.2	8.0-8.7	6.3-8.0	1.3-3.6	5.4-7.6	6.4-8.6	4.9-5.6
Standard Deviation		5.074t	4.958t	4.580t	5.067t	3.503t	3.966t	5.32t	7.44t	3.94t	4.233t	2.333t

Table 4. Efficacy of herbicides in rape crop after 40 days of treatment

Treatment name	Dose l or kg/ha	Weeds										
		Efficacy - % control in comparison with the untreated plots										
		Dălga					Tămădău					
		<i>PAPRH</i>	<i>GALAP</i>	<i>POLPE</i>	<i>CIRAR</i>	<i>SONSS</i>	<i>VIOAR</i>	<i>GALAP</i>	<i>EPHY</i>	<i>MATIN</i>	<i>CIRAR</i>	<i>RAPRA</i>
Untreated (ground %)		22	17.5	12.2	15.5	17.2	27.5	15	7.5	20.5	19	10.3
Untreated	-	0.0d	0.0c	0.0b	0.0c	0.0d	0.0c	0.0c	0.0c	0.0c	0.0c	0.0c
Clopyralid 30 SL	0.2	70.0c	72.3b	73.7a	69.4b	73.4c	53.8b	72.8b	86.2b	69.3b	63.0b	60.6b
	0.3	74.9 bc	78.8ab	78.0a	74.3b	78.0bc	61.3ab	78.0ab	91.1ab	74.1ab	69.4ab	62.1b
	0.4	97.9b	88.2a	88.5a	81.6	83.8abc	70.2a	82.5a	96.3a	82.8a	77.9a	70.7a
Clopyralid 72 SG	0.083	81.7a	71.4b	74.3a	68.5b	72.9c	54.6b	73.6b	85.7b	68.6a	63.5b	58.1b
	0.125	71.1c	78.2ab	83.2a	74.6b	77.9bc	60.9ab	75.9ab	91.9ab	73.6ab	68.6ab	62.9b
	0.167	82.0a	83.7ab	88.8a	83.7a	89.4a	73.0a	80.2ab	91.7ab	82.9a	76.8a	71.4a
Lontrel 300 SL	0.4	79.9 ab	87.8	87.8a	83.0a	86.4ab	70.3a	83.3ab	92.1ab	79.3ab	75.6a	71.76
LSD (P=.05)		4.7-5.2	8.6-9.9	11.1-12.0	5.4-6.3	7.3-8.8	8.3-8.9	5.1-5.5	3.5-5.0	7.6-8.3	7.1-7.7	4.5-5.6
Standard Deviation		2.306t	4.609t	5.907t	2.735t	4.134t	3.529t	2.536t	3.072t	3.689t	3.203t	2.980t



Figure 3. Experimental plot Dălga, 2018



Figure 4. Experimental plot Tămădău, 2019

CONCLUSIONS

The herbicides Clopyralid 30 SL and Clopyralid 72 SG provided a good result in rape against dicotyledonous weeds similar to standard reference.

At the dose of 0.4 l/ha Clopyralid 30 SL and at the dose of 0.167 kg/ha Clopyralid 72 SG, the herbicidal effect of active ingredient was maintained throughout the growing season of rapeseed.

In the case of *R. raphanistrum* from *Brassicaceae* family, the efficacy was lower.

This unique mode of action makes Clopyralid excellent for use in control strategies and resistance against broad-leaved weeds.

No phytotoxicity symptoms have been shown in experimental plots (Figures 3 and 4).

No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with clopyralid.

The research results indicated that the efficacy in controlling weeds of clopyralid formulated as soluble granules was slight higher than soluble concentrate.

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REFERENCES

- Blackshaw, R.E. (1989). Control of Cruciferae weeds in canola (*Brassica napus*) with DPX A7881. *Weed Sci.*, 37, 706–711.
- Berca, M. (2004). *Integrated weed management*. Ceres Publishing House, Bucharest.
- Brown, H., Cussans, G., Devine, M., Duke, S., Fernandez-Quintanilla, C., Helweg, A., Labrada, R., Landes, M., Kudsk, P., Streibig, J.C. (eds.).- Slagelse (Denmark), 969–974.
- Delchev, G., Gergiev, M. (2015). Achievements and problems in the weeds control in oilseed canola (*Brassica napus* L.). Series A. Agron., LVIII, 174–180.
- Deligios, P.A., Carboni, G., Farci R., Solinas S. and Ledda L. (2018). Low-input herbicide management: Effects on rapeseed production and profitability. *Sustainability* 2018, 10, 2258. doi: 10.3390/su10072258, 1–16.
- Grădîlă, M. (2017). Management of some noxious Dicotyledonous weeds in rape crops in Southern Romania. *Acta Zoologica Bulgarica, Supplement*, 9, 219–223.
- Grădîlă, M., Jalobă, D. (2018). Control measures of *Xanthium italicum* – an invasive species in maize crop (ROMANIA). *Acta Oecologica Carpatica*, XI, 73–84.
- Harker, K.N., O'Donovan, J.T., Turkington, T.K., Blackshaw, R.E., Lupwayi, N.Z., Smith, E.G., Klein-Gebbinck, H., Dossdall, L. M., Hall, L.M., Willenborg, C.J., Kutcher, H.R., Malhi, S.S., Vera, C.L., Gan, Y., Lafond, G.P., May, W.E., Grant, C.A., McLaren, D.L. (2012). High-yield no-till canola production on the Canadian prairies. *Canadian Journal of Plant Science*, 92(2), 221–23.
- Kaur, S., Bhullar, M.S., Singh, S., Singh. T. (2015). Control of weeds in canola gobhisarson cultivars and their tolerance to herbicides. *Indian J. Weed Sci.*, 47, 376–378.
- Khan, I. A., Hassan, G., Khan, M. A. (2003). Efficacy of post-emergence herbicides for controlling weeds in canola. *Asian J. Plant Sci.*, 2(3), 294–296.
- Klaus, M. (1992). Integrated weed control illustrated in the case of winter rape. *Gesunde Pflanzen*, 44(8), 251–254.
- Leyhe, A., Trozelli, H., Knaus, K. (1994). Lontrel 100 - new prospects for selective weed control in beet, oilseed rape and maize. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz*, 14, 577–581.
- Lukacs, P., Halasz, G. (1987). Realization of the research results achieved in the cultivation of oil-seed plants (sunflower and rape). *Olaj, Szappan. Hungary*, 36(4), 97–101.
- Lutman, P.J., (2018). Sustainable weed control in oilseed rape. *Weed Control: Sustainability, Hazards, and Risks in Cropping Systems Worldwide*, p.325.
- Mekki B.B., A. Faida, A. Sharara, Kowthar G. El-Rokiek. (2010). Effect of weed control treatments on yield and seed quality of some canola cultivars and associated weeds in newly reclaimed sandy soils. *American-Eurasian J. Agric. Environ. Sci.*, 7, 202–209.
- Mohamed, I.A. (2017). Efficiency of some post emergence acetyl coenzyme a carboxylase-inhibitor herbicides against certain grassy weeds in canola fields. *Egyptian Academic Journal of Biological Sciences, H. Botany*, 8.2, 49–58.
- O'Donovan, J.T. (1991). Quackgrass (*Elytrigia repens*) interference in canola. *Weed science*, 39(3), 397–401.
- O'Donovan, J.T., Newman, J.C. (1996). Manipulation of canola plant density and herbicide rate for economical and sustainable weed management. *Proceedings: Second international weed control congress*.
- Roshdy, A. M., Shams El-Din, G. M., Mekki, B.B., Elewa. T.A.A. (2008). Effect of weed control on yield and yield components of some canola varieties (*Brassica napus* L.). *American Eurasian J. Agric. Environ. Sci.*, 4, 23–29.
- Saskevich, P.A., Tibets, J.L., Gurikova, E.I. (2009). Agroecological assessment of plant protection products in the cultivation of spring rape. *Bulletin of*

- he Belarusian State Academy of Agriculture: Scientific-Methodical Magazine*, 2, 83–8.
- Singh, H., Singh B.P., Prasad. H. (2001). Weed management in *Brassica* species. *Indian J. Agron.*, 46(3), 533–537.
- Tibets, J.L., Saskevich, P.A. (2006). Comparative efficacy of herbicides with vozdelivanii spring rape. *Agroecology*, 4, 182–185.
- Wei, Y., Guo, Q., Feng, J. (2010). The occurrence regularity of weeds and its chemical control techniques in spring rape fields of no tillage with furrow sowing. *Journal of Northwest Agriculture and Forestry University*, 38(2), 184–190.
- *(2014) - <http://pp1.eppo.org/list.php>, PP1/135(4), *Phytotoxicity assessment. Efficacy evaluation of plant protection products Evaluation biologique des produits phytosanitaires. Bulletin OEPP/EPPO Bulletin*, 44(3), 265–273. DOI: 10.1111/epp.12134

RESEARCH ON THE GRAIN YIELD AND ITS QUALITY TRAITS IN SEVERAL WINTER WHEAT (*Triticum aestivum* L.) GENOTYPES GROWN UNDER THE CONDITIONS OF DĂLGA – CĂLĂRAȘI

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Abstract

The breeding programs of hybrid wheat are aimed at increasing the grain yield and ecological plasticity, as well as the stress tolerance to drought and heat that have an unfavourable effect on the harvest quality parameters. As a result, this brings up the question whether or not these hybrids can compete or even replace the wheat cultivars which are considered high in terms of bread-making value. In order to elucidate this question, comparative research was established in the 2014-2015 growing season. The genotypes studied consisted of four winter wheat varieties (cultivars: Dropia, Glosa, Katarina, Mulan) and four hybrids wheat (Hyfi, Hystar, Hywin, Hybiza). The hybrids that clearly produced higher grain yield than the winter wheat varieties. The results of the research have shown that the quality index, the technological indicators of the flour and the rheological attributes of the dough, all eight winter wheat genotypes tested and analysed recorded values that fit into the "very good" category regarding the milling-bakery properties. Of all studied hybrids, the Hyfi hybrid wheat was high to the other hybrids regarding the bread-making suitability.

Key words: winter wheat, grain yield, hybrid, quality index, bread-making value.

INTRODUCTION

Considering both the economic importance of wheat production and the special role it plays in human nutrition, the increase of the worldwide wheat production is the current approach and the future trend (Popa et al., 2013; Guta et al., 2015).

Besides the existing trend in increasing wheat yield levels through the use of hybrids, which allows the use of heterosis to meet the increasing global food demands (Longin et al., 2012; Çukadar, 2001), other objectives are being taken into account, such as the introduction on the market of varieties with high protein content, increased nutritional value and high bakery qualities (Kadar et al., 2009).

The emphasis on quality breeding is highlighted by numerous international research and meetings on wheat quality (Has et al., 2010; Cociu, 2014).

Nevertheless, in recent decades it has been found that the progress made in obtaining genotypes with high protein content and high bakery qualities is less obvious than expected due to the fact that wheat breeding activity is

directed mainly towards quantitative increase and less towards improving its quality parameters (Hrušková et al., 2012; Luo et al., 2000).

The possibility of developing new winter wheat genotypes by combining high yields and a higher nutritional quality is fully achievable (Egesel et al., 2012), taking into account that quality traits, although strongly influenced by environmental conditions, are also based on a quite wide intraspecific genotypic variability (Mustatea et al., 2003). The evolution of the breeding programs for developing new winter wheat genotypes that are better than the existing ones, involves continued genetic recombination. The number and value of the combinations widely depend on biodiversity and on the value of the already available genes (Yousif et al., 2015).

Knowing the contribution of genetic factors in the phenotypic realization of the quantitative traits is of major importance. Each winter wheat variety has specific genetic particularities such as morphological and physiological traits, grain yield, yield stability, lodging, pre-harvest sprouting, tolerance

diseases, protein content (Săulescu et al., 2010).

The most important quality index are protein content, wet gluten content, dry gluten content, bread final volume and weight, and farinograph measurements (Borghi et al., 1994; Egesel et al., 2012; Dumbrava et al., 2019).

Besides high yield potential, winter wheat genotypes must have high protein content, high nutritive value of the grains, tolerance to unfavourable environmental factors, and tolerance to diseases and pests (Blum, 1996; Longin et al., 2012).

The continuous improvement of crop technologies, the use of high yielding genotypes, with high-quality index adapted to the particular climatic and soil conditions of the various agricultural areas of Romania (Marin et al., 2015) are very important from the economic perspective, as well as for the future destination of the grain yield (Cociu, 2014; Lupu, 2010).

The phytosanitary treatments performed during the vegetation period ensure high expression of the biological yield potential and of the quality index (Cristea et al., 2015).

MATERIALS AND METHODS

Research was carried out in the 2014-2015 growing season on the chernozemic soil specific to the Dâlga area, Călărași county, Romania. The comparative study was aimed to analyses the local and foreign winter wheat genotypes (cultivars and hybrids varieties) regarding grain yield, quality index and bread-making value.

In the 2014-2015 agricultural growing season, the total amount of rainfall was 455.7 mm, accumulated from sowing to harvest. During the vegetation period from spring to summer, the amount of rainfall accumulated was 163.5 mm, with 54.8 mm rainfall accumulated in the critical water phase for winter wheat (May-June), and a daily precipitation average of 0.89 mm/day at this stage (Table 1).

The average daily temperature during the vegetative period (from October 2014 until June 2015) was 9.63°C. In these conditions, due to the efficient use of precipitation water under non-irrigation conditions, the tested wheat hybrids achieved yield performances that

proved that they were competitive, compared with the best winter wheat varieties cultivated on the Romanian Plain nowadays.

Table 1. Climatic conditions during the 2014-2015 growing season

Month/ Growing Season	2014- 2015 Temp. (°C)	Normal average of area (°C)	2014- 2015 Rainfall (mm)	Normal average rainfall of area (mm)
October	13.0	12.99	65.6	44.25
November	7.33	7.45	49.0	39.88
December	1.79	1.38	109.0	40.79
January	-0.1	0.04	30.5	40.73
February	3.1	2.08	38.0	26.64
March	6.8	7.29	95.3	32.54
April	12.7	12.06	13.45	34.78
May	19.2	18.15	4.8	58.32
June	22.9	21.38	50.0	69.76
Average/ Amount	9.63	9.20	455.7	387.4

The biological material in this study consisted of four local and foreign winter wheat varieties (cultivars: Dropia, Glosa, Katarina, Mulan) and four wheat hybrids (Hystar, Hyfi, Hywin, Hybiza).

The experiment was mono-factorial and was located in the field according to the randomized blocks method, in three replications, within eight genotypes of winter wheat.

The pre-crop plant was sunflower (*Helianthus annuus* L.). Soil preparation started immediately after harvesting with a disk harrow, followed by plowing at 25 cm in depth. The seedbed cultivator was used after the application of complex chemical fertilizers in a dose of 40 kg N active ingredient (a.i.)/ha and 40 kg P a.i./ha one day before sowing.

Sowing was carried out in the favourable timeframe, i.e. 10th October 2014, with a density sowing of 500 kernels/m² for the wheat varieties and 200 kernels/m² for the wheat hybrids, at a space of 12.5 cm between rows and 4-5 cm sowing depth.

In spring, as soon as the climatic conditions were favourable for entering the field (30th March 2015), phase fertilization 200 kg/ha of Urea (92 kg N a.i./ha) was administered to the fields, the second phase fertilization 150 kg/ha of Ammonium nitrate (49.5 kg N a.i./ha). Weed control was performed with Mustang (Florasulam 6.25 g/l + 2.4 D EHE 300 g/l) in a dose of 0.6 l/ha and Helmstar 75WG (tribenuron-metil 750 g/kg), in dose of 15 g/ha.

The phytosanitary treatments with fungicide Duet Ultra (310 g/l tiofanat metil + 187 g/l epoxiconazol) in a dose of 0,5 l/ha performed on 4th April 2015. The second fungicide treatment was Amistar Xtra 280 SC (azoxistrobin 200 g/l + ciproconazol 80 g/l) in a dose de 0.5 l/ha combined with insecticide Lamdex 5 EC (Lambda-cihalotrin 50 g/l) in a dose de 0.2 l/ha.

Harvesting was carried out separately for each experimental variant on 8th July 2015. The production and yield of each studied genotype was determined, and average seed samples were taken for laboratory analysis. The analysis was focused on the main physical indicators showing the quality of the harvest, as follows: 1000 seed weight TKW (g) and volumetric weight HM (kg/hl), moisture content M (%); it also included determinations regarding the quality of the raw material for baking, as follows: the technological indicators of the flour and the rheological traits of the dough obtained. The analytical section was performed with the INFRATECTM 1241 grain analyser.

The analysis and the interpretation of the experimental results was based on variance analysis, according to the experimental method of field setting (Săulescu et al., 1967), considering the average of the winter wheat

varieties and the average of the analysed wheat hybrids as reference points.

RESULTS AND DISCUSSIONS

The analysis of the yield potential for all the winter wheat genotypes studied (Table 2) showed higher values of the winter wheat hybrids compared to the winter wheat varieties tested. The yield grains registered values ranging from 7386 kg/ha in Drobia to 10137 kg/ha in Mulan, the latter recording the highest yield of all the analysed winter wheat varieties, compared to the average of the varieties taken as control 1 (9139.2 kg/ha).

The analysis of the yield obtained from varieties compared to the average of the hybrids, we noticed a decrease in production with values between 450.8 kg/ha in Mulan and 3201.8 kg/ha in Drobia.

The winter wheat hybrids tested were characterized by high yield compared to the wheat varieties taken as control, with a yield increase between 1070.8 kg/ha and 2051.8 kg/ha. Analysing the grain yield of the four hybrids tested in the experiment, we recorded variations between 10210 kg/ha and 11191 kg/ha, with the Hystar hybrid being the most valuable.

Table 2. Grain yield obtained in winter wheat genotypes, Dâlga area, Călărași County

Var. No.	Genotype cultivated	Yield (kg/ha)	(%)	Difference from Control 1 (kg/ha)	Significance	Difference from Control 2 (kg/ha)	Significance
1	GLOSA	9277	102	137.8	-	-1310.8	ooo
2	DROPIA	7386	81	-1753.2	ooo	-3201.8	ooo
3	KATARINA	9757	107	617.8	xx	-830.8	oo
4	MULAN	10137	111	997.8	xxx	-450.8	o
VARIETY AVERAGE (Control 1)		9139.2	100	-	(Ct. 1)	-	
LSD 5% = 357; LSD 1% = 462; LSD 0.1% = 956							
Nr. Crt.	Genotype cultivated	Yield (kg/ha)	(%)	Difference from Control 2 (kg/ha)	Significance	Difference from Control 2 (kg/ha)	Significance
5	HYSTAR	11191	106	603.2	xx	2051.8	xxx
6	HYFI	10554	100	-33.8	-	1414.8	xxx
7	HYWIN	10210	96	-377.8	o	1070.8	xxx
8	HYBIZA	10396	98	-191.8	-	1256.8	xxx
HYBRID AVERAGE (Control 2)		10587.8	100	-	(Ct. 2)	-	
LSD 5% = 233; LSD 1% = 446; LSD 0.1% = 974							

Thousand-kernel weight (TKW) values for the analyzed winter wheat varieties (Table 3) ranged from 42 to 53 g, with the highest value being recorded in Drobia, which is known as one of the most qualitatively valuable variety. The TKW values for hybrids varied between 43 g in Hywin and 50 g in Hybiza, the latter being very close to the quality of the Drobia variety. The hectolitre mass (HM) of the grains (Table 3) recorded in the wheat varieties tested ranged between 80 kg/hl in Mulan and 83 kg/hl in Glosa, with higher values in the analysed varieties than in the hybrids. Thus, in the four wheat hybrids tested in the experiment, the hectolitre mass ranged from 77 kg/hl in Hywin and 79 kg/hl in Hystar and Hyfi.

Table 3. Experimental results of physical quality indexes in winter wheat, Dâlga area, Călărași County

Genotype cultivated	TKW (g)	(%)	HM (kg/hl)	(%)	Bread-making quality*
GLOSA	49	104	83	102	VG
DROPIA	53	112	82	101	VG
KATARINA	45	95	81	99	VG
MULAN	42	89	80	98	VG
VARIETY AVERAGE (Ct. 1)	47.3	100.0	81.5	100.0	-
HYSTAR	48	101	79	101	VG
HYFI	49	103	79	101	VG
HYWIN	43	91	77	98	VG
HYBIZA	50	105	78	100	VG
HYBRID AVERAGE (Ct.2)	47.5	100.0	78.3	100.0	-

Bread-making quality* SR EN ISO 7971-3: 2010 (VG = very good)

Analysing the bread-making quality of the eight winter wheat genotypes studied (Table 3) they met the baking requirements as they exceeded the minimum amount of hectolitre mass for this purpose, i.e. 75 kg/hl.

In all the wheat variety tested in the experiment, the grain moisture determinations (Table 4) had values ranging from 12 to 14%, i.e. the corresponding limits established for bread-making wheat varieties. The protein content of the grains (Table 4) varied between 11.7% and 14.7%. The average of the regular varieties was higher than the average of the hybrids. Since there was no significant variation between the winter wheat varieties studied, they could be successfully used for bakery purposes.

The determinations related to the starch content of the grain (Table 4) in the wheat varieties ranged between 66.7% and 69.8%, with an average of 67.9%. In the wheat hybrids it varied between 68.6% and 69.9%, with an average starch content of 69.1%, which indicated that the hybrids were higher in quality when compared with the regular varieties.

The wet gluten content of the wheat strains tested in the experiment (Table 4) showed values between 29.7% and 35.9%. Glosa and Drobia contained an exceeding amount of gluten (32%), which included these varieties in the category of “very good” winter wheat variety for bakery purposes. With a wet gluten content ranging between 30.4% and 29.7%, Katarina and Mulan were considered “good” for this purpose.

Table 4. Moisture, protein and starch content in wheat varieties, Dâlga area, Călărași County

Genotype cultivated	Moisture (%)	%	Crude protein (%)	%	Starch (%)	%	Wet Gluten (%)	%
GLOSA	13.4	102	14.0	104	66.7	98	34.6	106
DROPIA	12.9	98	14.7	109	67.0	99	35.9	110
KATARINA	13.3	100	12.9	96	69.8	103	30.4	93
MULAN	13.2	100	12.3	91	68.1	100	29.7	91
VARIETY AVERAGE (Ct. 1)	13.2	100	13.5	100	67.9	100	32.7	100
HYSTAR	12.9	99	12.0	98	69.1	100	27.7	98
HYFI	13.1	101	12.9	106	68.8	99	31.2	110
HYWIN	13.1	101	11.7	96	68.6	99	27.1	96
HYBIZA	12.9	99	12.0	98	69.9	101	27.3	96
HYBRID AVERAGE (Ct. 2)	13.0	100	12.2	100	69.1	100	28.3	100

In the wheat hybrids, the analysed wet gluten content ranged from 27.1% to 31.2%; according to the standards (STAS 6283/1-1983), there were no restrictions for the baking purpose under the threshold of 22%.

The technological indicators of the flour and the rheological characteristics of the dough were interpreted on the basis of the indexes: Grain hardness (endosperm texture), Zeleny Index, deformation index, alveographic parameters - mechanical work (bakery force), etc.

Table 5. Rheological traits of wheat varieties, Dâlga area, Călărași County

Var. No.	Genotype cultivated	Grain hardness	%	Zeleny Index (ml)	%	Deflexion Index (mm)	%	Alveographic Mechanical Work W (cm ²)	%
1	GLOSA	52.5	103	72.5	99	6	92	274	112
2	DROPIA	52.6	103	72.0	99	7	108	281	115
3	KATARINA	34.1	67	80.0	110	5	77	217	89
4	MULAN	63.9	126	65.0	89	8	123	204	83
VARIETY AVERAGE (Ct. 1)		50.8	100	72.8	100	6.5	100	244.0	100
5	HYSTAR	24.0	76	66.5	94	5	100	187	97
6	HYFI	39.6	126	75.5	106	5	100	220	114
7	HYWIN	33.2	106	74.5	105	7	140	194	101
8	HYBIZA	28.8	92	67.0	94	3	60	168	87
HYBRID AVERAGE (Ct. 2)		31.4	100	70.9	100	5.0	100	192.3	100

The grain hardness of the winter wheat varieties studied (Table 5) ranged between 34.1 and 63.9 whereas the analysed wheat hybrids recorded values ranging from 24 to 39.6.

The Zeleny test is based on the swelling of wheat flour protein in a specially diluted lactic acid solution and for the Romanian grains the following scale is considered to be appropriate: Category I, over 50; category II, over 50-35.01; Category III, over 35-20.01; category IV, under 20. For the Zeleny index all eight wheat genotypes studied were included in the first class of quality, with values above 50 according to the scale of gradation (Table 5).

Following the determination of the deformation index of the dough (Table 5), the Hybiza hybrid recorded a deformation index of 3 mm, i.e. good but too strong gluten, which requires flour amelioration. In the other genotypes studied in the experiment, this parameter varied between 5 and 13 mm, and thus all were considered "very good" for bakery.

In the winter wheat varieties, the values of the baking strength (Table 5) ranged from 204 to 281, with an average of 244, which included them in the category of flours for manufacturing fast-growing products. In the winter wheat hybrids, the baking power (W) ranged from 168 to 220, with an average of 192.3, with Hyfi recording higher values than the other hybrids tested in terms of baking value.

CONCLUSIONS

Based on the experimental results obtained in the 2014-2015 agricultural growing season, the following conclusions were drawn:

For the productivity potential of the eight winter wheat genotypes studied, the higher value of the hybrids was observed compared to the average of winter wheat varieties tested, i.e. 9139.2 kg/ha.

In terms of productivity, the Mulan variety was higher than the others, with a grain yield of 10137 kg/ha, and the Hystar wheat hybrid recorded a yield of 11191 kg/ha.

Although the tested hybrids recorded higher grain yield than the wheat cultivars varieties, they recorded lower values of physical quality index, 1000 grains weight and volumetric weight, compared to the wheat cultivars varieties studied in the experience.

All the winter wheat genotypes tested during the experiment exceeded the minimum volumetric weight for milling-bakery, i.e. 75 kg/hl, being considered very good for this purpose.

All winter wheat genotypes tested in the experiment had grain moisture values ranging from 12% to 14%, i.e. the corresponding limits for baking wheat.

The protein content of the grains ranged between 12.9% and 13.4%, with no significant variation between the winter wheat genotypes studied, which could be successfully used for bakery purposes.

The starch content ranged from 66.7% to 69.8% in the wheat cultivars varieties and from 68.6% to 69.9% in the hybrids, as the latter were inferior compared with the former.

The wet gluten content of the eight winter wheat genotypes tested in the experiment ranged from 27.1% to 35.9%, thus classifying them as "very good" genotypes for bakery destination.

In terms of the Zeleny index, all eight wheat genotypes studied were included in the first grade of quality, with values above 50 according to grading scale.

According to the deformation index of the dough, in the Hybiza hybrid the deformation index was 3 mm, indicating good but too strong gluten and requiring slight improvement. All the other genotypes showed values between 5 and 13 mm, thus being considered "very good" for the milling-bakery purposes.

The technological indicators of the flour obtained from the grain milling of the eight winter wheat genotypes fell into the category of flours for manufacturing fast-growing products. For the rheological traits of the dough, the winter wheat varieties and hybrids tested during the experiment were part of the "very good" category for bakery purposes.

REFERENCES

- Blum, A. (1996). Yield potential and drought resistance: Are they mutually exclusive. In: P. Reynolds et al. (eds.). *Increasing yield potential in wheat: Breaking the barriers*. CIMMYT, Mexico, D.F. 90–100.
- Cociu, A.I. (2014). Influence of genotype x tillage interaction on wheat yield, its components and characteristics. *An. I.N.C.D.A. Fundulea, LXXXII*. 171–191.
- Cristea, M., Cristea, S., Toader, M. (2015). The microflora influence on wheat seeds on the quality indicators. *Lucrări științifice, 2, Seria Agronomie USAMV Iași*, 167–170.
- Çukadar, B., Pena, R.J., Ginkel, V.M. (2001). Yield potential and bread-making quality of bread wheat hybrids produced using Genesis, a chemical hybridizing agent. In *Wheat in a Global Environment* (p. 541–550). Springer, Dordrecht.
- Egesel, C.Ö., Kahrıman, F., Tümer, A.İ., Çolak, Ç. (2012). Yield and quality characteristics of some foreign bread wheat (*Triticum aestivum* L.) varieties in Turkey. *Romanian Agricultural Research*, 29, 31–38.
- Guță, B.A., Marin, D.I., Carrier, P.L. (2015). Comparative research on several wheat (*Triticum aestivum* L.) genotypes grown under the conditions of Dâlga - Călărăși. *AgroLife Scientific Journal*, 4(2).
- Has, V., Has, I., Antohe, I., Copândeian, A., Nagy, E. (2010). Variability of the grain yield and quality potential of maize hybrids in different FAO maturity groups. *An. I.N.C.D.A. Fundulea, LXXVIII(1)*.
- Hrušková, M., Švec, I., Karas, J. (2012). Relations between the grain hardness and other quality parameters of wheat. *Scientia Agriculturae Bohemica*, 43(2), 70–77.
- Dumbrava, M., Ion, V., Basa, A.G., Dusa, E.M., Epure, L.I. (2019). Study regarding the yield components and the yield quality at some wheat varieties. *Scientific Papers. Series A. Agronomy, LXII(2)*, 77–82.
- Kadar, R., Moldovan, V., Moldovan, M. (2009). Genetic progress in breeding of bread-making quality in winter wheat at ARS Turda: opportunities and constraints. *Annual Wheat Newsletter*, 47, 149–150.
- Longin, C.F., Muhleisen, J., Maurer, H.P., Zhang, H., Gowda, M., Reif, J.C. (2012). Hybrid breeding in autogamous cereals. *Theor Appl Genet*, 125, 1087–1096. doi: 10.1007/s00122-012-1967-7.
- Luo, C., Branlard, G., Griffin, W.B., McNeil, D.L. (2000). The Effect of Nitrogen and Sulphur Fertilisation and their Interaction with Genotype on Wheat Glutenins and Quality Parameters. *Journal of Cereal Science*, 31(2), 185–194.
- Lupu, C. (2010). Influence of basic soil tillage on wheat yield and of some soil features at ARDS Secuieni. *An. I.N.C.D.A. Fundulea, LXXVIII(2)*, 79–87.
- Marin, D.I., Rusu, T., Ciontu, C., Mihalache, M., Ilie, L., Nistor, E., Bolohan, C. (2015). Influence of soil tillage upon yield of several winter wheat varieties grown in the Ilfov area - Romania. *ProEnvironment*, 8(22), 136–141.
- Mustatea, P., Saulescu, N.N., Ittu, G., Paunescu, G., Stere, I., Tanislav, N., Zamfir, M.C., Voinea, I. (2003). Genetic variation in the resistance of wheat to drought, highlighted in the year 2002. *An. I.N.C.D.A. Fundulea, LXX*, 8–15.
- Borghı, B., Perenzin, M. (1994). Diallel analysis to predict heterosis and combining ability for grain yield, yield components and breadmaking quality in bread wheat (*T. aestivum*). *Theor. Appl. Genet.*, 89, 975–981.
- Popa, M., Motcă, G. (2013). Genotype influence upon production and quality of wheat grown in Găvanu-Burdea plain. *Scientific Papers. Series A. Agronomy, LVI*.
- Săulescu, N.A., Săulescu, N.N. (1967). *Câmpul de experiență*. Edit. Agro-Silvica, Bucuresti.
- Săulescu, N. et al. (2010). Diversifying germplasm as a basis of genetic progress in wheat breeding. *An. I.N.C.D.A. Fundulea, LXXVIII(L)*, 7–20.
- Yousif, S.A., Jasim, H., Abas, A.R., Yousef, D.P. (2015). Some Yield Parameters of Wheat Genotypes. *World Academy of Science*, 9(3).

EVAPOTRANSPIRATION OF SOYBEAN, GROWING AT DIFFERENT IRRIGATION REGIME

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Abstract

The aim of the research is establishment of soil moisture level influences on the evapotranspiration (ET) and water use efficiency (WUE) of soybean. The field experiment was conducted in period 2009-2010. Three treatments were applied: T1 without irrigation, T2 irrigation at 80% of field capacity for the soil layer 0-0.6 m, T3 irrigation at 90% of field capacity. Evapotranspiration of soybean under rain-fed conditions is 272 mm. In irrigation regime T2ET increases by 50% and in irrigation regime T3 - by 63%, respectively 407 and 443 mm. The maximum ET daily values were observed during period of flowering and bean forming. During the same period 48-49% of ET is formed, irrespective of irrigation regime. Soybean consumes the most intense water from the layer 0-0.2 m (more than 20%). The total irrigation rate provides 50-52% for ET of T2 and 55-56% for T3. Water use efficiency is higher at lower soil moisture (80% of FC) - 1.843 kg/m³, while at 90% of FC WUE is 1.856 kg/m³. It is recommends irrigation, when soil moisture drops to 80% of the field capacity.

Key words: soybean, evapotranspiration, water use efficiency.

INTRODUCTION

The question of establishing a biologically optimal and economically justifiable crop irrigation regime has been on the agenda for several decades. An integral part of the research in this direction is related to the study of evapotranspiration at different levels of water supply, focusing on the establishment of the total and daily average values. Knowledge of its formation is required to predict irrigation and establish an appropriate irrigation regime. Comparative studies to determine the influence of the soil moisture level before irrigation on soybean ET are in the range between 60 and 80% FC, since within these limits are the biologically optimal and economically justified irrigation regimes (Gorbanov, 1977; Eneva & Valchanov, 1986; Dimitrova & Dimitrov, 1987; Chervenкова & Matev, 2005). Maintaining excess soil moisture above 80% of FC according to Dimitrova & Dimitrov (1987) is justified when soybeans are cultivated for green mass. The data presented by Matev & Zhivkov (2005) and Georgiev et al. (2009) for central northern Bulgaria and by Zhivkov & Matev (2004) - for the Sofia region are similar. The use of drip irrigation in major arable crops (including soybean) has increased significantly

over the last decade. In the specialized scientific literature for drip irrigated soybean, limits of pre-irrigation soil moisture in the range of 70-80% FC are proposed (Pavlenko et al., 2010; Shuravilin et al., 2009; 2015; Borodychev et al., 2008; Babayan, 2018).

The purpose of this study is to establish soybean evapotranspiration under optimal and intensive irrigation conditions and to evaluate its effectiveness.

MATERIALS AND METHODS

During the period 2009-2010, a field experiment was conducted in the Agricultural University of Plovdiv on an alluvial-meadow soil. Three treatments were applied: T1 rain-fed only - control; T2 irrigation at 80% of FC; T3 irrigation at 90% of FC. The soil moisture was monitored by gravimetric method (every 0.10 m) to a depth of 1 m. Evapotranspiration is determined by water balance calculations, To determine its distribution during the growing season, it is conditionally divided into three periods, with the I period covering the time from sowing to the beginning of flowering, II period is the period of flowering and bean-forming, and IIIth is the period of grain filling. Irrigation was carried out using a drip system,

with a dripper's distance of 0.30 m. The experiment is based on the method of long plots in three repetitions, with the size of the experimental plot of 40 m², and of the harvest plots - 14 m². The middle-grade Bulgarian Srebrina variety was used.

RESULTS AND DISCUSSIONS

The meteorological parameters of the year influence the evapotranspiration (ET) and the elements of the irrigation regime. The first year (2009) was considered as an average dry in terms of rainfall of 190 mm/69% security), while 2010 is an average with security of 52% and rainfall of 234 mm. The distribution of rainfall during the first experimental year was uneven, when from mid-July to the end of August, rainfall is only 20 mm. In the second year of experience, the situation is more favourable. In addition to the significant quantities, there is a large number and rainfall of up to 5 mm, which also have a positive effect on the microclimate of the crop and on the intensity of ET. In terms of air temperature, the two experimental years are favourable for the normal development of soybean plants. Under these conditions irrigation practice is necessary in order to keep soil moisture above 80% of FC. The irrigations rates applied are shown in Table 1.

Table 1. Irrigation rates IR, mm

Number	T2 80% of FC		T3 90% of FC	
	period	mm	period	mm
2009				
1	II	55.1	II	32.1
2	II	44.4	II	25.5
3	III	57.5	II	45.1
4	III	59.2	II	31.2
5	-	-	III	44.4
6	-	-	III	60.0
IRaverage		54.1		39.7
IR total		216.2		238.3
2010				
1	II	66.0	II	66.0
2	II	39.0	II	26.0
3	III	49.0	II	23.0
4	III	47.5	II	25.0
5	-		III	15.0
6	-		III	25.0
7	-		III	25.0
IRaverage		50.4		29.3
IR total		201.5		205.0

Maintaining soil moisture up of 80% of FC for the experimental conditions was accomplished with 4 irrigations with an average irrigation rate of 52 mm. The irrigation period in this variant includes the time from the beginning of flowering to the filling of the grain, i.e. the whole reproductive period.

At higher soil moisture (90% of FC) the number of irrigation is 6-7, but with lower irrigation rates. The difference between the irrigation rates at T2 and T3 ranges from 26 to 42%. The irrigation period for this variant of irrigation is longer, because it starts about a decade earlier, and the inter-irrigation period is much shorter. The difference in total irrigation rate for the two treatments is in the range of 2-10%.

Evapotranspiration for growing season

Irrigation is a major factor influencing the values of evapotranspiration ET, according to Eneva (1986) under non-irrigation conditions ET reaches 250 mm and under optimal irrigation (up to 80% FC) it increases significantly and reaches 580-590 mm. Lower are the values stated by Mayaki et al. (1976). Dimitrova & Dimitrov (1987) and Chervenкова & Matev (2005) - between 460 and 500 mm. Studying the ET of soybean irrigation on the basis of different soil moisture levels, Kravchuk et al. (2015) find that ET is maximum (415 mm) when applying a 70-80-70% FC irrigation scheme. In the range of 60-80% of FC, ET of soybeans reaches 475 mm (Just et al., 2017).

The total ET data for the conditions of this experiment are presented in Table 2.

Table 2. Evapotranspiration ET for growing season

Treatment	ET (mm)	to rain fed		to 80% FC		to 90% FC	
		± mm	%	± mm	%	± mm	%
2009							
T1	260.9	st	100.0	-153.4	63.0	-172.9	60.1
T2	414.3	153.4	158.8	st	100.0	-19.5	95.5
T3	433.8	172.9	166.3	19.5	104.7	st	100.
2010							
T1	282.0	st	100.0	-117.9	70.5	-169.1	62.5
T2	399.9	117.9	141.8	st	100.0	-51.2	88.6
T3	451.1	169.1	160.0	51.2	112.8	st	100.
Average for 2009-2010 period							
T1	271.5	st	100.0	-135.7	66.7	-171.0	61.4
T2	407.1	135.7	150.0	st	100.0	-35.4	92.0
T3	442.5	171.0	163.0	35.4	108.7	st	100.

For non-irrigated soybean, ET reaches 261 mm during the average dry year 2009 and 282 mm in 2010. Higher values in the second year are due to better natural water supply to the plants, but the difference from the previous year is relatively small, due to the very frequent and low rainfall, which reduce the tension of the weather factors, increase the humidity of the ground layer air and reduce the intensity of ET. Irrigation applied to keep soil moisture above 80% of FC increases water consumption by an average of 50%. In the drier 2009, ET under non-irrigation conditions is 63% comparing with T2, and in the more favorable 2010 - 70.5%. Keeping soil moisture up to 90% FC increases the soybean water consumption between 5 and 13% (average 8.7%), reaching values 434 and 451 mm. At 90% FC easily accessible water is more, which is a prerequisite for its intensive consumption, but on the other hand, the greater number of irrigations increases the duration of the period during which the surface soil layer is humidly close to the FC. Under these conditions, evaporation from the soil surface is increasing, and it is an integral part of ET.

Evapotranspiration by periods

The values of total ET give a general idea of the water needs of the plants throughout the growing season. However, it has been proven that, under equal conditions, the plants consume water at different intensities during the different phases of the growing season. According to researches by Gorbanov (1977), from the emergence to the beginning of flowering ET of soybeans is from 90 to 110 mm, and for the whole period of flowering and bean formation between 300 and 330 mm. From the end of flowering to the harvest, the ET moves in the range of 70-110 mm. These values and deadlines depends of crop water supplies, soil depth, irrigation methods etc.

For the conditions of this study, the data of evapotranspiration, presented in Figures 1 and 2 are valid for the 0-0.60 m soil layer (in absolute and relative values, respectively). Under non-irrigation conditions, during the I period, average value of soybean ET is 70 mm, varying from 60 to 80 mm.

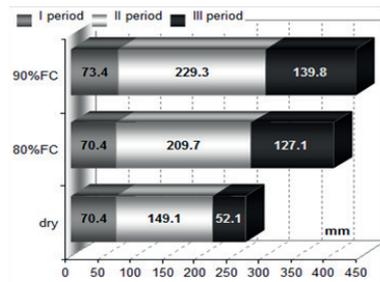


Figure 1. Evapotranspiration of soybean upon periods of vegetation, in mm

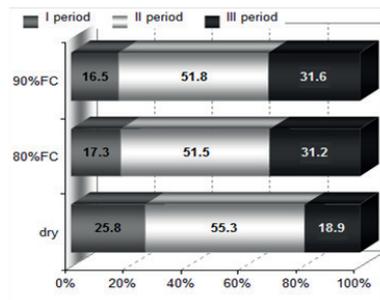


Figure 2. Evapotranspiration in relative values upon periods of vegetation, in %

This period is of long duration (between 4 and 5 decades), but due to the smaller leaf area and less pressure of meteorological factors, the relative ET is on average 26%, varying between 23 and 29%. The same results are received for the ET during this first period of crop vegetation for the irrigated treatments, because of high soil moisture level and lack of irrigation. The flowering and beanning period is critical to the soybean requirements for water and, at the same time, falls calendar-wise into the hottest and often driest part of the year (from the second half of June to the end of July). Under these conditions, easily accessible water in the active soil layer depletes rapidly, which limits the intensity of ET, especially in the absence of rainfall. Therefore, ET under non-irrigation conditions is relatively low - between 130 and 170 mm (average 150 mm). The relative share of the second period is high (46-65%) against the background of relatively low absolute values. Compensating for the deficit of rainfall, irrigation leads to an increase in the intensity of ET, while maintaining a soil moisture up of 80% FC. Its absolute values during the flowering and bean-forming period

are in the range 205-215mm. This means that irrigation only increases ET in the second period by an average of 40%, depending on the conditions of the year between 27 and 58%. This irrigation regime stabilizes the water supplies of the crop, there is no difference between the two experimental years and the relative share of the period amounts to 51-52%. Treatment T3 showed Et increasing between 4 and 15% (on average by 9.3% or by 20 mm. During this part of the growing season, soybean respond most strongly to the different levels of water supply. The differences in the absolute values of the ET between the two irrigation variants are proof of this. They are mostly due to the difference in the total ET. Evapotranspiration during the third period depends to a large extent on the water supply conditions of the previous two periods. For a period of 40-50 days an average of just over 50 mm is consumed under non-irrigation conditions; in the drier 2009, only 33 mm are spent, and in the wetter 2010, just over 70 mm. As a result, the relative ET during this period is low and averages 19%, varying between 13 and 25% over the years. In order to maintain soil moisture up of 80% FC, two irrigations were supplied during this period, as much as during the flowering and bean-forming period, and the environmental conditions also did not differ significantly. Nevertheless, the ET in this irrigation mode is 114-140 mm, ie. it decreased significantly compared to what was reported in the previous period. This is mainly due to the gradual attenuation of the processes in the plant organism leading to a decrease in water consumption. During this period, between 29 and 34% or approximately 1/3 of total ET is formed. At higher soil moisture (90% FC), irrigation during the grain filling period is 2-3. Thanks to them, ET increases by 8-12%, while maintaining the relative share within approximately the same limits (29-35%). These results show that during this phase soybeans respond positively to high soil moisture by increasing the amount of water consumed by 10-15 mm.

Daily average ET values depending on soil moisture

Information about the average daily ET values for soybean, establish in the same region, was

published by Dimitrova & Dimitrov (1987) and Chervenkova & Matev (2005). The authors find that the maximum of 6.0-6.5 mm is during the period of mass flowering - bean formation. Selitsky (2002) establishes approximately the same values of ET, when applying a rational irrigation regime during flowering - bean filling it reaches over 5 mm. The author found that at the beginning and at the end of the growing season it was respectively 56 and 42% smaller. Figure 3 shows the average daily flow of ET depending on irrigation regime.

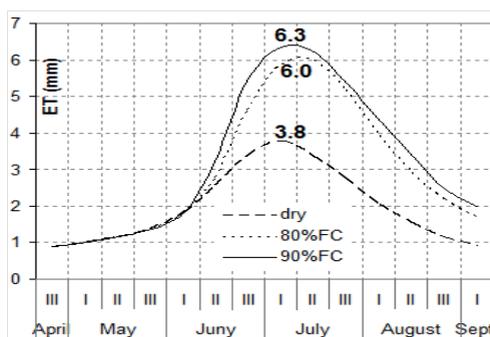


Figure 3. Daily average values of evapotranspiration for the season

In line with the distribution of ET by periods are also the results concerning the change of daily average values. Thus, under rain-fed conditions, the maximum of 3.8 mm per day is recorded in the first decade of July during the period of mass flowering, after which the values begin to decrease gradually. For the conditions of T2 ET increases very rapidly, reaching 4 mm per day at the beginning of the reproductive period, and a maximum of 6 mm in the second decade of July, confirming the outflow from other authors information. These high values are retained for about 1 ten days, after which the intensity of ET begins to gradually decrease, but remains significantly higher than that under non-irrigation conditions. When soil moisture was maintained to 90% of FC, the average daily ET values exceed those at T2 throughout the irrigation period. The maximum of 6.3 mm is also in the second decade of July, but values above 6 mm are retained for about 2 ten days (from 1 to 20 July). As the graph clearly shows, the most significant differences between the two irrigation regimes are in the second period of

soybean growing. The differences between the two irrigation variants during the third period are smaller but remain relatively constant over time. Although our experimental years are extreme, under the irrigation conditions there is a characteristic shift of the ET maximum by about ten days later. During very dry years, this difference can be increased to 2 ten days for obvious reasons. Soil level moisture before irrigation does not affect the time of maximum occurrence.

Layer formation of ET, depending on the pre-humidity

The distribution of ET in depth (by soil layers) is influenced mainly by two factors. The first is the distance of a given soil layer from the surface, in connection with the possibility of influence of abiotic factors, as well as absorption of different amounts of water precipitation and irrigation. The second factor is based on the distribution of the root system in depth, on which the contribution of the given soil layer to the formation of ET largely depends. According to Mayaki, et al. (1976), in the 0-0.30 m layer 67% of the root system of non-irrigated soybean is placed, and more than 70% under irrigation conditions. Benjamin (2006), reported an even greater concentration of roots in the surface soil layer-about 97% of the soybean roots are located in the 0-0.23 m layer. According to Willatt & Olsson (1982), very small is the proportion of roots located at depths below 0.70 m. The authors found that the bulk of ET formed in the layer between 0.15 and 0.50 m, with water being reduced significantly below this depth, even when maintaining high soil moisture. The main reason is the small amount of roots. Figures 4 and 5 illustrate the layered formation of ET under non-irrigation conditions, as well as irrigation on the basis pre-irrigation soil moisture 80% and 90% of FC. The results generally confirm the information presented above by other authors, but at the same time they are specific in terms of soil and climatic conditions and variety specificity. Under non-irrigation conditions, the surface soil layer (0-20 cm) absorbs most of the rainfall, which is why over 50% of the ET is formed by it (Figure 4).

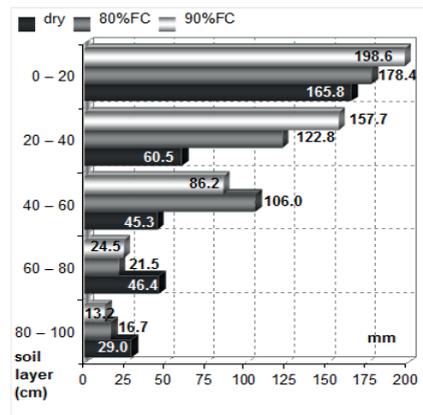


Figure 4. Layer formation of seasonal ET, in mm

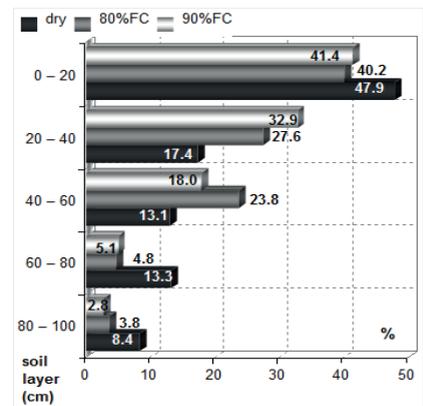


Figure 5. Layer formation of seasonal ET, in %

With increasing depth, ET in this variant decreases significantly, but in the 20-80 cm layer varies within relatively narrow limits (between 45 and 60 mm). Irrigation at soil moisture 90% FC is associated with the supply of more irrigations with lower irrigation rates, which is a prerequisite for higher water consumption from the 0-40 cm layer. Therefore, the ET of the 40-60 cm layer in this variant is inferior to 25% (average 20 mm), to the 80% FC. Because irrigation rates are calculated for wetting depths up to 60cm, there is practically no difference between the two irrigation options below this depth. Data on the relative distribution of water flow over soil layers shows that, regardless of water supply, more than 40% of ET is formed from water in soil layer 0-20 cm (Figure 5). In general, 80% of the total ET is formed in the active soil layer (0-60 cm) of the non-irrigated soybean, and

20% in the 60-100 cm layer. Under irrigation conditions water consumption from the 20-40 cm layer is much more intensive than that of non-irrigated soybean, with 80% FC accounting for almost 28% and 90% FC for almost 33%. The difference is only 5%, but it cannot be considered insignificant since it equals 35 mm of water consumed. Conversely, in the 40-60 cm layer, ET is more intense at 80% FC, exceeding 6% (20 mm) at 90% FC. Under irrigation conditions, 91-92% of the total ET for 0-100 cm formed in the active soil layer of soybean (0-60 cm). All of the above is confirmed by the graphs in Figures 6, 7 and 8, which illustrates the formation of ET in soil layers in dynamics in the three variants of the experiment. Under non-irrigation conditions, the 0-20 cm layer provides the main amount of water throughout the growing period (Figure 6).

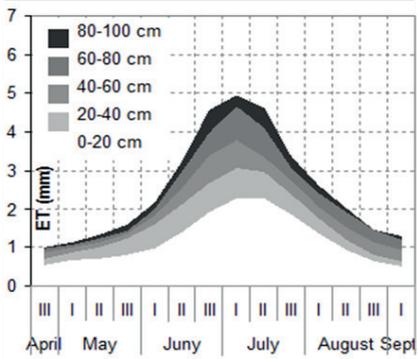


Figure 6. Layer formation of ET during the season, rain-fed condition

The involvement of 20-40 cm after mid-May is also visible, with the intensity of ET of this layer remaining until the end of flowering. The maximum ET of the underlying layers 40-60 and 60-80 cm below is during flowering and then gradually decreases and remains relatively constant during the seeding period. When maintaining soil moisture up to 80% FC, after the beginning of the irrigation period ET from the active soil layer increases rapidly, at the beginning of flowering water consumption is basically from 0-40 cm, but during the period of mass flowering and bean formation ET from 40- 60 cm reaches the upper two layers as values (Figure 7). As a result, during this part of the growing season (until July 20), water

flow in the individual layers (within 0-60 cm) is close in value. The ET begins to decrease during the seeding period, but remains relatively high, as is the case for each of them. Consumption of water below 60 cm depth is insignificant.

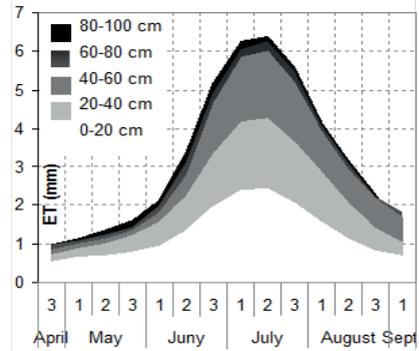


Figure 7. Layer formation of ET during the season, T2 treatment

When applying irrigation regime with high soil moisture up to 90% (T3), ET is formed from soil layer 20-40 cm more that 40-60 cm, the difference increasing substantially during the irrigation period and remains significant until the end of the growing season (Figure 8).

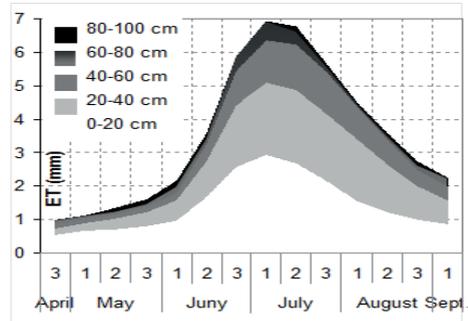


Figure 8. Layer formation of ET during the season, T3 treatment

The dynamics of water consumption from the 60-100 cm layer is similar to that of the previous variant.

Rainfall participation, irrigation rate and initial soil water content in the formation of evapotranspiration

Under rain-fed conditions, ET is formed due to the initial water supply and rainfall, and under irrigation conditions the irrigation rate is added.

According to Eneva & Valchanov (1986) rainfall compensates for an average of 73% of the ET of non-irrigated soybeans. When maintaining soil moisture more than 80% of FC, 43% of ET is provided by vegetation rainfall, 41% by irrigation rates, and 16% by soil moisture accumulated during the autumn-winter and early-spring periods. According to Dimitrova & Dimitrov. (1987), Chervenkova & Matev (2005), the irrigation rate participates with 40-60%, depending on the nature of the year. In the range of pre-irrigation soil moisture between 60 and 80% of FC, the relative participation of the components does not differ significantly, with the share of rainfall varying between 74-77% and the irrigation rate between 23 and 27% (Yust & Gorbacheva, 2017).

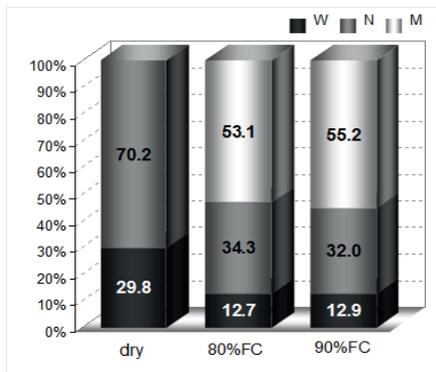


Figure 9. Relative participation of initial water content (W), rainfall (N) and irrigation rates (M)

Figure 9 shows the relative involvement of the three components in the formation of ET in the three variants of the experiment. Data refer to the 0-60 cm layer. Under non-irrigation conditions, rainfall provides an average of 70% of the ET, and the initial water supply falls to 30%. During the season of 2009 year, precipitation accounted for 63%, and the initial water supply - 37%, while the next year 2010, the relative share of the two components was 78 and 22%, respectively. The irrigation rate under irrigation is at the expense of the other two components. The differences between the two irrigated variants are minimal (about 2%) and have no practical significance. The irrigation rates provide more than 50% of ET, varying between 52 and 56% for T2 and T3,

regardless of the nature of the year. Rainfall provides about 1/3 of the total ET, and the initial water supply occupies an average of 13%.

Water use productivity (WUP)

This indicator gives an idea of what yield is obtained for 1 m³ of water consumed. It is calculated by dividing the yield (Table 3) by the total ET (Table 2). Figure 10 presents data by years and average experience. The average data show no significant difference between the two irrigation treatments.

Table 3. Yield in kg/da

Year	Treatment		
	T1 rain-fed	T2 80% FC	T3 90% FC
2009	103.7	192.2	196.7
2010	195.8	249.6	285.5
average	149.8	220.9	241.1

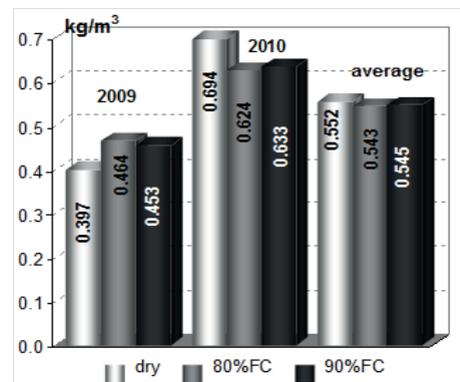


Figure 10. Effectiveness of ET depending of water supplying of soybean

CONCLUSIONS

A field experiment conducted with soybean, irrigated at two levels of soil moisture showed:

1. Evapotranspiration values depend on the irrigation regime applied and are affected by the maintained soil moisture level. Irrigation with soil moisture level of 80% FC increases water consumption by an average of 50% comparing with non irrigated crop.
2. The dynamics of change of evapotranspiration as a function of the growing season is not significantly affected by the maintaining soil moisture level before irrigation.

3. The maximum daily values of evapotranspiration are reached during the grain filling period under both irrigation and non-irrigation conditions, but they differ in values. ET reaches a maximum of 6 mm and 6.3 mm in the second decade of July for treatments T2 and T3, respectively.
4. Irrigation rates have a major share in the formation of ET - up to 50% of the total amount of water available to the plants.
5. The major amount of water used by soybeans is located in the 0-0.6 m soil layer, especially during the most active growing season. Over 40% of the total ET is from the layer 0-0.20 m
6. The average productivity of the water used by the plants during the experiment period does not differ substantially, both under rain-fed and irrigation conditions - 0.55 kg/da for rain-fed treatment and 0.54 kg/da for irrigated soybean.

REFERENCES

- Babayan, B.R., (2018). Assessment of the possibility of cultivating soybeans in Pridnestrovie, taking into account the use of irrigation and fertilizers. Biodiversity and factors affecting the ecosystem of the Dniester basin, Tiraspol: Eco-TIRAS, Materials of the scientific-practical conference, 18–21.
- Benjamin, J.G. (2006). Water deficit effects on root distribution of soybean, field pea and chickpea. *Field Crops Research*, 97, 248–253.
- Borodychev, V.V., Lytov, M.N., Shuravilin, A.V. (2008). Drop irrigation of soybean in the Nighniy Povolghye Region. AGRO XXI, Publishing house "Agrorus" (Moscow), 1-3, 42–43.
- Chervenкова, Z., Matev, A. (2005). Soybeans Evapotranspiration – Total Rate, Formation and Water Use Production Coefficient in Sadovo Region. Scientific Reports, Jubilee Scientific Conference “Breeding and Technological Aspects in Production and Processing of Soybean and Other Legume Crops”, Soybean Research Station - Pavlikeny, 260–269.
- Dimitrova, Z., Dimitrov, Z. (1987). Soybean water regime studies. In support of technical progress in water management, 2, 13–14.
- Eneva, St., Valchanov, D. (1986). Study on the biological optimum irrigation levels for irrigation of soybeans by sprinkling of humus-vertisol. *Bulgarian Journal of Crop Science*, 2, 40–45.
- Georgiev, G., Sabev, V., Georgiev, Iv., Goranova, K., Serafimov, P., Aleksieva, A., Todorova, R., Nikolova, I., Matev, A. (2009). Soybean production technology. Technologies for scientific support of agricultural production. *Agricultural Academy*, 1, 15–35.
- Горбанов, Н. (1977). Irrigation technique as a means of realizing the irrigation regime of maize and soybean. PhD thesis, Sofia.
- Kravchuk, A.V., Bondarenko, Y.V., Serebrennikov, V., Lapshova, A.G. (2015). Total water consumption of soy on dark chestnut soils of the dry steppe. Scientific Life, Education Science Publishing House (Moscow), 6, 127–133.
- Zhivkov, Zh., Matev, A. (2004). Growing soybean under irrigation water deficit. *Field Crop Studies*, 1(3), 470–477.
- Matev, A., Zhivkov, Zh. (2005). Water Deficit and its Influence on the Productivity of Soybean, Cultivated in Conditions of Pavlikeny. Scientific Reports, Jubilee Scientific Conference “Breeding and Technological Aspects in Production and Processing of Soybean and Other Legume Crops”, Soybean Research Station - Pavlikeny, 134–140.
- Mayaki, W. C., Teare, I.D. , Stone, L.R. (1976). Top and Root Growth of Irrigated and Nonirrigated Soybeans. *Crop Science*, 16(1), 92–94.
- Mayaki, W.C., Stone, L.R. , Teare, I.D. (1976). Irrigated and Nonirrigated Soybean, Corn, and Grain Sorghum Root Systems. *Agronomy Journal*, 68(3), 532–534.
- Pavlenko, V.N., Nesterenkom, D.I., Lyubimova, G.A., Pavlenko, V.A. (2010). Matematical statistics methods in the researches of drip irrigation efficiency at soybean growing. *Theoretical and Applied Problems of Agro-Industrial Complex*, 4, 28–31.
- Selitsky, S.A. (2002). Resource-saving technology for soybean cultivation during irrigation in the conditions of the Rostov region. PhD thesis, Novocherkassk, 214.
- Shuravilin, A.V., Borodychev, V.V., Lytov, M.N., Belik, O.A. (2009). Drip irrigation of the soya on heavy soils. *Scientific Bulletin of Peoples' Friendship University, Agriculture and Livestock*, 3, 21–25.
- Shuravilin, A.V., Borodychev, V.V., Lytov, M.N. (2015). Ways of irrigation and methods of cultivation of soy in lower Volga area. AGRO XXI, Publishing house "Agrorus" (Moscow). 37–40.
- Willatt, S.T., Olsson, K.A. (1982). Root distribution and water uptake by irrigated soybeans on a uplexsoil. *Australian Journal of Soil Research*, 20(2), 139–146.
- Yust N.A., Gorbacheva, N.A. (2017). Preirrigation Humidity Bridge Impact on the Structure of Total Water Requirement for Soy at Estuary and Chernozem-Like Soils. *Scientific Life, Education Science Publishing House (Moscow)*, 2, 41–48.

PYRAFLUFEN-ETHYL AND FLORASULAM EFFICACY AGAINST GLYPHOSATE RESISTANT HORSEWEED (*Conyza canadensis*) BIOTYPES

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Abstract

The confirmation of glyphosate resistance (GR) biotypes of *Conyza canadensis* (horseweed) in orchards and vineyards in Greece highlighted the need of alternative methods and tools for the management of this important species. Herbicides with a different mode of action ought to be studied in terms of their efficacy against the herbicide resistant biotypes. The aim of this study was the efficacy evaluation of the herbicides pyraflufen ethyl, florasulam, MCPA and glyphosate, against several horseweed biotypes. Seeds from several biotypes of *C. canadensis* were sampled and sown in pots. Assessments of fresh and dry weight of horseweed seedlings were carried out 7, 14, 21 and 28 days after treatment (DAT). Pyraflufen ethyl alone or in combination with florasulam, resulted in an exceptional efficacy (>95%) against horseweed, even at 7 DAT. It was also noticeable that the mean accumulation of fresh weight in the treatments with pyraflufen ethyl was significantly reduced compared to the untreated plants (44 to 59% lower at 3 DAT). The findings of the present study reveal the high potential of pyraflufen-ethyl alone or with florasulam for the effective management and control of GR *Conyza* spp. biotypes. Further research on pot and field experiments is expected to highlight the dynamics of PPO inhibitors against GR horseweed.

Key words: glyphosate, herbicides, horseweed, pyraflufen ethyl.

INTRODUCTION

Conyza canadensis (L.) Cronq. (henceforth horseweed) is a summer annual weed species belonging to the genus *Conyza* (Travlos & Chachalis, 2013). The prolific fecundity of this species, with up to 200,000 seeds per individual plant (Palma-Bautista et al., 2020), the wide seed dispersal due to wind and the capacity of germination in a wide range of environmental conditions (Nandula et al., 2006) makes the management of this weed a serious challenge for farming systems globally. Nowadays, horseweed poses a major threat for the sustainability of orchards, vineyards and field crops (Weaver, 2001), especially in no-tillage or minimum tillage farming systems (Travlos et al., 2009).

In the globe and specifically in countries of the Mediterranean basin, farmers have reported several herbicide applications failures to control *Conyza* spp. (Travlos & Chachalis, 2013). This was mainly the result of over-reliance on glyphosate and subsequently the

consecutive use of this herbicide as the only reliable solution for mitigation of horseweed infestations (Amaro-Blanco et al., 2018).

Glyphosate [N-(phosphonomethyl) glycine] is a non-selective, post-emergent, water-soluble and broad-spectrum herbicide (VanGessel, 2001), which blocks the shikimic acid pathway by inhibiting the 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS; EC 2.5.1.19) (González-Torralva et al., 2012).

This active ingredient has been characterized as the most important herbicide globally (Travlos et al., 2017b), while the last decades has been consecutively used in many agricultural and non-agricultural situations (Gonzalez-Torralva et al., 2010).

Nevertheless, the repeated applications of glyphosate, even 2-3 times in the same cultivation period, have contributed to the persistent evolution of glyphosate resistance cases in many weed species (Sansom et al., 2013). Currently, there are 48 glyphosate resistant weed species (GR) globally (Heap, 2020).

Therefore, alternative to glyphosate techniques and methods are required, in order to reduce the glyphosate reliance and mitigate GR evolution (Duke & Powles, 2008). Recently, extensive research focuses on long-term management of GR through evaluation of herbicide rotation regimes (Amaro-Blanco et al., 2018) or improvement of glyphosate applications through optimized use of adjuvants (Palma-Bautista et al., 2020), along with thorough research about the sustainability of farming systems through integration of novel integrated weed management techniques such as false seedbed (Kanas et al., 2020a) and proper decision-making for weed management (Kanas et al., 2020b).

Under this context, the scope of this study was the evaluation of the efficacy of alternative to glyphosate chemical herbicides against GR horseweed biotypes in Greece. This research included previously reported important active ingredients against dicot weeds, including pyraflufen ethyl, florasulam and MCPA. Pyraflufen ethyl is an effective, post-emergent, contact herbicide, inhibitor of protoporphyrinogen oxidase (PPO), which causes rapid necrosis in plant tissues and has been used against broad-leaf weeds in cereals and as desiccant in potato crop (Ivany, 2005; Miura et al., 2003). Florasulam is a post-emergent herbicide belonging to the triazolopyrimidine chemical family, inhibiting the acetolactate synthase enzyme (ALS) (Travlos et al., 2014). MCPA is a systemic, post-emergent herbicide used for the control of several annual and perennial weeds.

This is among the first researches to our knowledge that highlight several strands of alternative herbicide control in confirmed glyphosate-resistant *C. canadensis* in Greece. The objectives of this study were as follows: (1) to determine the susceptibility of GR horseweed biotypes at the rosette stage to chemical herbicides in comparison to untreated plants and plants treated with glyphosate; and (2) to evaluate the efficacy of pyraflufen-ethyl alone or in tank mix with florasulam, and MCPA plus florasulam against the GR horseweed biotypes.

MATERIALS AND METHODS

A pot experiment was conducted in a glass-greenhouse in the Agricultural University of Athens during September 2019. The prior activities to the herbicide treatments included the collection of seed samples of *C. canadensis* from perennial crops in Askri and Larisa region and then seed sowing. Overall, three horseweed biotypes, potentially glyphosate-resistant, were sampled from vineyards (A1, A2, A3) and two more from apple orchards (L1, L2).

For each weed population, 18 plastic pots were used. Each 11-cm-deep and 9.5-cm-wide pot was first filled with a mix of herbicide-free soil from the field of the Agricultural University of Athens and common peat substrate (1:1, v/v) and then 20 seeds were placed on their substrate surface. Following the sowing, pots were properly irrigated, organized into 5 groups (one for each population) of 6 pots each and maintained in the greenhouse. When the seedlings reached the rosette stage (7 to 12 cm diameter, 10 to 15 leaves), each pot of the aforementioned groups was treated with the herbicides reported in Table 1.

The plants were sprayed using a custom-built, compressed-air, low-pressure flat-fan nozzle experimental sprayer, calibrated to deliver 300 l ha⁻¹ at 250 kPa.

Table 1. Herbicide treatments tested *in situ* for efficacy against glyphosate-resistant *Conyza canadensis* biotypes

Active ingredient	Treatment	HRAC group	Dose (g a.e. or a.i. ha ⁻¹)
Control	Untreated	-	-
Glyphosate	T1	G	1800*
Pyraflufen ethyl	T2	E	22
Pyraflufen ethyl + florasulam	T3	E + B	22 + 5
Pyraflufen ethyl (x/2) + florasulam	T4	E + B	11 + 5
MCPA + florasulam	T5	O + B	1200 + 5

g a.i. ha⁻¹, grams of active ingredient per hectare.
 *g a.e. ha⁻¹, grams of acid equivalent per hectare.
 HRAC, Herbicide-Resistance Action Committee; G, EPSPS inhibitors; B, ALS inhibitors; O, synthetic auxins; E, PPO inhibitors

Three pots from each group was left as untreated control. Fresh and dry weight assessments were conducted at 3, 7, 14, 21 and 28 days after treatment (DAT) with cutting of the above ground biomass of 5 individual plants per treatment. Samples were then dried at 60°C for 48 hours and their dry weight was recorded in order to determine the biomass and thus evaluate the efficacy of each herbicide.

The experimental design was a completely randomized design with a split-plot arrangement, and biotypes were the main plots and herbicide treatments were the subplots. The experimental data were analyzed using the STATGRAPHICS Centurion XVII Version statistical software (Statpoint Technologies Inc., The Plains, VA, USA). All data were subjected to multiple ANOVA. Treatment means were separated using Fisher's protected LSD test at $P < 0.05$.

RESULTS AND DISCUSSIONS

The fresh and dry weight measurements revealed that there were significant differences among the treatments. According to the results, all surveyed horseweed biotypes were quite resistant to glyphosate, while the L1 biotype demonstrated the higher levels of resistance.

Table 2. Data records of dry weight of untreated and glyphosate treated *C. canadensis* plants for the six populations (A1, A2, A3, L1 and L2) over time. Data are presented as the average weight of 5 samples for each treatment and population over time

Average weight (g)		Control	Glyphosate
		Dry weight	Dry weight
14 DAT	A1	0.51	0.24
	A2	0.52	0.48
	A3	0.77	0.67
	L1	0.52	0.47
	L2	1.10	0.98
21 DAT	A1	0.71	0.49
	A2	0.58	0.53
	A3	1.07	0.98
	L1	0.89	0.77
	L2	0.83	0.78
28 DAT	A1	0.39	0.26
	A2	0.83	0.77
	A3	0.83	0.75
	L1	1.25	0.94
	L2	0.91	0.81

Several horseweed plants survived glyphosate application at 14 and 28 DAT, indicating the

glyphosate resistance of these biotypes (Figure 1). Although the glyphosate-treated plants managed to survive better than the pyraflufen-ethyl treated ones, the former displayed a reduction in their biomass over time when compared to the control, as estimated by using the dry-weight data presented in Table 2. In particular, four biotypes (A2, A3, L1 and L2) displayed resistance to glyphosate, while A1 was rather susceptible population.



Figure 1. Visual evaluation of glyphosate efficacy at 14 DAT

Regarding T5 (MCPA + florasulam), this treatment showed similar results to T1, indicating that this tank mix of post-emergent herbicides was not very effective for the control of GR horseweed. The mean accumulation of fresh weight at 21 DAT in T5 was only 19% lower than the glyphosate-treated plants (data not shown), while progressively the control was higher. This finding is in accordance with the results of Travlos et al. (2014) who recommended that florasulam in mix with penoxsulam provided long-term control of *C. canadensis* even four months after the application. Promising and relevant are also the results about florasulam when combined with aminopyralid against broad-leaf weeds in maize (Travlos & Apostolidis, 2017a).

Seedlings treated either with pyraflufen-ethyl alone or in tank mix with pyraflufen-ethyl and florasulam failed to survive 7 DAT, while the plant injury was visible even at 3 DAT (Figure 2).

Our data suggest that survival of *C. canadensis* was significantly affected by the contact herbicide pyraflufen-ethyl, which provided high control of the weed. This result is opposite to the conclusion of Shrestha et al. (2008) who stated that pyraflufen-ethyl provides partial control of *C. canadensis* and *C. bonariensis* in California.



Figure 2. Comparison of the several treatments at 3 DAT on A1 biotype

The factors of treatment and biotype had a statistically significant effect ($P=0.0001$, $P=0.0472$, respectively) on fresh weight of plants at 3 DAT (Figure 3).

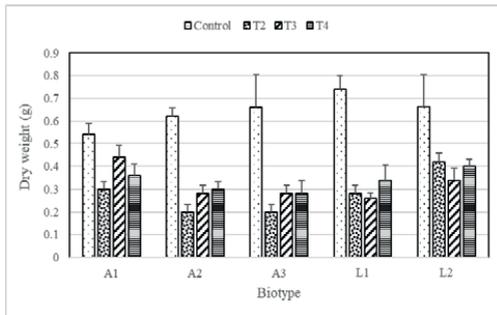


Figure 3. Comparison of the efficacy of pyraflufen-ethyl alone (T2) or in mixture with florasulam (T3, T4) to untreated plants at 3 DAT. The bars indicate standard errors of the means

There was a statistically difference between the untreated control and the treatments with pyraflufen-ethyl (T2, T3, T4) the same day. In particular, the mean accumulation of fresh weight in plants treated only with pyraflufen-ethyl (T2) was significantly reduced by 59% compared to the untreated plants. The application of pyraflufen-ethyl at recommended rate plus florasulam (T3) was strongly depended on biotype characteristics and led to moderate reduction of fresh weight

accumulation between 26 and 71% compared to control, while the half rate of pyraflufen-ethyl plus florasulam (T4) provided only 44% mean biomass reduction 3 DAT (Figure 3). Nevertheless, the results of this study show that a dose of 11 g a.i. ha⁻¹ of pyraflufen-ethyl, when this herbicide is combined with florasulam, provides adequate efficacy against GR horseweed, indicating that increasing the application doses give little advantage. Ivany (2005) recommended that an application rate of pyraflufen-ethyl between 10 and 15 g a.i. ha⁻¹ can provide acceptable desiccation results in *Solanum tuberosum* cultivars. Our results are partially in agreement to those of Amaro-Blanco et al. (2018) who concluded that pyraflufen ethyl at a rate of 22 g a.i. ha⁻¹ can effectively reduce weed soil cover, while it doesn't provide satisfactory reduction of horseweed seed production.

Unfortunately, we can't rely on the discovery of new herbicide modes of action soon, so the integration of innovative herbicide mixtures and the adoption of herbicide rotation regimes are imperative (Heap & Duke, 2018). The evaluation and utilization of effective alternatives to glyphosate for the control of GR *C. canadensis* has been proposed by Sansom et al. (2013) and Mora et al. (2019). Under this context, our results suggest that the active ingredients pyraflufen-ethyl and florasulam may be important substitutes or additives to glyphosate. Another PPO inhibitor, oxyfluorfen, has been also reported to be effective against *C. canadensis* (Norsworthy et al., 2009). The application of various chemical herbicides is critical for horseweed, since this species' germination period is extended throughout spring and summer (Bajwa et al., 2016). The timing of treatment is important as well, because horseweed plants are more susceptible in younger rather than in more advanced growth stages (Shrestha et al., 2008). Finally, we propose the utilization of different herbicide modes of action and their appropriate rotation, along with adoption of more integrated weed management technologies, for the management of the noxious weed *C. canadensis* (Travlos et al., 2014; Palma-Bautista et al., 2020).

CONCLUSIONS

Of the herbicides evaluated, pyraflufen-ethyl provided 100% control of glyphosate-resistant *C. canadensis* 7 DAT when either applied alone or in combination with the ALS inhibitor, florasulam.

This study suggests that glyphosate resistant weeds can be managed through an integrated weed management scheme that includes novel tank mixtures with both contact and systemic herbicides.

The susceptibility of horseweed biotypes from annual and perennial crops to pyraflufen-ethyl poses a significant tool for mitigation of this species infestations.

Glyphosate was the least effective herbicide even when applied at high rate of 1.8 kg ha⁻¹, while the mix of MCPA with florasulam provides acceptable efficacy only 21 DAT.

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REFERENCES

Amaro-Blanco, I., Fernández-Moreno, P. T., Osuna-Ruiz, M. D., Bastida, F., & De Prado, R. (2018). Mechanisms of glyphosate resistance and response to alternative herbicide-based management in populations of the three *Conyza* species introduced in southern Spain. *Pest management science*, 74(8), 1925–1937.

Bajwa, A.A., Sadiq, S., Ali, H. H., Jabran, K., Peerzada, A. M., & Chauhan, B.S. (2016). Biology and management of two important *Conyza* weeds: a global review. *Environmental Science and Pollution Research*, 23(24), 24694–24710.

Duke, S.O., & Powles, S.B. (2008). Glyphosate: a once-in-a-century herbicide. *Pest Management Science: formerly Pesticide Science*, 64(4), 319–325.

Gonzalez-Torralva, F., Cruz-Hipolito, H., Bastida, F., Mülleder, N., Smeda, R. J., & De Prado, R. (2010). Differential susceptibility to glyphosate among the *Conyza* weed species in Spain. *Journal of Agricultural and Food Chemistry*, 58(7), 4361–4366.

González-Torralva, F., Rojano-Delgado, A. M., de Castro, M. D. L., Mülleder, N., & De Prado, R. (2012). Two non-target mechanisms are involved in glyphosate-resistant horseweed (*Conyza canadensis* L. Cronq.) biotypes. *Journal of plant physiology*, 169(17), 1673–1679.

Heap, I. (2020). The International Herbicide-Resistant Weed Database. Online. Wednesday, April 15. Available www.weedscience.org

Heap, I., & Duke, S.O. (2018). Overview of glyphosate-resistant weeds worldwide. *Pest Management Science*, 74(5), 1040–1049.

Ivany, J.A. (2005). Response of three potato (*Solanum tuberosum*) cultivars to pyraflufen-ethyl used as a desiccant in Canada. *Crop Protection*, 24(9), 836–841.

Kanatas, P.J., Travlos, I. S., Gazoulis, J., Antonopoulos, N., Tsekoura, A., Tataridas, A., & Zannopoulos, S. (2020). The combined effects of false seedbed technique, post-emergence chemical control and cultivar on weed management and yield of barley in Greece. *Phytoparasitica*, 1–13.

Kanatas, P., Travlos, I.S., Gazoulis, J., Tataridas, A., Tsekoura, A. & Antonopoulos, N. (2020). Benefits and limitations of Decisions Support Systems (DSS) with a special emphasis on weeds. *Agronomy*, 10, 548.

Miura, Y., Mabuchi, T., Higashimura, M., & Amanuma, T. (2003). Development of a new herbicide, pyraflufen-ethyl. *Journal of Pesticide Science*, 28(2), 235–240.

Mora, D.A., Cheimona, N., Palma-Bautista, C., Rojano-Delgado, A.M., Osuna-Ruiz, M.D., de la Cruz, R.A., & De Prado, R. (2019). Physiological, biochemical and molecular bases of resistance to tribenuron-methyl and glyphosate in *Conyza canadensis* from olive groves in southern Spain. *Plant Physiology and Biochemistry*, 144, 14–21.

Nandula, V.K., Eubank, T.W., Poston, D.H., Koger, C.H., & Reddy, K.N. (2006). Factors affecting germination of horseweed (*Conyza canadensis*). *Weed Science*, 54(5), 898–902.

Norsworthy, J.K., McClelland, M., & Griffith, G.M. (2009). *Conyza canadensis* (L.) Cronquist response to pre-plant application of residual herbicides in cotton (*Gossypium hirsutum* L.). *Crop protection*, 28(1), 62–67.

Palma-Bautista, C., Vazquez-Garcia, J.G., Travlos, I., Tataridas, A., Kanatas, P., Dominguez-Valenzuela, J. A., & Prado, R.D. (2020). Effect of adjuvant on glyphosate effectiveness, retention, absorption and translocation in *Lolium rigidum* and *Conyza canadensis*. *Plants*, 9(3), 297.

Sansom, M., Saborido, A.A., & Dubois, M. (2013). Control of *Conyza* spp. with glyphosate—a review of the situation in Europe. *Plant Protection Science*, 49(1), 44–53.

Shrestha, A., Hembree, K., & Wright, S. (2008). *Biology and management of horseweed and hairy fleabane in California*. University of California, Agriculture and Natural Resources.

Travlos, I.S., & Chachalis, D. (2013). Assessment of glyphosate-resistant horseweed (*Conyza canadensis* L. Cronq.) and fleabane (*Conyza albida* Willd. ex Spreng) populations from perennial crops in Greece. *International Journal of Plant Production*, 7(4).

Travlos, I.S., Lysandrou, M., & Apostolidis, V. (2014). Efficacy of the herbicide GF-2581 (penoxsulam+

- florasulam) against broadleaf weeds in olives. *Plant, Soil and Environment*, 60(12), 574–579.
- Travlos, I., & Apostolidis, V. (2017a). Efficacy of the herbicide Lancelot 450 WG (aminopyralid+ florasulam) on broadleaf and invasive weeds and effects on yield and quality parameters of maize. *Agriculture*, 7(10), 82.
- Travlos, I., Cheimona, N., & Bilalis, D. (2017b). Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. *Agronomy*, 7(3), 60.
- Travlos, I.S., Chachalis, D., & Economou, G., 2009. Characters for the *in situ* recognition of some *Conyza* species and glyphosate resistant populations from Greece. P 63, in Proceedings of the 2nd International Conference on Novel and Sustainable Weed Management in Arid and Semi-arid Agroecosystems. Santorini, Greece: European Weed Research Society.
- VanGessel, M.J. (2001). Glyphosate-resistant horseweed from Delaware. *Weed Science*, 49(6), 703–705.
- Weaver, S. E. (2001). The biology of Canadian weeds. 115. *Conyza canadensis*. *Canadian Journal of Plant Science*, 81(4), 867–875.

PLANT GROWTH STIMULANTS INFLUENCE ON *Miscanthus x giganteus* BIOMASS INDEXES IN FOREST - STEPPE ZONE OF UKRAINE

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Abstract

Miscanthus (*Miscanthus x giganteus*) forms the largest number of stems in the first year of the life cycle. It was found that the height of the main shoot increased with the number of sprayings. Active growth and development of miscanthus plants begins from the tillering phase, which occurs after the appearance of the first true leaves (from May to September) immediately. It was found that the number of shoots increased (from 8 to 12 units per bush) after each foliar sprayings with plant growth stimulants. The largest leaf-stem biomass in the first year of vegetation was obtained in the trial of double foliar treatment soaking in a Quantum gold solution. It was found that the yield of the miscanthus rhizome was slightly higher in 2016-2017 than in 2018-2019. Excessive rainfall of 150 mm in May in 2016 gave a significant impetus to the formation of the underground part of the miscanthus. The highest yield of miscanthus 4.6 t ha⁻¹ and energy yield-80 GJ ha⁻¹ was obtained in the trial when soaking in a solution of Quantum gold with two-time treatment of plants during the growing season.

Key words: *miscanthus*, growth stimulator, biomass, indexes.

INTRODUCTION

Miscanthus (*Miscanthus x giganteus*) has a very good potential in terms of biomass yield compared with other varieties (Da Borso et al., 2018; Brosse et al., 2012). High adaptation potential make this cultivar very suitable for marginal lands (Heaton et al., 2004; Milovanovic et al., 2012). Giant miscanthus is a C-4 photosynthetic plant. It is characterised by greater carbon dioxide (CO₂) absorption (Kazimierowicz & Dzienis, 2015). Environmental limitations and the greenhouse gas balance of the miscanthus plantations have been estimated. During the combustion of the biomass of *Miscanthus giganteus*, less carbon dioxide is released than was absorbed by plants during photosynthesis. It was established that *Miscanthus* genotypes are able to reduce emissions and emit 40-99% less N₂O compared to annual conventional crops (Di Vita et al., 2017). Miscanthus can be grown on different well drained soil types with pH in the range from 5.5 to 7.5 and with a medium and high fertility level (Heaton et al., 2004; Kharytonov et al., 2019). It was established that *Miscanthus*

x giganteus crops achieve full potential yield, regardless of the soil, from the third year of vegetation (Matyka & Kus, 2016; Alexopoulou et al., 2015). It was shown also that miscanthus biomass parameters can be positively influenced with two plant growth regulators (PGRs) when the plant was grown in soil with good agricultural characteristics (Nebeska et al., 2019). Almost all studies on nutrition management of miscanthus (*Miscanthus x giganteus*) to date have focused on three fertilizer elements. Joint N, P, K fertilization increased yield during fourth and fifth year of development on marginal lands (Drazic et al., 2017). It was established, that any nutrient response is highly dependent on the soil on soil physiochemical properties, climatic conditions, and planting location (Souri, 2016; Souri et al., 2017; Hatamian et al., 2019) and planting location (Anderson et al., 2011). The need to develop innovative technological approaches for second generation crops arises in connection with the increase in the area of miscanthus plantations in Ukraine. The main objective of research was to study plant growth stimulants influence on

miscanthus (*Miscanthus x giganteus*) biomass parameters in forest - steppe zone of Ukraine.

MATERIALS AND METHODS

These studies were conducted on experimental fields of Veselopodilsky experimental station of the Institute of bioenergy crops and sugar beet NAAS of Ukraine for 2016-2019. The subzone of the left bank of the forest-Steppe of Ukraine is marked by a moderate continental climate with warm summers and mild winters and insufficient moisture in some dry years. Most of the precipitation fell in early spring (mainly in May 2016 and 2019). Lack of rain was observed in Spring of 2017 and 2018, which negatively affected the growth and development of plants later (Figure 1).

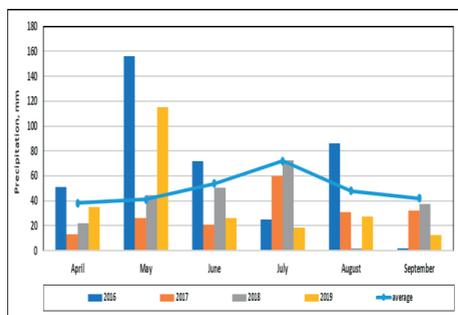


Figure 1. The precipitation (2016-2019)

The average annual precipitation was 511 mm with large variations from 306 to 500 mm. 326 mm of precipitation fell during the growing season (4-10 months). The average long-term air temperature data for the last four years are shown in Figure 2.

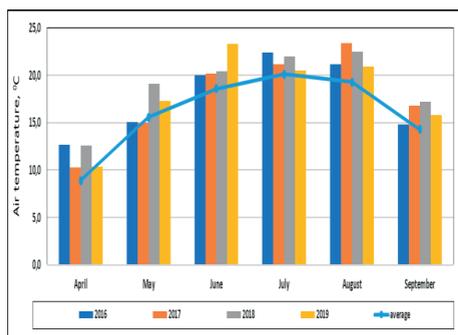


Figure 2. Air temperature data (2016-2019)

The average sum of the effective temperatures during vegetation period was 2200°C-2400°C. High air temperatures (above 25°C) and soil surfaces (up to 60°C) accompanied by winds (between May and August) were a negative prerequisite for restraining the growth and development of miscanthus plants. Local soils are represented by typical chernozem, slightly saline, low-humus, mid-loam. The depth of the humus horizon varies from 35 to 45 cm, the humus content from 3.6 to 4.2%. The reaction of the soil solution of the arable layer is weakly alkaline (pH 7.2-7.4).

The field experiment was conducted according to a two-factor scheme, which involved soaking with rhizomes and subsequent foliar treatment. Water as a control was selected to determine the effectiveness of two growth stimulators (Quantum gold and Vypel-K) to treat miscanthus rhizomes before planting. Quantum gold is a universal micronutrient containing a large number of macro and microelements, as well as a complex of biologically active substances, in particular a highly effective non-toxic phytohormone auxin type. The composition of the PGRs Vypel – K includes polyatomic alcohols - up to 300 g l⁻¹, humic acids - 30 g l⁻¹ and carboxylic acids of natural origin - 3 g l⁻¹. Foliar feeding of plants of miscanthus with growth stimulators was carried out in the phase 3-4 of real leaves and with the density of standing 90% of plants in the area. The first and second treatment was done at the end of the day. The total area of each plot is 50 m², the total area is 10 m². The number of repetitions - 4. Planting was done during the first half of April.

RESULTS AND DISCUSSIONS

The study of growth and development rates of miscanthus plants during the growing season allowed us to reveal the most important dependencies of the process of formation of high productivity of miscanthus. The height of the plant and the number of shoots are the most important signs of the plants organogenesis phases. It was found that the height of the main shoot increased with the number of foliar treatments from 125 to 135 cm on average (Table 1).

Table 1. Height of miscanthus plants in the first year of vegetation, cm

Factor A	Factor B	Height of the main shoot				Average
		2016	2017	2018	2019	
Water	Water	182	90	100	140	128
	Quantum Gold ¹	182	107	100	120	127
	Quantum Gold ²	190	86	100	120	124
	Vympel - K ¹	188	92	100	130	128
	Vympel - K ²	190	100	100	120	128
Quantum gold	Water	210	76	100	110	124
	Quantum Gold ¹	180	80	90	110	115
	Quantum Gold ²	202	100	90	130	131
	Vympel - K ¹	194	78	80	120	118
	Vympel - K ²	210	65	80	120	119
Vympel-K	Water	190	102	100	120	128
	Quantum Gold ¹	200	94	100	140	134
	Quantum Gold ²	196	84	100	130	128
	Vympel - K ¹	196	88	80	130	124
	Vympel - K ²	187	100	80	130	124
LSD _{0.05}		9.7	4.5	4.7	6.2	6.3

¹single foliar spraying; ²two time foliar spraying

It was established that the number of shoots increased after each foliar treatment with the PGRs from 8 to 12 units. The largest number of stems of miscanthus forms in the first year of the life cycle (Table 2).

Table 2. The number of miscanthus shoots in the first year of vegetation

Factor A	Factor B	The number of shoots, units				Average
		2016	2017	2018	2019	
Water	Water	9	8	8	7	8
	Quantum Gold ¹	19	6	10	5	10
	Quantum Gold ²	14	4	8	5	8
	Vympel - K ¹	22	7	12	7	12
	Vympel - K ²	20	10	13	5	12
Quantum gold	Water	16	5	9	7	9
	Quantum Gold ¹	14	2	8	5	7
	Quantum Gold ²	20	5	11	12	12
	Vympel - K ¹	21	5	11	5	11
	Vympel - K ²	19	3	10	6	9
Vympel-K	Water	16	6	10	7	10
	Quantum Gold ¹	16	4	9	6	9
	Quantum Gold ²	15	6	9	8	9
	Vympel - K ¹	18	6	11	5	10
	Vympel - K ²	24	7	14	7	11
LSD _{0.05}		0.9	0.3	0.5	0.3	0.5

Active growth and development of miscanthus plants begins from the tillering phase, which occurs immediately after the appearance of the first true leaves (from May to September).

The formation of new shoots depends on foliar treatment directly. Due to soaking rhizome in Quantum gold solution and subsequent single treatment received 8 shoots per bush. The number of stems increased to 12 units after the

second treatment. The number of stems in the bush varied from 10 to 11 after the use of the PGRs Vympel K (after soaking and one time foliar treatment) compared with double treatment.

The largest leave-stem biomass in the first year of vegetation was obtained in the trial of double foliar treatment soaking in a Quantum gold solution (Table 3).

Table 3. Productivity of aboveground miscanthus biomass in the first year of vegetation, t ha⁻¹

Factor A	Factor B	Productivity of aboveground miscanthus biomass				Average
		2016		2017		
		2016	2017	2018	2019	
Water	Water	5.9	2.8	3.5	3.1	3.8
	Quantum Gold ¹	7.3	2.8	3.9	1.5	3.9
	Quantum Gold ²	7.1	4.6	2.6	1.6	4.0
	Vympel - K ¹	6.0	4.2	1.6	2.3	3.5
	Vympel - K ²	8.0	2.3	1.3	1.5	3.3
Quantum gold	Water	6.4	4.2	1.6	1.5	3.4
	Quantum Gold ¹	6.9	2.3	2.6	1.2	3.3
	Quantum Gold ²	11.2	1.4	2.9	2.7	4.6
	Vympel - K ¹	6.8	1.4	1.2	1.3	2.7
	Vympel - K ²	10.0	2.8	1.7	1.3	3.9
Vympel-K	Water	4.3	3.7	3.6	2.0	3.4
	Quantum Gold ¹	6.7	4.6	2.2	2.5	4.0
	Quantum Gold ²	6.1	4.6	3.5	1.7	4.0
	Vympel - K ¹	8.7	3.7	3.5	2.0	4.5
	Vympel - K ²	8.2	2.8	2.3	2.0	3.8
LSD _{0.05}		0.4	0.2	0.1	0.1	0.2

The yield of miscanthus was 2.7 t ha⁻¹ when combined with two stimulating drugs (soaking in Quantum gold and one time foliar spraying with Vympel K). The branched root system of miscanthus plants in the first year of vegetation deepens to a depth of 40-60 cm, and underground stems (rhizomes) are placed at a depth of 5 to 30 cm.

The best underground yield of miscanthus 7.9 t ha⁻¹ was recorded after double treatment with Quantum gold solution (Table 4). It was found that the yield of the rhizome of miscanthus in 2016-2017 was slightly higher than in 2018-2019. Excessive rainfall of 150 mm in May in 2016 gave a significant impetus to the formation of the underground part of the miscanthus. The largest yield of solid biofuel 5 t ha⁻¹ was obtained when using the Quantum gold by soaking rhizome before planting in the soil and two time treatment of miscanthus plants during the growing season. Similar data were obtained after treatment of miscanthus with two plant growth stimulators Stimp and

Regoplant (Malinská et al, 2020). The results of energy output calculation with miscanthus biomass are shown in Table 5.

Table 4. Roots biomass yield in the first year of vegetation, t ha⁻¹

Factor A	Factor B	Roots biomass yield				Average
		2016	2017	2018	2019	
Water	Water	5.8	7.4	4.0	4.1	5.3
	Quantum Gold ¹	18.5	5.5	5.4	1.5	7.7
	Quantum Gold ²	13.5	10.2	5.6	2.2	7.9
	Vympel - K ¹	9.3	6.0	2.1	3.5	5.2
	Vympel - K ²	19.9	3.2	1.4	2.2	6.7
Quantum gold	Water	11.6	3.2	2.0	2.5	4.9
	Quantum Gold ¹	5.1	4.6	5.2	2.2	4.3
	Quantum Gold ²	15.9	3.7	5.4	5.7	7.7
	Vympel - K ¹	7.8	2.8	1.9	2.1	3.6
	Vympel - K ²	14.5	5.5	2.6	3.2	6.5
Vympel-K	Water	6.5	6.0	5.0	2.0	4.9
	Quantum Gold ¹	9.6	7.4	2.6	4.9	6.1
	Quantum Gold ²	15.0	4.6	4.7	3.3	7.7
	Vympel - K ¹	9.4	6.0	6.6	4.8	6.7
	Vympel - K ²	7.5	5.1	3.2	4.5	5.1
LSD _{0.05}		0.6	0.3	0.2	0.2	0.3

Table 5. The energy output with miscanthus biomass in the trials

Factor A	Factor B	Yield, ha ⁻¹	Solid biofuel, t ha ⁻¹	Energy output, GJ ha ⁻¹
Water	Water	3.8	4.2	67.1
	Quantum Gold ¹	3.9	4.2	67.8
	Quantum Gold ²	4.0	4.4	70.4
	Vympel - K ¹	3.5	3.9	61.7
	Vympel - K ²	3.3	3.6	57.6
Quantum gold	Water	3.4	3.8	60.2
	Quantum Gold ¹	3.3	3.6	57.6
	Quantum Gold ²	4.6	5.0	80.3
	Vympel - K ¹	2.7	3.0	47.3
	Vympel - K ²	3.9	4.3	69.4
Vympel-K	Water	3.4	3.7	59.8
	Quantum Gold ¹	4.0	4.4	70.4
	Quantum Gold ²	4.0	4.4	69.8
	Vympel - K ¹	4.5	4.9	78.4
	Vympel - K ²	3.8	4.2	67.3
LSD _{0.05}		0.2		

All PGRs contain various compounds and extracts which were expected to stimulate plant growth (Schmidt et al., 2017; Verma et al, 2016). The influence of PGRs “Regoplant” was higher and the best results were obtained with combined treatment: application to rhizomes before planting and spraying on the biomass during vegetation (Nebeská et al., 2019).

It is known that both Regoplant and Quantum gold contains synthetic analogue of plant auxin (1-NAA).

The maximum energy yield per unit area was achieved by soaking in growth stimulators with one-time and two-time treatment, as following: 80 GJ ha⁻¹ in Quantum gold, and 78 GJ ha⁻¹ - in Vympel K. Slightly lower volume of solid biofuel at 0.1 t ha⁻¹ was obtained by soaking rhizome in the preparation Vympel K.

CONCLUSIONS

Miscanthus forms the largest number of stems in the first years of the life cycle. The yield of miscanthus roots differs from the above-ground mass, since the formation of shoots in plants occurs throughout its growing season. First of all, the plant forms an underground part of the biomass. It was found that the number of shoots increased (from 8 to 12 units per bush) after each foliar treatment with growth stimulants. The highest yield of miscanthus 4.6 t ha⁻¹ and energy yield - 80 GJ ha⁻¹ was obtained in the trial when soaking in a solution of Quantum gold with two time treatment of plants during the growing season.

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REFERENCES

- Alexopoulou, E., Zanetti, F., Scordia, D., Zegada-Lizarazu, W., Christou, M., Testa, G., Cosentino, S.T., Monti, A. (2015). Long-Term Yields of Switchgrass, Giant Reed, and Miscanthus in the Mediterranean Basin, *BioEnergy Research*, 8, 1492–1499.
- Anderson, E., Arundale, R., Maughan, M., Oladeinde, A., Wycislo, A., Voigt, T. (2011). Growth and agronomy of *Miscanthus x giganteus* for biomass production, *Biofuels*, 2(1), 71–87.
- Brosse, N., Dufour, A., Meng, G X., Sun Q., Ragauskas, A. (2012). Miscanthus: a fast-growing crop for biofuels and chemicals production. Review. *Biofuels, Bioproducts & Biorefining*, 1–19.
- Da Borso, F., Di Marzo, C., Zuliani, F., Damiso, F., Baldini, M. (2018). Harvest time and ensilage suitability of giant reed and miscanthus for bi methane production and characterization of digestate for agronomic use. *Agronomy Research*, 16(1), 22–40.

- Di Vita, G., Pilato, M., Pecorino, B., Brun, F., D'Amico, M. A. (2017). Review of the Role of Vegetal Ecosystems in CO₂ Capture. *Sustainability*, 9, 1840.
- Drazic, G., Milovanovic, J., Ikanovic, J., Petric, I. (2017). Influence of fertilization on *Miscanthus × giganteus* (Greef et Deu) yield and biomass traits in three experiments in Serbia. *Plant Soil Environ*, 63, 189–193.
- Hatamian M., Rezaie Nejad A., Kafi M., Souri M.K., Shahbazi K. (2019). Growth characteristics of ornamental Judas tree (*Cercis siliquastrum* L.) seedling under different concentrations of lead and cadmium in irrigation water. *Acta Scientiarum Polonorum Hortorum Cultus*, 18(2), 87–96.
- Heaton, E.A., Clifton-Brown, J., Voigt, T.B., Jones, M.B., Long, S.P. (2004). Miscanthus for renewable energy generation: European Union experience and projections for Illinois. *Mitigation and Adaptation Strategies for Global Change*, 9, 433–451.
- Kazimierowicz, J. and Dzienis, L. (2015). Giant Miscanthus as a substrate for biogas production. *Journal of Ecological Engineering*, 16(4), 139–142.
- Kharytonov, M., Pidlisnyuk, V., Stefanovska, T., Babenko, M., Martynova, N., Rula, I. (2019). The estimation of *Miscanthus × giganteus* adaptive potential for cultivation on the mining and post-mining lands in Ukraine. *Environ Sci Pollut Res Int.*, 26(3), 2974–2986.
- Malinská H., Pidlisnyuk V., Nebeská D., Erol A., Medžová A., Trögl J. (2020). Physiological Response of *Miscanthus x giganteus* to Plant Growth Regulators in Nutritionally Poor Soil. *Plants*, 9, 194.
- Matyka, M. and Kus, J. (2016). Influence of soil quality for yielding and biometric features of *Miscanthus × giganteus*. *Polish Journal of Environmental Studies*, 25(1), 213–219.
- Milovanović, J., Dražić, G., Ikanović, J., Jurekova, Z. & Rajković, S. (2012). Sustainable production of biomass through *Miscanthus x giganteus* plantation development. *Annals of Faculty Engineering Hunedoara – International Journal of Engineering*, 10, 79–82.
- Nebeská, D., Pidlisnyuk, V., Stefanovska, T.R., Trögl, J., Shapoval, P., Popelka, J., Černý, J., Medkow, A., Kwak, V. & Malinska, H. (2019). Impact of plant growth regulators and soil properties on *Miscanthus x giganteus* biomass parameters and uptake of metals in military soils. *Reviews on environmental health*, 34(3).
- Schmidt, C.S., Mrnka, L., Frantík, T., Motyka, V., Dobrev, P.I., Vosátka, M. (2017). Combined effects of fungal inoculants and the cytokinin-like growth regulator thidiazuron on growth, phytohormone contents and endophytic root fungi in *Miscanthus × giganteus*, 120, 120–131.
- Souri, M.K. (2016). Aminocheilate fertilizers: the new approach to the old problem; a review. *Open Agriculture*, 1, 118–123.
- Souri, M.K., Sooraki, F.Y. & Moghadamyar, M. (2017). Growth and quality of cucumber, tomato, and green bean under foliar and soil applications of an aminocheilate fertilizer. *Hortic. Environ. Biotechnol.*, 58(6), 530–536.
- Verma, V., Ravindran, P. & Kumar, P.P. (2016). Plant hormone – mediated regulation of stress responses. *BMC Plant Biol*, 16, 86.

GREEN FORAGE PRODUCTIVITY AND YIELD COMPONENTS OF TRITICALE VARIETIES (*×Triticosecale* Wittm.) UNDER THE INFLUENCE OF DIFFERENT NITROGEN FERTILIZATION LEVELS

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Abstract

A three-year field experiment for determination of the green fodder yield of triticale varieties has been carried out on the experimental field of the Crop Science Department at the Agricultural University of Plovdiv. The experiment has been conducted in block method in 4 replications after predecessor sunflower. The study used the varieties Lasko - standard, Boomerang, Respect and Attila, created in the breeding center of triticale in Bulgaria - Dobruja Agricultural Institute, Gen. Toshevo. Triticale is grown under two levels of nitrogen fertilization - 60 and 180 kg ha⁻¹ nitrogen. As a result of the experiment, the main structural elements of the yield and some quality parameters of the plants have been established, depending on the variety and the nitrogen fertilization level. For the three years of the study, the average yield of green mass of all triticale varieties, fertilized with 180 t ha⁻¹ nitrogen is higher than the lower fertilizer rate. Under the influence of both fertilization rates, the variety Lasko has proven as less productive than all other varieties created in the Dobruja Agricultural Institute, Gen. Toshevo, Bulgaria.

Key words: triticale, nitrogen fertilization, fodder yield.

INTRODUCTION

Triticale is a grain crop, artificially created from a cross between wheat and rye. Apart from grain, rye genome involvement in its creation, makes triticale crop suitable for use in various feed derived from the green mass. Harvested after full spike formation, the crop is suitable for silage and production of various feed hay. Several studies identify the triticale crop as appropriate for participation in grass-forage mixtures with legumes or self (Aguilar-López et al., 2013; Bilgili et al., 2009; Jacobs and Ward, 2012; Zannat et al., 2012; Demidova et al., 2015; Liu et al., 2017; Maisak, 2017; Ostos Garrido et al., 2018).

An important point in the production of green forage of triticale is the choice of the variety. In a world selection of triticale varieties, designed specifically for green mass are created (Kina et al., 2009; Li et al., 2012; Royo and Blanco, 1999). Bulgaria is one of the first countries in the world that creates its own varieties of triticale. Almost all Bulgarian varieties, however, are designed for grain. The only one official recognized Bulgarian triticale variety of green mass is Belitsa1, created in the early '80s of the 20th Century (Tzvetkov, 1989; Giurova

et al., 1993). However, variety Belitsa1 has been long unsupported and no seeds are produced from it. This requires the production of green forage from triticale to use modern varieties, intended mainly for grain.

Besides choosing the variety, obtaining maximum biological yield is greatly influenced by the nitrogen fertilization, which is a powerful factor in the growth of vegetative organs of the plants (Cazzato et al., 2012; Nogalska et al., 2012; Georgieva, 2019).

According to Wysokinski and Kuziemska, (2019) following the application of a single nitrogen dose, spring triticale took up 65.05 kg N ha⁻¹ from the fertilizer, and the utilization coefficient of N amounted to 54.3%. The split application of the total amount of fertilizer divided into two and three doses increased the amount of nitrogen taken up from the fertilizer by 7.31 and 14.90 kg N ha⁻¹, respectively, and the value of the coefficient of its utilization rose by 6.0 and 12.4%, respectively. The amount of this element taken up from the soil reserves was not dependent on the test variants of nitrogen fertilization. A significant increase in the uptake and utilization of nitrogen from the fertilizer by triticale was obtained from BBCH stage 22 to stage 65. From BBCH stage

65 to stage 92, no significant increase in the amount of nitrogen taken up from these sources or in the value of the coefficient of utilization was noted.

At the experimental base of the Department of Plant production at Trakia University, Stara Zagora, nitrogen assimilation from fertilization increases with the increase of the nitrogen fertilizer rates. With the obtained yield from triticale an average of 96.53 kg ha⁻¹ N is extracted from the soil with the grain, 18.97 kg ha⁻¹ N with the straw or a total of 115.50 kg ha⁻¹ N. When growing triticale after leguminous predecessors the utilization of nitrogen is 35.39% and after predecessors sunflower, wheat and triticale - 28.76%. Nitrogen required for 100 kg yield of the grain of triticale is 1.9 kg of nitrogen when growing after legume predecessors and 2.8 kg of nitrogen after the other predecessors (Gerdzhikova et al., 2017).

The aim of this study is to identify the genotypic specifics of triticale varieties in terms of feedstock productivity at two rates of nitrogen fertilization.

MATERIALS AND METHODS

The experiment has been carried out during the 2017-2019 years in the experimental field of the Crop Science Department, Agricultural University of Plovdiv. Four varieties of triticale - Lasko (international triticale standard) the first Polish hexaploid triticale variety, obtained at the Laski Breeding Station from the cross Triticale 57 - winter wheat C 1218/67 x 6TA 206 (Wolski and Tymieniecka, 1983), Boomerang, Respect and Attila, created in the Dobruja Agricultural Institute, Gen. Toshevo, Bulgaria have been tested. Varieties have been grown at two levels of nitrogen fertilization - 60 and 180 kg ha⁻¹ nitrogen, introduced in early spring. The experiment was conducted after the predecessor sunflower and arranged according to the split-plot method in four replications.

The yield of green mass (t ha⁻¹) has been recorded at the end of the heading stage (BBCH 59) of the harvest plots with a size of 10 m². The following structural elements of the plants have been determined: plant height (cm);

the number of spikes per plant, length of spikes (cm). Depending on the amount of the accumulated biomass, the share of the main three organs (stems, leaves, and spikes) in the formation of the green mass is calculated.

Statistical analyses of individual factors were performed with a software package SPSS 16.0.

RESULTS AND DISCUSSIONS

Weather conditions during the vegetation are one of the factors that strongly influence the productivity of the tested varieties (Figure 1).

Although the sowing during the first year of the study has been carried out at the optimum crop time (11.10), the autumn drought delayed the onset of the crop and emergence phases was observed on 10.11 (one month later after sowing). The autumn of 2016 has indicators close to the minimum temperatures for the growth and development of the crop. During the sharp cold of the weather at the end of November, the growing season ended and the crops came to rest in the third leaf phase. December is warm with average monthly temperatures of 2.2°C. January is characterized as cold as the average monthly temperature (-3.9°C) is lower than the long-time average. The months were characterized by sharp and short-term decreases in temperatures up to -10.3°C and the lack of snow cover, which represented a real threat to sowing, but despite the low temperatures, no frost was observed. The onset of permanent spring vegetation is recorded under the conditions of the third ten days of March with values of average daily temperatures of the order of 9.7°C. The period of filling and ripening of the grain - May and June is characterized by values of temperatures close to the optimum. The amounts of winter rainfall were less than typical for the Plovdiv area, except for January, with a minimum of 2.4 mm recorded in December. In March, the amount of rainfall is 47.9 mm, while in May and June, when the maximum rainfall occurs, the amount of rainfall is respectively 87.3 mm and 124.6 mm below the normal. The total rainfall during the triticale vegetation is 288.4 mm, which is 271.6 mm below the climatic norm of the investigated area.

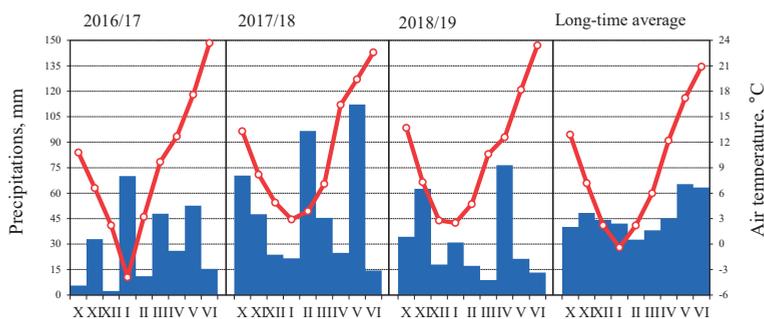


Figure 1. Meteorological conditions (climatogramme) during the years of the investigation

During the second year of the study, the temperatures during the growing season of triticale not only were higher than the previous year but also exceeded the climatic norm of the area, with the sum of average monthly temperatures of 11°C, which is 2.1°C above the long-term average. Unlike the 2016/2017 drought, the second harvest year is characterized by sufficient rainfall, which contributes to better soil moisture conservation and optimum crop development. Positive monthly average temperatures in November (8.2°C), as well as low rainfall (47.6 mm) close to the climatic conditions, create the conditions for the beginning of the tillering in the first ten days of December. The total rainfall in the second year exceeds the area's climate norm (417.0 mm) by 40 mm. Heavy rainfall in March (112.3 mm) prolongs the period of pouring and ripening of the grain.

In climatic terms, the third year is approaching the second year, with positive temperatures in the autumn and winter period exceeding the

climatic norm. The period from sowing to germination is 10 days, as in the second year. Although the rainfall in October is 5.8 mm below the region's climatic norm, the average monthly temperatures (13.7°C) exceed the 0.8°C long-term average, which contributes to the onset of the emergence phase. Positive average monthly temperatures in November (7.3°C) and heavy rainfall (62.5 mm), which exceed the climatic norm, contribute to the beginning of the tillering in early December. Although in 2017/18 the amount of rainfall during the same period exceeds the climatic norm, temperature plays a decisive role in the occurrence of the stem elongation phase. In 2018/19, the highest monthly average temperatures over the test period (23.4°C) are observed in June, which contributes to the earliest entry into the ripening phase.

The yield of green mass of the studied triticale varies depends on the conditions during the years and is greatly influenced by the tested levels of nitrogen fertilization (Table 1).

Table 1. Green mass yield, t ha⁻¹

Years	2017		2018		2019		Average	
	N ₆₀	N ₁₈₀	N ₆₀	N ₁₈₀	N ₆₀	N ₁₈₀	N ₆₀	N ₁₈₀
Lasko	2.4 ^a	4.2 ^a	4.2 ^a	4.9 ^a	3.5 ^a	4.2 ^a	3.4 ^a	4.4 ^a
Boomerang	2.9 ^c	4.6 ^b	4.7 ^b	5.6 ^b	3.6 ^b	5.2 ^b	3.7 ^b	5.1 ^c
Respect	2.6 ^b	4.5 ^b	4.6 ^b	5.6 ^b	3.5 ^b	4.9 ^b	3.6 ^b	5.0 ^b
Attila	2.5 ^b	4.6 ^b	4.6 ^b	5.6 ^b	3.6 ^b	4.8 ^b	3.6 ^b	5.0 ^b
LSD 5%	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2

*Values with the same letters do not differ significantly.

For the tested period, the yields of all varieties are the lowest at the first fertilization rate (N₆₀). During the three years of study, the lower yield is derived from the variety Lasko - 2.4, 4.2 and 3.5 t ha⁻¹, respectively in 2017, 2018 and 2019

years in the conditions of the fertilization rate N₆₀.

Differences between the international standard variety Lasko and varieties selected in Dobruja Agricultural Institute, Gen. Toshevo, Bulgaria

in the first year of study sets the standard in the lowest group of productivity of green mass and varieties Respect and Attila prove to be high yielding. Variety Boomerang proved as more productive than both the standard and the other varieties in the first year when fertilized with N₆₀. In 2018 and 2019 years, the differences in grain yield were demonstrated only between Lasko and the varieties created in Bulgaria. Both levels of fertilization established no differences between the three Italian varieties of triticale. This is probably due to the closest genotype origin of the varieties Boomerang, Respect and Attila. The reason for the differences proven in the first year for the lowest nitrogen rate can be attributed to poor rainfall conditions during the spring of 2017, from which it can be concluded that in terms of

drought years and lower rates of fertilization, genotypic differences in triticale are more pronounced.

Average for the three years of the study, the average yield of green mass of all varieties of triticale, fertilized with 180 t kg ha⁻¹ nitrogen is higher than the lower fertilizer rate by 1.33 t ha⁻¹. Under the conditions of both fertilization rates, the variety Lasko has proven as less productive than all other varieties created in the Dobruja Agricultural Institute, Gen. Toshevo, Bulgaria.

The main structural components directly influencing the formation of crop and productivity of triticale varieties can be considered independently by factors - variety, conditions of the year and nitrogen fertilization (Table 2).

Table 2. Differences between the main productivity components

Indices*	Plant height, cm	Number of spikes	Length of spike, cm
Varieties			
LASKO	105 ^a	2.1 ^a	11.5 ^a
Boomerang	115 ^b	3.3 ^b	16.0 ^c
Respect	120 ^c	3.1 ^b	14.5 ^b
Attila	118 ^b	3.1 ^b	13.5 ^b
Years			
2017	110 ^a	2.6 ^a	13.2 ^a
2018	119 ^b	3.2 ^b	14.0 ^a
2019	118 ^b	2.9 ^b	13.6 ^a
Nitrogen fertilization			
N ₆₀	110 ^a	2.5 ^a	13.8 ^a
N ₁₈₀	119 ^b	3.2 ^b	14.0 ^a

*Values with the same letters do not differ significantly.

The height of the stems varies depending on the variety from 105 cm in cultivar Lasko to 120 cm in Respect variety. Differences between varieties were statistically significant, except by Boomerang and Attila, where the difference in height of the stem is not proven. The influence of the conditions of the year is shown only between the first and the remaining two years of the study. The higher rate of nitrogen fertilization has been proven to increase the height of the crop by 9 cm.

The formation of productive tillers (number of spikes) is one of the main factors influencing directly the crop productivity. The amount of spikes per plant varieties varies between 2.1 and 3.3 per plant. These differences, however, are only proven compared with Lasko, and the remaining three varieties can be grouped together in terms of their productive tillering.

In the second and third year, an equal number of productive tillers (spikes) are formed and in 2017 when are observed the lowest yields of green mass, the tillering is lowest. The higher nitrogen rate leads to an increase in productive tillering with 0.7 spikes per plant compared to the fertilization with 60 kg ha⁻¹ N and the differences between both fertilization rates are statistically proven.

According to the spike length, there is a statistically proven difference between Lasko and all tested varieties, only between the varieties Respect and Attila, the difference remains unproven, which set them in the same statistical group. Longest spike gives the variety with the highest average yield - Boomerang. Weather conditions during the three years of study do not lead to statistically proven differences in the length of the spikes.

Because of the genetic determination of the length of the spike, the increasing nitrogen fertilization has no proven positive impact on the indicator.

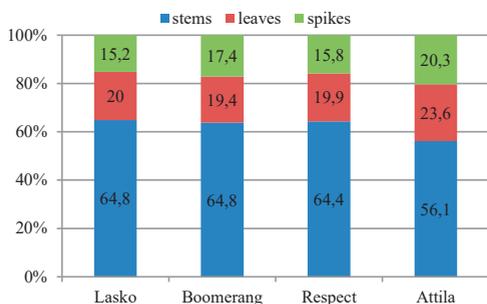


Figure 2. Part of the organs of the plant in the formation of the yield

The organs of the plants that form the total biomass of triticale in the medium milk stage are the stems, leaves, and spikes (Figure 2). The highest proportion of plants in all triticale varieties occupy the stems (62.5% of the plant mass on average for all triticale varieties). The lowest is the proportion of stems in the Attila variety - 56.1% and the largest in the varieties Lasko and Boomerang - 64.8%. The leaves occupy an average of 20.7% of the mass of the plants. The highest proportion of leaves is in the Attila variety - 23.6%, followed by the Lasko (20%) Respect (19.9%) and the lowest in the Boomerang variety - 19.4%. The share of spikes by all varieties of triticale occupies 17.2% of the mass of the whole plant. The highest share of the spikes is in the Attila variety - 20.3% and the smallest in the Lasko variety - 15.2%.

Relations between yield and the various structural elements, expressed by the correlation coefficient indicates that an increase in each of the three indicators influences positively the yield of green mass of triticale (Table 3). In most influence on the yield of a crop has a density, expressed as the number of spikes ($r = 0.648$), followed by plant height ($r = 0.620$) and the length of the spike ($r = 0.592$).

CONCLUSIONS

Average for the three years of the study, the yield of green mass of all triticale varieties, fertilized with 180 t ha^{-1} nitrogen is higher than the lower fertilizer

rate. Under the conditions of both fertilization rates, variety Lasko has proven as less productive than all other varieties created in the Dobruja Agricultural Institute, Gen. Toshevo, Bulgaria.

The largest share of the plants of all triticale varieties is occupied by the stems (62.5%) followed by the leaves (20.7%) and the share of the spikes occupies 17.2% of the mass of the whole plant.

Relations between yield and the various structural elements expressed by the correlation coefficient indicates that an increase in each of the three indicators influences the yield of green mass of triticale. The productive tillering influences the yield of a crop to the largest extent, followed by plant height and length of the spike.

Table 3. Correlation analysis

r_{xy}	1. Green mass yield	2. Plant height	3. Number of spikes	4. Length of spike
1	1			
2	0.620*	1		
3	0.648*	-0.112	1	
4	0.592*	0.957*	-0.016	1

Correlation is significant at the $p = 0.05$ level.

REFERENCES

- Aguilar-López, E.Y., Bórquez, J.L., Domínguez, I.A., Morales-Osorio, A., Gutiérrez-Martínez, M., Ronquillo, M. (2013). Forage Yield, Chemical Composition and In Vitro Gas Production of Triticale (*Triticosecale* Wittmack) and Barley (*Hordeum vulgare*) associated with Common Vetch (*Vicia sativa*) Preserved as Hay or Silage. *Journal of Agricultural Science*, 5(2), 227–238.
- Bilgili, U., Cifci, E.A., Hanoglu, H., Yagdi, K., Acikgoz, E. (2009). Yield and quality of triticale forage. 2009. *Journal of Food, Agriculture and Environment*, 7(3-4), 556–560.
- Cazzato, E.E., Laudadio, V.V., Tufarelli, V.V. (2012). Effects of harvest period, nitrogen fertilization and mycorrhizal fungus inoculation on triticale (*Triticosecale* Wittmack) forage yield and quality. *Renewable Agriculture & Food Systems*, 27(4), 278–286.
- Demidova, A.G., Kuleshova, I.V., & Gubina, T.G. (2015). Forage productivity of spring triticale in pure and mixed sowings as green feed in the conditions of Belgorod region. *Vestnik OrelGAU*, 1, 67–74.
- Gerdzhikova, M., Grozeva, N., Pavlov, D., & Tzanova, M. (2017). Effect of nitrogen fertilization in Triticale (*Triticosecale* Wittm.), cultivated after different predecessors. Nitrogen uptake and efficiency. *AGROFOR International Journal*, 2(3), 147–56.
- Georgieva, R. (2019). Alteration of yield components of triticale depending on treatment with plant stimulants in the condition of different soil nutrition regime. *Journal of Mountain Agriculture on the Balkans*, 22 (1), 130–138.

- Giurova, M., Georgiev, S., Ivanova, V., Dimitrov, L. (1993). Agroclimatic zoning of rye and triticale for green mass. *Rastenievadni nauki*, 5-6, 80–83 (Bg).
- Jacobs, J.L., Ward, G. N. (2012). Effect of intercropping forage peas (*Pisum sativum* L.) with winter wheat (*Triticum vulgare* L.) or triticale (*Triticale hexaploide* Lart.) on DM yield, nutritive characteristics when harvested at different stages of growth. *Animal Production Science*, 52(10), 949–958.
- Kina, B.B., Ayhan, V.V., Akman, Z.Z., Adiyaman, E.E. (2009). Determination of silage quality, herbage and hay yield of different triticale cultivars. *Asian Journal of Animal & Veterinary Advances*, 4(3), 167–171.
- Li, H., Lin, C., Li, C., Kong, G., Wang, Y., Wu, Q., Cao, L. (2012). Analysis of stability of forage triticale varieties with AMMI model. *Xinjiang Agricultural Sciences*, 49(5), 802–807.
- Liu, W., Maurer, H., Leiser, W., Tucker, M., Weissmann, S., Hahn, V., & Würschum, T. (2017). Potential for marker-assisted simultaneous improvement of grain and biomass yield in triticale. *BioEnergy Research*, 10(2), 449–455.
- Maisak, G.P. (2017). Winter crops ensilage in Permskii Krai. *Perm Agrarian Journal*, 20(4), 91–96.
- Nogalska, A.A., Czapla, J.J., & Skwierawska, M.M. (2012). The effect of multi-component fertilizers on spring triticale yield, the content and uptake of macronutrients. *Journal of Elementology*, 17(1), 95–104.
- Ostos Garrido, F.J., Pistón, F., Gómez, L.D., & McQueen-Mason, S.J. (2018). Biomass recalcitrance in barley, wheat and triticale straw: Correlation of biomass quality with classic agronomical traits. *PLoS ONE*, 13(11), 1–14.
- Royo, C., Blanco, R. (1999). Growth analysis of five spring and five winter triticale genotypes. *Agronomy Journal*, 91(2), 305–311.
- Tzvetkov, S. (1989). Triticale. *Zemizdat*, Sofia (Bg).
- Wolski, T., Tymieniecka, E. (1983). The first Polish triticale variety. *Cereal Research Communications*, 11(2), 139–141.
- Wysokinski, A., & Kuziemska, B. (2019). The dynamics of uptake and utilization of nitrogen from mineral fertilizer by spring triticale calculated by isotopic dilution method. *Journal of Elementology*, 24(1), 7–18.
- Zannat, M.K., Alam, M.R., Sarker, N.R., Huque, K.S., Khan, M.I., & Islam, M.A. (2012). Comparative study on the biomass yield, chemical composition and in-sacco dry matter degradability of different cultivars of triticale fodder. *Bangladesh Journal of Animal Science*, 41(1), 24–28.

STUDY OF THE EFFECT OF FERTILIZATION AND SOWING RATES ON THE YIELD CAPACITY OF DENI DURUM WHEAT

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Abstract

At the Experimental and Implementation Base of the Agricultural University of Plovdiv, Bulgaria, a field experiment was conducted to determine the impact of two factors on the yield of Deni durum wheat. Four levels ($N_0P_0K_0$; $N_{60}P_{40}K_0$; $N_{120}P_{80}K_0$; $N_{180}P_{120}K_{60}$ /ha) were tested for fertilization rate, being Factor A, and three levels (400, 500, 600 germinating seeds per m^2) were tested for sowing rate, being B. The experiment was performed after the precursor of rapeseed by the method of fractional plots in four repetitions during the period 2015 - 2018. As a result of the experiment, it was proved that: Deni wheat durum wheat shows its yield capacity to the greatest extent under the soil and climatic conditions of Plovdiv region when grown with fertilization rate: $N_{120}P_{80}K_0$ and sowing rate of 500 germinating seeds m^2 . Grain yield increased from 3.29 t/ha to 4.75 t/ha or an average of 4.02 t/ha (13.6%) over the three-year experimental period 2015-2018. Structural elements of the yield, i.e. productive bushiness, number of spikelets, number of grains and mass of grains in the wheat-ear have the highest values at fertilization level with $N_{120}P_{80}K_0$ and sowing rate of 500 germinating seeds per m^2 .

Key words: durum wheat, fertilization rates, sowing rates, yield capacity.

INTRODUCTION

Sowing rate is an important factor on which grain yield and grain quality depend. More and more manufacturers consider this factor as an instrument for maximizing profits. Subject to all technological practices, if optimal germination and spouting of the sown land is not achieved, maximum yield of good quality would be difficult to obtain, and even if the optimal sowing rate for dry years was determined, it would not be the same if the year is a wet one. A precautionary measure against lodging of the crops is to ensure optimal germination and sprouting of the plants, ensuring uniformity and moderate growth. The favourable distribution of plants on the nutritional surface leads to a better supply of light, water and nutrients. In very dense crops, the plants are depressed and yields are unsatisfactory, and in sparse crops, due to insufficient use of nutritional resources, less yields are obtained by the varieties compared to the potential yields. The issue of optimum density is up to date for every new variety at the appropriate place of cultivation.

Lloveras et al. (2004) determine sowing density as a factor of practical importance for durum wheat production since it can be controlled and recommend optimal sowing rates for the Mediterranean region from 371 to 508 germinating seeds/ m^2 .

Of all the components of the yield, sowing rate has the least effect on the mass of the grains in the ear according to Ozturk et al. (2006). The same author found an increase of 10% in the number of grains in the ear with decreasing the sowing rate from 625 to 325 germinating seeds/ m^2 .

In Diyarbakir, the effect of sowing rates of 50, 150, 250, 350, 450 and 550 germinating seeds/ m^2 on the yield and its elements of the Aydin 93 and Firat 93 durum wheat varieties was studied. The highest productivity of 5102 kg/ha was obtained at a sowing rate of 250 germinating seeds/ m^2 , which is determined to be optimal (Kilic et al., 2010).

An experiment was conducted in Poland (Bobrecka-Jamro et al., 2013) with winter wheat of the Rywalka variety, in the years 2008/2009, 2009/2010 and 2010/2011 on a farm located in south-eastern Poland. It was

fertilized with nitrogen rates of 40 to 120 kg/ha and foliar application compared to the control crops without nitrogen fertilization. Winter wheat production of the Rywalka variety has varied over the years of study. The highest yield was obtained after administration of nitrogen at a dose 120 kg/ha.

The concentrations of nitrogen, phosphorus and potassium in nine malting barley genotypes grown at four nitrogen levels 0, 40, 80, 120 kg N/ha were studied under a field fertilizing experiment. Genotypic reaction in regard to crude protein concentrations was stronger at unfertilized plants and it was considerably varying from 7.0% ("Kristi" and "70412296") to 10.0% ("2390300"). The higher nitrogen levels (N₈₀ and N₁₂₀) led to similar values of grain crude protein in studied barley genotypes (Kostadinova et al., 2012).

A field experiment was carried out on the experimental field of Field Crop Institute, Chirpan, during the period 2004-2007 the following varieties were studied: Progres, Neptun 2, Beloslava, Saturn 1 and Vozhod under four norms of nitrogen fertilization - N₀, N₆, N₁₂, N₁₈ kg/da. The results showed that nitrogen fertilization is a factor with a strong influence on the formation of leaf area of durum wheat. Compared with the impact of the variety, the effect of nitrogen fertilization on leaf area formation of durum wheat is more pronounced in the three phenological phases (spindling, ear formation and lactic ripeness). (Semkova and Saldzhiev, 2014). No research has been conducted on certain technology elements of the new Deni variety durum wheat. With this study we set ourselves the goal of establishing the optimum values of mineral fertilization and sowing rate for the cultivation of Deni variety durum wheat.

MATERIALS AND METHODS

The study was conducted in the period 2015-2018 in the Study, Experimental and Implementation base of the Agricultural University of Plovdiv using the method of fractional plots, repeated four times, with the size of the harvest plot of 15 m². The nutrient content of the soil in the layer up to 20 cm was respectively: N – 23.5 mg/1000 g, P₂O₅ -

39.3 mg/100 g, K₂O - 27.5 mg/100 g, humus 2.19%, CaCO₃ - 7.3% (Popova et al., 2010).

We investigated the effect of two factors on the productivity of Deni durum wheat. Four levels (N₀P₀K₀; N₆₀P₄₀K₀; N₁₂₀P₈₀K₀; N₁₈₀P₁₂₀K₆₀ kg/ha) were tested for factor A - fertilization, and three levels (400, 500, 600 germinating seeds per m²) were tested for factor B. The experiment was carried out after the precursor rapeseed by the method of fractional plots, repeated four times during the period 2015-2018. Durum wheat was grown after rapeseed as a precursor. For the variants with phosphorus fertilizers - 40, 80 and 120 kg/ha (the total amount of superphosphate) and the variants fertilized with potassium 60 kg/ha (the total amount of potassium sulphate), the fertilizers were introduced during the pre-sowing soil processing, while the nitrogen fertilizer (ammonium nitrate) - 1/3 of the quantity before sowing and the remaining 2/3 in early spring as nourishment. Sowing was carried out during the optimal for southern Bulgaria period from 20th October to 5th November. Weed, disease and pest control was carried out according to the established cultivation technology (Yanev et al., 2008).

RESULTS AND DISCUSSIONS

During the growing season of durum wheat, the amount of rainfall was as follows: 2015/2016 - 396.5 mm, 2016/2017 - 278.3 mm/m², 2017/2018 - 457.2 mm/m², compared to the climatic norm of 419,6 mm/m² (Figure 1 and Figure 2). The total rainfall in the second year of the experiment is less than the climatic norm, but the harvest year of 2016/2017 was more favourable for the growth and development of durum wheat due to its better distribution during the critical stages of development of the plants and then the values of the structural elements of the yield were higher in the Deni variety. Adverse to the development of plants is the harvest year 2015-2016 due to the less rainfall during the period from February to June compared to the climatic norm, which had a negative impact on the productivity of durum wheat.

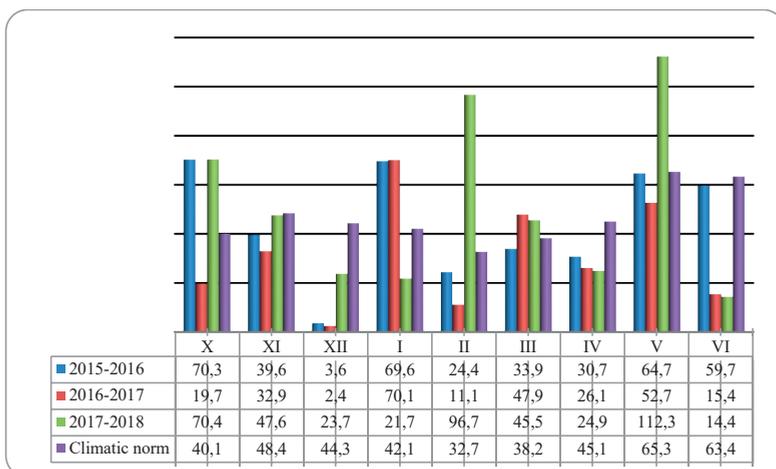


Figure 1. Precipitation by months, sum mm/m²

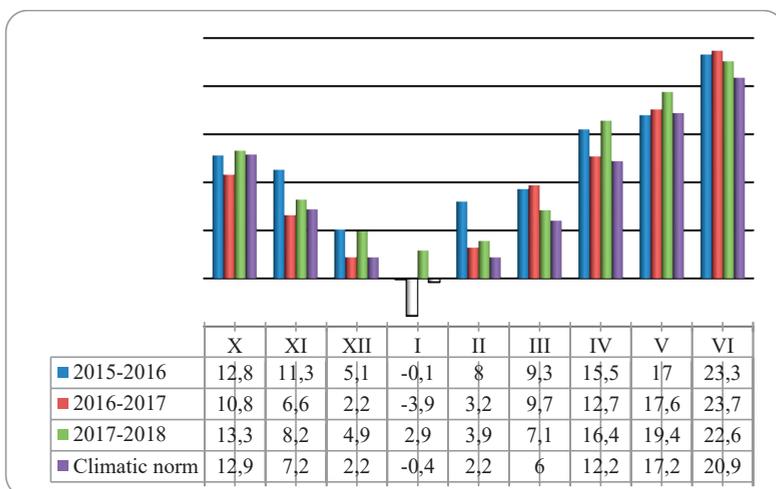


Figure 2. Monthly temperatures (average)

Plant development by phenophases

Individual phenophases were reported for 75% of the plants. The occurrence of phenophases in the Deni variety is presented in Table 1. What will be the duration of the period between the phenophases from sowing to germination

depends on the amount of rainfall before sowing, but is also affected by the air temperature, with higher average daily temperatures causing faster and united sprouting of wheat.

Table 1. Plant development by phenophases (2015-2018)

Plant development by phenophases	2015-2016	2016-2017	2017-2018
Sowing	20.10	21.10	26.10
Sprouting	31.10	29.10	06.11
3 rd leaf	18.11	16.11	24.11
Tillering	10.03	07.03	12.03
Shooting-up	14.04	05.04	09.04
Ear formation	08.05	03.05	08.05
Ripening	02.07	31.06	04.07

What will be the duration of the period between the phenophases from sowing to germination depends on the amount of rainfall before sowing, but is also affected by the air temperature, with higher average daily temperatures causing faster and united sprouting of wheat.

In the autumn of 2015, the amount of rainfall of 70.3 mm in October increased the soil moisture reserves and the sowing of durum wheat was carried out on 20.10.2015 (Table 1). This rainfall, as well as higher temperatures compared to the multiannual period of time (Figure 1 and Figure 2) were sufficient for germination of seeds and plants sprouted on 31.10.2015. The plants entered the tillering phenophase (autumn) on 18.11.2015. The average monthly temperatures in November and December were respectively by 4.10°C and 2.90°C higher than the monthly average norm, but as a result of the smaller amount of precipitation respectively by 8.8 mm and 40.7 mm, they had a retarding effect on the growth and development of durum wheat. Gradually, with the decrease in temperatures in the second and third 10-day periods of December, the growth processes in durum wheat also subsided. In January, the amount of precipitation was higher by 27.5 mm, while in February and March was less by 8.3 mm and 4.3 mm than the norm for the multiannual period. The month of February was also characterized by higher average monthly temperatures of 8.00°C (compared to 2.20°C in February for an average of thirty years), but the

plants were in winter rest. During the first ten days of March, as the temperature rose, the plants resumed their vegetation. Rainfall in April was by 14.4 mm below the norm and this deferred the growth of durum wheat. Rainfall in May and June was less than the norm by 0.6 mm and 3.7 mm, respectively, while the average monthly temperature in June was by 2.4°C higher than the norm, which led to accelerated development of durum wheat. The grain filling and ripening phases occurred under favourable conditions of rainfall. Harvesting of durum wheat was carried out on 02.07.2016.

Under the climatic conditions of Central Southern Bulgaria, varieties that complete their growing season faster have the advantage of avoiding the later hot periods, and this could indicate good adaptability of Deni variety to be grown in Plovdiv region. The longer growing season of a wheat variety means higher yields unless grain filling occurs in extreme drought.

At high temperatures and water deficiency, factors known to accelerate phenophase development, it takes several days less to undergo the respective phenophase (Garsia del Moral et al., 1991). The same author (Garsia del Moral et al., 1991) found that a longer period of grain formation had a positive effect on obtaining more grains.

Adverse factors have the greatest effect on plants through the phenophase of ear formation when the processes of gametogenesis are completing and the pollen and ova are formed. The data in Table 2 shows the duration of the

interphase periods expressed in number of days, the sum of active and average t°C for the

occurrence of the individual phases in 2015-2016.

Table 2. Duration, temperature sum and precipitation during interphase periods (2015-2016)

Year	Interphase periods	Number of days	$\sum t$ active, °C	t average, °C	Rainfall, mm/m ²
2015 - 2016	Sowing - sprouting	12	116.9	9.74	39.6
	Sprouting - 3 rd leaf	19	225.1	11.85	0.0
	3 rd leaf - tillering	113	688.7	5.65	145.0
	Tillering - shooting up	36	399.7	11.1	45.0
	Shooting up - ear formation	25	373.6	14.9	35.7
	Ear formation - ripening	57	1187.0	20.82	103.9

The sprouting of plants occurs in 12 days at the sum of active temperatures t°C - 116.9°C, and at an average temperature of 9.74°C with a rainfall for the period of 39.6 mm. The interphase period of “sprouting - 3rd leaf” runs for 19 days at a temperature sum of 225.1°C, an average temperature for the period 11.85°C, and quantity of rainfall of 0.0 mm. The longest is the duration of period “3rd leaf - tillering”, 113 days, with the sum of active temperatures 688.7°C. The average temperatures t°C for the period are 5.65°C and the rainfall quantity is 145.0 mm. The interphase period “tillering-shooting up” is 36 days. The sum of active temperatures is 399.7°C. The average temperatures are 11.1°C and the quantity of rainfall is 45.0 mm. The duration of the interphase period “shooting up - ear formation” is 25 days. It occurs at a sum of active temperatures of 373.6°C, and quantity of rainfall of 35.7 mm. The average temperature t°C for the period is 14.9°C. The period between the phenophases of “ear formation-

ripening” takes 57 days, at a sum of the active temperatures of 1187.0°C, and the average temperatures for the period and the rainfall quantity are 20.82°C and 103.9 mm, respectively. The vegetation period of the Deni variety wheat is 262 days.

In the autumn of 2016, the rainfall quantity of 19.7 mm in October was by 33.3 mm less than the average for the multiannual period (Table 2) and hindered the additional soil processing before sowing. The sowing of wheat took place on 21.10.2016. The rainfall quantity in November was less by 17.1 mm compared to the thirty-year period, but sufficient (Figure 1) for germination of seeds, and plants sprouted on 29.10.2016. In December, the rainfall quantity was only 2.4 mm, which is by 43.6 mm less than the multiannual period, and gradually with the decrease of temperatures during the second and third 10-day period of November, the growth processes in wheat also subsided. In early winter, wheat is in the third leaf phase and early tillering.

Table 3. Duration, temperature sum and precipitation during interphase periods (2016-2017)

Year	Interphase periods	Number of days	$\sum t_{active}, ^\circ\text{C}$	$t_{average}, ^\circ\text{C}$	Rainfall, mm/m ²
2016 - 2017	Sowing - sprouting	9	371.8	41.3	0.4
	Sprouting - 3 rd leaf	19	174.3	9.17	9.5
	3 rd leaf - tillering	113	238.3	2.11	113.6
	Tillering - shooting up	30	307.7	10.3	41.3
	Shooting up - ear formation	29	388.2	13.4	28.4
	Ear formation - ripening	59	1224.9	20.76	65.8

In January, precipitation (snow only) exceeded by 29.1 mm the multiannual norm, while in February it was less by 20.9 mm, and in March exceeded by 9.9 mm the multiannual norm. January was characterized by very low temperatures and their maintenance for a long time, the average temperature for January being -3.9°C compared to the norm of -0.4°C. The measured minimum temperature for this month was 18.40°C, the plants were in winter rest, and the presence of thick snow cover protected the plants from frost. During the third 10-day period of February, as the temperatures rose, the plants resumed their vegetation. Rainfall in

April was by 14.9 mm less than the norm, which had a retarding effect on the growth of durum wheat. The rainfall quantity in May of 52.7 mm was approximately equal to the norm, while in June - by 45.6 mm less than the norm, while the average monthly temperature in May and June was around the norm. The grain filling and ripening phases took place under less favourable conditions of rainfall. Wheat was harvested on 31.06.2017. The vegetation period of Deni variety durum wheat was 259 days for the harvest year of 2016-2017.

Table 4. Duration, temperature sum and precipitation during interphase periods (2017-2018)

Year	Interphase periods	Number of days	$\sum t_{active}, ^\circ\text{C}$	$t_{average}, ^\circ\text{C}$	Rainfall mm/m ²
2017 - 2018	Sowing - sprouting	12	105.3	8.775	9.8
	Sprouting - 3 rd leaf	19	169.1	8.9	16.6
	3 rd leaf - tillering	110	338.0	3.07	180.0
	Tillering-shooting up	29	277.4	9.565	56.8
	Shooting up - ear formation	30	529.2	17.64	18.6
	Ear formation-ripening	58	1223.6	21.10	221.4

In the autumn of 2017, the rainfall quantity of 70.4 mm in October was by 30.3 mm more than the average for the multiannual period (Figure 1). This made it possible to carry out quality processing and optimum sowing. The sowing of the wheat took place on 26.10.2017. The rainfall in November was 47.6 mm, which is around the climatic norm for this month. This

rainfall was sufficient for the optimal germination and sprouting of the seeds (Table 4). The plants sprouted on 06.11.2017. In December, precipitation was 23.7 mm, which is by 20.6 mm less than the norm of the multiannual period, but completely enough due to the accumulation of soil moisture in the previous months. Gradually, with the decrease

in temperatures, the wheat growing processes also subsided in January. In January, the amount of precipitation was 21.7 mm, which is less than the climatic norm for the period. However, in February the precipitation was 96.7 mm, which is 64 mm more than the norm for this month and in March by 7.3 mm more than the multiannual period norm. The month of January was characterized by high temperatures with warm days with around 10°C, and an average temperature for the month of 2.9°C (Figure 2). The plants were in winter rest, there was almost no snow. During the third 10-day period of February, as the temperature rose, the plants resumed their vegetation. Precipitation in April was by 20.2 mm below the norm. This did not affect the wheat, which was very-well developed during this period and was entering the shooting up phase. The amount of precipitation in May was a record for the period, 112.3 litres, which was

by 47 mm more than the norm for the period. Despite the heavy rainfall, no diseases were observed. Precipitation decreased gradually in June and was 14.4 mm, which is by 49 mm below the norm, while the average monthly temperature in May and June was slightly above the norm. The grain filling and ripening phases occurred under favourable conditions of rainfall. The wheat was harvested on 04.07.2018. In the third year of the experiment the duration of the vegetation period was 258 days.

The data presented in Table 5 show that, with respect to the tested factor of the mineral fertilizer, the best productivity results are obtained at the level of N₁₂₀P₈₀K₀ kg/ha. The grain yield of durum wheat at the lower fertilization level is less, however satisfactory. Durum wheat productivity decreases at the highest fertilization level which is N₁₈₀P₁₂₀K₆₀ kg/ha.

Table 5. Grain yield, t/ha

Fertilization rate	Cannopy density, seed/m ²	Years			Average (2015-2018)	
		2016	2017	2018	t/ha	%
		t/ha	t/ha	t/ha		
N ₀ P ₀ K ₀	400	2.95	4.13	3.50	3.53	100.0
	500	3.15	4.21	3.55	3.64	103.1
	600	3.23	4.27	3.62	3.71	105.1
N ₆₀ P ₄₀ K ₀	400	3.19	4.23	3.59	3.67	104.0
	500	3.28	4.31	3.67	3.75	106.2
	600	3.37	4.45	3.78	3.87	109.6
N ₁₂₀ P ₈₀ K ₀	400	3.31	4.39	3.70	3.80	107.6
	500	3.39	4.60	4.05	4.01	113.6
	600	3.34	4.48	3.85	3.89	110.2
N ₁₈₀ P ₁₂₀ K ₆₀	400	3.29	4.43	3.76	3.81	107.9
	500	3.20	4.35	3.63	3.73	105.7
	600	3.14	4.30	3.54	3.66	103.7

A B AXB A B AXB A B AXB
 GD 5% 22.3 34.1 50.8 27.1 39.3 48.1 25.2 42.6 61.7

With respect to the other factor tested, which is sowing rate, an increase in grain yield was observed at sowing with 600 germinating seeds/m², and mineral fertilization level of N₆₀P₄₀K₀ kg/ha, however at a sowing rate of 500 germinating seeds/m² and a fertilization level of N₁₂₀P₈₀K₀ kg/ha the highest yield increase was achieved. At a sowing rate of 400 germinating seeds/m², the highest fertilization rate of N₁₈₀P₁₂₀K₆₀ kg/ha is most appropriate.

The greatest increase of the yield of Deni variety durum wheat under the interaction of the tested factors is achieved with mineral fertilization level of N₁₂₀P₈₀K₀ kg/ha, and a sowing rate of 500 germinating seeds/m². The obtained greater quantity of yield in this variant is within the range from 80 kg/ha in 2016 to 350 kg/ha in 2018, or an average of 210 kg/ha (13.6%) over the three-year study period. Second comes, in terms of average figures, the variant treated with N₁₂₀P₈₀K₀ kg/ha, and a

sowing rate of 600 germinating seeds kg/ha, and on third place is the variant with fertilization N₁₈₀P₁₂₀K₆₀ kg/ha, and a sowing rate respectively of 400 germinating seeds/m², as grain yield increased on average, respectively by 360 kg/ha (10.2 %), 280 kg/ha (7.9 %).

The higher grain yield of the aforementioned variants can be explained by the optimal combination of mineral fertilization and sowing rate, which in turn led to an increase in the values of some of the structural elements of the crops (Table 6).

Table 6. Structural elements of the yield (average for 2015-2018)

Fertilization	Cannopy density, seed/m ²	Productivity tillering	Spikelets per spike, number	Grains per spike, number	Mass of the grains per spike, g
N ₀ P ₀ K ₀	400	1.5	18.6	38.2	1.85
	500	1.4	18.4	36.7	1.59
	600	1.2	17.7	34.3	1.44
N ₆₀ P ₄₀ K ₀	400	2.0	19.5	39.7	1.94
	500	1.8	19.2	37.3	1.88
	600	1.6	18.5	36.9	1.69
N ₁₂₀ P ₈₀ K ₀	400	2.3	20.2	40.9	2.04
	500	2.7	21.1	42.1	2.15
	600	2.4	18.7	37.8	1.53
N ₁₈₀ P ₁₂₀ K ₆₀	400	2.5	19.9	40.6	1.99
	500	2.1	19.6	40.1	1.71
	600	1.7	18.9	37.2	1.50

The highest are the values of the structural elements of the yield, as follows: productive tillering capacity - 2.7 tillers, number of spikelets in an ear - 21.1 pcs, number of grains in an ear - 42.1 pcs, and weight of grains in an ear - 2.15 g of Deni variety durum wheat are reported for the variant with mineral fertilization N₁₂₀P₈₀K₀ kg/ha and a sowing rate of 500 germinating seeds/m².

Increasing the sowing rate over 500 germinating seeds/m² and fertilizing with N₁₈₀P₁₂₀K₆₀ kg/ha does not lead to an increase in grain yield due to the excessive density of the crops and the high level of fertilization, which results in the plants lodging.

CONCLUSIONS

The highest grain yield of Deni variety durum wheat was obtained at the mineral fertilization level of N₁₂₀P₈₀K₀ kg/ha and the sowing rate of 500 germinating seeds/m², from 3.39 t/ha to 4.60 t/ha, or averagely for the three years - 4.01 t/ha, which is by 13.6% more than the control variant.

Under this level of fertilization and sowing rate, the highest values of the structural elements of the yield were obtained -

productive tillering capacity - 2.7 pcs; number of spikelets in an ear - 21.1 pcs; number of grains in an ear - 42.1 pcs, and weight of grains in an ear - 2.15 g.

The growing period of Deni variety wheat durum wheat varies from 258 days to 262 days.

ACKNOWLEDGEMENTS

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REFERENCES

- Bobrecka-Jamro, D., Kruczek, G., Romaniak, M., Jarecki, W., Buczek J. (2013). Effect of the dose and method of top-dressing with nitrogen on the yield and quality of winter wheat grain. *Acta Sci. Pol., Agricultura*, 12(4), 19–30.
- Garcia del Moral, M., Tejada, J., Garcia del Moral, L., Molina-Cano, J. (1991). Apex and ear development in relation to the number of grains on the main-stem ears in spring barley (*Hordeum distichon*). *Journal of Agricultural Science*, 117(01), 39–45.
- Kiliç, H., Yağbasanlar, T. (2010). The Effect of Drought Stress on Grain Yield, Yield Components and some Quality Traits of Durum Wheat (*Triticum turgidum*

- ssp. *durum*) Cultivars. *Not. Bot. Hort. Agrobot. Cluj*, 38(1), 164–170.
- Kostadinova S., Ganusheva, N. (2012). Macronutrients content of new malting barley genotypes *Field Crops Studies*, 8(1), 11-14.
- Lloveras, J., Manent, J., Viudas, J., Lopez, A., Santiveri, P. (2004). Seeding Rate Influence on Yield and Yield Components of Irrigated Winter Wheat in a Mediterranean Climate. *Agronomy Journal*, 96, 1258–1265.
- Ozturk, A., Caglar, O., Bulut, S. (2006). Growth and Yield Response of Facultative Wheat to Winter Sowing, Freezing Sowing and Spring Sowing at Different Seeding Rates. *Journal Agronomy & Crop Science*, 192, 10–16.
- Popova, R., Sevov, A. (2010). Soil characteristic of experimental field of Crop Production Department as a result of the cultivation of grain, technical and forage crops. Agricultural University of Plovdiv. *Scientific Works, LV(I)*, 151–156.
- Semkova N., Saldzhiev, I. (2014). Influence of nitrogen fertilization over leafarea indicator and photosynthetic potential of durum wheat varieties. *Agro technical research for the sustainable production of arable crops*, 219–225.
- Yanev, Sh., D. Dechev, Ts. Lalev, I. Saldzhiev, G. Panayotova, G. Delchev, T. Kolev and S. Rashev. (2008). Technology for growing Durum wheat. *Publishing Temko*, Stara Zagora.

AGROECOLOGICAL EVALUATION OF APPLICATION THE MICROBIOLOGICAL FERTILIZERS IN LENTIL CULTIVATION TECHNOLOGY

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Abstract

In a stationary field experiment on leached chernozem and in laboratory studies, an agroecological assessment of the influence of the microbiological fertilizers Azotovit and Phosphatovit on the productivity of lentil plants was carried out. It was established that inoculation of seeds with microbiological fertilizers at a dose of 2 liters per ton of seeds, in the technology of growing the crop in the soil and climatic conditions of the forest steppe of the Middle Volga region, provides intensive growth and development of plants at the initial stages of ontogenesis, which leads to accelerated formation of cotyledons and true leaves and activation bean-rhizobial apparatus. The resistance of lentil plants to adverse soil and climatic conditions increases (germination energy increases by 2.3%, laboratory germination - by 2.0%, plant safety for harvesting - by 9.6%). Lentil grain productivity from seed inoculation increased by 26.3%, protein collection - by 37.2%. The data obtained make it possible to rationally use the method of t with microbiological fertilizers in lentil cultivation technology.

Key words: lentil plants, microbiological fertilizers Azotovit and Phosphatovit, pre-sowing seed treatment, lentil cultivation technology.

INTRODUCTION

The development of new techniques in the technology of cultivation of lentil plants largely determines the possibility of obtaining a high and sustainable crop of this valuable legume crop (Antipova et al., 2010). Of particular interest in this light is the increase in the agricultural background in the implementation of a range of agrotechnical and organizational measures, the development of a science-based zonal farming system (Gursoy, 2010; Muehlbauer et al., 2006).

An important factor in increasing the yield of lentils is the improvement of mineral nutrition and the activation of legume-rhizobial symbiosis from the use of biological bacterial fertilizers. The interaction of factors and the identification of their mechanisms plays an important role in increasing the productivity of crops, obtaining ecologically clean products and increasing soil fertility (Koryagina et al., 2017; Kulikova et al., 2015; Chebotar et al., 2015; Tsyganova et al., 2013).

It is important to use new microbiological fertilizers that increase the biological activity of

the soil, improve nitrogen fixation, protect plants from diseases and pests with increased immunity and stress resistance of plants, and also stimulate plant growth.

The positive effects of biological products on crop yield and plant quality have been reported in many studies (Koryagin et al., 2018; Koryagin, 2014). However, work on the physiological evaluation of the stimulation effect during seed treatment with microbiological fertilizers (in the Middle Volga region, Russia) was carried out either at the initial stages of vegetation, or the quality of the crop was analyzed.

Since lentils in our area are a promising leguminous crop and have high plasticity to the effects of external environmental factors, studies of physiological changes affecting its productivity and new microbiological preparations are of great interest (Zavarzin, 2012).

In this regard, the article presents material on the agroecological assessment of the use of new microbiological fertilizers "Azotovit" and "Phosphatovit" in the technology of cultivating lentils in the soil-climatic conditions of the forest-steppe of the Middle Volga (Russia).

MATERIALS AND METHODS

Microbiological fertilizers “Azotovit” and “Phosphatovit” (manufactured by the company “Industrial Innovations”) have State Registration (certificate of State Registration No. 1085 and No. 1086 issued by the Federal Service for Veterinary and Phyto-Supervision). The active substance of the drug “Azotovit”: live bacterial cells (*Beijerinckia fluminensis*), a concentration of at least 1×10^9 CFU/cm³. Bacteria fix molecular nitrogen and, during a series of transformations, convert it to ammonium, nitrite, and nitrate forms, which are easily absorbed by plants; prevent the loss of mineral nitrogen during its conversion in the soil.

The active substance of the drug “Phosphatovit”: spores and living cells of bacteria *Paenibacillus mucilaginosus*, the concentration of not less than 0.12×10^9 CFU/cm³. Organic acids secreted by the bacteria *Paenibacillus mucilaginosus* mobilize inaccessible phosphorus (20 to 30 kg/ha per season) and potassium (15 to 20 kg/ha per season) from insoluble compounds in the plant rhizosphere; interfere with the processes of the enrichment of soils with assimilable phosphates.

Beijerinckia fluminensis and *Paenibacillus mucilaginosus* are not genetically modified strains, belong to microorganisms that are non-pathogenic for humans, do not require special precautions during operation.

The influence of the “Azotovit” and “Phosphatovit” microbiological fertilizers was studied on lentils of the CDC Redcliff variety (The origin of the variety is Crop Development Center, University of Saskatchewan, Canada).

The studies were carried out in a stationary field experiment on leached chernozem and in laboratory studies, accompanied by observations, counts and analyzes in accordance with the methodology and technique of setting up field experiments in the soil and climatic conditions of the Penza region (Kulikova et al., 2019).

The experience was laid on plots with a total area of 15,000 m², the accounting area was 10,000 m², triplicate three times, the placement of options randomized (random).

Scheme of experience:

1. Inoculation of seeds with water (control);
2. Inoculation of seeds “Azotovit”;
3. Inoculation of seeds “Phosphatovit”;
4. Inoculation of seeds “Azotovit” + “Phosphatovit”.

The seeds of lentil plants were treated at a dose of 2 liters per ton before sowing with microbiological fertilizers.

Meteorological conditions for the entire period of research (2016-2018) were generally favorable for the growth and development of lentils. The average air temperature was in the range of long-term average values. The highest rainfall during the growing season (239 mm) was noted in 2016. Compared with long-term average values, the deviation of the indicator was + 24 mm.

In 2017, the amount of rainfall during the growing season was 175 mm, which is 40 mm below the long-term average.

In 2018, the amount of precipitation during the growing season was 137.3 mm, which is 18.7 mm lower than the annual average.

RESULTS AND DISCUSSIONS

The quality of the sowing material of grains and leguminous crops largely depends on weather conditions during the growing season of plants and seed ripening. It is known that timely, friendly and full-fledged seedlings of optimal density are one of the main factors for obtaining high and stable yields with good quality products. In the practice of seed production, to obtain high-quality seeds, various techniques are recommended: maintaining the harvested crops in sheaves, ensuring their drying and ripening due to the influx of additional substances from the vegetative organs into the reproductive ones; pre sowing treatment with various growth substances and organo-mineral mixtures. Of great importance is the treatment of seeds before sowing with solutions of salts of trace elements, especially when seeds are obtained when grown under conditions of a deficiency of certain trace elements in the soils.

The initial changes that occur in seeds after treatment lead to processes associated with the intensity and direction of metabolism in the

early stages of plant development during the period of its greatest plasticity and susceptibility, could have a decisive influence on the passage of the further stage of development of the adult body. Inoculation of lentil seeds with “Azotovit” and “Phosphatovit” microbiological fertilizers increased the seed germination energy by an

average of 2.3% and laboratory germination of lentil seeds by 1.3-2.0% compared with inoculation of seeds with water, which ultimately determined the field germination of seeds, which increased by 3.4-6.3%. The combined use of drugs was most effective (Table 1).

Table 1. Characterization of the growth processes of CDC Redcliff lentil plants in laboratory and field conditions (2016-2018)

Experience option	Germination, %		Density of plants, thousand units/ha		Vegetation period, days	Crop thinning %
	laboratory	field	in the germination phase	before harvesting		
Inoculation of seeds with water (control)	92.9	85.5	1711.0	1295.9	84	24.4
Inoculation of seeds “Azotovit”	94.4	89.2	1785.0	1464.3	82	18.0
Inoculation of seeds “Phosphatovit”	94.2	88.9	1778.0	1432.9	83	19.4
Inoculation of seeds “Azotovit” + “Phosphatovit”	94.9	91.8	1836.0	1564.4	81	14.8

Our long-term experiments have found that changes in some aspects of the metabolism and increased growth processes ultimately affect yield, as it is an integral indicator of all physiological and biochemical processes during the individual development of lentil plants.

In our research, inoculation of lentil seeds with microbiological fertilizers did not have a significant effect on the duration of interphase periods; the growing season was 81-84 days according to the experimental variants. Observations of the growth of lentil plants showed that the growth of the vegetative mass went most intensively from the seedling phase to the onset of seed maturation of lentil plants.

The full germination phase began 6-11 days after sowing the seeds, the number of plants per 1 ha in varieties ranged from 1711.0 to 1836.0 thousand, and before harvesting, from 1295.9 to 1564.4 thousand per hectare.

The sowing thinness varied during the years of research from 14.8% to 24.4%: the greatest was observed in the variant with the treatment of seeds of lentil plants before sowing with water (control), the smallest - in the variant with joint treatment of seeds with drugs.

Accordingly, in the control variant, the lentil grain yield was lower than in the variant with the pre-sowing treatment of lentil seeds with the “Azotovit” microbiological fertilizer together with “Phosphatovit”.

Studies have shown that the highest grain yield of plants was obtained with the combined use of microbiological fertilizers “Azotovit” and “Phosphatovit”, where it was 1.73 t/ha, which is 26.3% higher than in the control variant. Separate use of the preparations ensured a yield increase of 21.2 and 18.2% to the control, respectively (Figure 1).

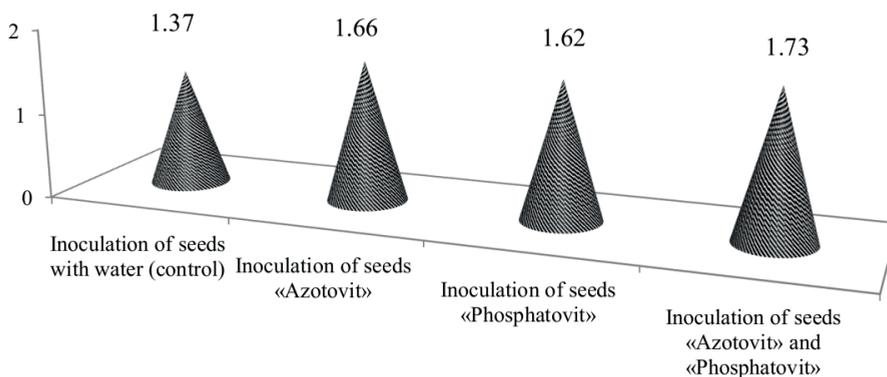


Figure 1. The effect of microbiological fertilizers on the yield of lentil grain (2016-2018), t/ha

When using the microbiological fertilizer “Azotovit” together with “Phosphatovit”, a synergistic effect is manifested, that is, an increase in their interaction.

According to the data obtained in the course of scientific research over the years, it can be stated that the reaction of plants to microbiological fertilizers “Azotovit” and “Phosphatovit” is more dependent on the growing conditions of the plant, since they determine the nature of metabolic processes, and the action of microorganisms only enhances or weakens the pace of these processes.

Therefore, our studies indicate the effectiveness and feasibility of using pre-sowing seed treatment, both in terms of synergy and interaction coefficient. Presowing seed treatment with the “Azotovit” microbiological fertilizer together with “Phosphatovit”, as an agrotechnical technology, fits organizationally easily into the technology of growing lentil plants.

Seed productivity of leguminous crops depends primarily on the number of beans in the plant, the seeds in the bean and the mass of 1000 seeds. Structural and morphological analysis of the crop of lentil plants showed that microbiological fertilizers positively affect all elements of the yield structure. The combined

use of the “Azotovit” and “Phosphatovit” microbiological fertilizers increases the plant height by 11.8 cm, the attachment height of the lower beans by 11.3 cm, the weight of 1000 seeds by 2.7 g and the nature of lentil grain by 7 g/l.

The following physiological features are inherent in high-protein nature: the ability of the plant's root system to absorb nitrogen from the soil throughout the entire individual development; high nitrate reduction potential; high photosynthetic activity; more complete outflow of nitrogenous compounds from vegetative organs into seeds.

These processes are activated under the influence of the drugs used, due to which protein accumulates.

The use of microbiological fertilizers in the processing of lentil seeds before sowing leads to an increase in protein by 1.7-2.4% compared with the control variant.

This increase in the protein content of lentil grains leads to an increase in protein collection from the hectare of crops of lentil plants by 25.6-37.2% (Table 2).

With the combined use of the “Azotovit” and “Phosphatovit” preparations, the maximum effect is achieved: the protein content increases by 2.4%, protein collection - by 37.2% of the control.

Table 2. The effect of microbiological fertilizers on the content and collection of protein in lentil grain (2016-2018)

Experience option	The protein content, %	Deviation from the control, %	Protein harvest, kg/ha	Deviation from the control	
				kg/ha	%
Inoculation of seeds with water (control)	27.1	–	369.5	–	–
Inoculation of seeds “Azotovit”	29.2	2.1	481.8	112.3	30.4
Inoculation of seeds “Phosphatovit”	28.8	1.7	464.1	94.6	25.6
Inoculation of seeds “Azotovit” + “Phosphatovit”	29.5	2.4	506.9	137.4	37.2

CONCLUSIONS

Lentil production cannot occur only due to the expansion of sown areas, but should be carried out as a result of intensification of production and the search for new ways to further increase the yield of leguminous crops.

Among the promising agricultural practices that provide a further increase in yield and improve product quality should include the method of pre-sowing treatment of seeds before sowing with microbiological fertilizers.

Inoculation of seeds with “Azotovit” and “Phosphatovit” at a dose of 2 liters per ton of seeds, in the technology of growing the crop in the soil and climatic conditions of the forest steppe of the Middle Volga region (Russia), provides intensive growth and development of plants at the initial stages of ontogenesis, which leads to accelerated formation of cotyledons and true leaves and activation of the legume-rhizobial apparatus.

Thus, pre-sowing treatment of lentil seeds with microbiological fertilizers, as an agrotechnical technology, fits easily into the technology of cultivating lentils and has a positive effect on the germination and preservation of lentil plants before harvesting, which leads to increased yield and increased protein collection.

REFERENCES

- Antipova, L.V., Kurchaeva, E.E., Manzhosov, V.I. (2010). Lentils: prospects for use in food technology: *a monograph*. Voronezh, FGOU VPO Voronezh State Agricultural University.
- Zavarzin, G.A. (2012). Azotovit and Fosfatami. Take mineral nutrition from nature. *Moscow, Industrial Innovations*.
- Koryagina, N.V., Koryagin, Yu.V., Efremova, S.Yu., Koryagina, E.Yu. (2017). Assessment of the use of environmentally safe biological bacterial preparations in resource-saving agriculture. *XXI century: results of the past and problems of the present*, 05(39)/06(40), 49–56.
- Koryagin, Yu.V., Koryagina, N.V. (2018). Influence of microbiological fertilizers on productivity and sowing qualities of winter wheat seeds. *The Volga Region Farmland*, 4(49), 71–78.
- Koryagin, Yu.V. (2014). Influence of application of biological preparations and microelements on sowing qualities of spring wheat seeds. *Achievements of Science and Technology in Agriculture*, 10, 29–30.
- Kulikova, E.G., Velikanova, G.S. (2015). Preservation of soil fertility in the Penza region as a basis for food security in Russia. *Food Policy and Security*, 2(2), 77–86.
- Kulikova, E.G., Koryagin, Yu.V., Koryagina, N.V. (2019). Plant physiology and biochemistry. *Penza, Russia, RIO PGAU*.
- Tsyganova, E.N., Zvyagintsev, D.G., Lysak, L.V., Stepanov, A.L. (2013). Effect of bacterial-humus preparation on the biological activity of soils. *Soil Science*, 46(7), 867–871.
- Chebota, V.K., Laktionov, Yu.V., Yakhno, V.V. (2015). Microbiological preparations in the system of ecological agriculture. *Regional Ecology*, 6(41), 41–47.
- Gursoy, S. (2010). Increasing the productivity of red lentil in the South East Anatolia region of Turkey. In: *Legumes for Global Health. Legume Crops and Products for Food, Feed and Environmental Benefit: 5th International Food Legumes Research Conf. (IFLRC V) & 7th European Conf. on Grain Legumes (AEP VII)* Antalya, Turkey, 300.
- Muehlbauer, F.J., Cho, S., Sarker, A. (2006). Application of biotechnology in breeding lentil for resistance to biotic and abiotic stress. *Euphytica*, 147, 149–165.

THE PRODUCTION TENDENCIES OF MAIN GRAIN CROPS IN BULGARIA UNDER CONTEMPORARY AGROMETEOROLOGICAL CONDITIONS AND CLIMATE CHANGES

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Abstract

Global and regional climate change is disturbing all sectors of the national economy. The most affected is agriculture, which takes place under open sky. Bulgaria is located in area with insufficient atmospheric and soil moisture. Climate change cause changes in agro-meteorological conditions and agro-climatic resources, depending on the geographical location. All agro technical activities - sowing, soil cultivation, fertilization, plant protection and harvesting, are highly dependent on weather conditions. The crops development, quantity and quality of production are determined to a great extent by the weather conditions, in particular, by those which have an adverse effect on plants and production processes such as drought, torrential rain and hail. Agricultural production is connected with many risks. Therefore, solving problems in this production area must be linked to agro-climatic and agro-meteorological research to improve agro-technologies with aim to mitigate the effects of climate change and adapt the agriculture sector to these changes. The purpose of this study is to define trends in production and average yields of basic agricultural crops in Bulgaria over the last two decades in climate change and different weather conditions.

Key words: climate change, planning region Bulgaria, production, yields, trends.

INTRODUCTION

Bulgaria is a country with great opportunities and traditions in agricultural production. However, in climatic terms, it falls into the zone of unstable humidity (Kouzмова, 2003). The average annual precipitations in Bulgaria are 550-600 mm, which, however, there are extremely unevenly distributed, and during the critical period for agricultural crops, they are extremely insufficient.

Another unfavourable moment is related to climate change in the direction of warming and drought, which have led to changes in the agroclimatic resources of a large part of the country's agricultural territories. This leads to fluctuations in the yields by years, their decrease and sometimes their complete compromise. Indicative of this was the drought in Bulgaria in 2000 (Kouzмова, 2001; Bogoev and Kouzмова, 2002).

The level of average yields is extremely important for the final economic results. It is

noteworthy that the yields from some agricultural crops are significantly lower than biologically possible. The reasons for this are complex and related to both technology non-compliance and global warming and climate change.

The purpose of this study is to analyse the area under cultivation, production and yields of main agricultural crops in Bulgaria over the last two decades and to define trends against the background of contemporary agrometeorological conditions and climate change.

MATERIALS AND METHODS

The period analysed in this study was 2001-2018. The data collected by Ministry of Agriculture, Food and Forestry on areas under cultivation (ha), production (tons) and yields (kg/ha) from 6 main crops for the country (wheat, barley, grain maize, sunflower, rapeseed, alfalfa) by years and by planning regions.

The regions used in the study are in accordance with the European Union Regulation on the general classification of territorial units for statistical purposes (NUTS) (Mihailov and Kouzмова, 2019): Northeast (NE), Northcentral (NC), Northwest (NW), Southeast (SE), Southcentral (SC) and Southwest (SW). Meteorological information from Synoptic station-Plovdiv has been collected over the last three decades (1990-2018) for one of the most intensive agricultural regions in Bulgaria Plovdiv. The main factors of the climate are the air temperature and sum of precipitations, based on which the main agro-climatic indicators by months, years and average for 29-years period are determined: average monthly air temperature, absolute maximum and absolute minimum air temperatures, monthly and seasonal sum of precipitations. For comparison, the climate norms published in the Climate Reference Book of Bulgaria, Volumes 3 and 6 are used.

The data were processed on Excel using the approved methods in agroclimatology: statistical methods, correlation and regression analysis (Gulinova, 1974).

RESULTS AND DISCUSSIONS

In recent decades, more and more often we hear about record high temperatures, record floods and droughts, storms and a record number of tornadoes. Northern Europe is already wetter and Southern Europe is drier than in previous centuries. The world's largest insurance companies have paid a lot of money in recent decades for damage caused by natural disasters. Even more troubling are the forecasts of scientists who say that these weather anomalies will become more intensive, causing disease and death to the population. Heat waves and flooding will become a normal climate event, not an exception. More and more people will have to leave their homes and emigrate, pressured by the natural disasters.

Climate change and the related impact on the lives and human health is a global problem that national and international organizations are fully aware and try to find a solution.

In Bulgaria, the problem of water scarcity is becoming more tangible. Bulgaria is relatively poor in surface water resources. According to

WWF data from December 7, 2018, they make up only 0.3% of the country's territory, which ranks it among the countries with the least resource in the world. However, Bulgaria falls in latitudes that are sensitive to climate change (WWF-Rivers in Bulgaria, 2018).

The quality and quantity of crop production are to a large extent conditioned by unfavorable meteorological conditions such as drought, heavy precipitations, hailstorm, etc.

Agriculture is one of the most vulnerable sectors of global warming and climate change. Any agro-technical activity, such as sowing, soil cultivation, harvesting and, ultimately, the yield obtained, are highly dependent on the conditions in which they are carried out. In turn, the development of agricultural crops is also dependent on these conditions.

The world economic losses and human life due to extreme weather events have increased many times over. In the last 30 years, weather conditions in Bulgaria have also been a prerequisite for natural disasters, both in terms of extreme temperatures and heavy precipitations.

Climate change in Bulgaria and what are the parameters of these changes are always topical issues, but at certainly, usually when there is a large deviation from the normal weather conditions, they emerge with renewed vigor. Over the last three decades, both in the atmospheric circulation and in the air temperature, there have been significant differences from the period up to the 1970s. In a significant part of research on climate change focuses on changes in temperature and precipitation. There is also a tendency for heavy rainfall to increase, even when precipitation total generally decrease (Spiridonov and Balabanova, 2017).

The results of the last three decades in Plovdiv show that global warming is a fact. It's most noticeable mainly in winter (January and February), early in the spring (March), and in the summer (June, July and August), when the deviation of air temperatures from the climatic norms is above 1°C (Figure 1). About normal are average monthly temperatures only in November, December and April. The average annual air temperature over the last three decades has been about 1°C above the normal (0.9°C). The whole period since 1990 till now, the average annual air temperatures in Plovdiv have been higher than normal, except for 1993,

which turned out to be the coldest year and 2008, when the average annual temperature was around the normal (according by 0.4°C and 0.1°C below the normal). The warmest year turned out to be 2000, with an average annual temperature of 1.5°C above the normal, and absolute maximum of 44°C was registered in July 2000. The temperatures during the summer of 2007 were also high with an absolute maximum of 42.6°C.

However, minimum air temperatures increased significantly faster than the maximum throughout the all year (Figure 2). The lowest temperature was recorded in the coldest year of 1993, when an absolute minimum temperature of -27.5°C was recorded in January. However, the absolute record for Plovdiv since 1942 has not been exceeded (-31.5°C).

A different trend is observed for rainfall, which gradually increases but fluctuates greatly by years (Figures 3 and 4). The driest year was 2000, which is the warmest year for the entire 30 years period, with only 251.6 mm precipitations (47% of normal), followed by 1993, which, in addition to being the most the coldest year turned out to be one of the driest years (321.1 mm or 59% of normal). Most precipitation fell in 2007. 842.4 mm (156% of normal), however, these are not evenly distributed throughout the year. There is an increase in the spring-summer precipitations, at the expense of the winters, which are decreasing (Figure 4). This feature shows that increases torrential summer precipitations, which also damage agricultural crops, create a risk of flooding, as we had in Plovdiv also in June 2019 (196.7 mm, which is 322% of the normal for the month).

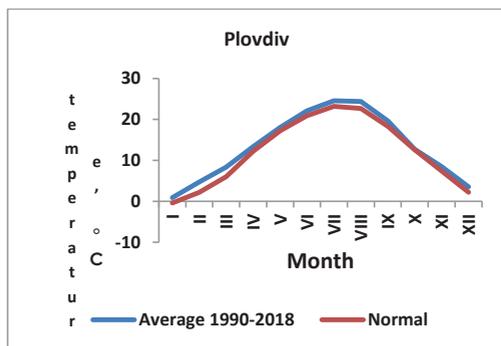


Figure 1. Changes in mean monthly air temperatures in Plovdiv

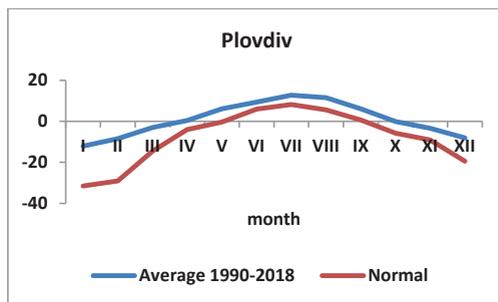


Figure 2. Changes in average of monthly absolute minimum temperatures in Plovdiv

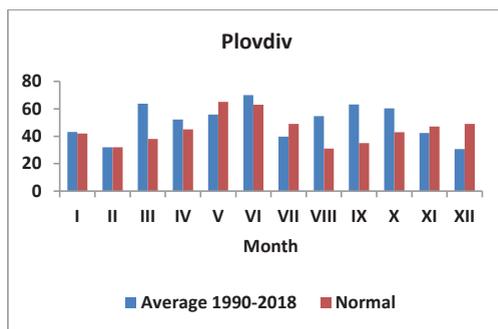


Figure 3. Changes in monthly sum of precipitations in Plovdiv

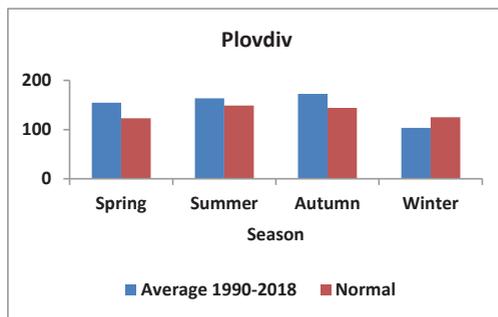


Figure 4. Changes in seasonal sum of precipitations in Plovdiv

Grain and oilseed technical crops have historical and strategic importance for the food needs of the population, the enlargement of national economy and overseas trade of our country (Ivanov et al., 2013).

Production of main cereals and oilseeds crops depends on the area under cultivation and yields. The analysis of the area under cultivation with main crops in Bulgaria shows that the highest positive trend is observed in sunflower and rapeseed (Figures 5a and 5b),

while in barley there is a significant negative trend (Figure 5c). Wheat and grain maize do not show an increase in areas under cultivation in the long term period, but rather stabilization. There is no mathematically proven variation in areas under cultivation of wheat and grain maize. In alfalfa, there is a significant decrease until 2010, after which a sharp increase (positive trend) is observed (Figure 5d).

Unlike the area under cultivation, the trend in yield is positive in all agricultural crops (Figure 6 a, b, c, d, e, f). With the exception of alfalfa, extremely close correlation and regression dependencies were found.

The increase in yield has a direct impact on the total production of these crops (Figure 7 a, b, c, d, e).

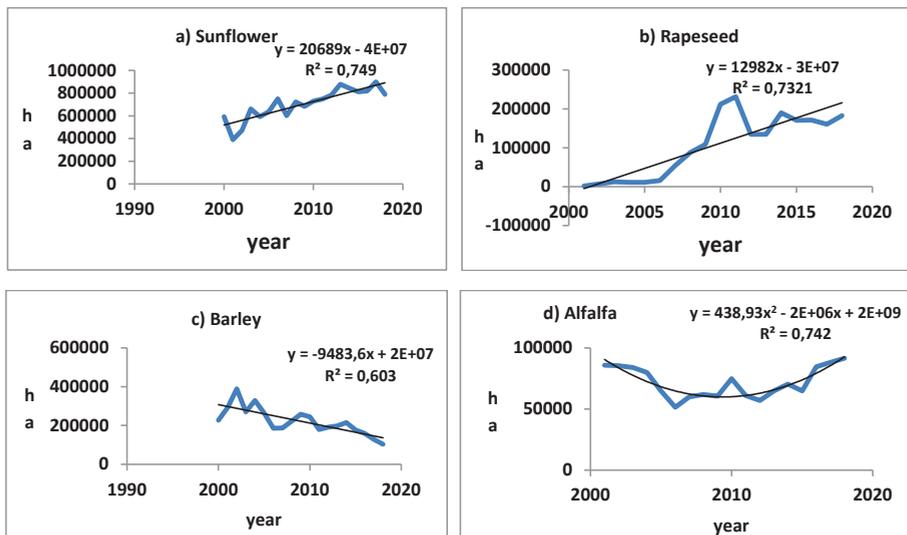
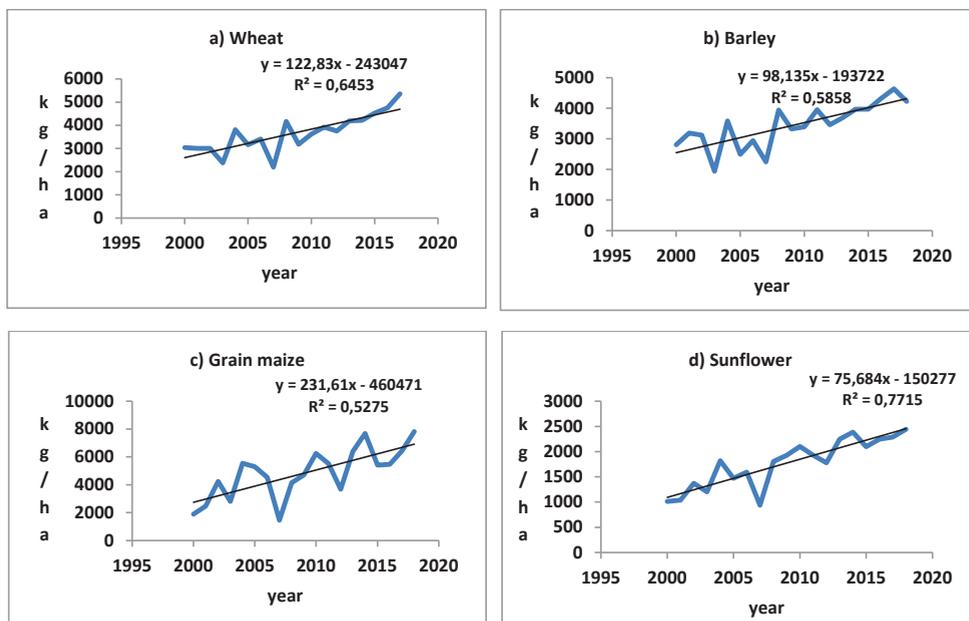


Figure 5. Trends in area under cultivation (ha) of main crops for the period 2001-2018:

a) sunflower; b) rapeseed; c) barley; d) alfalfa

Source: Data by Ministry of Agriculture, Food and Forestry



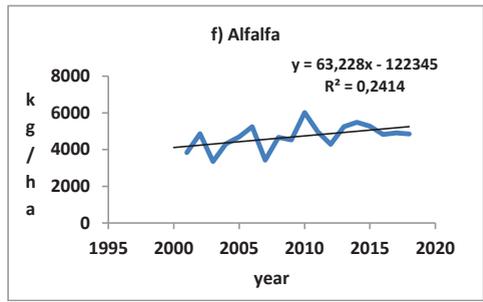
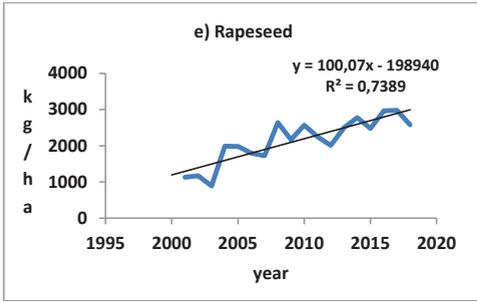


Figure 6. Trends in crop yields (kg/ha) for the period 2001-2018:
a) wheat; b) barley; c) grain maize; d) sunflower e) rapeseed; f) alfalfa
Source: Data by Ministry of Agriculture, Food and Forestry

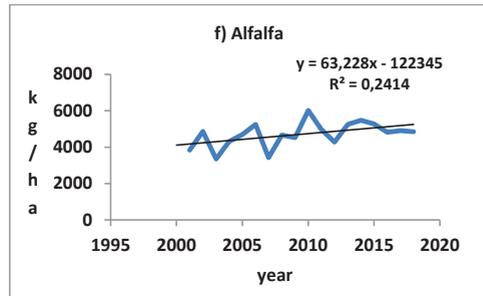
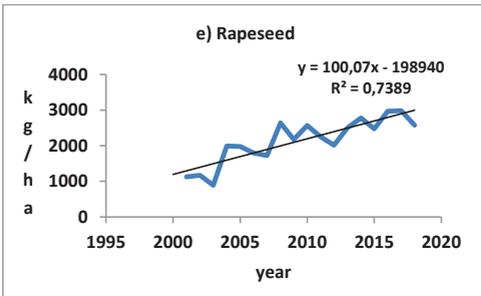
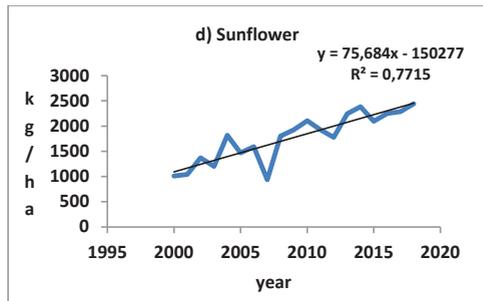
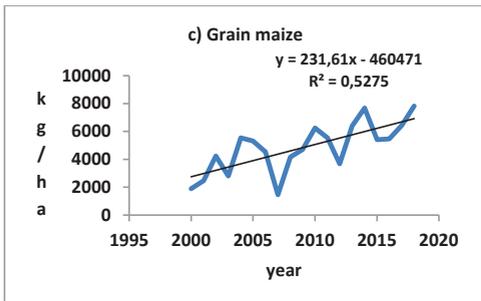
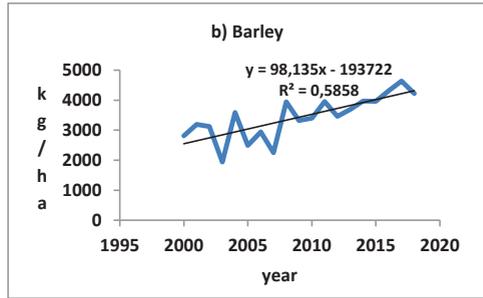
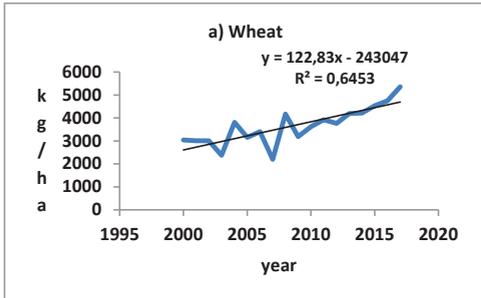


Figure 6. Trends in crop yields (kg/ha) for the period 2001-2018:
a) wheat; b) barley; c) grain maize; d) sunflower e) rapeseed; f) alfalfa
Source: Data by Ministry of Agriculture, Food and Forestry

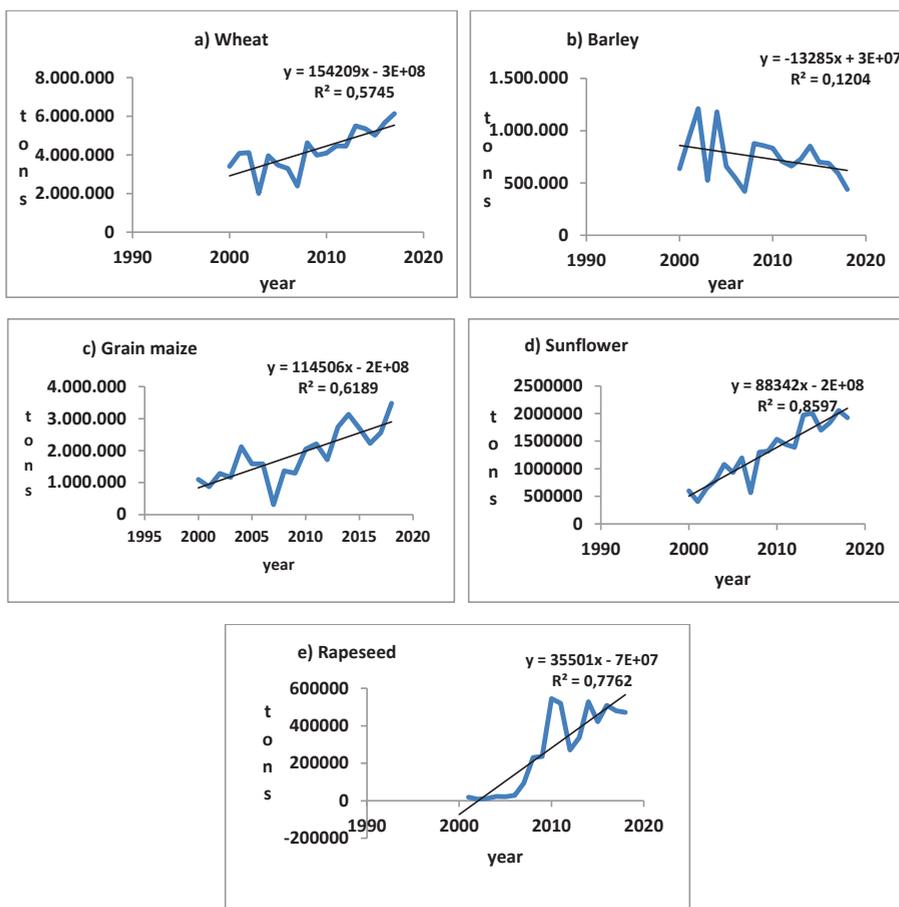


Figure 7. Trends in production (tons) of main crops for the period 2001-2018:

a) wheat; b) barley; c) grain maize; d) sunflower e) rapeseed

Source: Data by Ministry of Agriculture, Food and Forestry

There is no proven correlation in alfalfa. Against the background of the general trends, differences in planning regions according to NUTS have been found. Tables 1, 2 and 3

presented average data for 18 years period for areas under cultivation (Table 1), production (Table 2) and yields (Table 3) by planning regions.

Table 1 Average area under cultivation of main crops in Bulgaria by planning regions (2001-2018)

Planning regions	Area under cultivation, ha					
	wheat	barley	grain maize	sunflower	rapeseed	alfalfa
NW	263663	29461	102030	148040	26789	7029
NC	307362	50001	98016	162149	23548	16030
NE	393283	45475	143642	190411	29292	20137
SE	258924	61160	8392	115113	23149	9451
SW	54466	6940	8364	18939	1539	3300
SC	168872	28873	11824	76592	7177	14954
Total	1446570	221911	372267	711243	111493	70902

Source: Data by Ministry of Agriculture, Food and Forestry

Table 2. Average production of main crops in Bulgaria by planning regions (2001-2018)

Planning regions	Production, tons					
	wheat	barley	grain maize	sunflower	rapeseed	alfalfa
NW	762260	107730	559407	312106	68864	33681
NC	955946	175465	514636	328499	65202	78187
NE	1264731	163501	714297	373308	75875	92424
SE	718073	200435	35865	189387	50297	43406
SW	129804	16604	33313	27498	3318	15895
SC	440492	81406	53848	108227	14884	73891
Total	4271306	745140	1911365	1339024	278439	337483

Source: Data by Ministry of Agriculture, Food and Forestry

Table 3. Average yield of main crops in Bulgaria by planning regions (2001-2018)

Planning regions	Yield, kg/ha					
	wheat	barley	grain maize	sunflower	rapeseed	alfalfa
NW	3603	3520	4847	1871	2243	4648
NC	4001	3733	5058	1997	2358	4925
NE	4153	3798	5069	1956	2327	4756
SE	3390	3323	4379	1531	1883	4598
SW	2678	2378	3944	1333	1803	4904
SC	2997	2885	4649	1408	1866	4980
Total	3686	3466	4994	1815	2205	4763

Source: Data by Ministry of Agriculture, Food and Forestry

The highest yields of wheat and barley were obtained in the NE region and the lowest in the SW region. The highest yields of grain maize, sunflower and rapeseed were obtained in the NE and NC planning regions, and the lowest in the SW region. The highest yields of alfalfa were obtained in the SC and NC planning regions, and the lowest in the SE region.

The largest production of all crops, except barley, is concentrated in the NE region and the smallest in the SW region. The largest production of barley is concentrated in the SE region.

In barley, the highest yields on average in the country and by planning region were in 2017, with the exception of the NE region, where the highest yields were in 2016 and 2018. The lowest yields on average in the country and in most of the planning regions were obtained in 2007. An exception to this trend is SE and SC, where the minimum is in 2003.

The highest yields for grain maize average in the country and by planning region were established in 2018 and 2014. The lowest yields on average in the country and in the regions of Northern Bulgaria with continental climate (NE, NC, NW) were obtained in 2007, while in the regions of Southern Bulgaria (SE, SC, SW) - in 2012.

The highest yields of sunflower average in the Bulgaria and by planning regions were established in 2018, with the exception of NC, where the maximum is 2013. The lowest average yields for the country and by planning regions in 2007. Only in the SE area the minimum yield is in 2001.

The highest rapeseed yields on average in the country and in most of the planning regions were obtained in 2016 and 2017. In the SW region the maximum is in 2012 and 2014 and in the SC region in 2010. The lowest yields in the country and in most of the planning regions were obtained in 2003. In the SW region the maximum was in 2002, in the SC region in 2006.

The highest alfalfa yields on average in the country and in SE, NW and NC were obtained in 2010, in NE - 2014, in SC - 2002, in SW - 2008. The lowest yields on average in the country and in SE and SC were obtained in 2003, in other planning regions they were in 2007.

CONCLUSIONS

The nature of climate change has been identified and it has been proven that warming in Plovdiv is a fact. It is most sensitive in the

winter months, early in the spring (March) and in the three summer months (June, July and August). However, the minimum air temperatures rise much faster than the maximum.

A positive trend in yields over the last two decades in all main agricultural crops in Bulgaria, with the exception of rapeseed where there is no statistically proven addition.

There is a trend to increase the production of main cereals wheat, grain maize and sunflower as well as rapeseed, but the most rapidly growing production of sunflower and rapeseed.

The greatest production of all agricultural crops (wheat, grain maize, sunflower, rapeseed, and alfalfa) without barley is concentrated in the Northeast (NE) region, and at least in the Southwest region (SW). Barley production is concentrated in the Southeast region (SE).

The highest yields of all crops, excluding alfalfa, are in the Northeast (NE) and Northcentral (NC) regions, while alfalfa yields - in the Central part of Bulgaria: Southcentral (SC) and Northcentral (NC) regions.

It is necessary to continue research by looking link between planning regions and climate change.

ACKNOWLEDGEMENTS

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REFERENCES

- Bogoev G., Kouzмова, K. (2002). Drought - particularities and ecological problems in the Plovdiv region of Bulgaria, *Scientific Papers University of Agricultural Sciences, Timisoara, Romania, XXXIV*, 161–165.
- Climatic Reference Book of Bulgaria (1983). *Air Temperature, Soil Temperature, Frost*, vol.3, Sofia, BAS, 440 pp. (in Bulgarian).
- Climatic Reference Book of Bulgaria (1990). *Rainfall in Bulgaria*, Volume 6, Sofia, BAS, 169 pp. (in Bulgarian).
- Gulinova, N.V. (1974). *Methods of agroclimatic processing of observations*, Leningrad, Gidrometeoizdat, 151 pp. (in Russian).
- Ivanov, B., Sokolova, E., Kirovski, P. (2013). *Trends in the development of main crops in the grain sector*. Agricultural Academy, Institute for Market Economics, 25 pp. (in Bulgarian).
- Kouzмова, K. (2001). Analysis of drought in 2000 year in the Plovdiv region for agricultural purposes, Academic Session "Civil Society in Action, *Proceeding of articles on interdisciplinary national academy INGA*, NTS of Mechanical Engineering, Sofia, Bulgaria, 219–225 (in Bulgarian).
- Kouzмова, K. (2003). *Agrometeorology*, Academic Publishing of Agricultural University, Plovdiv, Bulgaria, 198 pp. (in Bulgarian).
- Mikhailov, P., Kouzмова, K. (2019). *456 questions on the Geography Test*, Academic Publishing House of the Agricultural University, Plovdiv, 111 pp. (in Bulgarian).
- Ministry of Agriculture, Food and Forestry (2018). *Agrostatistical Reference Book 2000-2017*, 268 pp., (in Bulgarian)
- Ministry of Agriculture, Food and Forestry (2019). *Yields from field crops - harvest 2018. Agrostatistics Bulletin*, 361 (in Bulgarian).
- Spiridonov, V., Balabanova, S. (2017). Impact of climate change (by 2050) on the intense precipitations in Bulgaria, *Bulgarian Journal of Meteorology and Hydrology*, 22(5), 26–37 (in Bulgarian).
- WWF (2018). *Rivers in Bulgaria, online* (in Bulgarian) https://wwf.panda.org/wwf_news/?339819/WWF-Rivers.

MATHEMATICAL APPROACH FOR ASSESSMENT OF THE IMPACT OF GROWTH REGULATORS ON BASIC MORPHOLOGICAL INDICATORS IN MULTIFOLIUM 1 AND LEGEND ALFALFA VARIETIES

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Abstract

The current study aims at using a mathematical approach (correlation and regression analysis) in order to establish a correlation between the examined biometric indicators, to make a more objective assessment, and to express the influence between indicators and trends for yield improvement by means of the regression analysis. Data of a three-year field study are used. It was conducted in Plovdiv in the period 2017-2019. As a result of the correlation analysis, correlations between the examined indicators were established. The strongest positive correlation was found between the indices showing the number of internodes per stalk and the weight of a single stalk $r = 0.993$ for Legend variety, followed by Multifolium 1 variety with a correlation between the indices of weight of multifolium leaves and green mass yield $r = 0.959$.

Key words: alfalfa, correlation, regression analysis, ANOVA.

INTRODUCTION

Increasing in green fodder production in Bulgaria and many countries around the world is mainly related to alfalfa production.

It is one of the most widespread and highly productive crops, which occupies a prime place among herbaceous plants.

According to FAO, around 436 million tones of total alfalfa production are produced annually, with the largest producers being the United States, Canada, Australia and more.

The wide application of this crop is due to a number of its biological and economic features such as high productivity, excellent quality of green mass and hay, long durability, low cost of production and more.

Its biological potential is to provide more than 100 tons of green biomass weight per hectare and more than 20 tons of hay under favorable conditions.

Main directions of the selection for getting high yields of green mass and hay is to create new varieties with improved characteristics. An achievement in this direction is the creation of alfalfa with 4, 5 or more leaflets per leaf.

The production, quality and long life of alfalfa depend on both external (environmental conditions) and internal (genetically determined) factors. These factors are in

complex relationships and if eliminating any of them, it would reduce the effect of the others and would have a negative effect on the yield and the long life of the alfalfa crop.

Research on alfalfa show that yields have increased by 20% over the last hundred years (Kertikova, 2000), only 10% of this rise was due to genetic improvements (Riday and Brummer, 2002).

Therefore, most studies are related to the search of opportunities for increasing the green mass yield and hay in alfalfa through the application of various agro-technical means - balanced fertilization, treatment with leaf fertilizers and growth regulators, optimal density, intensity of use (Berg et al., 2005).

The use of Reni preparations alone and in combination with trace elements in other crops (Kertikov, 2005; Jimotudis, 2008; Minev et al., 2009; Minev et al., 2011) leads to positive changes in yield and quality. It is assumed that these preparations can also have a good effect on the multifoliate alfalfa.

Using the mathematical approach in this study, we set out the following goals: to establish a correlational dependence between the biometric indicators in order to make a more objective assessment; using the regression analysis capabilities to express the impact between indicators and trends for yield improvement.

MATERIALS AND METHODS

During the period 2017-2019 a study was carried out at the experimental field of. Its task was to determine the impact of preparations with regulatory effect - RENI. They were applied independently and in combination with additional trace elements. The results were studied as follows: their effect on the enzyme activity, the emergence of multifolium leaves, the productivity and the quality of the multifolium alfalfa varieties.

RENI preparations were created at the Agricultural University of Plovdiv as a means of the nitrogen exchange regulation. They are used for combinations of the trace elements molybdenum, manganese, magnesium in different concentrations and ratios (Popov 1995). Each RENI combinations consist of additional ingredients, namely: cobalt (Popov et al., 2010) and boron. The activities of enzymes like nitrogenase, nitrate reductase, glutamine synthetase and asparagine synthetase serve as a diagnostic feature for determining the exact concentration and ratio of the individual components in the various combinations.

RENI is applied by foliar treatment of alfalfa with a solution containing the composition. The experimental methods and the examined indicators are mentioned in some other works (Jimotudis et al., 2008; Popov et al., 2007; Popov et al, 2010).

The experiment was set on the method of fractional plots, in 4 repetitions and size of the experimental plot of 10 m².

Two alfalfa varieties: *Multifolium 1* and *Legend* were tested for this particular research.

RENI preparations were created at the Department of Crop Production at the Agricultural University of Plovdiv.

The tested options were evaluated by the following indicators: height, number of internodes per stem, total number of leaves per stem, number of multifolium leaves per stem, weight of leaves per stem, weight of multifoliate leaves and weight of one stem, yield of green mass. A correlation analysis was conducted (Lakin, 1990), aiming to establish the existence of statistically significant correlational dependencies between the examined indicators. The correlation coefficient risen by the square - R² (R Square) is called a *coefficient of*

determination. It shows the variance percentage of the resultative variable that is explained by the factor variable effect. The linear regression models, expressing the influence of an indicator on the total green mass yield, allow us to determine theoretically how and in what direction the change of these indicators contributes to yield improvement. This approach has been used for establishing the relation between important agronomic indicators in maize hybrids (Stoyanova, A. and Gr. Delchev. 2014) and common wheat (Delchev Gr. and A Stoyanova. 2015).

Data processing was conducted through the SPSS statistical program and ANOVA.

RESULTS AND DISCUSSIONS

Correlation coefficients expressing the relationship between the studied indicators are shown in the correlation matrices (Tables 1 and 2). For *Multifolium 1* variety, a strong positive correlation was found between the weight of leaves and the green mass yield $r = 0.959$. Strongly negative are the following correlations: between the number of nodes per stem and leaf weight; between leaf weight and single stem weight, with correlation coefficients of $r = -0.906$ and $r = -0.851$, respectively. All correlational coefficients are statistically proven at a reliability level $\alpha = 0.001$.

The correlations between height, total number of leaves, number of multifolium leaves and other monitored indicators are mathematically unproven.

Legend variety showed stronger correlational dependence; there were both negative and positive correlations between the examined indicators. A negative correlation was observed between height and weight of the multifolium leaves; number of internodes per stem and leaf weight; leaf weight and weight of a single stem, with coefficients $r = -0.944$, $r = -0.952$ and $r = -0.923$, respectively. A strong positive correlation was registered between the number of internodes per stem and the weight of a single stem $r = 0.993$; between the number of multifolium leaves and their weight $r = 0.884$. The correlations between the total number of leaves per stem and the rest of the examined indicators remained mathematically unproven.

Table 1. A correlational matrix for *Multifolium 1* variety

	Height	Number of internodes per stem	Total number of leaves	Number of multifolium leaves	Weight of leaves	Weight of multifolium leaves	Weight of a single stem	Yield of green mass
Height	1	0.313	0.307	-0.199	-0.509	0.631	0.457	0.623
Number of internodes per stem		1	-0.458	0.581	-0.906*	0.099	0.831	0.173
Total number of leaves			1	-0.386	0.555	0.803	-0.485	0.663
number of multifolium leaves				1	-0.332	-0.298	0.684	-0.427
Weight of leaves					1	-0.018	-0.851*	-0.142
Weight of multifolium leaves						1	-0.090	0.959*
Weight of a single stem							1	-0.112
Yield of green mass								1

The determination coefficient indicates the percentage of the scattering of the resultative variable as it is explained by the action of the factor variable. In our case, $R^2 = 0.9188$, i.e.

91.9% of the yield depends on the weight of the multifolium leaves for *Multifolium 1* variety (Figure 1), and for *Legend* variety it is $R^2 = 0.3068$, i.e. 30.7% (Figure 2).

Table 2. A correlational matrix for *Legend* variety

	Height	Number of internodes per stem	Total number of leaves	Number of multifolium leaves	Weight of leaves	Weight of multifolium leaves	Weight of a single stem	Yield of green mass
Height	1	-0.112	-0.501	-0.803	-0.106	-0.944*	-0.122	-0.449
Number of internodes per stem		1	-0.672	0.155	-0.952*	-0.105	0.993**	0.256
Total number of leaves			1	0.620	0.835	0.725	-0.605	0.474
Number of multifolium leaves				1	0.132	0.884*	0.233	0.866
Weight of leaves					1	0.360	-0.923*	-0.026
Weight of multifolium leaves						1	-0.068	0.554
Weight of a single stem							1	0.361
Yield of green mass								1

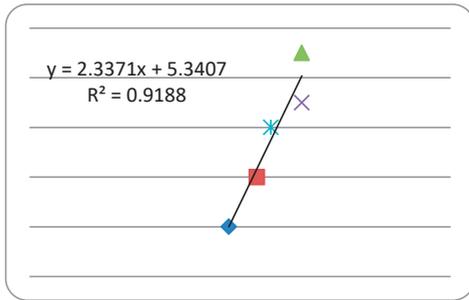


Figure 1. A linear regression model between the weight of multifolium leaves and the green mass yield for *Multifolium 1* variety

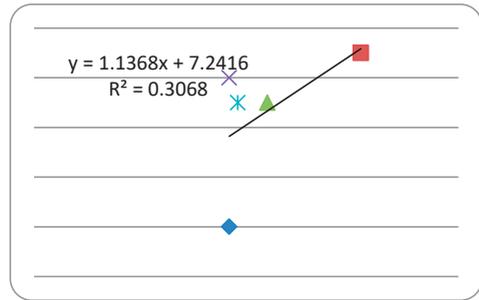


Figure 2. A linear regression model between the weight of multifolium leaves and the green mass yield for *Legend* variety

Table 3 presents the data of the multifolium leaves weight.

In 2017, there is an increase in the multifolium leaves weight at all treated variants, compared to the untreated in both studied varieties which is statistically proven. However, this is due to the increased number of leaflets, as a result of the treatment rather than their weight. This indicator also does not show that the application of the Reni preparations leads to a change in leaves weight in the direction of increase or decrease. If comparing the results obtained and calculate one leaf weight from the untreated variants (controls) with the treated with the most leaves, the following is obtained: At *Multifolium 1* variety for the first, second and third swaths in the control, the weight of one leaf is average 0.11 g, 0.07 g and 0.05 g, and the variant with the largest number of leaflets (treated with RENI + B; b4, with RENI + (RENI + B) and RENI + Co across different slopes) - 0.09 g, 0.08 g, and 0.06 g. In 2018, the unidirectionality of the results is maintained, with the difference that at *Multifolium 1* variety is observed a proven higher leaf weight in the treated variants compared to the control, whereas in the Legend variety this is not the case. Here again, it can be seen that there is no varietal difference in the weight of the leaves before and after RENI treatment.

For the first, second and third swath at untreated variants, one leaf weighs an average of 0.1 g, 0.09 g and 0.08 g, and the variant with

the most multifoliate leaves has an average weight of one leaf in the same sequence of swaths, respectively (the one treated with RENI + RENI + B) - 0.15 g, 0.07 g and 0.083 g. In the Legend variety, the untreated control forms leaves with a mean weight of 0.19 g, 0.17 g and 0.1 g, but the most multifoliate variant (RENI treatments) respectively - 0.12 g, 0.07 g and 0.11 g. Interesting here is the record highs for the average mass of Legend leaves on untreated variants. Given that this is the wettest year and it is normal for the leaves to be heavier with better moisture content of the crop, it would follow that the application of Reni under such conditions acts in the direction of getting smaller leaves at the American variety.

This explanation is logical also due to the fact that during the remaining years of the experiment no such drastic difference in leaf mass was observed in the treated and untreated variants. There is no such difference with *Multifolium 1* variety. This may be due to the lack of evidence and the balanced weight values of the multifaceted leaves in the Legend variety, despite the proven increase in the number of complex leaves resulting from the treatment.

It was conducted an one-way ANOVA. The difference between the average of the options were assessed by test smallest permissible difference in levels of significance. By Duncan's test to cross comparison is grouping the variants.

Table 3. Multifolium Leaf Weight/1 Step (g) Mowing for 2016 -2019

Variants	Multifolium leaves weight/1 stem (g)								
	2017			2018			2019		
	I swath	II swath	III swath	I swath	II swath	III swath	I swath	II swath	III swath
Mnogolistna I									
b1	1.1 ^a	1.0 ^{ab}	0.3 ^a	0.5 ^a	0.3 ^a	0.1 ^a	1.4 ^a	0.6 ^a	0.4 ^a
b2	1.2 ^a	0.9 ^a	0.4 ^b	0.8 ^b	0.5 ^c	0.2 ^b	2.1 ^c	0.7 ^b	0.5 ^b
b3	1.3 ^b	1.1 ^b	0.5 ^b	0.9 ^b	0.4 ^b	0.2 ^b	1.9 ^b	0.7 ^b	0.5 ^b
b4	1.3 ^b	1.3 ^c	0.6 ^c	1.2 ^c	1.3 ^d	0.6 ^d	2.0 ^b	1.0 ^c	0.5 ^b
b5	1.3 ^b	1.25 ^c	0.5 ^b	1.1 ^b	0.5 ^c	0.3 ^c	1.9 ^b	0.7 ^b	0.6 ^{bc}
LSD 5%	0.14	0.14	0.07	0.12	0.07	0.04	0.18	0.11	0.07
Legend									
b1	0.5 ^a	0.6 ^a	0.3 ^a	0.5 ^b	0.1 ^a	0.1 ^a	1.4 ^a	0.2 ^a	0.4 ^c
b2	1.0 ^c	0.9 ^c	0.5 ^c	0.8 ^c	0.2 ^b	0.2 ^b	2.2 ^c	0.7 ^c	0.5 ^d
b3	0.6 ^{ab}	0.7 ^{ab}	0.4 ^b	0.4 ^a	0.1 ^a	0.1 ^a	2.0 ^c	0.5 ^{b,c}	0.2 ^a
b4	0.6 ^{ab}	0.8 ^b	0.4 ^b	0.7 ^c	0.1 ^a	0.1 ^a	1.8 ^b	0.3 ^b	0.3 ^b
b5	0.5 ^a	0.7 ^{ab}	0.4 ^b	0.6 ^b	0.1 ^a	0.1 ^a	2.1 ^c	0.4 ^b	0.4 ^c
LSD 5%	0.4	0.1	0.03	0.06	0.02	0.02	0.14	0.04	0.04

b1 - Control; b2 - RENI; b3 - RENI + B; b4 - c 1/2 RENI + (1/2 RENI + B); b5 - RENI + C

CONCLUSION

As a result, the correlation analysis led to the registration of correlations between the studied indicators. The strongest positive correlation was found between the number of internodes per stalk and the weight of a single stalk $r = 0.993$ for *Legend* variety, followed by *Multifolium I* variety between the weight of multifolium leaves and the green mass yield $r = 0.959$. The correlations showed the degree of influence of each indicator in the formation of yields for both alfalfa varieties.

The linear regression models, which express the influence of the weight index on the multifolium leaves, allow us to determine theoretically how and in what direction the change of these indicators contributes to the improvement of green mass yield. The received results could be a basis for determining the more promising alfalfa varieties.

REFERENCES

- Berg, W.K., Cunningham, S.M., Brouder, S.M., Joern, B.C., Johnson, K.D., Volenc, J.J. (2005). Influence of phosphorus and potassium fertilization on alfalfa yield and yield components. *Crop Science*, 45. 297–304.
- Delchev, Gr., Stoyanova, A. (2015). Stability Valuation of Some Mixtures between Retardants and Antigrass Herbicides for Grain Yield of Durum Wheat. *Bulgarian Journal of Agricultural Science*, 2. 358–362.
- Dimotudis, A., (2008). Effect of Reni preparations on nitrogen exchange efficiency, productivity and quality of garden peas (*Pisum sativum* L.). Thesis.
- Kertikov, T. (2005). Effect of RENI biostimulator on grain yield and quality in spring fie varieties depending on the phenophases of treatment. *Plant Science*, 42(5), 407–412.
- Kertikova, D., Yancheva, H. (2000). Variability of some traits in Lucerne within one breeding cycle. *Genetics and Breeding*, 30(1-2), 1–2.
- Lakin, G.F., (1990), Biometrics, M., High School, 352 pp.
- Minev, N., Popov, N., Yancheva, H. 2009. Investigated on the effect from a third-party with Reni varchu the quality indicators for alfalfa. *Journal of Mountain Agriculture on the Balkans*, 12(6), 1354– 1365.
- Minev, N., Yancheva, H., Popov, N. (2011). Research on the productivity and quality of forage pea varieties treated with growth regulators. Climate change: challenges and opportunities in agriculture. *Agrisafe Final Conference, Budapest, Hungary*, 430–433.
- Popov, N., Dzimotudis, A. (2007). Study of the effect of “RENY” on the quality and productivity of Garden peas. *Journal Central European Agriculture*, 8(4), 413–418.
- Popov, N., Dzimotudis, A., Krastev, S., Kalapchieva, S. (2010). The effect of RENI products on enzymatic activity of nitrogen metabolism in garden peas. *Bulgarian Journal of Agricultural Science*, 16(5), 609–614.

- Riday, H., and Brummer, E.C. (2002). Forage yield heterosis in alfalfa. *Crop Science*, 42, 716–723.
- Stoyanova, A. and Delchev, Gr. (2014). Testing of Various Regimes of Irrigation Furrows in Grain Maize. *Bulgarian Journal of Agricultural Science*, 20 (3), 631–639.
- Ventroni M., Volenec, J., Cangiano, C. (2010). Fall dormancy and cutting frequency impact on alfalfa yield and yield components. *Field Crop Research*, 119(2-3), 252–259.

DISEASES AND YIELD OF NEW VARIETIES OF BARLEY AND WHEAT IN DOBROGEA REGION

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Abstract

The paper presents the behavior of some barley and wheat varieties under 2018 and 2019 conditions in Dobrogea area. In demonstrative plots carried out at S.C. SPORT AGRA SRL Amzacea, Constanța County is presented data about the main pathogens and yield results. Climatic conditions of these 2 years, favoured a major attack of pathogens. For barley a high attack of Rhynchosporium secalis with an attack rate (AR) of 6-30% and Pyrenophora teres in a lower attack rate of 3-20% in most varieties was reported in the April observations. For wheat, Septoria tritici showed an AR between 17-27% and Pyrenophora tritici-repentis showed a low attack (AR = 4.5-17.5%). The pathogen Puccinia striiformis was present in low percentage (AR = 0-13.5%) in the 2018 April observations. For crop protection against pathogens, 2 foliar treatments with fungicides were applied, Artea 330 EC (ciproconazol 80 g/l + propiconazol 250 g/l) 0.4 l/ha, in March and Priaxor EC (fluxaproxad 150 g/l + piraclostrobin 75 g/l) 1 l/ha and in April, respectively. After the last treatment with very good efficacy, there was noticed a decrease in the attack on most pathogens recorded in May observations. Fungal treatments have stopped the development of foliar and ear diseases. The beneficial effects were found in the good yields obtained. For barley crop, the highest yields were 8000 kg/ha for Predator and 8500 kg/ha for Panonic variety. Wheat cultivation yields ranged from 7500 kg/ha for Miranda and 8000 kg/ha for Avenue variety. Quality indices ranged from 70 to 76% for hectolitre weight and 11-14% for protein. All these special yields have been obtained under non-irrigated conditions.

Key words: barley, wheat, main pathogens, yields.

INTRODUCTION

In the last years, various grain varieties created by foreign companies have been placed in cultivation without being deeply known their behavior in the climatic conditions of the Romanian district, Dobrogea. Romanian varieties of winter grains occupy a percentage more than 70% of the cultivated area (Roman et al., 2011).

Even if new varieties of wheat and barley have more yield to offer, breeders must take into account the resistance to pathogens attack (Stadlmeier et al., 2019) and quality indices (Đekić et al., 2017; Luković et al., 2017). These tools may vary depending on the cultivated area (Kaya & Akcura, 2014), so there is a need for demonstration plots in different areas. The increase of draught areas made necessary the testing of drought-resistant barley and wheat lines (Jinga et al., 2010; 2017) identified through extensive field testing and selection in a decentralized participatory breeding programme (Ceccarelli et al., 2007). The paper

aimed to present the main pathogens of barley and wheat crops varieties, quality indices and the yields obtained in 2018 and 2019 at S.C. SPORT AGRA SRL Amzacea, Constanța County, on demonstrative plots.

MATERIALS AND METHODS

The experimental plots were organised in 2018 and 2019, in the field of SC SPORT AGRA SRL Amzacea, Constanta County (South-East of Romania) (Figure 1). The studied crops were winter crops: barley and wheat. The experience was situated on a land belonging to the South Dobrogea Plateau, represented by cambic chernoziom with a profile deeper than other chernozioms, a blackish-brown soil of 40-50 cm thickness, medium texture (Demeter, 2009). The content of nutrients was: mobile P index - 72; N index - 4; K index - 200; humus - 3.11%; neutral pH - 7.2. The climate is deeply temperate continental, with an average annual temperature of 10.7-11.7°C, with a high temperature in the period 20th June to 15th

August. This area is the most arid in the country, with 69 years multiannual average rainfall of 401 litters. Sowing was carried out on 7th October 2017 and 10th October 2018. Treatment of seeds was carried out with Yunta Quattro 373.4 FS (clotianidin 166.7 g/l + imidacloprid 166.7 g/l + prothioconazol 33.3 g/l + tebuconazol 6.7g/l) in dose of 1.6 l/ton. Twelve wheat varieties and three barley varieties were analyzed regarding their behavior under climatic conditions from Amzacea area, in agricultural year 2017-2018 and nine wheat varieties and six barley varieties in the next year, 2018-2019. The climatic conditions of autumn in 2017, 2018 and in spring in 2018, 2019, favored a strong attack by pathogens. Quantity of precipitations during the vegetation period for 2017-2018/2018-2019 years, are presented in Table 1. Due to the climatic conditions of the years 2018 and 2019, for the prevention and control of foliar and ear diseases, 2 treatments were performed. For the first year (2018), were applied Treatment I (March17): Artea 330 EC (cyproconazole 80

g/l + propiconazol 250 g/l) 0.4 l/ha; Treatment II (April20): Priaxor EC (piraclostrobin 150 g/l + fluxaproxad 75 g/l) 1 l/ha; Karate Zeon 50 CS (lambda-cyhalothrin 50 g/l), at a dose of 0.75 l/ha and for the second year (2019), were applied Treatment I (March 10): Artea 330 EC (cyproconazole 80 g/l + propiconazol 250 g/l) 0.4 l/ha; Treatment II (April 20): Priaxor EC (piraclostrobin 150 g/l + fluxaproxad 75 g/l) 1 l/ha; Karate Zeon 50 CS (lambda-cyhalothrin 50 g/l), at a dose of 0.75 l/ha and both were used for specific pest control.

The attack rate (AR) was calculated with the formula $AR = F \times I/100$ (F % - frequency of the attacked organs, I % - intensity of organs' attack). Observations on phytosanitary status of winter crops and collections of biological samples were made on April 17 and May 8, 2018 and April 18, May 25, 2019. Some of technological elements such as seed norm, plant density in autumn and spring, inflorescence emergence date, flowering date, plant height, yields and quality indices were evaluated.

Table 1. Precipitation during 2017/2018 and 2018/2019 growing season for wheat and barley (Amzacea, Constanta)

	Month											
	Oct.	Nov.	Dec.	Jan.	Febr.	March	April	May	June	July	Aug.	
Days	The growing season 2017/2018: Precipitation (mm) for 10-day periods											Sum
1-10	41	18	9	0	9	6	2	64	35	98	0	282
11-20	0	30	23	44	31	37	0	28	0	2	0	195
21-31	14.5	17	18	19	80	26	0	28	41	47	0	290.5
Sum	55.5	65	50	63	120	69	2	92	76	147	0	739.5
Days	The growing season 2018/2019: Precipitation (mm) for 10-day periods											Sum
1-10	0	10	20	10	0	10	19	0	10	12	7	98
11-20	3	10	12	26	8	0	1	6	4	22	0	92
21-31	0	37.5	15	0	0	6	15.5	12	0	10	0	96
Sum	3	57.5	47	36	8	16	35.5	18	14	44	7	286
Days	Average 1961-1990: monthly values of precipitation (mm)											Sum
1-31	34.3	42.4	41.0	27.7	24.0	29.1	31.8	37.7	47.1	38.9	37.4	464.0

RESULTS AND DISCUSSIONS

Observations on phytosanitary status of winter crops and collections of biological samples were made at two time points in order to see the pathogens behaviour to the fungicides (Figure 1 and Figure 2).

Due to the high rainfall during March and May, the attack of pathogens that cause diseases in cereal crops was very aggressive, requiring the

2 pesticides treatments. The last treatment with Priaxor EC reduced the intensity of the attack at a low level.

The highest AR at barley was 30% to *Rhynchosporium secalis* and the lowest AR was 2.0% to *Pyrenophora graminea*. For wheat the highest average of AR was 21.06% attributed to *Rhynchosporium secalis* and the lowest average of AR was 1.76% attributed to *Pyrenophora tritici-repentis* (Table 2).



Figure 1. Autumn grain crops - field trial



Figure 2. Phytosanitary status

Table 2. Autumn cereals phytosanitary status - April 17, 2018

Two-rowed Autumn Barley + Autumn Barley									
Variety	<i>Rhynchosporium secalis</i>			<i>Pyrenophora teres</i>			<i>Pyrenophora graminea</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
Bingo	100	30	30	30	10	3	70	15	10.5
Panonic	40	15	6	90	20	18	40	5	2.0
Predator	60	10	6	80	25	20	80	30	2.4
Wheat									
Variety	<i>Septoria tritici</i>			<i>Pyrenophora tritici-repentis</i>			<i>Puccinia striiformis</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
Avenue	100	20	20	70	20	14	25	5	1.25
Katarina	90	25	23.7	25	20	5	0	0	0
Miranda	100	20	20	70	25	17.5	0	0	0
Litera	70	30	21	45	20	9	0	0	0
Kraljca	80	25	20	35	15	5.3	20	5	1.0
Spranjca	75	25	18.7	45	10	4.5	30	5	1.5
Fiji	80	25	20	80	15	12	90	15	13.5
Silvja	90	30	27	70	25	17.5	0	0	0
Bubimir	80	25	20	40	30	12	20	7	1.4
El Nino	85	25	21.3	45	30	13.5	25	10	2.5
Tata Mata	80	30	24	35	25	8.7	0	0	0
Pepeljura	85	20	17	40	30	12	0	0	0

For barley, the attack of all pathogens were decreased up to 0% and were the attack does not been eradicated entirely the AR were under 1%. For wheat the results were the same (Table 3).

Table 3. Autumn cereals phytosanitary status -May 8, 2018

Two-rowed Autumn Barley + Autumn Barley									
Variety	<i>Rhynchosporium secalis</i>			<i>Pyrenophora teres</i>			<i>Pyrenophora graminea</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
Bingo	6	2	0.1	3	1	0.01	10	5	0.5
Panonic	0	0	0	0	0	0	0	0	0
Predator	0	0	0	2	1	0.01	8	5	0.4
Wheat									
Variety	<i>Septoria tritici</i>			<i>Pyrenophora tritici-repentis</i>			<i>Puccinia striiformis</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
Avenue	5	2	0.1	0	0	0	5	1	0.05
Katarina	0	0	0	5	2	0.1	0	0	0
Miranda	0	0	0	0	0	0	0	0	0
Litera	0	0	0	10	5	0.5	0	0	0
Kraljca	8	2	0.1	0	0	0	2	1	0.02
Spranjca	5	2	0.1	0	0	0	5	2	0.1
Fiji	0	0	0	10	5	0.5	7	1	0.07

Wheat									
Variety	<i>Septoria tritici</i>			<i>Pyrenophora tritici-repentis</i>			<i>Puccinia striiformis</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
Silvja	5	3	0.1	0	0	0	0	0	0
Bubimir	8	2	0.1	0	0	0	2	1	0.02
El Nino	5	2	0.1	10	4	0.4	5	1	0.05
Tata Mata	10	3	0.3	0	0	0	0	0	0
Pepeljura	5	2	0.1	0	0	0	0	0	0

After the 2 fungicide treatments, foliar diseases were stopped at basal leaves. For barley attack decreased completely for all three pathogens analyzed. Also, the fungicide applied to the wheat stopped the pathogen attack both on the last leaf and on ear, very significant. In 2018, the average yield, in Romania at wheat was

4.8 tons/ha (INS, 2018) which means the yield obtained at S.C. SPORT AGRA was significantly greater than it. Ten of the varieties had over 7 tons/ha and 4 of these had over 8 tons/ha. All the cultivars tested had a hectolitre mass over 70 kg/hl (Table 4).

Table 4. Technological sheet for autumn crops in 2018

Variety	Seed norm (kg/ha)	Plant density in the autumn 14.11.2017	Plant density in the spring 11.01.2018	Inflorescence emergence date	Flowering date	Plant height (cm)	Yield (kg/ha)	Quality index	
								M HI (kg/hl)	Protein (%)
Two-rowed Autumn Barley + Autumn Barley									
Bingo	220	520	888	April 20	April 28	73	7375	70.5	-
Panonic	220	522	868	April 26	May 3	101	8500	70.6	-
Predator	220	534	848	April 23	May 2	86	7875	70.6	-
Wheat									
Avenue	250	440	772	April 27	May 4	71	8026	74.6	11.9
Katarina	250	422	828	April 30	May 7	70	7475	76.9	12
Miranda	250	468	660	May 3	May 8	94	7425	75.6	12.3
Litera	250	495	684	May 4	May 8	95	7125	74.2	12.4
Kraljica	250	484	812	May 1	May 5	68	8300	74.5	12.3
Spranjka	250	534	784	May 1	May 9	65	8106	75.2	11.9
Fifi	250	472	732	May 3	May 9	76	6666	77.0	14.5
Silvja	250	445	672	May 2	May 7	80	7675	77.2	12.7
Bubimir	250	432	764	May 2	May 7	71	6575	77.5	12.6
El Nino	250	476	796	April 30	May 5	75	8125	76.5	12.5
Tata Mata	250	502	772	May 4	May 9	87	7475	70.4	12.5
Pepeljura	250	464	784	May 4	May 9	91	7920	73.1	11.9

In 2019, for barley, the most dangerous pathogens were *Pyrenophora teres*, with an AR of 18 and 20 for Predator and OSK 6.2/3-13

respectively. For wheat only two cultivars had an AR over 2% to *Puccinia striiformis* (Table 5).

Table 5. Autumn cereals phytosanitary status -April 18, 2019

Two-rowed Autumn Barley + Autumn Barley									
Variety	<i>Rhynchosporium secalis</i>			<i>Pyrenophora teres</i>			<i>Pyrenophora graminea</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
PLETER	40	20	8	30	10	3	70	15	10.5
PREDATOR	40	10	4	90	20	18	40	5	2.0
OSK 6.2/3-13	20	10	2	80	25	20	80	30	2.4
OSK	20	15	3	10	30	3	50	20	10
OSK	30	10	3	20	40	8	30	30	9
PANONIC	30	20	6	10	20	2	40	20	8

Wheat									
Variety	<i>Septoria tritici</i>			<i>Pyrenophora tritici-repentis</i>			<i>Puccinia striiformis</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
GLOSA	80	20	16	70	20	14	25	5	1.25
AVENUE	90	25	23.7	25	20	5	20	10	2
RENATA	70	20	14	70	25	17.5	0	0	0
OSK 51.117	70	30	21	45	20	9	0	0	0
OSK 110/17	80	25	20	35	15	5.3	20	5	1.0
OSK 159/17	75	25	18.7	45	10	4.5	30	5	1.5
OSK 84/116	80	25	20	80	15	12	90	15	13.5
BOREALIS	90	30	27	70	25	17.5	0	0	0
ICONA 2S	80	25	20	40	30	12	20	7	1.4

The attack of pathogens reported in the experimental plots, showed a very high degree of attack, and the treatment was performed with a fungicide with effect in stopping the attack and to protecting the last leave and the spike, with the product PRIAXOR on 20 April.

For barley, the attack of all pathogens were decreased up to 0% and were the attack does not been eradicated entirely the AR were under 1%. For wheat the results were the same (Table 6).

Table 6. Autumn cereals phytosanitary status - May 25, 2019

Two-rowed Autumn Barley + Autumn Barley									
Variety	<i>Rhynchosporium secalis</i>			<i>Pyrenophora teres</i>			<i>Pyrenophora graminea</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
PLETER	6	2	0.1	3	1	0.01	0	0	0
PANONIC	0	0	0	0	0	0	0	0	0
OSK6.2/3-13	5	10	0.5	10	5	0.5	0	0	0
OSK	10	2	0.2	10	2	0.2	0	0	0
OSK	0	0	0	5	4	0.2	0	0	0
PREDATOR	0	0	0	2	1	0.01	0	0	0
Wheat									
Variety	<i>Septoria tritici</i>			<i>Pyrenophora tritici-repentis</i>			<i>Puccinia striiformis</i>		
	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)	F (%)	I (%)	AR (%)
GLOSA	5	2	0.1	10	3	0.3	5	1	0.05
AVENUE	10	8	0.8	5	2	0.05	10	5	0.5
RENATA	20	3	0.6	0	0	0	0	0	0
OSK 51.117	10	4	0.4	0	10	2	0.2	0	0
OSK 110/17	8	2	0.1	10	5	0.5	2	1	0.02
OSK 159/17	5	2	0.1	8	5	0.4	5	2	0.1
OSK 84/116	10	5	0.5	0	0	0	7	1	0.07
BOREALIS	15	5	0.7	10	8	0.8	0	0	0
ICONA 2S	8	2	0.1	0	0	0	2	1	0.02

In 2018/2019 growing season the rainfall were less compared with 2017/2018 and that is why the yield had lower values. The values for

hectolitre mass werelower for barleyandhigher for wheat, four of themreachingvaluesover 80 kg/hl (Table 7).

Table 7. Technological sheet for autumn crops, 2019

Variety	Seed norm (kg/ha)	Plant density in the spring 01.02.2019	Inflorescence emergence date	Flowering date	Yield (kg/ha)	Quality index	
						M HI (kg/hl)	Protein (%)
Two-rowed Autumn Barley + Autumn Barley							
PLETER	220	440	May 7	May 12	6200	67.9	-
PREATOR	220	424	May 7	May 12	6275	68.6	-
OSK6.2/3-13	220	484	May 7	May 12	7012	69.1	-
OSK	220	436	May 9	May 12	6812	69.8	-
OSK	220	424	May 9	May 12	6450	65.9	-
PANONIC	220	420	May 9	May 12	6587	66.1	-
Wheat							
GLOSA	255	468	May 15	May 17	6187	80.7	11.6
AVENUE	260	512	May 12	May 15	6000	78.9	10.8
RENATA	260	488	May 14	May 17	5750	81	12.1
OSK 51.117	260	480	May 15	May 19	6375	79.3	10.8
OSK 110/17	260	440	May 15	May 19	5750	78.9	11.7
OSK 159/17	260	504	May 15	May 19	6125	80.2	12.1
OSK 84/116	260	516	May 16	May 19	6187	80.2	12.4
BOREALIS	260	496	May 13	May 17	5562	61.9	12.9
ICONA 2S	260	524	May 14	May 17	5287	79	13.6

CONCLUSIONS

Therefore, we can firmly assert that the heavy rainfall from March 2018 and 2019, favored the rise of pathogens. Both in April 2018 and in May 2019, after the application of the product PRIAXOR EC, could be observed that the degree of attack was significantly reduced.

To prevent and control the pathogens that cause diseases in autumn cereal crops, two treatments with fungicides were necessary under climatic conditions of 2018 and 2019. The beneficial effects were found in the good yields obtained. For barley crop, pathogen *Pyrenophora graminis*. showed reduced attack rates, compared to *Pyrenophora teres* or *Rhynchosporium secalis* in both years of experience.

For wheat crop, pathogens *Septoria tritici* and *Pyrenophora tritici-repentis* showed a reduced attack rates, compared to *Puccinia striiformis*., in both years of experience.

The productions obtained these years were very good, considering that they were obtained with non-irrigation technology.

Thereby, under 2018 conditions, the yields obtained ranged between 7375 and 8500 kg/ha and good quality index (hectolitre weight = 70.6 kg/hl) for barley and for wheat yields recorded were between 6575 and 8300 kg/ha.

In 2019, the yields obtained were contained between 6200 and 7012 kg/ha for barley, and between 5287 and 6375 kg/ha for wheat.

REFERENCES

- Ceccarelli, S., Grando, S., Baum, M. (2007). Participatory plant breeding in water-limited environments. *Experimental Agriculture*, 43(4), 411–435.
- Đekić, V., Milivojević, J., Popović, V., Branković, S., Jelić, M., Luković, K., Terzić, D. (2017). Yield components and genetic potential of winter barley. *Proceedings of the VIII International Agricultural Symposium „AGROSYM 2017“*, 643–649.
- Demeter, T. (2009). *Pedologie generala*, Bucharest, RO: Credis Publishing House.
- INS (2018). *Producția vegetală la principalele culturi în anul 2018*.
- Jinga, V., Iliescu, H., Stefan, S., Sandru, L., Andrei, A. M., Lixandru, M., Lazureanu, C. (2010). Pathogens in early stages of winter barley crops - prevention and control. *Romanian Jurnal for Plant Protection*, 2, 52–56.
- Jinga, V., Lupu, C., Giumba, A., Manole, D. (2017). Yields and pathogens of new varieties of barley and wheat during 20016 in Dobrogea region of Romania. *Proceedings of the VIII International Agricultural Symposium „AGROSYM 2017“*, 1290–1294.
- Kaya, Y., Akcura, M. (2014). Effects of genotype and environment on grain yield and quality traits in bread wheat (*T. aestivum* L.). *Food Science and Technology, Campinas*, 34(2), 386–393.

- Luković, K., Zečević, V., Prodanović, S., Milivojević, J., Đekić, V. (2017). Effect of growing season and genotype on winter wheat quality. *Proceedings of the VIII International Agricultural Symposium „AGROSYM 2017“*, 536–542.
- Roman, Gh., V., Tabără, V., Robu, T., Pirșan, P., Ștefan, M., Axinte, M., Morar, G., Cernea, S. (2011). *Fitotehnie-Vol I, Cerealesileguminoasepentruboabe*. Bucharest, RO: University Publishing House.
- Stadlmeier, M., Jørgensen, L., N., Corsi, B., Cockram, J., Hartl, L., Mohler, V. (2019). Genetic dissection of resistance to the three fungal plant pathogens *Blumeria graminis*, *Zymoseptoria tritici*, and *Pyrenophora tritici-repentis* using a multiparental winter wheat population. *G3-Genes Genomes Genetics*, 9(5), 1745–1757.

POTENTIAL OF JERUSALEM ARTICHOKE (*Helianthus tuberosus* L.) AS A BIOMASS CROP

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Abstract

Jerusalem artichoke is a species with high production potential, resistance to diseases and pests, with a good tolerance to the variations of climatic factors. The upper part of the Jerusalem artichoke can be used for the production of biofuel, for the manufacture of briquettes and pellets. In addition to the above-ground biomass yield, Jerusalem artichoke also produces a high yield of tubers. The research carried out by us in the climatic conditions from ARDS Caracal showed that both the applied fertilization level and the nutrition space influenced the quantity of biomass generated by the Jerusalem artichoke, with values that ranged between 36.2 t/ha in the unfertilized variant, with planting at 40 cm between plants/row and 60.1 t/ha at the variant with the fertilization dose of N₁₆₀P₁₆₀K₈₀ at the planting distance of 50 cm between plants/row.

Key words: *Jerusalem artichoke, biomass yields, fertilization, density.*

INTRODUCTION

The Jerusalem artichoke is a perennial plant that has a grassy stem that can reach a height of over 3 m, the vegetative part of the plant drying in autumn (Matei et al., 2018). In next spring emerges again from the underground tubers. It has a high nutritional value due to the presence of almost all essential amino acids (Rakhimov et al., 2003), is used as feed in animal feed (Seiler and Campbell, 2004), and biomass is considered as a rich source of ethanol (Denoroy, 1996). The biogas production in the Jerusalem artichoke is much higher compared to other energy crops (Emmerling, 2007).

According to Cabral et al. (2018), Jerusalem artichoke stems are an alternative raw material for cement composites being suitable to be used to produce cement-bonded boards for construction applications.

In the literature, there are reports on the feed value of Jerusalem artichokes, the effect of fertilization, variety and date of harvest on the yield of production and the chemical composition of the tubers (Sawicka, 1998;

Prośba-Białczyk 2007). However, there are fewer information on optimal plant density (Tabin and Pawłowski, 1956). Research studies have shown that plant density increases the yield of tubers and the upper part. The production of fresh tubers ranged from 40 to 100 t/ha in the researches mentioned by Iburguren et al. (2013). Liu et al. (2015), obtained yields between 20 and 60 t/ha for different Jerusalem artichokes clones in the semi-arid region of China. As a non-grain crop, Jerusalem artichoke possesses a number of desirable characteristics that make it a valuable feedstock, for bio refinery, such as inulin content, rapid growth, strong adaptability, and high yields. Qiu et al. (2018), provides a comprehensive introduction to renewable Jerusalem artichoke-based biomass resources and recent advances in bio-based product conversion.

From the agronomic point of view, the Jerusalem artichokes is considered drought-resistant and can be cultivated at low cost without irrigation by harnessing poor soils (Monti et al., 2005), exhibits very high

adaptability at the extremes of the unfavourable factors - drought resistance at extremely high temperatures.

MATERIALS AND METHODS

The research was carried out at Agricultural Research and Development Station Caracal (ARDS), during the 2018 year in the conditions of a chernozem soil, medium rich in nutrient and with a humus content which varied between 3% to 4%. The soil in the arable layer (0-20 cm) has a lutearic texture with a clay content (particles below 0.002 mm) of 36.2%, an apparent density of 1.42 g/cm³, a total porosity of 47% and one medium penetration rate (penetration resistance of 42 kg/cm²).

From the point of view of the hydric features in the superficial layer, the wilting coefficient records the value of 12.3%, the field capacity 24.5% and the hydraulic conductivity is 9.2 mm/h.

The main aim of the research was to establish the most valuable variant of fertilization on the best density on Jerusalem artichokes. As experimented genotype we use a rustic variety - Rares, with provenience from ARDS Bacau, Romania. The crop was planted in early of March and the experiment had two factors:

A factor - distance between plants/row:

- a1 - 40 cm;
- a2 - 50 cm.

B factor - fertilization - with five graduations:

- b1 - unfertilized variant;
- b2 - N₄₀P₄₀K₄₀;
- b3 - N₈₀P₈₀K₈₀;
- b4 - N₁₂₀P₁₂₀K₈₀;
- b5 - N₁₆₀P₁₆₀K₈₀;

The collected data in the field were analysed using statistical ANOVA program.

RESULTS AND DISCUSSIONS

Climatic conditions (Figure 2) show that the 2018 agricultural year was one excessively warm and these conditions were strongly influenced the main evolutions of the rhythm of plant's development and their capacity for production. Compared to the multiannual registrations, an average temperature of 12.6°C was achieved, with 2.0°C, higher than the multiannual average of 10.6°C. Regarding the

months of the warm period of the year (April - September), we find that in no month were recorded temperatures lower than the multiannual average. The deviations were positive, ranging from 0 to +5.2°C. April was remarkably hot, recording a thermal surge of +5.2°C, the highest temperature ever recorded in April from this area. Daily average temperatures exceeded 32°C in the middle of the month. Also in May were exceptional temperatures, with monthly deviation of +3°C, all of which led to a record: the warmest spring since there were meteorological records in the area. It is also remarkably hot in August and September, with a thermal surplus of +2.4°C and respectively +2.0°C related to multiannual average.

From the point of view of precipitations, the 2018 year was on with a high level of rainfall, especially in the second part of plant's vegetation. The precipitation in this agricultural year reach totals of 843.6 mm, with 306.2 mm higher than the multiannual average for the Caracal Plain area, of 537.4 mm.

The lack of precipitations at the beginning of stage vegetation of plants had negatively influenced the emergence of plants, which made the period with high temperatures in the summer overlap with the period when the requirements of the plants for water were maximum, that fact going to a decrease of the potential of plants to ensure large amount of biomass. These conditions lead to another negative situation for emerge of plants - crust on the soil surface (Figure 1).



Figure 1. Crust on the soil surface in spring of 2018 at ARDS Caracal

Jerusalem artichoke is a plant that ideally suits for efficient biomass production and can be grown on very weak, also degraded, soils

which are not used for the cultivation of crops for consumption. In a study who aims to evaluate the energy efficiency of Jerusalem artichoke in various processing technologies in terms of use in biogas plants: green mass silage, straw and bulbs fermentation, and then comparison of energy efficiency and

profitability in the biogas plants with the most popular type of substrate used in European biogas plants - maize silage - Kowalczyk-Jusko et al. (2017), showed that the total energy value of the aerial parts of dried Jerusalem artichoke was 4.472 kWh/kg DM, which represents approx. 65% of energy value of typical coal.

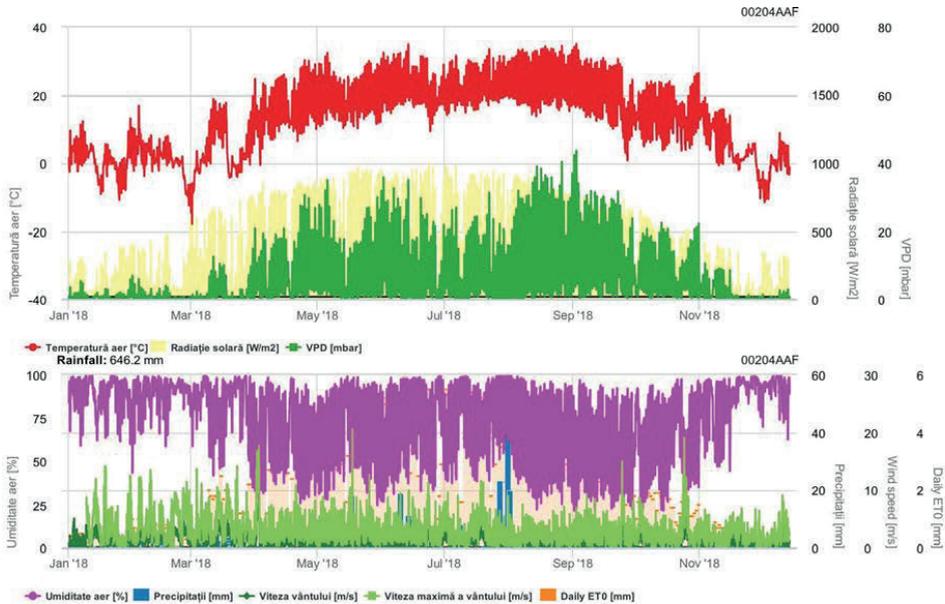


Figure 2. Climatic conditions in 2018 at ARDS Caracal

The yield formation on Jerusalem artichoke was studied by Lv et al. (2019), in order to describe the mechanism based on its growth and photosynthetic characteristics and to identify reliable criteria for high yield cultivar screening, and to guide the breeding of new high yield cultivars. High yield group (MH) cultivars exhibited vigorous above-ground and root growth, higher photosynthetic capacities (especially in terms of their ability to utilize weak light and minimize dark respiration) and lower rates of sexual reproduction than low yield group (ML) cultivars.

Information from the literature is discussed to define guidelines for a model of the growth and development of *Helianthus tuberosus* (L.) in non-limiting conditions. Dynamics of the leaf area index and the distribution of growth between structural growth and reserves (in stem, stolons and tubers) are the most crucial processes (Denoroy, 1996). The potential as feedstock and tuber yield was investigated by

Gao K. et al. (2019), using different leaf removal treatments in 2013 and 2014 in Inner Mongolia, China. Tuber yield was significantly higher in plants from which the lower 1/3 and 1/4 of the leaves were removed than that in control plants.

In our case, the main morphological features of Jerusalem artichoke under the influence of the density and fertilization levels were presented in Table 1. The plant's height was directly correlated with the fertilization and increase simultaneous with levels on both tested distances between plants/row. On the variant with 40 cm between plants/row the height varied from 247.6 cm on the unfertilized variant to 298.6 cm registered at the highest level of fertilization of $N_{160}P_{160}K_{80}$, with an average of 273.2 cm. A similar situation we recorded on the second tested density where the plants height ranged from 287.1 cm on the variant with no fertilizers applied to 308.1 cm on the $N_{160}P_{160}K_{80}$ variant. The height plant's

average on the second density was 295.3 cm. The value of the length of the plants from both densities tested in the experiment for the 2018 year was 284.3 cm.

One of the main aerial biomass components are leaves. The numbers and their dimensions had a very large influence to the amount of biomass on Jerusalem artichoke. Related to this aspect, we can observe that *on the variant with 40 cm between plants/row*, the average dimensions of the leaves were 15.7 cm length and 9.5 cm wide, with a minimum of 10 cm registered in case of length and a maximum of 23 cm for the same

parameter. Related the wide of leaves, those had a minimum values of 4.8 cm and a maximum registered of 15.5 cm. *On the variant* where we test the density *with 50 cm between plants/row*, the average dimensions of leaves had 16.9 cm of length and 9.4 cm of wide. Easy can be observed that the large space of nutrition for each plant lead to modify the leaf geometry, forming longer and narrower leaves. Also, on this density we found a large variations of leaf dimensions, from 8.5 cm to 26 cm for length of leaves and starting from 4 cm to 16 cm in case of leaf's wide.

Table 1. The influence of fertilization and distance between plant/row on development of Jerusalem artichoke

A Factor	B Factor	Plant's height	Height average	Leaf dimensions		Average number of ramification
Distance between plants/row	Fertilization	cm	cm	Length cm	Wide cm	
40 cm	Unfertilized	247.6	273.2	Av.: 15.7	Av.: 9.5	39.0
	N ₄₀ P ₄₀ K ₄₀	262.8				
	N ₈₀ P ₈₀ K ₈₀	270.1				
	N ₁₂₀ P ₁₂₀ K ₈₀	286.8				
	N ₁₆₀ P ₁₆₀ K ₈₀	298.6				
50 cm	Unfertilized	287.1	295.3	Av.: 16.9	Av.: 9.4	45.0
	N ₄₀ P ₄₀ K ₄₀	290.0				
	N ₈₀ P ₈₀ K ₈₀	292.6				
	N ₁₂₀ P ₁₂₀ K ₈₀	298.8				
	N ₁₆₀ P ₁₆₀ K ₈₀	308.1				
Av./experiment		284.3				

Not only the dimensions of the leaves were influenced by the nutritional space of the plant, but entire architecture of the plant. Thus, the number of ramifications of the plant's stem had a large variation due this factor and varied, in average on each tested distance between plants/row, between 39 ramifications in case of 40 cm and 45 ramifications on the second variant with 50 cm. One example of the recorded characters is exemplified in Figure 3. All this presented morphological features of the Jerusalem artichoke were finally influenced the biomass yield: underground and upper ground ones.

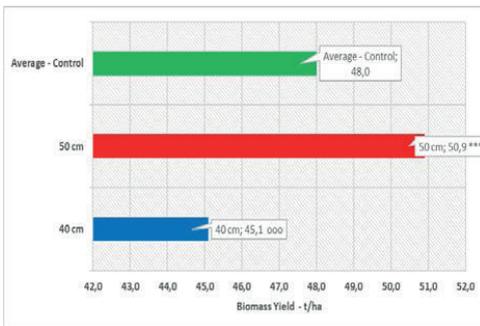
Gao K. et al. (2019), showed that fresh and dry weights of tubers and underground biomass were higher when harvested after freezing; the dry yields of leaves and stems decreased with harvest time. In addition, irrigation significantly enhanced the yields of underground biomass, aboveground biomass and tubers, compared with non-irrigation conditions ($p <$

0.05). The results obtained from the data recorded on the experimented variety - Rares - and presented in the Figure 4, conduct us to conclude that, for the climatic conditions of 2018 year, the most valuable density generate by the distance between plants/row was 50 cm, with a total fresh biomass of 50.9 t/ha and an increase of 2.9 t/ha, statistically ensured as distinct significant related to the average, used as Control. On the second distance between plants/row of 40 cm we recorded a smaller yield of fresh biomass, of 45.1 t/ha.

Even though it is considered by many to be a rustic species, Jerusalem artichoke has a powerful reaction to the applied fertilizers, a fact we registered and presented in Figure 5. The total fresh biomass had an increasing trend from the unfertilized variant, where we record a value of 40.7 t/ha, to highest level of fertilization where we obtained 55.5 t/ha aboveground fresh biomass.

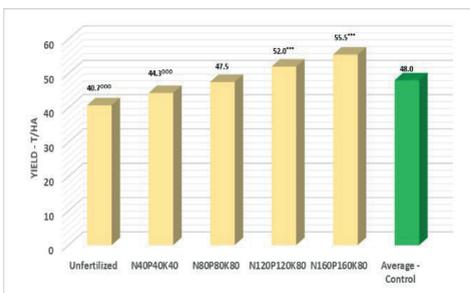


Figure 3. Influence of distance of plants/row on the branches degree



LSD 5% - 1.3 t/ha; LSD 1% - 1.7 t/ha; LSD 0.1% - 2.3 t/ha

Figure 4. Influence of the A factor - distance of plants/row - on the Jerusalem artichoke biomass yields



LSD 5% - 1.6 t/ha; LSD 1% - 2.3 t/ha; LSD 0.1% - 3.1 t/ha

Figure 5. Influence of the B factor – fertilization - on the Jerusalem artichoke biomass yields

Due the climatic conditions of this year, mostly related the rainfall regime, the unfertilized variant had a large amount of biomass. Comparing its value with other fertilized variants we can easy see that the increases were statistically

ensured as very significant. Taking in account this particularly situation we used as Control the average of fresh biomass on the entire experiment. Related to this mark the most valuable variants prove to be $N_{120}P_{120}K_{80}$ and $N_{160}P_{160}K_{80}$ with values of 52.0 t/ha fresh biomass and respectively 55.5 t/ha fresh biomass.

Regarding fertilization, the results obtained can be ambiguous (Denoroy, 1996). However, Kays and Nottingham (2008), recommend for fertilization 70-100 kg/ha of N, about 80 to 100 kg/ha of P and 150-250 kg/ha of K. These authors point out that nitrogen fertilizers can cause overgrowth of the aerial part to the detriment of tuber yield when there is a high level of nitrate in the soil.

Rebora et al. (2011), obtained a different answer, where the increase of density by decrease the distance between plants conduct in a decrease of the weight of the tubers and a compensation of the yield by increasing the number of tubers.

In our experiment the response of the Jerusalem artichoke to the combined interaction of the two factors - plant density and fertilization - is presented in Table 2. Thus, on the smallest distance between plants/row, of 40 cm, the average of the tested density was 45.1 t/ha fresh biomass, with limits that falls between 36.2 t/ha on the unfertilized variant and 50.9 t/ha on the level of $N_{160}P_{160}K_{80}$. Compared these values with Control – the average/experiment, who's production of fresh biomass was of 48.0 t/ha, very significant increases of 4.7% and 6% were observed on variants of $N_{120}P_{120}K_{80}$ and respectively of $N_{160}P_{160}K_{80}$. Negative differences, statistically ensured as very significant related to the Control, has been recorded to the first two variants: unfertilized (with a diminution of fresh biomass yield of 11.8 t/ha) and $N_{40}P_{40}K_{40}$ (with a diminution of fresh biomass yield of 6.0 t/ha).

On the second distance between plants/row of 50 cm, the average of fresh biomass yield recorded was of 50.9 t/ha. The most valuable levels of fertilization for that second graduation of A factor proved to be the same as those from the first graduation: $N_{120}P_{120}K_{80}$ and respectively of $N_{160}P_{160}K_{80}$ with yields of 53.7 t/ha and respectively 60.1 t/ha. Their increases of 5.7 t/ha and 12.1 t/ha were statistically

ensured as very significant in comparison with the Control - the average/experiment. From the results that we recorded is obviously that on the

second distance tested we obtained increases starting from the third level of fertilization of N₈₀P₈₀K₈₀.

Table 2. The influence of interaction of density (A) and fertilization (B) on Jerusalem artichoke biomass yield

A Factor Distance between plants/row	B Factor Fertilization	Yield		Differences	Signification
		t/ha	%	t/ha	
40 cm	Unfertilized	36.2	75.4	-11.8	OOO
	N ₄₀ P ₄₀ K ₄₀	42.0	87.5	-6.0	OOO
	N ₈₀ P ₈₀ K ₈₀	46.0	95.9	-2.0	
	N ₁₂₀ P ₁₂₀ K ₈₀	50.3	104.7	2.3	**
	N ₁₆₀ P ₁₆₀ K ₈₀	50.9	106.0	2.9	**
<i>Average</i>		<i>45.1</i>			
50 cm	Unfertilized	45.2	94.2	-2.8	O
	N ₄₀ P ₄₀ K ₄₀	46.6	97.2	-1.3	
	N ₈₀ P ₈₀ K ₈₀	48.9	102.0	0.9	
	N ₁₂₀ P ₁₂₀ K ₈₀	53.7	111.9	5.7	***
	N ₁₆₀ P ₁₆₀ K ₈₀	60.1	125.3	12.1	***
<i>Average</i>		<i>50.9</i>			
<i>Average/experiment</i>		48.0	100.0	CONTROL	CONTROL
LSD 5%		2.1			
LSD 1%		2.8			
LSD 0.1 %		3.7			

That situations conduct us to believe that on the higher density (with small distance between plants/row), is not justified to apply higher levels of nutrients of N₁₆₀ or P₁₆₀ due the feedback of yield increases. On the other part, the increases registered on the second density of factor A of 11.9% or 25.3% fully justifies the increases of nutrients amount.

CONCLUSIONS

From the above presented data, we can highlight, the follow important conclusions:

- climatic conditions of 2018 year from ARDS Caracal allow to the Jerusalem artichoke plants to express their productive potential;
- the main morphological features of plants were strongly influenced by the density of plant and fertilization regime, height of plants reaching over 3 meters in the situation of large nutritional space on the smallest density, with 50 cm between plants/row;
- the number of branches and leafs form were also modified in the variants, with impact on the total aboveground fresh biomass yields recorded;
- the best density of Jerusalem plants, not only from the point of view of morphological features, but from the fresh

biomass yields also, proved to be the distance of 50 cm between plants/row, with an average of 50.9 t/ha in the experiment;

- the fresh biomass yield, in average/experiment, had a value of 48 t/ha;
- from the fertilization factor the most valuable variants prove to be N₁₂₀P₁₂₀K₈₀ and N₁₆₀P₁₆₀K₈₀ with values of 52.0 t/ha fresh biomass and respectively 55.5 t/ha fresh biomass;
- if we take in account the *combined interaction of the two factors – plant density and fertilization* of the Jerusalem artichoke, very significant increases of 4.7% and 6% were observed on variants of N₁₂₀P₁₂₀K₈₀ and respectively of N₁₆₀P₁₆₀K₈₀ which proved to be the most valuable in the conditions of density with 50 cm between plants/row.

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REFERENCES

- Cabral R., Nakanushi, M. E. Y., Marmol, G., Palacios, J., Godbout, S., Lagace, R., Savastano Junior H., Fiorelli, J. (2018). Potencial of Jerusalem artichoke (*Helianthus tuberosus* L.) stalks to produce cement-bonded particleboards. *Industrial Crops & Products*, 122, 214–222;
- Denoroy. P. (1996). The crop physiology of *Helianthus tuberosus* L.: A model oriented view. *Biomass and Bioenergy*, 11(1), 11–32.
- Emmerling, C., Barton, J. (2007). Anaerobic co-digestion of topinambour (*Helianthus tuberosus* L.) and properties of the remaining biogas manure. *Archives of Agronomy and Soil Science*, 53(6), 683–690.
- Gao, K., Zhang, Z.X., Zhu, T.X., Tian, X., Gao, Y., Zhao, L.X., Li, T.Q. (2019). The influence of leaf removal on tuber yield and fuel characteristics of *Helianthus tuberosus* L. in a semi-arid area. *Industrial crops and products*, 131, 8–13.
- Ibarguren, L., Rebora, C. (2013). El cultivo de topinambur: generalidades sobre su ecofisiología y manejo. *Horticultura Argentina*, 32(77), 35–41.
- Kays, S.J., Nottingham, S.F. (2008). Chapter 6: Value in human and animal diets. *Biology and Chemistry of Jerusalem Artichoke Helianthus tuberosus* L.
- Kowalczyk-Jusko, A., Kupryaniuk, K., Oniszczyk, T., Wojtowicz, A., Janczak, D., Smurzynska, A., Jozwiakowski, K., Czechlowski, M. (2017). Applicability of Jerusalem artichoke in agricultural biogas plants as maize silage alternative. *Proceedings of the 3rd International Conference on Energy and Environment (ICEE 2017). Book Series: ICEE International Conference on Energy & Environment*, 89–94.
- Liu, Z.X., Steinberger, Y., Chen, X., Wang, J.S., & Xie, G.H. (2015). Chemical composition and potential ethanol yield of Jerusalem artichoke in a semi-arid region of China. *Italian Journal of Agronomy*, 10(1), 34–43.
- Lv, S.Q., Wang, R.X., Xiao, Y.M., Li, F.C., et al., (2019). Growth, yield formation, and inulin performance of a non-food energy crop, Jerusalem artichoke (*Helianthus tuberosus* L.), in a semi-arid area of China. *Industrial crops and products*, 134, 71–79.
- Matei Gh., Vlăduț V.N., Ștefan M., Constantinescu E., Sălceanu, C. (2018). Variability of some morphological characters to the Jerusalem artichoke crop under the conditions of the ARDS Caracal. *ISB INMA TECH' 2018 Agricultural and mechanical engineering*, 1023–1028.
- Monti A., Amaducci M.T., Venturi G., (2005). Growth response and leaf gas exchange and fructans accumulation of Jerusalem artichoke (*Helianthus tuberosus* L.) as affected by different water regimes. *Eur. J. Agron.*, 23, 136–145.
- Prošba-Białczyk, U. (2007). Produkcyjność topinamburu (*Helianthus tuberosus* L.) uprawianego bez nawożenia. *Fragm. Agron.*, XXIV, 4(96), 106–112.
- Rakhimov D.A., Arifkhodzhaev A.O., Mezhlumyan L.G., Yuldashev O.M., Rozikova U.A., Aikhodzhaeva N., Vakil M.M. (2003). Carbohydrates and proteins from *Helianthus tuberosus*. *Chem. Nat. Comp.*, 39(3), 312–313.
- Rebora, C., Ibarguren, L., Lelio, H., Gomez, L. (2011). Effect of plant population density on tuber yield of Jerusalem artichoke (*Helianthus tuberosus* L.) urban waste water irrigated. *Revista de la Facultad de Ciencias Agrarias*, 43(2), 83–90.
- Sawicka, B. (1998). Wartość pastewna słonecznika bulwiastego (*Helianthus tuberosus* L.) w warunkach nawożenia azotem. *Ann. Univ. Mariae Curie-Skłodowska, Sect. E. LII. 11*, 97–108.
- Seiler G.J., Campbell L.G. (2004). Genetic variability for mineral element concentrations of wild Jerusalem artichoke forage. *Crop Sci.*, 44, 289–292. *Buletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, 73(2).
- Tabin, S., Pawłowski, F. (1956). Wpływ rozstawy i obsypywania na plon bulwy (*Helianthus tuberosus* L.). *Ann. Univ. Mariae Curie-Skłodowska*, 11(9), 243–257.
- Qiu, Y.B., Lei, P. Zhang, Y.T, Sha, Y.Y., Zhan, Y.J., Xu, Z.Q., Li, S., Xu, H., Ouyang, P.K. (2018). Recent advances in bio-based multi-products of agricultural Jerusalem artichoke resources. *Biotechnology for biofuels*, 11(151).

INFLUENCE OF NITROGEN FERTILIZATION RATES ON THE PRODUCTIVITY OF MAIZE UNDER NON-IRRIGATION CONDITIONS

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Abstract

The aim of the study is to study the effect of different nitrogen fertilization rates on the productivity of maize grown under non-irrigation conditions in the Plovdiv area. The experiment was conducted in the period 2004-2007 in the AU-Plovdiv on alluvial-meadow soil. The following fertilization levels N_0 , N_8 , N_{16} and N_{24} were tested. As a source of nitrogen, ammonium nitrate (NH_4NO_3) was used. The results show that as the fertilizer rate increases, the yield increases, with N_8 increasing between 1 and 5% and N_{16} between 2 and 8%. The difference between N_{16} and N_{24} is less than 2%, with the average for the four years practically coinciding (907 and 908 kg/da, respectively). A square relationship between the fertilization rate and the yield was established at $R^2 = 0.732$. From an economic point of view, the best results are obtained at N_0 and N_{16} , which gives reason to believe that in the Plovdiv area, fertilization of maize under non-irrigation conditions is ineffective. The highest nitrogen fertilizer use efficiency (NFUE) was obtained at N_{16} , with an average yield of 2,375 kg/da from every 1 kg/da of nitrogen. Slightly lower (5.5%) is NFUE at N_8 . Nitrogen fertilization rates have little impact on the 1000 seeds weight and test weight.

Key words: maize, yield, nitrogen fertilization.

INTRODUCTION

Increasing the efficiency of cultivation of different crops is related to the optimization of agro-technical activities for the certain soil and climatic conditions. Hristov & Tashkov (1970) consider fertilization to be the second most important agro-technical activity after irrigation. This makes it one of the main agrotechnical components of maize cultivation. Studies in this area have identified scheme and rates of fertilization, which give the highest yield. However, they are not always optimal in terms of economic point of view. For example, according to Liangl & MacKenzie (1994), in order to obtain the maximum yield of maize for grain, it is necessary to fertilize it with 30-35 kg/da nitrogen, but the economically optimal rate is in the range 17.9-27.3 kg/da. Similar to these are the results reported by Halvorson et al. (2005). The authors note that with increasing nitrogen the NFUE decreases. They found that for conditions of "Arkansas River Valley" in Colorado the best economic results are obtained at the rate of 26.5 kg/da. Nitrogen fertilization rates, which are recommended for growing corn in central Italy, are also relatively high (Mazzoncini et al.,

2008). Based on results of long term field experiment, the authors found an increase in yield to the rate of 30 kg/da. High nitrogen fertilization rates significantly and positively affect the harvest index and the 1000 seeds weight (Wajid et al., 2007). The authors recommend a nitrogen rate of 25 kg/da, in which the yield increases by 22.8% and the absolute seeds weight - by 7.8%, compared to those at the rate of 15 kg/da. Another group of researchers found that corn productivity was maximized when applying significantly lower nitrogen fertilization rates - between 10 and 17 kg/da (Cambouris et al., 2016; Gehl et al., 2005; Torbert et al., 2001; Matev, 2001; Arif et al., 2010; Siam et al., 2008). Dawadi & Sah (2012) found a maximum yield when fertilizing with N_{20} , but recommended N_{16} because of better economic results due to the minimum yield losses (2.8%). The results of a number of studies have proved that corn with high nitrogen norms is ineffective in regard to grain yield, and it has been found that they should not exceed 10 kg/da. For example, Woldesenbet & Haileyesus (2016) found that grain yield increased to the rate of 9.2 kg/da, with negligible difference between the results at rate 6.9 and rate 9.2 kg/da, i.e. the smaller

rate is optimal. In support of this are the data published by Raicheva-Mehandzhieva (1971), Wilhelm et al. (1987), Adediran & Banjoko (1995), Liu & Wiatrak (2011; 2012). For maximum yields, Onasanya et al. (2009) recommended fertilization with $N_{12}P_4$ and economically rates N_6P_4 .

It is clear from the references made that the optimization of nitrogen fertilization in maize for grain cannot be done on a global scale, but must be done for specific soil and climatic conditions, in accordance with other agrotechnical activities.

The aim of the study was to determine the effect of increasing rates of nitrogen fertilization on yield and its components for grain corn, as well as to explore the possibilities to increase the economic efficiency of fertilization with nitrogen by optimizing the rates.

MATERIALS AND METHODS

The experiment is carried out during the period 2004-2007 at the Agricultural University of Plovdiv (Bulgaria) on alluvial-meadow soil. The Knezha-613 hybrid was used. Different levels of nitrogen fertilization are tested under non-irrigation conditions as follows: T0- N_0 , T1- N_8 , T2- N_{16} and T3- N_{24} . Ammonium nitrate (NH_4NO_3) is used. The experiment is based on the method of split-plots design in three repetitions. The size of the experimental plots is 20 m², and the harvest - 10 m². The sowing density is 6500 plants/da, with a row spacing of 0.7m. Nitrogen fertilization was performed once in a phase of 5-6 leaves, just before the last inter-row treatment. Fertilization with phosphorus and potassium is carried out in the autumn. The rates are determined according to the soil reserve. Harvesting was performed at technical maturity with grain moisture of 13-14%. The data for yield, 1000 seed weight and test weight by variants and repetitions are processed by analysis of variance using the ANOVA1 program to establish the warranties of differences. The relationship between the nitrogen rate and the yield is determined using the least squares method. The NFUE coefficient is determined in two reciprocal ways: 1) as an additional yield of 1 kg/da nitrogen; 2) amount of nitrogen to produce 1 kg

of additional yield. On the basis of current cost prices and production prices, an analysis has been made to determine the optimum nitrogen rate from an economic point of view. All agrotechnical activities (soil tillage, plant protection, etc.) are observed during the experiment. The corn was grown after soybean.

RESULTS AND DISCUSSIONS

Meteorological characteristics of the experimental years

In terms of precipitations during vegetation period, the experimental years are very different, and under non-irrigation conditions the amount of yield and the effect of nitrogen fertilization depend to a large extent on the amount and distribution of the precipitations during vegetation period. With regard to this indicator, the first year of the experiment (2004) is characterized as average, with a total rainfall of May-September of 234 mm and a 45% probability. During the same period of the second (2005) year, the amount of precipitation is 456 mm. It is humid with a 6% probability. The third year of the experiment (2006) is also an average with 49% probability and amount of precipitations 228 mm. The most humid (463 mm) with 4% probability is 2007. That, however, is extremely uneven distribution of rainfall, especially in July when practically lacking. During the other three years, precipitations are distributed more evenly.

In terms of air temperature, the first experimental year is average with 55% probability. Averages warm are 2005 and 2006 with probability respectively 34 and 28%. Warmest is the fourth year of experience (2007) which has a 9% probability. Despite those differences, all experienced years are favorable for normal growth, development and timely ripening.

Grain yield

The yield data for variants and years, as well as the average for the all experimental period, are presented in Table 1. The amount and distribution of precipitations during the growing season significantly affect the yield, regardless of the level of nitrogen fertilization. This influence is very well expressed in the wet 2005. The yield for all four values of nitrogen fertilization exceeds 1100 kg/da, while in

extreme 2007 it is more than twice as low (approximately 500 kg/da). In average in terms of rainfall years (2004 and 2006) yields are comparable in quantity - more than 900 kg/da.

Table 1. Grain yield depending on nitrogen rate

Variant	Yield kg/da	to N ₀			to N ₂₄		
		±Y kg/da	%	w	±Y kg/da	%	w
2004							
N ₀	931	St.	100.0	St.	-52	94.7	a
N ₈	949	18	101.9	n.s.	-34	96.5	n.s.
N ₁₆	970	39	104.2	n.s.	-13	98.7	n.s.
N ₂₄	983	52	105.6	a	St.	100.0	St.
LSD (kg/da)		5% = 41	1% = 62	0.1% = 100			
2005							
N ₀	1175	St.	100.0	St.	-45	96.3	n.s.
N ₈	1187	12	101.0	n.s.	-33	97.3	n.s.
N ₁₆	1210	35	103.0	n.s.	-10	99.2	n.s.
N ₂₄	1220	45	103.8	n.s.	St.	100.0	St.
LSD (kg/da)		5% = 119	1% = 180	0.1% = 290			
2006							
N ₀	889	St.	100.0	St.	-43	95.4	n.s.
N ₈	928	39	104.4	n.s.	-4	99.6	n.s.
N ₁₆	959	70	107.9	n.s.	27	102.9	n.s.
N ₂₄	932	43	104.8	n.s.	St.	100.0	St.
LSD (kg/da)		5% = 79	1% = 120	0.1% = 193			
2007							
N ₀	488	St.	100.0	St.	-19	96.3	c
N ₈	493	5	101.0	a	-14	97.2	c
N ₁₆	498	10	102.0	b	-9	98.2	b
N ₂₄	507	19	103.9	c	St.	100.0	St.
LSD (kg/da)		5% = 4	1% = 7	0.1% = 11			
W - warranty; Y - yield							

In general, for the conditions of the present experiment, the fertilization of maize under non-irrigation conditions has a relatively small effect on its productivity. Differences compared to non-fertilized variant are not statistically warranted for three of total four experimental years (2004, 2005 and 2006) but for the last one (2007) are warranted due to the extremely low LSD values. Fertilization with N₈ increased the yield by an average of 10 kg/da or 1.1% compared to the non-fertilized control (Figure 1). Depending on the conditions of the year, this increase ranges from 1.0 to 4.4%. With an increase in the rate of another 8 kg/da (N₁₆), maize responded positively, with a yield increase of 3-8% in the more favorable years and only 2% in the extreme 2007. On average, over four years, applying N₁₆ nitrogen fertilization, yield increases by 4.4% or 38 kg/da. Compared to N₈, at N₁₆ the yield is on average 28 kg/da or

just over 1% and is not statistically warranted. Studies on the fertilization of grain corn in different regions of Bulgaria show that the optimum nitrogen rate varies within a range close to N₁₆ (Nikolov, 1970; Dimitrov, 1973; Furdzhev, 1973; Zhivkov & Matev, 2002; 2005). Further increasing the nitrogen rate to N₂₄ practically no influence on the size of the yield, as compared to that of N₁₆ rate difference is only 1 kg/da (0.1%) and not be warranted statistically. Compared to the non-fertilized control, the application of the N₂₄ rate increases the yield from 3.9 to 5.6% or 43-52 kg/da and it is not statistically warranted too. During extremely dry reproductive periods additional yield is less than 20 kg/da but is statistically warranted.

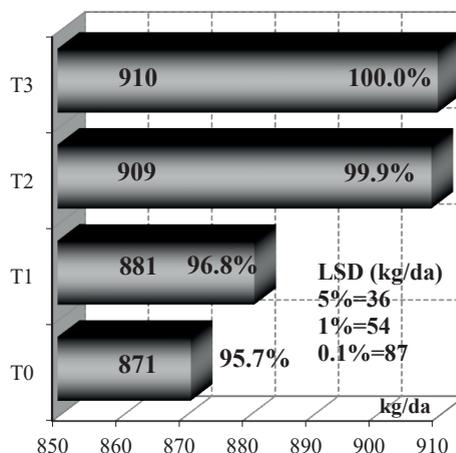


Figure 1. Grain yield depending on nitrogen rate - average for 2004-2007

There is a square relationship between the relative yield and the relative nitrogen rate (Figure 2). It is established on the basis of data from all variants and years. It is graphically represented as a curve representing a convex parabola that approximates the experimental points at $R^2 = 0.732$. According to this relationship, fertilization with N₁₂ (or 50% of the maximum nitrogen rate) gives 99% of the maximum yield. In the range of 85 to 120% (between N₂₀ and N₃₀) of the maximum rate, the yield is maximum, after which it begins to decrease. Figure 3 illustrates the yield change with increasing relative nitrogen rate in the range 0 to 1.4 or between N₀ and N₃₄. The

curve is drawn according to the equation of Figure 1, and the results can be used to optimize nitrogen fertilization for grain corn grown under non-irrigation conditions.

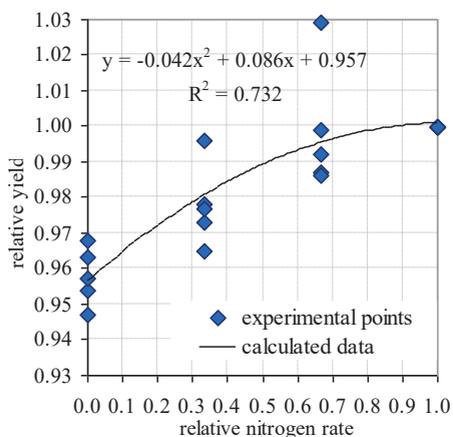


Figure 2. Crop relationship between relative yield and relative nitrogen rate

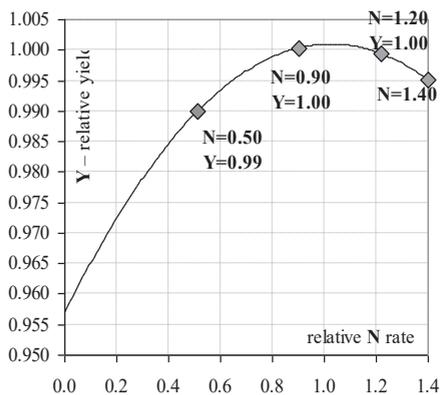


Figure 3. Yield change with increasing relative nitrogen rate

Nitrogen fertilizer use efficiency (NFUE), depending on the rate value

In this study the NFUE is expressed in two ways:

1) As an additional yield (kg/da), obtained from 1 kg/da nitrogen. It is calculated by the formula:

$$NFUE(1) = \frac{\Delta Y}{N_i}$$

Where: $\Delta Y = Y_{N_i} - Y_{N_0}$ is the additional yield due to nitrogen fertilization (kg/da); Y_{N_i} - yield

at N_i rate (kg/da) and Y_{N_0} - yield without fertilization (kg/da);

2) As the amount of nitrogen (kg/da) required to obtain 1 kg/da of additional yield. Is calculated by a reciprocal of the previous formula:

$$NFUE(2) = \frac{N_i}{\Delta Y}$$

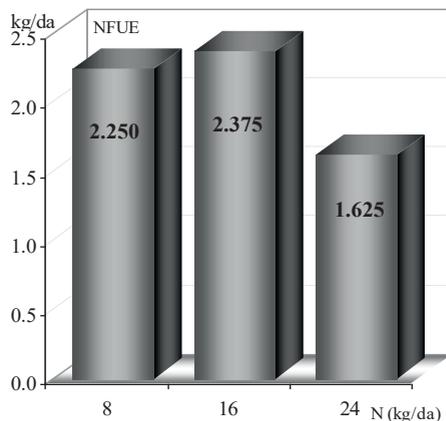


Figure 4. NFUE (1) - average for 2004-2007

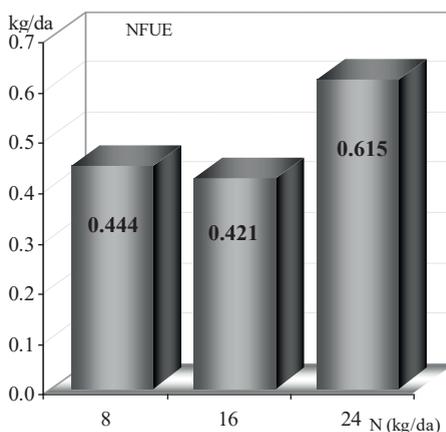


Figure 5. NFUE (2) - average for 2004-2007

As a measure of yield and nitrogen rates is kg/da, the NFUE is obtained and presented as a coefficient.

The results regarding the nitrogen fertilization efficiency are illustrated in Figures 4 and 5. Both graphs show clearly that the highest NFUE is at N_{16} , with an average additional yield of 2.375 kg/da for each 1 kg/da of nitrogen and 0.4211 kg/da of nitrogen is

required to obtain 1 kg/da of additional yield. Slightly lower (5.5%) is the fertilization efficiency with N₈, while at N₂₄ the difference is more significant (average 46.1%) than that at N₁₆. These results suggest that, under the conditions of the experiment, grain corn under non-irrigation conditions should not be fertilized at rates higher than N₁₆.

The 1000 seeds weight

Table 2. 1000 seeds weight depending on nitrogen rate

Var.	1000 s weight g	to N ₀			to N ₂₄		
		±Y /g/	%	w	±Y /g/	%	w
2004							
N ₀	321	St.	100.0	St.	-24	93.0	b
N ₈	323	2	100.6	n.s.	-22	93.6	b
N ₁₆	340	19	105.9	b	-5	98.6	n.s.
N ₂₄	345	24	107.5	b	St.	100.0	St.
LSD (g) 5% = 12 1% = 19 0.1% = 30							
2005							
N ₀	338	St.	100.0	St.	-3	99.1	n.s.
N ₈	332	-6	98.2	n.s.	-9	97.4	n.s.
N ₁₆	357	19	105.6	n.s.	16	104.7	n.s.
N ₂₄	341	3	100.9	n.s.	St.	100.0	St.
LSD (g) 5% = 47 1% = 71 0.1% = 114							
2006							
N ₀	320	St.	100.0	St.	34	111.9	n.s.
N ₈	289	-31	90.3	n.s.	3	101.0	n.s.
N ₁₆	270	-50	84.4	n.s.	-16	94.4	n.s.
N ₂₄	286	-34	89.4	n.s.	St.	100.0	St.
LSD (g) 5% = 59 1% = 89 0.1% = 143							
2007							
N ₀	225	St.	100.0	St.	-25	90.0	a
N ₈	233	8	103.6	n.s.	-17	93.2	n.s.
N ₁₆	218	-7	96.9	n.s.	-32	87.2	a
N ₂₄	250	25	111.1	a	St.	100.0	St.
LSD (g) 5% = 23 1% = 35 0.1% = 57							
Average for 2004-2007 period							
N ₀	301	St.	100.0	St.	-5	98.4	n.s.
N ₈	294	-7	97.7	n.s.	-12	96.1	n.s.
N ₁₆	296	-5	98.3	n.s.	-10	96.7	n.s.
N ₂₄	306	5	101.7	n.s.	St.	100.0	St.
LSD (g) 5% = 27 1% = 40 0.1% = 65							
Y - weight; W - warranty							

The absolute weight of maize seeds under non-irrigation conditions is more influenced by the conditions of the year and, regardless of the nitrogen fertilization rate, values during the more favorable years are within the range characteristic of the hybrid used for the purposes of the study. Significantly smaller absolute weight of seeds is obtained during the extreme 2007 (Table 2).

With regard to fertilization rates, there is little and is not unidirectional impact, without

statistical warranty. An exception is the results of the first test year, which show a statistically warranted gradual increase in values with increasing of nitrogen rates. On average over the four experimental years, all differences are not statistically warranted. This gives reason to believe that, under irrigation conditions, the fertilization of maize does not affect the absolute weight of the seeds. These results are confirmed by Wajid et al. (2007) and Siam et al. (2008).

Test weight of the seeds depending on nitrogen rate

Table 3. Test weight of the seeds depending on nitrogen rate

var.	test weight kg/m ³	to N ₀			to N ₂₄		
		±Y kg/m ³	%	w	±Y kg/m ³	%	w
2004							
N ₀	671	St.	100.0	St.	-32	95.4	a
N ₈	679	8	101.2	n.s.	-24	96.6	n.s.
N ₁₆	706	35	105.2	a	3	100.4	n.s.
N ₂₄	703	32	104.8	a	St.	100.0	St.
LSD (kg/m ³) 5% = 31 1% = 47 0.1% = 76							
2005							
N ₀	708	St.	100.0	St.	3	100.4	n.s.
N ₈	700	-8	98.9	n.s.	-5	99.3	n.s.
N ₁₆	710	2	100.3	n.s.	5	100.7	n.s.
N ₂₄	705	-3	99.6	n.s.	St.	100.0	St.
LSD (kg/da) 5% = 25 1% = 37 0.1% = 59							
2006							
N ₀	889	St.	100.0	St.	-42	95.5	n.s.
N ₈	928	39	104.4	n.s.	-3	99.7	n.s.
N ₁₆	959	70	107.9	n.s.	28	103.0	n.s.
N ₂₄	931	42	104.7	n.s.	St.	100.0	St.
LSD (kg/m ³) 5% = 79 1% = 120 0.1% = 193							
2007							
N ₀	710	St.	100.0	St.	-7	99.0	n.s.
N ₈	711	1	100.1	n.s.	-6	99.2	n.s.
N ₁₆	721	11	101.5	n.s.	4	100.6	n.s.
N ₂₄	717	7	101.0	n.s.	St.	100.0	St.
LSD (kg/m ³) 5% = 19 1% = 28 0.1% = 46							
Average for 2004-2007 period							
N ₀	745	St.	100.0	St.	-19	97.5	a
N ₈	755	10	101.3	n.s.	-9	98.8	n.s.
N ₁₆	774	29	103.9	b	10	101.3	n.s.
N ₂₄	764	19	102.6	a	St.	100.0	St.
LSD (kg/m ³) 5% = 18 1% = 27 0.1% = 43							
Y - test weight; W - warranty							

The data for the effect of nitrogen fertilization on the test weight are presented in Table 3.

With respect to this indicator, the variation by year is more significant than that is accounted over the year when fertilizing with different rates of nitrogen. However, the same trend has been observed here in all the experimental

years, namely - the highest test weight of seeds from plants fertilized with N₁₆, despite the fact that the differences are not statistically warranted in three of the experimental years. On average over the experimental period, there is a statistically warranted increase in test weight at N₁₆ and N₂₄ compared to N₀. The differences between N₈, N₁₆ and N₂₄ are below 2% and are not statistically warranted. The results in Table 3 give reason to believe that nitrogen fertilization has a relatively small effect on the test weight of maize seed grown under non-irrigation conditions, with the increase in norm in the N₈-N₂₄ range practically not changing the values.

Economical analysis

The costs of growing corn are valid for Bulgaria to 2019 and include all the major agricultural activities (tillage, sowing, plant protection and fertilization, harvesting and labor costs). Prices are valid in the presence of own equipment (tractors, combines, sowing machines and more). Revenue is determined on the basis of the exchange rate of the grain in 2019 (577 BGN/t or 0.577 BGN/kg). The summarized results of the economic analysis show that total production (revenue from the sale of production) increases steadily with an increase in the rate of nitrogen fertilization, with the absolute difference between N₀ and N₂₄ being 22.5 BGN/da or 4.5% (Table 4). In terms of this indicator, the results at N₁₆ and N₂₄ are comparable. Production costs also increase steadily with the increase in the nitrogen rate, in which case the rate of increase is the same (11 BGN/da). As a result, an increase in the rate of fertilization increases the cost price of production. It turns out that it is lowest in the variant without fertilization (0.07 BGN/kg) and highest at N₂₄ (0.10 BGN/kg). The non-fertilizing variant has a significantly higher rate of profitability than the fertilized variants, with an average difference of 57% over, compared to N₂₄. Of all included economic indicators, the most important for farmers is net income (profit). It is practically the same at fertilization with N₁₆ and without fertilization (N₀). Upon fertilization with N₈ and N₂₄ net income is lower by 5 to 10 BGN/da. These results suggest that fertilizing maize with nitrogen (using NH₄NO₃) is an

economically inefficient activity when it is grown for grain in non-irrigated conditions.

Table 4. Indicators of economic efficiency depending on the rate of nitrogen fertilization

Nitrogen rates (kg/da)			
N ₀	N ₈	N ₁₆	N ₂₄
Grain Yield (kg/da)			
871.0	881.0	909.0	910.0
Production cost BGN/kg			
0.577	0.577	0.577	0.577
Total production (sale production revenue) BGN/da			
502.57	508.34	524.49	525.07
Production costs BGN/da			
62	73	84	95
Cost price BGN/kg			
0.07	0.08	0.09	0.10
Rate of profitability %			
710.59	596.35	524.40	452.71
Net income (profit) BGN/da			
440.57	435.34	440.49	430.07

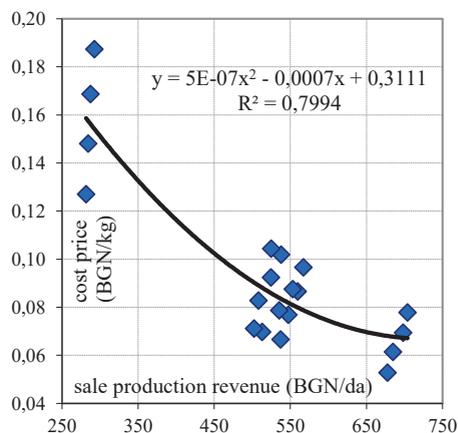


Figure 6. Relationship between sale production revenue and cost price for non-irrigated grain

Based on the economic parameters obtained in all variants and experimental years, some useful for science and practice relationships have been established. Figure 6 shows the relationship between revenue from the sale of production and its cost price, according to which, with the increase in revenue, the cost price gradually decreases and reaches minimum values (0.06 BGN/kg) in the range 695-705 BGN/da. The relationship is square and is graphically expressed by the concave parabola at $R^2 = 0.799$.

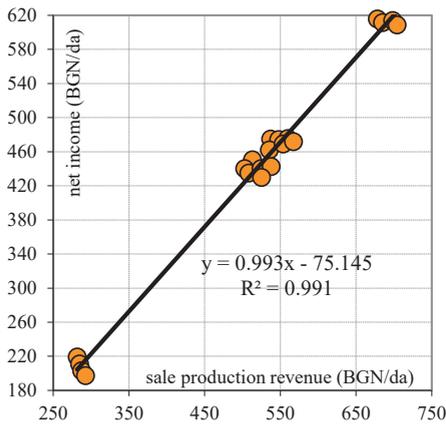


Figure 7. Relationship between sale production revenue and net income for non-irrigated grain

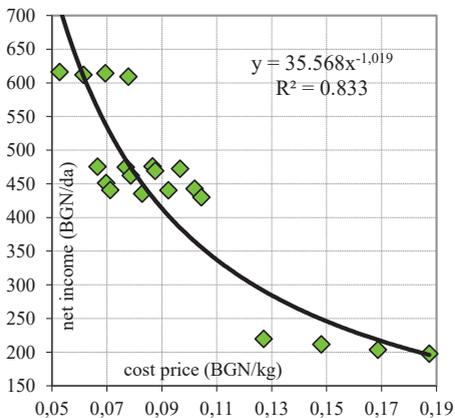


Figure 8. Relationship between cost price and net income for non-irrigated grain

Figure 7 shows the relationship between revenue and net income. It is linear at $R^2 = 0.991$. There is also a close mathematical relationship between production cost price and net income. It is most accurately expressed as power equation: $Y = 35.568x^{-1.019}$, where Y is the net income and x is the cost price. Graphically, this dependence is a concave curve that approximates the empirical points at $R^2 = 0.833$ (Figure 8).

CONCLUSIONS

The fertilization of non-irrigated corn has a relatively small effect on its productivity and differences from the non-fertilized variant are not statistically warranted. Fertilization with N_8 increases yield by an average of 10 kg/da or

1.1% over the non-fertilized control. With N_{16} fertilization, the yield increase is in the range of 3-8% in the more favorable years and only 2% in the dry years. Average for the four years, the application of N_{16} increases yields by 4.4% or 38 kg/da. Compared to N_8 , at N_{16} the yield is on average 28 kg/da or just over 1% and is not statistically warranted. The further increase of the nitrogen rate to N_{24} has practically no effect on the yield, and compared to N_{16} the difference is only 1 kg (0.1%) and is not warranted.

There is a square relationship between relative yield and relative nitrogen rate at $R^2 = 0.732$. It can be used to solve optimization tasks.

Fertilization rates have little or no impact on the 1000 seeds weight and their test weight.

From an economic perspective, the best results are obtained by N_0 and N_{16} , which gives reason to believe that for the region of Plovdiv, fertilization of grain corn under non-irrigation conditions is ineffective activity.

ACKNOWLEDGEMENTS

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REFERENCES

- Adediran, J.A., Banjoko, V.A. (1995). Response of maize to nitrogen, phosphorus, and potassium fertilizers in the savanna zones of Nigeria. *Communications in Soil Science and Plant Analysis*, 26(3-4), 593–606.
- Arif, M., Amin, I., Jan, M. T., Munir, I., Nawab, K., Khan, N.U., Marwat, K.B. (2010). Effect of plant population and nitrogen levels and methods of application on ear characters and yield of maize. *Pak. J. Bot.*, 42(3), 1959–1967.
- Cambouris, A.N., Ziadi, N., Perron, I., Alotaibi, K.D., Luce, M. St., Tremblay, N. (2016) Corn yield components response to nitrogen fertilizer as a function of soil texture. *Can. J. Soil Sci.*, 96, 386–399.
- Dawadi, D.R., Sah, S.K. (2012). Growth and yield of hybrid maize (*Zea mays* L.) in relation to planting density and nitrogen levels during winter season in Nepal. *Tropical Agricultural Research*, 23(3), 218–227.
- Dimitrov, D.A. (1973). Irrigation, Mineral Fertilizing and Planting Density as Affecting the Grain Yield of Maize Grown in the Area of Rouse Irrigation System. *Plant Science*, 10(1), 61–71.

- Furdzhev, I. (1973). Value of irrigation rate and annual irrigation rate of sprinkling irrigated grain corn for the conditions of Pazardzick irrigation system. *Plant Science*, 10(3), 53–63.
- Gehl, R.J., Schmidt, J.P., Maddux, L. D., Gordon, W.B. (2005). Corn yield response to nitrogen rate and timing in sandy irrigated soils. *Agronomy Journal*, 97(4), 1230–1238.
- Halvorson, A.D., Schweissing, F.C., Bartolo, M.E., Reule, C.A. (2005). Corn response to nitrogen fertilization in a soil with high residual nitrogen. *Agronomy Journal*, 97, 1222–1229.
- Hristov, A., Tashkov, G. (1970). Comparative Evaluation of the Importance of Individual Measures in the Total Agrotechnical Complex of Maize Irrigation. *Plant Science*, 7(10), 61–71.
- Liu, K., Wiatrak, P. (2012). Corn production response to tillage and nitrogen application in dry-land environment. *Soil and Tillage Research*, 124, 138–143.
- Liu K., Wiatrak P. (2011). Corn production and plant characteristics response to N fertilization management in dry-Land conventional tillage system. *International Journal of Plant Production*, 5(4), 405–416.
- Liangl, B. C., Mac Kenzie, A. F. (1994). Corn yield, nitrogen uptake and nitrogen use efficiency as influenced by nitrogen fertilization. *Canadian Journal of Soil Science*, 74(2), 235–240.
- Mazzoncini, M., Di Bene, C., Coli, A., Risaliti, R., Bonari, E. (2008). Long-term tillage and nitrogen fertilisation effects on maize yield and soil quality under rainfed Mediterranean conditions: a critical perspective. *DJF Plant Science*, 137, 13–16.
- Nikolov, G. (1970). Effect of the crop density on the growth, development and yield of irrigated maize in the region of the South Bulgarian chernozem smolnitza soils. *Plant science*, 7(9), 69–81.
- Onasanya, R.O., Aiyelari, O.P., Onasanya, A., Oikeh, S., Nwile, F.E., Oyelakin, O.O. (2009). Growth and yield response of maize (*Zea mays L.*) to different rates of nitrogen and phosphorus fertilizers in Southern Nigeria. *World Journal of Agricultural Sciences*, 5(4), 400–407.
- Raicheva-Mehandzhieva, A. (1971). Irrigation regimen of maize grown on leached cinnamon forest soil in the region of Stara Zagora irrigation system at various levels of fertilizing. *Plant science*, 8(1), 49–69.
- Siam, H.S., Abd-El-Kader, M.G., El-Alia, H.I. (2008). Yield and yield components of maize as affected by different sources and application rates of nitrogen fertilizer. *Research Journal of Agriculture and Biological Sciences*, 4(5), 399–412.
- Torbert, H.A., Potter, K.N., Morrison, J.E. (2001). Tillage system, fertilizer nitrogen rate and timing effect on corn yields in the Texas Blackland Prairie. *Agronomy Journal*, 93, 1119–1124.
- Wajid, A., Ghaffar, A., Maqsood, M., Hussain K., Nasim, W. (2007). Yield response of maize hybrids to varying nitrogen rates. *Pak. J. Agri. Sci.*, 44(2), 217–220.
- Wilhelm, W.W., Schepers, J.S., Mielke, L.N., Doran, J.W., Ellis, J.R., Stroup, W.W. (1987). Dryland maize development and yield resulting from tillage and nitrogen fertilization practices. *Soil and Tillage Research*, 10(2), 167–179.
- Woldesenbet, M., Haileyesus, A. (2016). Effect of nitrogen fertilizer on growth, yield and yield components of maize (*Zea mays L.*) in Decha district, Southwestern Ethiopia. *International Journal of Research - Granthaalayah*, 4(2), 95–100.
- Zhivkov, Zh., Matev, A. (2002). Productivity of irrigating norm and evapotranspiration, depending on applied irrigation regime and the value of nitrogen fertilization by grain corn. *Agricultural University - Plovdiv, Scientific Works*, XLVII(2), 181–188.
- Zhivkov, Zh., Matev, A. (2005). Possibilities for Decrease of Nitrogen Fertilization Rate by Grain Corn, Cultivated in Conditions of the Sofia by Ddifferent Water Regime. *Agricultural University - Plovdiv, Scientific Works*, L(1), 59–64.

REACTION TO FERTILIZATION OF ROMANIAN VARIETIES OF WINTER TRITICALE, UNDER THE CONDITIONS OF TRANSYLVANIA PLAIN, BETWEEN THE YEARS 2012-2019

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Abstract

In the last decades, due to the genetic advances obtained in the plant breeding sector, a series of triticale varieties with high production potential and stability have been created, more productive than wheat varieties and even maize cultivated in the hilly areas with poorly fertile soils and low pH. This research aims to follow the behaviour of several winter triticale varieties created by INCDA Fundulea, namely: Plai, Titan, Stil, Haiduc, Negoiu and TF 2, on a period of 8 experimental years, in condition specific to Transylvania Plain. Research covered two levels of fertilization $N_{50}:P_{50}$ and $N_{100}:P_{50}$. Results show different reaction of the winter triticale varieties to fertilization treatment. The older variety TF 2 delivered an average yield of 6954 kg ha⁻¹ in the plots fertilized with 100 kg ha⁻¹ N. The highest yields were recorded in the year 2014 by Haiduc variety, delivering a yield of 9203 kg ha⁻¹ in treatments with 100 kg ha⁻¹ N. Results pointed out that an important genetic progress was achieved in which concerns the potential yield of this species.

Key words: triticale, variety, fertilization, yield potential.

INTRODUCTION

Triticale is a new kind of wheat stubble, created by the hybridisation of wheat and rye (Lalević et al., 2012). According to the opinion of the most researchers, triticale is a plant species characterized by a high genetic potential for grain yield, as well as good nutritive properties of its grain, so it is regarded as a very promising crop (Borojević, 1981; Cvetkov, 1982; Đokić, 1988; Biberdžić et al., 2012). In their willing to create a species with great adaptability and stable yields, scientists combined the best properties of wheat (high and stable yield and early maturity) with the positive properties of rye (good resistance to diseases, pests, cold, drought, a large number of spikelet per spike etc.). The advantages of triticale are applied also in environments where conditions limit the productivity of other cereals; it can be grown on more marginal land (arid, acidic, etc.) and adapts to reduced tillage systems (Ciftci and Eleroglu, 2012). Triticale is suitable for cultivation even at high altitudes, in soils with poor physical and chemical properties, such as acidic and saline

soils (Naeem et al, 2002; Lalević et al., 2012). Thanks to its valuable characteristics and agronomic advantages, the number of areas for triticale cultivation in the world increased great in the last decades, from 232631 ha in 1985 to 3809192 ha in 2018 (FAO, 2020). Yield also varied from 2575 kg/ha in 1985 to 3361 kg/ha in 2018 (FAO, 2020). Modern triticale cultivars show higher yields and superior adaptation to soil quality and environments than wheat (Ugarte et al., 2007; Janušauskaitė, 2014). In Romania the area cultivated with triticale increased from 1214 ha in 1997 to 78887 ha in the year 2018 (FAO, 2020). The yield of triticale increased from 3012 kg/ha in 1997 to 4277 kg/ha in the year 2018. These increases could be explained by the increase of the cultivated area, by the evolution of scientific research in this field and also by improving the crop management techniques. One of the most important crop management techniques for spring triticale production is N fertilization (Janušauskaitė, 2008; Obuchowski et al., 2010; Janušauskaitė, 2013). Positive effects of N fertilization on grain yield have been reported by several researchers (Gibson et al., 2007;

Lestingi et al., 2010). The success of any treatment stands in adoption of optimum mineral fertilizer doses along with an optimum application moment. Nitrogen stress at critical growth stages may lead to irreversible yield loss (Janušauskaitė, 2013).

In Romania, an increase interest in triticale research programs has been showed by the agricultural research institute - R.I.C.I.C. Fundulea. Scientists from this institute conducted several amelioration programs in triticale since the year 1971 and they recorded so far 13 varieties (Ittu et al., 2006). Their achievements showed to be very competitive

with other international programs, fact highlighted also thru recording the Titan variety in Canada, France and Hungary or Negoiu variety in Moldavia (www.incda-fundulea.ro/60ani/INCDA60.pdf). Between the years 1982-2003, the first intensive varieties of Romanian triticale were recorded (Table 1, after Ittu et al., 2007): Plai (1992), Colina (1993), Titan (1998) and Tril (2001) and later Stil (2003), varieties which showed to be a remarkable genetic progress compared to the first Romanian variety, TF2, both from production potential and its stability point of view (Ittu et al., 2004).

Table 1. Triticale varieties created by R.I.C.I.C. Fundulea between the years 1984-2007

No. crt.	Variety	Registration year	Authors
1	TF2	1984	Gh. Ittu, N.N. Săulescu, C. Țapu, N. Ceapoiu
2	PLAI	1992	Gh. Ittu, N.N. Săulescu, Mariana Ittu, M. Verzea, P. Mustăța
3	COLINA	1993	Gh. Ittu, N.N. Săulescu, Mariana Ittu, P. Mustăța
4	TITAN	1998	Gh. Ittu, N.N. Săulescu, Mariana Ittu, P. Mustăța
5	TRIL	2001	Gh. Ittu, N.N. Săulescu, Mariana Ittu, P. Mustăța
6	STIL	2003	Gh. Ittu, N.N. Săulescu, Mariana Ittu, P. Mustăța
7	GORUN	2005	Gh. Ittu, N.N. Săulescu, Mariana Ittu, P. Mustăța
8	HAIUC	2006	Gh. Ittu, N.N. Săulescu, Mariana Ittu, P. Mustăța

Ittu et al. (2001) pointed out that the triticale cultivars created by R.I.C.I.C. Fundulea proved to be more adapted than other small grain cultivars in areas with acid soils from hilly region, but the yield recorded with Titan variety, showed that this cultivar can be successfully cultivated, even in plain area with fertile soils.

The objective of this study is to follow the behaviour of several winter triticale varieties created by R.I.C.I.C. Fundulea, on a period of 8 experimental years and to elaborate a proper fertilizer management for conditions specific to Transylvania Plain.

MATERIALS AND METHODS

The field research was conducted over an eight-year period (2012-2019) at the Agricultural Research and Development Station Turda. The experiment area is located in Turda city, Cluj County, in Western Transylvania Plain, Romania.

The soil is a deep alluvial - clay soil with neutral reaction and a medium humus supply

(3.5%). The climate is continental having 4 distinct seasons (after Koppen system).

Average annual rainfall is 540 mm from which 68% are recorded during the growing season (Deac et al., 2016).

The medium average rainfalls recorded in the experimental period is 599.51 mm (Figure 1). The highest monthly sum of rainfalls were recorded in May, September and October months while the highest sum of rainfalls were recorded in the year 2016 (816.8 mm).

The lowest sum of rainfalls were recorded in the year 2012 (504.4 mm). High variation in mean annual rainfall were registered between the eight study years, the difference in rainfall distribution over the growing season being very diverse, as well.

Monthly and annual mean temperatures recorded high variations among the experimental period (Figure 2).

The highest annual average temperature was recorded in the year 2019 (11.35°C) while the smallest annual average temperature was recorded in the year 2016 (9.99°C).

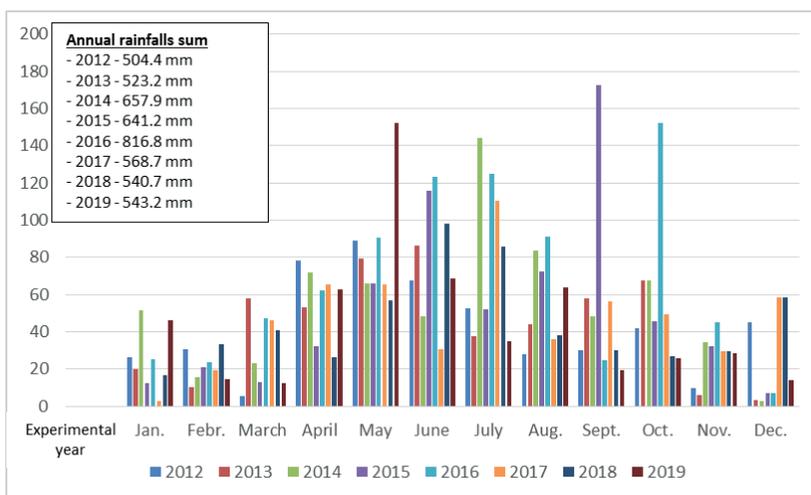


Figure 1. Monthly sum of rainfalls recorded over eight-year experimental trial (Source of data: Weather station from Turda - longitudinal: 23^o47'; latitudinal 46^o35'; altitudinal 427 m)

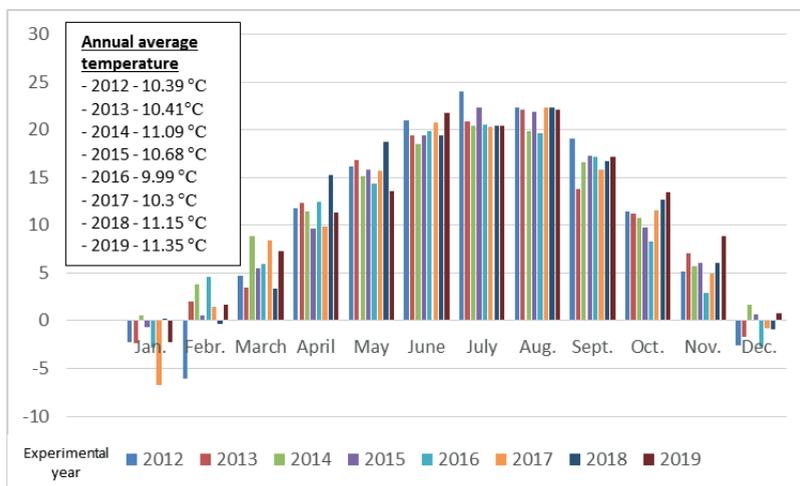


Figure 2. Monthly average temperatures recorded over eight-year experimental trial (Source of data: Weather station from Turda - longitudinal: 23^o47'; latitudinal 46^o35'; altitudinal 427 m)

As split plot design was used in each of the eight years, with six varieties of triticale in the main plots and two rates of N fertilization in the subplots, in 3 replications. The biological material consists in the following varieties of triticale (created at R.I.C.I.C. Fundulea): Plai, Titan, Stil, Haiduc, Negoiu and TF 2. The species were sown in the first decade of

October month - each year. The two N fertilization rates were N₅₀ and N₁₀₀ (kg N ha⁻¹). The treatments were applied to the same plots in each crop season. Harvest of triticale was carried out during full ripeness. The experimental data obtained were statistical analysed using the program Statistica vs. 10.

RESULTS AND DISCUSSIONS

The experimental trial analysed in this study started in the year 2012 for a period of eight

experimental years. The results recorded could be considered therefore representative for long-term trials. The importance of such studies is outstanding since during recent decades the global climate is changing, being recognized as

a serious environmental issue with high impact on crop production (Marton et al., 2007). The aim of our study was to follow grain yield behaviour of six triticale varieties in condition specific to Transylvania Plain. Conducting these trials over a period of eight experimental years validates the results recorded since the changes in weather patterns have been demonstrated through recent studies to have a greatest effect on crop yield (Barrow et al., 2000). The aim of every agricultural crop system is to achieve high and stable yields. These objectives can be fulfilled by adoption of a proper in-field technology, proper choice of sown genotypes and favourable agroclimatic conditions (Biberdžić et al., 2012). The significant differences ($p < 0.001$) found in grain yield of triticale was affected by variety, experimental year and nitrogen doses. The grain yield of Romanian varieties of winter triticale as affected by year are provided in Table 2. Results show high variations among the eight-year experimental trial. The average productions varied from 3942 kg ha⁻¹ to 8572.83 kg ha⁻¹. The highest grain yields were recorded in the year 2014 on all triticale varieties tested in this trial. The best reaction was recorded on Haiduc variety which registered the highest trial mean (6933.14 kg ha⁻¹). In some experimental years Haiduc variety recorded yields higher than the average

production (6950 kg ha⁻¹) reported by the producer (<https://samanta.ro/triticale-haiduc-soi-toamna-romanesc/#>). Comparing Haiduc variety with other five triticale varieties, Pochiscanu et al. (2013) concluded also that Haiduc recorded the highest yields. The smallest trial mean yields (average yields among the eight experimental years) were recorded on TF2 (6217.76 kg ha⁻¹). Results pointed out that the smallest yields were recorded in drought years, like the year 2012, on all triticale varieties studied. The average grain yield is comparable to that obtained by Villegas et al. (2010) and Pecio (2010). All the results were very significant from statistically point of view. Many reports showed the effect of weather conditions on nutrients utilization (Jelić, 1998; Biberdžić et al., 2012). Racz (2013) studied some varieties of triticale created at R.I.C.I.C. Fundulea in condition specific to Transylvania Plain. The experiment trial developed for a period of 3 experimental years, pointed out a good capacity for adaptation of the species analysed to different climate conditions. The author also highlighted a very significant influence of the experimental factor year on triticale production. Similar aspects were highlighted also by Voica (2011) who concluded that the variability of climatic conditions had significant influence on the behaviour of triticale.

Table 2. Grain yield of Romanian varieties of winter triticale as affected by year

Experimental year	Grain yield [kg ha ⁻¹]					
	Triticale varieties					
	Plai	Titan	Stil	Haiduc	Negoiu	TF2 (control)
2012	4733.66***	4795.33***	4747.83***	5382.00***	5399.66***	3942.00
2013	6405.66***	6343.55***	6288.00***	6786.66***	6111.66***	5734.16
2014	8523.00***	8568.00***	8572.83***	8321.16**	7861.33	7772.50
2015	7872.83***	6507.16***	7109.83	7473.83	7672.83**	7150.00
2016	6599.00 ⁰⁰	6830.00	7250.33	7356.00	7322.00	7093.00
2017	5315.16	5243.66	5874.33***	5646.66**	5905.50***	5033.50
2018	7995.83***	7571.33**	7845.00***	7975.83***	7798.66***	6985.33
2019	6490.83*	6749.16***	7099.83***	6523.00**	6610.00**	6031.66
Mean of variety (Y)	6741.99	6576.02	6848.49	6933.14	6835.20	6217.76

Notes: ** - $p < 0.01$ - significant; *** - $p < 0.001$ - highly significant (HS, confidence 99.9%)

The effect of N fertilization on yield and qualitative characters of cereal grain has been reported in various studies (Lestingi et al., 2010; Delogu et al., 1998; Riley). The amount of nitrogen needed by triticale crop to reach high yields and quality depends greatly on the

seasonal conditions, soil type, and rotational history of the soil as well as the potential yield of the cultivars. Nitrogen is the key nutrient input for achieving higher yield of triticale (Alazmani, 2015). The grain yield of Romanian varieties of winter triticale as affected by

fertilization are provided in Table 3. Results show high variation among the eight-year experimental trial, showing variations very significant from statistical point of view. The results increased in parallel with dose increase for all triticale varieties studied. The average productions varied from 6217.77 kg ha⁻¹ to 6933.14 kg ha⁻¹.

The highest yields were recorded on fertilization with N₁₀₀ on all triticale varieties. The highest production on both fertilization doses were recorded by Haiduc variety while the smallest production were recorded on TF2 (the oldest variety created at NARDI Fundulea, and was considered control variant).

Table 3. Grain yield of Romanian varieties of winter triticale as affected by fertilization

Source	Grain yield [kg ha ⁻¹]					
	Plai	Titan	Stil	Haiduc	Negoiu	TF2
N ₅₀ (control)	6115.95	5999.08	6187.29	6323.87	6163.87	5481.33
N ₁₀₀	7368.04***	7152.95***	7509.70***	7543.29***	7506.54***	6954.20***
Trial mean	6742.00	6576.02	6848.50	6933.14	6835.20	6217.77

Notes: ***: p < 0.001 - highly significant (HS, confidence 99.9)

Our results subscribe to previous studies which revealed increases in yield of triticale grain at increased N fertilizer levels. Lewandowski and Kauter (2003) reported that fertilization with 70 kg N ha⁻¹ significantly increased triticale crop yields. The highest reaction to fertilization (production increase from N₅₀ to N₁₀₀) was reported by TF2 (1473 kg ha⁻¹) followed by Negoiu (1343 kg ha⁻¹), Stil (1322 kg ha⁻¹), Plai (1253 kg ha⁻¹) and Haiduc (1220 kg ha⁻¹). The smallest reaction to fertilization was observed on Titan variety (1153 kg ha⁻¹). Moreno et al. (2003), which proceeds that the N fertilizer highly depends on growing season's variations conditioned by environmental factors. Biberdžić et al. (2012) conducted a study meant to follow the reaction of some triticale varieties in different agroclimatic conditions and concluded that application of fertilizers had a positive effect on triticale grain yield increase. Oral (2018) pointed out that the best grain yield and majority components results were obtained on the experimental plots fertilized with 120 kg N ha⁻¹. Moreover, the author recommended that the application of higher doses of nitrogen should be considered taking into account several factors like the climatic data of seasons.

CONCLUSIONS

Results show a positive reaction to the conditions of Transylvania plain of Romanian varieties of winter triticale tested in this trial. Despite high variations in the climatic condition specific to the eight-year experimental period, the varieties tested

recorded high yields in all experimental years. The significant differences (p < 0.001) found in grain yield of triticale was affected by variety, experimental year and nitrogen doses. Nitrogen (N) fertilization gave grain yield increase, the best results being achieved on treatment with 100 kg N ha⁻¹. The most productive variety for the agroclimatic condition tested in this trial is Haiduc.

Considering the results recorded in this experimental trial we recommend Haiduc variety for condition specific to those experimented in this study- in order to achieve maximum yields. Monthly and annual mean temperatures recorded high variations among the experimental period. Because the aim of this manuscript was to follow the reaction of several triticale varieties to fertilization within a specific experimental period we tracked only the influence of the experimental factor year into consideration reason why we didn't provide a deeper analyse of the correlation between climatic variations and triticale yield achievements. Yet we consider that this should be a further study objective since during recent decades the global climate is changing, being recognized as a serious environmental issue with high impact on crop production.

REFERENCES

- Alazmani, A. (2015). Evaluation of yield and yield components of barley varieties to nitrogen. *International Journal of Agriculture and Crop Sciences IJACS*, 8(1), 52–54.
- Barrow, E.M., Hulme, M., Semenov, M.A., Brooks, R.J. (2000). Climate change scenarios. In: Downing, T.E.,

- Harrison, P.A., Butterfield, R.E., Lonsdale, K.G. (eds.). Climate Change, Climatic Variability and Agriculture in Europe. *European Commission*. Brussels, Belgium.
- Biberdžić, M., Jelić, M., Delečić, N., Barać, S., Stojković S. (2012). Effects of agroclimatic conditions at trial locations and fertilization on grain yield of triticale. *Research Journal of Agricultural Science*, 44(1), 3–8.
- Borojević, S. (1992). *Principi Metodi Oplemenji vanja Bilja*. (pp. 384), NaučnaKnjiga, Beograd.
- Cvetkov, S.M. (1982). Selekcija Na ZimniTritikale ($2n=6x=42$) V Bulgaria. (pp. 74) Phd. Thesis, Agricultural Academy, Sofia, Bulgaria.
- Deac, V., Rotar, I., Vidican, R., Mălinaş, A., Păcurar, F. (2016). Researches Concerning the Productivity and Quality of Onix Soybean. *Agriculture and Agricultural Science Procedia*, 10, 112–117. Retrieved February, 22, 2020, from <https://www.sciencedirect.com/science/article>.
- Đokić, A. (1988). *Biljna Genetika* (pp. 488). NaučnaKnjiga, Beograd.
- Ciftci, Yenice, E. and Eleroglu, H. (2003). Use of triticale alone and in combination with wheat or maize: effects of diet type and enzymesupplementation on hen performance, egg quality, organ weights, intestinal viscosity and digestive system characteristics. *Anim Feed Sci. Technol.*, 105, 149–161.
- Gibson, L.R., Singer, J.W., Vos, R.J., Blaser, B.C. (2008). Optimum stand density of spring triticale for grain yield and alfalfa establishment. *Agron J.*, 100, 911–916.
- Ittu, Gh., Săulescu, N.N., Ittu, M., Mustăţea, P. (2001). Advances in triticale breeding program from R.I.C.I.C. Fundulea. *Romanian Agricultural Research*, 16, 1–4.
- Ittu, Gh., Săulescu, N.N., Ittu, M., Mustăţea, P. (2006). Progrese în ameliorareala triticale pentru obţinerea de soiuri cu talia scurtă. *Analele I.N.C.D.A., LXXII*, 19–28.
- Ittu, Gh., Săulescu, N.N., Ittu, M., Mustăţea, P. (2007). Realizări în ameliorarea la triticale (x *Triticosecale* Witt.). I.N.C.D.A. Fundulea, LXXV, Jubiliar Volume. Retrieved February, 12, 2020. from <http://www.inceda-fundulea.ro/anale/75/75.4.pdf>.
- Janušauskaitė, D. (2008). Winter triticale yield and quality depending on nitrogen fertilization and meteorological conditions. *Žemėsūkiomokslai*, 15(4), 21–27.
- Janušauskaitė, D. (2013). Spring triticale yield formation and nitrogen use efficiency as affected by nitrogen rate and its splitting. *Zemdirbyste-Agriculture*, 100(4), 383–392.
- Jelić, M., Milovanović, M., Stojanović, J. (1998). Proučavan jenekih agrotehnič kihmeraneophodnih u proizvodnjizrnajarog tritikalea, Zimskaškolaža agronome. *Čačak*, 2(2), 29–32, Srbija.
- Lalević, D., Biberdžić, M., Jelić, M., Barać, S. (2012). The characteristics of triticale cultivated in rural areas. *Agriculture & Forestry*, 58(2), 27–34.
- Lestingi, A., Ventrella, D., Bovera, F., De Giorgio, D., Tateo, A. (2010). Effects of tillage and nitrogen fertilisationon triticale grain yield, chemical compositionand nutritive value. *J. Sci. Food. Agric.*, 90, 2440–2446, DOI 10.1002/jsfa.4104.
- Márton, L., Pilar, P.M., Grewal, M.S. (2007). Long term studies of crop yields with changing rainfall and fertilization. *Agricultural Engineering Research*, 13, 37–47. Retrieved February, 22, 2020, from https://www.researchgate.net/profile/Marton_Laszlo3/publication.
- Naeem, H.A, Darvey, N.L., Gras, P.W. and MacRitchie, F. (2002). Mixing properties, baking potential, and functionality changes in storage proteins during dough development of triticale wheat flour blends. *Cereal Chem.*, 79, 332–339.
- Obuchowski, W., Bbanaszak, Z., Makowska, A., Łuczak, M. (2010). Factors affecting usefulness of triticale grain for bioethanol production. *Journal of the Science of Food and Agriculture*, 90(14), 2506–2511 <http://dx.doi.org/10.1002/jsfa.4113>.
- Oral, E. (2018). Effect of nitrogen fertilization levels on grainyield and yield components in triticale based on AMMI and GGE biplot analysis. *Applied Ecology and Environmental Research*, 16(4), 4865–4878.
- Pochişcanu, S.F., Buburuz, Al., Pomohaci, Tr., Leonte, Al., Naie, M. (2013). Behavior of some Romanian triticalevarieties in the climatic conditions of the Center of Moldavia, Romania. *Cercetări Agronomice în Moldova*, XLVI(4), 13–20.
- Pecio, A. (2010). Productivity of triticale affected by nitrogen fertilization and weather conditions. *Fertilizer and Fertilization*, 40, 101–116.
- Racz, I. (2013). Cercetări privind formarea producţiei și evaluarea însușirilor de calitate la unele cereal panificabile în Câmpia Transilvaniei. PhD thesis. Cluj-Napoca, Romania. Retrieved February 3, 2020, from <http://www.usamvcluj.ro/files/teze/2013>.
- Ugarte, C., Calderini, D.F., Slafer, G.A. (2007). Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. *Field Crops Research*, 100, 240–248. <http://dx.doi.org/10.1016/j.fcr.2006.07.010>
- Villegas, D., Casadesus, J., Atienza, S., Martos, V., Maalouf, F., Karam, F., Aranjuelo, I., Nogues, S. (2010). Tritordeum, wheat and triticale yield components under multi-local Mediterranean drought conditions. *Field Crops Research*, 116, 68–74 Retrieved February, 12, 2020, from <http://dx.doi.org/10.1016/j.fcr.2009.11.012>.
- Voica, M. (2011). Behaviour of some triticale cultivars in Hilly Region of Muntenia. *I.N.C.D.A. Fundulea*, LXXXIX (1), 21–30. <http://www.fao.org/faostat/en/#data/QC> <https://www.inceda-fundulea.ro/60ani/INCDA60.pdf> <https://samanta.ro/triticale-haiduc-soi-toamna-romanesc/#>.

THE QUALITY OF THE PLANTING MATERIAL FOR POTATO CROPS - FACTOR IN INSURING PROFITABLE PRODUCTS

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Abstract

Planting material quality is a complex notion supported by the phytosanitary, biological and physical quality of potato tubers. Phytosanitary quality refers to health status of tubers of adherent soil to tubers through which can be transmitted a lot number of diseases caused by viruses, bacteria, mycosis and soil pests, quarantine organism phytosanitary quarantine organism transmitted by tubers and soil. Potato diseases and pests may diminish the yield through: reducing or blocking photosynthesis; reducing or blocking transport of assimilated products from leaves to tubers; stain, wriggle, necrosis of leaves and plant death; rot of tubers in the field or storage. The biological quality of the planting material is characterized by the plants' capacity to sprout, emerge, grow and to make tubers, being influenced by physiological degeneration. Physiological degeneration is determined by high temperatures during the vegetation, by increasing the intensity of the aphids' flight transmitting viruses, but also by the condition of storage unventilated warehouses with high temperatures and high humidity. The physical quality of potato tubers is conferred by the external appearance and the size. The tubers which have other shapes and colors nonspecific to the variety are eliminated during sorting for batching together with the tubers that present deformations or infections with different diseases and pests. Depending on the size of the tubers the density and the planting norm are established. Due to the complexity of the factors involved in the making of a planting material with appropriate biological, physical and phytosanitary characteristics and the current state of potato production at national level, specific researches were initiated aimed to increase the areas cultivated with certified potato material and implicitly increase the average productions.

Key words: potato, planting material multiplying, biological and physical quality.

INTRODUCTION

The potato is one of the most important cultivated plants, with a high ecological plasticity, being grown on all the continents to be used fresh, as food, or processed as raw material for starch and alcohol, etc. but also as animal feed.

The degeneration of the potato has been, for a long time, a phenomenon manifesting itself as a weakening of the plant's entire vegetative system, leading to yield loss.

Parmentier (1786) was the first scientist to notice the degeneration of the potato cultivars grown. Ever since then, the potato degeneration has remained of interest for scientists, and the relation between climate and the degeneration of the potato is essential; this is why, in Romania and throughout the world, dedicated areas were set out, specialized in the cultivation and reproduction of tubers.

Ensuring the quality of the planting material to obtain the planned yield represents the most important activity in this sector, and is carried out by business operators authorised for the production, processing and marketing of planting material, and checked by the regional inspectorates in charge of the tuber's quality verification.

Tuber zoning represents a priority in potato growing. That is why, in countries with a seed growing tradition, there are areas with specific conditions dedicated to seed crops, areas where only tubers are grown, humid, cool areas, with as few aphids as possible.

The recent climatic changes led to a decrease in the areas tilled for seed, as well as to the change of the cultivar's conveyor in favour of the early and extra-early cultivars, with a shorter vegetation period, requiring less water and reaching maturity after 76-80 days of vegetation.

MATERIALS AND METHODS

Scientific literature and the legislation governing the growth, processing and sale of tubers were studied for this paper.

The areas that are favourable to the growth of potato planting material are cool areas with good air circulation, which don't encourage aphid proliferation, with a long interval from the first flight to the start of the maximum summer flight of aphids.

The depreciation of potato cultivars under the conditions of the favourable and very favourable areas is caused by viral degeneration where, after three years of propagation, the viral infection can increase from 34% to 85%, as a result of planting non-certified material.

In our country, the area necessary for the cultivation of seed potatoes spanned in the beginning over 5 regions (Figure 1): Harman and Rasnov in the Brasov county, Ciuc and Lazarea in the Harghita county, Suceava in the Suceava county, Targu Secuiesc in Covasna and Neamt in the Neamt county, and it subsequently extended with areas in the Botosani, Bacau, Iasi, Sibiu, Hunedoara counties, in humid, cool areas, for a total of 30,000 ha.

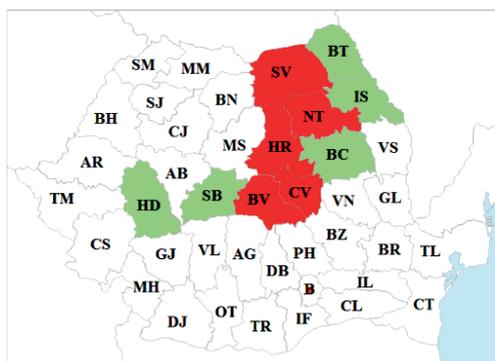


Figure 1. Closed areas for growing tubers

The delimitation of closed areas and the creation of the best conditions for the distribution of the tuber crops within the country proved to be a particularly useful and technically and economically effective measure. The distribution must be further refined by measures ensuring the most effective use of the favourable ecological conditions, where the source of viral infection and the plants' potential of viral infection are minimized.

Table 1. The evolution of cultivated areas (ha) in regions favourable to tuber crops for planting

County	Year	2009	2011	2013	2015	2016	2017	2018	2019
	-ha-	-ha-	-ha-	-ha-	-ha-	-ha-	-ha-	-ha-	-ha-
Bacau	9	-	-	-	-	-	-	-	-
Botosani	50	-	-	-	-	-	-	-	-
Brasov	407	265	109	200	126	167	148	150	
Covasna	455	159	111	284	311	290	238	286	
Harghita	338	114	37	108	134	110	75	103	
Hunedoara	14	-	-	-	-	-	-	-	-
Iasi	69	84	-	23	33	10	8	-	
Neamt	56	-	11	32	27	10	7	6	
Sibiu	27	15	26	32	28	28	28	34	
Suceava	536	117	25	20	26	44	57	81	
Total	1961	754	319	699	685	659	561	660	

Table 1 shows an alarming decrease in the potato tubers areas in regions closed for the cultivation of planting material from 1961 ha in 2009 to only 319 ha in 2013; we see a slight increase in the following years, reaching 660 ha in 2019, also as a result of the implementation of joint support for this crop as of 2015.

The yield obtained on the 660-ha certified country level is around 16,500 tons of certified planting material. This quantity is enough to plant certified material only on 4700 ha, i.e. 2.7% of the 170,000 ha of potatoes declared by the National Institute of Statistics for 2019, well under the level of the European states, where an average of 2200 kg/ha of certified seed is used on the total area; for us, the certified material used for the crops represents merely 470 kg/ha.

As a result of planting material of non-conforming quality, the national average yield is of only 14500 kg/ha, and the cultivates areas are steadily declining.

RESULTS AND DISCUSSIONS

The depreciation of the biological yield potential is a consequence of two major causes, the viral infection and the decreased growth rate due to the physiological age.

The main diseases affecting the phytosanitary quality of the seed potato are: viruses, bacterioses and mycoses.

The most common viruses for potato crops are: **The potato leafroll virus - PLRV)**

Potato leafroll was the first viral disease of the potato to be studied (Figure 2). The first symptoms are seen at the tip of the plant and consist of the leaf margins curling or rolling towards the upper side of the leaf blade, and leaflets chlorosis. Later on, the rolling extends to

the basal leaves, which become thick, leathery, rigid, because of the physiological age of the planting material.

In the field, this virus is persistently spread only by aphids. The *Myzus persicae* aphid is the most effective vector.



Figure 2. Potato leafroll virus - PLRV
(<http://kb-dev.gramophone.in/tag/leaf-roll-virus/?lang=en>)

Potato virus Y/PVY, leads to the formation of mosaic patterns, curling, spotting and ringspot disease (Figure 3). Potato virus Y is easily spread and can lead to major yield loss up to 70%. The most frequent vector is the green peach aphid (*Myzus persicae*).

The symptoms are more often than not the formation of marble and mosaic patterns. Virus Y^O strains usually encourage the curling of the leaves and even to the necrotic spotting of the leaves of some cultivars. Many cultivars infected with virus Y^C have symptoms of necrotic spotting.



Figure 3. Potato virus Y/PVY
(<https://www.agroscope.admin.ch/agroscope/fr/home/actualite/kurznews/2017/livre-PVY.html>)

Virus Y^{NTN} or the superficial necrotic spotting of the tuber (Potato virus Y^{NTN}/PVY^{NTN})

Potatoes cultivated in Europe are increasingly more affected by a virus Y strain with typical symptoms on the tubers, i.e. superficial necrotic ringspots. At first glance, they may be mistaken for the rings caused by the “tuber spotting” or the mop - top virus. This virus, named *Potato virus Y* (Y^{NTN}/PVY^{NTN}) (Figure 4), can sometimes overcome PVY resistance. During ELISA testing, virus Y^{NTN} is identified as virus Y and is not mentioned as a distinct strain.

Symptoms: upon harvesting, tubers show superficial irregularities shaped as arcs or rings. Sometimes, such semicircular defects have a pink hue. Later on, they sink in, become necrotic and brown. At this stage, it is difficult to differentiate them from the tuber necrosis caused by “tuber spotting” and the thickening of the tip of the potato plant (the mop - top virus). Finally, the entire area inside the ring becomes necrotic, brown and sinks in.

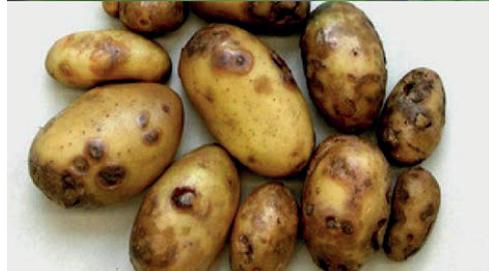


Figure 4. Potato virus Y^{NTN}/PVY^{NTN}
(<https://www.akkerwijzer.nl/artikel/85725-yntn-virus-velop-in-consumptieaardappelen/>)

Potato leaf rolling mosaic - Potato virus M (PVM)

All European cultivars are prone to infection, but the economic significance is not clearly

defined, albeit believed to be insignificant. Potato virus M can lead to yield loss of up to 40%.

Potato virus M is transmitted by contact, but in the field, it is generally spread by aphids, non-persistently (Figure 5). *Myzus persicae* and *Aphis nasturtii* are the most important vectors.



Figure 5. Potato virus M (PVM)

(<http://ephytia.inra.fr/en/C/21040/Potato-Potato-virus-M-PVM>)

Yield losses caused by viral diseases may be prevented by using certified planting material. The infection of the seed potato is particularly prevented by the early removal of infected plants, chemical treatment for aphid control and the destruction of the diseased plants removed.

Main potato pests affecting the phytosanitary quality of the seed potato

Aphids (*Aphids* spp.) are a major threat for tuber crops, because they spread viruses from one plant to the other.

Aphids are found in particular on the lower side of the leaf. The most dangerous species for potatoes are *Myzus persicae*, *Aphis nasturtii*, *Aulacorum solani* and *Myzus ascalonicus*.

The Colorado potato beetle (*Leptinotarsa decemlineata*)

Beetles coming from outside the crop sit on the young plants feeding on the leaf margins. They lay orange eggs, 1-2 mm in length, in distinctive groups of 15-80 eggs. The larvae are rapacious and can eat the entire leaf, leaving only the leafstalk.

Control is possible only with the help of pesticides destroying the adults as well as the larvae.

Potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*)

The first sign of their presence in the field are areas with slow growing plants. The infected plants are darker and flower later. The radicular

system is much more extended, because the infected roots branch out more. Swollen cysts can be seen on the roots, like needle point, 6-8 weeks after planting (Figure 6).



Figure 6. *Globodera* spp.

(<https://www.forestryimages.org/browse/detail.cfm?imgnum=5393019>)

Potato stalk and tuber nematode

(*Ditylenchus destructor*) is a pest the attacks of which have serious consequences for potato crops (Figure 7). This pest generated serious damage to the potato crop, both on the quantity and on the quality of the crop. The nematode is a worm which attacks only the underground parts of the plant. No signs of the attack are visible on the surface. On the potato tubers, the nematode attack created small discolored areas, where the epidermis is discolored and starts to crack. The tubers attacked by the nematode become brown and have a texture characteristic for the rot attack. The attack of these worms affects the crop quality and the yield.



Figure 7. *Ditylenchus destructor*

The seed potato is cultivated only on soil free of potato cyst nematodes.

The biological quality of the planting material is defined by a high germination, springing, growth and tuberization potential, and is influenced by the physiological aging of tubers (Draica, 1980).

Regarding the physiological aging (Draica, 1980), mentions that the phenomenon is manifested by the tubers' loss of the potential to produce high yield plants, leading to viral degeneration.

Physiological aging is determined by high temperatures during vegetation as well as during storage.

Research showed that the planting material produced in areas or under conditions with lower temperatures has a higher yield potential, the tubers have more sprouts and more main stalks/hole, respectively. In general, it is unanimously accepted that biologically as well as phytosanitary, the tubers grown in humid, cool areas is superior to the ones grown in areas with high temperatures, where the pilosity phenomenon frequently occurs.

In areas with high temperatures, when the conditions are unfavourable during vegetation: spurious precipitation, and in particular high temperatures exceeding the biological limits

(over 24°C), certain physiological and biochemical processes take place leading to the tubers' arrested development, deformation, decreased rest period and the sprouting of the parent tubers, the creation of secondary and even tertiary tubers, phenomena which depreciate the biological quality as well as the culinary and industrial processing quality (Draica, 1980).

For tubers obtained and stored under optimal environmental conditions, at the end of the rest period comes the incubation stage.

Therefore, the physiological age of the tubers can be assessed based on the sprouting, i.e. based on the aspect of the sprouts when planted.

The biological conditions used in Europe and in our country, according to Law no. 266/2002 on the production, processing, marketing and quality certification, sale of seeds and seed material, and Order no. 1266/2005 are (Table 2):

- Pre-basic seed;
- Basic seed;
- Certified seed;
- Marketed seed.

Table 2. Requirements for cultivar purity and phytosanitary condition (Order no. 1266/2005)

Biological harvest category	Maximum accepted ratio %							
	of alien plants during field inspection	of atypical plants during field inspection	during a field inspection - total viroses	during the latest field inspection – blackleg and tuber wet rot	<i>Rhizoctonia solani</i>	in direct line		
						Virus testing	Purity	
						Atypical	Other cultivars	
1	2	3	4	5	6	7	8	9
Pre-basic seed	0.0	0.0	0.1	0.0	3	0.5*	0.01	0.0
CEE 1 Basic seed class SE; CEE 2	0.1	0.25	0.5*)	0.5	5	4.0*	0.25	0.1
Basic seed class E; CEE 3	0.1	0.25	1.0*)	1.0	5**)	4.0*	0.25	0.1
Certified seed class A	0.2	0.5	2.0*)	1.5	10**)	8.0**)	0.5	0.2
Certified seed class B	0.2	0.5	5.0*)	2.0	10**)	10.0**)	0.5	0.2

*) Both serious and simple viroses are taken into account.

***) Serious viroses are taken into account, but not simple mosaic patterns where the symptoms are discoloration without leaf deformation.

The field inspection of seed crops is carried out by the approving inspector, and the minimum number of field inspection during vegetation is 4 for pre-basic and basic seed and 3 for certified seed.

Tuber crops must be free of black scab (*Synchytrium endobioticum*), ring rot (*Clavibacter michiganensis* subsp. *sepedonicus*) and other quarantine organisms.

The removal of atypical, alien plants and of plants infected by pests, is mandatory, beginning with the start of vegetation, until the destruction of the stems.

The removal is carried out by pulling out the diseased plant, including all tubers, removing them from the crop in plastic bags and destroying them.

Physical quality

From this perspective, the exterior aspect and size are highly relevant.

During screening and calibration by size, the physical quality is certified by the approving inspector.

Tubers with a shape or colour atypical for the soil are eliminated during planting.

As regards the tuber size, considering the latest fast reproduction techniques, it is arguable that all the tubers can be used as planting material, regardless of size, provided that they come from certified potato batches, officially approved.

We must note that the planting distance and norm, as well as the cost of the planting material, depend on the size of the tubers.

Seed tubers must comply with the following calibration requirements:

- a) Fractional calibration: 25-35 mm; 35-45 mm; 45-55 mm; over 55 mm; without exceeding 75 mm;
- b) Simple calibration: 25-45 mm; 35-55 mm; 55-75 mm;

For the Pre-basic yield; Basic and Certified in all classes, harvested and certified in Romania, the following deviations are admitted (Table 3).

Table 3. Deviations admitted for the certified tuber yield

Pos.	Impurities, defects and pests	Maximum % admitted per weight		
		Pre-basic	Basic Class SE and E, CEE 1, 2 and 3	Certified Class A and B
1	Foreign objects	1.0	1.0	2.0
2	External defects	3.0	3.0	3.0
3	Wet and dry rots, unless caused by <i>Synchytrium endobioticum</i> , <i>Corynebacterium sepedonicum</i> or <i>Pseudomonas solanacearum</i>	0.2	0.5	1.0
4	Common scab	5.0	5.0	5.0
5	Powdery scab	1.0	3.0	3.0
6	Rhizoctoniosis	1.0	5.0	5.0
7	Total deviations from items 2, 3, 4, 5 and 6	5.0	6.0	6.0

For the release of the phytosanitary passport, before the potato delivery by the grower, samples are taken during harvest and conditioning, to determine the presence of quarantine organisms.

CONCLUSIONS

Growing seed potatoes requires a complex approach and can only be carried out by professionals.

Climatic changes and land division led to a decrease in the certified potato areas and to the planting of non-conform material.

The large number of phytosanitary treatments required to prevent and control diseases and pests of the tubers leads to high production costs which oftentimes cannot be reclaimed.

The increase of the average potato yield is conditioned upon the provision of certified planting material.

The extension of the areas requires the reorganisation of the national tubers growing system.

The production of the planting material according to the national, European and international standards cannot be achieved without awareness of the relevant legislation.

Viral diseases lead to yields decreased by up to 80%, therefore seed potato crops are located in areas with low intensity aphid flight.

BIBLIOGRAPHY

- Donescu, D. (2001). *Biologia și ecologia comunităților de afide dăunătoare din culturile de cartof pentru sămânță în contextul protecției integrate*. Doctoral dissertation, USAMV Cluj-Napoca.
- Draica, C., Mike, L., Dima, E.L., Chiru, N., Rosu, R., Molnar, Z. (2004). *Modernizarea tehnologiei de producere a cartofului pentru sămânță*. Editura Phoenix Brasov, ISBN 973-8416-5-9.
- Draica, C. (1980). *Cercetări privind mărimea coeficientului de înmulțire la cartoful pentru sămânță*. Doctoral dissertation, USAMV Cluj-Napoca.
- Man, S. (1975). *Contribuții la producerea cartofului pentru sămânță în zonele închise*. Doctoral dissertation, USAMV Cluj-Napoca.
- Parmentier, A.A. (1786). Lecture read before de Royal Society of Agriculture, France.
- Iacob, V., Ulea, E., Puiu, I. (1998). *Fitopatologie Agricolă*, Editura Ion Ionescu de la Brad, Iasi.
- Law no. 266/2002 on potato growth and processing, quality certification, seed and planting material marketing and cultivar registration. Issuer: The Parliament of Romania, published in the Official Journal no. 343 of 23 May 2002, republished in the Official Journal no. 7 of 4 January 2011, republished in the Official Journal no. 239 of 3 April 2014.
- Commission Decision 2004/842/EC of 01.12.2004. published in <https://eur-lex.europa.eu/eli/dec/2004/842/2017-04-01>
- Order no. 1266/2005 of the Ministry of Agriculture, Forests and Rural Development, approving the rules and technical standards for the production for sale, quality certification and sale of seed potatoes. Issuer: The Ministry of Agriculture, Forests and Rural Development, Published in the Official Journal no. 14 bis of 6 January 2006.
- <http://ephytia.inra.fr/en/C/21040/Potato-Potato-virus-M-PVM>
- <http://kb-dev.gramophone.in/tag/leaf-roll-virus/?lang=en>
- <https://www.agroscope.admin.ch/agroscope/fr/home/actualite/kurznews/2017/livre-PVY.html>
- <https://www.akkerwijzer.nl/artikel/85725-yntn-virus-volop-in-consumptieaardappelen/>
- <https://www.foresstryimages.org/browse/detail.cfm?imgnum=5393019>

EFFECTS OF FOLIAR FERTILIZATION ON GROWTH, DEVELOPMENT AND PRODUCTION OF FLOWERS AND ESSENTIAL OIL ON LAVENDER (*Lavandula angustifolia* Mill.)

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Abstract

The purpose of this study was to determine the effect of foliar fertilization on the growth, development and productivity of lavender Jubileyna variety - the first and second year of cultivation. It is known that the soil and climate conditions are major factors in terms of growth and productivity in lavender. Important for science and practice is to determine the effect of different combinations of nutrients on yield and its components for this essential oil culture. They are used fertilizers with a different composition in terms of macro and micronutrients. The variants of the experiment are four (three leaf fertilizers + untreated controls) and the size of the experimental plot is 500 m². The application of leaf fertilizers is carried out in the budding phase of lavender. The results of some of the products show that foliar fertilization has a significant impact on the growth and productivity of the lavender. Some nutrients help to overcome physiological stress due to adverse weather factors. This leads to the formation of higher yields of flowers and oil of lavender.

Key words: lavender, lavender fertilization, essential oil crops.

INTRODUCTION

Lavender (*Lavandula angustifolia* Mill.) is one of the most widely grown plants with essential oil in the world. Lavender (*Lavandula angustifolia*) is a shrub of the *Lamiaceae* family and originated in the Mediterranean region. The material used for herbal purposes includes lavender flowers (*Lavandula flores*) containing essential oil (3%), anthocyanins, phytosterols, sugars, minerals and tannins. The qualitative and quantitative composition of lavender essential oil is different and depends on the genotype, place of cultivation, climatic conditions, reproduction and morphological features. The essential oil contains over 300 chemical compounds. Dominant components are linalool, linalilacetat, terpinene-4-ol, acetate lavandulol, ocimene and cineole. The essential oil of lavender has a good antioxidant and antimicrobial activity and significant positive effect on the digestive and nervous system. Lavender extract prevents dementia and can inhibit cancer cell growth, while lavender hydrolate is recommended for treating skin problems and burns. Lavender essential oil is widely used in aromatherapy as a holistic relaxant, antioxidant and antimicrobial agent

(Sabara and Kunicka-Styczynska, 2009). The industrial cultivation of lavender (*Lavandula angustifolia* Mill.) and the production of lavender essential oil in Bulgaria has grown rapidly over the last 10 years (Stanev et al., 2013).

The genetic characteristics of plants vary under the influence of many environmental factors (Sevik et al., 2016; Ozel et al., 2015). Probably among the most important of these factors is the content of nutrients in the soil and the methods of fertilization that change soil fertility. Problems arise in the development of plants when soil nutrients are deficient and in this case need to add nutrients to the soil. In this case, the content of nutrients in the soil can be increased by importing various fertilizers. The effect of fertilizers on the yield of flowers and essential oil and its components, is little known. The application of fertilizers and the absorption and accumulation of minerals are some of the most important factors that increase the yield and productivity of plants (Almeida et al., 2015). The production of essential oil in aromatic plants can be influenced positively or negatively by the form, type and quantity of fertilizers (Yadegari, 2015).

According to a Biesiada et al. (2008), the most appropriate level of N for the yield of lavender has an average N application 100 kg N/ha. The author notes the importance of an appropriate ratio of minerals (N: K, N: P) for plant nutrition. In various regions of the world with heavy fertilizer use (given the capacity to retain ions soil), overuse of N leads to groundwater pollution, i.e. nitrates, the most mobile form of N in any ecosystem.

High concentrations of P generally have a positive effect on plant growth parameters. Plants grown at the highest P concentration (70 mg/L P) show the highest biomass of 19.47 g/plant, while plants grown at 40 mg/L P show the lowest biomass, 12.69 on average g/plant. Application of 50 mg/L P keeps and increases root fresh weight and dry matter content, but reduce the ratio of overhead biomass/root system. Furthermore, plant height, length of the leaves and the thickness of the stem have not been influenced by the increased concentration of P in the nutrient solution. The leaf biomass of sage (*S. of inalcinalis* L.) and essential oil content increased with the addition of P fertilizer (Nelletal, 2009), as reported at 70 mg/L P in this study. It has been found that high concentrations of P in *Calendula officinalis* (L.) do not increase the yield of the flowers, but instead produce considerably more biomass of leaves (Stewart and Lovett-Doust, 2003). Lavender plants grown at different concentrations of P represent the following sequencing order of accumulation of macronutrients: N> K> Ca> Mg> P> Na and trace elements: Fe> Al> Mn> B> Zn> Cu. It is reported that the concentrations of P (5, 30 and 60 mg/L) in the solution affect the extraction of essential oil in *O. dictamnus* (Economakisetal., 2002). The application of phosphorus significantly increases the content of basil essential oil, but the fresh and dry weight of the above-ground mass remains unchanged (Ramezanietal., 2009).

Experiments were conducted in order to determine the effect of the amount of potassium (K: 275-300-325-350-375 mg L⁻¹) on the morphological and biochemical characteristics of lavender grown hydroponic (Antonios Chrysargyris et al). The main components of the essential oil from the leaves (1,8-cineol, borneol, camphor, α -terpineol, mirtenal) and

mineral accumulation is influenced by ottetiraniyata K. This study found that lavender treated with 300 mg L⁻¹ of K is suitable for the production of essential oil, while 325 mg L⁻¹ of K is more suitable for growing lavender for flowers use in fresh and dry condition. Minerals such as nitrogen (N), phosphorus (P) and potassium (K) can affect the growth and synthesis of an essential oil in aromatic plants and are used by plants to build many organic compounds such as amino acids, proteins, enzymes and nucleic acids. These mineral elements affect the function and levels of the enzymes involved in terpenoid biosynthesis (Hafsietal, 2014). Monovalent cations such as K, in the activation of enzymes that play a role in helping to substrate binding by lowering the energy barrier and/or transition states, and not causative of causing catalysis (Pageand Di Cera, 2006).

From an reference shows that the effect of the application of mineral fertilizers on the yield of the flowers and essential oil and its constituents is poorly known, as almost no literature regarding the effect of leaf nutrition on lavender.

The aim of this work is to determine the effect of foliar fertilizers on growth, development and productivity in lavender.

MATERIALS AND METHODS

The experiment was carried out during the period 2018-2019 in Agricultural University of Plovdiv with lavender Jubileyna variety. A production experiment with a plot size of 0.05 ha is carried out, with the following scheme: 1) Untreated control; 2) Fertiactyl Trium + Fertileader Vital - 1.5 + 1.5 l/ha; 3) Fertileader Vital - 3 l/ha; 4) Fertileader Alpha - 3 l/ha. The treatments were made in two successive vegetations of lavender. The first was carried out in the first growing year after transplanting, and the second in the following growing season.

Plant material

The lavender plantation was established in November 2017 with certified seedlings of the Bulgarian Jubileyna variety using conventional technology. The number of plants planted per 1 ha is 20000, with an interlinear space 35 cm and inter-row distance of 1.4 m. Due to the

quality of seedlings in the autumn and due to the optimum rainfall during the autumn-winter period of 2017-2018, the plant cover rate was quite high - 96-97%. Jubileyna variety is created through hybridization. There are rounded tufts, up to 56 cm high with about 460 stalks, dark purple flowers. The average yield of fresh flowers is 5540 kg/ha, the content of essential oil in flowers averages 2% and the yield is 52.8.

Used fertilizers

To test the effect of foliar fertilization of lavender were used the following commercial products:

- 1) Fertiaetyl Trium (5% N; 5% P₂O₅; 7% K₂O; 1.5% Zn)
- 2) Fertileader Vital (9% N; 5% P₂O₅; 4% K₂O; 0.02% Fe; 0.01% Mo; 0.05% Zn; 0.1% Mn; 0.05% B)
- 3) Fertileader Alpha (6% N; 12% P₂O₅; 4.2% B)

In November, in both experimental years, the granular product TOP 34 (5% N; 19% P₂O₅; 10% K₂O; 19% SO₃; 0.1% Zn; 0.1% B) is imported to the entire experimental area. The dose is 200 kg/ha. Spring nitrogen feeding is done with the Sulfamo product (25% N; 27% SO₃; 4% MgO). The dose for the first vegetation is 30 kg/ha and for the second one - 60 kg/ha. Leaf fertilizers in corresponding doses are imported in phase budding of lavender.

Soil analyses

Mineral nitrogen (ammonium and nitrate) in extraction with 1% KCl; Mobile phosphates by Egner-Reim method; Digestible potassium in extraction with 2N HCl acid; Soil reaction (pH) - potentiometrically in water extraction.

Plant analyses

The plant samples were taken in the full flowering phase of the inflorescences (95-100%). The fresh flowers are harvested manually, weighed and distilled by steam distillation separately for each variant of the

experiment. For this purpose, a specially adapted device with a capacity of 100 l is used and florentine vessel for separating essential oil from water. The distillation time is 80 minutes in all variants of the experiment.

In the phenological phase of full flowering, biometric parameters were also considered by variants, namely: shrub height (cm), shrub diameter (cm), number of flowering stems, length of inflorescence (cm) and number of flowering vertebrae.

Harvest index

The Harvest index (HI) is an indicator that shows what percentage or what proportion of all raw materials used have been converted into the desired pure product.

Soil and Climatic Characteristics

The soil in the Training-and-Experimental Fields of the Agricultural University of Plovdiv is alluvial-meadow. Geographically the site is located in the Thracian-Strandja region. The alluvial-meadow soils are formed on sandy-loam and sandy-gravel quaternary deposits. According to the International Classification of FAO they refer to Mollic fluvisol. They are formed on alluvial deposits, they have a well-formed humus-accumulative horizon, which gradually passes into C horizon and a gleization process is observed deeply down (below 100 cm) in the soil forming material - the A-C-G profile. The humus content is usually not high - no more than 1-2%.

The contents of the digestible forms of the nutrients nitrogen, phosphorus and potassium, as well as the soil reaction during the two years of the experiment are presented on Table 1.

Based on the generally accepted limits for contents of macronutrients to the soil, it was found that it is poor to average stock with nitrogen and well-stocked with phosphorus and potassium. It is also noteworthy that soil acidity has fallen by almost half a point as a result of intensive fertilization, which must be taken into account when lavender is grown on more acidic soils.

Table 1. Soil reaction, mineral nitrogen content and mobile forms of phosphorus and potassium

Depth, 0-30cm	pH water	NH ₄ -N mg/kg	NO ₃ -N mg/kg	Nmin mg/kg	P ₂ O ₅ mg/100g	K ₂ O mg/100g
2018	7.93	14.0	16.8	30.8	20.0	36.0
2019	7.6	8.30	33.2	41.50	28.2	56.0

The climate in the region of Plovdiv is transitional-continental. Climatic conditions have a decisive influence on productivity in lavender. The total precipitations amount in the January-July period for 2018 and 2019 is 517.4 and 418.9 mm, respectively, which characterizes them as relatively humid compared to the 1971-2000 multi-year period (307.9 mm). In the first experimental year with precipitations above normal are February, May, June and July, which largely coincides with the active growing season and flowering lavender (Figure 1). However, the relatively dry weather during the first two days of June for southern Bulgaria creates favorable conditions for timely and quality color harvesting. The second year is also characterized by precipitation above normal. It was particularly rainy in June with 196mm, which was four times more than the average quantity in the period 1971-2000. This coincided with the period of mass flowering and had an adverse effect on the harvest and on the yield.

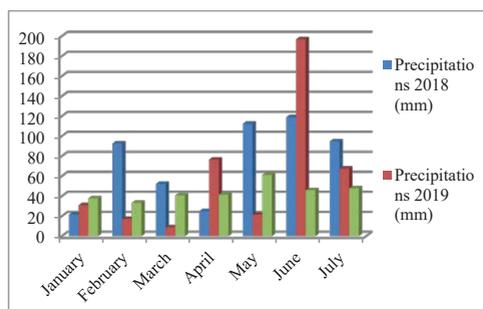


Figure 1. Amount of monthly precipitation during the study period

The reported temperature sums by the months (Figure 2) are similar to those of the multiannual period tended with slightly increase. Because of thermophilic nature of

lavender this is beneficial in terms of accumulating the required temperature sum for the formation of higher yields of essential oil per unit area.

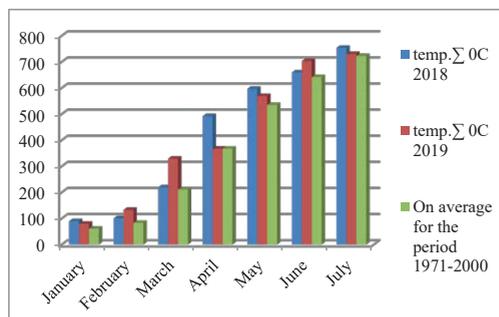


Figure 2. Average monthly temperatures during the study period

RESULTS AND DISCUSSIONS

In both experimental years (2018 and 2019) biometric indicators are established in the mass flowering phase of lavender. Were measured height and diameter of the bush (Table 2) as well as the parameters of the yield - number of floral stems, length of the inflorescence and the number of flowering vertebrae (Table 3).

Table 2. Height and diameter of the shrubs, cm in lavender Jubileyna variety

Variants	Shrub height, cm		Shrub diameter, cm	
	2018	2019	2018	2019
Control variant	23.7	40.4	25.6	64.9
FeriactylTrium+Fertileader Vital	24.9	40.2	25.0	73.7
Fertileader Vital	25.4	40.0	24.3	54.6
Fertileader Alpha	24.9	39.9	30.9	72.0

The results show that the foliar treatment with different products does not significantly influence the height of the lavender plants. However, the diameter of the bush is affected by the use of leaf fertilizers. The variation of this indicator is better expressed in the second

year of the experiment (Table 2) with the variants Feriactyl Trium + Fertileader Vital 1.5 + 1.5 l/ha and Fertileader Alpha - 3 l/ha the width of bushes increases by 13.6 and 10.9% respectively. This is due to the positive influence of these fertilizers in the first year of the experiment and led to the formation of a greater number of branches and, hence, a greater number of flowering stems in these variants (Table 3). It was with Fertileader Alpha and with the combination of Feriactyl Trium + Fertileader Vital that the highest lavender flower yields, respectively, were 6659 and 6556 kg/ha, or 7.2 and 5.6%, respectively more fresh flowers compared to the untreated variant (Table 4).

The positive effect of the Feriactyl Trium + Fertileader Vital combination as well as the Fertileader Alpha leaf preparation is most likely due to the increased amounts of P₂O₅ and K₂O applied to the leaves in the budding phase. Also a positive influence on the second leaf fertilizer turns and the combination of phosphorus and boron. These two elements are synergistic and in the conditions of a more alkaline soil, such as the one in experimental field of Agricultural University - Plovdiv, their impact on the branching and flower formation processes is quite effective.

Table 3. Yield parameters - Number of inflorescences, Length of inflorescences (cm). Number of flowering vertebrae in lavender Jubileyna variety

Variants	Number of inflorescences		Length of inflorescences, cm		Number of flowering vertebrae	
	2018	2019	2018	2019	2018	2019
Control variant	75.6	504.6	6.0	6.6	5	6.4
Feriactyl Trium+Fertileader Vital	88.3	551.7	6.3	6.2	6	6.1
Fertileader Vital	101.8	347.9	6.0	6.2	6	6.0
Fertileader Alpha	95.6	801.3	6.1	6.8	6	6.2

Therefore, adequate foliar application of certain macro and micronutrients during the first year may have an indirect effect on the degree of the flower formation and the yield of fresh flowers during the next growing season of lavender. The length of the inflorescence and the number of flowering vertebrae are not significantly affected by the applied leaf fertilizers. The

results in terms of these indicators have varied over the years of the experiment and no finding can be made as to their values.

The amount of essential oil can be influenced by a number of factors such as genotype, environmental factors, fertilization and more. The time of distillation can also significantly change the yield and composition of the essential oil of lavender (Zheljzakovet et al., 2013).

Table 4. Yield of inflorescences and essential oil in lavender Jubileyna variety, kg/ha

Variants	Inflorescences yield, kg/ha				Essential oil yield, kg/ha			
	2018		2019		2018		2019	
Control variant	703	100%	6209	100 %	15	100%	86	100 %
Feriactyl Trium+Fertileader Vital	910	129.4 %	6556	105.6 %	21	140%	110	127.9%
Fertileader Vital	897	127.6 %	6154	99.1 %	23	153.3 %	59	68.6 %
Fertileader Alpha	880	125.2 %	6659	107.2 %	22	146.7 %	70	81.4 %

From the analysis of the results in Table 4, it is clear that the higher amount of lavender flowers produced does not mean a greater amount of essential oil. Fertilizers applied into the budding phase have a significant effect on the yield of flowers and essential oil during the first vegetation of the lavender. The average increase in flower yield is from 25.2 to 29.4% and that of oil from 40 to 53.3% above the values of the untreated variant. However, a detailed review of the results of the first experimental year shows that despite the lower flower yield (897 kg/ha) of the Fertileader Vital treated plants, 13.3% more essential oil was obtained compared to the Feriactyl Trium + Fertileader Vital combination (910 kg/ha flowers).

During the first vegetation of the lavender, the flowerers are harvested in dry and hot weather conditions, while in the second year the extreme rainfall coincides with the mass flowering and harvesting of the lavender flower (Figure 1). The established yield of fresh flowers in the second experimental year is truly

impressive - from 6209 kg/ha under control to 6659 kg/ha under the Fertileader Alpha variant. However, significant amount of precipitations has a clear negative impact on the oil formation process. From the results presented in Table 4 it can be seen that in the Fertileader Alpha and Fertileader Vital lavender plants treated, the yield of essential oil is significantly lower than that of the untreated variant. The exception is made only combination Feriactyl Trium + Fertileader Vital. The combined foliar application of these two fertilizers causes an increase in oil yield of 27.9% (24 kg/ha) above the control values. This is most likely due to the increased potassium content whose total amount for this combination was 11%.

Therefore, it can be stated that in conditions of adverse environmental factors such as increased humidity and lower temperatures, the foliar application of higher concentrations of potassium plays the role of an anti-stress factor and leads to a more optimal flow of the lavender oil-forming process.

The Harvest index (HI) is an indicator that shows what percentage or what proportion of all raw materials used have been converted into the desired pure product. In our case, this indicator shows how much kilograms of flowers are required to produce one kilogram of lavender essential oil (Figure 3). The analysis of the data shows that the yield is influenced by both environmental factors (humidity, temperature, wind during harvest, etc.) and the fertilizers applied during budding. It can be seen that in the conditions of quiet, hot, dry and sunny weather during flowering and flower harvesting in 2018, the Harvest index on the variants treated with Fertileader Alpha and Fertileader Vital is below 40 (39.1 and 39.4). In unfavorable for flowering and synthesis of essential oil 2019 required raw material for the production of one kilogram of lavender oil for the variant Fertileader Vital was over 100 kg. However, the application of the Feriactyl Trium + Fertileader Vital combination gives a significant reduction in the amount of flowers (59.6) required to produce one kilogram of essential oil.

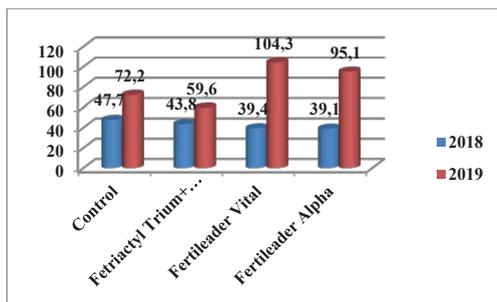


Figure 3. Harvest index for treated variants (2018 and 2019 year)

CONCLUSIONS

The application of Feriactyl Trium + Fertileader Vital 1.5 + 1.5 l/ha and Fertileader Alpha - 3 l/ha leaf fertilizers increased by 13.6% and 10.9%, respectively. This is due to the positive effect of these fertilizers during the first year of the experiment and consequently leads to the formation of a greater number of branches and, hence, a greater number of flower stalks in these variants.

Fertileader Alpha and the combination of Feriactyl Trium + Fertileader Vital have the highest lavender flower yields of 6659 and 6556 kg/ha, respectively, with 7.2 and 5.6% more fresh flowers than the untreated variant.

Appropriate foliar application of certain macro and micronutrients during the first year may have an indirect effect on the degree of flower formation and the yield of fresh flowers during the next growing season of lavender.

The leaf fertilizers applied in the budding phase have a significant effect on the yield of flower and essential oil during the first vegetation of the lavender. The average increase of flower yield is from 25.2 to 29.4% and oil from 40 to 53.3% above the values of the untreated variant.

Simultaneous foliar application of Feriactyl Trium + Fertileader Vital results in an increase in oil yield of 27.9% (24 kg/ha) above the control values under unfavourable flowering and oil-forming conditions. This is probably due to the increased potassium content, whose total amount in this combination is 11%. Therefore, it may be considered that, in adverse environmental conditions, the foliar application of higher potassium concentrations plays the

role of an anti-stress factor and leads to a more optimal flow of lavender oil synthesis.

REFERENCES

- Almeida, H.J., Pancelli, M.A., Prado, R.M., Cavalcante, V.S., Cruz, F.J.R. (2015). Effect of potassium on nutritional status and productivity of peanuts in succession with sugarcane. *J. Soil Sci. Plant Nutr.*, 15(1), 1–10.
- Antonios, Ch., Chrissyola. D., Nikos, T. (2017). Optimization of potassium fertilization/nutrition for growth, physiological development, essential oil composition and antioxidant activity of *Lavandula angustifolia* Mill. *Journal of Soil Science and Plant Nutrition*, 17(2), 291–306.
- Biesiada, A., Sokol-Letowska, A., Kucharska, A. (2008). The effect of nitrogen fertilization on yield- ing and antioxidant activity of lavender (*Lavandula angustifolia* Mill.). *Acta Sci. Pol.*, 7, 33–40.
- Economakis, C., Skaltsa, H., Demetzos, C., Sokovic', M.C.A. (2002). Effect of phosphorus concentration of the nutrient solution on the volatile constituents of leaves and bracts of *Origanum dictamnus*. *J. Agric. Food Chem.*, 50, 6276–6280.
- Hafsi, C., Debez, A., Abdelly, C. 2014. Potassium deficiency in plants: effects and signaling cascades. *Acta Physiol. Plant.*, 36, 1055–1070.
- Nell, M., Votsch, M., Vierheilig, H., Steinkellner, S., Zitterl-Eglseer, K., Franz, C., Novak, J., 2009. Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). *J. Sci. Food Agric.*, 89, 1090–1096.
- Ozel, H.B., Kirdar, E., Bilir, N. (2015). The effects of magnetic field on germination of the seeds of oriental beech (*Fagus orientalis* Lipsky.) and growth of seedlings. *Agriculture & Forestry/Poljoprivreda i Sumarstvo*, 61(3).
- Page, M.J., Di Cera, E. (2006). Role of Na⁺ and K⁺ in enzyme function. *Physiol. Rev.*, 86, 1049–1092.
- Ramezani, S., Rezaei, M.R., Sotoudehnia, P. (2009). Improved growth, yield and essential oil content of basil grown under different levels of phosphorus sprays in the field. *J. Appl. Biol. Sci.*, 3, 105–110.
- Sabara D, Kunichka-Styczynska, A. (2009). Lavender oil – flavouring or active cosmetic ingredient? *Food Chemistry and Biotechnology*, 73, 33–40.
- Sevik, H., Cetin, M., Kapucu, Ö. (2016). Effect of Light on Young Structures of Turkish Fir (*Abies nordmanniana* subsp. bornmulleriana). *Oxidation Communications*, 39(1–II), 485–492.
- Stanev, S, Zagorcheva, T., Atanassov, I. (2013). Industrial cultivation of *Lavandula angustifolia* and lavender oil production in Bulgaria in the 21st century: challenges and possible molecular genetics and breeding solutions. *Biotechnology and Biotechnological Equipment* (in press).
- Stewart, C.L.S., Lovett-Doust, L. (2003). Effect of phosphorus treatment on growth and yield in the medicinal herb *Calendula officinalis* L. (Standard Pacific) under hydroponic cultivation. *Can. J. Plant Sci.*, 4, 611–617.
- Valtcho D, Zheljazkov, Charles L. Cantrell, Tess Astatkie and Ekaterina Jeliazkova, Distillation Time Effect on Lavender Essential Oil Yield and Composition. *Journal of Oleo Science*, 62(4), 195–199.
- Yadegari, M. 2015. Foliar application of micronutrients on essential oils of borago, thyme and mari- gold. *J. Soil Sci. Plant Nutr.*, 15(4), 949–964.

BIOLOGICAL EFFICACY AND SELECTIVITY OF HERBICIDES FOR BROADLEAF WEEDS CONTROL IN MAIZE (*Zea mays* L.)

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Abstract

In 2017 and 2018 a field trial with the maize hybrid "P 9537" on the experimental field of the Agricultural University of Plovdiv, Bulgaria was conducted. The following herbicide products were evaluated: Kabadex Extra (267 g/l mesotrione + 16.7 g/l florasulam), Starane Gold SE (1 g/l florasulam + 100 g/l fluroxypyr), Derby Super WG (300 g/kg aminopyralide-potassium + 150 g/kg florasulam), Mustang 306,25 SC (300 g/l 2,4D + 6.25 g/l florasulam), Casper 55 WG (500 g/kg dicamba + 50 g/kg prosulfuron) and Arat WG (500 g/kg dicamba + 250 g/kg tritosulfuron). The application of Kabadex Extra + Dasoil, Derby Super WG (in rates of 0.033 kg ha⁻¹ alone, 25 and 0.033 kg ha⁻¹ + Dasoil), as well as Starane Gold in rate of 1.5 l ha⁻¹ caused low phytotoxicity symptoms for the crop. The highest maize yield (984.19 kg da⁻¹) as well as the highest herbicide efficacy against *Chenopodium album* L., *Amaranthus blitoides* L., *Xanthium strumarium* L., *Abutilon theophrasti* Medic., *Datura stramonium* L. and *Solanum nigrum* L. after the application of Kabadex Extra in rate of 0.033 l ha⁻¹ + Dasoil -1.0 l ha⁻¹ were found.

Key words: maize, weeds, herbicides, selectivity, efficacy.

INTRODUCTION

To obtain high yields from the spring crops it is necessary to apply all agro technical measures effectively.

Such measures are proper crop, rotation, combine fertilization, effective pest control and especially efficient weed control (Dimitrova et al., 2019; Tonev et al., 2018; Neshev and Manolov, 2016; Manolov et al., 2015; Hristeva et al., 2014; Tonev et al., 2007). Weeds occur everywhere every year and cause enormous damage to cultivated plants, reducing yields and the production quality (Kostadinova et al., 2016; Kalinova and Yanev, 2015; Yanev, 2015; Yanev et al., 2014a; Yanev et al., 2014b; Tonev et al, 2007).

Studies by a number of authors show that, depending on the type and degree of weed infestation, the maize grain yield may be decreased from 24% to 96.7% (Dimitrova et al., 2013; Mukherjee and Debnath, 2013; Najafi and Tollenar, 2005; Oerke and Dehne, 2004; Khan et al., 2003; Zhaltov and Raikov, 1996)

A great number of weed species infest the maize fields. Some of the most distributed are *Amaranthus* spp., *Chenopodium album* L., *Abutilon theophrasti* Medik., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., etc (Tonev

et al., 2011; Tonev T., 2008; Nikolov et al., 2005; Mousavi, 2001).

Today, high-yield agriculture is highly dependent on herbicides as they are a vital and integral component of weed control practices (Goranovska and Yanev, 2016; Rao, 2000).

In maize, chemical weed control is usually done using broad-leaved and soil herbicides. In recent years weed control in maize with post emergence herbicide application is increased (Whaley et al., 2006; Airoldi, 2000) because of several reasons: 1. Restrictions on the use of terbutylazine; 2. Low efficacy of soil herbicides applied before germination because of not enough soil moisture; 3. Spread of triazine resistant weeds; 4. Introduction of effective broad-spectrum post-emergence herbicides. Considering the herbicides used in monoculture cultivation of maize, mesotrione is an interesting triketone that inhibits the HPPD enzyme (p-hydroxyphenyl pyruvate decarboxylase) and provides control of the major annual broad leaf weeds (Armel et al., 2003a; Johnson et al., 1999). Mesotrione provides a useful and flexible addition to the products already available, as it allows good control of some noxious weeds (Sutton et al., 2002).

Phytotoxicity data indicate that mesotrione can be considered relatively safe for the crop,

which appears to be significantly related to herbicide doses. However, the symptoms of phytotoxicity were always transient and dissipated after 4-5 weeks, without reducing maize grain yields. Similar results are obtained by Whaley et al. (2006), Stephenson et al. (2004) and Waltz et al. (1999). Nicosulfuron, for example is a postemergence sulfonylurea herbicide that even in low rates can control many difficult-to-control weeds at maize (Green and Hale, 2005). Integrating the intercrop tillage with contemporary herbicides at maize is a perspective way for obtaining high efficacy of weed control and decreasing the harmful after-effect of the products for plant protection (Ljubenov, 1988).

Travlos and Apostolidis (2017) found that Lancelot 450 WG (aminopyralid 300 g/kg + florasulam 150 g/kg) could be proposed as a very efficient herbicide for the control of the major broadleaf weeds, as well as alien and invasive species in the maize crop.

In a two year study conducted by Arnold et al. (2005) it was found that when nicosulfuron plus rimsulfuron and foramsulfuron were applied in combination with diflufenzopyr plus dicamba, dicamba plus atrazine, mesotrione, or dicamba the control of broadleaf weeds increased significantly.

The objective of the study is to determine the efficacy and selectivity of combined herbicides for broadleaf weeds control in maize.

MATERIALS AND METHODS

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 4 replications. In both experimental years the grown maize hybrid was P9537. The maize was grown as monoculture under irrigated conditions. The size of the experimental plot is 28 m². The study included the following treatments: 1. Untreated control; 2. Kabadex Extra (267 g/l mesotrione + 16.7 g/l florasulam) - 0.3 l ha⁻¹; 3. Kabadex Extra + Dasoil (adjuvant) - 0.3 l ha⁻¹ + 1.0 l ha⁻¹; 4. Starane Gold SE (1 g/l florasulam + 100 g/l fluroxypyr) - 1.2 l ha⁻¹; 5. Starane Gold SE - 1.5 l ha⁻¹; 6. Derby Super WG (300 g/kg

aminopyralide-potassium + 150 g/kg florasulam) - 0.025 kg ha⁻¹; 7. Derby Super WG - 0.033 kg ha⁻¹; 8. Derby Super WG + Dasoil - 0.025 kg ha⁻¹ + 1.0 l ha⁻¹; 9. Derby Super WG + Dasoil - 0.033 kg ha⁻¹ + 1.0 l ha⁻¹; 10. Mustang 306,25 SC (300 g/l 2,4D + 6.25 g/l florasulam) - 0.5 l ha⁻¹; 11. Casper 55 WG (500 g/kg dicamba + 50 g/kg prosulfuron) - 0.3 kg ha⁻¹; 12. Arat WG (500 g/kg dicamba + 250 g/kg tritosulfuron) - 0.2 kg ha⁻¹.

The herbicides were applied in 3rd-5th true leaf stage of maize (BBCH 13-14). The size of the spraying solution was 250 l ha⁻¹.

On the trial field deep ploughing, two times disc harrowing and two times cultivation before sowing were done. On the whole experimental area basic combine fertilization with 250 kg ha⁻¹ NPK (15:15:15) and dressing with 300 kg ha⁻¹ NH₄NO₃ was performed. The efficacy of the herbicides was recorded by visual scale of EWRS (European Weed Research Society) on the 14th, 28th and 56th day after application. The selectivity of the studied herbicide products was evaluated by the 9-score scale of EWRS - at score 0 - no damage on the crop and at score 9 - Severe damage up to complete destruction of the crop is found. The maize grain yield is determined by harvesting the whole experimental plot of each treatment in all four replications. On the trial field 6 dicotyledonous weeds typical for the maize crop were found. Their average density per 1 m² was as follows: *Chenopodium album* L. - 39 specimens; *Amaranthus blitoides* L. - 9 specimens; *Xanthium strumarium* L. - 11 specimens; *Abutilon theophrasti* Medic. - 7 specimens; *Datura stramonium* L. - 6 specimens and *Solanum nigrum* L. - 21 specimens or 93 weeds per 1 m² in total.

Statistical analysis for the yield data was performed by using Duncan's multiple range test by the software SPSS 19. Statistical differences were considered proved at p<0.05.

RESULTS AND DISCUSSIONS

On Table 1 is shown the dynamics for the efficacy of the studied herbicide products against the weed *Ch. album* average for both years of the study. Average for the period, on the 14th day after treatments 100% efficacy only for the combine application of Kabadex

Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹ was recorded. After the application of Casper 55 WG - 0.3 kg ha⁻¹ and Arat WG - 0.2 kg ha⁻¹ the efficacy on the 14th day after treatments was 90% and 95%, respectively. Lower efficacy for the treatments with Kabadex Extra - 0.3 l ha⁻¹, Derby Super WG + Dasoil - 0.033 kg ha⁻¹ + 1.0 l ha⁻¹ and Mustang 306,25 SC - 0.5 l ha⁻¹ was recorded - 75-85%. For the other treatments the efficacy was very low and varied from 10 to 55%. On the last reporting date (56th day after application) the herbicide efficacy increased and reached 100% for treatments Kabadex Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹, Casper 55 WG - 0.3 kg ha⁻¹ and Arat WG - 0.2 kg ha⁻¹. The other two treatments with satisfactory efficacy on the 56th day were Derby Super WG + Dasoil - 0.033 kg ha⁻¹ + 1.0 l ha⁻¹ and Mustang 306,25 SC - 0.5 l ha⁻¹ - 95%.

Table 1. Efficacy of the evaluated herbicide products against *Ch. album* average for 2018-2019 (%)

Treatments	Days after treatment		
	14 th	28 th	56 th
1. Untreated control	-	-	-
2. Kabadex Extra - 0.3 l ha ⁻¹	75	75	85
3. Kabadex Extra + Dasoil - 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
4. Starane Gold SE - 1.2 l ha ⁻¹	40	60	60
5. Starane Gold SE - 1.5 l ha ⁻¹	50	70	75
6. Derby Super WG - 0.025 kg ha ⁻¹	10	40	40
7. Derby Super WG - 0.033 kg ha ⁻¹	25	50	70
8. Derby Super WG + Dasoil - 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	55	70	85
9. Derby Super WG + Dasoil - 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	85	90	95
10. Mustang 306,25 SC - 0.5 l ha ⁻¹ ;	80	90	95
11. Casper 55 WG - 0.3 kg ha ⁻¹	90	95	100
12. Arat WG - 0.2 kg ha ⁻¹	95	100	100

Table 2. Efficacy of the evaluated herbicide products against *A. blitoides* average for 2018-2019 (%)

Treatments	Days after treatment		
	14 th	28 th	56 th
1. Untreated control	-	-	-
2. Kabadex Extra - 0.3 l ha ⁻¹	100	100	100
3. Kabadex Extra + Dasoil - 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
4. Starane Gold SE - 1.2 l ha ⁻¹	50	80	90
5. Starane Gold SE - 1.5 l ha ⁻¹	50	85	95
6. Derby Super WG - 0.025 kg ha ⁻¹	50	80	90
7. Derby Super WG - 0.033 kg ha ⁻¹	60	85	95
8. Derby Super WG + Dasoil - 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
9. Derby Super WG + Dasoil - 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
10. Mustang 306,25 SC - 0.5 l ha ⁻¹ ;	100	100	100
11. Casper 55 WG - 0.3 kg ha ⁻¹	100	100	100
12. Arat WG - 0.2 kg ha ⁻¹	100	100	100

On Table 2 is shown the efficacy of the studied herbicides against *A. blitoides*. On the 14th day after the herbicide application unsatisfactory efficacy only for the products Starane Gold SE - 1.2 l ha⁻¹, Starane Gold SE - 1.5 l ha⁻¹, Derby Super WG - 0.025 kg ha⁻¹ and Derby Super WG - 0.033 kg ha⁻¹ was achieved - 50-60%. On the next evaluation dates the efficacy for these treatments increased and reached 90-95% on the 56th day. In all other variants 100% efficacy in the three reporting dates was observed.

In the filed crop rotation, one of the most difficult to control late spring weeds is *Xanthium strumarium* L. (Tonev et al., 2007; Tonev et al., 2011). All studied herbicide products in the study showed excellent control of this difficult-to-control dicotyledonous weed species. The results are shown on Table 3. Although the lower efficacy of Starane Gold SE - 1.2 l ha⁻¹ and Starane Gold SE - 1.5 l ha⁻¹ on the 1st evaluation date - 80%, on the last reporting date the efficacy reached 95%. All other treatments showed excellent efficacy against this weed.

Table 3. Efficacy of the evaluated herbicide products against *Xa. strumarium* average for 2018-2019 (%)

Treatments	Days after treatment		
	14 th	28 th	56 th
1. Untreated control	-	-	-
2. Kabadex Extra - 0.3 l ha ⁻¹	90	95	100
3. Kabadex Extra + Dasoil - 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
4. Starane Gold SE - 1.2 l ha ⁻¹	80	90	95
5. Starane Gold SE - 1.5 l ha ⁻¹	80	90	95
6. Derby Super WG - 0.025 kg ha ⁻¹	90	100	100
7. Derby Super WG - 0.033 kg ha ⁻¹	95	100	100
8. Derby Super WG + Dasoil - 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
9. Derby Super WG + Dasoil - 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
10. Mustang 306,25 SC - 0.5 l ha ⁻¹ ;	100	100	100
11. Casper 55 WG - 0.3 kg ha ⁻¹	100	100	100
12. Arat WG - 0.2 kg ha ⁻¹	100	100	100

The weed *A. theophrasti* was relatively resistant to some of the tested herbicide products of the experiment in both trial years (Table 4). After the application of Kabadex Extra - 0.3 l ha⁻¹, Kabadex Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹ and Casper 55 WG - 0.3 kg ha⁻¹ the efficacy of 100% was found on the three reporting dates. The application of Derby Super WG + Dasoil - 0.033 kg ha⁻¹ + 1.0 l ha⁻¹ showed 95% efficacy on the 14th day after application, but its efficacy also reached 100%

on the next reporting dates. The other treatments had lower efficacy that varied from 80 to 95% on the 56th day after treatments.

Table 4. Efficacy of the evaluated herbicide products against *A. theophrasti* average for 2018-2019 (%)

Treatments	Days after treatment		
	14 th	28 th	56 th
1. Untreated control	-	-	-
2. Kabadex Extra – 0.3 l ha ⁻¹	100	100	100
3. Kabadex Extra + Dasoil – 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
4. Starane Gold SE – 1.2 l ha ⁻¹	50	70	80
5. Starane Gold SE – 1.5 l ha ⁻¹	50	75	85
6. Derby Super WG – 0.025 kg ha ⁻¹	60	85	85
7. Derby Super WG – 0.033 kg ha ⁻¹	75	90	90
8. Derby Super WG + Dasoil – 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	90	90	95
9. Derby Super WG + Dasoil – 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	95	100	100
10. Mustang 306,25 SC – 0.5 l ha ⁻¹ ;	60	75	85
11. Casper 55 WG – 0.3 kg ha ⁻¹	100	100	100
12. Arat WG – 0.2 kg ha ⁻¹	80	85	85

Against the weed *D. stramonium* the efficacy of Starane Gold SE in both evaluated rates as well as that of Mustang 306,25 SC - 0.5 l ha⁻¹ was 70-80% on the 14th day after herbicide application. The efficacy against the concrete weed data is shown on Table 5. The efficacy of these treatments was increased on the next reporting days and reached 95-100% on the 56th day. For all other treatments, the obtained efficacy was 100% on the three reporting dates.

Table 5. Efficacy of the evaluated herbicide products against *D. stramonium* average for 2018-2019 (%)

Treatments	Days after treatment		
	14 th	28 th	56 th
1. Untreated control	-	-	-
2. Kabadex Extra – 0.3 l ha ⁻¹	100	100	100
3. Kabadex Extra + Dasoil – 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
4. Starane Gold SE – 1.2 l ha ⁻¹	70	85	95
5. Starane Gold SE – 1.5 l ha ⁻¹	70	95	95
6. Derby Super WG – 0.025 kg ha ⁻¹	100	100	100
7. Derby Super WG – 0.033 kg ha ⁻¹	100	100	100
8. Derby Super WG + Dasoil – 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
9. Derby Super WG + Dasoil – 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
10. Mustang 306,25 SC – 0.5 l ha ⁻¹ ;	85	95	100
11. Casper 55 WG – 0.3 kg ha ⁻¹	100	100	100
12. Arat WG – 0.2 kg ha ⁻¹	100	100	100

The efficacy results against the weed *S. nigrum* are presented on Table 6. The treatments from 2 to 9 had efficacy reaching 90%-100% against this weed. On the first reporting date the efficacy of Mustang 306,25 SC - 0.5 l ha⁻¹ was

70%, but on the next two days of reporting the efficacy reached 90%. The application of Casper 55 WG - 0.3 kg ha⁻¹ and Arat WG - 0.2 kg ha⁻¹ low efficacy ranging from 55 to 75% on the tree dates of evaluation.

Table 6. Efficacy of the evaluated herbicide products against *S. nigrum* average for 2018-2019 (%)

Treatments	Days after treatment		
	14 th	28 th	56 th
1. Untreated control	-	-	-
2. Kabadex Extra – 0.3 l ha ⁻¹	100	100	100
3. Kabadex Extra + Dasoil – 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
4. Starane Gold SE – 1.2 l ha ⁻¹	90	95	100
5. Starane Gold SE – 1.5 l ha ⁻¹	100	100	100
6. Derby Super WG – 0.025 kg ha ⁻¹	100	100	100
7. Derby Super WG – 0.033 kg ha ⁻¹	100	100	100
8. Derby Super WG + Dasoil – 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
9. Derby Super WG + Dasoil – 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	100	100	100
10. Mustang 306,25 SC – 0.5 l ha ⁻¹ ;	70	90	90
11. Casper 55 WG – 0.3 kg ha ⁻¹	55	60	60
12. Arat WG – 0.2 kg ha ⁻¹	70	75	75

Regarding the selectivity of the studied herbicide products it was found that some of them caused temporary phytotoxicity. For the treatments with Kabadex Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹, Derby Super WG - 0.033 kg ha⁻¹ and 8. Derby Super WG + Dasoil - 0.025 kg ha⁻¹ + 1.0 l ha⁻¹ the visual phytotoxicity on the 14th day after the herbicide application was found to be score 1. For the treatments with Starane Gold SE - 1.5 l ha⁻¹ and Derby Super WG + Dasoil - 0.033 kg ha⁻¹ + 1.0 l ha⁻¹ the phytotoxicity was score 2. The phytotoxicity symptoms were expressed in twisting of the leaves of single maize plants and in stunting the growth of the crop. On the 28th day after application the phytotoxicity was completely overcome. In all other variants visual symptoms of phytotoxicity were not observed. On Table 7 are presented the results for the obtained maize grain yield average for the period of the study. The differences in yields are determined by the herbicidal efficacy of the products and by their ability to control the weeds available in the experiment. The natural weed infestation with highly competitive weed species for 2017 and 2018 resulted in the lowest average yield for untreated control - 616.34 kg da⁻¹).

Table 7. Maize grain yield average for 2017 and 2018

Treatments	Yields, kg da ⁻¹
1. Untreated control	616.34 a
2. Kabadex Extra – 0.3 l ha ⁻¹	855.60* d
3. Kabadex Extra + Dasoil – 0.3 l ha ⁻¹ + 1.0 l ha ⁻¹	984.19* g
4. Starane Gold SE – 1.2 l ha ⁻¹	758.13* b
5. Starane Gold SE – 1.5 l ha ⁻¹	760.49* b
6. Derby Super WG – 0.025 kg ha ⁻¹	766.91* b
7. Derby Super WG – 0.033 kg ha ⁻¹	808.06* c
8. Derby Super WG + Dasoil – 0.025 kg ha ⁻¹ + 1.0 l ha ⁻¹	895.75* e
9. Derby Super WG + Dasoil – 0.033 kg ha ⁻¹ + 1.0 l ha ⁻¹	940.08* f
10. Mustang 306,25 SC – 0.5 l ha ⁻¹ ;	851.60* d
11. Casper 55 WG – 0.3 kg ha ⁻¹	898.51* e
12. Arat WG – 0.2 kg ha ⁻¹	854.14* d

Legend: All values with a * sign have significant differences with the result of the untreated control. All values followed by different letters are with proved difference according to Duncan's test at P < 0.05.

In terms of mathematical proof, seven distinct groups of herbicides are distinguished (a, b, c, d, e, f, g). Here is also noted that the treatment with Kabadex Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹ is from group - g, and the most distinct group is for the untreated control - a. That means that this treatment has the highest yield.

CONCLUSIONS

The highest herbicide efficacy against all weeds in the study after the application of Kabadex Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹ was recorded

The herbicide product Starane Gold SE in both evaluated rates showed lower efficacy against *Xa. strumarium* and *D. stramonium* in comparison to the other evaluated herbicide products in the study.

The addition of the adjuvant Dasoil to the herbicide product Derby Super WG applied in rates of 0.025 and 0.033 kg ha⁻¹ increased the efficacy against the weeds *Ch. album*, *A. blitoides* and *A. theophrasti*.

The application of Kabadex Extra + Dasoil - 0.3 l ha⁻¹ + 1.0 l ha⁻¹, 7. Derby Super WG - 0.033 kg ha⁻¹, Derby Super WG + Dasoil - 0.025 kg ha⁻¹ + 1.0 l ha⁻¹, Derby Super WG + Dasoil - 0.033 kg ha⁻¹ + 1.0 l ha⁻¹ and Starane Gold SE - 1.5 l ha⁻¹ caused temporary phytotoxicity that was completely overcome.

Mathematically proven differences in maize grain yield were reported in favor of all herbicide-treated variants versus the untreated control.

The highest maize grain yield after the application of Kabadex Extra in rate of 0.033 l ha⁻¹ + Dasoil – 1.0 l ha⁻¹ was recorded - 984.19 kg da⁻¹.

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REFERENCES

- Airoldi, M. (2000). Lottimizzazione del diserbo del mais. *Proceedings of the 12st Convegno Societa Italiana per la Ricerca sulla Flora Infestante, 5-6 December 2000, Milano, Italy*, 93–109.
- Armel, G., Wilson, H., Richardson, R., Hines, T. (2003). Mesotrion alone and in mixtures with glyphosate in glyphosate-resistant corn (*Zea mays*), *Weed Technoogy*, 17, 680–685.
- Arnold, R., Smeal, D., O'Neill, M. (2005). Broadleaf weed control in field corn with postemergence herbicides. *Crop Management*. doi:10.1094/CM-2005-0224-01-RS.
- Dimitrova M., Minev N., Yordanova N., Valcheva V., Yanev M. (2019). Effect of planting density of different maize hybrids on crop growth and yield. *Scientific Papers. Series A. Agronomy*, LXII(2), 73–76.
- Dimitrova, M., Dimova, D., Zhaltov, Iv., Zovorski, P., Zhelyazkov, I., Valcheva, E., Popova, R. (2013). The influence of new herbicides on the growth and the some structural elements of the yield of fodder maize. *Scientific Papers. Series A. Agronomy*, LVI.
- Goranovska, S., Yanev, M. (2016). Economic efficiency of the chemical control of the weeds in maize. *Proceedings of Science-Technical Conference with International Participatipon - Ecology and Health*, 82–85.
- Hristeva Ts, Yanev, M., Kalinova, Sht., Bozukov, H. (2014). Comparative Analysis of Some Herbicides from Amide and Dinitroaniline Families on the Soil Microorganisms. *Turkish Journal of Agricultural and Natural Sciences, Special Issue*, 2, 1447–1454.
- Green, J., Hale, T. (2005). Increasing and decreasing pH to enhance the biological activity of nicosulfuron. *Weed Technology*, 19(2), 468–475.
- Jonson, J., Wait, J., Holman, C. (1999). Mesotrione programs. *N. Cent. Weed Sci. Soc., Res., Rep.*, 56, 225–226.
- Kalinova, Sht., Yanev, M. (2015). Influence of soil herbicides on technological parameters of oriental tobacco. *Scientific Works of the Agricultural University of Plovdiv*, LIX(3), 65–70.
- Khan, M., Marwat, K., Khan, N. (2003). Efficacy of different herbicides on the yield and yield components of maize. *Asian J. Plant Sci.*, 2(3), 300–304.

- Kostadinova, S., Kalinova, Sht., Yanev, M. (2016). Sunflower productivity in response to herbicide diflufenican (Pelican 50 SC) and foliar fertilizing. *Agriculture & Food*, 4, 122–128.
- Ljubenov, Ya. (1988). Integrated Weed Control – 1st and 2nd Volume. Publisher “Zemdzat” Sofia (In Bulgarian).
- Manolov, I., Neshev, N., Chalova, V., Yordanova, N. (2015). Influence of potassium fertilizer source on potato yield and quality. *Proceedings. 50th Croatian and 10th International Symposium on Agriculture, Opatija, Croatia*, 363–367.
- Mousavi, M. (2001). Integrated Weed Management: Principles and Methods *Meiad Press*. (first edition).
- Mukherjee, P., Debnath, P. (2013). Weed control practices in maize (*Zea mays* L.) under conventional and conservation tillage practices. *Proceedings of the 24th Asian-Pacific Weed Science Society Conference, Bandung, Indonesia, October 22-25, Bandung: Weed Science Society of Indonesia*, 302–311.
- Najafi, H., Tollenaar, T. (2005). Response of corn at different leaf stages to shading by redrat pigweed (*Amaranthus retroflexus* L.). *Iranian J. Weed Sci.*, 1, 127–140.
- Neshev, N., Manolov, I. (2016). Effect of Fertilization on Soil Fertility and Nutrient Use Efficiency at Potatoes. *Geophysical Research Abstracts*, 18, 139. EGU General Assembly.
- Nikolov, P., Baeva, G., Milanova, S., Nakova, R., Chavdarov, L., Velichkov, A., Balchev, B., Maneva, S., Tonev, T., Dimitrova, M. (2005). Weed diversity assessment in different regions in Bulgaria. *Proceedings of I-st Congress of Plant Protection – Environmental concern and food safety – Ohrid, 28.XI.-2.XII*.
- Oerke, E., Dehne, H. (2004). Safequarding production–losses in major crops and the role of crop protection. *Crop Protection*, 23, 275–285.
- Rao, V. (2000). Principles of Weed Science (Second ed.) *Science Publishers, Inc., New Hampshire*.
- Stephenson, D., Bond, J., Walker, E., Bararpour, M., Oliver, R. (2004). Evaluation of mesotrione in Mississippi delta corn production. *Weed Technology*, 18, 1111–1116.
- Sutton, P., Richards, C., Buren, L., Glasgow, L. (2002). Activity of mesotrion on resistant weeds in maize. *Pest Manag. Sci.*, 58, 981–984.
- Tonev T., Tityanov, M., Vassilev, A. (2011). Guide to integrated weed management and proficiency in agriculture. Publisher “Biblioteka Zemedelsko Obrazovanie” (in English).
- Tonev, T. (2008). Problems and challenges of weed control in Bulgaria. *Proceedings of Symposium on Weed Science in Eastern Europe Held on February 4 and 5, in Bucharest, Romania*.
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Spasov, V. (2007). Herbology. *Academic publisher of the Agricultural University of Plovdiv*. (in Bulgarian)
- Tonev, T., Tityanov, M., Mitkov, A., Yanev, M., Neshev, N. (2018). A Guidebook for Exercises on General Agriculture and Herbology. Publisher: “Biblioteka Zemedelsko Obrazovanie” (Guidebook in English).
- Travlos, I., Apostolidis, V. (2017). Efficacy of the Herbicide Lancelot 450 WG (Aminopyralid + Florasulam) on Broadleaf and Invasive Weeds and Effects on Yield and Quality Parameters of Maize. *Agriculture*, 7, 82. doi:10.3390/agriculture7100082.
- Waltz, A., Martin, A., Spotanski, J. (1999). Weed control with ZA-1296 in field corn at Lincoln, NE in 1999. *N. Cent. Weed Sci. Soc., Res. Rep.*, 56, 228–230.
- Whaley, C., Armel J., Wilson, H., Hines, T. (2006). Comparison of mesotrione combinations with standard weed control programs in corn. *Weed Technology*, 20, 605–611.
- Yanev, M., Bozukov, H., Kalinova, Sht. (2014a). Distribution of *Orobanche ramosa* L. and *Orobanche mutellii* Sch. in the Main Tobacco Producing Regions of Bulgaria. *Plant Science*, LI(1), 114–117.
- Yanev, M., Kalinova, Sht., Bozukov, H., Tahsin, N. (2014b). Technological Indexes of Oriental Tobacco Treated with Glyphosate for the Control of Broomrape. *Turkish Journal of Agricultural and Natural Sciences, Special Issue, 1*, 1025–1029.
- Yanev, M. (2015). Study on weed infestation of tobacco fields in South Bulgaria. *Plant Science*, LII(3), 90–95.
- Zhalnov, I., Raikov, S. (1996). Influence of different infestation levels of *Sorghum halepense* L. on maize development. *Plant Science*, XXXIII(8), 64–66.

AGRONOMIC PERFORMANCE OF TRITICALE VARIETIES (× *Triticosecale* Wittm.) GROWN UNDER FERTILIZATION WITH ORGANIC MANURE FROM RED CALIFORNIAN WORMS (*Lumbricus rubellus*)

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Abstract

The aim of the study was to evaluate a grain yields of triticale varieties and some parameters of productivity - plant height and structural elements of spike, as well as possible relations between the studied traits under biological cultivation. In the period 2014–2017 three-factor field experiment was set up with block design method with 4 replicates, plot size of 18 m² with 550 germinated seeds per m². Three varieties of triticale, three fertilizing rates with organic fertilizer and two predecessors (sunflower and durum wheat) were tested on Vertisols soils. The most productive was Respect variety - 1,293 kg/ha. The greatest increase on yields was observed at a rate of 1,750 kg/ha Lumbrical - 1,416.4 kg/ha. After a predecessor of sunflower higher yield was established - 1,350.9 kg/ha. A positive correlation between grain yields and plant height for Colorit and Boomerang varieties were observed. A positive interdependence between length of spike, number of grains per spike and weight of grains per spike was founded in all the investigated varieties.

Key words: organic farming, organic fertilization, predecessors, triticale.

INTRODUCTION

In 1888 the German scientist Rimpau (Rimpau, 1891) created the first triticale plants. Since triticale sustained an evolutionary progress and is diffused in the world, due to the high productivity of grain and biomass. In 1967 the first Bulgarian 42-chromosomal triticale T-AD is created by Prof. Stoyan Tsvetkov. Bulgaria became the seventh country in the world selecting the culture. According to Tsvetkov (1982), this plant species is promising because exhibits high genetic potential for yields and favorable nutritional values. Bulgaria is located in the zone of the suitable area for the sowing of winter triticale forms. According to Salmon et al. (2004), sowing of triticale in Eastern and Western Europe, where the climate is cool, completely responds with the culture's requirement to pass its vernalization stages. According to FAOSTAT data over the last 20 years, triticale areas in the world are grown. Since the middle of 90's to the beginning of the 21st century, the triticale area ranged from two million to nearly two million and half hectares.

Since the beginning of the century there has been an increase of area, with over 3.8 million hectares in 2005. It followed a slight decrease in 2006 and a further increase to reach the absolute maximum of triticale areas in the world in 2009 - more than 4.3 million hectares. On average for 20 years, the area of triticale in the world is 3.4 million hectares (FAO, 2018). In Bulgaria, the area cultivated with triticale in 2017 compared to 2015 is increased with 45.4%, the average yields marked an increase with 5.0%, the production is increased with 54.0% (MAF, 2019). The main use of the grain and green mass of triticale is as a source of fodder. According to Grain Advisory Council, the area planted in 2018 is increased with 15.34% compared to 2017 (MAF, 2018). Organic farming is an important priority in agricultural development policy and one of the focus of the Common Agricultural Policy for the period 2014–2020. According to a strategy for development of this direction in Bulgaria to 2020, one of the goals is to conduct an oriented to the practice research based on organic farming (Atanasov, 2019). The grain

production without a negative impact on environmental with a long-term trend of sustainability is identified as crucial for the development of agriculture (Foresight, 2011). The selection of modern varieties is related to cultivation of varieties growing under conventional system. They are not suitable for organic farming, because are created in order to combine high productivity and standard quality, growing under high agrophone (Bozhanova and Dechev, 2009). The different requirements of the biological system limit their use, because the plants have difficulty adapting. Döring et al. (2012) and Butler et al. (2007) have confirmed that a few varieties are created specifically for organic farming. It is possible, triticale as a new culture to be adapted better to the requirements of organic farming. The tolerance against disease does not require the use of pesticides, which results in ecological production (Golovkov, 1969). In areas characterizing by unfavorable abiotic conditions such as drought and extreme temperatures, the triticale proves to be a competitive crop compared to other cereals (Salmon et al., 2004; Martinek et al., 2008). Compared to other cereals, especially common wheat, it is an interesting alternative in environment, where the conditions of farming are associated with low inputs (Erekul and Köln, 2006). Because of the high grain quality, triticale has great potential for use as a food for humans and a source of fodder (Green et al., 2002). Its advantages are the higher values of a lysine content in protein. According to Georgieva et al. (2016), the triticale is characterized by a higher digestibility of dry and organic matter of straw and grain (55.23, 46.65) compared to rye (44.68, 38.25). Under organic farming conditions Bozhanova et al. (2014) have received the highest yields of dry biomass from triticale. Pogonets (2015) has developed a science-based recipe for production of bakery products with triticale flour, defining a rational ratio of wheat flour and triticale of 40: 60. In Bulgaria, triticale studies have been conducted by many scientists (Kirchev et al., 2007; Kolev et al., 2011; Atanasova et al., 2014; Gerdjikova, 2015; Dobрева et al., 2018a; Dobрева et al., 2018b; Kirchev et al., 2018;

Stoyanov, 2018; Stoyanov and Baychev, 2018), but few experiments have been carried out to determine productivity of varieties under biological system, especially against the background of various predecessors and levels of organic fertilization. This creates necessary to conduct field trials to provide information on the most appropriate variety. Because organic management practices continue to be tested (Doltra and Olesen, 2013), the purpose of the study was to evaluate grain yields of triticale varieties and some parameters of productivity-plant height and structural elements of spike following different predecessors and fertilization rates, as well as possible relations between the specified parameters under biological cultivation.

MATERIALS AND METHODS

In the period 2014-2017 three-factor field experiment was set up with block design method with perpendicular arrangement of the degrees of the tested factors with 4 replicates, plot size of 18 m² with 550 germinated seeds per m². Three varieties of triticale (Colorit, Boomerang and Respect), three fertilizing rates with organic fertilizer (0, 1,400 and 1,750 kg/ha) and two predecessors (sunflower and durum wheat) were tested. The soil treatment before sowing included a double processing with heavy disc harrows at a depth of 8-12 and 6-8 cm followed by manual fertilization and cultivation. The plant samples were taken and processed according to the accepted methodology (Topalov et al., 1994). The organic fertilizer Lumbrical is product from processing of manure and other organic waste from Red Californian worms (*Lumbricus rubellus* and *Eisenia foetida*), was used. The commercial product contains: organic substance 45-60%; humic-acids up to 14%; fulvic-acids 7%; ammonium nitrogen (NH₄-N) - 33.0 ppm; nitric nitrogen (NO₃-N) - 30.5 ppm; P₂O₅ - 1410 ppm; K₂O - 1910 ppm; useful microflora of 2 x 10¹² pg/g and a large number of biologically active substances. pH = 6.5-7.0. In accordance with EU Regulation 889/2008 for organic farming was applied. The Colorit variety exceeds a yields of all varieties created from Dobrudja Agricultural Institute

and since 2015 was selected for a standard in Bulgaria for productivity in Executive Agency on Aproabation and seed control (IASAS). The cold resistance of Boomerang variety is close to wheat № 301 or slightly above it. The Respect variety demonstrates a cold resistance as the most cold-resistant standard-common wheat Mironovska 808.

The soil (PellicVertisols) is low to medium provided with mineral nitrogen, with low content of mobile phosphates and good supply of digestible potassium.

Field Crops Institute (42°11'58"N, 25°19'27"E) is located in temperate-continental subarea zone (Sabev and Stanev, 1963). The Chirpan area is characterized by strong variability of temperature conditions during the growing season. During the winter is possible the temperatures to decrease to -20°C...-30°C). The main precipitation maximum is observed in summer, as average

over a multi-year period is 160.7 mm. The amount of annual rainfall more than 750 mm is observed average once every 10-12 years. The vegetation of triticale for the first and the second year occurred under conditions of temperature amounts above average for 86 years (2,009.7°C), respectively 2,264.1°C and 2,530.4°C, and during the last year of the study the temperature sum was lower than a multi-year period - 1,843.5°C (Figure 1).

The fallen rainfall during 2014/15 period were 183.2 mm higher than multi-year period (395 mm), and during the next two years the rainfall were 71.6 mm and 19.8 mm less.

In order to establish statistically significant influences of the studied factors and differences between the tested variants BIostat was applied on date for a period 2015-2017 (Penchev et al., 1989-1991). The software Statistica 13 was used to establish correlation dependencies.

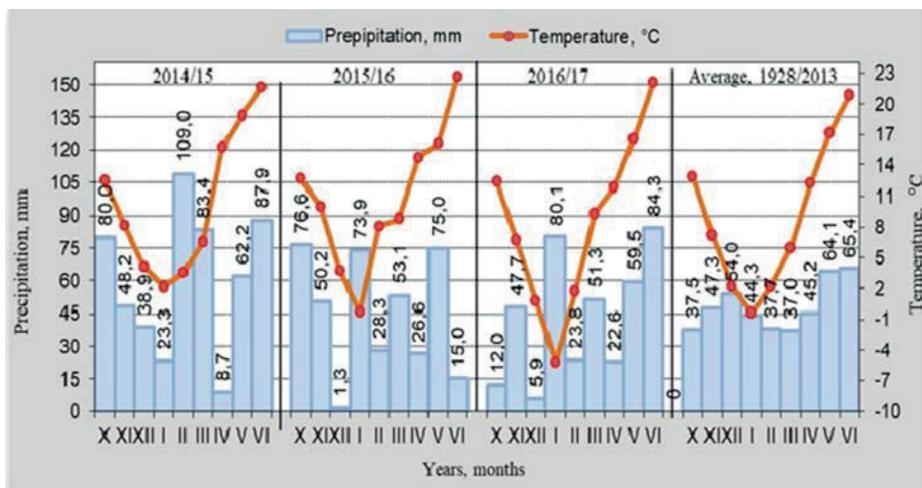


Figure 1. Temperatures and precipitation condition during vegetation of triticale

RESULTS AND DISCUSSIONS

The grain yields from triticale is result of a number of complex morphological and physiological processes that influence each other and occur in different stages of growth during vegetation (Janušauskaite, 2014). During the three years of the study, the triticale realized its productive potential through the

amount of grain yields in varying degrees under the influence of the studied factors. The data, both in productivity and studied parameters showed that the varieties are differed in vary degrees.

According to results on Table 1, the test of increasing fertilizer rates of 1,400 kg/ha and 1,750 kg/ha increased a grain yields and spike

Table 1. Results of ANOVA analysis of grain yields, structural elements of spike and plant height.

Interaction of the factors A × B × C

Factors and levels		GY (kg/ha)	LS (cm)	NGS	WGS (g)	PH (cm)
after predecessor of sunflower						
Colorit	0	929.0	8.4	46.0	1.84	77.3
	1,400 kg/ha	1,203.0 ^{ns}	9.3 ^{ns}	50.7 ^{ns}	2.14 ^{ns}	81.5 ^{ns}
	1,750 kg/ha	1,427.0 ^{**}	9.9 ^{**}	59.3 ^{**}	2.62 ^{**}	92.7 [*]
Boomerang	0	1,116.0 ^{ns}	8.3 ^{ns}	42.2 ^{ns}	1.65 ^{ns}	81.6 ^{ns}
	1,400 kg/ha	1,431.0 ^{**}	8.6 ^{ns}	46.3 ^{ns}	1.87 ^{ns}	87.5 ^{ns}
	1,750 kg/ha	1,597.0 ^{***}	9.4 ^{ns}	52.0 ^{ns}	2.17 ^{ns}	94.2 [*]
Respect	0	1,117.0 ^{ns}	7.9 ^{ns}	43.0 ^{ns}	1.75 ^{ns}	77.9 ^{ns}
	1,400 kg/ha	1,499.0 ^{***}	8.7 ^{ns}	49.8 ^{ns}	1.97 ^{ns}	82.8 ^{ns}
	1,750 kg/ha	1,780.0 ^{***}	9.7 [*]	58.7 ^{**}	2.63 ^{**}	94.6 [*]
after predecessor of durum wheat						
Colorit	0	949.0 ^{ns}	8.9 ^{ns}	42.8 ^{ns}	1.56 ^{ns}	76.2 ^{ns}
	1,400 kg/ha	1,115.0 ^{ns}	8.6 ^{ns}	45.6 ^{ns}	1.82 ^{ns}	87.9 ^{ns}
	1,750 kg/ha	1,294.0 ^{ns}	9.3 ^{ns}	51.9 ^{ns}	2.07 ^{ns}	92.6 [*]
Boomerang	0	883.0 ^{ns}	8.7 ^{ns}	43.3 ^{ns}	1.78 ^{ns}	76.0 ^{ns}
	1,400 kg/ha	1,066.0 ^{ns}	9.0 ^{ns}	47.8 ^{ns}	2.08 ^{ns}	85.2 ^{ns}
	1,750 kg/ha	1,228.0 ^{ns}	9.5 [*]	52.5 ^{ns}	2.27 [*]	92.3 [*]
Respect	0	1,006.0 ^{ns}	8.2 ^{ns}	42.7 ^{ns}	1.86 ^{ns}	71.0 ^{ns}
	1,400 kg/ha	1,130.0 ^{ns}	9.0 ^{ns}	48.7 ^{ns}	1.88 ^{ns}	74.7 ^{ns}
	1,750 kg/ha	1,172.0 ^{ns}	9.2 ^{ns}	53.2 ^{ns}	2.20 ^{ns}	80.3 ^{ns}
LSD	5.0%	298.0	1.1	8.5	0.4	13.6
	1.0%	400.0	1.4	11.4	0.6	18.3
	0.1%	527.0	1.8	15.0	0.8	24.2

A - variety; B - fertilizing; C - predecessor; ns - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%, respectively; GY - Grain yields; PH - Plant height; SL - Spike length; NGS - Number of grain per spike; WGS - Weight of grains per spike.

Table 2. Results of ANOVA analysis of triticale grain yields

Source	LSD			Levels of factors		
	(kg/ha)					
	5.0 %	1.0 %	0.1 %	Control	2	3
A	121.5	163.1	215.2	1,152.7	1,220.2 ^{ns}	1,293.9 [*]
B	121.5	163.1	215.2	1,009.9	1,240.6 ^{***}	1,416.4 ^{***}
C	99.2	133.2	175.7	1,350.9	1,093.7 ^{ns}	-

A - variety; B - fertilizing; C - predecessor; ns - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%, respectively.

elements values for all triticale varieties after of two predecessors. The highest values was observed at maximum fertilizer rate - 1,750 kg/ha. The greatest increase of grain yields compared to a control option was received after a predecessor of sunflower (29.5-91.6%) with high significance for Boomerang and Respect varieties when was applied 1,750 kg/ha Lumlrical, and for Respect variety was in range of 61.4-91.6%. After a predecessor of durum wheat the increase was between 14.7% and 39.3%, but the differences were not statistically significant. In the study LS, NGS, WGS and HP modified under influence of the test factors for all varieties. According to date on Table 1, after predecessor of sunflower the LS values was from 7.9 to 9.9 cm, NGS varied

from 42.2 to 59.3, the WGS was between 1.65 to 2.63 g and the PH was in the range of 77.3 and 94.6 cm. After durum wheat the increase of the values changed as follows: LS (8.2-9.5 cm); NGS (42.7-53.2); WGS (1.56-2.27 g); PH (76.0-92.6 cm). According to Alheit et al. (2014) has no observed large variation in LS for triticale. Gerdgikova et al. (2008) have obtained under biological system for triticale after two predecessors - soybeans and winter peas -, higher average values for PH and LS, but lower results for NGS and WGS. Stoyanov and Baychev (2018) have received higher results under conditions of Northeastern Bulgaria in regard to PH for Colorit, Boomerang and Respect varieties - 122, 131 and 123 cm, respectively.

Maximum average yields was achieved for Respect variety (1,293.9 kg/ha), which completely characterized productive capacity of a variety compared to other two Colorit and Boomerang - 1,152.7 kg/ha and 1,220.2 kg/ha, respectively (Table 2). It can be suppose, that the Respect variety exhibits better adaptability to environmental conditions, realizes higher productivity under low supply of soil nitrogen and manifest high responsiveness to the applied biological fertilizer. In detail study of Respect variety (Bajchev and Petrova, 2011) has pointed the better ecological plasticity which has evidenced by the higher relative yields during unfavorable years, higher productivity (8,330 kg/ha), compared to average standard from all varieties included in the study.

The average grain yields after a sunflower was 1,350.9 kg/ha and after a durum wheat was lower - 1,093.7 kg/ha or 257.2 kg/ha less than a control option (Table 2). Obviously, the varieties showed same reaction to the two

predecessors. This is confirmed by the results presented on Table 4 where is shown, that 11.89% from the total yield dispersion is due to the influence of a factor predecessor. In contrast to our results, higher yields values under organic farming (2.60-4.84 t ha⁻¹) have obtained by testing triticale varieties in Lithuania (Kronberga et al., 2013). In another study, the authors has obtained 4.76 t/ha average yields from triticale lines growing on the same technological system (Kronberga, 2008).

From Table 2 is obviously, that the fertilizing significantly increased the grain yields in rate of 1,400 and 1,750 kg/ha Lumbrical, respectively 1,240.6 and 1,416.4 kg/ha or 230.7 and 406.5 kg/ha, respectively over the control option.

According to the data on Table 3, 19.92% from the total dispersion is due to a fertilizing and significantly influence of a factor fertilizing was observed in all the yields parameters.

Table 3. Results of ANOVA analysis of investigating parameters

Source	df	GY		LS		NGS		WGS		PH	
		η	MS	η	MS	η	MS	η	MS	η	MS
A	2	2.3	898.0 ^{ns}	0.3	0.3 ^{ns}	0.6	24.2 ^{ns}	0.8	2.78 ^{ns}	3.8	171.4 ^{ns}
B	2	19.9	7,482.9 ^{***}	16.4	15.2 ^{***}	14.2 ^{***}	574.6 ^{***}	45.9	1.5 ^{***}	20.9	942.0 ^{***}
C	1	11.9	8,929.4 ^{***}	7.6	0.1 ^{ns}	0.8	62.6 ^{ns}	2.9	0.28 ^{ns}	2.1	191.4 ^{ns}
A × B	4	0.1	11.0 ^{ns}	0.1	3.9 ^{ns}	0.3	6.8 ^{ns}	1.5	2.58 ^{ns}	0.3	7.0 ^{ns}
A × C	2	3.4	1,267.2 [*]	1.5	1.3 ^{ns}	1.1	43.8 ^{ns}	8.6	0.3 [*]	3.4	151.0 ^{ns}
B × C	2	1.8	668.0 ^{ns}	0.3	0.3 ^{ns}	0.3	13.5 ^{ns}	3.4	0.18 ^{ns}	0.5	21.3 ^{ns}
A × B × C	4	0.6	116.2 ^{ns}	0.9	0.4 ^{ns}	0.1	2.5 ^{ns}	1.6	2.68 ^{ns}	0.8	17.0 ^{ns}

A - variety; B - fertilizing; C - predecessor; ns - no significant; *, **, *** significant at P = 5%, P = 1% and P = 0.1%, respectively; GY - Grain yields; PH - Plant height; SL - Spike length; NGS - Number of grain per spike; WGS - Weight of grains per spike.

Ugarte et al. (2007) have established that WGS of triticale is influenced by environmental conditions. In terms of the present study WGS was most affected by fertilization - 45.91% of the total dispersion. In regard to a predecessor, it should be noted that its biological role in crop rotation is undisputed, from a point of view of the amount of plant residues which subsequently are mineralized. On the other hand, the quantity of the exported nitrogen from the predecessors is different. Durum wheat assimilates less nitrogen to realize 100.0 kg grain (3.2-4.9 kg N), unlike sunflower—about 6.0 kg N (Terziev et al., 2007). Such conclusions about the role of

predecessor, fertilizing and meteorological conditions have expressed other authors. Delibaltova and Kirchev (2016) have founded that the predecessor and the nitrogen fertilization in combination with meteorological conditions are crucial factors for formation of the productivity of winter wheat. A low significant interaction between the factors variety and predecessor on GY and WGS was observed, 3.73% and 8.58%, respectively from the total dispersion.

Each variety exhibits a specific response to the environmental conditions. This determines differences in a character of the correlation relations between the individual components of

yield in the various studies. Additional correlation analysis was applied to estimate all possible relations of yields with studied agronomic characteristics, which results are presented on Table 4. It is known that the yields is integral value, this value has a complex character and is related with other parameters. Rachovska and Ur. (2010) have observed a relation between yields and spike parameters, Nikolova and Panayotov (2008) have found a relation of yields with number of productive tillers and Stoyanov (2013) with photosynthetic activity of crops. The established positive correlations not necessarily to give explanation for causality dependence between the studied indicators and yields, as there are additional, uncontrolled factors in the study. It is known that triticale is characterized with many features of its phenology, which depend mostly

of the the environment conditions (Giunta et al., 1993).

Different values of the coefficients as well as the different character of the relations between the considered components of spike, YG and PH were observed. For Colorit variety, a positive and significant correlation was founded between GY ($r = 0.586^*$) and LS ($r = 0.513^*$) with fertilization. A low dependence of PH with fertilization was established ($r = 0.487^*$). The same tendency was also observed for Boomerang variety - the PH correlated with fertilization ($r = 0.535^*$). The common in both varieties is that the LS showed equal coefficient between GY and PH ($r = 0.540^*$). This finding is in agreement with results in triticale study which have indicated that in reference to correlation, one of the main components associated with increasing the grain yield of triticale is the plant height (Gulmezoglu et al., 2010).

Table 4. Correlation coefficients between investigating parameters

	Fertilizing	GY	LS	NGS	WGS	PH
Colorit						
Fertilizing	1					
GY	0.586*	1				
LS	0.513*	-0.096	1			
NGS	0.314	-0.172	0.827**	1		
WGS	0.308	-0.140	0.775**	0.971**	1	
PH	0.487*	0.540*	-0.158	-0.511*	-0.548*	1
Boomerang						
Fertilizing	1					
GY	0.438	1				
LS	0.402	-0.210	1			
NGS	0.304	-0.315	0.894**	1		
WGS	0.286	-0.368	0.891**	0.984**	1	
PH	0.535*	0.540*	-0.283	-0.454	-0.504*	1
Respect						
Fertilizing	1					
GY	0.361	1				
LS	0.404	-0.356	1			
NGS	0.425	-0.198	0.957**	1		
WGS	0.321	-0.174	0.904**	0.939**	1	
PH	0.326	0.271	0.164	0.171	0.182	1

*, **significant at P = 5% and P = 1%, respectively; GY - Grain yields; PH - Plant height; SL - Spike length; NGS - Number of grain per spike; WGS - Weight of grains per spike.

Agronomic performance of seven lines and two spring triticale varieties presented by Dogan et al. (2009), have confirmed the results obtained regarding to expressed correlation between grain yields and plant height. The same dependence have obtained with other cereal crops. Ivanova and Tsenov

(2009) and Dragov et al. (2019) have reported a good defined positive correlation between yields and plant height for durum wheat. Base on the significant of the phenotypic correlation between plant height and grain yields it can be assumed that the better leafiness, respectively the vegetative

mass, corresponds to grain yield. No correlation was established between YG and considered spike components. In previous triticale studies have suggested that WGS is of the least importance in determining grain yields (Singh and Sethi, 1974; Kamboj and Mani, 1983). However, there are authors who have established a high correlation between grain yields with number of grain per spike and weight of grains per spike (Dogan et al., 2009). Concerning Colorit and Boomerang varieties was observed a significant, negative relations ($r = -0.548^*$ and $r = -0.504^*$, respectively).

A high and significant positive correlation was established between SL with NGS and WGS for the three tested varieties. The NGS was in strongly expressed, positive correlation with WGS, $r = 0.971^{**}$, $r = 0.984^{**}$, $r = 0.939^{**}$, respectively for Colorit, Boomerang and Respect varieties. These data showed that, the spike productivity of studied triticale varieties directly depends on the length of spike. Based on similar correlation coefficients, it can be assumed that the spike productivity is determined of the same biometric and physical characteristics for the three tested varieties.

In respect to Respect variety, established interdependencies between spike length, number of grain per spike and weight of grains per spike, have confirmed in report by Stoyanov and Baychev (2015). The correlation coefficients in their research for Respect variety as follows: $r = 0.761^{**}$ between SL and NGS; $r = 0.792^{**}$ between SL and WGS; $r = 0.933^{**}$ between WGS and NGS. No significant negative correlation between GY with NGS ($r = -0.315$) and WGS ($r = -0.368$) for Boomerang variety was observed. These results are in contradiction with Shimelis data (2006), who has established that the number of grain per spike and the weight of grains per spike are positively correlated with grain yields in durum wheat.

A negative relation was observed between NGS and PH for Colorit variety ($r = -0.511^*$). It should be noted, that as opposed to Respect variety PH correlated negatively

with WGS, $r = -0.548^*$ and $r = -0.504^*$, respectively for Colorit and Boomerang varieties.

The results received give rise to grounds for further work with triticale, to analyze of more agronomic characteristics and additional yields estimation with a view to increase of the areas and their participation in the structure of the arable land.

CONCLUSIONS

The results on the influence of the three triticale varieties, the fertilization rates and the predecessors on grain yields allow the following conclusions to be formulated: the greatest significant effect on yields, the structural components of spike and the plant height had the fertilization; the highest yields was obtained after a fertilization in rate of 1,750 kg/ha Lumbrical - 1,416.4 kg/ha; after a predecessor of durum wheat the yields was lower with 257.2 kg/ha compared to a predecessor sunflower; the Respect variety realized highest productivity - 1,293.9 kg/ha; the complex influence of the factors variety and predecessor on yields and weight of grains per spike was low; a positive, significant correlation between grain yields and the plant height for the Colorit and Boomerang varieties and a negative between the weight of grains per spike and plant height was established; for Colorit variety the relation between number of grain per spike and plant height was significantly negative, and the fertilization correlated positively with grain yields and spike length; a high, positive and significant interdependence of length of spike, number of grains per spike and weight of grains per spike was founded in all the investigated varieties. The results of the present one and future researchs can be used for formation of a triticale cultivation technology under organic farming.

REFERENCES

- Alheit, K.V., Busemeyer, L., Liu, W., Maurer, H.P., Gowda, M., Hahn, V., Weissmann, S., Ruckelshausen, A., Reif J.C., Wuerschum, T.

- (2014). Multiple-line cross QTL mapping for biomass yield and plant height in triticale (*×Triticosecale* Wittmack). *Theor Appl Genet*, 127, 251–260.
- Atanasov, A. (2019). *Strategy for sustainable development of agriculture in Bulgaria*. Zahariy Stoyanov, Sofia, 73–77. ISBN 978-954-09-1372-8.
- Atanasova, D., Maneva, V., Zarkov, B. (2014). Influence of predecessor at productivity on rye and triticale in organic farming. In: National Conference with International Participation “Organic Farming, Livestock and Food”, 27–28 Nov. Troyan, 204–207 [http://www.agriacad.bg/images/novini/Site_2_2%20\(1\).pdf](http://www.agriacad.bg/images/novini/Site_2_2%20(1).pdf)
- Baychev, C., Petrova T. (2011). Triticale "Respect" – a new productive variety with a unique cold tolerance. *Field Crops Studies*, VII, 63–70.
- Bozhanova, V., Koteva, V., Savova, T., Marcheva, M., Panayotova, G., Nedjalkova, S., Rachovska, G., Kostov, K., Mihova, G. (2014). Choice of appropriate cereals varieties and seed production for the needs of organic farming in Bulgaria. <http://uni-sz.bg/truni1/wp-content/uploads/biblioteka/file/TUNI10042835.pdf>
- Bozhanova, V., Dechev, D. (2009). Plant breeding oriented to the needs of the organic agriculture—reasons, problems and perspectives. *Agricultural Science*, 42(3), 14–22.
- Butler, G., Newton, H. Bourlakis, M. Leifert, C. (2007). *Factors influencing supply and demand for organic foods. Food supply chain management*. Blackwell, Oxford: Chapter 13, 199–210.
- Delibaltova, V., Kirchev, H. (2016). Productivity of common wheat (*Triticum aestivum* L.) depending on predecessor and the level of nitrogen fertilization. *International Journal for Research in Agricultural Research*, 1(6), 1–10.
- Dobrova, S., Kirchev, H., Muhova, A. (2018a). Influence of nitrogen fertilization in combination with foliar fertilization on the structural elements of the spike in triticale varieties (*× Triticosecale* Wittm.). *Research Journal of Agricultural Science*, 50(4), 116–121.
- Dobrova, S., Kirchev, H., Muhova, A. (2018b). Grain yield of triticale varieties depending on the foliar fertilization in the conditions of increasing norms of fertilization of the soil with nitrogen. *Research Journal of Agricultural Science*, 50(4), 122–126.
- Dogan, R., Kacar, O., Coplu, N., Azkan, N., Azkan, N. (2009). Characteristics of new breeding lines of triticale. *African Journal of Agricultural Research*, 4(2), 133–138.
- Doltra, J., Olesen, J. (2013). The role of catch crops in the ecological intensification of spring cereals in organic farming under Nordic climate. *European Journal of Agronomy*, 44, 98–108.
- Dragov, R., Dechev, D., Taneva, K. (2019). Genetic distance of new bulgarian durum wheat varieties and breeding lines of FCI–Chirpan, Bulgaria. *International Journal of Innovative Approaches in Agricultural Research*, 3(3), 402–410.
- Ereku, O., Kohn, W. (2006). Effect of weather and soil conditions on yield components and breadmaking quality of winter wheat (*Triticum aestivum* L.) and winter triticale (*Triticosecale* Wittm) varieties in North-East Germany, *J. Agron. Crops Sci.*, 192, 452–464.
- FAO, (2018). www.fao.org/faostat/en/#data/QC.
- Foresight, (2011). *The Future of Food and Farming: Challenges and Choices for Global Sustainability*. The Government Office for Science, London: UK. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/288329/11-546-future-of-food-and-farming-report.pdf
- Georgieva, N., Nikolova, I., Kolev, T., Tahsin, N., Naydenova, Y., Kosev, V. (2016). Feeding value of new wheat, rye and triticale cultivars. *Journal of Mountain Agriculture on the Balkans*, 19(4), 57–69.
- Gerdjikova, M., Videva, M., Eneva, S. (2008). *Influence of the species of the leguminous predecessor upon the productivity of triticale*. Union of scientist, International scientific conference. *Issues in Plant Studies*. ISBN 9789549329445
- Gerdjikova, M. (2015). Influence of some predecessors and nitrogen fertilization on the productivity, the chemical composition and the nutritional value of triticale (*× Triticosecale* Wittmack) in the region of Stara Zagora. PhD Thesis.
- Giunta, F., Motzo, R., Deidda, M. (1993). Effect of drought on yield and yield components of durum wheat and triticale in a Mediterranean environment. *Field Crops Research*, 33(4), 399–409.
- Golovkov, A.M. (1969). *Formation of wheat yields depending on the agrotechnical practices*. <https://istina.msu.ru/dissertations/3225713/>
- Green, C., Arseniuk, E., Osinski, R. (2002). The competitive position of triticale in Europe. *Proceedings of the 5th International Triticale Symposium, Poland, I*.
- Gulmezoglu, N., Alpu, O., Ozer, E. (2010). Comparative performance of triticale and wheatgrains by using path analysis. *Bulgarian Journal of Agricultural Science*, 16(4), 443–453.
- Janušauskaite, D. (2014). Analysis of grain yield and its components in spring triticale under different N fertilization regimes. *Zemdirbyste-Agriculture*, 101(4), 381–388.
- IASAS. <https://iasas.government.bg/bg/>.
- Ivanova, A., Tsenov, N. (2009). Biological and economic characteristics of varieties of common wheat according to the conditions of cultivation. *Field Crops Studies*, 5(1), 173–182.

- Kamboj, R.K., Mani, S. C. (1983). Correlation and path analysis in hexaploid triticale. *Indian J. Agric. Sci.*, 53, 394–397.
- Kirchev, H., Muhova, A., Georgieva, R. (2018). Approach for organic triticale (*x Triticosecale* Wittm.) farming i. genotypic specific in the accumulation of biomass. *Research Journal of Agricultural Science*, 50(1), 101–106.
- Kirchev, H., Terziev, J., Tonev, T., Semkova, N. (2007). Formation of biological extraction in different triticale varieties under conditions of nitrogen deficiency. *Field Crops Studies*, 4(2), 293–298.
- Kolev, T., Todorov, J., Nenkova, D. (2011). Testing of new biologically active substances at triticale. *Plant Breeding Sciences*, 48(5), 491–494.
- Kronberga, A., Legzdiņa, L., Strazdiņa, V., Vicupe, Z. (2013). Comparison of selection results in organic and conventional environments for winter triticale. *Proceedings of the Latvian Academy of Sciences*, 67(3), 268–271. DOI: 10.2478/prolas-2013-0047
- Kronberga, A. (2008). Formation of triticale crop ideotype for organic farming. *Options Méditerranéennes, Series A.(81)*, 391–393.
- MAF. (2018). *Information for Grain Advisory Council*. www.mzh.government.bg/media/uploads/2018/09/11/info_kons_savet_septemvri_2018.doc.
- MAF. (2019). www.mzh.government.bg/bg/statistika-i-analizi/izsledvane-rastenievadstvo/danni/
- Martinek, P., Vinterov, M., Buresova, I., Vyhnanek, T. (2008). Agronomic and quality characteristics of triticale (*x Triticosecale* Wittmack) with HMW glutenin subunits 5 + 10. *Journal of Cereal Science*, 47, 68–78.
- Nikolova, E., Panayotov, I. (2008). Inheritance of quantitative traits in F₁ hybrid winter common wheat (*Triticum aestivum* L.). *Agricultural Sciences*, 10, 11–18.
- Penchev, E., Bankov, L., Koev, A. (1989-1991). *BIOSTAT. Statistical software Biostat© version 1.0. Dobrich*.
- Pogonets, E. V. (2015). Technological advantages of triticale grain for food purposes and elaboration directions for its use. <http://tekhnosfera.com/view/595986/a?#?page=16>
- Rachovska, G., Ur, Z. (2010). Inheritance, heterosis and variability of quantitative traits, related to productivity in F₁ hybrids of durum wheat. *Field Crop Studies*, 6(3), 361–367.
- Rimpau, W. (1891). Kreuzungsprodukte landwirtschaftlicher Kulturpflanzen. *Landwirtschaftlichen Jahrbuch*, 20, 335–371.
- Sabev, L., S. Stanev, 1963. *Climatic regions in Bulgaria and their climate*. Zemizdat, Sofia.
- Salmon, D.F., Mergoum, M., Gomez M., H. (2004). *Triticale production and management*. Food and Agriculture Organization of the United Nations Rome. ISBN 92-5-105182-8, 27–36.
- Shimelis, H. A. (2006). Associations of yield and yield components among selected durum wheats (*Triticum turgidum* L.). *South African Journal of Plant and Soil*, 23(4), 305–309.
- Singh, H.B, Sethi, G.S. (1974). Path and regression analysis in triticale. *Plant Sci.*, 6, 10–16.
- Statistica 13. (2018) TIBCO Software.
- Stoyanov, H. (2013). Correlation between the spike characteristics in common winter wheat varieties (*Triticum aestivum* L.). *Scientific works of the Institute of Agriculture – Karnobat*, 2(1), 95–104.
- Stoyanov, H., Baychev, V. (2015). Correlations between spike parameters of first generation direct and reciprocal crosses of triticale (*x Triticosecale* Wittm.). *Agricultural sciences*, 7(18), 25–34.
- Stoyanov, H. (2018). Reaction of triticale (*x Triticosecale* Wittm.) to abiotic stress. <http://www.dai-gt.org/bg/acadr/avtoref/avtoref46.pdf>.
- Stoyanov, H., Baychev, V. (2018). Tendencies in the yield and its components of the bulgarian varieties of triticale, grown under contrasting conditions of the environment. *Bulgarian Journal of Crop Science*, 55(3), 16–26.
- Terziev, Zh., Yancheva, H., Yanchev, I., Georgieva, T., Yankov, B., Ivanova, R., Dimitrov, I., Kolev, T. (2007). *Plant Growing*. Academic Publishing House of Agricultural University. Plovdiv. Bulgaria. ISBN 978-954-517-034-8, 48–54.
- Topalov, V., Yankov, B., Stoyanova, S., Dimitrov, I., Slavcheva, K. (1994). *Guidance for exercises on plant breeding*. Zemizdat. Sofia. Bulgaria. ISBN 954-05-0160-1, 48–51.
- Tsvetkov, S.M., 1982. *Selection of winter triticale in Bulgaria*. Agricultural Academy. Sofia, 1–74.
- Ugarte, C., Calderini, D. F., Slafer, G.A. (2007). Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. *Field Crops Research*, 100, 240–248.

EXPERIMENTAL RESULTS ON COMMON WHEAT GRAINS QUALITY PRODUCED IN ORGANIC FARMING

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Abstract

Consumer demand for the quality of agricultural products has grown constant in recent decades, representing a set of particularities: hygiene (or sanitary), nutritional and dietetic, organoleptic, use (ease of preparation and preservation), regulatory (compliance with the rules in force), commercial. The quality of an organic product must meet all these requirements and is subject to strict regulations regarding the conditions of production, processing and marketing. In this context, the present paper presents the results of the quality analyses performed on the common wheat produced in organic farming in Romania, regarding: the content in pesticide residues, nitrates and nitrites, mycotoxins, as well as the nutritional quality (content in carbohydrates, proteins, lipids, ash, fibres, energy value). The results illustrated the absence of toxic compounds and that this product meets the requirements of FAO quality standards in terms of biochemical components and nutritional value.

Key words: wheat, organic farming, grain quality.

INTRODUCTION

The development of agriculture in the second half of the XXth century resulted in a considerable increase of agri-food production, so that they began to meet the consumption needs of human collectives, in economically developed countries in Europe and North America. As a result, since the 8th decade of the century, the demands of consumers in these countries have increased towards the quality of agri-food products, while intensifying concerns about diminishing the negative effects of agricultural activities on the environment (Roman et al., 2011). These trends led to the initiation and development of new concepts regarding agricultural production systems and the formulation of stricter regulations regarding the quality of agri-food products (Nastase and Toader, 2016). Compared to the intensive agricultural production system, based on the mechanization and chemization of technologies, the new concepts regarding agricultural production systems - called *sustainable, conservative, organic farming* - promoted the limitation or even prohibition of the use of chemical fertilizers and synthetic

pesticides, in order to reduce the polluted impact on the environment and the quality of agricultural products.

Among the new concepts, organic farming has undergone an important evolution, especially in economically developed countries, and for organic products has developed its own market, which is currently in full development (Nastase et al., 2018). The organic production system is regulated by very strict laws and directives, issued by national, regional and global authorities, who concern the whole chain, from the production processes, to the processing and marketing of products. In Romania, the organic farming system has developed considerably in the last decades and is regulated by decisions issued by national and European Union authorities (Roman et al., 2015).

For this purpose, there are control and certification bodies for operators in organic farming, which operate under the authority of the Ministry of Agriculture and Rural Development.

The operators - the economic agents that work in this field - are registered in the official records and are permanently monitored in compliance with the legal provisions in force, and the organic products are compulsory

analyzed in terms of quality, with priority of the content in pesticide residues.

The present paper presents the results of the quality analyzes performed at the common wheat crops (wheat for bakery) produced in organic farms on the territory of Romania, in 2016 and 2017. The nutritional value (the contents in biochemical compounds and the energy value) were analyzed, as well as and the contents in compounds with potentially negative effect on product quality - pesticide residues, nitrites, nitrates and mycotoxins.

MATERIALS AND METHODS

There were analyzed samples of common wheat (wheat for bakery) produced in certified organic crops, taken from important areas of winter wheat cultivation on the territory of Romania. The paper presents the results obtained by analyzing the wheat samples from the organic crops from Meadows of Prut River (Vaslui County), Tecuci Plain (Galati County), Gavanu-Burdea Plain (Teleorman County), Balacitei Plain, south of the Getic Plateau (Dolj County), Arad Plateau (Arad County), Barcau Valley (Bihar County) (Figures 1 and 2). The sampling for the analyzes was carried out in accordance with the regulations in force (Standard SR EN ISO/IEC 17065:2013 and Regulations (EC) 834/2007 and 889/2008), specific to organic farming (Figure 3). The quality analyzes were performed in specialized and accredited laboratories, from Germany and Bulgaria.



Figure 1. Organic winter wheat in Bihor County, 2016



Figure 2. Organic winter wheat at harvesting, in Bihor County, 2017



Figure 3. Samples of wheat grains for analysis

The following methods of analysis were used to determine the nutritional value (content in biochemical compounds): for *carbohydrates* - High Performance Liquid Chromatography (HPLC) with RI detection (refractive index); for *proteins* - Dumas Method; for *lipids* - Nuclear Magnetic Resonance Method - MRI; for *mineral substances* - Gravimetric method; for *food fibers* - Enzymatic method; for *dry matter* - by drying in the oven. Based on the results of these analyzes, the *nutritional value* was calculated, using the relation:

$W = P \text{ (g)} \times 4.1 \text{ (kcal/g)} + L \text{ (g)} \times 9.3 \text{ (kcal/g)} + G \text{ (g)} \times 4.1 \text{ (kcal/g)}$, in which: P = proteins content; L = lipids content; G = carbohydrates content.

To recalculate the results in joule, the relationship was used:

$$1 \text{ kcal} = 4.18 \text{ joule}$$

Also, the *lipid energy* was calculated, starting from the fact that 1 g of lipids generates 9.3 kcal.

The presence of *pesticide residues* was analyzed according to Standard EN 15662: 2008 ("Foodstuffs of vegetable origin. Determination of pesticide residues by GCMS and/or LC-MS/MS after extraction/separation of acetonitrile and cleaning by SPE dispersion-QuEChERS method"), elaborated by the EU.

The content of *mycotoxins* was analyzed by High Performance Liquid Chromatography (HPLC) with UV detection (PDA detector), with purification on the immunoaffinity column. The content of *nitrites and nitrates* was analyzed by High Performance Liquid Chromatography (HPLC) with UV detector.

RESULTS AND DISCUSSIONS

The nutritional value of common wheat produced under ecological conditions. The moisture content of the wheat samples analyzed (Tables 1 and 2) was on average 11.15% and 12.16%, respectively, values at which good conditions for the conservation of the crops are ensured. In one case, a slightly higher humidity of 13.90% was found in 2016, in Bihor County.

The analysis of the obtained results shows that, in all cases, the protein content exceeded the value of 11% - minimum admitted to the wheat for baking. The average values were 12.59% in 2016 and 13.03% in 2017.

It should be noted that, in 2016 - a favorable year for autumn wheat crops in terms of natural conditions - the organic wheat harvested in Galati and Arad Counties contained 13.70 to 13.71% proteins.

The other biochemical compounds (Figure 4) analyzed were within the normal limits for wheat for baking. Specifically, the analyzes revealed for **carbohydrates** contents of 65.65% on average, with variations between 60.10 and 67.44% in 2016 and of 66.22%, on average, with variations between 65.10 and 67.11% in 2017.

Table 1. The nutritional value of common wheat grains produced under organic agriculture conditions in 2016

No. crt.	Biochemical compound	U.M.	Region of organic wheat production						Average
			North-East (Vaslui County)	South-East (Galați County)	South (Teleorman County)	South-West (Dolj County)	West (Arad County)	North-West (Bihor County)	
1.	Carbohydrates	g/100 g	66.45	67.44	65.80	67.32	66.78	60.10	65.65
2.	Proteins	g/100 g	12.66	13.70	12.60	11.06	13.71	11.80	12.59
3.	Lipids	g/100 g	1.32	1.23	1.83	1.22	1.42	1.73	1.46
4.	Mineral Substances (Ash)	g/100 g	1.47	1.47	1.56	1.46	1.88	1.70	1.59
5.	Dietary Fibers	g/100 g	1.34	1.33	2.10	2.65	2.54	1.80	1.96
6.	Dry Matter	g/100 g	88.81	87.90	87.90	88.43	87.90	86.10	87.84
7.	Water	g/100 g	11.19	12.10	12.10	11.57	12.10	13.90	12.16
8.	Nutritional value	Kj/100 g	1388	1438	1451	1438	1435	1370	1420
9.		Kcal/100 g	332	344	338	333	343	328	336
10.	Lipid Energy	Kj/100 g	51	48	71	47	55	64	56
11.		Kcal/100 g	12	11	17	11	13	15	13

Table 2. The nutritional value of common wheat grains produced under organic agriculture conditions in 2017

No. crt.	Biochemical compound	U.M.	Region of organic wheat production			
			North-East (Vaslui County)	West (Arad County)	North-West (Bihor County)	Average
1.	Carbohydrates	g/100 g	66.45	67.11	65.10	66.22
2.	Proteins	g/100 g	11.56	13.73	13.80	13.03
3.	Lipids	g/100 g	1.32	1.22	1.73	1.42
4.	Mineral Substances (Ash)	g/100 g	1.47	1.51	1.80	1.59
5.	Dietary Fibers	g/100 g	1.34	2.04	1.80	1.72
6.	Dry Matter	g/100 g	88.81	88.94	88.79	88.85
7.	Water	g/100 g	11.19	11.06	11.21	11.15
8.	Nutritional value	Kj/100 g	1388	1433	1421	1414
9.		Kcal/100 g	332	343	340	338
10.	Lipid Energy	Kj/100 g	51	47	67	55
11.		Kcal/100 g	12	11	16	13

Lipids represented 1.46% on average in 2016, with variations between 1.22 and 1.85%, and 1.42% on average in 2017, with variations between 1.22 and 1.73%.

The content in **mineral substances** (ash) was on average 1.59% in both years, with variations between 1.46 and 1.88% in 2016, and between 1.47 and 1.80% in 2017.

Fibers represented 1.96% on average in the case of organic wheat harvested in 2016 (variation limits 1.33-2.65%) and 1.72% in 2017 (variation limits 1.34-2.04%).

The calculations regarding the **nutritional value** of the products harvested in the organic crops analyzed in 2016 revealed values of 1370-1451 kJ/100 g, on average 1420 kJ/100 g or 328-344 kcal/100 g, on average 336 kcal/100 g.

In the second experimental year (2017), the values resulting from the calculations were 1388-1433 kJ/100 g, on average 1414 kJ/100 g, or 332-340 kcal/100 g, on average 338 kcal/100 g.

The **lipid energy** of the organic wheat harvested in 2016 was 47-71 kJ/100 g on average 56 kJ/100 g or 11-17 kcal/100 g on average 13 kcal/100 g in 2016. In 2017, the values results of calculation for lipid energy were similar, namely 55 kJ/100 g, with variations between 47 and 67 kJ/100 g or 13 kcal/100 g, with variations between 11 and 16 kcal/100 g.

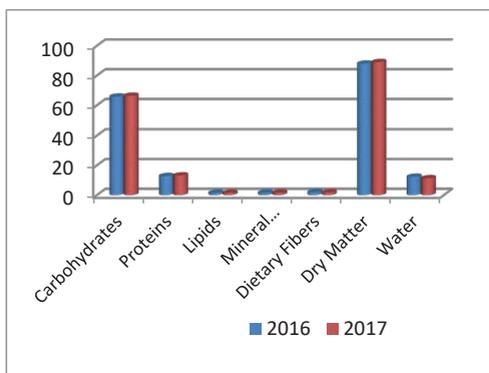


Figure 4. Biochemical compounds of organic winter wheat, on period 2016-2017 (%)

Analyzing the presence of **pesticide residues** from organic wheat samples is very important because their absence from the harvest is an essential condition to enable the use of organic

products on a specific market. The analysis of the obtained results showed that in all cases (Table 3), the presence of pesticide residues in the studied wheat samples was not reported, which reflects the correctness of the technologies applied by organic farmers.

Also, the analysis of the contents of the organic wheat samples in **nitrites** and **nitrates** have relevant absence of these compounds in most cases. In two cases, in 2016, the analyzes reported the presence of nitrite content of 6.6 mg/kg in Arad County and 5.1 mg/kg in Bihor County, which exceeded the maximum allowed limit of 1.0 mg/kg (Table 4).

Table 3. Results of analyzes on the pesticide residues content of wheat grains produced under organic conditions

Substances residues	2016	2017	Maximum permissible limit for products conventional (Regulation (EC) No 396/2005)
Glyphosat+Glufosynat	None product detected	None product detected	0.01 mg/kg
Acid aminomethylphosphoric			0.01 mg/kg
Chlormequat			0.01 mg/kg
Mepiquat			0.01 mg/kg

Table 4. Results of analyzes on the nitrate and nitrite content of wheat grains produced under ecological conditions

Compound	Production region of wheat	2016	2017	Maximum allowable limit for conventional products (Directive 91/676 / EEC and Reg. (EC) no. 396/2005)
Nitrite	West (Arad County)	6.6 mg/kg	No nitrites were detected in the others samples analyzed	1.0 mg/kg
	North-West (Bihor County)	5.1 mg/kg		
Nitrate	All regions analyzed	No nitrates were detected in the samples analyzed		50 mg/kg

The analysis of the causes revealed that, on the respective surfaces, organic fertilizers administered and the high fertility of the respective lands determined nutrition with

excess nitrogen. The respective products were directed to other non-food uses; at the same time, recommendations were made to avoid excess nitrogen nutrition in the future.

The analysis of **mycotoxins** contents was also considered a priority of the research carried out because, in the absence of treatments with synthetic chemicals for the control of pathogens, it is considered that there is a danger of occurrence, in the organic crops, as well as on the storage-processing chains-commercialization of some pathogens-microorganisms - phytopathogens or epiphytes (mold fungi) - that lead to contamination of the products with mycotoxins.

Table 5. Results of analyzes on the mycotoxin content of wheat grains produced under organic conditions

Compound	Production region of wheat	2016	2017	Maximum allowable limit for conventional products (Directive 91/676/CEE), Reg.(EC) 396/2005)
Deoxynivalenol (DON)	North-Wwest (Bihor County)	146 $\mu\text{g}/\text{kg}$	No compounds were detected in the other organic crops analyzed	750 $\mu\text{g}/\text{kg}$
Aflatoxin B ₁	No compounds were detected in the other organic simples analyzed			2 $\mu\text{g}/\text{kg}$
Aflatoxin B ₁ +B ₂ +G ₁ +G ₂				4 $\mu\text{g}/\text{kg}$
Fumonisin B ₁ +B ₂				1900 $\mu\text{g}/\text{kg}$

The analysis of mycotoxins contents from the organic wheat produced in different areas on the Romanian territory in 2016 and 2017 did not reveal the presence of mycotoxins. In one case, in 2016 at an organic wheat sample from Bihor County, a content of deoxynivalenol (DON) of 146 $\mu\text{g}/\text{kg}$ was found, but does not exceed the maximum allowed limit of 750 $\mu\text{g}/\text{kg}$ (Table 5). As a result, the respective product was directed to consumption in human nutrition.

CONCLUSIONS

Research conducted in 2016 and 2017 on the quality of common wheat crops (wheat for bakery) produced in organic agriculture conditions revealed the superior quality of the

crop in terms of nutritional value, as well as the absence of compounds that can negatively influence the quality of the harvest.

In terms of nutritional value, the average contents were as follows: 87.84-88.85% dry matter, 65.65-66.22% carbohydrates, 12.59-13.03% protein, 1.42-1.48% lipids, 1.59% mineral substances, 1.72-1.96% dietary fibers. The nutritional value was 1414-1420 kJ/100 g or 336-338 kcal/100 g and the lipid energy of 55-56 kJ/100 g or 13 kcal/100 g. These values correspond to the recommendations for wheat intended for baking.

The analyzes carried out are relevant, in all cases, the absence of pesticide residues from organic wheat produced in Romania, which illustrates that the recommendations regarding the technologies of cultivation of organic wheat have been respected.

Also, the analyzed samples, with two exceptions, did not contain nitrites and nitrates or mycotoxins. In two cases, nitrite contents resulted above the allowed limits; the origin of which was determined by the high fertility of the lands on which the wheat was grown, as well as the application of high doses of organic fertilizers.

The presence of mycotoxins (deoxynivalenol) was found in only one case, in 2016 at an organic wheat crop produced in Bihor County. This indicates a *Fusarium* attack, but the determined values are well below the maximum limits allowed for wheat intended for baking.

Following the research carried out, it can be concluded that the wheat produced in 2016 and 2017 in the organic crops from the main cultivation areas in Romania meets higher quality conditions and is not contaminated with compounds that diminish their quality.

REFERENCES

- Nastase, P.I., Toader, M. (2016). Study regarding organic agriculture and certification of products. *Scientific Papers. Series A. Agronomy*, LIX, 344–349.
- Nastase, P.I., Toader, M., Sonea, C. (2018). Study on legislation of organic products quality. *Lucrări Științifice, Seria Agronomie*, 61(2), 151–156.
- Roman, Gh.V., Toader, M., Epure L.I., Ion, V., Mihalache, M., Mihalache, D., Georgescu, M.I., Dobrin, I. (2011). *Dictionar ecologic de agricultura ecologica*. Editura "Universitara", Bucuresti.

- Roman, Gh.V., Pirsan, P., Ștefan, M., Robu, T., Duda, M.M., Tabără, V. (2015). *Fitotehnie. Cereale și Leguminoase pentru boabe*. Editura "Universitara" București.
- BS EN 15662: 2008. Foodstuffs of vegetable origin. Determination of pesticide residues by GCMS and/or LC-MS/MS after extraction/separation of acetonitrile and cleaning by SPE dispersion-QuEChERS method.
- Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.
- Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources.
- Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.
- Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC with EEA relevance. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32005R0396>
- SR EN ISO/CEI 17065: 2013. Evaluarea conformității. Cerințe pentru organisme care certifică produse, procese și servicii.

WINTER WHEAT MUTATIONS BY PLANT HEIGHT AND STRUCTURE CAUSED BY CHEMICAL SUPERMUTAGENS

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Abstract

The objectives of our investigations are to describe the variation by mutations of stem architecture of the modern Ukrainian winter wheat varieties due to their interactions with mutagen concentration and genotype-mutagen interaction specific. Agronomic-value mutations like as short stem, dwarfs and semi-dwarfs have been investigated too. New perspective mutant lines have been obtained in terms of mutation breeding program. Main components for mutation breeding successful was genotype-mutagen interaction (due to factor analyses). By mutation occurs (in sense of mutation rate and spectra) genotypes can be subdivided on two groups. At the first group only varieties, which obtained with nitrosoalkylurea were observed. At second group other six varieties. Nitrosoalkylurea were less useful for obtaining mutations by plant architecture for any varieties. Higher level of short-stem and semi-dwarfs mutation were induced by NMU 0.025%. Semi-dwarfs as mutations significance responded to nitrosoalkylurea action by genotype-mutagen interaction.

Key words: winter wheat, chemical mutagenesis, plant height and structure, nitrosoalkylureas.

INTRODUCTION

Experimental mutagenesis has been used successful in main crops for obtaining agronomical important traits. Induced mutations in wheat have been obtained for morphological and quantitative characters by treatment with different mutagens (Nazarenko et al., 2018). The main purpose of using mutagens has been to induce genetic variation of agronomic-value traits. Grain yield, a complex polygenic trait is highly affected due to complex of difference traits of plants architecture (Nazarenko, 2017). More than 3500 varieties of plants obtained either as direct mutants or derived from their crosses and 2700 mutant varieties of different plants including cereal crops have been released throughout the world through direct or indirect use of mutation breeding (IAEA, 2018).

Bread wheat (*Triticum aestivum* L.) with the annual production of about 757 million tons (in 2017) (USDA, 2018), is one of the world's most important cereal crops. Winter wheat is the world's leading cereal grain and the most important food crop, occupying first position in Ukraine. Ukrainian agriculture takes about 48%

area under cereals and contributing 38% of the total food grain production in the country (Nazarenko, 2016).

The improvement of grain yield and yield components of wheat through application of mutagens leads towards improvement of new cultivars with improved traits. The use of induced mutations has become an important approach to optimize plant structure for bioproductivity (Naveed et al., 2015).

The present studies were therefore undertaken to investigate the effects of chemical supermutagens (nitrosoalkylureas) on so yield associated trait as plant height and structure of stem.

Plant height is important agronomic traits related to plant architecture and grain yield in wheat. Tiller number and plant height are pointed out as two major agronomic traits in cereal crops affecting plant architecture and grain yield (Ellis et al., 2004). In investigation of chines researches of NAUH167, a new mutant of common wheat landrace induced by ethylmethyl sulfide treatment, exhibits higher tiller number and reduced plant height was attributed to the decrease in the number of cells and their length. Genetic analysis showed that

the high-tillering number and dwarf phenotype were related and controlled by a partial recessive gene (Xu et al., 2017).

Dwarfing and semi-dwarfing mutations have a mutual effects. As for example, dwarfing gene Rht-5 was associated with a plant height reduction, delaying heading date by 1 day, increasing the number of fertile tillers plant⁻¹, while reducing the number of spikelets spike⁻¹ and number of grains spike⁻¹. The results of this study could be useful for proper use of Rht-5 dwarfing gene in breeding programs to improve lodging tolerance, yield potential in wheat and increase efficiency of marker assisted selection for agronomic traits (Daoura et al., 2014).

One strategy to meet this challenge is to raise wheat productivity by optimizing plant stature. As a sample of this investigation, the reduced height 8 (Rht8) semi-dwarfing gene is one of the few, together with the Green Revolution genes, to reduce stature of wheat (*Triticum aestivum* L.), and improve lodging resistance, without compromising grain yield. Rht8 is widely used in dry environments where it increases plant adaptability. Morphological analyses show that the semi-dwarf phenotype of Rht8 lines is due to shorter internodal segments along the wheat culm, achieved through reduced cell elongation (Gasperini et al., 2012).

The development of wheat mutants not only provided new genetic resources for wheat improvement, but also facilitated our understanding of the regulation of these traits at the molecular level. Identification of a dwarf mutant with a compact spike, NAUH164, produced from ethyl methyl sulfonate treatment of wheat variety Sumai 3, has reduced plant height and shortened spike length. Dwarfness and compact spike were controlled by a single dominant gene that was designated Rht23 (Chen et al., 2015).

Regarding 47 wheat cultivars carrying different Rht alleles screening for their ability to emerge from deep sowing, and for detailed physiological characterization in the field the modern wheat lines have been shown differences in early developmental stages were associated with grain yield, as indicated by a reduction of 37.3% in the modern cultivars (Amram et al., 2015). But reducing by grain

productivity at modern investigations not always characteristic for dwarf winter wheat varieties with typical gibberellin-responsive (GAR) dwarfing genes, such as Rht12. In investigations of chine sciences (Chen et al., 2014) plant height of the tall lines was not affected significantly by GA3 treatment. Plant biomass and seed size of the GA3-treated dwarf lines was significantly increased compared with untreated dwarf plants while there was no such difference in the tall lines. This effect has addictive value effect Rht12 dwarf plants developed faster than control plants and reached double ridge stage 57 days, 11 days and 50 days earlier and finally flowered earlier by almost 7 days while the tall lines. Both possibilities are confirmed by several investigations (Bachir et al., 2014; Fellahi et al., 2018; Hans et al., 2019; Lingling et al., 2019).

The objectives of our investigations are to describe the genotypic variation of new mutant winter wheat lines by plant height and structure, investigation of role genotype-mutagen interactions at formation of new trait. The most target objects are developing relations between genotype and nature of chemical mutagen, mutagen concentration. Second our purpose to estimate new lines and their suitability as direct new varieties or components for future breeding crosses.

MATERIALS AND METHODS

Dried seeds (approx. 14% moisture content, in brackets method of obtaining varieties or used mutagens) of 'Favoritka', 'Lasunya', 'Hurtovina' (mutation and mutation-recombination varieties regarding IAEA classification, radiomutans), line 418, 'Kolos Mironovschiny' (hybrid varieties), 'Sonechko' and 'Kalinova' (mutation varieties, chemomutant), 'Voloshkova' (mutation variety, termomutagenesis - low plus temperature at plant development stage of vernalizaion) of winter wheat (*Triticum aestivum* L.) were treated with solutions of chemical mutagens - nitrosomethylurea (NMU) 0.0125 and 0.025%, nitrosoethylurea (NEU) - 0.01 and 0.025%. Each treatment was comprised of 1,000 wheat seeds. Exposition of chemicals mutagens was 18 hours. These

concentrations and exposure are trivial for the breeding process that has been repeatedly established earlier (Nazarenko, 2016a). Non-treated varieties and national standard by grain yield Podolyanka were used as a control for mutation identified purpose by all traits changes.

Treated seeds were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, to raise the M₁ population. The untreated seeds of mother varieties and standard (parental line/variety) were also planted after every ten rows as control for comparison with the M₁ population. M₁ plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval (Nazarenko, 2017).

In M₂-M₃ generations mutation families have been selected via visual estimation. 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, 1-2 rows for sample with control-rows of untreated varieties and standard in every twenty-sample interval.

Estimation of total characteristics and heritability of changed traits was conducted from 2014 to 2018 years (M₄-M₈ generations). The controls were national standard by productivity 'Podolyanka' and initial variety. The working-methods in the breeding trials are satisfied to state variety exam requests. The trial was set up as a randomized block design method with three replications and with a plot size of from 5 to 20 m² in 2-3 replications (Shu et al., 2013).

Experiments were conducted on the experiment field of Dnipro State Agrarian-Economic University (village Oleksandrovka, Dnepropetrovsk district, Dnipro region, Ukraine). Normal cultural practices including fertilization were done whenever it is necessary. Weeds were manually removed where necessary, and fungicides and insecticides were applied to prevent diseases and insect damage. Evolution was conducted during 2011-2018 years.

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, the grouping mutants cases was performed by cluster and

discriminant analysis, factor analyses was conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

RESULTS AND DISCUSSIONS

Total size of population 20000 families at second-third generation (include controls) and represented by variants of mutagen treatment at Table 1. Investigators are conducted with trivial mutagen concentrations for breeding purposes (Nazarenko, 2016a; Nazarenko, 2017b).

From M₂-M₃ generations (from all experiments, included all variants with other mutagens) 1,482 potential productivity winter wheat mutation lines and 5,862 lines with mutation changes were determined overall. In all variants 500 families have been investigated, all concentrations are optimal for plant surviving. General rate of mutations was up to 14.2% under NEU action (Sonechko) and to 15% under NMU action (Voloshkova) (Table 1).

The lowest general mutation rate was 3.6% (NEU) and 4.2% (NMU) (at both cases for chemomutant Sonechko, which obtained with nitrosoalkylureas action). NMU is more active as mutagen by general rate of mutations, but its depends on genotype-mutagen interaction and can be changed according to initial variety.

Regarding rate of plant structure mutations action of both mutagens was equal at average and depended on initial genotype only.

From these investigations fact of decreasing general mutation rates and number of mutation traits (level of changeability) for chemomutants after nitrosoalkylureas action has been developed.

Regarding dates of Tables 2-4 any statistically reliable difference between rates in this group between three types of genotypes has been observed for varieties Sonechko and Kalinova, but line 418 is close to these genotypes by this parameter and cluster and discriminant analysis has been used for more precision classification of material.

Table 1. General rate of mutations at second – third generations (rate of mutations by plant structure in brackets)

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Control	0.4(0.4)	1.2(1)	1.8(1.4)	0.8(0)	0.6(0.2)	0.8(0)	1.4(0.8)	0.8(0.2)
NEU 0.01%	9.6(1)	6.4(0.6)	8.8(1.4)	4.2(1.4)	8.8(1.6)	7.0(0.6)	8.49(1.8)	9.4(0.8)
NEU 0.025%	13.4(1.8)	10.8(0.6)	15.0(2.6)	7.8(0.6)	12.4(2.2)	11.2(1)	13.8(2.6)	12.0(2.6)
NMU 0.0125%	8.8(1.4)	5.0(1)	7.8(1.6)	3.6(0.6)	7.6(1.8)	8.0(1.6)	8.0(1.2)	7.6(1.2)
NMU 0.025%	13.6(2)	7.0(1.6)	15.8(2.2)	4.8(1.8)	12.0(2.8)	10.2(2.6)	12.0(2)	14.2(1.4)

Table 2. Spectrum of mutations under nitrosoalkylureas action (radiomutants), %

N	Trait	Check	NEU 0.01%	NEU 0.025 %	NMU 0.012 5%	NMU 0.025%
variety Favoritka						
1	high stem	0.0	0.6	0.8	0.6	1
2	short stem	0.2	0	0.2	1	0.8
3	semi-dwarf	0.0	0	0	0.2	0.6
4	dwarf	0.0	0	0	0	0.2
5	thick stem	0.0	0	0	0	0.2
6	thin stem	0.0	1	1.2	0	0
variety Hurtovina						
1	high stem	0	0.4	0.6	1	1.2
2	short stem	0	0.2	0.2	0.6	0.6
3	semi-dwarf	0	0	0.2	0	0.4
4	thin stem	0	0	0	0	0.4
variety Lasunya						
1	high stem	0.4	1	1.6	0.4	0.4
2	short stem	0.4	0.8	0.6	0.6	0.8
3	semi-dwarf	0.0	0	0.2	0.2	0.6
4	dwarf	0.0	0	0	0	0.2
5	thin stem	0.0	0	0.2	0	0

Table 3. Spectrum of mutations under nitrosoalkylureas action (chemomutants), %

N	Trait	Check	NEU 0.01%	NEU 0.025 %	NMU 0.0125 %	NMU 0.025 %
variety Kalinova						
1	high stem	0.8	0.6	0.6	0.6	1
2	short stem	0.2	0	0	0.4	0.4
3	semi-dwarf	0.0	0	0	0	0.2
variety Sonechko						
1	high stem	0.0	0.6	0.2	0.4	1
2	short stem	0.0	0.4	0.2	0.2	0.4
3	semi-dwarf	0.0	0.2	0.2	0	0.4
4	dwarf	0.0	0.2	0	0	0

Rate of this type of mutations varied from 0.6 (Kalinova, Sonechko, Hurtovina) to 2.6% (Voloshkova, line 418) for NEU and from 0.6 (Sonechko) to 1.4% (line 418) for NMU. As we can see from the tables, higher rates and more types of this group mutations were characterized for recombination and radiomutant varieties, chemomutants like Kalinova and Sonechko (at higher level) were less sensitive to this type of mutagen action.

Table 4. Spectrum of mutations under nitrosoalkylureas action (hybrid varieties), %

N	Trait	Check	NEU 0.01%	NEU 0.025 %	NMU 0.012 5%	NMU 0.025%
variety Kolos Mironivschini						
1	high stem	0.2	0.8	0.6	0.8	0.6
2	short stem	0.2	0.2	0.2	0.4	1
3	semi-dwarf	0.0	0	0.2	0	0.2
4	thin stem	0.0	0	0.8	0.2	0.2
variety Voloshkova						
1	high stem	0.0	0.4	0.2	0.8	0.4
3	short stem	0.0	1	1.6	0.8	1.6
4	semi-dwarf	0.6	0	0.6	0	0
5	dwarf	0.8	0	0.2	0	0.2
line 418						
1	high stem	0.2	0.8	1.8	1	0.6
2	short stem	0.0	0	0.6	0.2	0.6
3	semi-dwarf	0.0	0	0.2	0	0.2

Cluster analyses (Figure 1) confirmed complicated and complex character of mutagen-genotype interaction. Only one group has been identified with statistically reliability.

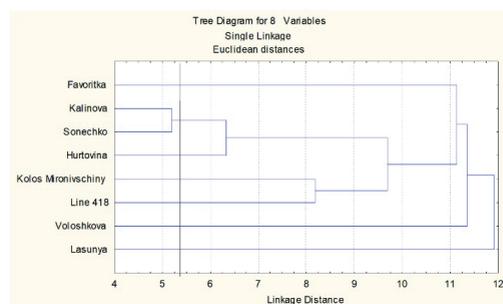


Figure 1. Results of cluster analyze

General mutation rate to all types mutation has been increased concentration growth. High level of changeability was corresponded to higher concentrations of NEU and NMU. Rate of mutations by plants structure obeys this tendency (excluded varieties Sonechko (decreasing of rate) and Kaliniova (rate is

lower). In spite of gamma-rays from previous investigations dates were enough clearly for this conclusion, may be due to more site-specific action of chemical mutagens.

We can subdivided initial material by the method of breeding as radiomutants (Favoritka, Hurtovina, Lasunya), chemomutants (Kalinova and Sonechko), thermomutants (low plus temperature at plant development stage of vernalizaion has been used as mutagen factor) (Voloshkova) and forms, obtained after hybridization (Kolos Mironivschini, line 418). For first group (Table 2) similar number and types of mutations was characterized to all mutagens and concentrations, but reaction of genotypes was differing for all three genotypes. Rates of mutations are not high, variety Favoritka characterized by more types of mutations, dwarfs mutations were seldom and only for two genotypes under NMU 0.025% fcton. Lower mutability was inherited for variety Hurtovina. Seldom mutations of stem thickness in spite of gamma-rays can be observed at all cases, and were appeared at all coccentrations and mutagens, but not genotypes. Nitrosoalkylureas action are more useful for this type of mutations then gamma-rays.

For second group (Table 3) lower rate of mutations was developed for all varieties and concentrations. We observed only mutations by plant height.

Regarding Table 4 the same situation was observed as for radiomutants from table 1. At all cases for all genotypes concentration NMU 0.025% was more suitable for mutation induction by plant structure.

Thus, mutants with thick stem for NEU - for a thick stem of mutations is completely not marked; for unlikely, arose with a very high frequency for the variety Kolos Mironivshchyni - the frequency of occurrence was 0.8% and one case for the variety Lasunya, that is, the mutation is rather specific; high-stem mutants on average 0.8%, high-frequency mutation occurring in any variant with a frequency from 0.2 to 1.8%, but mainly for Favoritka, Lasunya, line 418; short stem - high probability of occurrence, but more rarely than high- frequency, an average of 0.5%, the frequency in some variants up to 1.6%, which is considerably lower than in the case of

gamma rays, this mutation is completely absent for the variety Kalinova, but unlikely for the varieties Sonechko and Kolos Mironivschini; semi-dwarfs - mutation average probability, much less frequent than gamma rays, up to 0.6%, on average - 0.1%, characteristic for higher concentrations of NEU, almost absent at NEU 0.01% (except for the Sonechko) and absent from the Kalinova; dwarf - for NEU, in contrast to gamma rays almost absent, only one case for varieties Voloshkova and Sonechko.

Table 5. Results of discriminant analyze

Variables at model	Wilks Lambda λ	Partial Lambda	F-remove (4.02)	p-level
high stem	0.289232	0.783392	2.212000	0.089858
short stem	0.258270	0.877308	1.118807	0.364900
semi-dwarf	0.416547	0.634530	2.657860	0.043019
dwarf	0.277161	0.817512	1.785787	0.156017
thick stem	0.336470	0.867650	2.545489	0.132456
thin stem	0.317843	0.965437	2.097654	0.245678

For NMU for this group - only one case of a mutant with a thick stem (Favoritka, NMU 0.025%) was noted for the thickness of the stem; thin stem - very rare mutation. the frequency of occurrence from 0 to 0.2%, arose for only three variants; high-stem mutants on average 0.7% variations, high-frequency mutation occurring in any variant with a frequency from 0.4 to 1.2%, more or less evenly in all varieties, which significantly differs from NEU; short stem - high probability of occurrence, in all cases, an average of 0.7%, the frequency in individual variants up to 1.6%, which is much lower than in the case of gamma-rays, approximately uniform and on average more frequent than in NEU; semi-dwarf - mutation is also highly probable, comparable frequency to the frequency of gamma rays, up to 0.6%, on average 0.2%, characterized by a higher concentration of NMU, for the Voloshkova this mutation is absent, which is partially coincides with NEU; dwarfs occur only in three cases - for the varieties Voloshkova NMU 0.025%, Favoritka and Lasunya variety, that is, unlike NEU, isolated cases occur.

Regarding analyze of these groups it has been developed that rate of these types of mutations was significantly lower for first group, than for others. According to ANOVA analyses number

of mutations was depended on concentrations at all cases, relation with genotype and mutation rate has been identified with significance reliability for only one case semi-dwarf mutations ($F_{3.01}$, $F_{critical}$ 2.15). In spite of this fact, genotype and mutagen interaction are statistically reliable for all cases and traits, just the same for gamma-rays at previous investigations.

CONCLUSIONS

Due to results of our investigations NEU and NMU as a mutagens for creation new variation material on plant height and stem structure has been shown as less successful than gamma-rays.

Large number of material has been obtained both as for perspective new varieties and as the sources for future winter wheat breeding program for changing plant architecture. Only one trait appeared significant influence of genotype as a key component for mutation breeding success but at all times genotype-mutagen interaction regarding results of ANOVA analyze was significance in its influence on mutation rates. Genotype-mutagen interaction and classification of mutant material is possible by rate of semi-dwarf forms

Nitrosoalkylureas were less effective to mutants, which obtained with same action, for mutations by stem high. NMU 0.025% were more preferable to short-stem and semi-dwarfs mutation obtaining, no concentrations of NEU or NMU useful of dwarfs mutations.

REFERENCES

Fellahi, Z., Hannachi, A., Oulmi, A., Bouzerzour, H. (2018). Analyse des aptitudes générale et spécifique à la combinaison chez le blé tendre (*Triticum aestivum* L.). *Revue Agriculture*, 9(1), 60–70. Retrieved November 14, 2019, from https://www.researchgate.net/publication/330957165_Analyse_des_aptitudes_generale_et_specifique_a_la_combinaison_chez_le_ble_tendre_Triticum_aestivum_L

Amram, A., Fadida-Myers, A., Golan, G., Nashef, K., Ben-David, R., Peleg, Z. (2015). Effect of GA-sensitivity on wheat early vigor and yield components under deep sowing. *Frontiers in Plant Science*, 6 (487). Retrieved November 14, 2019, from <https://www.frontiersin.org/articles/10.3389/fpls.2015.00487/full>

Chen, L., Hao, L., Condon, A.G., Hu, Y-G. (2014). Exogenous GA3 Application Can Compensate the Morphogenetic Effects of the GA-Responsive Dwarfing Gene *Rht12* in Bread Wheat. *PLoS ONE*, 9(1), e86431. Retrieved November 14, 2019, from doi:10.1371/journal.pone.0086431.

Chen, S., Gao, R., Wang, H., Wen, M., Xiao, J., Bian, N., Zhang, R., Hu, W., Cheng, S., Bie, T., Wang, X. (2015). Characterization of a novel reduced height gene (*Rht23*) regulating panicle morphology and plant architecture in bread wheat. *Euphytica*, 203, 583–594. Retrieved November 14, 2019, from <https://link.springer.com/article/10.1007/s10681-014-1275-1>

Daoura, B., Chen, L., Du, Y., Hu, Y. (2014). Genetic effects of dwarfing gene *Rht-5* on agronomic traits in common wheat (*Triticum aestivum* L.) and QTL analysis on its linked traits. *Field Crop Research*, 156(1), 22–29. Retrieved November 14, 2019, from <https://www.sciencedirect.com/science/article/pii/S0378429013003572>

Ellis, M., Rebetzke, G., Chandler, P., Bonnett, D., Spielmeier, W., Richards, R. (2004). The effect of different height reducing genes on the early growth of wheat. *Functional Plant Biology*, 31, 583–589. Retrieved November 14, 2019, from <https://www.publish.csiro.au/FP/FP03207>

Gasperini, D., Greenland, A., Hedden, P., Dreos, R., Harwood, W., Griffiths, S. (2012). Genetic and physiological analysis of *Rht8* in bread wheat: an alternative source of semi-dwarfism with a reduced sensitivity to brassinosteroids. *Journal of Experimental Botany*, 63, 4419–4436. Retrieved November 14, 2019, from <https://www.ncbi.nlm.nih.gov/pubmed/22791821>

Hans, D., Anthony, G., Matthew, H. (2019). Artificial selection causes significant linkage disequilibrium among multiple unlinked genes in Australian wheat. *Evolutionary Applications*, 19(4), 194–205. Retrieved November 14, 2019, from <https://onlinelibrary.wiley.com/doi/full/10.1111/eva.12807>

International Atomic Energy Agency (2018). Mutant varieties database. [Online] Vienna: IAEA. Available at: <https://mvd.iaea.org> [Accessed 16 July 2019].

Lingling, C., Zhaoyan, C., Ruolin, B., Huijie, Z., Xuejiao, C., Huiru, P., Yingyin, Y., Zhaorong, H., Mingming, X., Weilong, G., Qixin, S., Aiju, Z., Zhongfu, N. (2019). Dissection of two quantitative trait loci with pleiotropic effects on plant height and spike length linked in coupling phase on the short arm of chromosome 2D of common wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 132(6), 1815–1831. Retrieved November 14, 2019, from <https://www.ncbi.nlm.nih.gov/pubmed/30267114>

Naveed, A., Nazir, A., Abdul, H., Raza, S., Muhammad, A. (2015). Mutation breeding: a tool to improve wheat yield and yield components. *Life Science*, 9(1), 3274–3279. Retrieved November 14, 2019, from <https://pdfs.semanticscholar.org/d004/781230da65be859e55fabde6c24b49daa650.pdf>

- Nazarenko, M. (2016). Identification and characterization of mutants induced by gamma radiation in winter wheat (*Triticum aestivum* L.). *Scientific Papers. Series A. Agronomy, LIX*, 350–353. Retrieved November 14, 2019, from <http://agronomyjournal.usamv.ro/index.php/scientific-papers/past-issues?id=644>
- Nazarenko, M.M. (2017). The influence of radiomimetic chemical mutagen on chromosomal complex of winter wheat cells. *Regulatory Mechanisms in Biosystems*, 8(2), 283–286. Retrieved November 14, 2019, from <https://medicine.dp.ua/index.php/med/article/view/021744>
- Nazarenko, M. (2017). Specific Features in the Negative Consequences of a Mutagenic Action. *Russian Journal of Genetics: Applied Research*, 7(2), 195–196. Retrieved November 14, 2019, from <https://link.springer.com/article/10.1134/S2079059717020083>
- Nazarenko, M. (2017). Influence of nitrosoalkylureas on winter wheat plants at first generation after mutagen action. *Agriculture and Forestry*, 63(1), 319–328. Retrieved November 14, 2019, from http://www.agricultforest.ac.me/paper.php?journal_id=201&id=2678
- Nazarenko, M., Lykholat, Y., Grigoryuk, I., Khromykh, N. (2018). Optimal doses and concentrations of mutagens for winter wheat breeding purposes. Part I. Grain productivity. *Journal of Central European Agriculture*, 19(1), 194–205. Retrieved November 14, 2019, from <https://jcea.agr.hr/en/issues/article/2037>
- Shu, Q.Y., Forster, B.P., Nakagava, H., (2013). *Plant mutation breeding and biotechnology*, Vienna: CABI publishing.
- World Agricultural Supply and Demand Estimates*. USDA, Washington, 2018. Retrieved November 14, 2019, from <https://www.usda.gov/oce/commodity/wasde/latest.pdf>.
- Xu, T., Bian, N., Wen, M., Xiao, J., Yuan, C., Cao, A., Zhang, S., Wang, X., Wang, H. (2017). Characterization of a common wheat (*Triticum aestivum* L.) high-tillering dwarf mutant. *Theoretical Applied Genetic*, 130(3), 483–494. Retrieved November 14, 2019, from <https://link.springer.com/article/10.1007/s00122-016-2828-6>

EFFICACY AND SELECTIVITY OF IMAZAMOX-CONTAINING HERBICIDES AT CLEARFIELD® AND CLEARFIELD® PLUS SUNFLOWER HYBRIDS

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Abstract

In 2018 a field experiment with the sunflower hybrids SY Bacardi CLP and SY Diamantis CL was conducted. The experiment included the following treatments: 1. Untreated control; 2. Pulsar 40 - 1.25 l ha⁻¹; 3. Pulsar 40 - 2.50 l ha⁻¹; 4. Pulsar Plus - 1.20 l ha⁻¹, 5. Pulsar Plus - 2.40 l ha⁻¹. The herbicides were applied in phenophase 4th-6th true leaf of the crop. The efficacy and the selectivity of the studied herbicides were evaluated. The highest efficacy against *S. halepense* from rhizomes was recorded for treatment 5 (Pulsar Plus - 2.40 l ha⁻¹). *S. halepense* developed from seeds, *S. viridis*, *A. retroflexus*, *Xa. strumarium*, *S. nigrum* and *S. arvensis* were successfully controlled by application of Pulsar 40 or Pulsar Plus in the low examined rates. *Ch. album* and *A. theophrasti* can be controlled by Pulsar Plus at the lower rate - 1.20 l ha⁻¹. The absolute seed mass of 1000 seeds and the yield as well as the seed oil content from the plants treated with the registered and double herbicide rates, independently the herbicide product and studied hybrid, were higher than those of the untreated controls highly infested with weeds.

Key words: efficacy, selectivity, Imazamox, sunflower, yields.

INTRODUCTION

Bulgaria and Romania are the largest sunflower seed producers in the EU, with sunflower yields increasing twice over the past ten years. The results in the sector are good starting point for a debate over its competitiveness and efficiency (Hristov et al., 2019).

In order to achieve high yields, along with the optimization of the main vegetation factors, it is necessary to effectively control the weeds (Tonev, 2000). Weed control at sunflower should be performed in the optimal phases of the crop before the critical period for decreasing the morphological parameters is reached (Simic et al., 2011).

In Bulgaria, the most common weeds in the sunflower fields are *Amaranthus* spp., *Sinapis arvensis* L., *Chenopodium album* L., *Cannabis ruderalis* Janisch, *Setaria* spp., *Echinochloa crus-galli* L., *Sorghum halepense* (L.) Pers., *Cirsium arvense* Scop., *Convolvulus arvensis* L., some new races of *Orobanche cumana* Wallr. etc. (Manilov and Zhaltov, 2015; Tonev et al., 2010).

In the region of the cities of Plovdiv and Stara Zagora, Bulgaria it is found that in the sunflower fields *Amaranthus blitoides* L. and

Amaranthus albus L. occupy much of the total weed infestation with annual weeds. The authors report that the most common perennial weeds is *Convolvulus arvensis* L. (Moskova et al., 2016).

Breeding of hybrids resistant to imazamox and tribenuron-methyl enabled an efficient control of the main broadleaf and grassy weeds, including parasitic species from genus *Orobanche* (Fernandez-Martinez et al., 2009; Malidza et al., 2003).

At the Clearfield® technology, the grown hybrids are resistant to the imidazolinone herbicide imazamox. The active substance inhibits the acetolactate synthase - ALS, the enzyme common to the biosynthesis of the amino acids valine, leucine and isoleucine. By its mode of action, the active substance controls the broadleaf weeds, as well as the grass weeds. For optimization of the existing technology, control of the new broomrape races and increasing the tolerance of the sunflower hybrids to imazamox, from 2016 there was released more developed version of the technology in Bulgaria. The name of the new technology is Clearfield Plus®. This system provides sunflower growers a better tool to manage weeds (Pfenning et al., 2012).

The aim of the current study is to evaluate the efficacy and selectivity of imazamox-containing herbicides at two sunflower hybrids bred to be grown by the Clearfield® and Clearfield® Plus technologies.

MATERIALS AND METHODS

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. The trial was conducted by the randomized block design in 4 replications. The size of the experimental plot was 28 m². The sowing date is on the 18th of April 2018 with planting distance 25 x 70 cm. The grown sunflower hybrids were SY Bacardi CLP and SY Diamantis CL. The experiment included the following treatments: 1. Untreated control; 2. Pulsar 40 - 1.25 l/ha; 3. Pulsar 40 - 2.50 l/ha; 4. Pulsar Plus - 1.20 l/ha, 5. Pulsar Plus - 2.40 l/ha. Both sunflower hybrids were treated with the same rates and herbicides products. Pulsar® 40 is containing 40.0 g/l Imazamox and Pulsar® Plus is containing 25 g/l Imazamox. The herbicides were applied in phenophase 4th-6th true leaf of the sunflower (BBCH 14-16).

A predecessor of the sunflower was winter wheat. On the trial field deep ploughing, two times disc harrowing and two times cultivation before sowing were done. Basic combine fertilization with 250 kg ha⁻¹ NPK 15:15:15 and spring dressing with 200 kg/ha NH₄NO₃ was performed.

The efficacy of the studied herbicides and rates was evaluated by the 10 score scale of EWRS was evaluated on the 14th, 28th and 56th day after application as described by Zhelyazkov et al. (2017). The weed infestation was presented by Johnson grass (*Sorghum halepense* Pers.) developed from rhizomes, Johnson grass (*S. halepense*) developed from seeds, Green bristle grass (*Setaria viridis* L.), Fat-hen (*Chenopodium album* L.), Common amaranth (*Amaranthus retroflexus* L.), Rough cocklebur (*Xanthium strumarium* L.), Velvetleaf (*Abutilon theophrasti* L.), Black nightshade (*Solanum nigrum* L.) and Wild mustard (*Sinapis arvensis* L.). The selectivity by the 9 score scale of EWRS as described by Zhelyazkov et al. (2017) was evaluated on the

7th and on the 14th day after the herbicide application (at score 0 there are not damages on the crop, and at score 9 the crop is completely destroyed).

The hectoliter seed mass was measured by weighing two parallel samples of 100 dm³ air dry seeds. The hectoliter mass is calculated, as the arithmetic means of the established mass of the two samples (in grams) multiply by 100 and the resulting is divided into 1000 to obtain the mass in kilograms (Tonev et al., 2018).

The absolute seed mass of 1000 clean, air-dry seeds, expressed in grams was also measured (Tonev et al., 2018).

The oil content in the sunflower seeds was determined by the Soxhlet method as described by Ivanov and Popov (1994).

Statistical analysis of collected data was performed by using Duncan's multiple range test by the software SPSS 19. Statistical differences were considered significant at p<0.05.

RESULTS AND DISCUSSIONS

Because of the fact that the efficacy of both herbicide products is performed parallel for both sunflower hybrids the results are going to be presented together independently the hybrids.

Table 1. Efficacy against Johnson grass (*Sorghum halepense* Pers.) developed from rhizomes (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	20	35	45
3. Pulsar 40 - 2.50 l ha ⁻¹	20	40	50
4. Pulsar Plus - 1.20 l ha ⁻¹	30	40	55
5. Pulsar Plus - 2.40 l ha ⁻¹	40	55	65

On the 14th day after the treatments, the highest efficacy against Johnson grass (*S. halepense*) developed from rhizomes was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 40%, followed by variant 4 (Pulsar Plus - 2.40 l ha⁻¹) - 30% (Table 1). The efficacy on this evaluation date against this difficult-to-control weed was unsatisfactory.

On the next evaluation dates the efficacy increased insignificantly. The efficacy data showed that Johnson grass developed from rhizomes cannot be controlled by application of Pulsar 40 or Pulsar Plus independently the

examined rate. In situation with infestation of Johnson grass developed from rhizomes a partner grass herbicide product should be applied. Such a herbicide product is Stratos Ultra/Focus Ultra (100 g/l Cycloxydim) at rate of 2.00 l/ha.

On the 14th day after the treatments, the highest efficacy against the Johnson grass (*S. halepense*) developed from seeds was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 90% (Table 2).

Table 2. Efficacy against Johnson grass (*S. halepense*) developed from seeds (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	85	100	100
3. Pulsar 40 - 2.50 l ha ⁻¹	85	100	100
4. Pulsar Plus - 1.20 l ha ⁻¹	80	100	100
5. Pulsar Plus - 2.40 l ha ⁻¹	90	100	100

For the other treatments the efficacy was also satisfactory from 80 to 85%.

On the 28th and on the 56th day after application the efficacy increased and reached 100% for all treatments. The efficacy data showed that Johnson grass (*S. halepense*) developed from seeds can be successfully controlled by application of Pulsar 40 or Pulsar Plus.

On the 14th day after the treatments, the highest efficacy against the Green bristle grass (*S. viridis*) was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 90% (Table 3). For the other treatments the efficacy was also satisfactory - 85%. On the 56th day after application the efficacy increased and reached 100% for all treatments. The efficacy data showed that Green bristle grass developed from seeds can be controlled by Pulsar 40 or Pulsar Plus.

Table 3. Efficacy against Green bristle grass (*Setaria viridis* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	85	90	100
3. Pulsar 40 - 2.50 l ha ⁻¹	85	90	100
4. Pulsar Plus - 1.20 l ha ⁻¹	85	90	100
5. Pulsar Plus - 2.40 l ha ⁻¹	90	100	100

On the 14th day after the treatments, the highest efficacy against the Fat-hen (*Ch. album*) was recorded for variants 5 (Pulsar Plus - 2.40 l ha⁻¹). For the other treatments, accept for

variant 2 (Pulsar 40 - 1.25 l ha⁻¹) - 65%, the efficacy was also satisfactory - from 80 to 85% (Table 4).

Table 4. Efficacy against Fat-hen (*Chenopodium album* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	65	75	80
3. Pulsar 40 - 2.50 l ha ⁻¹	85	95	95
4. Pulsar Plus - 1.20 l ha ⁻¹	80	100	100
5. Pulsar Plus - 2.40 l ha ⁻¹	95	100	100

On the 56th day after application the efficacy increased and reached 95-100% for all treatments accept treatment 2 (Pulsar 40 - 1.25 l ha⁻¹) which stayed the lowest - 75% on the 28th day and 80% on the 56th day after the herbicide treatment.

The efficacy data showed that the Fat-hen can be successfully controlled by application of Pulsar Plus even from the lowest of 1.20 l/ha, and Pulsar 40 cannot assure efficient control of this weed species.

On the 14th day after the treatments, the highest efficacy against the Common amaranth (*A. retroflexus*) was recorded for treatments 5 (Pulsar Plus - 2.40 l/ha) and 3 (Pulsar 40 - 2.50 l ha⁻¹) - 95% (Table 5). For the other treatments, the efficacy was also satisfactory - from 80 to 85%.

Table 5. Efficacy against Common amaranth (*Amaranthus retroflexus* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	85	95	100
3. Pulsar 40 - 2.50 l ha ⁻¹	95	100	100
4. Pulsar Plus - 1.20 l ha ⁻¹	80	95	100
5. Pulsar Plus - 2.40 l ha ⁻¹	95	100	100

On the 56th day after application the efficacy increased and reached 100% for all treatments. The efficacy data showed that the Common amaranth can be successfully controlled by application of Pulsar 40 or Pulsar Plus applied even in the lowest examined rates.

Tan et al. (2005) stated that using IMI herbicide resistant hybrids gave farmers, the opportunity to control broadleaf weeds such wide spread *Xanthium* sp., *Cirsium* sp. and the root parasite broomrape. The statement is confirmed in our study. On the 14th day after the treatments, the

highest efficacy against the Rough cocklebur (*Xa. strumarium*) was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 95% (Table 6). On the 56th day after application the efficacy increased and reached 100% for all treatments.

Table 6. Efficacy against Rough cocklebur (*Xanthium strumarium* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	85	95	100
3. Pulsar 40 - 2.50 l ha ⁻¹	90	100	100
4. Pulsar Plus - 1.20 l ha ⁻¹	85	100	100
5. Pulsar Plus - 2.40 l ha ⁻¹	95	100	100

The efficacy data in our trial also showed that the Rough cocklebur can be successfully controlled by Pulsar 40 or Pulsar Plus used even in the lowest rates.

On the 14th day after the treatments, the highest efficacy against the Velvetleaf (*A. theophrasti*) was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 95% (Table 7).

Table 7. Efficacy against Velvetleaf (*Abutilon theophrasti* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	75	85	90
3. Pulsar 40 - 2.50 l ha ⁻¹	85	95	100
4. Pulsar Plus - 1.20 l ha ⁻¹	85	95	100
5. Pulsar Plus - 2.40 l ha ⁻¹	95	100	100

For the other treatments, except for variant 2 (Pulsar 40 - 1.25 l ha⁻¹) - 75%, the efficacy was satisfactory - 85%. On the 28th and 56th day after application the efficacy reached 95 - 100% for all treatments except for treatment 2 which stayed the lowest - 85% on the 28th day and 90% on the 56th day.

The efficacy data showed that the Velvetleaf can be successfully controlled by application of Pulsar Plus even from the lowest rate of 1.20 ha⁻¹, and Pulsar 40 showed lower efficacy against this weed species.

On the 14th day after the treatments, the highest efficacy against the Black nightshade (*S. nigrum*) was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 100% (Table 8). For the other treatments the efficacy was also satisfactory - from 85 to 95%.

On the 56th day after application the efficacy increased and reached 100% for all treatments.

The efficacy data showed that the Black nightshade can be successfully controlled by application of Pulsar 40 or Pulsar Plus in the lowest examined rates.

Table 8. Efficacy against Black nightshade (*Solanum nigrum* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	90	95	100
3. Pulsar 40 - 2.50 l ha ⁻¹	95	100	100
4. Pulsar Plus - 1.20 l ha ⁻¹	85	100	100
5. Pulsar Plus - 2.40 l ha ⁻¹	100	100	100

On the 14th day after the treatments the efficacy against the Wild mustard was the highest for variant 5 (Pulsar Plus - 2.40 l ha⁻¹) - 100% (Table 9). For the other treatments the efficacy on this reporting date was also satisfactory - from 85 to 90%.

On the 56th day after application the efficacy increased and reached 100% for all treatments. The efficacy data showed that the Wild mustard can be also successfully controlled by the low rates of both studied herbicide products.

Table 9. Efficacy against Wild mustard (*Sinapis arvensis* L.) (%)

Treatments	14 day	28 day	56 day
1. Untreated control	-	-	-
2. Pulsar 40 - 1.25 l ha ⁻¹	85	95	100
3. Pulsar 40 - 2.50 l ha ⁻¹	90	100	100
4. Pulsar Plus - 1.20 l ha ⁻¹	85	100	100
5. Pulsar Plus - 2.40 l ha ⁻¹	100	100	100

The visual phytotoxicity 7 and 14 days after the treatments for hybrid SY Bacardi CLP is on Figure 1, and for hybrid SY Diamantis CL is on Figure 2.

Balabanova and Vassilev (2015) concluded that the treatment with the herbicide imazamox causes an inhibition of growth and photosynthetic performance in IMI-R Clearfield sunflower hybrids. The inhibition is less pronounced in the plants treated with the recommendable dose (120 ml/da Pulsar 40) and significantly higher in the plants treated with the exceeded imazamox dose.

The highest phytotoxicity for hybrid SY Bacardi CLP was observed for variant 3 (Pulsar 40 - 2.50) l ha⁻¹ - score 2. It was determined as low phytotoxicity. This was due to the treatment of the doubled rate of Pulsar 40. The

content of the active substance in the herbicide product is higher and it was more aggressive to the sunflower hybrid that is bred to be grown by the Clearfield Plus technology. After the treatments for variant 2 the phytotoxic symptoms were classified as very weak - score 1. Phytotoxic symptoms were not reported after the application of Pulsar Plus even in the double rate of 2.50 l ha^{-1} .

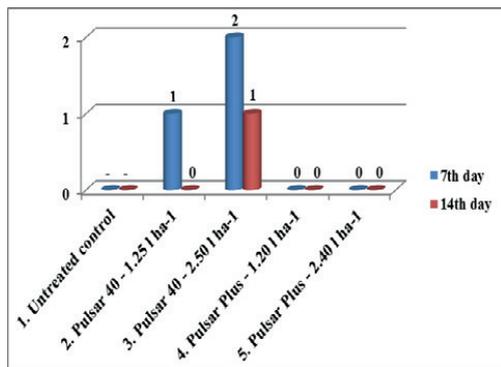


Figure 1. Visual phytotoxicity 7 and 14 days after the treatments for hybrid SY Bacardi CLP (Scores)

In our trial the phytotoxicity was higher for the hybrid SY Diamantis CL and it was determined as weak (score 2) after the application of the doubled Pulsar 40 rate (variant 3). After the treatments Pulsar Plus the phytotoxic symptoms for all variants were determined as weak (score 2) to very weak (score 1). On the second evaluation date the phytotoxicity decreased independently the hybrid, herbicide and application rate.

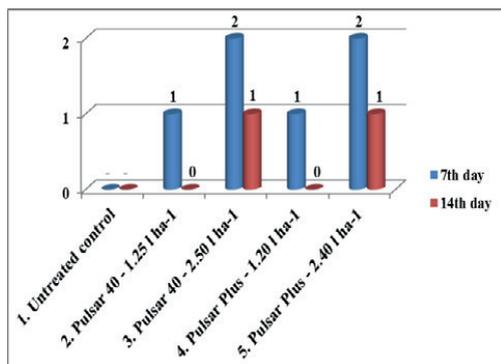


Figure 2. Visual phytotoxicity 7 and 14 days after the treatments for hybrid SY Diamantis CL (Scores)

The absolute seed mass of 1000 seeds is a very important quality indicator. The results from the current study are presented in Table 10. The seeds with higher values of the indicator have a higher price. According to a lot of authors this indicator is crucial for the formation of the yields (Georgiev et al., 2014).

The highest absolute seed mass of 1000 sunflower seeds for the hybrid SY Bacardi CLP was reported for treatments 2 and 4 (Table 10). The differences of the obtained results for these treatments are with proved differences according to Duncan's multiple range test ($p < 0.05$) in comparison to the rest of the treatments.

Table 10. Absolute seed mass of 1000 seeds (g)

Treatments	SY Bacardi CLP	SY Diamantis CL
1. Untreated control	55.47 c	57.22 c
2. Pulsar 40 - 1.25 l ha^{-1}	61.50 a	61.02 a
3. Pulsar 40 - 2.50 l ha^{-1}	59.30 b	60.63 b
4. Pulsar Plus - 1.20 l ha^{-1}	61.35 a	61.71 a
5. Pulsar Plus - 2.40 l ha^{-1}	58.89 b	60.10 b

All values with different letters are with proved difference according Duncan's test, $p < 0.05$

The lowest absolute seed mass of 1000 sunflower seeds for the untreated control was recorded - 55.47 g.

For the hybrid SY Diamantis CL similar results were obtained. The plants treated with doubled herbicide rates independently the studied herbicide product had lower absolute seed mass.

The lowest absolute seed mass of 1000 seeds for the untreated highly infested with weeds control was recorded - 57.22 g. The result concerning this indicator for the control plants was with a proved difference in comparison to all treated variants according to Duncan's multiple range test at $p < 0.05$.

High hectoliter mass is thus preferred by the industry (Abraham Nel, 2001). The lowest hectoliter mass of the sunflower seeds was the lowest for the control for both sunflower hybrids - 41.17 and 40.83 kg for SY Bacardi CLP and SY Diamantis CL respectively (Table 11). The magnitude of the hectoliter seed mass is determined by the grain size, the presence of impurities, etc. (Tonev et al., 2018).

The highest hectoliter mass of the sunflower seeds for SY Bacardi CLP was recorded at variants 2, 4 and 5 (Table 11).

The differences of the obtained results for these treatments were with proved differences with the treatment 3. This could be a result of the doubled Pulsar 40 rate and the fact that this is a hybrid bred to be grown by the Clearfield Plus technology.

The lowest results were recorded for the untreated control - 40.10 kg.

Table 11. Hectoliter mass of the sunflower seeds (kg)

Treatments	SY Bacardi CLP	SY Diamantis CL
1. Untreated control	40.10 c	40.80 c
2. Pulsar 40 - 1.25 l ha ⁻¹	43.60 a	43.60 a
3. Pulsar 40 - 2.50 l ha ⁻¹	42.60 b	42.30 b
4. Pulsar Plus - 1.20 l ha ⁻¹	43.35 a	43.60 a
5. Pulsar Plus - 2.40 l ha ⁻¹	43.60 a	43.30 a

All values with different letters are with proved difference according Duncan's test, $p < 0.05$.

The highest hectoliter mass of the sunflower seeds for SY Diamantis CL was recorded for variants 2, 4 and 5 (Table 11). The differences of the obtained results for these treatments were with proved differences regarding the data for treatment 3 according to Duncan's test. This could be also a result of an herbicide stress caused by the double Pulsar 40 rate.

As well as for the 1000 seeds mass the values and for the indicator hectoliter seed mass, a decrease in the values after the application of double herbicide rates for both studied sunflower hybrids was recorded. This could be a result of herbicide stress caused by the doubled herbicide doses.

In our previous study similar results were gained. The values of the hectoliter mass after the application of double herbicide rates independently the phenophase of the sunflower were lower (Mitkov et al., 2019).

Yield can generally decrease with increased duration of weed interference (Elezovic et al., 2012). In order to obtain maximum yields, along with the use of high-yield hybrids it is necessary to effectively control the weeds (Manilov and Zhaltov, 2015).

The results in our study correspond with these statements. The highest sunflower seed yield for the hybrid SY Bacardi CLP was obtained for variant 4 (Pulsar Plus - 1.20 l ha⁻¹) - 3.38 t

ha⁻¹. The yield of the untreated control was the lowest - 1.85 t/ha (Table 12). This in turn shows how harmful the weed infestation in the sunflower fields can be.

The variants treated with doubled imazamox rates had lower yields in comparison to those treated with lower doses.

Table 12. Seed yield of SY Bacardi CLP (t/ha)

Treatments	t ha ⁻¹
1. Untreated control	1.85 d
2. Pulsar 40 - 1.25 l ha ⁻¹	2.96 b
3. Pulsar 40 - 2.50 l ha ⁻¹	2.21 c
4. Pulsar Plus - 1.20 l ha ⁻¹	3.38 a
5. Pulsar Plus - 2.40 l ha ⁻¹	2.42 c

All values with different letters are with proved difference according Duncan's test, $p < 0.05$.

The highest sunflower seed yield for the hybrid SY Diamantis CL was recorded for variant 4 (Pulsar Plus - 1.20 l ha⁻¹) - 3.51 t ha⁻¹. The difference of the gained results for the sunflower seed yield at this variants was with proved differences according to Duncan's multiple range test ($p < 0.05$) in comparison to the other treatments. The yield of the untreated control was the lowest for this hybrid also - 1.89 t ha⁻¹ (Table 13).

Table 13. Seed yield of SY Diamantis CL (t/ha)

Treatments	t ha ⁻¹
1. Untreated control	1.89 d
2. Pulsar 40 - 1.25 l ha ⁻¹	2.96 b
3. Pulsar 40 - 2.50 l ha ⁻¹	2.32 c
4. Pulsar Plus - 1.20 l ha ⁻¹	3.51 a
5. Pulsar Plus - 2.40 l ha ⁻¹	2.35 c

All values with different letters are with proved difference according Duncan's test, $p < 0.05$.

Oil quality is determined by the fatty acid composition and the levels of tocopherols, sterols, carotenoids and other compounds. Sunflower is regarded as one of the most promising crops when it comes to the genetic alteration of oil quality (Scharp, 1986). The high weed infestation can decrease the oil content in the sunflower seeds, so it is important to effectively control the weeds (Tonev et al., 2007).

In the recent research we also reported decrease of the oil content in the sunflower seeds from the untreated control for both studied hybrids (Table 14) 48.43% for SY Bacardi CLP and 44.98 % for hybrid SY Diamantis CL. The results were the lowest in the study.

Table 14. Sunflower seed oil content (%)

Treatments	SY Bacardi CLP	SY Diamantis CL
1. Untreated control	48.43 e	44.98 d
2. Pulsar 40 - 1.25 l ha ⁻¹	54.01 b	49.36 a
3. Pulsar 40 - 2.50 l ha ⁻¹	51.43 d	46.86 c
4. Pulsar Plus - 1.20 l ha ⁻¹	55.48 a	49.91 a
5. Pulsar Plus - 2.40 l ha ⁻¹	53.47 c	48.11 b

All values with different letters are with proved difference according Duncan's test, $p < 0.05$.

There are differences in the sunflower seed oil content between the varieties (Clagett et al., 1951; Cummins et al., 1967; Pehlivanov et al., 1998; Khoufi et al., 2014). It is also found in our study. The oil content of the seeds for the hybrid SY Bacardi CLP was higher than the oil content of hybrid SY Diamantis CL (Table 14). The highest oil content of SY Bacardi CLP was recorded for treatment 4 (Pulsar Plus - 1.20 l ha⁻¹) – 55.48%. In comparison to the other treatments, the difference of the obtained results for the sunflower seed oil content at treatment 4 (Pulsar Plus - 1.20 l/ha) was with proved differences according to Duncan's multiple range test ($p < 0.05$). It was also reported that the seed oil content for the treatments with doubled herbicide rates was decreased (Table 14).

The highest oil content of SY Diamantis CL was recorded for treatments 2 (Pulsar 40 - 1.25 l ha⁻¹) and 4 (Pulsar Plus - 1.20 l ha⁻¹) - 49.36-49.91%. In comparison to the other treatments, the difference of the obtained results for the sunflower seed oil content at treatments 2 and 4 were with proved differences according to Duncan's multiple range test.

It was also reported that the seed oil content for the treatments with doubled herbicide rates was also decreased (Table 14).

CONCLUSIONS

The highest efficacy against the Johnson grass (*S. halepense*) developed from rhizomes was recorded for variant 5 (Pulsar Plus - 2.40 l ha⁻¹). If there is infestation with this difficult-to-control weed a partner grass herbicide should be applied.

The Johnson grass (*S. halepense*) developed from seeds, the Green bristle grass (*S. viridis*), the Common amaranth (*A. retroflexus*), the Rough cocklebur (*Xa. strumarium*), the Black

nightshade (*S. nigrum*) and the Wild mustard (*S. arvensis*) can be successfully controlled by application of Pulsar 40 or Pulsar Plus even in the low examined rates.

The Fat-hen (*Ch. album*) and the Velvetleaf (*A. theophrasti*) can be successfully controlled by application of Pulsar Plus even from the lowest of 1.20 l ha⁻¹.

Pulsar 40 is more selective for the Clearfield Plus hybrid SY Bacardi CLP than Pulsar Plus for the Clearfield hybrid SY Diamantis CL.

The absolute seed mass of 1000 seeds, the hectoliter seed mass and the yield as well as the seed oil of the plants from the doubled herbicide rates, independently the herbicide product and studied hybrid, were higher than those of the untreated controls highly infested with weeds.

REFERENCES

- Abraham Nel, A. (2001). Determinants of Sunflower Seed Quality for Processing. *Submitted in partial fulfillment of the requirements for the degree PhD Plant Production: Agronomy*. 122 pp.
- Balabanova, D., Vassilev, A. (2015). Response of Sunflower Clearfield Hybrids to both Recommendable and Higher Doses of the Imazamox Herbicide. *Agricultural Sciences*, VII(18), 41–46.
- Clagett, C., Hoffman, G., Sollin, I. (1951). Oil and Protein Content of Sunflower Seed". *North Dakota Ag. Exp. Sta. Bimonthly Bulletin*, XIII(4), 173.
- Cummins, D., Marion, J., Craigmiles, J., Burns, R. (1967). Oil content, fatty acid composition, and other agronomic characteristics of sunflower introductions. *Journal of the American Oil Chemists' Society*, 44(10), 581–582.
- Elezovic, I., Datta, A., Vrbnicanin, S., Glamoclija, D., Simic, M., Malidza, G., Knezevic, S. (2012). Yield and yield components of imidazolinone-resistant sunflower (*Helianthus annuus* L.) are influenced by pre-emergence herbicide and time of post-emergence weed removal. *Field Crops Research*, 128, 137–146.
- Fernández-Martínez, J., Domínguez J., Pérez-Vich, B., Velasco, L. (2009). Current research strategies for sunflower broomrape control in Spain. *HELIA*, 32(51), 47–56.
- Georgiev, G., Encheva, V., Nenova, N., Peevska, P., Encheva, Y., Valkova, D. Georgiev, G., Penchev, E. (2014). Characterization of the yield components of sunflower lines under the conditions of North-East Bulgaria. *Scientific Works*, 3(1), 121–131.
- Hristov, K., Beluhova-Uzunova, R., Shishkova, M. (2019). Competitive advantages of Bulgarian sunflower industry after the accession into the European Union. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 19(2), 197-202.

- Ivanov, K. and Popov, N. (1994). A Guidebook for Exercises on Biochemistry of the Plants. *Publisher "Zemizdat"*, Sofia, 88 pp. (In Bulgarian).
- Khoufi, S., Khamassi, K., Teixeira Da Silva, J., Rezgui, S., Ben Jeddi, F. (2014). Watering regime affects oil content and fatty acid composition of six sunflower lines. *Journal of New Sciences*, 7(1), 1–9.
- Malidza, G., Jocic, S., Skoric, D. (2003). Weed and broomrape (*O. cernua*) control in Clearfield in Sunflower. *Proceeding of European Weed Research Society 7th Mediterranean Symposium*. 6-9 May. *Cukurova University, Adana, Turkey*, 51–52.
- Manilov, T. and Zhalnov, Iv. (2015). Weed control in sunflower cultivation by a conventional technology. *Proceedings of Jubilee Science Conference with International Participation Traditions and Challenges of Agricultural Education, Science and Business*. Agricultural University-Plovdiv. *Scientific Works, LIX(3)*, 167–174.
- Mitkov, A., Yanev, M., Neshev, N., Tonev, T. Joița-Păcureanu M., Cojocaru F. (2019). Efficacy Against Broomrape and Selectivity of Imazamox-containing Herbicides at Sunflower. *Romanian Agricultural Research*, 36.
- Moskova, C., Koleva, N., Dimitrov, S., Tityanov, M., Traykova, B. (2016). Distribution and extent of weeding of Amaranth species and other weeds in sunflower crops in Plovdiv and Stara Zagora region. *Journal of Mountain Agriculture on the Balkans*, 19(4), 84–94.
- Pehlivanov, M., Moskov, G., Yankov, B., Terziev, Zh., Zhelyazkov, V., Yancheva, Hr. (1998). Field Crop Production. *Academic Publisher of the Agricultural University of Plovdiv*, 342 pp. (Textbook in Bulgarian).
- Pfenning, M., Tan, S., Perez-Brea, J. (2012). Improved weed Control in Clearfield-Plus Sunflowers with superior herbicide solutions. *Proceedings of 18th International Sunflower Conference, Mar del Plata, Argentina*, 535–538.
- Scharp, W. (1986). Opportunities for biotechnology in the development of new edible vegetable oil products. *Journal of the American Oil Chemists' Society*, 63(5), 594–600.
- Simic, M., Dragičević, V., Dolianovic, Z., Jug, D., Brankov, M., Stipešević, B. (2011). Effect of applied herbicides (fluchloridone + s-metolachlor) on weeds in different stage of sunflower growth. *Proceedings of 46th Croatia and 6th International Symposium on Agriculture, Opatija, Croatia, 14-18 February, Zagreb*, 704–708.
- Tan, S., Evans, R., Dahmer, M., Singh, B., Shaner, D. (2005). Imidazolinone-tolerant crops: history, current status and future. *Pest Management Sci.*, 61(3), 246–257.
- Tonev T., Tityanov M., Mitkov M. (2010). Integrated weed control in sunflower. *Jubilee Science Conference with International Participation Traditions and Challenges of Agricultural Education, Science and Business*. Agricultural University-Plovdiv. *Scientific Works, LV(2)*, 127–132.
- Tonev, T. (2000). Handbook for integrated weed management and culture of agriculture. *Publisher "Biblioteka Zemedelsko obrazovanie"*, VSI Plovdiv. Book 2 (In Bulgarian).
- Tonev, T., Tityanov M., Mitkov, A., Yanev, M., Neshev, N. (2018). A Guidebook for Exercises on General Agriculture and Herbology. *Publisher: "Biblioteka Zemedelsko Obrazovanie"*, pp. 71-72.
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Spasov, V. (2007). Herbology. *Academic publisher of AU-Plovdiv* (Textbook in Bulgarian).
- Zhelyazkov, I., Mitkov, A., Stoychev, D. (2017). A Guidebook for Exercises on Herbology. *Academic Publisher of the Agricultural University of Plovdiv*, 188 pp. (In Bulgarian).

ALLELOPATHIC ACTIVITY OF RHIZOSPHERE SOIL OF ALFALFA (*Medicago sativa* L.)

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Abstract

In the laboratory condition at the Institute of Forage Crops - Pleven was studied allelopathic potential of soil from the rhizosphere of alfalfa zone on germination and initial development of the test plants - *Cucumis sativus* L. It was found that concentrations of the rhizosphere soil zone on alfalfa have a stimulatory or inhibitory effect on the independent seedling growth of test plants. The rate of growth and accumulation of fresh biomass (μ), as well as the dynamic development index (DDI), response index (RI) and seedling vigor index (SVI) of seedlings depend mainly on applied concentration and do not depend on the type of the growth media. The observed differences in the values of the GI regarding the growth media - "distilled water" or "agar" can be explained by their different ability to dissolved and absorbed the available allelochemicals from rhizosphere soil samples of alfalfa as the comparisons between them are made under controlled conditions.

Key words: alfalfa, allelopathic effect, rhizosphere soil, inhibition.

INTRODUCTION

Allelopathic relationships in agrophytocenoses are determined by a variety of factors occurring simultaneously and/or sequentially, having direct or indirect effects on plant species through the synthesis of various chemical substances (allelochemicals) released into the environment (Rice, 1984; Seigler, 1996; Aleksieva & Serafimov, 2008; Ravlić et al., 2012; Kalinova et al., 2012; Ravlić et al., 2014; Baličević et al., 2015; Treber et al., 2015; Marinov-Serafimov, 2015a; 2015b).

Alfalfa (*Medicago sativa* L.) is well known as a plant species with significant allelopathic potential, probably due to the accumulation and release of allelochemicals in the surrounding soil as a remedy against pests (Miller, 1983; Ells & McSay, 1991; Chon & Nelson, 2010; Mousavi et al., 2013). Many studies revealed that part of the allelochemicals (polyphenols, terpenoids, saponins, etc.), synthesized in the life cycle of alfalfa, are formed from the root system and accumulate into the rhizosphere zone (Ells & McSay, 1991; Wink & Mohamed,

2003; Wink, 2013). Other surveys have proved that the increment of allelochemicals concentration can influence their toxicity (Chung & Miller, 1995; Mousavi et al., 2013).

In the experimental work Wyman-Simpson (1991) found that alfalfa has a strong auto and hetero-toxic allelopathic effect, which is often used as a biological method for reducing the level of weed infestation in agricultural practice. It has been experimentally found that alfalfa root exudates strongly suppress the initial development of *H. vulgare*; *R. sativus*; *L. sativum*; *L. esculentum* and a number of monocotyledonous and dicotyledonous weed species (Rice, 1984; Ming Chung & Miller, 1995; El-Dariera & El-Dienb, 2011).

Oleszek & Jurzysta (1987) reported that high amounts of glycosides of the medicagenic acid accumulate in the alfalfa roots, accounting for about 6% of the dry underground biomass. Assuming that, they calculated that the alfalfa crops can accumulate up to 10 t per ha of root biomass in the soil, which is equivalent to introducing about 600 kg per ha of highly

active allelopathic compounds, which could affect the cultivation of subsequent crops.

The aim of the present study was to determine the allelopathic activity of soil from the alfalfa rhizosphere zone on the initial development of test plant species *Cucumis sativus* L.

MATERIALS AND METHODS

The study was conducted at laboratory conditions in the Institute of Forage Crops, Pleven, Bulgaria. Two factors were studied: Factor A - growth media: a1 - Distilled water; a2 - Agar-agar, and Factor B - concentration: b1 - Control; b2 - 2.5% w/v; b3 - 5.0% w/v; b4 - 10.0% w/v and b5 - 5.0% w/v.

Sampling of soil samples from the rhizosphere zone was carried out in a five-year alfalfa stand (Dara variety) in phenophase BBCH 65-6.

Each soil sample, consisted of 5 subsamples, was air-dried and stored in polyethylene containers at 4°C. Two screening methods were used to detect the presence of allelochemicals in the alfalfa rhizosphere soil: 1) Cold Aqueous Extracts Method; 2) Rhizosphere Soil Method (RSM).

The experimental method of cold aqueous extract was applied as follows: 20 g of the alfalfa rhizosphere soil were soaked in 100 ml of distilled water. These samples were cold extracted at $22 \pm 2^\circ\text{C}$ for 24 h. The extracts obtained were decanted, filtered through Filtrak 389 filter paper and brought to final concentrations of 2.5, 5.0, 15.0 and 20.0% w/v. $\text{C}_{10}\text{H}_{14}\text{O}$ was added to each of the extracts (Marinov-Serafimov et al., 2007). Ten seeds of *Cucumis sativus* L. (Longo da China variety) were placed into petri dishes (90 mm diameter) between Filtrak 389 filter paper disks. The seed surface was pre-sterilized for one minute with a 0.3% KMnO_4 solution and then dried. The experimental extracts have been added at the quantity of 20 ml per petri. Distilled water was used for the control. The petri dishes were placed in a thermostat at $22^\circ\text{C} \pm 2^\circ\text{C}$ for five days. Each variant is set in four replications.

The adapted method of RSM, proposed by Fujii et al. (2005) and Karmegam et al. (2014), was applied in order to determine the presence of allelochemicals, separated from the root system in the alfalfa rhizosphere soil zone: 2.5, 5.0, 10.0 and 20.0% w/v of the alfalfa rhizosphere

soil (equivalent to 3.3, 6.7, 20.0 and 26.7 cm^3 volume) were placed into Petri dishes (90 mm diameter). Then 10 ml agar (1%) with 1 ml/L^{-1} $\text{C}_{10}\text{H}_{14}\text{O}$ have been added into each Petri (Marinov-Serafimov et al., 2007). After gelation, a second layer of 10 ml agar (1%) was pipetted. 1% agar with sequential pipetting of two 10 ml layers was used for the control. All petri dishes were placed into a heat shaker for 24 h at $22^\circ\text{C} \pm 2^\circ\text{C}$. After that, 10 seed of *Cucumis sativus* L. (Longo da China variety) were added and all petri dishes were placed in a thermostat at $22^\circ\text{C} \pm 2^\circ\text{C}$ for five days. Each variant is set in four replications.

The following parameters were determined for all variants of the experiment:

- percentage of germinated seeds (%);
- seedling length (root + shoot), cm;
- fresh biomass of seedling, g;
- Dynamic Development Index (DDI):

$$DDI = \left\{ \frac{t \log^2}{\log b - \log a} \right\}$$
, where: a is the percentage of germinated seeds (%), length (cm) and/or biomass (g) of seedlings in the control variant; b - seed germination (%), length (cm) and/or biomass (g) of seedlings in the experimental variant; t - duration, days².

- Allelopathic Effect Index (RI), according to the formula of Williamson & Richardson (1988): $RI = \frac{T}{C} - 1$, where: C - parameter in the control variant; T - parameter in the experimental variant;

- Rate of growth and accumulation of fresh seedlings biomass was determined by the adapted formula of Dauta et al. (1990): $\mu = \left\{ \frac{\ln N_t - \ln N_0}{t} \right\}$, where: N_t - length (cm) or biomass (g) of seedlings in the experimental variant; N_0 - length (cm) or biomass (g) of seedlings in the control variant; t - duration, days;

- Growth Rate (GR) was calculated as follows - $GR\% = \left(1 - \frac{(N_t - C_n)}{(N_c)} \right) \cdot 100$, where: N_t - percentage of the germinated seeds in the experimental variant, %; N_c - germinated seeds in the control, %; C_n - concentration, %;

- Development Index (GI) according to Gariglio et al. (2002): $GI = \left[\left(\frac{G}{G_0} \right) \cdot \left(\frac{L}{L_0} \right) \right] \cdot 100$, where: G - germinated seeds in experimental variant, %; G_0 - germinated seeds in the control variant, %; L - length of seedlings in

experimental variant, converted to percentage; L_0 - length of seedlings in the control variant, accepted for 100%;

- Seedling vigour index (SVI) according to Islam et al. (2009) : $SVI = \left(\frac{S.G}{100}\right)$, where: S - length (cm) or biomass (g) per seedling; G - germinated seeds,%;

- Coefficient of Allometry (CA) according to Nasr & Shariati (2007): $CA = \frac{L_s}{L_r}$, where: L_s - length (cm) or biomass (g) per shoot; L_r - length (cm) or biomass (g) per root.

The percentage of germinated seeds was transformed according to the formula of Hinkelmann & Kempthorne (1994): $Y = \arcsin \sqrt{x\%/100}$.

The results obtained were processed mathematically and statistically with the software products STATGRAPHICS Plus for Windows Version 2.1 and Statistica 10.

RESULTS AND DISCUSSIONS

A general tendency to decrease the percentage of germinated seeds of the test plants with increasing the concentration (Factor B) in the growth media was observed (Table 1). The degree of inhibition of seed germination, depending on the type of growth media (Factor A) - distilled water or agar, varied from 0 to 23.6% in the distilled water variants (a1) and from 0 to 30.5% in the agar variants (a2). No

significant differences in the seed germination rate (GR%) according to the growth media type have been found, whereas the dynamic development index (DDI), the allelopathic effect index (RI) and the growth rate (μ) differed significantly (from 0.2 to 4.3 times) (Table 2). These differences probably could be explained by the different solubility of allelochemicals in the growth media.

Data from the biometric measurements on root, shoot and seedling length (cm) make it possible to objectively evaluate the allelopathic potential of alfalfa rhizosphere soil (Tables 1 and 2), depending on the concentrations applied and the type of growth media. Two highest concentrations of 10 and 20% w/v of alfalfa rhizosphere soil have a significant inhibitory effect ranging from 3.7 up to 89.9% ($P = 0.05$), regardless of the type of media. By reducing the concentration to 2.5 and 5.0% w/v, the inhibitory effect on the root, shoot and seedling (cm) also was reduced and practically exerted an indifferent and/or weak stimulating effect from 1.0 to 20.2% on both growth media when compared to the control variants. This dependence could be explained by the presence of secondary metabolites - glycosides, tannins, polyphenols, terpenoids, saponins, and other allelopathically active substances, synthesized in the life cycle of alfalfa and separated from the root system in the rhizosphere zone.

Table 1. Allelopathic effect of rhizosphere soil of alfalfa on germination and initial development of *Cucumis sativus* L.

Variants		Parameters						
Growth medium/ Concentration, % w/v	Germination,% ± SE	Length, cm			Biomass, g			
		root ± SE	shoot ± SE	seedling ± SE	root ± SE	shoot ± SE	seedling ± SE	
Distilled water	0.0	83.4a ±6.65	1.04 bc ±0.02	0.83b ±0.07	1.87b ±0.09	0.08a ±0.006	0.11b ±0.010	0.19c ±0.014
	2.5	83.4 a ±6.65	1.20 cd ±0.05	0.74b ±0.1	1.93b ±0.14	0.04b ±0.009	0.10b ±0.008	0.14b ±0.009
	5.0	76.7 a ±6.68	1.32 d ±0.08	0.78b ±0.08	2.10b ±0.16	0.04b ±0.007	0.10b ±0.010	0.14b ±0.016
	10.0	76.7 a ±6.68	0.87 ab ±0.15	0.30a ±0.02	1.17a ±0.03	0.03b ±0.004	0.06a ±0.009	0.08a ±0.010
	20.0	63.7 a ±9.24	0.78 a ±0.12	0.26a ±0.05	1.04a ±0.17	0.03b ±0.004	0.05a ±0.011	0.08a ±0.010
Agar-agar	0.0	83.4a ±3.31	1.14b ±0.03	0.99b ±0.06	2.13b ±0.09	0.09b ±0.013	0.15b ±0.029	0.24b ±0.032
	2.5	83.4a ±3.31	0.91b ±0.07	1.00b ±0.18	1.91b ±0.21	0.08b ±0.007	0.15b ±0.021	0.23b ±0.025
	5.0	58.0a ±4.22	1.29b ±0.63	0.92b ±0.10	2.21b ±1.03	0.03a ±0.02	0.06a ±0.058	0.09a ±0.077
	10.0	58.0a ±4.22	0.38a ±0.05	0.27a ±0.11	0.65a ±0.13	0.02a ±0.0061	0.04a ±0.029	0.06a ±0.028
	20.0	58.0a ±4.22	0.18a ±0.15	0.10a ±0.20	0.28a ±0.35	0.02a ±0.019	0.01a ±0.025	0.03a ±0.044

Legend: a, b, c, d LSD at $P = 0.05$ level, ± SE standard error

Table 2. Indexes of germination and initial development of the test plant depending on the two studied factors

Variant	Germination				Seedling length					Seedling biomass						
	DDI	RI.10 ²	μ .10 ²	GR%	DDI	RI.10 ²	μ .10 ²	SVI.10 ² cm	CA cm	DDI	RI.10 ²	μ .10 ²	SVI.10 ² g	CA g	GI	
Distilled water	0.0	0.0	0.0	100.0	0.0	0.0	0.0	3.5	79.8	0.0	0.0	0.0	3.61	1.38	100.0	
	2.5	0.0	0.0	97.0	35.6	-9.4	3.2	3.7	61.7	-3.7	-26.3	-6.1	1.96	2.50	103.2	
	5.0	-13.4	-8.0	-1.7	86.0	9.7	-1.4	12.3	4.4	59.1	-3.7	-26.3	-6.1	1.96	2.50	103.3
	10.0	-13.4	-8.0	-1.7	80.0	-2.4	-45.1	-37.4	1.4	34.5	-1.3	-57.9	-17.3	0.64	2.00	57.5
	20.0	-4.2	-11.8	-5.4	52.4	-1.9	-51.2	-44.0	1.1	33.3	-1.3	-57.9	-17.3	0.64	1.67	42.5
Agar-agar	0.0	0.0	0.0	100.0	0.0	0.0	0.0	4.5	0.87	0.0	0.0	0.0	5.76	1.67	100.0	
	2.5	0.0	0.0	97.0	-10.3	-10.3	-2.2	3.6	1.10	-26.4	-4.2	-0.9	5.29	1.88	89.7	
	5.0	-3.1	-30.5	-7.3	63.5	30.5	3.8	0.7	4.9	0.71	-1.1	-62.5	-19.6	0.81	2.00	72.2
	10.0	-3.1	-30.5	-7.3	57.6	-0.9	-69.5	-23.7	0.4	0.71	-0.8	-75.0	-27.7	0.36	2.00	21.2
	20.0	-3.1	-30.0	-7.3	45.6	-0.6	-87.0	-40.6	0.1	0.56	-0.5	-88.0	-41.6	0.09	0.50	9.1

It is well known that alkaloids, tannins, and some other secondary metabolites exhibit a toxicity with protoplasmic and haemolytic activity (Li, 2010; Zohaib et al., 2014). At higher concentrations (10.0 and 20.0% w/v) they caused an inhibitory effect on the test plants development, while at lower concentrations (2.5 and 5.0% w/v) they exerted an indifferent and/or stimulating effect to different extent.

The dynamics in the accumulation of fresh biomass in g per seedling through the initial stages of *C. sativus* development depended on the same factors and followed the observed dependences on root, shoot and seedling growth (cm) (Table 1).

The highest amount of fresh biomass in g per seedling was accumulated at the lower concentrations of 2.5 and 5.0% w/v. A tendency to weight decrease was found with concentration increment to 10.0 and 20.0% w/v as follows: in the distilled water media the biomass inhibition ranged from 1.1 up to 2.3 times, while in the agar media it ranged from 1.1 to 8.0 times ($P = 0.05$). An exception of this tendency was found at the lowest concentration of 2.5% w/v in agar variant, as well as at the two lower concentrations of 2.5 and 5.0% w/v in the distilled water variant.

Statistical evaluation of the results showed that the applied concentrations of alfalfa rhizospheric soil have a stimulating, inhibitory or indifferent effect on the seedling growth. The growth rate and accumulation of fresh biomass (μ), development index (DDI), allelopathic effect index (RI) and seedling vigour index (SVI) in test seedlings depended mainly on the concentration applied and are independent of the type of growth media (Table

2). Similar results have been obtained in relation to the coefficient of allometry (CA) with respect to the seedling length - increasing of concentration led to the CA decrease from 1.3 to 2.4 times, regardless of the type of media. When regarding the CA with respect to the biomass formation, its value increased from 0.5 to 3.3 times compared to the control variant. The analysis of the results obtained showed that the coefficients of depression according to Factor A (growth media) and Factor B (concentration) are correlated only at higher concentrations (r varies from -0.757 to -0.992).

The complex assessment of tested rhizosphere soil extracts showed that depending on the GI values, they could be arranged in the following order: distilled water media - from 103.2 to 42.5%, and agar media - from 89.7 to 9.1%. Therefore, the observed differences could be explained by their different ability to dissolve and absorb the available allelochemicals from soil samples, collected in the alfalfa rhizosphere zone, since comparisons between them were made at controlled conditions.

Data from the dispersion analysis express the hierarchical distribution of variation to determine the weight of factors η^2 on the laboratory germination. They indicate that Factor A (growth media) and Factor B (concentration) have a relatively equal proportion of the total variation, and η^2 is in the range of 5.05 to 5.78 (Figure 1). The interaction of the studied A x B factors accounts for a relatively small proportion of the total variation $\eta^2 = 1.23$, with no significant differences.

With regard to the hierarchical distribution of variation between the factors determining growth (cm) and the accumulation of fresh

biomass in g per seedling, the weight of factor B accounts for the largest proportion of the total variation and the weight of the factor (η^2) is in the range of 55.9 to 75.32. The influence of the media (Factor B) occupies a relatively

small share of the total variation, with a factor weight (η^2) from 0.03 to 9.95. The variance due to the A x B relationships have a relatively high proportion of the total variation, and the η^2 range is from 16.93 to 23.76 (Figure 1).

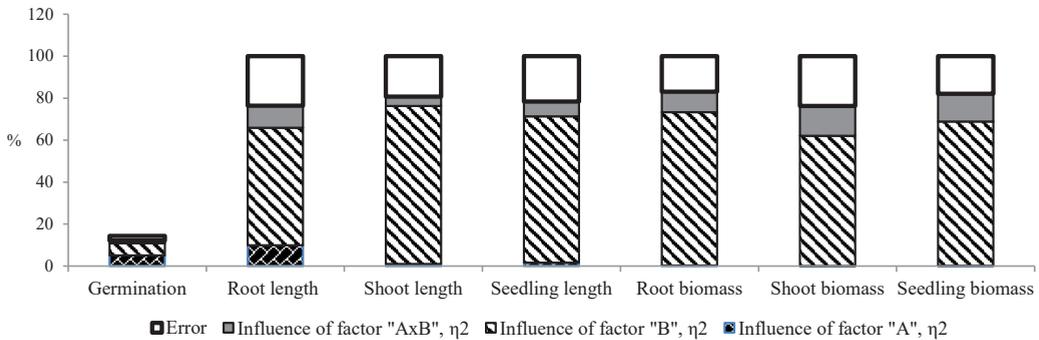


Figure 1. Dispersion analysis of the two factors

CONCLUSIONS

Soil from the rhizosphere zone of alfalfa (Dara variety) has a slight inhibitory effect on seed germination of the test plants, depending on the type of growth media. The applied concentrations of the alfalfa rhizosphere soil have a stimulating, inhibitory or indifferent effect on the seedlings growth. The rate of growth and accumulation of fresh biomass (μ), the development index (DDI), allelopathic effect (RI) and seedling vigour index (SVI) depend mainly on the concentration applied and are independent of the type of media.

The complex assessment of tested rhizosphere soil extracts showed that the allelopathic inhibition was stronger in the distilled water media - from 103.2 to 42.5%, while in the agar media it ranged from 89.7 to 9.1%. Therefore, the observed differences could be explained by their different ability to dissolve and absorb the available allelochemicals from soil samples, collected in the alfalfa rhizosphere zone, since comparisons between them were made at controlled conditions.

REFERENCES

Aleksieva, A., Serafimov, P. (2008). A study of allelopathic effect of *Amaranthus retroflexus* (L.) and *Solanum nigrum* (L.) in different soybean genotypes. *Herbologia*, 9(2), 47–58.
 Balićević, R., Ravlić, M., Mišić, M., & Mikić, I. (2015). Allelopathic effect of *Aristolochia clematitis* L.

Proceedings of the 50th Croatian and 10th International Symposium on Agriculture, 54–58.
 Chon, S., Nelson, C. (2010). Allelopathy in Compositae plants. A review. *Agronomy for Sustainable Development*, 30(2), 349–358.
 Chung, I., Miller, D. (1995). Effect of alfalfa plant and soil extracts on generation and seedling growth. *Agronomy Journal*, 87, 762–767.
 Dauta, A., Devraux, J., Piquemal, F., Boumich, L. (1990). Growth rate of four freshwater algae in relation to light and temperature. *Hydrobiologia*, 207, 221–226.
 El-Dariera, S., El-Dienb, M. (2011). Biological activity of *Medicago sativa* L. (alfalfa) residues on germination efficiency, growth and nutrient uptake of *Lycopersicon esculentum* L. (tomato) seedlings. *Journal of Taibah University for Science*, 5, 7–13.
 Eills, J., McSay, A. (1991). Allelopathic Effects of Alfalfa Plant Residues on Emergence and Growth of Cucumber Seedlings. *Horticultural Science*, 26(4), 368–370.
 Fujii, Y., Furubayashi, A., Hiradate, S. (2005). Rhizosphere soil method: a new bioassay to evaluate allelopathy in the field. *Proceedings of the 4th World Congress on Allelopathy "Establishing the Scientific Base"*, Wagga Wagga, 490–492.
 Islam, A., Anuar, N., Yaakob, Z. (2009). Effect of genotypes and pre-sowing treatments on seed germination behavior of *Jatropha*. *Asian Journal of Plant Science*, 8, 433–439.
 Gariglio, N, Buyatti, M., Pillati, R, Rossa, D., Acosta, M. (2002). Use a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *New Zealand Journal of Crop and Horticulture Science*, 30, 135–139.
 Hinkelmann, K., Kempthorne, O. (1994). *Design and analysis of experiments*. New York: Wiley and Sons.

- Kalinova, S., Golubina, I., Hristoskov, A., Ilieva, A. (2012). Allelopathic effect of aqueous extract from root systems of Jonson grass on seed germination and the initial development of soybean, pea and vetch. *Ratarstvo i povrtarstvo*, 49(3), 250–256.
- Karmegam, N., Kalpana, M., Prakash, M. (2014). Allelopathic effect of aqueous root bark extract of *Tamarindus indica* L. and rhizosphere soil on germination and seedling growth of *Oryza sativa* L. *International Journal of Current Microbiology and Applied Sciences*, 3(3), 505–514.
- Li, Z.H., Wang, Q., Ruan, X., Pan, C.D., & Jiang, D.A. (2010). Phenolics and Plant Allelopathy. *Molecules*, 15, 8933–8952.
- Marinov-Serafimov, P., Dimitrova, T., Golubina, I., Ilieva, A. (2007). Study of suitability of some solutions in allelopathic researches. *Herbologia*, 8(1), 1–10.
- Marinov-Serafimov, P., Golubina, I., Katova, A. (2015a). Allelopathic activity of rhizosphere soil at some annual cereal forage crops. *Proceedings of The Union of Scientists - Ruse, Section "Agriculture & Veterinary Sciences"*, 3(7), 202–208.
- Marinov-Serafimov, P., Katova, A., Golubina, I. (2015b). Allelopathic activity of rhizosphere soil in some perennial grasses. *Proceedings of The Union of Scientists - Ruse, Section "Agriculture & Veterinary Sciences"*, 3(7), 209–215.
- Miller, D.A. (1983). Allelopathic effects of alfalfa. *Journal of Chemistry and Ecology*, 9(8), 1059–1072.
- Ming Chung, I., Miller, D. (1995). Natural Herbicide Potential of Alfalfa Residue on Selected Weed Species. *Agronomy Journal*, 87(5), 920–925.
- Mousavi, S., Alami-Saeid, K.H., Moshatati, A. (2013). Effect of leaf, stem and root extract of alfalfa (*Melilotus indicus*) on seed germination and seedling growth of wheat (*Triticum aestivum*). *International Journal of Agriculture and Crop Sciences*, 5(1), 44–49.
- Nasr, M., Mansour, S. (2005). The use of allelochemicals to delay germination of *Astragalus cycluphyllus* seeds. *Journal of Agronomy*, 4(2), 147–150.
- Oleszek, W., Jurzysta, M. (1987). The allelopathic potential of alfalfa root medicagenic acid glycosides and their fate in soil environments. *Plant and Soil*, 98(1), 67–80.
- Ravlić, M., Baličević, R., Knežević, M., Ravlić, I. (2012). Allelopathic effect of scentless mayweed and field poppy on seed germination of winter wheat and winter barley. *Herbologia*, 13(2), 1–7.
- Ravlić, M., Baličević, R., Lucić, I. (2014). Allelopathic effect of parsley (*Petroselinum crispum* Mill.) cogermination, water extracts and residues on hoary cress (*Lepidium draba* (L.) Desv.). *Poljoprivreda*, 20(1), 22–26.
- Rice, E.L. (1984). *Allelopathy*. 2nd Edition. Orlando: Academic Press.
- Seigler, D. (1996). Chemistry and Mechanisms of Allelopathic Interactions. *Agronomy Journal*, 88, 876–885.
- Treber, I., Baličević, R., Ravlić, M. (2015). Assessment of allelopathic effect of pale persicaria on two soybean cultivars. *Herbologia*, 15(1), 31–38.
- Wink, M., Mohamed, G.I.A. (2003). Evolution of Chemical Defense Traits in the Leguminosae: Mapping of Distribution Patterns of Secondary Metabolites on a Molecular Phylogeny Inferred from Nucleotide Sequences of the *rbcL* Gene. *Biochemical Systematics and Ecology*, 31, 897–917.
- Wink, M. (2013). Evolution of Secondary Metabolites in Legumes (Fabaceae). *South African Journal of Botany*, 89, 164–175.
- Williamson, G., Richardson, D. (1988). Bioassays for allelopathy: measuring treatment responses with independent controls. *Journal of Chemistry and Ecology*, 14, 181–188.
- Wyman-Simpson, C., Waller, G., Jurzysta, M., McPherson, J., Young, C. (1991). Biological activity and chemical isolation of root saponins of six cultivars of alfalfa (*Medicago sativa* L.). *Plant and Soil*, 135(1), 83–94.
- Zohaib, A., Tanveer, A., Khaliq, A., Safdar, M. (2014). Phytotoxic effect of water soluble phenolics from five Leguminous weeds on germination and seedling growth of Rice. *Pakistan Journal of Weed Scientific Research*, 20(4), 417–429.

ASSESSMENT THE IMPACT OF FERTILIZATION ON POTATOES YIELDS IN SMOLYAN REGION OF BULGARIA

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Abstract

An assessment has been made of the impact of fertilisation on potato yields. A four-year field fertiliser experiment was carried out in the region of Smolyan, Bulgaria during the period 2009-2012. Nine fertilisation variants were investigated: N, P, K, NP, NK, NPK, NPKMg with fertiliser rates of 120 kg N/ha, 60 kg P₂O₅/ha, 100 kg K₂O/ha and 33 kg Mg/ha. It has been found that nitrogen fertilisation was an essential factor for yield formation. However, adding either phosphorus or potassium did not result in a statistically different yield compared to the one received only using nitrogen. Balanced fertilisation with the three primary nutrients (NPK) ensures the highest returns. The costs of adding both phosphorus and potassium fertilisers were small compared to the value of additional production. This makes the combination of the three fertilisers economically justified. Adding magnesium did not result in a statistically proven increase in yield, but magnesium may improve the quality of production and the resistance of plants to biotic and abiotic stress.

Key words: potatoes, fertilisation, yields, revenue, conditional profit.

INTRODUCTION

Potatoes are a traditional crop for agriculture in Bulgaria, especially in mountain areas where the alternatives for other crops are limited. In recent years, however, the area planted and production in the country has declined. Average yields are relatively unchanged, but there has been significant variation over the years. Reducing production is a sign that producers are losing interest in this crop due to low and unstable profits.

Potato production is concentrated in two regions of Bulgaria - Southwest and South Central, where 86% of the areas planted are located and for 88% of the potatoes are produced. Soils in both regions are relatively weak fertile, but the climate conditions are favourable for cultivating this crop. Growing potatoes in mountain areas is an essential element of crop rotation and income generation for farmers (Beluhova-Uzunova, 2018).

The purpose of this article was, based on a field experiment conducted during the period 2009-2012, to assess the best combination of fertilisers for potatoes production.

To do so, field experiments were conducted in the South Central Region, Smolyan area (GPS coordinates 41°36'48.6" N 24°40'00.1" E)

during the period 2009-2012 on shallow brown forest soil (Cambisols - coarse). Smolyan area is located in the Rhodopi Mountains, and it is one of the leading potato growing areas in the country

MATERIALS AND METHODS

The trial was organised in the randomised block design in 4 replications, on 25 m² plots. The experiment included nine variants: the control (not fertilised) - N₀P₀K₀ and eight fertilisers combinations N₁₂₀P₀K₀; N₀P₆₀K₀; N₀P₀K₁₀₀; N₁₂₀P₆₀K₀; N₁₂₀P₀K₁₀₀; N₀P₆₀K₁₀₀; N₁₂₀P₆₀K₁₀₀; N₁₂₀P₆₀K₁₀₀Mg₃₃. The rate of the fertilizers application were N -120 kg/ha, P₂O₅ - 60 kg/ha, K₂O - 100 kg/ha, MgO - 33 kg/ha. The potatoes planting distance was 25 x 70 cm. Ammonium nitrate (NH₄NO₃), triple superphosphate (Ca(H₂PO₄)₂) and potassium sulphate (K₂SO₄) was used for fertilisation. For the ninth variant, potassium magnesium sulfate (K₂O - 30%, MgO - 10%) was used as a source of potassium and magnesium. The entire fertiliser rates were introduced into the soil before planting. Every year, the experiment was conducted on a new terrain to avoid monoculture. The potatoes were grown on non-

irrigated conditions. The English hybrid, mid-early to mid-late potato variety ‘Picasso’, was grown without irrigation.

To study the effect of the various fertilizer combinations, the method of the comparison of the mean values of independent samples with equal variances was employed (one-tailed test). The null hypothesis (H_0) was that the average yields of the fertilized trials are equal or smaller than the one of control. The alternative hypothesis (H_1) was that the average yields of the fertilized trials are larger compared to the control.

$$H_0: \mu_f \leq \mu_c \quad H_0: \mu_f - \mu_c \leq 0$$

$$H_1: \mu_f > \mu_c \quad H_1: \mu_f - \mu_c > 0$$

The hypotheses were tested with, EXEL software. The economic effect of the different fertilisers combinations was estimated by comparing the resulting revenue with the costs of fertilization.

RESULTS AND DISCUSSIONS

The descriptive statistics of the experiment are presented in Table 2.

For the four-year study period, the yields ranged from 12280 kg/ha in the non-fertilized control to 23109 kg/ha in the eighth variant, which included the fertilisation with the three NPK elements plus magnesium. The yields of all variants were abnormally low during the fourth year - 2012.

This was due to the extremely low rainfalls during the potato growing season (Table 1). They were particularly low in July (only 13 mm), the period of intense plant growth and the formation of potato tubers.

Table 1: Amount of precipitation during vegetation period of potatoes by months and years of experience (mm)

Years /mont	VI	VII	VIII	IX	Total
2009	140.0	171.6	6.6	106.6	424.8
2010	184.0	203.1	24.5	74.7	486.6
2011	94.5	43.7	131.0	54.7	324.0
2012	46.5	13.4	49.6	37.3	146.8

Source: Rozhen meteorological station (nearest meteorological station to experimental fields)

The tests for the differences in the average yields of the experiments are presented in Tables 3, 4 and 5.

When comparing the control (0) and all other variants (Table 3), we can reject the null hypothesis of no differences in the average yields for the variants that include nitrogen fertiliser - variants 1, 4, 5, 7, 8. However, we cannot do so for the other variants 2, 3 and 6. These variants include phosphorus or potassium fertiliser or both fertilisers, but no nitrogen. This means that we can find statistical support that the average yields are higher for the trials that include nitrogen, but not for the experiments where nitrogen is absent, regardless of what other fertilisers are used. The conclusion that can be made here is that the use of nitrogen fertiliser was crucial for the formation of the yield. This was confirmed by other authors who have investigated the influence of nitrogen fertilisation on potato development and productivity (Joern & Vitosh 1995; Sharifi et al., 2005; Kavvadias et al., 2012; Manolov et al., 2014).

The use of phosphorus and potassium fertilisers in the absence of nitrogen may not guarantee a higher yield compared to the not-fertilized control, but Kansay and Tejada (2019) found out that the most limiting factor of potato production in Ethiopia was not nitrogen but phosphorus. Also, potassium is a nutrient that may not affect the yield, but it has a strong effect on the quality parameter of tubers - dry matter, specific gravity, starch and vitamin C content (Khan et al., 2012; Manolov et al., 2016).

Following this line of thought, the interesting question is, if a nitrogen fertiliser was used, what would be the combination of phosphorus and potassium that would help to receive higher yields. To answer this question, variant one was assumed as a control, (applying only nitrogen), and comparison was made for a difference in yields with the other variants where the other two fertilisers (phosphorus and potassium) were also used (Table 4). In this case, we can reject the null hypothesis of no differences in the average yields for the variants that include all three fertilisers - variants 7 and 8. However, we cannot do so for the variants 4 and 5, where except nitrogen only phosphorus or potassium was used.

Table 2. Descriptive statistics of the potatoes yields

Variants	Indicators		2009	2010	2011	2012	Average
0	Average yield	kg/ha	13 600	16 490	12 603	6 429	12 280
N ₀ P ₀ K ₀	Min	kg/ha	13 200	15 440	10 429	4 018	4 018
	Max	kg/ha	14 400	17 360	14 598	8 482	17 360
1	Average yield	kg/ha	17 200	23 860	20 295	9 241	17 649
N ₁₂₀ P ₀ K ₀	Min	kg/ha	16 400	22 600	16 723	4 911	4 911
	Max	kg/ha	18 000	24 920	23 652	13 393	24 920
2	Average yield	kg/ha	15 200	22 440	15 335	6 830	14 951
N ₀ P ₆₀ K ₀	min	kg/ha	14 400	21 200	13 152	5 893	5 893
	max	kg/ha	15 600	23 600	18 938	7 857	23 600
3	Average yield	kg/ha	15 200	18 260	10 594	6 022	12 519
N ₀ P ₀ K ₁₀₀	min	kg/ha	14 000	16 960	5 589	4 911	4 911
	max	kg/ha	16 800	19 200	17 214	7 143	19 200
4	Average yield	kg/ha	17 600	22 440	19 969	11 924	17 983
N ₁₂₀ P ₆₀ K ₀	min	kg/ha	16 400	20 760	19 420	8 286	8 286
	max	kg/ha	18 800	23 920	20 464	16 518	23 920
5	Average yield	kg/ha	17 200	21 710	17 346	13 214	17 368
N ₁₂₀ P ₀ K ₁₀₀	min	kg/ha	16 000	20 800	13 125	10 179	10 179
	max	kg/ha	18 000	23 440	21 179	16 429	23 440
6	Average yield	kg/ha	14 000	20 340	13 359	6 763	13 616
N ₀ P ₆₀ K ₁₀₀	min	kg/ha	13 200	18 840	11 027	2 679	2 679
	max	kg/ha	15 200	21 480	14 491	12 500	21 480
7	Average yield	kg/ha	19 600	27 310	24 528	16 004	21 861
N ₁₂₀ P ₆₀ K ₁₀₀	min	kg/ha	18 800	25 840	20 188	14 286	14 286
	max	kg/ha	20 400	29 160	28 304	18 571	29 160
8	Average yield	kg/ha	20 400	28 030	26 997	17 009	23 109
N ₁₂₀ P ₆₀ K ₁₀₀ Mg ₃₃	min	kg/ha	19 200	26 200	20 991	15 179	15 179
	max	kg/ha	21 600	30 080	32 750	19 286	32 750

Source: Calculated with data on from the experiments

Table 3. Test for difference in the average yields of fertilised variants compared to the not fertilised control

Number of variants	0	1	2	3	4	5	6	7	8
Fertilization	N ₀ P ₀ K ₀	N ₁₂₀ P ₀ K ₀	N ₀ P ₆₀ K ₀	N ₀ P ₀ K ₁₀₀	N ₁₂₀ P ₆₀ K ₀	N ₁₂₀ P ₀ K ₁₀₀	N ₀ P ₆₀ K ₁₀₀	N ₁₂₀ P ₆₀ K ₁₀₀	N ₁₂₀ P ₆₀ K ₁₀₀ Mg ₃₃
Mean	12 280	17 649**	14 951	12 519	17 983**	17 368**	13 616	21 861**	23 109**
Variance	16 094 627	35 787 756	34 512 433	29 048 771	19 540 977	13 874 598	29 113 844	23 808 452	29 809 950
Observations	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Hyp.Mean Diff.		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Df		26.00	26.00	28.00	30.00	30.00	28.00	29.00	28.00
t Stat		2.98	1.50	0.14	3.82	3.72	0.79	6.07	6.39
P(T<=t) one-tail		0.00	0.07	0.44	0.00	0.00	0.22	0.00	0.00
T Critical one-tail		1.71	1.71	1.70	1.70	1.70	1.70	1.70	1.70

*Significant at 0.05; **Significant at 0.01

Table 4. Test for difference in the average yields of fertilised with NPK variants compared to the control fertilised only with N

Number of variants	2	4	5	7	8
Fertilization	N ₁₂₀ P ₀ K ₀	N ₁₂₀ P ₆₀ K ₀	N ₁₂₀ P ₀ K ₁₀₀	N ₁₂₀ P ₆₀ K ₁₀₀	N ₁₂₀ P ₆₀ K ₁₀₀ Mg ₃₃
Mean	17 649	17 983	17 368	21 861*	23 109**
Variance	35 787 756	19 540 977	13 874 598	23 808 452	29 809 950
Observations	16	16	16	16	16
Hyp.Mean Diff.		0	0	0	0
Df		28.00	25.00	29.00	30.00
t Stat		0.18	-0.16	2.18	2.70
P(T<=t) one-tail		0.43	0.44	0.02	0.01
t Critical one-tail		1.70	1.71	1.70	1.70

*Significant at 0.05; **Significant at 0.01

Table 5. Test for difference in the average yields of the variant with Mg compared to the control fertilised with NPK

Number of variants	7	8
<i>Fertilization</i>	N ₁₂₀ P ₆₀ K ₁₀₀	N ₁₂₀ P ₆₀ K ₁₀₀ Mg ₃₃
Mean	21 861	23 109
Variance	23 808 452	29 809 950
Observations	16	16
Hyp.Mean Diff.		0
Df		30.00
t Stat		0.68
P(T<=t) one-tail		0.25
t Critical one-tail		1.70

This means that we find statistical support that the average yields were higher for the trials that include all the three fertilisers together, but not for the variants where nitrogen was applied along with only one of the other two fertilisers. The conclusion that could be made was that adding only one of the fertilisers (phosphorus or potassium) to the nitrogen do not guarantee higher yields. Only the joint application of all three fertilizers can ensure such yields.

Melkamu, (2010) and Rana et al. (2017) found that fertilisation with N, P, K, and Mg provides higher yields of potatoes with the highest efficiency of nutrient use. Both variants 7 and 8, includes all three types of fertilisers, but in the variant 8 also magnesium was included. To test for a difference in yields between these variants, the variant 7 was assumed as a control. In this case, we can not reject the null hypothesis of no differences in the average yields. The conclusion that can be made is that adding magnesium did not guarantee higher yields. Magnesium, however, has a positive effect on the quality of production and the resistance of plants to abiotic and biotic stress.

The economic assessment was done with the following prices: ammonium nitrate - 1.41 BGN/kg; the triple superphosphate - 1.25 BGN/kg; the potassium sulphate - 2.65 BGN/kg and potassium-magnesium sulphate - 2.80 BGN/kg. The price of potatoes was assumed to be 0.70 BGN/kg. (one Euro = 1.95583 BGN lev).

Since we did not find statistical support for the differences in the average yields between the control and the variants with no nitrogen fertiliser (2, 3, 6) (Table 3), the average yields of all of them were assumed to be equal to the control - 12 280 kg/ha (variant 0).

Also, we did not find statistical support for the difference in the average yields between the variant with only nitrogen fertiliser (2) and the variants where the nitrogen was combined with phosphorus or potassium (4, 5). Therefore, for these variants, the average yields were assumed to be equal to 17649 kg/ha (variant 2). Following the same logic for the variants 7 and 8, we assume an average yield of 21861 kg/ha (variant 7).

It is clear from Table 6 that the best option, highest conditional profit (the difference between revenues and costs of fertilisers only) was achieved for variant 7.

Table 6: Evaluation of applied fertiliser rates

Variants	Average yields kg/ha	Revenue BGN lev	Costs of fertilisers BGN lev	Conditional profit BGN lev
0 N ₀ P ₀ K ₀	12 280	8596	0	8596
1 N ₁₂₀ P ₀ K ₀	17 649	12354	169	12185
2 N ₀ P ₆₀ K ₀	12 280	8596	75	8521
3 N ₀ P ₀ K ₁₀₀	12 280	8596	265	8331
4 N ₁₂₀ P ₆₀ K ₀	17 649	12354	244	12110
5 N ₁₂₀ P ₀ K ₁₀₀	17 649	12354	434	11920
6 N ₀ P ₆₀ K ₁₀₀	12 280	8596	340	8256
7 N ₁₂₀ P ₆₀ K ₁₀₀	21 861	15303	509	14794
8 N ₁₂₀ P ₆₀ K ₁₀₀ Mg ₃₃	21 861	15303	524	14779

CONCLUSIONS

Several conclusions can be drawn from this study. First, the results from the experiment indicated that nitrogen fertilisation was an essential factor for the formation of the yield in the region of Smolyan, Bulgaria, where the soil is relatively weak. Second, focusing primarily on nitrogen fertilisation was not always the best strategy. What guarantees higher yields was the balanced fertilisation with all three main fertilisers: nitrogen, phosphorus and potassium. Third, the low profits from potato production could not be a result of high fertiliser prices. The costs for fertilisation were negligible compared to the extra yield they generate. If the profits of potato production are low, this could not be a result of the fertilisers' prices, but rather a result of the other production factors, not included in the experiment. Fourth, calculating the revenue for the different

experiments, methodologically, is better to be done with yields that we have proved that was different compared to the control.

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REFERENCES

- Abdelaziz, H.H., Emam, A.A., Taha, E.E. (2010). Economics of Potato Production in the Northern Part of Khartoum State. *Journal of Science and Technology*, 11(2). March 2010 Sudan University of Science and Technology. Available at: http://www.sustech.edu/staff_publications/20120505134235159.pdf
- Atanasova, E. (2005). Quality of potatoes in dependence of mineral fertilization. *Bulgarian Journal of Soil Science, Agrochemistry and Ecology*, 15(1), 45–48.
- Beattie, B., Taylor, Ch.R. (1985) The economics of production. Wiley.
- Beluhova-Uzunova, R., Hristov, K. Shishkova M. (2018). Structure of Bulgarian agriculture 10 years after the accession to the EU. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 18(2), 29–34.
- Borisova T., VanSickle, J. (2015). Role of economics in developing fertilizer best management practices Serhat Ascii. *Agricultural Water Management*, 152, 251–261. <https://doi.org/10.1016/j.agwat.2015.01.021>
- Debertin, D. (1986). *Agricultural Production Economics*. Macmillan Publishing Company, a division of Macmillan Inc.
- Grebenicharski, S. (2015). Potatoes production in Bulgaria - market analysis and prospects. INTELIAGRO. Available at: <http://inteliagro.bg/article/88/Proizvodstvoto-na-kartofi-v-Bulgaria>
- Joern, B., Vitosh, M. (1995). Influence of applied nitrogen on potato. Part I: Yield, quality, and nitrogen uptake. *American Potato Journal*, 72(1), 51–63. Available at: <https://link.springer.com/article/10.1007/BF02874379>
- Kavvadias, V., Paschalidis, C., Akrivos, G., Petropoulos, D. (2012). Nitrogen and Potassium Fertilization Responses of Potato (*Solanum tuberosum*) cv. Spunta. *Communications in Soil Science and Plant Analysis*, 43(1-2), 176–189. DOI: <https://doi.org/10.1080/00103624.2012.634711>
- Kahsay, W.S., Tejada, M.M. (2019). Effects of nitrogen and phosphorus on potatoes production in Ethiopia: a review. *Cogent Food & Agriculture*, 5(1), 1–7. <https://doi.org/10.1080/23311932.2019.1572985>
- Khan, M.Z., Akhtar, M.E., Mahmood-ul-Hassan M., Mahmood M.M., Safdar M.N. (2012). Potato tuber yield and quality as affected by rates and sources of potassium fertilizer. *Journal of Plant Nutrition*, 35(5), 664–677. <https://doi.org/10.1080/01904167.2012.653072>
- MAFF (2018). Vegetable production in Bulgaria–harvest 2017, 340, April 2018. Available at: http://www.mzh.government.bg/media/filer_public/2018/04/27/ra340_publicationvegetables2017.pdf
- Manolov I., Neshev, N., Yordanova, N. (2014). Effect of nitrogen fertilization on nutrient content in plant biomass and productivity of potatoes. 3rd Conference with International Participation, Conference VIVUS, Slovenia, 216–222.
- Manolov, I., Neshev, N., Chalova V. (2016). Tuber quality parameters of potato varieties depending on potassium fertilizer rate and source, *Agriculture and Agricultural Science Procedia*, 10, 63–66. <https://doi.org/10.1016/j.aaspro.2016.09.010>
- Melkamu, J. (2010). Long-term effect of balance mineral fertilizer application on potato, winter rye, and oat yields; nutrient use efficiency; and soil fertility. *Archives of Agronomy and Soil Science*, 56(4), 421–432. DOI: <http://dx.doi.org/10.1080/03650340903512579>
- Ogola, O., Ayieko, W., Orawo, O., Kimani, W. (2011). Analysis of fertiliser use in potato production in Nakuru district, Kenya. *African Journal of Agricultural Research*, 6(16), 3672–3677. DOI: <https://doi.org/10.5897/AJAR10.464>
- Rana, L., Banerjee, H., Dutta, S.K., Ray, K., Majumdar, K., Sarkar, S. (2017). Management practices of macronutrients for potato for small holder farming system at alluvial soil (Entisols) of India. *Archives of Agronomy and Soil Science*, Publish ed online: 17 Apr 2017. DOI: 10.1080/03650340.2017.1317922
- Shahriar, M., Hasan K., Kamruzzaman M. (2013). Farm level potato (*Solanum tuberosum* L.) cultivation in some select ed sites of Bangladesh. *Bangladesh Journal of Agricultural Research*, 38(3), 455–466. DOI: <https://doi.org/10.3329/bjar.v38i3.16972>
- Sharifi, M., Zebarth, B., Hajabbasi M., Kalbasi, M. (2005). Dry matter and nitrogen accumulation and root morphological characteristics of two clon al selections of ‘russetnorkotah’ potato as affected by nitrogen fertilization. *Journal of plant nutrition*, 23(12), 2243–2253. DOI: <https://doi.org/10.1080/01904160500323552>

VARIABILITY OF SEMINAL ROOTS ANGLE IN SOME ROMANIAN BARLEY GENOTYPES

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Abstract

Drought tolerance of plants as a complex character, makes the choice of physiological traits, selection methods and breeding to be very difficult. Breeding of barley (and not only) focused on higher production under water stress, which led to relatively good progress. Now, however, when a so-called 'plateau' of production has been reached, the focus is on the search for those secondary features that can ensure further progress. Root phenotyping is as important as shoot phenotyping, because plant's ability to uptake moisture and nutrients mainly depends on root architecture. Therefore, root phenotyping is important for crop breeding, although under field conditions, screening roots by phenotyping is a very difficult task. Our previous research shows that in case of winter wheat for most of Romania and for regions with similar conditions, winter wheat breeding should aim at creating mainly cultivars with large seminal roots angle that could better use rainfall falling during the vegetation season, but also cultivars with a small seminal roots angle that can improve the access to water in the soil depth profile during severe drought conditions. In this study we evaluated the root system architectural traits for several Romanian barley genotypes and association with yield. Our results shown that the angle of seminal roots varied from about 44° in cultivar F-8-3-01 to more than 120° in some inbred lines. Will be discussed if breeding new barley cultivars with efficient root systems carries great potential to enhance resource use efficiency and plant adaptation to unstable climate from Romania.

Key words: winter barley, seminal root angle, yield.

INTRODUCTION

In Romania, climate change has led in recent years to an intensification of water deficits (often associated with heat), in almost all areas of the country, with negative effects on crops. Creating of winter barley varieties resistant to drought and heat is an important goal of breeding from the world and in Romania as well.

It is known that drought tolerance is a complex trait making the search for efficient selection, breeding and screening methods difficult. The traits related to root architecture are more less studied but are important for known the use of moisture from the deep layers of the soil and can be used to improve the adaptation of barley genotypes to soil water deficiency. Root system architecture is also important for nutrient use efficiency (Lynch, 2019). By exploring the subsoil, a steep and deep root system is beneficial not only for accessing water from the soil depth (Singh et al., 2011), but also for N capture from the soil profile. In contrast, a shallow but dense root system is not only better for using rainfall during the vegetation season

(Liao et al., 2006), but also has advantages regarding P capture and should also be useful for capture of K, Ca, and Mg in acid soils (Lynch, 2019).

Finally, the root system is important in barley for lodging resistance, by its effect on anchorage strength. Crook and Ennos (1994) reported that plants with stronger, more widely spread coronal roots produced larger soil cones during anchorage failure and resisted larger forces, while Pinthus (1967) found high correlations between root spreading angles and lodging rates from a series of field trials grown under various environmental conditions.

Several studies on wheat, rice, sorghum, etc. have shown that a small angle in seedlings is a precursor of a deep root system and large branches in soil depth. These characteristics are advantageous for terminal drought conditions, when there is water stored in the soil depth (Manschadi et al., 2006; Uga et al., 2011; Mace et al., 2012; Christopher et al., 2013). Every extra millimetre of water extracted during filling grain has produced a plus yield of 55 kg ha⁻¹ (Manschadi et al., 2008). In sorghum, a small root angle was associated with the

phenotype “stay-green”, due to improving the access of water in the soil depth profile (Singh et al., 2011).

Recent study (Petcu et al., 2020) shown than in Romanian conditions winter wheat breeding should aim at creating mainly cultivars with large seminal roots angle that could better use rainfall falling during the vegetation season, but also cultivars with a small seminal roots angle that can improve the access to water in the soil depth profile during severe drought conditions.

In case of winter barley, previous studies shown that the availability of barley mutants affecting seminal root angle and number is limited. A mutant with a highly geotropic root system was identified through a chemically mutagenized barely population; however, the population was reported to be unstable and display inconsistent phenotypes (Bovina et al., 2011). As a result of the challenges associated with accurately and efficiently phenotyping roots, the relationship between root traits and yield is still uncertain in barley (Robinson, 2016).

The objectives of this study is to established the variability of several winter barley genotypes for root angle and an attempt to evaluate the relationship between seminal root angle and grain yield of winter barley grown in several years under the continental climate of Romania.

MATERIALS AND METHODS

Seminal root angle was measured in several winter barley cultivars, which has performed in NARDI Fundulea.

For determination the seminal root angle, we were inspired by the work of Richard et al. (2015) and used 1 L transparent pots. The transparent pots were filled with two types of soils mixture (70% turba and 30% chernozem soil). Seeds were sown at a depth of 2 cm every 2.5 cm along the pot wall. The seeds were carefully placed vertically, embryo downwards and facing the wall to facilitate root growth along the transparent wall. Three grains of each genotype were sown, 3 seeds x 4 replications. After sowing, the clear pots were wrapped in aluminum foil and placed in dark-colored paper bags to exclude light from the developing roots. The pots were watered after sowing and no additional water or nutrients were supplied there after this.

The roots were photographed at 10 days after sowing, then foto images were transferred in PC. The angle between the first pair of seminal roots was measured with ImageJ software (<http://rsb.info.nih.gov/ij/>). The exemple of seminal root angle measurement is give in Figure 1.



Figure 1. Illustration of seminal root angle measurement of the first pair of seminal roots

Available data about grain yield recorded in 24 yield trials in several locations during 2014-2019, which included at least ten of the cultivars characterized for seminal root angle, were used for computing correlation coefficients.

Data about grain yield in yield trials were available from the National Agricultural Research and Development Institute Fundulea (44°26'N latitude and 26°31'E longitude), and seven Agricultural Research Stations (ARDS) from different regions of the country: ARDS Teleorman (44°07'N - 25°45'E), ARDS Șimnic (44°36'N - 25°45'E), ARDS Valu lui Traian (44°16'N - 28°48'E), ARDS Livada (47°50'N - 21°93'E), ARDS Mărculești (44°40'N - 27°50'E), ARDS Secuieni (44°78'N - 24°85'E) and ARDS Brăila (45°28'N - 27°97'E). These included a large variation of weather and soil conditions, as well as crop management. For example, soil conditions varied from chernozem to luvisol, and crop management included various preceding crops, and sowing dates.

Concerning the rainfall, at Fundulea average annual rainfall is 571 mm, of which 72% during the vegetation period, especially in May-June.

In the summer season, only 35% of the total annual precipitation falls, these being torrential. The frequency of droughty years is over 40%. At Șimnic annual precipitation is about 540-550 l/sqm, very unevenly distributed during the vegetation season.

Data were analysed using the statistical analysis of variance and correlation analysis was used to study the relationship between seminal root angles and recorded grain yield.

RESULTS AND DISCUSSIONS

A significant variability was found between the studied genotypes for seminal roots angle. The analysis of variance showed a significant effect of barley cultivars for the probability of 95 and 99% (Table 1).

Table 1. The analysis of variance for seminal root angle of studied barley cultivars

Source	Sum of Squares	DF	S ²	Calculated F	Ft 5%	Ft 1%
Total	12599.3	29				
Replicates	37.620	2				
Plots	10057.4	9	1117	8.1	2.4	3.5
Error	2464.2	18	136			

The seminal roots angle varied from about 44° in cultivar F 8-3/01 (Figure 3) to more than 92° (Figure 4) in cultivar Simbol (Figure 2).

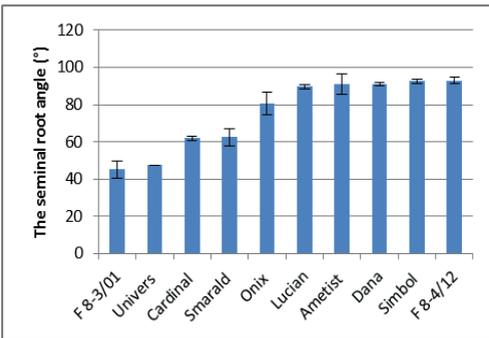


Figure 2. Genotypic variation of the seminal root angle in studied barley genotypes



Figure 3. Seminal root angle for F 8-3/01 genotype (44°)



Figure 4. Seminal root angle for Simbol genotype (93°)

A high degree of variation in phenotypes for root angle was observed in the panel of 59 barley genotypes (Figure 5). Seminal root angle ranged between 42° and 120° with a mean of 78°. Three respectively four breeding line had the lowest and highest seminal root angle, respectively.

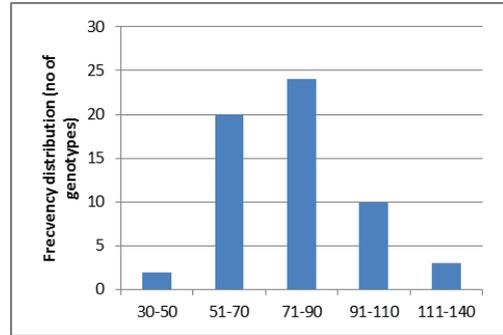


Figure 5. Frequency distribution for the seminal root angle in different barley breeding lines

Studies show that, wild and landrace barley germplasm tend to have a narrow root angle (Bengough et al., 2004; Hargreaves et al., 2009; Sayed et al., 2017), which is thought to be a consequence of originating in water-limited environments where access to deep-stored soil moisture was critical for survival. In addition, wild and landrace lines appear to produce fewer roots than their modern counterparts. For example, the study by Bengough et al. (2004) reported a mean root angle for wild barley of 40° with an average root number of three. In comparison, modern cultivars were reported to have a wider angular spread of up to 120° and a higher root number of up to seven (Bengough et al., 2004). On the other hand Arifuzzaman et al. (2014, 2016) shown that barley genotypes with a winter growth habit, and thus a longer vegetative growth phase, had a larger more vigorous root system.

The diversity of conditions included in the study was reflected in the yields of the analysed cultivars, which varied from 3664 kg ha⁻¹ at Şimnic in 2015 to 8720 kg ha⁻¹ at Fundulea in 2014. The smallest yields were obtained in 2015, a year characterized by drought, especially during the grain filling and in Şimnic, characterized as a dry area (Table 2).

Table 2. The grain yield of 10 winter barley cultivars in 8 locations and 6 years (48 environments)

Location	Year					
	2014	2015	2016	2017	2018	2019
Fundulea	8720	7073	6530	6249	7060	7015
Mărculești	5972	5885	7457	7375	6665	8689
Valu lui Traian	5533	6487	6951	4632	5495	4782
Teleorman	5965	5457	6706	7638	4414	7190
Șimnic	4097	3664	5152	5783	4615	-
Secuieni	6423	4382	4851	6018	7748	4865
Brăila	5386	5185	5812	5009	5205	5906
Livada	7268	4366	4515	5146	4331	5013
Average yield/year	5709	4946	5554	5981	5692	6209

Grain yield of the studied cultivars averaged across the 48 environments varied from 5602 to 6298 kg/ha, with highest yields obtained in new cultivars (Smarald, Cardinal and Lucian) and lowest yield in the old cultivar Dana (Table 3). Maximum yields varied from 5967 kg/ha in Ametist to 6672 kg/ha in Cardinal, while minimum yield varied from 4324 kg/ha in Dana to 5561 kg/ha in Ametist. Average yield was correlated with maximum yield but not with minimum yield and seminal root angle (Table 4).

Table 3. Average, maximum and minimum grain yields and yield amplitude in 10 winter barley cultivars

Cultivars	Average yield	Maximum yield	Minimum yield	Amplitude
Dana	5602	6118	4324	1794
Cardinal	6291	6672	5116	1556
Univers	5843	6456	4865	1591
Ametist	6088	5967	5561	406
Smarald	6298	6231	5458	773
Simbol	6124	6438	4818	1619
Onix	6027	6438	5148	1290
Lucian	6176	6544	5053	1491
F8-3-01	5735	6174	4659	1515
F8-4-12	5926	6542	4808	1734

Yield amplitudes were very large, from 406 to 1796 kg/ha and were correlated with minimum and maximum yield, but not with average yield and seminal root angle (Table 4). Similar result were obtained by Mustățea et al. (2009).

Table 4. Correlations between seminal root angle, average yield and several stability parameters

Parameters	Seminal root angle	Average yield	Maximum yield	Minimum yield	Amplitude
Seminal root angle	1				
Average yield	0.12	1			
Maximum yield	-0.015	0.38	1		
Minimum yield	0.013	0.78**	-0.07	1	
Amplitude	-0.018	-0.46	0.58*	-0.86***	1

The coefficients of correlation between the seminal root angle of wheat cultivars and grain yield of the respective cultivars varied very much, from -0.07 to +0.46, when all studied cultivars were taken into consideration (Figure 3). On the other hand excluding the cultivar Dana those correlation was insignificant, expressed by a correlation coefficient from 0.10 to 0.51 suggesting the tendency of positive correlation between yield and seminal root angle (Figure 6).

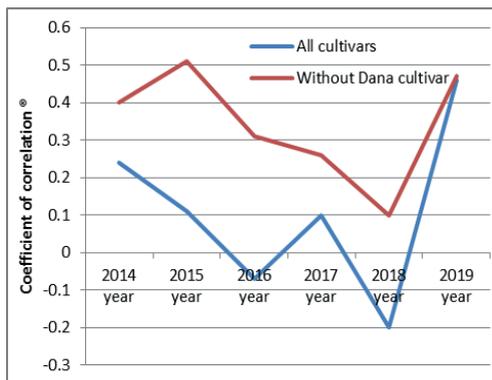


Figure 6. Histogram of the correlation coefficients between seminal root angle and grain yield

Our results suggest that, in the environmental conditions represented by most of the 48 analysed yield trials, a small seminal root angle, corresponding to a deep root system was not associated with higher grain yield but there is a tendency of positive correlation between large seminal angle and barley yield.

CONCLUSIONS

The seminal root angle of Romanian barley genotypes varied from 43° to 120°. This diversity of seminal root angle of Romanian barley germplasm offers large opportunity for breeding of winter barley.

Yields recorded for several Romanian winter wheat cultivars in a representative sample of 48 yield trials, covering a wide range of environments from Romania, showed not significant correlation with seminal root angles. With all of this there was a tendency of positive correlation between large seminal angle and yield of our barley cultivars.

Many studies propose that root traits are essential for drought adaptation and will pave the way to a second green revolution in low-input systems.

In this context we suggest that cultivars with large seminal roots angle could better use rainfall during the vegetation season, from superficial soil layers and will be suitable for most of Romania and for regions with similar conditions.

Breeding of winter barley should aim at creating diverse cultivars, including mainly cultivars with large seminal roots angle that could better use rainfall during the vegetation season, from superficial soil layers.

This could be beneficial in capitalizing small amounts of precipitation during periods of drought or to capture more nutrients with minimal metabolic costs, thus freeing up energy for the crop to invest in other developmental process, such as above-ground biomass, but also cultivars with a medium seminal roots angle that can improve the access to water in the soil depth profile during severe drought conditions.

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REFERENCES

- Arifuzzaman, M., Sayed, M.A., Muzammil, S., Pillen, K., Schumann, H., Naz, A., Leon, J. (2014). Detection and validation of novel QTL for shoot and root traits in barley (*Hordeum vulgare* L.). *Mol Breed*, 34, 1373–1387.
- Arifuzzaman, M., Gunal, S., Bungartz, A., Muzammil, S., Afsharyan, N.P., Leon, J., Naz, A. (2016). Genetic mapping reveals broader role of Vrn-H3 gene in root and shoot development beyond heading in barley. *PLoS ONE*, 11, e0158718.
- Bengough, A.G., Gordon, D.C., Al-Menaie, H., Ellis, R.P., Allan, D., Keith, R., Thomas, W.T.B., Forster, B.P. (2004). Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant Soil*, 262, 63–70.
- Bovina, R., Talame, V., Ferri, M., Tuberosa, R., Chmielewska, B. (2011). Identification of root morphology mutants in barley. *Plant Genet Resour-C*, 9, 357–360.
- Christopher, J., Christopher, M., Jennings, R., Jones, S., Fletcher, S., Borrell, A., Manschadi, A.M., Jordan, D., Mace, E., Hammer, G. (2013). QTL for root angle and number in a population developed from bread wheats (*Triticum aestivum*) with contrasting adaptation to water-limited environments. *Theor. Appl. Genet.*, 126, 1563–1574.
- Crook, M.J., Ennos, A.R. (1994). Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. *Journal of Agricultural Science, Cambridge*, 123, 167–174.
- Liao, M., Palta, J.A., Fillery, I.R.P. (2006). Root characteristics of vigorous wheat improve early nitrogen uptake. *Australian Journal of Agricultural Research*, 57, 1097–1107.
- Lynch, J.P. (2019). Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. *New Phytologist*, doi: 10.1111/nph.15738.
- Hargreaves, C., Gregory, P., Bengough, A.G. (2009). Measuring root traits in barley (*Hordeum vulgare* ssp. *vulgare* and ssp. *spontaneum*) seedlings using gel chambers, soil sacs and X-ray microtomography. *Plant Soil*, 316, 285–297.
- Mace, E.S., Singh, V., vanOosterom, E.J., Hammer, G.L., Hunt, C.H., Jordan, D.R. (2012). QTL for nodal root angle in sorghum (*Sorghum bicolor* L. Moench) collocate with QTL for traits associated with drought adaptation. *Theor. Appl. Genet.*, 124, 97–109.
- Manschadi, A.M., Christopher, J., DeVoi, P., Hammer, G.L. (2006). The role of root architectural traits in adaptation of wheat to water-limited environments. *Funct. Plant Biol.*, 33, 823–837.
- Manschadi, A.M., Hammer, G.L., Christopher, J.T., DeVoi, P. (2008). Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). *Plant and Soil*, 303, 115–129.
- Mustăţea, P., Săulescu, N.N., Ittu, Gh., Păunescu, G., Voinea, L., Stere, I., Mirlogeanu, S., Constantinescu, E., Năstase, D. (2009). Grain yield and yield stability of winter wheat cultivars in contrasting weather conditions. *Rom. Agr. Journal*, 26, 1–8.
- Petcu, E., Lazăr, C., Marinciu, C., Săulescu, N.N. (2020). Relationship of seminal roots angle and grain yield of winter wheat cultivars under the continental climate of Romania. *Rom. Ag. Journal*, 37, 1–5.
- Pinthus, M.J. (1967). Spread of the root system as indicator for evaluating lodging resistance of wheat. *Crop Science*, 7, 107–110.
- Richard, C., Hickey, L., Fletcher, S., Jennings, R., Chenu, K., Christopher, J. (2015). High-throughput phenotyping of seminal root traits in wheat. *Plant Methods*, 11, 13.
- Robinson, H., Hickey, L., Richard, C., Mace, E., Kelly, A., Borrell, A., Franckowiak, J., Fox, G. (2016). Genomic regions influencing seminal root traits in barley. *Plant Genome*, 9.

- Sayed, M., Hamada, A., Léon, J., Naz, A. (2017). Genetic mapping reveals novel exotic QTL alleles for seminal root architecture in barley advanced backcross double haploid population. *Euphytica*, 213, 2.
- Singh, V., van Oosterom, E.J., Jordan, D.R, Hunt, C.H., Hammer, G.L. (2011). Genetic variability and control of nodal root angle in sorghum. *Crop Sci.*, 51, 2011–2020.
- Uga, Y., Okuno, K., Yano, M. (2011). Dro1, a major QTL involved in deep rooting of rice under upland field conditions. *J. Exp. Bot.*, 62, 2485–2494.

DEFICIT IRRIGATION FOR GREEN BEANS - LATE FIELD PRODUCTION

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Abstract

The aim of this work is to establish the influence of water deficit during different vegetative stages on the yield of green beans grown at late field production. The field experiment was conducted during 2010-2012 in Agricultural University of Plovdiv. Treatments are as follows: 1) without irrigation; 2) optimum irrigation (by 80% FC for 0-40 cm soil layer); 3) without irrigation during vegetative period; 4) during budding and flowering period; 5) during the pod development period. The number of irrigations is 2-4 with amount of irrigation rate 50 mm. The yield by optimum irrigation treatment is 1609 kg/da. It is triple and more than non-irrigated (519 kg/da). The Yield losses caused of irrigation cancelling during vegetative period are 13%. More sensitive is the period of pod development with 11-33% losses. The most sensitive is budding and flowering period with average yield losses of 36% (17-58%).

Key words: green beans, irrigation, water deficit, yield.

INTRODUCTION

The short growing season and tolerance for abiotic factors allow green beans to be sown during the period from the second half of April to mid-July (under the conditions of Bulgaria). Thus, it is used as the main, second or an intermediate crop in crop rotations, giving high and stable yields under irrigated conditions and when applying the correct agricultural technology. The scarcity of water resources for irrigation, as well as the high cost of irrigation water, is prerequisites for the application of deficit irrigation. In practice it is usually done most often done by the cancellation of one or more irrigations. The purpose of the studies in this research direction is to determine the sensitivity to soil drought during different parts of the vegetation period on the one hand, and responsiveness to irrigation on the other (with very limited water resources). The sensitivity of green beans to drought during different periods of vegetation has been the subject of many years of research in various parts of the world. According to the publication of Poryazov (2001), green beans are very sensitive to soil and air humidity, as well as to the ambience temperature and the plant stresses caused by the fluctuation of soil moisture are reflected differently in different phenophases, as follows:

water stress before flowering leads to a decrease in the number of flowers and pods, and if there is a shortage of soil water during the pod development, they remain with less weight. The author thinks that the irrigation of the beans is also a means for regulating the temperature of the crop. Oliveira, et al. (2008) found that deprivation of sufficient water during any phase of green bean vegetation results in a decrease in yield. Concerning sensitivity to soil drought, Acosta Gallegos & Shibata (1989) separate the bean vegetation period into two sub periods - vegetative and reproductive. For the Mexico conditions, cancellation of irrigation during the vegetative sub period and optimal irrigation during the reproductive sub period results in a decrease in yield of 37-39% and by irrigation through vegetative period and drought during the reproductive period, the yield decreases by 42-50%. Also comparable to these are the results published by Gunton & Evenson (1980), according to which, in Queensland (Australia), the cancellation of watering to flowering leads to a decrease in yield by 31 - 44%, and, if cancelled, irrigation during the flowering period losses are 31 - 55%. Khah et al. (2005) found that, under greenhouse conditions, cancellation of irrigation during the reproductive period of green beans leads to

significant water savings, but also to a significant decrease in yield. According to Nielsen & Nelson (1998) water stress during the vegetative phases reduces plant height and leaf area and, when apply it during the reproductive period, yield reduces due to the reduction in the number of pods per plant and their size. Nuñez-Barrios et al. (2005) also reported a significant decrease in yields following the interruption of irrigation during the reproductive period. The authors reported a 27% reduction in the number of flowers, reducing their number by twigs to 50%. Ucar et al. (2009) confirm the criticality of the period of flowering, formation and development of pods in relation to soil moisture, noting that irrigation before and after the water stress experienced by the plants during this part of the vegetation period is not able to increase the yield. Based on field experiments conducted in California (USA) Shen & Webster (1986) concludes that beans are most sensitive to soil moisture deficiency during the transition from vegetative to reproductive vegetation. Acosta Diaz et al. (1997); Albient (1976); Tawadros et al. (1983) and Calvache et al. (1997) define the flowering period as the most critical, and except that the yields at cancellation of irrigation during this phase significantly decreases and the WUE is very low. Zerbi & Chiaranda (1984) found that, for Naples (Italy), reducing the soil water potential to - 5 bars in the flowering period reduced yield by 10% and the number of beans by 30%. According to Isk et al. (2004) water stress after flowering has the most negative effect on the yield, thus the authors recommend that irrigation water be provided primarily during this part of the growing season when irrigation water is scarce. This view is also confirmed by a publication by Ritter & Scarbrou (1992). According to the authors irrigation only during the reproductive period increases the yield by an average of 47%, compared to non-irrigated beans. Based on studies conducted in Zimbabwe, Manjeru et al. (2007) found that water stress significantly reduced bean yield, as the flowering period (duration 2 weeks from the beginning of flowering) being the most sensitive and the least sensitive to drought – vegetative period. The negative effect of water stress on the structural elements of the yield is confirmed (a

significant reduction in the number of beans per bean). To obtain maximum yields, water stress during flowering and pod development should be avoided. Under non-irrigation conditions, the authors recommend sowing as early as possible. For the conditions of Bulgaria there is no information on the possibilities for applying deficit irrigation regime of the green beans by cancellation of watering. At the same time, scientists' opinions are not unidirectional in terms of phase sensitivity to drought stress. This means that studies on different soil and climatic conditions, different in character years and different (if possible contrasting in sensitivity, requirements and productivity) bean varieties are needed, to achieve a correct refinement of irrigation parameters and the possibilities for applying rational irrigation according to the specificity of the individual phenophases in terms of sensitivity to water stress.

The aim of this study is to determine the effect of cancellation of irrigation during the different phases of the growing season on the quantity and quality of yield of green beans, grown in the Plovdiv region.

MATERIALS AND METHODS

The experiment was conducted in the period 2010-2012 in the Agricultural University of Plovdiv, on alluvial-meadow soil. The *Strike* variety was used. To determine the sensitivity of green beans to drought, the growing season is separated into 3 sub periods: I period - vegetative (from germination to budding); II period - budding and flowering; III period - pod formation and development (including harvesting period). On the basis of this division of the growing season, the variants of the experiment were determined as follows: 1) without irrigation throughout the growing season (000); 2) without irrigation during the I subperiod (0++); 3) without irrigation during the II subperiod (+0+); 4) without irrigation during the III subperiod (++0); 5) optimum irrigation throughout the growing season (+++), at pre-irrigation soil moisture of 80% FC (field capacity) for the 0-40 cm layer. The irrigation rates in the optimal variant are calculated for wetting the layer 0-60 cm. The experiment was based on the block method in 4 repeats with the

size of the experimental plots 18 m² and the harvest 10 m². Irrigation was done gravity by short closed furrows. The yield data and its structural components were processed by the ANOVA1 software to establish the warranty of differences between the different variants of the experiment. During the experiment, all agrotechnical activities related to the cultivation of the crop were observed.

RESULTS AND DISCUSSIONS

Meteorological conditions

Precipitations during vegetation period

The growing season in late field production of green beans concurs with the period with probability of prolonged droughts. This has been observed in all three experimental years, with a minimum precipitations amount of 36.5 mm during 2012. Although the first two years their quantity is larger (156.5 mm in 2010 and 113.3 mm in 2011), their distribution is unevenly. In 2010 the majority of the precipitations fall during the third decade of July, providing the germination of plants. In the second experimental year (2011), the peak of rainfall is at the beginning of August, as a result of which the development of plants is ensured until the flowering begins. During the critical periods in the development of the beans, namely during the flowering and pod development, the experimental years are dry.

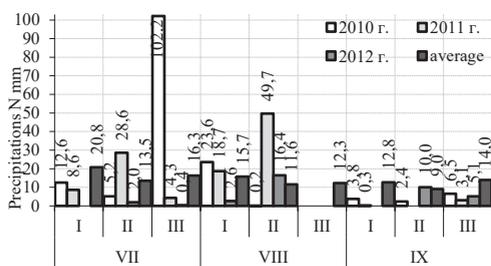


Figure 1. Precipitations amount by decades for the VII – IX period

Figure 1 shows the amount of the precipitation by decade for the period VII-IX during the three experimental years, compared with those for a multiyear period. With few exceptions, the average multiannual ten-day precipitation amounts are higher than those in the experimental years, i.e. they are drier than the

norm for the area. The largest deviations from this tendency were found in the third ten days of July 2010, when the precipitation amount was 102.2 mm. They practically provide for the emergence of the plants and also store the soil with water at a greater depth than the active soil layer (0-60 cm). In the second ten days of August 2011, the rainfall is 49.7 mm, which is commensurate with the size of the irrigation rate. This results in the shift of the beginning of the irrigation period to a later phase of the growing season.

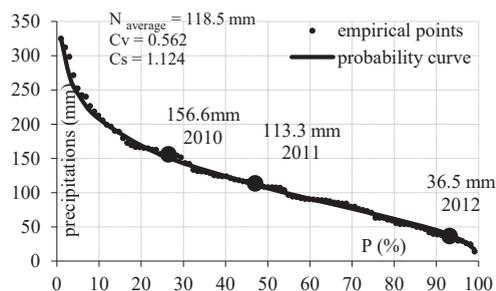


Figure 2. Precipitations probability (P) for the VII-IX period

According to the graph of Figure 2, the first (2010) year is a medium moist with a probability of 26% and the second (2011) medium with a probability of 47%. The most extreme in terms of rainfall is 2012, which is characterized as dry with 93% probability.

Air temperature

Temperature is a major factor affecting the duration of a phenophases, i.e. the duration of the all growing season (Figure 3).

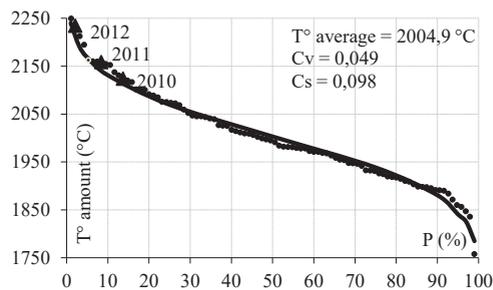


Figure 3. Probability (P) of air temperature amount for the VII-IX period

For the period July-September in the first experimental year (2010) it is 2124°C, in 2011

it is 2158°C, and in 2012 it is the highest - 2234°C. The highest is the average daily temperature in July and August and the lowest - in the period of pod development (end of September). According to the graph in Figure 3, the three experimental years are warm with a probability of 14% for 2010, 8% for 2011 and 2% for 2012, respectively. According to the meteorological characteristics, the experimental years are relatively suitable for growing of green beans.

Irrigation regime components for green beans - late field production

Data on the number of irrigations, irrigation rates and phases during which they were realized under optimal irrigation conditions are presented in Table 1. In mid-2010, a total of 3 irrigations are needed to optimize soil moisture throughout the growing season. The inter-irrigation period is 13-14 days. The irrigations are, respectively, in the phases 5-6 triple leaf, flowering and beginning of pod formation and pod development. The annual irrigation rate is 149.3 mm. The water saving is about 50 mm at practically the same irrigation rates (100 mm). During the vegetative period of the medium 2011, amount of the precipitations is sufficient to maintain optimum soil moisture. Therefore, irrigation was not carried out and this year the 0++ variant was practically not realized. Subsequently, two irrigations were applied during the inter-irrigation period again for 14 days and the irrigation rate for optimally irrigated beans 98.6 mm (Table 2).

Table 1. Number of irrigations, irrigation rates and phase of irrigation implementation under optimum irrigation conditions

№	m (mm)	Phase of the development
2010		
1	49.9	3-5 triple leaf
2	49.3	first pod set
3	50.1	pod formation and pod development
2011		
1	49.3	beginning of flowering
2	49.3	pod formation and pod development
2012		
1	53.4	3-5 triple leaf
2	57.5	budding
3	51.0	flowering
4	50.0	pod formation and pod development
m - irrigation rate (mm)		

In the +0+ and ++0 variants, irrigation was applied in the “pod development” and “flowering”, respectively, so that the irrigation and irrigation rates are equal (49.3 mm). Due to the small amount of precipitations, in the dry year 2012, the number of irrigations to ensure optimal irrigation regime is 4 and the first one being during the vegetative period. During the “budding” and “flowering” period, 2 irrigations are required and the last is during the fruit development. The annual irrigation rate is 211.9 mm and 6 - 7 days period between irrigations. When thus distributed irrigations, for realizing the variants 0++ and ++0 were done three irrigations with practically uniform irrigation rates (160 mm). As the second and third irrigations are during “budding” and “flowering” for variant +0+ have been missed two irrigations, so irrigation rate is 100 mm. The irrigation regime described in this way provides a wealth of scientific information on the impact of water deficits during in different phases on the productivity of green beans - late field production.

Table 2. Irrigation regime components for green bean

date	Irrigation rates			
	+++	0++	+0+	++0
2010				
27VIII	49.9	–	49.9	49.9
09IX	49.3	49.3	–	49.3
23IX	50.1	50.1	50.1	–
M (mm)	149.3	99.4	100.0	99.2
2011				
30VIII	49.3	49.3	–	49.3
14IX	49.3	49.3	49.3	–
M (mm)	98.6	98.6	49.3	49.3
2012				
21VIII	53.4	–	53.4	53.4
29VIII	57.5	57.5	–	57.5
05IX	51.0	51.0	–	51.0
11IX	50.0	50.0	50.0	–
M (mm)	211.9	158.5	103.4	161.9
M – annual irrigation rate (mm)				
* on the day of sowing is carried watering germination rate of 20 mm				

Influence of irrigation and cancellation of irrigation on the yield of Green Beans

Tables 3 and 4 present data on yields during different years, as well as average data on the productivity of green beans under non-irrigation conditions, optimum irrigation and phase irrigation cancellation. Optimizing soil moisture throughout the all growing season

provides a statistically warranted increase in yield by about 3 times or more. It is confirmed that optimum irrigation contributes significantly to mitigating the impact of meteorological factors on the productivity of green beans. According to the summarized data, the yield increase is also more than 3 times, as under non-irrigation conditions it is 519 kg/da and under optimal irrigation - 1609 kg/da. The difference is 1090 kg/da or 310%. These results demonstrate the qualities of the variety used which and in this production direction gives an average yield under non-irrigated conditions of nearly 520 kg/da or 32% of the maximum.

Table 3. Irrigation regime influence on the yield

variant	yield kg/da	to 000			to +++		
		±Y	%	w	±Y	%	w
2010							
000	524.0	St.	100.0	St.	-951.6	35.5	C
+++	1475.6	951.6	281.6	C	St.	100.0	St.
0++	1028.2	504.2	196.2	C	-447.4	69.7	C
+0+	1226.0	702.0	234.0	C	-249.6	83.1	B
++0	1305.6	781.6	249.2	C	-170.0	88.5	A
GD (kg/da) 5% = 160.5 1% = 221.1 0.1% = 303.9							
2011							
000	356.1	St.	100.0	St.	-981.0	26.6	C
+++	1337.1	981.0	375.5	C	St.	100.0	St.
0++	1328.3	972.2	373.0	C	-8.8	99.3	n.s.
+0+	562.3	206.2	157.9	C	-774.8	42.1	C
++0	895.5	539.4	251.5	C	-441.6	67.0	C
GD (kg/da) 5% = 105.3 1% = 145.1 0.1% = 199.5							
2012							
000	676.3	St.	100.0	St.	-1338.5	33.6	C
+++	2014.8	1338.5	297.9	C	St.	100.0	St.
0++	1826.7	1150.4	270.1	C	-188.1	90.7	n.s.
+0+	1297.4	621.1	191.8	C	-717.4	64.4	C
++0	1368.8	692.5	202.4	C	-646.0	67.9	C
GD (kg/da) 5% = 227.9 1% = 314.0 0.1% = 431.7							
W - warranty							

Table 4. Irrigation regime influence on the yield - average

Var.	yield kg/da	to 000		to +++	
		±Y	%	±Y	%
000	518.8	St.	100.0	-1090.4	32.2
+++	1609.2	1090.4	310.2	St.	100.0
0++	1394.4	875.6	268.8	-214.8	86.7
+0+	1028.6	509.8	198.3	-580.6	63.9
++0	1190.0	671.2	229.4	-419.2	73.9

Drought during early vegetation periods adversely affects the yield due to the inhibition of growth and the development of sufficient photosynthetic leaf area. However, subsequent optimization of soil moisture ensures that approximately 90% of the maximum yield is obtained for the specific conditions. The

cancellation of watering in the third period of vegetation has a more pronounced negative effect on the yield, in the middle of the wet years it reaches almost 90% of the maximum, but in the average dry and dry years - it is only 70% (67-68%). In confirmation of the results published by a number of authors, drought during the flowering period is most critical in terms of yield. Although this period has a short duration, cancellation of irrigation results in the most significant decrease in yield. In favorable years it reaches just over 80% of the maximum, but in average dry and dry years it may not reach even up to 50% of it (42-64%).

Providing irrigation only during the reproductive period increases yields by at least twice, and in dry years this increase can reach and exceed 300%. In more favorable from a meteorological standpoint years, irrigation during the reproductive period and the period of pod development can increase yields by over two times, but in dry years this increase is relatively small (below 60%), considering results under non-irrigation conditions. While maintaining the soil moisture in the optimum range of the beginning of the vegetation until the end of flowering, the yield increased by 230% compared to that in non-irrigated conditions. Regardless of the conditions of the year, this increase is at least 200% (202-252%).

Irrigation water use efficiency (IWUE) and yield losses

The results of the yield losses resulting from the cancellation of irrigation, as well as the additional yield of each 1 m³ of irrigation water, depending on the conditions of the year, are presented in Table 5. The cancellation of irrigation during the growing season, combined with prolonged drought, can lead to a significant loss of yield (up to 30%). In the presence of rainfall and shortening of the deficit period, the cancellation of this irrigation has a less pronounced negative effect on the yield, with losses between 1 and 9%. In meteorological-favorable years, the negative effect on yield of irrigation cancellation during flowering is expressed by a yield loss of 17%. However, if there is no rainfall and no irrigation during this period, the losses are significant and are in the range of 36-58%. A less pronounced negative effect is observed in

the cancellation of watering during the period of pod development and harvests. In years where there is also rainfall, losses from unrealized irrigation are just over 10%, and in years with significant drought, they are also significant (32-33%). In conclusion it can be said that irrigation cancellation during the growing season results in the lowest yield losses, followed by cancellation during the pod development and harvest period, and the largest are irrigation cancellation losses during the period of budding and flowering, which is actually the shortest as duration.

Table 5. Irrigation water use efficiency (IWUE) and yield losses

variant	Additional yield		Yield losses		M	IWUE
	kg/da	kg/da	%	mm		
2010						
000	St.	-951.6	-64.5	0.0	-	-
+++	951.6	St.	St.	149.3	6.374	
0++	504.2	-447.4	-30.3	99.4	5.072	
+0+	702.0	-249.6	-16.9	100.0	7.020	
++0	781.6	-170.0	-11.5	99.2	7.879	
2011						
000	St.	-981.0	-73.4	0.0	-	-
+++	981.0	St.	St.	98.6	9.949	
0++	972.2	-8.8	-0.7	98.6	9.860	
+0+	206.2	-774.8	-57.9	49.3	4.183	
++0	539.4	-441.6	-33.0	49.3	10.941	
2012						
000	St.	-1338.5	-66.4	0.0	-	-
+++	1338.5	St.	St.	211.9	6.317	
0++	1150.4	-188.1	-9.3	158.5	7.258	
+0+	621.1	-717.4	-35.6	103.4	6.007	
++0	692.5	-646.0	-32.1	161.9	4.277	
average						
000	St.	-1090.4	-67.8	0.0	-	-
+++	1090.4	St.	St.	153.3	7.114	
0++	875.6	-214.8	-13.3	118.8	7.368	
+0+	509.8	-580.6	-36.1	84.2	6.052	
++0	671.2	-419.2	-26.1	103.5	6.487	

*M - annual irrigation pate

The IWUE is related to the influence of the period with water reduction and in combination, the favorable situation after the cancellation of the irrigation within the respective period is reflected. These circumstances are due to variations in values for a given irrigation regime over different meteorological conditions. With optimal irrigation, the impact of the year is the least pronounced. The indicators for applying the 0++ variant are high and stable, which is also in sync with the data on yields and losses. This irrigation regime also gives the highest averages. The cancellation of irrigation through

flowering significantly reduces the value of the IWUE.

Influence of irrigation regime on the quality of production

Tables 6, 7 and 8, as well as Figures 4, 5 and 6 present the results concerning some structural elements of the yield, such as the mass of one bean, the length and diameter of the beans.

Table 6. Influence of irrigation regime on the beans weight

variant	g	to 000			to +++		
		±Y	%	W	±Y	%	W
2010							
000	3.8	St.	100.0	St.	-1.1	77.6	C
+++	4.9	1.1	128.9	C	St.	100.0	St.
0++	4.3	0.5	113.2	B	-0.6	87.8	C
+0+	4.4	0.6	115.8	C	-0.5	89.8	B
++0	4.9	1.1	128.9	C	0.0	100.0	n.s
GD (g):	5% = 0.3	1% = 0.4	0.1% = 0.5				
2011							
000	3.1	St.	100.0	St.	-1.4	68.9	C
+++	4.5	1.4	145.2	C	St.	100.0	St.
0++	4.4	1.3	141.9	C	-0.1	97.8	n.s.
+0+	3.5	0.4	112.9	A	-1.0	77.8	C
++0	4.2	1.1	135.5	C	-0.3	93.3	n.s.
GD (g):	5% = 0.3	1% = 0.4	0.1% = 0.5				
2012							
000	4.3	St.	100.0	St.	-1.0	81.1	C
+++	5.3	1.0	123.3	C	0.0	100.0	St.
0++	4.6	0.3	107.0	n.s.	-0.7	86.8	B
+0+	4.6	0.3	107.0	n.s.	-0.7	86.8	B
++0	4.9	0.6	114.0	A	-0.4	92.5	n.s.
GD (g):	5% = 0.4	1% = 0.6	0.1% = 0.8				

W - warranty

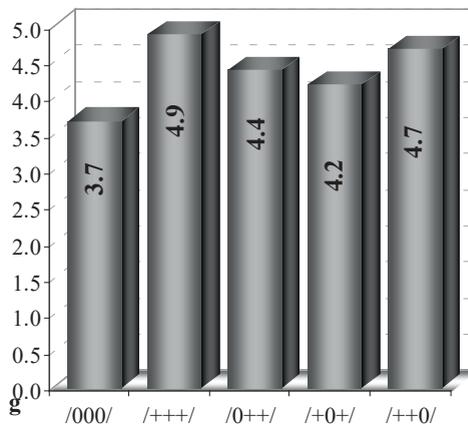


Figure 4. Influence of irrigation regime on the beans weight (average)

Under non-irrigation conditions, the mass of the beans is 3.7 g (3.1 to 4.3 g) and they are on average 1.2 g lighter than those under optimum

irrigation. This difference is warranted statistically, regardless of the conditions of the year. During dry years, the cancellation of irrigation during the vegetative period or during flowering leads to weight of beans close to those under non-irrigation conditions, even though the necessary irrigations were made during the period of formation and development of the beans. The difference in weight of beans against optimum irrigation is significant and statistically warranted, regardless of the conditions of the year. In more favorable years it is 10% and in the case of drought it reaches 22% (average 15%). Irrigation affects the length of the pods of beans, regardless of the conditions of the year, but it is less than the impact on the mass of pods. The best results are obtained with optimum irrigation (14.1 cm), which corresponds to the specified characteristics variety. The increase in the length of the beans compared to those under non-irrigation conditions was 2.1 cm or 15%, and this increase was statistically warranted (Table 7).

Table 7. Influence of irrigation regime on the beans length

variant	cm	to 000			to +++		
		±Y	%	w	±Y	%	w
2010							
000	12.3	St.	100.0	St.	-1.7	87.9	C
+++	14.0	1.7	113.8	C	St.	100.0	St.
0++	12.9	0.6	104.9	A	-1.1	92.1	C
+0+	13.0	0.7	105.7	A	-1.0	92.9	C
++0	13.5	1.2	109.8	C	-0.5	96.4	n.s.
GD:	5% = 0.5 cm		1% = 0.7 cm		0.1% = 0.9 cm		
2011							
000	11.8	St.	100.0	St.	-1.9	86.1	C
+++	13.7	1.9	116.1	C	St.	100.0	St.
0++	13.5	1.7	114.4	C	-0.2	98.5	n.s.
+0+	11.9	0.1	100.8	n.s.	-1.8	86.9	C
++0	12.5	0.7	105.9	C	-1.2	91.2	C
GD:	5% = 0.3 cm		1% = 0.4 cm		0.1% = 0.6 cm		
2012							
000	11.9	St.	100.0	St.	-2.7	81.5	C
+++	14.6	2.7	122.7	C	St.	100.0	St.
0++	12.8	0.9	107.6	A	-1.8	87.7	C
+0+	13.5	1.6	113.4	C	-1.1	92.5	B
++0	14.0	2.1	117.6	C	-0.6	95.9	n.s.
GD (cm):	5% = 0.7		1% = 0.9		0.1% = 1.3		
W - warranty							

The most pronounced negative effect on the length of the beans is observed when the irrigation is cancelled during flowering, resulting in the length of the beans reaching between 87 and 93% of the maximum. When the irrigation is cancelled during the vegetative

period, the beans reach an average of 93% of the maximum length, but in favorable years it is commensurate with that of optimal irrigation. However, in dry years the difference is significant - over 12%. The length of the beans is least affected by the meteorological conditions of the year when the irrigation is cancelled during the third period, resulting in 91-96% of the maximum length.

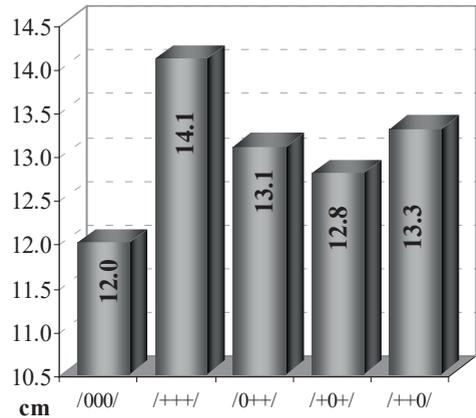


Figure 5. Influence of irrigation regime on the beans length (average)

Table 8. Influence of irrigation regime on the beans diameter

variant	mm	to 000			to +++		
		±Y	%	w	±Y	%	w
2010							
000	7.8	St.	100.0	St.	-0.4	95.1	C
+++	8.2	0.4	105.1	C	St.	100.0	St.
0++	7.8	0.0	100.0	n.s.	-0.4	95.1	C
+0+	7.8	0.0	100.0	n.s.	-0.4	95.1	C
++0	7.8	0.0	100.0	n.s.	-0.4	95.1	C
GD (mm):	5% = 0.1		1% = 0.2		0.1% = 0.3		
2011							
000	8.1	St.	100.0	St.	-0.5	94.2	C
+++	8.6	0.5	106.2	C	St.	100.0	St.
0++	8.6	0.5	106.2	C	0.0	100.0	n.s.
+0+	8.2	0.1	101.2	n.s.	-0.4	95.3	C
++0	8.4	0.3	103.7	B	-0.2	97.7	A
GD (mm):	5%=0.1		1%=0.2		0.1%=0.3		
2012							
000	7.9	St.	100.0	St.	-0.9	89.8	C
+++	8.8	0.9	111.4	C	St.	100.0	St.
0++	8.1	0.2	102.5	n.s.	-0.7	92.0	C
+0+	8.3	0.4	105.1	A	-0.5	94.3	B
++0	8.6	0.7	108.9	C	-0.2	97.7	n.s.
GD (mm):	5% = 0.3		1% = 0.4		0.1% = 0.6		
W - warranty							

Regarding the diameter of the beans, the trend of the previous two indicators is maintained,

but the overall impact of both irrigation and drought is even less pronounced. The averaged data show that under optimal irrigation, the bean diameter is 8.5 mm and is 0.6 mm larger than that under non-irrigation conditions (Table 8). The cancellation of watering slightly changes the diameter of the beans within about 5%, but this relative decrease is statistically warranted.

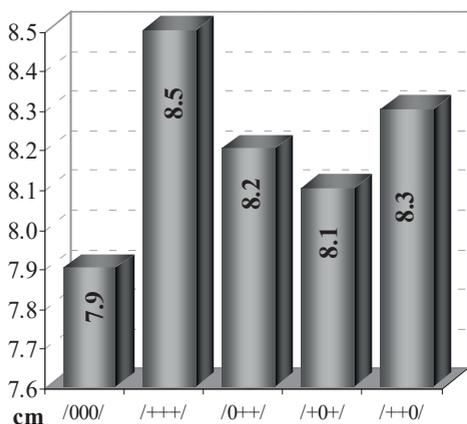


Figure 6. Influence of irrigation regime on the beans diameter (average)

Figure 6 summarizes data on the influence of irrigation regime on the diameter of the beans. With regard to irrigation cancellation options, the most significant relative decrease in this indicator value was in the case of cancellation during flowering (5%). Under non-irrigation conditions, it is 7%, and under the variants 0++ and ++0 - respectively 4 and 3%.

CONCLUSIONS

To optimize soil moisture, for green bean late production, it is necessary to supply 2-4 irrigations with an irrigation rate of 50 mm, with a maximum of 1 irrigation during the vegetative period and 1-2 during flowering. During the development of beans and harvests, it is usually given 1 irrigation, and in dry years - 2.

Regardless of which phase is irrigation cancelled, this has a negative effect on the rate of linear growth, as drought during flowering having the most pronounced negative impact,

and subsequent optimization of soil moisture cannot compensate for this lag.

Drought during early vegetation periods adversely affects yields, but with subsequent optimization of soil moisture, yields are around and above 90% of the maximum yield for the specific conditions. The cancellation of irrigation in the third period of vegetation has a more pronounced negative effect on the yield, in the mid-wet years it reaches almost 90% of the maximum, but in the average dry and dry years - it is only 70%. The cancellation of irrigation through flowering leads to the most significant decrease in yield. In favorable years it reaches just over 80% of the maximum, but in average dry and dry years it may not reach even up to 50% of it (42-64%).

The irrigation water use efficiency is highest when irrigation is cancelled during the vegetative period. The values of the indicator at optimum irrigation vary the least. The cancellation of irrigations during flowering most significantly reduces the values of the indicator.

With enough water for irrigation, it is best to apply a biologically optimal irrigation regime as it coincides with the economically optimal one. In case of shortage of irrigation water, it should to apply rational irrigation regime, recommending cancellation of irrigation during the vegetative period. The cancellation of irrigation during the reproductive period significantly worsens economic performance, especially if it occurs during the flowering period.

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REFERENCES

- Acosta-Diaz, E., Kohashi-Shibata, J., Acosta-Gallegos, J.A., Alberto J. (1997). Rendimiento y sus componentes en frijol bajo condiciones de sequia. *Agricultura Tecnica en Mexico*, 23(2), 139–150.
- Gallegos, J. A.A., Shibata, J.K. (1989). Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus Vulgaris*) cultivars. *Field crops research*, 20(2), 81–93.

- Albient, E. (1976). Rezultate în cultura irigată a unor soiuri de fasole de grădină. *Prod. Veget. Hort.*, 25(5), 10–12.
- Calvache, M., Reichardt, K., Bacchi, O., Dourado-Neto, D. (1997). Deficit irrigation at different growth stages of the common bean (*Phaseolus vulgaris* L., cv. *imbabello*). *Scientia Agricola*, 54, 1–16.
- Gunton, J., Evenson, J. (1980). Moisture stress in Navy beans. 1. Effect of withholding irrigation at different phenological stages on growth and yield. *Irrigation Science*, 2(1), 49–65.
- Isk, M., Önceler, Z., Cakr, S., Altay, F. (2004). Effects of different irrigation regimes on the yield and yield components of dry bean (*Phaseolus vulgaris*). *Acta Agronomica Hungarica*, 52(4), 381–389.
- Khah, E., Akoumianakis, K., Passam, H. (2005). Greenhouse Seed Production of Two Dwarf Green Bean Cultivars in Relation to the Duration of Irrigation. *Hort. Science*, 40(2), 443–445.
- Manjeru, P., Madanzi, T., Makedza, B., Nciizah, A., Sithole, M. (2007). Effects of water stress at different growth stages on components and grain yield of common bean (*Phaseolus vulgaris* L.). *8Th African Crop Science Society Conference, El-Minia, Egypt*, 299–303.
- Nielsen, D.C., Nelson, N.O. (1998). Black bean sensitivity to water stress at various growth stages. *Crop science*, 38(2), 422–427.
- Nuñez-Barrios, A., Hoogenboom, G., Nesmith, D. (2005). Drought stress and the distribution of vegetative and reproductive traits of a bean cultivar. *Scientia Agricola*, 62(1), 18–22.
- Oliveira, R., Lima, J., Reis, E., Pezzopane, J., Silva, A. (2008). Levels of water deficit at different growth phases of bean (*Phaseolus vulgaris* L.) cv. Capixaba precoce. *Engenharia Na Agricultura*, 16(3), 343–350.
- Poryazov, I. (2001). Garden beans. *Agriculture*, 10, 1–16.
- Ritter, W.F., Scarbrough, R.W. (1992). Response of lima beans to irrigation on the Delmarva Peninsula. *Applied Engineering in Agriculture (USA)*, 8(1), 23–27.
- Shen, X.Y., Webster, B.D. (1986). Effects of water stress of pollen of *Phaseolus vulgaris* (L.). *Journal of the American Society for Horticultural Science*, 111(5), 807–810.
- Tawadros, H.W., Ageeb, O.A.A., Mohamed, G.E., Salih, F.A., El Nadi, A.M.H. (1983). Water relations Faba bean in the Nile valley. *Martinus Nijhoff Publishers*, 87–94.
- Ucar, Y., Yilmaz, H.I., Yardimci, N., Kadayifci, A., Tuylu, G.I. (2009). The effect of deficit irrigation on the grain yield of dry bean (*Phaseolus vulgaris* L.) in semiarid regions. *Spanish Journal of Agricultural Research*, 7(2), 474–485.
- Zerbi, G., Chiaranda, F.Q. (1984). L'importanza della gestione irrigua per il fagiolo in ambiente meridionale. *L'informatore Agrario*, 40(48), 45–47.

COMPARATIVE PERFORMANCE OF SOME MAIZE HYBRIDS FOR YIELD AND OTHER AGRONOMIC TRAITS

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Abstract

A field experiment was conducted at Agricultural Research and Development Station (ARDS) Simnic - Craiova during 2019 in rain fed condition, consisting of eight Romanian maize hybrids, to evaluate for grain yield and other agronomic traits. According to results: plant height between 189.5 cm (Turda 201) and 264 cm (Olt), ear height 79.5 cm (Turda 200) and 113.5 cm (F 423), thousand grain weight 230 g (Turda 200, Turda 201) and 306 g (Olt), hectolitre mass 61.1 kg/hl (Turda 332) and 74.4 kg (Turda Star), grain yield 4285.7 kg/ha (Turda 165) and 5432.1 kg/ha (Turda 200), was found. The hybrids Turda 200 (5432.1 kg/ha), Olt (5428 kg/ha) and F 376 (5378.6 kg/ha) produced significantly highest grain yield as compared to the average (control). Thus, it was concluded from the study that, the hybrids Turda 200, Olt and F376 have been found suitable for this region.

Key words: grain yield, maize (*Zea mays* L.), thousand seed weight, hectolitre mass.

INTRODUCTION

The applying of different types and doses of fertilizers with field crops has had a beneficial effect on the yield (Dodocioiu et al., 2009).

Maize or corn (*Zea mays* L.) is a major cereal crop in many parts of the world and it used as an important source of food, animal feeds and industrial raw materials.

In Romania, the area under maize cultivation was 2371 thousand hectares with the total production of 18.353 thousand tons (MADR, 2018).

The changing environmental conditions affects the performance of maize genotypes, therefore, breeding programs must take into account the consequences of environment and exploring and developing more competitive maize genotypes (Ferdoush et al., 2017) evidence the evolution of the number of dairy cows, milk yield and total milk production in the period 1990-2010.

The drought tolerance traits of crops is probably the most difficult to identify with great precision, since grain yield is a complex traits and is influenced by the genotype by environment interaction and other mechanisms associated with heterosis (Farre et al., 2000; Messmer, 2006; Pereira 2016). Thus, in the

absence of precise information regarding the complete genetic mechanism of drought tolerance in maize, some secondary traits of the plant have been used as selection criteria, such as morpho-physiological mechanisms (Wattoo et al., 2018) and agronomic traits (Mikić et al., 2016).

The Oltenia area is often affected by drought and heat, only two years out of ten are favourable to agricultural crops. Maize yield is highly influenced by environmental factors, especially by increasing temperature and the uneven distribution of rainfall. For this area, the rainfall during sowing to anthesis period and temperature during grain-filling period had a decisive role in defining the production capacity of maize (Bonea and Urechean, 2020; Urechean and Bonea, 2012).

The maize crop is particularly sensitive to drought one week before and two weeks after flowering resulting in an average yield loss of 20% to 50%, and at grain-filling period, the high temperatures, shorten this period and reduce grain weight, resulting in 10% reduction in grain yield (Meseka et al., 2018).

The grain yield reduction of maize due to the drought effects is varied between 1 to 76 % depending on the timing and stage of occurrence and severity (Mostafavi et al., 2011;

Khodarahmpour and Hamidi, 2012; Moradi et al., 2012; Adebayo and Mendkir, 2014).

Therefore, the identifying of genotypes with tolerance to drought and their use for high and stable productions are very important issues in this region (Urechean et al., 2010; Bonea, 2016; Bonea and Urechean, 2017; 2019).

One of the factors that determine the reduction of maize yields in farms is the use of low yielding hybrids.

Assessment of maize response to agro-climatic factors in each crop area, may be the basis for providing farmers with adequate information, so that they to properly plan the specific production management strategies (Farooq et al., 2011; Bonea and Urechean, 2019).

The aim of this paper is to compare the grain yield and other agronomic traits at an assortment of Romanian maize hybrids cultivate under agro-climatic conditions from central part of Oltenia and to select suitable hybrids.

MATERIALS AND METHODS

The experiment was performed under conditions of the reddish preluvosol in Agricultural Research and Development Station (ARDS) Simnic - Craiova area, Dolj County, and in the context of the climate conditions of the year 2019 (Figure 1). This station is located in central part of Oltenia area. The experiment was laid out in randomized block design with three replications.

Sowing was performed on 5th of April. Fertilisation was performed with 250 kg/ha of

complex fertilizer 7:21:21. For weed control was used DUAL GOLD in a rate of 1.5 l/ha.

At harvesting, determinations and analyses were performed regarding: the plant and ear height (cm), the thousand grain weight (g), the hectolitre mass (kg/hl) and the grain yield (kg/ha) at 15% moisture content.

The obtained data were processed by analyses of variance (ANOVA) and significant differences were determined at probability levels of 1%.

Differences in trait means were determined at $p \leq 0.05$ the least significant difference.



Figure 1. View from a experimental field in ARDS Simnic

In this study, eight Romanian maize hybrids (Turda 125, Turda 200, Turda 201, Turda 332, Turda Star, F 376, F423 and Olt) were used.

The year of the investigation was considered a dry year. The rainfall deficiency was largely pronounced in May (-39.7 mm), July (-23.2 mm) and August (-38.0 mm). The mean monthly temperatures were over the multiannual average in June (+1.2°C), August (+2.6°C) and September (+2.4°C) (Table 1).

Table 1. Climatic conditions at ARDS Simnic in 2019

Months	Temperature (°C)		Precipitation (mm)	
	2019	Multiannual average	2019	Multiannual average
April	11.9	12.2	42.0	53.1
May	16.2	17.5	32.0	71.7
June	22.7	21.5	136.0	73.6
July	22.9	23.8	59.0	82.2
August	25.1	22.5	9.0	47.0
September	20.2	17.8	0	61.8
Total			278	389

RESULTS AND DISCUSSIONS

Analysis of Variance

The analysis of variance (ANOVA) for yield and other agronomic traits is presented in Table 2. Results indicated that means squares due to hybrids were highly significant ($P < 0.01$) for grain yield and all traits studied.

Plant height

The average of plant height at the studied maize hybrids was of 223 cm (Figure 2).

Table 2. Analysis of variance (mean squares) for different traits of maize genotypes

Traits	df	MS	F
Plant height	7	2641.5	64.2**
Ear height	7	415.6	17.2**
1000-grain weight	7	1762.1	66.8**
Hectolitre mass	7	53.9	11.66**
Grain yield	7	802970.8	16.07**

**Significant at 1% level of probability

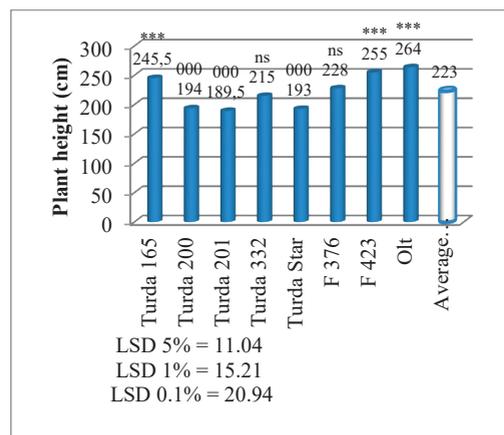


Figure 2. Plant height at the studied maize hybrids

The hybrids Olt, F423 and Turda 165 gave maximum mean values of 264 cm, 255 cm and 245.5 cm, respectively. However, these hybrids also produced significantly higher plant height than the average (control), while Turda 200 (194 cm), Turda Star (193 cm) and Turda 201 (189.5 cm) produced lowest plant height (125.03 g). The difference in plant height could be due to variation in genetic composition of the maize cultivars (Ali et al., 2006).

These results are in accordance with the results of Bonea and Urechean (2019) and Urechean and Bonea (2017), who also reported significant difference of plant height in different maize hybrids cultivated at ARDS Simnic.

Ear height

The average of ear height at the studied maize hybrids was of 94.1 cm (Figure 3).

The maximum ear height was observed in F423 (113.5 cm) and Turda 332 (104 cm) which are very significantly and significantly higher than the average (control) while the hybrids Turda 200 (79.5 cm), Turda 2001 (83.5 cm) and Turda Star (84.5 cm) are significant and distinctly significant negative, respectively.

The shorter ear heights are, generally, undesirable because the aeration and low transmission of sun light to the lower parts may result in drastic reduction in grain yield (Ali et al., 2011; Bawa et al., 2015). On the other hand, the ear placement at a greater height from the ground level is also undesirable since it may exert pressure on plants during grain filling and physiological maturity and may cause severe lodging, which could ultimately affect the final yield to the farmer (Menyonga et al., 1987; Younas et al., 2002).

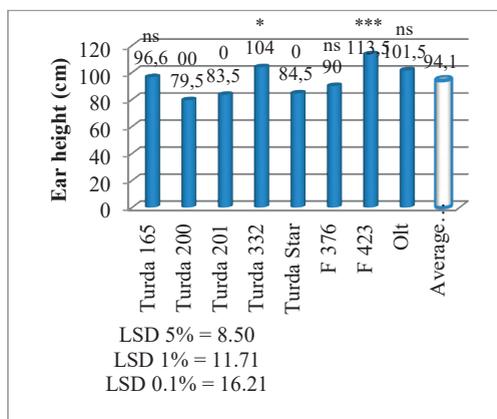


Figure 3. Ear height per cob at the studied maize hybrid

In our study, the maximum ear height is of 113.5 cm which can be considered as acceptable, while the lowest was of 79.5 cm which is good height.

The difference in ear height might be attributed to genetic diversity of tested maize hybrids (Muneeb et al., 2017).

Thousand grain weight (TGW)

The average 1000-grain weigh to eight hybrids was 248.7 g (Figure 4).

Compared with the average (control), the hybrid Olt (306 g) had achieved very significant positive TGW differences while the hybrids Turda 200 and Turda 2001 had very significant negative TGW differences. This was due to the fact that 1000-grain weight is a genetically controlled factor (Tahir et al., 2008).

Urechean and Bonea (2017) found significant differences among six Romanian maize hybrids cultivated at ARDS Simnic, for 1000-grain weight which strongly supports this result. They reported an average value of 259.16 g for 1000-grain weight.

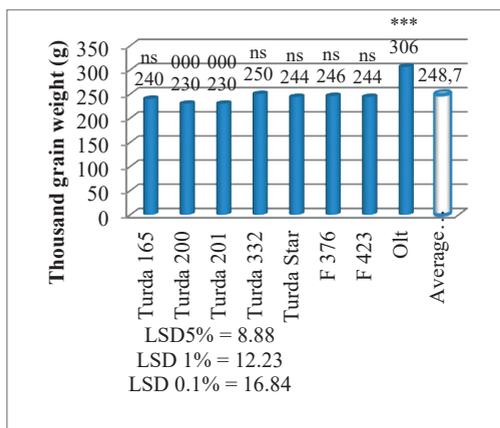


Figure 4. Thousand grain weight at the studied maize hybrids

Hectolitre mass

The average hectolitre mass to eight hybrids was 71.2 kg/hl (Figure 5).

Compared with the average (control), all other hybrids had achieved similar hectolitre mass assured from the point of view statistically, excepted the Turda 332 hybrid (61.1 kg/hl) which had registered, a difference very significant negative.

The results obtained by Toader et al. (2018) showed that the hectolitre mass ranged from 70.3-76.8 kg/hl for eight maize genotypes

cultivated under non-irrigated conditions in a farm from Rovine village, Ialomita County.

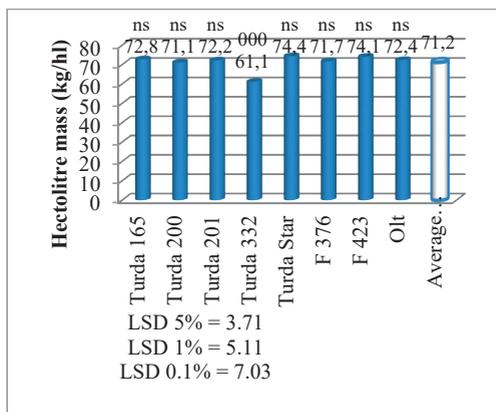


Figure 5. Hectolitre mass at the studied maize hybrids

Grain yield

The average of grain yield at the studied maize hybrids was of 4847.6 kg/ha (Figure 6).

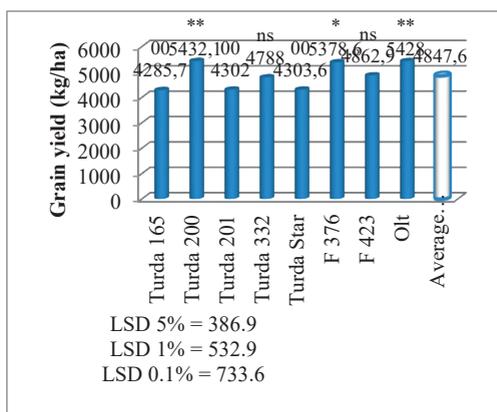


Figure 6. Grain yield at the studied maize hybrids

The grain yield was high, 5432.1 kg/ha for Turda, 5428 kg/ha for Olt and 5378.6 kg/ha for F376, with a yield difference compared to the control being distinct significant positive and significant positive, respectively.

Many researchers found that there were significant differences among hybrids for grainyield and other traits which strongly support the present finding (Ali et al., 2011; Bonea, 2016; Urechean and Bonea, 2017).

According to Cooper et al. (2014), obtaining hybrids with good grain yields in environments with water restriction and a significant increase

in environments without water restriction has been the aim of many plant breeding programs.

CONCLUSIONS

For identifying superior hybrids, the highest grain yield was one of the basic criteria. The hybrids Turda 200 (5432.1 kg/ha), Olt (5428 kg/ha) and F 376 (5378.6 kg/ha) were found superior in their grain yield potentiality. Thus, it was concluded from the study that, the hybrids Turda 200, Olt and F376 have been found suitable hybrids for this region.

REFERENCES

- Adebayo, M.A., Menkir, A. (2014). Assessment of hybrids of drought tolerant maize (*Zea mays* L.) inbred lines for grain yield and other traits under stress managed conditions. *Nigerian Journal of Genetics*, 28, 19–23.
- Ali, Z., Haqqani, A.M., Saleem, A., Bakhsh, A. (2006). Growth and yield components of maize cultivars in Khushab district. *Pak. J. Agric. Res.*, 19, 55–58.
- Ali, F., Muneer, M., Rahman, H., Noor, M., Durrishahwar, Shaukat, S., Yan, J. (2011). Heritability estimates for yield and related traits based on testcross progeny performance of resistant maize inbred lines. *Journal of Food, Agriculture and Environment*, 9, 438–443.
- Bawa, A., Abdulai, M.S., Addai, I.K. (2015) Evaluation of in-bred lines and hybrid maize (*Zea mays* L.) for tolerance to *Striga hermonthica* (Del.) Benth in the guinea savanna agro-ecological zone of Ghana. *American Journal of Agricultural and Biological Sciences*, 10, 128–136.
- Bonea, D. (2016). The effect of climatic conditions on the yield and quality of maize in the central part of Oltenia. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, 46(1), 48–55.
- Bonea, D., Urechean, V. (2017). Study on the selection for drought tolerance of some semi-late maize hybrids cultivated at A.R.D.S. Simnic. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, 47(1), 41–49.
- Bonea, D., Urechean, V. (2019). Evaluation of maize hybrids under local conditions of Craiova, Oltenia region. *Annals of the University of Craiova, Agriculture, Montanology, Cadastre Series*, 49(2), 44–49.
- Bonea, D., Urechean, V. (2020). Response of maize yield to variation in rainfall and average temperature in central part of Oltenia. *Romanian Agricultural Research*, 37, 1–8.
- Cooper, M., Gho, C., Leafgren, R., Tang, T., Messina, C. (2014). Breeding drought-tolerant maize hybrids for the US Corn-belt: discovery to product. *Journal of Experimental Botany*, 65, 6191–6204.
- Dodocioiu, A.-M., Mocanu, R., Susinski, M. (2009). *Agrochimie*. Editura Sitech, 344–345.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy and Sustainable Development*, 29, 185–212.
- Farre, I., Oijen, M.V., Leffelaar, P.A., Faci, J.M. (2000). Analysis of Maize growth for different irrigation strategies in northeastern Spain. *European Journals of Agronomy*, 12, 225–238.
- Ferdoush, A., Haque, M.A., Rashid, M.M., Bari, M.A.A. (2017). Variability and traits association in maize (*Zea mays* L.) for yield and yield associated characters. *J Bangladesh Agril Univ.*, 15(2), 193–198.
- Khodarahmpour, Z., Hamidi, J. (2012). Study of yield and yield components of corn (*Zea mays* L.) inbred lines to drought stress. *African Journal of Biotechnology*, 11, 3099–3105.
- MADR (2018). Date privind evoluția suprafețelor și a producției în România. <https://www.madr.ro/culturi-de-camp/cereale/porumb.html>
- Menyonga, J., Tay, B., Anthony, Y. (1987). Food grain production in semi-arid Africa. *Proceedings of an International Drought Symposium*, Nairobi, Kenya, 191–208.
- Meseka, S., Menkir, A., Bossey, B., Mengesha, W. (2018). Performance assessment of drought tolerant maize hybrids under combined drought and heat stress. *Agronomy*, 8, 274.
- Messmer, R.E. (2006). The genetic dissection of key factors involved in the drought tolerance of tropical maize (*Zea mays* L.). A dissertation submitted to the Swiss Federal Institute of Technology Zurich. Available online at: <http://e-collection.library.ethz.ch/eserv/eth:29035/eth-29035-01.pdf>
- Mikić, S., Zorić, M., Stanisavljević, D., Kondić-Špika A., Brbaklić, L., Kobiljski B., Nastasić A., Mitrović, B., Šurlan-Momirović, G. (2016). Agronomic and molecular evaluation of maize inbred lines for drought tolerance. *Spanish Journal of Agricultural Research*, 14, 1–13.
- Moradi, H., Akbari, G.A., Khorasani, S.K., Ramshini, H.A. (2012). Evaluation of drought tolerance in corn (*Zea mays* L.) new hybrids with using stress tolerance indices. *European Journal of Sustainable Development*, 1, 543–560.
- Mostafavi, Kh., Shoahosseini, M., Sadeghi Geive H. (2011). Multivariate analysis of variation among traits of corn hybrids traits under drought stress. *International Journal of Agricultural Sciences*, 1(7), 416–422.
- Muneeb, K., Kamran, K., Sami, U.A., Nawab, A., Muhammad, M.A., Hazrat, U., Muhammad, O.I. (2017). Yield performance of different maize (*Zea mays* L.) genotypes under agro climatic conditions of Haripur. *Int.J. Environ. Sci. Nat. Res.*, 5(5), 555–572.

- Pereira, A. (2016). Plant abiotic stress challenges from the changing environment. *Frontiers in Plant Science*, 7, 1123.
- Tahir, M., Tanveer, A., Ali, A., Abbas, M., Wasaya, A. (2008). Comparative yield performance of different maize (*Zea mays* L.) hybrids under local conditions of Faisalabad-Pakistan. *Pak. J. Life Soc. Sci.*, 6(2), 118–120.
- Toader, M., Georgescu, E., Ionescu, A.M., Năstase, P.I. (2018). The productivity elements, chemical composition and energetical value (caloric) of some maize hybrids in Ialomita county conditions. *Agriculture for Life, Life for Agriculture Conference Proceedings*, 1(1), 122–125.
- Urechean, V., Bonea, D., Borleanu, C.I. (2010). The influence of climate on maize production in the centre of Oltenia. *Maize Genetics Cooperation Newsletter*, 1, 14–15.
- Urechean, V., Bonea, D. (2012). Aspects regarding the behaviour of some sorghum (*Sorghum bicolor* L. Moench) grain hybrids in the soil and climate conditions of Oltenia central. *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series, XLII(2)*, 264–267.
- Urechean, V., Bonea, D. (2017). Estimate of drought tolerance at some maize hybrids grown in the central Oltenia zone with using stress tolerance indices. *17th International Multidisciplinary Scientific GeoConference SGEM, Conference Proceedings*, 17(61), 681–688.
- Wattoo, F.M., Rana, R.M., Fiaz, S., Zafar, S.A., Noor, M.A., Hassan, H.M., Bhatti, M.H., ur Rehman, S., Anis, G.B., Muhammad Amir, R. (2018). Identification of drought tolerant maize genotypes and seedling based morpho-physiological selection indices for crop improvement. *Sains Malaysiana*, 47, 295–302.
- Younas, M., Rahman, H., Hayder H., (2002). Magnitude of variability for yield and yield associated traits in maize hybrids. *Asian Journal of Plant Science*, 1, 694–696.

MICROBIAL SYMBIONTS AND NUTRIENTS (N AND P) SHARING: EFFECT ON SOIL MICROBIAL ACTIVITY IN THE UPLAND RICE (*Oriza sativa*) AND BEAN (*Phaseolus vulgaris*) INTERCROPPING

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Abstract

The symbiotic association (plant-soil-microorganisms) has an important role in nitrogen (N) and phosphorus (P) uptake. The main objective of this study is to assess the potential of fungal and rhizobial symbionts as well as the importance of plant-soil-microorganism interactions on microbial dynamics. The upland rice and the beans were cultivated in mono or in co-culture using the rhizospheric soil of the upland rice and beans collected on plots treated with different levels of organic and mineral fertilizers. What about saying: Microbial (fungal and rhizobial) inoculate were constituted by root fragments (rice or bean) collected from previous crop and coded as I1 (root fragments from rice), I2 (root fragments from bean) and I3 (mixed root fragments from rice and bean). The results showed that soils inoculated with I1 and I3, were characterized by high phosphatase activity. These two treatments enhance also the amount of nitrogen and phosphorus in the aerial part of upland rice intercropped with bean. These results suggest that the bean with its symbiont can be considered as ecological engineers that stimulate the biological functioning of soils and is beneficial for upland rice cultivation.

Key word: bean, co-culture, inoculum, microbial symbionts, upland rice.

INTRODUCTION

About 45% of the hillsides, or "tanety", of the highland regions of Madagascar are potentially cultivable with an average slope of almost 9.23% (Ramifehiarivo et al., 2016). The increased use of tanety soils could alleviate the saturation of the lowlands, especially for flooded rice cultivation (Rasoamampionona et al., 2008) and provide local populations with new cultivable land. However, these tanety are characterized by ferralitic soils rich in iron and aluminium oxyhydroxides, which strongly adsorb phosphorus (P) (Hinsinger, 2001; Morel, 2002; Smith et al., 2003; Rabeharisoa, 2004; Richardson et al., 2009; Gérard, 2016)

limiting its uptake by plant species (Holford, 1997; Jarosch et al., 2015). These soil properties are also accompanied, by low nitrogen (N) content which, like phosphorus, is well known as a factor limiting plant production (Vance et al., 2003). The use of ferralitic soils for agriculture requires then prior improvements to reduce these deficiencies in available P and N. Direct inputs of P and N and crop rotation practice which contributes mainly to the intensification of major biological processes that influence nutrient acquisition, plant growth and plant productivity are the best and usual ways to correct such deficiency. It is also possible in some case to combine these options, i.e. by providing immediately available

nutrients and optimizing biological, processes. These different alternatives allow the improvement of nutrients availability, particularly P, and soil quality.

Several authors have demonstrated the importance of nitrogen-fixing rhizobia and mycorrhizal fungi, two major microbial groups involved in soil fertility improvement and plant productivity (Abdelkader et al., 2017). The nitrogen-fixing rhizobia are involved in atmospheric nitrogen (N₂) fixation, which can reach more than 200 to 300 kg N ha⁻¹ per year in some legumes (Mohammadi et al., 2012). Mycorrhizal fungi (mainly vesicular arbuscular mycorrhizal fungi or VAM), which are obligate symbionts (Duponnois et al., 2010), improve directly (mycorrhizae formation at the root level) or indirectly (extension of extramatrical hyphae in the soil), P acquisition by plant species (Smith and Read, 1997, 2008). Generally, legumes, except the lupines species (*Lupinus albus*), are able to contract mycorrhizal and nitrogen-fixing symbiosis at the same time (Jensen and Hansen, 1968; Amarger et al., 1977). These two forms of association are complementary.

Plants throughout their development cycle benefit from the positive contributions of these microorganisms but also constitute an indispensable support for their propagation in different forms resulting from sexual or asexual multiplication (Bonfante and Anca, 2009), such as cells, spores, hyphae (Hartnett and Wilson, 1999) and root pieces with nodules or mycorrhizae (Scheublin et al., 2004). Indeed, these beneficial microorganisms (rhizobia and mycorrhizal fungi) in addition to their symbiotic potential, also excrete in the soil different substances (enzymes, natural antibiotics, growth hormones...) (Bashan, 1998; García-Garrido and Ocampo, 2002) that by their activities strongly contribute to the maintenance of soil fertility. Thus, on the one hand, a gap in terms of microbial density in the soil should be corrected by introducing active populations of beneficial microorganisms (technique called inoculation), and on the other hand, a significant wealth could be used to enrich other soils according to compatibilities (Koide and Mosse, 2004). In this case, the inoculum consists essentially of propagules (germs, spores, hyphae, mycorrhizal root

pieces) whose effectiveness has already been proven (Adholeya, 2003; Smith and Read, 2010).

In the highlands of Madagascar, the most adopted technique is the leguminous-cereal crop rotation (most often voandzou- upland rice) (Andriamananjara, 2011) and the crop association involving upland rice and maize, rarely upland rice-bean. The latter one deserves a particular attention in order to better understand the mechanism of the "soil-microorganism-plant" interaction. Thus, the main objective of this study was to assess the potential of the "rice-bean" intercropping system to promote the propagation of microbial symbionts and the sharing of nitrogen and phosphorus while stimulating the activity of soil microorganisms under different controlled fertilization.

MATERIALS AND METHODS

Two different plant species were used in this study: the upland rice (*Oryza sativa* L.) *Poaceae* family and the common bean (*Phaseolus vulgaris* L.), *Fabaceae* family.

Soil preparation

The soils used during this study were taken from "rice-bean" plots located in Lazaina, an agronomic experimental site owned by the University of Antananarivo. These plots were divided according to the type of fertilizer used, i.e. Triple SuperPhosphate with 45% P₂O₅ (S2), manure (S3) and Triple Super Phosphate + manure (S4) and Control (without any fertilizer input) (S1).

The soils (S1, S2, S3 and S4) were respectively mixed with sterilized sandy soil (120°C for 40 min) and distributed into 2 liter buckets. Microbial (fungal and rhizobial) inoculate were constituted by root fragments (rice or bean) collected from previous crop and coded as I1 (root fragments from rice), I2 (root fragments from bean) and I3 (mixed root fragments from rice and bean).

Greenhouse experiment

Each soil (S1 to S4), were respectively inoculated with I1, I2 and I3. Three techniques were adopted for the experiment: monoculture

of rice (MoR), monoculture of bean (MoH) and coculture rice and bean (CoRH).

Nitrogen and phosphorus content of above-ground biomasses

The determination of soil phosphorus content was carried out according to the ammonium molybdate blue-ascorbic acid method described by Murphy and Riley (1962) and the total plant nitrogen was determined by the Kjeldahl method.

Soil microbial enzymatic activities

Acid phosphatase activity and total microbial activity through Fluorescein diacetate or FDA hydrolysis activities were assessed *in vitro*. For the first one, the para- Nitrophenyl Phosphate (pNPP) substrate in contact with the soil was hydrolyzed to p-Nitrophenol by phosphatase enzymes and the FDA in contact with the soil was hydrolyzed by different enzymes producing the fluorescein (Alef, 1998; Schnurer and Rosswall, 1982).

Mycorrhization rate evaluation

Fresh roots were stained with Trypan Blue (Phillips and Hayman, 1970) and the percentage of root length colonized by the mycorrhizal fungus was quantified by observing 30 root segments under microscope and calculating the mycorrhization rate according to the method of Giovannetti and Mosse (1980).

RESULTS AND DISCUSSION

Effect of soil type/fertilizer on the acquisition of mineral elements (nitrogen and phosphorus) in the aerial parts of rice and bean.

In this study, high levels of fertilization [S3 (manure), S4 (TSP+manure)] increase the N and P content in the aerial part of each plants, rather than their growth. With regard to N acquisition, a 20 and 10% increase was observed in rice and beans in S3 and S4 soil, respectively. For P content, this increase was 16 and 24% in rice and beans, respectively. These effects can be explained by a direct supply of P from fertilizers applied in the mineral form of TSP. Manure is both an additional intake of P, an intake of N (14 g N

kg⁻¹) (Henintsoa, 2018) and other nutrients such as macronutrients (K, Ca...) and micronutrients, necessary for plant growth. S3 soil (manure) is the source of N, mainly in mineral form, which is immediately available to the plants.

Effect of soil type/fertilizer on the mycorrhization rate of plants

The results obtained (Tables 1 and 2) showed that P input to S3 and S4 soils slightly increase mycorrhization rate, which increase by about 10% (65-75% on rice and 70-80% on beans). These high mycorrhization rate values could explain better phosphate nutrition of the plants and thus a higher P content. Moreover, it has been confirmed by Boukcim and Mousain (2001) that the level of mineral or organic fertility in the soil or growing medium and/or the nutritional status of the host plant has a major influence on plant mycorrhization. Compared to the roots of the two plants grown with the control soil without fertilizer, mycorrhizal infection of plants grown on the fertilized soil is significantly high. However, generally, the intensity of root colonization by symbiotic fungi decreases when the level of phosphorus in the soil increases (Dickson et al., 1999; Wang et al., 2014; Guo et al., 2016). But, the establishment of mycorrhizal symbiosis on plants within an ecosystem generally promotes the availability of major soil nutrients, especially phosphorus (Boullard, 1968; Mosse, 1981; Strullu, 1985; Vassilev et al., 2001; Khasa et al., 1992) and improves plant health. This subsequently maintains growth and productivity of the host plant (Van Der Heijden et al., 1998; Scheublin et al., 2007; Shah et al., 2009).

Effect of the inoculum on the acquisition of mineral elements (nitrogen and phosphorus) from the aerial parts of rice and bean.

Tables 1 and 2 give the results on the phosphorus and nitrogen contents in the aerial part of plants inoculated with I1, I2 and I3. Results showed that I1 (1112.48 mg·kg⁻¹) and I3 (1131.77 mg·kg⁻¹) increase significantly the phosphorus content of the aerial part of bean. A similar result was obtained with I3 (1040.93 mg·kg⁻¹) for rice aerial part. Maximum nitrogen gain of the aerial part of beans and rice was observed with I3.

Table 1. ANOVA results and comparison of means for bean productivity parameters as a function of soil/fertilizer type, inoculum and cropping system

Parameters Factors	P (mg·kg ⁻¹)		N (mg·kg ⁻¹)		Mycorrhization rate (%)		
	F	P(F)	F	P(F)	F	P(F)	
Soil type/fertilizer (S)	21,91	< 0,0001	12,51	< 0,0001	18,31	< 0,0001	
Inoculum (I)	4,34	0,02	17,42	< 0,0001	15,31	< 0,0001	
Cultivation system (MoH/CoRH)	57,37	< 0,0001	0,73	0,39	16,85	0,00	
S*I	2,61	0,02	2,91	0,01	4,75	0,00	
S*MoH/CoRH	4,17	0,01	2,61	0,06	5,12	0,00	
I*MoH/CoRH	18,93	< 0,0001	1,69	0,19	6,53	0,00	
S*I*MoH/CoRH	1,29	0,27	3,03	0,01	2,01	0,08	
Comparison of means		Mean	group	Mean	group	Mean	group
Soil type (S)	S1	916,30	c	18025,14	b	70,71	b
	S2	1089,64	b	18386,48	b	68,62	b
	S3	1217,12	a	19665,77	a	83,33	a
	S4	1152,50	ab	20099,57	a	81,86	a
Inoculum (I)	I1	1112,48	a	19141,05	b	75,98	b
	I2	1037,43	b	17982,36	c	82,17	a
	I3	1131,77	a	20009,31	a	70,24	c
Cultivation system	MoH	1198,53	a	19164,79	a	72,52	b
	CoRH	989,25	b	18923,69	a	79,75	a

Table 2. ANOVA results and comparison of averages for rice productivity parameters as a function of soil/fertilizer type, inoculum and cropping system

Parameters Factors	P (mg·kg ⁻¹)		N (mg·kg ⁻¹)		Mycorrhization rate (%)		
	F	P(F)	F	P(F)	F	P(F)	
Soil type/fertilizer (S)	16,45	< 0,0001	37,76	< 0,0001	8,76	< 0,0001	
Inoculum (I)	54,02	< 0,0001	88,79	< 0,0001	13,38	< 0,0001	
Cultivation system (MoR/CoRH)	252,95	< 0,0001	14,87	0,00	0,05	0,82	
S*I	5,95	< 0,0001	5,94	< 0,0001	1,72	0,13	
S*MoR/CoRH	3,05	0,03	3,23	0,03	1,92	0,13	
I*MoR/CoRH	20,93	< 0,0001	0,64	0,53	0,51	0,60	
S*I*MoR/CoRH	3,28	0,01	7,54	< 0,0001	1,79	0,11	
Comparison of means		Mean	group	Mean	group	Mean	group
Soil type (S)	S1	772,85	b	14337,64	b	64,84	b
	S2	952,21	a	14846,51	b	65,71	b
	S3	929,85	a	16404,38	a	72,92	ab
	S4	972,93	a	17169,35	a	78,71	a
Inoculum (I)	I1	923,59	b	15286,36	b	74,99	a
	I2	756,35	c	14171,29	c	74,17	a
	I3	1040,93	a	17610,76	a	62,48	b
Cultivation system	MoR	728,28	b	15274,83	b	70,30	a
	CoRH	1085,63	a	16104,11	a	70,79	a

This increase in P and N content may be closely linked to the abundance of beneficial microorganisms which have the ability to release or transform complex elements into some mineral forms that are immediately available to plant species. In this case,

arbuscular and vesicular mycorrhizae have a high affinity P absorption mechanism that improves the P nutrition of the plants. Arbuscular and vesicular mycorrhizae are able to improve the availability of P through their hyphae, which explore large areas of soil and

act as a bridge between the soil and plant roots for P transfer (Liu et al., 2000; Bianciotto and Bonfante, 2002). It was reported also that soil inoculation with rhizobia improved the acidification of the rhizosphere which subsequently stimulated P mobilization from a poorly soluble P source (Ögüt et al., 2011) and improved N nutrition of plants (Peoples et al., 1995). In addition to the effects of rhizobia and mycorrhizae, other groups of beneficial microorganisms can play an important role in improving plant nutrition. These include PGPR bacteria, whose association with plant roots plays a key role in P nutrition in many agroecosystems, particularly in P-deficient soils (Goldstein, 2007; Jorquera et al., 2008). Considering that some PGPRs have a capacity to solubilize phosphate, they could be useful in improving bean production by increasing soil P content and improving nodulation and nitrogen fixation. The inoculation of plants with PGPRs could increase the native population through various mechanisms that convert insoluble inorganic and organic P into the available form and thus improve plant nutrition (Guiñazu et al., 2010; Qureshi et al., 2012; Sharma et al., 2013; Singh, 2013). Thus, these PGPRs have enormous potential in biofertilizer formulations to be exploited to increase crop yields through the solubilization of soil-fixed P (Hayat et al., 2012).

Effect of inoculum on plant mycorrhization rate

Our results showed that the presence of a high diversity of microorganisms at the root level explains the response to inoculation. Thus, the high presence of arbuscular and vesicular mycorrhizae colonizing the roots of legumes and cereals, which cause highly stimulated plant growth, has already been reported (Youpensuk et al., 2005; Scheublin and Van Der Heijden, 2006; Malina Singha and Sharma, 2013).

Effect of cropping system on the acquisition of mineral elements (nitrogen and phosphorus) in the aerial parts of rice and bean.

The nitrogen rate of the bean was slightly reduced by competitiveness in crop association, that of the rice gave an excellent yield (I don't understand what you are saying here? Could

you please reformulate this phrase?). These results show the role of legumes and symbiotic nitrogen-fixing rhizobacteria in the leguminous-grass system, as well as the possibility of nitrogen transfer from one plant to another in the intercropping system (He et al., 2007; Shen et al., 2004). In addition, according to Zheng and Song (2000) vesicular-arbuscular mycorrhizae cooperate with legume N-fixing bacteria by affecting nodulation. Therefore, there would be an effect on nitrogen acquisition. In an intercropping system, mycorrhizal symbiosis transfers nutrients such as nitrogen between plants via hyphae networks (Johansen and Jensen, 1996). Thus, mycorrhizal symbiosis allows better phosphate nutrition and may play an important role in the sustainability of our agricultural systems (Plenchette et al., 2005; Hijri et al., 2006).

Effect of cropping system on plant mycorrhization rate

Mycorrhizal infection rates in beans and rice in monoculture or co-culture reach values almost 80% and 70%, respectively (Tables 1 and 2). It is well known that arbuscular and vesicular mycorrhizae can easily colonize legume roots (Xiao et al., 2010). Many previous studies have also shown that most legume crops, including beans, are hosts for MVA fungi (Haugen and Smith, 1992; Khasa et al., 1992; Lin et al., 2001; Kasiamdari et al., 2002; Sprent and James, 2007). In co-culture, the rate of mycorrhization was more marked on bean roots under soil treated with manure alone.

Effect of soil type/fertilizer on the microbial activities of the crop soil.

Fertilizer inputs to the soil can be expressed indirectly through their effects on soil microbial communities. In this study, soil type/fertilizer did not have a significant effect on phosphatases activity and does not contribute to the P uptake. Indeed, the inorganic phosphate available to the plant is often released by phosphate-solubilizing bacteria that secrete phosphatases (Smith and Read, 1997). Results showed a significant increase in global microbial activity (FDA) for high fertility levels (S3-S4 > S1-S2) in soils under beans (Table 3) and rice (Table 4). Indeed, some authors explain that microbial

biomass activities increase after the addition of energy sources such as organic amendments

(Bolton et al., 1985; Goyal et al., 1993; Höflich et al., 2000).

Table 3. ANOVA results and comparison of averages for parameters related to soil microbial activities under beans as a function of soil/fertilizer type, inoculum and cropping system

Parameters Factors	P.ase Ac ($\mu\text{g-pNPh}^{-1}\text{g}^{-1}$)		P.ase Al ($\mu\text{g-pNPh}^{-1}\text{g}^{-1}$)		FDA ($\mu\text{g-h}^{-1}\text{g}^{-1}$)	
	F	P(F)	F	P(F)	F	P(F)
Soil type/fertilizer (S)	4,751	0,006	1,412	0,251	2,958	0,042
Inoculum (I)	154,750	< 0,0001	25,993	< 0,0001	2,855	0,067
Cultivation system (MoH/CoRH)	0,679	0,414	22,165	< 0,0001	1,374	0,247
S*I	2,696	0,025	4,318	0,001	0,250	0,957
S*MoH/CoRH	5,667	0,002	2,769	0,052	1,821	0,156
I*MoH/CoRH	5,497	0,007	4,764	0,013	0,386	0,682
S*I*MoH/CoRH	2,694	0,025	3,150	0,011	0,153	0,988
Comparison of means	Mean	group	Mean	group	Mean	group
S1	300,966	ab	122,407	a	88,705	b
Soil type (S) S2	252,447	b	117,077	a	112,509	ab
S3	289,656	ab	142,778	a	113,446	ab
S4	329,524	a	119,008	a	121,811	a
Inoculum (I) I1	245,308	b	117,738	b	109,449	a
I2	164,940	c	85,585	c	96,842	a
I3	469,196	a	172,629	a	121,061	a
Cultivation system MoH	287,116	a	101,852	b	113,969	a
CoRH	299,180	a	148,783	a	104,266	a

*P.ase Ac: Acid phosphatase activities; P.ase Al: alkaline phosphatase activities; FDA: Fluorescein diacetate hydrolysing activities

Table 4. ANOVA results and comparison of averages for soil microbial activity parameters under rice according to soil/fertilizer type, inoculum and cropping system

Parameters Factors	P.ase Ac ($\mu\text{g-pNPh}^{-1}\text{g}^{-1}$)		P.ase Al ($\mu\text{g-pNPh}^{-1}\text{g}^{-1}$)		FDA ($\mu\text{g-h}^{-1}\text{g}^{-1}$)	
	F	P(F)	F	P(F)	F	P(F)
Soil type/fertilizer (S)	2,637	0,060	1,322	0,278	5,988	0,001
Inoculum (I)	218,216	< 0,0001	23,459	< 0,0001	4,123	0,022
Cultivation system (MoR/CoRH)	0,085	0,772	17,712	0,000	3,505	0,067
S*I	2,621	0,028	2,897	0,017	0,055	0,999
S*MoR/CoRH	1,909	0,141	1,541	0,216	2,816	0,049
I*MoR/CoRH	1,804	0,176	8,220	0,001	0,082	0,922
S*I*MoR/CoRH	1,382	0,241	2,482	0,036	0,753	0,610
Comparison of means	Mean	group	Mean	group	Mean	group
S1	325,450	a	129,643	a	79,311	b
Soil type (S) S2	276,548	a	114,352	a	93,957	ab
S3	278,214	a	140,873	a	101,282	ab
S4	307,950	a	113,016	a	117,936	a
Inoculum (I) I1	200,893	b	81,657	b	101,707	a
I2	177,034	b	114,673	b	85,214	b
I3	513,194	a	177,083	a	107,445	a
Cultivation system MoR	294,901	a	100,159	b	91,978	a
CoRH	299,180	a	148,783	a	104,266	a

*P.ase Ac: Acid phosphatase activities; P.ase Al: alkaline phosphatase activities; FDA: Fluorescein diacetate hydrolysing activities

Effect of inoculum on the microbial activities of the crop soil.

It has also been shown by our results (Tables 3 and 4) that the effect of the inoculum of rice

roots (I2) and bean roots (II) is less important than the inoculum of rice-bean mixed roots (I3). The presence of a high diversity of

microorganisms at the root level justifies this inoculation response.

Many studies have led to the identification of rhizobacterial microorganisms such as the Proteobacteria and Endobacteria group (Sun et al., 2008; Joshi and Bhatt, 2011; Pereira et al., 2011), vesicular-arbuscular mycorrhizae (Youpensuk and Yimyam, 2005; Scheublin and Van Der Heijden, 2006; Malina Singha and Sharma, 2013) that colonize the roots of legumes and cereals. Thus, the composition of the microbial community in the root zone depends on root type, plant species, plant age and soil/fertilizer type (Campbell, 1985).

Effect of cropping system on the microbial activities of the crop soil.

According to the results (Tables 3 and 4), alkaline phosphorus activities and global microbial activities were significantly affected by the cropping system. The highest values were found on CoRH (combined rice-bean crop) for alkaline phosphorus activity and on MoH (bean monoculture) for global microbial activity. Studies have already shown significant increases in microbial community activity in the rhizosphere of legume alone or associated with other plant families (Song et al., 2007; Wang et al., 2007; Li et al., 2010).

In addition, Wang et al. (2007) pointed out that in an associative system, legume root mixtures lead to a larger microbial community through the mixing of the respective communities of each of the two species. This root mixing zone is richer in organic compounds that are derived from root exudates in equally important quantities. Hartmann et al. (2009) and Dennis et al. (2010) thus confirm that the quantities of root exudates and rhizodepots have a significant influence on the diversity of the microbial community and their activity. These facts demonstrate the importance of community abundance and microbial activity in the soils studied under associational cultivation. According to Barea et al. (2005), soil biological quality refers to the abundance, diversity, and activity of the living organisms in the soil. Indeed, microorganisms associated with bean roots, such as arbuscular endomycorrhizae and rhizobacteria or nitrogen-fixing bacteria, are thus strongly involved in the coexistence

mechanism of plants as well as in maintaining soil fertility.

CONCLUSIONS

In conclusion, the choice of an associative crop between rice and bean, the inoculation technique using roots pieces, and the rational use of chemical and organic fertilizers at optimal doses could improve the yield of upland rice. In this study, we recorded that the P and N contents of the aerial parts of upland rice were positively affected by the combined cultivation of rice and beans, and by the inoculum from bean roots. In addition, the involvement of soil microorganisms (rhizobacteria and endomycorrhizae) as well as the tripartite interaction, arbuscular endomycorrhizae - nitrogen-fixing bacteria - legumes, in the mechanism of plant coexistence showed particularly better N and P acquisition and improved nutrient sharing in rice plants. It has also been shown that soils under the associative cropping system maintain soil fertility that corresponds to the abundance of community and soil microbial activities.

REFERENCES

- Adholeya, A. (2003). Commercial production of AMF through industrial mode and its large scale application. In *Abstracts, 4th International Conference on Mycorrhizae. Montreal, Canada*, 240.
- Abdelkader, M., Btissam, B.M., Laila, N., et Jamal, I. (2017). Dénombrement des populations naturelles de rhizobium du pois chiche (*Cicer arietinum*) dans différents sols du Maroc. *European Scientific Journal*, 13(6), 273.
- Alef K. (1998). *Methods in applied soil microbiology and biochemistry*. Academic Press, London, pp. 232–233.
- Amarger, N., Macheret, V., Laguerre, G. (1997). *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* nodules. *International Journal of Systematic and Evolutionary Microbiology*, 47(4), 996–1006.
- Andriamananjara, A. (2011). Système de culture à rotation voandzou-riz pluvial (*Oryza sativa*) sur les hautes terres de Madagascar: rôle du voandzou (*Vigna subterranea*) sur la biodisponibilité du phosphore dans les ferrasols (Thèse pour l'obtention du diplôme de doctorat en Sciences Agronomiques. Université d'Antananrivo. 162 p.).
- Barea, J.M., Pozo, M.J., Azcon, R., Azcon-Aguilar, C. (2005). Microbial co-operation in the rhizosphere. *Journal of Experimental Botany*, 56(417), 1761–1778.

- Bashan, Y. (1998). Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnology Advances*, 16(4), 729–770.
- Bianciotto, V., Bonfante, P. (2002). Arbuscular mycorrhizal fungi: a specialised niche for rhizospheric and endocellular bacteria. *Antonie van Leeuwenhoek*, 81(1–4), 365–371.
- Bolton H., Elliot L.F., Papendick R.I., Bezdick D.F. (1985). Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. *Soil Biology and Biochemistry*, 17, 297–302.
- Bonfante, P., Anca, I.A. (2009). Plants, mycorrhizal fungi, and bacteria: a network of interactions. *Annual Review of Microbiology*, 63, 363–383.
- Boukcim, H., Mousain, D. (2001). Effets de la fertilisation phosphatée sur la mycorrhization, la croissance et la nutrition en phosphore et en azote de semis de Cèdre (*Cedrus atlantica* Manetti) inoculés en pépinière par *Tricholoma tridentinum* Sing. var. *cedretorum* Bon. *Annals of forest science*, 58(3), 289–300.
- Boullard, B. (1968). Les mycorrhizes. *Mycorrhiza*, 2, 135.
- Campbell, R. (1985). *Plant Microbiology* (p. 191). Baltimore: Edward Arnold.
- Dennis, P.G, Miller, A.J. Hirsch, P.R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology*, 72, 313–327.
- Dickson, S., Smith, S.E., Smith, F.A. (1999). Characterization of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: inflow and flux of phosphate across the symbiotic interface. *New Phytologist*, 144(1), 173–181.
- Duponnois, R., Bâ, A. M., Prin, Y., Baudoin, E., Galiana, A., Dreyfus, B. (2010). Les champignons mycorrhiziens : une composante majeure dans les processus biologiques régissant la stabilité et la productivité des écosystèmes forestiers tropicaux. pp. 421–440.
- García-Garrido, J.M., Ocampo, J.A. (2002). Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. *Journal of Experimental Botany*, 53(373), 1377–1386.
- Guo, Y., Du, Q., Li, G., Ni, Y., Zhang, Z., Ren, W., Hou, X. (2016). Soil phosphorus fractions and arbuscular mycorrhizal fungi diversity following long-term grazing exclusion on semi-arid steppes in Inner Mongolia. *Geoderma*, 269, 79–90.
- Gérard, F. (2016). Clay minerals, iron/aluminum oxides, and their contribution to phosphate sorption in soils—A myth revisited. *Geoderma*, 262, 213–226.
- Giovannetti, M., Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 489–500.
- Goldstein, A.H. (2007) Future trends in research on microbial phosphate solubilization: one hundred years of insolubility (91-96). Springer Dordrecht.
- Goyal, S., Mishra, M.M., Dhankar, S.S., Kapoor, K.K., Batra, R. (1993). Microbial biomass turnover and enzyme activities following the application of farmyard manure to field soils with and without previous long-term applications. *Biology and Fertility of Soils*, 15(1), 60–64.
- Guñazú, L.B., Andrés, J.A., Del Papa, M.F., Pistorio, M., Rosas, S.B. (2010). Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti*. *Biology and Fertility of Soils*, 46(2), 185–190.
- Hartmann, A., Schmid, M., van Tuinen, D. Berg, G., (2009). Plant-driven selection of microbes. *Plant Soil*, 321, 235–257.
- Hartnett, D.C., Wilson, G.W. (1999). Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, 80(4), 1187–1195.
- Haugen, L.M., Smith, S.E. (1992). The effect of high temperature and fallow period on infection of mung bean and cashew roots by the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. *Plant and Soil*, 145(1), 71–80.
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant Signaling and Behavior*, 7(11), 1456–1466.
- He, X., et Nara, K. (2007). Element biofortification: can mycorrhizas potentially offer a more effective and sustainable pathway to curb human malnutrition? *Trends in Plant Science*, 12(8), 331–333.
- Henintsoa, M. (2018). Dynamique et disponibilité du phosphore : Cas d'un sol ferrallitique malgache. (Thèse en cotutelle pour l'obtention du diplôme de doctorat en Sciences Agronomiques; Université d'Antananarivo; Université de Montpellier Supagro, 148 p).
- Hijri, I., Sýkorová, Z., Oehl, F., Ineichen, K., Mäder, P., Wiemken, A., Redecker, D. (2006). Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology*, 15(8), 2277–2289.
- Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil*, 237(2), 173–195.
- Höflich G., Tauschke M., Kühn G., Rogasik J. (2000). Influence of agricultural crops and fertilization on microbial activity and microorganisms in the rhizosphere. *Journal of Agronomy and Crop Science*, 184, 49–54.
- Holford, I.C.R. (1997). Soil phosphorus: its measurement, and its uptake by plants. *Soil Research*, 35(2), 227–240.
- Jarosch, K.A., Doolette, A.L., Smernik, R.J., Tamburini, F., Frossard, E., Bünemann, E.K. (2015). Characterisation of soil organic phosphorus in NaOH-EDTA extracts: A comparison of 31P NMR spectroscopy and enzyme addition assays. *Soil Biology and Biochemistry*, 91, 298–309.
- Jensen, H.L., Hansen, A.L. (1968). Observations on host plant relations in root nodule bacteria of the *Lotus anthyllis* and the *Lupinus ornithopus* groups. *Acta Agriculturae Scandinavica*, 18(3), 135–142.

- Johansen, A., Jensen, E.S. (1996). Transfer of N and P from intact or decomposing roots of pea to barley interconnected by an arbuscular mycorrhizal fungus. *Soil Biology Biochemistry*, 28, 73–81.
- Jorquera, M.A., Hernández, M.T., Rengel, Z., Marschner, P., de la Luz Mora, M. (2008). Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. *Biology and Fertility of Soils*, 44(8), 1025.
- Joshi P., Bhatt, A.B. (2011). Diversity and function of plant growth promoting Rhizobacteria associated with wheat Rhizosphere in North Himalayan Region. *International Journal of Environmental Sciences*, 1(6), 1135–1143.
- Kasiandari, R.S., Smith, S.E., Smith, F.A., Scott, E.S. (2002). Influence of the mycorrhizal fungus, *Glomus coronatum*, and soil phosphorus on infection and disease caused by binucleate Rhizoctonia and *Rhizoctonia solani* on mung bean (*Vigna radiata*). *Plant and Soil*, 238(2), 235–244.
- Khasa, P., Furlan, V., Fortin, J.A. (1992). Response of some tropical plant species to endomycorrhizal fungi under field conditions. *Tropical Agriculture*, 69(3), 279–283.
- Koide, R.T., Mosse, B. (2004). A history of research on arbuscular mycorrhiza. *Mycorrhiza*, 14(3), 145–163.
- Li, H.G., Shen, J.B., Zhang, F.S., Marschner, P., Cawthray, G., Rengel, Z. (2010). Phosphorus uptake and rhizosphere properties of intercropped and monocropped maize, faba bean, and white lupin in acidic soil. *Biology Fertility Soils*, 46, 79–91.
- Lin, C.C., Wu, S.J., Wang, J.S., Yang, J.J., Chang, C. H. (2001). Evaluation of the antioxidant activity of legumes. *Pharmaceutical Biology*, 39(4), 300–304.
- Liu, J., Bollinger, D.W., Ledoux, D.R., Venum, T.L. (2000). Effects of dietary calcium: phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. *Journal of Animal Science*, 78(1), 106–109.
- Malina Singha, F., Sharma, G.D. (2013). Biodiversity of Rhizospheric Soil Bacteria and Arbuscular Mycorrhizal (AM) Fungi in Some of the Wild Medicinal Legumes of Barak Valley. *Current World Environment*, 8(1), 123–126.
- Mohammadi, K., Sohrabi, Y. (2012). Bacterial biofertilizers for sustainable crop production: a review. *ARPJ Agric Biol. Sci.*, 7(5), 307–316.
- Morel, C. (2002). Caractérisation de la phytodisponibilité du phosphore du sol par la modélisation du transfert des ions phosphates entre le sol et la solution. Mémoire d'HDR, INPL Nancy, 80 p.
- Mosse, B., Stribley, D.P., LeTacon, F. (1981). Ecology of mycorrhizae and mycorrhizal fungi. *Advances in Microbial Ecology*, 137–210.
- Murphy J., Riley J.P. (1962). A modified single solution method for determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36.
- Ögüt, M., Er, F., Neumann, G. (2011) Increased proton extrusion of wheat roots by inoculation with phosphorus solubilising microorganism. *Plant Soil*, 339, 285–297.
- Pereira, P., Ibanez, F., Rosenblueth, M., Etcheverry, M. & Martinez-Romero, E. (2011). Analysis of the Bacterial Diversity Associated with the Roots of Maize (*Zea mays* L.) through Culture-Dependent and Culture-Independent Methods. International Scholarly Research Network.10 p.
- Peoples, M.B., Herridge, D.F., Ladha, J.K. 1995. Biological nitrogen-fixation: an efficient source of nitrogen for sustainable agricultural production. *Plant Soil*, 174, 3–28.
- Phillips, J.M., Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. British Mycological Society*, 55, 158–161.
- Plenchette, C., Clermont-Dauphin, C., Meynard, J.M., Fortin, J.A. (2005). Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal of Plant Science*, 85(1), 31–40.
- Qureshi, M.A., Ahmad, Z.A., Akhtar, N., Iqbal, A., Mujeeb, F., Shakir, M.A. (2012). Role of phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. *Journal of Animal and Plant Sciences*, 22(1), 204–210.
- Rabeharisoa, L. (2004). Gestion de la fertilité et de la fertilisation phosphatée des sols ferrallitiques des hautes terres de Madagascar. These de Doctorat d'Etat. Université d'Antananarivo, Madagascar, 199 p.
- Ramifehiarivo, N., Brossard, M., Razakamanarivo, H. (2016). Challenges in establishing digital maps of soil organic carbon in Madagascar. *Pedometron*, 39, 26–31.
- Rasoamampionona, B., Rabeharisoa, L., Andrianjaka, A., Michellon, R., Moussa, N., Razafimbelo, T., Plenchette, C. (2008). Evaluation du potentiel infectieux mycorrhizogène du sol en semis direct du dispositif d'Andranomanelatra de l'ONG Tafa Antsirabe. *Terre Malgache*, 26, 69–71.
- Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil*, 321(1-2), 305–339.
- Scheublin, T.R., Van Der Heijden, M.G.A. (2006). Arbuscular mycorrhizal fungi colonize non-fixing root nodules of several legume species. *New Phytologist*, 172, 732–738.
- Scheublin, T.R., Ridgway, K.P., Young, J.P.W., Van Der Heijden, M.G. (2004). Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. *Applied and Environmental Microbiology*, 70(10), 6240–6246.
- Scheublin, T.R., Van Logtestijn, R.S., Van Der Heijden, M.G. (2007). Presence and identity of arbuscular mycorrhizal fungi influence competitive interactions between plant species. *Journal of Ecology*, 95(4), 631–638.
- Schnurer, J., Rosswall, T. (1982). Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.*, 43, 1256–1261.

- Shah, M.A., Reshi, Z.A., Khasa, D. (2009). Arbuscular mycorrhizal status of some Kashmir Himalayan alien invasive plants. *Mycorrhiza*, 20(1), 67–72.
- Sharma, A., Shankhdhar, D., Shankhdhar, S.C. (2013). Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. *Plant, Soil and Environment*, 59(2), 89–94.
- Shen Q.R., Chu G.X., Cao J.L., Cao Y., Yin X.Y. (2004). Yield advantage of groundnut intercropped with rice cultivated in aerobic soil from the viewpoint of plant nitrogen nutrition. *Scientia Agricultura Sinica*, 37, 117–1182.
- Singh, Y., Ramteke, P.W., Shukla, P.K. (2013). Characterization of Rhizobium isolates of pigeon pea rhizosphere from Allahabad soils and their potential pppr characteristics. *International Journal of Research in Pure and Applied Microbiology*, 3(1), 4–7.
- Smith, S.E., Read, D.J. (1997). *Mycorrhizal symbiosis* 2nd eds (605 p.). Academic Press. London.
- Smith, S.E., Read D.J. (2008). *Mycorrhizal symbiosis* 3rd eds (815 p.). New York: Academic Press.
- Smith, S.E., Read, D.J. (2010). *Mycorrhizal symbiosis*. Academic Press.
- Smith, S.E., Smith, F.A., Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, 133(1), 16–20.
- Song, Y.N., Zhang, F.S., Marschner, P., Fan, F.L., Gao, H.M., Bao, X.G., Sun, J.H., Li L. (2007). Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and faba bean (*Vicia faba* L.). *Biology and Fertility of Soils*, 43, 565–574.
- Sprent, J.I., James, E.K. (2007). Legume evolution: where do nodules and mycorrhizas fit in? *Plant Physiology*, 144(2), 575–581.
- Strullu, D.G. (1985). Les mycorrhizes. *Handbuch der Pflanzenanatomie*, 13, 2.
- Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X. Song, W. (2008). Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology*, 55(3), 415–424.
- Van Der Heijden, M.G., Boller, T., Wiemken, A., Sanders, I.R. (1998). Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, 79(6), 2082–2091.
- Vance, C.P., Uhde-Stone, C., Allan, D.L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157(3), 423–447.
- Vassilev, N., Vassileva, M., Fenice, M., Federici, F. (2001). Immobilized cell technology applied in solubilization of insoluble inorganic (rock) phosphates and P plant acquisition. *Bioresource Technology*, 79(3), 263–271.
- Wang, D.M., Marschner, P., Solaiman, Z., Rengel, Z. (2007). Growth, P uptake and rhizosphere properties of intercropped wheat and chickpea in soil amended with iron phosphate or phytate. *Soil Biology and Biochemistry*, 39, 249–256.
- Wang, M., Moore, T.R., Talbot, J., Richard, P.J. (2014). The cascade of C: N: P stoichiometry in an ombrotrophic peatland: from plants to peat. *Environmental Research Letters*, 9(2), 024003.
- Xiao, T., Yang, Q., Ran, W., Xu, G., Shen, Q. (2010). Effect of inoculation with Arbuscular Mycorrhizal Fungus on Nitrogen and Phosphorus utilization in Upland Rice-Mungbean intercropping system. *Agricultural Sciences in China*, 9(4), 528–535.
- Youpensuk, S., Yimyam, N., Rerkasem B., Dell, B. (2005). Arbuscular mycorrhizal fungi associated with upland rice in a rotational shifting cultivation system. *International Rice Research Note*, 30, 22–23.
- Zheng W.W., Song Y.N. (2000). Effect mycorrhiza fungi and Rhizobium on wingbean growth and nitrogen fixation. *Fujian Journal of Agricultural Sciences*, 15, 50–55.

RESEARCH ON THE INFLUENCE OF THE CROP SYSTEM ON YIELD AND ITS QUALITY IN WHEAT, TRITICALE AND BARLEY CROPS IN THE CONDITIONS OF THE LUVISSOIL OF THE AGRICULTURAL RESEARCH AND DEVELOPMENT STATION OF SIMNIC

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Abstract

The paper presents the results of testing of five varieties of wheat, triticale and barley species, cultivated in the conventional and ecological luvisoil systems at the Agricultural Research and Development Station of Simnic (ARDS) between 2016 and 2018. The following factors were the subject of biometric determinations: grain production, plant height, test weight (TW), 1000 grains weight (TGW), no. of grains / ear, weight of grains / ear and the protein content. The results obtained were analyzed regarding the influence of the three experienced factors (A factor - species, B factor - variety, C factor - crop system) and their interaction. All varieties tested were more productive in the conventional system rather than in the ecological system. The obtained results suggest that the TW was influenced by the interaction between the three factors, while the TGW was largely influenced by the variety within a species, but less influenced by the crop system. The crop system had a major influence on the protein content in all species and all varieties.

Key words: wheat, triticale, barley, crop systems, yield.

INTRODUCTION

Prospectively, in the context of the evolution towards ecological agriculture, as well as of the ongoing climatic changes, the research on further diversification of the genetic basis of variability becomes a priority. Agricultural biotechnologies play an important role in facilitating adaptation to climate changing conditions, and help farmers to tailor their production to this new challenge (Bonciu, 2019).

The demand for environmentally certified products is steadily growing. Currently, the internal market for organic products is expanding.

The environmentally friendly way of production, based on the non-use of synthetic chemicals and concern for animals' welfare, is a sustainable solution. Generally, the chemicals used in conventional agriculture can have cytotoxic effects on crop plants which can affect the health of the consumers and the environment (Bonciu et al., 2018).

The 2010-2014 data show that the Romanian agricultural surface area cultivated with

ecologic cereals or vegetables increased by almost 60% (www.finantariagricole.ro).

The number of ecological operators registered with the Romanian Ministry of Agriculture and Rural Development (RMARD) was 3,155 in 2010 and 14,470 in 2014. In 2014 the total surface area occupied by organic farms was of 301,148.1 ha, which represented an increase of 61% compared to 2010 (Burghelea et al., 2016; www.madr.ro).

The latest report of the Research Institute of Organic Agriculture FiBL indicates a 14.2% increase in the ecological surface area, Romania ranking the 6th in Europe in terms of growth dynamics. This shows an increased interest of the Romanian farmers in ecological products when compared to other countries in Europe. Over the last years, this increase has not placed Romania in leading positions in the share of organic products resulting from the total agricultural surface area at the national level (only 2% in Romania), being much lower in comparison with other countries (Estonia 20.5%, Czech Republic 12.2%, Slovakia 10%,

Lithuania 8.1%, Germany 8.2%, Hungary 4.3%, Poland 3.4%, Bulgaria 2.9%).

A large part of the products obtained from ecological farming was export-oriented. A percentage of approximately 70-80% of Romania's ecological products are exported annually.

In Romania, the competitiveness of ecological products is determined by the following factors: the number of operators registered in this sector constantly increases; the market of ecological products is expanding; now more than ever, consumers are aware that together with the quality and the value of ecological products for health, ecological farming has also a major contribution to sustainable development (Săucă, 2010). Sustainable development of agricultural systems from production to consumption contributes to enhancing sustainable food production and limiting the negative impact on the environment (Bonciu, 2017).

As far as conventional wheat is concerned, the average production in the European Union was 5757 kg/ha in 2017. Among the producing countries, the European Union top-ranks, six of the Member States (The Netherlands, Belgium, Denmark, Germany, Sweden, France) being in the first ten with an average production of 6,757 to 9,094 kg/ha in 2017 (FAOSTAT).

In Romania, the average production per hectare in the period 1925-1938 was of 9.4 q/ha, with variation limits of 5.1 to 13.1 q/ha. Between 1960-1970 the average wheat production ranged from 12.11 to 14.36 q/ha. Wheat production per hectare has increased in our country over the recent years compared to the inter-war period by 300%. In 2017, the national average production was 4,888 kg/ha.

The FAO data reported in 2017 regarding barley yields shows that Russia is the main conventional producer (7,847,738 ha), followed by Australia (4,834,102 ha).

In 2017, the surface area cultivated with barley in Romania was of 455,460 ha, for which an average production of 4,186 kg/ha and a total production of 1,906,700 tons was yielded.

Genetic and cytogenetic research on barley was carried out in numerous fields. Studies on the heritability of the main components of the production capacity have highlighted the effects of the additivity and the dominant

actions of the genes involved for both barley and two-row barley (Popescu, 1985).

A special program of diversification of the genetic basis was developed for the peeled barley, an initial biological material represented by DH lines and well adapted to ecological agriculture (Mihăilescu, 2007).

Over the recent years, the interest of cultivators in triticale has increased, as the new cereal has proved to be a good alternative to traditional cereals. Thus, significant progress has been made in Europe. In Germany, triticale was grown on 389,000 ha in 2017. In Poland, the growth was more than significant, going from 66,700 ha in 1993 (Wolsky and Pojmaj, 1995) to 1,352,013 ha in 2014 (FAOSTAT). The surface area cultivated in Romania in 2017 was of 80,120 ha.

With regard to the triticale, real interest genetic stocks were created for introgression works (Giura and Mihăilescu, 2000; Giura et al., 2007).

The first wheat-rye hybrid was obtained by Săulescu in our country in 1927, and the first form of octoploid triticales ($2n = 56$) by Priadencu in 1939 (Priadencu, 1952).

An important step in favour of the diversification of the genetic basis for triticale was achieved by transferring the genes to reduce the height plant of wheat and rye. The trait of resistance to sprouting (low amylolytic activity) was transferred from wheat, obtaining the first forms of triticale with a Hagberg index over 300 (Ittu et al., 2006).

A comparative study in the ecological system at SCDA Pitesti led to the following conclusions: varieties of the two species, with better adaptability to unfavorable environmental conditions, were identified: FDL Miranda, Izvor, Delabrad 2, Dropia, Faur F, Plai, varieties well adapted to favorable environmental conditions: Alex, Litera, Boema1, Glosa, Haiduc, Cascador F, and varieties with high adaptability to contrasting environmental conditions, such as Trivale, varieties that produce high yields both under favourable and unfavourable environmental conditions; by cultivating varieties with high adaptability to different environmental conditions, the risks of decreasing yield in years with less favourable climatic conditions can be reduced (Voica, 2014).

Globally, studies have been much more diverse. Breeders in France, particularly those belonging to INRA, have made this new species - triticale very appealing to farmers to use as animal feed. Currently, triticale has yielded crops that are equivalent or even better than those obtained from wheat. The breeding program for triticale started in 1971, the first variety being registered in France - Clercal created in the laboratories at Clermont Ferrand in 1983 (Bernard and Bernard, 2005).

Montana (USA) triticale crops consistently show higher yields than spring and winter wheat grown under irrigation and drought conditions. With reference to Montana, under non-irrigated conditions between 1990-1993, the winter triticale registered a 9% increase in yield compared to the winter wheat and to the spring triticale 12% compared to the spring wheat (Karpstein-Machan and Heyn, 1992). Under irrigation conditions, the increase obtained in the spring triticale compared to the spring wheat was of 22%. The authors conclude that triticale use the soil and nitrogen more effectively than winter wheat.

Comparative triticale - wheat experiments were carried out in North Dakota (USA) between 1981-1983. Triticale were 4-6 days earlier and more susceptible to rust than common wheat and durum wheat, their height being equal to that of the common wheat and durum wheat, displaying lower test weight, and slightly lower protein content (Oelke et al., 1989).

Comparative winter wheat and triticale experiments were conducted at the University of Ontario in the United States. The crop yield was within the range: 3,452 kg/ha in the durum wheat variety, and 5,201 kg/ha for a common wheat variety. The experienced triticale variety (Bogo) recorded the highest production - 8,661 kg/ha. In contrast, the protein content was the lowest for triticale (8.6%), while the highest value (11.4%) was associated with the common winter wheat variety. With regard to the heading date, the earliest was the variety of triticale, the wheat varieties registering the heading date even 2 weeks later (Eldredge et al., 1999).

Barley - oats - rye - triticale comparative tests were performed in Texas, Oklahoma, Pennsylvania, Virginia and Georgia. Barley recorded a 5% increase in yield, while rye

recorded a 9% decrease. In the rye case, a yield loss of 20% compared to wheat was observed in the same experiment (Ortiz-Monasterio et al., 2002).

Gibson et al. (2004) evaluated 2 varieties of triticale: Trical 815 and Danko Presto at 4 planting dates: 15.09, 24.09, 5.10, 15.10. The yields obtained during the first three planting dates were not differentiated. However, sowing at 15.10 induced a 19% yield reduction compared to sowing at 15.09.

MATERIALS AND METHODS

The research was carried out on the Luvisoil of the Agricultural Research and Development Station of Simnic (ARDS).

The experiment with three factors was set according to the method of the subdivided plots, at 3 replications. The factors studied were: factor A (species) with three graduations: wheat, triticale, barley; factor B (variety) with 5 graduations and factor C (crop system) with 2 graduations: ecological system and conventional system.

The aim of the research was to identify which of the cereal species cultivated in the two crop systems behaved best by comparing the species among them. At the same time it was studied how the varieties tested, regardless of the species, are placed in relation to the most widespread wheat variety in Romania, namely the Glosa variety.

The Glosa, Boema, Otilia, Adelina and Alex varieties were tested for wheat; the varieties Plai, Negoiu, Utrirom, Utrifun, Vifor for triticale, while the Dana, Cardinal, Univers, Ametist and Smarald varieties were tested for barley.

The experimental research carried out focused on how the varieties of these species behaved in an ecological system and in a conventional system, in terms of yield, plant height, number of grains/ear, weight of grains/ear, test weight, 1000 grains weight and protein content.

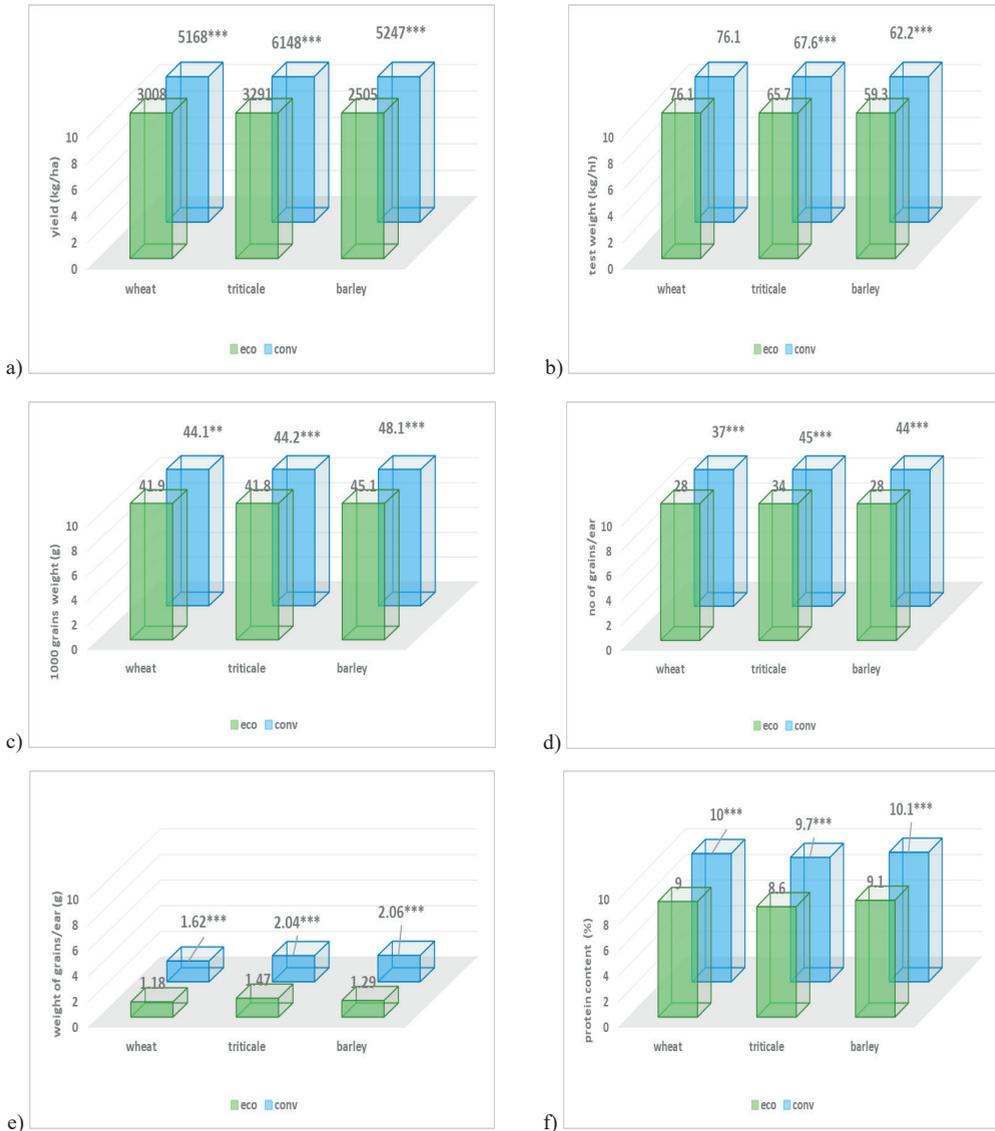
The following were analyzed: the influence of the species, regardless of variety and crop system; the influence of the variety, regardless of species and crop system; the influence of the crop system, regardless the species and variety; species x crop system interaction; variety x species x crop system interaction; crop system

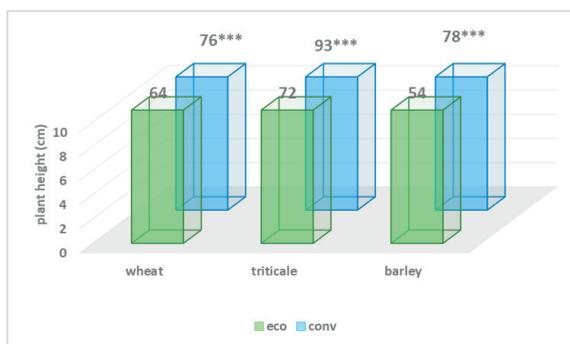
x species x variety interaction. The reporting was done with respect to the Glosa variety - the most widespread variety in Romania.

The practical implications of this paper refer to drawing up recommendations regarding the most adapted species and varieties of cereals depending on the studied crop systems in the central area of Oltenia.

RESULTS AND DISCUSSIONS

The crop system x species interaction. For all the studied characters, regardless of the variety, the interaction showed that the values obtained in the conventional system were significantly higher than in the ecological system for all species (Figure 1).





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Figure 1. The interaction crop system x species, regardless of the varieties tested:

- a) Yield: DL 5% = 271 kg/ha; DL 1% = 365 kg/ha; DL 0.1% = 485 kg/ha
- b) Test weight: DL 5% = 0.9 kg/hl; DL 1% = 1.2 kg/hl; DL 0.1% = 1.6 kg/hl
- c) 1000 grains weight: DL 5% = 1.2 g; DL 1% = 1.7 g; DL 0.1% = 2.3 g
- d) Number of grains/ear: DL 5% = 4 grains/ear; DL 1% = 5 grains/ear; DL 0.1% = 6 grains/ear
- e) Weight of grains/ear: DL 5% = 0.19 g/ear; DL 1% = 0.26 g/ear; DL 0.1% = 0.34 g/ear
- f) Protein content : DL 5% = 0.2%; DL 1% = 0.3%; DL 0.1% = 0.4%
- g) Plant height: DL 5% = 7 cm; DL 1% = 9 cm; DL 0.1% = 12 cm

The exceptions registered were those related to the test weight for wheat, which was identical in both crop systems, and in the 1000 grains weight for wheat, in the conventional system the difference being distinctly positive compared to the ecological system.

Regarding the obtained yield, the results show that this is influenced by the species. On an average of 3 years, the triticale species was significantly more productive than wheat, while the barley species with an average yield of 3,876 kg/ha registered a significant decrease compared to the wheat species (Table 1).

Table 1. The influence of species (A factor) on the yield

Species	Yield kg/ha	Dif. Ct	Significance
Wheat	4,088	Ct	
Triticale	4,719	631	***
Barley	3,876	-212	o

DL 5% = 187 kg/ha; DL 1% = 309 kg/ha; DL 0.1% = 578 kg/ha

The influence of variety. The minimum yield in this experiment was recorded for the Smarald barley variety - 2,402 kg/ha in the ecological system, whereas the maximum for the Vifor triticale variety - 6,739 kg/ha in the conventional system (Table 2).

Comparative results between the yield of the wheat variety Glosa, taken as a control variety, and the other varieties, regardless of species and the crop system, showed that three of the varieties of barley (Dana, Univers and Ametist), were inferior, with statistical

assurance. All the tested wheat varieties were at the control level. In contrast, the varieties of triticale were distinctly superior to the Glosa variety (Table 2).

Table 2. The influence of varieties (B factor) on the yield

Varieties	Yield kg/ha	Dif. ct	Significance
GLOSA	4,102	Ct	
BOEMA	3,925	-177	
OTILIA	3,944	-158	
ADELINA	4,240	138	
ALEX	4,229	127	
PLAI	4,697	595	***
NEGOIU	4,386	284	**
UTRIROM	4,619	517	***
UTRIFUN	4,759	657	***
VIFOR	5,135	1,033	***
DANA	3,793	-309	Oo
CARDINAL	4,010	-92	
UNIVERS	3,842	-261	Oo
AMETIST	3,687	-416	Ooo
SMARALD	4,048	-54	

DL 5% = 187 kg/ha; DL 1% = 254 kg/ha; DL 0.1% = 340 kg/ha

The influence of the crop system showed that under the conditions of the conventional system, the yield increase of 2,586 kg/ha in relation to the ecological system is highly significant (Table 3).

The variety x species x crop system interaction showed that in the ecological system no variety was highlighted for any of the species, whereas in the conventional system, the varieties reacted slightly differently within each species.

Table 3. The influence of the crop system (C factor) on the yield

Crop system	Yield kg/ha	Dif. Ct	Significance
Ecological system (ct)	2935	Ct	
Conventional system	5521	2586	***

DL 5% = 157 kg/ha; DL 1% = 211 kg/ha; DL 0.1% = 280 kg/ha

Thus, for triticale, the Vifor variety was distinctly higher than the Plai control variety, with an increase of 799 kg/ha; for barley, the Smarald variety was significantly higher than the control variety Dana, with an increase of 571 kg/ha. For wheat, the Adelina variety recorded an increase of 376 kg/ha compared to Glosa, but it did not have statistical assurance (Table 4).

The influence of the **crop system x variety x species interaction** was highlighted through

Table 4. The influence of the variety x species x crop system interaction and the crop system x variety x species interaction on the yield

Crop system	Species	Variety	Yield kg/ha	Dif. ct	Significance	Dif. ct	Significance
Variety x species x crop system interaction						Crop system x variety x species interaction	
Ecological systems	WHEAT	GLOSA	3,042	ct		ct	
		BOEMA	2,887	-155		ct	
		OTILIA	2,867	-175		ct	
		ADELINA	2,943	-99		ct	
		ALEX	3,303	261		ct	
	TRITICALE	PLAI	3,454	ct		ct	
		NEGOIU	3,047	-407		ct	
		UTRIROM	3,052	-402		ct	
		UTRIFUN	3,368	-86		ct	
		VIFOR	3,532	78		ct	
		BARLEY	DANA	2,463	ct		ct
		CARDINAL	2,755	292		ct	
		UNIVERS	2,502	39		ct	
		AMETIST	2,403	-60		ct	
		SMARALD	2,402	-61		ct	
Conventional systems	WHEAT	GLOSA	5,162	ct		2120	***
		BOEMA	4,963	-199		2076	***
		OTILIA	5,021	-141		2154	***
		ADELINA	5,538	376		2595	***
		ALEX	5,156	-6		1853	***
	TRITICALE	PLAI	5,940	ct		2485	***
		NEGOIU	5,725	-215		2679	***
		UTRIROM	6,186	246		3133	***
		UTRIFUN	6,150	210		2782	***
		VIFOR	6,739	799	**	3207	***
		BARLEY	DANA	5,123	ct		2660
		CARDINAL	5,266	143		2511	***
		UNIVERS	5,181	58		2679	***
		AMETIST	4,970	-153		2568	***
		SMARALD	5,694	571	*	3292	***

Variety x species x culture system interaction: DL 5% = 537 kg/ha; DL 1% = 726 kg/ha; DL 0.1% = 967 kg/ha

Crop system x species x variety interaction: DL 5% = 606 kg/ha; DL 1% = 817 kg/ha; DL 0.1% = 1,084 kg/ha

the fact that, with no exception, all varieties of wheat, triticale and barley recorded significantly higher yields in the conventional system compared to the ecological system on an average timeframe of over 3 years, the differences being mostly over 2 t/ha and even over 3 t/ha for the varieties of triticale Utrirom and Vifor and for the barley variety Smarald. Basically, on the Luvosoil of the Agricultural Research and Development Unit of Simnic (ARDS), the yield in the ecological system is halved in comparison to the conventional system. Even if the price of ecological wheat is almost double, the recommendation that it should be grown must be accompanied by calculations of economic efficiency, based on the gross and net product index (Table 4).

The other studied characters showed different results depending on the factors taken into consideration. The analysis was made only from the point of view of the influence of each factor separately, regardless of their interaction (Table 5).

The influence of species (A factor). Plant height, the number of grains/ear and the

weight of the grains/ear were superior with the statistical assurance at triticale in relation to wheat, regardless of variety and crop system. The weight of the grains/ear, the 1,000 grains weight and the protein content were higher with barley statistical assurance in comparison to wheat, also, regardless of variety and crop system.

Table 5. The influence of the studied factors on other characters determined for the three species in different crop systems

Studied factors		Plant height (cm)	No. grains/ear	Weight grains/ear (g)	1000 grains weight (g)	Test weight (kg/hl)	Protein content (%)
Between species (A factor)	Wheat (ct)	70	33	1.40	43.0	76.1	9.5
	Triticale	82***	40**	1.76**	43.0	66.7 ^{ooo}	9.1 ^{oo}
	Barley	66	36	1.68**	46.6***	60.8 ^{ooo}	9.7*
	DL 5%	5	4	0.16	1.0	0,3	0,2
	DL 1%	7	7	0.26	1.6	0,4	0,4
DL 0.1%	8	14	0.50	3.0	0,8	0,7	
Between varieties (B factor)	GLOSA (ct)	68	35	1.62	46.8	75.8	9.8
	BOEMA	70	34	1.44 ^o	42.5 ^{ooo}	75.3 ^o	9.7 ^o
	OTILIA	69	32 ^o	1.37 ^{oo}	42.8 ^{ooo}	77.4***	9.4 ^{ooo}
	ADELINA	68	30 ^{oo}	1.30 ^{ooo}	42.7 ^{ooo}	76.8***	9.4 ^{ooo}
	ALEX	75***	33	1.29 ^{ooo}	40.2 ^{ooo}	75.2 ^{oo}	9.2 ^{ooo}
	PLAI	99***	38*	1.71	41.3 ^{ooo}	68.0 ^{ooo}	9.1 ^{ooo}
	NEGOIU	86***	41***	1.93**	45.9	64.7 ^{ooo}	9.2 ^{ooo}
	UTRIROM	75***	38*	1.50	39.8 ^{ooo}	65.9 ^{ooo}	9.4 ^{ooo}
	UTRIFUN	75***	41***	1.88**	44.9 ^{ooo}	66.9 ^{ooo}	9.1 ^{ooo}
	VIFOR	78***	41***	1.77	43.2 ^{ooo}	67.7 ^{ooo}	9.0 ^{ooo}
	DANA	69	34	1.64	47.8*	59.6 ^{ooo}	9.8
	CARDINAL	65	39**	1.77	45.2 ^{oo}	61.1 ^{ooo}	9.2 ^{ooo}
	UNIVERS	65	34	1.58	47.4	62.2 ^{ooo}	10.0**
	AMETIST	68	35	1.67	47.7	60.4 ^{ooo}	10.0**
SMARALD	65	39**	1.74	44.8 ^{ooo}	60.4 ^{ooo}	9.3 ^{ooo}	
Between crop systems (C factor)	Ecological system (ct)	63	30	1.31	42.9	67	8.9
	Conventional system	82***	36***	1.61***	44.2***	68.6***	10.0***
	DL 5%	4	2	0.11	0.7	0.4	0.1
	DL 1%	6	3	0.15	1.0	0.5	0.2
DL 0.1%	8	4	0.20	1.3	0.7	0.3	

For both species, triticale and barley, the test weight was significantly lower compared to wheat. The protein content in triticale was significantly lower in relation to wheat.

The influence of varieties (B factor). The varieties of triticale, without exception, were significantly higher than the Glosa variety, although the architecture of the new varieties of triticale is directed towards varieties of smaller height. Of the wheat varieties, the Alex variety was significantly higher than Glosa.

As for the number of grains/ear, the varieties of triticale, with no exception, and the Cardinal and Smarald barley varieties were superior, with

statistical assurance, compared to wheat. The Otilia and Adelina wheat varieties were inferior to the Glosa variety with 3 and 5 grains/ear less. The weight grains/ear of all wheat varieties was lower with statistical assurance compared to Glosa, while the Negoiu and Utrifun - triticale varieties were significantly higher.

The 1,000 grains weight and the test weight were lower, with statistical assurance for all varieties of triticale and barley, with the exception of the Dana barley variety (MMB = 45.4 g), significantly higher compared to the Glosa wheat variety. From the point of view of the 1,000 grains weight, as well the varieties of

wheat tested were significantly lower than the control variety. For the same character, the Negoiu (triticale), Univers and Ametist (barley) varieties were highlighted, which were at the control variety level.

In most varieties, the protein content was inferior to the Glosa variety, being exceeded, with statistical assurance, only by the Univers and Ametist barley varieties.

The influence of crop systems (C factor). For all the studied characters, in the conventional system, the determined values were significantly higher than those in the ecological system. The higher value of the yield obtained in the conventional system is doubled by the higher value of all the studied characters, the most important being the protein content, an important indicator of the quality of the yield.

CONCLUSIONS

The influence of the system x variety x species interaction resulted in, with no exception, all varieties of wheat, triticale and barley, which recorded significantly higher yields in the conventional system compared to the ecological system, on an average timeframe of over 3 years, the differences being mostly over 2 t/ha and even over 3t / ha in the Utrirom and Vifor varieties of triticale and the Smarald barley variety. Basically, on the Luvisoil of the Agricultural Research and Development Station of Simnic (ARDS) the yield in the ecological system is halved compared to the conventional system. Even if the price of ecological wheat is almost double, the recommendation that it be grown must be accompanied by calculations of economic efficiency based on the gross and net product index.

Comparative results between the yield of the Glosa wheat variety, taken as the control level and the other varieties, regardless of species and crop systems, showed that three of the varieties of barley (Dana, Univers and Ametist), were inferior, with statistical assurance. All the wheat varieties tested were at the control level. In contrast, the varieties of triticale were distinctly significant and significantly higher in the Glosa variety.

The crop system had a strong influence on the protein content for all species and all varieties.

For all the studied characters in the conventional system (plant height, number of grains/ear, weight of grains/ear, 1000 grains weight, test weight, protein content), the values determined were significantly higher than those of the ecological system.

REFERENCES

- Bernard, M., Bernard, S. (2005). Molecular Analysis of the Triticale Lines with Different Vrn Gene Systems Using Microsatellite Markers and Hybridization In Situ. *Genome*, 5, 289–295.
- Bonciu, E. (2019). The climate change mitigation through agricultural biotechnologies. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, 49(1), 36–43.
- Bonciu, E., Roşculete, E., Oлару, A.L., Roşculete, C.A. (2018). Evaluation of the mitodepressive effect, chromosomal aberrations and nuclear abnormalities induced by urea fertilization in the meristematic tissues of *Allium cepa* L. *Caryologia*, 71(4), 350–356.
- Bonciu, E. (2017). Food processing, a necessity for the modern world in the context of food safety: a Review. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, 47(1), 391–398.
- Burghilea, C., Uzlău, C., Ene, C.M., Topor, D. (2016). Prospects for organic farming in Romania. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 16, 73–78.
- Eldredge, E.P., Karow, S.R., Shock, C.C., Marx, E., Saunders, L. (1999). *Cereal grain variety trial*. Oregon State University.
- Gibson, L.R., Schwarte, A.J., Sundberg, D., Karlen, D. (2004). Planting Date Effects on Winter Triticale Grain and Forage Yield. Iowa State Research Farm Progress Reports.
- Giura, A., Mihăilescu, Alexandrina (2000). Metode de cercetare in cultura plantelor. *Metode moderne de reducere a duratei programelor de ameliorare la grâu și orz*. (17-36) Edit. Agris, București.
- Giura, A., Mihăilescu A., Verzea, M., Ittu, Gh. (2007). Cercetări de genetică efectuate la Fundulea. *Analele I.N.C.D.A. FUNDULEA, LXXV*, Volum jubiliar genetică și ameliorarea plantelor.
- Ittu, Gh., Săulescu, N.N., Ittu, Mariana, Mustătea, P. (2006). Progrese în ameliorarea la triticale pentru obținerea de soiuri cu talia scurtă. *Analele I.N.C.D.A. Fundulea, LXXII*, 19–28.
- Karpstein-Machan, M., Heyn, J. (1992). Yield and yield structure of the winter cereals triticale and wheat in the middle mountain areas of northern Hessen. *Agribiol. Res.*, 45, 88–96.
- Mihăilescu, A. (2007). Orzul golaș – nouă provocare pentru programele de ameliorare. *Probl. Genet. Teor. Aplic.*, XXXIX.
- Oelke, E.A., Oplinger, E.S., Brinkman, M.A. (1989). Alternative field crops manual. *Triticale*. Univ.

- Wisconsin, CES, Madison, WI, Univ. Minnesota CES, St.Paul.
- Ortiz-Monasterio, J.I., Pena, R.J., Pfeiffer, W.H., Hede, A.H. (2002). Phosphorus use efficiency, grain, yield, and quality of triticale and durum wheat under irrigated conditions. *Proceedings of the 5th International Triticale Symposium, Annex Radzikow, Poland*.
- Popescu, C. (1985). Cercetări privind ereditatea principalelor elemente ale capacității de producție la orzul și orzoaica de toamnă. *Probl. Genet. Teor. Aplic.*, XVIII(3), 153–173.
- Priadcencu, A. (1952). Contribuții la studiul hibridilor îndepărtați. *Analele I.C.A.R.*,XX:1.
- Săucă, Florentina, (2010). *Agricultura ecologică și biodiversitate*. Ed. Ex Ponto. ISBN: 978-973-644-988-8
- Voica, Maria, (2014). Reacția unor soiuri de grâu și triticale cultivate în sistem ecologic. *Anale Fundulea LXXXII*. 39-47
- Wolsky, T., Pojmaj, M.S. (1995). Some prospect of hybrid triticale breeding. *Current opinion for cereal improvement*, 185-192.
- ***, faostat.fao.org
- ***, *Agricultura ecologică. Dinamica operatorilor și a suprafețelor în agricultura ecologică*.www.madr.ro
- ***, *Agricultura ecologică crește, dar merge majoritar la export*. www.finantariagricole.ro

THE FORMATION OF CORN GRAIN YIELD WHEN USING SILICON-CONTAINING PREPARATIONS

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Abstract

Studies to substantiate the species and timing of use of silicon-containing preparations providing the highest productivity of the early ripening hybrid of corn were carried out on heavy loamy leached chernozem. Non-root treatment with NanoSilicon, contributed to an increase in the number of grains in the cob by 13.6-26.7% compared with the control (water treatment), and it was more effective to use the drug in a phase of seven to eight leaves and double treatment. When using Kelik Potassium-Silicon and Microvit-6 Silicon, the number of grains increased by 10.0-19.3%. Double processing with NanoSilicon additionally produced 16.2 g of grain from the ear, which is 28.4% more than in the control. During non-root treatment, Kelik Potassium-Silicon and Microvit-6 Silicon growth compared to the control was 23.3-26.7%. Against the background of foliar treatment with drugs, grain yield increased by 37.5-39.3%, and the best anti-stress effect was achieved by double treatment with NanoSilicon and the use of MicroVit-6 Silicon in the phase of five leaves.

Key words: grain, silicon, corn, structure, productivity.

INTRODUCTION

In modern technologies, great importance is given to various methods of treating plants with environmentally friendly drugs, which stimulate the growth and development of plants, increase their productivity and resistance to stress. In modern agriculture, the search for ways to increase the productivity of agrobiogeocenosis is relevant.

Among the possible ways to solve this problem and increase the profitability of agricultural production, it is of interest to use silicon-containing compounds that not only improve soil fertility, but also have a direct stimulating effect on plants, accelerating their growth and development, as well as increasing resistance to adverse environmental factors.

Silicon is necessary to improve the consumption of nitrogen, phosphorus and potassium. It stimulates growth processes, accelerates the onset of the sweeping and ripening phases, which is associated with an increase in energy for metabolic processes and sugar synthesis.

It was established that plants can absorb low molecular weight silicic acids and their anions not only through the root system, but also

through the surface of the leaves if they are sprayed with silicon-containing solutions.

It is important to note that the absorption of silicon by leaves is about 30-40%, while through the root system it does not exceed 1-5%. In this case, a double cuticular-silicon protective layer forms on the leaf surface (Radkowski et al., 2017).

In addition, silicon accumulation also occurs in the epidermis and conducting tissues of the stem, leaves, roots, and shell of grains (Ma, 2004; Fauteux, 2005; Ma, Yamaji, 2006; Heather et al., 2007).

These accumulations of silicon allow plants to survive under abiotic and biotic stresses (Liang et al., 2007).

One of the important functions of the active forms of silicon is to stimulate the development of the root system (Savithramma et al., 2012; Suriyaprabha et al., 2012; Pavlovskaya et al., 2017).

Of great importance for the country's agro-industrial complex is the willingness to use in its production fertilizer materials obtained using nanotechnology elements. Such substances belong to a new class of modern fertilizers, the fertilizer value of which should be identified first.

The use of silicon-containing preparations in which silicon is in the form of nanoparticles contributes to the greatest assimilation of nutrients by the plant (Gumilar et al., 2017). The best way to make silicon-containing preparations is foliar feeding, which is carried out by spraying with an aqueous solution of fertilizers. When applying this method of feeding, most of the nutrients fall directly on the surface of the leaves of the plant.

In this regard, the study of various types of silicon-containing preparations and the timing of their use in corn crops in the conditions of the forest-steppe of the Middle Volga (Russia) is relevant in the scientific and applied plan, which determined the purpose of the study.

MATERIALS AND METHODS

The experiments were conducted in 2018-2019 under the conditions of the Closed joint-stock Company "Konstantinovo" in the Penza istrict within the Penza region (Russia) on heavy loamy leached chernozem with the following agrochemical characteristics: humus content - 5.1-5.3%; N_{alkali} - 105-112; P_2O_5 - 108-126; K_2O - 148-157 mg/kg of soil; S - 28.8- 29; H_g - 4.6-5.0 mEq/100 g of soil; pH_{HCl} - 5.30-5.41. Field experience was laid down in four repetitions in accordance with generally accepted methods.

We studied the preparations Kelik Potassium-Silicon (1.5 l/ha), Nano Silicon (150 g/ha), Microvit-6 Silicon (0.5 l/ha) and in the control they were treated with water. Non-root treatment was carried out in the phase of 3-5 leaves, 7-8 leaves and double treatment in the phase of 3-5 and 7-8 leaves of corn. The area of the second-order plots is 28 m².

Under the first pre-sowing cultivation, mineral fertilizers were introduced in the norm

$N_{90}P_{60}K_{60}$. The object of research is an early ripe hybrid of corn ROSS 191 MV (FAO 190). Sowing was carried out with aisles of 70 cm. The plant stand density (70 thousand units/ha) was formed in the phase of full germination. The predecessor is winter wheat in pure steam.

An analysis of weather conditions showed that the vegetation of 2018 took place with insufficient moisture supply against a background of moderately low air temperature. The insufficient number of active air temperatures did not contribute to the full realization of the potential of the hybrid and to a high yield of corn grain.

Precipitation during the growing season of 2019 was uneven, but most of it fell in the second decade of July and the first decade of August, during the period of active growth and development of corn, which contributed to the formation of a higher yield of corn compared to the previous year of the study.

RESULTS AND DISCUSSIONS

Two-year studies found that the formation of generative organs was influenced by both the use of preparations containing silicon and weather conditions during the growing season. So, in conditions of insufficient active temperatures and moisture in 2018, in each experiment, one ear per plant was formed.

In a more heat-supplied 2019, an increase in the number of developed cobs was noted with the use of preparations with silicon. On average, over two years of observation during foliar treatment with silicon-containing preparations, the number of developed cobs per 100 plants increased by 13.3-21.8% compared with water treatment (Table 1).

Table 1. Elements of the structure of grain productivity, average for 2018-2019

A drug	Term	Processing number cobs at 100 plants, pieces	Ear			Weight 1000 grains, g
			Length, Cm	Number grains from the ear, pcs	Weight grains from the ear, g	
Nodrug (treatment water)	5 leaves	119	14.8	461	56.9	122
	7-8 leaves	120	14.8	460	57.0	122
	5 leaves + 7-8 leaves	119	15.0	460	57.0	123
Kelik Potassium - Silicon	5 leaves	140	18.3	527	72.1	137
	7-8 leaves	138	17.6	539	65.7	122
	5 leaves + 7-8 leaves	140	19.2	550	70.3	128
NanoSilicon	5 leaves	143	16.9	562	64.6	114
	7-8 leaves	136	18.7	572	65.3	115
	5 leaves + 7-8 leaves	145	19.0	584	73.2	126
Mikrovit-6 Silicon	5 leaves	141	17.5	531	70.4	131
	7-8 leaves	142	17.7	533	65.1	120
	5 leaves + 7-8 leaves	141	19.0	525	68.1	118

The greatest stimulating effect was noted with the use of the drug NanoSilicon. When processing crops in the phase of 5 leaves of corn, the growth of ears per 100 plants amounted to 24 pieces or 20.2% of the option with water treatment. Spraying with NanoSilicon in a phase of 7-8 leaves provided an increase of 13.3%, and double application contributed to an increase in the number of ears of corn by 26 pieces, or 21.8% compared to the version without the drug. Slightly smaller results were obtained with foliar treatment with the Kelik Potassium-Silicon. Spraying in the phase of 5 leaves and binary use of the drug allowed to increase the number of ears of corn by 17.6%, and treatment in the phase of 7-8 leaves - by 15.0%. Treatment with the Microvit-6 Silicon preparation on all

experimental variants contributed to an 18.3-18.5% increase in the number of ears of corn per 100 corn plants.

The grain size depends to a certain extent on the linear dimensions of the cob. Measurements showed that on average over the years of research, the length of the ears in variants with silicon-containing preparations increased by 3.2-3.5 cm or 21.5-23.5%. The best results in both years of research were observed on the options for double treatment of plants with preparations with silicon. The increase in the length of the cob was 5 cm. A smaller result was obtained on the application of the drug NanoKremny in the phase of 5 corn leaves - 2.2 cm. On other options, the difference with the variant without the drug was about 3-4 cm.

In 2019, an increase in the length of ears on all versions of leaf processing of corn plants with various preparations with silicon amounted to 1.1-2.8 cm or 7-18%. The best indicators are recorded on the options for the use of Kelik Potassium-Silicon and Nano Silicon preparations in the phase of 7-8 corn leaves and double foliar treatment of plants. In variants with Microvit-6 Silicon, an increase in the length of the cob by 1.4-2.4 cm was noted.

The number of grains in the cob is an important element of the yield structure. It was revealed that in 2018 the use of all preparations with silicon led to an increase in the cob grains. The earliest ones were the cobs during double sheet treatment with NanoKremny. The number of grains in this embodiment was 603 pieces with 396 grains in the variant with water treatment. The use of the Kelik Potassium-Silicon increased the number of grains in the cob by 91-135 pieces, and the maximum increase was obtained on the option with double processing of crops.

Mikrovit-6 Silicon provided an increase in grains in the cob of 40-90 pieces, and the best result was obtained with the use of this drug in the phase of 7-8 corn leaves. In the growing season of 2019, variants with the use of Microvit-6 Silicon were distinguished, which provided an increase in the number of grains of 10.6-15.0%, and the best results were obtained with a double treatment with the drug. NanoSilicon and Kelik Potassium-Silicon showed greater efficiency when using 7-8 corn leaves in the phase, the increase was 114-12.7% compared with water treatment. On average, over two years of experience, more grainy ears were obtained by double treatment with NanoKremny. The addition to the water treatment option was 26.7%.

The use of NanoSilicon in the phase of 5 leaves contributed to an increase in the coarse grains of the ears by 21.7%, and in the phase of 7-8 leaves of corn - by 24.3%. Spraying plants with Kelik Potassium-Silicon in the 5-leaf phase increased the cob grazing by 14.3%. The use of this drug in the phase of 7-8 corn leaves contributed to an increase of 17.4%, and with double treatment, the increase in the number of grains was 19.6%. Processing of Microvit-6 Silicon crops in a phase of 5 leaves led to an increase in cob grazing by 15.2%, in a phase of

7-8 leaves it provided an increase in the number of grains in the cob of 15.9%, and with binary use of the preparation, an increase in grazing was noted, compared to the version with water treatment by 14.1%.

The mass of 1000 grains depends on the performance of the grain and is an indicator characterizing the effectiveness of cultivation techniques. The experimental data obtained indicate that, on average, over two years of the experiment, an increase in the mass of 1000 grains, compared with water treatment, by 7.4% and 12.3% was obtained with the use of Microvit-6 Silicon and Kelik Potassium-Silicon in phase 5 of corn leaves. In other options for improving this indicator of grain quality is not fixed.

Productivity is largely determined by the number of ears of corn on a plant and the weight of grain from one ear. It was established that the most full-bodied ear was obtained by foliar treatment of crops in the five-leaf phase with the preparations Kelik Potassium-Silicon and Microvit-6 Silicon, as well as in the variants with double treatment with NanoKremny and Kelik Potassium-Silicon. The increase in the option with water treatment ranged from 23.3% to 26.7%.

The main quantitative sign of the effectiveness of the applied technological technique is grain yield. The results obtained indicate that the studied preparations showed better adaptogenic properties under the conditions of elevated vegetation temperatures in 2019. The use of NanoKremny in the 5-leaf phase increased grain yield by 2.50 t/ha or 41.7%, and in the 7-8 leaf phase by 2.31 t/ha or 38.8% compared with the version without the drug.

The best result was obtained by double treatment with NanoSilicon, the grain increase was 4.25 t/ha. With foliar treatment of crops with Kelik Potassium-Silicon in a phase of 5 leaves, the yield increased by 3.37 t/ha compared with the version with water treatment, and when using the drug in a phase of 7-8 leaves - by 2.24 t/ha or 38.0%. Against the background of double processing, an additional 2.24 t/ha of grain was obtained. The use of the Microvit-6 Silicon preparation in a phase of 5 leaves and a phase of 7-8 leaves was equivalent and contributed to an increase in grain yield by 3.82 t/ha. Binary treatment with

the drug contributed to an increase in productivity by 3.24 t/ha or 54.4% in relation to the variant with water treatment.

In less favorable conditions for the growing season of 2018, the best results were obtained when processing crops with Kelik Potassium-Silicon. So, foliar treatment in the phase of 5 leaves provided an increase in grain yield of 1.26 t/ha or 40.6% to the variant with spraying with water, and in the phase of 7-8 leaves - 0.87 t/ha or 27.6%. With double treatment with the drug, grain yield increased by 1.37 t/ha or 43.9%. When using NanoSilicon, the yield increase varied from 0.66 t/ha when using the drug in the phase of 7-8 corn leaves, to 1.03 t/ha - in the double-treated version. Leaf treatment in phase 5 of corn leaves with Microvit-6 Silicon increased grain yield by 0.74 t/ha or 23.9% compared with water treatment, in the phase of 7-8 leaves by 0.90 t/ha or 28.6%, and double use 0.83 t/ha or 26.6%.

On average, over two years of observation, the use of the preparation Kelik Potassium-Silicon in the 5-leaf phase contributed to an increase in grain yield by 2.32 t/ha, and in the 7-8 leaf corn phase by 1.56 t/ha or 34.0% compared with water treatment. When spraying twice, the increase was 1.80 t/ha. The use of NanoSilicon in the phase of 5 corn leaves made it possible to obtain an additional 1.62 t/ha of grain or 35.7% in relation to the variant with water. The foliar treatment of crops in the phase of 7-8 corn leaves ensured an increase in grain yield by 1.49 t/ha and double treatment by 2.64 t/ha. In the variant with treatment with Mikrovit-6 Silicon in a phase of 5 leaves, the grain yield growth was 2.28 t/ha or 50.2%, and in a phase of 7-8 leaves and when applied twice, the increase was 43.0% of the variants with water treatment.

CONCLUSIONS

During the foliar treatment with silicon-containing preparations, the number of developed cobs per 100 plants increased by 13.3-21.8% compared with the treatment with water, and the greatest stimulating effect was noted with the use of the drug Nano Silicon.

More blackened ears were obtained by double sheet treatment with NanoKremny; the addition to the variant with water was 26.7%. During

foliar treatment, Kelik Potassium-Silicon Mikrovit-6 Silicon, the cob gravel content increased by 14.1-17.4%.

During leaf treatment of crops in the five-leaf phase with Kelik Potassium-Silicon and Microvit-6 Silicon preparations, as well as in variants with double treatment with NanoKremny and Kelik Potassium-Silicon, the grain weight from the ear increased by 23.3-26.7%.

Against the background of foliar treatment with drugs, grain yield increased by 34.0-51.1%, and the best anti-stress effect was achieved by double treatment with NanoSilicon and the use of MicroVit-6 Silicon and Kelik Potassium-Silicon in the phase of five corn leaves.

REFERENCES

- Fauteux, F. (2005). Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiol. Lett.*, 249, 1–6.
- Gumilar, Tony Abdillah, Prihastanti, Erma, Haryanti, Sri (2017). Utilization of Waste Silica and Chitosan as Fertilizer Nano Chisil to Improve Corn Production in Indonesia. *International Workshop on Intelligent Information Technology*, 23(3), 2447–2449.
- Heather, A. Currie, C., Perry, C. (2007). Silica in Plants. *Biological, Biochemical and Chemical Studies, Ann Bot., December; 100(7)*, 1383–1389.
- Liang, Y., Sun, W., Zhu, Y-G., Christie, P. (2007). Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environmental Pollution*, 147, 422–428.
- Ma, J.F., Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends Plant Sci.*, 11(8), 392–397.
- Ma, J.F. (2004). Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.*, 50, 11–18.
- Pavlovskaya, N.E., Borodin, D.P., Khoroshilova, A.A., Yakovleva, I.V. (2017). Study of the action of nanosilicon on the photosynthetic productivity of spring wheat. *Bulletin of Altai State Agrarian University*, 7(153), 12–18.
- Radkowski, A., Sosin-Bzducha, E., Radkowska, I. (2017). Effects of silicon foliar fertilization of meadow plants on nutritional value of silage fed to dairy cows. *Journal of Elementology*, 22(4), 1311–1322
- Savithramma, N., Ankanna, S., Bhumi, G. (2012). Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic and endangered medicinal tree taxon. *Nano Vision*, 2, 61–68.
- Suriyaprabha, R. (2012). Silica nanoparticles for increased silica availability in maize (*Zea mays* L.) seeds under hydroponic conditions. *Curr Nanosci.*, 8, 902–908.

ALLELOPATHIC ACTIVITY OF SUNFLOWER BROOMRAPE (*Orobanche cumana* Wallr.) ON SUNFLOWER (*Helianthus annuus* L.) VARIETIES

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Abstract

It has been found that dry biomass of sunflower broomrape (Orobanche cumana Wallr.) has an inhibitory effect on seed germination and the initial development of five tested sunflower varieties. Sunflower broomrape with the highest overall allelopathic potential (OAP) can be identified with originating in Tyulenovo and Radnevo with average OAR 0.22. Sunflower broomrape with the lowest overall allelopathic potential (OAP) can be identified with originating in the ecological areas of Senokos and Shumen with average OAR 0.10. High allelopathic tolerance was observed in the seed germination and in the initial development of the sunflower varieties San Luka and Diveda. Their index of initial plant development (GI) ranged from 72.9 to 127.0 at higher studied concentrations 16.00% w/v and 32.0% w/v. The lowest allelopathic tolerance was observed in the seed germination and in the initial development of the sunflower variety Enigma. Its index of initial plant development (GI) ranged from 39.4 to 68.5. It has been found an equivalent between the allelopathic effect of dry biomass of Orobanche cumana Wallr. and the allelopathic tolerance of Helianthus annuus L. varieties, according to the common allelopathic potential of Orobanche cumana Wallr. and the Initial Development Index (IDI) of the sunflower plants.

Key words: allelopathic activity, allelopathic effect, sunflower broomrape, sunflower.

INTRODUCTION

Weed species are a constant and ubiquitous companion of agricultural production, inflicting huge damage on it, which often exceeds the total losses caused by diseases and pests (Kubiszewski and Cleveland, 2012).

Research on weed species in recent years has focused mainly on development of highly efficient systems for their control (Rubiales, 2012).

Synthetic herbicides are an essential means of combating weeds in sunflower, but their intensive use is a prerequisite for creating resistance to some weeds and for a wider spread of the sunflower broomrape (*Orobanche cumana* Wallr.) (Mitkov et al., 2019; Masliiiv et al., 2018; Molinero-Ruiz et al., 2015; Shindrova, 2006; Venkov and Shindrova, 2000; Venkov and Bozoukov, 1994).

Generalized studies of (Habimana et al., 2014; Plakhine et al., 2012; Runyon et al., 2009) show that the technological solutions and the effective ways to control of the sunflower

broomrape by applying conventional methods are limited, due to the nearby physiological link between the parasite and the host plant (Matusova, 2014; Abbes et al., 2008).

Currently, there is a growing interest to allelopathy in agriculture, because allelopathy could offer promising alternative methods to control of weeds and to help for reduce of the application of synthetic herbicides (Singh et al., 2003).

Alelochemicals could replace partially used synthetic herbicides or to be used as a prototype for the synthesis of biodegradable herbicides. Due to their biological nature, they will be safer for the environment than synthetic herbicides (Takemura et al., 2013).

In recent decades, research has focused on the detection of species and varieties with allelopathic tolerance to typical weed flora. Chou (1999), Chon and Nelson (2010) have found that there are varietal differences in allelopathic tolerance with respect to the allelopathic effect of some typical weeds in different crops. According to summary studies

of Parvatha (2017) and Jabran (2017) in breeding programs, allelopathy can be considered as a means of biological control against weeds.

Scientific reportings of Belz (2007), Ferguson et al. (2013) and Fragasso et al. (2013) to determine the allelopathic effect of parasitic weed species are controversial. Concerning allelopathic tolerance, specific data are lacking. The purpose of the study is through use of standard methods in laboratory conditions to be determined and compare the allelopathic tolerance of sunflower varieties to the allelopathic effect of aboveground biomass of *Orobanche cumana* Wallr. as components in future breeding programs.

MATERIALS AND METHODS

During the period 2017-2018 at the Institute of Forage Crops - Pleven, Bulgaria using standard methods in laboratory conditions the allelopathic tolerance of five sunflower varieties (Enigma, Favorit, San Luka, Markesa and Diveda) to the allelopathic effect of aboveground biomass of *Orobanche cumana* Wallr. was studied and compared.

The following factors have been studied: Factor A - sunflower varieties (*Helianthus annuus* L.), selected in Dobroudja Agricultural Institute, General Toshevo, Bulgaria (DAI): a₁- Favorit; a₂ - San Luka; a₃ - Enigma; a₄ - Diveda; a₅ - Markesa; Factor B - concentrations: b₁ - 0.08% w/v; b₂ - 0.16% w/v; b₃ - 0.32% w/v of parasitic weed biomass of sunflower broomrape (*Orobanche cumana* Wallr.). The aboveground biomass of sunflower broomrape (*Orobanche cumana* Wallr.) is collected in phenophase flowering (BBCH 65-69) in the infectious field of Dobroudja Agricultural Institute, General Toshevo. The plant material of *Orobanche cumana* Wallr. is dried to constant dry weight at temperature 50°C ± 5°C.

To evaluate the allelopathic tolerance of the tested sunflower varieties to the allelopathic effect of the aboveground biomass of *Orobanche cumana* Wallr. under laboratory conditions was used the adapted method of (Fujii et al., 2005) "Rhizosphere Soil Method" (RSM). A dry parasitic weed biomass of sunflower broomrape (*Orobanche cumana* Wallr.) was placed in Petri dishes (90 mm),

according to factor B, into which 20 ml (0.8%) agar were pipetted, as added 1 ml/l C₁₀H₁₄O. The thus prepared Petri dishes were placed in a thermostat in the dark for 72 h at a temperature of 18°C ± 2°C.

In each Petri dish, 10 numbers of seeds of *Helianthus annuus* L. were seeded according to factor A, and then they was incubated in the dark into a thermostat at temperature 23°C ± 2°C for five days. Each experimental option is pledged in seven repetitions. 0.8% agar whit added 1 ml/l C₁₀H₁₄O was used for control.

The percentage of germinated seeds (GR%) and the length in cm of the sprouts for each variant of the experiment was determined.

Dynamic Development Index (DDI):

$$DDI = \left\{ \frac{t \log^2}{\log b - \log a} \right\}$$

where: *a* is the sprouted seeds in %; *b* is the length of the sprouts in the control and the experimental variants in cm; *t* is duration of the exposure in days.

Allelopathic Effect Index (RI):

The Percent of Inhibition (IR) is determined by the formula:

$$IR\% = \frac{C-T}{C} \times 100$$

where: *C* is an indicator, reported in the control variant and *T* is an indicator, reported in the treated variants.

The rate of growth and accumulation of fresh sprout biomass is determined by the adapted formula of Dauta et al. (1990):

$$\mu = \left\{ \frac{\ln N_t - \ln N_0}{t} \right\}$$

where: *N_t* is the length of the sprouts in the experimental variants in cm; *N₀* is the length of the sprouts in the control variant in cm; *t* is duration of the exposure in days.

The Development Index (GI) is determined by the formula of Gariglio et al. (2002):

$$GI = \left[\left(\frac{G}{G_0} \right) \cdot \left(\frac{L}{L_0} \right) \right] \cdot 100$$

where: *G* is the percentage of germinated seeds for the treated variants; *G₀* is the percentage of germinated seeds for the control variant; *L* is the length of the sprout in the treated variants, in percentage; *L₀* is the length of the sprout in the control variant accepted for 100%.

The viability of a sprout (SVI) is determined by the formula of Islam et al. (2009):

$$SVI = \left(\frac{S \cdot G}{100} \right)$$

where: S is the length of the sprout in cm; G is the percentage of germinated seeds.

The mathematical and statistical processing of the experimental data has been done after preliminary transformation of the percentage of germinated seeds by the formula: $Y = \arcsin\sqrt{(x\%/100)}$.

The data from the laboratory experiment are processed mathematically and statistically with the software products Statgraphics Plus for Windows Version 2.1 and Statistica 10.

RESULTS AND DISCUSSIONS

The laboratory germination of seeds in the tested varieties (*Helianthus annuus* L.) in the control varieties ranged from 56.8% to 90.0%. The highest percentage of germinated seeds was reported for the Enigma variety, and relatively lower for the San Luka and Diveda

varieties. The Favorit and Markesa varieties occupy an intermediate position, the differences between them being statistically proven at ($P = 0.05$) compared to Enigma variety.

The applied aboveground dry sunflower broomrape biomass (*Orobanche cumana* Wallr.) has a low stimulating effect with IR of 4.2% to 9.7% and/or an inhibitory effect with IR of 18.1% to 24.5% on the laboratory seed germination of the tested sunflower varieties. Depending on the degree of inhibition (IR), the tested sunflower varieties can be conditionally divided into three groups: I. Group with stimulating effect ($IR \leq -10\%$) varieties Diveda and Markesa; II. Group with inhibitory effect ($IR \leq 20\%$) variety Favorit; III. Group ($IR \geq 20\%$) varieties San Luka and Enigma (Table 1).

Table 1. Allelopathic effect of sunflower broomrape (*Orobanche cumana* Wallr.) on the germination and the initial development of sunflower (*Helianthus annuus* L.) varieties

Variety	Concentration, % w/v	Germination				Seedling length			
		GR%	IR	DDI	μ	cm	IR	DDI	μ
Favorit	Control	63.4c				8.7b			
	0.08	60.0c	5.4	-20.41	-0.01	8.1b	6.4	-16.89	-0.01
	0.16	50.8b	19.9	-5.08	-0.04	7.8b	10.2	-10.41	-0.02
	0.32	45.0a	29.0	-3.28	-0.07	7.2a	17.5	-5.85	-0.04
Average		54.8	18.1	-9.59	-0.04	8.0	11.4	-11.05	-0.02
San Luka	Control	56.8b				8.8b			
	0.08	56.8b	0.0	0.00	0.00	9.0c	-2.4	47.65	0.01
	0.16	39.2a	31.0	-3.03	-0.07	7.8a	11.3	-9.41	-0.02
	0.32	39.2a	31.0	-3.03	-0.07	7.9a	10.1	-10.54	-0.02
Average		48.00	20.67	-2.02	-0.05	8.38	6.33	9.23	-0.01
Enigma	Control	90.0c				10.8b			
	0.08	63.4a	29.6	-3.21	-0.07	8.8a	18.6	-5.46	-0.04
	0.16	77.1b	14.3	-7.27	-0.03	9.3a	14.0	-7.49	-0.03
	0.32	63.4a	29.6	-3.21	-0.07	9.0a	16.3	-6.33	-0.04
Average		73.48	24.50	-4.56	-0.06	9.48	16.30	-6.43	-0.04
Diveda	Control	56.8ab				10.8b			
	0.08	63.4c	-11.6	10.23	0.02	14.4c	-33.4	3.904	0.058
	0.16	63.4c	-11.6	10.23	0.02	15.4c	-42.9	3.154	0.071
	0.32	50.8a	10.6	-10.08	-0.02	8.7a	19.7	-5.137	-0.044
Average		58.60	-4.20	3.46	0.01	12.33	-18.87	0.64	0.03
Markesa	Control	63.4a				8.6a			
	0.08	77.1b	-21.6	5.75	0.04	9.3b	-8.2	14.229	0.016
	0.16	71.6b	-12.9	9.25	0.02	8.5a	1.5	-74.115	-0.003
	0.32	60.0a	5.4	-20.41	-0.01	8.5a	1.5	-74.115	-0.003
Average		68.03	-9.70	-1.80	0.02	8.73	-1.73	-44.67	0.00

Legend: GR% - seed germination; IR - percent of inhibition; DDI -dynamic index development

A specific varietal response to the allelopathic effect of the sunflower broomrape (*Orobancha cumana* Wallr.) has been established. Relatively high allelopathic tolerance to the allelopathic effect of *Orobancha cumana* Wallr. to the seed germination was reported in the varieties Diveda and Markesa, relatively lower in the variety San Luka and variety Enigma.

The applied concentrations have a significant effect on the laboratory germination of the sunflower seeds. By increasing of the applied concentration of *Orobancha cumana* Wallr. there is a general tendency to decrease the laboratory germination of the seeds from 5.40% to 31.0% for the Favorit, San Luka and Enigma varieties.

The differences in the laboratory germination of the seeds were statistically proven at ($P = 0.05$) only at the higher concentrations of 0.16% v/w and 0.32% v/w.

The lower concentrations 0.08% v/w and 0.16% v/w of *Orobancha cumana* Wallr. have a statistically proven stimulating effect on the laboratory germination of the seeds of the varieties Diveda and Markesa.

Significant differences in the dynamic index (DDI) and the rate of seed germination (g) were found, depending on the manifested allelopathic tolerance of the sunflower varieties to the allelopathic effect of the sunflower broomrape.

These differences can be explained by the diffusion of the soluble allelochemicals from the aboveground of sunflower broomrape biomass (*Orobancha cumana* Wallr.) into the carrier - agar and with the allelopathic tolerance of the tested sunflower varieties, because the comparisons between them were made under controlled conditions (Sangeetha and Baskar, 2015).

Similar specific variety reaction with respect to the allelopathic interference has been found in other cultures (Kruse et al., 2000; Wu et al., 1999).

Data from the biometric measurements of the length of the sprout (cm) make it possible to objectively compare and evaluate the allelopathic tolerance of the tested sunflower varieties to the allelopathic effect of the sunflower broomrape (*Orobancha cumana* Wallr.).

The attached parasitic weed biomass from *Orobancha cumana* Wallr. has no a statistically proven inhibitory effect on the sprouting and the growth of the Enigma, Markesa, and Favorit varieties. In the Diveda variety, the applied parasitic weed biomass of *Orobancha cumana* Wallr. elicited a statistically proven stimulating effect at the lower concentrations 0.08% v/w and 0.16% v/w, respectively IR - 33.4% and IR - 42.9% (Table 1).

Regarding to the concentration dependences, it is evident that with an increase of the content of the parasitic weed biomass from 16.0% w/v to 32.0% w/v, there is no disproportionate reduction of the length of the sprout in the tested sunflower varieties except Enigma. The differences were statistically unproven at ($P = 0.05$) compared to the lowest concentration of 0.08% v/w. Similar results have been reported by (Labrousse et al., 2001), according to the authors the allelopathic tolerance is species and variety specific.

The results for the growth rate (μ) and the development index (DDI) of sprouts in the studied sunflower varieties are similar. They also depend on the applied concentrations of the parasitic weed biomass of *Orobancha cumana* Wallr.. The relatively lowest allelopathic effect (RI) was found from the lowest concentration of the parasitic weed biomass of *Orobancha cumana* Wallr. - 0.08% w/v. With increasing concentration of the parasitic weed biomass of *Orobancha cumana* Wallr. to 32.0% w/v, RI and μ increase respectively from 0.4 to 1.3 times and from 0.3 to 1.3 times. The Dynamic Plant Development Index (DDI) decreases from 0.7 to 2.9 times only in the Favorit and Enigma varieties. An exception to the described dependence is found in the varieties San Luka, Diveda and Markesa, where lower concentrations have a stimulating effect up to 42.9%.

The results for determining of the viability (SVI) in cm of the tested sunflower varieties are similar (Figure 1). Relatively most sensitive are the Favorit and San Luka varieties. In these, the viability (SVI) in cm is in the range from 3.10 to 5.51, followed by Enigma and Markesa, with (SVI) in cm from 5.10 to 9.68 and relatively least sensitive is Diveda with (SVI), in cm in the range from 6.12 to 9.76.

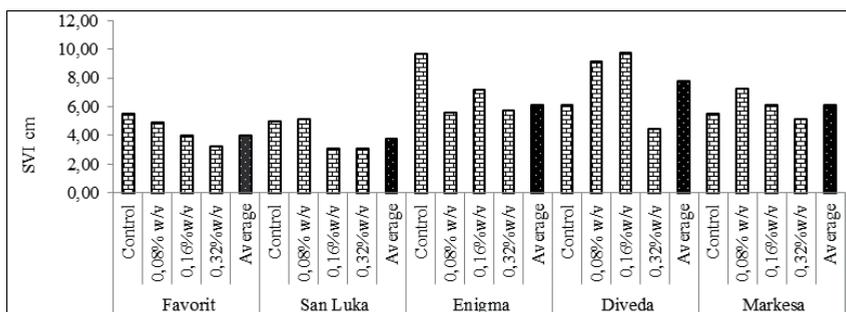


Figure 1. Allelopathic effect of sunflower broomrape (*Orobanche cumana* Wallr.) on seedling vigour index $SVI_{(cm)}$ of sunflower (*Helianthus annuus* L.)

The plant development index (GI) depends on the same factors and follows the observed dependencies in terms of the laboratory germination and the dynamics of sprouting in the tested sunflower varieties (Figures 1 and 2). Depending on the GI values, they can be grouped conditionally in the following ascending order: Enigma → Favorit → San Luka → Markesa → Diveda. Therefore, the

observed differences with respect to the viability SVI (cm) of the sprouts and the plant development index (GI) can be explained by varietal differences, since the comparisons between them are made under the same conditions, which also determine the allelopathic tolerance of the sunflower varieties to the allelopathic effect of the sunflower broomrape.

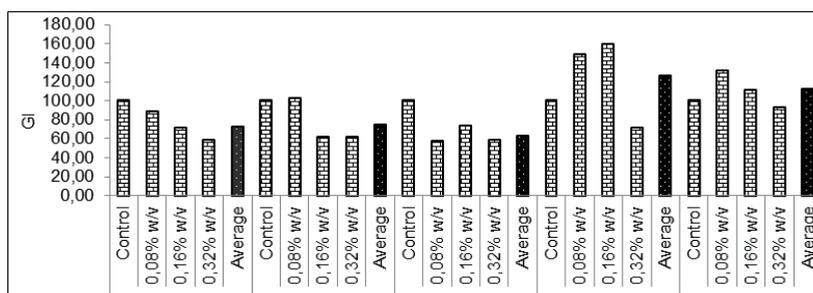


Figure 2. Index of development (GI) on the seed germination and the initial development of sunflower (*Helianthus annuus* L.) depending on the allelopathic effect of (*Orobanche cumana* Wallr.)

CONCLUSIONS

Concentrations from 0.08% w/v to 0.32% w/v of the aboveground biomass of the sunflower broomrape (*Orobanche cumana* Wallr.) exert an inhibitory effect on the seed germination and the initial development of the plants of the tested sunflower varieties (*Helianthus annuus* L.). They can be conditionally grouped into three groups: I. Group with $IR \leq -10\%$ for Diveda and Markesa varieties; II. Group with $IR \leq 20\%$ for Favorit variety; III. Group with $IR \geq 20\%$ for San Luka and Enigma varieties.

The relatively high allelopathic tolerance to the allelopathic effect of *Orobanche cumana* Wallr. on the seed germination and in the initial

development of the tested sunflower varieties has been reported in the Diveda and Markesa varieties, which may be used as components in future breeding programs. Relatively lower allelopathic tolerance was reported in the San Luka and Enigma varieties.

REFERENCES

- Abbes, Z., Kharrat, M., Chaibi, W. (2008). Seed germination and tubercle development of *Orobanche foetida* and *Orobanche crenata* in presence of different plant species. *Tunisian Journal of Plant Protection*, 3, 101–109.
- Belz, R.G. (2007). Allelopathy in crop/weed interactions - an update. *Pest Management Science*, 63, 308–326.

- Chon, S., Nelson, U. (2010). Allelopathy in Compositae plants. A review. *Agronomy for Sustainable Development*, Springer Verlag /EDP Sciences /INRA, 30(2), ffl0.1051/agro/2009027ff.fhhal00886545f.
- Chou, C.H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Crit. Rev. Plant Sci.*, 18, 609–636.
- Ferguson, J.J., Rathinasabapathi B., Chase C.A. (2013). Allelopathy: How plants suppress other plants. *HS994*. <http://edis.ifas.ufl.edu>.
- Fragasso, M., Iannucci, A., Papa, R. (2013). Durum wheat and allelopathy: toward wheat breeding for natural weed management. *Frontiers in Plant Science*, 4, 375. doi:10.3389/fpls.2013.00375.
- Fujii, Y., Furubayashi, A., Hiradate S. (2005). Rhizosphere soil method: a new bioassay to evaluate allelopathy in the field. *Proceedings of the 4th World Congress on Allelopathy, Establishing the Scientific Base, Wagga*, 490–492.
- Gariglio, N.F., Buyatti, M., Pillati, R., Gonzales, R.D., Acosta, M. (2002). Use a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *New Zealand Journal of Crop of Horticultural Science*, 30, 135–139.
- Habimana, S., Nduvumuremy, A., Chinama, R. (2014). Management of *Orobanche* in field crops – a review. *Journal of Soil Science and Plant Nutrition*, 14, 43–62.
- Islam, A., Anuar, N., Yaakob, Z. (2009). Effect of genotypes and pre-sowing treatments on seed germination behavior of *Jatropha*. *Asian Journal of Plant Sciences*, 8, 433–439.
- Jabran, K. (2017). Maize allelopathy for weed control. *Manipulation of Allelopathic Crops for Weed Control*, 29–34. Doi:10.1007/978-3-319-53186-1_4.
- Kruse, M., Strandberg, M., Strandberg, B. (2000). Ecological effects of allelopathic plants - a review. *National Environmental Research Institute, Silkeborg, Denmark*. 66 pp. - NERI Technical Report No. 315.
- Kubiszewski, I., Cleveland, C. (2012). United Nations Conference on Environment and Development (UNCED). Rio de Janeiro. Brazil. Retrieved from <<http://www.eoearth.org/view/article/156773>>.
- Labrousse, P., Arnaud, M., Serieys, H., Bervillé, A., Thalouarn, P. (2001). Several mechanisms are involved in resistance of *Helianthus* to *Orobanche cumana* Wallr. *Annals of Botany*, 88(5), 859–868. <http://www.jstor.org/stable/42771103>.
- Masliiov, S., Macai, N., Beseda, O., Stepanov, V. (2018). Control of broomrape *Orobanche cumana* Wallr. *Ukrainian Journal of Ecology*, 8(2), 74–80. DOI:10.15421/2018_312.
- Matusova, R., Kullačová, D., Tóthq, P. (2014). Response of wild and weedy broomrapes to synthetic strigolactone analogue GR24. *Journal of Central European Agriculture*, 15(4), 72–82.
- Mitkov, A., Yanev, M., Neshev, N., Tonev, T., Joița-Păcureanu, M., Cojocar, F. (2019). Efficacy against broomrape and selectivity of imazamox-containing herbicides in sunflower. *Romanian Agricultural Research*, 36, 201–207.
- Molinero-Ruiz, L., Delavault, Ph., Pérez-Vich, B., Pacureanu-Joita, M., Bulos, M., Altieri, E., Dominguez, J. (2015). History of the race structure of *Orobanche cumana* and the breeding of sunflower for resistance to this parasitic weed: A review. *Spanish Journal of Agricultural Research*, 13, 1–19.
- Parvatha, P. (2017). Allelopathy. *Agro-ecological Approaches to Pest Management for Sustainable Agriculture*, 10.1007/978-981-10-4325-3_18.
- Plakhine, D, Tadmor, Y., Ziadne, H., Joel, D. (2012). A maternal tissue is involved in stimulant reception by seeds of the parasitic plant *Orobanche*. *Annals of Botany*, 109, 979–986.
- Rubiales, F. (2012). Innovations in parasitic weeds management in legume crops. A review. *Agronomy for Sustainable Development*. Springer Verlag, 32(2), 433–449.
- Runyon, J.B, Tooker, J., Mescher, M., De Moraes, C. (2009). Parasitic plants in agriculture: chemical ecology of germination and host-plant location as targets for sustainable control. A review. In: *Lichtouse E (ed) Sustainable agriculture reviews*, Springer, 1, 123–136.
- Sangeetha, C., Baskar, P. (2015). Allelopathy in weed management: a critical review. *African Journal of Agricultural Research*, 10(9), 1004–1015.
- Shindrova, P. (2006). Broomrape (*Orobanche cumana* Wallr.) in Bulgaria-distribution and race composition. *Helia*, 29(44), 111–120.
- Singh, H.P., Batish, D.R., Kohli R.K. (2003). Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. *Critical reviews in plant sciences*, 22(3-4), 239–311.
- Takemura, T., Sakuno, E., Kamo, T., Hiradate, S., Fujii, Y. (2013). Screening of the growth-inhibitory effects of 168 plant species against lettuce seedlings. *American Journal of Plant Science*, 4(5), 1095–1104.
- Venkov, V., Bozoukov, H. (1994). Influence of different species of *Orobanche* on cultivated sunflower and tobacco. *Biology and management of Orobanche: Proceedings of the 3-rd International Workshop on Orobanche and Striga Research, Amsterdam, The Netherlands*, RTI, 349–350.
- Venkov, V., Shindrova P. (2000). Durable resistance to broomrape (*Orobanche cumana* Wallr./*Orobanche cernua* Loefl.) in sunflower. *Helia*, 23(33), 39–44.
- Wu, H., Pratley, J., Lemerle, D., Haig, T. (1999). Crop cultivars with allelopathic capability. *Weed Research*, 39, 171–180.

INFLUENCE OF NEW BIOSTIMULANTS ON AMINO ACID SYNTHESIS IN ALFALFA UNDER CLIMATE CHANGING CONDITIONS

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Abstract

Discussion on the impact of climate change on various aspects of life is of increasing concern to the scientific community. Providing food for the growing population in adverse climatic conditions is a major challenge. This includes the provision of high-quality animal feed. Applying environmental approaches in this direction is key to restoring ecological balance. In this study, we investigated the effect of novel biostimulants on the synthesis of amino acids in alfalfa. Green mass and alfalfa hay are high in protein and essential amino acids, the main indicators that determine the quality of the biomass produced. The use of biostimulants in this study leads to an improvement in the biological value of proteins - the total amount of essential amino acids is increased and the ratio of essential amino acids to the other proteinogenic amino acids is changed in favor of the essential and increased the nutritional value of feed. The results obtained are crucial for the successful implementation of green practices in agriculture.

Key words: alfalfa, amino acid content, biostimulants, climate change, green agriculture.

INTRODUCTION

Current methods in the development of alfalfa as a crop are directed at the use of mechanisms for direct impact on production and quality. In the cultivation of various high quality alfalfa varieties, techniques are used to directly influence the protein content, fiber (Petkova et al., 2018; Toktarbekova et al., 2020) or by increasing the percentage of leaves (Kephart et al., 1990; Huset et al., 1991). Modern varieties and hybrids have high productive potential, which is not fully realized in production conditions. The factors limiting the expression of their productive capacities are various, but among the most important are the environmental conditions and the applied agricultural technology. The use of modern techniques to improve the adaptability of plants to changing environmental conditions is an important prerequisite for nature conservation in the development of sustainable agriculture. Different products are used, which are a variety of chemicals applied to plants or soils to improve crop viability, yield, quality and resistance to abiotic stress. Biostimulants influence by improving plant metabolism leading to increased yields; increasing the

resistance of plants to abiotic factors (meteorological factors, pollution, pesticides, etc.); facilitate the assimilation of trace elements and digestibility of nutrients; increase the quality of production and more, (Niewiadomska et al., 2019; 2020). Feeding plants is a specific process, the primary place where nitrogen occupies. In legumes, the basic amount of the element is ensured by biological fixation of atmospheric nitrogen. The advantage of legumes is that they can grow on soils with a very low concentration of bound nitrogen, where other plants cannot. By increasing mineral nitrogen in the soil (ammonium and nitrate), its absorption from nitrogen-fixing plants increases and symbiotic nitrogen fixation decreases (Blumenthal et al., 1999; Campbell, 1999; Ruselle and Birr, 2004). In addition to environmental factors, productive potential is also influenced by the age of the alfalfa crop and the intensity of use (Brink et al., 2010). Two different enzymatic systems may be involved in the uptake of nitrogen by legumes. One is the nitrogenase system, which is part of the arsenal of symbiotic microorganisms and provides the fixation of atmospheric nitrogen (Ruselle and Birr, 2004; Hristozkova et al., 2010; 2011). The second system involves

nitrate reductase, which is responsible for the reduction of nitrate nitrogen from soil (Ruselle and Birr, 2004; Hristozkova et al., 2011; Petkova et al., 2018; Nedyalkova et al., 2019). In the subsequent steps for the primary incorporation of the resulting ammonium ions into an organic molecule, the enzymes glutamine synthetase and asparagine synthetase take part (Hristozkova et al., 2011). The end result is a large number of amino acids and amides, and their quality and quantity depend on environmental factors and the physiological state of the plants. Knowledge of the processes involved in their production enables them to be modulated and to improve the quality of the final output. For example, in addition to molybdenum, induction of nitrate reductase activity is induced by cytokinins, a combination of cytokinins and gibberellins, humic acids, carbohydrates, mainly glucose and sucrose, certain organic acids, ammonium sulfate, organic acids included in the Krebs cycle (Aranjielo et al., 2011).

The possibility of regulating the biosynthesis of amino acids and proteins in legumes through the use of biostimulants and leaf fertilizers (containing microelements, phytohormones and other growth regulators) in the face of changing climatic factors is a challenge in modern agriculture. This determined the purpose of this study on the effects of new biostimulants on the quantity and quality of amino acids in alfalfa.

During 2017-2019 period at the experimental field of the Agricultural University of Plovdiv it was conducted a field experiment under the following conditions: alluvial-meadow soil type of pH - 6.7-7.1 (H₂O) and medium level of basic nutritive elements.

The investigation was performed by the 4 replications block method in 10 m² lots.

The sowing was done in spring 2017 and the plants are treated by generally accepted technology for alfalfa forage production (Yankov et al., 1996).

During the investigation 4 variants were controlled: 1. treated by Tecamin max; 2. treated by Amino Bore; 3. treated by Plantafol; 4. treated by Fertigrain Foliar.

Tecamin max contains amino acids 14.4% of which free 12%, organic matter 60%, total nitrogen 7%. It activates the growth and development of crops, promotes the restoration

of plants after stressful situations – frost, hail, herbicide effect, phytotoxicity, and helps for the transport in plants of mineral nutrients, including trace elements. Also, it increases plant productivity and yield and improves product quality.

AminoBore is an organic biostimulant containing nitrogen 4%, water soluble boron (B) 9% and free amino acids 5%. It is used in oilseeds, alfalfa, fruits, vineyards and more (Meets organic farming standard NFU 42-003-2). It promotes faster absorption and movement of boron in the plant, restoration of cultures under stress of abiotic character (cold, drought, hail) stimulation of photosynthesis and fruit formation, overall balanced development of crops.

Plantafol is a N:P:K 20:20:20 mineral leaf fertilizer enriched with micro elements chelated with an EDTA chelating agent. Its content includes total nitrogen 20% (of which nitrate nitrogen - 4%, ammonium nitrogen - 2% and amide nitrogen - 14%), phosphorus (as diphosphorus pentoxide) - 20%, potassium (as potassium sulfate) - 20%. Of the trace elements, boron (B) - 0.02%, copper (Cu) - 0.05%, iron (Fe) - 0.1%, manganese (Mn) - 0.05% and zinc (Zn) - 0.05% are present.

Fertigrain Foliar - biostimulator for leaf application. It contains amino acids 10%, nitrogen 5%, organic matter 40%, zinc 0.75%, manganese 0.50%, boron 0.10%, iron 0.10%, copper 0.10%, molybdenum 0.02% and cobalt 0.01%. It has a powerful effect of stimulating plant growth and development of the plants due to the unique combination of organic nutrients in the form. Free amino acids and the most important trace elements in the form of chelates are the starting components for protein and enzyme biosynthesis.

The treatment of each swath was performed at stage by 2 l/ha of the preparations.

MATERIALS AND METHODS

Plant material

Mnogolistna 1. The variety is representative of the newest generation of multifaceted alfalfa. Over 50% of the leaves of the plants hold from 5 to 7 petals on a single leaf handle.

Legend. The variety is registered by the US company Land O'Lakes. It is part of the new generation of so-called multifaceted alfalfa

with more than 3 leaf handles, and has better in vitro digestibility than standard three-leaf sorts.

Used preparations

Four leaf treatments were used, with different contents and a combination of active substances at a dose of 3 l/ha twice. The products were Tecamine Max, Amino Boron, Plantafol and Fertigrain Foliar.

Samples for enzymatic analyzes (roots with nodules and aboveground part) were collected in the budding and flowering phases when nitrogen fixation was most intense. The activity of four key enzymes of nitrogen assimilation - nitrogenase, glutamine synthetase, asparagine synthetase and nitrate reductase - was investigated.

Nitrogenase activity (EC 1.7.99.2.) was determined by the method of Hardy et al. (1973) with modification (Popov et al., 1985).

The activity of glutamine synthetase (EC 6.3.1.2.) was determined by orthophosphate separated from ATP, which was determined by the Sumner method (Evstigneeva et al., 1980).

Asparagin synthetase activity (EC 6.3.5.4.) was determined by the same procedure as for the determination of the enzyme glutamine synthetase, except that glutamate is replaced by aspartate in the incubation mixture.

Nitrate reductase activity. Nitrate reductase (EC 1.6.6.2.) catalyzes the reduction of nitrates to nitrites. The method for determining the amount of nitrite is based on the color complex formed by the interaction of the nitrite ions with sulfanilamide in acetic acid and with N-(1-naphthyl)-ethylenediamine (Berova et al., 2013).

Samples for analysis of the type and amount of amino acids (roots with tubers and

aboveground part) were collected in the budding and flowering phases, when nitrogen fixation was most intense. The research was done in an accredited laboratory at the Agricultural University of Plovdiv. Measurement of amino acids was done with an automatic amino analyzer.

Statistical processing

The obtained data were mathematically processed by the method of variance analysis using the SPSS program, and the Duncan multivariate test with the smallest significant difference (LSD) - 0.05 (5%) was used to determine the differences between the tested variants. Correlation analysis was performed with the SPSS program.

RESULTS AND DISCUSSIONS

The production, quality and longevity of alfalfa depend on both external (environmental conditions) and internal (genetically determined) factors. These factors are in complex relationships and the elimination of any of them reduces the effect of the others and ultimately affects both the yield and the longevity of the alfalfa crops.

Research on alfalfa shows that yields have increased by 20% over the last hundred years (Kertikova, 2000), with only 10% of this increase being due to genetic improvements. As a protein culture, in addition to yield, protein content, and in particular the amino acid composition, is also essential.

Alfalfa green mass and hay are characterized by high protein content and essential amino acids, essential indicators that determine the quality of the produced biomass.

Table 1. Essential amino acids content in Mnogolista 1 biomass (% by weight of dry matter) average for the study period

Variants	Control	Tecamin Max	Amino Bore	Plantafol	Fertigreïn Foliar
Amino acids					
Lysine	1.71 ^b	1.72 ^b	1.56 ^c	1.57 ^c	1.92 ^a
Threonine	1.12 ^b	1.14 ^b	1.13 ^b	1.14 ^b	1.25 ^a
Valine	1.12 ^c	1.26 ^b	1.19 ^c	1.23 ^b	1.35 ^a
Methionine	0.13 ^a	0.11 ^b	0.09 ^c	0.09 ^c	0.11 ^b
Isoleucine	0.84 ^c	0.98 ^{ab}	0.89 ^{bc}	0.91 ^b	1.05 ^a
Leucine	1.71 ^b	1.78 ^b	1.57 ^c	1.57 ^c	1.86 ^a
Phenylalanine	1.22 ^b	1.24 ^b	1.19 ^c	1.21 ^{bc}	1.45 ^a
Total	7.85 ^c	8.23 ^b	7.62 ^c	7.72 ^c	8.99 ^a

The different letters (a, b, c) after the average show statistically significant differences between the analyzed variants.

A number of scientific studies show that the application of different growth regulators has a positive effect on the quality of alfalfa forage (Wang et al., 2003; Radu et al., 2010).

Proteinogenic amino acids in the alfalfa vegetative mass are related to the biological value and protein balance. Their amount in the individual protein fractions is under genetic control and difficult to change, but the ratio of protein fractions, and through them, the quality of the protein can change under external influences. The application of the tested products in the present study positively affects the total content of proteinogenic amino acids in both varieties. At Mnogolistna 1 variety, the application of different products significantly increased by 14.52% the content of essential amino acids in only one variant (Fertigrein Foliar), in Tecamin Max the increase was

insignificant, and in the variants Amino Bore and Plantafol it was lower than the control (Table 1).

The results are similar for the other proteinogenic amino acids, except for the Plantafol variant which has a 3.3% higher total amino acid composition, obtained mainly because of the aspartic acid. All tested amino acids, show higher content when Fertigrein Foliar is applied - aspartic acid by 27%, glycine by 21.8%, arginine by 21.4%, valine by 20.5% compared to the control (Table 2).

The reaction to the used products shows also variety differences. In the second alfalfa variety - Legend, a higher effect of treatment was observed with the Tecamin Max variant both for total proteinogenic and irreplaceable proteinogenic amino acids (Table 3).

Table 2. Total proteinogenic amino acids content in the alfalfa biomass at Mnogolistna 1 (% by weight of dry matter), averaged for the study period

Variants	Control	Tecamin Max	Amino Bore	Plantafol	Fertigrein Foliar
Amino acids					
Lysine	1.71 ^b	1.72 ^b	1.56 ^c	1.57 ^c	1.92 ^a
Histidine	0.57 ^b	0.61 ^a	0.59 ^{ab}	0.59 ^{ab}	0.64 ^a
Arginine	1.31 ^c	1.49 ^b	1.11 ^c	1.25 ^d	1.59 ^a
Aspartic acid	3.11 ^c	3.29 ^c	3.34 ^{bc}	4.24 ^a	3.95 ^b
Threonine	1.12 ^b	1.14 ^b	1.13 ^b	1.14 ^b	1.25 ^a
Serine	1.33 ^b	1.31 ^b	1.44 ^a	1.47 ^a	1.45 ^a
Glutamic acid	2.49 ^b	2.61 ^{ab}	2.29 ^c	2.36 ^b	2.84 ^a
Proline	1.99 ^a	1.97 ^a	1.99 ^a	1.83 ^b	1.83 ^b
Glycine	1.01 ^b	1.17 ^a	0.98 ^b	1.04 ^{ab}	1.23 ^a
Alanine	1.29 ^b	1.41 ^a	1.27 ^b	1.23 ^b	1.52 ^a
Cysteine	0.09 ^a	0.09 ^a	0.11 ^a	0.08 ^a	0.09 ^a
Valine	1.12 ^c	1.26 ^b	1.19 ^c	1.23 ^b	1.35 ^a
Methionine	0.13 ^a	0.11 ^b	0.09 ^c	0.09 ^c	0.11 ^b
Isoleucine	0.84 ^c	0.98 ^{ab}	0.89 ^{bc}	0.91 ^b	1.05 ^a
Leucine	1.7 ^b	1.78 ^b	1.57 ^c	1.57 ^c	1.86 ^a
Tyrosine	0.76 ^b	0.81 ^a	0.71 ^b	0.71 ^b	0.86 ^a
Phenylalanine	1.22 ^b	1.24 ^b	1.19 ^c	1.21 ^{bc}	1.45 ^a
Total	21.79 ^c	22.99 ^b	21.45 ^c	22.52 ^b	24.99 ^a

The different letters (a, b, c) after the average show statistically significant differences between the analyzed variants.

Table 3. Average content of essential and nonessential amino acids in aboveground biomass of alfalfa Legend variety (% by weight of dry matter)

Variants	Control	TecaminMax	AminoBore	Plantafol	FertigreinFoliar
Amino acids					
Lysine	1.52 ^c	1.86 ^a	1.66 ^b	1.61 ^b	1.66 ^b
Threonine	0.97 ^b	1.23 ^a	1.12 ^a	1.07 ^b	1.06 ^b
Valine	1.09 ^c	1.46 ^a	1.27 ^b	1.32 ^b	1.22 ^{bc}
Methionine	0.04 ^b	0.09 ^a	0.06 ^{ab}	0.07 ^a	0.03 ^b
Isoleucine	0.88 ^c	1.11 ^a	0.91 ^b	1.06 ^a	0.92 ^b
Leucine	1.51 ^{bc}	1.91 ^a	1.56 ^b	1.8 ^a	1.61 ^b
Phenylalanine	1.11 ^b	1.31 ^a	1.26 ^a	1.24 ^{ab}	1.16 ^b
Total	7.12 ^b	8.97 ^a	7.84 ^b	8.17 ^a	7.66 ^b

The different letters (a, b, c) after the average show statistically significant differences between the analyzed variants.

The total irreplaceable amino acids content increases from 7.58% at Fertigrein Foliar up to 26% at Tecamin Max. The valine content increased the most - by 34% and the lowest of phenylalanine - by 18% (Table 3).

For other proteinogenic amino acids, the increase was between 8.09% for Fertigrein Foliar and 24.54% for Tecamin Max. The content of aspartic acid was significantly increased (by 77%) in the Tecamin Max treated variant, 57.8% in the Amino Bore variant, 31.0% in the Plantafol variant, 46.3% in the Fertigrein Foliar variant (Table 4).

At Legend variety, from all tested 17 proteinogenic amino acids, only proline reduced its content, and it significantly decreased from 25.2 to 28.73%. The most significant decrease was observed when Plantafol was applied (Table 4). Proline is thought to be a stress indicator amino acid and its amount increases with different types of stress. For water stress, for example, the higher the content of endogenous proline is in bean leaves, the greater is the decrease in water potential (Zlatev, 2005).

Table 4. Total proteinogenic amino acids content in the biomass of alfalfa Legend variety (% by weight of dry matter), average for the study period

Variants	Control	Tecamin Max	AminoBore	Plantafol	Fertigrein Foliar
Amino acids					
Lysine	1.52 ^c	1.86 ^a	1.66 ^b	1.61 ^b	1.66 ^b
Histidine	0.54 ^b	0.66 ^a	0.66 ^a	0.57 ^b	0.58 ^{ab}
Arginine	1.21 ^c	1.47 ^a	1.29 ^c	1.29 ^c	1.31 ^b
Aspartic acid	2.42 ^c	4.3 ^a	3.82 ^{ab}	3.17 ^b	3.54 ^b
Threonine	0.97 ^b	1.23 ^a	1.12 ^a	1.07 ^b	1.06 ^b
Serine	1.03 ^d	1.45 ^a	1.34 ^b	1.21 ^c	1.23 ^c
Glutamic acid	2.17 ^c	2.74 ^a	2.35 ^b	2.39 ^b	2.38 ^b
Proline	2.82 ^a	2.01 ^b	2.18 ^b	1.91 ^c	2.11 ^b
Glycine	0.94 ^c	1.21 ^a	0.97 ^c	1.08 ^b	0.95 ^c
Alanine	1.11 ^c	1.38 ^a	1.16 ^c	1.29 ^b	1.19 ^c
Cysteine	0.09 ^a	0.09 ^a	0.09 ^a	0.07 ^a	0.08 ^a
Valine	1.09 ^c	1.46 ^a	1.27 ^b	1.32 ^b	1.22 ^{bc}
Methionine	0.04 ^b	0.09 ^a	0.06 ^{ab}	0.07 ^a	0.03 ^b
Isoleucine	0.88 ^c	1.11 ^a	0.91 ^b	1.06 ^a	0.92 ^b
Leucine	1.51 ^{bc}	1.91 ^a	1.56 ^b	1.8 ^a	1.61 ^b
Tyrosine	0.68 ^b	0.79 ^a	0.72 ^a	0.73 ^a	0.73 ^a
Phenylalanine	1.11 ^b	1.31 ^a	1.26 ^a	1.24 ^{ab}	1.16 ^b
Total	20.13 ^c	25.07 ^a	22.42 ^b	21.88 ^{bc}	21.76 ^{bc}

The different letters (a, b, c) after the average show statistically significant differences between the analyzed variants.

In both alfalfa varieties, the content of aspartic acid and the irreplaceable amino acid valine is significantly increased. Aspartic acid is a starting substrate in the biosynthesis of the essential amino acids lysine, threonine, isoleucine and methionine, as well as asparagine, directly related to the activity of aspartic acid. In addition, asparagine is one of the transport forms of amino acids due to the low C:N ratio in its molecule. If we compare this data with the results of the effect of the tested products on the efficiency of nitrogen fixation (unpublished data), the logic behind this is: Tecamin Max increases the activity of the enzymes related to the efficiency of nitrogen fixation in the tested varieties of alfalfa. As a result, larger amounts of glutamine and asparagine, respectively glutamic and

aspartic amino acids, are obtained, as our results show. The trends presented here give rise to the following summary: TecaminMax treatment improves the biological value of proteins - increases the total amount of essential amino acids and changes the ratio of essential amino acids to other proteinogenic amino acids in favor of the essential ones.

As a result of the treatment, there was detected a difference between varieties regarding the amount of proteinogenic amino acids. At Legend variety, the highest amount of proteinogenic amino acids was when treated with Tecamin Max, and at Mnogolistna 1 variety, when Fertigrein Foliar was applied.

Mnogolistna 1 variety has higher crude protein content in the biomass compared to Legend variety in all studied variants. The tendency of

protein increase is kept in all swats. The crude protein content at Legend variety is highest when Tecamin Max is applied (21.65, 21.92 and 21.02% by weight of dry matter, respectively, for first, second and third swats). At Mnogolistna 1 variety, the highest protein content is after Fertigrejn Foliar treatment in all three swaths was 23.86, 22.23 and 23.89% to absolutely dry matter (Table 5).

Cellulose content data by swaths shows that Fertigrejn Foliar treatment influences this indicator, which is kept at all three swaths at Mnogolistna 1 variety.

As a result of this research, it is clear that the use of leaf products can significantly increase the content of irreplaceable proteinogenic

amino acids as well as the quality of the protein in the alfalfa biomass. The product containing molybdenum and cobalt (Fertigrain Foliar) has the most significant effect in increasing the amount of amino acids and protein in the biomass. This can be explained by its stimulating effect on the enzymes of nitrogen metabolism – nitrogenase, glutamine synthetase and asparagine synthetase (unpublished data). These results are in agreement with those obtained by other authors working with alfalfa and leaf products (Niewiadomska et al., 2020; Toktarbekova et al., 2020; Niewiadomska et al., 2019; Petkova et al., 2018).

Table 5. Crude protein and cellulose content by swaths, average for the experimental period

Variants	1 st swat		2 nd swat		3 rd swat	
	% by weight of dry matter					
Mnogolistna 1	Protein	Cellulose	Protein	Cellulose	Protein	Cellulose
Control	19.58 ^c	22.28 ^c	20.38 ^b	29.39 ^{bc}	23.12 ^a	33.41 ^a
Tecamin Max	21.94 ^b	25.55 ^b	20.43 ^b	30.84 ^b	23.60 ^a	33.84 ^a
Amino Bore	23.10 ^a	25.96 ^b	21.94 ^{ab}	31.5 ^{ab}	23.69 ^a	28.15 ^c
Plantafol	21.76 ^b	27.74 ^a	20.39 ^b	29.94 ^b	23.63 ^a	31.74 ^b
Fertigrejn Foliar	23.86 ^a	27.59 ^a	22.23 ^a	32.41 ^a	23.89 ^a	34.27 ^a
Legend						
Control	18.11 ^c	28.08 ^a	20.75 ^b	30.48 ^b	20.20 ^b	35.17 ^a
Tecamin Max	21.65 ^a	27.24 ^b	21.92 ^a	31.76 ^a	22.02 ^a	33.41 ^b
Amino Bore	20.13 ^b	28.77 ^a	21.01 ^a	31.41 ^a	21.67 ^a	35.14 ^a
Plantafol	20.22 ^b	27.14 ^b	21.13 ^a	30.83 ^b	21.19 ^{ab}	33.91 ^b
Fertigrejn Foliar	21.24 ^a	29.31 ^a	21.90 ^a	29.94 ^c	21.86 ^a	32.02 ^b

In the lines, the numbers followed by the identical letters (a, b, c) are not statistically proven.

CONCLUSIONS

The use of Tecamine Max and Fertifrain Foliar significantly increases the total content of proteinogenic amino acids and protein, including essential and nonessential amino acids.

There are variety differences in response of plant reaction to the products. Mnogolistna 1 variety has higher crude protein content in the biomass compared to Legend variety in all studied variants.

All used growth regulators led to a decrease in proline content in both studied varieties. Proline is considered to be a stress indicator amino acid, on the basis of which the applied products can be used at multifolium alfalfa varieties to support the culture to overcome various types of stress.

Cellulose content is also influenced by the treatment - all used products led to an increase of the researched indicator.

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REFERENCES

- Aranjuelo, I., Molero, G., Erice, G., Avice, J., Nogues, S. (2011). Plant physiology and proteomics reveals the leaf response to drought in alfalfa. *Journal of Experimental Botany*, 62, 111–123.
- Berova, M., Pevicharova, G., Stoeva, N., Zlatev, Z., Karanatsidis, G. (2013). *Journal of Elementology*, 18(4), 565–576.

- Blumenthal, J., Babyak, M., Doraiswamy, P., Watkins, L., Hoffman, B., Barbour, K., Herman, S., Craighead, W., Brosse, A., Waugh, R., Hinderliter, A., Sherwood, A. (2007). Exercise and pharmacotherapy in the treatment of major depressive disorder. *Psychosomatic Medicine*, 69, 587–596.
- Brink, G., Marvin, H., Glen, Sh., Dan, U., Neal, M., Richard, W. (2010). Changes in alfalfa yield and nutritive value within individual harvest periods. *Agronomy journal*, 102(4), 1274–1282.
- Campbell, W. (1999). Nitrate reductase structure, function and regulation: Binding the gap between biochemistry and physiology. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 277–303.
- Evstigneeva, Z., Gromyko, E., Aseeva K. (1980). Biochemical methods. *M. Science*, 84–86.
- Hardy, R., Burus, R. Holsten, R. (1973). Applications of the acetylene-reduction assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry*, 5, 47–81.
- Hristozkova, M., Geneva, M., Stancheva, I. (2009). Effect of foliar feeding on nitrogen assimilation in alfalfa plants at insufficient molybdenum supply. *Acta Biologica Hungarica*, 60(2), 211–219.
- Hristozkova, M., Geneva, M., Stancheva, I. (2011) Plant Growth and Health Promoting Bacteria. *Microbiology Monographs*, 18, 417–431.
- Huset, D.E., Schneble, D.A., Kugler, J.L., and Peterson, M.A. (1991). Registration of 'WL 322 HQ' alfalfa. *Crop Science*, 31, 1699–1670.
- Kertikova, D., Yancheva, H. (2000). Variability of some traits in Lucerne within one breeding cycle. *Genetics and Breeding*, 30, 1–2.
- Kephart, K., Twidwell, E., Bortnem, R. Boe, A. (1992). Alfalfa yield component responses to seeding rate several years after establishment. *Agronomy Journal*, 84, 827–831.
- Nedyalkova, K., Petkova, G., Valchovski, H., Donkova, R. (2019). Influence of bio-inoculants (earthworms and nitrogen-fixing bacteria) on rhizosphere microflora of alfalfa. *Pochvoznane, agrokhimiya i ekologiya/ Bulgarian Journal of Soil Science, Agrochemistry and Ecology*, 53(1), 27–34.
- Niewiadomska, A., Sulewska, H., Wolna-Maruwka, A., Ratajczak, K., Waraczewska, Z., and Budka, A. (2020). The influence of bio-stimulants and foliar fertilizers on yield, plant features, and the level of soil biochemical activity in white lupine (*Lupinus albus* L.) cultivation. *Agronomy*, 10(1), 150.
- Niewiadomska, A., Sulewska, H., Wolna-Maruwka, A., Ratajczak, K., Waraczewska, Z., Budka, A., Głuchowska, K. (2019). The influence of biostimulants and foliar fertilisers on the process of biological nitrogen fixation and the level of soil biochemical activity in soybean (*Glycine max* L.) cultivation. *Applied Ecology and Environmental Research*, 17(5), 12649–12666.
- Peoples, M., Herridge, D., Ladha, J. (1995). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. *Plant and Soil*, 174, 3–28.
- Petkova, G., Nedyalkova, K., Perfanova, J., & Eftimova, D. (2018). Effect of Microbial Inoculants on Plant Biomass of Lucerne. *Journal of Balkan Ecology*, 21(4), 370–377.
- Popov, N., Nikolov, N., Krastev, A., Mashev, N. (1985). Application of the acetylene method for determination of nitrogenase activity in nitrogen-fixing systems, N. *Proceedings ALL "V. Kolarov"*, Plovdiv, 30(3), 51–56.
- Radu, F., Ahmadi, M., Cojocariu, I., Marian, F., Bostan, C., Borozan, A. (2010). Genotype-biostimulations interactions in some high quality active principles appearance for alfalfa. *Research journal of agricultural science*, 42(1), 526–530.
- Russelle, M., Birr, A. (2004). Large-scale assessment of symbiotic dinitrogen fixation by crops: soybean and alfalfa in the Mississippi River basin. *Agronomy Journal*, 96, 1754–1760.
- Toktarbekova, S., Meirman, G., Yerzhanova, S., Abayev, S., Umbetov, A. (2020). Productivity of the green mass of new alfalfa cultivars depending on the effect of macro- and microfertilizers on various phosphorous backgrounds. *Journal of Ecological Engineering*, 21(2), 57–62.
- Wang, J., Zhai, Z., Duan, S., Tian, L., Wang, B. (2003). Effects of DTA – 6 on crude protein and amino acid content of alfalfa (*Medicago sativa* L.). *Journal of China Agricultural University*, 55(8), 25–28.
- Yankov, B., Yancheva, H., Petkova, D. (1996). Contribution to the selection of multifaceted alfalfa. *Plant Sciences*, 8, 15–18.
- Zlatev, Z. (2005). Comparative study of water exchange and photosynthesis of different bean genotypes in drought conditions. Thesis.

INVESTIGATION OF THE GRAIN SEEDER OPENER OPERATION FOR ENVIRONMENTAL FRIENDLY TECHNOLOGIES OF CROPS PRODUCTION

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Abstract

Resource-saving technologies for sowing crops involve minimizing tillage with scientifically-based husbandry and rational use of resources. Such technologies include minimal and zero tillage based on mulched and direct sowing, with simultaneously applying fertilizers at different levels. The authors suggest the fundamental directions of an integrated farming system in relation to crops production, which, along with the use of all the positive environmental features of the organic, biological, biodynamic systems, allow using the chemical fertilizers within a limited framework. Consequently, it could lead to the dynamic development and increase rural sustainability as a whole. Based on these research materials, prototypes of seeders with experimental paws for different levels of fertilizing and sowing seeds were developed and manufactured. Field experiments with that grain seeder opener have shown steady work of combines in sowing grain crops.

Key words: grain seeder opener, environmental friendly technology, ecology, fertilizer.

INTRODUCTION

Currently, the questions of technical support for high-quality sowing with minimal labor and production resources, as well as the rational use of mineral fertilizers remain relevant, this is due to increased intensification of production, as well as the development of an integrated approach to the cultivation of grain crops, all this would increase the environmental friendliness of products. The optimal conditions for the growth and development of plants depend on methods of sowing, in which a sufficient amount of nutrients, light, moisture, heat is provided, as well as providing the most favorable nutritional area and the lowest production costs. Therefore, the method of sowing is primarily chosen depending on climatic conditions, soil and the ratio of nutrients in it, the sowing qualities of seeds, as well as the needs of the cultivated crop in various nutrients. To achieve maximum yield when sowing, it is necessary to place the seeds at a given depth and observe the optimal area of their nutrition (Kalabushev et al., 2019).

MATERIALS AND METHODS

Laboratory studies were carried out with the aim of determining the optimal design parameters of a grain seeder opener at different

levels in fertilizer application and sowing seeds, using multi-factorial design of the experiment, as well as conducting experiments according to the one-factor plan.

The basis of the research adopted methodology, Maintenance Standard (MS) AIST 5.6-2010. "Tests of agricultural machinery. Sowing and planting machines. Destination metrics. General requirements" and Interstate Standard (IS) R 52778-2007 "Testing of agricultural machinery. Operational and technological assessment methods". The coefficient of variation was taken as a criterion for the uneven distribution of seeds over the sieving area.

Investigations of the grain seeder opener at different levels in fertilizing and sowing seeds were carried out with the laboratory equipment (Figures 1, 2). This equipment consists of a drive trolley (11), including a frame of the drive trolley (10), which are mounted on the soil channel (4). The tested grain seeder opener (15) is fixed on the drive trolley (11) and mounted the sowing system, consisting of a hopper for seeds (12), sowing apparatus (13) and seeds tube (14). The trolley (11) is driven through a cable using a gear motor (5). The motor gearbox (8) using chain gears drives the shaft (13) of the sowing coils. Management of the soil canal is carried out from the remote control (18).

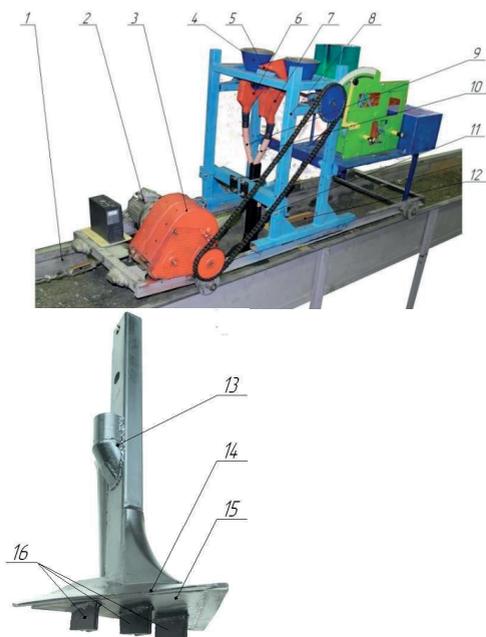


Figure 1. General view of the laboratory equipment and experimental grain seeder opener:

- 1 - soil channel; 2 - gear motor; 3 - chain gear; 4 - seed hopper; 5 - fertilizer hopper; 6 - seed sowing apparatus; 7 - fertilizer sowing apparatus; 8 - drive trolley; 9 - seeds tube; 10 - piping; 11 - grain seeder opener; 12 - screening surface; 13 - rack pipe line; 14 - lancet paw; 15 - sole; 16 - hollow wedges

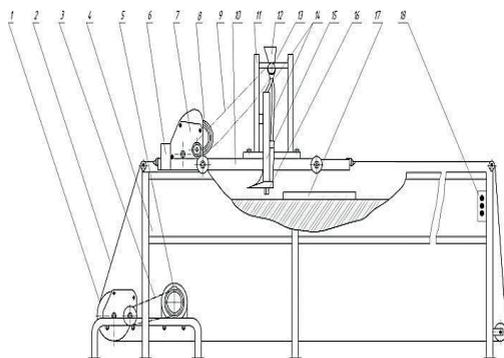


Figure 2. Scheme of the laboratory equipment:

- 1 - the system of pulley blocks; 2 - cable; 3, 9 - chain transmission; 4 - soil channel; 5, 8 - gear motor; 6 - frequency converter; 7 - gear; 10 - drive trolley; 11 - hitch; 12 - hopper; 13 - seed and fertilizer sowing machines; 14 - seeds tube; 15 - grain seeder opener; 16 - hollow wedges; 17 - screening surface; 18 - control panel

To bring the experimental conditions closer to the real ones, the grain seeder opener (15) was mounted on the drive carriage (11) so that the

hollow wedges (16) of the fertilizer were deepened into the soil to the working depth, and the cutting plane of the grain seeder opener practically touched the soil surface. Seeds were poured into the hopper (12) (at least $\frac{3}{4}$ of its total volume) and the sowing apparatus was filled with seeds, starting it for a while. Next, the seeding rate was set using the frequency converter (6). From the control panel (18) the trolley and sowing apparatus (13) were turned on. When the grain seeder opener sole moved (15), the hollow wedges (16) were buried in the soil, forming lines for fertilizing 3 cm below the soil surface, then the sole sealed the lines and leveled the surface of the seed bed. In this case, the seeds passing through the sowing apparatus (13) and seeds tube (14), falling on the seed distributor, were distributed over the soil surface prepared by the grain seeder opener sole. Furthermore, a frame (17) with 5 x 5 cm cells was placed on the sieving surface. To get as close as possible to real conditions, the grain seeder opener (15) was mounted on the drive carriage (10) so that the plane of movement of the grain seeder opener (15) with hollow wedges (16) practically touched the sieving surface (box for collecting fertilizers) (17).

The drive carriage (11) is driven by a gear motor (5) by means of a chain hoist (1) and a chain gear (3). The shaft of the fertilizer metering device (13) of the reel and pin type, is driven by a gear motor (8) by means of chain gears (3), a multi-stage gearbox (7). Rotational speed of the drive shaft (13) is carried out using a frequency converter. The control panel (18) controls the equipment.

The experiments were carried out in the following sequence: they fill the hopper with granular fertilizers (at least $\frac{3}{4}$ of its total volume) and made the fertilizer metering machine to fill it with fertilizers. Then, in turn, the fertilizer distributors under study are installed in the fertilizer rack, and at the same time they include the drive of the cart and fertilizer sowing devices. Fertilizers moving from the hopper enter the fertilizer line through a fertilizer meter, passing through the fertilizer spreader and the hollow wedges (16) fall into the fertilizer container.

The variation coefficient of the seeds distribution over the sieving area with the grain

seeder opener depends on many factors and their interaction with each other, which cannot be fully covered during the research.

When using grain seeders with reel sowing machines and grain seeder opener, the distribution of seeds over the sieving area can be described by Poisson's law:

$$D_m = \frac{\lambda^m}{m!} \cdot e^{-\lambda}$$

λ - the average number of seeds on the accounting row length; m - a random number of seeds ($0 = 1$); e - the base of the natural logarithms ($e \approx 2,718$).

The uneven distribution of seeds over the sieving area is evaluated by choosing squares with the same number of seeds ($W = 0, 1, 2, \dots, n$) and counting their number n_w .

The frequency of the squares is calculated by the formula:

$$\bar{P} = \frac{n_w}{n}$$

n_w - the number of seeds located in squares of 0, 1, 2 or more pieces; n - the total number of accounting squares (at least 300 pcs.) (Shumaev et al., 2016).

Average density \bar{m} (average number of seeds) is found from the expression:

$$\bar{m} = \frac{n_c}{n}$$

n_c - total number of seeds squared.

We calculate the variation indicators: uneven distribution (coefficient of variation) (ν); standard deviation (σ), accuracy indicator (P) and the main mistake (ε).

Analyzing the uneven distribution of seeds, it is necessary to find out if there are any coincidences of the optimal, experimental frequencies of empty squares and squares with one plant.

Frequency Probability Dependencies P_0 and P_1 from the seeding rate, taking into account the field germination R_{ec} are presented in the form of nomograms. Optimal values P_0 and P_1 is determined after implementation of the R coefficient based on the seeding density \bar{m} in pieces per square 5x5 cm.

To determine the dependence of the design of the grain seeder opener at different levels in fertilizer application and sowing of seeds on the uneven distribution of seeds over the sieving area, conclusions are drawn by comparing the calculated frequency values with the experimental ones.

The studies were carried out at a seeding rate of 240 kg/ha, the grain seeder opener was moved at a speed of 2.5 m/s, while the sowing unit was installed at a height of 0.95 m.

The optimization criterion was the non-uniformity (coefficient of variation) of the distribution (ν) seeds and fertilizers according to the sifting area. Since it is impossible to take into account the influence of all factors when studying the process of seed distribution over the sieving area, therefore, 14 factors that most affect the grain seeder opener work were initially identified. Then, based on the specific tasks of the study, as well as a priori information, more significant factors were identified: the length of the wedges, the angle of inclination of the wedges in the longitudinal-vertical plane, the distance between the rows of wedges in the longitudinal-vertical plane, the width of the wedges, the width of the working part of the sole of the grain seeder opener necessary for closing grooves of wedges, the height of the sole relative to the cutting edge of the paw, the height of the seed distributor relative to the cutting edge of the paw, the speed of the grain seeder opener. To identify more significant factors, a screening experiment was carried out, which made it possible to reduce the number of further experiments by screening further insignificant factors. Further, the basis of a three-factor experiment was laid D-optimal plan, as a result of which regression equations were obtained for the process of sowing seeds of grain crops with simultaneous multi-level fertilizer application. The research results were processed on a personal computer using computer programs: Statistica 6.0 RUS, MathCAD 15, Microsoft Office and etc.

RESULTS AND DISCUSSIONS

To solve the problem, a grain seeder opener of different levels of fertilizer application and seed distribution was developed, manufactured and tested.

When determining the effect of the installation of hollow wedges (Figure 3) on the sowing quality, the coefficient of distribution of seeds variation over the sifting area was taken as an optimization criterion (v , %). This parameter depends on many factors. In this regard, laboratory studies were carried out using the methodology for planning a multifactor experiment in a laboratory setting.

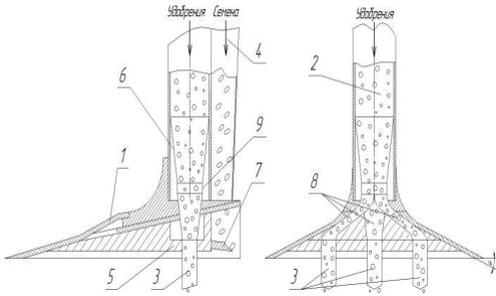


Figure 3. Grain seeder opener at different levels in fertilizer application and seed distribution:
 1 - lancet paw; 2 - rack-pipe line; 3 - hollow wedges (knives); 4 - seeds tube; 5 - sole; 6 - guide funnel; 7 - seed distributor; 8 - supply channels; 9 - fat stream divider

After processing the results of a multivariate PC experiment in the Statistika 6.0 program, we obtained an adequate second-order mathematical model describing the dependence $v = f(\psi, \gamma, b)$ in encoded form:

$$Y = 46,635 - 0,129x_1 + 0,324x_2 + 0,257x_3 - 1,069x_1^2 - 1,639x_2^2 + 0,949x_3^2 + 0,355x_1x_2 - 1,797x_1x_3 - 0,099x_2x_3$$

To describe the response surface by a second-order equation, the theory of planning a multifactor experiment of uniform-uniform table design was used. After processing the results, an adequate model of the coefficient of variation of seed distribution (v , %) was obtained, which in decoded form will be written as:

$$v = 44,2256 + 0,047 \cdot \psi + 10,3378 \cdot \gamma - 0,0888 \cdot b - 0,0005 \cdot \psi^2 - 11,1983 \cdot \gamma^2 + 0,0014 \cdot b^2 - 0,0008 \cdot \psi \cdot \gamma - 0,0021 \cdot \psi \cdot b + 0,0676 \cdot \gamma \cdot b$$

To study the response surface, two-dimensional sections with contour lines were constructed (Figure 4).

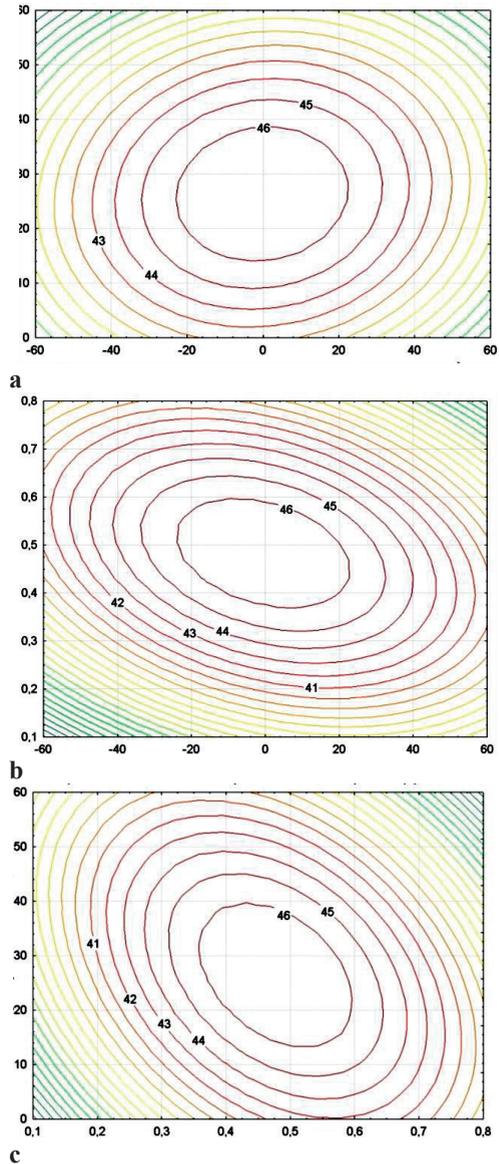
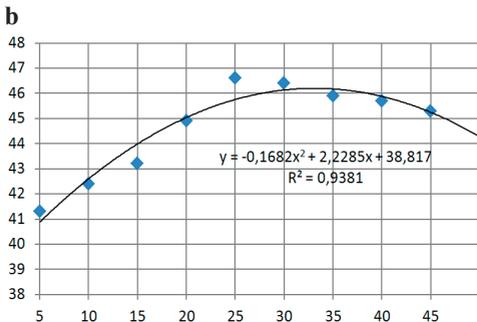
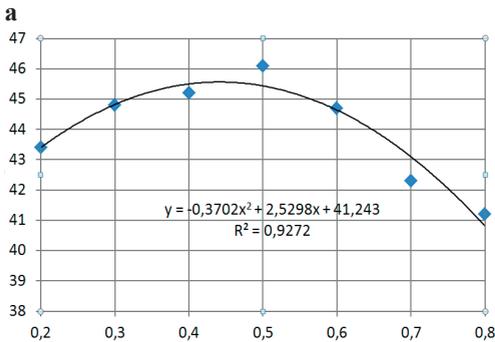
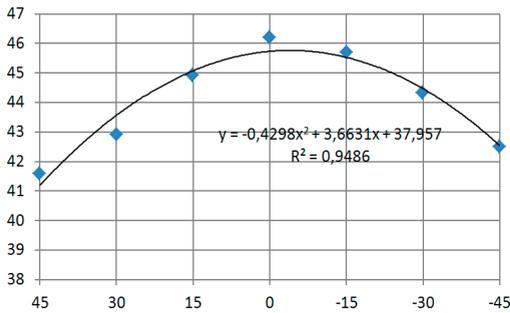


Figure 4. Two-dimensional cross-section of the response surface, characterizing the dependence of the coefficient of variation of the distribution of seeds over the sieving area (V):

a) from the angle of inclination of the hollow wedges in the longitudinal vertical plane (ψ) and the width of the working part of the sole of the opener necessary for sealing furrows of hollow wedges (b); b) from the angle of inclination of the hollow wedges in the longitudinal vertical plane (ψ) and the tangent of the half angle of the solution of hollow wedges (γ); c) from the tangent of half the angle of the solution of hollow wedges (γ) and the width of the working part of the sole of the opener necessary for sealing furrows of hollow wedges (b)



c

Figure 5. Dependence of the uniform distribution of seeds over the sieving area on the angle of inclination of hollow wedges in a longitudinally vertical plane (a), the tangent of the half angle of the solution of the hollow wedges (b), the width of the sole of the opener necessary for filling the grooves of the hollow wedges (c)

Analyzing the graphic image of two-dimensional sections (Figure 4), we could conclude that the optimal values of the studied factors are in the intervals:

$$\psi = -22.7 - 21.5 \text{ deg}$$

$$\gamma = 0.37 - 0.59$$

$$b = 14.2 - 38.3 \text{ mm}$$

in this case, the optimization parameter (v is the coefficient of variation of the seeds distribution over the sieving area) would accordingly be equal to 46%.

Based on the results of the field experiment, the dependences of the uniform distribution of seeds on the angle ψ of the inclination of the hollow wedges in the longitudinal-vertical plane, the tangent γ of the half angle of the solution of the hollow wedges, the width b of the working part of the sole of the opener necessary for filling the grooves of the hollow wedges were plotted and presented in Figure 5 (Larushin et al., 2016).

According to studies, the optimal uniformity of the distribution of seeds over the sifting area was obtained with an angle of installation of hollow wedges in the longitudinally vertical plane (forward-backward) $\psi = -7-10$ deg, the tangent of half the angle of the solution of hollow wedges $\gamma = 0.39-0.47$, the width of the sole necessary for filling the furrows behind the hollow wedges - $b = 27-39$ mm, with a distribution variation coefficient of at least 45.5%.

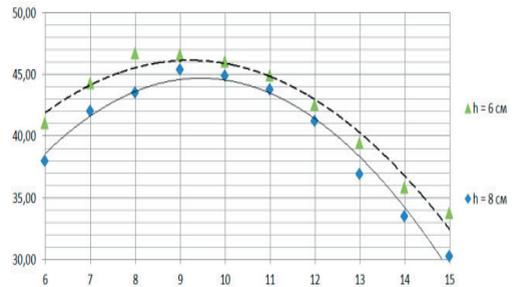


Figure 6. Effect of aggregate speed and sowing depth on the value of the coefficient of the seeds distribution variation over sifting area

It was also determined the influence of the speed of movement and the depth of tillage on the coefficient of the seeds distribution variation over the sowing area of the grain seeder opener (Figure 6). As a result of research, it was found that the optimal speed of the experimental grain seeder opener with a sowing depth of 6 cm should be 8.4-9.7 km/h with a coefficient of variation in the distribution of seeds of at least 45.5%.

Industrial studies have shown that the experimental grain seeder opener at different levels of fertilizer application and seed distribution compared with the base seeder provides an increase in spring wheat productivity to 0.27 t/ha.

At the same time, it ensures the introduction of the starting dose of fertilizers together with the seeds and the main dose of fertilizers below the sowing bed with a soil layer of 21.5-25.3 mm. The uniform distribution of seeds by an experimental grain seeder opener will be at least 45.5% (uniform distribution of seeds by a basic seeder is 40%).

CONCLUSIONS

In the course of studies the developed grain seeder openers, it was found that the use of an experimental grain seeder opener provides an increase in spring wheat productivity to 0.27 t/ha.

Economic calculations confirm that the use of the grain seeder opener with coulter at different levels of fertilizer application and seed distribution is economically feasible. The annual economic effect at a standard annual

load of 160 hours amounted to 412824.55 rubles. on one seeder, with a payback period of 1.43 years.

It was found that due to the use of the proposed design, the dose required for applying mineral fertilizers is reduced to 40%, which would undoubtedly have a beneficial effect on reducing the environmental load on the soil.

REFERENCES

- Kalabushev, A.N., Laryushin, N.P., Shumaev, V.V. (2019). Theoretical calculation of some parameters of the grain seeder opener. *Volga Region Farmland*, 1(50), 151–156.
- Shumaev, V.V., Polikanov, A.V., Machnev, A.V. (2016). *Research Methods: Study Guide*. Penza: RPO PSAA.
- Larushin, N.P., Shukov, A.V., Shumaev, V.V. (2016). The theoretical basis for calculating the working bodies of sowing machines. *Scientific Papers. Penza: RPO PSAA*.

THE INFLUENCE OF CLASSICAL AND FOLIARE FERTILIZATION WITH HUMIC ACIDS ON THE PRODUCTIVITY ELEMENTS OF GROUNDNUTS

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Abstract

The purpose of this study was the analysis of the organic foliar fertilization influence on the groundnut yield. The experience was located at SD Banu Mărăcine, Tâmburești farm, on sandy soil, in 2015-2017. The experiment included two factors. The graduations of A factor (A_1 and A_2) were consider fertilization type. First graduation A_1 - normal fertilization of soil with NPK complex fertilizers (20:20:0), 200 kg/ha and the second graduation A_2 - same fertilization of soil and three extra leaf fertilization with Humusoil, 2 l/ha. B factor (genotype) had 6 graduations (genotypes). The analyzed characters were: pods yield, efficiency, total number of pods/plant, percent of total mature pods/plant, weight of 1000 pods/plant and weight of 1000 seeds/plant. Fertilization and genotype and their interaction statistically influenced all characters, except percent of total mature pods/plant. In the case of analysis of the interaction of the two factors, concerning the pods yield, the best results are obtained from the genotypes Solar and Venus at the A_2 graduation (2884.24 kg/ha pods yield respective 2895.45 kg/ha pods yield), by additional foliar fertilization the genotypes registering superior results compared with the basic fertilization.

Key words: pods yield, yields elements, PCA, characters correlations, genotype, fertilization type.

INTRODUCTION

Groundnuts (*Arachys hypogaea*) are considered one of the most important tropical plants. They are mainly cultivated for their grains rich in fat and protein as well as for their high quality oil. The demand for groundnut is increasing due to its many uses and high nutritional value. Also, different parts of the groundnut can be utilized for different purposes, such as, groundnuts leaves, which provide good-quality roughage after the crops was harvested. Groundnut haulms constitute approximately 45% of the total plant biomass and provide excellent forage for livestock (Özyiğit and Bilgen, 2013). Higher quantities of chemical fertilizers have been used in our country since the 1970s and the rapid growth of agricultural fertilizer has played an irreplaceable role in promoting the development of agricultural production, but there are many problems. With the transition to the intensive agriculture and the use of higher doses of fertilizers it became necessary to create new varieties able to use superior quantities of nutrients (Iancu et al., 2019b). Excessive introduction of chemical fertilizer not only results in a great waste of resources,

but also increases the risk of environmental pollution. It is therefore necessary to limit the use of fertilizers and synthetic chemical pesticides because of the harmful effect on the environment (Soare et al., 2018).

The intensive application of the synthetic fertilizing substances determines the decrease of the organic matter content in the soil, the base of its fertility. The application of humic acids and fulvic acids at optimum concentrations can be an alternative to chemical fertilization, with an effect on plant growth and development, as well as changing the bioavailability of nutrients in the soil. These products and methods of fertilization are modern technologies with a positive impact both economically and on the environment.

Organic fertilizers take the place of the chemical. Dinu et al. (2017) showed that the tomatoes cultivated in ecological system and fertilized in vegetation with the Folicist organic product had a good quantitative and qualitative yield.

The intensification of agriculture has conducted a critical point where the negative impact derived from this activity are now resulting in

irreversible global climate change and loss in many ecosystems, and one alternative solution maybe the use of plant biostimulants based on humic substances (Canellas et al., 2015).

The application of humic and fulvic acids at optimal concentrations can be an alternative to chemical fertilization, with an effect on plant growth and development, as well as changing the bioavailability of nutrients in the soil. These products and methods of fertilization are modern technologies with a positive impact both economically and on the environment.

Many studies conducted both in the open field and in the laboratory have provided experimental evidence on the beneficial action of humic substances (HS) on plant growth. In recent years, research has focused on the use of humic acids as foliar fertilizers in various crops: tomatoes (Dinu et al., 2015; Dinu et al., 2013), melon (Soare et al., 2018), cabbage (Soare et al., 2017), vines and sunflower (Pârvan et al., 2013), sugar beet, maize and sunflower (Sîrbu et al., 2016). Yield components as well as quality parameters of sweet potato tubers grown into biocyclic humus soil were determined in comparison with tubers grown conventionally and showed significant increases (Eisenbach et al., 2018).

The purpose of this study was to compare the influence of classical complex fertilizers and extra foliar fertilization with the Humusoil product on the morphological and productivity characteristics of groundnuts cultivated in the conditions of southern Romania.

MATERIALS AND METHODS

Yield potential and some yield elements evaluation of groundnuts was conducted at Tâmburești Research Station of University of Craiova, Romania, located at latitude of 44°1'40" N, longitude 23°56'9" E, and 52 meters above sea level, on sandy soil. The quantity of humus is low (0.3-0.5%). The sands are very poor in nitrogen and phosphor and mediocre supplied with potassium (0.03-0.05% total nitrogen; 2-5 mg/100 g soil phosphor assimilable and approximately 12 mg/100 g soil assimilable potassium).

The experiment was carried out during 2015-2017 and has the aim to study the influence of foliar fertilization with Humusoil upon

groundnut crop. Humusoil is a natural organic growth stimulator produced from Leonardite of plant origin 100% and contain 12.5% humic acid and 2.5% fulvic acid. The sow was made at the beginning of May, at a distance of 50 cm between rows and 20 cm between plants/row. Biological material was sowed after randomized block design with three replicates. In this experience, the technological sequences specific to groundnut crop were applied. Humusoil was applied in 3 stages: at the vegetative growth, at flowering and at pods formation.

The analyzed characters were: pods yield, efficiency, total number of pods/plant, percent of total mature pods/plant, average weight of 1000 pods and average weight of 1000 seeds.

The experiment included two factors. The graduations of A factor (A₁ and A₂) were considered: fertilization type. First graduation A₁-normal fertilization of soil with NPK complex fertilizers (20:20:0), 200 kg/ha and the second graduation A₂ - same fertilization of soil and three extra leaf fertilization with Humusoil, 2 l/ha. B factor (genotype) had 6 graduations (6 genotypes): Tâmburești, Virginia B, Argentine, Jelud, Solar and Venus. From the combination of the two factors resulted the next variants:

A factor	B factor	A x B
A ₁ 200 kg/ha NPK fertilizers	Tâmburești	a ₁ b ₁
	Virginia B	a ₁ b ₂
	Argentine	a ₁ b ₃
	Jelud	a ₁ b ₄
	Solar	a ₁ b ₅
	Venus	a ₁ b ₆
A ₂ 200 kg/ha NPK fertilizers and extra Humusoil leaf fertilization	Tâmburești	a ₂ b ₁
	Virginia B	a ₂ b ₂
	Argentine	a ₂ b ₃
	Jelud	a ₂ b ₄
	Solar	a ₂ b ₅
	Venus	a ₂ b ₆

Statistical methods

Data were statistically analyzed and means were compared by least significant differences (LSD), $p \leq 0.05$ (Ciulcă, 2006).

Correlation analysis and coefficients were compare after Pearson significance values and Principal component analysis (PCA) was performed based on the analyzed indices. Both correlation and PCA were performed by IBM SPSS Version 2011 and MS Office Excel 2016.

RESULTS AND DISCUSSIONS

The number of pods that are formed by a plant is an important element of yield and of these the mature pods have an important role.

As concern the influence of A factor (fertilization), except for the character percent of total mature pods/plant, in all other characters the average values registered at graduation A₂ have significant differences compared with the average values registered at graduation A₁.

Regarding the number of pods character, it showed small amplitude of variation for both the fertilized variants and for the additional fertilized variants with the Humusoil product.

It is noted also that the variants fertilized in addition to the product Humusoil showed an increase in the percent of mature pods, so it can be seen that the product Humusoil had a positive effect on the processes of fruiting and maturing of groundnuts. In the case of percent of total mature pods/plant, no statistically significant difference was identified between the two average values recorded for the two factors graduations (Table 1). In a similar experiment analysis of yield indicate that the mean of the irrigated variants recorded significant differences compared to the average of non-irrigated variants and drought can lead to a substantial decline in yield, but this reduction depends on genotype (Iancu et al., 2019a).

Table 1. The influence of factor A on the studied characters

A Graduation \ Value	Pods yield (kg/ha)	Efficiency (%)	Total number of pods/plant	Total mature pods/plant (%)	Weight of 1000 pods (g)	Weight of 1000 seeds (g)
A ₁	2237.278 ^b	63.38 ^b	16.63 ^b	72.09 ^{ns}	568.17 ^b	403.94 ^b
A ₂	2580.909 ^a	67.20 ^a	18.88 ^a	75.81 ^{ns}	599.52 ^a	426.04 ^a
LSD 5%	122.89	1.53	0.44	8.99	14.95	10.93

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \leq 0.05$

Regarding the influence of factor B (genotype), in the case of pods yield kg/ha, the highest results are obtained by the genotypes Solar and Venus, which have significant differences compared to all other genotypes analyzed. The last classified genotype is Argentine, which shows significant negative differences compared to 4 of the 6 analyzed genotypes (Table 2). Concerning efficiency character, the best results are obtained by the Argentine, Jelud and Tâmburești genotypes, which record positive differences compared to the other three genotypes, between them there are no statistically significant differences.

In case of total number of pods/plant character, the best results are recorded by the genotypes Argentine and Venus, which record positive differences compared to the other four genotypes, between them there are no statistically significant differences.

Regarding the analysis of the percent of total mature pods/plant character, no statistically appreciated differences were recorded.

Regarding the average weight of 1000 pods/plant character, the best results are obtained by the genotypes Virginia B, Solar and Venus, which register significant differences

compared to the other three genotypes, between them there are no statistically appreciated differences. The accumulation of biomass is intense in groundnut plants, especially in July and August, which is corresponding to the formation and maturation of pods (Iancu et al., 2014b).

Concerning average weight of 1000 pods character, the average values recorded by the first two genotypes, respectively Solar and Venus, obtain significant differences compared to all the other average values recorded. Also, they differ significantly in value, the last one being the Tâmburești genotype.

Fertilizers and variety represents adequate agricultural practices to increase yield to groundnut crop (Iancu et al., 2019b).

In the case of analysis of the interaction of the two factors, concerning the pods yield, the best results are obtained from the genotypes Solar and Venus at the A₂ graduation (2884.24 kg/ha pods yield respective 2895.45 kg/ha pods yield), the values recorded by them differing significantly from all the other values, in general, at the foliar fertilization the genotypes registering superior results compared with the basic fertilization (Table 3).

Table 2. The influence of factor B on the studied characters

B Graduation (genotype) \ Value	Pods yield (kg/ha)	Efficiency (%)	Total number of pods/plant	Total mature pods/plant (%)	Weight of 1000 pods (g)	Weight of 1000 seeds (g)
Tâmburești	2354.39 ^{bc}	67.64 ^a	17.10 ^b	74.43 ^{ms}	484.10 ^c	300.52 ^c
Virginia B	2499.06 ^b	61.64 ^c	15.69 ^c	68.50 ^{ms}	672.17 ^a	334.66 ^d
Argentine	2036.12 ^d	67.22 ^a	19.69 ^a	76.15 ^{ms}	444.53 ^d	353.28 ^c
Jelud	2103.80 ^{cd}	67.93 ^a	17.70 ^b	76.01 ^{ms}	527.62 ^b	439.06 ^b
Solar	2734.39 ^a	62.75 ^{bc}	17.17 ^b	73.73 ^{ms}	684.87 ^a	525.00 ^a
Venus	2726.79 ^a	64.56 ^b	19.20 ^a	74.62 ^{ms}	689.77 ^a	537.41 ^a
LSD 5%	212.86	2.64	0.76	15.57	25.89	18.93

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \leq 0.05$

Some authors in their researches with groundnuts cultivated on sands reported 2359 kg/ha pods yield, applying nitrogen fertilizer in dose of N90 on two occasions, the first at sowing, the second in vegetation (Dima et al., 2013).

Other studies indicate that yield is also influenced by the type of fertilization and irrigation and non-irrigation, being until 2020.95 kg/ha pods yield (Iancu et al., 2019b). Humidity is a key factor of the groundnuts crop technology, the presence or absence of this factor influencing other factors such as nitrogen fertilization (Iancu et al., 2015).

The increase of yield and its components according to adding organic fertilizer may provides nutrient rich in organic carbon for the microbial biomass which converts unavailable nutrient in plant residues to one's available (Zaki et al., 2017).

It can say that groundnut yield is influenced by many factors: genotype, fertilization, irrigation or non-irrigation and also by drought. Pod yield in some genotypes decrease in drought conditions, reduced number of pods and height of plants (Soare et al., 2016).

Regarding the efficiency, the best result is recorded by the genotypes Tâmburești, Jelud, Argentine and Venus on the A₂ graduation.

In the case percentage of total pods/plant, the best results are obtained by the most productive genotypes at the A₂ graduation, while the last classified variants are found in the A₁ graduation at the genotypes with the lowest biological potential.

Regarding the percent of total mature pods/plant, there was no statistical difference, that is, not only that the single action of the two factors does not influence this index, but also the interaction of the two does not influence this character.

The average weight of 1000 pods reaches the highest values by the variants of the Solar and Venus genotypes at A₂ graduation, while the lowest values are registered at the A₁ graduation by the least productive genotypes. In others studies, moderate doses of nitrogen increases the number of pods/plant and phosphorus, potassium and especially irrigation, have contributed in making a larger number of seed/plant (Iancu et al., 2014a).

In the case of average weight of 1000 seeds, the highest values are recorded in the genotypes with the highest productive potential at graduation A₂, while the lowest values are also found in the genotypes weakly productive at graduation A₁.

Table 3. The influence of A and B factors interaction on the studied characters

a _i b _j \ Value	Pods yield (kg/ha)	Efficiency (%)	Total number of pods/plant	Total mature pods/plant (%)	Weight of 1000 pods (g)	Weight of 1000 seeds (g)
a ₁ b ₁	2186.67 ^{fbh}	65.75 ^{bcd^{df}}	15.98 ^f	72.89 ^{ms}	469.29 ^{bh}	325.11 ^{fg}
a ₁ b ₂	2302.67 ^{de}	59.80 ^g	14.01 ^g	66.43 ^{ms}	654.77 ^d	427.50 ^d
a ₁ b ₃	1868.00 ^h	65.58 ^{cd^{df}}	18.90 ^b	74.05 ^{ms}	434.11 ^h	292.75 ^h
a ₁ b ₄	1923.67 ^{gh}	66.22 ^{abcd}	16.69 ^{def}	74.66 ^{ms}	514.51 ^{ef}	344.10 ^{ef}
a ₁ b ₅	2573.33 ^{cd}	60.73 ^g	16.30 ^{ef}	72.08 ^{ms}	666.19 ^{cd}	510.83 ^c
a ₁ b ₆	2569.33 ^{cd}	62.22 ^{fg}	17.91 ^{bc}	72.39 ^{ms}	670.17 ^{bcd}	523.34 ^b
a ₂ b ₁	2522.12 ^{cd}	69.53 ^{ab}	18.21 ^{bc}	76.75 ^{ms}	498.92 ^{fg}	344.20 ^{ef}
a ₂ b ₂	2695.46 ^{ab}	63.48 ^{d^{fg}}	17.38 ^{cd}	70.57 ^{ms}	689.57 ^{abcd}	450.63 ^d
a ₂ b ₃	2204.24 ^{ef^{gh}}	68.85 ^{abc}	20.49 ^a	78.25 ^{ms}	454.95 ^h	308.29 ^{gh}
a ₂ b ₄	2283.94 ^{def}	69.65 ^a	18.70 ^b	77.37	540.74 ^c	362.46 ^c
a ₂ b ₅	2895.45 ^a	64.77 ^{df}	18.03 ^{bc}	75.38 ^{ms}	703.55 ^{ab}	539.16 ^{ab}
a ₂ b ₆	2884.24 ^a	66.91 ^{abcd}	20.48 ^a	76.85 ^{ms}	709.37 ^a	551.48 ^a
LSD 5%	301.03	3.73	1.07	22.02	36.63	26.76

Values represent the mean in the same column followed by different superscript letters are significantly different at $p \leq 0.05$

Regarding the influence of Humus soil treatment on average weight of 1000 seeds/plant, it is observed that the additional fertilized variants with two doses, achieved higher values for this character in all experienced genotypes.

Chemical fertilizers along irrigation ensure high yield increases (Iancu et al., 2014b). Nitrogen fertilizer is an important factor in achieving better growth and development of vegetative and reproductive organs of groundnut and sequently the yield components (Awadalla and Abbas, 2017).

Regarding the analysis of the variation of the correlation coefficients for the studied characters in the variants resulting from the AxB interaction, in the case of pods yield, very significant values of the coefficients were

identified in the case of average weight of 1000 pods and respectively in the case of average weight of 1000 seeds (Table 4).

The efficiency character, records very significant values in the case of correlations with total number of pods/plant, percent of total mature pods/plant and average weight of 1000 pods.

Related to total number of pods/plant, this records a very significant value in the case of correlation with the percentage of total mature pods/plant.

Also, the average weight of 1000 pods is in very close correlation with the character average weight of 1000 seeds, a fact proved by the very significant value of the correlation coefficient.

Table 4. Correlation coefficients variation for the studied characters on the analyzed variants in the case of A x B interaction

Specification	Pods yield (kg/ha)	Efficiency (%)	Total number of pods/plant	Percentage of total mature pods (g)	Weight of 1000 pods (g)
efficiency	-0.130				
total number of pods/plant	0.220	0.725***			
percent of total mature pods/plant	0.068	0.902***	0.873***		
average weight of 1000 pods	0.798***	-0.589***	-0.160	-0.389*	
average weight of 1000 seeds	0.851***	-0.480**	0.011	-0.189	0.949***

P 5%=0.325, P1%=-0.418, P 0.1%=-519

Regarding PCA analysis, the first two components account for 93.708% of the total version, of which the first component registers

55.797 and the second component 37.910% (Table 5).

Table 5. Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.348	55.797	55.797	3.348	55.797	55.797	2.846	47.438	47.438
2	2.275	37.910	93.708	2.275	37.910	93.708	2.776	46.270	93.708
3	0.218	3.641	97.349						
4	0.091	1.512	98.860						
5	0.060	1.001	99.861						
6	0.008	0.139	100.000						

Extraction Method: Principal Component Analysis

Regarding the analysis of the first component, positive values record for pods yield and efficiency, this component can be considered as high biological yield potential of producing pods and peanuts with high weight (Table 6).

For the second component, positive values are recorded for efficiency, total number of

pods/plant and percent of total mature pods/plant so this component can be considered as high biological potential of producing a high number of mature pods/plant and with high efficiency.

Table 6. Character Component score Method: Principal Component Analysis - 2 components extracted

Character	Component	
	1	2
Pods yield (kg/ha)	0.938	0.168
efficiency (%)	-0.348	0.898
total number of pods/plant	0.130	0.934
total mature pods/plant (%)	-0.086	0.977
weight of 1000 pods (g)	0.934	-0.309
weight of 1000 seeds (g)	0.974	-0.132

Extraction Method: Principal Component Analysis

The first group consists of 2 variants that have both positive components, these variants being: a_{2b6} and a_{2b5}. This group is characterized by the fact that there are the genotypes with highest yield potential on A₂ graduation and has the highest values for pods yield, average weight of 1000 pods and average weight of 1000 seeds (Figure 1) and high value for the other characters.

The second group consists of 4 variants respectively a_{1b2}, a_{1b5}, a_{1b6}, and a_{2b2}, variants having the first component positive and the second negative. Characteristic for this group is that it is the group with the highest yield potential after the first group. Also, this group has high value for average weight of 1000 pods and average weight of 1000 seeds.

The third group consists of 2 variants respectively a_{1b1} and a_{1b4}, variants that have

both negative components. Characteristic for this group is that it has the lowest values for pods yield and total number of pods/plant and low value for average weight of 1000 pods and average weight of 1000 seeds.

The fourth group consists of 4 respective variants a_{1b3}, a_{2b1}, a_{3b3}, a_{2b3} and a_{2b4}, variants having the first negative component and the second positive. Characteristic for these groups is the fact that it has high value for efficiency, total number of pods/plant and percent of total mature pods/plant and low value for pods yield, weight of 1000 pods and weight of 1000 seeds. Results obtained previously indicate the possibility of using peanut cultivars with medium but stable yields for cultivation and those with higher but fluctuating yields as sources of breeding genes (Soare et al., 2011).

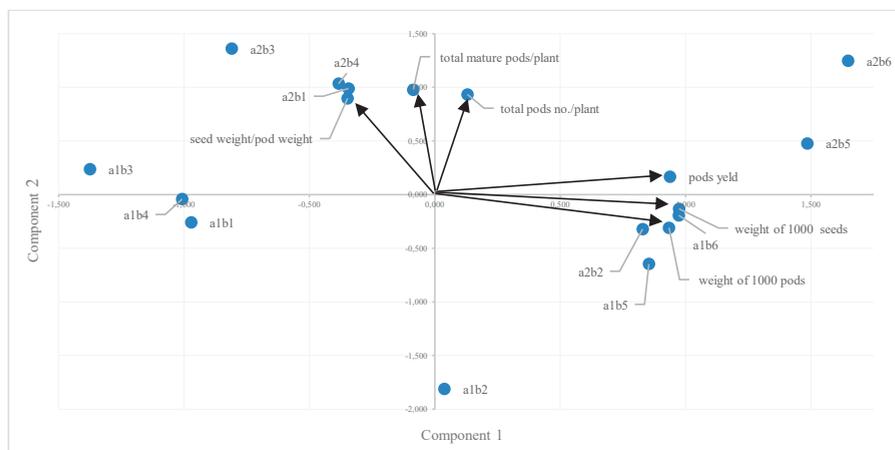


Figure 1. Distribution of the analyzed variants by groups according to PCA analysis

CONCLUSIONS

Concerning the influence of A factor (fertilization), except for the character of percentage of total mature pods/plant, in all

other characters the average values registered at graduation A₂ have significant differences compared with the average values registered at graduation A₁.

Regarding the influence of factor B (genotype), in the case of pods yield, the highest results are obtained by the genotypes Solar and Venus, which have significant differences compared to all other genotypes analyzed. The last classified genotype is Argentine which shows significant negative differences compared to 4 of the 6 analyzed genotypes.

In the case of analyzing the interaction of the two factors on the most studied characters, the best results are obtained on the A₂ graduation. The percent of total mature pods/plant character is the only character that wasn't influenced by the two analyzed factors or by the interaction of those ones.

Regarding the analysis of the variation of the correlation coefficients for the studied characters in the variants resulting from the A x B interaction there were calculated statistical values coefficient for the next cases:

- between efficiency and total number of pods/plant, percent of total mature pods/plant, average weight of 1000 pods and average weight of 1000 seeds;
- between total number of pods/plant and percent of total mature pods/plant;
- between percent of total mature pods/plant and average weight of 1000 pods;
- between average weight of 1000 pods and average weight of 1000 seeds.

Regarding the PCA analysis, the first component had positive values record for pods yield, average weight of 1000 pods and average weight of 1000 seeds, so this component can be considered as high biological yield potential of producing pods and groundnuts with high weight. For the second component, positive values are recorded for efficiency, total number of pods/plant and percent of total mature pods/plant, so this component can be considered as high biological potential of producing a high number of mature pods/plant and with high efficiency. The four groups that resulted from the PCA analysis were the next: the group with both positive components has 2 variants (a_{2b6} and a_{2b5}), the second group with first component positive and the second one negative consists of 4 variants respectively a_{1b2}, a_{1b5}, a_{1b6}, and a_{2b2}, the third group with both negative components consists of 2 variants respectively a_{1b1} and a_{1b4} and the fourth group with first component negative and the second

one positive consists of 4 respective variants a_{1b3}, a_{2b1} a_{3b3}, a_{2b3} and a_{2b4}. Based on all these characteristics and traits studied, it can be noted that the extra application of Humus soil product had favorable effects on the genotypes of groundnuts cultivated on sandy soil from South of Romania.

REFERENCES

- Awadalla, A.O., Abbas, M.T. (2017). Peanut (*Arachis hypogaea* L.) yield and its components as affected by N-fertilization and diazotroph inoculation in Toshka desert soil-South Valley-Egypt. *Environ Risk Assess Remediat.*, 1(3), 40–46.
- Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P. et al. (2015). Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulture*, 30(196), 15–27.
- Ciulcă, S. (2006). *Experimental methodologies in agriculture and biology*. Agroprint Publishing House, Timișoara.
- Dima, M., Croitoru, M., Drăghici, R. (2013). Research on application of NPK fertilizers in peanuts grown on sandy soils. *Scientific Papers. Series A. Agronomy, LVI*, 223–225.
- Dinu, M., Soare, R., Dumitru, M.G. (2017). The effect of organic fertilization on fruit production and quality of tomatoes grown in the solar. *Annals of the University of Craiova, Agriculture, Montanology, Cadastre Series*, 47(2), 112–118.
- Dinu, M., Dumitru, M.G., Soare, R. (2015). The effect of some biofertilizers on the biochemical components of the tomato plants and fruits. *Bulgarian Journal of Agricultural Science*, 21(5), 998–1004.
- Dinu, M., Soare, R., Dumitru, M.G. (2013). Effect of the humic acids and their combination with boron and polyphenols extracted from the seeds of *Vitis vinifera* to culture of tomatoes in solar. *Annals of the University of Craiova, Series Biology, XVIII(LIV)*, 157–163.
- Iancu, P., Păniță, O., Soare, M. (2019a). Response of some new wheat genotypes to nitrogen fertilization and prospects of yield breeding based on yield elements. *Romanian Agricultural Research*, 36(1), 1–9.
- Iancu, P., Păniță, O., Soare, M. (2019b). Evaluation of Drought Tolerance Indices and Nitrogen Fertilization for Some Groundnut (*Arachis hypogaea* L.) Genotypes. *Agricultural Science*, 1(1), 18–29.
- Iancu, P., Păniță, O., Soare, M., Bonciu, E., Soare, R. (2015). Humidity and Nitrogen Dose Influence on Yield Capacity of Some Groundnut Genotypes in Southern Oltenia. *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM I*, 443-450.
- Iancu, P., Soare, M., Matei, G. (2014a). Experimental results concerning the influence of fertilizers and x rays upon quantity and quality of groundnuts yield, in

- irrigated conditions, on sands. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, 43(1), 161–165.
- Iancu, P., Soare, M., Matei, G. (2014b). The influence of chemical fertilisers and soil upon the crop formation to groundnuts and the correlation with the phenomenon of withering. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, 44(1), 107–112.
- Özyiğit, Y., Bilgen, M. (2013). Forage potential of some groundnut (*Arachis hypogaea* L.) cultivars. *Romanian Agricultural Research*, 30, 57–63.
- Pârvan, M.D., Sîrbu, C., Cioroianu, T. (2013). Fertilizer with humic substances. *Romanian Agricultural Research*, 30, 205–211.
- Sîrbu, C., Cioroianu, T., Birescu, G., Grigore, A., Stănescu, A., Rujoi, B., Burtan, L., Mărin, N., Mihalache, D., Iancu, M., Dumitraşcu, M., Nicu, E. (2016). Fertilizers with Humic Substances - Development and Characterization of New Products. *Lucrări Ştiinţifice, Seria Agronomie*, 59(2), 257–260.
- Soare, M., Iancu, P. (2011). Selection indices used to groundnut for choosing the initial material of breeding by irradiation. *Annals of the University of Craiova, Agriculture, Montanology, Cadastre Series*, XLI(2), 231–236.
- Soare, M., Iancu, P., Matei, Gh. (2016). Drought and its influence upon groundnut crop. *SGEM 16th International Multidisciplinary Scientific Geoconference. Advances in Biotechnology*, 6(1), 517–524.
- Soare, R., Dinu, M., Băbeanu, C. (2017). The influence of foliar fertilization with humic acids on the production of White Cabbage. *Annals of the University of Craiova, Agriculture, Montanology, Cadastre Series*, 47(2), 246–252.
- Soare, R., Dinu, M., Băbeanu, C., Soare, M. (2018). The influence of alternative technological sequences on the quality of melon production. *Scientific Papers. Series B. Horticulture*, LXII, 477–483.
- Zaki, N.M., Hassanein, A.M.S., Mohamed, M.H. (2017). Effect of organic and bio-fertilizer on yield and some chemical composition of two peanut cultivars under newly reclaimed sandy soil condition. *Middle East Journal of Applied Sciences*, 7(4), 937–943.

SEED RETAINING MODEL OF NON-DEHISCENCE SESAME (*Sesamum indicum* L.) GENOTYPES AT RIPENING

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Abstract

The retention of seeds in capsules of 25 sesame genotypes intended for mechanized harvesting was investigated. The mentioned share is determined for capsules which when ripe open tops, but retain their seeds because they have attached placenta and narrowing. Before opening, the capsules stay with the tip down to reach 12% humidity of the seeds under natural conditions. A statically adequate regression model for the percentage of seeds retained in the capsules was obtained. The model testifies that the distance of the capsule narrowing relative to its base is most strongly influenced by said percentage, followed by the force by which the placenta retains the seeds. Least but significantly and directly proportional effect on the percentage of retained seed has the average number of branches of a plant. As the number of branches in one plant increases, a greater degree of capsules narrowing are observed. The genetic progress and the opportunities to increase the number of branches in the future sesame crop is shown for breeding improvement.

Key words: sesame (*Sesamum indicum* L.), breeding, seed retention, modelling.

INTRODUCTION

The mechanized harvesting of sesame is an unsolved problem worldwide and is of paramount importance for preserving the culture. Scientists from several countries are working hard on the issue, giving priority to the breeding of varieties suitable for mechanized harvesting (Stamatov et al., 2018). They have to meet three basic requirements:

- Varieties must retain their seeds in the capsules upon ripening until they enter the threshing apparatus. The capsule retains mature seeds by the presence of a placenta attached and due to anatomical features that determine its shape.
- Plants should dry under natural field conditions. If the moisture content of seeds is above 8%, they suffer significant mechanical damage and loss of germination when threshing with a conventional combine. Therefore, inertial threshing is recommended under these conditions (Ishpekov et al., 2016).
- The variety must have high seed yield. The relatively low yields of sesame globally

require directing the selection process towards increasing it (Furat and Uzun, 2010). The wide range of plant gene-plasma provides great possibilities for the genetic improvement of the crop plants. Introduction of gene-plasma allows extension of this range and genetic improvement on valuable economic signs of culture (Zhang et al., 2011).

The purpose of the study is to model the retention of seeds in the capsules when ripening non-dehiscent genotypes of sesame from Bulgarian breeding program.

MATERIALS AND METHODS

Plant material

25 sesame accessions from the national collection in IPGR - Sadovo have been evaluated. Their capsules open the tops but retain their seeds at maturation, because they have a placenta attached and narrowing of the walls. The accessions are the result of eight hybrid combinations, also derived from international exchange and personal correspondence.

Morphological measurements

The accession's indicators of three groups were tested. The first group includes the percentage of retained seeds in the capsules when their tips are turned down under natural conditions. Each accession dries out for a different number of days up to 12% humidity. This position allows the seeds to leave the capsules when they are not attached to the placenta or when their walls do not narrow. During the drying the wind speed changes from 2.0 to 10.3 m/s.

The second group of indicators is related to the yield and includes:

- the seed mass of one plant - ms_1 , g;
- the height of the central stem - hst , cm;
- the number of branches - Nbr ;
- the number of capsules on the central stem - $Ncst$;
- the number of capsules on the branches - $Ncbr$.

The third group of indicators characterizes the shape and dimensions of the seminal chamber (carpel) and includes:

- seminal chamber length - lc , mm;
- the width of chamber at the base, middle and top - bb , bm , bt , mm;
- the dimensions of narrowing's the capsule walls - bn , mm and its distance from the base of the capsule - bn in natural (mm) and relative units (%).

The rate of capsule narrowing was calculated constriction - R_n by the expression:

$$R_n = 100 \frac{b_b - b_n}{b_b}, \% \quad (1)$$

All measurements were made in 10 replicates with an electronic micrometer caliper with an accuracy of 0.01 mm.

Statistical methods

The genetic advance of the number of branches in a plant was estimated using the variance analysis. Evaluation of variation components, phenotypic and genotypic variants was performed according to the method proposed by Burton and Devane (1953). The following statistical estimates are used:

1. Environmental variance:

$$\sigma_e^2 = MSE \quad (2)$$

2. Phenotypic variance:

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \quad (3)$$

3. Genotypic variance:

$$\sigma_g^2 = MST - MSE \quad (4)$$

where:

MSE is the mean square error;
 MST - the mean square treatment;
 r - the replications.

4. Phenotypic coefficient of variation:

$$PCV = 100 \frac{\sqrt{\sigma_p^2 x}}{x} \quad (5)$$

5. Genotypic coefficient of variation:

$$GCV = 100 \frac{\sqrt{\sigma_g^2 x}}{x} \quad (6)$$

where:

σ_p^2 is the phenotypic variance;
 σ_g^2 - the genotypic variance;
 x - the grand mean of a character.

By Johnson et al (1955) genetic advancement and genetic advancement was determined as a percentage of the mean value:

$$GA = \frac{K \sigma_g^2 \sqrt{\sigma_p^2}}{\sigma_p^2} = \frac{K \sigma_g^2}{\sqrt{\sigma_p^2}} \quad (7)$$

where:

GA is the expected genetic advance;
 K - the standardized selection differential at 5% selection intensity ($K = 2.063$).

$$GAM = 100 \frac{GA}{x} \quad (8)$$

where:

GAM is the genetic advance as percentage of mean, %;
 GA - the expected genetic advance.

The analyzed results determine the most important factors that affect seed retention in the capsules. They are included in a regression analysis for obtaining equations in coded and natural form. All statistical analyses are performed at a level of significance $\alpha = 0.05$.

RESULTS AND DISCUSSIONS

The values obtained for the parameters h_n and R_n show that the capsule walls of all genotypes tested have a narrowing. It is located from the base at a height of 46.0 to 75.4% of its length. In two of the accessions (464 and 361-7-3-2-1) capsule walls have narrowing at the base of the seminal chamber and are located from the base

at a height of 24.7 to 27.5% of their length. For this reason, the distance of the capsule narrowing relative to its base and the rate of capsule narrowing are included as factors in regression analysis.

The seed retention in capsules in some varieties is due to the force with which the placenta attached them. That ability is explained by the i_3 index (Ishpekoy and Stamatov, 2015) and therefore its value is also included as a factor in the regression analysis.

The dependencies in Table 1 show that there is no connection between the yield elements, the seed chamber sizes, and the ability of the genotype to retain its seeds in the capsules at ripening. Besides, the mass of seeds from a plant is directly related to the elements of the yield. The sizes of the seed chamber have a strong correlation between each other.

The correlation coefficients obtained indicate a direct dependency between the parameters studied. The results of the PATH analysis reveal the existence of an indirect link between them. The retention of seeds is due to the architecture of the capsules that define the shape of seeds chamber (Langham, 2014). Narrowing the capsule walls causes the seeds retaining in the period of maturing. In Table 2 is seen that the ability of capsules to retain seeds decreases when there are no walls narrowing (direct PATH = - 0.547). This ability increases when the narrowing is closer to the tip, as evidenced by the value of the indirect coefficient (0.596). It is obtained from the

correlation between the length of the seminal chamber and the distance of the narrowing from the base of the capsule. The width at the base of chamber indirectly affects the retention of the seeds (indirect factor 1.821) by the correlation between it and the width in the middle part. Reducing the width in the middle part of the seminal chamber leads to an increase in seed retention (direct PATH = 2.119). Reducing the tip width also leads to seed retention (indirect coefficient 1.570). This is due to the correlation that exists between the width of both the middle of the seminal chamber and tip. Reducing the width in the middle of the chamber leads to increasing of seed retention (direct PATH = 2.119). The absence of narrowing in the seed chamber leads to an increase in the proportion of released seeds (direct PATH = - 1.966). This lack is a reason for their retention, if any (indirect factor 2.00) and is explained from the correlation existing between the width of the middle part of the chamber and the narrowing of capsule. When the constriction is located near the tip, it retains the seeds in the capsule (direct PATH = 1.260). When it is close to the base, its seed retention effect is greatly reduced (direct PATH = -1.206).

The tested accessions have an average of 2.29 branches. This is a sufficient reason to include the average number of branches in a sesame plant as another factor in the regression analysis.

Table 1. Correlation between the surveyed signs

	Ds	msl	hst	Nbr	Ncst	Ncbr	lc	bb	bm	bt	bn	hn	hn (%)
	%	g	cm	number	number	number	mm	mm	mm	mm	mm	mm	
Ds	1	-.339	-.338	-.208	-.374	-.269	.184	-.098	.170	.049	-.054	-.143	-.258
msl		1	.753**	.615**	.510**	.817**	-.350	.037	-.100	.199	.024	-.142	-.021
hst			1	.706	.363	.677**	-.338	-.074	-.101	.195	.058	-.381	-.297
Nbr				1	.301	.783**	-.200	-.354	-.430*	-.212	-.299	-.149	-.081
Ncst					1	.674**	.301	-.051	-.206	-.020	-.161	.273	.137
Ncbr						1	-.059	-.157	-.309	-.148	-.221	.103	.120
lc							1	-.058	-.016	-.162	-.134	.473*	.072
bb								1	.859**	.633**	.881**	.406*	.457*
bm									1	.741**	.944**	.104	.086
bt										1	.791**	-.241	-.231
bn											1	.066	.096
hn												1	.908*
hn (%)													1

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Table 2. Direct and indirect connections between the structural elements of the yield with the anatomical characteristics of the seminal chamber

	lc	bb	bm	bt	bn	hn	hn (%)	Phenotypic correlation
	mm	mm	mm	mm	mm	mm		
lc	-0.547	0.014	-0.034	-0.025	0.267	0.596	-0.087	0.184
bb	0.032	-0.250	1.821	0.097	-1.758	0.511	-0.551	-0.098
bm	0.009	-0.215	2.119	0.114	-1.884	0.131	-0.104	0.170
bt	0.089	-0.158	1.570	0.153	-1.580	-0.303	0.278	0.049
bn	0.073	-0.220	2.000	0.121	-1.996	0.083	-0.116	-0.054
hn	-0.258	-0.101	0.221	-0.037	-0.132	1.260	-1.095	-0.143
hn (%)	-0.040	-0.114	0.183	-0.035	-0.191	1.144	-1.206	-0.258

Dependent variant: % retained of seeds

The relationship between the number of branches in a plant and the width of the chamber is presented in Table 1 ($r = -0.430$). The increase in the number of branches is accompanied by contraction of the walls of the seed chamber. This is the reason for including the average number of branches in one sesame plant as a factor in the regression analysis. The accessions studied formed an average of 2.29 branches.

The analysis of genetic progress for the number of branches in a plant is presented in Table 2. It shows that the phenotypic coefficient of variation (PCV) is 98.2% and significantly higher than the genotypic (GCV) with a value of 8.1%.

PCV and GCV values greater than 20% are considered high, whereas values of less than 10% are considered low and values between 10 and 20% are average (Deshmukh et al., 1986). Based on this argument, the number of branches in a plant is strongly influenced by growing conditions.

In the accessions examined, the low GCV value refers to the conservative nature of the sign and the genes expressing it are in the homozygous state. Johnson et al. (1955) classify the values of genetic advance as a percentage of the mean (GAM) in the following way: values of 0-10% are low, 10-20% is moderate and over 20% are high.

The resulting GAM for our collection has a value of 27.7% (Table 3).

Table 3. The genetic advance of number branches in a plant

	Mean	σ_e^2	σ_g^2	σ_p^2	GCV, %	PCV, %	GA	GAM, %
Nbr	2.29 ± 0.144	2.00	2.21	0.21	8.1	98.2	0.64	27.7

This fact, separately from genetic advance, also points to the possibility of genetic improvement of the indicator in future breeding-improvement work.

A regression analysis was carried out in which the response variable was the percentage of seed retained in the capsules - D_s , % and the following factors:

- The distance from seminal chamber narrowing to the base of the capsule - hn , %;
- The degree of capsules narrowing - R_n , %;
- The value of the index i_3 for each tested genotype;
- The average number of branches per plant - N_{br} ;

The following regression equations were obtained:

In coded form:

$$D_s = 0.1636.N_{br} + 1.2691.h_n - 0.6984.h_n^2 + 0.2931.i_3 \quad (9)$$

In natural form:

$$D_s = 4.5241.N_{br} + 1.4657.h_n - 0.0120.h_n^2 + 41.8993.i_3 \quad (10)$$

The equations have coefficient of determination $R^2 = 0.936$ and probability $pF = 0.00001 < 0.05$. These values testify that regression models include all significant factors that affect the percentage of retained seeds in capsules.

The factors take values from -1 to +1 in the coded regression equation (9). This allows an assessment of the influence power of each factor on the dependant variable by the value of the regression coefficient in front of it. The highest value has the coefficient in front of hn followed by the one in front of i_3 which testify that their influence on the change of the response - D_s is the strongest and most proportionate due to the equal signs in front of the factors. The least impact on the percentage

of retained seeds in the capsules - D_s has the number of branches - N_{br} . The degree of narrowing of the capsule walls - R_n is not included as a predictor in the above equations because it does not significantly affect the response - D_s . Obviously no matter how much narrow the capsules are. It is important to have a narrowing and be closer to the top of capsules to prevent the seeds from releasing. The natural form of regression equation (10) is used to visualize the results of the regression analysis. The statistical significance of the number of branches in a plant as a factor affecting the proportion of retained seeds in the capsules is confirmed by two different statistical criteria. One is PATH coefficient analysis, and the second one is the Student criterion for evaluating the significance of coefficients in the regression equation. In the Figure 1 is shown the effect of the morphological sign - N_{br} on the response - D_s . It is clear that the proportion of retained seeds in the capsules increases proportionally to the average number of branches in a plant. If the average number of branches in a plant is greater than 3, can be expected larger proportion of retained seed in capsules.

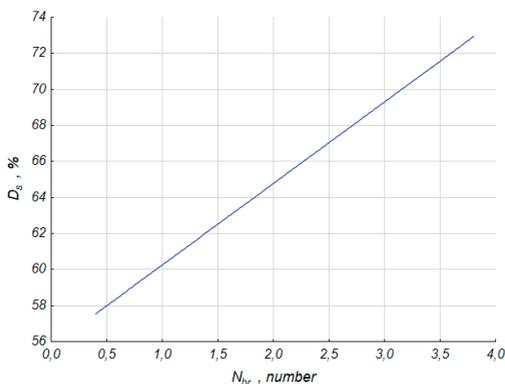


Figure 1. The percentage of retained seeds in the capsules - D_s depending on the number of number of branches in a plant - N_{br} when the distance of narrowing from the base of capsule is $h_n = 54.7\%$ and the index $i_3 = 0.345$

The effect of the two factors characterizing the shape of the capsules on the response - D_s is presented in Figure 2. Obviously the position of the narrowing relative to the base of the capsule and its second degree influences significantly and positively the percentage of retained seeds

in the capsules. The response - D_s grows steeply with the increase in the index i_3 because the strength of the placenta attachment greatly affects the percentage of seeds remaining in the capsules. This percentage reaches to 100% when the genotype had an index $i_3 \geq 1.5$ and its capsules have a narrowing spaced at a distance $h_n \geq 60\%$ relative to the base (Figure 2).

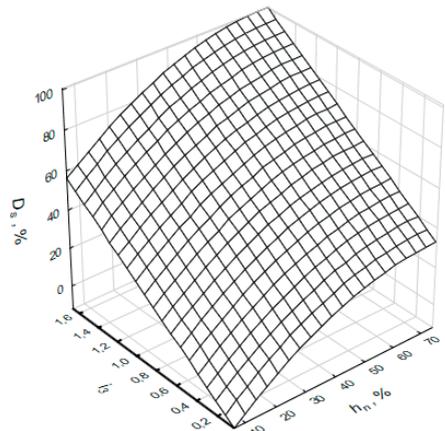


Figure 2. The percentage of retained seeds in the capsules - D_s , depending on the distance of the narrowing from the base - h_n and the index i_3 at an average number of branching from one plant $N_{br} = 2.2$

The results obtained may be implemented in the breeding program associated with the preparation of sesame forms suitable for mechanized harvesting. The justification of a morphological sign that significantly influences seed retention in capsules in the maturation of sesame genotypes can be used to select parent pairs and to provide an additional express method in creating future progenies.

CONCLUSION

A statistically adequate regression model was obtained that included all factors that significantly affect the percentage of seeds that are retained in the capsules when their tips are upside down when drying to 12% humidity in natural conditions. The model testifies that the distance of the capsule's narrowing relative to its base influences the strongest to the seed retention, followed by the strength with which the placenta retains the seeds. Less, but significant and proportional impact on the

proportion of retained seeds in the capsules is the average number of branches per plant.

REFERENCES

- Burton, W.G., Devane, E.H. (1953). Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agron. J.*, 45, 478–481.
- Furat, S., Uzun, B. (2010). The use of agromorphological characters for the assessment of genetic diversity in sesame (*Sesamum indicum* L). *Plant Omics J.*, 3, 85–91.
- Deshmukh, S.N., Basu, M.S., Reddy, P.S. (1986). Genetic variability, character association and path coefficient analysis of quantitative traits in Virginia bunch varieties of ground nut. *Ind. J. Agric. Sci.*, 56, 515–518.
- Ishpekov, S., Stamatov, St. (2015). Method for assessment the susceptibility of sesame genotypes for mechanized harvesting of the seed. *Bulgarian Journal of Agricultural Science*, 21(6), 1230–1233.
- Ishpekov, S., Zaykov, R., Petrov, P., Ruschev, R. (2016). Indices of flow fruit detacher with angular vibrations at inertial threshing of sesame. *Agricultural Engineering International: CIGR Journal*, 18(2), 94–102.
- Johnson, H.W., Robinson, H.F., Comstock, R.E. (1955). Estimates of genetic and environmental variability in soybeans. *Agron. J.*, 47, 314–318.
- Langham, D.R. (2014). Method for mechanical harvesting of improved non-dehiscent sesame US - Patent US8656692 B2.
- Stamatov, St., Andonov, B., Chipilski, R., Deshev, M. (2018). Genetic Variability and Genetic Advance of the Parameters of Water Exchange in Peanut Varieties (*Arachis hypogaea* L.) from the Bulgarian Selection. *JOJ Horticultural*, 1(3): JOJHA. MS. ID 555563, 1–4.
- Zhang, HL., Zhang, DL., Wang, MX., Sun, JL, Qi YW., Li, JJ., Wei, XH., Han, LZ., Qiu, ZE., Tang, SX., Li, ZC. (2011). A core collection and mini core collection of *Oryza sativa* L. in China. *Theor Appl Genet*, 122, 49–61.

EVALUATION OF GENETIC DIVERSITY IN CHICKPEA (*Cicer arietinum* L.) GERMPLASM

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Abstract

The paper aimed to estimate the degree of genetic diversity in 42 chickpea genotypes. The research was carried out between 2016-2017 in the experimental field of the University of Agronomic Sciences and Veterinary Medicine of Bucharest and it is based on the multivariate analysis of several quantitative and qualitative traits. By developing the dendrogram using the AHC algorithm (Agglomerative Hierarchical Clustering), the varieties and lines tested have been divided into two large groups, depending on the TSW (thousand seed weight), the colour and shape of seeds. It was estimated that 73.81% belong to the *Desi* type and 26.19% to the *Kabuli* type. Principal Component Analysis (PCA) showed a significant correlation between the number of pods per plant and the number of seeds per plant. The yield was significantly correlated with the number of pods per plant, number of seeds per plant and TSW (thousand seed weight). The first principal component of the PCA analysis, accountable for a 45.5% share of the total variation between genotypes, has been shown to be an association between the following variables: number of pods per plant, number of seeds per plant and seeds yield per plant. These findings can provide practical information for the selection of parental material and thus assist in planning breeding strategies.

Key words: chickpea, genetic diversity, cluster analysis, phenotypic correlation, Principal Component Analysis.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an annual grain legume or “pulse crop” that is used extensively for human consumption. It is a self-pollinating, diploid with $2n = 2x = 16$ chromosomes (Arumuganathan and Earle, 1991) and has an estimated genome size of 740 Mbp (Varshney et al., 2013). Chickpea is a cool season legume in the Mediterranean region including North Africa, West Asia and South Europe, but tolerates high temperature when mature. Chickpea is playing a leading role in food safety in the world by covering the deficit in proteins of daily food ration of Indian and African Sub Sahara populations (Merga et al., 2019). However, chickpea is an interesting crop not only for the developing African countries and the Asiatic region. It is an important niche crop in Europe and the USA (Lavrenko et al., 2019). The area in the EU is about 172294 ha (out of a world total of 17.8 million ha) with 70609 ha in Spain (www.fao.org, 2018). The chickpea yield in Europe and worldwide is about 1 t/ha (Murphy-Bokern et al., 2014).

Two distinct forms of cultivated chickpeas are *desi* and *kabuli*, depending mainly on their seed size, shape and colour. The *desi* type chickpea is grown in the semi-arid tropics (Muehlbauer and Singh, 1987) and is characterized by small seeds, angular shape, and coloured seeds with a high percentage of fibre. The *kabuli* type has generally large seeds, owl-head shape, beige coloured seeds with a low percentage of fibre. *Kabuli* chickpea is usually utilized as whole grains, while *desi* is processed into flour.

The genetic diversity of genotypes makes them an important resource of genes for breeding programs, developing more diversified farming systems, and new quality products (Jing et al., 2010; Sharifi et al., 2018).

The analysis of genetic diversity of chickpea germplasm can provide practical information for parental selection strategies in plant breeding programs. A significant heterosis effect has long been associated with the allelic richness of hybrids (Ramanujam et al., 1974; Parameshwarappa et al., 2012). Thus, highlighting the genetic distance between the varieties of a given collection facilitates the

identification of a hybrid vigor, by providing insights for a correct and quick selection of divergent forms to be crossed.

Genetic diversity has been traditionally assessed by measuring the variation of qualitative and quantitative phenotypic traits, which are of direct interest to users.

The main drawback of this approach is that the genetic information provided by morphological characters is often limited, as the expression of quantitative characteristics is strongly influenced by environmental conditions.

DNA-based techniques can be used in complementarity with traditional approaches, allowing the identification of polymorphisms at DNA level without the influence of environmental factors. Due to advances in molecular biology, a diverse array of methods to analyze genetic diversity has been developed over the last decade (Ahmar et al., 2020). The use of DNA markers for marker-assisted selection (MAS) is the current trend in modern breeding.

The genetic base of chickpea has narrowed substantially during the domestication process (Thudi et al., 2016). Therefore, increasing the genetic diversity of chickpea has been a major goal for breeders.

Most chickpea breeding programs have been limited to intraspecific hybridization, and the crossbreeding between parents belonging to the *desi* and *kabuli* types has been widely used for the exploitation of genes present in one type. For example, *desi* type parents have contributed to the improvement of *kabuli* types with important genes for resistance to *Fusarium oxysporum* and *Ascochyta* and with drought tolerance genes (Gaur et al., 2006). On the other hand, *kabuli* type parents represented the genetic source for improved seed quality, especially for large seed size in *desi* type breeding programs. Highly productive progenies have been consistently obtained through the *desi* x *kabuli* crosses, representing a source for many new cultivars.

An analysis of the diversity pattern in the global chickpea collection revealed several conclusions about plant traits in relation to their origin (Dwivedi et al., 2009). The European resources produced the largest seeds, having more pods per plant and highest grain yield, while the varieties originating in Africa had the smallest seeds. The African varieties exhibited

the earliest flowering, contrasting the latest flowering varieties originating in East Asia. In addition, the seed colour was found to be the character with greatest diversity among the analyzed pool.

The results of the principal component analysis in various studies using clustering techniques to assess genetic diversity in chickpea revealed the potential of the main quantitative characters for chickpea breeding (Nie et al., 2015; Agrawal et al, 2018; Mahmood et al., 2018).

Nie et al. (2015) classified 100 chickpea genotypes into four groups and indicated the 73.9% of total variance explained by the top four principal components. In a recent study, Sharifi et al. (2018) revealed the clustering of twenty-five chickpea genotypes in two main groups and four clusters. The PCA showed the following characteristics to be the main responsible for the variation within the chickpea genotypes: number of days to flowering, flowering period, number of days to maturity, canopy height, canopy width, biological yield, and number of pods per plant.

The present study was undertaken to estimate the extent of genetic diversity in 42 varieties and chickpea lines through multivariate analysis which will help to select potential parents to develop transgressive segregants in the future breeding program.

MATERIALS AND METHODS

Plant material and field experiments

This study was conducted during the 2016 and 2017 growing seasons at the University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania. Forty-two chickpea genotypes (*Cicer arietinum* L.) were analyzed, of which 40 varieties and lines collected/developed by SCDA Teleorman and 2 chickpea lines improved by USAMV Bucharest (Table 1). The field experiment was performed according to the method of randomized complete block design (RCBD) with 42 variants in three replications. The sowing spacing was 50 cm between rows and 6 cm between plants per row, with 6 m² for an experimental plot.

Several of traits including seed colour, seed shape, plant height (PH), number of pods per plant (NPP), number of seeds per pod (NSP), number of seeds per plant (NSP), thousand

seed weight (TSW) and seed yield per plant (SY) were determined in every year.

Table 1 Name and source of chickpea genotypes

Entry no.	Genotype name	Source*	Entry no.	Genotype name	Source
1	CICERO	SCDA T	22	PP 137	SCDA T
2	KINELSKII 17	SCDA T	23	PP 138	SCDA T
3	DOLINSKII 1	SCDA T	24	NR. 309	SCDA T
4	VISOCOROS LII 30	SCDA T	25	NR. 231	SCDA T
5	STEPNOI I	SCDA T	26	NR. 207	SCDA T
6	ZERNOGRAS KII 30	SCDA T	27	NR. 203	SCDA T
7	KUBANSKII 199	SCDA T	28	NR. 300	SCDA T
8	KOSTILKOV A	SCDA T	29	NR. 303	SCDA T
9	NIGRUM TABOR	SCDA T	30	NR. 183	SCDA T
10	AGONRAE	SCDA T	31	STEPNO VOI	SCDA T
11	DOBRUJANS KII 7	SCDA T	32	PI 468946	SCDA T
12	MALKO GRADISTE 6	SCDA T	33	PI 468938	SCDA T
13	SVOBODINO VA	SCDA T	34	N 279/99	SCDA T
14	KALOFRER	SCDA T	35	N 294/99	SCDA T
15	NR. 2 RUSE	SCDA T	36	N 323/99	SCDA T
16	NR. 45 RUSE	SCDA T	37	N 191/98	SCDA T
17	PP 87	SCDA T	38	N 681/01	SCDA T
18	KUBANSKII 16	SCDA T	39	BURNAS	SCDA T
19	PP 117	SCDA T	40	RODIN	SCDA T
20	PP 130	SCDA T	41	AGRO-N 1-08	USAMV B
21	PP 136	SCDA T	42	AGRO-N 2-08	USAMV B

* SCDA T - Agricultural Research and Development Station Teleorman, Romania; USAMV B - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania

Statistical analysis

The statistical analysis was performed with the Microsoft Excel program and its statistical software, XLStat version 2017. In order to achieve the objectives of the paper, correlation tests, Principal Components Analysis - PCA and Agglomerative Hierarchical Clustering - AHC, were performed.

RESULTS AND DISCUSSIONS

Cluster analysis

Using the AHC - Agglomerative Hierarchical Clustering algorithm based on Euclidean distances and Ward's method, a dendrogram was developed which divided the 42 chickpea genotypes, according to TSW, seed colour and seed shape, into two large groups, marked with A and B (Figure 1). Within group A, two clusters were identified (1 and 2) (Figure 1).

Cluster 1 encompasses a single chickpea genotype, namely Kostilkova, characterized by large seeds, cream colour and edged shape. In the cluster 2 there are 10 chickpea genotypes: PP 117, PP 137, Dolinskii 1, Nigrum Tabor, Agro-N1-08, Kubanskii 199, Cicero, Dobrujanskii 7, No. 2 Ruse and Agro- N2-08.

All chickpea varieties included in the group A are characterized by medium (MMB = 200 - 300 g) and large (MMB = 301-400 g) seeds, cream-colored, round or edged (angular) shape. In group B, 31 genotypes were grouped.

Within group B, two clusters were detected (3 and 4) (Figure 1). In cluster 3, 17 chickpea genotypes are concentrated, characterized by small seeds (MMB < 200 g), with variability in colour: black, cream, reddish-brown and round or edged (angular) shape. Cluster 4 includes 14 genotypes of chickpeas with medium seeds (MMB = 200-300 g), light yellow (cream) to black in colour and edged shape, except for the genotype Malko Gradiste 6, which has a round shape.

According to this analysis we could appreciate that the varieties and lines of chickpeas tested are classified into two types as follows: *kabuli* type (group A) - represents 26.19% and *desi* type (group B) is predominant and represents 73.81%.

In the future breeding programs, crosses between chickpea genotypes from these two major groups (A and B) might lead to a high heterosis, and improved genotypes could be obtained in terms of resistance to biotic and abiotic stress and thus with higher yield potential.

Principal component analysis

Analysis of the main components (PCA) was carried out in order to find the correlations and associations between the six traits and to identify those that describe in a significant proportion the variance between the 42 chickpea genotypes.

Phenotypic correlations between seed yield elements of chickpea genotypes

The correlation matrix generated by the PCA analysis reveals information on phenotypic correlation coefficients and those useful in the study of multicollinearity of variables (Table 2). The results indicates a significant positive correlation among number of pods per plant and number of seeds per plant, as well as

between seed yield per plant and three yield components: number of seeds per plant, number of pods per plant and TSW. The plant height and the number of seeds per pod have recorded correlation coefficients very

close to zero, which means that these traits do not correlate with the other quantitative traits. These results suggest that these two variables could be removed from the study without effect on the quality of the outcomes.

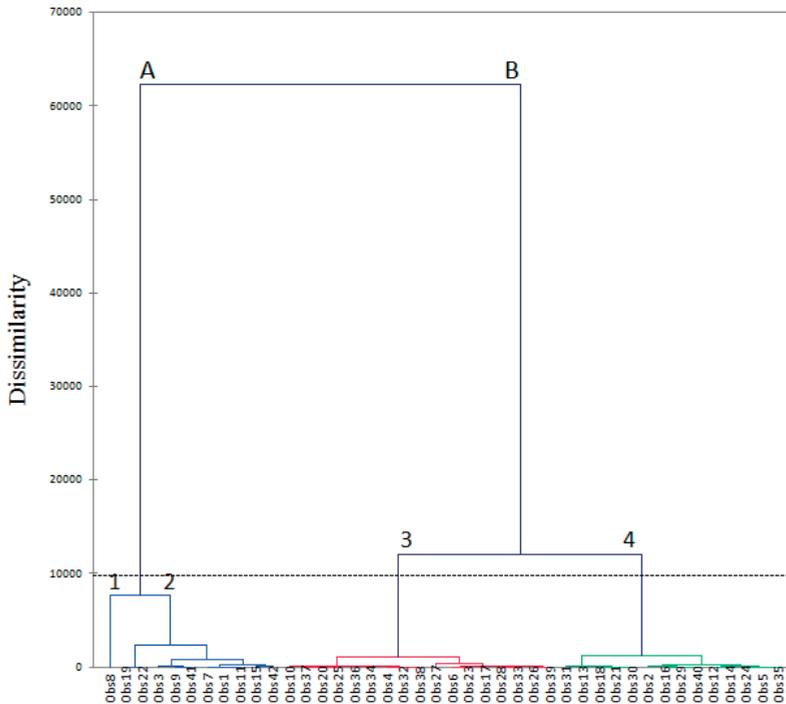


Figure 1. Cluster analysis - dendrogram of 42 chickpea genotypes (*Cicer arietinum* L.) using Euclidean dissimilarity and Ward's method (the name of genotypes given in Table 1)

Table 2. Correlation matrix (Pearson)

Trait	PH	NPP	NSP	NSPL	TSW	SYPL
PH	1	0.090	-0.023	0.075	-0.288	-0.073
NPP	0.090	1	-0.140	0.971	-0.168	0.819
NSP	-0.023	-0.140	1	0.056	-0.030	0.078
NSPL	0.075	0.971	0.056	1	-0.144	0.870
TSW	-0.288	-0.168	-0.030	-0.144	1	0.338
SYPL	-0.073	0.819	0.078	0.870	0.338	1

Bold values are different from 0 at a significance level $\alpha = 0.05$

PH - plant height, NPP - number of pods per plant, NSP - number of seeds per pod, TSW - thousand seed weight (g), NSPL - number of seeds per plant; SYPL seed yield per plant (g).

Factor and biplot analysis

Six principal components (PCs) axes are shown in Table 3 and Figure 2. A trait with coefficient greater than 0.3, was considered as an important trait. Traits having less than 0.2 coefficient value were considered to have no effect on the overall variation (Adebisi et al., 2013). The results show that only the first four

factors need to be taken into account for analysis, as they are responsible for more than 99% of the variation between the 42 genotypes. Similar results have been reported by several researchers who studied chickpea genotypes by PCA (Sharifi et al., 2018; Malik et al., 2014; Shiv et al., 2012).

Table 3. Principle component analysis of chickpea genotypes

Trait	PCs					
	F1	F2	F3	F4	F5	F6
PH	0.150	-0.589	-0.670	0.426	-0.001	0.001
NPP	0.961	-0.200	-0.037	-0.180	0.006	0.055
NSP	-0.431	-0.456	0.695	0.349	-0.003	0.024
NSPL	0.845	-0.457	0.262	-0.046	-0.067	-0.031
TSW	0.264	0.906	-0.030	0.323	-0.054	0.015
SYPL	0.902	0.256	0.245	0.236	0.071	-0.022
Eigenvalue	2.729	1.692	1.064	0.498	0.013	0.005
Variability (%)	45.478	28.193	17.736	8.298	0.209	0.087
Cumulative %	45.478	73.671	91.406	99.704	99.913	100.00

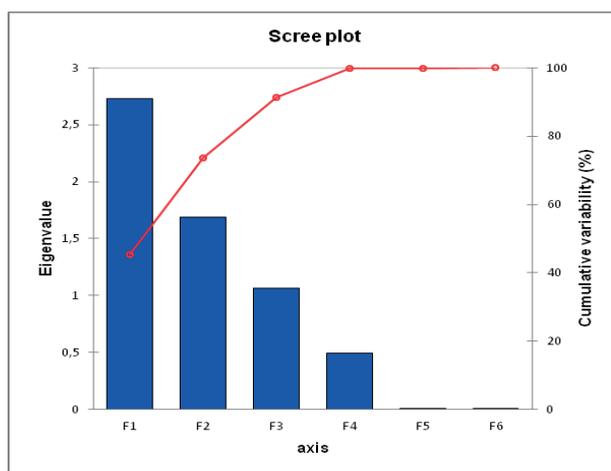


Figure 2. Diagram of eigenvalues in response to number of components for the estimated variables of chickpea genotypes

Our results also indicate that the first main component, which practically explains in the largest measure the genetic variability between the 42 genotypes (greater than 45%), is a combination of the three traits: number of pods per plant, number of seeds per plant and seed yield per plant (Table 4).

Table 4. Loading of the first main component of the PC analysis

Trait	F1
PH	0.150
NPP	0.961
NSP	-0.431
NSPL	0.845
TSW	0.264
SYPL	0.902

PH - plant height, NPP - number of pods per plant, NSP - number of seeds per pod, TSW - thousand seed weight, NSPL - number of seeds per plant; SYPL seed yield per plant.

The findings suggest that these traits mentioned above are of the utmost importance for setting chickpea breeding objectives. The choice, as a parent material, for crosses of contrasting genotypes could lead to the identification of hybrid vigor in offspring, knowing that a significant heterosis is often associated with the allelic richness of hybrids (Ramanujam et al., 1974; Parameshwarappa et al., 2012).

The two-dimensional arrangement indicates a significant positive correlation between seed yield per plant, number of pods per plant, number of seeds per plant and TSW.

Of all the studied traits, the number of pods per plant has the greatest positive effect on seed yield per plant.

Similar results were reported by Sharifi et al (2018), in their study on 25 chickpea genotypes.

Two characters are positively correlated if the angle between vectors is $< 90^\circ$, negatively correlated if the angle is $> 90^\circ$ and independent if the angle is 90° (Yan et al., 2002).

In addition, the obtuse angle between the vectors shows that the plant height is negatively correlated with the TSW, as confirmed by the correlation matrix (Figure 3).

The vectors representing the traits plant height and the number of seeds per pod are closer to the origin, which means that any interpretation regarding their correlation with the other traits could be erroneous.

The number of seeds per pod has previously been shown not to exhibit sufficient variation between genotypes so as to have an influence on the other traits.

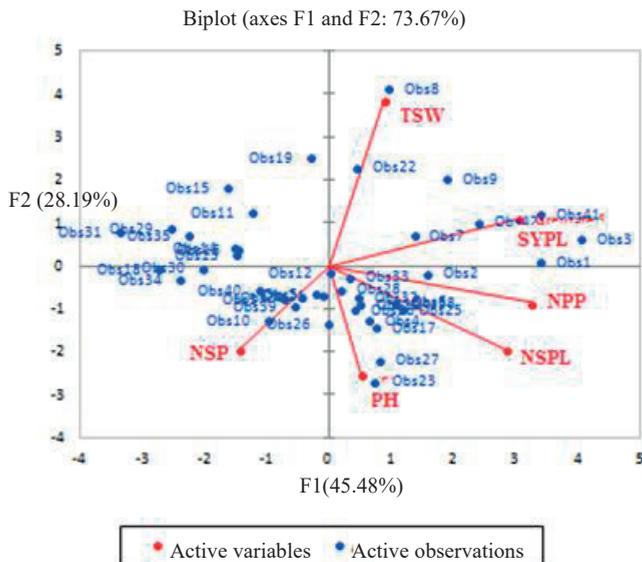


Figure 3. Two-dimensional ordination of six traits in chickpea genotypes on principal component axes
 PH - plant height, NPP - number of pods per plant, NSP - number of seeds per pod, TSW - thousand seed weight, NSPL - number of seeds per plant;
 SYPL seed yield per plant

CONCLUSIONS

Overall, these results show that PCA and cluster analysis are very effective for assessing the genetic variation of chickpea genotypes.

This study, which used multivariate techniques to assess the genetic diversity in 42 varieties and chickpea lines, was a first step in gaining an insight into the germplasm divergence, which is an important step towards an efficient exploitation of genetic resources of chickpea genotypes.

Based on the dissimilarity analysis, using the AHC (Agglomerative Hierarchical clustering) algorithm, the dendrogram distributed the 42 chickpea genotypes into two large groups: *desi* (A) and *kabuli* (B), characterized by a wide variability in terms the seed size, shape and colour. In the future breeding programs,

hybridization of genotypes across groups/clusters could lead to a high heterosis in cross progenies.

Our results also show a significant positive correlation between seed yield per plant and number of pods per plant, number of seeds per plant and thousand seed weight. Similar results were reported by Kumar et al. (2003).

The principal component analysis recognized number of pods per plant, number of seeds per plant and seed yield per plant as traits that mainly described the variation within the chickpea genotypes.

The components such as number of seeds per pod and plant height did not contribute considerably to the variation within the entries and could be dropped in similar analysis.

These results can provide practical information for the selection of parental material and thus

assist in planning chickpea breeding strategies for yield improvement.

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REFERENCES

- Adebisi, M.A., F.S. Okelola, M.O. Ajala, T.O. Kehinde, I.O. Daniel, O.O. Ajani (2013). Evaluation of variations in seed vigour characters of west African rice (*Oryza sativa* L.) genotypes using multivariate technique. *Am. J. Plant Sci.*, 4, 356–363.
- Agrawal, T., Kumar, A., Kumar, S., & Kumar, A. (2018). Assessment of Genetic Diversity in Chickpea (*Cicer arietinum* L.) Germplasm under Normal Sown Condition of Bihar. *International Journal of Current Microbiology and Applied Science*, 7(4), 3552–3560.
- Ahmar, S., Rafaqat, A.G., Ki-Hong, J., Faheem, A., Qasim, M. U., Mubeen, M., Zhou, W. (2020). Conventional and molecular techniques from simple breeding to speed breeding in crop plants: Recent advances and future outlook. *International Journal of Molecular Sciences*, 21(7), 2590.
- Arumuganathan, K. and Earle, E.D. (1991). Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rept.*, 9, 208.
- Donal Murphy-Bokern et al. (2014). Outlook for knowledge and technology for legume-supported cropping systems. *Legume-supported cropping systems for Europe* (Legume Futures). http://www.legumefutures.de/_Report_5.3.pdf.
- Dwivedi, S.L., Upadhyaya, H.D., vGowda, C.L.L. (2009). *Approaches to enhance the value of genetic resources in crop improvement*. In: Strengthening information on Plant Genetic Resources in Asia, Bangkok, Thailand.
- Gaur, P.M., Gowda, C.L.L., Knights, E.J., Warkentin, T., Açıkgöz, N., Yadav, S.S. (2006). Chickpea breeding and management. 1st ed. pp. 321, 392. UK: CABI *International.*, ed. S.S. Yadav. <https://www.researchgate.net>.
- Jing, R, Vershinin, A., Grzebyta, J., Shaw, P., Smýkal, P. Marshall, D., Ambrose, M.J., Ellis, T.N., Flavell, A.J. (2010). The genetic diversity and evolution of field pea studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. *BMC Evol. Biol.*, 10, 1–20.
- Kumar, S., Arora, P.P., Jeena, A.S. (2003). Correlation studies for yield and its component in chickpea. *Agric. Sci. Digest.*, 23, 229–230.
- Lavrenko, S., Nataliia Lavrenko, Oleksandr Kazanok, Gennadiy Karashchuk, Mykhailo Kozychar, Yevhenii Podakov, Alina Sakun (2019). Chickpea yields and water use efficiency depending on cultivation technology elements and irrigation. *AgroLife Scientific Journal*, 8(2).
- Mahmood, M.T., Ahmad, M., Ali, I., Hussain, M., Latif, A., Zubair, M. (2018). Evaluation of chickpea genotypes for genetic diversity through multivariate analysis. *Journal of Environmental and Agricultural Sciences*, 15, 11–17.
- Malik, S.R., Shabbir, G., Zubir, M., Iqbal, S.M., Ali, A. (2014). Genetic diversity analysis of morpho-genetic traits in Desi chickpea (*Cicer arietinum* L.). *Int. J. Agric. Biol. Eng.*, 16, 956–960.
- Merga, B., Jema, H. (2019). Economic importance of chickpea: Production, value, and world trade. *Journal Cogent Food & Agriculture*, 2331–1932.
- Muehlbauer, F.J., Singh, K.B. (1987). Genetics of chickpea. In: M.C. Saxena & K.B. Singh (Eds.), *The Chickpea*, pp. 99–125. CAB International, London, U.K.
- Nie, D.H., Pieng, L., Wang, X., Ji, L. (2015). Genetic diversity of agronomic traits in chickpea (*Cicer arietinum* L.) germplasm resources. *Journal of Plant Genetic Resources*, 16(1), 64–70.
- Parameshwarappa, S.G., Salimath, P.M., Upadhyaya, H. D., Kajjidoni, S.T., Patil, S.S. (2012). Heterosis in relation to genetic divergence in minicore collections of Chickpea (*Cicer arietinum* L.). *Indian Journal of Genetics and Plant Breeding*, 72(3), 303–308.
- Ramanujam, S., Tiwari, A.S., Mehra, R.B. (1974) Genetic divergence and hybrid performance in mung bean. *Theoret. Appl. Genetics*, 45, 211–214.
- Sharifi, P., Astereki, H., Poursmael, M. (2018). Evaluation of variations in chickpea (*Cicer arietinum* L.) yield and yield components by multivariate technique. *Annals of Agrarian Science*, 16(2), 136–142.
- Shiv, S., Iqbal, M.A., Singh, N.P., Solanki, R.K. (2012). Genetic diversity studies in chickpea (*Cicer arietinum* L.) germplasm. *J. Food Leg.*, 25, 31–36.
- Thudi, M., Chitikineni, A., Liu, X., He, W., Roorkiwal, M., Yang, W., Samineni, S. (2016). Recent breeding programs enhanced genetic diversity in both desi and kabuli varieties of chickpea (*Cicer arietinum* L.). *Scientific Reports*, 6, 38636.
- Varshney, R.K., Chi Song, Douglas R Cook (2013). Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nature Biotechnology*, 31, 240–246.
- Yan, W., Rajcan, I. (2002). Biplot analysis of test sites and trait relations of soybean in Ontario, *Crop Sci.*, 42, 11–20.
- www.fao.org, 2018.

EFFICACY ASSESSMENT OF SYNTHESIS PYRETHROIDS ON *Ostrinia nubilalis* (Hübner) POPULATION REDUCTION FROM CORN AGRO-ECOSYSTEM

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Abstract

Ostrinia nubilalis is the most important corn pest, in las years a aggressivity increasing was observed, causing harvest losses of up to 40%. The aim of this study was to evaluate the efficacy of synthetic pyrethroids (alpha-cypermethrin 50 g/l; alpha-cypermethrin 100 g/l; deltamethrin 25 g/l, acetamiprid 100 g/kg + lambda-cyhalotrin 30 g/kg) in reducing population of *Ostrinia nubilalis* from the corn agroecosystem (consisting of two semi-early hybrids DKC 4590; DKC 4670). The treatments were applied when the maximum flight curve was reached, assessments were made 10 days after the treatments and prior harvesting. The effectiveness was determined through aggressivity and yield obtained. The aggressivity of *Ostrinia nubilalis* was higher in the variants drilled with the DKC 4590 hybrid (80%) compared to 71.25% in the variants drilled with DKC 4670; the situation being similar at the second generation. Among synthetic pyrethroids tested alpha-cypermethrin 100 g/l proved superiority by reducing the attack of *Ostrinia nubilalis* up to 11.25% in the DK 4590 hybrid and 10% respectively in DKC 4670. The yield was correlated with the treatments efficacy (6.67-12.05 t/ha).

Key words: *Ostrinia nubilalis*, synthesis pyrethroids, efficacy, aggressivity, corn.

INTRODUCTION

In recent years (2016-2018), Romania has occupied the first positions of the European Union ranking on production (27.8% of total production is provided by Romania) and the area cultivated with corn (2.49 million hectares) according to the National Institute of Statistics.

Diseases, pests and weeds are the main biotic factors that reduce corn production. *Ostrinia nubilalis* (Lepidoptera: Crambidae) being known as one of the most important pests of corn (*Zea mays*) (Grozea et al., 2019). The species is native to Europe, later reported in North Africa, North America, Canada, and is now found in almost all maize-producing regions (Bereš, 2016).

Ostrinia nubilalis is a special polyphagous (Bengtsson et al., 2006), it attacks a very large number of plants. Research on ECB host plants has been the subject of several studies: Hodgson (1928) stated that this insect feeds on

plants of 131 genera from 40 botanical families; Ponsard et al. (2004) estimate that the ECB attacks more than 200 plants; Filip Franeta (2018) points out that the number of ECB host species on the European continent is significantly lower than on the North American continent.

Filip Franeta (2018) states that the plants attacked by the species *Ostrinia nubilalis* are part of the families: *Poaceae*, *Polygonaceae*, *Amaranthaceae*, *Solanaceae*, *Fabaceae*, *Malvaceae*, *Cannabaceae*, *Iridaceae*, *Cucurbitaceae* and *Apiaceae*.

In Romania, the ECB larva attacks corn, hemp, sorghum, millet, sunflower, but also develops on spontaneous plants such as Johnson grass, Sudan grass (Grozea, 2006).

Ostrinia nubilalis attacks all the above-ground organs of maize plants.

The researchers (Papp, 1990; Szőke et al., 2002) divided the damage caused by the ECB into direct (disruption of nutrient transport by destroying vascular tissue) and indirect

(damage reflected by grain damage, stem breakage, presence of *Fusarium* pathogens).

Production losses caused by the ECB have values in the range of 15-50% (Papp, 1990; Szőke et al., 2002; Pálffy, 1983; Magg et al., 2002). In Romania, production losses vary between 1.3-17.7% according to studies conducted by Paulian (1976); while Champrag (1994) indicates a decrease in yield between 10% and 30%. More recent data suggest that the average production loss due to the ECB attack was 7.5% (Popov and Roșca, 2007 cited by Georgescu E. et al., 2019).

The economic losses caused by *Ostrinia nubilalis* in the USA counts up to 1 billion dollars (Huang et al. 2014), in Germany according to estimates made by the Federal Biological Research Center for Agriculture and Forestry caused losses of 11-12 million euros/year (Gaspers Claudia, 2009), and worldwide 1.6 billion euros/year (FAOSTAT, 2016, cited by Kárpáti Z. et al., 2016).

The damage and losses caused by this pest have led to numerous studies in order to obtain a more effective method of control.

Direct control (chemical or biological) of *Ostrinia nubilalis* larvae from the maize agroecosystem is difficult to achieve due to the short period of exposure to treatments (from hatching until the larvae enter the stems) (Mason et al., 1996; Degenhardt et al., 2003).

Studies up to date show that the correct determination of the period of application of the insecticide is an essential condition for achieving success in ECB control. Insecticides are most effective when applied immediately after egg larvae hatch (Bartels and Hutchison 1995; Rinkleff et al., 1995) because mature larvae become inaccessible to chemical treatments by penetrating the stem of the host plant (Pélozuelo L. et al., 2006).

This is one of the reasons that led researchers to speed up their research towards an IPM (Integrated Pest Management) program:

- Mechanical control of *Ostrinia nubilalis* to be performed by using the rotary cultivator (to destroy the larvae) (Ackermann et al., 2003); observance of rotation; gathering plant debris; performing deep plowing to increase the mortality of larvae in diapause over the winter (Zellner, 2001).

- Biological control of the species *Ostrinia nubilalis* is frequently used by the application of parasitic wasps *Trichogramma evanescens* and *Trichogramma brassica* (parasitizes eggs and thus avoids the damage caused by larvae). Bioproducts such as *Bacillus thuringiensis* var. *kurstaki* (B.t.k.), are also widely used, Spinosad and insect growth regulators (IGR).

- The ECB's chemical control has been efficient achieved with insecticides from the organophosphorus (OP) and synthetic pyrethroid (P) families (Rinkleff et al., 1995; Musser and Shelton, 2003).

MATERIALS AND METHODS

Research on the control of the species *Ostrinia nubilalis*, with synthetic pyrethroids, was carried out in 2019, in the western part of Romania (Șag locality, Timiș county) (Figure 1).

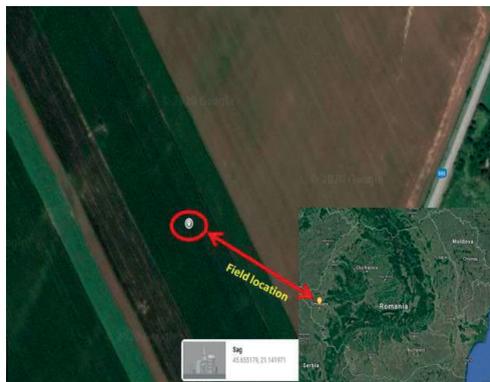


Figure 1. The geographical location of the experimental field

Aiming the aggressivity of the species *Ostrinia nubilalis*, the maize hybrids DKC 4590 and DKC4670 (FAO 350-390) were drilled on 28.04.2019.

The research regarding the evaluation of the efficacy of synthetic pyrethroids, on population of *Ostrinia nubilalis* reduction, was setup using randomized blocks method, including five treatments (for each hybrid) in four replicates. The experimental plots, drilled with maize, had an area of 30 m² (length of 10 m and a width of 3 m), according to EPPO standard.

Synthetic pyrethroids tested in experience were alpha-cypermethrin 50 g/l; alpha-cypermethrin 100 g/l; deltamethrin 25 g/l, acetamiprid 100 g/kg + lambda-cyhalothrin 30 g/kg (Table 1).

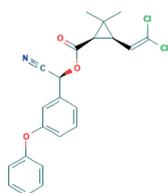
Table 1. Active substances used in the study on the control of the species *Ostrinia nubilalis* from the maize agroecosystem

Trt.	Active substance	A.i. content	Commercial name	Dose
1	Alpha-Cypermethrin	50 g/l	Fastac Active ME	0.6 l/ha
2	Alpha-Cypermethrin	100 g/l	Alfadone 10 EC	0.15 l/ha
3	Deltamethrin	25 g/l	Poleci	0.05 l/ha
4	Acetamiprid + lambda-Cyhalothrin	100 g/kg +30 g/kg	Inazuma WG	0.15 l/ha
5	Control		untreated	

The active substances (from the group of synthetic pyrethroids) used in the control of the species *Ostrinia nubilalis* have contact and ingestion action.

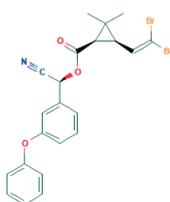
Three simple insecticides, as well a compound one (Inazuma), were tested in the study.

It contains acetamiprid 100 g/kg, from the group of neonicotinoids with translaminar penetration and lambda-cyhalothrin 30 g/kg, from the group of synthetic pyrethroids having non-systemic action, blocking the central nervous system.



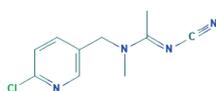
Alpha-Cypermethrin

(<https://pubchem.ncbi.nlm.nih.gov/compound/alpha-Cypermethrin#section=Structures>)



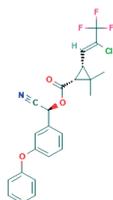
Deltamethrin

(<https://pubchem.ncbi.nlm.nih.gov/substance/53788229#section=2D-Structure>)



Acetamiprid

(<https://pubchem.ncbi.nlm.nih.gov/compound/6440554#section=2D-Structure>)



lambda-Cyhalothrin

(<https://pubchem.ncbi.nlm.nih.gov/compound/6440554#section=2D-Structure>)

The optimal time to apply the treatments, in respect of species *Ostrinia nubilalis* control, was established with the help of pheromone type traps CSALOMON®VARL (Figure 2) (28.06.2019).



Figure 2. Pheromone trap CSALOMON®VARL, used to establish the flight curve

At 36 DAA of the insecticides, the first assessment was performed, regarding the effectiveness of the synthetic pyrethroids and of the insecticide compound Inazuma (acetamiprid + lambda-cyhalothrin) throughout the number of plants attacked (the number of larvae in the stems was determined, the number of holes, as well as the attack of the corn stalker on the cob) (Figure 3).



Figure 3. Assessing the aggressivity of European corn borer in experimental variants

The second assessment of the *Ostrinia nubilalis* aggressivity was performed a day before harvest, At 80 DAA tacking the number of plants attacked (broken above the cobs; broken under the cobs; broken cobs, cobs damaged) in account (Figure 4). In order to determine the frequency of attack on the maize stalk grower, in maize agroecosystem, 80 plants/variant were taken into consideration at first assessment and

at the time of second assessment the number of samples was 200.



Figure 4. Assessment of *Ostrinia nubilalis* damage on maize plants, before harvesting

After establishing the aggressiveness of the European corn borer, the yield of the two maize hybrids used in the experiment was determined. The insecticides efficacy was determined using ABBOT formula:

$$\text{Efficacy after Abbott \%} = \left(\frac{C_a - C_t}{C_a} \right) \times 100$$

where C_a untreated infestation percent, C_t treated infestation percent

The climatic conditions (Figure 5) recorded, in the experimental year 2019, were favorable for the development of the polyphagous pest, *Ostrinia nubilalis*.

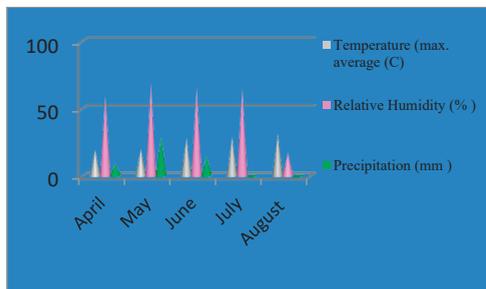


Figure 5. Trial climatic conditions for control of the species *Ostrinia nubilalis* in maize agroecosystem

The average temperature of 20.06°C, recorded in May, shows that the prolificity threshold (14.2°C) (Grozea, 2006) of the species *Ostrinia nubilalis* has been exceeded. The average temperatures recorded in June (28.21°C), July (29.13°C) and August (31.42°C) were still favorable for the development of the species, according to literature data (Grozea, 2006) temperature of 29°C and the relative high humidity being favorable.

RESULTS AND DISCUSSIONS

Table 2 shows the results on the efficacy of synthetic pyrethroids in the reduction of the *Ostrinia nubilalis* population in the maize agroecosystem (hybrid DKC 4590), 36 days after the application of the treatments.

The frequency of ECB larvae attack on the DKC 4590 maize hybrid treated with synthetic pyrethroids, in the climatic conditions of 2019, had values of 15-77.5%.

Synthetic pyrethroid, alpha-cypermethrin applied at a dose of 0.6 l/ha successfully controlled ECB larvae (80.65%), the aggressivity being 15.0%. Low values regarding the frequency of attack (21.25%) of the species *Ostrinia nubilalis* were also registered in the variants treated with the compound insecticide Inazuma WG, showing an efficacy of 72.58%. At the first evaluation, the highest agresivty (77.5%) of ECB larvae was recorded in the control variant (untreated).

Table 1. Efficacy of synthetic pyrethroids in the control of ECB larvae (hybrid DKC 4590 - 1st assessment)

Active ingredient	Doza	Σ attacked plants/trt	ECB larvae agresivty/trt	Insecticides efficacy
Alpha-Cypermethrin	0.6 l/ha	12	15.0	80.65
Alpha-Cypermethrin	0.15 l/ha	32	40.0	48.39
Deltamethrin	0.05 l/ha	44	55.0	29.03
Acetamiprid + lambda-Cyhalothrin	0.15 l/ha	17	21.25	72.58
Control	-	62	77.5	-

The efficacy of insecticides used in the experiment was studied by other researchers too. Blandino et al. 2010 emphasizes that the efficacy of insecticides in control of *Ostrinia nubilalis* is greatly influenced by their applications timing. Studies performed by him show a high efficacy of synthetic pyrethroids if applied: 7-10 days before reaching the maximum flight curve or 2-3 days after reaching the maximum flight curve; if applied at the end of flowering the efficacy will be significantly lower. Mason et al. (1996) believe that insecticides should be applied early because the attack of wintering larvae may be more dangerous.

After Rinkleff et al. (1995) the application period of synthetic pyrethroids is flexible due to the good effect in the control of ECB larvae.

Blandino et al. 2010 support the idea of choosing the timing of application of insecticides used in the control of *Ostrinia nubilalis* in respect of the active substance.

After determining the frequency of attack on the variant, the maize plants were analyzed (cutting the stems) and the average number of larvae in the stem was determined for each variant. At 36 days after the application of insecticides, the average number of larvae varied between 0.01-0.3 larvae/plant (Figure 6). By applying deltamethrin treatments, there was a reduction in the average number of larvae (0.2 larvae/plant) compared to the untreated control.

The study showed that corn plants had a higher frequency of attack under cobs (average/exp. 6.25%). The damage through the presence of perforation on the corn leaves recorded the lowest values (3.5%) (Figure 6).

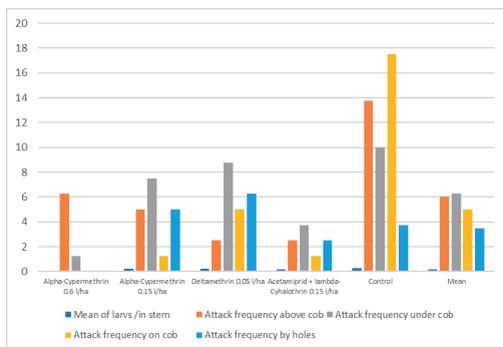


Figure 6. Aggressivity of *Ostrinia nubilalis*, on maize plants, related to plant parts, 36 days after treatment

80 days after the application of the treatments, regarding the reduction of the population of *Ostrinia nubilalis*, the second assessment was performed (Table 3), it showed that the insecticides had lower efficacy in the control of the species *Ostrinia nubilalis*, their effectiveness being of 25.33-54.67% (but with same hierarchy).

The application of synthetic pyrethroids led to a decrease in the percentage of broken plants (1.5-4.5%) compared to the untreated control (7.0%). The analysis of the results by the graph (Figure 7) shows that the ECB larvae showed a higher aggressivity on the cob (11.0%) and above the cob (14.5%).

Table 3. Efficacy of synthetic pyrethroids in the control of ECB larvae (hybrid DKC 4590-2nd assessment)

Active ingredient	Dose	Σ attacked plants/trt	ECB larva aggressivity	Insecticides efficacy
Alpha-Cypermethrin	0.6 l/ha	68	34	54.67
Alpha-Cypermethrin	0.15 l/ha	85	42.5	43.33
Deltamethrin	0.05 l/ha	112	56	25.33
Acetamiprid + lambda-Cyhalothrin	0.15 l/ha	84	42	44.00
Control	-	150	75.0	-

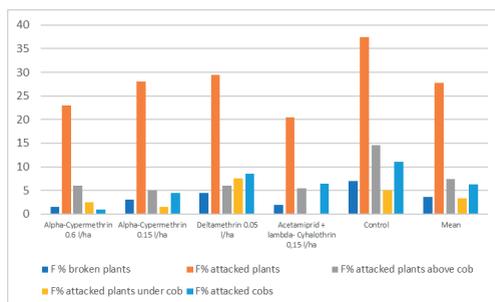


Figure 7. The attack of ECB larvae on maize plants (hybrid DKC 4590-2nd assessment)

The aggressivity of ECB larvae (20-73.75%) on the DKC 4670 hybrid (Table 4) was lower compared to that recorded on the DKC 4590 hybrid. The plants from the untreated control variant showed an attack frequency of (73.75%) (Table 4).

Table 4. Efficacy of synthetic pyrethroids in reducing the *Ostrinia nubilalis* population in the maize agroecosystem (hybrid DKC 4670 - 1st evaluation)

Active ingredient	Dose	Σ attacked plants/var	Frequency %	Efficacy %
Alpha-Cypermethrin	0.6 l/ha	16	20	72.88
Alpha-Cypermethrin	0.15 l/ha	24	30	59.32
Deltamethrin	0.05 l/ha	40	50	32.20
Acetamiprid + lambda-cyhalothrin	0.15 l/ha	18	22.5	69.49
Control	-	59	73.75	-

The hierarchy of insecticides in the control of *Ostrinia nubilalis* is maintained in the case of the hybrid DKC 4670, the first position is held by alpha-cypermethrin 0.6 l/ha (72.88%), followed by: acetamiprid + lambda-cyhalothrin 0.15 l/ha (69.49%), alpha-cypermethrin 0.15 l/ha (59.32%) and deltamethrin 0.05 l/ha (32.20%). The results obtained from this study are also supported by research conducted by Bažok R. et al., 2009.

They tested synthetic pyrethroids alone or in combination with ethyl chlorpyrifos to control

Ostrinia nubilalis. Bažok R. claims that the efficacy of deltamethrin was satisfactory, that lambda-cyhalothrin had a very poor efficacy, and the reason for the low efficacy may be the late application. The combination of cypermethrin and ethyl chlorpyrifos has been satisfactory, which is why this combination is very often used in the fight against ECB in many European countries (Bažok, 2009).

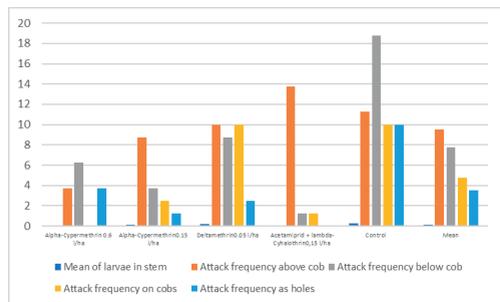


Figure 8. Frequency of attack of ECB larvae on maize plants (hybrid DKC 4670 - 1st assessment)

The study showed that for the DKC 4670 hybrid the highest aggressivity was recorded above the cobs with an average percentage/trial of 9.5% (Figure 8). ECB larvae attacked the cobs with a frequency of 4.75%.

The synthetic pyrethroids used in the experiment since not being systemic insecticides did not offer a very good control of the species *Ostrinia nubilalis* over a long period of time, so that at the second evaluation of their efficacy (Table 5) decreased compared to the first evaluation.

Table 5. Efficacy of synthetic pyrethroids in control of *Ostrinia nubilalis* population in the maize agroecosystem (hybrid DKC 4670 - 2nd assessment)

Active ingredient	Dose	Σ attacked plants/var	Frequency %	Efficacy %
Alpha-Cypermethrin	0.6 l/ha	40	20.0	66.10
Alpha-Cypermethrin	0.15 l/ha	67	33.5	43.22
Deltamethrin	0.05 l/ha	91	45.5	22.88
Acetamiprid + lambda-Cyhalothrin	0.15 l/ha	57	28.5	51.69
Control	-	118	59.0	-

The control of the ECB population, 80 days after the application of the treatments, ranged between 22.88 - 66.10% (Table 6).

In this growth stage (before harvesting) the most dangerous way of damage, caused by the ECB, is the breaking of plants and the attack of cobs, but by applying the four treatments it was

possible to decrease the percentage of broken plants (1.5-5.0%). and attacked cobs (0-2.5%) compared to the results obtained in the control variant (untreated) (Figure 9).

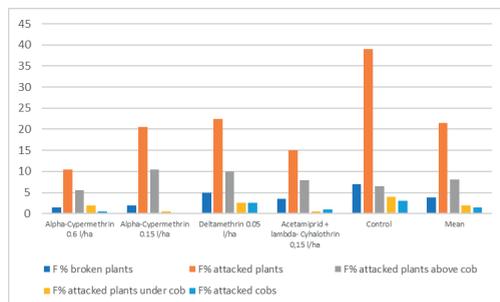


Figure 9. Graphical representation of the frequency of attack of ECB larvae on maize plants (hybrid DKC 4670 - 2nd assessment)

The successful control of ECB by the application of synthetic pyrethroids has been demonstrated by other authors (Gingera et al., 1998; Zhekova et al., 2015; Bartels et al., 1995; Hutchison, 2000). According to Foster (2001) the efficacy of pyrethroids is due to the very fast contact action. Lisowicz (1998; 1999), and Adamczewski et al. (2002) confirmed the high efficacy of pyrethroids in controlling ECB in maize. According to studies by Lisowicz (1998), two applications of synthetic pyrethroids would completely protect maize plants against ECB. Research by Lisowicz (2003), Mazurek et al. (2005) and Bereś (2008) confirm the efficacy of lambda-cyhalotrin in controlling ECB, and the highest efficacy was obtained when plants were treated twice with this active substance.

The maize yields, for the DKC 4590 hybrid, registered in the experimental variants varied between 8.71 t/ha (untreated control) and 11.72 t/ha (in the variants treated with alpha-cypermethrin 0.6 l/ha). By applying the treatments, production increases of 1.96-3.01 t/ha were obtained, the results being very significantly positive compared to the results recorded in the untreated variant.

The yield results (Table 6) for the DKC 4670 maize hybrid were lower compared to those obtained for the DKC 4590 hybrid, which is explained by the lower germination density/ha (69000). Yields higher than those recorded in

the untreated control variant (7.57 t/ha) were obtained in the variants treated with 0.6 l/ha alpha-cypermethrin (10.82 t/ha) acetamiprid + lambda-cyhalothrin 0.15 l/ha (10.18 t/ha), alpha-cypermethrin 0.15 l/ha (9.33 t/ha), these differences being very significant and distinctly significantly positive.

Table 6. Insecticides efficacy of in the control of the *Ostrinia nubilalis* population in maize cultivation expressed in terms of production

Hybrid	Treatment	Mean t/ha	Relative. diff.	significance
DKC 4590	Alpha-Cypermethrin 0.6 l/ha	11.72	3.01	***
	Alpha-Cypermethrin	10.89	2.18	***
	Deltamethrin	10.673	1.96	***
	Acetamiprid + lambda-Cyhalothrin	11.20	2.49	***
	Control	8.71	0	-
	LSD (p5%) = 0.58 LSD (p5%) = 0.84 LSD (p5%) = 1.27			
DKC 4670	Alpha-Cypermethrin	10.82	3.25	***
	Alpha-Cypermethrin	9.33	1.76	**
	Deltamethrin	8.44	0.87	-
	Acetamiprid + lambda-Cyhalothrin	10.18	2.61	***
	Control	7.57	0.00	-
	LSD (p5%) = 0.91 LSD (p5%) = 1.32 LSD (p5%) = 1.98			

Grain maize production, recorded in the variant treated with deltamethrin 0.05 l/ha (8.44 t/ha), did not show significant differences compared to the untreated control.

CONCLUSIONS

The efficacy of synthetic pyrethroids in the control of ECB larvae was satisfying at the first evaluation (36 days after treatment).

The control rate of *Ostrinia nubilalis* in the maize agroecosystem (consisting of the two hybrids DKC 4590 and DKC 4670) has decreased 80 days after treatment, this result is explained by the fact that non-systemic insecticides did not protect the maize plants over a longer period.

Pyrethroid alpha-cypermethrin (0.6 l/ha) showed the best efficacy in reducing the population of *Ostrinia nubilalis*.

The aggressivity of the attack of ECB larvae on maize plants was ascending, from the first assessment to the second.

Maize production has been correlated with the efficacy of treatments against *Ostrinia nubilalis*.

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REFERENCES

- Ackermann, R., Pönitz, K., Rochel, J., Holschowsky I. (2003). Larve gegen Stängel. *Bauernzeitung*, 17, 21–23.
- Adamczewski, K., Matysiak, K. (2002). Kukurydza *Zea mays* L. [In:] Klucz do określenia faz rozwojowych roślin jedno- i dwuliściennych wskaźniki BBCH, K. Adamczewski i K. Matysiak (tłumaczenie i adaptacja), Wyd. I, *Inst. Ochr. Roślin Poznań*, 20–21 [in Polish].
- Bartles, D.W., Hutchison, W.D., Udayagiri, S. (1997). Pheromone trap monitoring of Z-strain European corn borer (Lepidoptera: *Pyralidae*): optimum pheromone blend, comparison with blacklight traps, and trap number requirements. *J. Econ. Entom.*, 90, 449–457.
- Bažok, R., Igrc, Barèia, J., Kos, T., Gotlin Èuljak, T., Šiloviae, M., Jelovean, S., Kozina, A. (2009). Monitoring and efficacy of selected insecticides for European corn borer (*Ostrinia nubilalis* Hubn., Lepidoptera: *Crambidae*) control. *Journal of Pest Science*, 82, 311–319.
- Bengtsson, M., Karpati, Z., Szöcs, G., Reuveny, H., Yang, Z., Witzgall, P. (2006). Flight tunnel responses of Z strain European corn borer females to corn and hemp plants. *Environ. Entomol.*, 35(5), 1238–1243.
- Bereš, P.K. (2016) Monitoring of occurrence and notifying dates for European corn borer (*Ostrinia nubilalis* Hbn.) control measures in Poland – current situation and perspective. *Progress in Plant Protection*, 54(3), 276–282
- Bereš, P.K., Pruszyński G. (2008). Pest management in integrated maize production. *Acta Scientiarum Polonorum Series Agricultura*, 7(4), 19–32.
- Blandino, M., Saladini, M.A., Alma, A., Reyneri, A. (2010). Pyrethroid application timing to control European corn borer (Lepidoptera: *Crambidae*) and minimize *Fumonisin* contamination in maize kernels. *Cereal Research Communications*, 38(1), 75–82
- Čamprag, D.S. (1994). Integralna zaštita kukuruza od štetočina. Novi Sad: Feljton.
- Degenhardt, H., Horstmann, F., und N. Müllerder. (2003). Bt-Mais in Deutschland. *Mais* 2, 75–77.
- EPPO (2004): Guidelines for the efficacy evaluation of plant protection products: Efficacy evaluations of insecticides – *Ostrinia nubilalis* – PP 1/152(2), in EPPO Standards: Efficacy evaluation of insecticides & Acaricides, volume 3, European and Mediterranean Plant Protection Organization - EPPO, Paris, 22–24.
- Foster, R.E. (2001). Managing insects in commercially grown sweet corn. *Vegetables*. Department of Entomology. Purdue University Cooperative Extension Service. *West Lafayette* 4790, 8.

- Franeta, F.M. (2018). Uticaj insekticida na mortalitet i fiziološki stres gusenica kukuruznog plamena (*Ostrinia nubilalis* Hbn.) i pojavu sekundarnih gljivičnih infekcija na kukuruзу, Novi Sad, Serbia.
- Gaspers, C. (2009). The European corn borer (*Ostrinia nubilalis*, Hbn.), its susceptibility to the Bt-toxin Cry1F, its pheromone races and its gene flow in Europe in view of an Insect Resistance Management, *USDA Technical Bulletin*, 77.
- Georgescu, E., Toader, M., Cană, L., Rîşnoveanu, L. (2019). Researches concerning european corn borer (*Ostrinia nubilalis* Hbn.) control, in South-East of the Romania, *Scientific Papers. Series A. Agronomy, LXII*(1), 301–308.
- Gingera, G.J., Subramanyam, Bh., Hutchison, W.D. (1998). Insecticides used in Minnesota processors to control European corn borer and corn earworm in sweet corn. University of Minnesota Extension Service. FO-6322-GO, 4.
- Grozea, I. (2006) Entomologie specială, Editura Mirton, Timişoara, 51–53.
- Grozea, I., Horgos, H., Stef, R., Carabet, A., Virteiu, A. M., Butnariu, M., Molnar, L. (2019). Assessment of population density of insect species called "species problem", in lots with different maize hybrids, *Research Journal of Agricultural Science*, 51(1), 132–137.
- Hodgson, B.E. (1928). The host plants of the European corn borer in New England.
- Huang, F. (2014) Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. *Plos One* 9, e112958.
- Kárpáti, Z., Tóth, A.F., Bognár, C., Szóke, C., Bónis, P., Marton, C.L, Molnár, B.P. (2016). Pheromone-based monitoring of the European corn borer (*Ostrinia nubilalis*) in Hungary, *Maydica*, 61, 1–7.
- Lisowicz, F. (1998). Efekty chemicznego zwalczania omacnicy prosowianki (*Ostrinia nubilalis* Hbn.) na kukurydzy. *Prog. Plant Protection/Post. Ochr. Roślin* 38(2), 362–364.
- Lisowicz, F. (1999). Badania nad terminami chemicznego zwalczania omacnicy prosowianki (*Ostrinia nubilalis* Hbn.) nakukurydzy. *Prog. Plant Protection / Post. Ochr. Roślin*, 39(2), 490–492.
- Lisowicz, F. (2003b). The occurrence and the effects of European corn borer (*Ostrinia nubilalis* Hbn.) control on corn crop in Przeworsk region in 2001–2002. *J. Plant Protection Res.*, 43(4), 91–96.
- Magg, T., Melchinger, A.E., Klein, D., Bohn, J.M. (2002). Relationship between European corn borer resistance and concentration of mycotoxins produced by *Fusarium* spp. In grains 96 of transgenic Bt maize hybrids, their isogenic counterparts, and commercial varieties. *Plant breeding*, 121(2), 146–154.
- Mason, C.E., Stromdahl, E.Y., Pesek, J.D. (1996). Placement of pheromone traps within the vegetation canopy to enhance capture of male European corn borer (Lepidoptera: Pyralidae). *J Econ Entomol.*, 90, 795–800.
- Mazurek, J., Hurej, M., Jackowski, J. (2005). The effectiveness of selected chemical and biological insecticides in control of European corn borer (*Ostrinia nubilalis* Hbn.) on sweet corn. *Journal of Plant Protection Research*, 45(1), 41–47.
- Mazurek, J., Hurej, M., Jackowski, J. (2005). The effectiveness of selected chemical and biological insecticides in control of European corn borer (*Ostrinia nubilalis* Hbn.) on sweet corn. *J. Plant Protection Res.*, 45(1), 41–47.
- Musser, F.R., Shelton, A.M. (2003). Bt sweet corn and selective insecticides: impacts on pests and predators. *J. Econ. Entomol.*, 96(1), 71–80.
- Pálffy, C. (1983). A kukoricamoly és kártétele. (The corn borer and its damage.) *Növényvédelem*, 19. 515–517.
- Papp I. (1990). A kukorica növényvédelmi technológiája. (Maize Plant Protection Technology.) Komárom-Esztergom megyei Növényegészségügyi és Talajvédelmi Állomás.
- Paulian, F., Mustea, D., Burdea, V., Baniţă, E., Enica, D., Peteanu, S., Petcu, L., Sapunaru, T., Sandru, I. (1976). Evolution of European corn borer-*Ostrinia nubilalis* Hb and damaging potential registered in 1971–1975 in R.S. Romania. *Problems of Plant Protection*, 4(1), 23–51.
- Paweł, K., Bereś, Sławomir Drzewiecki, Łukasz Siekaniec, Łukasz Kontowski (2019). Alternative host plants for *Ostrinia nubilalis* Hbn. and *Diabrotica v. virgifera* LeConte beetles in southern Poland, *Progress in Plant Protection*, 59(1), 69–75.
- Pélozuelo, L., Faghhih, A.A., Espahbodi, A., Genestier, G., Hervé Guénégo, M., C., Frérot, B. (2006). Efficiency of pheromone baited traps for monitoring of the European corn borer *Ostrinia nubilalis* (Lep.: Crambidae) in Mazandaran province, *Appl. Ent. Phytopath.*, 73(2), 19–31.
- Ponsard, S., Bethenod, M.T., Bontemps, A., Pélozuelo, L., Souqual, M.C., Bourguet, D. (2004). Carbon stable isotopes: a tool for studying the mating, oviposition, and spatial distribution of races of European corn borer, *Ostrinia nubilalis*, among host plants in the field. *Canadian Journal of Zoology*, 82, 1177–1185.
- Rinkleff, J.H., Hutchison, W.D., Campbell, C.D., Bolin, P.C., Bartels, D.W. (1995). Insecticide toxicity in European Corn Borer (Lepidoptera: Pyralidae): ovicidal activity and residual mortality to neonates. *J. Econ. Entomol.*, 88, 246–253.
- Szóke, C., Zsubori, Z., Pók, I., Rác, F., Illés, O., Szegedi, I. (2002). Significance of the European corn borer (*Ostrinia nubilalis* Hübn.) in maize production. *Acta Agronomica Hungarica*, 50, 447–461.
- Zellner, M. (2001). Maßnahmen gegen den Maiszünsler Wirkungsweise und Erfolgsaussichten. *Maisinformation*, 1, 3–5.
- Zhekova E. D., Nikolova I. M. (2015). Insecticide products of different groups against *Plagionotus floralis* Pall, *Journal of Biotechnology Banat*, VI(12), 69–73.
- ***<https://pubchem.ncbi.nlm.nih.gov/compound/6440554#section=2D-Structure>
- ***<https://pubchem.ncbi.nlm.nih.gov/compound/6440554#section=2D-Structure>
- ***<https://pubchem.ncbi.nlm.nih.gov/compound/alpha-Cypermethrin#section=Structures>
- ***<https://pubchem.ncbi.nlm.nih.gov/substance/53788229#section=2D-Structure>

EFFECTIVE SOLUTIONS FOR CONTROL OF *Convolvulus arvensis* L. IN WINTER WHEAT (*Triticum aestivum* L.)

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Abstract

One of the most difficult to control weeds in winter wheat is *Convolvulus arvensis* L. In wheat, chemical weed control is preferred of its better efficiency. The aim of the current study is to find effective solutions for *Convolvulus arvensis* L. The experiments were situated in the experimental field of Agricultural University in Plovdiv. The trials were conducted in 3 replications. The following treatments are included: 1. Untreated; 2. Aminopyralid+Florasulam- 33 g/ha; 3. Florasulam +Fluroxypyr- 1.5 l/ha; 4. Florasulam +Fluroxypyr- 1.8 l/ha; 5. Fluroxypyr- 0.7 l/ha; 6. Fluroxypyr- 0.9 l/ha; 7. Amidosulfuron +Iodosulfuron - 100 ml/ha. The herbicides were applied in phenophase BBCH 32-33. The efficacy of the studied herbicides and rates against *Convolvulus arvensis* L. was evaluated by the scale of EWRS on the 14th, 28th and 56th day after application. External symptoms of phytotoxicity were not observed. The highest efficacy was recorded for Florasulam +Fluroxypyr-1.8 l/ha; and Fluroxypyr- 0.9 l/ha.

Key words: weeds control, efficacy, herbicides, phytotoxicity.

INTRODUCTION

Weeds remain one of the most significant agronomic problem associated in the cultivation of winter wheat (*Triticum aestivum* L.). It is recognised to the crop as it provides food and habitat for a range of beneficial organisms. The infestation of the crop with different broad leave weeds can reduce the yield. Winter wheat is generally invaded by both grass and broad leaved weeds but major challenge offered is by broad leave weeds. One of the key weed infesting the crop is *Convolvulus arvensis* L. The aim of the current study is to find effective solutions for control of *Convolvulus arvensis* L. in winter wheat (*Triticum aestivum* L.). It is a prostrate plant unless it climbs on an object for support. The root system has both deep vertical roots and shallow horizontal lateral roots. The vertical roots can reach depths of 20 feet or more. Experiments on *Convolvulus arvensis* L. have shown that its root and rhizome growth can reach to 5 tons per acre. Control of the weed isn't easy and it can't be accomplished with a single treatment or in a single season. Effective

control requires prevention of seed production, reduction of stored carbohydrates by deep tillage of the root system, competition for light from other plants and constant vigilance in removing top growth. Application of herbicides, which reduce the weed growth and kill germinating seedlings, can also be part of an integrated weed management.

MATERIALS AND METHODS

The common winter wheat variety that was an object in the recent research is Enola (*Triticum aestivum* L. subsp. *vulgare* var. *erythrospermum*). The variety is bred in Dobrudzha Agricultural Institute - General Toshevo, Bulgaria. Certificate No: 10595/30.11.2004. The plants are characterized with 80-90 cm of stem height, high tolerance to lodging, very high productive tillering. The ear is with awns. The hectolitre masses 81-83 kg on average, and the absolute seed mass of 1000 clean, air-dry seeds is 41-44 g on average. The wheat variety is early. It has very good cold and winter resistance, high drought tolerance, good resistance to Powdery mildew (*Blumeria*

graminis f. sp. *tritici*), Yellow rust (*Puccinia striiformis*), Black rust (*Puccinia triticina*) and Leaf Blotch (*Septoria tritici*) as well as high resistance to Fusarium head blight (*Fusarium graminearum*). The wheat variety owns high and stable yield (8.50 t/ha on average) and it is suitable for intensive growing without special requirements (<http://www.dai-gt.org>).

The experiment was situated in the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria during 2018-2019. The trials was conducted by the randomized block design in 3 replications. The size of the experimental plot was 20 m². The sowing date was on the 6th of October 2017 with. The sowing is performed in the optimal time for the region. The experiment included the following treatments: 1. Untreated control; 2. Derby Super WG - 33 g/ha; 3. Starane Gold - 1.5 l/ha; 4. Starane Gold - 1.8 l/ha; 5. Flurostar 200 - 0.7 l/ha; 6. Flurostar 200 - 0.9 l/ha and 7. Sekator OD - 100 ml/ha. Derby Super WG contains Florasulam + Aminopyralid-potassium Starane Gold 1.2 l/ha. Starane Gold contains 1 g/l florasulame + 100 g/l fluroxypyr; Flurostar 200 contains 200 g/l fluroxypyr; Sekator OD 100 g/kg is containing amidosulfuron + 25 g/kg iodosulfuron.

The herbicides were applied in crop stage 2nd – 3rd stem node (BBCH 32-33) on 20.04.2018. The plant density was 450 plants/m². Predecessor of Winter wheat in the crop rotation was sunflower (*Helianthus annuus* L., hybrid P 64 LE 25). The performed soil tillage before the wheat sowing is deep ploughing followed by disking and cultivation. Fertilization with 300 kg ha⁻¹ with NPK 15:15:15 applied before sowing and spring dressing with 300 kg ha⁻¹ NH₄NO₃ is done. The efficacy of the studied herbicides and rates against the Field bindweed (*Convolvulus arvensis* L.) was evaluated by the 10 score scale of EWRS was evaluated on the 14th, 28th and 56th day after application as described by Zhelyazkov et al. (2017). The selectivity by the 9 score scale of EWRS as described by Zhelyazkov et al. (2017) was evaluated on the 7th and the 14th day after the herbicide application (at score 0 there are not damages on the crop, and at score 9 the crop is completely destroyed). The hectoliter seed mass was

measured by weighing two parallel samples of 100 dm³ air dry seeds. The hectoliter mass is calculated, as the arithmetic means of the established mass of the two samples (in grams) multiply by 100 and the resulting is divided into 1000 to obtain the mass in kilograms (Tonev et al., 2018). The absolute seed mass of 1000 clean, air-dry seeds, expressed in grams was also measured (Tonev et al., 2018). The seed moisture content was measured at the time of harvest with portable moisture meter model “DRAMINSKI GMM mini”. The height of the winter wheat plants was determined as 10 plants were measured and the results were divided to 10 to obtain average values. That was performed for each replication of every treatment. The length of the winter wheat ears was determined as the ears of 10 plants were measured and the results were divided to 10 to obtain average values. That was also performed for each replication of every treatment. Statistical analysis of collected data was performed by using Duncan’s multiple range test by the software SPSS 19. Statistical differences were considered significant at p < 0.05.

On the basis of the analysis of the meteorological data we can indicate the vegetation period of 2017-2018 as favorable for the growth, development and realization of the productive abilities of the winter wheat plants from the trial.

RESULTS AND DISCUSSIONS

During the 7 days before, 7 days after and during the herbicide treatment, no temperature values were reported that could contribute to a wrong assessment of the efficacy, selectivity and phytotoxicity of the studied herbicides (Figure 1).

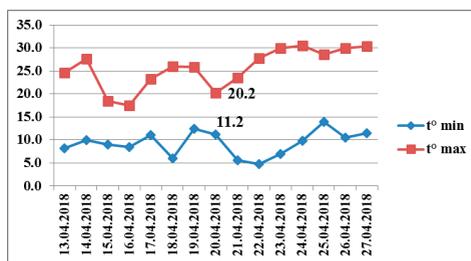


Figure 1. Minimum and maximum air temperatures 7 days before and 7 days after the herbicide application on 20.04.2018 (°C)

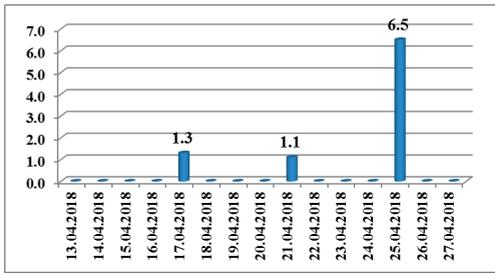


Figure 2. Precipitation 7 days before and 7 days after the herbicide application on 20.04.2018 (mm)

A rainfall during or immediately after treatment can wash the unabsorbed amount of the herbicide (Tonev et al., 2007; Tonev et al., 2011). On the day of the treatment the amount of precipitation is 0.0 mm. The total rainfall 7 days after the herbicide application is 7,6 mm (Figure 2). However, we do not take into account the effect of this precipitation on the efficacy, selectivity and phytotoxicity of the studied herbicides.

Due to the late application time of the herbicides (BBCH 32-33 of the winter wheat) the efficacy rates of all tested products and their doses are lower than the actual ones. It was reported that the most resistant weed species in the trial conducted by Mitkov et al. (2017) was the Field bindweed.

There were no visible signs of phytotoxicity on the crop caused by any of the studied herbicide products and the phytotoxicity score by EWRS was 0.

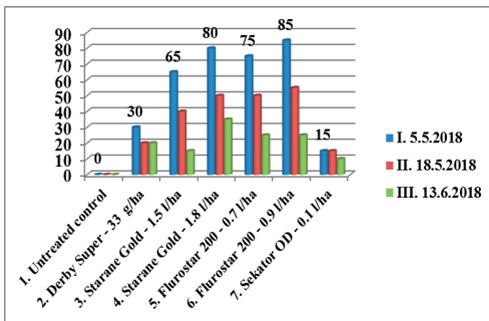


Figure 3. Efficacy of the studied herbicide products against *Convolvulus arvensis* L.

The efficacy of the studied herbicide products against the Field bindweed is shown on Figure 3. Derby Super WG applied at rate of 33 g/ha affects this difficult -to-control weed but the

efficacy is extremely low in the order of 30% on the 1st reporting date (05.05.2018). On the 2nd and the 3rd reporting dates 18.05 and 13.06.2018, respectively, the efficacy decreased was more severe. Delchev (2013) did not found any efficacy of Derby Super WG against Field bindweed in his study.

On the 1st reporting date the efficiency of the herbicide product Starane Gold against the weed was higher independently the studied rates. At the rate of 1.5 l/ha the efficacy was 65% and at the rate of 1.8 l/ha the efficacy reached 85%. On the 2nd and the 3rd reporting dates the efficacy of the herbicide product was also decreased, but for the application of the higher rate of 1.5 l/ha the efficacy was the highest for the concrete trial conditions.

On the 1st reporting date the efficiency of the herbicide product Flurostar 200 against *C. arvensis* was higher independently the studied rates. At the rate of 0.7 l/ha the efficacy was 75% and at the rate of 0.9 l/ha the efficacy reached 85%. On the next two reporting dates the efficacy of the herbicide product was also decreased. Similar efficacy (76%) results against this noxious weed war recorded by Radivojević et al. (2006) after the application of Starane 250 at rate 0.8 l/ha.

We do not report any satisfactory efficacy from the herbicide product Sekator OD at all three evaluation dates independently the reporting dates. The same results were obtained by Delchev (2013) and Mitkov et al. (2017) where Secator OD did not control the weed Field bindweed. Practically the weed was not controlled by any of the products used in the study.

On the last reporting date, the weed recovered to a great extent (around 80%) thanks to the amounts of rainfall during the reporting period after herbicide treatments. Similar results were obtained in study conducted by Culhavi and Manea (2011). In their experiment all the variants treated with herbicides, 30 days after treatment and mainly 60 days after treatment *Convolvulus arvensis* L. tended to regenerate shooting new sprouts.



Picture 1. Untreated control



Picture 2. Derby Super 33 g/ha - 13.06.2018



Picture 3. Starane Gold 1.8 l/ha - 13.06.2018



Picture 4. Flurostar 200 - 0.9 l/ha - 13.06.2018

On Figure 4 is shown the data for the height of the winter wheat plants at harvesting time. The shortest plants in the study were the plants of the untreated control (variant 1) (75.56 cm) as well as the plants from treatment 7 (Secator OD

- 0.1 l/ha) (75.67 cm) where the efficacy was negligible.

That is probably due to the infestation with Field bindweed and its concurrence with the winter wheat for space, water and nutrients. It is a relatively poor competitor for light (Wiese and Phillips, 1976).

The plants with the higher reported efficacy after the herbicide application are higher. For the treatments 4 (Starane Gold - 1.8 l/ha) and 6 (Flurostar 200 - 0.9 l/ha) the height reached 86.27 and 87.43 cm respectively.

The values for these two treatments were with proved difference according to Duncan's test in comparison to the other treatments in the experiment.

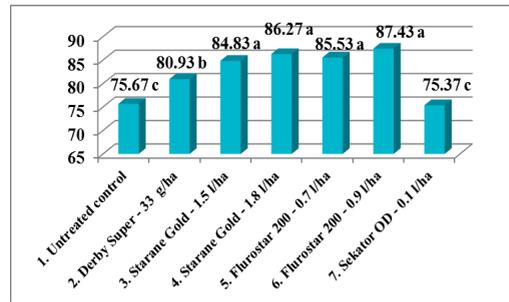


Figure 4. Height of the winter wheat plants at harvesting time, cm.

The variants treated with higher rates of the studied herbicides had higher yields in comparison with those treated with lower doses.

This was probably due to the highest efficacy of the herbicides (Figure 5).

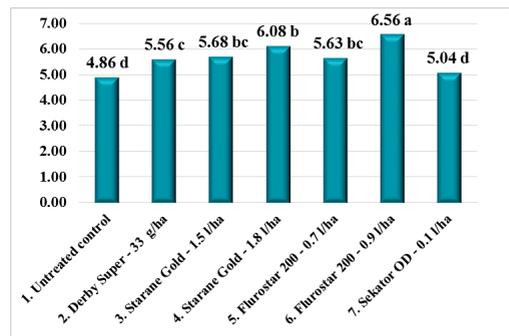


Figure 5. Winter wheat grain yield, t/ha

CONCLUSIONS

The experimental conditions for the growing season of 2018-2019 were favorable for the growth, development and realization of the productive abilities of the winter wheat variety Enola grown in the study.

The temperature values and precipitation during the 7 days before, 7 days after and during the herbicide treatment were suitable for appropriate assessment of the efficacy, selectivity and phytotoxicity of the active substances.

The highest efficacy against *Convolvulus arvensis* L. was recorded for Starane Gold and Flurostar 200. The efficacy of Derby Super WG is extremely low. No satisfactory efficacy from Sekator OD was found.

On the last reporting date, the weed recovered to a great extent (approximately 80%) thanks to the amounts of rainfall during the vegetation; There were no visible signs of phytotoxicity on the crop caused by any of the studied herbicide products.

The plants treated with Starane Gold and Flurostar 200 were the highest and had the longest ears in the study.

The absolute seed mass of 1000 seeds, the hectoliter seed mass and the yield were higher when Starane Gold and Flurostar 200 are applied in high rates.

The plants of the untreated control and those treated with Sekator OD were the shortest and had the shortest ears as well as the lowest grain yield.

The plants of the untreated control had the lowest absolute seed mass of 1000 seeds and hectoliter seed mass.

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REFERENCES

- Briggle, L., Curtis, B. (1987). Wheat Worldwide. In *Wheat and Wheat Improvement*, EG Heyne, ed. Ed. 2nd. American Society of Agronomy/Inc. Publishers (2 more societies), Madison, Wisconsin USA. pp 4-31.
- Malik, R., Singh, S. (1993). Evolving strategies for herbicide use in wheat. Resistance and integrated weed management. *Proceedings of Indian Society of Weed Science International Symposium on Integrated Weed management for Sustainable Agriculture*, 18-20 November. Hisar, India, 1, 225-289.
- Mitkov, A., Neshev, N., Yanev, M., Tonev, T. (2017). Efficacy and selectivity of broadleaf herbicides applied in higher rates at Winter wheat (*Triticum aestivum* L.). *Proceedings of 52 Croatian and 12 International Symposium on Agriculture*, 12-17 February, Dubrovnik, Croatia, 371-375.
- Petrova, Z. (2017). Effect of treatment with foliar herbicides on common winter wheat cultivar Dragana. *Rastenievadninauki*, 54(5), 36-40.
- Tityanov, M., Mitkov, A., Yanev M., Rankova, Z. (2015). Ergon - a new opportunity for an efficient chemical control of BL weeds in wheat. *Аграрна наука*, 18(7), 89-94.
- Tonev, T., Tityanov, M., Mitkov, A., Yanev, M., Neshev, N. (2018). *A Guidebook for Exercises on General Agriculture and Herbology*. Publisher: Biblioteka Zemedelsko Obrazovanie, 71-72.
- Tonev, T., Dimitrova, M., Kalinova, S., Jalnov., Zhelyazkov., Vasilev, A., Tityanov, M., Mitkov, A., Yanev M. (2019). *Herbology*, Plovdiv (in Bulgarian language).
- Vogelgsang, S., Watson, A.K., Ditommaso, A., Hurlle, K. (2008). Effect of the pre-emerge bioherbicide *Phomopsis convolvulus* on seedlings and established plant growth of *Convolvulus arvensis*. *Weed Research*, 38, 175-182.
- Velinova, E., Tityanov, M., Rankova Z., Moskova, C., Dimova, M., Mitkov, A., Yanev M., Neshev, N. (2018). *Innovative products of plant protections and Fertilizers - part I*. Biblioteka Zemedelsko obrazovanie (in Bulgarian language).
- Wiese, A., Salisbury, C., Bean, B., Schoenhals, M., Amosson, S. (1996). Economic Evaluation of Field Bindweed (*Convolvulus arvensis*) Control in a Winter Wheat-Fallow Rotation. *Weed Science*, 44(3), 622-628.

WEED CHEMICAL CONTROL IN THE WINTER WHEAT PLANTING AFTER NON FALLOW PREDECESSORS IN THE NORTHERN STEPPE OF UKRAINE

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Abstract

*The use of alfalfa as predecessors for winter wheat leads to the increase of weed infestation of its cultivation in the result of frequent cutting out of the culture during three years of use. It was shown that of winter wheat yields have had a higher level of weed infestation after non fallow predecessors with inadequate coverage of soil surface. Therefore they demand high-priority protection from weed sprouts. None of the studied herbicides was able to provide reliable protection of wheat crops from bromes (*Bromus tectorum* L.), as well as a sticky weed (*Galium aparine* L.). The tank mix of herbicides (Esteron – 0.8 l/ha + Puma Super – 0.8 l/ha) provides a reliable protection for winter wheat crops from many types of short-lasting weeds including the cattail grass (*Setaria pumila* L.), water grass (*Echinochloa crus-galli* L.). A significant suppressive effect on cereal weeds Super was registered for the herbicide Ellay.*

Key words: winter wheat, weeds, herbicides, grain yield.

INTRODUCTION

Winter wheat distinguishes with high productivity and relates to the plants with high ability to suppress weeds especially due to its cultivation after the best predecessors (clean, early, occupied fallow, perennial herbs, pea, etc.) and growing by intensive technologies (Toigildinv et al., 2016; Gerdzhikova, 2014; Stagnari et al., 2017). A considerable part of winter wheat plantings is cultivated after non fallow crops (sunflowers, stubble cereals) in the result of changing priorities of management, technologies, crop rotation and cropping system.

Depending on the species composition and density of weeds in the crops, delays with the measures to limit harmfulness of weeds may lead to losses of winter wheat grain yield from 40 to 100 % It is a well-known fact that weeds are transmitters of diseases and pests, they complicate the process of crop harvesting and increase costs on product cleaning and drying (Durgan and Gunsolus, 2013; Shaner and Beckie, 2014). Therefore, it is important promptly to carry out recommended different activities on weeds control in order to obtain a planned and quality grain harvest in time

(Hossein and Ismail, 2018; Hossard et al., 2014; Varshney et al., 2012; Ashiq et al., 2007).

The main criteria and arguments due to the herbicide assortment organization are high biological efficiency against a wide variety of weeds, an increased selectivity to cultivation, maximum independence on weather, a wide variety of terms of use, adaptability, security for personnel and environment, the absence of after-action on sensitive crops, and affordable cost of each chemical (Barberi et al., 2010; Benoit et al. 2004; José-Maria and Sans, 2011; Tsyliuryk et al., 2017). All above-mentioned features are appropriate for sulfonylureas (El-Kholy, 2013; Arif et al., 2004; Pacanoski and Mehmeti, 2018).

High activity of sulfonylureas and their significant selectivity allow applying these substances in chemical weeds withdrawal on cereal fields (barley, wheat) and other crops. Analysis of technical and economic efficiency of sulfonylurea drugs in the field conditions showed that cereals are high resistant from the stage of the two leaves before their transformation into a tube (Ferreira et al., 1990). The most effective is achieved in case of spraying of young actively growing weeds. Effectiveness of sulfonylureas does not depend

on weather conditions (Song et al., 2004; Khan et al., 2003). Technical crops treatment can be carried out with these chemicals with a temperature of 5 °C that determines their larger choice of terms and applications (Yin et al., 2008; Hamada et al., 2013).

Tank mixes of sulfonylurea substances are highly effective and selective (Tkalic et al., 2018). On the other hand, there is a fact of after-action of herbicides not only on weeds but on culture plants in the field conditions too. It is important to use mixed combinations of industrial production or tank with the addition of already known substances such as chlorsulfuron or triasulfuron in the form of small additions to other herbicides (Dalga et al., 2014; Modhej and Kaihani, 2013).

The main objective of the research are to determine a biological efficiency of derivative sulfonylureas herbicides and their tank mixes for the protection of winter wheat crops from weeds in the Steppe zone of Ukraine.

MATERIALS AND METHODS

The experiment regarding efficiency of tank mixes and economic threshold of harmfulness of weeds (ETH) was carried out in 2014-2017 with winter wheat ("Spivanka" variety) in the field crop rotation of State Enterprise "Dnipro" of the State Institute of Grain Culture of the Agricultural Science National Academy of Ukraine (Dnipropetrovsk region). Alfalfa, feeding vetch and oat mix were predecessors for winter wheat. Generally, during the years of the experiment, weather conditions were favorable for the growing and development of plants. The soil of the experiment was ordinary, low-humic and hard-loamy chernozem (Kharytonov et al., 2016). The perennial weeds (field bindweed, blue lettuce, field thistle and field milk thistle) characterized average density (25-42000 pcs/m²). Short-annual seeds have got high density (310-460 million pcs/m²). The scheme of the experiment to study the biological efficiency of herbicides on the background of the two predecessors (alfalfa, mix of vetch and oats) included 10 trials: a) control (without herbicides); b) Granstar - 25 g/ha; c) Esteron 60-0.6 l/ha; d) Banvel 4C - 0.3 l/ha; e) Grodyl Maxi - 100 ml/ha; f) Ellay Super - 0.3 l/ha + PAR Trend 90-0.3 l/ha; g) Esteron - 0.8 l/ha; h) Esteron - 0.8 l/ha + Puma super -

0.8 l/ha; i) Mastak - 0.5 l/ha; j) Granstar Gold - 18 g/ha. Herbicides and their tank mixes were applied by spraying along consumption rate of spray solution in amount of 250-300 l/ha. The plot area of the experiment was 100 m² (20 m × 5 m), accountable is 43 m² triple repeatability. Accountability of weed infestation was undertaken before herbicide application, after 30 days of the treatment and before winter wheat crops harvesting. Efficiency of the herbicides which were used to protect the grain yields from weeds was calculated by formula:

$$E=100\% - \left(\frac{K_2}{K_1}\right) \times 100, (\%), \quad (1)$$

where:

E - part of eliminated weeds of the total number in the yields before spraying process, %;

K₂ - a number of weeds in the winter wheat plantings in case of the maximum effect of the applied herbicide, pcs/m²;

K₁ - amount of weeds in crops sowing before the spraying process, pcs/m².

A new methodology of the ETH determination was developed: a) from 50% to 84% - inadequate coverage; b) from 85% to 95% - adequate coverage; c) from 96% and more - best coverage.

RESULTS AND DISCUSSIONS

It was established that underdeveloped plants of winter wheat are formed after non fallow predecessors, especially in dry years with lack of autumn precipitations. The full projective cover of the ground surface in next spring period occurs on the level of 35-45%, encourages the increase of lower layer of plant stand, and affects the increase of weed infestation (Table 1). Sowings after non fallow predecessors were weeded particularly with common ragweed (*Ambrosia artemisiifolia* L.), as well as with white goosefoot (*Chenopodium album* L.). These weeds create a potential threat to the yield reduction. Therefore, they were eliminated at first. Except for these three main weeds, sporadically more 10-12 species of early weeds were found in the crops. The herbicide Esteron 60.85% CU (2-Ethylhexyl ester 2.4-D, 850 g/l) showed the best results regarding to control of common ragweed (*Ambrosia artemisiifolia* L.) and white goosefoot (*Chenopodium album* L.) in three trials.

Table 1. Quantitative and species composition of short - annual weed shoots of winter wheat planting, pcs/m²

Botanical name of weeds	Agrobiologic group and other features	Density of shoots, (pcs/m ²)	
		Alfalfa	Vetch and oats
Common ragweed (<i>Ambrosia artemisiifolia</i> L.)	An early spring annual plant with a late fruitage. Quarantine allergen weed	105.2	73.6
Dropping brome (<i>Bromus tectorum</i> L.)	An early cereal weed in winter wheat crops, herbicide resistant	18.4	1.3
Purple dead-nettle (<i>Lamium purpureum</i> L.)	An annual weed with kidney-shaped leaves: first on the petioles and standing upper	0.1	0.2
Field gromwell (<i>Lithospermum arvense</i> L.)	An annual dioecious weed that has winter and spring shape	3.5	0.2
Shepherd's purse (<i>Capsella bursa-pastoris</i> L.)	A ruderal weed with winter and spring shapes and a long-term (35 years) grain viability	6.7	2.1
Herb-Sophia (<i>Descurainia sophia</i> L.)	An early spring weed with winter and spring shapes	4.6	1.2
Ragworts (<i>Senecio vernalis</i> Waldst)	A ruderal spring weed able to develop as a winter one	1.3	0.4
Goosefoot (<i>Chenopodium album</i> L.)	A dioecious early ruderal and segetal weed with a high (up to 700,000) fruitage	67.8	52.5
Sticky weed (<i>Galium aparine</i> L.)	A spiniferous plant with climbing stems, herbicide 2.4-D resistant	3.9	0.7
Fumewort (<i>Fumaria Schleicheri</i> Soy.)	An annual ruderal spring poisonous weed with pinnatisected leaves	1.2	0.3
Rocket-larkspur (<i>Consolida arvensis</i> L., <i>Delphinium consolida</i> L.)	Spring or wintering annual weed which infects mainly crops of winter wheat	0.7	0.1
Small tumbleweed mustard (<i>Sisymbrium loeselii</i> L.)	Mainly two-year-old ruderal plant, 70-130-centimeter height. It produces a lot of seeds which contaminates soil	0.2	0.0
Field pennycress (<i>Thlaspi arvense</i> L.)	An early spring and wintering annual plant	0.4	0.5
Wild buckwheat (<i>Fallopia convolvulus</i> L.)	An early ruderal and segetal weed with climbing stems	0.3	0.3
Giant sumpweed (<i>Cyclachaena xanthiifolia</i> (Nutt.) Fressen, <i>Iva xanthiifolia</i> Nutt.)	An early spring one-year-old, allergen weed with the height of stems from 0.6-0.8 metres to 2.5-3 metres	0.8	0.1
Total:		214.5	133.5

Both destroyed and damaged by the chemical weeds, as well as those which came up of the potential stock of seeds in the soil after spraying were included in the registration.

The data on winter wheat crops infestation before the herbicides application are shown in the Figure 1. The number of vegetative plants of common ragweed (*Ambrosia artemisiifolia* L.) and white goosefoot (*Chenopodium album* L.) in the winter wheat crops was the least after 24 days of the appliance of the herbicide, particularly, at the areas of the experiment (Figure 2).

None of the studied herbicides was able to provide reliable protection of wheat crops from bromes (*Bromus tectorum* L.), as well as a sticky weed (*Galium aparine* L.). The tank mix of herbicides (Esteron - 0.8 l/ha + Puma Super - 0.8 g/ha) provides a reliable protection for winter wheat crops from many types of short-lasting weeds including the cattail grass (*Setaria pumila* L.), water grass (*Echinochloa crus-galli* L.). However it does not guarantee proper control of cheatgrass (*Bromus inermis* Leyss L.).

It should be pointed out that after the second register of infestation in the winter wheat crops

regarding control (without herbicides), particularly less amount of short-annual shoots of weeds was identified (Figure 3). The density of cheat grass in the winter wheat crops sowed after third year of perennial crops use is gradually increasing during the vegetation period (Figure 4).

An important stage to provide development of winter wheat crops after non fallow predecessors is the time of temporary interruption or remission of biological (technical) influence on weeds by applied herbicides to suppress weeds and prevent their fruitage and regeneration. A malicious quarantine weed common ragweed (*Ambrosia artemisiifolia* L.) created the main agrotpe of infestation on the whole herbicide

backgrounds even in the conditions of the last register of weed infestation.

An amount of ambrosia plants reached 57.6 pcs/m² comparative to control (without herbicides) after the last register of weed infestation. Unfortunately, sustainable control strategies to mitigate its spread into areas not yet invaded and to reduce its abundance in badly infested areas are lacking (Gerber et al., 2011). Application of herbicides and their tank mixes on the winter wheat crops particularly had an effect on grain yield of winter wheat (Figure 5). The highest winter wheat grain yield (6.2 t/ha) was registered in trial h with the appliance of tank herbicide mixes (Esteron - 0.8 l/ha + Puma Super - 0.8 l/ha).

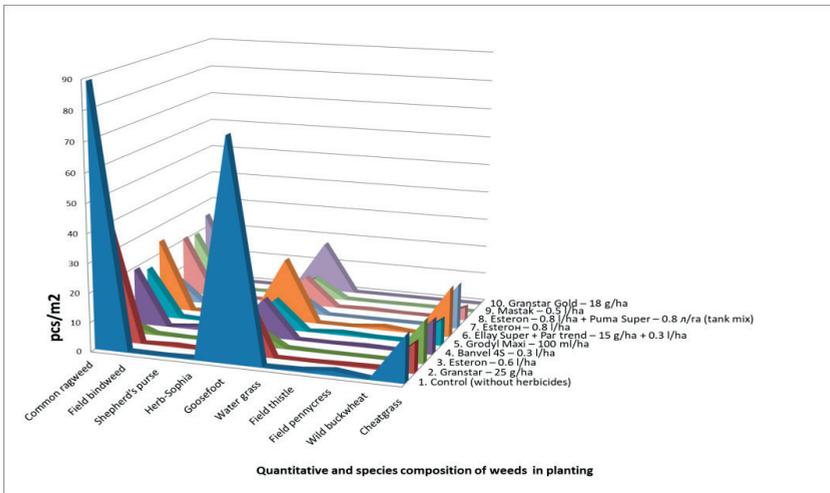


Figure 1. Winter wheat crops infestation before the herbicides application, pcs/m²

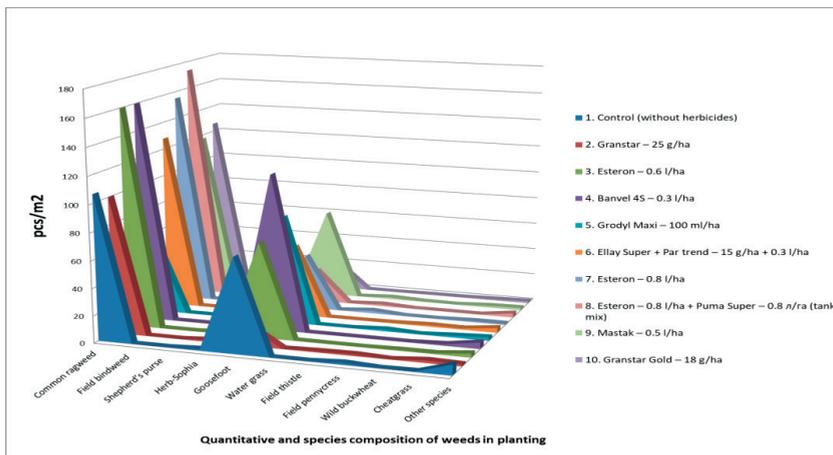


Figure 2. Winter wheat crops infestation in 24 days after the herbicides application, pcs/m²

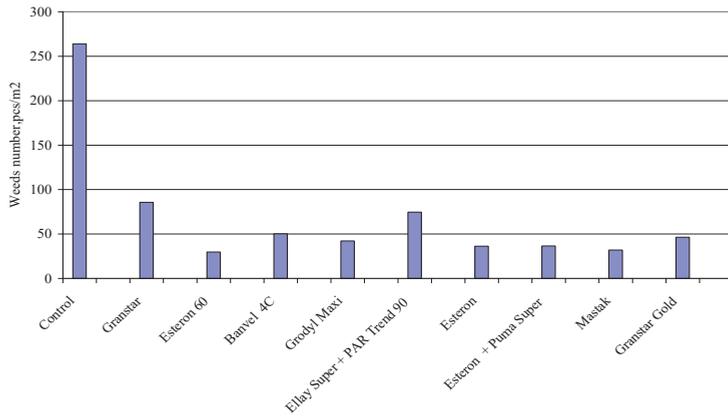


Figure 3. Weed control with herbicide

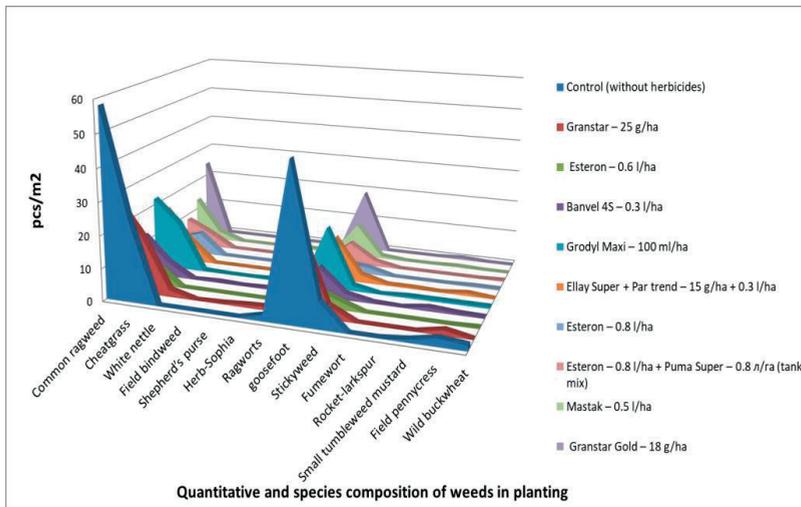


Figure 4. Winter wheat crops infestation before harvesting, pcs/m²

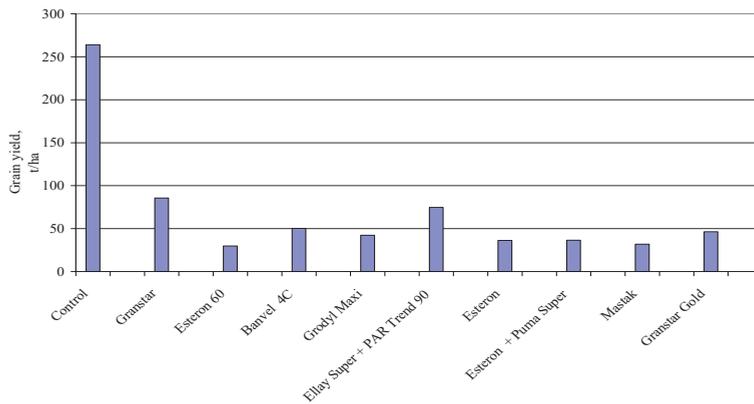


Figure 5. Winter wheat grain yield in the trials with different weed control (2014-2017, t/ha)

Furthermore, during harvesting, the mass of weeds in the air-dried basis was 16 g/m². Weeds did not pass to the upper and even middle layer, as well as there were in suppressed state and did not create a threat of crop losses. It should be mentioned also that high winter wheat yield (6.2 t/ha) on the area where the efficacy of herbicide Esteron - 0.8 l/ha was not worse than the tank mix of the trial (Esteron - 0.8 l/ha + Puma Super - 0.8 l/ha). A significant suppressive effect on cereal weeds Super was registered for the herbicide Ellay. Herbicides Granstar (25 g/ha) and Granstar gold (18 g/ha) were ineffective and practically did not stop the growth and development of ragweed (*Ambrosia artemisiifolia* L.) and white quinoa (*Chenopodium album* L.).

It was established that a number of weed shoots per unit area are not always related to their effect on the grain yield. It was shown that of winter wheat yields have had a higher level of weed infestation after non fallow predecessors with inadequate coverage of soil surface. Therefore they demand high-priority protection from weed sprouts. In general, the problem of effective protection from weeds of winter wheat after non fallow predecessors minimises to solving of the two main objectives which are to prevent seed bank growth of their short-annual species and vegetative regeneration of perennial rooted weeds.

CONCLUSIONS

The use of alfalfa as a predecessor for winter wheat leads to the increase of weed infestation of its cultivation in the result of frequent cutting out of the culture during three years of use, its gradual "drop-out" from agrocenosis and filling ecological niche with harmful weeds in the result of lower layer lightness of crops stands especially rooted and rooted-stem species. Highest level of weeds control was provided due to the herbicide Esteron 60.85% CU (2-Ethylhexyl ester 2,4-D, 850 g/l) both in individual and tank mixed application (Esteron - 0.8 l/ha + Puma Super - 0.8 l/ha).

The maximum winter wheat yield was registered in trial with tank mixes of herbicides (Esteron - 0.8 l/ha + Puma Super - 0.8 l/ha) - 6.2 t/ha.

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REFERENCES

- Arif, M., Awan, I.U., Khan, H.U. (2004). Weed management strategies in wheat (*Triticum aestivum* L.). *Pak. J. Weed Sci. Res.*, 10(1-2), 11–16.
- Ashiq, M., Muhammad, N., Ahmad, N. (2007). Comparative efficacy of different herbicides against broad leaf weeds in wheat. *Pak. J. Weed Sci.*, 13(3-4), 149–156.
- Bärberi, P., Bburgio, G., Dinelli, G., Moonen, A.C., Otto, S., Vazzana, C., Zanin, G. (2010). Functional biodiversity in the agricultural landscape: relationships between weeds and arthropod fauna. *Weed Research*, 50, 388–401.
- Benoit, D.L., Kenkel, N.C., Cavers, P.B. (2004). Factors influencing the precision of soil seed bank estimate. *Canadian Journal of Botany*, 67, 2833–2840.
- Dalga, D., Sharma, J., Tana, T. (2014). Evaluation of Herbicides and Their Combinations for Weed Management in Bread Wheat (*Triticum Aestivum* L.). *International Journal of Novel Research in Life Sciences*, 1(1), 31–47.
- Durgan, B.R., Gunsolus, J.L. (2013). Developing weed management strategies that address weed species shifts and herbicide resistant weeds on wheat crop. *Extension Weed Scientists*, Department of Agronomy and Plant Genetics University of Minnesota, 23–31.
- El-Kholy, R.M.A., Abouamer, W.L., Ayoub, M.M. (2013). Efficacy of some herbicides for controlling broad-leaved weeds in wheat fields. *Journal of Applied Sciences Research*, 9(1), 945–951.
- Ferreira, K. L., Baker, T.K., Peeper, T.F. (1990) Factors Influencing Winter Wheat (*Triticum aestivum*) Injury from Sulfonylurea Herbicides. *Weed Technology*, 4(4), 724–730.
- Gerber, E., Schaffner, U., Gassmann, A., Hinz, H.L., Seier, M., Müller-Schärer, H. (2011). Prospects for biological control of *Ambrosia artemisiifolia* in Europe: learning from the past. *Weed Research*, 6, 559–573.
- Gerdzhikova, M. (2014). Influence of N fertilization and predecessors on Triticale yield structure characteristics. *Turkish Journal of Agricultural and Natural Science*, 2, 1922–1932.
- Hamada, S.H.E., Abdel-Lateef, M.F., Abdelmonem, A.E., El-Kholy, R.M.A., Helalia, A.A.R. (2013). Efficiency of certain clodinafop-propargyl formulations in controlling annual grassy weeds in wheat. *Annals of Agricultural Science*, 58(1), 13–18.
- Hossard, L., Philibert, A., Bertrand, M., Colnenne-David, C., Debaeke, P., Munier-Jolain, N., Jeuffroy, M. H., Richard G., Makowski D. (2014). Effects of halving pesticide use on wheat production. *Sci Rep.*, 4, 4405.
- José-Maria, L. Sans, F.X. (2011). Weed seedbanks in arable fields: effects of management practices and

- surrounding landscape. *Weed Research*, 51(6), 631–640.
- Hossein, M., Ismail, B.S., (2018). Effect of Herbicides on the Density of Broad Leaf Weeds and their Effect on the Growth and Yield Components of Wheat (*Triticum aestivum* L.). *Journal of Agronomy*, 17, 11–17.
- Khan, N, Hassan, G, Khan, M.A., Khan, I. (2003). Efficacy of different herbicides for controlling weeds in wheat crop at different times of application-I. *Asian J Plant Sci.*, 2, 305–309.
- Kharytonov, M.M., Pashova, V.T., Bagorka, M.O., Kozechko, V.I., Dudar, T.O. (2016). Arable lands degradation in the northern steppe zone of Ukraine. *Agriculture and Forestry Journal*, 62(2), 071–080.
- Modhej, A. Kaihani, A. (2013). Effect of nitrogen fertilizer and herbicides on weed control and wheat grain yield under subtropical conditions. *International Journal of Biosciences*, 3(10), 73–81.
- Pacanoski, Z., Mehmeti, A. (2018). Post herbicide programme for effective weed control in winter wheat (*Triticum aestivum* L.). *Agronomy Research*, 16(4), 1796–1808.
- Shaner, D.L., Beckie, H.J. (2014), The future for weed control and technology. *Pest Management Science*, 70, 1329–1339.
- Song, N.H, Yin, X.L, Chen, G.F, Yang, H. (2007). Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. *Chemosphere*, 68, 1779–1787.
- Stagnari, F., Maggio, A., Galieni, A., Pisante, M.(2017). Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and biological Technologies in Agriculture*, 4(2).
- Tkalich, Yu.I., Tsyliuryk, A.I., Masliiov, S.V., Kozechko, V.I. (2018). Interactive effect of tank-mixed post emergent herbicides and plant growth regulators on corn yield. *Ukrainian Journal of Ecology*, 8(1), 961–965.
- Toigildinv, A.V., Morozov, V.I., Podsevalov, I.I., Isaev, M.Y., Toigildina, I.A. (2016). Selection of winter wheat predecessors in crop rotations of the Volga region forest steppe. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7(6), 2203–2209.
- Tsyliuryk, A.I., Tkalich, Yu.I., Masliiov, S.V., Kozechko, V.I. (2017). Impact of mulch tillage and fertilization on growth and development of winter wheat plants in clean fallow in Northern Steppe of Ukraine. *Ukrainian Journal of Ecology*, 7(4), 511–516.
- Varshney, S., Hayat, S., Alyemeni, M.N., Ahmad, A.. (2012). Effects of herbicide applications in wheat fields. *Plant Signal Behav.*, 7(5), 570–575.
- Yin, XL, Jiang, L, Song, NH, Yang, H. (2008). Toxic reactivity of wheat (*Triticum aestivum*) plants to herbicide isoproturon. *J Agric Food Chem.*, 56, 4825–4831.

COMPOSITION AND QUALITY OF RAPESEED OIL (*Brassica napus oleifera biennis*) DEPENDING ON SOWING TIME AND TREATMENT WITH LEAF FERTILIZERS

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Abstract

The study was conducted in the period 2012-2015 in the area of Training, Experimental and Implementation centre of the Department of Crop Production at the Agricultural University - Plovdiv. The experiment was based on a block method, in 4 repetitions, with the size of the experimental plot of 20 m² with Visby hybrid. The leaf fertilizers Lactofol B, Litovit® and Fertiactyl Starter were applied. The purpose of the study was to determine the content and fatty acid composition of winter rapeseed oil sown at different sowing times and treated with several leaf fertilizers. The fat content is determined by the residual method with the Soxhlet apparatus and the fatty acids - by extraction. Depending on the variants, the crude fat content varies from 29.51 to 35.79. The amount of saturated fatty acids is relatively low from 4.85% to 6.56%. The amount of unsaturated fatty acids was highest in the Fertiactyl Starter treated variants (93.77 to 95.24%), while the lowest was in the controls (93.18 to 93.63%).

Key words: rapeseed, sowing dates, leaf fertilizers, fats, fatty acids.

INTRODUCTION

The continuous climate change in recent years leading to global warming in turn has a negative impact on the weather conditions necessary for the optimal development of field crops.

In years with minimal or no rainfall during the sowing and germination of rapeseed, plant development slows down, necessitating the use of different ways to accelerate these processes. The use of leaf fertilizers is one of them.

Rapeseed is a crop that responds very well to foliar fertilization, which is usually applied under stressful conditions during the growing season, or when necessary to stimulate specific growth and physiological processes when they are disturbed. (Gillingham et al., 2011).

That is why we have set ourselves the goal of testing several leaf fertilizers and the stages of their application in rapeseed sown at different times, on the quality of the oil in the seeds obtained in these variants.

MATERIALS AND METHODS

The experiment was conducted during the period 2012-2015 in the area of Training, Experimental and Implementation centre of the

Department of Crop Production at the Agricultural University - Plovdiv. The experiment is based on a block method, repeated 4 times, with a plot size of 20 m², with Visby hybrid, originating in Germany, in the following variants:

- Factor A - sowing dates;
- Factor B - leaf fertilizers;
- Factor C - phases of treatment.

I. Sowing dates, 1-10.IX.; 1-10.X.

- Untreated variant;
- Spraying with Lactofol B - 400 ml/da, phenophase - 2-4th leaf;
- Spraying with Litovit® - 200 g/da, phenophase - 2-4th leaf;
- Spraying with Fertiactyl Starter - 300 ml/da - 2-4th leaf.

II. Sowing dates, 10-20.X.; 20-30.X.

- Untreated variant;
- Spraying with Lactofol B - 400 ml/da, phenophase - 1-2nd leaf;
- Spraying with Litovit® - 200 g/da, phenophase - 1-2nd leaf;
- Spraying with Fertiactyl Starter Spray - 300 ml/da - 1-2nd leaf.

The study was conducted according to the conventional rapeseed technology following

wheat precursor. The seeds for analysis were obtained from the averaging of seed obtained from rapeseed grown at different sowing times and treated with different leaf fertilizers. The fat content is determined by the residual method with the Soxhlet apparatus, while the fatty acids - by extraction.

The soil on which the experiment was carried out is a former meadow swamp soil, slightly saline, sandy clay with an "A" humus horizon thickness of 25-28 cm. In mechanical composition it is a clayey, heavy soil (Popova et al., 2012).

The agro-meteorological characteristics of the region of Southern Bulgaria show that no significant deviations from the average monthly temperatures are observed compared to the multiannual period. Greater differences are reported in terms of moisture. The years of the study were characterized by a sufficient amount of moisture in the critical phases of rapeseed development, with the exception of the sowing-germination period during the first two years.

RESULTS AND DISCUSSIONS

The fat content of the seeds depends on the type of plant, the degree of ripening of the seeds and also on the growing conditions of the plants. A number of studies show that both the fat content and the qualitative composition are influenced by humidity, temperature, and geographical factor, chemical and physical properties of the soil, organic and mineral fertilizers, and the whole system of agrotechnical activities.

Data on the percentage fat content in the variants tested are presented in Table 1. It can be seen that the crude fat content is low and ranges from 29.51 to 35.79%.

The lowest fat content was observed in control variants and later sowing dates, while highest - in the first sowing date (30.45%). A decrease was observed in the second (30.24%) and third sowing date (29.51%), while the last (20-30.10.) there were no plants.

The reduction of fat at later sowing dates is most likely due to the later entry of the plants into the developmental stages and the poor maturation of the seeds.

In all variants treated with leaf fertilizers, the values of fatty acids increase. The strongest

stimulating effect on seed quality is that of the leaf fertilizer Lactofol B, where the fat content is highest and ranges from 35.07 to 35.79%. Both in the control variant and in these variants, at later sowing dates, the values slightly decrease, and in the last sowing date, there are no plants.

The stimulating effect on plant development is strongest in the variant treated with Fertiactyl Starter leaf fertilizer. It is the only variant in which at the latest sowing date the plants pass through all the stages up to ripening.

Differences in the fat content of this variant between the different sowing dates are minimal, from 33.44% at the first date; 32.99%, at the second; 32.77%, at the third date, to 32.57%, at the latest date.

The ratio of saturated and unsaturated fatty acids determines the line of use of the oil obtained. The low content of saturated fatty acids (6-11%) makes rapeseed oil one of the most preferred vegetable oils in the world.

The meta-analyses carried out by Mitrović, P. et al. (2009) have found a close relation between high consumption of these fats and elevated levels of "bad" cholesterol in the blood and the risk of ischemic heart disease. However, the consumption of small amounts of saturated fat is extremely important, both for the proper functioning of the body and brain and for optimizing metabolism.

The data in Table 1 shows that in the variants studied the amount of saturated fatty acids is relatively low and meets the quality standards. In the variants tested, their oil content varies from 4.85% to 6.56%, indicating that it can be used for nutritional purposes without any health problems. The highest content of saturated fatty acids was recorded in the control variants, from 6.14% to 6.56%.

Foliar fertilizer treatment has a positive effect on reducing their content. This is most strongly influenced by the treatment of plants with Litovit®. In this variant, the saturated fat values are the lowest (5.04; 4.90 and 4.85%). The content of saturated fat does not differ significantly between the different sowing dates.

The fatty acid composition of rapeseed oil in terms of saturated fatty acids is presented in Table 2.

The data in the table shows that the share of the palmitic acid (4.02-4.36%) is the largest, followed by stearic (0.27 to 1.61%). A number of studies have found that amounts of 2.5 to 7.0% for palmitic and 0.8 to 3% for stearic acids do not have a harmful effect on human (Shpaara, 2007).

In the variants we tested we did not find quantity above these values. In the control variants, the palmitic acid content ranged from 4.17% to 4.36% and the stearic acid - from 1.31% to 1.61%. The difference in content at different sowing dates is negligible.

Table 1. Fat content, saturated and unsaturated fatty acids in the oil of the tested variants

Content of fat, saturated and unsaturated fatty acids, %	VARIANTS			
	Control	Fertiactyl Starter	Litovit ®	Lactofol B
1-10.IX.				
Crude fat content	30.45	33.44	32.15	35.79
Saturated fatty acids, %	6.56	5.23	5.04	5.15
Unsaturated fatty acids, %	93.18	94.53	94.28	93.35
1-10.X.				
Crude fat content	30.24	32.99	31.75	35.31
Saturated fatty acids, %	6.14	5.00	4.90	6.03
Unsaturated fatty acids, %	93.01	95.24	94.01	93.63
10-20.X.				
Crude fat content	29.51	32.77	30.44	35.07
Saturated fatty acids, %	6.23	5.11	4.85	6.13
Unsaturated fatty acids, %	92.40	94.86	94.66	93.46
20-30.X.				
Crude fat content	-	32.57	-	-
Saturated fatty acids, %	-	5.13	-	-
Unsaturated fatty acids, %	-	93.77	-	-

Table 2. Fatty acid composition of rapeseed oil - saturated fatty acids

Content of saturated fatty acids, %	VARIANTS			
	Control	Fertiactyl Starter	Litovit ®	Lactofol B
1-10.IX.				
C16:0- Palmitic acid	4.36	4.11	4.12	4.12
C18:0- Stearic acid	1.61	0.63	0.37	0.55
C20:0- Arachidic	0.59	0.49	0.55	0.48
C22:0- Behenic acid	-	-	-	-
1-10.X.				
C16:0- Palmitic acid	4.29	4.12	4.13	4.10
C18:0- Stearic acid	1.41	0.45	0.29	0.42
C20:0- Arachidic	0.46	0.49	0.48	0.52
C22:0- Behenic acid	0.59	-	-	-
10-20.X.				
C16:0- Palmitic acid	4.17	4.12	4.02	4.01
C18:0- Stearic acid	1.31	0.39	0.27	0.28
C20:0- Arachidic	0.61	0.49	0.56	1.11
C22:0- Behenic acid	-	-	-	-
20-30.X.				
C16:0- Palmitic acid	-	4.19	-	-
C18:0- Stearic acid	-	0.38	-	-
C20:0- Arachidic	-	0.46	-	-
C22:0- Behenic acid	-	-	-	-

In the variants treated with foliar fertilizers, the content of these acids decreases compared to the control crops.

The palmitic acid values of the treated variants of all sowing dates are almost the same and the differences with the control crops are minimal. In the case of stearic acid, the treatment with leaf fertilizers has a stronger effect on reducing its values. Its content in the oil of the control variants exceeds several times that of the treated variants.

Rapeseed oil is a high quality vegetable oil characterized by its low content of saturated and high content of unsaturated fatty acids.

Unsaturated fatty acid content data are reported in Table 1. They show that at all sowing dates their content is the lowest in the

control variants, from 92.40% to 93.18%. Their decrease at later sowing dates is negligible.

Their quantity in all fertilized variants increased, with the highest value reported in the variant treated with Fertiaetyl Starter, from 93.77% - at the latest sowing date to 95.24% - at the second sowing date, followed by the variant treated with Litovit®. The least stimulating effect was achieved in the treatment of the plants with Lactofol B, where the values of unsaturated fatty acids were almost as high as in the control crops (93.35% to 93.63%).

The high quality of rapeseed oil is guaranteed by the high content of oleic and linoleic acid and the low content of linolenic and erucic acid (Table 3).

High levels of oleic acid are very important for human health. It participates in the composition of the cell membranes, stimulates the cell receptors for "bad" cholesterol and reduce its amounts in the blood, participates in the formation of the protective envelope of nerve endings, improves the immune function, protects the body from the development of cardiovascular disease, reduces insulin resistance thus increasing glucose metabolism (Gillingham, LG et al., 2011). Therefore, the purpose of the selection is to continuously increase it to that of olive oil, above 80%.

In other experiments in the region, Visby varieties reported oleic acid values, from 69.70% to 76.8% (Todorov et al., 2012)

In this variants, oleic acid ranges from 63.04% to 68.21%. Its lowest values are in the control crops, from 63.04% to 66.90%. It is noteworthy

that treating the variants with foliar fertilizers increased its content.

At the first sowing date, the highest amount of oleic acid was reported in the variant treated with Fertiaetyl Starter, 67.54%, in the other variants the effect of the treatment was not reported.

At the second sowing date, the treated variants most strongly exceeded the oleic acid content compared to that of the control crops, by 4.51% when treated with Fertiaetyl Starter, by 4.29, when treated with Litovit® and by 1.98%, with Lactofol B.

At the third sowing date, the strongest stimulation effect was observed in the Litovit ® treated variant, in which the oleic acid content exceeded the control by 2.44%.

At the latest sowing date, oleic acid content was reported only in the Fertiaetyl Starter treated variant (66.54%).

The second most important unsaturated fatty acid is linoleic acid. Due to its high specific biological activity, it is often called vitamin F in dermatology. Certain minimal doses of linoleic acid, introduced into the body, regulate hormonal metabolism, have antagonistic action on cholesterol, are important in the prevention and treatment of atherosclerosis and other heart disease.

When testing different varieties of rapeseed its content varies from 12.3% to 17.7% (Mariana Petkova-Andonova et al., 2012).

In this experiment, the linoleic acid content of the various variants varied from 16.89% to 19.04%.

At the first sowing date, better results for the linoleic acid content were obtained from foliar fertilization than the control. The strongest stimulating effect was observed with the variant treated with Lactofol B, which exceeded the control with 1.34%.

At the second sowing date, the highest linoleic acid content was reported in the control variant (19.04%). In the treated variants, no stimulating effect was reported and the values of this acid were significantly lower (17.85; 17.46; 17.30%).

At the third sowing date, there was almost no difference in content between the variants.

Almost the same amount (17.89%) was reported for the last sowing date in the only

Table 3. Fatty acid composition of rapeseed oil - unsaturated fatty acids

Content of unsaturated fatty acids, %	VARIANTS			
	Control	Fertiactyl Starter	Litovit ®	Lactofol B
1-10.IX.				
C18:1-Oleic acid	66.90	67.54	66.71	66.51
C18:2-Linoleic acid	16.89	17.69	17.39	18.23
C18:3-Linolenic acid	6.53	7.87	7.69	7.98
C18:3-3-Alpha-linolenic acid	-	-	-	-
C 20:1-Gadoleic acid	1.24	1.16	1.04	1.18
C20:2- Eicosadienoic acid	1.34	-	-	0.10
C22:1- Erucic acid	-	-	-	-
1-10.X.				
C18:1-Oleic acid	63.04	67.55	67.33	65.02
C18:2-Linoleic acid	19.04	17.85	17.46	17.30
C18:3-Linolenic acid	7.21	7.80	7.83	7.53
C18:3-3-Alpha-linolenic acid	-	0.49	-	-
C 20:1-Gadoleic acid	1.13	1.10	1.12	2.90
C20:2- Eicosadienoic acid	2.95	0.22	-	-
C22:1- Erucic acid	-	-	-	-
10-20.X.				
C18:1-Oleic acid	65.77	67.40	68.21	63.58
C18:2-Linoleic acid	17.23	17.87	17.49	16.90
C18:3-Linolenic acid	7.10	8.0	7.49	7.31
C18:3-3-Alpha-linolenic acid	-	-	-	0.55
C 20:1-Gadoleic acid	2.83	1.13	1.33	-
C20:2- Eicosadienoic acid	0.26	-	-	3.81
C22:1- Erucic acid	-	-	-	-
20-30.X.				
C18:1-Oleic acid	-	66.54	-	-
C18:2-Linoleic acid	-	17.89	-	-
C18:3-Linolenic acid	-	7.87	-	-
C18:3-3-Alpha-linolenic acid	-	-	-	-
C 20:1-Gadoleic acid	-	1.09	-	-
C20:2- Eicosadienoic acid	-	0.10	-	--
C22:1- Erucic acid	-	-	-	-

variant that reached full maturity phase treated with Fertiactyl Starter

Another fatty acid present in rapeseed oil is linolenic acid. It is a valuable fatty acid, but it is easily oxidized and reduces the quality of the food when stored. Reducing its content makes rapeseed oil more resistant to rancid. A high-linolenic fat oil is better used for technical purposes and combustion mixed with other oils. The purpose of selection for this acid is to reach the theoretically desired "0".

Although the content varies slightly between variants, the amount is much higher than other studies of the same variety (3.7%), (from 6.53% to 8%). (Todorov et al., 2012; Ivanova et al., 2009; Perfanova & Mechenov, 2001).

The content of the other two unsaturated fatty acids (Gadoleic and Eicosadienic) in all tested variants and sowing dates is insignificant.

The variants we tested did not account for the presence of harmful erucic acid.

CONCLUSIONS

The content of fat and unsaturated fatty acids was the lowest in the control variants and higher in the treated variants.

The strongest stimulating effect on fat content was observed in Lactofol B treated variant (from 35.07% to 35.79%) and on the unsaturated fatty acids in Fertiactyl Starter treated variant (from 93.77 to 95.24%).

The tested variants did not account for the presence of harmful erucic acid. In all variants, the content of saturated fatty acids is low and varies from 4.85% to 6.56% and can be easily used for nutritional purposes. The only variant where the plants go through all the stages to full maturity is the one treated with the leaf fertilizer Fertiactyl Starter. The highest content of all indicators studied was reported at the first sowing date, 1-10.IX.

REFERENCES

- Gillingham, L.G., Gustafson, J.A., Khan, S.Y., Jassal D.S., Jones, P.J.H. (2011). High-oleic canola (rapeseed) oil and flax seed oil changes erumlipids and inflammatory biomarkers in hypercholesterol aemicsubjects, *British Journal*, NA, p.417.
- Ivanova, R., Todorov, Zh., Delibaltova, V. (2009). Quality of seeds and oil obtained from the cultivation of several varieties and hybrids of rapeseed in two regions of Southern Bulgaria. Ninth Scientific Conference Ecology, Agriculture, Animal Husbandry, Agroeco 2009. *Scientific Papers, LIV*, Agrarian University Academic Publishing House, 59–64.
- Mitrović, P., Milovac, Ž., Marinković, R. (2009). Current problems in the protection of winter rapeseed (*Brassica napus* var. *napus* L.) Marinković, R., Institutzaratarstvoipovrtarstvo, Novi Sad (Serbia), Plant Protection.
- Perfanova, M., Mechenov, G. (2001). Comparative characteristics of Canola type rapeseed used for oilseed production. *Scientific Papers of the Union of Scientists - Plovdiv, Series B. Engineering and Technology, Physics and Mathematics, II*, 145–148.
- Petkova-Andonova M., Ivanova, R., Todorov, Zh. (2012). Fatty acid composition of the oil from several hybrids of winter oilseed rape grown in Central Southern Bulgaria. *Agrarian Sciences, II*, 39–43.
- Popova, R., Zhalnov, I., Valcheva, E., Zorovskiand, P., Dimitrova, M. (2012). Assessment of the Ecological Status of Soil sin the Plovdiv Region with Application of New Herbicides for Weed Control in Main Field Crops. *Journal of Central European Agriculture, 13*(3), 604–609.
- Shpaara, D., 2007. Rapeseed and woad, 2nd and expanded edition, book, Moscow.
- Todorov, Zh., Ivanova, R., Petkova-Andonova, M. (2012). Fatty acid composition of the oil of several hybrids winter oilseed rape. *Field Crops Studies, VIII*(1), 97–102.

PRODUCTIVITY OF OIL-BEARING ROSES UNDER ORGANIC AND CONVENTIONAL SYSTEMS

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Abstract

Organic production and markets are expanding rapidly. A field study was carried to compare effects of different agriculture soil system on productivity of *Rosa damascena* Mill. and *Rosa damascena* x *Rosa gallica*. The experiment was conducted on six private arable areas with oil bearing rose in Rose valley, Southern Bulgaria. The selected study area size was 5000 m² from each private territory. The flowers of *R. damascena* were picked up in the morning (6:00-8:00 a.m.), in 3, 4 and 5 phases. The productivity was determined as essential oil content in the blossoms. Soil samples were also collected from every study area from surface horizon 0-30 cm. The samples were analyzed for several soil parameters as, organic matter content, pH values, available nitrogen, phosphorus and potassium content. Statistical analysis was done with Unscrambler (Camo, Norway) software packages. The agricultural system of the oil bearing rose (*Rosa damascena* Mill.) grown has an effect on the essential oil content. The mean value of essential in organic production = 0.026% is statistically proven lower than conventional production = 0.038%.

Key words: rose oil, organic system, conventional system, low input system.

INTRODUCTION

Growing oil-bearing roses and production of rose oil is a tradition with a long history of over 300 years in Bulgaria. Bulgaria together with Turkey supply around 80-90% of the rose essential oil consumed worldwide by the perfumery and cosmetics industry (Kovacheva et al., 2010). Almost 95% of rose fields in Bulgaria are based on a single genotype of *R. x damascena* f. *trigintipetala* Dieck (the thirty petalled rose). In more recent years new hybrids based on crosses between *R. x damascena* and *R. gallica* showing increased resistance to diseases and good quality of the rose oil were also spreaded (Kovacheva, 2007). The Bulgarian rose oil is a key ingredient used by leading companies like Kenzo, Chanel, Dior, Fendi, Bulgari, Faberge and many others. (Kovacheva et al., 2010). Recently, the application of rose oil has been increasing. In addition to its perfuming effects, it possesses a wide range of biochemical activities, such as analgesic, hypnotic, antispasmodic, anti-

inflammatory and anticonvulsant. (Kumar et al, 2013) The essential oil content of the oil-bearing rose (*Rosa damascena* Mill.) is relatively low, around 0.3-0.4 ml kg⁻¹ in fresh flowers. In the scientific literature there are various methods have been examined to improve essential oil extraction efficiency and ultimately essential oil yields. For instance the investigation of Kumar et al., 2013 are connected with the effect of various agronomic interventions viz., harvesting date, harvesting conditions and storage duration of damask rose (*Rosa damascena* Mill.) flowers on essential oil content and composition in western Himalayas. Tintchev et al. (2012) demonstrated that the yield could be significantly improved after treatment of raw material with pulsed electric fields. However, studies related to irrigation and fertilization of *Rosa damascena* are limited. Based on the results obtained of Nedkov et al. (2014) work team concluded that the additional yield (for blossom and oil) depending on the amount of irrigation depth. The debatable topic is also chemical versus organic cultivation in medicinal and aromatic

plants (Malik and Ahmad, 2011). According to Nurzynska-Wierdak (2013), biofertilization, balanced mineral fertilization of medicinal plants is an important cultivation factor determining essential oil quantity and quality. *R. damascena* grows well and produces high yields on deep, aerated soils, with organic matter content and neutral soil reaction. Penkov and Kovacheva (2013) defined Chromic Luvisols as one of the most suitable for growing the oil-bearing rose with values of pH between 6.7 and 6.9). More efficient agricultural practices is a key factor for further increase of the overall rose production and reduction of the production costs. Regardless of the numerous challenges faced by organic rose growers, the increasing worldwide demand and price for organic rose oil provide a promising future for the organic rose sector in Bulgaria (Chalova et al., 2017).

The aim of our study to perform monitoring of several private oil-bearing farms in Southern Bulgaria, with the same climate and soil conditions and different agriculture systems in order to find the best actual and tested agriculture systems in oil-bearing roses cultivation producing high quantity and quality rose oil. Here are presented the initial results, with the focus on soil fertility, soil available nutrients, rose essential oil and the relationship between them.

MATERIALS AND METHODS

Study area and soil sampling

The study was conducted on six private oil bearing roses farms in Kazanlak (Rose) valley called, Southern Bulgaria in 2019. The Rose valley is situated in the middle of the country between the Stara Planina Mountain on the North and Sredna Gora Mountain on the South. The valley is around 90 km long and around 10 km wide. The climate is transitional continental, relatively mild with altitude between 400 and 500 m. The winters are warmer and the summers cooler, in this area the average annual temperature is around 11°C, and the annual precipitation is 540 mm. Three of oil rose private plantations are certified as organic farming and have been applied an organic agriculture system and the rest of them are characterized as

conventional farming. The detailed characterization of agricultural practices of studied farms is presented in Table 1.

The study area size of every farm was 5000 m². The geographical coordinates of every study area was measured by GPS Garmin. Soil samples were collected from every area from surface horizon 0-30 cm using an Eijkkelkamp soil sampling equipment. The soils were air-dried, ground and sieved with a particle less than 2 mm. The samples were analyzed for several soil parameters as, organic matter content by loss of ignition, pH values by potentiometric method, mineral nitrogen content using spectrophotometer JENWAY 6705 UV/VIS, available potassium and available phosphorus content by the Egner-Riem method. The concentration of available potassium was determined by AAS using Analyst 800 Atomic Absorption Spectrometer, Perkin Elmer. Visual soil assessment of soil texture and soil structure in the field was performed (Houšková, 2005).

Plant sampling and analysis

Rosa damascena Mill and *Rosa damascena* x *Rosa gallica* genotypes have been observed. In general, harvesting time and flowering stage are critical factors for obtaining high yields and quality of the oil. Harvesting takes place early in the morning, from 06.00 to no later than 11.00, to avoid temperature increase during the day, which negatively affects the yield and the quality of the rose oil. Harvesting time starts in the middle of May and continues for less than a month (Chalova, 2017). The flowers in our study are picked up in the morning (6-8 a.m.), in the most appropriate development phases (IV-V), with half to full-open petals (Staikov et al., 1975)

The essential oil content in the blossoms was determined after water distillation in Clevenger-type microapparatus. The process parameters were: sample amount 200 g; roses:water ratio = 1:4; distillation rate 3-4 ml/min; duration of the distillation process: 2.5 h. The essential oil was measured to the graduated part of the apparatus in milliliters and is calculated as a percentage by volume (v/w). For better accuracy, a relative density recalculation is made and is presented as a percentage by weight (w/w).

Table 1. The geographical data, soil type, variety and agricultural practices of the studied farms

Farm's number	Area	Soil type	Variety	Mineral fertilization	Organic fertilization	Irrigation	Soil tillage
Conventional farming							
01	Damascena 1, Skobelevo	Fluvisols	<i>R. x damascena f. trigintipetala</i> Dieck	yes	-	yes	-
02	Damascena 2, Skobelevo	Fluvisols	<i>R. x damascena f. trigintipetala</i> Dieck	yes	-	yes	-
03	Koprinka	Luvissols	<i>R. x damascena f. trigintipetala</i> Dieck	yes	-	-	yes
Organic farming							
04	Skobelevo	Fluvisols	<i>Rosa damascena x Rosa gallica</i>	-	yes	yes	-
05	Asen	Fluvisols	<i>Rosa damascena x Rosa gallica</i>	-	yes	yes	-
06	Yasenovo	Fluvisols	<i>R. x damascena f. trigintipetala</i> Dieck	-	yes	-	yes

Data analysis

The statistical analysis was done with Unscrambler (Camo, Norway) software packages.

RESULTS AND DISCUSSIONS

The Rose Valley represents the deluvial non-calcareous sediments. Colluvium is an assorted mixture of material with or without rock fragments consisting of pebbles, sand, silt, and clay that accumulates at the base of steep slopes. According to Revised Legend of the World Soils, the soil type in the all arable areas is Fluvisols. Fluvisols (Deluvial soils) are formed by downhill creep, where the sorting of materials comes about through gravity. Creep is the slow movement of soil masses down slopes that are usually steep.

The process takes place in response to gravity where there is pronounced water saturation (Shishkov and Kolev, 2014). In the deluvial soils, organic matter content naturally decreases over time.

The soil in the region have ochric and nondiagnostic features, typical for fluvisols. The soils samples in the all six arable areas were characterized with acid reaction, typical for that soil. The range of values of pH (H₂O) was between 4.20 and 6.10. The distribution of values of pH in the soil among the studies areas is presented on Figure 1, both farms with organic with conventional farming cultivate soil with acid reaction. The soil organic matter (OM) content varied between low and high content with values from 0.86 to 4.03%, where mean OM, % values was 2.80 (Figure 2).

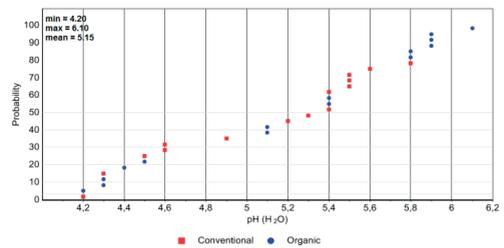


Figure 1. Normal probability distribution of pH (H₂O) values in soils samples of organic and conventional farming

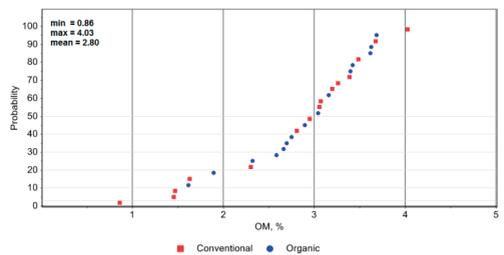


Figure 2. Normal probability distribution of organic matter content in soils samples of organic and conventional farming

The samples with the lowest organic matter was found in farm 04 with conventional farming. It was not found statistically significant of OM, % values in the soils with organic and conventional farming. It was found statistically significant of values of mineral nitrogen, $p < 0.05$ between soil samples taken from organic farms and the rest - conventional farms. The all studies areas were characterized with high mineral nitrogen and potassium content, as soils belonged to conventional farming were shown higher total mineral nitrogen content (N, mg/kg) than areas

with organic farming. That difference is shown in Figure 3, which graphically illustrated the visible difference between the two studied agricultural systems, with mean values = 57.61 mg/kg at conventional farming versus 50.38 mg/kg at organic farming. The values of available potassium varied between 20.69 and 31.96 mg/100 g, with mean values = 27.7 mg/100 g at conventional farming to 22.37 mg/100 g at organic farming. The values of available phosphorus varied between 5.95 and 12.30 mg/100 g, as the lowest values was found in Farm 06 - organic farming. The highest values soil nutrients content were obtained in samples taken from the all tree farms with conventional farming (Figure 4).

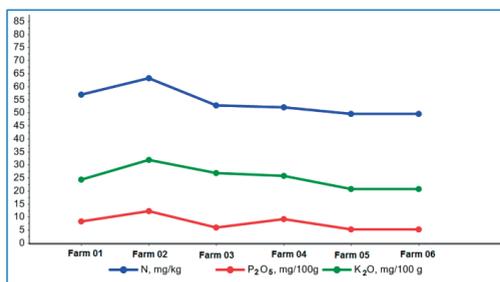


Figure 3. Statistical data of N mg/kg, P₂O₅ mg/100 g and K₂O mg/100 g values in soils samples of organic and conventional farming

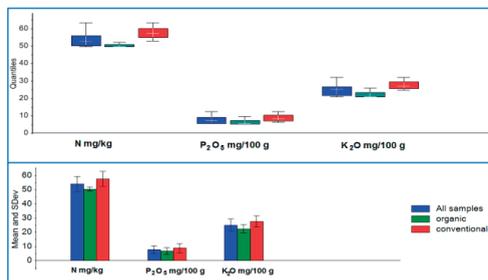


Figure 4. Distribution of available nitrogen, phosphorus and potassium content in soils of all studied farms

The reasons of that are several, as an annual mineral fertilization, the built-in drip irrigation system supply the plant with water and one important fact was mulching as an alternative technique for weed management in the same farms. In the farm 01, 02 conventional farming and 04 and 05 have been not applied soil tillage, but mulching as an alternative technique has been applied for weed management in that

farms. Therefore the Farm 01 and Farm 02 could be classified as farms with a low input farming systems, as a kind of conventional farming. According to Parr et al. (1990) “the definition of Low Input Farming Systems (LIFS) seek to optimise the management and use of internal production inputs (i.e., on-farm resources) and to minimise the use of production inputs (i.e., off-farm resources), such as purchased fertilizers and pesticides, wherever and whenever feasible and practicable, to lower production costs, to avoid pollution of surface and groundwater, to reduce pesticide residues in food, to reduce a farmer’s overall risk, and to increase both short- and long- term farm profitability”.

Such kind of conservation agriculture is a way to increase soil fertility, reduce soil erosion, increase organic matter and improve water buffer capacity. Conservation agriculture refers to several practices which permit management of the soil. Crop residues are left in place as soil cover instead of ploughing them into the ground. Therefore that both farms not belonged to typical conventional agricultural systems, but take place between typical conventional and organic farming.

The obtained values of rose essential oil, % in all studied farms with organic and conventional farming are presented in Table 2.

The rose essential oil, % values varied between 0.260 and 0.460% in all six farms. The rose essential oil productivity in conventional farming is statistically proven higher than organic production with values of 0.038% versus 0.026%, respectively. The data show that there is no statistically proven difference in oil production in the organic cultivation of different types of roses. The agricultural system of cultivation of the oil bearing rose (*Rosa damascena* Mill.) has an effect on the essential oil content (Figure 5).

The farms 01 and 02, characterized with low input system, with mulch system - without soil tillage and irrigation obtained significant higher oil productivity than others farms. The mean values of rose essential oil in the both farms are between 0.043 and 0.046% in compare to others. Tabaei-Aghdaei et al. (2007) reported that the essential oil content of *Damask Rose* from 28 provinces in Iran varied between 0.034 and 0.051%. According to Kovacheva et al., 2010

the essential oil content of cultivated *Rosa damascena* Mill. in the territory of Bulgaria is between 0.045 and 0.054%. It is not clear which

kind of cultivation in that report the data belonged to - organic, conventional or both agricultural system for cultivation.

Table 2. Statistical data of rose essential oil, % values in studied farms with organic and conventional farming

Farm's number	Area	Rose essential oil, % (v/w)	Rose essential oil,% (w/w)	Rose essential oil, % average
Conventional farming				
01	Damascena 1	0.0533	0.0430	0.043±0.005
02	Damascena 2	0.0533	0.046	0.046±0.008
03	Koprinka	0.02833	0.0260	0.026±0.004
	min			0.0260
	max			0.0460
	mean			0.0383*
	SD			0.0091
Organic farming				
04	Skobelevo	0.0283	0.0247	0.025±0.002
05	Asen	0.0367	0.0313	0.031±0.002
06	Yasenovo	0.0250	0.0220	0.022±0.000
	min			0.0220
	max			0.310
	mean			0.0260*
	SD			0.0039

*statistically significant ($p < 0.05$)

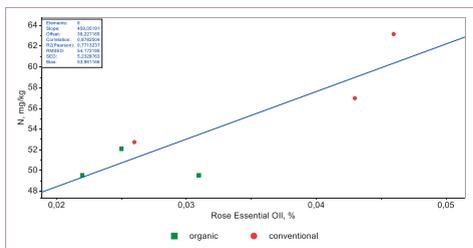


Figure 5. Statistical data of rose essential oil,% values between organic and conventional farming

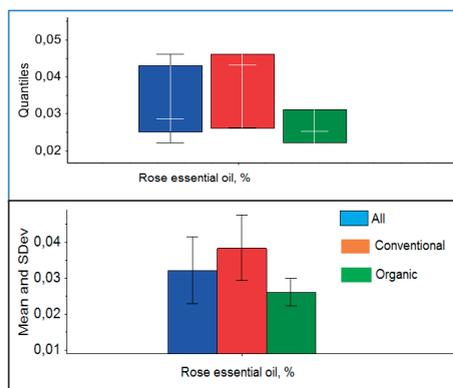


Figure 6. Scatter plots of correlation between mineral N, mg/kg and rose essential oil, %

The data obtained in our study confirmed the results of Ucar et al. (2017) that oil yields were significantly affected by irrigation water

amounts, nitrogen levels, and irrigation water amounts × nitrogen levels interaction. Figure 6 graphically illustrated the correlation between soil mineral nitrogen and rose essential oil % productivity, with high Pearson's correlation coefficients, $r = 0.88$.

The nutrition plays a key role in the growth and development of all crop plants. In the case of medicinal plants that synthesize essential oils, nutrients can effectively increase oil yield and quality. According to Nurzyska-Wierdak (2013) nitrogen, one of essential minerals, is used by plants to build many organic compounds: amino acids, proteins, enzymes, and nucleic acids.

Amino acids and enzymes play a key role in the biosynthesis of numerous compounds which are essential oil constituents.

A second important nutrient for plants is potassium, which usually occurs in the plant at quite a high concentration, in particular in the meristematic tissues and in the phloem. For instance rosemary oil yield is significantly dependent on N and K application. (Nurzyska-Wierdak, 2013)

CONCLUSIONS

The agricultural system of the oil bearing rose (*Rosa damascena* Mill.) grown has an effect on the essential oil productivity. The mean value of

essential in organic production = 0.026% is statistically proven lower than conventional production = 0.038%. The conventional farming has different shades and should not be placed under a common denominator, and to take into account every conservation agriculture practice which increase soil fertility, reduce soil erosion, with a higher productivity as a results of all of that. It was found that there are private oil-bearing farms in Southern Bulgaria, characterized as farms with low input systems applied good agriculture practices in oil-bearing roses cultivation producing high quantity rose oil and keeping soil fertility, simultaneously.

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REFERENCES

- Chalova, V., Manolov, I., Manolova, V. (2017). Challenges for commercial organic production of oil-bearing rose in Bulgaria. *Biological Agriculture & Horticulture*, DOI: 10.1080/01448765.2017.1315613
- Houšková, B. (2005). Visual soil assessment. 3rd European Summer School on Soil Survey. Co-organized by TAIEX, JRC and SZIV.
- Kovacheva, N. (2007). Characterization gene found of oil-bearing roses. Productivity and variability of some symptoms. Thesis. Plovdiv, 171.
- Kovacheva, N., Rusanov, K., Atanassov, I. (2010). Industrial Cultivation of Oil Bearing Rose and Rose Oil Production in Bulgaria During 21st Century, Directions and challenges. *Biotechnology & Biotechnological Equipment*, 24(2), 1793–1798, DOI: 10.2478/V10133-010-0032-4
- Kumar, Rakesh., Sharma, S., Sood, S., Agnihotri, K. (2013). Agronomic interventions for the improvement of essential oil content and composition of damask rose (*Rosa damascena* Mill.) under western Himalayas. *Industrial Crops and Products*, 48, 171–177.
- Malik, A., Suryapani S., Ahmad., J. (2011). Chemical Vs Organic Cultivation of Medicinal and Aromatic Plants: the choice is clear. *Int. J. Med. Arom. Plants*, 1(1), 5–13.
- Nedkov, N., Matev, Al., Ovcharova, A. (2014). Additional yield - irrigation depth relationship for white bearing rose (*Rosa alba* L.). Ovidius University Annals Series: *Civil Engineering*, 16, 91–104.
- Nurzyska-Wierda, R. (2013). Does mineral fertilization modify essential oil content and chemical composition in medicinal plants? *Acta Sci. Pol., Hortorum Cultus*, 12(5), 3–16.
- Parr J.F., Stewart B.A., Hornick S.B., Singh R.P. (1990) Improving the Sustainability of Dryland Farming Systems: A Global Perspective. In: Singh R.P., Parr J.F., Stewart B.A. (eds) *Advances in Soil Science. Advances in Soil Science*, 13, Springer, New York, NY.
- Penkov, M., Kovatcheva, N. (2013). The soils and their influence on the development and productivity of oil bearing rose. *Journal of Mountain Agriculture on the Balkans*, 16(6), 1585–1601.
- Shishkov, T., Kolev, N. (2014). Springer Science+Business Media Dordrecht.
- Staikov, V., Decheva, R., Balinova-Tsvetkova, A. (1975). Studies on the composition of rose oil obtained from the flowers in different stages of their development. *Riv. Italiana*, 57(4), 192–195.
- Tabaei-Aghdaei, Seyed Reza, Alireza Babaei, Morteza Khosh-Khui, Kamkar Jaiman, Mohammad Bagher Rezae, Mohammad Hassan Assare, Mohammad Reza Naghavi. (2007). Morphological and oil content variations amongst Damask rose (*Rosa damascena* Mill.) landraces from different regions of Iran. *Scientia Horticulturae*, 113, 44–48.
- Tintchev, F., Dobрева, A., Schulz, H., Toepfl, S. (2012). Effect of Pulsed Electric Fields on Yield and Chemical Composition of Rose Oil (*Rosa damascena* Mill.). *Journal of Essential Oil Bearing Plants*, 15(6), 876–884.

WEED ASSOCIATION DYNAMICS IN THE SUNFLOWER FIELDS

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Abstract

The data analyses from the research showed that in the sunflower fields of Bulgaria a significant dynamics of the weed species and densities that form the weed associations has occurred. In the beginning of the monitoring the dominating weed species were *Xanthium strumarium* L., *Sinapis arvensis* L., and on separate fields *Cannabis ruderalis* Janisch. in high densities was prevailing. The reasons for the mass distribution of these weed species are the violated crop rotations, seeding of sunflowers in short period of time – in 1-2 years on the same field, insufficient quality of the soil tillage, the limited choice of herbicides for their control, etc. The implementation of the alternative cropping technologies like Clearfield® and ExpressSun®, their constant improvement, as herbicide content and selection process showed positive effect for decreasing the density and range of distribution of these three weeds in the sunflower fields. From the other hand led to clearly expressed compensatory processes and mass distribution *Chenopodium album* L. and *Portulaca oleracea* L. to a lower extent. It is known that imazamox and tribenuron-methyl have limited efficacy against *Chenopodium album* and *Portulaca oleracea*.

Key words: weed associations, dynamics, Clearfield® and ExpressSun®, sunflower.

INTRODUCTION

It is considered, that the sunflower is the fourth oilseed crop in the world (Nisar et al., 2011). It is established, that the percentage of seed oil and protein is 40-55% and 23% respectively (Jadaan et al., 1999). The seeds also content linoleic acid, oleic acid and linoleic acid (Nasralla et al., 2014). In Bulgaria sunflower is main oilseed crop and in 2018 the total harvested area was 788 656 ha with average seed yield of 2,443 t/ha (www.mzh.government.bg). In order to achieve high yields, in addition to optimizing the main vegetation factors, it is necessary to effectively control weeds.

Under intensive agriculture, a key element of weed control is their systematic reporting and in the agricultural areas. A number of authors have examined weed infestation in sunflower fields. The most important weed species in Slovakia are *Agropyron repens* (L.) P. Beauv., *Iva xanthiifolia* Nutt., *Echinochloa crus-galli* (L.) Beauv., *Chenopodium album* L., *Chenopodium hybridum* L., *Abutilon theophrasti* Medik., *Datura stramonium* L., *Convolvulus arvensis* L., *Panicum miliaceum* L., *Cirsium arvense* (L.) Scop., *Fallopia convolvulus* (L.) Á. Löve, *Amaranthus* spp.,

Persicaria spp. and *Polygonum* spp. (Týr and Vavrik, 2015).

In Hungary, the most distributed weed species are *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Convolvulus arvensis* L., *Xanthium italicum* Moretti, *Echinochloa crus-galli* (L.) Beauv., *Panicum miliaceum* L., and *Setaria pumila* (Poir.) Roem. & Schult. (Pinke et al., 2013; Pinke and Karácsony, 2010).

In Dagestan, Russia the most important role in suppressing the normal development of sunflower *Convolvulus arvensis* L., *Sonchus arvensis* L., *Cynodon dactylon* (L.) Pers., *Equisetum arvense* L., *Phragmites vulgaris* (Lam.) Crép., *Chenopodium album* L., *Setaria viridis* (L.) P. Beauv., *Echinochloa crus-galli* (L.) Beauv., *Xanthium strumarium* L., *Amaranthus retroflexus* L. and *Capsella bursa-pastoris* (L.) Medik. (Kurbanov et al., 2018).

In Bulgaria, the most distributed weed species in the sunflower fields are *Amaranthus* spp., *Sinapis arvensis* L., *Chenopodium album* L., *Setaria* spp., *Echinochloa crus-galli* (L.) Beauv., *Sorghum halepense* (L.) Pers., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L. etc. (Manilov and Zhalnov, 2015; Tonev et al., 2010). In the region of Plovdiv and Stara Zagora (Bulgaria) the most common species are *Amaranthus blitoides* S. Wats.,

Amaranthus albus and *Convolvulus arvensis* L. (Moskova et al., 2016). Not only in sunflower, but also in other trench crops the main weeds are the annual late-spring weeds (Sevov et al., 2014; Dimitrova and Laleva, 2003; Tonev, 2002; Tonev and Valeva, 1989).

Another important biotic factor limiting sunflower production is the root parasite broomrape. *Orobancha cumana* Wallr. is a obligate parasite without chlorophyll, as a result of which it cannot do photosynthesis. The broomrape supplies its nutrients and water entirely from the host. It is widespread in all sunflower-growing countries in Central-Eastern and Eastern Europe as well as in Western Asia. The sunflower broomrape populations are classified in races that as more and more aggressive new more virulent races occur (Encheva, 2018; Masliiov et al., 2018; Fernandez-Aparicio, 2016; Amri et al., 2012). Crucial to successful weed control is the early plowing after harvesting of the predecessor and the additional summer and autumn tillage operations. From the spring pre-sowing tillage applications, the most important is the first early cultivation with harrowing, which is carried out in order to destroy any soil bark formed and reduce weed infestation. The second harrowing is recommended to be performed after the formation of the first pair of true leaves (Tonev et al., 2019).

Incorporating sunflower into science-based crop rotations, which alternate mainly with winter cereals, facilitates the control of the noxious weeds (Tonev et al., 2007).

The crop is most sensitive to weed infestation in the early development stages. Golipour et al. (2009) established that in the presence of weeds in the sunflower fields, the critical period for performing weed control measures is from 1 to 9.5 weeks after crop germination. The control of the weeds in this crop is performed mainly by herbicides (Prashant et al., 2017).

There are several sunflower cropping systems that determine the choice of the used herbicides. Good to excellent control (81-100%) against the broadleaf weeds in sunflower is performed by oxyfluorfen application (Osman et al., 2014). Jursík et al. (2011) found that 88 to 95% efficacy against *Chenopodium album* after application of oxyfluorfen. For grass and broadleaf weed

control Pannacci et al. (2007) recommend the application of s-metolachlor+aclonifen and s-metolachlor+oxyfluorfen. Tonev et al. (2010) recorded that *pendimethalin* controls the annual weeds, as the grass weed control was more pronounced. The authors also found that *oxyfluorfen* has excellent broadleaf weed control and limited grass weed control.

The herbicide products Stomp Aqua, Gardoprim Plus Gold, Wing-P and Pledge 50 WP performed much better control of the main annual monocotyledonous and dicotyledonous weeds, except *Xanthium strumarium* L. The application of the herbicide combinations Wing-P at 400 cm³/da+Stratos Ultra in rate of 200 cm³/da and Stomp Aqua at 350 cm³/da+Stratos Ultra at rate of 200 cm³/da ensured 84.4 to 88.0% efficacy against the mixed weed infestation in sunflower (Manilov and Zhalnov, 2015). When growing conventional sunflower hybrids, the selective herbicides have no efficacy against *Xanthium strumarium* L. and *Cirsium arvense* (L.) Scop. In the recent years, these two weeds multiplied in high density. An alternative for control of these noxious weeds is the ExpressSun[®] technology at sunflower (*Helianthus annuus* L.) (Mangiapan et al., 2012; Tonev et al., 2009). At this technology the main herbicide is tribenuron-methyl - Express 50 SG. It should be applied at sunflower hybrids bred to be resistant to the active substance. At this technology main and difficult-to-control broadleaf weeds are controlled. Manilov and Zhalnov (2018) reported that the herbicide combinations of Express 50 SG at 40 g ha⁻¹ + Stratos Ultra in a rate of 2.0 l ha⁻¹ and Express 50 SG at 40 g ha⁻¹ + Fusilade Forte at rate of 1.3 l ha⁻¹ ensured 87.2 and 83.3% efficacy.

In the recent years the interest to the herbicide tolerant sunflower hybrids is increased. Other widely spread alternative for broadleaf and grass weeds as well as broomrape control is the Clearfield[®] technology. At this technology the sunflower hybrids are bred to be resistant to herbicides from the group of imidazolinones (Pfenningl et al., 2008; Istilart, 2005).

Manilov and Zhalnov (2016) observed that the treatment of the sunflower hybrid EC Candimis CL Plus with Pulsar 40 without Dash, significantly reduces the efficiency against *Sorghum helepense* L., *Cirsium arvense* L. and

Chenopodium album L. However, the efficacy against against *Amaranthus* spp., *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Solanum nigrum* L. is 100%. The pre-emergence herbicides, used immediately after sowing show very good control against some annual weeds in the early development stages of crop. At hybrid ES Candimis CL Plus Mitkov et al. (2015) also found that the application of Pulsar

40 without Dash significantly reduces the efficacy against *S. halepense*, *C. arvense*, *C. arvensis*, *C. ruderalis*, *Xa. strumarium*, *Ch. album* and *Or. cumana*. The efficacy against *A. retroflexus*, *S. arvensis*, *R. raphanistrum* is 100%.

The aim of the research is to study the weed association dynamics in sunflower fields for the ecological conditions of Bulgaria.

MATERIALS AND METHODS

Table 1. Description of the investigated areas with sunflower in the period 2001-2019

Year/Municipalities	Settlements	Hybrid	Investigated Area, da	Total area, da
2001/Shumen	Village of Marash	Albena	320	800
2001/Shumen	Village of Salmanovo	Albena	320	800
2002/Novi Pazar	Village of Voivoda	Albena	200	650
2003/Brezovo	Village of Streltsi	LG 54.78	360	540
2003/Kaloyanovo	Village of Suhozem	LG 54.78	90	310
2004/Parvomay	Village of Poroina	Talento	130	700
2005/Tsar Kaloyan	Village of Ezerche	PR63E82	150	400
2006/Radnevo	Town of Radnevo	San Luka	200	800
2006/Yambol	Village of Ovchi Kladenets	Almansor	120	360
2007/Ruen	Village of Lyulyakovo	Albena	400	700
2007/G. Toshevo	Village of Petleshkovo	San Luka	200	500
2008/G. Oryahovitsa	Town of D. Oryahovitsa	Armada CL	450	1000
2008/Popovo	Village of Popovo	PR 63 E 82	300	800
2009/Vidin	Village of Dimovo	Armada CL	300	1000
2009/Pazardzhik	Village of Dragor	Sumiko	500	1500
2010/Radnevo	Village of Beli Bryag	Meldimi	100	1050
2010/Nova Zagora	Village of Pet Mogili	Armada CL	100	460
2010/Opan	Village of Pastren	PR63E82	100	1050
2011/Plovdiv	City of Plovdiv	PR63E82	60	200
2011/Rodopi	Village of Krumovo	Rimisol CL	200	600
2012/Haskovo	Village of Dinevo	PR63E82	140	400
2012/Tundzha	Village of Roza	Rimisol CL	400	1200
2012/Tundzha	Village of Miladinovtsi	Armada CL	200	500
2013/Tundzha	Village of Tenevo	P64LE25	300	800
2013/Tundzha	Village of Dryanovo	Neoma	140	400
2014/Harmanli	Village of Ivanovo	P64LE25	20	65
2014/Harmanli	Village of Leshnikovo	P64LE25	140	440
2015/Dryanovo	Village of Gostilita	P64LE25	90	310
2015/Sadovo	Town of Sadovo	P64LE25	130	1000
2016/G. Toshevo	Village of Samoilovo	P63LE113	80	190
2016/ G. Toshevo	Village of Malina	P63LE113	130	700
2017/Svishtov	Village of Tsarevets	P64LC108	50	400
2017/Svishtov	Village of Sovata	P64LC108	100	150
2017/Svishtov	Village of Hadzhidimitrovo	P64LC108	200	550
2018/Burgas	Village of Vetren	Bacardi	160	520
2018/Aytos	Town of Aytos	Neostar	150	500
2019/Pavlikeni	Village of Slomer	Bacardi	200	600
2019/Pavlikeni	Village of Duskot	Neostar	120	290
2019/Svishtov	Village of Kozlovets	P64LE25	100	300
Total - 27	Total - 39		7 450 da 31.65%	23 535 da 100%

The study was performed during the period of 2001-2019 in 10 districts, 27 municipalities and the lands of 39 settlements in the Republic of Bulgaria. The criteria for choosing the pigeonholed areas were to be typical for the sunflower production and with optimal soil-climatic conditions. The weed infestation monitoring of the fields on which conventional sunflower hybrids are grown is in period of 20 years and the weed infestation monitoring of the areas with Clearfield® and ExpressSun® sunflowers is in period of 10 years.

The weed mapping was performed by “Methodology for reporting and mapping of the weed infestation in main field crops” (Dimitrova et al., 2004).

The efficacy of imazamox and tribenuron-methyl was reported by the 10-score visual scale of EWRS.

The data from table 1 shows that, the committed study of sunflower weed infestation in an area of 23 535 da. The mapping and determination of the species composition of weed associations covers an area of 7450 da, to 31.65% of the total area and the study of the dynamics of weed associations in sunflower is representative.

RESULTS AND DISCUSSIONS

The weed species in the areas with sunflower in the different regions of Bulgaria is very

diverse. The data is presented on Table 2. 21 species of weeds from 6 biological groups have been identified. The predominant species are late-spring weeds - 65% of the total weed infestation-, which is explained by the fact that their mass development and reproduction coincides with the sunflower growing season. The weed species Fat hen (*Chenopodium album* L.), Mat amaranth (*Amaranthus blitoides* L.), Common amaranth (*Amaranthus retroflexus* L.), Common purslane (*Portulaca oleracea* L.), Black nightshade (*Solanum nigrum* L.), Rough cocklebur (*Xanthium strumarium* L.), Green foxtail (*Setaria viridis* (L.) P.B.), etc., are widespread. In separate regions in Bugaria the Wild hemp (*Cannabis ruderalis* Janisch.), and Barnyard millet (*Echinochloa crus-galli* (L.) Beauv) are also observed. From the early-spring weeds Wild mustard (*Sinapis arvensis* L.) is widely distributed. The most widely found weeds from the winter-spring group is Wild radish (*Raphanus raphanistrum* L.) which is found to be in low densities in comparison to the perennial weeds like Johnson grass developed from seeds and rhizomes (*Sorghum halepense* (L.) Pers.) and Creeping thistle (*Cirsium arvense* (L.) Scop.). Field horsetail (*Equisetum arvense* L.), Bermuda grass (*Cynodon dactylon* (L.) Pers.) and Field bindweed (*Convolvulus arvensis* L.) are more common in northern Bulgaria. With respect to diversity, perennial

Table 2. Weed species in sunflower fields not treated with herbicides in the period 2001- 2019

	Grass weeds	Broadleaf weeds
I. Annual		
A/ Winter-spring		Wild radish – <i>Raphanus raphanistrum</i> L.
B/ Early spring		Wild mustard – <i>Sinapis arvensis</i> L.
C/ Late spring	Barnyard millet - <i>Echinochloa crus-galli</i> (L.) Beauv	Common amarants – <i>Amaranthus retroflexus</i> L.
	Hairy crabgrass – <i>Digitaria sanguinalis</i> (L.) Scop.	Fat hen – <i>Chenopodium album</i> L.
	Green foxtail – <i>Setaria viridis</i> L.	Common purslane – <i>Portulaca oleracea</i> L.
	Yellow foxtail - <i>Setaria glauca</i> L.	Rough cocklebur – <i>Xanthium strumarium</i> L.
		Velvetleaf – <i>Abutilon theophrasti</i> Medic
		Black nightshade – <i>Solanum nigrum</i> L.
		Mat amaranth – <i>Amaranthus blitoides</i> L.
		Jimson weed – <i>Datura stramonium</i> L.
		Wild hemp – <i>Cannabis ruderalis</i> L.
II. Perennial		
A/ Rhizome	Johnson grass – <i>Sorghum halepense</i> (L.) Pers.	
	Field horsetail – <i>Equisetum arvense</i> L.	
	Bermuda grass – <i>Cynodon dactylon</i> (L.) Pers.	
B/ Root-sprouted		Creeping thistle – <i>Cirsium arvense</i> (L.) Scop.
		Field bindweed – <i>Convolvulus arvensis</i> L.
III. Parasite		Broomrape – <i>Orobanche cumana</i> Wallr.

weeds take second place of the total weed infestation of sunflower in Bulgaria - 25%. Data from twenty-year mapping about the root parasite Broomrape (*Orobancha Cumana* Wallr.) do not lead to an accurate algorithm for its distribution. An exception is observed in the Haskovo and Harmanli regions. There, because of impaired crop rotation and sunflower

growing on the same area in 2-3 years period the Broomrape infection is increased. During the period 2016-2019 in eight regions in the country, a race appurtenance of the Broomrape was performed. Race H predominates and was followed by race E. in one of the regions race D was found (Table 3).

Table 3. The sunflower broomrape races (*Or. cumana* Wallr.)

Origin	Year	Race
Location		
Kardam	2016	H
Dobroudja Agricultural Institute, General Toshevo, Bulgaria (DAI) (Infectious disease)	2016	H
Dobroudja Agricultural Institute, General Toshevo, Bulgaria (DAI) (Experimental field)	2017	E
Kardam	2018	H
Tyulenovo	2018	H
Dyakovo	2017	H
Selanovtsi	2017	D
Svishtov	2019	E
Radnevo	2016	E
Radnevo broomrape on <i>Artemisia maritima</i> L .	2017	E

The data regarding the efficacy of the Clearfield® technology showed that average for the period of investigation the following weed species in high density were reported: from the dicotyledonous late spring weeds Fat hen, Rough cocklebur, Velvetleaf, Common amaranth, Black nightshade and Common purslane.

From the dicotyledonous early spring weeds only Wild mustard was found. Observed species from the monocotyledonous group were the late spring weeds Green foxtail and Barnyard millet.

The most widely spread perennial weed species were found to be Johnson grass and Creeping thistle.

The root parasite Broomrape is also observed in very high densities but only in some regions of the country.

From the abovementioned weeds after the herbicide application at the Clearfield® system the following species are successfully controlled: Wild mustard, Common amaranth, Black nightshade, Rough cocklebur and Barnyard millet (Figure1).

When the rhizome weed Johnson grass is in high density could not be efficiently controlled and a partner herbicide product should be

applied. Such herbicides are karo cycloxiidim, fluazifop-P-butyl, fluazifop-P-ethyl, etc. The efficacy of imazamox against Creeping thistle is relatively good - 85% on average. At this technology the main problems are the weeds Fat hen and Common purslane. The reason is that in years with less precipitation in the spring, no soil herbicides are used that have excellent efficacy against these two weeds and they multiply in great density. In the presence of Fat hen and Common Purslane, as well as Wild hemp, the efficacy of imazamox is even lower, especially when administered without the adjuvant Dash.

Against the Broomrape in the sunflower fields the Clearfield® technology relies entirely on the mode of action of the herbicide products containing imazamox. The efficacy of the herbicide imazamox is high enough and reaches 90%.

In some regions of Bulgaria on the 56th day after the herbicide application secondary parasitaion in low density can be observed.

In many scientific studies it has been proven that the broomrape that remains in the field after treatment with imazamox containing herbicide products forms sterile seeds, which is also economically important.

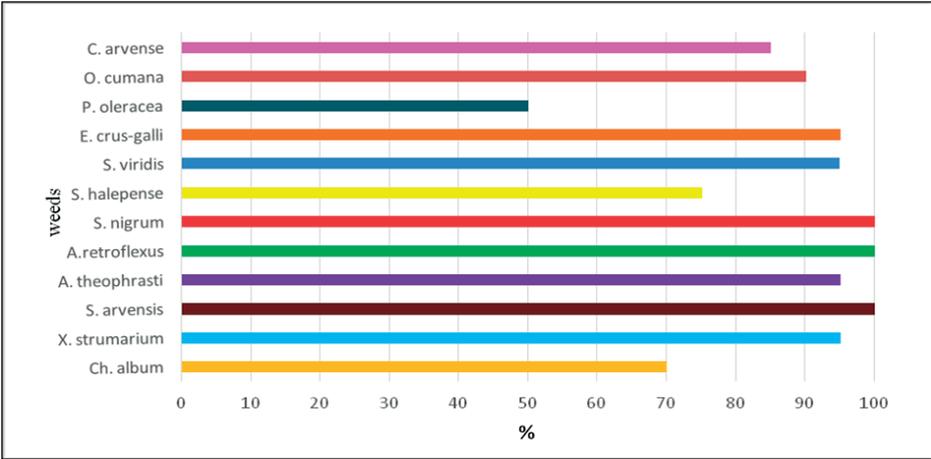


Figure 1. Efficacy of imazamox against the weeds average for the period of the study

For the ExpressSun[®] technology (Figure 2), the tribenuron-methyl efficacy is slightly higher against some of the major dicotyledonous weeds that are controlled by using imazamox in the Clearfield[®] system. Despite this fact, in the ExpressSun[®] technology again difficult-to-control weeds are Fat hen and Common purslane. If these two weeds as well as Wild hemp are present on the sunflower fields it is obligatory the active substance tribenuron-methyl with the adjuvant Trend 90 in concentration 0.1% to be applied. This measure is necessary for increasing the efficacy against these noxious weeds. Tribenuron-methyl cannot control the dicotyledonous weeds. That

is why if there are grass weeds on the field it is mandatory graminicides as cycloxdim, cletodim, fluzifop-P-butyl, fluzifop-P-ethyl, propaquizafop, etc. to be applied as partner herbicides. To avoid the risks of phytotoxicity under the different meteorological conditions each year, it is better to apply them separately rather than in tank mixture with herbicides containing tribenuron-methyl. At the ExpressSun[®] technology the control of the broomrape relies entirely on the genetic resistance of some homozygous hybrids to the "G" race. The active ingredient tribenuron-methyl cannot control the root parasite in any extent.

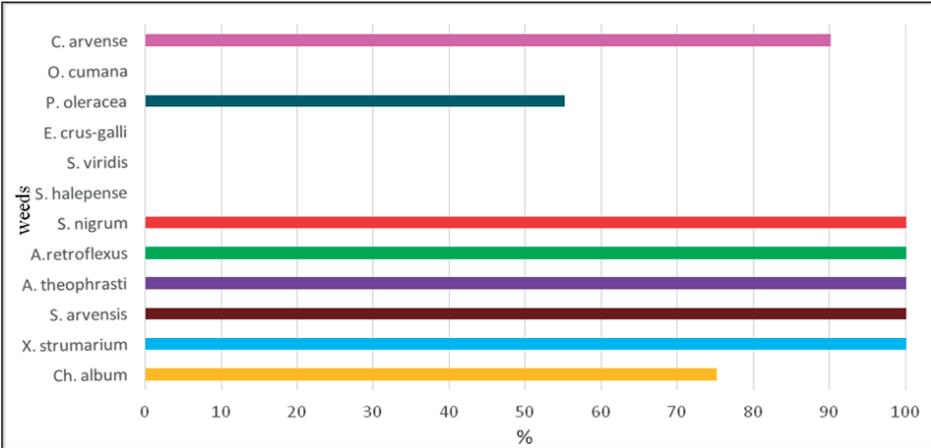


Figure 2. Efficacy of tribenuron-methyl against the weeds average for the period of the study

In conclusion, it can be said that in the last 10-15 years the Clearfield® and ExpressSun® technologies took 85 to 90% of the fields seeded with sunflower plants in Bulgaria. The share of conventional sunflower hybrids during the investigation period significantly reduces from 100% in the period of 2001-2005 to 7-10% during the last several years. The control of the weeds at conventional sunflower production in the period of our study is performed by a great number of soil herbicides as acetochlor, oxyfluorfen, s-metolachlor, flumioxazin, diflofenican, pendimethalin, dimethenamid, etc. For grass weeds control cycloxdim, cletodim, fluazifop-P-butyl, propaquizafop, etc. during the vegetation can be applied.

CONCLUSIONS

The data from the research showed that in the sunflower fields of Bulgaria a significant dynamics of the weed species and densities has occurred. In the beginning of the monitoring period the dominating weed species were Rough cocklebur (*Xanthium strumarium* L.), Wild mustard (*Sinapis arvensis* L.), and on separate fields the Wild hemp (*Cannabis ruderalis* Janisch.).

The implementation of the Clearfield® and ExpressSun® technologies showed positive effect for decreasing the density and range of distribution the weeds in the sunflower fields. The growing share of the Clearfield® and ExpressSun® technologies - from 80 to 85% of the total sunflower production in Bulgaria, compared to the conventional technology - 7 – 10% had led to compensatory processes and distribution of Fat-hen (*Chenopodium album* L.) and Common purslane (*Portulaca oleracea* L.). It is also known that imazamox and tribenuron-methyl have limited efficacy against *Ch. album* and *P. oleracea*. This is also contributed by the frequent application of both herbicides without adjuvants - Dash and Trend 90, respectively.

REFERENCES

- Amri, M., Abbes, Z., Youssef, S.B., Bouhadida, M., Salah, H., Kharrat, M. (2012). Detection of the parasitic plant, *Orobancha cumana* on sunflower (*Helianthus annuus* L.) in Tunisia. *African Journal of Biotechnology*, 11(18), 4163–4167. Nairobi: Academic Journals.
- Dimitrova, M., Laleva, E. (2003). Distribution and density of weeds in major cotton producing areas of the country. Sadovo, *Scientific Reports*, III, 78–84.
- Dimitrova, M., Zhalnov, I., Kalinova, Sht., Tonev, T., Milanova, S., Nikolova, V., Baeva, G., Nakova, R. (2004). Methodology for reporting and mapping of the weed infestation in main field crops. *Publisher MZG, NSRZ, Softia* (Book In Bulgarian).
- Encheva, V. (2018). Distribution and race composition of broomrape (*Orobancha cumana* Wallr.) in Bulgaria during 2008-2017. *4th International Symposium on Broomrape in Sunflower Bucharest, Romania*, 2-4 July 2018, 58.
- Fernandez-Aparicio, M., Reboud, X., Gibot-Leclerc, S. (2016). Broomrape Weeds. Underground Mechanisms of Parasitism and Associated Strategies for their Control: A Review. *Frontiers in Plant Science*, 7, 135; DOI: 10.3389/fpls.2016.00135.
- Golipour, H., Mirshekari, B., Moghbeli, A., Hanifian, S. (2009). Critical period of weeds control in sunflower, *Helianthus annuus* L. *Journal of New Agricultural Science*, 5(17). en11.
- https://www.mzh.government.bg/media/filer_public/2019/06/27/ra361-publicationcrops2018_new.pdf
- Istilar, C. (2005). Evaluation of imazapir herbicide on annual and perennial weeds in sunflower (*Helianthus annuus* L.). *Proceedings of XVII Congreso de la Asociación Latinoamericana de Malezas (ALAM) I Congreso Iberoamericano de Ciencia de las Malezas, IV Congreso Nacional de Ciencia de Malezas, Matanzas, Cuba, 8 al 11 de noviembre del 2005*. 451–452.
- Jadaan, H., Murjana, F., AlFalahi, H. (1999). Analysis of qualitative characteristics of different genotypes of the sunflower seeds. *Iraqi J. Agric. Sci.*, 30(1), 25–34.
- Jursik, M., Andr, J., Holec, J., Soukup, J. (2011). Efficacy and selectivity of post-emergent application of flumioxazin and oxyfluorfen in sunflower. *Plant, Soil and Environment*, 57(11), 532–539.
- Kurbanov, S.A., Magomedova, D.S., Karaeva, L.Yu. (2018). Effect of plant density of sunflower on weed infestation of its crops and yield. *Agrarnaya Rossiya* (10) Moscow: Izdatel'ski Dom "Folium", 28–31
- Mangiapan, S., Pasquini, S., Lodi, G., Bonacini, F. (2012). Express® sun for weed control in post emergence of sunflower. *Università di Bologna*, 579–586.
- Manilov, T., Zhalnov, I. (2015). Weed control in sunflower cultivation by a conventional technology. Jubilee Science Conference with International Participation Traditions and Challenges of Agricultural Education, Science and Business. Agricultural University-Plovdiv. *Scientific Works*, LIX(3), 167–174.
- Manilov, T., Zhalnov, I. (2016). Weed control in sunflowers with Clearfield Plus technology. *Proceedings of VII International Scientific Agriculture Symposium, Agrosym 2016, 6-9 October. Jahorina, Bosnia and Herzegovina*, 1401–1407.
- Manilov, T., Zhalnov, I. (2018). Weed control in ExpressSun® sunflower (*Helianthus annuus* L.). *Zbornik radova 53. hrvatski i 13. meunarodni*

- simpozij agronoma, 18. do 23. veljače 2018., Vodice Hrvatska*, 304–308.
- Masliiov, S., Macai, N., Beseda, O., Stepanov, V. (2018). Control of Broomrape *Orobanche cumana* Wallr. *Ukrainian Journal of Ecology*, 8(2), 74–80; DOI: 10.15421/2018_312.
- Mitkov A., Yanev M., Tonev T., Tityanov, M. (2015). Weed control in sunflower fields by Clearfield technology. *Agricultural Sciences*, 7(18), 167–173.
- Moskova, C., Koleva, N., Dimitrov, S., Tityanov, M., Traykova, B. (2016). Distribution and extent of weeding of Amaranth species and other weeds in sunflower crops in Plovdiv and Stara Zagora region. *Journal of Mountain Agriculture on the Balkans*, 19 (4), 84–94.
- Nasralla, A., Al-Hilfy, I., AlAbodi, M., Mohammed, O., Mhmood, M. (2014). Effect of spraying some plant extractions and anti-oxidant on growth and yield of sunflower. *The Iraqi J. of Agric. Sci.*, 45(7), 651–659.
- Nisar, M., Hussain, Nausheen, S., Khan, N., Siddique, M. (2011). Chemical composition of open pollinated and hybrid population of sunflower (*Helianthus Annus* L.), *Pakistan J. Botany*, 43(1), 157–163.
- Osman, A., El-Habieb, R., Elkhawad, M. (2014). Herbicidal efficacy of oxyfluorfen (Sharoxy 24% EC) for pre-emergence weed control in sunflower. *Persian Gulf Crop Protection*, 3(4), 37–44.
- Pannacci, E., Graziani, F., Covarelli, G. (2007). Use of herbicide mixtures for pre and post-emergence weed control in sunflower (*Helianthus annuus*). *Crop Protection*, 26(8), 1150–1157. Amsterdam: Elsevier.
- Pfenning, M., Palfay, G., Guillet, T. (2008). The Clearfield® technology – A new broad-spectrum post-emergence weed control system for European sunflower growers. *Journal of Plant Diseases and Protection, Special Issue, XXI*, 647–651.
- Pinke, G., Karácsony, P. (2010). Weed survey of sunflower fields in Hungary. *Növényvédelem*, 46(9), 425–429. Budapest: Agroinform Kiadó.
- Pinke, G., Karácsony, P., Botta-Dukát, Z., Czúcz, B., (2013). Relating *Ambrosia artemisiifolia* and other weeds to the management of Hungarian sunflower crops. *Journal of Pest Science*, 86(3), 621–631.
- Prashant, S., Shrinivas M., Jagadish C. (2017). Weed management in sunflower - a review. Trends in Biosciences. *Kalyanpur: Society for Advancement of Science and Rural Development*, 10(34), 7201–7207.
- Sevov, A., Yankov, B., Zorovski, P., Valcheva, E., Popova, R., Palagacheva, N., Stoychev, D. (2014). *Technology for sweet corn production*, Academic publisher of Agricultural University of Plovdiv. 1–44. ISBN 978-954-517-208-3 (Book In Bulgarian).
- Tonev, T. (2002). Optimization of weed control in corn using technology Rayndap piecing. *Scientific Papers Item, XLVII*(1).
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Spasov, V. (2007). *Herbology*. Academic publisher of Agricultural University of Plovdiv. ISBN 978-954-517-047-8 (Textbook in Bulgarian).
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Zhelyazkov, I., Vasilev, A., Tityanov, M., Mitkov, A., Yanev, M. (2019). *Herbology*. Publisher Vidinov & son. ISBN: 978-954-8319-75-1 (Textbook in Bulgarian).
- Tonev, T., Mitkov, A., Dochev, Ch., Tityanov, M. (2009). Possibilities of SU-technology for an efficient weed control in sunflower. *Agricultural Academy – Bulgaria. Plant Science, XLVI*(2).
- Tonev, T., Tityanov, M., Mitkov, M. (2010). Integrated weed control in sunflower. *Scientific Works of the Agricultural University-Plovdiv, LV*(2), 127–132.
- Tonev, T., Valeva, R. (1989). Biological and technological aspects of using some new herbicides in sunflower. *Scientific Papers Item, XXXIV*(2).
- Týr, Š., Vavřík, D. (2015). Chemical weed control of sunflower stands. *Research Journal of Agricultural Science*, 47(1), 243–251.

THE QUALITY OF GREEN MASS AND SILAGE FROM *Amaranthus hypochondriacus* GROWING UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The identification of alternative crops that need less water and produce increased yields of organic matter per unit of water is important for agricultural sustainability. Amaranthus species are C₄ dicotyledonous plants characterized by effective photosynthesis, intensive nitrogen metabolism and good adaptability. The aim of this paper was to evaluate some biological peculiarities, the quality of green mass and produced silage from Amaranthus hypochondriacus, as well as the possibility of using it as feed for ruminant animals and as feedstock for the production of biomethane. The results of our research revealed that the dry matter of harvested whole plants contained 172 g/kg CP, 330 g/kg ADF, 462 g/kg NDF, 55 g/kg ADL, 68 g/kg TSS and 88 g/kg ash, 275 g/kg Cel and 132 g/kg HC with 10.22 MJ/kg metabolizable energy and 6.23 MJ/kg net energy for lactation. The prepared silage was characterized by agreeable colour with pleasant smell and pH 3.86, it contained 13.4 g/kg DM lactic acid, 5.8 g/kg DM acetic acid, 877.6 g/kg DM organic matter, 167 g/kg CP, 348 g/kg ADF, 516 g/kg NDF, 45 g/kg ADL, 303 g/kg Cel and 156 g/kg HC with 10.05 MJ/kg metabolizable energy and 6.02 MJ/kg net energy for lactation. It has been found that the biomethane potential of the Amaranthus hypochondriacus biomass varied from 282 to 303 l/kg ODM. Amaranthus hypochondriacus contains many nutrients, which make it suitable to be used as fodder for animals and has potential as feedstock for biomethane production.

Key words: *Amaranthus hypochondriacus*, biochemical composition, biomethane potential, fodder value, green mass, silage.

INTRODUCTION

The ever-growing human population is expected to reach nine billion persons in the coming decades, imposing the need for urgent solutions to increase food supplies. Human activities require energy to power the systems of production, transportation, heating and cooling, at the same time, fossil fuel consumption increased atmospheric CO₂ concentrations with important impacts on the global climate. Climate change generates serious problems in the environment causing soil degradation and erosion, water pollution and biodiversity decline. One critical measure to ensure future food availability for all is to provide more diverse food sources and develop agricultural systems that are resistant to climate change.

The diversification of crops and of the systems in which they grow are essential for agriculture and can make it sustainable, resilient, and suitable for local environments and soils. The

cultivation of neglected and underused crops and the domestication of new species would promote agricultural diversity and could provide a solution to many of the problems associated with food security, nutrition, healthcare, medicine and industrial needs. A leading strategy is to fulfill the potential of plant species with C₄ photosynthetic pathway. Most of these species have high nutritional value, are water use efficient and are able to withstand drought, flooding, extreme temperatures, and pests and diseases.

Amaranthus are among the group of dicotyledonous C₄ plants, characterized by atriplicoid type Kranz anatomy of leaves, cotyledons and bracts (Sage et al., 2007). They are characterized by effective photosynthesis, intensive nitrogen metabolism and a good adaptability, which can grow in the poor soils and areas with high temperature and limited rainfall.

Amaranth was cultivated by early civilisations over 2000 years ago in America and continues to be used commonly worldwide up to our days. Sauer (1967) reports the introduction of amaranth into Spain in 16th century, from where it had spread throughout the Europe, around 1700s, it was known as a minor grain plant in central Europe and Russia.

The genus *Amaranthus* L. belongs to the *Amaranthoidae* subfamily of the *Amaranthaceae* family, *Caryophyllales* order and includes 105 species. Some of the amaranth species domesticated for grain production: *Amaranthus hypochondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus*; while those grown for leaf vegetables are: *Amaranthus dubius*, *Amaranthus blitum*, *Amaranthus tricolor*, *Amaranthus viridis* and *Amaranthus hybridus* (Das, 2016). *Amaranthus* seeds being a rich source of fatty acids, proteins, micronutrients, vitamins and squalene, are used as cereals, which are consumed whole toasted or milled into flour. The pigments obtained from all the parts amaranth plants can be used as a food or pharmaceutical dye. Many species of the *Amaranthus* genus are medicinally important and bear antiallergic, anticancer, antihypertensive and antioxidant properties, thus being used in the treatment of several ailments (Assad et al., 2017). *Amaranthus* has also been used in many countries as a grain, forage or silage crop for many animals, including cattle, chickens, pigs and rabbits as an alternative protein and fibre source and as a bioactive component (essential fatty acids, flavonoids, stanols, tocotrienols and squalene) source (Peiretti, 2018). Feeding amaranth silage to cows increases the milk productivity by 11.9%, milk fat by 0.46% and milk protein by 0.18 % as compared with corn silage (Pavlenkova et al., 2019). Von Cossel (2019) observed that the flowers of amaranth attracted numerous insect species such as wild bees, honey bees and bumblebees, are expected to have a higher benefit of amaranth stands compared to maize stands.

As a result of the research conducted in Botanical Garden Chişinău it has been found that the species *Amaranthus cruentus* and *Amaranthus mangostanus* are tolerant to

drought and have a high productivity of fresh mass that allows obtaining 5.31-6.68 t/ha nutritive units and 841-926 kg/ha digestible protein (Teleuță, 1995; Țiței & Teleuță, 1995). Marin et al. (2011) reported that, in the central part of the Romanian Plain, the recorded green mass yield of the studied cultivars was 43.1 t/ha for *Amaranthus hypochondriacus* and 46.7 t/ha for *Amaranthus cruentus*; the grain productivity of *Amaranthus hypochondriacus* was 4576 kg/ha and *Amaranthus cruentus* - 4609 kg/ha. Toader & Roman (2009) found that the chemical composition of grains *Amaranthus hypochondriacus* cultivars was following: 15.73-17.83% proteins, 60.75-62.83% starch, 5.17-6.49% lipids, 4.34-4.93% fibres and 3.31-3.93% ash. Rivelli et al. (2008) mentioned that, under the irrigation conditions of southern Italy, the total aboveground dry matter of tested *Amaranthus* species ranged from 15 t/ha (*Amaranthus cruentus*) to 23 t/ha (*Amaranthus hypochondriacus*).

The aim of this study was to evaluate some biological peculiarities, the quality of green mass and silage produced from *Amaranthus hypochondriacus*, as well as the possibility of using it as feed for ruminant animals and as feedstock for the production of biomethane.

MATERIALS AND METHODS

The non-native species *Amaranthus hypochondriacus*, which was cultivated in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute) Chişinău N 46°58'25.7" latitude and E 28°52'57.8" longitude, served as subject of the research, and the C₄ fodder crops corn, *Zea mays*, and Sudangrass, *Sorghum sudanense*, were used as controls.

The green mass of the *Amaranthus hypochondriacus* and *Sorghum sudanense* was mowed in early flowering stage (July), but the *Zea mays* - in kernel milk-wax stage (middle August). The green mass productivity was determined by weighing the yield obtained from a harvested area of 10 m², which was afterwards transformed per hectare. The leaves/stems ratio was determined by separating leaves and panicles from the stem, weighing them separately and establishing the

ratios for these quantities, samples of 1.0 kg harvested plants were used. For chemical analyses, the samples were dried at $65 \pm 5^\circ\text{C}$. The dry matter content was detected by drying samples up to constant weight at 105°C . The silage was prepared and evaluated in accordance with the Moldavian standard SM 108. Some assessments of the main biochemical parameters: protein, ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research and Development Institute for Grasslands Braşov, România. The concentration of hemicellulose (HC) and cellulose (Cel), the relative feed value (RFV), the digestible energy (DE), the metabolizable energy (ME) and the net energy for lactation (NEL) were calculated according to standard procedures.

The carbon content of the substrates was calculated from data on volatile solids, using an empirical equation indicated by Badger et al. (1979). The biochemical biogas potential (Y_b) and the methane potential (Y_m) were calculated according to the equations of Dandikas et al. (2014) based on the chemical compounds – acid detergent lignin (ADL) and hemicellulose (HC) values:

$$Y_b = 727 + 0.25 \text{ HC} - 3.93 \text{ ADL}$$

$$Y_m = 371 + 0.13 \text{ HC} - 2.00 \text{ ADL}$$

RESULTS AND DISCUSSIONS

The results of our study revealed that the seedlings of *Amaranthus hypochondriacus* emerged uniformly on the soil surface 3-5 days after sowing. In early stages, amaranth plantlets were characterized by slower growth of the stem and leaves, than corn and Sudangrass plantlets. We noticed that the growth and development rate of the aerial part increased 25 days after the emergence of the seedlings. The *Amaranthus hypochondriacus* root system developed actively from the seedling to the flowering period, the strong taproot grew over 1.7-2.0 m in depth and the lateral roots on the upper part - 35-40 cm. Plant height, stem thickness and leaves/stems ratio have significant

impact on the yield, but also affect the quality of the phytomass. Results regarding some biomorphological characteristics of the studied species and the structure of the harvested phytomass are presented in Table 1. At the time of the harvest, the *Amaranthus hypochondriacus* plants (160 cm) were shorter than *Zea mays* (239 cm) and *Sorghum sudanense* plants (212 cm), but amaranth stems, at the base, were about as thick as corn stems and three times thicker than Sudangrass. The productivity of *Amaranthus hypochondriacus* harvested in early flowering stage, late July, achieved 6.85 kg/m² green mass or 1.04 kg/m² dry matter, the annual yield of Sudan grass from two harvests (cuts) – 3.63 kg/m² green mass or 0.94 kg/m² dry matter, but the productivity of *Zea mays* reached 4.09 kg/m² green mass or 1.22 kg/m² dry matter, due to the high content of cobs in plant structure of the harvested phytomass in middle August. The harvested amaranth biomass was characterised by higher content of leaves (54%), but lower amount of dry matter (18%) in contrast with the control variants.

The biochemical composition, the nutritive and energy value of the green mass of the tested C₄ species are shown in Table 2. Analyzing the results of the green mass quality, we could mention that the concentrations of nutrients in *Amaranthus hypochondriacus* plants were 172 g/kg CP, 330 g/kg ADF, 462 g/kg NDF, 55 g/kg ADL, 68 g/kg TSS and 88 g/kg ash; *Sorghum sudanense* contained 85-101 g/kg CP, 372-413 g/kg ADF, 593-656 g/kg NDF, 39-41 g/kg ADL, 136-138 g/kg TSS and 95-97 g/kg ash, but in *Zea mays* - 62 g/kg CP, 310 g/kg ADF, 510 g/kg NDF, 51 g/kg ADL, 210 g/kg TSS and 60 g/kg ash, respectively. Thus, amaranth green mass contained a very high amount of crude protein and lower content of total soluble sugars than Sudangrass and corn. The digestibility of amaranth dry matter reached 57.1%, higher than Sudangrass, but significantly lower than corn dry matter. The nutritive and energy value of *Amaranthus hypochondriacus* was RFV= 127, 12.44 MJ/kg DE, 10.22 MJ/kg ME and 6.23 MJ/kg NEL, but the controls: *Sorghum sudanense* - RFV=80-94, 10.39-11.92 MJ/kg DE, 8.52-9.79 MJ/kg ME, 5.29-5.75 MJ/kg NEL and *Zea mays* RFV= 116, 12.72 MJ/kg DE, 10.75 MJ/kg ME, 6.46 MJ/kg NEL, respectively. The calculated annual crude

protein yield of amaranth natural fodder may be 1910 kg/ha, Sudangrass - 830 kg/ha and corn fodder - 760 kg/ha, respectively. Several literature sources describe the nutritional value of *Amaranthus hypochondriacus* plants. The results obtained

by Pисаоикова et al. (2006) in the Czech Republic, varied in the advancing stage of regrowth, from 80 to 120 days: 183.6-113.1 g/kg CP, 14.8-33.2 g/kg EE, 163.5-233.7 g/kg CF, 445.9-481.6.0 g/kg NFE, 192.2-138.4 g/kg ash, 17.2-18.4 MJ/kg GE.

Table 1. Some bio-morphological characteristics and the structure of the harvested phytomass of the studied species

Indices	<i>Amaranthus hypochondriacus</i>	<i>Sorghum sudanense</i>		<i>Zea mays</i>
		First cut	Second cut	
Plant height, cm	160	212	163	239
Stem thickness, mm	20	7	8	21
Leaf fresh mass, g/tiller	440.1	12.5	21.2	151.6
Leaf dry matter, g/tiller	87.6	3.2	6.1	46.4
Stem fresh mass, g/tiller	449.8	33.1	44.4	320.2
Stem dry matter, g/tiller	63.9	7.1	11.1	94.5
The yield :				
- fresh mass, kg/m ²	6.85	2.79	0.84	4.09
- dry matter, kg/ m ²	1.04	0.72	0.22	1.22

Table 2. The biochemical composition, the nutritive and the energy value of the green mass

Indices	<i>Amaranthus hypochondriacus</i>	<i>Sorghum sudanense</i>		<i>Zea mays</i>
		First cut	Second cut	
Crude protein, g/kg	172	85	101	62
Acid detergent fibre, g/kg DM	330	413	372	310
Neutral detergent fibre, g/kg DM	462	656	593	520
Acid detergent lignin, g/kg DM	55	41	39	51
Total soluble sugars, g/kg DM	68	138	136	210
Crude ash, g/kg DM	88	95	97	60
Digestible dry matter, % DM	57.1	51.7	60.3	72.3
Digestible organic matter, % DM	51.4	50.6	58.1	68.3
Digestible energy, MJ/kg DM	12.45	10.39	11.92	12.72
Metabolizable energy, MJ/kg DM	10.22	8.52	9.79	10.75
Net energy for lactation, MJ/kg DM	6.23	5.28	5.75	6.46
Relative feed value	127	80	94	116
Potential crude protein, kg/ha	1910	610	220	760

According to Rahnama & Safaie (2017), *Amaranthus hypochondriacus* can produce 75.86-90.30 t/ha fresh and 11.0-13.05 t/ha dry forage yield with 11.5-12.00% protein, 2.1-2.4% fats, 67.4-69.1% DMD, RFV 157.1-171.5, RFQ 158-174.6. Leukebandara et al. (2015) mentioned that the *Amaranthus hypochondriacus* plants harvested the mid-bloom stage contained 13.20% DM, 18.43% CP, 3.17% EE, 24.50% CF, 16.83% ash; *Amaranthus cruentus* - 13.87% DM, 16.00% CP, 3.60% EE, 24.50% CF, 15.33% ash; *Amaranthus caudatus* - 13.43% DM, 14.30% CP, 2.90% EE, 21.80% CF, 13.60% ash, and *Zea mays* - 18.27% DM, 8.13% CP, 2.43% EE, 25.70% CF, 5.07% ash. The results obtained by Biel et al. (2017) on feed value of *Amaranthus hypochondriacus* aerial part were as follows: 101 g/kg CP, 17.6 g/kg EE, 240

g/kg DM CF, 440 g/kg NDF, 332 g/kg ADF, 63.1 g/kg lignin, 269 g/kg Cel, 109 g/kg HC, 144 g/kg ash, 13.7 g/kg calcium and 6.8 g/kg phosphorus, 63.8% DMD, 10.8 MJ/kg DE and 8.7 MJ/kg ME. Pospisil et al. (2009) studied the nutrient and fibre composition in flowering stage and found that *Amaranthus hypochondriacus* contained 85-113 g/kg CP, 60-91 g/kg DP, 13-20 g/kg EE, 239-290 g/kg CF, 423-478 g/kg NDF, 301-366 g/kg ADF, but sorghum forage contained 64-92 g/kg CP, 43-65 g/kg DP, 19-21 g/kg EE, 295-329 g/kg CF, 637-659 g/kg NDF, 352-389 g/kg ADF. Abbasi et al. (2018) reported that fresh amaranth forage contained 233 g/kg DM, 187 g/kg CP, 420 g/kg NDFom, 275 g/kg ADFom, 44.6 g/kg ADL, 57 g/kg EE, 62.5 g/kg water-soluble carbohydrates, 145 g/kg ash, 11.1 g/kg

calcium, 6.6 g/kg phosphorus and 8.4 MJ/kg ME.

Silage is commonly used as fodder, as high-quality roughage for farm animals, but in recent decades, it has also been used as substrate in biogas production. During the sensorial assessment, it was found that the prepared silage from *Amaranthus hypochondriacus* green biomass had a pleasant smell, specific to pickled vegetables, the consistency of the silage was retained, in comparison with the initial green mass, without mould and mucus. The fermentation quality of prepared silages is illustrated in Table 3. The performed analysis helped determining that the pH index varied unessentially from 3.61 in corn silage to 3.86 in amaranth silage. The pH index of the prepared silages from the studied species met the standard SM 108 for the first class quality. The greatest differences were obtained in concentrations of organic acids. It has been determined that the content of total organic acids was low in *Amaranthus hypochondriacus* silage – 19.2 g/kg, in comparison with corn and Sudangrass silages. Butyric acid was not detected in the amaranth and corn silages. The *Amaranthus hypochondriacus* silage was characterised by high content of acetic acid (5.8 g/kg) and lower concentration of lactic acid (13.4 g/kg). The dry matter content in the silage prepared from *Amaranthus hypochondriacus* was low (184 g/kg) in comparison with *Zea mays* silage (314.5 g/kg). The results of the study on nutrient concentrations indicate that *Amaranthus hypochondriacus* silage contained 167 g/kg CP, 122 g/kg ash, 516 g/kg NDF, 348 g/kg ADF, 45 g/kg ADL, 12 g/kg TSS. *Sorghum sudanense* silage prepared after the first cut contained 57 g/kg CP, 109 g/kg ash, 402 g/kg ADF, 652 g/kg NDF, 39 g/kg ADL, 108 g/kg TSS and *Zea mays* silage – 53 g/kg CP, 50 g/kg ash, 514 g/kg NDF, 303 g/kg ADF, 276 g/kg TSS. It was found that during the process of ensiling, the concentrations of crude protein in *Amaranthus hypochondriacus* silage had not modified in comparison with the initial mass, but it had decreased essentially in controls silages. In both silages, the lignin content decreased and the digestibility of nutrients was higher in comparison with the initial mass. In

Amaranthus hypochondriacus silage, the concentrations of energy reached very acceptable values: 10.05 MJ/kg ME and 6.02 MJ/kg NEL, as compared with *Sorghum sudanense* silage - 9.38 MJ/kg ME and 5.54 MJ/kg NEL, however, they were lower than in *Zea mays* silage - 10.52 MJ/kg ME and 5.54 MJ/kg NEL.

Some authors mentioned various findings about the silage quality of studied species. According to Herrmann et al. (2016), the biochemical composition of *Amaranthus* silages were 9.4-9.6% CP, 3.3-3.6% EE, 42.9-50.5% NFE, 35.2-44.7% NDF, 30.2-37.6% ADF, 4.0-6.3% ADL, 5.8-9.1% lactic acid, 1.1% acetic acid, pH 4.1-4.2; *Zea mays* silage - 4.4-12.1% CP, 1.0-3.9% EE, 53.8-71.4% NFE, 26.8-53.7% NDF, 14.3-37.1% ADF, 1.0-6.1% ADL, 3.5-9.5% lactic acid, 0.2-3.6% acetic acid, pH 3.1-4.1; *Sorghum bicolor* x *S. sudanense* silage – 5.3-17% CP, 0.5-3.2% EE, 40.8-58.4% NFE, 48.2-69.1% NDF, 28.5-42.7% ADF, 2.9-7.2% ADL, 2.5-12.0% lactic acid, 0.3-2.4% acetic acid, pH 3.2-5.5. Abbasi et al. (2018) found that the ensiled amaranth forage, without additives, was characterized by 250 g/kg DM, pH 4.49, 57 g/kg lactic acid, 17.1 g/kg acetic acid and 0.3 g/kg butyric acid, 171 g/kg CP, 400 g/kg NDFom, 267 g/kg ADFom, 43.0 g/kg ADL, 103 g/kg EE, 19.7 g/kg water-soluble carbohydrates, 170 g/kg ash, 10.8 g/kg calcium, 5.8 g/kg phosphorus and 8.4 MJ/kg ME. Rezaei et al. (2015) reported that amaranth silage had pH 3.99, 23.5% DM, contained 69.1 g/kg lactate, 19.3 g/kg acetate, 10.3 g/kg butyrate, 114 g/kg CP, 451g/kg NDFom, 310 g/kg ADFom, 35.6 g/kg lignin with 676 g/kg DOM and 9.34 MJ/kg ME, the corn silage - pH 4.0, 22.4% DM, 71.8 g/kg lactate, 20.4 g/kg acetate, 0.87 g/kg butyrate, 83 g/kg CP, 510 g/kg NDFom, 335 g/kg ADFom, 45.7 g/kg lignin with 661 g/kg DOM and 9.71 MJ/kg ME. Rahjerdi et al. (2015) compared the ensilability of *Amaranthus hypochondriacus* and corn and pointed out that, the amaranth silage had pH 4.10-4.25 and 23.0-23.4% DM, contained 6.10-6.24% lactic acid, 1.18-1.20% acetic acid, 0.031-0.047% butyric acid, 12.0-12.8% CP, 40.0-41.9% NDFom, 26.0-27.2% ADFom, 3.13-3.21% ADL, 2.36-2.68% EE, 13.6-14.0% ash, the corn silage - pH 3.82 and 22.0% DM,

it contained 8.02% lactic acid, 1.74% acetic acid, 0.005% butyric acid, 6.98% CP, 52.89% NDFom, 32.7% ADFom, 4.88 % ADL, 1.98% EE, 6.28% ash. Ma et al. (2019) concluded that the dry matter and the chemical

composition of amaranth silage varied depending on the time when the plants had been harvested and ensiled: 15.31-22.00% DM, 13.21-11.51% CP, 47.88-54.14% NDF, 29.79-39.03% ADF, 2.53-4.67% ADL.

Table 3. The fermentation quality of the silages from the studied species

Indices	<i>Amaranthus hypochondriacus</i>	<i>Sorghum sudanense</i>	<i>Zea mays</i>
pH index	3.86	3.82	3.61
content of organic acids, g/kg DM	19.2	30.0	32.8
free acetic acid, g/kg DM	2.7	2.3	1.3
free butyric acid, g/kg DM	0.0	0.0	0.0
free lactic acid, g/kg DM	6.0	10.2	13.7
fixed acetic acid, g/kg DM	3.1	2.1	2.0
fixed butyric acid, g/kg DM	0.0	0.2	0.0
fixed lactic acid, g/kg DM	7.4	15.2	15.8
total acetic acid, g/kg DM	5.8	4.4	3.3
total butyric acid, g/kg DM	0.0	0.2	0.0
total lactic acid, g/kg DM	13.4	28.4	29.5
acetic acid, % of organic acids	30	15	10
butyric acid, % of organic acids	0	1	0
lactic acid, % of organic acids	70	84	90

Table 4. The biochemical composition and the feed value of the silages from the studied species

Indices	<i>Amaranthus hypochondriacus</i>	<i>Sorghum sudanense</i> (first cut)	<i>Zea mays</i>
Crude protein, g/kg DM	167	57	53
Acid detergent fibre, g/kg DM	348	402	303
Neutral detergent fibre, g/kg DM	516	652	514
Acid detergent lignin, g/kg DM	45	39	46
Total soluble sugars, g/kg DM	12	108	276
Crude ash, g/kg DM	123	109	50
Digestible dry matter, %	61.8	57.5	72.4
Digestible organic matter, %	51.3	53.8	68.5
Digestible energy, MJ/ kg DM	12.2	11.43	12.82
Metabolizable energy, MJ/ kg DM	10.2	9.38	10.52
Net energy for lactation, MJ/ kg DM	6.03	5.54	6.54
Relative feed value	111	82	118
Potential crude protein, kg/ha	1650	390	620

Biomass is an important source for the production of multi-purpose renewable energy. The production of biogas using lignocellulosic biomass as a renewable energy source is both sustainable and environmentally friendly. The stability and the productivity of biogas digesters are mostly influenced by biochemical composition, biodegradability and ratio of carbon and nitrogen of substrate. The optimal C/N ratio in biomass should range from 10 to 30, which does not affect the development of microflora involved in anaerobic digestion. The results regarding of the quality of the substrate and its biochemical methane potential from the studied species are shown in Table 5. We would like to mention that in the green mass and silage substrates from

Amaranthus hypochondriacus plants, the carbon and nitrogen ratio was 18, but in Sudangrass substrates C/N = 39-41 and in corn substrates C/N = 46-51. It has been found that the concentration of hemicellulose in amaranth silage substrates increases by 26 g/kg, but lignin decreases by 10 g/kg DM in comparison with amaranth green mass substrates, and thus it has a positive impact on methane yield. The calculated biomethane potential of the amaranth silage substrates was 302 l/kg, but – of amaranth green mass substrates 278 l/kg, the annual methane potential productivity achieved 2760 m³/ha. The biomethane potential of the Sudangrass substrates was 319-326 l/kg, but of corn substrates 296-306 l/kg.

Table 5. The biochemical composition and the biomethane production potential of substrates from the studied species

Indices	<i>Amaranthus hypochondriacus</i>		<i>Sorghum sudanense</i>		<i>Zea mays</i>	
	green mass	silage	green mass	silage	green mass	silage
Nitrogen, g/kg DM	27.52	26.72	13.62	9.12	9.92	8.51
Carbon, g/kg DM	50.66	48.77	50.27	49.50	52.22	54.00
Ratio carbon/nitrogen	18.41	18.25	37.00	54.28	52.61	63.45
Cellulose, g/kg DM	275	303	272	363	259	257
Hemicellulose, g/kg DM	132	158	243	250	210	211
Acid detergent lignin, g/kg DM	55	45	41	39	51	46
Biogas potential, l/kg VS	544	590	627	637	580	599
Biomethane potential, l/kg VS	278	302	319	326	296	306

Several literature sources describe the methane potential of amaranth substrate. Seppälä (2013) found that in boreal conditions the methane yield from amaranth biomass reached 290 l/kg VS or 2 700 m³/ha, but from Sudangrass – 330 l/kg VS or 2 500 m³/ha.

Mursec et al. (2009) remarked that methane production from amaranth silage was 125 l/kg VS, maize and sorghum silages 187-188 l/kg VS. Eberl et al. (2014) reported that the amaranth silage was characterized by a poor dry mater content (23.6%) accompanied by high contents of ash (13.7% of VS), ADL (5.8% of VS) and cellulose (26% of VS), which caused a much lower specific methane yield (270 l/kg) compared to maize (350 l/kg). Based on the batch experiments, Dubrovskis & Adamovics (2015) found that the average methane yield per unit of dry organic matter added from digestion of amaranth silage was 403 L/kg, with catalyst Metaferm 434-484 L/kg.

Herrmann et al. (2016), reported that the tested amaranth silage substrate had C/N = 27-27 and 4.0-6.3% lignin, methane content in biogas 58.4-59.1%, biochemical methane potential 268.9-287.9 l/kg VS; corn silages - C/N = 23-61 and 1.0-6.1% lignin, the methane content in biogas 53.1-58.4%, biochemical methane potential 294.5-376.2 l/kg; Sudangrass hybrid silages had C/N = 15-54 and contained 1.0-6.1% lignin, methane content in biogas 53.6-61.1%, biochemical methane potential 248.4-348.52 l/kg.

According to Von Cossel (2019), the specific methane yield of *Amaranthus hypochondriacus* substrate was on average 266 l/kg VS, which was negatively affected by the high contents of ash (13.6%), lignin (6.5%) and cellulose (32.9%).

CONCLUSIONS

The yield of *Amaranthus hypochondriacus* harvested in early flowering period is about 6.85 kg/m² green mass or 1.04 kg/m² dry matter.

The concentrations of nutrients and energy in *Amaranthus hypochondriacus* plants harvested in early flowering stage are 172 g/kg CP, 330 g/kg ADF, 462 g/kg NDF, 55 g/kg ADL, 68 g/kg TSS and 88 g/kg ash, RFV = 127, 12.44 MJ/kg DE, 10.22 MJ/kg ME and 6.23 MJ/kg NEL.

The *Amaranthus hypochondriacus* silage contains 13.4 g/kg DM lactic acid, 5.8 g/kg DM acetic acid, 167 g/kg CP, 123 g/kg ash, 516 g/kg NDF, 348 g/kg ADF, 45 g/kg ADL, 12 g/kg TSS, 158 g/kg HC and 303 g/kg Cel, 10.05 MJ/kg ME and 6.02 MJ/kg NEL.

The biochemical methane potential of amaranth green mass substrates is 278 l/kg ODM and silage substrates - 302 l/kg ODM.

The green mass and silage obtained from *Amaranthus hypochondriacus* contain many nutrients, which make them suitable to be used as fodder for animals and have high potential as feedstock for biomethane production.

REFERENCES

- Abbasi, M., Rouzbehan, Y., Rezaei, J., Jacobsen, S.E. (2018). The effect of lactic acid bacteria inoculation, molasses, or wilting on the fermentation quality and nutritive value of amaranth (*Amaranthus hypochondriacus*) silage. *Journal of Animal Science*, 96, 3983–3992.
- Assad, R., Reshi, Z.A., Jan, S., Rashid, I. (2017). Biology of Amaranths. *Botanical Review*, 83, 382–436.
- Badger, C.M., Bogue, M.J., Stewart, D.J. (1979). Biogas production from crops and organic wastes. *New Zealand Journal of Science*, 22, 11–20.
- Biel, W., Jendrzyczak, E., Jaroszewska, A., Witkowicz, R., Piatkowska, E., Telesinski, A. (2017). Nutritional

- content and antioxidant properties of selected species of *Amaranthus L.* *Italian Journal of Food Science*, 29(4), 728–740.
- Dandikas, V., Heuwinkel, F., Drewes, J.E., Koch, K. (2014). Correlation between biogas yield and chemical composition of energy crops. *Bioresource Technology*, 174, 316–320.
- Das, S. (2016). *Amaranthus: A promising crop of future*. Springer. 208p. <https://doi.org/10.1007/978-981-10-1469-7>
- Dubrovskis, V., Adamovics, A. (2015). Anaerobic digestion of hemp, sunflower silage and amaranth silage with catalyst metaferm. 23rd European Biomass Conference and Exhibition, 845–850 DOI: 10.5071/23rdEUBCE2015-2DV.1.45
- Eberl, V., Fahlbusch, W., Fritz, M., Sauer, B. (2014). Screening und Selektion von Amaranthsorten und -linien als spurenelementreiches Biogassubstrat (No. 37), Berichte aus dem TFZ. Technologie- und Förderzentrum im Kompetenzzentrum für Nachwachsende Rohstoffe, Straubing, 114 s.
- Herrmann, C., Idler, C., Heiermann, M. (2016). Biogas crops grown in energy crop rotations: Linking chemical composition and methane production characteristics. *Bioresource Technology*, 206, 23–35.
- Leukebandara, I.K., Premaratne, S., Peiris, B.L. (2015). Nutritive quality of thampala (*Amaranthus* spp.) as a forage crop in Sri Lanka. *Tropical Agricultural Research*, 26(4), 624–631.
- Ma, J., Sun, G., Shah, A.M., Fan, X., Li, S., Yu, X. (2019). Effects of different growth stages of amaranth silage on the rumen degradation of dairy cows. *Animals (Basel)*, 9(10), 793. doi:10.3390/ani9100793
- Marin, D.I., Bolohan, C., Mihalache, M., Rusu, T. (2011). Research on *Amaranthus cruentus* L. and *Amaranthus hypochondriacus* L. species grown in South-Eastern Romania (Moara Domnească - Ilfov). *Scientific Papers, USAMV Bucharest, Series A*, 54, 297–303.
- Mursec, B., Vindis, P., Janzekovic, M., Brus, M., Cus, F. (2009). Analysis of different substrates for processing into biogas. *Journal of Achievements in Materials and Manufacturing Engineering*, 37(2), 652–659.
- Pavlenkova, S.V., Shuvaeva, G.P., Miroshnichenko, L.A., Sviridova, T.V., Korneeva O.S., Tolkacheva, A.A. (2019). Comparative characteristics of amaranth and corn silage qualitative indicators. *Vestnik VGUIT [Proceedings of VSUET]*, 81(3), 174–179. (in Russian). doi:10.20914/2310-1202-2019-3-174-179
- Peiretti, P.G. (2018). Amaranth in animal nutrition: A review. *Livestock Research for Rural Development*. 30. <http://www.lrrd.org/lrrd30/5/peir30088.html>
- Pisaoikova, B., Peterka, J., Trakova, M., Moudr, J., Zral, Z., Herzig, I. (2006). Chemical composition of the above-ground biomass of *Amaranthus cruentus* and *A. hypochondriacus*. *Acta Veterinaria Brno*, 75, 133–138.
- Pospisil, A., Pospisil, M., Macesic, D., Svecnjak, Z. (2009). Yield and quality of forage sorghum and different amaranth species (*Amaranthus* spp.) biomass. *Agriculturae Conspectus Scientificus*, 74(2), 85–89.
- Rahjerdi, N.K., Rouzbehan, Y., Fazaeli, H., Rezaei, J. (2015). Chemical composition, fermentation characteristics, digestibility, and degradability of silages from two amaranth varieties (*Kharkovskiy and Sem*), corn, and an amaranth-corn combination. *Journal of Animal Science*, 93, 5781–5790.
- Rahnama, A., Safaeie, A.R. (2017). Performance comparison of three varieties of amaranth (*Amaranthus hypochondriacus* L.) at different harvest time. *Journal of Asian Scientific Research*, 7(6), 224–230.
- Rezaei, J., Rouzbehan Y., Zahedifar, M., Fazaeli, H. (2015). Effects of dietary substitution of maize silage by amaranth silage on feed intake, digestibility, microbial nitrogen, blood parameters, milk production and nitrogen retention in lactating Holstein cows. *Animal Feed Science and Technology*, 202, 32–41.
- Rivelli, A.R., Gherbin, P., De Maria, S., Pizza, S. (2008). Field evaluation of *Amaranthus* species for seed and biomass yields in Southern Italy. *Italian Journal of Agronomy/ Rivista di Agronomia*, 3, 225–229.
- Sage, R.F., Sage, T.L., Percy, R.W., Borsch, T. (2007). The taxonomic distribution of C₄ photosynthesis in Amaranthaceae sensu stricto. *American Journal of Botany*, 94, 1992–2003.
- Sauer, J.D. (1967). The grain amaranths and their relatives: a revised taxonomic and geographic survey. *Annals of Missouri Botanical Garden*, 54, 103–137.
- Seppälä, M. (2013). Biogas production from high-yielding energy crops in boreal conditions. Jyväskylä University Printing House. 92p.
- Teleuță, A. (1995). Particularitățile biologice și valoarea nutritivă a biomasei la unele specii din genul *Amaranthus* L. *Buletinul Academiei de Științe a Moldovei. Științe Biologice și Chimice*, 4, 3–10.
- Țiței, V., Teleuță A. (1995). Toleranța la secetă și productivitatea speciilor din genul *Amaranthus* în condițiile Moldovei. In. *Apele Moldovei: seceta și măsurile complexe de combatere*. Chișinău, p.251
- Toader, M., Roman, Gh.V. (2009). Experimental results regarding morphological, biological and yield quality of *Amaranthus hypochondriacus* L. species under the Central part of Romanian Plain conditions. *Research Journal of Agricultural Science*, 41(1), 54–57.
- Von Cossel, M. (2019). Agricultural diversification of biogas crop cultivation. Dissertation, University of Hohenheim, Stuttgart, Germany. <https://opus.uni-hohenheim.de/volltexte/2019/1600/>
- *SM 108:1995 (1996). Siloz din plante verzi. Condiții tehnice. Moldovastandard. 10.

COMPARATIVE TESTING OF COMMON WINTER WHEAT LINES AND THEIR SUITABILITY FOR CHANGING ENVIRONMENTAL CONDITIONS

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Abstract

The trial was carried out on the field from IPGR, Sadovo, during the period 2017-2019. The test was performed by means of a block method with four repetitions; experimental field area - 10 m² after leguminous predecessor. The aim of the study was to establish the mass of 1000 grains, hectolitre mass, plant height and yield from 31 lines and candidate varieties obtained by the method of the variety and remote hybridization were tested. In the experiment were involved two standard varieties - Sadovo 1 and Enola. The adopted in IPGR - Sadovo technology for growing of winter common wheat was used. The grain yield is determined with standard grain moisture of 13%. The indices: thousand kernel weight (g), test weight (kg), and grain yield (kg/ha) were determined. Data obtained for the plant height, the grain yield and the physical properties of the grain were statistically processed by the method of dispersion and correlation analyses. The results show: environmental factors have the most influence on the grain yield, 1000 grain weight and test weight; was found that the plant height is significantly influenced by the genotype. The highest average yield for the period is reported on the lines MX 286-1777, MX 258-3355 and candidate variety Yilzla

Key words: common winter wheat, lines, suitability, plant height, grain yield.

INTRODUCTION

In the near future agriculture, especially field plant production, will face various challenges, one of these will be the need to satisfy the food requirements of a growing population while the available freshwater reserves are declining steadily (Paks & Reynolds, 2013). Wheat production plays an important role in the food supply not only today but also in the future, as this sector is highly sensitive to the climatic and environmental changes (Semenov & Stratonovitch, 2013). Extreme weather is occurring more frequently due to climate change in many parts of the world, including changes in precipitation patterns. The decreasing precipitation in combination with increased air temperature is the most important yield-limiting factors and these are threatening food security worldwide (Daryanto et al., 2017). Drought is one of the most important stressors for cereals (Fahad et al., 2017), it can reduce the amount of biomass by more than 25% and can cause a large yield loss (Zhang et al., 2018). When endeavouring optimum yields with limited water supplies, farmers must choose to use new water-saving technologies or

growing varieties that use water more efficiently (Jabran et al., 2015). Besides the deficit of rainfall, the unusually high amount of precipitation can also affect the plant growth negatively. Waterlogging affects 25% of the wheat plantation worldwide (Powell et al., 2012). Wheat can tolerate the waterlogging for different time of period and its resistance depends on both the plant's maturity stage and the temperature (Ding et al., 2018). The use of different breeding methods for the creation of great genetic diversity, its evaluation, the use of new technological solutions is a prerequisite for the creation of varieties suitable for changing conditions.

The present study aims to determine the suitability of advanced lines of common winter wheat to changing environmental conditions

MATERIALS AND METHODS

The experiment was conducted in the period 2017-2019 by a block method in four replicates with a plot size of 10 m² after a bean precursor to the experimental field of IRGR, Sadovo - central southern Bulgaria. The adopted in IPGR - Sadovo technology for growing of winter

common wheat was used. 31 lines and candidate varieties obtained by the method of the variety and remote hybridization were tested. Two standard varieties - Sadovo 1 and Enola - were included in the experiment. All the stages of the established technology for wheat growing were followed. Soil tillage included single disking (10-12 cm) after harvesting of the previous crop, and double disking after the main fertilization (Dallev and Ivanov, 2015). The area was treated by N₁₂₀P₈₀ and the whole quantity of the phosphorous fertilizer and 1/3 of the nitrogenous fertilizer were applied before main soil tillage. The remaining amount from the nitrogen norm was applied before the beginning of permanent spring vegetation. Triple super phosphate and ammonia nitrate were used. Sowing was completed within the agrotechnical term optimal for this region at sowing norm 550 germinating seeds/m².

Control of weeds, diseases and pests was done with suitable pesticides when necessary (Mitkov et al., 2017). Harvesting was done at full maturity. The grain yield is determined with standard grain moisture of 13%.

The indices grain yield (kg da⁻¹); test weight (kg); thousand kernel (grain) weight (g), and plant height were determined.

The analysis of the variance, the effect of the individual factors on the studied traits and the correlations between them was made using the statistical program SPSS ver. 19.

The period of the research (2016-2019) is characterized with variety of temperature and rainfall conditions which enables to evaluate the reaction of the studied lines in accordance with their yields and quality characteristics under different climatic conditions (Figures 1, 2).

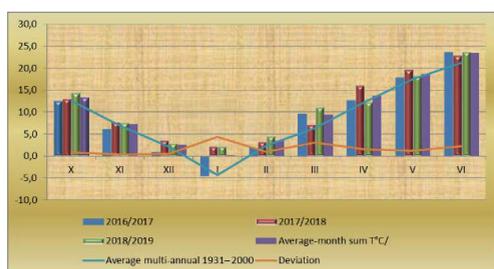


Figure 1. Average monthly temperature during three vegetation years, t°C

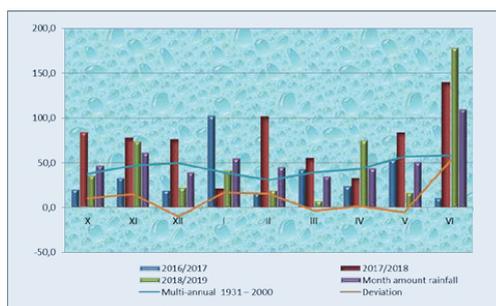


Figure 2. Monthly rainfall sum during three vegetation years (mm/m²)

The most favorable for plant growth and development was the first experimental year (2016-2017), followed by the second (2017-2018), and unfavorable was the third year (2018-2019), of the experiment, having an effect on yield and grain quality of winter wheat.

RESULTS AND DISCUSSIONS

The growth and development of common winter wheat plants took place under different meteorological conditions. During the three years of the survey the environmental conditions were not typical. This also applies to the development of wheat plants by phase. In agroclimatic terms, the harvest year 2017 can be described as "very different". Rainfall in October /21.4 mm/ was a favourable factor for /allowed for/quality soil tillage. We sowed at the end of the optimal period October 27 to November 2. Winter damage and frost in the area were not observed as the crops had not emerged. The average monthly air temperatures in January and February 2017 are close to normal. Due to the low temperatures in November and December, massive and heavy snow cover until February 15th reported on February 21st. This resulted in a shortening of all interphase periods. The plants formed lower than the typical height. In March, average monthly temperatures were higher than the multi-annual values, with precipitation falling by about 20 mm below normal. The wilting and flowering phase passed very quickly until the first ten days of May and passed at temperatures close to normal and very good moisture supply (+26.2 mm). The flowering to milky maturity has taken place in about 10-15

days. The dough maturity was marked on June 20, and full maturity was reported in late June and early July.

In agrometeorological terms, the 2017-2018 growing season can also be defined as "very different". Rainfall in October /84.6/ impeded soil tillage. We sowed at the end of the optimal period - 23.10. An emergence was reported on 3.11.2017. Their development proceeded normally and in appropriate weather conditions for the vernalization. In the months of November and December, the average maximum daily temperatures reached, respectively, 11.5°C and 8.2°C, and the average daily temperatures reached 8.0°C and 3.8°C. The average minimum temperatures were positive in November (3.46°C) and slightly negative in December (-0.44°C), with negative average daily temperatures occurring in 4 days in December and snow cover lasting only two days, also in December. Meteorological conditions in January and February did not differ from those in December, with longer lasting snow cover in the second half of January. The average monthly air temperature in January was much higher than normal and in February 2018 it was closer to normal. Winter damage and frost in the area were not observed, as winter was mild. During the harvesting period the plants were well supplied with moisture and the formed plants were higher than the previous year. The phasing-out phases (26 April-2 May) and flowering (early May) were almost merged due to the warm weather. Full maturity was reported in the third ten days of June - 25.06. Due to heavy rainfall, the harvest was delayed between June 26 and June 29 and early July. The quality of the grain was impaired. The monthly rainfall was 139.9 l/m².

In the last year of the study, the autumn was very dry and the plants were late on 14.11.2018 after the fall of rain. The tillering phase was also delayed. The fallen rain after 40 days of drought led to the normal development of the plants and the jointing was reported. Heading was observed in the period 30.04.-04.05.2019, and milk maturity on 20-22.05.2019 and dough

maturity on 29-31.05.2019. We can summarize that the average monthly temperatures for all the months of vegetation were higher than the annual average and the rainfall was not evenly distributed.

The three-year competitive varietal yield test included 31 lines and candidate varieties obtained by the method of variety and remote hybridization (crosses with durum wheat). Grain yield is an integral indicator that expresses the complex of qualities that a variety possesses, including resistance to stress factors. Therefore, the primary task of any breeding program for common wheat is to increase the yield potential of new varieties.

The average yield for the period by variety varies from 410.3 to 672.0 kg / da. (Table 1). The value of the indicator for all varieties is 538.7 kg / da and for the average standard is 501.2. The highest values were reported at MX 258/3355 - 672 kg / da, Ayilzla - 655.2 kg/da and MX 286/1777 - 633.9 kg/da and differences against the standard were statistically proven. During the three-year period, 2017 is the most favourable year and then the highest yields are formed. They range from 469.2 kg/da to 840.5 kg/da. The lines PY 48/2553, MX 258/3355, MX 274/711, MX 286/1777, PY 49/2300, MX 289/2048 and Ayilzla have best expressed their productivity. In 2018, due to the rainfall at the end of June, the crops fell and high losses were reported, however, the candidate Ayilzla yielded 714.0 kg, MX 258-3355 - 650 kg/da, MX 260 / 1175-645.0 and Sashez - 631 kg/da. The table shows that the lowest yields are in 2019.

For the three-year period the 1000 grains weight is from 36.0 to 47.5 g, and the average for all varieties is 43.5 g. With proven largest grain average for the period are varieties Sashets - 47.5 and MX 270 / 3462- 47.4 g. In 2017 and 2018, the average values are higher than in 2019 and the average for the period.

A test weight is an important indicator of wheat. The average for 3 years is 71.6 kg/hl, ranging from 65.4 to 75.9 kg/hl. (Table 1)

Table 1. Complex characteristic of winter common wheat lines for the period 2017-2019

№	Name	Yield, kg							1000 kernel weight, g					
		2017	2018	2019	mean	± D	Sign.	%St	2017	2018	2019	mean	± D	Sign.
1.	MX 265-3430	633.3	472.4	551.4	552.4	50.5	n.s.	110.1	46.6	44.5	38.4	43.2	0.4	n.s.
2.	MX 270-3461	658.8	462.3	431.2	517.4	15.5	n.s.	103.2	50.1	48.0	42.8	47.0	4.2	n.s.
3.	MX 270-3462	606.5	378.1	499.3	494.6	-7.3	n.s.	98.7	51.1	51.0	40.0	47.4	4.7	+
4.	MX 270-3463	617	392.9	497.3	502.4	0.5	n.s.	100.2	52.0	47.7	39.8	46.5	3.8	n.s.
5.	MX 270-3464	623.5	496.6	411.0	510.4	8.4	n.s.	101.8	52.1	49.5	38.0	46.5	3.8	n.s.
6.	MX 274-717	618.4	486.6	367.1	490.7	-11.2	n.s.	97.9	50.3	47.9	43.4	47.2	4.5	n.s.
7.	MX 286-1759	761.8	516.6	460.3	579.6	77.7	n.s.	115.6	47.5	46.2	38.1	43.9	1.2	n.s.
8.	MX 286-1777	777.1	567.1	557.6	633.9	132.0	+	126.5	50.0	48.1	40.6	46.2	3.5	n.s.
9.	MX285-1058	592.9	459.6	580.8	544.4	42.5	n.s.	108.6	40.0	35.0	36.0	37.0	-5.7	+
10.	MX289-2048	774.9	347.1	574.3	565.4	63.5	n.s.	112.8	48.2	46.0	44.0	46.1	3.3	n.s.
11.	MX295-2524	694.2	562.5	474.5	577.1	75.2	n.s.	115.1	52.0	49.2	40.0	47.1	4.3	n.s.
12.	MX298-2582	673.6	379.5	459.1	504.1	2.1	n.s.	100.6	50.6	38.0	36.0	41.5	-1.2	n.s.
13.	MX298-2622	694.3	330.0	483.1	502.5	0.6	n.s.	100.3	37.1	35.8	38.0	37.0	-5.8	-
14.	MX298-2580	717.2	320.1	521.0	519.4	17.5	n.s.	103.6	45.6	35.7	35.0	38.8	-4.0	n.s.
15.	7621x Demetra 611-4	757.8	486.4	341.8	528.7	26.7	n.s.	105.5	43.3	36.9	33.6	37.9	-4.8	-
16.	7621x Demetra 612-1-2p	469.2	433.1	328.6	410.3	-91.6	n.s.	81.9	49.0	43.1	40.3	44.1	1.4	n.s.
17.	7621x Demetra 612-4-2p	476	457.1	512.9	482.0	-19.9	n.s.	96.2	48.3	43.0	34.0	41.8	-1.0	n.s.
18.	7621x Demetra 613-1	662.9	494.3	330.7	496.0	-5.9	n.s.	99.0	41.9	40.8	39.1	40.6	-2.1	n.s.
19.	7621x Demetra 613-2	479.3	406.4	481.7	455.8	-46.1	n.s.	90.9	46.6	47.1	38.6	44.1	1.4	n.s.
20.	MX 270-24	704.3	461.6	494.4	553.4	51.5	n.s.	110.4	49.4	43.9	36.9	43.4	0.7	n.s.
21.	MX 270-27	634.1	510.0	385.7	509.9	8.0	n.s.	101.7	47.1	52.0	37.9	45.7	3.0	n.s.
22.	MX 270-28	679.3	434.6	378.7	497.5	-4.4	n.s.	99.3	48.3	48.5	36.6	44.5	1.8	n.s.
23.	MX 270-50	638	304.5	472.1	471.5	-30.4	n.s.	94.1	44.8	46.7	39.2	43.6	0.8	n.s.
24.	MX 270-86	673.8	438.0	416.0	509.3	7.3	n.s.	101.6	45.9	51.3	40.0	45.7	3.0	n.s.
25.	Sashez	683.3	631.0	523.3	612.5	110.6	n.s.	122.2	47.9	52.9	41.6	47.5	4.8	+
26.	MX 260-1175	646.5	645.0	527.1	606.2	104.3	n.s.	120.9	46.6	52.0	40.7	46.4	3.7	n.s.
27.	Ayilzla	730.6	714.0	520.9	655.2	153.3	+	130.7	52.8	46.0	38.9	45.9	3.2	n.s.
28.	py 48-2553	840.5	540.0	499.9	626.8	124.9	n.s.	125.1	43.2	45.5	33.0	40.6	-2.2	n.s.
29.	py 49-2300	787.6	541.0	484.8	604.5	102.6	n.s.	120.6	47.8	47.1	38.0	44.3	1.6	n.s.
30.	MX 258-3355	801.3	650.0	564.8	672.0	170.1	++	134.1	43.1	44.4	37.2	41.6	-1.2	n.s.
31.	MX 274-711	795.3	438.3	527.0	586.9	85.0	n.s.	117.1	39.5	35.2	33.4	36.0	-6.7	--
32.	Sadovo 1	612.2	477.0	439.0	509.4	7.5	n.s.	101.6	51.1	47.3	39.5	46.0	3.3	n.s.
33.	Enola	649.3	345.4	488.6	494.4	-7.5	n.s.	98.6	44.4	40.0	34.0	39.5	-3.3	n.s.
Mean for Standarts		630.8	411.2	463.8	501.9				47.8	43.7	36.8	42.7		
mean		671.7	472.1	472.3	538.7				47.1	45.0	38.3	43.5		
min		469.2	304.5	328.6	410.3				37.1	35.0	33.0	36.0		
max		840.5	714.0	580.8	672.0				52.8	52.9	44.0	47.5		
GD		GD 5.0%=125,7 GD 1.0%=167,1 GD 0.1%=217,0							GD 5.0%=4,6 GD 1.0%=6,1 GD 0.1%=7,9					

Table 1 – continued. Complex characteristic of winter common wheat lines for the period 2017-2019

№	Name	Test weight, kg/hl						Plant height, cm					
		2017	2018	2019	mean	± D	Sign.	2017	2018	2019	mean	± D	Sign.
1.	MX 265-3430	79.2	69.2	71.1	73.2	-	n.s.	92.0	90.0	90.0	90.7	4.3	n.s.
2.	MX 270-3461	76.0	67.1	67.5	70.2	-	--	78.0	90.0	90.0	86.0	-0.3	n.s.
3.	MX 270-3462	77.5	68.3	67.0	70.9	-	n.s.	80.0	87.0	94.0	87.0	0.7	n.s.
4.	MX 270-3463	75.8	65.9	68.2	70.0	-	--	83.0	90.0	86.0	86.3	0.0	n.s.
5.	MX 270-3464	76.7	65.8	67.0	69.8	-	--	80.0	86.0	83.0	83.0	-3.3	n.s.
6.	MX 274-717	82.1	71.5	73.3	75.6	0.9	n.s.	107.0	105.0	90.0	100.7	14.3	+
7.	MX 286-1759	84.3	67.4	75.9	75.9	1.1	n.s.	78.0	72.0	90.0	80.0	-6.3	n.s.
8.	MX 286-1777	82.6	68.6	74.9	75.4	0.6	n.s.	80.0	72.0	83.0	78.3	-8.0	n.s.
9.	MX285-1058	80.3	64.5	71.6	72.1	-	n.s.	73.0	80.0	90.0	81.0	-5.3	n.s.
10.	MX289-2048	82.0	66.9	68.0	72.3	-	n.s.	85.0	82.0	82.0	83.0	-3.3	n.s.
11.	MX295-2524	80.0	65.9	69.5	71.8	-	n.s.	87.0	85.0	93.0	88.3	2.0	n.s.
12.	MX298-2582	80.0	66.9	68.2	71.7	-	-	75.0	75.0	75.0	75.0	-	n.s.
13.	MX298-2622	78.9	66.2	67.5	70.9	-	-	79.0	70.0	74.0	74.3	-	-
14.	MX298-2580	82.0	64.2	68.9	71.7	-	-	75.0	80.0	81.0	78.7	-7.7	n.s.
15.	7621x Demetra	75.3	65.4	68.9	69.9	-	--	78.0	79.0	78.5	78.5	-7.8	n.s.
16.	7621x Demetra	79.0	65.0	67.1	70.4	-	--	104.0	115.0	84.0	101.0	14.7	+
17.	7621x Demetra	79.7	65.1	68.9	71.2	-	-	108.0	115.0	90.0	104.3	18.0	++
18.	7621x Demetra	80.5	65.3	69.5	71.8	-	n.s.	80.0	80.0	85.0	81.7	-4.7	n.s.
19.	7621x Demetra	79.4	64.4	68.0	70.6	-	-	105.0	105.0	84.0	98.0	11.7	+
20.	MX 270-24	76.1	63.6	66.1	68.6	-	---	75.0	72.0	90.0	79.0	-7.3	n.s.
21.	MX 270-27	79.2	69.1	66.5	71.6	-	-	82.0	95.0	83.0	86.7	0.3	n.s.
22.	MX 270-28	77.3	67.0	71.0	71.8	-	n.s.	88.0	87.0	94.0	89.7	3.3	n.s.
23.	MX 270-50	72.9	62.5	67.1	67.5	-	---	83.0	85.0	90.0	86.0	-0.3	n.s.
24.	MX 270-86	72.2	60.7	63.4	65.4	-	---	85.0	85.0	92.0	87.3	1.0	n.s.
25.	Saschez	78.9	66.9	70.0	71.9	-	n.s.	82.0	98.0	96.0	92.0	5.7	n.s.
26.	MX 260-1175	80.0	67.4	73.2	73.5	-	n.s.	78.0	90.0	90.0	86.0	-0.3	n.s.
27.	Ayilzla	83.2	69.6	71.0	74.6	-	n.s.	84.0	104.0	100.0	96.0	9.7	n.s.
28.	py 48-2553	82.6	64.5	68.9	72.0	-	n.s.	68.0	80.0	86.0	78.0	-8.3	n.s.
29.	py 49-2300	80.2	64.1	68.6	71.0	-	n.s.	82.0	93.0	92.0	89.0	2.7	n.s.
30.	MX 258-3355	81.4	66.9	69.6	72.6	-	n.s.	65.0	85.0	82.0	77.3	-9.0	n.s.
31.	MX 274-711	80.7	58.4	63.4	67.5	-	---	75.0	83.0	82.0	80.0	-6.3	n.s.
32.	Sadovo 1	83.3	70.9	70.7	75.0	0.2	n.s.	90.0	100.0	90.0	93.3	7.0	n.s.
33.	Enola	82.0	70.3	71.3	74.5	-	n.s.	75.0	76.0	87.0	79.3	-7.0	n.s.
Mean for Standarts		82.7	70.6	71.0	74.8			82.5	88.0	88.5	80.1		
mean		79.4	66.2	69.1	71.6			83.0	87.6	87.2	85.9		
Min		72.2	58.4	63.4	65.4			65.0	70.0	74.0	74.3		
Max		84.3	71.5	75.9	75.9			108.0	115.0	100.0	104.3		
GD		GD5.0%=3.0 GD1.0%=4.0 GD0.1%=5.3						GD5.0%=11.4 GD1.0%=15.1					

The highest test weight are expressed by MX 286/1759, MX 274/717 and MX 286/1777, but the differences to the standard Sadovo 1 are insignificant. The highest values of this indicator were determined in 2017 whereas the ones for 2018 are the lowest (Table 2).

Plant height is a major feature of breeding, which is crucial for the lodging resistance, especially at higher fertilization levels. The average value for the period is 85.9 cm and they are in the range 74.3 for MX 298/2622 and 104.3 cm 7621 x D 612-4-2p, ie the deviations

are from -11.6 to 18.4 cm. The highest plants were formed in 2018 - 70-115.0 cm. It should be noted that the highest values were measured at crosses with durum wheat. According to this criterion, a selection was made and plants of smaller height were selected.

For the region of Sadovo, south Bulgaria, the analysis of variance revealed that grain yield, 1000 grains weight and test weight were most strongly influenced by environmental conditions (Table 3).

Table 2. Effect of sources of variation on yield, 1000 kernel weight, test weight, and plant height of winter common wheat lines for the period 2017-2019 (ANOVA)

Productivity Elements	Sources of variation	SS	df	MS	F exp.	F tab.	η
Yield	Genotype -A	354121.1	32	11066.	1.9*	1.6	22.0
	Enviromental condition,	875273.8	2	437636	73.7***	7.7	54.4
	Error	380148.9	64	5939.8			23.6
	Total	1609543.	98				100
1000 kernel weight	Genotype -A	1116.3	32	34.9	4.4***	2.5	36.8
	Enviromental condition,	1410.9	2	705.5	89.2***	7.7	46.5
	Error	506.4	64	7.9			16.7
	Total	3033.0	98				100
Test weight	Genotype -A	544.4	32	17.0	4.9***	2.5	13.8
	Enviromental condition,	3176.3	2	1588.2	456.6***	7.7	80.5
	Error	222.6	64	3.5			5.6
	Total	3943.3	98				100
Plant height	Genotype -A	5865.2	32	183.3	3.8***	2.5	62.3
	Enviromental condition,	426.5	2	213.2	4.4*	3.1	4.5
	Error	3122.0	64	48.8			33.2
	Total	9413.7	98				100

SS - sum of squares; gf - degrees of freedom; MS variant; F exp. - F experienced; F tab. - F is tabular; η - force of influence of the factor (%); *, **, *** - proved respectively at $p < 0.05$, $p < 0.01$, $p < 0.001$, n.s.- not proven

Table 3. Correlation coefficients between the investigated indicators

Indicators	Yield	1000 kernel weight	Test weight	Plant hight
Yield	1			
1000 kernel weight	0.037	1		
Test weight	0.316	0.171	1	
Plant height	-0.325	0.491**	0.091	1

**Correlation is proven at significance level $\alpha = 0.01$

On the basis of the correlation analysis, a positive, medium-strength relationship (Connection) between plant height and 1000 kernel weight was found during the study period (Table.3). Correlations between yield and other indicators have not been proven.

In other studies, carried out by us, for the period 2005-2009, it was also found that meteorological conditions had a greater influence than genotype (Rachovska et al., 2011). Other authors report that as a result of their studies in northern Bulgaria, it was found that meteorological conditions over the years have been decisive for both indicators plant height and test weight. (Nankova and Penchev, 2006; Penchev and Popova, 2005).

It is therefore worth further testing and studying the response of varieties under

different environmental conditions. For breeding of modern varieties, it is extremely important to have a low and non -logging stem and at the same time to have the biomass at the level of the old varieties (Tsenov and Tsenova, 2004).

Plant height is significantly influenced by genotype. By studies of other winter common wheat lines was found that the height of the plants was most influenced by the environment (53.4%), followed by the genotype (28.6%) (Dimitrov, 2017).

CONCLUSIONS

The highest values were reported at MX 258/3355 - 672 kg/da, Ayilzla - 655.2 kg/da and MX 286/1777 - 633.9 kg/da and

differences against the standard were statistically proven.

Analysis of variance revealed that yields, 1000 grains weight, and test weight were most strongly influenced by environmental conditions. Plant height is significantly influenced by genotype.

A positive, medium-strength relationship (connection) between plant height and 1000 kernel weight was found during the study period. Correlation between yield and other indicators have not been proven.

REFERENCES

- Dallev, M., Ivanov, I. (2015). Study of body for surface tillage in heavy soils with low humidity. *Scientific Papers. Series A. Agronomy, LVIII*, 45–48.
- Daryanto, S., Wang, L., Jacinthe, P.A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. *Agric. Water Manag.*, 179, 18–33.
- Dimitrov, Ev. (2017). Research on immune reaction of breeding lines winter common wheat (*Triticum aestivum* L.) to the causes brown roast, powdery mildew and fusariosis by spike, Phd thesis.
- Ding, J., Huang, Z., Zhu, M., Li, C., Zhu, X., Guo, W. (2018). Does cyclic water stress damage wheat yield more than single stress? *Plos One*, 13 4: e0195535.
- Fahad, S., Bajwa, A.A., Nazir, U., Anjum, S.A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S. (2017). Crop Production under Drought and Heat Stress: Plant Responses and Management Options. *Front. Plant Sci.*, 8, 1147.
- Mitkov, A., Yanev, M., Neshev, N., Tonev, T. (2017). Opportunities for single and combine application of herbicides at winter wheat. *Scientific Papers-Series A, Agronomy*, 60, 314–319.
- Jabran, K., Ullah, E., Hussain, M., Farooq, M., Zaman, U., Yaseen, M., Chauhan, B.S. (2015): Mulching improves water productivity, yield and quality of fine rice under water-saving rice production systems. *J. Agror. Crop Scim.*, 201, 389–400.
- Nankova, M., Penchev, E. (2006). Influence of continuous mineral fertilization on the process of formation of productivity and physical qualities of the grain of the Enola variety (*Triticum aestivum* L.). *Field Crops Studies*, 3(1), 125–135.
- Paks, A.J.D., Reynolds, M.P. (2013). Breeding for yield potential has increased deep soil water.
- Penchev, P., Popova, Z. (2005). Comparative testing of winter wheat varieties selected at IRGR, Sadovo. *Balkan Scientific Conference, Karnobat*, 494–496.
- Powell, N., Ji, X., Ravash, R., Edlington, J., Dolferus, R. (2012). Yield stability for cereals in a changing climate. *Funct. Plant Biol.*, 39, 539–552.
- Rachovska, G. et al. (2011). Evaluation of yield and stability of Bulgarian varieties of common winter wheat. *Agricultural Science*, 1, 111–114
- Semenov, M.A., Stratonovitch, P. (2013). Designing high-yielding wheat ideotypes for a changing climate. *Food Energy Secure*, 3, 185–360.
- Tsenov, N., Tsenova, E. (2004). Combining ability of some bread wheat varieties. I Yield and grain yield related characters. *Research Communications of U.S.B. branch Dobrich*, 6(1), 29–36.
- Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., Jin, J. (2018): Effect on drought on agronomic traits of rice and wheat: a meta-analysis. *Int. J. Environ. Res. Public Health*, 155, E839.

YIELD EXTRACT ESTIMATION OF ROMANIAN WINTER BARLEY GENOTYPES

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Abstract

This paper aimed at estimating the yield extract of some Romanian winter barley genotypes (six and two-row varieties and breeding lines), using different growing conditions (nitrogen fertilizer rate). All the experiments were performed at NARDI Fundulea and during the 2013-2016 period, winter six-row barley and two-row barley genotypes were tested under different N rates (NR0, NR1 and NR2). The obtained results were used to determine yield extract of each genotype (using Bishop's formula), perform the variance analysis, and assess the correlations of the studied parameters (grain weight, protein content, and yield extract). ANOVA showed a genotype and N fertilizer rate significantly influenced on grain weight, protein content, and yield extract for both six and two-row genotypes and for all nine tested conditions. For all winter six-row barley quality parameters under two years an insignificant influence of genotype x N rate interaction on these was showed while winter two-row barley revealed a different behavior. This source of variation was significant only one experimental year for protein content, two years for yield extract and one year for grain weight. The yield extract, grain weight, and protein content were assessed separately among winter six-row and two-row barley. Under all N rate (except NR2 for winter six-row barley and NR0 for winter two-row barley) there was a significant negative correlation between yield extract and protein content (from -0.54 to -0.89 and -0.61 to -0.89 respectively). The same comparison showed that the grain weight was not correlated with protein content for winter two-row barley under any of the experimental year conditions; while this was positive in two years for winter six-row barley (from 0.42 to 0.77). During the 2013-2016 period, the yearly average yield extract has varied due to change of the protein content and grain weight, namely was noticed an increasing tendency from NR0 to NR1 and a decrease one from NR1 to NR2.

Key words: six and two - row winter barley genotype, nitrogen rate, grain weight, protein content, yield extract.

INTRODUCTION

Micro-malting techniques had begun with small barley samples at a small scale in the past, about 125 years ago (Meredith et al., 1962). The raw industry has evaluated the malting barley quality by "micro malting" (small scale grain malt producing) and then the malt is analyzed for desired traits (Haslemore et al., 1985). The pioneer of barley yield extract estimation was L. R. Bishop, which began to study barley protein content in different barley varieties in 1930 and continued in other papers in 1933 and 1934 years. In this work, he stated that protein content is negatively correlated with yield extract due to the fractions of protein named hordeins.

The extract can be defined as the percentage of dry matter, which is solubilized from the grist during the mashing process (Kunze, 1999). Yield/malt extract is very important as quality parameter for maltsters and brewers when selecting malting barley (Dráb et al., 2014), because the amount of extract obtained from a variety is economically appreciated and also determines the amount of produced beer (Schwarz et al., 2007). So, the breeders are focused on bred new barley varieties with a high yield and high yield/malt extract for maltsters and brewers (Li et al., 2008). The quality of the extract is influenced by the environment (fertilizer, temperature, and rainfall) affecting the varieties traits which influence the protein and starch content (Fox et

al., 2003). Other traits are the type of barley (six/two-row), the fines of husk, and also the grain weight. In the latest, there is a huge interest to do varieties selection on the molecular assisted markers (MAS) so, two QTLs had been found that accounting for 35.7-53.6% of the yield extract variance (Zhou et al., 2012) and a genetic base of breeding barley with malting quality was provided by Fang et al. (2019), which identified controlling malting quality QTLs or genes located on 1, 2, 4, 5 and 7 barley chromosomes.

Nowadays, in the Czech Republic, barley malting quality is assessed on the basis of malting quality index (MQI) according to the index from the 2002 year (Psota and Kosař, 2002; Psota et al., 2019). They evaluated the quality of some spring barley varieties (Cosmopolitan, Ismena, Klarinette, Laureate, LG Aurus, Runner) and also some six-row winter barley varieties (Azrah, Impala, Journey, Laurin, SU Jule and the hybrid variety SU Hylona).

A typical yield is quantified under laboratory conditions after barley malting. Other mash methods or correlations between different qualitative parameters have been taken into account in order to predict the malt fermentability or extract yield because its variability is a concern for both brewers and barley breeders (Kunze, 1999). The most variation in yield extract (74.3%) has been explained on the three quality barley parameters by Li et al. in 2008 which used a predicted extract equation based on protein content (Pr), 1000 kernel weight (1000 KW) and diastatic power (DP).

The form of the equation is:

$$\text{Extract} = 89.3 - 1.64 \text{ Pr} + 0.16 \text{ KW} + 0.019 \text{ DP}$$

and here we can notice that we need diastatic power value of barley variety which only can be obtained after the grain sample micro malting.

According to the European Brewery Convention (EBC) for a Lager malt, the minimum value for yield extract has to be >80.5% (O'Rourke, 2002).

For a barley breeding programme the micro malting method is expensive and meanwhile, the behavior of barley varieties and lines (F₇ and F₈ generations) have to be made fast, before the next cropping year.

This study aims at evaluating the potential yield extract of some varieties and breeding lines of Romanian winter barley, based on a calculation formula, in order to determine the yield extract without malting under specific growing conditions.

MATERIALS AND METHODS

Seventeen winter barley genotypes (11 six-row and 6 two-row winter barley varieties and breeding lines in 2013-2014) and fifty winter barley genotypes (25 six-row and 25 two-row winter barley varieties and lines in 2015 and 2016), developed at NARDI Fundulea, were tested during 2013-2014, 2014-2015, 2015-2016 years under three experimental conditions (without applied nitrogen rate - NR0, 100 kg/ha - NR1 and 200 kg/ha - NR2, the nitrogen doses were applied every year in March).

After the harvest and seeds conditioning, the protein content (P%) was determined by Infratech 1241 (NIR method), the grain weight (GW) with Contador seed, and all three replications (1000 seeds each) were weighted for each barley samples (500 g per sample).

On the basis of the modified Bishop's mathematical formula (Gregor et al., 2011), the yield extract of each sample was calculated accordingly to the following formula:

$$E = K - (0.85 * B) + (0.15 * G), \text{ where:}$$

E - extractivity of barley grain;

K - a constant value equal with 83;

B - protein content (P%);

G - grain weight (GW).

The experimental data have been assessed through statistical analysis (ANOVA) and the obtained results were the subject of correlation analysis and expressed as a minimum, mean and maximum values. Shares of genotype (G), nitrogen rate (NR0, NR1, NR2), and genotype x nitrogen rate interaction (G x NR) in the phenotypic expression of grain weight (GW), protein content (P) and yield extract (EXT) were performed in Microsoft Excel Software.

The yield extract, grain weight and protein content were assessed and analyzed separately among six-row and two-row winter barley, due to the phenotypic differences among them and also the nitrogen utilization efficiency.

RESULTS AND DISCUSSIONS

ANOVA showed that genotype (G) and N fertilizer rate (NR) significantly influenced the grain weight (GW), protein content (P), and yield extract (EXT) for both six and two-row genotypes and for all nine tested conditions. For all six-row winter barley quality parameters under two years (2015 and 2016) an insignificant influence of genotype x nitrogen rate interaction (G x NR) on these was showed while two-row winter barley revealed a different behavior. This source of variation was significant for only one experimental year for protein content (2014), two years for yield extract (2014 and 2016) and one year (2016) for grain weight (Table 1). The differences between six and two-row winter barley were due to the different years' climatic conditions and probably nitrogen utilization efficiency.

Share of factors (%) in achieving yield extract in six and two-row winter barley (Fundulea, 2014-2016), indicated that these components are strongly influenced by nitrogen rate (Figures 1, 3 and 5) in the case of six-row winter barley (72% in 2014, 97% in 2015, and 89% in 2016) and a little bit less in the case of two-row winter barley (Figures 2, 4 and 6), 66% in 2014, 48% in 2015, and 68% in 2016. Also, the genotype as a factor (%) describes a different influence on both, this was not so close comparing with nitrogen dose and their interaction, which means that in variable climatic conditions and management practices the yield extract presents a high degree of vulnerability (16% in 2014, 2% in 2015 and 41% in 2016 for six-row barley and 20% in 2014, 10% in 2015, and 27% in 2016 for two-row barley).

Table 1. P-value for six and two-row winter barley genotypes, 2014-2016 period (grain weight, protein content and yield extract)

2014 year		Grain weight		Protein content		Yield extract	
		P-value		P-value		P-value	
Source of variation	df*	six-row	two-row	six-row	two-row	six-row	two-row
Nitrogen rate (NR)	2	0.011	0.001	0.000	0.000	0.000	0.000
Genotype (G)	10 (5)	0.000	0.000	0.000	0.000	0.000	0.001
G x NR	20 (10)	0.000	0.413	0.000	0.000	0.000	0.007
2015 year		Grain weight		Protein content		Yield extract	
		P-value		P-value		P-value	
Source of variation	df	six-row	two-row	six-row	two-row	six-row	two-row
Nitrogen rate (NR)	2	0.000	0.007	0.000	0.000	0.000	0.000
Genotype (G)	24	0.000	0.000	0.000	0.000	0.000	0.000
G x NR	48	0.163	0.980	0.947	0.258	0.624	0.757
2016 year		Grain weight		Protein content		Yield extract	
		P-value		P-value		P-value	
Source of variation	df	six-row	two-row	six-row	two-row	six-row	two-row
Nitrogen rate (NR)	2	0.000	0.000	0.003	0.000	0.001	0.000
Genotype (G)	24	0.000	0.000	0.000	0.000	0.000	0.000
G x NR	48	0.595	0.000	0.317	0.986	0.954	0.000

* 11 six-row genotypes and 6 two-row genotypes

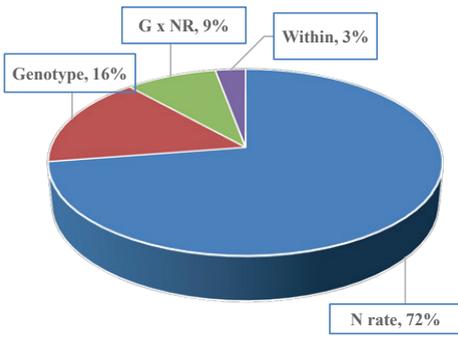


Figure 1. The influence of genotype, nitrogen rate and their interaction on yield extract, 2014 (six-row barley)

had the same percentage contribution in 2015 (0%), higher with 2% in 2014 (11%), and lower with just 1% in 2016 (3%). The share of factors (%) showed that always the extract yield will be different among the six and two-row barley under different climatic and technological sequences (nitrogen rate).

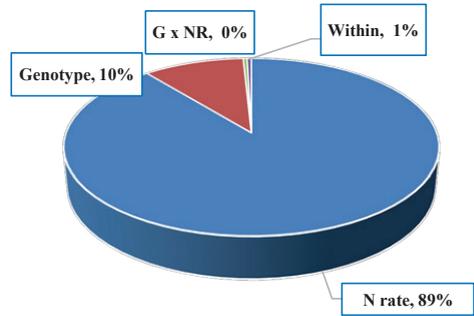


Figure 4. The influence of genotype, nitrogen rate and their interaction on yield extract, 2015 (two-row barley)

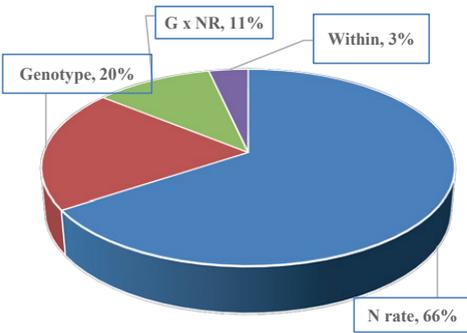


Figure 2. The influence of genotype, nitrogen rate and their interaction on yield extract, 2014 (two-row barley)

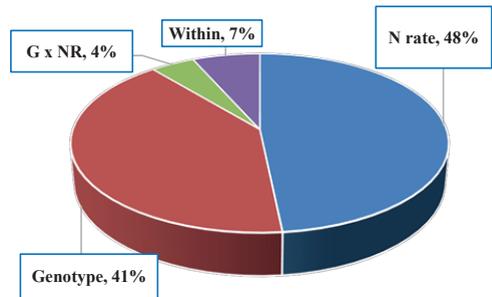


Figure 5. The influence of genotype, nitrogen rate and their interaction on protein content, 2016 (six-row barley)

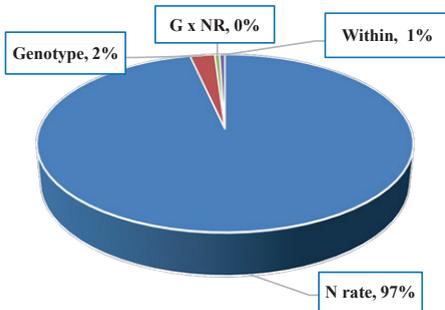


Figure 3. The influence of genotype, nitrogen rate and their interaction on protein content, 2015 (six-row barley)

Regarding the interaction of genotype and nitrogen rate (G x NR) was noticed a different yearly and row barley type contribution in achieving yield extract. This ranged from 0% (2015) to 4% (2016) and 9% (2015) for six-row barley. Comparing the two-row with six-row barley type in all tested years, the interaction

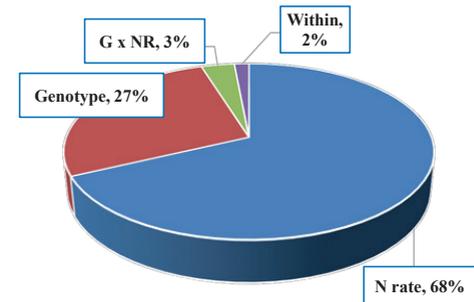


Figure 6. The influence of genotype, nitrogen rate and their interaction on yield extract, 2016 (two-row barley)

Depending on genotypes and growing conditions (nitrogen rate) in the 2014 year (Table 2), six-row barley grain weight (GW) ranged from 33.0 g (NR0) to 42.4 g (NR2), protein content from 11.0% (NR0) to 14.1% (NR2) and yield extract decreased from 77.3% (without nitrogen) to 79.2% (NR1).

The grain weight values of two-row barley ranged from 38.3 g (NR0) to 48.5 g (NR1 and NR2), whilst the protein content varied between 11.6% (NR1) to 14.7% (NR2). Regarding two-row barley yield extract, the minimum values were higher than six-row barley genotypes value (for all three cultivation conditions) and the higher value was equal just under the NR2.

Table 2. The minimum, mean and maximum value of grain weight, protein content and yield extract, 2013-2014 cropping year

Grain weight (g)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	33.0	38.3	34.3	42.0	33.2	40.0
Mean	37.3	43.1	38.8	45.5	37.9	45.1
Maxim	41.2	46.0	41.5	48.5	42.4	48.5
Protein content (%)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	11.0	11.9	11.3	11.6	12.0	13.2
Mean	12.4	13.0	12.1	12.7	13.1	14.0
Maxim	13.6	13.8	13.0	14.2	14.1	14.7
Yield extract (%)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	77.3	78.0	77.9	78.1	76.2	76.8
Mean	78.0	78.4	78.5	79.0	77.5	77.9
Maxim	79.0	79.3	79.2	79.8	78.8	78.8

In the second year of testing (2015), two-row barley genotypes had higher GW (minimum value from 48.3 to 48.9 g and maximum from 63.6 to 64.5 g) for all experimental conditions comparing six-row barley (Table 3) and higher protein content (minimum 11.6% under NR0 and maximum 16.6% under NR2). The yield extract ranged from 76.7 to 80.1% under NR2, and from 78.2 to 81.8% under NR0. The type six-row achieved lower values of GW and protein content under all nitrogen rates but a maximum extract value was registered for six-row barley without nitrogen addition at

cultivation with a similar behavior under NR1 and NR2 (from 82.2% under NR0 to 81.9% under NR1 and 80.6% under NR2).

Table 3. The minimum, mean and maximum value of grain weight, protein content and yield extract, 2014-2015 cropping year

Grain weight (g)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	38.2	48.3	40.0	48.5	40.3	48.9
Mean	49.2	56.4	49.6	55.5	50.9	56.5
Maxim	53.5	63.8	54.7	64.5	56.1	63.6
Protein content (%)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	9.4	11.6	10.3	11.9	11.7	13.5
Mean	10.7	12.9	11.2	13.3	13.1	14.8
Maxim	11.5	14.2	12.3	14.6	14.6	16.6
Yield extract (%)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	80.5	78.2	79.9	77.9	78.6	76.7
Mean	81.3	80.5	80.9	80.0	79.5	78.9
Maxim	82.2	81.8	81.9	81.5	80.6	80.1

Table 4. The minimum, mean and maximum value of grain weight, protein content and yield extract, 2015-2016 cropping year

Grain weight (g)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	36.3	30.4	39.3	34.6	36.1	33.2
Mean	41.2	37.0	44.6	41.9	44.3	40.2
Maxim	46.6	47.6	50.5	49.1	51.8	46.8
Protein content (%)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	11.7	12.8	12.2	13.3	12.9	14.1
Mean	14.3	14.5	14.5	15.0	15.1	15.7
Maxim	15.9	16.5	16.1	17.1	16.2	17.9
Yield extract (%)						
N dose	NR0		NR1		NR2	
No. of row	six row	two row	six row	two row	six row	two row
Minim	76.0	74.1	76.3	74.2	75.9	73.2
Mean	77.0	76.1	77.3	76.5	76.9	75.7
Maxim	78.6	78.1	79.0	78.2	78.0	76.9

As is show in Table 4, the third year was characterized by an increase of minimum GW for six-row barley comparing with the two-row barley (36.1-39.3 g for six-row and 30.4-34.6 g

for two-row) and also of maximum value under NR1 and NR2 (50.5-51.8 g for six-row and 46.8 g at NR2 and 49.1 g at NR1 for two-row). Grain protein content was higher under all growing conditions for both type of row with a maximum of 16.2% for six-row (NR2) and 17.9% for two-row (NR2). The minimum and maximum values of yield extract were lowest than 2014 and 2015 due to considerable increased protein content. Also, Molina-Cano et al. (2000) performed a large experiment (346

trials, with many barley varieties harvested across all the EBC countries during 1980-1990 and 1993-1995 periods) and had concluded that the negative correlation between these two variables (extract yield and barley protein content) has been more or less significant, the trends being affected by regional peculiarities (2000 year). A common point of these trends was registered, namely that yield extract decreased when barley protein content increased with some variations.

Table 5a. Correlations between the six-row winter barley analyzed parameters, 2013-2014

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1								
NR1 Ext	0.80	1							
NR2 Ext	0.14	-0.02	1						
NR0 GW	0.17	-0.04	-0.14	1					
NR1 GW	0.19	0.35	-0.35	0.75	1				
NR2 GW	0.04	-0.08	0.41	0.62	0.49	1			
NR0 Prot	-0.79	-0.74	-0.21	0.47	0.29	0.35	1		
NR1 Prot	-0.56	-0.60	-0.28	0.67	0.54	0.48	0.92	1	
NR2 Prot	-0.12	-0.02	-0.80	0.56	0.70	0.22	0.46	0.62	1

Table 5b. Correlations between the two-row winter barley analyzed parameters, 2013-2014

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.94	1.00							
NR2 Ext	0.89	0.86	1.00						
NR0 GW	0.70	0.67	0.58	1.00					
NR1 GW	0.72	0.73	0.60	0.95	1.00				
NR2 GW	0.68	0.67	0.62	0.97	0.94	1.00			
NR0 Prot	-0.61	-0.56	-0.61	0.13	0.04	0.13	1.00		
NR1 Prot	-0.61	-0.68	-0.63	0.03	0.00	0.02	0.87	1.00	
NR2 Prot	-0.43	-0.40	-0.63	0.24	0.17	0.22	0.89	0.79	1

Table 6a. Correlations between the six-row winter barley analyzed parameters, 2014-2015

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.83	1.00							
NR2 Ext	0.70	0.69	1.00						
NR0 GW	-0.23	-0.31	-0.17	1.00					
NR1 GW	-0.40	-0.31	-0.21	0.91	1.00				
NR2 GW	-0.50	-0.52	-0.19	0.87	0.88	1.00			
NR0 Prot	-0.85	-0.77	-0.60	0.71	0.78	0.83	1.00		
NR1 Prot	-0.72	-0.89	-0.66	0.66	0.67	0.77	0.88	1.00	
NR2 Prot	-0.75	-0.75	-0.71	0.69	0.76	0.77	0.92	0.90	1

Table 6b. Correlations between the two-row winter barley analyzed parameters, 2014-2015

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.94	1.00							
NR2 Ext	0.89	0.86	1.00						
NR0 GW	0.70	0.67	0.58	1.00					
NR1 GW	0.72	0.73	0.60	0.95	1.00				
NR2 GW	0.68	0.67	0.62	0.97	0.94	1.00			
NR0 Prot	-0.61	-0.56	-0.61	0.13	0.04	0.13	1.00		
NR1 Prot	-0.61	-0.68	-0.63	0.03	0.00	0.02	0.87	1.00	
NR2 Prot	-0.43	-0.40	-0.63	0.24	0.17	0.22	0.89	0.79	1

Table 7a. Correlations between the six-row winter barley analyzed parameters, 2015-2016

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1.00								
NR1 Ext	0.83	1.00							
NR2 Ext	0.70	0.69	1.00						
NR0 GW	-0.23	-0.31	-0.17	1.00					
NR1 GW	-0.40	-0.31	-0.21	0.91	1.00				
NR2 GW	-0.50	-0.52	-0.19	0.87	0.88	1.00			
NR0 Prot	-0.85	-0.77	-0.60	0.71	0.78	0.83	1.00		
NR1 Prot	-0.72	-0.89	-0.66	0.66	0.67	0.77	0.88	1.00	
NR2 Prot	-0.75	-0.75	-0.71	0.69	0.76	0.77	0.92	0.90	1

Table 7b. Correlations between the two-row winter barley analyzed parameters, 2015-2016

Parameters	NR0 Ext	NR1 Ext	NR2 Ext	NR0 GW	NR1 GW	NR2 GW	NR0 Prot	NR1 Prot	NR2 Prot
NR0 Ext	1								
NR1 Ext	0.78	1.00							
NR2 Ext	0.55	0.82	1.00						
NR0 GW	0.60	0.35	0.12	1.00					
NR1 GW	0.13	0.44	0.33	0.58	1.00				
NR2 GW	-0.01	0.29	0.47	0.44	0.82	1.00			
NR0 Prot	-0.63	-0.60	-0.54	0.24	0.40	0.44	1.00		
NR1 Prot	-0.72	-0.72	-0.61	0.07	0.30	0.33	0.95	1.00	
NR2 Prot	-0.59	-0.62	-0.65	0.25	0.35	0.37	0.95	0.93	1

The obtained results of correlation coefficients are presented separately for six and two-row barley in Tables 5a, 5b, 6a, 6b, 7a, and 7b. In all three years, for six-row barley, yield extract (Tables 5a, 6a, 7a, red cells) and grain weight were correlated neither. Meanwhile, the grain weight is strongly negatively correlated with protein content (green cells). In the case of two-row barley, the situation is changed, in all the tested years extract is positively correlated with grain weight (Table 5b, 6b and 7b, green cells)

and strongly negatively correlated with protein content at all NR (green cells). In 2014, there was no correlation between grain weight and protein content at all NR for six and two-row and in 2015 and 2016 the correlations among grain weight and protein content were positively for six-row (Tables 6a and 7a) and did not correlate for two-row barley (Tables 6b and 7b). According to Mohammadi et al. (2015), protein content is strongly related to yield extract and is negatively correlated, high

protein content can lead to reduced yield extract. During the malting process is produced the hydrolysis of starch, malt protein provides a-amylase and b-amylase enzymes for starch degradation (Elía et al., 2010).

CONCLUSIONS

The potential yield extract generally shows a decreasing trend from one experimental condition to another, their variation being closely related to grain weight (GW) and protein content (P).

Protein content (P) tends to be stable for some genotypes, especially for six-row winter barley genotypes, no matter the growing conditions.

Increasing the dose of nitrogen (NR) used during cultivation leads to higher protein content (P) in two-row winter barley in comparison with the six-row winter barley, which significantly affects the extract yield (EXT).

The "Bishop's law" on yield extract decrease with barley protein increase became a general statement also in the case of Romanian six and two-row winter barley varieties and lines.

A pattern of yield extract variation with changes in barley protein content has been observed, the extract decreases progressively with increases in barley protein content more for two-row and less for six-row barley.

When the extract yield (EXT) is estimated on the grain weight and protein content basis it would be necessary to take into account the temperatures and rainfall from the growing geographical region and the negative protein content deviation from regression, not only the genotype and technology.

REFERENCES

Bishop, L.R. (1930). The nitrogen content and "quality" of barley. *Journal of the Institute of Brewing*, 36(4), 352–369.

Bishop, L.R., Day, F. E. (1933). The effect of variety on the relation between nitrogen content and extract. *Journal of the Institute of Brewing*, 39(5), 505–559.

Dráb, Š., Francáková, H., Psota, V., Solgajová, M., Ivanišová, E., Tóth, Ž., Mocko, K., Balková, H. (2014). The malt extract, relative extract and diastatic power as a varietal characteristic of malting barley. *J.*

Microbiol. Biotech. Food Sci., 3 (Special Issue), 206–209.

Elía, M., Swanston, J.S., Moralejo, M., Casas, A., Pérez-Vendrell, A.M., Ciudad, F.J. (2010). A model of the genetic differences in malting quality between European and North American barley cultivars based on a QTL study of the cross Triumph _ Morex. *Plant Breed.*, 129, 280–290.

Fang, Y., Zhang, X., Xue, D. (2019) Genetic Analysis and Molecular Breeding Applications of Malting Quality QTLs in Barley. *Front. Genet.*, 10, 352.

Fox, G.P., Panozzo, J.F., Li, C.D., Lance, R.C.M., Inkerman, P.A., Henry, R.J. (2003). Molecular basis of barley quality. *Australian Journal of Agricultural Research*, 4(12), 1081–1101.

Gregor, T., Cerkal, R., Hřivna, L., Sottníková, V. (2011). Determination of extract in barley grain by the enzymatic way. *Kvasný Průmysl*, 57(7-8), 236–241.

Haslemore, R.M., Slack, C.R., Brodrick, K.N. (1985). Assessment of malting quality of lines from a barley breeding programme. *New Zealand Journal of Agricultural Research*, 25(4), 497–502.

Kunze, W., (1999). *Technology Brewing and Malting*, 3rd ed., Berlin: Ed. VLB Berlin.

Li, Y., Schwarz, P.B., Barr, J.M., Horsley, R.D. (2008). Factors predicting malt extract within a single barley cultivar. *Journal of Cereal Science*, 48(2), 531–538.

Meredith, W.O.S., Anderson, J.A., Hudson, L.E. (1962). Evaluation of malting barley. In: *Barley and Malt: Biology, Biochemistry, Technology* (edited by A.H. Cook), p. 207-269. London: Academic Press, Inc.

Mohammadi, M., Blake, T.K., Budde, A.D., Chao, S., Hayes, P.M., Horsley, R.D. (2015). A genome-wide association study of malting quality across eight US barley breeding programs. *Theor. Appl. Genet.*, 128, 705–721.

Molina-Cano, J.L., Rubio, A., Igartua, E., Gracia, P., Montoya, J.L. (2000). Mechanisms of Malt Extract Development in Barleys from Different European Regions: I. Effect of Environment and Grain Protein Content on Malt Extract Yield. *Journal of The Institute of Brewing*, 106(2).

O'Rourke, T. (2002). Malt specifications and brewing performance. *The Brewer International*, 2, 27–30.

Psota, V., Dvořáčková, O., Nečas, M., Musilová, M. (2019). Barley varieties registered in the Czech Republic after harvest 2018. *Kvasný Průmysl*, 65, 97–105.

Psota, V., Kosař, K. (2002). Malting Quality Index. *Kvasný Průmysl*, 47, 142–148.

Schwarz, P.B., Li, Y., Barr, J., Horsley, R.D. (2007). Effect of operational parameters on the determination of laboratory extract and associated wort quality factors. *J. Am. Soc. Brew. Chem.*, 65, 219–228.

Zhou, T., Takashi, I., Ryouichi, K., Naohiko, H., Makoto, K., Takehiro, H. (2012). Malting quality quantitative trait loci on a high-density map of Mikamo golden Harrington cross in barley (*Hordeum vulgare* L.). *Mol. Breed.*, 30, 103–112.

THE EFFECT OF NITROGEN RATE ON RICE TILLERING AND GRAIN YIELD UNDER WATER SAVING IRRIGATION

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Abstract

In order to study the best nitrogen regime under water saving irrigation condition, an experiment was conducted based on completely randomized block design with 3 replications on rice at Caofeidian, north of China in 2018. There were six treatments, that was five levels of nitrogen (CK0 = 0, T1 = 217.8, T2 = 254.1, T3 = 290.4 and CK1=363 kg ha⁻¹ i.e. farmers' traditional practice) under water saving condition of optimize alternate wetting and drying irrigation (OAWD) water 10,800 m³ ha⁻¹, compared with farmers' practice nitrogen level of 363 kg ha⁻¹ under farmers' traditional irrigation practice water 13,800 m³ ha⁻¹ (CK2) as check. The results clearly indicated that excess nitrogen and water influenced negatively the productive tillers. Whereas proper nitrogen rate under OAWD irrigation enhanced the grains number of per panicle, filled grains percentage, grain yield and benefit. Compared to CK2, the treatment T2 was of good economic and ecological benefits with a significant higher of population panicles by 5.37%, of productive tillers percentage by 18.81%, of filled grains per panicle percentage by 6.88%, of grain yield by 8.72% and of benefit by 4146.15 yuan ha⁻¹, in addition, it was of an decrease of nitrogen dose by 30%, of fertilizer cost by 261.36 yuan ha⁻¹ and of irrigation water by 21.7%.

Key words: rice, nitrogen regime, optimize alternate wetting and drying irrigation, tillering, grain yield.

INTRODUCTION

Rice is the most water consumption crop under the condition of farmers' traditional growing experience, 80% of irrigation water was lost by evaporation and leaching. Whereas, it is leading to a lot of waste on both fertilizer and irrigation water resources due to over-using on fertilizers and water resources with the decreasing nitrogen use efficiency. Especially in north China rice growth area, the contradiction between excess nitrogen fertilizer, excess irrigation water and shortage of water for rice production will be problem limiting rice development (Bolun, 2008; Ningning, 2011). Earlier reports observed limiting some water increased tillers and grain yield (Dordas, 2008; Sulin, 2018; Baocheng, 2011), while interaction of limiting water and nitrogen levels will be an effectively help for the growth of rice. The present study mainly compared the tillering, grain yield and benefit at variables doses of nitrogen under water saving irrigation with a view to better understanding for the best

management of rice fertilization and irrigation in China north rice growth area.

MATERIALS AND METHODS

Study area and experiment design

A field experiment was conducted in the China season of 2018 at Caofeidian, Tangshan city, north of China. There was six treatments, that was five levels of nitrogen (CK0 = 0, T1 = 217.8 kg ha⁻¹, T2 = 254.1 kg ha⁻¹, T3 = 290.4 kg ha⁻¹ and CK1 = 363 kg ha⁻¹ i.e. farmers traditional practice) under water saving condition of optimize alternate wetting and drying irrigation (OAWD), compared with farmers' practice nitrogen level of 363 kg ha⁻¹ under farmers' traditional irrigation practice as check. The treatment was arranged in random design and replicated three times. Unit plot was 48 m². Seedlings were planted on June 10, 2019 and harvested on October 30 in raw 29.6 cm and the distance 18.5 cm with 3 to 5 plants. A blanket rate fertilizer at 60 kg P₂O₅, 75 kg K₂O ha⁻¹ was applied uniformly in all the plots

and thoroughly incorporated into the soil at the time of final land preparation. Nitrogen was applied as per treatments in the form of urea in 5 times (first the time final land preparation, the next was continues 3 times top dressing from the first time that is 10 days after transplant with 10 days intervals up to the maximum tillering stage, the last top dressing was done at panicle initiation stage), Phosphorous was applied in the form of superphosphate and potassium was applied in the form of potassium sulfate.

Refer to the irrigation, the farmers' traditional practice irrigation maintained a layer of water of 7 to 10 cm after transplanting, irrigated 18 times during the growth period with irrigation water $13,800 \text{ m}^3 \text{ ha}^{-1}$, whereas OAWD irrigation regime operated be consistent with the need of different growth stages of rice tried to encourage or controlling tillering and growth. For example, the depth of the water layer kept 2 to 3 cm at transplant stage, followed 3 to 5 cm after planting, then 2 to 3 cm water layer at the beginning of tillering, next limited water and aired field in the late tillering stage to control the vegetative growth, after that at the panicle differentiation initiation stage 2 ~ 3cm was kept, followed aired field after the panicle differentiation, then kept water layer 2 to 3cm at milk initiation stage, and started airing field again at the medium dough stage to maturity. Total irrigation was $10,800 \text{ m}^3 \text{ ha}^{-1}$. The precipitation through the growth period of rice was 440.9 mm, and the annual rainfall was 542.9 mm, of which the rainfall was 421.3 mm in July, August, and September, accounting for 77.6% of the annual rainfall, and 95.55% of the growth period (Figure 1). In addition, intercultural protection measures were taken as and when necessary.

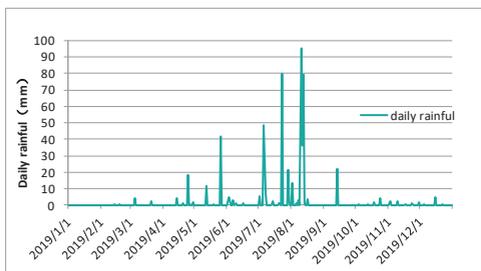


Figure 1. Daily rainfall of the year 2019 at study area

Sampling methods

For the determination of growth and other characters, plants were observed in the plots at regular intervals. At each sampling, 10 hills per plot were marked in the third row avoiding the border effect. The number of tillers in each 10 hills was counted and means of each hill were recorder, grain yield of rice was determined by harvesting a sample area of 30 m^2 in the middle of each plot.

RESULTS AND DISCUSSIONS

Tillering deveopment

Less or excess nitrogen fertilizer rate exerted significant influence on the tillers production, retention and the tillering peak time of reaching at the maximum tillers number through growth stages (Figure 2). All treatments started speed tillering stage on June 19. Tillers peak of CK0 emerged on July 1 with the largest tillers number $1628,900 \text{ ha}^{-1}$, whereas peaks of treatments T1 and T2 emerged on July 8 with tillers number of $2200,230 \text{ ha}^{-1}$ and $2310,700 \text{ ha}^{-1}$, respectively, then peaks of treatments T3, CK1 and CK2 emerged on July 15 with tillers number $2483,600 \text{ ha}^{-1}$, $2615,000 \text{ ha}^{-1}$ and $2556,600 \text{ ha}^{-1}$, respectively. This was rather expected because all the treatments applied nitrogen produced larger number of tillers than CK0 without nitrogen, meanwhile, under the uniform OAWD irrigation condition, the number of tillers per unit area increased gradually with the increase of nitrogen dose at the earlier tillering stage before July 15. Tiller mortality began past the maximum tillering stage and the number of tillers per unit area declined gradually reaching the lowest at maturity stage.

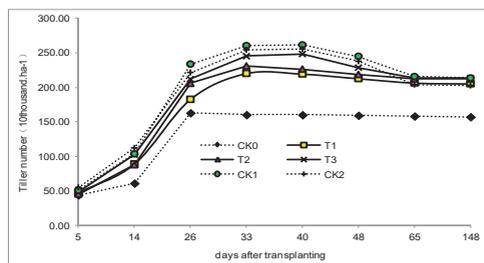


Figure 2. Tillering of different treatments

The percentage of unproductive tillers

It was discernible in Figure 3 that treatment CK0 was of the lowest percentage of unproductive tillers 2.32% at maturity, it was most likely caused by the minimum tillers number. Apart from CK0, treatment T2 was of a lower percentage of unproductive tillers of 6.80% and a higher productive tillers percentage of 93.20%, followed treatment T3, next CK1, then the percentage of unproductive tillers of the check treatment CK2 was highest. It implied both nitrogen dose and irrigation water exerted influence on the percentage of productive tillers and unproductive tillers, the productive tillers number i.e. panicles number per unit gradually increased with the nitrogen fertilizer rate increasing under the OAWD irrigation, excess nitrogen fertilizer dose negatively influenced the number of productive tiller. At the same time, excess irrigation water negative affected the number of panicle per unit population due to inhibition of tillering. The treatment T2 was of an increase productive percentage by 18.81% compared as the check CK2. It also shows proper nitrogen dose together with OAWD promoted productive tillering whereas decreased unproductive tillering.

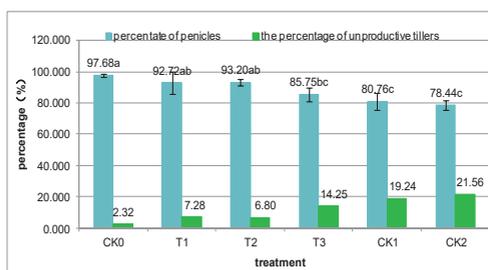


Figure 3. The percentage of unproductive tillers

Grain yield

Grain yield in rice as observed at maturity stages are shown in Table 1. Grain yield varied between 8404.20 kg ha⁻¹ and 11439.10 kg ha⁻¹. Treatment T2 produced the maximum grain yield, while CK0 produced the least. A closer look at the results reveals that the rice grain yield tended to increase linearly with increasing rate of applied nitrogen under OAWD irrigation at maturity stage, T2 treatments with application of 254.1 kg N produced statistically higher grain yield than other treatments. As for

treatment T3 and CK1 the further increase in N rates caused lower grain yield. Akita (1989) reported that excess nitrogen gave higher dry weight at heading causing a yield decline due to reduced ripening percentage. Compared to CK2, treatment T2 produced a significant higher grain yield by 8.72%. In additional, under the excess nitrogen fertilizer, the grain yield of OAWD irrigation CK1 was of a little higher than that of farmers' traditional practice irrigation of CK2 by 1.11%, which indicates that the optimized dry and wet alternating management way was of the effect both on saving water and stabilizing production output.

Table 1. Effects of different treatments on grain yield and yield components of rice

Treatments	Panicles number per ha (10 thousand ha ⁻¹)	Grains number per panicle	Filled grain percentage (%)	Grains weight per thousand (g)	Yield Theoretical Calculate d (kg ha ⁻¹)	Grain yield harvested (kg ha ⁻¹)
CK0	156.57d±4.21	195.25c±1.68	79.47c±1.27	33.09a±0.73	8037.10c±246.44	8404.20c±264.76
T1	203.85bc±2.85	239.81b±8.25	81.35b±0.98	31.51bc±1.21	12516.64b±778.93	10988.55a±317.23
T2	211.35a±3.12	252.59a±2.69	85.48a±1.24	32.10ab±0.36	14456.10a±345.10	11439.10a±246.86
T3	209.80ab±1.60	249.13a±7.64	82.91b±0.61	31.63bc±0.49	13701.46a±456.79	11038.85a±305.51
CK1	210.92ab±5.91	248.22ab±4.68	80.36c±0.14	30.47c±0.54	12814.11b±369.18	10638.65b±251.94
CK2	200.58c±5.09	247.71ab±2.11	79.97c±1.47	31.29bc±0.94	12430.55b±435.66	10521.88b±431.28

Grain yield factors

Proper nitrogen dose and irrigation allocation significantly increased the panicles number of per ha., grains number per panicle, and filled grain percentage at maturity stage was observed in Table 1.

Treatment T2 produced the highest 2113,600 panicles per ha. with a significant higher of 5.37% compared to the farmers traditional practice CK2. Followed by CK1, T3, T1, CK2 and CK0 gave the least. Variation of the panicles number of per ha. perhaps due to the increasing of nitrogen under the uniform OAWD irrigation. On the contrary, the excess water is of negative influence on the productive tillers number i.e. panicles number.

The sort order of grains number per panicle was observed as T2> T3> CK1> CK2> T1> CK0. Among them, the treatment CK0 produced the least panicle grain number, significantly lower than that of other treatment, whereas treatment T2 produced the biggest grain number with average 252.59 per panicle,

it was of a little higher by 1.97% compared with CK2, and the difference was not significant.

The filled grain percentage was ranked as T2> T3> T1> CK2> CK1> CK0, evidently proper nitrogen dose and OAWD irrigation interaction enhanced the filled grain percentage to a certain extent. Both excess or inadequate nitrogen fertilizer and water exerted a negative influence on the grain filling. The treatment T2 produced a significant higher filled grains percentage by 6.88% compared with CK2.

The sort order of grains weight per thousand was CK0> T2> T3> T1> CK2> CK1, and the difference between T2 and CK1 was significant, indicated that a reasonable combination of nitrogen and water increased the grain weight per thousand. Excess or insufficient nitrogen fertilizers affected the grains weight. Whereas irrigation helped produce grains weight per thousand.

Compared with farmers' traditional practice water and nitrogen management CK2, T2 significantly increased the number of population panicles by 5.37%, increased the grains number per panicle by 1.97%, significantly increased the filled grain percentage by 6.88%, and had no significant effect on the grain weight per thousand.

Benefit

Table 2 indicated the cost and benefits performance of rice from different treatments.

Table 2. Effects on benefit of rice of different treatments (Yuan·ha⁻¹)

Treatments	Gross income, Yuan · ha ⁻¹	Cost (Yuan · ha ⁻¹)			Benefit (Yuan · ha ⁻¹)
		fertilizer	irrigation	others	
CK0	21850.92	762.00	3000.00	20437.50	-2348.58
T1	28570.23	1458.96	3000.00	20437.50	3673.77
T2	29741.66	1371.84	3000.00	20437.50	4932.32
T3	28701.01	1284.72	3000.00	20437.50	3978.79
CK1	27660.49	1633.20	3000.00	20437.50	2589.79
CK2	27356.88	1633.20	4500.00	20437.50	786.17

Note: the price per kg of rice: 2.6 yuan, nitrogen fertilizer: 2.4 yuan, P₂O₅: 5.0 yuan and K₂O: 6.0 yuan. Others cost 20437.5 yuan per ha include 375.0 yuan for tilling plots, 525.0 yuan for harrowing plots, 375.0 yuan for making plots flat, 2062.5 yuan for seedlings, 450.0 yuan for pesticides, 150.0 yuan for making furrows, 12,000.0 yuan for field be rented, and 4500.0 yuan for labor.

Apart from CK0, benefit varied between 786.17 yuan per ha. to 4932.32 yuan per ha. The treatment T2 was of a higher benefit 4141.15 yuan per ha. compared to the farmers' practice CK2. In addition, a review from the experiment design showed the treatment T2 was of a decreased of nitrogen fertilizer by 30% with fertilizer cost of 261.36 yuan per ha., and saved irrigation water by 21.7% compared to CK2.

Water and nutrients are two important factors affecting rice tillering. If tillers were produced earlier and generated at a low position on stem, and it would be helpful to grain yield (Hanliang, Z.H., 2000; Qinghe, C.H., 2008). Rice tillering stage is more sensitive to water. If the water layer is too deep, it would affect normal tillering. Shallow irrigation is helpful for tillering. Reports indicated that when the temperature was around 26-33°C and the soil water holding capacity is 80%, tillers were the most, on the contrary, when the temperature was lower than 20°C and the soil water capacity reached 100%, tillers were the least (Shou, 2008; Manli and Guowei, 2017). OAWD irrigation promoted tillering by controlling the irrigation according to rice demand for water in different periods of rice, so tillers number for each growth stage and numbers of population panicles at maturity of CK1 were higher than that of CK2, it discernible a significant promotion effect on productive tillers of rice. In addition, nutrients are also important factors influencing tillering of rice. The rate of nitrogen applied to rice affects the number of tillers and productive tillering. If nitrogen applied is excessive, the peak of tillering is delayed, and when the rate of nitrogen applied increasing, the number of unproductive tillers growing, whereas the productive tillers decreasing, it affects the grain yield.

Reports showed that the water demand of rice plants would be 2310 m³ per ha. through the growth period, accounting for 11% of the total water consumption, and the evaporation would be 3735 m³ per ha., accounting for 17% whereas leakage would be 15660 m³ per ha, accounting for 72%. It can be seen that a large amount of irrigation water for rice is consumed by evaporation and leakage (Chu, 2016; Weiguang, 2013; Fuqiang, 1999). At the same time, with a lot of loss of irrigation water, the

production problem of excessive fertilizer loss, especially excessive nitrogen fertilizer loss, is also very prominent. The habitual nitrogen application rate of farmers in this study area is up to 360 kg to 450 kg per ha. The yield is between the range of 10500 kg to 11250 kg per ha, and the demand for nitrogen is around 255 kg per ha. This research has proved that the reasonable nitrogen reduction matched with water-saving cultivation will be the right way for solve the problem in rice production and is of practical significance.

CONCLUSIONS

Excess nitrogen and water influenced negatively the productive tillers. Whereas proper nitrogen rate under OAWD irrigation enhanced the grains number of per panicle, added filled grains percentage, increased grain yield and benefit. Compared with farmers' traditional practice water and nitrogen management, the right allocation of nitrogen fertilizer and irrigation significantly decreased the unproductive tillers, significantly increased the number of population panicles by 5.37%, increased the grains number per panicle by 1.97%, significantly increased the filled grain percentage by 6.88%, strength the productive tillers percentage by 18.81%, increased the grain yield 8.72% and benefit by 4146.15 yuan per ha. In addition, it was of an decrease of nitrogen dose by 30%, saving fertilizer cost by 261.36 yuan ha⁻¹ of fertilizer, and a saving of irrigation water by 21.7%.

ACKNOWLEDGEMENTS

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REFERENCES

- Akita, S. (1989). Improving yield potential in tropical rice. Cited from progress in irrigated rice research. International Rice Research Institute, Los Banos, Philippines, 41–73.
- Baocheng, W., Xinhai, W., Yongmei, Z., et al. (2011). High Efficiency Water Saving Technology and Measures of Rice in Hebei Province. *Journal of Hebei Agricultural Sciences*, 15(3), 18–20.
- Bolun, W., Baoyan, J., Ning, H. (2008). Analysis of Rice Production in Northern China. *North Rice*, 38(1), 1–5.
- Ningning, L., Lingyuan, M. (2011). Production status and technical analysis of rice in northern China. *Heilongjiang Science*, 27(7), 37–44.
- Chu ZH., Xiaochuang, C., Lianfeng, ZH. et al. (2016). A review on effects and regulation of paddy alternate wetting and drying on rice nitrogen use efficiency. *Transactions of the Chinese Society of Agricultural Engineering*, 32(19), 139–145.
- Dordas, C.A, Sioulas, C. (2008). Safflower yield, chlorophyll content, photo-synthesis, and water use efficiency response to nitrogen fertilization under rained conditions. *Industrial Crops and Products*, 27, 75–85.
- Fuqiang, T., Heping, H., Shixiu, Y., et al. (1999). Computer Simulation of Irrigation Water Requirement for Paddy Rice. *Transactions of the Chinese Society of Agricultural Engineering*, 15(4), 100–106.
- Guowei, X., Dake, L., Huizhong, S., et al. (2017). Effect of alternative wetting and drying irrigation and nitrogen coupling on rhizosphere environment of rice. *Transactions of the Chinese Society of Agricultural Engineering*, 33(4), 186–192.
- Hanliang, ZH., Xuelin, L., Qiuling, ZH. (2000). Studies on Tiller Regularity of Middle Tillering Part and Productive Forces of Rice. *Acta Agriculturae Boreall-Sinica*, 15(2), 112–117.
- Manli, ZH., Ying, CH., Shougui, H., et al. (2011). Effect of N-fertilizer Management on the Tiller, Dry Matter Weight and Yield of Rice. *Liaoning Agricultural Sciences*, 3, 6–8.
- Qinghe, CH., Genxing, P., Jianying, L. (2008). Effect of Single or Multiple N Applications under Different Total N Fertilization on Rice Yield in a Paddy Soil of North China. *Chinese Journal of Soil Science*, 39(1), 82–86.
- Shou, P., Le, L., Xiaohong, L., et al. (2008). Effects of nitrogen on rice tiller bud development and its mechanism. *Journal of Nanjing Agricultural University*, 06, 32–36.
- Sulin, X., Fanfan, M., Weijun, W., et al. (2018). Effects of Water Management on Yield, Quality of Rice and Nitrogen and Phosphorus Loss in Paddy Field. *China Rice*, 24(3), 16–20.
- Weiguang, W., Fengchao, S., Shizhang, P., et al. (2013). Simulation of response of water requirement for rice irrigation to climate change. *Transactions of the Chinese Society of Agricultural Engineering*, 29(14), 90–97.

WEED CONTROL IN OILSEED RAPE (*Brassica napus* L.)

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Abstract

During the period of 2017-2019 a field trial with Clearfield oilseed rape (hybrid PT 200 CL) on the experimental field of the Agricultural University of Plovdiv, Bulgaria was conducted. The experiment included the application of imazamox - containing herbicide products - Cleranda SC, Cleravis SC, Cleravo SC and Clentiga SC together with the adjuvant Dash. The herbicide products were applied at BBCH 11-12 (1st-2nd true leaf) of the crop. The efficacy of the studied herbicides was evaluated by the 10-score scale of EWRS. The highest efficacy against *Anthemis arvensis* L., *Papaver rhoeas* L., *Galium aparine* L., *Capsella bursa-pastoris* (L.) Medik, *Lolium temulentum* L., *Avena fatua* L. and the volunteer *Triticum aestivum* L. after the application of Cleranda SC + Dash in rates of 2.00+1.00 l/ha, followed by Cleranda SC + Dash in rates of 1.40+1.00 l/ha and Cleravis SC+ Dash in rates of 2.00+1.00 l/ha was reported. For these treatments, the values for plant height, number of brunches per plant, silique number per plant, length of the central silique and yield were the highest.

Key words: oilseed rape, weeds, herbicides, efficacy, yield.

INTRODUCTION

One of the main problems accompanying the agricultural production is the weeds. The weeds occur all over agricultural and non cultivated areas every year and are causing great damage to crops, reducing yields and the quality of the production (Kalinova et al., 2012; Tityanov et al., 2010; Tonev, 2000a). Furthermore weeds negatively affect the formation of the yield of agricultural crops as well the shortage of nutrients, which is often also caused by the weed infestation (Manolov and Neshev, 2017; Neshev and Manolov, 2016; Kostadinova et al., 2015; Manolov et al., 2015; Neshev and Manolov, 2014; Goranovska et al., 2014; Neshev et al., 2014; Karimi et al., 2010; Yanchev et al., 2002).

For obtaining optimal yield from the oilseed rape (*Brassica napus* L.) it is necessary to accomplish timely and precise weed control (Tonev et al., 2019; Pavlovic' et al., 2015; Dimitrova et al., 2014a; Hamzei et al., 2010; Maataoui et al., 2003).

Very important part for the efficient weed management in oilseed rape is determination of their species composition (Tonev, 2000b). There are dynamics in the weed species composition depending on the latitude in which oilseed rape is grown. On the experimental

field of the Agricultural University of Plovdiv, Bulgaria dominating weeds in the oilseed rape are *Sinapis arvensis* L., *Raphanus raphanistrum* L., *Anthemis arvensis* L. and *Papaver rhoeas* L. (Tityanov et al., 2009a). These weeds have been reported in various studies by other researchers in Bulgaria (Mitkov, 2014; Mitkov et al., 2009; Delibaltova et al., 2009; Tityanov et al., 2009b; Atanasova and Zarkov, 2005; Atanasova, 2000).

Studies on oilseed rape weeding abroad show the following. The most distributed weeds in Hubei province, China are: *Alopecurus aequalis* Sobol., *Veronica persica* Poir., *Polypogon fugax* Nees ex Steudel, *Malachium aquaticum* (L.) Fr., *Beckmannia syzigachne* (Steud.) Fernald, *Galium aparine* var. *tenerum*, *Poa annua* L., *Alopecurus japonicus* (Zhu Wen Da et al. 2008).

In Gorgan, Golestan Province, Iran the most commonly found weed species are *Phalaris minor* Retz., *Melilotus officinalis* (L.) Pall., *Rapistrum rugosum* (L.) All., *Avena sterilis* subsp. *ludoviciana*, *Veronica persica* Poir. and *Sinapis arvensis* L. (Ataie et al., 2018).

The weed infestation in Germany of oilseed rape is presented mainly by *Matricaria* spp., *Viola arvensis* Murray, *Capsella bursa-pastoris* (L.) Medik., *Stellaria media* (L.) Vill., *Thlaspi arvense* L., *Poa annua* L., *Apera spica-venti*

(L.) P. Beauv., etc. (Hanzlik et al., 2010). Hanzlik and Gerowitt (2012) reported that in the areas with intensive oilseed rape growing the infestation with *Geranium* spp., *Sisymbrium* spp. and *Anchusa arvensis* (L.) M. Bieb. has increased.

In order to protect the environment and human health, the efficient use of material and energy resources in the cultivation of oilseed rape for the control of weeds, non-chemical methods and biological products are applied (Marcinkevičiene et al., 2017; Velička et al., 2016; Marcinkevičiene et al., 2015; Velička et al., 2015).

Organic oilseed rape cultivation is not always efficient and easily applicable. That is why in the practice for unwanted weeds control the application of synthetic herbicides is mainly used. The application of herbicides to rapeseed is one of the most important and responsible points in its agricultural technology (Frisen et al., 2003; Heard et al., 2003; Harker et al., 2003; Senior and Dale, 2002; Tonev et al., 2000a). The successful weed control in oilseed rape depends on the application time of the herbicide product, i.e. from the optimal stages of the weeds and the crop. The soil or early-vegetation herbicide application is more effective in comparison to the traditional spring treatment. By these treatments the oilseed rape crop is released on time from the weed concurrence for water, light, nutrients, etc. (Freeman and Lutman, 2004; Franek, 1994).

The choice of herbicide depends on that if the grown oilseed rape hybrid is selected to be grown by the conventional or Clearfield® technology. In dependence of the weed species at the moment of application in the conventional oilseed rape production a great number of herbicides had been studied: propisochlor, trifluralin, haloxyfop-p-methyl; metazachlor; bifenox; clomazone, napropamide, dimethachlor; alachlor; isoxaben; halauxifen-methyl, picloram; propyzamide, aminopyralid; clopyralid; ethametsulfuron-methyl; clethodim and propaquizafop (Bardsley et al., 2018; Lourdet and Rougerie, 2016; Koleva-Valkova et al., 2016; Zotz et al., 2016; Koprivlenski et al., 2015; Dimitrova et al., 2014b; Dimitrova et al., 2014c; Werner, 2014; Duroueix et al., 2013; Lourdet, 2013; Drobny and Schlang, 2012;

Stormonth et al., 2012; Bijanzadeh et al., 2010; Majchrzak and Jarosz, 2010; Tityanov et al., 2009a; Majchrzak et al., 2008; Konstantinovic', 2007; Franek and Rola, 2002a; Franek and Rola, 2002b; Franek and Rola, 2001).

At the conventional oilseed rape hybrids some of the registered selective herbicides have no satisfactory efficacy against the cruciferous weeds like *Sinapis arvensis* L., *Raphanus raphanistrum* L. and *Descurainia sophia* (L.) Plantl. An alternative for solving this problem is the Clearfield® technology at oilseed rape (Pfenning et al., 2012). In this technology the oilseed rape hybrids are IMI-tolerant (Imidazolinone-tolerant). At these hybrids Cleranda SC (375 g/l metazachlor + 17.5 g/l imazamox) in rate of 1.50-2.00 l/ha + the adjuvant Dash can be applied (Tonev and Mitkov, 2015; Schönhammer et al., 2010; Ádámszki et al., 2010).

In the Clearfield® oilseed rape accept Cleranda SC, the herbicide products Clentiga (250 g/l quinmerac and 12.5 g/l imazamox) + adjuvant Dash in rates of 1.0 l/ha + 1.0 l/ha in autumn (BBCH 10-18) and in spring (BBCH 30-50) can be applied. The product Vantiga D is combined product with three active substances - metazachlor, quinmerac and imazamox. The herbicide is also applied with the adjuvant Dash®. Although the permitted period of use of the product is very long (BBCH 10-18) it is mainly used relatively early - as soon as most of the important weeds appear (Schönhammer and Freitag, 2014).

In comparison with Vantiga D, Clentiga has slightly less activity spectrum and less pronounced soil activity due to the lack of metazachlor, it provides greater flexibility in the choice of application dates and combinations with soil and foliar herbicides.

If there is high weed infestation and difficult soil conditions Schönhammer and Freitag (2014) recommend sequential application Butisan Kombi (metazachlor + dimethenamid-P) before or very early after germination of the oilseed rape followed by application Clentiga.

Schönhammer et al. (2018) reported that the application of Clentiga + Dash in rates of 1.0 l/ha + 1.0 l/ha in combination Runway in rate of 0.2 l/ha showed excellent efficacy against all cruciferous weed species in the study.

Under specific conditions, e.g. high infestation with blackgrass or cranesbill, Clearfield-Clentiga Runway Pack can also be used in spraying systems together with metazachlor, dimethenamid-P or propyzamide-containing herbicides. The aim of the current research is to study the possibilities of chemical weed control in oilseed rape (*Brassica napus* L.) at the agroecological conditions of Plovdiv district, Bulgaria.

MATERIALS AND METHODS

During the vegetation periods of 2017/2018 and 2018/2019 a field trial with the Clearfield® oilseed rape hybrid PT 200 CL on the experimental field of the Base for Training and implementation at the Agricultural University of Plovdiv, Bulgaria was conducted. The study was performed by the Split Plot Method, in 4 replication, with size of the trial plot 20 m².

The experiment included the following treatments: 1. Untreated control; 2. Cleranda SC (375 g/l metazachlor + 17.5 g/l imazamox) + Dash (adjuvant) - 1.40 + 1.00 l/ha; 3. Cleranda SC + Dash - 2.00 + 1.00 l/ha; 4. Cleravis SC (375 g/l metazachlor + 100 g/l quinmerac + 17.5 g/l imazamox) + Dash - 1.40 + 1.00 l/ha; 5. Cleravis SC + Dash - 2.00 + 1.00 l/ha; 6. Cleravo SC (35 g/l imazamox + 250 g/l quinmerac) + Dash - 0.70 + 1.00 l/ha; 7. Cleravo SC + Dash 1.00 + 1.00 l/ha; 8. Clentiga SC (12.5 g/l imazamox + 250 g/l quinmerac) + Dash - 0.60 + 1.00 l/ha; 9. Clentiga SC + Dash - 1.00 + 1.00 l/ha.

During both experimental years a predecessor of the oilseed rape was winter wheat. On the whole experimental field the following measures were performed: fertilization with 300 kg/ha with N:P:K = 15:15:15, followed by deep ploughing on 25 cm of depth. Before the sowing of the oilseed rape one disking on the depth of 15 cm and two harrowing operations of a depth of 8 cm was done. In spring, dressing with 250 kg/ha with ammonium nitrate was applied.

On the experimental field, before the sowing of the crop, artificial infestation with seed of *Anthemis arvensis* L., *Papaver rhoeas* L., *Galium aparine* L., *Sinapis arvensis* L., *Lamium purpureum* L., *Capsella bursa-pastoris* (L.) Medik, *Lolium temulentum* L.,

Avena fatua L., as well as seeds from winter wheat was accomplished.

The sowing of the oilseed rape in 2017 is done on the 22.09., and on the 18.09., in 2018.

The herbicide application was performed in 1st – 2nd true leaf stage of the crop (BBCH 11-12) with size of the working solution - 200 l/ha.

The biological efficacy was reported on 14th, 28th and 56th day after the herbicide application. The efficacy against the weeds was evaluated by the 10-score visual scale of EWRS.

The efficacy results were compared with the untreated control.

The selectivity of the studied herbicides was evaluated on the 7th, 14th, 28th and 56th day after the herbicide application by the 9-score visual scale of EWRS (at score 0 - there is no damage on the crop, and at score 9 there is complete death of the crop).

The biological yield as well as the following biometric indicators have been identified and analyzed: plant height, number of branches per plant, silique number per plant, length of the central silique.

Reported biometric indicators were processed with the software package SPSS 17 - module two-factor analysis of variance for Windows 8. The difference between the evaluated treatments was statistically analysed by ONE WAY ANOVA.

RESULTS AND DISCUSSIONS

On the experimental field of the Base for Training and Implementation at the Agricultural University of Plovdiv in both experimental years only annual weed species from two biological groups were reported. From the group of winter-spring weeds the three dicotyledonous weeds were found: *Anthemis arvensis* L., *Papaver rhoeas* L., *Capsella bursa-pastoris* (L.) Medik. The monocotyledonous winter-spring weeds were presented only by *Lolium temulentum* L. The early spring weeds were presented by the broadleaf species *Galium aparine* L., *Sinapis arvensis* L., *Lamium purpureum* L. and the grass weed *Avena fatua* L. on the trial area a volunteer winter wheat - *Triticum aestivum* L., was observed. The efficacy of the studied herbicides is presented on tables 1, 2 and 3. The presented data is average for both trial years.

From the obtained results on the 14th day after the herbicide application against *A. arvensis*, *P. rhoeas* and *S. arvensis* the efficacy of Cleranda SC + Dash - 2.00 + 1.00 l/ha was 88.3, 88.3 and 100%, respectively. Against the weeds *P. rhoeas* and *S. arvensis* the application of Cleranda SC + Dash - 1.40 + 1.00 also controlled the weed. An excellent control - 98.3% against the difficult-to-control weed in the *S. arvensis* after the treatment with Cleravis SC + Dash in both examined rates was recorded. The usage of Cleravis SC + Dash in the high rate - 2.00+1.00 l/ha ensured good efficacy against *P. rhoeas* - 85%. The lowest efficacy in the experiment after the application of Clentiga SC + Dash - 0.60 + 1.00 l/ha against *A. arvensis* and *P. rhoeas* was reported 21.7 and 38.3%, respectively (Table 1).

Regarding the weed *G. aparine* on the 14th day after the treatments average for both years, the highest efficacy after the application of Cleravo SC + Dash - 1.00 + 1.00 l/ha - 93.3%, followed by Cleravis SC + Dash - 2.00 + 1.00 l/ha - 90% was found. The lowest efficacy was recorded after the application Cleravis SC + Dash - 1.40+1.00 l/ha and Clentiga SC + Dash - 0.60 + 1.00 l/ha - 78.3% (Table 1).

Against the weed *L. purpureum*, at all studied treatments the efficacy was excellent. The lowest efficacy was observed after the application of Clentiga SC + Dash - 0.60 + 1.00 l/ha - 90%. The results about the efficacy against *C. bursa-pastoris* showed that it varies in a narrow range. The best control was recorded for treatment 5-91.7% (Table 1).

With regard to the control of *L. temulentum*, the best results on the 14th day after treatments were reported after the application of Cleranda SC + Dash - 2.00 + 1.00 l/ha - 83.3%. On the second place for efficacy was treatment 2 - 81.6%, followed by treatment 5 - 80%.

Cleranda SC + Dash - 2.00 + 1.00 l/ha showed the highest efficacy against *A. fatua* and *T. aestivum* volunteer was the highest on the 14th day after the application 83.3 and 90%, respectively. The lowest efficacy against *A. fatua* after the treatment with Clentiga SC + Dash - 1.00 + 1.00 l/ha was reported (Table 1). On the 28th day after the herbicide application in both experimental years the highest efficacy against *A. arvensis*, *P. rhoeas*, *L. purpureum*, *L. temulentum*, *A. fatua* and *T. aestivum*

volunteer after the treatment with Cleranda SC + Dash - 2.00 + 1.00 l/ha was observed 91.7, 91.7, 100, 88.3, 88.3 and 90%, respectively (Table 2). Very good efficacy against these weed species was also recorded for the treatment Cleranda SC + Dash - 1.40 + 1.00 l/ha. The lowest efficacy against this weed association with accept *A. fatua* after the application of Clentiga SC + Dash - 0.60 + 1.00 l/ha was found. The highest efficacy against *G. aparine* (93.3%) on the 28th day after treatments with Cleravis SC + Dash - 2.00 + 1.00 l/ha and Cleravo SC + Dash 1.00 + 1.00 l/ha was recorded. The lowest control was recorded for the treatment of Clentiga SC + Dash - 0.60 + 1.00 l/ha - 80%.

Under the conditions of the trial *S. arvensis* is the most sensitive of all weed species. On the 28th day at all variant 100% efficacy was recorded against *S. arvensis*. Similar results were found for *L. purpureum* (Table 2).

For the weed *C. bursa-pastoris* on the 28th day after application, the highest efficacy was observed for the treatments with Cleranda SC + Dash in both examined rates - 91.7%. Very good control against this weed (90%) was found for variants 3 and 6. The lowest control was recorded for the treatment Clentiga SC + Dash - 0.60 + 1.00 l/ha - 86.7%.

The results at day 56 after treatment from both trial years maintained the trend in efficacy of the previous two reporting dates. The herbicidal effect of all the variants studied was found to be highest during the third reporting date. This can be explained by the sufficient duration of action of the herbicides on the weeds and their ability to maximize their implementation.

In the third reporting date, the highest biological efficacy against *A. arvensis*, *P. rhoeas*, *G. aparine*, *C. bursa-pastoris*, *L. temulentum*, *A. fatua* and *T. aestivum* volunteer was recorded for the treatment with Cleranda SC + Dash - 2.00 + 1.00 l/ha - 95, 91.7, 100, 95, 91.7, 88.3 and 98.3% (Table 3). The efficacy of 100% against the weed *G. aparine* after the application of Cleravis SC + Dash - 2.00 + 1.00 and Cleravo SC + Dash 1.00 + 1.00 l/ha. It is correct to note that except for Cleranda SC + Dash - 2.00 + 1.00 l/ha, 95% efficacy against *C. bursa-pastoris* after the application of Cleravis SC + Dash - 1.40 + 1.00 l/ha was recorded (Table 3).

Average for both trial years, on the 56th day, 100% efficacy against *S. arvensis* and *L. purpureum* for all treatment was recorded.

Average for the period, the lowest efficacy on the 56th day against the weeds *A. arvensis*, *P. rhoeas*, *L. temulentum*, *A. fatua* and *T. aestivum* (Table 3) was observed for the treatment with Clentiga SC + Dash - 0.60 + 1.00 l/ha. The application of Clentiga SC + Dash - 1.00 + 1.00 l/ha against *G. aparine* and *C. bursa-pastoris* was with the lowest efficacy. The same efficacy was observed for the applications of Cleravo SC + Dashed in both evaluated rates against *C. bursa-pastoris*.

The visual observations of phytotoxicity over the two experimental years indicated that all imazamox-containing herbicides at the appropriate doses exhibited excellent selectivity for the oilseed rape in the study.

The productivity of the oilseed rape hybrid was also evaluated. The results show that for the treated variants there is a positive correlation between their biological efficacy against weeds and the crop yield.

As a result of the high weed infestation of the experimental field with competitive weed species, a low average yield of the untreated control was reported - 1.170 t/ha (Table 4).

The highest oilseed rape seed yield after the treatment with Cleranda SC + Dash - 2.00 + 1.00 l/ha - 3,520 t/ha. High yield was also reported for the treatments of Cleranda SC + Dash - 1.40 + 1.00 l/ha and Cleravis SC + Dash - 2.00 + 1.00 l/ha - 3.470 t/ha and 3.417 t/ha, respectively. It should be noted that, the difference between the yields obtained in the three variants were not statistically proved.

The lowest productivity among the treated variants after the treatment with Clentiga SC + Dash in both examined rates - 2.207 t/ha and 2,333 t/ha. There were not statistically proved differences between these two treatments. The reason for this is probably the low efficacy against *L. temulentum*, *A. fatua*, *A. arvensis*, *P. rhoeas* and the volunteer *T. aestivum* (Table 3). In all the variants treated, the yield obtained is higher than that of the untreated control and its increase is statistically proven at the level of significance $gD = 5\%$ (Table 4).

Accept biological efficacy, selectivity and yield some of the main biometrical indicators were tracked. For the indicator height of the plants at

the end of the vegetation it was found that the shortest were the plants from the untreated highly infested with weeds control. This difference is statistically proven at the level of significance 5%. The results showed that in the presence of weeds, the crop plants compete with them and as a consequence, in severe weed infestation the oilseed rape remains suppressed and plants were shorter in habitus - 124.8 cm. The highest plants were reported for the treatments with Cleranda SC + Dash - 1.40 + 1.00 l/ha, Cleranda SC + Dash - 2.00 + 1.00 l/ha and Cleravis SC + Dash - 2.00 + 1.00 l/ha - 162.9, 160.2 and 158.4 cm, respectively. There were no statistically proven differences between these three treatments. These results correlate with bio-efficiency and yield data. In the other variants treated, the values of the indicator ranged from 135,1 to 150,3 cm.

For the indicator number of branches per plant there was also observed difference. The lowest branch number was found for the control - 6,6. The highest number of branches was found to be for the treatments 3, 2 and 5 - 9,2; 9,0 and 8,8 branches per plant respectively. At the above mentioned three treatments there were no statistically proved differences. There were no significant differences in the number of branches per plant for variants 4, 6, 7, 8 and 9. Silique number per plant is one of the most important indicators influencing the production of oilseed rape. The comparative analysis of the untreated control with the other studied variants shows that there are statistically proven differences in favour of the herbicide-treated variants.

The highest results were obtained after the treatment Cleranda SC + Dash in rates of 2.00 + 1.00 l/ha and 1.40+1.00 l/ha, as well as after the treatment of Cleravis SC + Dash - 2.00 + 1.00 l/ha - 404.0, 398.0 and 377.3 siliques per plant respectively. No statistical difference was found between these treatments. Among the treated variants, the lowest was the result for this studied indicator after the application Clentiga SC + Dash - 0.60 + 1.00 l/ha - 286.7 siliques per plant. Among the other treatments the siliques number per plant per plant varied from 290.0 to 353.7 (Table 4).

Regarding the indicator length of the central silique it was reported that the lowest results were found to be for the untreated control - 3,9

cm. This difference is statistically proven at a significance level of 5%. At treatments 8 and 9

the length of the central silique was 4.6 and 4.7 cm.

Table 1. Efficacy of imazamox-containing herbicides on the 14th day after the treatments (% by EWRS)

Treatments	<i>A. arvensis</i>	<i>P. rhoeas</i>	<i>G. aparine</i>	<i>S. arvensis</i>	<i>L. purpureum</i>	<i>C. bursa-pastoris</i>	<i>L. temulentum</i>	<i>A. fatua</i>	<i>Volunteer T. aestivum</i>
1. Untreated control	–	–	–	–	–	–	–	–	–
2. Cleranda SC + Dash – 1.40 + 1.00 l/ha	83.3	88.3	85.0	100	95.0	90.0	81.6	81.6	83.3
3. Cleranda SC + Dash – 2.00 + 1.00 l/ha	88.3	88.3	88.3	100	95.0	90.0	83.3	83.3	90.0
4. Cleravis SC + Dash – 1.40 + 1.00 l/ha	78.3	81.7	78.3	98.3	93.3	86.7	61.7	68.3	75.0
5. Cleravis SC + Dash – 2.00 + 1.00 l/ha	71.7	85.0	90.0	98.3	100	91.7	80.0	71.7	85.0
6. Cleravo SC + Dash – 0.70 + 1.00 l/ha	53.3	81.7	88.3	93.3	100	90.0	31.7	46.7	76.7
7. Cleravo SC + Dash – 1.00 + 1.00 l/ha	71.7	76.7	93.3	96.7	100	88.3	53.3	60.0	81.7
8. Clentiga SC + Dash – 0.60 + 1.00 l/ha	21.7	38.3	78.3	88.3	90.0	88.3	3.3	25.0	33.3
9. Clentiga SC + Dash – 1.00 + 1.00 l/ha	38.3	38.3	85.0	95.0	95.0	83.3	13.3	21.7	43.3

Table 2. Efficacy of imazamox-containing herbicides on the 28th day after the treatments (% by EWRS)

Treatments	<i>A. arvensis</i>	<i>P. rhoeas</i>	<i>G. aparine</i>	<i>S. arvensis</i>	<i>L. purpureum</i>	<i>C. bursa-pastoris</i>	<i>L. temulentum</i>	<i>A. fatua</i>	<i>Volunteer T. aestivum</i>
1. Untreated control	–	–	–	–	–	–	–	–	–
2. Cleranda SC + Dash – 1.40 + 1.00 l/ha	88.3	90.0	90.0	100	98.3	91.7	83.3	83.3	88.3
3. Cleranda SC + Dash – 2.00 + 1.00 l/ha	91.7	91.7	91.7	100	100	90.0	88.3	88.3	90.0
4. Cleravis SC + Dash – 1.40 + 1.00 l/ha	78.3	78.3	85.0	100	98.3	88.3	63.3	70.0	83.3
5. Cleravis SC + Dash – 2.00 + 1.00 l/ha	76.7	85.0	93.3	100	100	91.7	81.7	71.7	88.3
6. Cleravo SC + Dash – 0.70 + 1.00 l/ha	56.7	81.7	90.0	100	100	90.0	31.7	46.7	76.7
7. Cleravo SC + Dash – 1.00 + 1.00 l/ha	73.3	83.3	93.3	100	100	88.3	53.3	60.0	85.0
8. Clentiga SC + Dash – 0.60 + 1.00 l/ha	21.7	38.3	80.0	100	93.3	88.3	3.3	25.0	33.3
9. Clentiga SC + Dash – 1.00 + 1.00 l/ha	38.3	40.0	85.0	100	98.3	86.7	13.3	21.7	43.3

Table 3. Efficacy of imazamox-containing herbicides on the 56th day after the treatments (% by EWRS)

Treatments	<i>A. arvensis</i>	<i>P. rhoeas</i>	<i>G. aparine</i>	<i>S. arvensis</i>	<i>L. purpureum</i>	<i>C. bursa-pastoris</i>	<i>L. temulentum</i>	<i>A. fatua</i>	<i>Volunteer T. aestivum</i>
1. Untreated control	–	–	–	–	–	–	–	–	–
2. Cleranda SC + Dash – 1.40 + 1.00 l/ha	88.3	90.0	98.3	100	100	93.3	86.7	85.0	91.7
3. Cleranda SC + Dash – 2.00 + 1.00 l/ha	95.0	91.7	100	100	100	95.0	91.7	88.3	98.3
4. Cleravis SC + Dash – 1.40 + 1.00 l/ha	78.3	78.3	95.0	100	100	95.0	68.3	70.0	93.3
5. Cleravis SC + Dash – 2.00 + 1.00 l/ha	80.0	86.7	100	100	100	95.0	81.7	76.7	90.0
6. Cleravo SC + Dash – 0.70 + 1.00 l/ha	65.0	80.0	98.3	100	100	90.0	40.0	53.3	76.7
7. Cleravo SC + Dash – 1.00 + 1.00 l/ha	80.0	83.3	100	100	100	90.0	58.3	63.3	88.3
8. Clentiga SC + Dash – 0.60 + 1.00 l/ha	31.7	45.0	93.3	100	100	91.7	3.3	25.0	48.3
9. Clentiga SC + Dash – 1.00 + 1.00 l/ha	38.3	50.0	91.7	100	100	90.0	13.3	30.0	53.3

Table 4. Yield and biometrical indicators of oilseed rape hybrid PT 200 Cl, treated with imazamox-containing herbicides in the period of 2017-2019

Treatments	Yield, t/ha	Plant height, cm	Number of branches per plant	Silique number per plant	Length of the central silique, cm
1. Untreated control	1.170 a	124.8 a	6.6 a	127.0 a	3.9 a
2. Cleranda SC + Dash – 1.40 + 1.00 l/ha	3.470 f	160.2 e	9.0 c	398.0 e	5.4 d
3. Cleranda SC + Dash – 2.00 + 1.00 l/ha	3.527 f	162.9 e	9.2 c	404.0 e	5.5 d
4. Cleravis SC + Dash – 1.40 + 1.00 l/ha	3.220 d	150.3 d	7.8 b	353.7 cd	5.3 d
5. Cleravis SC + Dash – 2.00 + 1.00 l/ha	3.417 ef	158.4 e	8.8 c	377.3 de	5.3 d
6. Cleravo SC + Dash – 0.70 + 1.00 l/ha	2.893 c	149.8 d	7.6 b	329.3 c	5.2 cd
7. Cleravo SC + Dash – 1.00 + 1.00 l/ha	3.200 d	148.9 d	7.6 b	333.3 c	5.3 d
8. Clentiga SC + Dash – 0.60 + 1.00 l/ha	2.207 b	135.1 b	7.4 b	286.7 b	4.6 b
9. Clentiga SC + Dash – 1.00 + 1.00 l/ha	2.333 b	141.4 c	7.5 b	290.0 b	4.7 b
	$gD_{5\%} = 19.6974$	$gD_{5\%} = 5.9796$	$gD_{5\%} = 0.8043$	$gD_{5\%} = 35.1769$	$gD_{5\%} = 0.5371$

CONCLUSIONS

The highest efficacy against *Anthemis arvensis* L., *Papaver rhoeas* L., *Galium aparine* L., *Capsella bursa-pastoris* (L.) Medik, *Lolium temulentum* L., *Avena fatua* L. and the volunteer *Triticum aestivum* L. after the application of Cleranda SC + Dash - 2.00 + 1.00 l/ha and followed by Cleranda SC + Dash - 1.40+1.00 l/ha and Cleravis SC + Dash - 2.00 + 1.00 l/ha was recorded.

From the analysis of biometric indicators for the oilseed rapeseed hybrid PT200 CL: plant height, number of brunches per plant, silique number per plant, length of the central silique and yield were the highest for the treatment of Cleranda SC + Dash in both examined rates 2.00 + 1.00 l/ha and 1.40+1.00 l/ha, as well as Cleravis SC + Dash - 2.00 + 1.00 l/ha. Of all the variants studied, the lowest values were reported in the untreated control.

REFERENCES

- Ádamszki, T., Kukorelli, G., Torma, M., Reisinger, P. (2010). Experiences in weed control of imidazolinon resistant winter oilseed rape. *Magyar Gyomkutatás és Technológia*, 11(2), 45–59.
- Ataie, S., Pirdashty, H., Kazemi, H., Younesabadi, M. (2018). Mapping the distribution and flora of the weeds in canola fields of Gorgan Township by Geographic Information System (GIS). *Journal of Plant Protection (Mashhad)*, 31(4), 605–616.
- Atanasova, D. (2000). Dynamics and weed species in winter cereal crops. *Plant Science*, 37(10), 965–969.
- Atanasova, D., Zarkov, B. (2005). Weed infestation of barley and wheat grown as monoculture. *Field Crops Studies*, 11(1), 93–97.
- Bardsley, E., Waite, P., Sellars, J. (2018). GF-3447 - a new herbicide concept containing halauxifen-methyl for the post emergence control of broadleaf weeds in winter oilseed rape. *The Dundee Conference. Crop Production in Northern Britain 2018, Dundee, UK, 27-28 February*, 139–144.
- Bijanazadeh, E., Ghadiri, H., Behpouri, A. (2010). Effect of trifluralin, pronamide, haloxyfop-p methyl, propaquizafop, and isoxaben on weed control and oilseed rape yield in Iran. *Crop Protection*, 29(8), 808–812.
- Delibaltova, V., Zheljzkov, I., Tonev, T. (2009). Effect of some herbicides on the weeds and productivity of the *Triticum aestivum* L. winter wheat. *Agricultural Sciences*, 1(2), 19–25.
- Dimitrova, M., Dimova, D., Zhalnov, I., Stoychev, D., Zorovski, P., Georgieva, T., Mitkov, A., Idirizova, E. (2014b). Influence of some herbicides on the growth and development of winter oilseed rape. Balkan Agricultural Congress, 8-11 September 2014, Edirne, Turkey, *Turkish Journal of Agricultural and Natural Science*, 1058–1062.
- Dimitrova, M., Dimova, D., Zhalnov, I., Zorovski, P., Georgieva, T., Mitkov, A., Idirizova, E. (2014a). The influence of some herbicides on the structural

- elements of the yield of winter oilseed rape. Balkan Agricultural Congress, 8-11 September 2014, Edirne, Turkey, *Turkish Journal of Agricultural and Natural Science*, 1054–1057.
- Dimitrova, M., Zhaltov, I., Stoychev, D. (2014c). Efficacy and selectivity of some herbicides on winter oilseed rape, *Agricultural Science and Technology*, 6(3), 297–300.
- Drobny, H.G., Schlang, N. (2012). SALSA® (Ethamsulfuron-methyl 75% WG): a novel selective herbicide for oilseed rape in Europe. *Julius-Kühn-Archiv*, 2(434), 540–543.
- Duroueix, F., Hebrail, M., Lacotte, J. (2013). Weed control in winter oilseed rape: solutions for progress with postemergence. *22e Conférence du COLUMA. Journées Internationales sur la Lutte contre les Mauvaises Herbes, Dijon, France, 10-12 décembre*, 249–259.
- Franek, M. (1994). Spring weeding treatments in winter oilseed rape. *Ochrona Roślin*, 38(4), 3–4.
- Franek, M., Rola, H. (2001). Effects of weed control in winter oilseed rape with the herbicide Colzor Trio 405 EC in lower Silesia conditions. *Rośliny Oleiste*, 22(1), 97–102.
- Franek, M., Rola, H. (2002a). Application of herbicides to weed control in winter oilseed rape in dependence on economic condition of farm. *Rośliny Oleiste*, 23(2), 365–372.
- Franek, M., Rola, H. (2002b). Efficacy of herbicide Nimbus 283 SE to weed control in winter oilseed rape on Lower Silesia. *Rośliny Oleiste*, 23(2), 351–356.
- Freeman, S., Lutman, P. (2004). The effects of timing of control of weeds on the yield of winter oilseed rape (*Brassica napus*), in the context of the potential commercialization of herbicidetolerant winter rape. *The Journal of Agricultural Science*, 142(3), 263–272.
- Frisen, L., Nelson, A., Van Acker, R. (2003). Fudence of contamination of pedigreed canola (*Brassica narus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal*, 95(5), 1342–1347.
- Goranovska, S., Kalinova, Sht., Valchinkova, P. (2014). Influence of herbicides and mineral nutrition on yield and photosynthetic pigments of maize hybrid Knezha 509. *Proceedings of Jubilee Scientific Conference 90 years Maize Research Institute „Breeding-genetic and technological innovations in cultivation of cultivated plants”*, 128–135.
- Hamzei, J., Nassab, A., Heidari, G., Baradaran, M., Esfandiari, E., Veisi, B. (2010). Comparison of the critical period of weed control in three winter oilseed rape (*Brassica napus*) cultivars. *Proceedings of 3rd Iranian Weed Science Congress, Volume 1: Weed biology and ecophysiology, Babolsar, Iran, 17-18 February 2010*, 319–322.
- Hanzlik, K., Gerowitt, B. (2012). Occurrence and distribution of important weed species in German winter oilseed rape fields. *Journal of Plant Diseases and Protection*, 119(3), 107–120.
- Hanzlik, K., Gerowitt, B., Schulte, M. (2010). What are weed species composition and species richness in oilseed rape influenced by? - Results from a weed survey on 1463 German oilseed rape fields. *Julius-Kühn-Archiv*, 428, 328–329.
- Harker, K., Clayton G., Blackshaw, R., O'Donovan, J., Stevenson, F. (2003). Seeding rate, herbicide timing and competitive hybrids contribute to integrated weed management iv canola (*Brassica napus*). *Canadian Journal of Nant Science*, 83(2), 433–440.
- Heard, M., Hawes, C., Champion, G., Clark, S. (2003). Weeds in fields with contrasting conventional and genetically modified herbicide – tolerant crops. II. Effects on individual species. *Philosophical Transactions of the royal society of London Series B-Biological sciences*, 358(1439), 1833–1846.
- Kalinova, Sht., Zhaltov, I., Dochev, G. (2012). Indirect Indication of Weed Damage as Incubators for Diseases and Pests of Cultivated Plants. *Scientific Works of the Agricultural University of Plovdiv, Bulgaria, LVI*. 291–294.
- Karimi, H., Kazemeini, S. A., Ghadiri, H., Hamidi, R. (2010). Effect of nitrogen on canola yield and yield component under wild oat densities. *Proceedings of 3rd Iranian Weed Science Congress, Volume 1: Weed biology and ecophysiology, Babolsar, Iran, 17-18 February*, 316–318.
- Koleva-Valkova, L., Vasilev, A., Dimitrova, M., Stoychev, D. (2016). Determination of metazachlor rezidues in winter oilseed rape (*Brassica napus* var.Xanon) by HPLC. *Emirats Journal of Food and Agriculture*, 28(11), 813–817.
- Konstantinovic', B., Meseldzija, M., Konstantinovic', B. (2007). Possibilities of weed control in oilseed rape. *Biljni Lekar (Plant Doctor)*, 35(4), 468–474.
- Koprivlenski, V., Dimitrova, M., Zhaltov I. (2015). Economic assessment of the herbicides for weed control in oilseed rape. *Indian Journal of Research*, 4(3), 6–8.
- Kostadinova, S., Kalinova, St., Hristoskov, A., Samodova, A. (2015). Efficiency of some foliar fertilizers in winter wheat. *Bulgarian Journal of Agriculture Science*, 21(4), 742–746.
- Lourdret, Y. (2013). GF-2540: a new product for post emergence weed control in oilseed rape. *22e Conférence du COLUMA. Journées Internationales sur la Lutte contre les Mauvaises Herbes, Dijon, France, 10-12 décembre*, 910–916.
- Lourdret, Y., Rougerie, I. (2016). GF-3447 a new product for post emergence control of broadleaf weeds in oil seed rape. *23e Conférence du COLUMA. Journées Internationales sur la Lutte contre les Mauvaises Herbes, Dijon, France, 6-8 décembre*, 700–708.
- Maataoui, A., Bouhache, M., Benbella, M., Talouizte, A. (2003). Critical period of weed control in oilseed rape in two Moroccan Regions. *Communications in Agricultural and Applied Biological Sciences*, 68(4a), 361–371.

- Majchrzak, L., Jarosz, A. (2010). Efficacy of propisochlor and its tank mix for weed control in winter oilseed rape. *Progress in Plant Protection*, 50(3), 1381–1385.
- Majchrzak, L., Pudeko, J., Skrzypczak, G. (2008). Influence of mixture of bifenoks and metazachlor in weed control in winter oilseed rape. *Progress in Plant Protection*, 48(1), 291–296.
- Manolov, I., Neshev, N. (2017). Growth and yields of potato varieties depend on potassium fertilizer rate and source. *Proceedings of 52nd Croatian and 12th International Symposium on Agriculture, Dubrovnik, Croatia in February*, 356–360.
- Manolov, I., Neshev, N., Chalova, V., Yordanova, N. (2015). Influence of potassium fertilizer source on potato yield and quality. *Proceedings. 50th Croatian and 10th International Symposium on Agriculture, Opatija, Croatia*, 363–367.
- Marcinkevičiene, A., Velička, R., Keidan, M., Butkevičiene, L. M., Kriauciuniene, Z., Kosteckas, R., Čekanauskas, S. (2017). The impact of non-chemical weed control methods and biopreparations on winter oilseed rape preparation for over-wintering and productivity. *Zemes ukio Mokslai*, 24(4), 119–132.
- Marcinkevičiene, A., Velička, R., Mockevičiene, R., Pupaliene, R., Kriauciuniene, Z., Butkevičiene, L. M., Kosteckas, R., Čekanauskas, S. (2015). Non-chemical weed control systems in organically grown spring oilseed rape. *Acta Fytotechnica et Zootechnica*, 18(Special Issue), 34–36.
- Mitkov, A. (2014). Biological efficacy of some leaf herbicides against economically important weeds in field experiments with wheat. *Agricultural University - Plovdiv, Scientific Works, LVIII*, 105–114.
- Mitkov, A., Tonev, T., Tityanov, M. (2009). Spreading of the major weeds in wheat in different agroecological regions of South Bulgaria. *Plant Science*, 46, 148–153.
- Neshev, N., Manolov, I. (2014). Influence of potassium fertilizer source on vegetative parameters of potatoes. *Scientific Works of Agricultural Academy*, 3(2), 213–218.
- Neshev, N., Manolov, I. (2016). Effect of fertilization on soil fertility and nutrient use efficiency at potatoes. *Geophysical Research Abstracts*, 18, EGU, 139. EGU General Assembly.
- Neshev, N., Manolov, I., Chalova, V., Yordanova, N. (2014). Effect of nitrogen fertilization on yield and quality parameters of Potatoes. *Journal of Mountain Agriculture on the Balkans*, 17(3), 615–627.
- Pavlovic', D., Mitrovic', P., Marisavljevic', D., Marjanovic'-Jeromela, A., Anelkovic', A. (2015). The effect of weeds on the yield and quality parameters of rapeseed. *Sixth International Scientific Agricultural Symposium "Agrosym 2015", Jahorina, Bosnia and Herzegovina, October 15-18, Book of Proceedings*, 914–918.
- Pfenning, M., Kehler, R., Bremer, H. (2012). New perspectives for weed control in winter oilseed rape due to the introduction of the Clearfield® system. *Julius-Kühn-Archiv*, 2(434), 435–442.
- Schönhammer, A., Bremer, H., Freitag, J. (2018). Clearfield®-Clentiga® Runway Pack: a flexible solution for complete post-emergence weed control in winter oilseed rape. *Julius-Kühn-Archiv*, 458, 334–344.
- Schönhammer, A., Freitag, J. (2014). Clearfield®-Clentiga® and Clearfield® Kombi-Pack: two new herbicides for targeted weed control in winter- and spring oilseed rape. *Julius-Kühn-Archiv*, 443, 543–551.
- Schönhammer, A., Pfenning, M., Chenevier, S. (2010). Innovative possibilities of weed control in oilseed rape with Clearfield. *Julius-Kühn-Archiv*, 428, 329–330.
- Senior, I.J., Dale, P.J. (2002). Herbicide – tolerance crops in agriculture oilseed rape as a case study. *Plant Breeding*, 121(2), 97–107.
- Stormonth, D., Moss, S., Guichard, A. (2012). Clethodim: evaluation in field experiments for grass-weed control in oilseed rape. *Aspects of Applied Biology*, 117, 47–51.
- Tityanov, M., Tonev, T., Mitkov, A. (2009). Advances in chemical weed control in rape. *Third International Symposium Ecological approaches towards the production of safety food*, 237–244.
- Tityanov, M., Tonev, T., Mitkov, A. (2009). New opportunities for efficient chemical control of weeds in wheat. *Plant Science*, 46, 154–160.
- Tityanov, M., Tonev, T., Mitkov, A. (2010). Chemical control of Field brome (*Bromus arvensis* L.) in wheat fields. *Agricultural University - Plovdiv, Scientific Works, LV(2)*, 139–142.
- Tonev, T. (2000b). *Color atlas of 100 economically most important weeds in Bulgaria*. Publisher: Bibliotheka Zemedelsko Obrazovanie.
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Zhelyazkov, I., Vasilev, A., Tityanov, M., Mitkov, A., Yanev, M. (2019). *Herbology*, Publisher: Vidinov & son, ISBN: 978-954-8319-75-1.
- Tonev, T., Mitkov, A. (2015). Chemical weed control in main field crops. *Zemedelie Plus*, 2(265), 33–44.
- Tonev, T. (2000a). *A guidebook for integrated weed management and proficiency of agriculture*. Publisher: Bibliotheka Zemedelsko Obrazovanie.
- Velička, R., Marcinkevičiene, A., Pupaliene, R., Butkevičiene, L.; Kosteckas, R., Čekanauskas, S., Kriauciuniene, Z. (2016). Winter oilseed rape and weed competition in organic farming using non-chemical weed control. *Zemdirbyste-Agriculture*, 103(1), 11–20.
- Velička, R., Mockevičiene, R., Marcinkevičiene, A., Pupaliene, R., Butkevičiene, L. M., Kriauciuniene, Z., Kosteckas, R., Čekanauskas, S. (2015). The comparison of non-chemical weed control methods efficiency in spring oilseed rape crop under the conditions of organic farming system. *Zemes ukio Mokslai*, 22(4), 189–197.

- Werner, B. (2014). Possibilities for a specific post-emergence weed control in winter oilseed rape. *Julius-Kühn-Archiv*, 443. 662–670.
- Yanchev, I., Zhalnov, I., Raykov, S. (2002). Influence of the nitrogen nutrition on grain yield at varieties of soft winter wheat in conditions of Plovdiv region. *Scientific Works of the Agricultural University of Plovdiv*, XLVII(1), 331–336.
- Zhu WenDa, Wei ShouHui, Zhang ChaoXian. (2008). Species composition and characterization of weed community in oilseed rape fields in Hubei Province. *Chinese Journal of Oil Crop Sciences*, 30(1), 100–105.
- Zotz, A., Bernhard, U., Koops, A., Gaujac, X. De. (2016). Milestone - a selective herbicide for the control of important grasses and broadleaved weeds in winter oilseed rape. *Julius-Kühn-Archiv*, 452. 355–358.

RESULTS OF RESEARCH ON JUSTIFICATION THE DEVICE FOR PRODUCING ECOLOGICALLY PURE BUTTER

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Abstract

A device for the production of environmentally friendly butter with a rotary-blade working body is presented. The research technique for substantiating the optimal parameters is presented. Based on the theory of probability and mathematical statistics, the regression equation describing the energy intensity of the process of whipping butter is determined. When solving it, the optimal design and kinematic parameters of the device for the production of environmentally friendly butter were revealed with a churning capacity of 13.4 kg/h, a butter yield of 59.5% and a fat loss of less than 0.4%. The rotational speed of the rotor-blade working body is $n_p=422 \text{ min}^{-1}$; the number of blades of the rotor-blade working body $z_p=3$; the capacity filling factor $\phi_{zap}=0.57$. Evaluation of the economic efficiency of introducing a device for the production of environmental friendly butter will reduce the energy intensity of churning by 24% compared with the mass-produced EMB-01 Salyut.

Key words: butter, environmental friendly, rotary-blade working body.

INTRODUCTION

At the present stage of the production of butter, the question of preserving traditional technologies that allow the production of various types of butter from cow's milk, as well as the use of innovative technologies that contribute to the intensification of the churning process, remains relevant for the butter industry. An important factor for obtaining a high-quality product is the use of modern oil-smelting equipment, which allows to obtain the maximum amount of the best product with the minimum energy intensity of whipping. As a result of the analysis of various designs of oil manufacturers from domestic to imported, it could be concluded that the most promising and productive are devices with non-moving horizontally mounted tanks and rotating working bodies of various designs. Their use leads to greater turbulization of the entire cream content, a decrease in stagnant zones and an increase in productivity.

In this case, the most promising are the mechanisms of knocking down with horizontally located blades, since during the churning process they provide a more thorough and intensive mixing of the cream.

MATERIALS AND METHODS

A batch-type oil producer with a rotor-blade working body (Figure 1) consists of a cylindrical horizontally located container 1, mounted on supports 2 and a rotor-blade working body 3, located with an eccentric relative to the axis of the tank (Yashin et al., 2018).

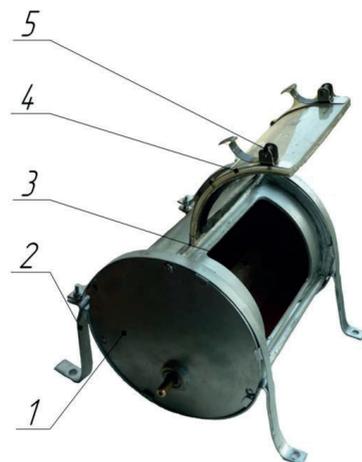


Figure 1. General view of the oil manufacturer of the periodic action: 1 - the capacity of the oil manufacturer; 2 - supports; 3 - rotor-blade working body; 4 - loading hatch; 5 - clamp

A loading hatch 4 is installed in the tank, made of transparent material (plexiglass) in order to control the churning process, as well as to supply cream before churning. The loading hatch is pressed against the container with clamps 5.

The degree of filling the tank during churning was 40-80% based on the fact that when filling the working tank with more than 80%, the cream churning process slows down and the waste of fat to buttermilk increases due to a decrease in the boundary surface of the air cream, and when filling the tank below 40 % of its volume of cream is sprayed with a rotary-blade working body onto the walls of the container and the churning process is not carried out.

The rotational speed of the rotor-blade working body was chosen at 200 min^{-1} and 600 min^{-1} based on the fact that the wave motion of the cream begins at 200 min^{-1} and ends at 600 min^{-1} . At a rotation frequency of more than 600 min^{-1} , in addition to the wave motion along the profile of the rotor-blade working body, its blades begin to be ejected by its blades (Yashin et al., 2018).

Based on the fact that the degree of collapsing and reduction of stagnant zones depends on the location of the rotor-blade working body, the eccentricity of the rotor-blade working body relative to the center of the tank was chosen to be 21.5 and 43 mm, respectively (Yashin,

Polivyannii et al., 2018; Polivyannii et al., 2017).

The principle of operation is the following. Before starting work, the installation is connected to the network via a switch.

Through the loading hatch, the tank 3 is filled with cream to 40-80% of its content. After that, the loading hatch is closed with the help of clamps. The churning mechanism 3 is brought into rotation by an electric motor, while setting the necessary rotational frequency with a frequency converter. When the rotary-blade working body 3 rotates, the cream stream is turbulized. As a result of the action of the blades, two wave fronts are formed. The front of the wave displaces the cream into their main stream, and the back pumps them out, which leads to the appearance of a "traveling wave", which makes the cream layer move along the generatrix of the rotor. With an increase in the peripheral speed of the cream, the resistance to mixing decreases and when the equality of the peripheral speed of the traveling wave and the cream is achieved, it decreases significantly, which leads to a decrease in the resistance to rotation of the churning mechanism (Yashin et al., 2018).

RESULTS AND DISCUSSIONS

The matrix of the three-factor experiment and its results are presented in Table 1.

Table 1. Matrix and results of a three-factor experiment

$N\bar{0}$	x_1	x_2	x_3	$Y, \frac{W \cdot h}{\text{butter, kg}}$
1	1	1	1	14.16
2	1	1	-1	11.45
3	1	-1	1	14.87
4	1	-1	-1	17.16
5	-1	1	1	16.06
6	-1	1	-1	12.2
7	-1	-1	1	15.16
8	-1	-1	-1	12.56
9	1	0	0	10.42
10	-1	0	0	13.11
11	0	1	0	10.41
12	0	-1	0	12.78
13	0	0	1	11.89
14	0	0	-1	9.53
15	0	0	0	7.36

Table 2. Significance Levels of Factors on Churning Energy Intensity

	a0	a1	a2	a3	a12	a13	a23	a11	a22	a33	a1122
Estimate	7.36	-0.103	-0.825	0.924	-0.87	-0.755	0.7825	4.405	4.235	3.35	-5.148

The results of experimental studies were processed by the Multiple Regression module of the Statistica 6.0 program.

As a result of processing the experimental data, a second-order regression equation is obtained that describes the dependence of the energy intensity of the whipping (W in h/kg) on the selected factors $E = f(z_n, n_p, \varphi_{3an})$ in encoded form:

$$E = 7,36 - 0,103000 \cdot x_1 - 0,825000 \cdot x_2 + 0,924000 \cdot x_3 - 0,870000 \cdot x_1 \cdot x_2 - 0,755000 \cdot x_1 \cdot x_3 + 0,782500 \cdot x_2 \cdot x_3 + 4,405 \cdot x_1^2 + 4,235 \cdot x_2^2 + 3,35 \cdot x_3^2 - 5,148 \cdot x_1^2 \cdot x_2^2 \quad (1)$$

The decoded regression equation (1) takes the form:

$$E = 17,67 - 0,193 \cdot z_n + 0,026 \cdot n_p - 15,205 \cdot \varphi_{3an} - 0,028 \cdot z_n \cdot n_p - 3,419 \cdot z_n \cdot \varphi_{3an} + 0,021 \cdot n_p \cdot \varphi_{3an} + 1,286 \cdot z_n^2 + 0,000003 \cdot n_p^2 + 18,193 \cdot \varphi_{3an}^2 + 0,000005 \cdot z_n^2 \cdot n_p^2 \quad (2)$$

The adequacy of the obtained regression equations (1) and (2) is confirmed by the multiple correlation coefficient $R_k = 0.97$ and

the convergence of the calculated and experimental data $F\text{-mecm} = 0.968$.

To determine the optimal constructive and kinematic parameters of the periodic oil producer with a rotor-blade working body, the extremum was determined when solving equation (1).

In this case, the optimal values of the factors in encoded form were: $x_1 = 0$; $x_2 = 0.112$; $x_3 = -0.15$. The obtained two-dimensional cross-sections (Figures 2, 3, 4) indicate the location of the extremum and obtaining the minimum energy capacity of knocking down the oil producer of periodic action with a rotor-blade working body.

The obtained values were interpolated for each factor according to the table. In decoded form, the optimal values of the factors are: the number of blades of the rotor-blade working body $z_p = 3$; rotor-blade working body rotation frequency $n_p = 422 \text{ min}^{-1}$; capacity factor $\varphi_{3an} = 0.57$. The energy intensity of the knocking down of the periodic manufacturer of oil with a rotor-blade working body is $E = 7.24 \text{ W} \cdot \text{h/kg}$ (Yashin et al., 2018).



Figure 2. Two-dimensional sections of the response surface, characterizing dependence of the energy intensity of whipping butter on the number of rotor blades (pcs) and rotor speed (min^{-1})

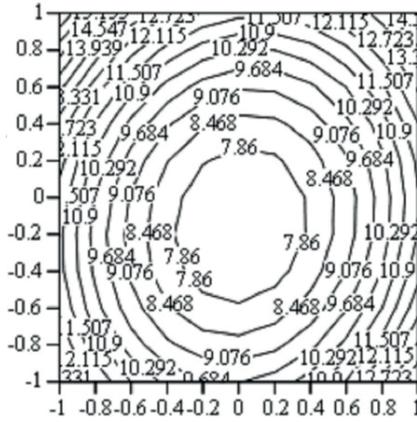


Figure 3. Two-dimensional sections of the response surface, characterizing dependence of the energy intensity of whipping butter on: the number of rotor blades (pcs) and the capacity filling factor

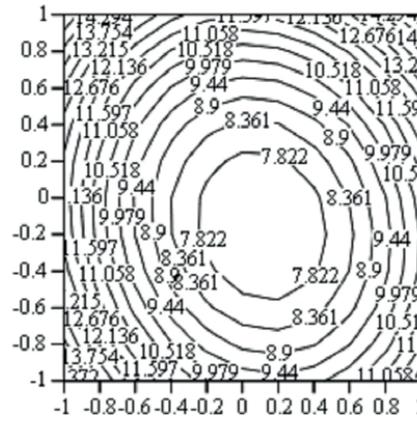


Figure 4. Two-dimensional sections of the response surface characterizing dependence of the energy intensity of whipping butter on rotor speed (min^{-1}) and fill factor capacities

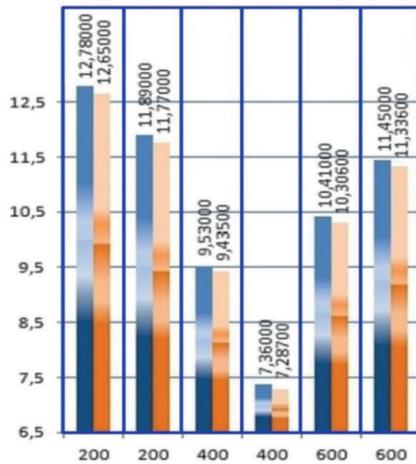


Figure 5. The dependence of the energy intensity of the churning oil manufacturer periodic action on the rotational speed of the rotor-blade working body (left columns according to the regression equation (2), right columns according to the theoretical dependence)

The dependences of the energy intensity of knocking down the oil producer of periodic action on the rotational speed of the rotor-blade working body, determined by the regression equation (2) and theoretical dependence, are shown in Figure 5 (Yashin et al., 2018). The proposed oil producer can be used both at enterprises with a large production capacity, and in farms with a small production program.

With small dimensions and weight, simplicity of the device of the oil manufacturer and its reliability in operation, it will be in demand especially for small volumes of production.

CONCLUSIONS

The analysis of theoretical and experimental values established sufficient convergence $F\text{-test} = 0.982$, and the spread of values does not exceed 1.02%. As a result, it could be argued that the application of theoretical dependence is fair. An experimental sample

of a batch-type oil manufacturer with a rotor-blade working body was developed and laboratory studies were carried out, after analysis of which the optimal design and kinematic parameters of the oil manufacturer were identified with a mass production of 13.4 kg/h, an oil yield of 59.5% and a waste of fat in buttermilk not more than 0.4%.

REFERENCES

- Polovyannii, Y.V., Parfenov, V.S., Chugunov, V.A., Yashin, A.V. (2017). *Patent* 2624925, The Russian Federation, MIIK A 01J15/00. Butter maker, № 2016104701; Published 11.07.2017, Bulletin № 20.
- Yashin, A.V., Polovyannii, Y.V., Kuderkin, N.S. (2018). *Patent* 182020, The Russian Federation, MIIK A 01J15/02, A 01J17/00. Oil press, № 2017145823; Published 31.07.2018, Bulletin № 22.
- Yashin, A.V., Polovyannii, Y.V., Mishanin, A.L., Horev, P.N. (2018). Determination of power on the drive of an oil manufacturer with a flexible vibrodrive. *Izvestiya of Samara State Agricultural Academy*, 4. 92–101.

STUDY OF THE SPREAD OF TEV IN VIRGINIA, BURLEY AND BASMI TOBACCO

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Abstract

The subject of study in the present work is the spread of TEV from the potyvirus group in Virginia, Burley and Basmi tobacco varieties. The aim is to monitor the occurrence and development of TEV in these varieties grown in the central parts of southern Bulgaria and to analyze their resistance to this virus. During the period 2010 - 2019 immunological studies were carried out to prove the new viral tobacco disease in Bulgaria. A serological identification kit of the French company SEDIAG S.A.S. was used to demonstrate the virus. To establish TEV, a TAS-ELISA was performed at 1: 100 dilution of IgG. The virus was found to be sporadic. From the eight years of the study, TEV has been proven in four. The 2010-2011 period is characterized by a significant prevalence of TEV in Burley Group tobacco (14.3% and 12.7%, respectively). The virus is most strongly developed in 2019. It is evidenced in 60% of the tested samples from the Virginia variety. During the eight-year study period, only in 2010 TEV was found in isolates from the three tobacco varieties - Virginia, Burley and Basmi. As a result of the single-factor analysis of variance and Duncan's test, it was proven that tobacco from the Basmi variety was the most susceptible to infection with the TEV virus (0.165), followed by Virginia (0.136). Burley tobacco was found most resistant to the disease of TEV (0.126).

Key words: ELISA - test, TEV, Burley, Virginia, Basmi, analysis of variance.

INTRODUCTION

Plant viruses are one of the most important pathogens causing diseases of major economic importance in many basic agricultural crops, including tobacco, reducing yield and quality (Maiss, 2004). Tobacco is a natural host for more than 20 viruses, economically the most important of which and causing significant damage to tobacco production are TMV (Tobacco mosaic virus) genus Tobamovirus; TSWV (Tomato spotted wilt virus) genus Tospovirus; CMV (Cucumber mosaic virus) genus Cucumovirus; AMV (Alfalfa mosaic virus) genus Alfamovirus; TRSV (Tobacco ringspot virus) genus Nepovirus; PVY (Potato virus Y); TEV (Tobacco etch virus) and TVMV (Tobacco vein mottling virus) genus Potyvirus, (Dukić et al., 2006; Dimitrov & Bozhukov, 2004). Viruses of the type Potyvirus are the most common tobacco viruses and cause serious economic losses in this crop. The major vector responsible for the spread of viruses in this group are the aphids of the species *Myzus persicae* Sulz (Lukas, 1975; Gooding, 1985;

Kovachevski et al., 1999). The three potyviruses PVY (Potato virus Y), TEV (Tobacco etch virus) and TVMV (Tobacco vein mottling virus) are often considered by some authors as a viral complex (Greenwell, 2011; Dietrich & Maiss, 2003; Yonchev, 2014). They have been described as viruses of major economic importance for tobacco production in the United States, especially in regions such as Tennessee, Kentucky, Virginia, and North Carolina (Pirone, 1989).

PVY has been established in a number of countries, but it is proving to be the most serious problem for tobacco production in Chile, Hungary, Spain, South Africa. Last but not least, it is a problem on the Balkan Peninsula (Gooding, 1985; Kovachevski et al., 1999) Dukić et al., 2006; Duduk & Krstic, 2006; Dekic et al., 2007).

TVMV was not described until 1970, when Gooding & Sun (1972) and Pirone et al. (1973) found a new virus attacking Burley tobacco in regions of the United States such as Tennessee, Kentucky, Virginia and North Carolina. The virus is spread in North America, Canada,

Mexico, USA, Alaska, Italy, Portugal, Zambia, Colombia and China (Kenedy, 2011; Horowitz & Ishaaya, 2004).

Tobacco Etch Virus was first reported in 1928 in the United States - Kentucky by Valleau and Johnson (1928). It was then found in Canada, Venezuela, Nicaragua, India and Japan. Mickowski (1984) believes that Tobacco Etch Virus does not occur on tobacco grown in the countries of the Balkan Peninsula. In Bulgaria, Kovachevski and others (1999) report that the disease is found in tomato and thorn apple. Dimitrov and Bozukov (2004), on the basis of symptomatic diagnosis, reported the presence of TEV in the tobacco in Bulgaria.

In 2010, Tobacco Etch Virus - TEV was diagnosed serologically by ELISA in Virginia tobacco in the Plovdiv area (Yonchev et al, 2010). The virus is commonly found in tobacco mainly in mixed infections with other viruses of the genus Potyvirus, but is most commonly found in mixed infection with PVY. Potyviruses found in tobacco cannot be distinguished solely on the basis of symptoms. Accurate diagnosis is possible by applying ELISA or PCR.

The purpose of this study is to trace the occurrence and development of TEV in the three varieties of tobacco grown in the central part of southern Bulgaria and to make a comparative assessment of their resistance to TEV.

MATERIALS AND METHODS

During the period 2010-2019, immunological studies were carried out to prove the new viral disease in Bulgaria, caused by TEV (Tobacco etch virus).

For this purpose, fresh material is collected annually in the years 2010-2014 and 2017 and 2019 from the tobacco grown on the experimental fields of the Institute of Tobacco and Tobacco Products - Markovo - leaves with symptoms characteristic of the TEV virus. Samples (isolates) were obtained from 208 tobacco plants - 92 from Virginia variety, 66 from Burley and 50 from Oriental tobacco. Samples collected were subjected to an ELISA test to determine the presence of TEV.

A serological identification kit of the French company SEDIAG S.A.S. was used to prove

the potyvirus. A TAS-ELISA was performed to determine TEV with a 1: 100 dilution of IgG. The study was performed according to the methodology of the manufacturer of the ELISA kit.

Optical density (OD) reading in the ELISA test was performed using a Biotek Elx 808 spectrophotometer at a wavelength of 405 nm, 60 minutes after substrate deposition. Signals with OD values at least twice as high as those of the negative control are considered positive.

The serological evidence of TEV was obtained from the laboratory for immunity to viral diseases of the Maritza Institute of Vegetable Crops.

C. frutescens cv *tobasco* and *D. stramonium* are used for biotest screening for TEV, which respond with specific symptoms of TEV contamination, with pepper reacting with necrosis of the plants, wilting and death of plants, and thorn apple responds to the infection simultaneously with severe deformation and wrinkling of the leaves. The latter can take on a filamentous form.

In the present study, a comparative assessment of the impact of TEV on the different varieties was made by ANOVA and Duncan test to evaluate the differences. The mathematical processing of the data was performed using the IBM Statistics SPSS 25 statistical software (Cronk, 2016; Field, 2013; McCormick et al., 2017; Meyers et al., 2013).

RESULTS AND DISCUSSIONS

According to bibliographic data, three potyviruses TVMV, TEV, and PVY are common in tobacco, which cause similar symptoms (Shew & Lucas, 1991; Kennedy, 2011; Uzest, 2007). On the basis of these data, samples were collected from plants with differing symptoms of Potato Virus Y, which were subjected to an ELISA test for the presence of the new in Bulgaria potyvirus TEV (Photos 1 and 2).

The results of the conducted analyzes strongly prove that tobacco complexes or self-contained viruses cannot be distinguished solely on the basis of symptoms (Yonchev, 2015). A number of other authors draw similar conclusions (Gooding and Lapp, 1980; Kennedy, 2011; Chi et al., 2011; Gooding & Sun, 1972). Accurate

diagnosis is possible through the use of ELISA or PCR (Kennedy, 2011).



Photo 1. Symptoms of a disease caused by TEV with tobacco isolate from the Basmi variety



Photo 2. Symptoms of a disease caused by TEV with tobacco isolate from the Virginia variety

Figures 1-5 show the results of the ELISA test performed on all 208 samples.

From Figure 5 it can be seen that the TEV potyvirus is sporadic throughout the study period. From the eight years of the study, TEV has been demonstrated in four of the reporting years. The virus was proven by ELISA - test in the test samples for two consecutive years - 2010 and 2011, after which in 2012, 2013, 2014 and 2017 the virus was not detected, but in 2018 and 2019, TEV recurred. In 2010, TEV was proven on 12 (24.5%) of the samples of the three varieties tested during the reporting year. In 2011, the virus was detected in 10 samples (15.9%), in 2018, 7 of the tested samples (20%) responded positively, and in 2019 the positive isolates were 6 (60%) (Figures 1, 2, 3 and 4).

The period 2010-2011 is characterized by a serious spread of TEV in the large-leaf tobacco of the Burley variety, 14.3% and 12.7%

respectively, while in 2018 the virus is detected in 20% of the tested samples of the Basmi variety. The virus develops most strongly in 2019. It is evidenced in 60% of the tested samples from the Virginia variety.

During the eight-year study period only in 2010, TEV was demonstrated in isolates from the three tobacco varieties - Virginia, Burley and Basmi (Figure 5).

The results from the study show that TEV does not infect tobacco annually in the study area, such as PVY. It can be concluded that when TEV develops, it can be found in all three varieties of tobacco.

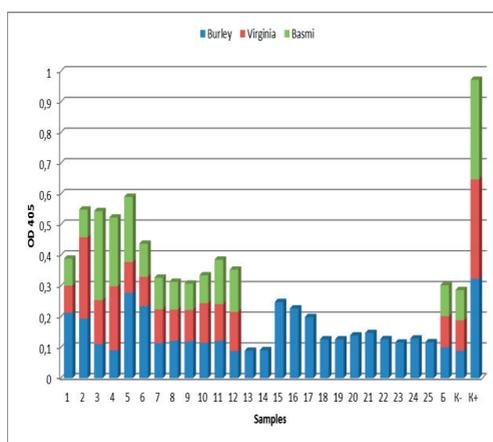


Figure 1. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2010

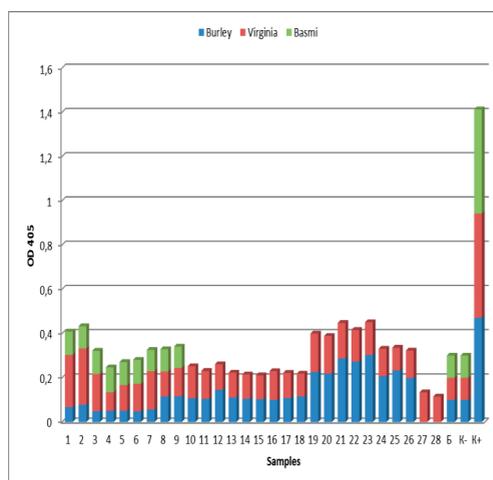


Figure 2. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2011

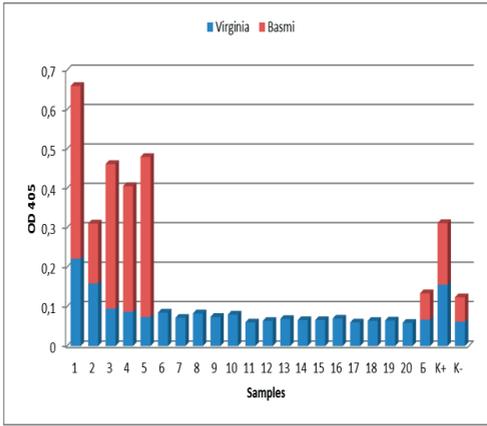


Figure 3. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2018

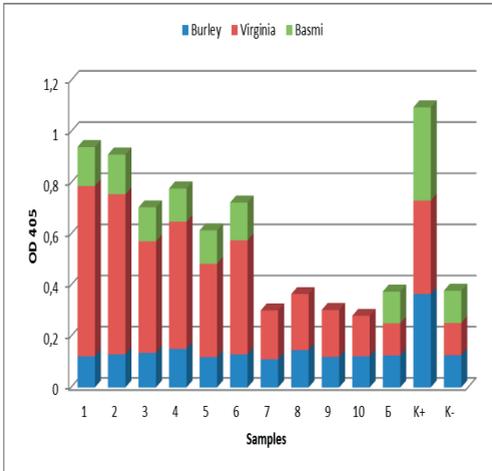


Figure 4. Identification of TEV in tobacco genotype plants of Burley, Virginia and Basmi varieties in 2019

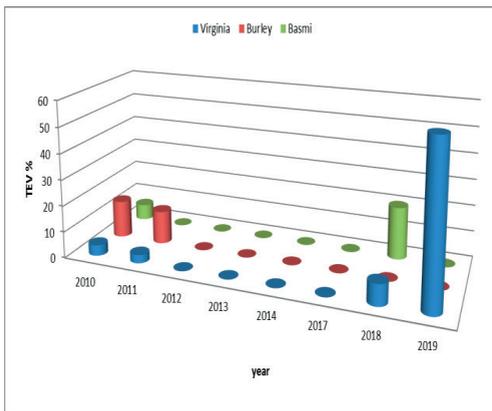


Figure 5. Percentage distribution of TEV by tobacco varieties in different years of the study

The spread of the TEV virus in different tobacco genotypes over the whole study period is at different scales, as evidenced by the comparative evaluation of Virginia, Burley and Basmi varieties. A prerequisite for its implementation is the homogeneity of the experimental data base. This was verified by the Levene’s Homogeneity Test (Test of Homogeneity of Variances, Levene Statistic). It was found that the varieties studied could be compared according to the severity of TEV.

As a result of the One-way Analysis of Variance and Duncan’s test, it was proved that tobacco from the Basmi variety was the most susceptible to infection with the TEV virus (0.165), followed by Virginia (0.136) (Table 1). The most resistant to TEV disease is Barley (0.126).

Taking into account the standard deviation values (0.042), it should be considered that Barley is not only the most resistant varietal group to TEV infection, but this stability is maintained over time. This fact proves that environmental factors do not have a significant effect on Barley morbidity. In Basmi it was proven that its high level of susceptibility to the virus is unstable over time, i.e. environmental changes have an impact on its sustainability. This fact warrants future research to determine the factors that have a positive effect on the prevention of tobacco from the Basmi variety from TEV infection.

Table 1. Comparative evaluation of tobacco varieties according to the disease rate of the TEV virus by one-way ANOVA and Duncan test at significance level $\alpha = 0.05$

Name	Duncan	Std. Deviation	Std. Error
Barley	0.126 ^b	0.066	0.008
Virginia	0.136 ^{ab}	0.042	0.007
Basmi	0.165 ^a	0.108	0.021

CONCLUSIONS

The TEV virus appears sporadically. From the eight years of the study, TEV has been demonstrated in four of the reporting years - 2010, 2011, 2018 and 2019.

The period 2010-2011 is characterized by a high prevalence of TEV in the large-leaf tobacco of the Burley variety, 14.3% and 12.7% respectively.

The highest spread of the virus is in 2019. It is evidenced in 60% of the tested samples from the Virginia varietal group. During the eight-year study period, only in 2010, TEV was proven in isolates from the three tobacco varieties - Virginia, Burley and Basmi. Basmi variety tobacco is the most susceptible to infection with the TEV virus, followed by Virginia, and the most resistant is Barley. In addition, environmental factors do not have a significant effect on Barley morbidity. TEV can be found in all three varieties of tobacco when it has the right conditions to manifest itself.

REFERENCES

- Chi, E., Hunter, M., Swanson, R. (2011). Engineered Plant Cysteine Proteases and Their Uses. US Patent Application Publication No. WO2011130533 A1.
- Cronk, B. (2016). How to Use SPSS: A Step-By-Step Guide to Analysis and Interpretation, Routledge.
- Đekić, I., Bulajić, A., Zindović, J., Berenji, J., Pauković, M., Krstić B. (2007). Identifikacija sojeva virusa crtičastog mozaika krompira na duvanu. *Pesticides. Phytomed. (Belgrade)*, 22, 155–163.
- Dimitrov, A., Bozhukov, H. (2004). Virus diseases in tobacco in Bulgaria and the fight against them. *Bulgarian Tobacco*, 5, 11–18.
- Dietrich, C., Maiss, E. (2003). Fluorescent labelling reveals spatial separation of potyvirus populations in mixed infected *Nicotiana benthamiana* plants. *J. General Virology*, 84, 2871–2876
- Duduk, B., Krstić, B. (2006). Nekrotični soj virus crtičastog mozaika krompira na duvanu u Srbiji. Zbornik Regime VIII savetovanja o zaštiti bilja, Zlatibor, 67–68.
- Dukich N., Bulajich A., Berenji J., Dekich I., Duduk B., Krstich B. (2006). Presence and Distribution of Tobacco Viruses in Serbia. *Pesticides. Phytomed. (Belgrade)*, 21, 205–214
- Field, A. (2013). Discovering Statistics using IBM SPSS Statistics, Mobile Study, London.
- Gooding, G. & Jr. (1985) Relationship between strains of potato virus Y and breeding for resistance, cross-protection and interference. *Tobacco Science*, 29, 99–104.
- Greenwell, R. (2011). Selecting a burley tobacco variety.
- Gooding, G., Jr., Sun M. (1972). A newly recognized virus disease of burley tobacco in North Carolina. *Phytopathology*, 62, 803.
- Gooding, G., Jr., Lapp N. (1980). Distribution, incidence and strains of potato virus Y in North Carolina. *Tob. Sci.*, 24, 89–92.
- Horowitz, A.R., Ishaaya, I. (2004) Insect pest management: field and protected crops, p. 37
- Kennedy, B. (2011). CORESTA integrated pest management taskforce, 31-34 pp. http://www.uky.edu/Ag/Agronomy/IPM_Taskforce/assets/jan2011_update/Final%20Report%20Jan-18-2011.pdf
- Kovachevski, I., Markov, M., Yankulova, M., Trifonov, D., Stoyanov, D., Kacharmazov, V. (1999). Virus and virus-like diseases of crop plants, Sofia, Pensoft.
- Lukas, G. (1975). Diseases of tobacco. Biological Consulting Associates, North Carolina, pp. 493-494.
- Maiss, E. (2004). Recombination and spatial separation of potyviruses in transgenic plants and in mixed infections, *The 8th International Symposium on the Biosafety of Genetically Modified Organisms, Montpellier, France, 26-30*, 39–41.
- McCormick, K., Salcedo, J., Peck, J., Wheeler, A. (2017). SPSS Statistics for Data Analysis and Visualization, John Wiley and Sons, Inc.
- Meyers, L., Gamst, G., Guarino, A. (2013). Performing Data Analysis Using IBM SPSS, Wile
- Mitskovsky, J. (1984). Tougnotic Diseases. Skopje, 223–229.
- Pirone, T., Gooding G., Jr., Smiley J. (1973). Tobacco vein mottling virus on burley tobacco in Kentucky. *Plant Dis. Repr.*, 57, 841–844
- Pirone T. (1989). Comparison of tobacco vein mottling and pepper veinal mottle viruses. *Plant Disease*, 73, 336–339.
- Shew, D., Lucas, G. (1991). Compendium of Tobacco Diseases. APS Press.
- Uzest, M., Gargani, D., Drucker, M., Hubrard, E., Garzo, E., Candres, T., Fereres, A., Blanc, S. (2007). A protein key to plant virus transmission at the tip of the insect vector stylet. *Proc. Natl. Acad. Sci.*, 104, 17959–64.
- Valleau, W., Johnson, E. (1928). Some virus diseases of tobacco in Kentucky. *Phytopathology*, 18, 132–133.
- Yonchev, Y., H. Bozukov, E. Stoimenova, Pasev, G. (2010). Tobacco ETCH virus - tev on tobacco in Bulgaria, 33–36.
- Yonchev, Y. (2014). Study of the prevalence of some viral diseases and their resistance to large-scale tobacco in southern Bulgaria. Dissertation.

INFLUENCE OF THE FIELD STORAGE ON THE CONTENT OF BASIC MOLASSES IN ROOTS OF VARIOUS SUGAR BEET HYBRIDS

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Abstract

An important criterion in assessing varieties and hybrids of sugar beet is their ability to long-term storage. Therefore, the selection of sugar beet hybrids, which have good keeping quality, is becoming one of the pressing issues of the sugar beet industry. Changes in the quality indicators of root mass during storage were monitored after 30 and 60 days according to the generally accepted method, which provides for laying mesh samples. As a result, it was found that in the root mass of hybrids of normal type the content of α -amino nitrogen increased after 60 days of storage by 2.5-2.9 times compared with the initial state, hybrids of normal yielding type - by 2.7-3.0 times, normal sugar type - 3.3 times, and sugar type - more than 4 times. The accumulation of sodium in root mass in all hybrids occurred approximately equally. For three years of research, after 60 days of storage, the most sodium was found in root mass of the normal compact and Hercules hybrids, and least of all - in the root mass of sugar type.

Key words: α -amino nitrogen, potassium, sodium, field storage, sugar beet.

INTRODUCTION

The solution to the problem of obtaining high and stable sugar beet crops with good technological properties of root mass is often limited to the use of foreign varieties and hybrids that are not adapted to local conditions. Recently, many sugar beet producers have switched to using other seed material for sowing, guided mainly by its high yield, not taking into account the yield of the main product - sugar after storage and processing (Loel et al., 2014).

In recent years, in the Russian Federation there has been a significant increase in the production of sugar beet and the volume of its preparation by sugar factories. In these conditions, the preservation of the grown crop and timely processing become one of the urgent issues of the beet-growing industry. During the mass-harvesting of beets, sugar plants accumulate a large amount of raw materials, which cannot be processed immediately. Since sugar factories cannot immediately accept the entire harvest, the root crops are stored for storage in kagats (Kostin et al., 2017; Stevanato et al., 2019). In the first 5-10 days after harvesting, a significant part of the sugar loss occurs (daily 0.15%). During this time,

approximately the same amount of sugar is lost as in the following 60-70 days of storage in the unloading units (Bobrivnyk et al., 2016).

An important criterion in assessing varieties and hybrids of sugar beet is the ability of root mass to long-term storage (Monteiro et al., 2018). The traditional method of storing sugar beet root mass before processing at sugar factories is to put them in kagats (Dolgopolova, 2011). This is not the best way, but in the soil and climatic conditions of Russia it justifies itself. With such storage, losses occur for unavoidable reasons associated with biochemical respiration reactions and for dependent reasons caused by the phytosanitary state of root mass and weather conditions.

MATERIALS AND METHODS

The studies were conducted in the conditions of the local company "Krasnaya Gorka" in the Kolyshleysky district of the Penza region (Russia). Sugar beet hybrids were studied in the experiment: 1 - F₁ PMC 120; 2 - F₁ Compact; 3 - F₁ Hercules; 4 - F₁ Spartak; 5 - F₁ XM 1820; 6 - F₁ Nero; 7 - F₁ Triad; 8 - F₁ Badia; 9 - F₁ Volga. Soil - medium-loamy leached chernozem, whose arable layer was characterized by the following agrochemical

parameters: humus content of 5.64%, mobile forms of nitrogen 103 mg/kg, phosphorus and potassium - 94 and 100 mg/kg, respectively; pHKCl - 5.5, Ng - 4.8 mEq/100 g, S - 29.8 mEq/100 g of soil. For storage, a site was selected at the edge of the field.

The reclaimed earth area before laying the beets was leveled, watered and treated with cannon-lime (0.2 kg/m²). Root mass laid for storage was assigned to category II, designed for medium storage periods (up to 2 months). Kagati without shelter were formed during the harvesting period, placed near a dirt road with orientation from north to south.

Kagat sizes: 3-4 m high and base width 12-16 m. Changes in the quality indicators of root mass were monitored after 30 and 60 days of storage in accordance with the generally accepted method that provides for the laying of mesh samples. For this, samples were formed that were homogeneous in mass, phytopathological state, and quality. The mass of each of them is 12 kg. Samples were weighed, labeled, analyzed in order to determine the initial quantitative and qualitative indicators of the beetroot being driven and placed in storage in the appropriate section of the beetroot to a depth from the upper part of 1.0-1.5 m. The quality indicators were determined in 30 and 60 days. After the expiration of the storage period, the nets were removed from the cages and the same indicators were determined as in the bookmark: the sucrose content was measured using an AP-05 polarimetric sugarmeter, potassium and sodium using a potentiometric method, and α -amine nitrogen was photometric method. The experiment was carried out in triplicate.

RESULTS AND DISCUSSIONS

After harvesting, sugar beet roots are a qualitatively different biological organism compared to vegetation. During storage, hydrolytic decay processes predominate and natural changes in the composition of sugar beet occur. All this leads to a deterioration in

the technological qualities of sugar beets, a decrease in the sucrose content and the accumulation of non-sugars. When storing intact root mass under optimal conditions, with a correctly trimmed head, sucrose is consumed primarily for respiration and the magnitude of these losses is negligible. But during storage of damaged root mass containing a large amount of green mass, earth and other impurities, breathing processes are enhanced and sucrose losses increase dramatically.

It is known that during storage in root mass complex physiological, biochemical, microbiological and other processes occur that lead to the loss of sucrose, to the accumulation of nob-sugar - mainly potassium, sodium and alpha-amine nitrogen, which interfere with traction of crystallized sugar remaining in molasses.

Researchers have found that after 30 days of storage, the sugar content of root mass decreased by 0.32-1.02%. The smallest sugar loss during storage - 0.32% - was observed in the F1 Triad hybrid, and the largest sugar loss was observed in the F1 Spartak hybrid (1.02%). In hybrids of the normal type, the loss of sugar content was 0.35-1.02%, of the normal-crop type - 0.93-0.94%, of the normal-sugar type - 0.32-0.36%, of the sugar-type type - 0.69%.

After 30 days of storage in the root crops of all the studied hybrids, an increase in the content of the main molas co-formers: K, Na, and α -amino nitrogen was observed. The potassium content increased by 0.016-0.038%, sodium - by 0.003-0.049%, depending on the hybrids. The maximum amount of sodium was accumulated in the root crops of the F1 Compact hybrid - 1.44 mmol/100 g, the minimum - of the sugar Volga F1 hybrid - 0.78 mmol/100 g.

The increase in the content of α -amino nitrogen was from 0.015% to 0.023% by weight of the root mass, and the maximum content of this molass former was in the hybrid of the normal type F1 PMC 120 and amounted to 3.43 mmol/100 g sugar beets (Figure 1).

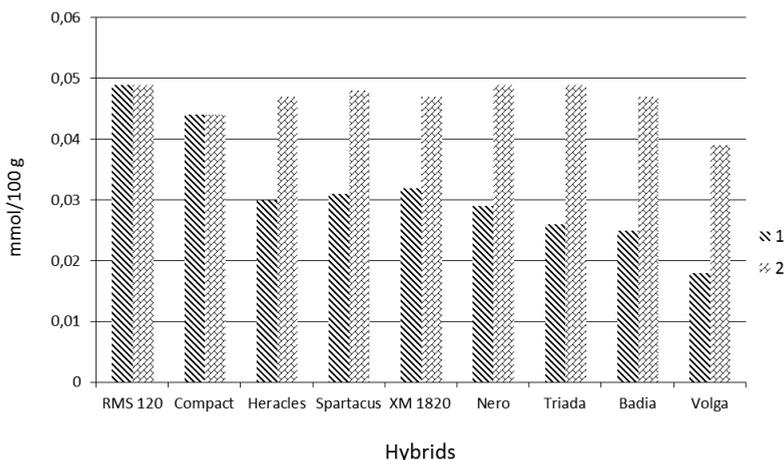


Figure 1. The content of α -amino nitrogen in the roots of sugar beets: 1 - before laying it for storage; 2 - 30 days after storage

It was found that during storage of the fluctuation of air temperature with a change from negative to positive, they significantly influenced the content of harmful nitrogen, since when thawing root mass, most of the sucrose turns into invert sugar, the amount of harmful nitrogen increases, the sugar yield decreases and the molasses yield.

After 60 days of storage, a tendency toward gradual stabilization of the technological parameters of sugar beet root mass was noted.

On average, over the years of observation after 60 days of storage, the following changes in the technological qualities of root fruits were noted. The potassium content in the root crops of the normal type increased by 17.2-26.9%, except for the F₁ Spartak hybrid, in which the content of this molass former increased by 9.5%. In the root mass of hybrids of the normally productive type, the amount of potassium was 1.03 mmol/100 g (F₁ Nero) and 0.897 mmol/100 g (F₁ XM 1820). Of all the studied hybrids, the minimum increase in the content of potassium during the storage period was observed in root-sugar-type root mass: in the Triad hybrid, the increase was 17%, in the F₁ Badia hybrid - 15.8%. After storage in the roots of the Volga hybrid F₁ (sugar type), the amount of potassium was 4.127 mmol/100 g, i.e. an increase of 27.8%.

During storage, there was an increase not only in potassium, but also in sodium. On average,

over four years, after 60 days of storage, the most sodium was found in root mass of normal hybrids F₁ Compact (1.590 mmol/100 g) and F₁ Heracles (1.580 mmol/100 g), and least of all in root mass of hybrids yes F₁ Volga (sugar type) - 1.023 mmol/100 g. The amount of α -amino nitrogen after storage in all hybrids ranged from 5.880 to 6.057 mmol/100 g, except for the F₁ Volga hybrid - in the roots of this hybrid it was found 5.323 mmol/100 g. Changes in the content of the main molasses co-formers in root mass after the entire storage period had a direct the influence on standard sugar losses during molasses formation. The results of the studies showed that in the initial storage period (up to 30 days), the main losses of sugar occur, gradually decreasing in the subsequent period. After root crops of F₁ RMS 120 hybrid, after storage for 60 days, the standard sugar loss, which is calculated based on the content of potassium, sodium and "harmful" nitrogen, was 2.78% (Table 1).

Hybrids F₁ Compact and F₁ Hercules (normal type) losses were 2.66 and 2.70%.

In hybrids of the normally productive type (F₁ XM 1820 and F₁ Nero) sugar losses were at the level of the domestic hybrid F₁ PMC 120, in normal-sugar hybrids (F₁ Triad and F₁ Badia) they were 0.17 and 0.21% less than the F₁ PMC 120 hybrid. The sugar-type hybrid has a loss of sugar of 2.38%.

Table 1. Standard sugar loss by sugar beet during the formation of molasses, %

Hybrid F ₁ Between	During harvesting	After 60 days of storage
Normal type		
PMC 120	1.79	2.78
Compact	1.61	2.66
Hercules	1.62	2.70
Spartacus	1.75	2.79
Normal yielding type		
XM 1820	1.72	2.77
Nero	1.69	2.77
Normal sugar type		
Triad	1.44	2.61
Badia	1.43	2.57
Sugar type		
Volga	1.22	2.38

One of the indicators of the quality of root mass is the content of purified sugar. The research results showed that hybrids of normal and normal-yield type in terms of sugar storage loss were approximately equivalent, while normal-sugar and sugar type had a positive tendency to reduce sugar loss. For 60 days of storage, on average, for three years of research, the advantage in the content of refined sugar is 15.25-15.80% for hybrids of the normal-sugar and sugar type.

Thus, as a result of the studies, it was found that hybrids of foreign selection related to normal-sugar and sugar types lose more refined sugar during storage than hybrids of normal and normal-yielding types. At the same time, the loss of refined sugar in the F₁ Triad hybrid was 1.11%, F₁ Badia - 1.27% and the F₁ Volga hybrid - 1.28%.

According to the total assessment, which includes the loss of mass of root mass and the amount of molasses forming substances and the final indicator of root crop processing - the yield of refined sugar from 1 ton of root fruits after 30 and 60 days of storage, hybrids of normal-sugar F₁ Triad, F₁ Badia and sugar type are distinguished F₁ Volga.

Hybrids F₁ PMC 120 and F₁ Compact, whose root mass is less adapted to storage conditions in the first 30 days of storage, are better stored in the next 30 days, and hybrids of normal-crop type - on the contrary - losses increase (Table 2 and Table 3).

The effectiveness of using hybrids of different breeding in sugar processing depends on the genetic characteristics and storage conditions.

Table 2. Adaptation features of hybrids when stored in the sacks within 30 days, score

Hybrid F ₁	Mass loss	Quantity			Total score	The yield of refined sugar from 1 ton of root mass
		K	Na	α- amino nitrogen		
30 days						
Normal type						
PMC 120	93.4	115.7	104.3	105.3	419	96.6
Compact	97.4	103.2	123.1	95.1	419	97.7
Hercules	102.9	98.5	121.9	101.2	425	99.6
Spartacus	106.6	112.8	119.4	103.2	442	97.5
Normal yielding type						
XM 1820	100.1	112.6	92.9	100.9	407	98.2
Nero	104.6	112.4	105.4	104.3	427	98.0
Normal sugar type						
Triada	97.4	83.6	81.2	105.2	367	102.3
Badia	99.6	83.5	81.5	101.3	366	102.8
Sugar type						
Volga	98.5	77.8	66.7	83.6	327	105.7
Average	100.0	100.0	100.0	100.0	400.0	100.0

Table 3. Adaptation features of hybrids when stored in the sacks within 60 days, score

Hybrid F ₁	Mass loss	Quantity			Total score	The yield of refined sugar from 1 ton of root mass
		K	Na	α - amino nitrogen		
60 days						
Normal type						
PMC 120	93.5	115.3	99.7	101.4	410	97.3
Compact	96.3	103.2	119.5	95.3	414	98.0
Hercules	102.0	98.5	118.8	100.6	420	99.5
Spartacus	103.2	112.6	117.8	100.9	435	97.3
Normal yielding type						
XM 1820	102.9	112.6	91.7	102.5	410	97.7
Nero	102.7	111.9	104.0	101.6	420	97.6
Normal sugar type						
Triada	99.8	84.2	85.2	104.4	374	101.8
Badia	99.7	84.6	89.0	100.9	374	102.3
Sugar type						
Volga	99.4	77,7	76,9	91,8	346	105,7
Average	100.0	100.0	100.0	100.0	400.0	100.0

The hybrids F₁ Triad, F₁ Badia and F₁ Volga are the most adapted to storage conditions in field piles and able to give the greatest yield of purified sugar from 1 ton of root mass after 60 days of storage.

CONCLUSIONS

Thus, the studies showed that in the roots of hybrids of normal type, the content of α -amino nitrogen increased after 60 days of storage by 2.5-2.9 times compared with the initial state, hybrids of the normal crop direction - by 2.7-3.0 times, normal-sugar direction - 3.3 times and sugar-more than 4 times. Studies have established that the content of "harmful" nitrogen increases sharply during storage, especially after 30 days of storage. Of all the studied hybrids, the minimum increase in potassium content over 60 days of storage was noted in normal-sugar-type root mass - 0.617-0.683 mmol/100 g. The accumulation in the root crops of sodium in all hybrids occurred approximately equally and amounted to 0.006 - 0.014% by weight depending on the hybrid. The root mass of the F₁ hybrid PMC 120 was more resistant to environmental factors, having an adverse effect on the technological performance of sugar beets. The loss of refined sugar during storage in this hybrid was only 0.91%, which is 1.2-1.4 times less than in other hybrids.

REFERENCES

- Bobrivnyk, L.D., Bovkun, N.P., Bobrovnyk, S.L. (2016). Beet saponin: Distribution in sugar beet root and in processing streams at beet sugar factories. *International Sugar Journal*, T. 118(1413).
- Dolgoplova, N.V. (2011). Storage and processing of crop products (for example, sugar beets). *Urals Agrarian Bulletin*, 3(82), 66–69.
- Kostin, V.I., Isaichev, V.A., Oshkin, V.A. (2017). Technologic qualities of sugar beet root crops in foliage application of melafen and trace elements. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, T., 8(1), 1780–1787.
- Loel, J., Kenter, K., Marlander, B., Hoffmann, C.M. (2014). Assessment of breeding progresses in sugar beet by testing old and new varieties in greenhouse and field conditions. *European Journal of Agronomy*, 52, 146–156.
- Monteiro, F., Frese, L., Castro, S., Duarte, M.C., Paulo, O.S., Loureiro, J. and Romeiras, M.M. (2018). Genetic and genomic tools to assist sugar beet improvement: The value of the crop wild relatives. *Frontiers in Plant Science*, 9.,74.
- Stevanato, P., Chiodi, C., Broccanello, C. (2019). Sustainability of the Sugar Beet Crop. *Sugar Tech.*, 21, 703–716.

INFLUENCE OF ORGANIC FERTILIZERS ON SOME QUANTITATIVE AND QUALITATIVE INDICATORS OF STRAW AND HARVEST INDEX (HI) ON WILD WHEATS (*Triticum dicoccum* Sch., *Triticum spelta* L. AND *Triticum monococcum* L.) IN ORGANIC FARMING

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Abstract

Worldwide, as in Bulgaria, the significance of crops as *Triticum dicoccum* Sch., *Triticum spelta* L., and *Triticum monococcum* L. consistently increases. This is due to the rich genome of crops on one hand and their importance as a healthy food for people (low gluten content, etc.) on the other. In the present study influence of five types of fertilizers on organic farming on some quantitative (straw yield), qualitative (content of cellulose) and the Harvest Index (HI) for three types of wheat - *Triticum dicoccum* Sch., *Triticum spelta* L., and *Triticum monococcum* L. has been researched. The field experiment was conducted during the 2014-2017 period at the Demonstration Center for Organic Farming at the Agricultural University of Plovdiv, Bulgaria. Treatment with Amalgerol and Baikal EM-1U increases the yield of straw at *Triticum dicoccum* Sch. (4538 kg/ha and 4527 kg/ha, respectively), and Tryven treatment in all three species of wild wheat. Tryven increases cellulose content in straw at *Triticum dicoccum* Sch. (42.1%), Baikal EM-1U (46.3%) at *Triticum spelta* L., Lithovit at *Triticum spelta* L. (45.8%) and *Triticum monococcum* L. (43.25%). Highest cellulose content in the straw was recorded at *Triticum spelta* L., followed by *Triticum monococcum* L., and *Triticum dicoccum* Sch. All biofertilizers increase HI at *Triticum monococcum* L.

Key words: organic farming, harvest index, *Triticum dicoccum* Sch., *Triticum spelta* L., *Triticum monococcum* L.

INTRODUCTION

Agroecosystems are open systems that exhibit certain stability in functioning of biological cycles. One of the aims of agroecosystems is to obtain higher biomass (i.e. biological productivity) (Vlahova and Popov, 2014a). In recent years, the cultivation of the ancient wheat species *Triticum spelta* L. (dinkel), *Triticum monococcum* L. (einkorn) and *Triticum dicoccum* Sch. (emmer) is of great interest in the conservation of genetic resources and biodiversity in agriculture. They are particularly suitable for organic farming because of their identity (Wolfe et al., 2008), and interest in producing organic and healthy foods draws attention to their study. *Triticum dicoccum* Sch. also grows on nutrient-poor soils. This makes the species suitable for cultivation of less fertile soils in mountainous areas (Marino et al., 2009; Giuliani et al., 2009).

Triticum dicoccum Sch. is tetraploid wheat (2n = 28). Their straw is not as coarse as that of *Triticum aestivum* L. or *Hordeum sativum* L. It

is suitable as rough forage for ruminants. The use of fertilizers leads to higher yields and grain quality, but also to a better development of the green mass, resulting in more straw. In recent years, biofertilisers have emerged as a promoting component of an integrated nutrient supply system in agriculture (Vlahova et al., 2014b). Strong variations in the straw:grain ratio are observed depending on the variety of wheat, and depending on the presence or absence of awns (Koutev et al., 2014). Another study indicates the role of nitrogen fertilizer norm and that the harvest index, characterizing the proportion of grain in total biomass, is lowest in the non-tiller variants. This is a consequence from the formation of fewer grains in the low mass class (Kirchev et al., 2014). According to Dai et al. (2016) though strong relationships may exist among HI and yield parameters, and high HI does not necessarily suggest high grain yield or low straw yield; and vice versa. The low HI, observed in ND in 2009 coincided with severe foliar fungal diseases caused by, *Septoria* spp. and leaf rust. Therefore it is possible that biotic

stresses may also affect HI. Other contributing environmental factors include pest and disease activity. Foliar fertilization is a necessary additional activity in the overall system of optimal mineral nutrition of plants, which supplements the effect and corrects.

When considering wheat straw as a commodity, it is important to understand the links between biomass, grain production and straw production.

Harvest index (HI), indicates directly the distribution of biomass to grain and indirectly the partitioning between grain and straw production. Grain yield is proportional to harvest index and their correlation is 1.00, because biological yield and harvest index are unrelated (Donald and Hamblin, 1976). HI values for modern wheat varieties usually range within 0.3-0.6 (Hay, 1995). A number of studies show that HI is influenced by genetic changes within a particular class of cereals and environmental changes in a particular climate region. Larsen et al. (2012) find, that the difference in straw production in the years and places of cultivation cannot be avoided, but the choice of varieties with higher straw yields can be successfully used as a way to increase the straw's resource in biofuel production.

HI is influenced by abiotic factors (Akram, 2011) as well as early or late sowing date (Donaldson et al., 2001).

Effective weed control in winter field crops is an essential part of successful and profitable agricultural production (Mitkov et al., 2017). Large-scale weeding in winter wheat (*Triticum aestivum* L.) can reduce yields by up to 70% (Tonev et al., 2019). The purpose of the study was to investigate the effect of some leaf fertilizers supplementing nutrition on straw yields, some quality indicators of straw and harvest index (HI) in *Triticum dicoccum* Sch., *Triticum spelta* L. and *Triticum monococcum* L. in organic farming conditions.

MATERIALS AND METHODS

The study was conducted at the experimental field in the Agroecological center - Demonstration center of organic farming the Agricultural University of Plovdiv (Bulgaria) during the 2014-2017 period.

A two-factor field experiment, based on block method has been set in three replications and with a plot size of 10.5 m². Sowing was carried out in mid-October with seed rate of 500 g.s./m², after a pepper as a predecessor. The main fertilization was carried out with organic fertilizer Agriorgan pellet at a dose of 1000 kg/ha.

The following factors have been studied: Factor A - wheat species A1 - *Triticum dicoccum* Sch.; A2 - *Triticum spelta* L.; A3 - *Triticum monococcum* L., and Factor B - Organic fertilizers: B1 - Amalgerol; B2 - Lithovit; B3 - Baykal EM - 1U; B4 - Tryven. Soil fertilizing Agriorgan pellet - 1000 kg/ha for all variants of the experiment; Amalgerol, Lithovit, Baykal EM - 1U, Tryven - foliar fertilizers, sprayed during the vegetation of the wheats. The following indicators were studied: straw yield (kg/ha); cellulose content in straw (%), harvest index (HI).

Fertilizers used: Amalgerol® is a liquid fertilizer rich in hydrocarbons and natural plant growth hormones. Contains extracts from seaweed, distilled paraffin oil, vegetable oils, distilled herbal extracts. Stimulates the growth of plants, increases the quality and quantity of crop yields in grain production.

Lithovit contains 79.19% (CaCO₃) Calcium Carbonate, 4.62% (MgCO₃) Magnesium Carbonate, 1.31% (Fe) Iron. It is applied as leaf manure for fodder crops, trenches, meadows and pastures.

Baykal EM - 1U - probiotic product containing useful microorganisms (lactic acid bacteria, yeast, bifidobacteria, ferments and spore bacteria) that are antagonists of pathogenic and conditionally pathogenic microflora. The preparation contains a large group of microorganisms that live in a mode of interaction with a nutrient medium.

Tryven contains Nitrogen (N) total 24.4%, Ammonium Nitrogen (N) 2.60%, Nitrate Nitrogen <0.01%, Carbamide Nitrogen 4.47%, Organic Nitrogen 17.3%, Phosphorus (P₂O₅) water-soluble 17.2%, potassium (K₂O) water-soluble 7.42%. It is a complex mixture of NPK intended for leaf-feeding use.

Agriorgan Pellet - organic fertilizer made of sheep fertilizer, enriched with microorganisms and microelements. Contains: Total Nitrogen (N) - 3.0%, Organic Nitrogen (N) - 2.5%,

Phosphorous Oxide (P₂O₅) Total - 3.0%, Organic (K₂O) carbon (C) - 28.5%, Humic acids - 6.0%. The biochemical analysis of the straw for cellulose content is made by Šchtoman and Heneberg method. Statistical processing of the data was carried out with the program SPSS for Microsoft Windows (SAS Institute Inc. 1999).

RESULTS AND DISCUSSIONS

Straw yield, kg/ha

Studies on the productive capacity of cereals in Bulgaria are focused mainly on grain production, however there is insufficient information on straw production and data on organic farming are lacking.

Determination of straw yields enables the production cycle to be closed, as well as for the agro-ecosystem (when used as an organic substance) to function better on the organic farm.

The study period is characterized as suitable for crop development, but no three-way trend for straw production is observed in all three wheat varieties (*Triticum dicoccum* Sch, *Triticum*

spelta L. and *Triticum monococcum* L.), since climatically in the three years they differ from each other both in the distribution of rainfall and in temperature.

Straw extraction is an indicator that is formed on the basis of the complex influence of soil-climatic conditions and factors that directly influence the absorption of nutrients, as well as the specific microclimate during the particular year.

Average over the study period, it can be seen that the type is a strong factor in the straw yield indicator. This influence is confirmed by a study by other authors (Koutev et al., 2014), according to which the ratio of straw and grain varies depending on the species, variety, acuminate or powerless. *Triticum monococcum* L., proven to realize the highest yield of 5738.2 kg/ha compared to the other two wheat species, with no difference between them (Table 1). There is no tendency in results across years, based on the preparations used. Only Tryven treatment on *Triticum dicoccum* Sch. and *Triticum spelta* L. show a trend in increasing straw yields. However, this effect in *Triticum monococcum* L. varies widely.

Table 1. Straw yield (kg/ha) according to the applied organic fertilizer and the type of wheat during the study period

Variant	Straw, kg/ha											
	<i>Triticum dicoccum</i> Sch.				<i>Triticum spelta</i> L.				<i>Triticum monococcum</i> L.			
	Year	2015	2016	2017	Average of the period	2015	2016	2017	Average of the period	2015	2016	2017
Controla (Agriorgan pellet)	3481c	4445c	4800b	4242ab	4089b	4167b	4587b	4281ab	5782bc	5812c	6987a	6194a
Amalgerol	4282b	4331c	5000b	4538ab	3447c	5081ab	3521c	4016ab	4499d	6764b	5533b	5599a
Lithovit	3605c	4479c	4200c	4095b	3454c	4585ab	3267c	3768b	6088a	6668b	5453b	6069a
Baykal EM	3596c	4773b	5213b	4527ab	3561c	2943c	4083b	3529b	5420c	3213d	4961c	4531a
Tryven	4782a	5296a	5840a	5306a	4217a	5407a	5760a	5128a	5903b	7151ab	5840ab	6298a
Wheat species	4541.5b				4144.6b				5738.2a			

*Means followed by the same letter are not statistically different (P<0,05) by Duncan's multiple range test

During the study period, the application of foliar fertilizers showed that Tryven treatment, proven leads to increasing in straw yields at

Triticum dicoccum Sch. - 5306 kg/ha and *Triticum spelta* L. - 5128 kg/ha, compared to controls, fertilized only with Agriorgan

pellets. This shows the positive effect of foliar Tryven feeding and the nitrogen contained in it to accumulate more biomass. The influence of nitrogen in the fertilizer rate is confirmed by another authors, (Kirchev et al., 2014). The straw yield in *Triticum monococcum* L. is also highest when Tryven was applied - 6298 kg/ha, but tis nis not statistically proven, compared to the control. The effect of the other foliar fertilizers varied over the years, taking into account the proven values of the indicator compared to the control after application of Amalgerol and Lithovit.

Cellulose content in straw, %

The cellulose content in straw varied across the wheat varieties in the range of 33.34-46.27% in the different variants, but compared to the cellulose content of the common wheat (*Triticum aestivum* L.) - 39.5% were quite

similar. This indicates that the straw of the ancient wheat species can be used as a substitute for that of common wheat.

With highest cellulose content is characterized the straw of *Triticum spelta* L., followed by the straw of *Triticum monococcum* L. Its lowest content is at *Triticum dicoccum* Sch. With few exceptions, the application of the studied biofertilizers increased the content of cellulose in leafs of all three wheat species relative to their controls (Table 2).

There is a species reaction of wheat, about the cellulose accumulation in straw due to fertilization. The strongest effect is reported at Lithovit-treated variants, namely, a decrease in cellulose % in straw in *Triticum dicoccum* Sch. - 33.34% and a significant increase in *Triticum spelta* L. - 45.88% and *Triticum monococcum* L. - 43.25% over their controls.

Table 2. Harvest index (HI) and cellulose content in straw of the studied species and variants averaged for the study period

Variant	Cellulose, %	Grain yield, kg/ha	Straw yield, kg/ha	Aboveground biomass, kg/ha	HI
<i>Triticum dicoccum</i> Sch.					
Wheat species	-	2218.3a	4541.5b	6759.8	0.489a
Control (Agriorgan pellet)	36.53	2175.5a	4242ab	6417.5	0.510a
Amalgerol	36.35	2182.8a	4538ab	6720.8	0.476a
Lithovit	33.34	2172.2a	4095b	6267.2	0.533a
Baykal EM	38.53	2180.4a	4527ab	6707.4	0.483a
Tryven	42.14	2380.4a	5306a	7686.4	0.443a
<i>Triticum spelta</i> L.					
Wheat species	-	2520.9a	4144.6b	6662.5	0.498a
Control (Agriorgan pellet)	43.79	2389.5a	4281ab	6670.5	0.550a
Amalgerol	40.11	2479.6a	4016ab	6495.6	0.446a
Lithovit	45.88	2568.7a	3768b	6336.7	0.483a
Baykal EM	46.27	2468.9a	3529b	5997.9	0.500a
Tryven	44.29	2697.8a	5128a	7825.8	0.510a
<i>Triticum monococcum</i> L.					
Wheat species	-	2655.6a	5738.2a	8393.8	0.476a
Control (Agriorgan pellet)	39.26	2597.7a	6194a	8791.7	0.416a
Amalgerol	41.34	2671.2a	5599a	8270.2	0.493a
Lithovit	43.25	2593.5a	6069a	8662.5	0.430a
Baykal EM	40.76	2538.3a	4531a	7069.3	0.576a
Tryven	39.99	2877.2a	6298a	9175.2	0.463a

*Means followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test

Harvest index (HI)

Harvest index (HI), characterizing the proportion of grains in total biomass is highest with *Triticum spelta* L. - 0.498, followed by *Triticum dicoccum* Sch. (0.489) and *Triticum monococcum* L. (0.476), but all the differences are not proven (Table 2). Fertilizer treatment increases the harvest index to varying degrees in the individual variants, but with no statistically confirmation. Higher values of the indicator were reported with *Triticum dicoccum* Sch. after Lithovit application - 0.533. All studied leaf fertilizers for *Triticum monococcum* L. increased the harvest index, but with no statistically effect. At Baykal EM treatment, the harvest index was 0.576, followed by variants treated with Amalgerol - 0.493, Tryven - 0.463 and Lithovit - 0.430. The values of the harvest index for *Triticum spelta* L. after are close to those of control.

CONCLUSIONS

At all three wheat species, *Triticum monococcum* L., demonstrates highest straw yield - 5738.2 kg/ha, compared to other two species.

Tryven treatment increase the straw yield at *Triticum dicoccum* Sch. - 5306 kg/ha and *Triticum spelta* L. - 5128 kg/ha, compared to controls.

The highest cellulose content in straw is reported at *Triticum dicoccum* Sch. when Tryven was applied (42.14%), and at *Triticum spelta* L., after application of Baikal EM (46.27%). At *Triticum monococcum* L. all studied biofertilizers increase cellulose content, the most significant increase was observed at Lithovit application (43.25%).

The harvest index (HI) is highest at *Triticum spelta* L. - 0.498, followed by *Triticum dicoccum* Sch. (0.489) and *Triticum monococcum* L. (0.476), but no statistically significance. Fertilizer treatment increases the harvest index to varying degrees in the individual variants.

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REFERENCES

- Akram, M. (2011). Growth and yield components of wheat under water stress of different growth stages. *Bangladesh Journal of Agricultural Research*, 36(3), 455–468.
- Dai, J., Bean, B., Brown, B., Bruening, W., Edwards, J., Flowers, M., Karow, R., Lee, C., Morgan, G., Ottman, M., Ransom, J., Wiersma, J. (2016). Harvest index and straw yield of five classes of wheat. *Biomass Bioenergy*, 85, 223–227.
- Donald, C., Hamblin, J. (1976). The biological yield and harvest index of cereals as agronomic and plant breeding criteria, *Advances in Agronomy*, 28(1), 361–411.
- Donaldson, E., Schillinger, W., Dofing, S. (2001). Straw production and grain yield relationships in winter wheat. *Crop Science*, 41(1), 100–106.
- Guliani, A, Karagöz, A, Zencirci, N. (2009). Emmer (*Triticum dicoccon*) production and market potential in marginal mountainous areas of Turkey. *Mountain Research and Development*, 29(3), 220–229.
- Hay, R. (1995). Harvest index: a review of its use in plant breeding and crop physiology. *Annals of Applied Biology*, 126(1), 197–216.
- Kirchev, Hr., Matev, A., Yanchev, I., Zlatev, Z. (2014). Productivity and its elements of triticale varieties depending on the nitrogen rate. *Governance and Sustainable Development*, 46(3), 67–71, Human Nature Society, LTU-Sofia.
- Koutev, V., Trifonova, T., Landjeva, S., Nenova, V., Kocheva, K. (2014). Straw: grains ratio study of 100 Bulgarian wheat varieties for the needs of balanced fertilization. *Scientific works the Institute of Agriculture – Karnobat*, 3(1), 261–265.
- Larsen, S., Bruun, S., Lindedam, J. (2012). Straw yield and saccharification potential for ethanol in cereal species and wheat cultivars. *Biomass Bioenergy*, 45(1), 239–250.
- Marino, S, Tognetti, R, Alvino, A. (2009). Crop yield and rain quality of emmer populations grown in central Italy, as affected by nitrogen fertilization. *European Journal of Agronomy*, 31(4), 233–240.
- Mitkov, A., Yanev, M., Neshev, N, Tonev, T. (2017). Opportunities for single and combine application of herbicides at winter wheat. *Scientific Papers, Series A. Agronomy*, 60, 314–319.
- SAS Institute Inc. 1999 *SAS Procedures Guide*, SPSS for Microsoft Windows, 9(4).
- Tonev, T., Dimitrova, M., Kalinova, Sh., Zhalnov, I., Zheliazkov, I., Vasilev, A., Titianov, M., Mitkov, A., Yanev, M. (2019). *Herbology*. Videnov & Son, ISBN 978-954-8319-75-1.
- Vlahova, V., Popov, V. (2014a). Biological efficiency of biofertilizers emosan and seazol on pepper (*Capsicum annum* L.) cultivated under organic farming conditions. *International Journal of Agronomy and Agricultural Research (IJAAR)*, 4(5), 80–95. Available at: <http://www.innspub.net/wp-content/uploads/2014/05/IJAAR-V4No5-p80-85.pdf>.

- Vlahova, V., Popov, V., Boteva, H., Zlatev, Z., Cholakov, D. (2014b). Influence of biofertilisers on the vegetative growth, mineral content and physiological parameters of pepper (*Capsicum annuum* L.) cultivated under organic agriculture conditions. *Acta Scientiarum Polonorum. Hortorum Cultus*, 13(4), 199–216.
- Wolfe, M., Baresel, J., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Ostergard, H., Lammerts Van Bueren, E. (2008). Developments in breeding cereals for organic agriculture. *Euphitica*, 163, 323–346. DOI 10.1007/s1081-008-9690-9.

HARVESTING FLAT CROPS WITH MINIMAL LOSS

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Abstract

The most responsible resource-consuming stage of grain production is harvesting. Improving the efficiency of harvesting can be achieved by equipping combine harvesters with stripper headers. Stripper headers can be operated at higher speeds, which contributes to a 35-50% increase in productivity and a 20-25% reduction in fuel consumption. Stripper headers are especially effective when harvesting high-yielding crops with high humidity and weeds. However, their use in harvesting flat crops leads to large losses of grain, since there are no devices that can raise flat stems and feed them to the stripper zone. This problem is especially acute in the production of seeds of elite crops. Laboratory studies substantiated the basic design parameters and the operating mode of the copying stem lifter. A production audit confirmed that with stripper header, losses are reduced by 70%.

Key words: *stripper header, combine harvesters, harvesting.*

INTRODUCTION

Harvesting is the most critical stage in the production of crops. To increase the efficiency of harvesting grain crops every year more and more, both in our country and abroad, find combing reapers. This is due to the fact that stripper headers can be operated at higher speeds, which contributes to an increase in productivity by 35-50% and a decrease in fuel consumption by 20-25%. Combine harvesters are especially effective when harvesting high-yielding breads with high humidity and weedy weeds. However, their use in harvesting lodged bread leads to large losses of grain, since there are no devices that could lift lodged stems and feed them to the tow zone (Kuhmazov et al., 2018).

Therefore, the aim of the study is to develop the stem lifter of the stripper header and justification of its design parameters and operating modes.

MATERIALS AND METHODS

Experimental studies were carried out in laboratory and field conditions on the basis of generally accepted methods in accordance with the current Interstate Standards (IS), as well as using the theory of planning a multifactor experiment.

To obtain a mathematical model of the process, the optimal design of the experiment was used. When planning an experiment, the optimization criterion is initially selected, that is, the parameter by which the studied object is evaluated and which links the factors into a mathematical model.

It is necessary to strive to ensure that the optimization criterion is one and has clear physical meaning and a quantitative assessment. Therefore, it is best to choose a criterion that would be the aggregate and comprehensive characteristic of the studying object. For any trimmer, the evaluation criteria of the process are completeness of removal, injury, productivity and energy intensity. In this case, as the optimization criterion, we adopted the value of grain losses, and the remaining criteria were used as limitations.

When researching the process, factors influencing the process of work were identified, and initially more than 12 were selected, which characterized the design and operating parameters of the working body, the technological conditions of the process, as well as the physical and mechanical properties of the materials. In research it is impossible to capture the influence of all factors and their interaction. Therefore, based on a priori information, as well as on the basis of specific research tasks, the most significant factors were identified.

Moreover, some of them did not change during the research process and were fixed at constant levels.

Subsequently, a screening experiment was carried out, according to its results, after processing, information was obtained on the significance of each parameter. This made it possible to exclude insignificant factors from further consideration and, consequently, the quantity of further studies was reduced.

The plans for the experiments, methods for processing the results are described in details in many sources. In accordance with them, experiments and mathematical data processing were organized (Kuhmazov and Tizov, 2018).

To realize a screening experiment, we composed a matrix taking into account the initially selected factors by randomly mixing two half-replicas of the type 2^{4-1} . One semi-replica attributed to factors $X_1 - X_4$, another - to factors $X_5 - X_8$. The number of experiments in the matrix should be a multiple of $2k$ and exceed the number $k + 1$ (k is the number of factors). Therefore, experiments 9 and 10, formed by a random selection of both half-replicas, were included in the matrix. The experimental design was randomized using random number tables. First of all, the reproducibility of plans was checked by testing the hypothesis of homogeneity of variances.

Since the number of repetitions in each series of experiments was the same ($n=3$), the homogeneity of a number of dispersions was determined by $G_{I/l}$ - Cochren's criterion. Processing of experimental data began with their graphical representation on the initial scattering diagram.

The influence degree of the factors was evaluated by the difference in the medians of the values of the experimental data at upper and lower levels of the factors and the number of distinguished points. After isolating factors X_1 and X_2 carried out the adjustment of the results of the screening experiment in order to more clearly identify the remaining factors and their pair interactions. The correction consisted of summing the experimentally obtained values of the optimization parameter in the matrix of the screening experiment of the found values of

the effects of factors X_1 and X_2 , taken with the opposite sign. Based on the adjusted results of the optimization parameter, a second scattering diagram was constructed and its analysis made it possible to distinguish two factors X_3 . At each stage of the research, scattering diagrams were used to select pair interactions using the "distinguished points" method. The significance of the effects of pairwise interactions was evaluated similarly to the effects of individual factors.

Together with the significance assessment of the effects of factors at each stage, a statistical analysis of the corrected observation results was made, as a result the need for further identification of significant factors was identified. If the calculated value of the Fisher criterion is less than the tabulated one, the screening of factors and their interactions can be stopped. Based on the results of adjusting the experimental data, a scatter plot of the distribution of optimization parameter values was built.

It was decided not to conduct a steep ascent along the response surface, since in the implementation of the planning matrix the values of the optimization parameter in most cases turned out to be in an almost stationary region.

In screening experiments, it is assumed that the response surface is described by a linear model of the form:

$$Y = b_0 + b_1x_1 + \dots + b_{n-1}x_{n-1} + d,$$

b_0, b_1, b_{n-1} - regression coefficients with selected linear terms;

n - the total number of linear factors;

l - the number of screening effects;

d - the response component related to the noise field along with the experimental error.

As a rule, it is not completely possible to approximate the response surface, and in this case it is necessary to switch to second-order plans.

To describe the response surface by a second-order equation, central compositional orthogonal second-order planning was used, which is simple and easy to calculate, and also quite economical in the number of experiments. Based on the results of the screening experiment, the main levels of variation of the selected factors were selected.

Before the experiment, factors were encoded according to the formula:

$$X_i = \frac{X_i - X_{0i}}{\varepsilon}$$

X_i - the coded value of the factor (dimensionless quantity), the upper level is designated +1, and the lower level -1 (in the center of the experiment there will be zero level); X_i - natural value of the factor; X_{0i} - the natural value of the factor at zero level; ε - the natural value of the interval of variation of the factor, determined by the formula:

$$\varepsilon = \frac{X_i^a - X_i^i}{2},$$

X_i^a - the value of the factor at the upper level; X_i^i - the value of the factor at the lower level.

To obtain a mathematical model of the process of removing onion and weed tops in the form of a polynomial of the second degree, we implemented an orthogonal compositional plan, the planning matrix of which with the obtained experimental data. The total number of experiments N depends on the number of factors k and is determined by the expression $N = 2k + 2k + n$. The magnitude of the "star shoulder" α and the number of experiments

n_0 in the center of the plan choose depending on the accepted criterion of optimality. If orthogonality is taken as a sufficient criterion for the optimality of the experimental design, then there is no restriction on the number of experiments in the center of the design, and usually $n_0=1$. And the value of the "star" shoulder with the number of factors $k = 3$ is $\alpha = 1,215$.

When processing the experimental results, the following formulas were used:

a) The arithmetic average value of the completeness of removal of tops of onions and weeds:

$$\bar{\delta} = \frac{\sum \delta}{n},$$

$\sum \delta$ - the sum of all measurement options;

n - the number of measurements.

b) standard deviation:

$$S = \sqrt{\frac{\sum (X - \bar{X})^2}{n-1}},$$

c) the coefficient of variation of the completeness of removal of tops of onions and weeds:

$$v = \frac{S}{\bar{X}} \cdot 100\%,$$

Upon receipt of an adequate mathematical model of the second order, it is necessary to determine the coordinates of the optimum and study the surface properties in the vicinity of the optimum.

To do this, we produce the canonical transformation of the obtained mathematical models. For analysis and systematization, second-order equations led to the canonical form:

$$Y - Y_S = B_{11}X_1^2 + B_{22}X_2^2,$$

Y - optimization criterion value;

Y_S - the value of the optimization criterion at the optimal point;

X_1, X_2 - new coordinate axes rotated relative to the old x_1, x_2 ;

B_{11}, B_{33} - regression coefficients in canonical form.

During the canonical transformation of the equations, the origin was transferred to a new point S and the old shafts were rotated by a certain angle in the factor space, as a result of which linear terms disappear and the value of the free term changes.

To carry out the transfer of the origin to a particular point on the response surface, we differentiated the response function with respect to each variable and, equating the partial derivatives to zero, solved the resulting system of equations, that is, found the values of factors optimizing the value of the optimization criterion.

After the canonical transformation and determination of the type of response surface, its analysis was carried out using two-dimensional sections.

For this, giving different values to the optimization criterion in the canonical equation, we built a series of equal output curves (isolines) in the range of permissible values of the variation of independent variables. Consideration of two-dimensional sections gives a visual representation of the values of the optimization criterion, which it takes when varying the levels of each pair of factors.

The main calculations and processing of experimental results were performed on a PC using standard programs MathCAD, Microsoft Excel and Statistica 6.0.

RESULTS AND DISCUSSIONS

To reduce losses during harvesting of laid-down breads by the towing method, we developed a copying stem-lifter of a combing header, consisting of a slide 1, which is pivotally connected to the base of the slide 2. Lift feather 3 is rigidly fixed to the slide 1, and the angle between the slide 1 and lifting pen 3 is 35°. Between the base 2 and the lifting pen 3 there is a gas stop 4 consisting of a pressure cylinder and a rod with a piston package. Gas under pressure in the rodless cavity of the pressure cylinder plays the role of an elastic element. The gas stop rod 4 is pivotally connected to the lifting pen 3, and the pressure cylinder with the base 2.

The copying window lifter is attached to a horizontal holder of 5 square sections. The right end of the horizontal holder 5 is bent up and fixed in the sleeve 6, welded to the lower pipe of the housing combing header.

To determine the optimal values of the design parameters and the operating mode of the copying window lifter of the combing header, we developed a laboratory setup consisting of frames 1 and 2 (Figure 1), on which a feeding belt conveyor 3, a cutting device 4 and a copying window lifter 5 are installed. A belt conveyor 3 is installed on the frame 1, and the cutting apparatus 4 and the stem lifter 5 on the frame 2. The feed belt conveyor 3 is driven from the gear motor 6 through a chain transmission. The speed of the feed belt conveyor 3 is regulated by interchangeable sprockets. To the conveyor belt 3 are attached the stalks 7 of a grain crop with a given

lodging. The height of the cutting apparatus 4 is installed in the area of the tow, and is driven by an electric motor 8, V-belt transmission 9 and the mechanism of the swash plate 10. To collect the cut stems under the cutting apparatus is a box-collector 11.

The laboratory machine works as follows: first, on the feed conveyor belt 3, we fix the stems 7 with a given lodging. On the frame 2, we install the tested copying window lifters 5. Then it is necessary to select the appropriate speed of the feeding belt conveyor 3. After that you should sequentially turn on the drive of the cutting device 4 and the feeding conveyor 3. When the conveyor belt 3 moves, the dead stems rise by the copying window lifter 5 into the tow area, cut off by the cutting device 4 and fall into the collection box 11. Not cut stems 12 remain on the conveyor belt 3.

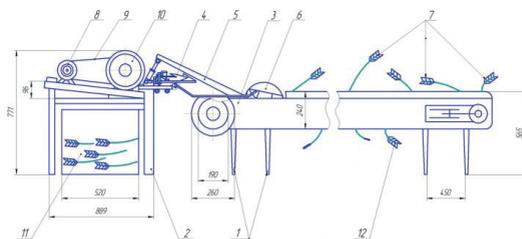


Figure 1. Laboratory machine diagram:

1,2-frames; 3-feed conveyor; 4-cutting unit; 5-copy stalk lifter; 6-gear motor; 7-stems; 8-electric motor; 9-belt drives; 10-mechanism swash plate; 11-box-compilation; 12 - uncut stems

The quality indicators of the copying power window of the combing header depend on many factors. Therefore, laboratory studies were organized with using the methodology of planning a multifactor experiment.

As an optimization criterion, we took the loss of crops ($G, \%$) in the form of uncut stems.

$$G = \frac{\sum_{i=1}^{100} (n_i - n_{cp})}{n_i} * 100,$$

n_i - the number of fixed stems before the experiment, pcs;

n_{cp} - the number of cut stems after the experiment, pcs.

Based on a priori information from the results of studies on the state of sowing crops during the harvesting period, as well as on the basis of specific research tasks, the most significant factors affecting the loss of crops during harvesting with a stripper header were

identified. During the study, some of them did not change and were fixed at constant levels (Tizov et al., 2018). The levels and ranges of variation of the three most significant factors are presented in Table 1.

Table 1. Factor affecting the loss of crops during harvesting with a stripper header with the copying stem lifter, their levels and variation intervals

Factors	Code value	Equation of variation			Range of variation
		+1	0	-1	
Long runner (skid) L, mm	x1	510	460	410	50
The value of the gas stop load (rod), P	x2	40	60	80	20
Working speed Vp, m/s	x3	2.8	2.2	1.6	0.6

For these factors, a matrix of a non-compositional plan of the second order was compiled.

After processing the experimental results, we obtained an adequate second-order mathematical model describing the dependence $G = F(\alpha, P, Vp)$ in encoded form:

$$Y = 0.58857 - 1.64000X_1 - 1.66000X_2 + 5.32857X_1^2 + 5.92857X_3^2 + 0.36250X_1 * X_2 + 0.28750X_1 * X_3 + 0.98750X_2 * X_3$$

The multiple correlation coefficient will be $R = 0.96$, the final balance is 0.026, and the F-test = 0.98. Consequently, the obtained model adequately describes the experimental results. The response surface was studied using two-dimensional cross sections (Figures 2, 3 and 4). The optimal values of the factors after solving equation 1 in encoded form have the following values: $X_1 = -0.207877$, $X_2 = -0.210412$, $X_3 = 0.403078$. In the decoded form, the factors will have the following values: length of the runner $L = 449.6$ mm, load of the gas stop, $P = 55.79176$, working speed $Vp = 0.403078$ m/s. Analyzing graphic images of two-dimensional sections, we can conclude that the optimal values of the studied factors are in the intervals: the length of the stem lifter $L = 465-480$ mm; the load of the gas stop $P = 58-66$ mm; the working speed of the combine with a stripper header $Vp = 1.6-2.56$ m/s.

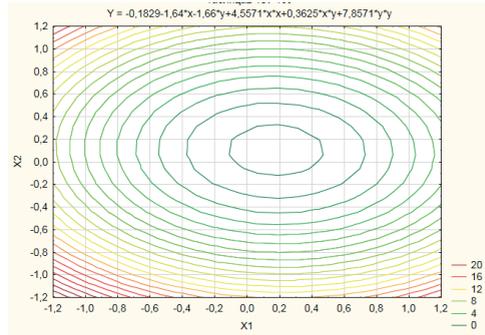


Figure 2. Two-dimensional cross-section of the response surface, characterizing the dependence of the losses of crops during harvesting with a combing header on the length of the runner (L) and the load of the gas stop (P)

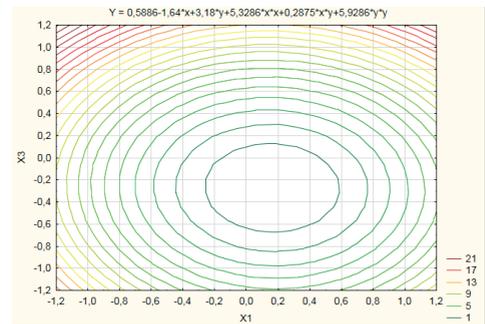


Figure 3. Two-dimensional cross-section of the response surface, characterizing the dependence of grain losses during harvesting with a combing header on the length of the runner (L) and the working speed (Vp)

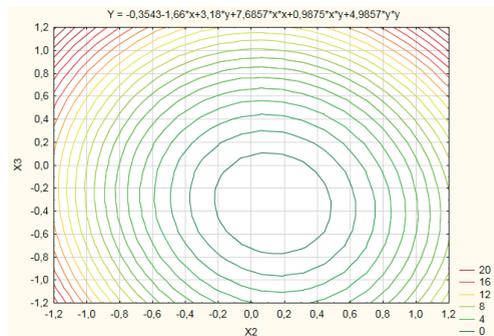


Figure 4. Two-dimensional cross-section of the response surface, characterizing the dependence of the loss of crops during harvesting with a combing header on the load of the gas stop (P) and operating speed (Vp)

Table 2. Planning matrix for a three-factor experiment of a plan close to D - optimal

Experience number	X1	X2	X3	Y _{ep}
1	+1	+1	+1	12.8
2	+1	+1	-1	5.9
3	+1	-1	+1	17.2
4	-1	+1	+1	18.3
5	+1	-1	-1	8.3
6	-1	+1	-1	6.6
7	-1	-1	+1	18.2
8	-1	-1	-1	16.4
9	+1	0	0	0.9
10	-1	0	0	2.0
11	0	+1	0	4.7
12	0	-1	0	4.8
13	0	0	+1	3.3
14	0	0	-1	0.8
15	0	0	0	1.2

CONCLUSIONS

Thus, the optimal values of the factors would have the following values: length of the runner $L = 449.6$ mm, load of the gas stop, $P = -55.79176$, working speed $V_p = 0.403078$ m/s, while losses of crops during harvesting reaper will not exceed 4%.

REFERENCES

- Kuhmazov, K.Z., Orehov, A.A., Tizov, V.M., Fedin, T.O. (2018) Decrease in grain losses when harvesting flat bread. *Volga Region Farmland*, 4(49), 126–131.
- Kuhmazov, K.Z., Tizov, V.M. (2018). Stem lifter of the stripper header. *Patent for utility model*, 180669, MPK A01D 65/00, Bulletin № 18.
- Tizov, V.M., Kuhmazov, K.Z. (2018). *Patent № 180669. МИК А01Д 65/00.*, The Russian Federation. Steam lifter of the stripper header. Published 21.06.2018, Bulletin № 18.

MISCELLANEOUS

CULTURAL ECOSYSTEM SERVICES IN AGROECOSYSTEMS - PERCEPTION OF STAKEHOLDERS - CASE OF BULGARIA

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Abstract

Cultural services as a part of Ecosystem Services represent intangible benefits of ecosystems related to their aesthetic and recreational value, their spiritual characteristics, their educational value, and the creation of an environment for the development of the spirit. Relationship between culture and nature is very important for sustainable agricultural practices and rural landscapes maintenance. Cultural ecosystem services (CES) are perceived strongly by local communities because they are identified with place of living and cultivation of land. The aim of current study was to obtain data on CES perceived by farmers and local residents, based on a raking from 1 to 10 in the Questionnaire to assess cultural ecosystem services and land-use management changes (LUMC) in selected regions in Bulgaria. This investigation was made in the frame of the STACCATO project.

Key words: Bulgaria, Cultural Agroecosystem Services, stakeholders.

INTRODUCTION

Ecosystem Services (ES) - briefly presenting all possible human benefits, identified in several major groups. Cultural services as a part of them represent intangible benefits of ecosystems (Sarukhán and Whyte, 2005). Based on the Millennium Ecosystem Assessment (MA, 2003; 2005) cultural ecosystem services (CES) related to aesthetic and recreational value of ecosystems, their spiritual characteristics, cultural identity and educational value.

Relationship between culture and nature is very important for sustainable agricultural practices and rural landscapes maintenance (Gullino and Larcher, 2013).

In case of change or loss of culture and traditions of local communities related to land management changes the environment and deeply affects the interconnection between humans and nature (Zheng et al., 2015).

In traditional communities CES are perceived strongly by local communities because they are identified with place of birth and traditional agricultural practices are essential for cultural identity (Milcu et al., 2013). This also applies to Bulgaria, whose population has for the most part preserved the rich history, traditions, agricultural practices and their transmission

from generation to generation (Borisova et al., 2015).

The aim was to obtain data on CES perceived by farmers and local residents, based on a raking from 1 to 10 in the Questionnaire To assessment cultural ecosystem services and land-use management changes (LUMC) in selected regions in Bulgaria.

MATERIALS AND METHODS

Case study regions for the assessment of cultural ecosystem services were selected and core issues of interest identified. A Questionnaire was developed for the evaluation of stakeholders' acceptance of cultural ecosystem services, and for the documentation of site characteristics (land management system) as well as social and economic structures of land use (e.g. ownership patterns, export orientation, demographic change). The survey was conducted from April to December 2016 in 11 different villages, in two different regions based on direct face-to-face interaction: South-Central and South-East Bulgaria (Table 1). The work was undertaken through the collaboration of STACCATO researcher partners from the Universitat Autònoma de Barcelona and the Agricultural University of Plovdiv.

Table 1. Study areas in Bulgaria

Bulgaria		
Region	Municipality	Village
South-Central: Plovdiv	Maritsa	Kostievo
	Kaloyanovo	Kaloyanovo
	Kaloyanovo	Duvanlii
	Rhodopi	Tsalapica
South-Central: Haskovo	Haskovo	Haskovo
South-East Yambol	Elhovo	Chernozezen
	Elhovo	Borisovo
	Straldza	Straldza
	Straldza	Malenovo
	Straldza	Kamenetz
North Central Pleven	Cherven Bryag	Suhache

On the base of methodological scheme by STACCATO protocols of stakeholders mapping the lists of stakeholders at the regional level was made (Fres Osmán, 2016). It's including the following types of organisations or entities:

- Agrarian Cooperatives Federations;
- Agribusiness companies;
- Agricultural engineers;

- Agriculture departments, including agricultural extension/technology transfer;
- Consumers' organisations;
- Development NGOs;
- Environmental organisation;
- Farmers union;
- Farming associations;
- Green Parties;
- Organic agriculture certification bodies;
- Organic agriculture engineers; developing and conserving local varieties;
- Organic farming associations;
- (Inter) Institutional Programmes/Platforms;
- Public research institutions.

The next step was categorising these organisations. For this an Interest-Influence Matrix (Ackermann and Eden, 2011), a method that classifies stakeholders based on their level of influence and interest, resulting in four types of positioning subsequent recommended strategies. The result of this process is presented in Figure 1.

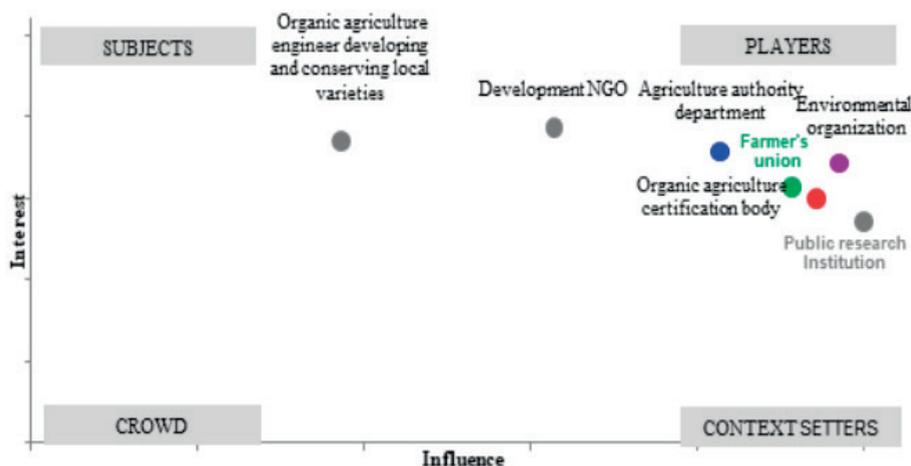


Figure 1. Interest and Influence Matrix of Group B (Plovdiv Region)

In group B (Plovdiv Region), 'Players' were represented by Agriculture authority department, Development NGO, Environmental organization, Organic agriculture Certification Body and Farmer's Union. In this case, Organic Agriculture engineer developing and conserving local varieties was categorized as a 'Subject'. In none of the groups there was any stakeholder that could be categorized as 'Crowd' i.e. 'Potential'.

RESULTS AND DISCUSSIONS

The representative from stakeholders' group was selected for Plovdiv Region (Group B) - based on their willingness to participate in the activity. The types of stakeholders selected, and their main interests and motivations are shown in Table 2.

Table 2. Participant stakeholders in the online consultation in Plovdiv (after Fres Osman, 2016)

Stakeholder type	Main activities	Plovdiv
Agriculture authority department	Formulation of agricultural policy, ensuring that natural resources are used sustainably, ensuring food supplies to the region, promoting the exchange of knowledge and training, promoting regional products.	B1
Development NGO	Protection of biodiversity and reduction of pollution through partnerships with several stakeholders and society.	B2
Environmental organization	Promotion and conservation of flora and fauna, promotion and encouragement of sustainable management approaches by campaigns and projects.	B3
Farmer's union	Representation of farmers interests in relation to the preservation of family farm, fair income, legal framework, and rational land use, production of quality products, protection of cultivated land and the protection of nature.	B4
Farming association	Representation of groups that produce the same type of product, to assure their economic, politic, legal interests, as well as provision of common services.	-
Organic agriculture certification body	Certification, inspection, pre-auditing, standard and regulation services to enhance organic agriculture.	B6
Organic agriculture engineer	Individuals, developing and conserving local varieties, with function of snow-how transference, technical consultation and training, partnerships with other stakeholders for organic agriculture.	B7
Organic farming association	Representation of organic farmers to develop common guidelines and standards for agriculture and processing, as well as knowledge exchange among its members.	-
Private research institution	Generate, support and exchange scientific knowledge regarding agriculture through private funds.	-
Public research institution	Generate, support and exchange scientific knowledge regarding agriculture through public funds.	B10

The survey involves researchers and agronomist that conducted previously a field work around the sites, book authors worked on the regions, through conversations, our observations, meetings and discussions. Especially for the farmers' cooperatives and individuals, important was to have a contact, such as a person that grow up there and is working on the fields, as well as collaboration with NGOs implementing projects, e.g., on biodiversity conservation or birds nesting. The workshop held by the Agricultural University of Plovdiv brought together 16 participants representing three main groups: the first group included members of the local NGOs and ecologists, the second one comprised agricultural technicians and scientists, and the third group was formed by local farmers and residents.

ECOSYSTEM SERVICES PERCEIVED BY STAKEHOLDERS

The different types of benefits perceived by consulted stakeholders in Bulgaria listed in Table 3 are based on both workshops with regional experts and direct interviews to farmers. The results show that all the stakeholder groups identified benefits across the four categories traditionally used to classify ES. In this research work the notion of service-generating structures (SGS) (Fischer and Eastwood, 2016) refer to the physical elements that, through human intervention and often involving the transformation of ecosystems, promote ES co-production.

Table 3. Ecosystem services identified in the study areas of Bulgaria

ES class	Authorities, Technicians, Agr. experts	Ecologists & NGOs	Farmers and rural inhabitants
Cultural	Aesthetics, Agro-tourism, Heritage intangible: traditional local language (names for crops and plants), Home gardens, Local festivals, Local flora and fauna, Religious beliefs related to local plants, Traditional cultivations, Traditional grazing, Traditional knowledge, Traditional local landscapes, Traditional rural lifestyle and way of living, Traditional vineyards and wine production, Traditional water channels	Customs, Home gardens, Homemade food, Local landscape, Tourism, Traditional houses with bird nesting roofs, Traditional local food markets, Traditional knowledge	Aesthetics, Community spirit, Education, Enjoyment, Heritage, Home gardens, Inspiration, Local festivals, Local production (e.g. artefacts), Medicinal plants, Motivation, Responsibility, Spiritual enrichment, Traditional food, Traditional knowledge, Traditional landscape, Traditional poems and stories, Traditional pottery from terra cotta clay in the region
Provisioning	Food, Natural resources (e.g. land), work	Biofuel, Food, Fuel, Natural resources (e.g. land), Pollination, Raw materials, Space for living	Food, Natural resources, Work, Income
Regulating	Air purification, Biotechnology, Climate regulation, Erosion control, Landscape, Local agr. production	Air purification, Local animal diversity, Erosion control, Local climate regulation, Local environment, Soil and land conservation	Air purification, Climate regulation, Climate regulation, Erosion control, Pollination, Soil conservation, Soil fertility
Supporting	Biodiversity, Genetic diversity, Local traditional crops	Biodiversity, Habitat	Biodiversity, Genetic diversity, habitat, Local animals and crops

In agro-ecosystems, CES depend on humans, and in that way, are sustained and maintained. Features in the landscape can be also abstract notions linked to its aesthetic and sensorial characteristics.

Hanaček and Rodríguez-Labajos (2018) review the types of structures used for that purpose and their relative importance in the literature. Rural landscapes have always been shaped by agriculture-based societies creating a build and nature-based heritage, as well as (agri)cultural and semi-natural landscapes. In turn, these become a means for CES generation and often for the

provision of other types of ES. The protection and maintenance of these structures is therefore crucial for the multifunctionality of agro-ecosystems.

Different SGS can be divided in agricultural landscapes: agricultural heritage systems, mosaic elements and semi-natural landscapes, depending on local environmental conditions for example or traditions related to farming activities of a given place (Fischer and Eastwood, 2016; Hanaček and Rodríguez-Labajos, 2018).

Here we present how different stakeholders groups relate CES that these farming landscapes hold (Table 4).

Table 4. Identified links between types of service generating structures and CES in Bulgaria, per stakeholder type

SGS class	Ecologists and NGOs:	Authorities, Experts, Technicians:	Farmers
Agricultural landscapes 	1. Aesthetic, 2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 5. Co-creation of Ecological values, 6. Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 9. Education, 12. History and historical memory, 13. Inspiration, 14. Outdoor Recreation and Cultural hunting, 15. Physical, intellectual, emotional sustenance, 16. Place shaping and attachment, 17. Social interaction, 18. Spiritual enrichment, 20. Traditional agricultural practices & Small-scale farming	1. Aesthetic, 2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 4. Celebrations, 6. Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 9. Education, 11. Heritage-intangible, 16. Place shaping and attachment, 17. Social interaction, 18. Spiritual enrichment, 20. Traditional agricultural practices & Small-scale farming	1. Aesthetic, 2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 5. Co-creation of Ecological values, 6. Connectedness to nature, 7. Sense of place – belonging, 9. Education, 10. Heritage-tangible, 16. Place shaping and attachment, 17. Social interaction, 20. Traditional agricultural practices & Small-scale farming, 21. Traditional knowledge
Heritage systems 	1. Aesthetic, 2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 4. Celebrations, 5. Co-creation of Ecological values, 6. Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 9. Education, 11. Heritage-intangible, 12. History and historical memory, 13. Inspiration, 15. Physical, intellectual, emotional sustenance, 16. Place shaping and attachment, 17. Social interaction, 18. Spiritual enrichment, 19. Tourism, 20. Traditional agricultural practices & Small-scale farming, 21. Traditional knowledge	1. Aesthetic, 2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 4. Celebrations, 5. Co-creation of Ecological values, 6. Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 11. Heritage-intangible, 12. History and historical memory, 15. Physical, intellectual, emotional sustenance, 16. Place shaping and attachment, 17. Social interaction, 18. Spiritual enrichment, 19. Tourism, 20. Traditional agricultural practices & Small-scale farming, 21. Traditional knowledge	2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 4. Celebrations, 6. Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 10. Heritage-tangible, 11. Heritage-intangible, 12. History and historical memory, 13. Inspiration, 16. Place shaping and attachment, 17. Social interaction, 18. Spiritual enrichment, 19. Tourism, 20. Traditional agricultural practices & Small-scale farming, 21. Traditional knowledge
Semi-natural landscapes 	1. Aesthetic, 2. Artistic creation, 3. Traditional local varieties and breeds (Biocultural Diversity), 5. Co-creation of Ecological values, 6. Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 9. Education, 10. Heritage-tangible, 13. Inspiration, 14. Outdoor Recreation and Cultural hunting, 15. Physical, intellectual, emotional sustenance, 16. Place shaping and attachment, 17. Social interaction, 20. Traditional agricultural practices & Small-scale farming	1. Aesthetic, 2. Artistic creation, 5. Co-creation of Ecological values, 6. Connectedness to nature, 9. Education, 10. Heritage-tangible, 11. Heritage-intangible, 12. History and historical memory, 13. Inspiration, 14. Outdoor Recreation and Cultural hunting, 15. Physical, intellectual, emotional sustenance, 18. Spiritual enrichment, 20. Traditional agricultural practices & Small-scale farming	1. Aesthetic, 3. Traditional local varieties and breeds (Biocultural Diversity), 6. Connectedness to nature, 8. Cultural transmission, 10. Heritage-tangible, 14. Outdoor Recreation and Cultural hunting, 15. Physical, intellectual, emotional sustenance, 17. Social interaction
Mosaic elements 	1. Aesthetic, 2. Artistic creation, 5. Co-creation of Ecological values, Connectedness to nature, 7. Sense of place – belonging, 8. Cultural transmission, 9. Education, 12. History and historical memory, 13. Inspiration, 14. Outdoor Recreation and Cultural hunting, 15. Physical, intellectual, emotional sustenance, 16. Place shaping and attachment, 17. Social interaction, 20. Traditional agricultural practices & Small-scale farming	1. Aesthetic, 4. Celebrations, 5. Co-creation of Ecological values, 6. Connectedness to nature, 7. Sense of place – belonging, 9. Education, 11. Heritage-intangible, 17. Social interaction, 20. Traditional agricultural practices & Small-scale farming	3. Traditional local varieties and breeds (Biocultural Diversity), 5. Co-creation of Ecological values, 13. Inspiration, 14. Outdoor Recreation and Cultural hunting, 16. Place shaping and attachment, 18. Spiritual enrichment, 19. Tourism

Authorities, technicians and agricultural experts perceive more CES to each SGS in comparison with other two stakeholders groups. In general, they relate CES mostly to heritage systems. Follow agricultural landscapes in CES perception and then semi natural landscapes. The least CES perception is related to mosaic elements. Similarly, farmers relate CES mainly to heritage systems. In lower degree farmers perceive CES to agricultural landscapes, and even less to semi-natural landscapes. Mosaic elements for farmers were also the least SGS related to CES. Ecologist and NGOs relate and perceive CES less than authorities, technicians, agricultural experts and farmers. However, similar to the previous findings, ecologists and NGOs relate CES mainly to agricultural heritage systems. Follow agricultural landscapes and semi-natural landscapes. The least CES, ecologists and NGOs relate as well to mosaic elements.

CES categories were found across all four SGS. However, stakeholders connect CES in higher or lower degree depending on SGS category. Aesthetics, arts, connectedness to nature are the main CES related to SGS. Follow nature connectedness, sense of place and belonging. In lower degree CES that relate to each SGS were biocultural diversity, place shaping and place attachment, traditional small-scale farming practices. Mainly intangible heritage, spiritual enrichment and cultural transmission were, for instance importantly related to heritage systems, including social interaction and traditional knowledge. In lower degree different stakeholders relate CES, such as history and memory, tangible heritage, education and inspiration. For semi-natural landscapes the most important CES is recreation.

All four categories of ES provided by agroecosystems were found to be perceived by all three stakeholders' groups at the regional level. Cultural services are dominantly perceived by authorities, technicians and agricultural experts with 56% and farmers with 51%. Ecologist and NGO's perceived CES with 31%, in lower degree when compared to other user groups. Regulating services follow in importance ranking in similar percentage for all three groups: 22% for authorities, technicians and agricultural experts; 23% for farmers, and 27% for ecologists and NGOs. Provisioning services were mainly perceived by ecologists and NGOs (31%), while

only from 11-12% for authorities, technicians, agricultural experts and farmers. All three groups perceived supporting services from 11-14%.

CONCLUSIONS

The results show that birthplace, sense of belonging, connection with nature, aesthetic and transmission of knowledge from generation to generation are the most important cultural ecosystem services on investigated villages in Bulgaria.

Stakeholders' evaluation of CES at both regional and community levels indicate a disrupted trend of CES evaluation, in which the value of some CES increase while others decrease. In particular, at the regional level there has been an increasing trend for the CES, connectedness to nature, sense of place, history, cultural memory and intangible agricultural heritage. On the other hand, stakeholders express the decreased relevance of traditional agricultural practices and traditional knowledge (Hanacek, 2019). Our results are similar to the study of Gómez-Baggethun et al. (2010) and demonstrate that traditional agricultural practices and knowledge are critical to sustainable farming systems (Burton and Riley, 2018; Gobattoni et al., 2015).

Frequent land-use changes found in the studied communities are agricultural intensification, expansion, and monocultures.

CES have huge importance for human and society well-being.

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REFERENCES

- Ackermann, F., Eden, C. (2011). Strategic management of stakeholders: theory and practice. *Long Range Planning*, 44(3), 179–196.
- Borisova, B., Assenov, A., Dimitrov, P. (2015). The Natural Capital of Selected Mountain Areas in Bulgaria, in: Luc, M., Somorowska, U., Szmanda, J.B. (Eds.), *Landscape Analysis and Planning*. Springer Geography, 91–108.

- Burton, R.J.F., Riley, M. (2018). Traditional Ecological Knowledge from the internet? The case of hay meadows in Europe. *Land Use Policy*, 70, 334–346. doi:10.1016/j.landusepol.2017.10.014
- Fischer, A., Eastwood, A. (2016). Coproduction of ecosystem services as human-nature interactions-An analytical framework. *Land Use Policy*, 52, 41–50. doi:10.1016/j.landusepol.2015.12.004
- Fres Osmán, L. (2016). Combining stakeholder analysis and relational ambiguity in natural resource management. The case of ecological intensification of agriculture. Master dissertation. Universitat Autònoma de Barcelona.
- Gobattoni, F., Pelorosso, R., Leone, A., Ripa, M.N. (2015). Sustainable rural development: The role of traditional activities in Central Italy. *Land Use Policy*, 48, 412–427. doi:10.1016/j.landusepol.2015.06.013
- Gómez-Baggethun, E., Mingorria, S., Reyes-García, V., Calvet-Mir, L., Montes, C. (2010). Traditional Ecological Knowledge Trends in the Transition to a Market Economy: Empirical Study in the Donana Natural Areas. *Conserv. Biol.*, 24, 721–729. doi:10.1111/j.1523-1739.2009.01401.x.
- Gullino P., Larcher, F. (2013). Integrity in UNESCO World Heritage Sites. A comparative study for rural landscapes. *Journal of Cultural Heritage*, 14(5), 389–395.
- Hanaček, K. (2019). Land-use changes, cultural ecosystem services, and environmental conflicts: Evidence from rural Bulgaria: PhD Dissertation.
- Hanaček, K., Rodríguez-Labajos, B. (2018). Impacts of land-use and management changes on cultural agroecosystem services and environmental conflicts. A global review. *Glob. Environ. Chang.*, 50, 41–59. doi:10.1016/j.gloenvcha.2018.02.016.
- MA. (2003). *Ecosystems and Human Well-being. A Framework for Assessment*. Washington, DC: Island Press. Accessed February 22, 2017.
- MA. (2005). *Ecosystems and Human Well-being Synthesis*. Island Press. Accessed February 22, 2017.
- Milcu, A. Ioana, J. Hanspach, D. Abson, and J. Fischer (2013). Cultural ecosystem services: a literature review and prospects for future research. *Ecology and Society*, 18(3), 44. <http://dx.doi.org/10.5751/ES-05790-180344>.
- Sarukhán, J., Whyte, A. (Ed.) (2003). *Ecosystems and human well-being: a framework for assessment*. Millennium Ecosystem Assessment Series. A report of the Conceptual Framework Working Group of the Millennium Ecosystem Working Group. Island Press, Washington, D.C., USA.
- Zheng, H., Lu, C., Shang, D., Cao, S. (2015). Cultural invasions and land degradation. *Reg. Environ. Chang.*, 15, 939–944. doi:10.1007/s10113-015-0785-2.

POTENTIAL OF *Miscanthus giganteus* AT INTRODUCTION IN THE MIDDLE VOLGA REGION

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Abstract

Along with industrial and food crops, energy crops as fast-growing species could form abundant biomass. One of such species of raw materials is *Miscanthus giganteus* which grows in one place for more than 25 years and annually could produce powerful ground mass even on poor lands since the third year of its life. To study the agrobiological features of *Miscanthus giganteus* and its introduction in the Middle Volga region, a plantation was laid on the light gray soil of the experimental field near Penza State Agrarian University (Russia) FSBEI HE Penza GAU in 2013. The accumulated snow cover contributed to a good wintering of the culture, and in the second year of life, its productivity increased by 15 t/ha. The next year of research, it was 36 t/ha, despite the arid conditions. Consequently, it should be mentioned that during the introduction of *Miscanthus giganteus* in zones with unstable moisture on poor soils, it is possible to obtain, if not a stable, but a sufficient high yield of raw materials.

Key words: *Miscanthus giganteus*, introduction, productivity.

INTRODUCTION

Currently, the world's energy sector, automobile, marine, rail transport and aviation, housing and communal services and other sectors of the economy are based on such natural mineral resources as oil, gas and coal. But their use is not unlimited, as well as wood. Therefore, humanity must work out all possible options for replacing hydrocarbons. In this regard, along with technical and food crops, energy crops begin to appear, which are combined by the term "biomass".

The plant world presents unlimited possibilities of choice. As objects are considered woody, reed, grassy plants. But, first of all, researchers pay attention to fast-growing species (Babich et al., 2019), which form abundant biomass subject to environmentally friendly technologies (Gismatulina et al., 2015).

In the coming decades, the use of renewable plant materials as sources of thermal energy will become the main component. *Miscanthus giganteus* could become one of such objects. This is a multi-purpose crop plant (Schroder et al., 2018), growing in one place for 25-30 years and producing annually powerful above-ground raw material mass, starting from the third year of life (Gushchina et al., 2018).

This plant is positioned to produce biofuels (Somerville et al., 2010). Being a group of plants with the mechanism of C4 photosynthesis, *Miscanthus* efficiently accumulates solar energy and produces such an important component of photosynthesis as cellulose (Morandi et al., 2016; Xue et al., 2017), which serves as a base product for many developments with improved physicochemical characteristics.

These include, for example, tissue paper and writing paper, bacterial cellulose with a hemostatic effect, a biodegradable product used to make packaging materials that make up a worthy replacement for plastic. For the production of ballistic powder, the raw materials are cellulose nitrates. *Miscanthus giganteus* short cellulose fiber provides a clear cross in the training targets of shooters and athletes, and in the textile industry its cellulose is used for the production of denim. Therefore, to obtain cellulose with high quality characteristics, it is necessary to grow *Miscanthus giganteus*, whose raw materials can be obtained without prejudice to food crops.

For agricultural producers, while expanding the range of cultural flora of the region, certain risks are assumed. To avoid this, it is necessary to study the agrobiological features of *Miscanthus giganteus* upon its introduction in the Middle Volga (Russia).

MATERIALS AND METHODS

The *Miscanthus giganteus* plantation was laid on the light gray soil of the collection area belonged to the institution of higher education Penza state Agrarian University (Penza, Russia) in the first decade of May, 2013. The precursor of the crop was spring wheat after harvesting which, stubble cultivation and plowing were carried out to a depth of 20- 22 cm. Soil preparation in the spring consisted of harrowing. Planting was carried out with rhizomes to a depth of 10-12 cm with a row spacing of 100 cm and a distance in the row of 50 cm. According to an agrochemical examination, the soil of the site was acidic - close to neutral (pH - 5.7) (Interstate Standard (IS) 26483-75), with a low humus content - 2.7% (IS 26213-91) and easily hydrolyzable nitrogen 102 mg/kg of soil (according to the Kornfield method), high - available to plants phosphorus - 188 mg/kg of soil and exchange potassium - 110 mg/kg of soil (IS 26204-91). All analyzes and surveys were carried out according to the methodology of the State variety testing of agricultural crops.

RESULTS AND DISCUSSIONS

When introducing the culture into the new climatic conditions, it is necessary to establish its response to the factors determining growth, development and productivity. One of the main

factors of decisive importance in the conditions of the Middle Volga region (Russia) is the amount of precipitation and their distribution over the growing season.

The annual rainfall from the planting of *Miscanthus giganteus* in 2013 to the second decade of May, 2014 was 664 mm, which is only 36 mm less than the culture requirements established. It should be noted that 58% of the precipitation occurred during the growing season. Hydrothermal Coefficient (HC) was 1.41, that is, the planting year of the plantation was characterized by sufficient moisture with a total of active temperatures of 2643°C (Table 1). The appearance of *Miscanthus giganteus* seedlings observed on May 27, first of all, depended on the quality of planting material, which was not visually infected with diseases and 3-4 regeneration buds were observed on each rhizome. Therefore, insufficient rainfall in May (28 mm at a norm of 43.4 mm) did not affect the germination of *Miscanthus giganteus*, and their almost triple norm in the second half of June contributed to the good development of shoots from 4 to 8 in the bulk of plants, in single plants - one stalk. Optimum humidification conditions at elevated temperatures from the third decade of July to mid-September led to an intensive growth of *Miscanthus giganteus* with a slowdown at the end of the month. With a foliage of 38%, the height of the plants even in the first year of life exceeded 180 cm due to developed leaves.

Table 1. Hydrothermal coefficient of the growing season *Miscanthus giganteus* in years of research

Month	Years						
	2013	2014	2015	2016	2017	2018	2019
May	0.52	1.08	0.25	1.80	0.64	0.54	0.5
June	1.40	0.98	1.14	0.43	1.15	0.39	0.35
July	0.79	0.60	1.40	1.64	1.41	1.08	0.76
August	1.15	0.51	0.14	0.36	0.19	0.25	1.38
September	3.20	1.17	0.15	2.10	3.20	0.72	0.77
For vegetation	1.41	0.87	0.62	1.27	1.32	0.60	0.75

With the decrease in the daily average temperature to 8°C, the growth of plants stopped and with the first frosts of October the leaves lost turgor, which subsequently drooped, remaining on the stems.

Therefore, another factor affecting the productivity of *Miscanthus giganteus* is the length of the frost-free period, which limits the limits of the growing season. In plants of the first

year of life, the period from germination to harvest lasted 133 days.

When determining the structure of the bush, it was found that by the end of the growing season, from each rhizome, on average, 11 stems were formed with a height of 85 cm and a thickness of 10 mm at the base. Their total weight was 335 g and with a stem density of 46 pcs/m², a high yield of aboveground mass of 14 t/ha was formed (Figure 1).

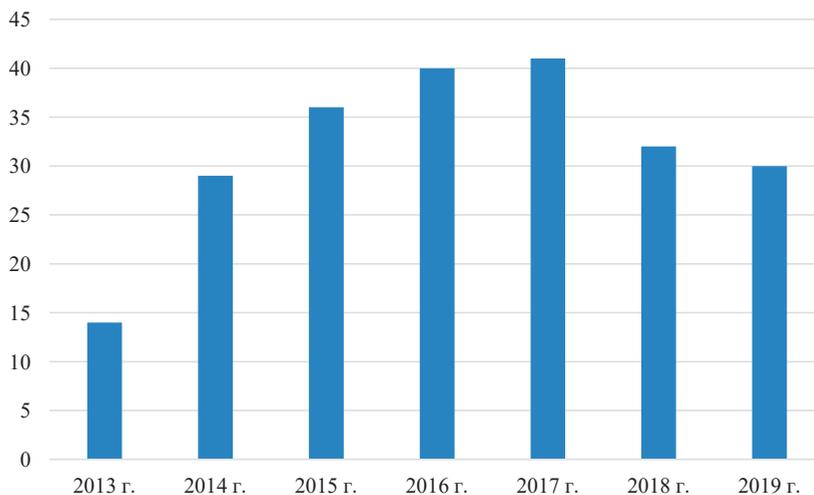


Figure 1. *Miscanthus* productivity by years of research

Important unregulated factors for perennial crops are winter air temperatures, the duration of the snow cover period and its thickness. The increased risk of successful *Miscanthus giganteus* overwintering (2013-2014) is associated with sharp temperature fluctuations and an unstable snow cover.

Precipitation in the form of snow in December fell 1.5 times less than normal at an average daily temperature of minus 3.8°C. Their significant fluctuations from +1.2°C to -31.7°C were observed in January. In the form of rain and snow, precipitation fell 2 times higher than normal.

After a three-day thirty-degree frost in February, by the end of the first decade, the temperature rose to minus 1.9°C with the amount of precipitation exceeding the norm by 37%. In the following decades of the month, as well as in March, sharp fluctuations in temperature and precipitation were observed. However, the remaining stems delayed the snow and the underground organs were not affected.

The moisture that formed on the surface during the thaw in April was gradually absorbed into the still loose soil layer. Therefore, in the spring of 2014, the soaking and milking of the organs of renewal of *Miscanthus giganteus*, which include root offspring with wintering buds, were not noted. In the third decade of April, the average daily temperature was established plus 10°C at which the *Miscanthus giganteus* continued its ontogenesis and its full regrowth was noted on

May 5. With a daily gain of 3.6 cm, already in early June, plants exceeded a meter height. This was also facilitated by moderate hydration during this period.

Elevated temperatures in July and August only contributed to the active process of photosynthesis and the economical consumption of water by *Miscanthus giganteus*. This led to a better development of the assimilation surface, and, consequently, to an increase in biological productivity.

September precipitation and temperature corresponding to the climatic norm did not reduce the intensity of plant development. Their height slightly exceeded 220 cm and the yield of the aerial mass of *Miscanthus giganteus* of the second year of life was 29 t/ha, and the more developed root system began to gradually colonize the soil space.

In the following autumn months, 64% of normal precipitation fell at temperatures below the annual average. However, in winter it was warmer by 1.5-6.6°C, and the amount of precipitation exceeded the climatic norm by 25 mm. This led to an increase in the thickness of the snow cover, an increase in the winter hardiness of the underground organs, and a decrease in the rate of snow melting. In this regard, in 2015, spring growth of *Miscanthus giganteus* was observed a week later than in the previous year. May drought (HC - 0.25) did not affect the growth of the crop, since *Miscanthus giganteus* used winter moisture

reserves. And in June and July there were conditions with sufficient moisture, when the HC amounted to 1.14 and 1.40, respectively. It was during this period that there was an intensive increase in the aerial mass with a stem density of 122 pieces/m² and a height of 385 cm. Plants of the third year of life showed high resistance to lodging, since the thickness of the stem at the base reached 1.5 cm.

Under very dry conditions in August and September (HC - 0.14), the lower leaves began to dry out, which no longer participated in the photosynthesis process due to their shading. The growth of plants in height also did not occur, but the productivity of the aboveground mass increased by 7 t/ha compared to the previous year. The next two years of the life of *Miscanthus giganteus* (fourth and fifth) according to moisturizing conditions corresponded to the first year of research. At the same time, in 2016 precipitation fell 67 mm more than normal, and in 2017 - at its level. It should be noted that the greatest number of them was 96 and 126 mm in September, when there was no increase in above-ground mass, as well as in the August drought (HC - 0.36 and 0.19, respectively).

However, at this time, the outflow of lamellar substances into the underground organs, which were responsible for the future crop, was actively passing. During the period of active growth of *Miscanthus giganteus* from May to July, HC for these years of research was 1.29 and 1.10, which affected its productivity, which was almost the same 40 and 41 t/ha with a stem density of 140 pieces/m² and height stems without leaves 242-250 cm. It would seem that, under the same conditions of plant growth, the spring of 2017 was cooler than the previous one. This led to a late spring regrowth of *Miscanthus giganteus*, which allowed him to avoid night frosts in mid-May and subsequently not to reduce the productivity of the aerial mass.

Thawing of the soil on the *Miscanthus giganteus* plantation in the spring of 2018 was delayed due to more severe winter conditions and the first spring month, when the average daily temperature was 2°C below normal. The amount of precipitation exceeded the annual average by 37 mm. Despite a sharp increase in temperature in May (up to 17.5°C), soil heating was weak, which led to the growth of *Miscanthus giganteus*

only in the third decade, when an almost double rainfall fell.

The June drought (HC - 0.39) reduced the growth rate of *Miscanthus giganteus*, but July rainfall of 71.7 mm and high average daily temperatures (21.9°C at a rate of 19.7°C) led to active assimilation of leaves and plants reached a height of 270 cm with stalk 132 pcs/m². This was facilitated by the temperature, which is considered optimal for adequate photosynthesis. However, in August and September there was no increase in above-ground mass, since dry weather was during this period (HC - 0.48). With good development of the leaves of the upper third of the stem, the lower leaves dried out and crumbled. The hydrothermal coefficient for the growing season was 0.60, which corresponds to insufficient moisture. The yield of the aerial mass of *Miscanthus giganteus* decreased by 9 t/ha compared to the crop of 2017, when the conditions of sufficient hydration of the State Customs Committee -1.32

In the seventh year of life of *Miscanthus giganteus*, i.e. in 2019, in conditions of insufficient moisture (HC - 0.75), the yield of the aboveground mass of *Miscanthus giganteus* at the level of the previous year was 30 t/ha. In April, after the liberation of *Miscanthus giganteus* from snow, a gradual heating of the soil began. The average daily temperatures in May, exceeding the norm by 4.1°C, stimulated the growth of plants, but this month the precipitation was 29% less than the average annual.

During the first two summer months, according to the biology of the culture, the vegetative organs of plants undergo intensive development, but the existing drought (HC - 0.5 and 0.76) caused a steady decrease in the relative growth rate and the daily growth of plants did not exceed 2 cm. Precipitation the first decade of August (70 mm with a climatic norm of 19 mm), increased the viability of the lower leaves, but the number of stems per square meter decreased to 86 pieces against 132 in the previous year. Probably, the aftereffect of the dry 2018 affected the formation of rhizomes with kidney renewal. However, the stems reached a height of 224 cm with a diameter at the base of up to 12 mm. Therefore, its productivity corresponded to the yield obtained in the years with the HC - 0.6, i.e. in the third and sixth year of *Miscanthus giganteus* life.

CONCLUSIONS

Miscanthus giganteus, being a promising renewable raw material in the field of alternative energy and having a number of economic and environmental advantages over other sources, can compete with wood and hydrocarbon raw materials. When introduced into the forest-steppe of the Middle Volga region (Russia), it exhibits high resistance to adverse moisture conditions.

However, as the duration of the drought conditions increases, the ability of plants to adapt decreases, which is clearly manifested when it repeats. At the same time, productivity decreases by 25% compared to years with increased moisture (2016-2017).

Nevertheless, starting from the third year of life, *Miscanthus giganteus* can produce 30- 41 t/ha of aerial mass annually, even on marginal lands.

REFERENCES

- Babich, O., Krieger, O., Chupakhin, E., Kozlova, O. (2019). Miscanthus plants processing in fuel, energy, chemical and microbiological industries. *Foods and Raw Materials*, 7(2), 403–411.
- Gismatulina, Y.A., Budaeva, V.V., Sakovich, G.V., Veprev, S.G., Shumny, V.K. (2015). Cellulose from various parts of Soranovskii *Miscanthus*. *Russian Journal of Genetics: Applied Research*, 5(1), 60–68.
- Gushchina, V.A., Volodkin, A.A., Agapkin, N.D., Ostroborodova, N.I. (2018). Introduction and adaptation of giant miscanthus to the conditions of the forest-steppe of the middle Volga region. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 9(5), 2292–2297.
- Morandi, F., Perrin, A., Ostergard, H. (2016). Miscanthus as energy crop: Environmental assessment of a miscanthus biomass production case study in France. *J. Clean. Prod.*, 137, 313–321.
- Schroder, P., Beckers, B., Daniels, S., Gnadinger, F., Maestri, E., Marmioli N., Menchd, M., Millan, R., Obermeier, M.M., Oustriere, N. Persson, T., Poschenrieder, C., Rineau, F., Rutkowska, B., Schmid, T., Szulc, W., Witters, N., Sæbo, A. (2018). Intensify production, transform biomass to energy and novel goods and protect soils in Europe - A vision how to mobilize marginal lands. *Sci. Total Environ.*, 616-617, 1101–1123.
- Somerville, C., Youngs, H., Taylor, C., Davis, S.C., Long, S.P. (2010). Feedstocks for lignocellulosic biofuels. *Science*, 329, 790–792.
- Xue S., Lewandowski I., Kalinina O. (2017). Miscanthus establishment and management on permanent grassland in southwest Germany. *Ind. Crop. Prod.*, 108, 572–582.

SILICON - AN ALTERNATIVE WAY TO CONTROL THE POPULATION OF THE TWO-SPOTTED SPIDER MITE ON CUCUMBER

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Abstract

The need for biological control of pests is increasing every day. Science is trying to minimize the use of conventional pest-control products. The aim of the current study was to examine the effect of Silicon on the population of the two-spotted spider mite Tetranychus urticae Koch. on cucumber (Cucumis sativus L.) cv. Gergana. The experiment was conducted in a laboratory under controlled conditions at the Department of Entomology, Agricultural University of Plovdiv, Bulgaria. Plants were infested with mites and treated with Si in form of orthosilicic acid. The results showed an effect of Si on the male/female ratio of the mite population, as well as the duration of the development of the different stages of the pest. Some biochemical parameters of the infested plants were also measured. There was an increase of the salicylic acid content and the activity of phenylalanine ammonia-lyase. The results obtained suggest that Silicon could be successfully used for reducing the population density of the two-spotted spider mites on cucumber and to improve the physiological status of the plants.

Key words: biotic stress, cucumber, mites, salicylic acid, silicon.

INTRODUCTION

Producing vegetables under variable biotic and abiotic stresses is a big challenge for the farmers nowadays. The need for more and more high-quality produce is rising along with the rise of the population worldwide. It is really a challenge for the common farmer to grow healthy and pesticide-free yield. The overuse of chemical pesticides led to the fast-growing development of resistance in targeted insect pests, as well as to severe effects on non-target organisms and human health as well (Lucchi and Benelli, 2018). In the last years, there is a sustainable tendency for reducing the levels not only of the pesticides but also of the mineral fertilizers. That's why scientists together with the leading producers of crop protection products discover new horizons in agriculture. Their efforts aim at testing new generation of plant protection and fertilization using substances that act as biostimulants. Some of these products not only have positive effect on the quantity and quality of crop production but also decrease the level of pest invasions.

Pest control is a serious issue especially in the greenhouse vegetable growing. Tetranychid mites (family Tetranychidae), some of which are

the spider mites, along with thrips, whiteflies and nematodes, are major pests of crops. They are the most important herbivore mites around the world (Hoy, 2011) and are among the most economically dangerous pests of greenhouse crops (Zhang, 2003). More than a thousand species of plants, representing more than a hundred families, are attacked (Van Leeuwen et al., 2010).

Before World War II, spider mites were a minor problem for crops. This changed dramatically in the 1950s and 1960s, when the use of synthetic organic pesticides and fertilizers increased sharply (Hoy, 2011). Tetranychid mites quickly manage to build up resistance to intensively applied pesticides, especially in greenhouse vegetables and flowers.

The small size of mites, rapid development, high reproductive potential, large number of generations and diapause (optional) allow mites to multiply for a short time in high density when environmental conditions are favorable. The attack of the common spider mite *Tetranychus urticae* represents a potential biotic stress for the host plant and seriously affects the physiological and biochemical processes in it (Sivritepe et al., 2009).

During feeding, the mite mechanically damages the epidermis of the leaves. This disturbs the

water regime of the plants and effects on the productivity of the photosynthetic apparatus (changes in CO₂-gas exchange, reduction of the chlorophyll content), which can cause premature foliage and flowering, even the death of whole plants (Park and Lee, 2002).

Meteorological conditions in Bulgaria are favorable for the development and intensive multiplication of mites, which is a problem both in the field and in greenhouses. In Bulgaria, the pest multiplies rapidly at the end of July and the beginning of August as the temperatures rise and the relative humidity decreases. In greenhouses under favorable conditions and in the presence of a host plant, the species develops without diapause (continuously). This makes the two-spotted spider mite one of the most dangerous pests of many vegetable crops, including cucumber and other species of the Cucurbitacea family.

Many synthetic organic pesticides kill not only the herbivores but also the beneficial species (predatory insects and mites) that control populations of pests. On the other hand, it is well known fact that the overuse of some fertilizers stimulates the multiplication of herbivore mites. Moreover, some pesticides have a stimulating effect on mite breeding when applied at low concentrations, leading to higher reproductive potential. This stimulating effect, called "hormoligosis," has been described as a physiological phenomenon that exhibit living organisms exposed to very low concentrations of essentially toxic substances (Hoy, 2011).

All the problems described above necessitate the research, development and implementation of alternative pest control approaches. A pest control strategy that relies only on plant protection chemicals for mites is unsustainable. To all these disadvantages of the use of chemical products for plant protection, we can add their negative effects on soil, water and crops. Consumers of plant products are also becoming increasingly concerned about pesticide residues in food.

Inspired by the lack of alternative of the chemical pest control researchers tested many unknown properties of some substances during the last years. Some of these substances are Silicon-containing products like different silicates (K₂SiO₃, Na₂SiO₃, Ca₂SiO₃), rice hulls, H₄SiO₄, etc. According to some researchers (Ma et al., 2001; Shetty et al., 2012; Debona et al., 2017; Harizanova and Koleva, 2019), silicon is

able to alleviate negative effects of variable abiotic and biotic stress factors. Plants commonly use monosilicic acid (H₄SiO₄) as the source of Si and H₄SiO₄ exists in soil in form of liquid (Meena et al., 2014; Carpinteri et al., 2013). Although there is a plenty of publications reporting about Silicon application advantages in agriculture, this element is not considered as an essential one. Silicon is classified as a quasi-essential element for plants (Epstein, 1999). Si application is recognized as an eco-friendly approach for crop production therefore for example the use of Si is commonly recommended under package and practices for cereals. Likewise, in vegetables, Si application has been documented to reduce the attack of diseases (Bakhat et al., 2018).

The efficacy of Si application has been reported against many fungal pathogens including powdery mildew, *Fusarium* sp., *Pythium* sp. etc. (Fauteux et al., 2005) especially in vegetable crops. Studies have shown that Si application is able also to increase the resistance of host plants to insect and non-insect pests. Several authors report about the effect of Si against *Bemisia tabaci* in tomato and cucumber (Correa et al., 2005; Callis-Duehl et al., 2017) and *Tetranychus urticae* also in cucumber (Harizanova et al., 2019). According to Bakhat et al. (2018) Si increases tolerance against insect pests such as brown plant hopper, stem borer, green leaf hopper, white backed plant hopper, and non-insect pests like spider mites (Debona et al., 2017).

MATERIALS AND METHODS

The experiments were performed under controlled environmental conditions in a laboratory at the Department of Entomology, Agricultural University of Plovdiv, Bulgaria: photoperiod 14/10 h (light/dark), photosynthetically active radiation (PhAR) - 250 μmol/m/s, air temperature 25°C (day)/ 22°C (night) and relative air humidity 50±5%. The cucumber seeds, cv. Gergana, were subjected to the process of imbibition for 24 h. They were then placed in inert material (perlite). After the full development of the cotyledons and the appearance of the first true leaf, the young plants were transferred in plastic containers with nutrient solution. Plants of all variants were grown in perlite containing ½ Hoagland nutrient

solution with all the necessary macro and microelements to phase third true leaf and then artificially infested with *Tetranychus urticae*. At the same time started the treatment with Si in form of H_4SiO_4 via leaf spraying (3 applications for 30 days). Experimental plants were grouped in 4 variants: 1 - control plants; 2 - mite-infested plants; 3 - Si-treated plants; 4 - mite-infested Si-treated plants. Each variant included 24 test plants. Thirty days after mite infestation test plants were analyzed.

Mite infestation

All plants from variants Mite and Mite + Si were artificially infested with mites from the stock colony reared under described above laboratory conditions. For infestation, each plant received small discs of cucumber leaves with 50 females in total, which were placed on the second and/or third true (mature) leaf of each cucumber plant. Infestation was done 15 days after planting. The population density was checked 30 days after infestation. Infested leaves from each plant were removed and checked under the stereomicroscope. The number of eggs, larvae, nymphs and adults of *T. urticae* was counted and recorded on twenty-four leaf discs (2 cm in diameter). Experiment for study the duration of developmental stages of *T. urticae* was carried out under the same laboratory conditions. Small leaf discs (3 cm in diameter each) of cucumber plants from two variants (treated and untreated with Si, respectively) were placed on wet cotton in Petri dishes (9 cm in diameter). Five females from these two variants were individually isolated and transferred to each leaf disc for laying eggs. The discs containing adult females were checked every two hours after mite transfer. The mites were removed if at least one egg was found. Immediately after the new egg deposition females were transferred to new leaf discs. The discs were checked twice a day and the duration of developmental stages was recorded. The leaf discs were changed to ensure their freshness. The immature were transferred to new discs very carefully with the help of a tin hair brush.

Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL) activity is determined spectrophotometrically by following the formation of trans-cinnamic acid which exhibits an increase in absorbance at 290 nm

according to Brueske (1980). One g leaf sample was homogenized in sodium borate buffer (pH = 7.0). After a 10 minutes centrifuge at 10,000 rpm 0.2 ml of the supernatant was mixed with 0.5 ml borate buffer (pH = 8.7) and 1.3 ml of distilled water to prepare the reaction mixture. In each test tube was added 0.5 ml l-phenylalanine followed by 30 minutes of incubation. The reaction was terminated by 0.5 ml of trichloroacetic acid (1 M). Finally the absorbance was recorded at 290 nm and PAL activity was measured in terms of amount of t-cinnamic acid (t-CA) formed.

Salicylic acid

Content of Salicylic acid in leaves of infested plants was determined according to Warriar et al. (2013) using 1 g fresh plant material. The sample extraction was made using distilled water followed by centrifuge at 10,000 rpm for 10 min and 100 μ l of the supernatant was mixed with 0.1% freshly prepared ferric chloride. The volume of the reaction mixture was made up to 3.0 ml and the complex formed between Fe^{3+} ion and SA, which is violet in color was determined by spectrophotometry, measuring the absorbance of the complex in the visible region (at 540 nm). The results were calculated using standard curve.

Statistical Analysis

The 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. The analysis of the mite population included 2 variants and independent sample t-test with $p < 0.05$ was used for data processing. All data were analyzed using IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSIONS

In order to evaluate the population build-up of the two-spotted spider mite on Si-treated and Si-untreated plants the number of eggs and mobile stages was recorded and analyzed. Living and dead individuals of each stage were separately recorded (Table 1). There is a decrease by 17% of the total number of the mobile stages on the

leaves of Si-treated plants compared to the Si-untreated. The number of the males is almost 15% less, and the number of the females is reduced by 72% on the silicon sprayed leaves. There is also a reduction of the number of the protonymphs - by 52%. The number of the other stages is higher in the Si-treated variant. Silicon treatment resulted in a higher number of dead

mites. The highest number of dead individuals is recorded for mobile immature stages - average number of dead larvae on Si-treated plants is 11.63, compared to 0 on Si-untreated plants. For adults the difference between two variants is not so significant but the number of dead mites on the Si-treated plants is still higher than the number on the Si-untreated ones.

Table 1. Number of living and dead individuals of each developmental stage of *T. urticae* on Si-treated and Si-untreated plants

Developmental stages of <i>T. urticae</i>	Variant	Living mites		Dead mites	
		Mean	Std. Error Mean	Mean	Std. Error Mean
Eggs	Mite+Si	24.63	7.91	0.00	0.00
	Mite	18.08	4.55	0.00	0.00
Larvae	Mite+Si	20.25	4.94	11.63	2.12
	Mite	19.42	5.50	0.00	0.00
Protonymphs	Mite+Si	3.13	0.94	0.50	0.21
	Mite	6.58	2.08	0.00	0.00
Deutonymphs	Mite+Si	12.00	2.37	0.12	0.07
	Mite	11.67	1.42	0.00	0.00
Mobile immature stages	Mite+Si	35.38	5.81	12.25	2.20
	Mite	37.67	8.10	0.00	0.00
Females	Mite+Si	2.25	0.86	4.12	0.58
	Mite	8.08	3.19	0.75	0.19
Males	Mite+Si	10.87	1.29	2.00	0.68
	Mite	12.75	1.39	0.25	0.12
Adults (females and males)	Mite+Si	13.13	2.01	6.13	1.06
	Mite	20.83	4.31	1.00	0.21
All mobile stages	Mite+Si	48.50	6.95	18.38	2.17
	Mite	58.50	8.88	1.00	0.21

Generally, the average number of all dead individuals from the mobile stages is 18.38 in the Si-treated variant compared to 1.00 in the Si-untreated variant. It is obvious that Si-treatment leads to a high mortality and respectively to a visible reduction of the number of mobile stages of the mite at the end of the experiment.

The percentage of dead individuals of each developmental stage is different for the two variants of the experiment. In the variant without Si only dead adults are recorded and the average number is very low, while in the Si-treated variant 67% of the dead mites are for mobile immature stages (Figure 1). One of the possible explanations of the higher mortality of larvae and nymphs of *T. urticae* in the Si-treated variant could be that these

stages cannot feed normally on the leaves treated with Si. The results of Independent sample t-test show that the differences between mean number of dead individuals from the two variants (with and without Si treatment) are statistically significant ($t_{23+24,776} = 1.813 \div 5.559$; $p = 0.000 \div 0.025$). The Si-treatment affects also the life cycle of the pest (Table 2). The analysis of the duration of each developmental stage of the pest shows differences among the two variants. More than 80 % of the mites in the Si-treated variant did not reach adulthood because they died as immature stages. Almost 70 % of them died at larval stage and 18 % died as nymphs. Only 11% of the hatched larvae on the Si- treated leaves reached adult stage.

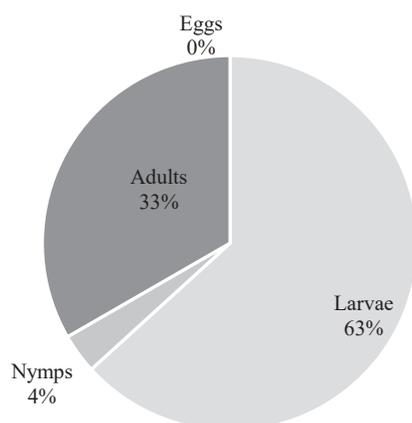


Figure 1. Percentage of dead eggs, larvae, nymphs and adults of *T. urticae* on Si-treated plants

Table 2. Duration of the different developmental stages (in days) of the two spotted spider mite *T. urticae*, reared on Si-treated and Si-untreated cucumber plants

Developmental stage	Variant	Number	Duration	Std. Error Mean
Egg	Mite+Si	53	3.60	0.08
	Mite	45	3.29	0.07
Larva	Mite+Si	16	3.19	0.26
	Mite	22	2.41	0.13
Protonymph	Mite+Si	12	2.08	0.15
	Mite	15	1.73	0.18
Deutonymph	Mite +Si	6	3.33	0.42
	Mite	11	2.09	0.16
Egg to adult	Mite+Si	6	10.50	0.56
	Mite	13	9.85	0.19

After analyzing the time needed for the individuals to enter the next developmental stage it was noted that on the Si-treated plant the mites needed a little bit longer to reach the next developmental stage compared to those feeding on Si-untreated plants. The differences are statistically significant for all stages ($t_{6,184+24,186} = 0.03 \div 0.312$). According to some authors higher silicon concentration in the soil or in the nutrient medium causes a decrease in the number of insect and non-insect pests of crop plants (Liang et al., 2005).

Some of the researchers suggest that the Si-treatment affects the feeding of the pests via mechanical injuries of the digestive system that makes them unable to feed and eventually leads to death (Han et al., 2017; Bakhat et al., 2018). Some other researchers suppose that silicon treatment triggers some biochemical mechanisms that activate the plant defence system. It is observed also that silicon supply affects the

duration of each developmental stage. In the Si-treated plants the time for entering the next developmental phase is longer in comparison to the Si-untreated plants. The reduced number of the feeding mites is probably related to the level of the mechanical and physiological injury, respectively of the physiological status of plant leaf tissues. Many studies confirm that Silicon treatment helps in alleviating plant stress. Most of the studies present positive results about the silicon properties in abiotic stress conditions (Ma et al., 2004; Song et al., 2014).

Most of them analyzed some abiotic stress factors as salinity (Harizanova et al., 2014; Harizanova and Koleva-Valkova, 2019), metal toxicity, nutrient imbalances etc.). There is also information about heat, freezing, and drought stress (Ma et al., 2004; Liu et al., 2009). According to Harizanova and Koleva-Valkova (2019) one of the possible effects of Si-treatment is the enhancement of the photosynthesis and the

activation of the enzymatic defence system of the plants. In the last years the number of manuscripts about the silicon influences in biotic stress conditions is increasing (Gomes et al., 2005; Han et al., 2017; Harizanova et al., 2019). Some authors suggest that the effect is caused by physical strengthen of the plant tissues that makes them unfavourable for feeding (Massey et al., 2006; Massey and Hartley, 2009). Others suppose that the silicon could be involved in the plant defence system (Gomes et al., 2005; Kvedaras et al., 2010; Ye et al., 2013). There are also many evidences about the ability of Si pre-treatment to improve the activities of the defense related enzymes such as peroxidase, phenylalanine ammonia-lyase (PAL) and polyphenoloxidase (Bakhat et al., 2018). Rahman et al. (2015) reported about higher activity of PAL in Si-treated plants infested with pathogens. All phenylpropanoids are derived from cinnamic acid, which is formed from Phenylalanine by the action of PAL. Phenylalanine ammonia-lyase is the branch-point enzyme between primary metabolism and the branch of secondary metabolism leading to the phenylpropanoid pathway, which is considered to be one of the most important metabolic pathways due to its responsibility for the synthesis of a large range of secondary metabolites, including phenolic acids and flavonoids (Shetty et al., 2011). Plants with low PAL activity have thinner cell walls in the secondary xylem (Elkind et al., 1990) and reduced lignin content. Twenty-five days after the mite infestation the activity of PAL in the plants was analyzed. The results are presented in table 4. The activity of the enzyme is lowest in the leaves of the control. The activity is higher in the mite infested plants but the difference is not statistically proven. Si treatment affected the activity of PAL in the leaves of mite-infested plants where its value increased by 2% compared to the mite infested untreated plants and the sole Si-treated plants had the highest activity of the enzyme. Kim et al. (2011) analyzed the effect of short-term treatment with Si combined with wounding stress in rice plants. The authors observed that the levels of endogenous salicylic acid level was significantly higher in sole Si-treated plants. However, a combined application of wounding stress and Si induced a significantly small quantity of endogenous salicylic acid (SA). In

our research the mite feeding induced the production of relatively low amount of SA compared to the control. But when treated with silicon the content of that phytohormone increased almost twice in mite-infested plants (Table 3). One of the possible ways of silicon action is namely the activation of plant defense system. It triggers protective mechanisms including the accumulation of lignin, phenolic compounds and phytoalexins (Epstein, 1999; Ma and Yamaji, 2006), formation of papillae, deposition of callose and stimulation of system stress signals (salicylic acid, jasmonic acid and ethylene) (Shetty et al., 2012).

Table 3. Activity of phenylalanine ammonia-lyase (PAL) ($\mu\text{M t-cinnamic acid/g FW/min}$) and content of Salicylic acid (SA) in the leaves on the test plants ($\mu\text{g/g}$)

Variant	PAL	SA
Control	154.6 ^c	1,228 ^b
Si	170.3 ^a	627 ^d
Mite	157 ^c	804 ^c
Mite+Si	161 ^b	1,430 ^a

CONCLUSIONS

The application of silicon in the form of H_4SiO_4 on cucumber is able to alleviate the negative effects of two-spotted spider mite feeding.

The data obtained show that Si treatment reduces the population of the spider mite. The number of larvae, nymphs and adults is reduced. The duration of the all of the analyzed developmental stages of the mite increased on the Si-supplied cucumber plants. This effect of Silicon on pest's life cycle could be due to the enhancement of the activity of some phytohormones involved in the biochemical defense response of the infested plants.

Silicon application enhances the content of salicylic acid in the infested plants but decreased in the Si-treated plants not infested with mites.

The activity of phenylalanine ammonia-lyase in the Si-supplied plants also increased.

The mechanisms which are involved in way of silicon action are not quite clear. The positive effect of silicon may be related to the accumulation of the element in the cell walls of the plants, which makes the tissues harder and less attractive to the pest, as well as to its stimulating effect on the formation of some protective substances activation of the enzymatic defense system of the plants.

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REFERENCES

- Bakhat, H., Bibi, N., Zia, Z., Abbas, S., Hammad, H., Fahad, S., Ashraf, M., Shah, Rabbani, G., Saeed, F. (2018). Silicon mitigates biotic stresses in crop plants: A review. *Crop Protection*, 104, 21–34.
- Brueske, C. (1980). Phenylalanine ammonia lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*, *Physiological Plant Pathology*, 16(3), 409–414.
- Callis-Duehl, K., McAuslane, H., Duehl, A., Levey, D. (2017). The effects of silica fertilizer as an anti-herbivore defense in cucumber. *Journal of Horticultural Research*, 25, 89–98.
- Carpinteri, A., Manuello, A. (2013). Reply to ‘Comments on ‘Geomechanical and Geochemical Evidence of Piezonuclear Fission Reactions in the Earth’s Crust’ by A. Carpinteri and A. Manuello’ by U. Bardi and G. Comoretto. *Strain*, 49, 548–551.
- Correa, S., Moraes, J., Auad, A., Carvalho, G. (2005). Silicon and acibenzolar-S-methyl as resistance inducers in cucumber. against the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotype B. *Neotropical Entomology*, 34(3), 429–433.
- Debona, D., Rodrigues, F., Datnoff, L. (2017). Silicon's role in abiotic and biotic plant stresses. *Annual Review of Phytopathology*, 55, 85–107.
- Elkind, Y., Edwards, R., Mavandad, M., Hedrick, S., Ribak, O., Dixon, R., Lamb, C. (1990). Abnormal plant development and down-regulation of phenylpropanoid biosynthesis in transgenic tobacco containing a heterologous phenylalanine ammonia-lyase gene. *Proceedings of the National Academy of Sciences of the United States of America*, 87, 9057–9061.
- Epstein, E. (1999). Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 641–664.
- Fauteux, F., Rémus-Borel, W., Menzies, J., Bélanger, R. (2005). Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiology Letters*, 249, 1–6.
- Gomes, F., de Moraes, J., dos Santos, C., Goussain, M. (2005). Resistance induction in wheat plants by silicon and aphids. *Scientia Agricola*, 6, 547–551.
- Han, Y., Gong, S., Wen, L., Hou, M. (2017). Effect of silicon addition to rice plants on *Cnaphalocrocis medinalis* feeding and oviposition preference. *Acta Ecologica Sinica*, 37(5).
- Harizanova, A., Koleva-Valkova, L. (2019). Effect of silicon on photosynthetic rate and the chlorophyll fluorescence parameters at hydroponically grown cucumber plants under salinity stress. *Journal of Central European Agriculture*, 20(3), 953–960.
- Harizanova, A., Koleva-Valkova, L., Stoeva, A., Sevov, A. (2019). Effect of Silicon on the activity of antioxidant enzymes and the photosynthetic rate of cucumber under mite infestation. *Scientific Papers. Series A. Agronomy*, 62(1), 519–528.
- Hoy, M. (2011). *Agricultural Acarology: Introduction to Integrated Mite Management*. CRC Press. ISBN 9781439817513.
- Kim, Y., Khan, A., Hamayun, M., Kang, S., Beom, Y., Lee, I. (2011). Influence of short-term silicon application on endogenous phytohormonal levels of *Oryza sativa* L. under Wounding Stress. *Biological Trace Elements Research*, 144(1-3), 1175–1185.
- Kvedaras, O., An, M., Choi, Y., Gurr, G. (2010). Silicon enhances natural enemy attraction and biological control through induced plant defences. *Bulletin of Entomological Research*, 100, 367–371.
- Liang, Y., Sun, W., Si, J., Römheld, V. (2005). Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. *Plant Pathology*, 54, 678–685.
- Liu, J., Lin, S., Xu, P., Wang, X., Bai, J. (2009). Effects of exogenous silicon on the activities of antioxidant enzymes and lipid peroxidation in chilling-stressed cucumber leaves. *Agricultural Sciences in China*, 8(9), 1075–1086.
- Lucci, A., Benelli, G. (2018). Towards pesticide-free farming? Sharing needs and knowledge promotes Integrated Pest Management. *Environmental Science and Pollution Research*, 25, 13439–13445.
- Ma, C., Li, Q., Gao, Y., Xin, T. (2004). Effect of Silicon Application on drought resistance of cucumber plants. *Soil Science and Plant Nutrition*, 50(5), 623–632.
- Ma, J., Miyake, Y., Takahashi, E. (2001). Silicon as a beneficial element for crop plants. In: Datnoff, L., Snyder, G., Korndorfer, G., (Eds.), *Silicon in Agriculture. Studies in Plant Science*, 8, 17–39.
- Meena, V., Dotaniya, M., Coumar, V., Rajendiran, S., Ajay, S., Kundu, S.; Rao, S. (2014). A Case for silicon fertilization to improve crop yields in tropical soils. *Proceedings of the National Academy of Sciences, India Section B: Biological Science*, 84(3), 505–518.
- Massey, F., Ennos, A., Hartley, S. (2006). Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *Journal of Animal Ecology*, 75, 95–603.
- Massey, F., Hartley, S. (2009). Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *Journal of Animal Ecology*, 78, 281–291.
- Park, Y., Lee, J. (2002). Leaf cell and tissue damage of cucumber caused by twospotted spider mite (Acari: Tetranychidae). *Journal of Economic Entomology*, 95, 952–957.
- Rahman, A., Wallis, C., Uddin, W. (2015). Silicon-induced systemic defense responses in perennial ryegrass against infection by *Magnaporthe oryzae*. *Phytopathology*, 105, 748–757.

- Shetty, R., Fretté, X., Jensen, B., Shetty, N., Due Jensen, J., Jorgensen, H., Newman, M., Christensen, L. (2011). silicon-induced changes in antifungal phenolic acids, flavonoids, and key phenylpropanoid pathway genes during the interaction between miniature roses and the biotrophic pathogen *Podosphaera pannosa*. *Plant Physiology*, 2194–2205.
- Shetty, R., Jensen, B., Shetty, N., Hansen, M., Hansen, C., Starkey, K., Jorgensen, H. (2012). Silicon induced resistance against powdery mildew of roses caused by *Podosphaera apannosa*. *Plant Pathology*, 61(1), 120–131.
- Sivritepe, N., Kumral, N., Erturk, U., Yerlikaya, C., Kumral, A. (2009). Responses of grapevines to twospotted spider mite mediated biotic stress. *Journal of Biological Sciences*, 9(4), 311–318.
- Song, A., Li, P., Fan, F., Li, Z., Liang, Y. (2014). The effect of silicon on photosynthesis and expression of its relevant genes in rice (*Oryza sativa* L.) under high-zinc stress. *PLoS One*, 9(11), 113782.
- Van Leeuwen, T., Vontas, J., Tsagkaracou, A., Dermauwa, W., Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochemistry and Molecular Biology*, 40, 563–572.
- Worrier, R., Paul, M., Vineetha, M. (2013). Estimation of salicylic acid in eucalyptus leaves using spectrophotometric methods. *Genetics and Plant Physiology*, 3(1-2), 90–97.
- Ye, M., Song, Y., Long, J., Wang, R., Baerson, S., Pan, Z., Zhu-Salzman, K., Xie, J., Cai, K., Luo, S., Zeng, R. (2013). Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proceedings of the National Academy of Sciences of the United States of America*, 38, 3631–3639.
- Zhang, S. (2003). Ecology of two *Encarsia formosa* strains and their control efficacy on tobacco whitefly, *Bemisia tabaci*. MS Thesis, Northwest Agricultural and Forestry University.

VARIATION OF CURRENT MAIZE (*Zea mays* L.) COBS BY MORPHOLOGICAL CHARACTERS

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Abstract

Currently in obvious progress, studies on the variability of morphological characters, along with the biochemical and molecular ones, are considered very useful for the improvement of new maize hybrids. The rich genetic endowment and the cultivation conditions of the maize lead to the characteristic expression of the plant morphology. In case of maize, the hybrids SUM 405 and DKC 4590, some new directions have been found, which have recently been improved, by morphological characters. Thus, the cobs had an average length of 18 to 20 cm, the thickness was 4.5 to 4.7 cm and weighed 210 to 261 g. The number of grains on the cob was 544 to 612, weighed 179 to 222 g, and the one thousand grains was 325 to 361 g. Compared to the cobs it had 14 to 16 rows of grains, and the proportion in grains of the cobs was 85% in both hybrids. The grains of hybrids were 12 to 13 mm long, 8.7 to 8.2 mm wide and 4.4 to 4.6 mm thick. Between the analyzed characters of the cobs of the two hybrids, most positive correlations were obtained. The new hybrids, from the semi-late category (FAO 400), cultivated, have shown a good adaptability for a new and efficient agriculture.

Key words: cob, grains, maize, variability.

INTRODUCTION

With a long history, maize (*Zea mays* L.) is one of the most important crop plants (Tokatlidis & Koutroubas, 2004). At world level, as a surface, it occupies the place between wheat and rice. The content of grains in nutrients is diverse and specific (Winkel-Shirley, 2001), having a special importance in animal nutrition, in industry, as well as in the human diet (maize flour). Over time, the plant evolved through different characters (Fasoula & Fasoula, 2002; Doebley, 2004; Haş et al., 2008; Haş et al., 2010). Thus, maize is still considered an important organism model for future genetics and biology (Duvick & Cassman, 2009; Haş et al., 2011). The origin of the plant is lost in time (Wilkes, 2004; Roney, 2009), so that the beginning moment constituted a rustic species that produced small cobs, with a single grain of 25 mm in length. This cultivated plant interspersed with *Zea mays mexicana*, or *teosinte*, has evolved, and in time it has been possible to obtain several small cobs (a few centimeters) on a single plant. From the period

there are still three species of *Zea*, namely: *Z. mays* - common maize, *Z. diploperennis-teosinte* perennial form and *Z. mays mexicana-teosinte* annual form. Corn is expressed in the world both by maize- originating in maize (Spanish), and by corn, which in some parts means cereal culture, with expression and in a culinary context. Elsewhere corn was developed from Indian corn = maize, referring to flint multicolored corn, used for decorations (you know with different colored grains and sheets woven and hung). The diploid plant contains $2n = 2x (2 \times 10) = 20$ chromosomes, fixes carbon to the C4 type, and also has a higher efficiency of water recovery (Widdcombe & Thelen, 2002; Tolleaar et al., 2004; Troyer, 2006). Being an unisexual monoecious species, maize has female flowers grouped in a spike-like inflorescence (Sarca et al., 1990; Schnable et al., 2009), with a much thickened axis (spadix). The corn spikelet has a long stigma with role in capturing pollen grains, an ovary from which the specific grains, glume and paleo at the base develop (Ştefan, 2004). The mature cobs have lengths of 3- 50 cm and a diameter of 1.5-6 cm,

being cylindrical, cylindrical-conical or fusiform. Their weight is between 50 and 500 g, and formed 8-20 rows. Grain is a caryopsis with a great variability in shape, size and color (Osorno & Carena, 2008). The literature shows maize grains 2.5-22 mm long, 3-18 mm wide and 2.7-8 mm thick, and the mass of one thousand grains of 30-1200 g. The studied SUM 405 and DKC 4590 hybrids have relatively medium size, with red spadix and characteristic yellow grains. Both hybrids belong to the *Zea mays indentata* (Sturtey.) L.H. Bayley.

The researches carried out to observe the variation of certain characteristics of the corm cobs included: total length, diameter in the central portion, absolute weight, total number of grains, number of rows, weight of grains/ cobs, TGW-thousand grain weight, the percent of grains, length, width and thickness of grains.

MATERIALS AND METHODS

The variants have been cultivated in recent years with hybrids SUM 405 and DKC 4590, semilate (Osorno & Carena, 2008). These hybrids with high productive potential could express a certain degree of adaptability in the analyzed area. The experience was established according to the block method, with 25 m² variants in 4 repetitions (replicates). The technology used was that recommended by the resort (Farnham, 2001; Tokatlidis et al., 2005). At full maturity, 25 cobs were randomly selected from each replicates (in total 100), cut and brought to the laboratory. The 100 cobs were measured and determined: total length, thickness in central area, weight, total number of grains, number of rows, total weight of grains, mass of one thousand grains, grains percent, grain length, width grain size and grain thickness.

The morphological characters obtained were analyzed by the histograms (or frequency polygons, FP,%). In their expression were used the class intervals established according to the specific string of values obtained. The study carried out highlighted several aspects, namely: i) the modal values (with the highest frequencies), ii) the limits of the intervals of variability of the studied characters and iii) the specificity of each character of the maize ecotypes in the analyzed area. Between the analyzed characters, the correlations were

established, with the help of which we could also observe their tendencies within the studied ecotypes. The Excel program was used to express the values. The significance of the correlation coefficients was obtained by comparing with the max values (Erba Weber, 1961) for the 5%, 1% and 0.1% levels of the transgression probabilities.

In the statistical calculation of all the obtained values the analysis of variance (Anova test) was used on the ranges of variation. Statistical parameters were calculated using formulas: $\bar{a} = \Sigma x/n$, where \bar{a} = media of determinations, and x = values, S^2 (variance) = $1/(n-1) \cdot [\Sigma x^2 - (\Sigma x)^2/n]$, S (standard error) = $\sqrt{S^2}$ and $S\%$ (variation coefficient) = $s \cdot \bar{a}^{-1} \cdot 100$.

RESULTS AND DISCUSSIONS

Variability of maize cobs. The appearance and dimensions of these corn hybrids are characteristic (Haş et al., 2008; Haş et al., 2010). Thus, their length was between 9 and 25 cm in hybrid 405 and between 17.1 and 23 cm in hybrid 4590. The lengths of 17-19 cm (37%) in 405 and those of 19-21 cm in 4590 (63%) were dominated (Figure 1). The sampling close in length were those of 19-21 cm (28%) in hybrid 405 and those of 21-23 cm (21%) in hybrid 4590. In the case of hybrid 4590 the longest samplings (25 cm) as well as those shorter (10-13 cm) made up 1% of the total. In the case of the lengths of these hybrids, there is a greater fluctuation of variability in the case of SUM 405 hybrid and much more restricted in the case of DKC 4590 hybrid, possibly due to the specific culture conditions.

The thickness of the cobs in the central portion was relatively wide (Figure 2). There were between 4.1 and 5.0 cm in hybrid 405 and between 4.2 and 5.2 cm in hybrid 4590. They dominated the specimens with thicknesses of 4.5 cm (23%) in the case of the first hybrid and 4.7 cm (23%) in the case of the second hybrid. They followed the specimens with a thickness of 4.4 cm (17%) and 4.6 cm (14%) at SUM 405 and those with 4.8 cm (20%) at DKC 4590. Smaller and larger thickness generally constituted 1%, with 5% exception for thinner cobs in the 405 hybrid. The adaptability and the culture conditions showed significant differentiations between the two hybrids (Figure 3 and Figure 4).

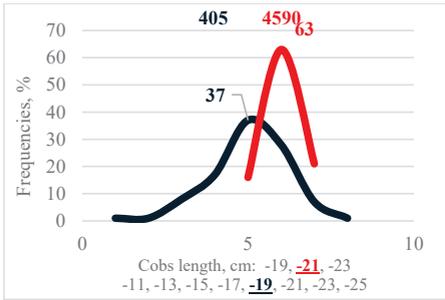


Figure 1. Frequencies of cob lengths

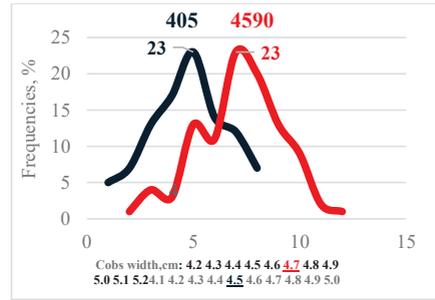


Figure 2. Frequencies of cob widths

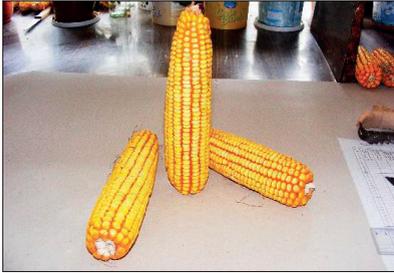


Figure 3. SUM 405 hybrid



Figure 4. DKC 4590 hybrid

The weight of the cobs has experienced relatively wide variability. In the case of the 405 hybrid, values ranging from 120 to 320 g were determined. The 4590 hybrid had limits between 180 and 340 g. It was dominated the cobs with 180-200 g (19%) in 405 hybrid and those with 260-280 g (29%) in 4590 hybrid (Figure 5). Close values were obtained at weights of 220-240 g (17%) in the case of the first hybrid and 240-260 g (21%) in the case of second hybrid. Values of 300-320 g constituted

only 3% at SUM 405 and 340 g (1%) at DKC 4590.

The number of grains on a cob had greater variability at SUM 405. In this case, between 250 and 750 grains were determined, and the dominant ones were the cobs with 500-600 grains (23-22%). DKC 4590 formed grains as a number between 450 and 700. They dominated the cobs with 600-650 grains (45%) and were followed by those with 550-600 grains (28%) (Figure 6).

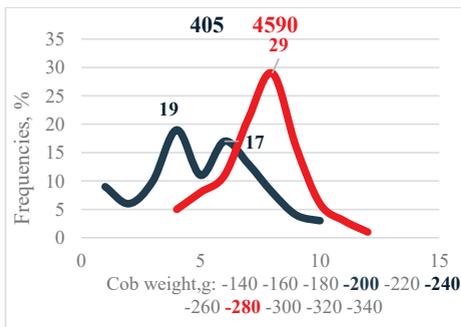


Figure 5. Frequencies of cob weights

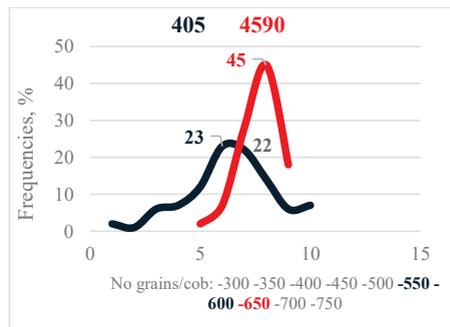


Figure 6. Frequencies of no. grains/cob

The weight of the grains on a cob was between 100 and 300 g on both hybrids. The modal value was 160-180 g (21%) in the SUM 405 hybrid.

The frequencies followed with 140- 160 g (16%) and those with 180-200 g (15%). In the case of the DKC 4590 hybrid, the modal value

was 220-224 g (36%). Close to it were the limits of 200-220 g (18%) and 240-260 g (17%). In the case of the SUM 405 hybrid, the maximum value from the 260-280 limit constituted only 1%. The DKC 4590 hybrid formed as a sample, only 2 cobs in total (2%) whose grain weights were within the maximum limit of 280-300 g (Figure 7).

The mass of one thousand grains (TGW) ranged between the limits of 200-220 g and 400-420 g in the hybrid 405 and between 260-280 g and

400-420 g in the hybrid 4590. However, the modal value were 300-320 g (28%) in the case of the SUM 405 hybrid and at 360-380 g (29%) in the DKC 4590 hybrid. The minimum values of TGW in the case of the 405 hybrid, in 4 intervals between 200 and 280 g represented only 1-2% (Figure 8). The maximum values for this hybrid were included in 4 other class intervals, between 280 and 360 g. the 4590 hybrid had the maximum grouping of TGW values between 340 and 420 g.

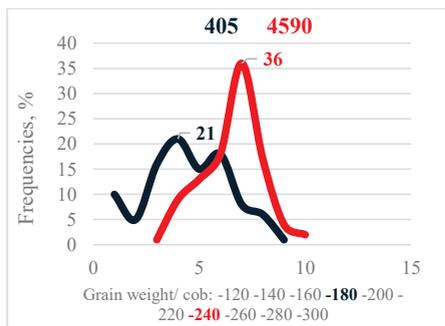


Figure 7. Frequencies of grains weight/cob

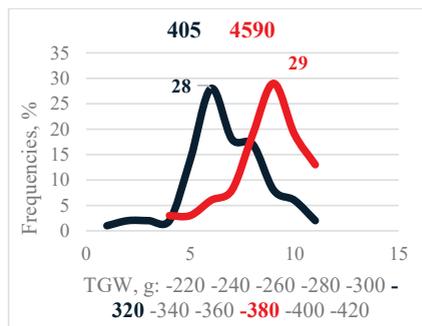


Figure 8. Frequencies of thousand grains weight

Variability of maize grains. Research has shown that both the cobs and the grains they form have a relatively high morphological diversity. From the data presented and in the case of hybrid SUM 405 and DKC 4590, the scientists had different and varied features, specific. And in case of grain morphology, some peculiarities have been found.

The degree of filling of the cobs with grains is characterized by special aspects (%). Both hybrids have proven very good coverage grades of grains on cobs. From the data it turned out that the percent ranged between 80 and 92% in the case of the SUM 405 hybrid and between 78 and 89 % in the DKC 4590 hybrid. The modal value was at 84-85% (20-24%) at 405 and 86% (31%) at 4590. The analyzed character proved quite wide limits (Figure 9), fact proven by the appearance of the proportion of the grains of the SUM 405 hybrid cobs (Figure 10). The cobs with the lowest grain percent, as well as those with the

highest ones, they made up only 1% of the total, on both hybrids.

Regarding the characteristics of the maize grains, determinations were made for length, width and thickness. The first character- the grain length, had values between 9 mm and 15 mm at the level of both hybrids. The hybrid 405 had the grain length between 9 and 14.5 mm, with the modal value at 11.5-12 mm (35%). The dimensions of 11-11.5 mm (15%) followed and those of 12-12.5 mm (15%) (Figure 11). Grains with shorter and larger lengths constituted 1-3%. The hybrid 4590 had grains between 11 and 15 mm in length. It dominated the range by 12.5-13 mm (29%).

The width of the grain was between equally wide limits, namely between 6.5 mm and 10 mm on both hybrids. The modal value was 8.9-9.1 mm (38%) in the hybrid 405. The grains of the 4590 hybrid had the modal value the width of 8-8.2 mm (35%). And with this character there is a great variability, with large oscillations between categories (Figure 12).

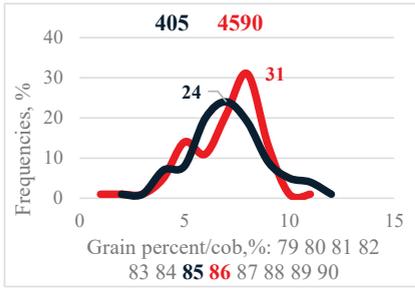


Figure 9. Frequencies of grain percent/cob



Figure 10. Hybrid SUM 405 grains aspect

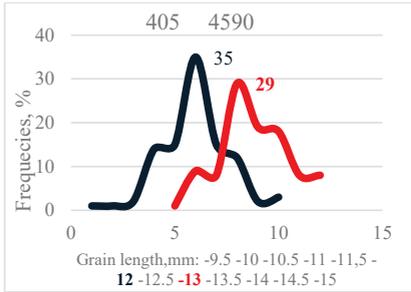


Figure 11. Frequencies of grains length

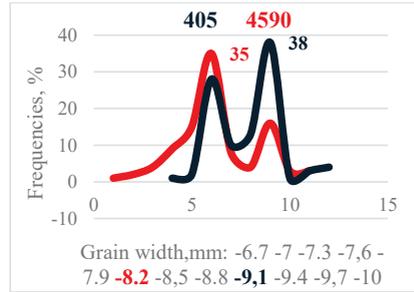


Figure 12. Frequencies of grains width

The third grain size, the thickness had values between 3.7 and 5.6 mm considering both hybrids. Thickness of 4.1-4.2 mm dominated, both in the case of SUM 405 hybrid (32%) and in the case of the DKC 4590 hybrid (22%). The grains with the smallest thicknesses were 1% at 405 and 5% at

4590, while the grains with the thickness of 5.3-5.4 mm were determined in the case of the first hybrid, and those with the thickness of 5.5-5.6 mm constituted 2% (Figure 13). Characteristic aspects of the grains were thus found in the case of the DKC 4590 hybrid (Figure 14).

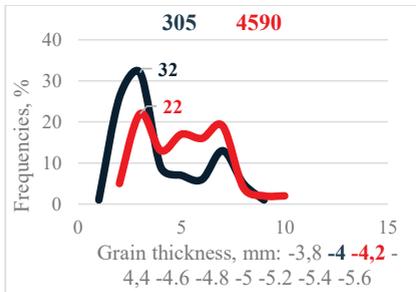


Figure 13. Frequencies of grains thickness



Figure 14. Hybrid DKC 4590 grains aspect

Correlations between the main characters. If we analyze the two sets of correlations between all the analyzed characters, we find both positive and negative situations (Haş et al., 2011). Very obvious positive correlations were observed between the characteristics of the cob: length, thickness, weight, all number of grains, total weight of grains, mass of one thousand grains

and size of grains (length, width, thickness). Negative correlations were observed between the grain percent of the cobs and the other characteristics of the cob. Also some negative correlations were observed between the grains thickness with some studied characters. The cause can be the condition of cultivation of these hybrids and of the climatic factor, both

determinants in the formation of the maize yields. Significant positive correlations were also found between the TGW with the other characters of the cobs (Table 1 and Table 2), which could demonstrate a high degree of adaptability to both maize hybrids in this area.

Statistical analysis of the variability of the morphological characters in maize. The results obtained in the morphological analysis of some maize characters showed specific aspects (Eberhart & Russel, 1966). Thus, the length of the cob measured 18.1 cm at SUM 405 and 20.1 cm at DKC 4590. The variability showed a slightly higher value at SUM 405 (13%). The width of the cob measured 4.48 cm on the first hybrid and 4.72 cm on the second hybrid, both with reduced variability, below 5%. The weight

of the cob ranged from 210 g (SUM 405) to 261 g (DKC 4590). The variation was large for the first hybrid (SUM 405 with 23%) (Table 3). The percent of grains was approximately equal, 85%, with reduced variability (2%). The grains consisted of 14-16 rows on the cobs. The average number of grains on the cob was 544 to SUM 405 compared to 612 to DKC 4590. The character variability was higher to the hybrid 405 (18%).

The weight of the grains from a cob ranged from 179 g to 222 g, the variability of the character was 22 to 12%. The absolute weight of the grains was 325 g at SUM 405 and 361 g at DKC 4590, with a slight variation (12%) (Table 4). SUM 405 had slightly longer grains, with smaller widths and more obvious thickness.

Table 1. Correlations between the main characters of maize cobs, SUM 405 hybrid

Character	Grain thickness, mm	Grain width, mm	Grain length, mm	% grains/cob	TGW, g	Grain weight, g	No.grains/cob	Grains weight, g	Cob width, cm	Cob length, cm
Cob length, cm	-0.77	.258	.539	-.376^a	.538	.881	.807	.883	.605	1
Cob width, cm	-0.60	.067	.597	-.345	.440	.812	.769	.816	1	
Cob weight, g	-1.00	.211	.702	-.396	.615	.996	.876	1		
No. grains/cob	-1.37	-.048	.517	-.265	.263	.879	1			
Grains weight, g	-1.04	.210	.703	-.317	.612	1				
TGW, g	.044	.460	.583	-.305	1					
% grains/cob	-0.28	-1.25	-.247	1						
Grain length, mm	-0.82	.361	1							
Grain width, mm	-0.47	1								
Grain thickness, mm	1									

DL 5 % = 0.19 DL 1 % = 0.25 DL 0.1 % = 0.32

Table 2. Correlations between the main characters of maize cobs, DKC 4590 hybrid

Character	Grain thickness, mm	Grain width, mm	Grain length, mm	% grains/cob	TGW, g	Grain weight, g	No.grains/cob	Grains weight, g	Cob width, cm	Cob length, cm
Cob length, cm	.137	.275	.308	-0.20	.437	.637	.533	.638	.365	1
Cob width, cm	.292	-1.23	.684	-0.77	.656	.836	.596	.843	1	
Cob weight, g	.204	.053	.732	-.169	.827	.977	.613	1		
No. grains/cob	.146	-.283	.385	.259	.114	.669	1			
Grains weight, g	.180	.028	.736	.017	.813	1				
TGW, g	.127	.257	.656	-.173	1					
% grains/cob	.144	-1.38	.002	1						
Grain length, mm	.082	-2.37	1							
Grain width, mm	-0.91	1								
Grain thickness, mm	1									

DL 5 % = 0.19 DL 1 % = 0.25 DL 0.1 % = 0.32

Table 3. Statistical indices of maize cobs

Indices	Cob length, cm	Cob thick, cm	Cob weight, g	% grains/cob	No. rows/ cob
SUM 405					
Mean, \bar{a}	18.11	4.48	210.25	85.08	14.8
Variance, s^2	5.672	0.049	2360.1	3.809	1.778
Std. error, s	2.382	0.222	48.581	1.952	1.333
Var. coef., s%	13.15	4.95	23.11	2.29	9.01
DKC 4590					
Mean, \bar{a}	20.14	4.72	261.15	84.86	16.8
Variance, s^2	1.309	0.038	1102.7	3.119	1.707
Std. error, s	1.144	0.196	33.206	1.766	1.307
Var. coef., s%	5.68	4.15	12.72	2.08	7.77

Table 4. Statistical indices of maize grains

Indices	No. grains/cob	Grains weight, g	TGW, g	Grain length, mm	Grain width, mm	Grain thick, mm
SUM 405						
Mean, \bar{a}	544.2	178.51	324.55	11.86	8.67	4.35
Variance, s^2	9414.7	1608.4	1536.6	0.687	0.260	0.150
Std. error s	97.029	40.105	39.199	0.829	0.510	0.387
Var. coef., %	17.83	22.47	12.08	6.99	5.88	8.90
DKC 4590						
Mean, \bar{a}	611.6	221.83	361.43	13.26	8.19	4.57
Variance, s^2	1546.7	786.65	1898.7	0.695	0.166	0.142
Std. error s	39.328	28.047	43.574	0.834	0.408	0.376
Var. coef., %	6.43	12.64	12.06	6.29	4.98	8.23

CONCLUSIONS

The morphological characteristics of the corn stalks were specific to the analyzed hybrids. Thus, the average length of the cob was in order of SUM 405- DKC 4590 from 18 to 20 cm, the thickness in the middle portion was 4.48 to 4.72 cm. The cobs weighted 210 g to 261 g, formed 544 to 612 grains, on 14-16 rows. The grains weighted 179 to 222 g and had a mass of one thousand grains from 325 to 361 g. the corn kernels were 12-13 mm long, 8.7-8.2 mm wide and 4.4-4.6 mm thick.

Simple correlations were established between all the studied characters, with some differentiations. Thus, between the characters of the cobs the

correlations were generally positive and significant, in both hybrids. Negative correlations were observed between the grains percent of the cobs with all the other characters, more evident in the hybrid SUM 405. By highlighting the characters of the two hybrids, they demonstrate the important productive possibilities that they have demonstrated in this area of culture.

The statistical indicators studied have shown two maize hybrids that form medium to big sized cobs, with many grains and with a mass of one thousand grains of 325 (SUM 405) and 361 g (DKC 4590). The grains specific to the var. indented, had the length, the width and the thickness to relatively close values.

REFERENCES

- Doebley, J.F. (2004). The genetics of maize evolution. *Annual Review of Genetics*, 38: 37–59.
- Duvick, D. N. & Cassman, K. G. (2009). Post-green-revolution trends in yield potential of temperate maize in the north-central United States. *Crop Science*, 39(6), 1622–1630.
- Eberhart, S. & Russel, W.A. (1966). Stability parameters for comparing varieties. *Crop Science*, 6, 36–40.
- Farnham, D.E. (2001). Row spacing, plant density, and hybrid effects on corn grain yield and moisture. *Agronomy Journal*, 93, 1049–1053.
- Fasoula, V.A, Fasoula, D.A. (2002). Principles underlying genetic improvement for high and stable crop yield potential. *Field Crops Research*, 75, 191–209.
- Haş, V., Tokaltidis, I., Haş, I., Mylonas, I. (2008). Analiza unor parametri determinanți în realizarea potențialului și capacității de producție la porumb. *Analele INCDA Fundulea*, 76, 35–41.
- Haş, V., Haş I., Antohe, I., Copândeian, A., Nagy, E. (2010). Variabilitatea capacității de producție și calității boabelor la hibridii de porumb din diferite grupe de maturitate FAO. *Analele INCDA Fundulea*, 78, 37–47.
- Haş, V., Haş, I., Chicinas, C., Schiop, T., Coste, I.D., Tritean, N. (2011). Valoarea fenotipică și genetică a unor linii consanguinizate isonucleare de porumb. II. Studiul fenotipic și genetic al unor elemente ale productivității. *Analele INCDA Fundulea*, 79(1), 49–66.
- Osorno, J.M., Carena, M.J. (2008). Creating groups of maize genetic diversity for grain quality: implications for breeding. *Maydica*, 53, 131–141
- Roney, J.W. (2009). The Beginnings of Maize Agriculture. *Archaeology Southwest*, 23(1), 4.
- Sarca, T., Ciocăzanu, I., Bica, N., Tanislav, N. (1990). Efectul interacțiunii citoplasmelor C și S și 3 surse de citoplasmă androsterilă de tip C cu genotipul, asupra unor însușiri agronomice la porumb (*Zea mays* L.). *Probleme de genetică teoretică și aplicată*, 22(2), 35–60.
- Schnable, P.S., Ware, D., Fulton, R.S. (2009). The B73 Maize Genome: Complexity, Diversity, and Dynamics. *Science*, 326(5956), 1112–1115.
- Ștefan, M. (2004). *Fitotehnie*, Ed. Ion Ionescu de la Brad, Iasi, 141–145.
- Tokatlidis, I.S., Koutroubas, S.D. (2004). A review of maize hybrids, dependence on high plant populations and its implications for crop yield stability. *Seed Field Crop Research*, 88, 103-114.
- Tokatlidis, I.S., Koutsika-Sotiriou, M., Tamoutsidis, E. (2005). Benefits from using maize density-independent hybrids. *Maydica*, 50, 9–17.
- Tolleaar, M., Ahmadzadeh A., Lee, E.A. (2004). Physiological basis of heterosis for grain yield in maize. *Crop Science*, 44, 2086–2094.
- Troyer, A.F. (2006). Adaptedness and heterosis in corn and mule hybrids. *Crop Science*, 46, 528–53.
- Weber, E. (1961). *Grundriss der biologischen Statistik für Naturwissenschaftler, Landwirte und Mediziner*, VEB Gustav Fischer, Verlag, Jena,

- Widdicombe, W.D., Thelen, K.D. (2002). Row width and plant density effects on corn grain production in the northern Corn Belt. *Agronomy Journal*, 94, 1020–1023.
- Wilkes, G. (2004). Corn, strange and marvelous: but is a definitive origin known? In C.W. Smith, J. Betrán, E.C.A. Runge, eds., *Corn: Origin, History, Technology, and Production* (pp. 3-63). USA: John Wiley & Sons.
- Winkel-Shirley, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiology*, 126(2), 485–493.

MICROALGAE EFFECTS ON THE BIOCHEMICAL PARAMETERS OF BARLEY GROWN ON SOIL CONTAMINATED WITH PETROLEUM PRODUCTS

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Abstract

A strategy to alleviate soil toxicity, which is gaining popularity in the last years, is bioremediation by inoculation with specific microorganisms. In the present study, we tested the influence of a mixture of four microalgae strains (Scenedesmus incrasatulus, Trachydiscus minutus, Chlorella sp. and Phormidium sp.) on some biochemical parameters of barley plants cultivated on soil contaminated with petroleum products. The aim was to evaluate the effect of microalgae suspension treatment on soil health and on the potential for phytoremediation. For the purpose, the nitrogen assimilation capacity, the levels of oxidative stress as well as the state of both enzymatic and non-enzymatic antioxidant systems in plants were measured. The results clearly show that petroleum-contaminated soil adversely affects the growth and development of the model culture, while treating the soil with the microalgae suspension significantly mitigates the negative impact. This is supported by the lower levels of stress markers and the increasing of some antioxidants in the plants grown on microalgae-treated soil. Therefore, the application of microalgae is an environmentally friendly strategy for improving soil health in areas affected by petroleum pollution.

Key words: microalgae, oxidative stress, soil petroleum contamination, soil remediation, stress response.

INTRODUCTION

Petroleum contamination, resulting from activities related to the petrochemical industry, is among the major environmental problems today. One of the reasons is the fact that petroleum-related components were shown to act as carcinogens and neurotoxic organic pollutants (Das & Chandran, 2011; Hatami et al., 2018). Moreover, the processes of natural purification of the affected soils are slow and take thousands of years to accomplish. Therefore, soils contaminated with petroleum products pose a permanent risk for the health of the human population as well as for the sustainable functioning of numerous ecosystems (Park & Park, 2011; Tang, 2019). In addition, some soil microorganisms, which are of particular importance for the proper biogeochemical cycles and maintenance of soil fertility, are sensitive to the presence of petroleum-based contaminants. The latter influence significantly the species distribution in the microbial community and generally have a negative impact on its biodiversity (Sutton et al., 2013). All these problems provoke serious scientific interest on the topic in the last years with the aim to develop technologies for safe and rational usage of such contaminated soils and

their remediation (Chen et al., 2015; Tang, 2019). Classic methods to treat petroleum-contaminated soil are expensive and disturb the ecological balance at the treated sites (Juck et al., 2000; Liste and Alexander, 2000; Liu et al., 2012). A sound alternative is offered by the bioremediation approach, which utilizes living organisms to remove the toxic agents in the polluted soil or to alleviate their negative effects (Das & Chandran, 2011). The main advantages of bioremediation are its non-invasiveness and the relatively low cost (April et al., 2000). Though microalgae are important members of the microbial community in soil ecosystems, the information in the literature about their involvement in hydrocarbon biodegradation is sparse. The presence of microalgae, however, may synergize with the activity of oil-degrading bacterial strains. These consortia of microalgae/bacteria may scavenge various pollutants more effectively than individual microorganisms (Subashchandrabose et al., 2011; Chen et al., 2015). For instance, inoculation with blue-green algae such as *Calothrix elenkinii* stimulated the phyllosphere and rhizosphere microbiomes of okra (Manjunath et al., 2016). One of the main mechanisms responsible for the improvement of soil microbial communities in

response to inoculation with blue-green algae relates to the production of exopolysaccharides. Exopolysaccharides secreted by many micro algal species provide organic carbon for the growth and development of beneficial microbes (Xiao & Zheng, 2016; Xia et al., 2020). Their association with soil elements helps in the solubilization, mineralization, and bioavailability of macro and micronutrients, thus improving crop performance (Chiaiese et al., 2018). Soil contamination with petroleum is unfavorable for plant growth as well, due to the significant decrease in the available nutrients (Adam & Duncan, 1999) and the rise in the concentrations of certain elements such as iron and zinc to toxic levels (John et al., 2011). The usual symptoms observed in plants cultivated on petroleum contaminated soils include degradation of chlorophyll, general reduction of the photosynthetic activity and respiration, accumulation of toxic substances, size and biomass decrease, which in the case of crops leads to the consecutive loss of yield (Vange et al., 2004; Bona et al., 2011). The evaluation of the phytotoxicity is most often based on indirect methods like the assessment of the total yield loss in comparison to neighboring non polluted regions. Other readily accessible indicators of phytotoxicity are the data for seed germination, dry weight or similar biometric characteristics (Baud-Grasset et al., 1993; Lu et al., 2010). However, more accurate approaches utilize functional physiological and biochemical indicators as well, since germination efficiency and growth parameters by themselves do not provide sufficiently objective information. Such functional parameters include activities of the main antioxidant enzymes like peroxidases, catalase and superoxide dismutase, membrane integrity, changes in the photosynthetic parameters etc. (Cartmill et al., 2014; Wyszowska et al., 2015; Hatami et al., 2018).

In the light of these actual problems, the major goal of this study was to perform a plant test, which combines growth and functional parameters, in order to assess the possibility for potential soil recovery by treatment with nonsterile microalgae cultures.

MATERIALS AND METHODS

Plant cultivation

The experiments were carried out with barley (*Hordeum vulgare* L.) as a model culture,

variety Veslets. Plants were cultivated on soil in four different variants: Soil 4.5% + MS – polluted with 4.5% content of petroleum products, supplemented with microalgae suspension; Soil 4.5% - polluted with 4.5% content of petroleum products, no supplementation with microalgae suspension; Control + MS - non-polluted, supplemented with microalgae suspension; Control - non-polluted, no supplementation with microalgae suspension. Each of these variants was grown in three repetitions five plants per repetition in the following controlled conditions: photoperiod 16/8 h (light/dark), 250 $\mu\text{mol}/\text{m}^2/\text{s}$ photosynthetic photon flux density (PPFD), 26/22°C day/night temperature and 60-65% relative air humidity. After 21 days of cultivation, the plants were subjected to analyses for determination of various physiological and biochemical parameters.

For the variants with microalgae supplementation, before sowing the soil was irrigated daily with an inoculation mixture of 4 microalgae strains (*Scenedesmus incrassatulus*, *Trachydiscus minutus*, *Chlorella* sp. and *Phormidium* sp.) with a final concentration of 0.5 mg/ml for each of the species for a period of 5 days. Cultures of these four strains were previously isolated from an oil-spill contaminated site near Sofia, Bulgaria, and were therefore considered a suitable candidate for the evaluation of microalgae-assisted bioremediation of oil-contaminated soil. These strains were kindly provided by colleagues from the Bulgarian Academy of Sciences, the Institute of Algology. For the non-supplemented varieties the irrigation was carried out with water.

Within the period of their cultivation the plants were subjected to the following watering regimes: the variants supplemented with microalgae suspension were given 50 ml daily per pot, with alternation of microalgae suspension and water. For the other variants was used only water with the same quantity. Both groups were additionally supplemented twice with 50 ml $\frac{1}{2}$ strength of modified nutrient solution: 0.505 mM KNO_3 , 0.15 mM $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.1 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.1 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 4.63 mM H_3BO_3 , 0.91 mM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.03 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.06 mM $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$, 0.16 mM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.64 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.81 mM $\text{Na}_2\text{-EDTA}$.

Lipid peroxidation

To determine the peroxidation state of membrane lipids the thiobarbituric acid (TBA) method (Heath & Packer, 1968) was used. It quantifies the malondialdehyde (MDA), considered to be one of the final products of lipid peroxidation. The spectrophotometric measurement is carried out at 532 nm wavelength, from the value of which is extracted the value of the non-specific absorption at 600 nm. The results are expressed as nmol MDA/g Fw. The quantity of the final MDA-TBA complex with red color is calculated with the specific molar extinction coefficient $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Enzymatic analyses

In these experiments the following antioxidant enzymes were included: peroxidases (EC 1.11.1.7) - guaiacol peroxidase (GPOD) and syringaldazine peroxidase (SPOD), determined by the methods of Bermeyer (Bergmeyer, 1974) and Imberty (Imberty et al., 1984), respectively. Frozen material (0.5 g FW) was homogenized in 5 ml ice-cold 0.1 M Tris-HCl buffer (pH 7.8) containing 1 mM DTT and 1 mM EDTA, and centrifuged at 13 500 g (at 4 °C for 10 min). The supernatant was collected and utilized for further experiments. Both methods for the antioxidant enzymes are spectrophotometric, with GPOD being measured at 436 nm and SPOD at 550 nm. In addition, the activity of the enzyme nitrate reductase (NR), which is crucial for nitrogen metabolism (NR; EC 1.7.99.4) was also assessed. NR activity was determined using *in vivo* assays following the methods of Al Gharbi & Hipkin (1984) and Downs et al. (1993). To determine the concentrations of nitrite produced during the assays, 1 ml samples were mixed with 1 ml sulphanilamide (1% weight/volume in 10 times diluted concentrated HCl) and 1 ml NEDA (0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride in distilled water). The solution was left to stand at room temperature for 10 min, until the colour development was complete, and the absorbance was measured at 543 nm using a spectrophotometer.

Measurement of free proline

Free proline content was determined by the methodology of Bates et al. (1973).

Measurement of polyphenols

Determination of polyphenols was carried out by measuring the absorption at 765 nm by a modified method of Singleton and Rossi (Singleton & Rossi, 1965). In brief, 10 g of each sample were mixed with 20 mL of 60% acidic methanol in an extraction tube and subjected to homogenization. The extraction continued 24 hours at room temperature. The supernatant was used for determination of total polyphenol content (TPC) by spectrophotometry, using gallic acid (99% purity, Sigma Argentina) as standard. The reagent mixture contained 40 μl extract, 3160 μl distilled water, 200 μl Folin - Ciocalteu's phenol reagent (Merck Chemicals) and after 15 minutes 600 μl 20% NaCO_3 were added. The tubes were then left at room temperature for 60 min and thereafter the absorbance at 765 nm was measured. The TPC was finally expressed as gallic acid equivalents (GAE) in g/100 g material.

Statistical analyses

One-way ANOVA (for $P < 0.05$) was used for all experiments. Based on the ANOVA results, a Tukey's test for main comparison at a 95% confidential level was applied.

RESULTS AND DISCUSSIONS

To assess the overall influence of petroleum contamination on barley plants, initially experiments to measure biochemical parameters were conducted. In order to determine whether petroleum contamination and microalgae supplementation interfere with the assimilation of nitrogen and general nitrogen metabolism the activity of the enzyme nitrate reductase was measured. As shown in Table 1, when grown in petrol contaminated soil the barley plants manifested the highest nitrate reductase activity ($100 \mu\text{g NO}_2/\text{g/h}$). The plants grown on control soil showed lower enzyme activity ($82 \mu\text{g NO}_2/\text{g/h}$) compared to the contaminated variant. In turn, the presence of microalgae seems to affect negatively the activity of nitrate reductase both in the plants cultivated on normal soil and those subjected to petroleum contamination. The observed differences are all statistically significant with $P < 0.05$. This is in concordance with numerous previous studies from authors worked with petrol contaminated soil (Anoliefo and Edegbai, 2000; Akaniwor et al., 2007).

Different stress factors (abiotic or biotic) often triggers development of oxidative stress as a second stress. Oxidative stress is associated also with large-scale peroxidation of polyunsaturated fatty-acids, which ultimately leads to the accumulation of malondialdehyde (MDA). Therefore, MDA levels are used as a direct marker for this kind of stress (Shulaev & Oliver, 2006; Xu et al., 2019). The measurement of MDA concentration revealed as expected, that

the lipid peroxidation was more expressed in the variants cultivated on contaminated soil (Table 1), confirming the stress on which these plants are subjected. This observation confirms the findings of other researchers worked with barley and petroleum contamination (Rajaei et al., 2016). On the other hand, supplementation with microalgae reduces lipid peroxidation as shown by the decreasing MDA quantities both in the controls and the contaminated variants.

Table 1. Measurements of the nitrate reductase activity (NR), lipid peroxidation state, expressed as malondialdehyde (MDA) content and free proline in young *Hordeum vulgare* L. plants, grown either on control non-polluted or polluted soil with petroleum products and treated or not with microalgae suspension (MS). Variants: polluted soil with 4.5% petroleum products + MS; polluted soil with 4.5% petroleum products; control soil + MS; control soil. The parameters were measured in leaves

Variants	NR activity ($\mu\text{g NO}_2/\text{g/h}$)	MDA (nmol/g FW)	Proline (mg/g FW)
Soil 4.5% + MS	75 \pm 11 b	18.3 \pm 1.2 b	0.19 \pm 0.08 a
Soil 4.5%	100 \pm 20 a	23 \pm 0.5 a	0.025 \pm 0.003 d
Control + MS	58 \pm 7 c	10 \pm 0.2 c	0.125 \pm 0.06 b
Control	82 \pm 4 b	12.4 \pm 0.5 c	0.073 \pm 0.002 c

The data in the columns followed by the same letter (a, b, c) are not statistically significant for $P < 0.05$.

A powerful biomarker for plant stress and the antioxidant status, which can be relatively easily determined, is the concentration of free proline. This amino acid is accumulated in plant cells in various stress conditions, mostly during drought, cold or salt stress factors (Miller et al., 2010; Hayat et al., 2012), and acts both as a compatible solute to facilitate water uptake and as a direct scavenger of reactive oxygen species (ROS) (Matysik et al., 2002). The analysis of free proline in our case demonstrates that when no microalgae are added barley plants contain almost three times as much free proline in normal conditions as when exposed to petroleum contamination (Table 1). This most probably reflects the state of stress of petroleum-treated plants which exhausts both carbon and nitrogen sources for the biosynthesis of this amino acid. The supplementation with microalgae significantly increased the amounts of proline in both variants. However, the microalgae-induced proline accumulation was much more pronounced in the contaminated samples than in the control ones reflecting the possible induction of defense mechanisms by the MS suspension.

Another non-enzymatic biomarker for stress is the accumulation of polyphenols (Krishnaiah et

al, 2011). Their quantity expressed as gallic acid equivalents was assessed separately in roots and leaves (Table 2). In leaves the amounts of gallic acid equivalents (GAE) did not vary a lot between the different variants. In roots when the plants were cultivated on clean soil, the addition of MS-induced only a slight increase in GAE. Much higher amounts of total polyphenols in the roots were detected in the polluted variant without microalgae. Similar results were reported by other authors as well (Noori et al., 2012). However, in the presence of petroleum contamination, the microalgae supplementation led to a noticeable reduction of gallic acid equivalents. This observation is in contrast with the effect of microalgae on the other non-enzymatic antioxidant compound - the proline. The status of the antioxidant system in the studied plants was investigated not only by means of non-enzymatic antioxidants like proline and polyphenols but also with analyses of the activities of the antioxidant enzymes guaiacol peroxidase (GPOD) and syringaldazine peroxidase (SPOD). These two enzymes perform similar functions in protecting cells against the oxidative damage caused by H_2O_2 , but can use different substrates – guaiacol and syringaldazine, respectively.

Table 2. Measurement of total polyphenols content, expressed as gallic acid equivalents (GAE), syringaldazine peroxidase (SPOD) activity and guaiacol peroxidase (GPOD) activity in young *Hordeum vulgare* L. plants, grown either on control non-polluted or polluted soil with petroleum products and treated or not with microalgae suspension (MS). Variants: polluted soil with 4.5% petroleum products + MS; polluted soil with 4.5% petroleum products; control soil + MS; control soil. The parameters were measured in leaves and in roots

Variants	GAE mg/g FW		SPOD U/g FW		GPOD U/g FW	
	roots	leaves	roots	leaves	roots	Leaves
Soil 4.5% + MS	0.62±0.03 b	0.52±0.02 b	92±15 b	10±1.1 b	52±1.2 b	4.5±1.2 b
Soil 4.5%	0.87±0.05 a	0.51±0.02 b	124±7 a	11±1.3 b	67±8 a	5.7±1.1 b
Control + MS	0.26±0.05 c	0.59±0.05 a	72±3 c	11±1.2 b	36±2 d	5.1±1 b
Control	0.18±0.03 d	0.41±0.03 c	84±5 b	20±1 a	44±2 c	10±1 a

The data in the columns followed by the same letter (a, b, c) are not statistically significant for $P < 0.05$.

In this case, the comparison between the four analyzed variants was also carried out separately for roots and leaves. The results for both enzymes were relatively similar (Table 2). The activities of both enzymes in the leaves were lower than those in the roots. The measured values representing the activity of SPOD in the leaves for all four variants were without significant differences. Only in the controls grown on clean soil without microalgae supplementation was observed a slightly higher activity (20 U/g FW). In the roots, contamination with petroleum products considerably induced the activity of SPOD. This is not surprising since roots are the organs that have direct contact with the petroleum derivatives and are thus subjected to stronger stress. On the other hand, the addition of microalgae suspension provokes a reduction of SPOD activities which suggests that treatment with microalgae mixtures may help mitigate the stress symptoms. A similar pattern of enzyme activity was observed for guaiacol peroxidase (GPOD) (Table 2) which is referred to as general peroxidase activity, while SPOD is associated with the specific activity in the apoplast and is related to the lignification of the cell walls. The first reaction of plants to the presence of the petroleum hydrocarbons in their growth environment and after the penetration of the contaminants into their tissues is the induction of internal defense mechanisms like enhancing antioxidant enzyme activity, which varies among plant species. The results presented here are in accordance with obtained from other authors who worked with soil contamination with gasoline (degree of contamination 0, 1, 2, 3, and 4%) and corn as a plant object. They also observed deviation of the activities of

superoxide dismutase, peroxidase, and malondialdehyde concentration (Ghalamboran et al., 2019).

According to the newest classification, the methods used for remediation of soil contaminated by petroleum hydrocarbons, are divided into three groups i.e. physical methods, chemical methods, and biological methods. Biological methods can be further classified into bioremediation and phytoremediation. Bioremediation applies microorganisms, especially bacteria and fungi to remove soil contaminants or break them down into harmless compounds via, for instance, mineralization during which contaminants are used to produce carbon and energy. Phytoremediation removes contaminants from the environment by using plants and their micro-symbionts (Tang, 2019). The rationale behind the current research is investigating the possibility of the establishment of productive plants/microorganisms symbiotic interactions in problematic areas contaminated with petroleum products. This would, in turn, stimulate and accelerate the soil detoxification by biological means and lead to future enhancement of the crop yield. The plant species chosen as a model for the study was barley because of its good growth in controlled conditions and because of a report that barley could be a good marker for phytoremediation of contaminated areas (Asiabadi et al., 2014). Microalgae pose numerous advantages as remediation agents since they have relatively low nutrient requirements, grow fast and produce a lot of biomass due to their autotrophic metabolism, and rarely produce toxic byproducts (Kumar & Oommen, 2012). Moreover, these kinds of microorganisms have been already shown to be effective for other

kinds of soil pollution, for example with heavy metals (Suresh & Ravishankar, 2004). One of the interesting observations in the present study was the fact that the microalgae actually seemed to provoke reduction of nitrate reductase activity (Table 1). A plausible explanation is a competition between the microalgae and the plants for inorganic nitrogen sources from the soil. The decrease in the available inorganic nitrogen for the plants, however, does not affect their growth parameters. Moreover, it should be noted that it has been already documented that low-molecular-weight organic nitrogenous compounds can be exuded by the algae (Adam, 1999) and in this case, they may compensate for the reduction of inorganic nitrogen. As far as the influence of oil-contamination on nitrate reductase activity is concerned, in barley, the oil products appear to slightly induce the enzyme activity. In other studies, the activity of nitrate reductase has been shown to be increased in some cases or inhibited in others. For example, in the herbaceous plant, *Melilotus albus* L. after treatment with diesel, nitrate reductase seems to react similarly to the enzyme in barley (Hernández-Ortega et al., 2012). On the other hand, the response in *Amaranthus hybridus* L. grown in soil soaked in engine oil is the opposite, i.e. a reduced activity of nitrate reductase, for all concentrations of the pollutant (Odjegba & Atebe, 2007). Therefore, the influence of the presence of microalgae seems to be at the functional, not the structural level, most probably due to the alleviation of stress symptoms (Chen & Wang, 2020). In soils polluted with oil-derived chemicals, plants are subjected to combined stress from nutritional deficiency and chemical toxicity (Martí et al., 2009). The result of long-acting stress factors is an accumulation of reactive oxygen species (ROS) like the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\bullet$) which cause oxidative damage in numerous cellular organelles (Petrov et al., 2015). On the other hand, plants use ROS, especially hydrogen peroxide, as a second messenger in numerous signaling cascades. Thus, regulation of ROS levels and proper maintenance of the redox- status is crucial for plant development and to the proper functioning of all defense systems (Gechev and Hille, 2005; Eluehike et al., 2019). The level of oxidative

stress in our barley plants was determined by measuring the concentrations of the markers MDA, free proline and phenolic substances while the status of the antioxidant systems was tested by quantification of the activities of two antioxidant enzymes GPOD and SPOD (Table 2). The results from all these experiments complement well each other and demonstrate that supplementation with microalgae suspension provides beneficial effects on the contaminated plants. This is illustrated by the reduced levels of lipid peroxidation, as determined by the decrease of MDA concentrations; the highly increased levels of the compatible solute with antioxidant properties proline; the lower activities of both antioxidant enzymes GPOD and SPOD in roots, which are directly exposed to the stress. The observed higher amounts of phenols in the roots of oil-treated plants, especially in the variant without microalgae supplementation, show that, in the absence of algae the root cells, which are in direct contact with the toxic substances in the soil, upregulate the synthesis of phenolic substances. The expected result is a thickening of the cell walls and limitation of the access of contaminants. This phenomenon correlates well with the data obtained for the activities of both antioxidant peroxidases, GPOD and SPOD because these enzymes are also involved in the synthesis of polyphenols and cell lignification. Similar results were obtained from other authors worked with soil contamination and remediation (Cuypers et al., 2002; Lu et al., 2010; Eluehike et al., 2019).

CONCLUSIONS

In conclusion, the supplementation of microalgae suspension had a positive effect on the growth and development of the barley plants cultivated on polluted soil. This is shown by the increase in the growth parameters, the overall boost of photosynthesis (data in press), the reduction of the levels of the stress marker MDA and the moderate activity of some of the main antioxidant enzymes (GPOD and SPOD). Therefore, inoculation with nonsterile microalgae cultures appears to be a promising approach to complement and accelerate phytoremediation in areas affected by oil spills. Follow-up studies on the topic would focus on

the development of suitable approaches to inoculate bacterial/microalgae cultures in affected soils, either on their own or by specific vectors, as well as comparison of the performance of barley and other plant species in order to select the most appropriate candidates to overcome the negative effect of petroleum contamination and to quicken soil recovery.

REFERENCES

Adam, M. (1999). The promotive effect of the cyanobacterium *Nostoc muscorum* on the growth of some crop plants. *Acta Microbiologica Polonica*, 48(2), 163–171.

Adam, G., Duncan, H. (1999). Effect of diesel fuel on growth of selected plant species. *Environmental Geochemistry and Health*, 21, 353–357.

Akaniwior J., Ayeleso A., Monago C. (2007). Effect of different concentrations of crude oil (Bonny light) on major food reserves in quinea corn during germination and growth. *Scientific Research and Essay*, 2, 127–131

Al Gharbi, A., Hipkin, C. (1984). Studies on nitrate reductase in British angiosperms: I. A comparison of nitrate reductase activity in ruderal woodlandedge and woody species. *New Phytologist*, 97(4), 629–639.

Anoliefo, Edegbai B. (2000). Effects of spent lubricating oil as a soil contaminant on the growth of two egg plant species *Solanum melongena* L. and *Solanum incanum* L. *Journal of Agriculture, Forestry and Fisheries*, 1, 21–25.

April, T., Foght, J., Currah, R. (2000). Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. *Canadian Journal of Microbiology*, 46(1), 38–49.

Asiabadi, F., Mirbagheri, S., Najafi, P., Moatar, F. (2014). Phytoremediation of Petroleum-Contaminated Soils Around Isfahan Oil Refinery (Iran) by Sorghum and Barley. *Current World Environment*, 9(1), 65–72.

Bates, L.S., Waldren, R.P. & Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*, 39, 205–207.

Baud-Grasset, F., Baud-Grasset, S., Safferman, S. (1993). Evaluation of the Bioremediation of a Contaminated Soil with Phytotoxicity Tests. *Chemosphere*, 26, 1365–1374.

Bergmeyer, H., Gawehn K., Grassl, M. (1974). Enzymes as Biochemical Reagents. In: *Methods in Enzymatic Analysis*, Bergmeyer, H.U. (Eds.), Academic Press, New York.

Bona, C., Rezende I., Santos, G., Souza L. (2011). Effect of soil contaminated by diesel oil on the germination of seeds and the growth of *Schinus terebinthifolius* Raddi (Anacardiaceae) seedlings. *Brazilian Archives of Biology and Technology*, 54(6), 1379–1387.

Cartmill, A., Cartmill, D., Alarcón A. (2014). Controlled release fertilizer increased phytoremediation of petroleum-contaminated sandy soil. *International Journal of Phytoremediation*, 16(3), 285–301.

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Chen, H., Wang, Q. (2020). Microalgae-based nitrogen bioremediation. *Algal Research*, 46, 101775.

Chen, M., Xu, P., Guangming Zeng, G., Yang, Ch., Huang, D., Zhang, J. (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. *Biotechnology advances*, 33, 745–755.

Chiaiese, P., Corrado, G., Colla, G., Kyriacou, M., Roupael, Y. (2018). Renewable Sources of Plant Biostimulation: Microalgae as a Sustainable Means to Improve Crop Performance. *Frontiers in Plant Science*, 9, 1782.

Cuypers, A., Vangronsveld, J., Clijsters, H. (2002). Peroxidases in roots and primary leaves of *Phaseolus vulgaris*. Copper and zinc phytotoxicity: A comparison. *Journal of Plant Physiology*, 159, 869–876.

Das, N., Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, *Biotechnology Research International*, Article ID 941810.

Downs, M., Nadelhoffer, K., Melillo, J., Aber, J. (1993). Foliar and fine root nitrate reductase activity in seedlings of four forest tree species in relation to nitrogen availability. *Trees*, 7(4), 233–236.

Eluehike, N., Oriakhi, K., Eromosele, A.I., Ikponmwosa-Eweka, O., Campbell, P. (2019). Physicochemical changes in maize plant (*Zea mays*) grown on contaminated soil exposed to sawdust treatment. *J. Appl. Sci. Environ. Manage.*, 23(3), 5190525.

Ghalamboran, M.R., Kordkheli, S.S., Bernard, F. (2019). Enzymatic response and metal ion content in roots of corn and broad beans planted in soil contaminated with gasoline. *International Journal of Environmental Science and Technology*, 17, 973–982.

Gechev, T. Hille, J. (2005). Hydrogen peroxide as a signal controlling plant programmed cell death. *Journal of Cell Biology*, 168(1), 17–20.

Hatami, E., Abbaspour, A., Dorostkar, V. (2018). Phytoremediation of a petroleum-polluted soil by native plant species in Lorestan Province, Iran. *Environ. Sci. Pollut. Res.*, 1–8.

Hayat, S., Hayat, Q., Alyemeny, MN., Wani, AS., Pichtel, J., Ahmad, A. (2012). Role of proline under changing environments: a review. *Plant Signal Behav*, 7, 1456–1466.

Heath, R., Packer L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives in Biochemistry and Biophysics*, 125, 189–198.

- Hernández-Ortega, H., Alarcón, A., Ferrera-Cerrato, R., Zavaleta-Mancera, H., López-Delgado, H., Mendoza-López, M. (2012). Arbuscular mycorrhizal fungi on growth, nutrient status, and total antioxidant activity of *Melilotus albus* during phytoremediation of a diesel-contaminated substrate. *Journal of Environmental Management Suppl.*, S3, 19–24.
- Imberty, A., Goldberg R., Catesson, A. (1984). Tetramethylbenzidine and p-phenylenediamine-pyrocatechol for peroxidase histochemistry and biochemistry: Two new non cancerogenic chromogens for investigating lignification processes. *Plant Science Letters*, 35, 103–108.
- John, R., Itah, A., Essien, J., Ikpe, D. (2011). Fate of Nitrogen-Fixing Bacteria in Crude Oil Contaminated Wetland Ultisol. *Bulletin of Environmental Contamination and Toxicology*, 87(3), 343–353.
- Juck, D., Charles, T., Whyte, L., Greer, C (2000). Polyphasic microbial community analysis of petroleum-contaminated soils from two northern Canadian communities. *FEMS Microbiology Ecology*, 33(3), 241–249.
- Krishnaiah, D, Sarbatly, R, Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89, 217–233.
- Kumar, J., Oommen, C. (2012) Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyalina*. *Journal of Environmental Biology*, 33(1), 27–31.
- Liste HH. Alexander, M. (2000). Plant-promoted pyrene degradation in soil. *Chemosphere*, 40(1), 7–10.
- Liu, R., Jadeja, R., Zhou, Q., Liu, Z. (2012). Treatment and Remediation of Petroleum-Contaminated Soils Using Selective Ornamental Plants. *Environmental Engineering Science*, 29(6), 494–501.
- Lu, M., Zhang, Z., Sun, S., Wei, X., Wang, Q., Su, Y. (2010). The use of goosegrass (*Eleusine indica*) to remediate soil contaminated with petroleum. *Water, Air, Soil Pollut.*, 209, 181–189.
- Manjunath, M., Kanchan, A., Ranjan, K., Venkatachalam, S., Prasanna, R., Ramakrishnan, B., Hossain, F., Nain, L., Shivay, Y., Rai, A., Singh, B. (2016). Beneficial cyanobacteria and eubacteria synergistically enhance bioavailability of soil nutrients and yield of okra. *Heliyon* 2. e00066.
- Martí, M., Camejo, D., Fernández-García, N., Rellán-Álvarez, R., Marques, S., Sevilla, F., Jiménez, A. (2009). Effect of oil refinery sludges on the growth and antioxidant system of alfalfa plants. *Journal of Hazardous Materials*, 171, 879–885.
- Matysik, J., Alia, Bhalu, B., Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Current Science*, 82, 525–532.
- Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R. (2010). Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant, Cell and Environment*, 33, 453–467.
- Noori, A., Zare Maivan, H., Alaie, E. (2012). Changes in total phenol and flavonoid contents in *Chrysanthemum leucanthemum* under crude oil contamination. *Advances in Environmental Biology*, 6(12), 3057–3064.
- Odjegba, V., Atebe, J. (2007). The effect of used engine oil on carbohydrate, mineral content and nitrate reductase activity of leafy vegetable (*Amaranthus hybridus* L.). *Journal of Applied Sciences Environmental Management*, 11(2), 191–196.
- Park, I., Park, J. (2011). Determination of a risk management primer at petroleum-contaminant sites: Developing new human health risk assessment strategy. *Journal of Hazardous Materials*, 185, 1374–80.
- Petrov, V., Hille, J., Mueller-Roerber, B., Gechev, T. (2015). ROS-mediated abiotic stress-induced programmed cell death in plants. *Frontiers in Plant Science*, 6, 69.
- Rajaei, S., Seyedi, S.M., Eaiesi, F., Shiran, B., Moazam Jazi, M. (2016). Effects of soil petroleum contamination on some physiological and molecular properties of plants. *J. of Molec. And Cellular Research (Iranian Journal of Biology)*, 29(2), 181–197.
- Shulaev, V., Oliver, D. (2006). Metabolic and Proteomic Markers for Oxidative Stress. New Tools for Reactive Oxygen Species Research. *Plant Physiology*, 141(2), 367–372.
- Singleton, V., Rossi, J. (1965). Colorimetry of Total Phenolics with Phosphomolybdic- Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
- Subashchandrabose, S., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K., Naidu, R. (2011). Consortia of cyanobacteria/microalgae and bacteria: Biotechnological potential. *Biotechnology Advances*, 29(6), 896–907.
- Suresh, B., Ravishankar, G. (2004). Phytoremediation – a novel and promising approach for environmental clean-up. *Critical Reviews in Biotechnology*, 24(2–3), 97–124.
- Sutton, N., Maphosa, F., Morillo, J., Al-Soud, W., Langenhoff, A., Grotenhuis, T., Rijnaarts, H., Smidt, H. (2013). Impact of long-term diesel contamination on soil microbial community structure. *Applied and Environmental Microbiology*, 79, 619–630.
- Tang, KHD. (2019). Phytoremediation of soil contaminated with petroleum hydrocarbons: A review of recent literature. *Glob J Civil Environ Eng.* 1, 33–42.
- Vange, V., Hevchand, I., Vandvik, V. (2004). Does seed mass and family affect germination and juvenile performance in *Knautia arvensis*? A study using failure time methods. *Acta Oecologia*, 25, 169–178.
- Wyszkowska, J., Borowik, A., Kucharski, J. (2015). Response of *Avena sativa*, microorganisms and enzymes to contamination of soil with diesel oil. *Plant Soil Environment*, 61(11), 483–488.
- Xia, M., Chakarborty, R., Terry, N., Singh, R.P., Fu, D. (2020). Promotion of saltgrass growth in a saline petroleum hydrocarbons contaminated soil using a plant growth promoting bacterial consortium. *Biodegradation*, 146, 104808.
- Xiao, R., Zheng, Y. (2016). Overview of microalgal extracellular polymeric substances (EPS) and their applications. *Biotechnology Advances.*, 4, 1225–1244.
- Xu, C., Yang, W., Wei, L., Huang, Z., Wei, W., Lin, A. (2019). Enhanced phytoremediation of PAHs-contaminated soil from an industrial relocation site by *Ochrobactrum sp.* *Environ. Sci. and Pollut. Res.*, doi.org/10.1007/s11356-019-05830-7

NEW APPROACHES FOR ANALYSIS METHODS OF THE INPUTS USED IN ECOLOGICAL AGRICULTURE

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Abstract

Ecological agriculture is nowadays considered a potential solution to obtain healthier and nutritious food products but also to minimize the environmental impact by using as inputs, natural resources without resorting synthetic chemicals. The inputs (organic and mineral fertilizers, soil amendments, plants protection products, etc.) accepted for ecological agriculture are regulated by Commission Regulation (EC) No 889/2008 and by Regulation (EC) No 2003/2003 of the European Parliament and of the Council. The quality of inputs is very important and therefore it is mandatory to subject them to chemical analysis, from two perspectives: to characterize their nutritional value for crops and also to evaluate the level of contaminants. Having in view the above mentioned aspects, this paper presents a systematic review of the methods used for nutrient (macro-, oligo-/microelements) and contaminants (heavy metals, pesticides) quantification.

Key words: *contaminant, ecological agriculture, input, nutrient, pesticide.*

INTRODUCTION

An important amount of the food produced worldwide is obtained from intensive agricultural farms, but even if the costs are lower, the impact may result by contribution to environmental pollution.

Recently, it has been observed that ecological agriculture has gained importance and this is due to consumer demand for healthy food products and to the necessity of environment protection by promoting sustainable farming practices (Saracin and Vasile, 2015). The restriction to use chemicals and pesticides contribute to minimizing pollution effects.

Ecological agriculture rely on organic and some mineral fertilizers, biofertilizers (Vlahova, 2014; Vlahova and Popov, 2014), soil amendments, use of natural pest controls that has to comply strict standards, the accepted products being regulated by Commission Regulation (EC) No 889/2008 and by Regulation (EC) No 2003/2003 of the European Parliament and of the Council.

Derived from plant or animal products by different techniques (decomposing, fermentation etc.), inputs destined for ecological agriculture present a great importance due to their beneficial

effects on soil structure, biological activity and represent an important lever to preserve soil quality and to provide healthy food products.

The inputs used for ecological agriculture must be subjected to chemical analysis to characterize their nutritional value for crops, to evaluate the level of contaminants and also to avoid the tendency of fraud in organic food sector that has been evidenced with increase of demand for ecological products.

Having in view the above mentioned aspects, this paper presents a systematic review of the methods used for nutrient (macro, oligo/microelements) and contaminants (heavy metals, pesticides) quantification.

Accordingly, literature studies indicate as suitable analytical methods for nutrient and heavy metals contents, the following: **atomic absorption spectrometry** (AAS), **inductively coupled plasma mass spectrometry** (ICP-MS), **inductively coupled plasma optical emission spectrometry** (ICP-OES).

Concerning pesticide residue analysis, recommended methods are **gas chromatography-mass spectrometry** (GC-MS), **high performance liquid chromatography** (HPLC).

METHODS USED FOR INPUT ANALYSIS

1. Atomic absorption spectrometry (AAS) is a very sensitive technique able to detect 70-80 elements in concentrations lower than 1 ppm from different samples (biological fluids, water, soil, drinks, food, pharmaceutical samples etc.) (Janusa and Beck, 2002).

This technique is based on measurable signal (proportional to the concentration) produced by free gaseous atoms of analyte which have absorbed electromagnetic radiation at a specific wavelength (Fernandez et al., 2018).

The components of AAS spectrometer are: (i) a source of radiation (commonly a hollow cathode lamp), (ii) atomizer (flame or furnace), (iii) monochromator and (iv) detection system (Fernandez et al., 2018).

In order to be subjected to analysis, the sample it has to be atomized, this requirement being solved by using flames or graphite furnace. Air-acetylene flame is sufficient hot for atomization of the majority of macro, micro/oligoelements found in inputs used for ecological agriculture. There are some exceptions, including Mo, for which it is recommended to use nitrous oxide-acetylene flame (Fernandez et al., 2018).

AAS present some chemical interference as (i) formation of less volatile compounds; (ii) formation of more volatile compounds; (iii) the analyte could be transformed into refractory species. All these inconvenient are surpassed using hotter flames, optimizing flame conditions, adding chelating agents etc. (Hill and Fisher, 2017).

AAS technique presents many advantages: low costs, easy to use, high precision and sensibility. As disadvantages it worth to be mentioned that can be analyzed solutions (excepting when graphite furnace is used as atomizer) and some problems with refractory elements.

Determination of metals from inputs for organic agriculture using AAS technique is preceded by sample digestion, the adopted methods being adapted to the nature of the input.

A sum of methods collected after literature review is presented in Table 1.

2. Inductively coupled plasma mass spectrometry (ICP-MS) is an elemental analysis technique able to determine low concentrations (ppb) and ultra-low-concentrations (ppt) of most elements

form periodic system. Sample solution is introduced with a peristaltic pump into the equipment, nebulized in spray chamber and converted into aerosols which typically constitute 2-5% from sample. The aerosols are injected into argon plasma (6000-8000K temperature) and suffer several transformations: desolvation, vaporization, atomization, ionization. Resulted ions are sorted in the basis of their mass.

ICP-MS techniques analyze samples in solution, this providing good control over homogeneity, ease calibration and good precision. This technique uses low volume of solution, has possibility to detect isotope composition of elements, present a large linear range and is capable of multi-element analysis. Disadvantages of the ICP-MS are spectral interferences caused by polyatomic ions or ions with the same mass as the analyte (Beauchemin, 2017). The solid sample is recommended to be destructured with acid solutions and microwave digested (Table 2).

3. Inductively coupled plasma optical emission spectrometry (ICP-OES) also named **inductively coupled plasma atomic emission spectroscopy (ICP-AES)** is a technique that allows determination simultaneously up to 70 elements in a single sample analysis. Detection limits for these elements are in the ppb range. This technique is recommended for refractory elements (silicon, aluminum etc.) but is not suitable for halogens and inert gases (Boss and Fredeen, 2004).

The analyzed samples, usually liquids, are converted into aerosols which are injected into the plasma where temperature reaches 7000K. Here, the aerosols are desolvated, vaporized, atomized, excited and/or ionized. The excited atoms and ions emit their characteristic radiation measured by a spectrometer (Boss and Fredeen, 2004).

For solid samples, direct analysis it is possible by means of vaporization are available (laser ablation or electrothermal vaporization) (Carey and Caruso, 1992). Otherwise, solid sample must be turned into solution, usually by acid digestion (Table 3). A special attention must be paid to siliceous samples when for digestion protocol is used hydrofluoric acid which requires a special sample introduction system (alumina injector) into ICP-OES (Arslan and Tyson, 2008).

Table 1. An overview of input analysis by AAS technique

Input	Sample preparation	Quantified elements	References
compost	microwave digestion with aqua regia (35% HCl and 65% HNO ₃)	<i>Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, Pb, Zn</i>	Bozym and Siemiatowski, 2018
compost	digestion with HNO ₃ and HClO ₄	<i>Ca, Cu, Mg, Zn</i>	Albuquerque Nunes et al., 2015
compost, vermicompost, manure	sample is dried at 105°C, treated with an acidic mixture HNO ₃ :H ₂ SO ₄ :HClO ₄ , 10:1:4 and heated at 200°C	<i>Cd, Cu, Pb, Ni, Zn</i>	National Center of Organic Farming, 1985
fertilizers containing organic matter	a sample of 5 g is heated at 450°C till calcination is done; the residue is treated with aqua regia, HNO ₃ :HCl, 1:3 (v/v).	<i>Cd, Cu, K, Ni, Pb, Zn</i>	FAMIC, 2016
fish waste compost	digestion with H ₂ SO ₄ and H ₂ O ₂ 30%	<i>Ca, Mg</i>	Lopez-Mosquera et al., 2011
fish waste compost	microwave digestion with HNO ₃ 1.4 g/cm ³	<i>Cd, Cu, Cr, Ni, Pb, Zn</i>	Radziemska et al., 2018
foliar fertilizers	sample is treated with concentrated H ₂ SO ₄ and concentrated HNO ₃ (1:6, v/v) and it is heated on sand bath until a white fume appear	<i>Cu, Fe</i>	Răuță and Dorneanu, 1984
foliar fertilizers	sample is treated with concentrated HNO ₃ until solution is clear and it is heated on sand bath	<i>Mn, Mo</i>	Răuță and Dorneanu, 1984
manure	a) calcination at 550°C for 4 hours; resulted residue is treated with HCl; b) sample (0.1-0.5 g for solid samples, 10-15 mL for liquid samples) is treated with 10 mL concentrated HNO ₃ and microwave digested; c) sample (0.1-0.5 g for solid samples, 10-15 mL for liquid samples) is treated with a mixture of HNO ₃ -H ₂ O ₂ -HCl.	<i>Ca, Cu, Fe, K, Mg, Mn, Zn</i>	Peters, 2003
mineral fertilizers	sample is treated with diluted HCl (HCl d = 1.18g/ml and distilled water, 1:1, v/v) and heated	<i>Ca, Co, Cu, Fe, Mg, Mn, Zn</i>	Regulation (EC) 2003/2003
mineral fertilizers	sample is treated with a mixture of HCl and HNO ₃ and then is boiled for 30 minutes	<i>Ca, Mg</i>	Răuță and Dorneanu, 1984
organic fertilizers	sample is calcinated at 500°C for 2 hours; the residue is treated with HCl and boiled for 30 minutes	<i>Ca, Mg</i>	Răuță and Dorneanu, 1984
pig manure	digestion with HCl 37%, HNO ₃ 69% and H ₂ O ₂ 30%	<i>Cd, Cu, Pb, Zn</i>	Lupascu et al., 2009
vermicompost from cow dung and biogas plant slurry	digestion with concentrated HNO ₃ and concentrated HClO ₄ (1:1, v/v)	<i>Cr, Cu, Fe, Zn</i>	Yadav et al., 2013

Contaminant elements are marked with italic.

Table 2. An overview of inputs analysis by ICP-MS technique

Input	Sample preparation	Quantified elements	References
animal manure compost	digestion with HNO ₃ and HClO ₄	<i>As, Cd, Cr, Cu, Ni, Pb, Zn</i>	Yang et al., 2017
ash	ash sample is digested with an acidic solution HNO ₃ 65%, HCl 35%, HF 40%	<i>Cd, Cu, Mn, Pb, Zn</i>	Yu et al., 2013
fluid sludge fertilizers	sample is treated with HNO ₃ and H ₂ O ₂ and is microwave irradiated	<i>Cd, Cr, Ni, Pb</i>	FAMIC, 2016
manure based fertilizers	sample is placed in a Teflon digestion vessel, treated with HNO ₃ , H ₂ O ₂ 30% and subjected to microwave-assisted digestion	<i>As, Cd, Cr, Cu, Zn</i>	Qian et al., 2016
manures, fertilizers, limes	samples are digested with HNO ₃ and HCl for 8 hours; then was added HF and microwave digested.	<i>B, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Zn</i>	McBride and Spiers, 2001
organic fertilizers	digestion with HNO ₃ , HClO ₄ and HF in Teflon vessels, at temperature	<i>Cd, Co, Ni, Mo, Pb</i>	Kawasaki and Arai, 1996
sediments, organo-mineral fertilizers, sludge	sample is treated with HNO ₃ 7 mol/l, H ₂ O ₂ 30% and microwave-assisted digestion	<i>Cd</i>	Machado et al., 2017

Contaminant elements are marked with italic.

Table 3. An overview of inputs analysis by ICP-OES technique

Input	Sample preparation	Quantified elements	References
compost	digestion with aqua regia, HNO ₃ :HCl, 1:3 (v/v).	<i>As, Cd, Co, Cr, Cu, Fe, K, Mn, Ni, Pb, Zn</i>	Mladenov, 2018
compost	digestion with aqua regia, HNO ₃ :HCl, 1:3 (v/v).	<i>Cd, Cr, K, Ni, P, Pb</i>	Becher et al., 2018
fish waste and seaweed compost	microwave-assisted digestion with nitric acid	<i>Cd, Cu, Cr, Ni, Pb</i>	Lopez-Mosquera et al., 2011
manure	a) calcination at 550°C for 4 hours; resulted residue is treated with HCl; b) sample (0.1-0.5 g for solid samples, 10-15 ml for liquid samples) is treated with 10 ml concentrated HNO ₃ and microwave digested; c) sample (0.1-0.5 g for solid samples, 10-15 ml for liquid samples) is treated with a mixture of HNO ₃ -H ₂ O ₂ -HCl.	P, K, Ca, Mg	Peters, 2003
chicken manure	microwave-assisted digestion with aqua regia, HNO ₃ :HCl, 1:3 (v/v).	<i>Ca, Cr, Cu, Mg, Ni, Pb, Zn</i>	Ravindran et al., 2017
sludge fertilizers	sample is heated in an electric furnace at 450°C till calcination is done; the resulted residue is treated with aqua regia, HNO ₃ : HCl, 1:3 (v/v).	<i>Cd, Cu, Ni, Zn</i>	FAMIC, 2016
wood ashes	digestion with HF and HClO ₄	<i>Ca, Cu, K, Mg, Ni, Zn</i>	Sinaj et al., 2015
ash	digestion with HF, HNO ₃ and H ₂ O ₂	<i>Ca, Fe, K, Mg, P</i>	Wang et al., 2012
phosphate products and organic fertilizers	microwave digestion with nitric acid at 200°C	<i>As, Cd, Co, Cr, Pb, Mo, Ni</i>	Kane and Hall, 2006

Contaminant elements are marked with italic.

4. Gas chromatography mass spectrometry (GC-MS) is a technique able to analyze volatile molecules which are sufficiently thermal stable, to separate complex mixtures and to quantify pesticides at low levels found in various types of samples. Usually, pesticides occur in complex environmental matrixes and before chromatographic analysis it is required a sample pretreatment (Table 4). After this procedure (matrix preparation, extraction, clean-up), sample is volatilized into GC-MS and separated using a capillary column (filled with stationary phase). The compounds are carried by mobile phase (represented by argon, helium or nitrogen) and when leaving the GC column are ionized using electron ionization or chemical ionization. Positively charged ions are accelerated through mass analyzer (quadrupole, ion trap, cyclotron resonance etc) and separated on the basis of their different mass-to-charge (m/z) ratios (Andreu and Pico, 2004; Sneddon et al., 2007).

5. High performance liquid chromatography (HPLC) is a versatile method used in pharmaceutical, environmental, forensic areas. The sample/analyte in a solvent (mobile phase) is pumped at high pressure through a column filled with stationary phase and carried by a

stream of nitrogen or helium. The time that a specific compound travels through column to the detector is retention time and depends on the pressure, nature of stationary phase, temperature of the column, used solvent/solvents. The detector recognizes the analytes that has passed through the column and sends the information to a computer to generate the chromatogram (Bottcher et al.). The most common is UV detector, but for highly sensitive measurements are also used photodiode array detectors, fluorescence detectors, refractive index detectors, conductivity detectors (<https://www.ssi.shimadzu.com/products/liquid-chromatography/nexera/uhplc-hplc-detectors/index.html>).

Ultra performance liquid chromatography (UPLC) is a superior technique, the main differences between UPLC and HPLC being the size of used particles filled into column (2 μm vs. 3-5 μm) and operating pressures (103.5 MPa vs. 35-40 MPa).

As result, UPLC present an advantage over HPLC in improved separation and detection of analyte, reduced time of analysis, lower solvent consumption, and reduced costs (Taleuzzaman et al., 2015). Some reported methods for pesticide analysis are presented in Table 5.

Table 4. An overview of pesticide analysis by GC-MS technique

Input	Pesticide	Sample preparation	References
compost	dioxins and furans	- extraction with toluene - clean-up using semi-automatic system equipped with three different columns (silica, alumina, carbon)	Conesa et al., 2009
poultry manure	organochlorine pesticides	- modified QuEChERS procedure: extraction followed by a d-SPE clean-up	Aznar et al., 2014
sediments	triazines	- QuEChERS procedure: extraction followed by SPE clean-up	Brondi et al., 2011
sewage sludge	polychlorinated biphenyls and organochlorine pesticides	- extraction with chloroform: resulted extract was dried with sodium sulphate - clean-up using silica gel column; polychlorinated biphenyls were eluted with hexane and organochlorine pesticides with toluene	Erickson and Pellizari, 1979
compost	carbaryl, chlorpyrifos, cypermethrins, diazinon, imazalil, malathion, mefenacet, pirimiphos methyl, thiobencarb	- extraction by pressurized liquid extraction - clean-up by a partition between hexane and acetonitrile followed by d-SPE extraction using a porous carbon made from Moso bamboo (<i>Phyllostachys pubescens</i>)	Kawata et al., 2005
compost	aldrin, endosulfan (α and β), lindane	- extraction by ultrasonication using dichloromethane and acetone - clean-up on silica gel column; elution with dichloromethane and acetone	Muntjeer et al., 2014

QuEChERS - Quick, Easy, Cheap, Effective, Rugged and Safe (Anastassiades et al., 2003); d-SPE - dispersive solid phase extraction, SPE - solid phase extraction

Table 5. An overview of pesticide analysis by HPLC technique

Input	Pesticide	Sample preparation	References
sludge	glyphosate	- extraction using aqueous solution of sodium phosphate and sodium citrate; extract was adjusted to pH 9 and contaminations were removed by washing with hexane; - purification by LLE method; - determination by HPLC with fluorescence detector	Sun et al., 2017
sewage	organochloride pesticides	- extraction: SPME procedure - determination employing HPLC with UV diode array detector	Torres Padron et al., 2006
cattle manure compost	clopyralid	- extraction: micro LLE - clean-up with SPE cartridge - determination by UPLC-MS/MS, electrospray ionization	Watanabe et al., 2019
compost	isoproturon, bentazone, metalaxyl, linuron	- extraction: SPE procedure - determination by HPLC with diode array detector	De Wilde et al., 2009
peat, compost	isoproturon, bentazone,	- extraction with methanol - determination by HPLC with UV detector	Coppola et al., 2009
poultry manures	bifenthrin, imidacloprid, fipronil	- extraction: SDIE procedure - clean-up by SPE - detection by UPLC	Ong et al., 2017

LLE – liquid liquid extraction; SPME – solid phase microextraction procedure; SPE – solid phase extraction; UPLC-MS/MS - ultraperformance liquid chromatography tandem mass spectrometry; SDIE – solvent direct-immersion extraction

CONCLUSIONS

To take the advantage of growing interest in ecological agriculture, many types of inputs have been promoted, in some cases the quality of them being questionable. As these products have to obey strict standards, their chemical analysis is a very important step to contribute for providing high quality products and to ensure environment protection.

The present paper described the most used methods for inputs analysis to characterize their nutritional value by assessing macro-, oligo-/microelements concentrations and to detect

potential contaminants (heavy metals, pesticides).

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REFERENCES

- Albuquerque Nunes, W.A.G., Scherrer Menezes, J.F., MeloBenites, V., Lima Jr., S.A., Santos Oliveira, A. (2015). Use of organic compost produced from slaughterhouse waste as fertilizer in soybean and corn crops. *Scientia Agricola*, 72(4), 343–350.
- Anastassiades, M., Lehota, S.J., Stajnbaher, D., Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residue in produce. *Journal of AOAC International*, 86(2), 412–431.
- Andreu, V., Pico, Y. (2004). Determination of pesticides and their degradation products in soil: critical review and comparison of methods. *Trends in Analytical Chemistry*, 23(10-11), 772–789.
- Arslan, Z., Tyson, J. (2008). Determination of trace elements in siliceous samples by ICP-MS after precipitation of silicon as sodium fluorosilicate. *Microchimica Acta*, 160(1-2), 219–225.
- Aznar, R., Albero, B., Sanchez-Brunete, C., Miguel, E., Tadeo, J. (2014). Multiresidue analysis of insecticides and other environmental contaminants in poultry manure by gas chromatography/mass spectrometry. *Journal of AOAC International*, 97(4), 1–9.
- Beauchemin, D. (2017). Inductively coupled plasma mass spectrometry methods. *Encyclopedia of Spectroscopy and Spectrometry, Third Edition*, 236–245. <https://doi.org/10.1016/B978-0-12-409547-2.11222-3>.
- Becher, M., Symanowicz, B., Jaremko, D., Trzcinska, E. (2018). Chemical composition of compost from municipal waste in the context of use as fertiliser. *Acta Agrophysica*, 25(3), 329–341.
- Boss, C., Fredeen, K. (2004). Concepts, instrumentation and techniques in inductively coupled plasma optical emission spectrometry. Perkin Elmer.
- Bottcher, J., Margraf, M., Monks, K., HPLC Basics - principles and parameters. Science Together, Knauer. <https://www.knauer.net/en/Systems-Solutions/Analytical-HPLC-UHPLC/HPLC-Basics---principles-and-parameters>
- Bozym, M., Siemiakowski G. (2018). Characterization of composted sewage sludge during maturation process: a pilot scale study. *Environmental Science and Pollution Research*, 25, 34332–34342.
- Brondi, S.H.G., Macedo, A.N., Vicente, G.H.L., Nogueira, A.R.A. (2011). Evaluation of the QuEChERS method and gas-chromatography-mass spectrometry for the analysis pesticide residues in water and sediment. *Bulletin of Environmental Contamination and Toxicology*, 86, 18–22.
- Carey, J., Caruso, J. (1992). Electrothermal vaporization for sample introduction in plasma source spectrometry. *Critical Reviews in Analytical Chemistry*, 23(5), 397–439.
- Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for implementation of Council Regulation (EC) No 834/2007 on organic production and labeling of organic products with regard to organic production, labeling and control.
- Conesa, J., Galvez, A., Marhuenda-Egea F., Bustamante, M.A., Moral, R. (2009). Dioxin content in compost samples. *Communications in Soil Science and Plant Analysis*, 40, 672–681. *Bulletin of Environmental Contamination and Toxicology*, 22, 688–694.
- Coppola, L., Pilar Castillo, M., Vischetti, C. (2010). Degradation of isoproturon and bentazone in peat - and compost-based biomixtures. *Pest Management Science*, DOI 10.1002/ps.2040.
- De Wilde, T., Mertens, J., Simunek, J., Sniegowski, K., Ryckeboer, J., Jaeken, P., Springael, D., Spanoghe, P. (2009). Characterizing pesticide sorption and degradation in microscale biopurification systems using column displacement experiments. *Environmental Pollution*, 157, 463–473.
- Erickson, M., Pellizari E. (1979). Analysis of municipal sewage sludge samples by GC/MS/Computer for polychlorinated biphenils and other chlorinated organics. *Bulletin of Environmental Contamination and Toxicology*, 22, 688–694.
- FAMIC, Incorporated Administrative Agency Food and Agricultural Materials Inspection Center, (2016). Testing methods for fertilizers.
- Fernandez, B., Lobo L., Pereiro, R. (2018). Atomic absorption spectrometry: fundamentals, instrumentation and capabilities. *Encyclopedia of Analytical Science, Third Edition*, <http://dx.doi.org/10.1016/B978-0-12-409547-2.14116-2>.
- Hill, S., Fisher, A. (2017). Atomic absorption, methods and instrumentation. *Encyclopedia of Spectroscopy and Spectrometry, Third Edition*, <http://dx.doi.org/10.1016/B978-0-12-803224-4.00099-6>.
- Janusa, M., Beck, J. (2002). Recent applications of flame atomic absorption spectrometry to environmental measurements. *Applied Spectroscopy Reviews*, 37(2), 137–186.
- Kane, P.F., Hall, W.L. (2006). Determination of arsenic, cadmium, cobalt, chromium, lead, molybdenum, nickel, and selenium in fertilizers by microwave digestion and inductively coupled plasma-optical emission spectrometry detection: collaborative study. *Journal of AOAC International*, 89(6), 1447–1466.
- Kawasaki, A., Arai, S. (1996). Evaluation of digestion methods for multi-elemental analysis of organic wastes by inductively coupled plasma mass spectrometry. *Soil Science and Plant Nutrition*, 42(2), 251–260.
- Kawata, K., Asada, T., Oikawa, K. (2005). Determination of pesticides in compost by pressurized liquid extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1090, 10–15.
- Lopez-Mosquera, M., Fernandez-Lema, E., Villares, R., Corral, R., Alonso, B., Blanco, C. (2011). Composting fish waste and seaweed to produce a fertilizer for use in organic agriculture. *Procedia Environmental Sciences*, 9, 113–117.

- Lupasca, N., Chirila, E., Munteanu, M. (2009). Heavy metal contaminants in organic fertilizers. *Ovidius University Annals of Chemistry*, 20(2), 232–234.
- Machado, R., Amaral, C., Schiavo, D., Nobrega, J., Nogueira, A.R. (2017). Complex samples and spectral interferences in ICP-MS: evaluation of tandem mass spectrometry for interference-free determination of cadmium, tin and platinum group elements. *Microchemical Journal*, 130, 271–275.
- McBride, M., Spiers, G. (2001). Trace element content of selected fertilizers and dairy manures as determined by ICP-MS. *Communications in Soil Science and Plant Analysis*, 32(1-2), 139–156.
- Mladenov, M. (2018). Chemical composition of different types of compost. *Journal of Chemical Technology and Metallurgy*, 53(4), 712–716.
- Muntjeer, A., Kazmi, A.A., Ahmed, N. (2014). Study on effects of temperature, moisture and pH in degradation and degradation kinetics of aldrin, endosulfan, lindane pesticides during full-scale continuous rotary drum composting. *Chemosphere*, 102, 68–75.
- National Center of Organic Farming, Department of Agriculture and Cooperation, India (1985).
- Ong, S.Q., Majib, A.H.A., Ahmad H. (2017). Insecticide residues on poultry manures: field efficacy on selected insecticides in managing *Musca domestica* population. *Tropical Life Sciences Research*, 28(2), 45–55.
- Peters, J. (2003). Recommended methods for manure analysis. University of Wisconsin Extension.
- Qian, M., Wu, H., Wang, J., Zhang, H., Zhang, Z., Zhang, Y., Lin, H., Ma, J. (2016). Occurrence of trace elements and antibiotics in manure-based fertilizers from Zhejiang Province of China. *Science of the Total Environment*, 559, 174–181.
- Radziemska, M., Vaverkova, M.D., Adamcova, D., Brtnicky, M., Mazur, Z. (2018). Valorization of fish waste compost as a fertilizer for agricultural use. *Waste and Biomass Valorization*, <https://doi.org/10.1007/s12649-018-0288-8>.
- Răuță, C., Dorneanu, E. (1984). Metode de analize chimice și control al calității amendamentelor calcaroase și a îngrășămintelor chimice produse industrial. Institutul de Cercetări pentru Pedologie și Agrochimie.
- Ravindran, B., Mupambwa, H., Silwana, S., Mnkeni, P. (2017). Assessment of nutrient quality, heavy metals and phytotoxic properties of chicken manure on selected commercial vegetable crops. *Heliyon*, 3, e00493.
- Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilizers.
- Saracin, V.-C., Vasile, A. (2015). An exploratory research regarding Romanian organic farming sector. *Agrolife Scientific Journal*, 4(2), 119–123.
- Sinaj, S., Maltas, A., Kebli, H., Turpault, M.P. (2015). Wood ashes – a new fertilizer for agriculture. Ramiran 2015, 16th Rural-Urban Symbiosis, 8-10th September 2015, Hamburg, Germany.
- Sneddon, J., Masuram, S., Richert, J.C. (2007). Gas-chromatography-mass spectrometry - basic principles, instrumentation and selected applications for detection of organic compounds. *Analytical Letters*, 40(6), 1003–1012.
- Sun, L., Kong, D., Gu, W., Guo, X., Tao, W., Shan, Z., Wang, Y., Wang, N. (2017). Determination of glyphosate in soil/sludge by high performance liquid chromatography. *Journal of Chromatography A*, 1502, 8–13.
- Taleuzzaman, M., Ali, S., Gilani, S.J., Imam, S.S., Hafeez, A. (2015). Ultra performance liquid chromatography (UPLC) - a review. *Austin Journal of Analytical and Pharmaceutical Chemistry*, 2(6), Id 1056, 1–5.
- TorresPadron, M.E., Sosa Ferrera, Z., Santana Rodriguez, J.J. (2006). Optimisation of solid-phase microextraction coupled to HPLC-UV for the determination of organochlorine pesticides and their metabolites in environmental liquid samples. *Analytical and Bioanalytical Chemistry*, 386, 332–340.
- Vlahova, V. (2014). The impact of biofertilizers on the quality parameters of the pepper fruit (*Capsicum annuum* L.) in organic agriculture conditions. *Scientific Papers, series B, Horticulture, LVIII*, 289–294.
- Vlahova, V., Popov, V. (2014). Impact of biofertilisers on vegetative growth and leaf gas-exchange of pepper seedlings (*Capsicum annuum* L.) in organic farming. *Agrolife Scientific Journal*, 3(1), 156–162.
- Wang, G., Shen, L., Sheng, C. (2012). Characterization of biomass ashes from power plants firing agricultural residues. *Energy and Fuels*, 26, 102–111.
- Watanabe, E., Seike, N., Namiki, S. (2019). Highly sensitive analytical method for herbicide clopyralid residue in cattle manure compost with ultraperformance liquid chromatography tandem mass spectrometry. *Journal of Pest Science*, 44(3), 186–191.
- Yadav, A., Gupta, R., Garg, V.K. (2013). Organic manure production from cow dung and biogas plant slurry by vermicomposting under field conditions. *International Journal of Recycling of Organic Waste in Agriculture*, 2(21), 1–7.
- Yang, X., Li, Q., Tang, Z., Zhang, W., Yu, G., Shen, Q., Zhao, F.J. (2017). Heavy metal concentrations and arsenic speciation in animal manure composts in China. *Waste Management*, 64, 333–339.
- Yu, J., Sun, L., Xiang, J., Jin, L., Hu, S., Qiu, J. (2013). Physical and chemical characterization of ashes from a municipal solid waste incinerator in China. *Waste Management Research*, 31(7), 663–673.

PLANT DIVERSITY AND PASTORAL VALUE OF SOME GRASSLANDS FROM ALPINE AND SUBALPINE AREAS OF SOUTH-WEST FĂGĂRAȘ MASSIF (ROMANIAN CARPATHIANS)

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Abstract

The Făgăraș Massif is situated in the central area of the Carpathian Mountains, aligned west to east and whose slopes mainly have north and south aspects. The habitat diversity is high, comprising grasslands from the foot of the mountains to their summits. These grasslands have been used for sheep grazing for centuries. Our study examined six private grasslands, recording the vegetation both within enclosures, which simulate ungrazed areas, and outside the enclosures in grazed areas. In each investigated grassland, an inventory of the plant species was made and the pastoral value calculated. From these observations, we can argue that the plant species diversity and pastoral value of the grasslands decreases with increased grazing intensity and higher altitude. The overgrazed grasslands are dominated by species with low palatability which might lead to starvation of the sheep and forcing the animals to consume even these species. Thus, a good management plan can lead to sustainable future usage of these grasslands.

Key words: species diversity, pastoral value, grasslands, Romanian Carpathians, Făgăraș Massif.

INTRODUCTION

The socio-economic and cultural development of human communities has been influenced and shaped over time by the use of natural resources at local and/or regional level. People used grasslands as renewable natural resources. Permanent grassland - natural and/or semi-natural - are an important part of the natural heritage and through their use for grazing represent the cheapest source of fodder. Grassland is a multifunctional system with different utilities: fodder production, plant and animal biodiversity, prevention of soil erosion, water storage, maintaining groundwater quality, ensuring landscape quality, storage of carbon, supplying soil with biologically fixed nitrogen, etc. (Bernués et al., 2015; Dragomir, 2017).

The quality of the soil and water of any agricultural system is intrinsically linked with its productivity. Maintaining healthy perennial vegetation aids the creation, improvement and protection of soil and maintains clean water (Jones, 1996).

In Romania 783 habitat types have been defined, of which almost 60% are natural grasslands (Doniță et al., 2005). Marușca et al. (2012), have

been identified 3700 plant species, which, based on IUCN red list categories (2012) can be framed as is follow: 74 species are extinct, 485 are critically endangered, 200 species are vulnerable, 23 species are declared nature monuments and 1253 are rare species. 70% from these species belong to the vegetation of permanent grassland (Marușca et al., 2010). In hilly and mountainous regions, permanent grasslands occupy large areas, but the land relief is more varied, the slopes are steeper and the local climate (microclimate) variable. The soils are thinner, skeletal and less fertile, and hence dry or with moisture excess. These factors lead to a high heterogeneity of plant species, high heterogeneity and uneven distribution of component habitats (phytocoenoses). This ecological variation leads in turn to variation in fodder production and quality i.e. different value of the permanent grasslands from one region to another and even within smaller areas. Their rational use, the implementation of appropriate work on improvement, maintenance and use can only be carried out in accordance with accurate knowledge of existing vegetation, the soil-climate conditions, as well as the natural and anthropogenic environmental factors that affect

them. Improvement of grassland quality for agriculture requires appropriate technologies adapted to the land relief and soil conditions (Motcă et al., 1994).

Pastoral Value (PV) is an integrated index derived from vegetation surveys, summarising forage yield, quality of a grassland, and palatability of the species for livestock, determined by floristic evaluation methods (Marușca et al., 2014). This index can provide a reliable estimate of the grassland carrying capacity in accordance with the maximum livestock in a specified grazing system (Pittarello et al., 2018). As it was reported by Moisuc et al. (2010), the influence of the altitude on the values of grasslands PV index in hill areas are heterogeneous due to the important presence of poor species, without nutritional value, and it is independent of altitude itself.

As it was described in the Management Plan of ROSCI0122 Făgăraș Mountain and ROSPA0098 Făgăraș Piedmont (2016), the Făgăraș Massif is situated in central Romania, extending over 2300 km² with clearly differing slopes on the northern and southern sides, the latter being more moderate. The climate of the Făgăraș Massif has special features due to the huge size and orientation of these mountains. The mountains act as a barrier, both for the cold and wet air masses coming from the Atlantic or the North seas, retaining them longer on its northern slope, and for Mediterranean or tropical masses that stop on its southern slopes (Ciulache, 2005). The result is a dynamic climate on the north side (humid, and cold) and a more moderate, calm and clear on the southern side. The elements of the climate (temperature, wind, rainfall) are influenced by altitude in the Făgăraș Massif. This is reflected in the existence of differing bioclimatic conditions favouring either deciduous forests, coniferous forests or alpine meadows. In addition, on the southern side, the forest rises to a higher altitude than on the northern, Transylvanian one (Kotek et al., 2006). Alpine grasslands (2300-2544 m altitude) are more extensive than grasslands in subalpine (1600-2300 m altitude) and submontane areas (650-1600 m altitude) where they are present in mosaics with coniferous, mixed and beech forests (Ielenicz and Pătru, 2005). In alpine areas, the grasslands are composed of short species (*Carex curvula*, *Juncus trifidus*, *Festuca*

supina) and the productivity is very low (about 0.8 t/year/ha) due to a) the lower temperature during the short growing period for alpine plants and b) the shallower, acidic soils poor in nutrients. At the subalpine level, the temperature is somewhat higher and the growing season longer, but the soils are still shallow, nutrient-poor and acidic. The subalpine vegetation is dominated by shrubs, except where the shrubs are clear cut, producing secondary pastures (with *Festuca supina*, *Nardus stricta*), and higher productivity (about 2-6 t/year/ha) (Pușcaru-Soroceanu et al., 1963; Doniță et al., 2005).

MATERIALS AND METHODS

The study area is situated in the South-West region of Făgăraș Mountains (Photo 1), in alpine and subalpine levels.



Photo 1. A general view of Făgăraș Massif with the study area framed by yellow

Our study was performed in four community private grasslands used as grazing pastures for sheep, spread over six mountains (Photo 2).



Photo 2. The localization of the grasslands in six mountains sites from Făgăraș Massif

In the grazing areas, the representatives of the communities have installed enclosures delimitating 10 x10 m plots (Photo 3) where grazing has been banned.



Photo 3. The image of enclosures used

The plant species were identified within and outside enclosures, and the species were recorded using the *pratological* method of Ivan and Doniță (1975) with coverage % of each species encountered and their classification in economic groups: grasses, legumes, sedges and rushes, other families, moss and lichens, woody species. This classification is recommended as a quick method for determining grassland vegetation (Blaj et al., 2014).

Where necessary, the species identification guide (Ciocârlan, 2009; Sârbu et al., 2013) was used for more precise identification of vascular plant species. We used the formula for determination of pastoral value described by Marușca et al. (2012) where PV is the indicator of pastoral value expressed as a percentage:

$$PV = \frac{\sum PC \times IC}{5}$$

PC = participation in herbaceous layer;
IC = forage quality index.

Following the methodology of Marușca et al (2014), in the table of the inventoried species (with percentage participation of the species) we added the forage quality index (IC) and calculated the pastoral value index that allows the framing of the condition type for each grassland (Table 1).

Table 1. The grassland condition type based on calculated PV

Grassland condition type	PV Intervals (%)
Degraded	0-5
Very poor	5-15
Poor	15-25
Medium	25-50
Good	50-75
Very good	75-100

The forage quality index is given by the agronomic traits of the species as it is given in the literature (Kovacs, 1979).

In each grassland, apart from plots within the enclosures, we inventoried plots in the open grazed

grassland: a) 5 plots in Sterminoasa (S1-S5) situated at 1740-1755 m altitude, with W aspect and slopes 15°; b) 4 plots in Budislavu (B1-B4) situated at 2073-2098 m altitude, with SW aspect and slopes 20-30°; c) 2 plots in Cocorciuc (C1-C2) situated at 1918-1919 m altitude, with S aspect and 15° slopes; d) 4 plots in Grohotișu (Gr1-Gr4) situated at 1746-1868 m altitude, with E and S-SE aspect and 20-25° slopes; e) 3 plots in Galbeana (Ga1-Ga3) situated at 1710-2054 m altitude, with W and W-SW aspect and 15° slopes; f) 4 plots in Vemeșoia (V1-V4) situated at 1747-2043 m altitude, with S and S-SE aspect and 15-20° slopes.

The data obtained after field inventory, was statistically analysed, and for multivariate Detrended Canonical analysis (DCA) - characteristic species/plot, we used the PAST program (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The inventory of plant species in the studied grasslands revealed that the species number is low (Figure 1) dominated by *Deschampsia cespitosa* (L.) P. Beauv., *Festuca rubra* L., *Nardus stricta* L., *Poa media* Schur, *Phleum alpinum* L. ssp. *alpinum*.

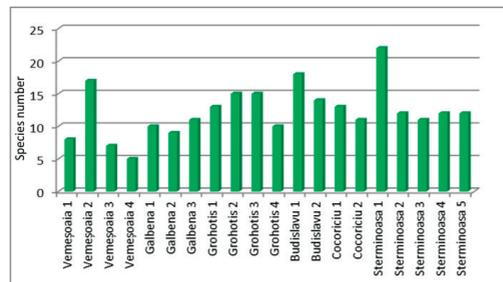


Figure 1. The number of the species inventoried in studied grasslands

In ungrazed enclosures, the species diversity is greater (V2, G3, G3, B1, C1, S1) and the percentage of those species with a high forage quality index was higher when compared to grazed areas. In grazed areas, the palatability of these species made them to be overgrazed and replaced by species without forage quality (*i.e. Deschampsia cespitosa*).

Species diversity differs in relation to the altitude and slope (Figure 2), but the impact of grazing is to produce uniform areas, becoming similar due to the dominance of some species (*Deschampsia cespitosa*, *Nardus stricta*).

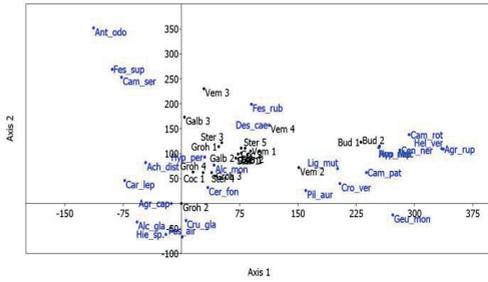


Figure 2. Detrended Canonical analysis (DCA) - characteristic species/plot

There was low variation in species composition in relation to site, but the species distribution correlated with altitude. Forage species with a forage quality index between 1 and 5 from Poaceae Family (*Festuca rubra* L. *Phleum alpinum* L. ssp. *alpinum*, *Poa media* Schur), Fabaceae Family (*Trifolium repens* L.) and Apiaceae Family (*Ligusticum mutellina* (L.) Crantz) are present at high altitude (i.e. B1-B4 at 2073-2098 m altitude). It is known that these forage plant species are cold tolerant (Filho et al., 2018) and thus the number of plant species preferred by grazing animals was not affected by the harsh climate at high altitudes in the Făgăraș mountain range.

Analysis of the forage quality of species recorded in this study showed that most of them are worthless, with no pastoral value for grazing animals (Figure 3).

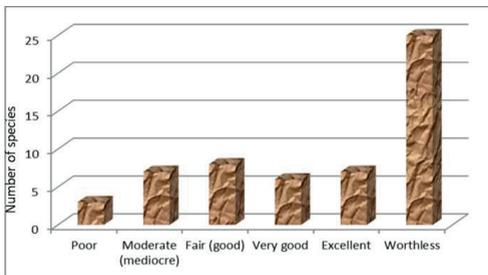


Figure 3. Forage plants species (used by grazing animals)

Our investigations on the altitudinal gradient showed that the entire South-West region of the Făgăraș Mountains is dominated by South-Eastern Carpathians grasslands with *Nardus stricta* and *Viola declinata* (Romanian Habitat RO3609). This type of grassland is degraded due to medium to high grazing impact. The impact

transforms the original habitat RO3609 into an anthropogenic habitat dominated by *Deschampsia caespitosa*. Where valuable forage species do survive, the overgrazing limits their growth at the soil level thus making them not accessible to the grazing animals.

The grasslands dominated by *Nardus stricta* are well developed in the areas. Among swards of *Nardus* it is difficult for other species to arise. The ecological factors that stimulate spread of *Nardus* habitat are acidity and lack of nutrients in the soil, combined with climate, relief and grazing intensity (Pușcaru-Soroceanu et al., 1963). Marușca et al. (2014) have classified these grasslands as the *Nardus stricta* series of sub-alpine level (of junipers) in high mountains.

This type of grassland belongs within the subalpine oligotrophic pastures of all high mountainous massifs that are characteristic of podzolic soils, acidic and poor in nutrients, occupying tens of hectares at altitudes between 1200 and 1800 m (Coldea et al., 2001). These grasslands develop well toward lower zone of the alpine level (Pușcaru-Soroceanu, 1963).

The pastoral value (Figure 4) of the studied grasslands is relatively low, in spite of the high proportion of grasses and leguminous species (Poaceae and Fabaceae).

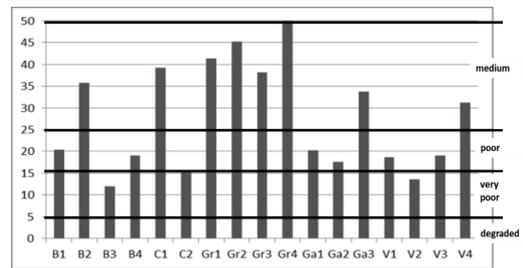


Figure 4. Pastoral Value of the grasslands of South-West Făgăraș Massif

Using the pastoral value intervals (%) of grasslands defined in Table 1, we can classify the studied grasslands as follows: a) very poor (B3 at 2098 m altitude, C2 at 1918 m altitude, V2 at 1887 m altitude); b) poor (B1 at 2090 m altitude, B4 at 2073 m altitude, Ga1 at 2054 m altitude, Ga2 at 2050 m altitude, V1 at 1747 m altitude, V3 at 2043 m altitude); c) medium (B2 at 2068 m altitude, C1 at 1919 m altitude, Gr1 at 1746 m altitude, Gr2 at 1747 m altitude, Gr3 at

1721 m altitude, Gr4 at 1868 m altitude, Ga3 at 1710 m altitude, V4 at 1933 m altitude).

Grassland type and quality are determined not by the altitude but by the grazing pressure which, under increased intensity, notably modifies the grassland type (positive feedback).

CONCLUSIONS

The grasslands have very poor to medium quality, due to both natural and anthropogenic impacts; they are overgrazed, which can have irreversible effects on the quality of grassland and thus on grazing animals (reducing the quality of livestock products). The main threats to the studied grasslands are the overgrazing and the burning of alpine scrub. This results in reduced plant species diversity, increased dominance of some non-forage species and increased distribution and density of some toxic species. Overgrazing and intensification will produce more uniform grasslands over a large area, regardless of climate, geology and natural soil diversity.

Local people should become aware of important new management actions, other than grazing, that support the other ecosystem services provided by grasslands. Conservation of grassland does not imply taking no action but adopting actions for sustainable use and expansion of the resources (e.g. genetic, soil quality & quantity).

Climate change mitigation for the eco-sustainability and biodiversity of production systems is the priority for the European agricultural agenda.

Minimising the damaging ecological impact of farms is a key factor for farmers seeking to obtain public incentives for enhancing the multifunctionality of agricultural systems expressed as services for society. Thus, the assessment and valuation of environmental performance may be a very important factor to improve the competitiveness of grassland-based farming, especially for those located in protected areas.

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REFERENCES

- Bernués, A., Rodríguez-Ortega, T., Alfnes, F., Clemetsen, M., Eik, L.O. (2015). Quantifying the multifunctionality of fjord and mountain agriculture by means of sociocultural and economic valuation of ecosystem services. *Land Use Policy*, 48, 170–178.
- Blaș, V.A., Marușca, T., Dragomir, N., Ichim, E., Rusu, M., Mocanu, V., Silistru, D., Rtoad, M.A., Ene, T.A. (2014). *Măsuri proactive zonale de ameliorare a valorii pastorale a pașiștilor permanente degradate sub acțiunea modificărilor climatice și a intervențiilor antropice*, Editura Capolavoro, Brașov.
- Ciocârlan, V. (2009). *Flora Ilustrată a României. Pterydophyta et Spermatophyta*, Editura Ceres, București.
- Ciulache, S. (2005). *Meteorologie și climatologie*, Ed. Credis, București.
- Coldea, Gh., Sârbu, I., Negrean, G., Sârbu, A. (2001). *Ghid pentru identificarea și inventarierea pașiștilor seminaturale din România*, Editura Alo, București.
- Doniță, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș, I.A. (2005). *Habitatele din România*, Editura Tehnică Silvică, București.
- Dragomir, N. (2017). Cum recunoaștem o pașiște degradată, *Ferma*, 20, 203.
- Filho, J.M., Gaspar de Oliveira, C.M., Caramori, P.H., Nagashima, G.T., Hernandez, F.B.T. (2018). Cold tolerance of forage plant species, *Ciências Agrárias, Londrina*, 39(4), 1469–1476.
- Hammer, R., Harper, D.A.T., Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1–9.
- Ielenicz, M., Pătru, I. (2005). *Geografia fizică a României*, Editura Universitară, București.
- IUCN Red List categories and criteria, version 3.1, second edition, <https://www.iucn.org/resources/conservation-tools/iucn-red-list-threatened-species>
- Ivan, D., Doniță, N. (1975). *Metode practice pentru studiul ecologic și geografic al vegetației*. Universitatea din București, Facultatea de Biologie.
- Jones, C.E. (1996). Pastoral value and production from native pastures, *New Zealand Journal of Agricultural Research*, 39(4), 449–456.
- Kotek, M., Greiser, J., Beck, C., Rudolf, B., Rubel, F. (2006). World Map of the Köppen-Geiger climate

- classification, *Meteorologische Zeitschrift*, 15(3), 259–263.
- Kovács, J.A. (1979). *Indicatorii biologici, ecologici și economici ai florei pajiștilor*, Stațiunea Centrală de Cercetări pentru cultura pajiștilor Măgurele-Brașov.
- Marușca, T., Mocanu, V., Cardașol, V., Hermenean, I., Blaj, V.A., Oprea, G., Tod, M.A. (2010). *Ghid de producere ecologică a furajelor de pajiști montane*, Editura Universității Transilvania.
- Marușca, T., Blaj, V.A., Rusu, M. (2012). *Tehnologii de creștere a valorii pastorale pentru pajiștile montane*. Academia de Științe Agricole și Silvicultură Gheorghe Ionescu Sisești.
- Marușca, T., Mocanu, V., Haș, E.C., Tod, M.A., Andreoiu, A.C., Dragoș, M.M., Blaj, V.A., Ene, T.A., Silistru, D., Ichim, E., Zevedei, P.M., Constantinescu, C.S., Tod, S.V. (2014). *Ghid de întocmire a amenajamentelor pastorale*, Editura Capolavoro.
- Moisuc, A., Sărățeanu, V., Samfira, I., Bărluț, R. (2010). Influence of altitude on pastoral value in hills' area from western Romania (case study). *Research Journal of Agricultural Science*, 42(1), 513–516.
- Motcă, Gh., Geamănu, L.-I., Oancea, I. (1994). *Pajiștile României (Tipologie și tehnologie)*, Editura Tehnică Agricolă.
- Pittarello, M., Lonati, M., Gorlier, A., Perotti, E., Probo, M., Lombardi, G. (2018). Plant diversity and pastoral value in alpine pastures are maximized at different nutrient indicator values, *Ecological Indicators*, 85, 518–524
- Pușcaru-Soroceanu, E., Pușcaru, D., Buia, Al., Burduja, C., Csuros, Șt., Grîneanu, A., Nie-Dermayer, K., Popescu, P., Răvăruf, M., Resmeriță, I., Samoilă, Z., Vasiliu, V., Velea, C. (1963). *Pășunile și fânețele din Republica Populară Română*, Editura Academiei Republicii Populare Române.
- Sârbu I., Ștefan N., Oprea A. (2013). *Plante vasculare din România. Determinator ilustrat de teren*, Editura Victor B. Victor.
- MP. (2016). The Management Plan of ROSCI0122 Munții Făgărașului and ROSPA0098 Piemontul Făgăraș, (<http://www.mmediu.ro>)

ISOLATION AND CHARACTERIZATION OF SOIL MICROORGANISMS DEGRADING THE HERBICIDE ISOXAFLUTOL

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Abstract

Chemical weed control is one of the most distributed weed management methods around the globe. As herbicide-resistant weeds often develop, dosage increases are required, leading to environmental pollution. An effective environmental strategy for the degradation of herbicides is to detect and apply microorganisms capable of breaking down and transforming the herbicides. The study aims to determine the degradation of the herbicide Merlin Flexx SC 480 (240 g/l isoxaflutole + 240 g/l cyprosulfamide - antidote) by the soil microorganisms. The current research was conducted with microbial communities from the maize rhizosphere that are resistant to isoxazoles. The soil was treated with two rates of isoxaflutole - 420 and 840 ml ha⁻¹. The biodegradation of isoxaflutole to diketonitrile and benzoic acid derivative was measured by HPLC. The main representatives of the microflora were bacteria, mold fungi, and nitrogen-fixing bacteria to a greater extent. A decrease in the number of bacteria and an increase in the number of mold fungi in the treated soils was found. The number of nitrogen-fixing bacteria increased by increasing the amount of Merlin Flexx in the soil.

Key words: herbicide, herbicide degradation, soil microflora.

INTRODUCTION

The most limiting factor in agricultural production is the weeds. They can decrease the maize yield, for instance, from 24% to 96.7% (Mukherjee and Debnath, 2013; Zhaltov and Raikov, 1996). In agriculture, the weed control is based mainly on chemical control (Tityanov, 2016; Tonev et al., 2007). The active substance isoxaflutol is isoxazole herbicide, which is applied for control of grass and broadleaf weeds in maize (Taylor-Lovell et al., 2002; Tonev et al., 2002).

The application of the herbicide Merlin Duo (37.5 g/l isoxaflutole + 375 g/l terbuthylazine) showed different efficacy against the weeds in comparison to the application rate. The most difficult-to-control annual broadleaf weed in the experiment was *Xanthium strumarium* L. (Mitkov et al., 2018). Very good efficacy against the weeds was found (94.6%) after the application of Merlin Flexx (240 g/l isoxaflutol + 240 g/l cyprosulfamide) in a rate of 42 ml/da (Dimitrova et al., 2013).

The herbicide isoxaflutol inhibits hydroxyphenylpyruvate dioxygenase, an enzyme that is involved in the carotenoids biosynthesis, as by

this way suppress the weeds growth and development.

In soil, the isoxaflutol is hydrolysed diketonitrile derivative, which is the active form of the herbicide and benzoic acid analogue (Rice et al., 2004; Spundjueva et al. 2001).

There is a strong relationship between the contamination of pesticides and their residual detection and accept they have toxic for humans, there is a high risk of contamination in ecosystems (Veiga et al., 2006; Calderbank, 1989). Biodegradation is widely used for the treatment of xenobiotics in soil. It is employed in many countries because it is being ecofriendly (Enrica, 1994; Ritmann et al., 1988).

Although a number of techniques are available for biodegradation: (i) Bacterial degradation; (ii) Fungal degradation; (iii) Enzymatic degradation (Javaid et al., 2016).

Therefore, it is necessary to evaluate the effect of persistent soil herbicides and their degradation products by rhizospheric and soil microorganisms, to study the biochemical pathways for their degradation. It is also necessary resistant microorganisms to be isolated and a study of their biochemical pathways for their transformation to be conducted.

The aim of this study is to investigate the degradation of the herbicide Merlin Flexx SC containing the active substance isoxaflutol by soil microorganisms.

MATERIALS AND METHODS

Herbicide application

The experiment was conducted in two consecutive years (2016 and 2017) on the experimental field of the base for training and implementation of the Agricultural University of Plovdiv, Bulgaria. On the area where the trial was performed, maize was grown in the two years. The herbicide product Merlin Flexx SC 480 (240 g/l isoxaflutol + 240 g/l cyprosulfamide - antidote) was applied with a sprayer for plot experiments with a spraying solution volume 300 l/ha. Treatments of the experiment were: 1) Untreated control; 2) Merlin Flexx SC 480-420 ml/ha; 3) Merlin Flexx SC 480-840 ml/ha. The size of each experimental plot was 30 m².

Soil samples

The soil of the experimental field is with the following agrochemical properties in the layer of 0-30 cm depth: pH (H₂O) - 8.10; NH₄ N - 19.30 mg/1000 g; NO₃ N - 16.60 mg/1000 g; P₂O₅ - 32.75 mg/100 g; K₂O - 31.80 mg/100 g. An average soil sample from the upper soil layer in depth of 5-15 cm was taken from all treatments. For greater accuracy of the experiment the average sample is obtained by taking soil by the diagonal of each plot.

Toxicity test

In 2016, in the scientific laboratory of the department of "Microbiology and ecological biotechnologies" at the Agricultural University of Plovdiv a pot trial with test crop lettuce (*Lactuca sativa* L.) was conducted. This plant is very sensitive to the active substance isoxaflutol. The used pots were with 8 cm of diameter and were filled with soil taken from the experimental area. Treatment 1 was with soil taken from the untreated plots. Treatments 2 and 3 were with soil taken from the treated plots - Merlin Flexx SC 480-420 and 840 ml/ha respectively. The pot trial was conducted with two lettuce varieties: "Cherna giumiurdjinska" (*L. sativa*) and "Lollo Rosso" (*L. sativa* var. *rosso*). In each pot 25 lettuce seeds were seeded. Each treatment was replicated three times.

Soil respiration

Fifty grams of air dry soil was weighted; it was previously sifted through a 2 mm sieve. The soil sample was placed in glass jar with a lid. At the bottom of the glass jar a Beaker glass with 20 ml 0.05 M KOH was placed. The soil was incubated for 6 hours at 25-27°C temperature. After the six hours, the soil was taken out and was immediately treated with 5 ml 0.05 M BaCl₂ for the sedimentation of carbamates. Glucose for inducing is added. A titration with 0.1% HCl with the phenolphthalein indicator till obtaining white coloration was performed. As a control a sample with KOH only was used.

Soil pH and EC (electroconductivity)

Five gram soil sample is placed in 25 ml of distilled water (dilution 1:5) and was well shaken for 10 minutes. It was followed by 120 minutes of rest for sedimentation of the soil particles. The sample was decanted and pH with pH meter was measured. The extract was also used to measure the soil EC.

Determination of β-glucosidase activity

One g soil sample was placed in 50 ml flask, 0.25 ml toluene, 4 ml buffer (21.1 g Tris, 11.6 g maleic acid, 14 g citric acid, 6.3 g boric acid, 500 ml 1 M NaOH and distilled water to 1 liter, as well as 1 ml p-nitrophenol-β-D-glucoside were added. It was intensively shaken for 1 hour at 37°C on a water bath. After that CaCl₂, tris buffer with pH 12 was added and the solution was filtered. The color reaction was analyzed spectrophotometrically (400 nm).

Isolation of resistant microorganisms

The influence of Merlin Flexx SC 480 on different groups of microorganisms was evaluated. Important groups of microorganisms resistant to isoxaflutol were isolated. For this purpose, 1 g soil sample is placed in 99 ml of sterile water (dilution 1:100) in flask. The solution is shaken for 10 minutes and is placed at resting for sedimentation of the soil particles. From the solution the other 3 dilutions by adding 1 ml of soil suspension to 9 ml sterile water (dilutions 10³, 10⁴, and 10⁵). Petri dishes with different nutrient media were prepared beforehand. The nutrient medias are on Table 1.

Table 1. Chemical composition of the media used

Nutrient media	Content
Mineral nutrient media	K ₂ HPO ₄ - 0.8 g/l, KH ₂ PO ₄ - 0.2 g/l, MgSO ₄ ·7H ₂ O - 0.2 g/l, CaSO ₄ - 0.1 g/l, (NH ₄) ₆ MoO ₂₄ ·4H ₂ O - 0.001 g/l, (NH ₄) ₂ SO ₄ - 5 g, agar - 20 g/l
LB (Laura-Bertani)	Tryptone - 10 g/l, yeast extract - 5 g/l, NaCl - 5 g/l, agar - 20 g/l, pH 7.0
Chapek	NaNO ₃ - 3.0 g/l, KH ₂ PO ₄ - 1.0 g/l, MgSO ₄ - 0.5 g/l, KCl - 0.5 g/l, FeSO ₄ - 0.01 g/l, sucrose 30 g/l, agar 20.0 g/l, pH 4.5-5
TSA	Caseinpeptone - 17.0 g/l, soypeptone - 3.0 g/l, NaCl - 5.0 g/l, K ₂ HPO ₄ - 2.5 g/l, dextrose - 2.5 g/l, agar - 20 g/l
Nutrient media for moulds with rosebengal	TSB - 6.8 g/l, yeast extract - 0.8 g/l, glucose - 9.4 g/l, MgSO ₄ - 0.5 g/l, rose Bengal - 0.05 g/l, agar - 24 g/l
Yeast agar	Yeast extract - 3.0 g/l, malt extract - 3.0 g/l, peptone - 5.0 g/l, glucose - 10.0 g/l, agar - 20.0 g/l
Nutrient media for ornitrogen-fixing bacteria	Glucose - 5.0 g/l, mannitol - 5.0 g/l, CaCl ₂ ·2H ₂ O - 0.1 g/l, MgSO ₄ ·7H ₂ O - 0.1 g/l, Na ₂ MoO ₄ - 0.5 mg/l, KH ₂ PO ₄ - 0.1 g/l, FeSO ₄ ·7H ₂ O - 0.01 g/l, CaCO ₃ - 5.0 g/l, agar - 20 g/l
Water agar	Agar 20 g was added per liter of distilled water
Nutri Agar	Meat extract - 1.0 g/l, yeast extract - 2.0 g/l, peptone - 5.0 g/l, agar - 15.0 g/l, pH - 7.4-7.5

Universal and selective nutrient media were used in order to isolate microorganisms from different groups that are resistant to izoxaflutol. For resistant microorganisms isolation, izoxaflutol (Merlin Flexx SC 480) was added to the nutrient media (mineral nutrient media, water agar and rose Bengal media) in concentrations of 0, 15, 75, 150 µg/L.

RESULTS AND DISCUSSIONS

Toxicity test

There are several methods for testing the toxicity of herbicides to test crops. Seed germination has been reported with acute herbicide toxicity. The reaction of the test plants to the two evaluated izoxaflutol rates was evaluated. The results are presented on Figure 1. The results of the pot trial showed that the rate of 420 ml/ha of izoxaflutol suppressed the growth and development of the test crop. The doubled rate of 840 ml/ha stopped the growth of the lettuce completely. The toxic effect was more severe for the plants of the “Lollo Rosso” lettuce variety than “Cherna giumiurdjinska” variety (Pictures 1 and 2).

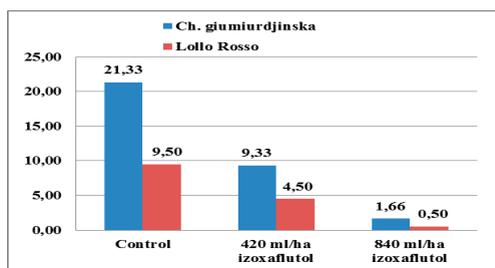
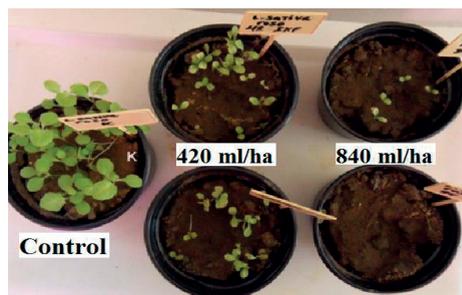
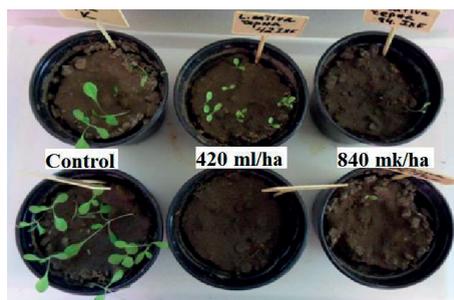


Figure 1. Test for toxicity - number of germinated lettuce plants of the two studied varieties



Picture 1. Test plants of “Lollo Rosso” lettuce variety



Picture 2. Test plants of “Cherna giumiurdjinska” lettuce variety

Soil respiration

To determine the influence of the herbicide rate on soil microorganisms, quantitative and functional characteristics by usage of soil respiration method were performed. Soil respiration indicates the current state of the soil by determining the amount of CO₂ released and indirectly serves to determine microbial biomass. The microorganisms present may not be active at this time, so the induction of microbial activity by glucose supplementation is used. The experiment was conducted in two stages - initial (immediately after field sampling) and a second period (after 6 months of soil storage). The obtained results indicated

that in the initial stage without induction, the total biological activity of the treated soils is higher than that of the control (Figure 2A). Glucose induction decreased the amount of CO₂ released from the treated soils (Figure 2 B). Differences in CO₂ emissions before and after induction are significant in untreated soil (Figure 2 C). In the second stage, the analyses performed with and without induction showed that the soils treated with Merlin Flexx SC 480 in both examined rates increased the biomass values twice as compared to the control (Figure 2 B). On Figure 2 D the difference in reported total biological activity reported at 180 days from sampling is presented. In the absence of glucose, there is a decrease and, after induction, an increase in the amount of microbial biomass is observed. On Figure 3 the differences in the biomass changes reported in the periods are presented. The amount of individual CO₂ is higher at the higher dose of the herbicide (840 ml/ha) compared to twice lower rate (420 ml/ha). This result proves that the microorganisms are resistant to izoxaflutol.

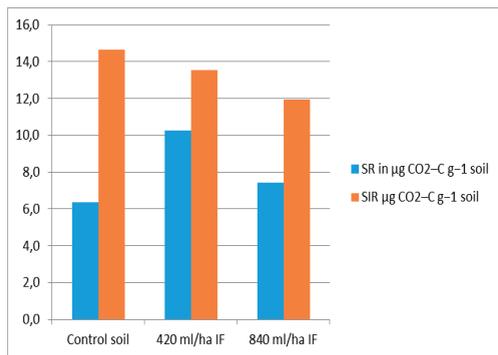


Figure 2A. CO₂ released from the treated soils without induction

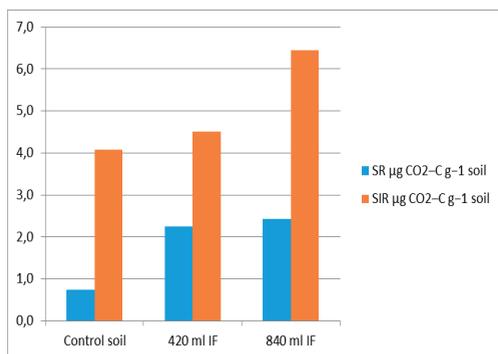


Figure 2B. Biomass increase after induction

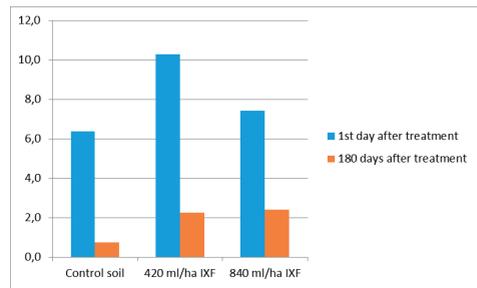


Figure 2C. Differences in CO₂ emissions before and after induction

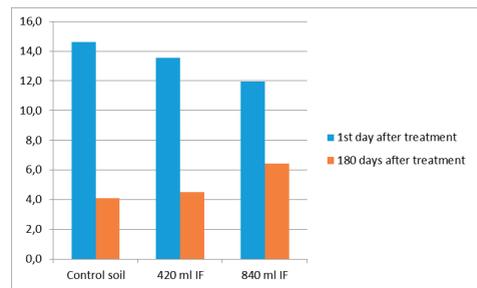


Figure 2D. Total biological activity

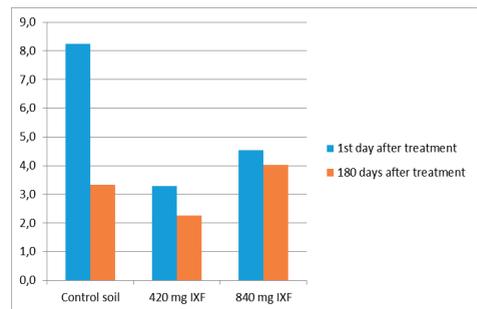


Figure 3. Differences in the biomass changes

Determination of β-glucosidase activity

The obtained data showed that the higher herbicide rate had simulative effect on the development and activity of the microorganisms (Figure 4).

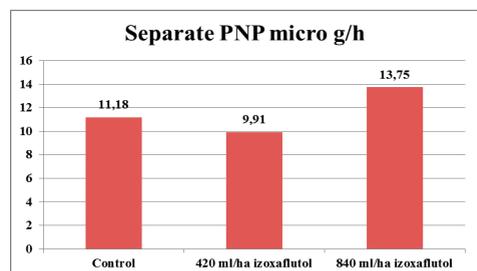


Figure 4. β-glucosidase activity

Soil pH and EC

The soil EC of treatment 2 (420 ml/ha izoxaflutol) was 103 mS/cm. For the doubled rate (840 ml/ha izoxaflutol) the EC values were 97 mS/cm. The data concerning the soil pH showed no considerable differences and varied from 8.23 to 8.32 (Table 2).

Table 2. Soil EC and pH

Soil samples	EC mS/cm	pH (H ₂ O)
Control	78	8.30
420 ml/ha izoxaflutol	103	8.32
840 ml/ha izoxaflutol	97	8.23

The obtained data regarding the biogenicity of the three evaluated soil samples showed that the main components of the microflora are, to a lesser extent, bacteria, molds, and to a much greater extent nitrogen-fixing bacteria. On Figure 5 it is shown that there is a decrease in the quantity of bacteria and an increased quantity of molds in the treated soils.

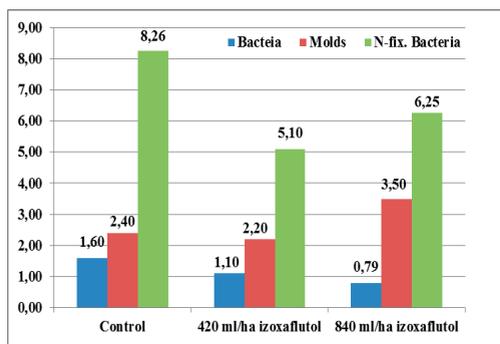


Figure 5. Number of colonies of bacteria, molds and nitrogen-fixing bacteria after treatments with izoxaflutol

The dynamics of the nitrogen-fixing bacteria are consistent with the results obtained from the total biological activity and β -glucosidase activity. It is worth to notice that the quantity of the nitrogen-fixing bacteria was increased with the increase of the izoxaflutol rate.

Isolation of resistant microorganisms

The influence of the herbicide on the growth and development of different groups of microorganisms by cultivation on three different nutrient media was studied (Table 1) in the presence of increasing concentration of izoxaflutol from 0, 15, 75 to 150 μ g/L.

The data obtained that is shown on Figure 6 were processed by three replicates and showed that as the concentration of izoxaflutol in the nutrient media increased, the number of soil microorganisms was decreased. The nutrient media with rose Bengal is used to cultivate molds. In the control soil, the quantity of the molds varies between 18.5×10^5 .

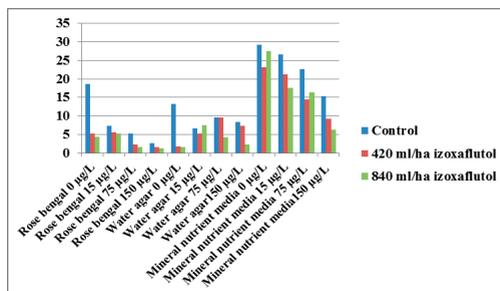


Figure 6. Total number of colonies forming units of microorganisms, isolated from the evaluated soils on three different medias

In the soil samples after treatment with 2 izoxaflutol rates (420 and 840 ml/ha) the number of microorganisms decreases with increasing dose of the herbicide added to the nutrient media.

Mold fungi developed on water agar in the presence of izoxaflutol, and bacterial number decreased. In the water agar the herbicide was used by the microorganisms as a carbon source. It was found that with the increase of the izoxaflutol rate in the nutrient media the total number of microorganisms was decreased.

CONCLUSIONS

The rate of 420 ml/ha suppressed the development of the test crop, and the doubled herbicide rate of 840 ml/ha stopped the growth and development of the test culture.

The reported quantity of released CO₂ was higher at the higher izoxaflutol rate (840 ml/ha) in comparison to the lower rate (420 ml/ha). This result shows that the microorganisms are resistant to the studied herbicide.

In treated with izoxaflutol soils the quantity of the bacteria was decreased, and the quantity of the molds was increased.

The quantity of nitrogen-fixing bacteria was increased with the increasing the izoxaflutol rate.

With the increase of isoxaflutol rate in the nutrient media the total number of microorganisms decreased.

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REFERENCES

- Calderbank, A. (1989). The occurrence and significance of bound pesticide residues in soil. *Reviews of Environmental Contamination and Toxicology*, 108, 71–103.
- Dimitrova M., Zhalnov I., Zhelyazkov I., Stoychev D. (2013). Efficiency and selectivity of new herbicides on fodder maize. *Agrolife Scientific Journal*, 2(1), 47–50.
- Enrica, G. (1994). The role of Microorganisms in environmental decontamination. In *Contaminants in the Environment: A Multidisciplinary Assessment of Risks to Man and Other Organisms*. A. Renzoni, 25, 235–246, Lewis Publishers, BocaRaton, Fla, USA.
- Javaid, M., Ashiq, M., Tahir, M. (2016). Potential of Biological Agents in Decontamination of Agricultural Soil. *Hindawi Publishing Corporation Scientifica. A Review Article*. Article ID 1598325, 9.
- Mitkov, A., Yanev, M., Neshev, N., Tonev T. (2018). Biological Efficacy of Some Soil Herbicides at Maize (*Zea mays* L.). *Scientific Papers. Series A. Agronomy*, LXI(1), 340–345.
- Mukherjee, P., Debnath, P. (2013). Weed control practices in maize (*Zea mays* L.) under conventional and conservation tillage practices. The role of weed science in supporting food security by 2020. *Proceedings of the 24th Asian-Pacific Weed Science Society Conference, Bandung, Indonesia, October 22-25, 2013 Bandung: Weed Science Society of Indonesia*, 302–311.
- Rice, P., Koskinen, W., Carrizosa, M. (2004). Effect of soil properties on the degradation of isoxaflutole and the sorption-desorption of isoxaflutole and its diketonitrile degradate. *J. Agric Food Chem*, 52, 7621–7627.
- Ritmann, B., Jacson D., Storck, S. (1988). Potential for treatment of hazardous organic chemicals with biological Process. *Biotreatment Systems*, 3, 15–64.
- Spundjueva, K., Kuzmanova, Yo., Kartalska, Yo., Tonev, T., Georgiev, S. (2001). Study on side effect of the herbicide Roundup Biopower on microbiological and biochemical activity in maize rhizosphere. *Jubilee Scientific Session: "80 Years of High Agronomical Education in Bulgaria"*. Book 3. 309–314.
- Taylor-Lovell, S., Sims, G., Wax, L. (2002). Effects of moisture, temperature, and biological activity on the degradation of isoxaflutole in soil. *J. Agric. Food Chem.*, 50, 5626–5633.
- Tonev, T., Tityanov, M., Mitkov, A. (2010). Integrated weed control during maize vegetation. *Scientific Works of Agricultural University of Plovdiv. Jubilee scientific Conference with International Participation: "Traditions and challengers of Agricultural education, science and bussines" book 2 Plovdiv*.
- Tonev, T., Dimitrova, M., Kalinova, Sht., Zhalnov, I., Spasov, V. (2007). Herbology. *Academic Publisher of the Agricultural University of Plovdiv, Bulgaria*, 227 (Text book in Bulgarian language).
- Veiga, M., Silva, D., Veiga, L., de CastroFaria, M. (2006). Pesticide pollution in water systems in a small rural community in Southeast Brazil. *Cadernos de Saude Prublica*, 22(11), 2391–2399.
- Zhalnov, I., Raikov, S. (1996). Influence of the different levels of infestation with *Sorghum halepense* L. on the development of maize. *Plant Science*, XXXIII, (8), 64–66.

RESEARCH ON TENSILE STRENGTH AND SPECIFIC FUEL CONSUMPTION FOR A MISCANTHUS RHIZOME PLANTER IN DIFFERENT EQUIPPING CONDITIONS AT THE FOUR ROW

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Abstract

A four-section miscanthus rhizome planter machine was equipped with a ploughshare and cover discs at the outer units and with ploughshare and compaction wheels arranged in V at the central units of the machine. Under these conditions, experimental determinations were made regarding the tensile strength (kN), the power required for towing (kW) and fuel consumption (l/ha), for three working depths (6, 9 and 12 cm) and different working speeds (between 1.27 and 2.65 m/s).

Field results showed traction force values between 4.90-8.51 kN for planting depths of 6 cm, respectively between 6.90-8.35 kN for depths of 9 cm and between 8.38-8.52 kN for 12 cm depths. Fuel consumption showed values between 2.96-5.18 l/ha at planting depths of 6 cm, between 3.30-4.07 l/ha for depths of 9 cm, respectively between 4.4-5.2 l/ha for planting depths of 12 cm. It was found that there is no well-defined rule regarding variation of traction parameters with planting depth, especially since the average travel speed could not be maintained at the same value for each sample, and the soil probably had wetter areas or with different resistance to the opening of the trench.

Key words: miscanthus rhizome planter, planting depth, working speed, tensile strength, specific fuel consumption.

INTRODUCTION

Among the species of energy plants there are also some species of *Miscanthus x giganteus* which are characterized by high biomass yields and relatively low costs, there is a growing interest for use in bioenergy production (Boersma et al., 2017; Daraban et al, 2015; Peric et al., 2018, Smeets et al., 2009; Dumitru et al., 2017).

There have written whole books and numerous papers on miscanthus culture and other energy crops capable of replacing petroleum energy and its products in the near future, but also other fossil fuels. Replacing lignite and wood with miscanthus briquettes would also lead to the protection of the environment through significant reduction of CO₂ equivalents (eq), SO₂ eq, P eq, N eq, 1,4 dichlorobenzene (1,4-DB) eq, non-methane volatile organic compound (NMVOC), PM₁₀ eq and U₂₃₅ eq emissions (Peric et al., 2018).

But many unknowns about these cultures remain, how they are set up, the land on which they should be cultivated, agrotechnics used, as well as the methods of use after harvest, either they are used as solid fuels, either by biorefinery in the form of liquid fuels (Xiu et al., 2017; Wang et al., 2018). By using the components and by-products resulting after biorefining, the economic efficiency of these plants can increase substantially.

Low division efficiency (1:10) it makes the demand for large planting rhizomes large, increasing the costs of setting up miscanthus crops. For this reason, various technologies have been developed to increase the rate of division or multiplication by seeds, but the costs are still relatively high. The authors analysed several published papers in the field and found that the highest costs are related to the establishment of miscanthus crops, the other costs of the management, harvesting, storage and transport phases (Pyter et al., 2010)

being relatively similar since the third year of growth. Establishing rhizome crops requires about average costs 3375.7 €/ha, compared to establishing crops by seeds that are about 1508.5 €/ha (Xue et al., 2015).

However, the method of setting up miscanthus crops through rhizomes remains the only method used in Romania (Sorica, 2015).

Because of the perennial character, the miscanthus does not enter the desolation, but it is cultivated extensively on certain soils less suitable for other crops, where it can remain 15-20 years, including on mining soils (Ussiri and Lal, 2014). Perennial culture has the advantage of reducing the costs of preparing the land and planting it. The miscanthus goes well after any crop plant (Sorica et al., 2009).

Rhizomes should be planted at the beginning of spring (March-April) when soil moisture is high, at a depth of 8-10 cm. The complete establishment of a miscanthus culture lasts 3 years. The oldest commercial rhizome planting machine was developed by the former company BICAL and Hvidsted Energy Forest (Huisman and Kortleve, 1994). Later farmers and private companies (ex. Tomax Ltd., Portlaw Co., ADAS Ltd.) developed automatic rhizome planters and rhizome mechanical harvesting machines (Anderson et al., 2011). On small areas, potato planters can also be successfully used (Adamchuk et al., 2019).

The preparation must be done in the fall, starting with the application of a broad spectrum herbicide, to combat any perennial weed, because weeds have a significant influence on plant development (implicitly also on biomass production), especially in the initial growth and development phase (Maksimovic et al., 2016).

The planting material consists of rhizomes between two and three years old. Rhizomes can be replanted to produce new plants, but it is preferable to have at least 2-3 sprigs, it is recommended to be commercially certified to avoid the transmission of pathogens in soil between different areas, that is, they are not affected by fungal species (*Fusarium avenaceum*, *Fusarium oxysporum* and *Mucor hiemali*) which causes rhizome rot (Covarelli et al., 2012). The best result is obtained when the rhizomes are replanted within 24 hours of harvesting (Figure 1).

Planting distance, respectively the density of plants, varies depending on soil fertility and the amount of nitrogen applied (Zivanovic et al., 2014). Thus, on fertile soils where the stems grow tall, it is recommended that the distance between rhizomes be 1 m, and between rows it should be 1 m, the middle ones 50/100 cm (15000 plants/ha.), and the poor 50/50 cm (20000 plants/ha) (Sorica, 2009; 2015).



Figure 1. Rhizomes of Miscanthus

The main operations to be performed by planters, regardless of culture, are:

- to ensure the opening of gutters, pits or nests;
- to distribute the planting material evenly at the regulated depth and distance;
- to ensure that the planting material is covered with soil and that soil is squeezed near it;
- not to cause injury to the planting material, when deposited in the soil.

In this regard, the planters are provided with ploughshares for opening the gutters, distribution systems for planting material in the gutter opened by ploughshare, coating systems and, eventually, soil compaction wheel cover. The highest energy consumption is given by the ploughshares, these being designed in different types of construction to reduce tensile strength (Altuntas et al., 2006; Troger et al., 2012; Benjaphragairat et al., 2010).

Thus, shoe type gutter opener with the distance between rows of 0.25 m showed tensile strengths of 10.5 N/row, for moving speed 1.68 km/h when planting garlic (Benjaphragairat et al., 2010). For a depth of planting of 0.12 m, tensile strength can increase more than 100 times, especially if the fence opener is not properly designed or is planted directly in the stem (Troger et al., 2012).

They cannot be neglected, like the other resistance to the movement of the planting machine.

In this context, the paper presents an analysis of the displacement resistances and the fuel consumption for a rhizome planting machine, on four rows, with different equipment in the marginal sections compared to the central sections. It is desired to highlight the energy indices of the planting machine for different planting depths, at three speed classes of machine, without being followed the indications of quality of the work, and possibly the participation of each working body in the energy index analysed.

MATERIALS AND METHODS

The MPM-4 machine was used to characterize the tensile behaviour of the miscanthus rhizome planting machine (designed and realized by the INMA Institute of Bucharest) in aggregate with the 80 HP New Holland TD80D tractor (Figure 3). The determinations were made on the experimental field of the institute (4000 m²) using a five-channel tensometric frame and Quantum MX 1615 - HBM data acquisition and amplification system between the tractor and the machine, and for the fuel consumption a device equipped with Flowtronic 215-217 type tester was used (Figure 2).



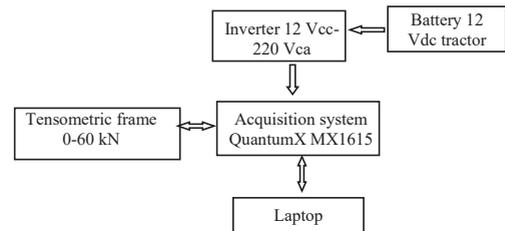
Figure 2. Tensometric frame for measuring tensile strength

The land was plowed in autumn at 0.25 m depth, and in the spring it was dug with a disc harrow at a depth of 0.15 m and levelled with a combiner. The soil of the experimental field of INMA Bucharest is of a reddish-brown type of forest, characteristic soil within the research

institute. It is characterized as having high saturated hydraulic conductivity (0.01-0.035 m/h), in the layer 0-0.25 m, low penetration resistance (1.0-2.5 MPa) and large field capacity (25-30% in volume), in the same top layer (Dumitru et al., 2011). At the time of the experiments, soil moisture had values between 13.4-20.9% (medium value 17.86%), and the penetration resistance was between 0.67-2.25 MPa (average 1.47 MPa), in layer 0-0.25 m.

The miscanthus rhizome planter was designed to work with a number of sections, each equipped in two variants: ploughshare-disc cover-compaction wheel, respectively ploughshare-disc cover-wheels with V-fins.

For the experimental samples were calibrated, first, tensometric frame transducers on the traction-compression machine inside the INMA laboratory, after which they were mounted on the miscanthus planting machine. Then the movement was made in the field, where the plots of 90 m long were laid out, on each plot, carrying out an experiment as trial.



The tests were carried out, the parameters being followed: tensile strength, fuel consumption per hectare and movement speed of tractor. There were several rehearsals, with different movement speeds, with different combinations of ploughshares and cover discs (or wheels with wings) in the processed agricultural land. Data processing was done using spreadsheet tools, EXCEL and MathCad math computing software. Diagrams of the tracked sizes were obtained, tensile strength, in relation to the time and the average values of these sizes for each repetition.



Figure 3. Equip of the planting machine on each work section

The determinations were made for three working depths (0.06, 0.09, 0.12 m) and three speed stages (with small variations within the step due to the specificity of the experiments), average 1.29, 1.78 and 2.6 m/s.

The tensile force was calculated from the force records of the two lateral and central straps, also representing itself graphically (Figures 4 and 5). Was obtained, thus, the variation of the tensile force during each experiment, in the equipment variant specified for miscanthus planting machine sections.

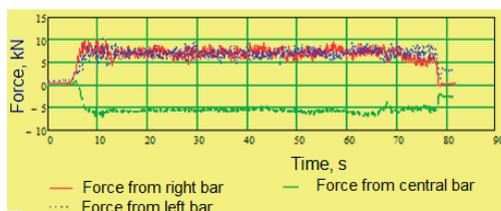


Figure 4. Signal recorded in experiment 20

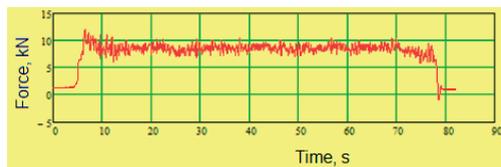


Figure 5. Variation of the total tensile strength

As for the power required for traction, it was calculated as a product between the travel speed and the traction force, for each working depth.

RESULTS AND DISCUSSIONS

The results obtained in the experiments are presented in Table 1.

Table 1. Traction force, fuel consumption and traction power at the planting unit

Sample	Work depth (m)	Work speed (m/s)	Traction force (kN)	Fuel consumption (l/ha)	Required power (kW)
1	0.06	1.27	7.039	5.18	8.92
2		1.28	6.919	4.44	8.88
3		1.32	5.971	4.44	7.89
4		1.76	6.423	3.33	11.33
5		1.76	7.309	3.70	12.89
6		1.84	6.857	3.33	12.59
7		2.57	6.056	2.96	15.56
8		2.57	4.898	3.33	12.59
9		2.65	8.513	3.33	22.54
10	0.09	1.38	7.682	4.07	10.63
11		1.32	6.113	4.07	8.08
12		1.34	7.240	3.70	9.71
13		1.73	8.283	4.07	14.33
14		1.73	7.701	4.07	13.33
15		1.76	6.911	3.70	12.19
16		2.37	8.053	3.70	19.06
17		2.64	6.338	3.30	16.76
18		2.50	8.318	3.70	20.80
19	0.12	1.32	8.380	5.18	11.08
20		1.30	8.516	4.40	11.09
21		1.27	8.379	4.40	10.61

Analysis on sets of experiments of the energy parameters of rhizome planting aggregate involves determining their average values, for average values of the working speed of the machine and the same working depth (Table 2).

Table 2. The experimental average values of the energy parameters of planting unit

Depth (m)	Speed (m/s)	Force (kN)	Consumption (l/ha)	Power (kW)
0.06 (9 sample)	1.290	6.643	4.687	8.563
	1.787	6.863	3.453	12.270
	2.597	6.489	3.207	16.897
0.09 (9 sample)	1.347	7.012	3.947	9.473
	1.740	7.632	3.947	13.283
	2.503	7.570	3.567	18.873
0.12 (3 sample)	1.297	8.425	4.660	10.927

From the analysis of data from Table 1 and Table 2 it is found that the traction force does not necessarily have a connection with the speed of movement, this having a random variation, but the power required to tow the machine increases with increasing working speed. The power required to tow the machine showed variations within the limits 7.9-22.5 kW for planting depths of 0.06 m and between 8.1-20.1 kW, at the depth of 0.09 m, for speeds from 1.29 to 2.6 m/s, respectively

between 10.6-11.1 kW for the depth of 0.12 m, but in the latter case the travel speeds were about 1.3 m/s.

Variation of the average values of the energetic and working parameters of the planting unit, obtained in the experiments, depending on the working depth, is shown in Figure 6.

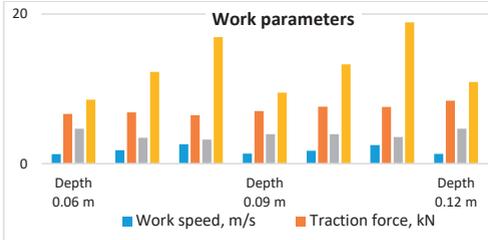


Figure 6. Variation of average energy parameters in experiments

One can easily observe the increasing variation of the average values of the working speed for the first two sets of experiments, as well as the power required to tow the planter. Also, the decreasing variation in fuel consumption per hectare is observed for each set of experiments (Figure 6).

Based on data from Table 1, the correlation table between the main working parameters with the planting unit was created (Table 3).

Table 3. Working parameters correlations matrix for the miscanthus planting unit

Parameter	Work speed	Traction force	Fuel consumption	Required power
Depth = 0.06 m				
Work speed	1	-0.057	-0.821	0.830
Traction force	-0.057	1	0.113	0.502
Fuel consumption	-0.821	0.113	1	-0.651
Required power	0.830	0.502	-0.651	1
Depth = 0.09 m				
Work speed	1	0.189	-0.714	0.925
Traction force	0.189	1	0.279	0.535
Fuel consumption	-0.714	0.279	1	-0.490
Required power	0.925	0.535	-0.490	1
Depth = 0.12 m (only working speed = 1.3 m/s)				
Work speed	1	0.121	0.803	0.910
Traction force	0.121	1	-0.495	0.521
Fuel consumption	0.803	-0.495	1	0.484
Required power	0.910	0.521	0.484	1

It can be stated that for values above 0.8, analysed parameters (two by two) has a strong correlation, for values between 0.5-0.8 the correlation is good, for values 0.3-0.5 the correlation is weak, and for values below 0.3 the two parameters have an insignificant correlation. It can be seen, so, that, for all working depths used, the traction force has a negligible correlation with the working speed, but it has a good correlation with the power required to move the machine. Furthermore, working speed correlates very well with specific fuel consumption (l/ha) and with the actuating power (values above 0.8 in most cases). Still, at the working depths of 0.06-0.09 m, the correlation of speed with fuel consumption per hectare is negative, that is, the two parameters are inversely proportional, as shown in Figure 7.

A strong correlation is found between the working speed and the power required to move the planting unit, in all cases it having values above 0.83 (mostly above 0.91). It is noticed, also, a good correlation of the power required for the traction force (values over 0.5), but also with fuel consumption per hectare (values over 0.49), though presented globally, for each working depth, the correlation seems mostly negative than positive (Table 3).

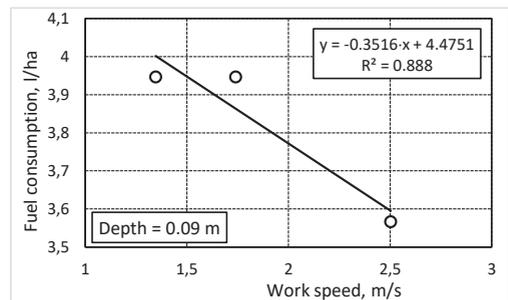
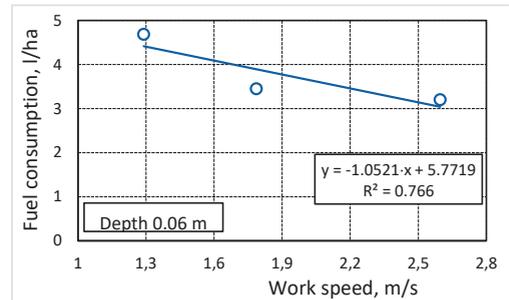


Figure 7. Variation of fuel consumption with working speed, at depths of 0.06 m and 0.09 m

Figure 8 shows the correlation of fuel consumption per hectare with the traction force, taking into account their average values within each planting depth, a correlation that seems logical, that is, a directly proportional variation of the two parameters.

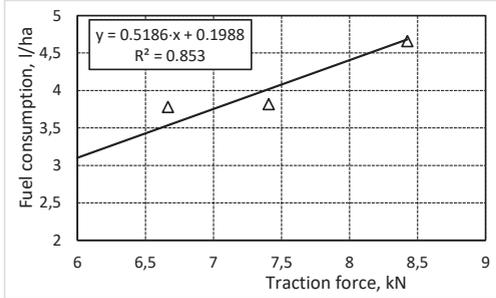


Figure 8. Variation of fuel consumption with traction force, for their average values

Also, taking into account the average values of the traction force (within each working depth), its variation with depth seems, again, a logical one: tensile strength increases with increasing working depth, no matter how fast the planter moves (Figure 9).

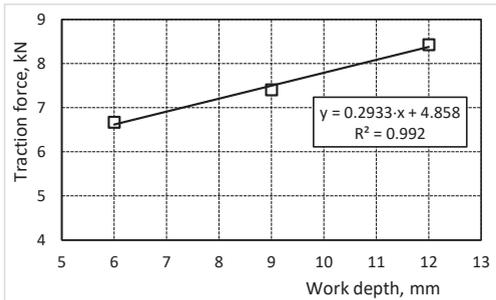


Figure 9. Traction force variation with working depth, at average force values for each depth

Matched with Voicu et al. (2015), tensile strength of the MPM-4 planter equipped only with ploughshare for opening gutters, to all four sections, is about average 7.761 kN, and the fuel consumption and power required for towing have average values of 4.44 l/ha, respective 10.56 kW, for the working depth of 0.06 m and travel speed of 1.36 m/s. Also, for the same speed regime and the same working depth, experiments show average values of 4.165 kN for tensile strength, respective 4.07 l/ha and 5.51 kW, for fuel consumption and

towing power, if the planting machine is provided with two ploughshares and two cover/compaction V-wheels (only at the outer sections). At the same speed and same equipment of the machine, but at a depth of 0.09 m, the results show average values of 5.085 kN, 3.33 l/ha and 6.84 kW, for the three specified energy parameters. If the machine is equipped with ploughshares and cover discs, only at the outer sections, at the working depth of 0.06 m and average speeds 1.4 m/s, experiments showed values of 3.737 kN, 3.33 l/ha and 5.25 kW, for tensile strength, specific fuel consumption, respectively the towing power.

It can be stated, in these conditions, that the covering discs require a lower traction force than the covering / tapping wheels, but for the planting process to be complete the work sections must be equipped with additional tapping wheels. However, a small surplus of speed can lead to greater traction power and increased fuel consumption, to the same way of equipping the sections.

It has been observed that at a lower working speed, fuel consumption per hectare increases, and the V-cover wheels with flaps have a lower fuel consumption than the machine ploughshares. The same can be said about the contribution of the covering discs to the fuel consumption of the machine in relation to the contribution of the ploughshares. More, the fuel consumption of the coating discs is lower compared to the fuel consumption of the coating/tapping V-wheels, at the same speed regime and the same working depth. Also, the aforementioned are also valid in the case of the traction power required, which is greater as the speed of work and depth of planting increases. Thus, if a ploughshare has a fuel consumption of 1.2-1.3 l/ha, at a speed of 1.3 m/s and a working depth of 0.06 m, then a pair of wheels with V-fins gives fuel consumption of 0.65-0.80 l/ha, and a pair of cover discs gives a fuel consumption of 0.55-0.65 l/ha. It must be said that the contribution to the overall fuel consumption of the weight of the machine and its rolling system was not taken into account.

The same way, having regard to the present paper and the papers presented by Voicu et al. (2015) and Poenaru et al. (2015), estimates could be made on the power required by the

working organs of the planting machine at towing, for the speed regime 1.3-1.35 m/s and working depth of 0.06 m. It is estimated that a ploughshare demands a power of 1.75-1.9 kW, while a pair of V-shaped wheels has a contribution of approximate 0.25-0.3 kW, and a pair of cover disks requires only a traction power of 0.1-0.2 kW. It is obvious that all these values of the energy indices presented by each working body increase with increasing working depth, but it shows variations and depending on the speed it is moving and the characteristics of the land where the planting takes place.

CONCLUSIONS

The estimation of the energy indices of a miscanthus rhizomes planting machine can be done only by experimental determinations in the field. The machine can be equipped in several variants, but in this paper the machine presented two sections with ploughshare and tapping / cover wheels arranged in V, with wings, and two sections with ploughshare and cover discs. For experiments performed at different planting depths (between 0.06-0.12 m) and speeds within limits 1.3-2.6 m/s, energy indices of the work (tensile strength, fuel consumption per hectare and the power required for towing) presented different variations, depending on the input parameters chosen.

A particular finding is that fuel consumption per hectare decreases with increasing working speed. (e.g. from 3.9 l/ha, for speeds of approx. 1.35 m/s, to 3.6 l/ha for speeds 2.5 m/s, at the planting depth of 0.09 m). Power required to tow the machine (calculated as a product between working speed and traction force) it has grown both with increasing planting depth, but also with increasing working speed (e.g. from 8.56 kW for planting depth of 0.06 m/s, to 9.47 kW for the depth of 0.09 m/s and at 10.92 kW for the depth of 0.12 m, all for the same speed regime - 1.3-1.35 m/s).

However, the values of the energy indices of a planting machine depend to a large extent on the way of equipping the machine, as well as the preparation of the land, for the same input parameters (travel speed and working depth).

REFERENCES

- Adamchuk, V., Bulgakov, V., Ivanovs, S., Prysyzhnyi, V., Boris, A. (2019). Experimental research of miscanthus planting technological process by means of upgraded potato planter, *INMATEH - Agricultural Engineering*, 57(1), 173–178.
- Altuntas E., Ozgoz, E., Taser, O.F., Tekelioglu, O. (2006). Assessment of different types furrow openers using a full automatic planter, *Asian Journal of Plant Sciences*, 5, 537–542.
- Anderson, E., Arundale, R., Maughan, M., Oladeinde, A., Wycislo, A., Voigt, T. (2011). Growth and agronomy of *Miscanthus x giganteus* for biomass production, *Biofuels*, 2, 167–183.
- Benjaphragairat, J., Sakurai, H., Ito, N. (2010). Design and control of metering system and furrow openers for garlic planter, *International Agricultural Engineering Journal*, 19(2), 39–47.
- Boersma, N., Tejera, M., Heaton, E. (2017). Long-term assessment of miscanthus productivity and sustainability, *Farm Progress Reports*, 2016(1), Article 145.
- Covarelli, L., Beccari, G., Tosi, L. (2012). Miscanthus rhizome rot: A potential threat for the establishment and the development of biomass cultivations, *Biomass and Bioenergy*, 46, 263–269.
- Daraban, A., Jurcoane, S., Voicea, I. (2015). *Miscanthus giganteus* - an overview about sustainable energy resource for household and small farms heating systems, *Romanian Biotechnological Letters*, 20(3), 10369–10380.
- Dumitru, C., Marin, E., Iuga, D. (2017). Researches on evolution of certain characteristics related to miscanthus sp. Crop springing, size and stress resistance, *International Symposium ISB-INMA TEH' 2017, Agricultural and mechanical engineering*, Bucharest, Romania, 757–762, ref. 19.
- Dumitru, M., Manea, A., Ciobanu, C., Dumitru, S., Vrinceanu, N., Calciu, I., Tanase, V., Preda, M., Risnoveanu, I., Mocanu, V., Eftene, M. (2011). *Monitoring the quality status of soils in Romania* (in Romanian), ICPA Bucharest, RO: Sitech Publishing House, Craiova.
- Huisman, G.W., Kortleve, W.J. (1994). Mechanization of crop establishment, harvest, and post-harvest conservation of *Miscanthus sinensis*, *Industrial Crops and Products*, 2(4), 289–297.
- Maksimovic, J., Pivic, R., Stanojkovic-Sebic, A., Vucic-Kisgeci, M., Kresovic, B., Dinic, Z., Glamoclija D. (2016). Planting density impact on weed infestation and the yield of Miscanthus grown on two soil types, *Plant Soil Environ.*, 62(8), 384–388.
- Milica, P., Mirko, K., Dragi, A., Branko, B., Zeljko, D. (2018). Life Cycle Impact Assessment of Miscanthus Crop for Sustainable Household Heating in Serbia. *Forests*, 9, 654; doi:10.3390/f9100654
- Peric, M., Komatina, M., Antonijevic, D., Bugarski, B., Dzeletovic Z. (2018). Life Cycle Impact Assessment

- of Miscanthus Crop for Sustainable Household Heating in Serbia, *Forests*, 9, 654–679.
- Poenaru, I.C., Cardei, P., Voicu, Gh., Paraschiv, G., Dinca, M., Vladut, V., Matache, G. (2015). Researches in the field of the energetics of the miscanthus planter. (1) - Determination of the traction force, *U.P.B. Sci. Bull., Series D*, 77(1), 245–256.
- Pyter, R.J., Dohleman, F.G., Voigt, T.B., (2010). Effects of rhizome size, depth of planting and cold storage on *Miscanthus x giganteus* establishment in the Midwestern USA, *Biomass and Bioenergy*, 34(10), 1466–1470.
- Singh, S., Tripathi, A., Singh, A.K. (2018). Performance evaluation of furrow openers for sugarcane planting in sub-tropical India, *AgricEngInt: CIGR Journal*, 20(1), 56–62.
- Smeets, E.M.W., Lewandowski, I.M., Faaij, A.P.C. (2009). The economical and environmental performance of miscanthus and switchgrass production and supply chains in a European setting, *Renewable and Sustainable Energy Reviews*, 13, 1230–1245.
- Sorica, C., Voicu, E., Manea, D., Schweighofer, K. (2009). Technology for promoting in Romania the energy crop of miscanthus, as a renewable source in order to increase competitiveness and energy security (in Romanian), *INMATEH - Agricultural engineering*, 29(3), 10–15.
- Sorica, E. (2015). Analysis of profitability of implementing the miscanthus energetic crop technology for rhizomes capitalization, *INMATEH - Agricultural Engineering*, 46(2), 155–164.
- Troger, H.C.H., Dos Reis, A.V., Machado, A.L.T., Machado R.L.T. (2012). Analyzing the efforts in furrow openers used in low power planters, *Engenharia Agricola. Jaboticabal*, 32(6), 1133–1143.
- Ussiri, D.A.N., Lal, R. (2014). Miscanthus agronomy and bioenergy feedstockpotential on minesoils, *Biofuels*, 5(6), 741–770.
- Voicu, Gh., Dinca, M., Matache, M.G., Poenaru, I.C., Cardei, P., Vladut, V. (2015). Researches regarding organization of the experiments for energetic characteristics determination when using miscanthus planter, 4th International Conference on Thermal Equipment, Renewable Energy and Rural Development, TE-RE-RD 2015, Posada-Vidraru, RO: 405–410.
- Wang, K.-T., Jing, C., Wood, C., Nagardeolekar, A., Kohan, N., Dongre, P., Amidon, T.E., Bujanovic, B.M. (2018). Toward complete utilization of miscanthus in a hot-water extraction-based biorefinery. *Energies*, 11, 39–60.
- Xiu, S., Zhang, B., Boakye-Boaten, N.A., Shahbazi, A. (2017). Green biorefinery of giant miscanthus for growing microalgae and biofuel production, *Fermentation*, 3, 66–77.
- Xue, S., Kalinina, O., Lewandowski, I. (2015). Present and future options for miscanthus propagation and establishment, *Renewable and Sustainable Energy Reviews*, 49, 1233–1246
- Zivanovic, L., Ikanovic, J., Popovic, V., Simic, D., Kolaric, L., Maklenovic, V., Bojovic, R., Stevanovic, P. (2014). Effect of planting density and supplemental nitrogen nutrition on the productivity of miscanthus, *Romanian Agricultural Research*, 31, 291–298.

AN INNOVATIVE APPROACH TO PRODUCE FORAGE CROPS: BARLEY FODDER IN VERTICAL FARMING SYSTEM

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Abstract

The rapid growth of the world's population, limited natural resources and environmental challenges caused by climate change have had brought up global food security to the agenda. Scientific researches and new practices considering sustainable food production and efficient use of natural resources are getting more attention during last decades all around the world. Furthermore, advanced technological applications that enable new agricultural production systems such as vertical farm have been starting to emerge to provide a solution on this issue. Vertical farming which is the method of growing crops in vertically stacked layers under controlled environment is one of the promising techniques to protect environmental resources, provide continuous and sustainable plant production. Applications of vertical farming have already experienced in many countries such as Japan, Singapore, England, USA, Netherlands and the vertical agricultural market is expected to increase by 25% by 2024 to reach 11.4 billion Euro. Plant groups that are widely grown with vertical farming system are mostly; carrot, radish, potato, tomato, pepper, pea, cabbage, spinach, lettuce and strawberries. Although, cereal grain production in the vertical systems is not economically profitable today, considering remarkable increase in demands on forage crops, barley fodder production seems to have great potential for vertical farming systems. The benefits of the system such as less water use (about 90%), no herbicides, pesticides and fertilizer application relative to conventional production would be more pronounced for barley fodder in vertical system comparison to other conventional forage crops production systems. In this review, potential of barley fodder production in vertical farming system was discussed.

Key words: barley fodder, sustainable agriculture, vertical farming.

INTRODUCTION

As stated by the United Nations (2017), the world population, which is currently about 8 billion, is expected to be 9.8 billion in 2050. In addition to limited natural resources such as agricultural land and water, environmental challenges caused by global climate change have increased the interest on global food security. According to the United Nations Food and Agriculture Organization (FAO) report, the most urgent problem faced with increasing needs on safe, adequate and appropriate food supply for the growing population (Besthorn, 2013).

Within the same report, it is underlined that, by maintaining existing agricultural practices and even with the best efforts in sustainable land use, there will not be enough space to meet the world's increasing food needs. Therefore problems such as climate change and drought and extreme weather events that are rapidly

increasing their impact reveal that agricultural resources should be used more effectively (Jia, 2019).

Totally, 70% of the clean water in the world is used for irrigation in agriculture.

Predictions indicate that water scarcity will be inevitable especially in the regions where the population is dense as a result of the continued use of clean water in the current situation (Despommier, 2009).

In this context, research and applications on implementation of developing technologies and technological equipments, which are becoming accessible, in order to efficient use of resources in agriculture and production of environmentally friendly and sustainable foods are increasing rapidly (Jia, 2019).

Beside, widespread use of technological applications integrated into existing agricultural systems such as agricultural machine control systems, equipment automations, remote sensing methods, drone use, agricultural

information system, farmer portal and electronic crowning, advanced technological applications which offer a different agricultural production system, such as vertical farming, have also begun to develop.

Vertical farming is an alternative method to efficient use of resources (e.g. 70-80% less water) to achieve much more efficient use of unit area than traditional agriculture, to ignore seasonal effects, and to remove substances such as herbicides and pesticides. Thus, it has promising potential to provide an environmental friendly and sustainable agricultural production (Despommier, 2013).

On the other hand, one of the most important agricultural activities affected by environmental restrictions such as limited resources and climate change is livestock production. High quality green feed should be provided to animals regularly for sustainable livestock production. However, despite the increase in animal husbandry, the decrease in land in forage production, insufficient water resources, inability to find the same quality product throughout the year, and input costs such as fertilizer and diesel fuel are among the biggest obstacle in production (Gebremedhin et al., 2015; Rajesh et al., 2018). Therefore, barley fodder production in vertical farming system using with advanced technology could be accepted as a future solution to provide sustainable and continues feed to livestock industry.

In this review, vertical farming system, which offers a different production technique against traditional agriculture and production of barley fodder potentially seen in livestock forage problem in vertical farming system, has been examined within the conceptual framework. It has been aimed to assist in the future studies on this subject by making national and international literature reviews about the subject.

Vertical Farming

The idea of vertical farming is based on the Hanging Gardens of Babylon in 600 BC (Despommier, 2014). On the other hand, the concept of vertical farming in its current sense was introduced in 1999 by an American professor of public health and microbiology, Dickson Despommier and his students, at the University of Columbia.

The vertical farming system is based on the principle of growing plants with low agricultural input, using the technological elements and stacking them vertically, and it is presented as an important potential by different disciplines such as agricultural experts and city planners (Despommier, 2009). It is also thought to produce concrete results that will contribute to food safety in the future (Besthorn, 2013).

Vertical farming types appear in three ways as roofs of buildings, multi-storey buildings and medium sized facilities according to the production target (Al-Kodmany, 2018). The vertical farms already exist and active are generally medium size enterprises. The success of these medium-sized enterprises will form the basis of more visionary multi-storey vertical farming systems that will be established in the future (Despommier, 2014).

Vertical farming promises to offer best growing conditions to plant using with technological equipments during the processes from seeding to harvest. Therefore, factors such as light, humidity, temperature and carbon dioxide should be optimized according to plant species (Banerjee & Adenauer, 2014). For that reason, fully controlled indoor systems instead of open or semi-open facilities are recommended to obtain satisfactory product amount and quality. Currently, commercial enterprises investing on vertical farming systems have been rapidly emerging in America, China, Japan, Singapore, Italy, the Netherlands, England, Jordan, South Korea, Saudi Arabia, the United Arab Emirates and Canada (Besthorn, 2013). Accordingly, the vertical farming global market is expected to increase by 25% by 2024 to reach around 11.5 billion Euros (Askew, 2019).

Why Vertical Farming?

The increasing demand for agricultural products over the years has been generally met by expanding cultivated areas (FAO, 2011). Given that traditional agricultural production is limited by land availability and production intensity, land per capita has been decreasing day by day due to rapidly increasing population, and as a result, approximately one billion people are malnourished especially in developing countries (Germer et al., 2011).

Traditional farming techniques, excessive use of water and unsustainable soil management, chemical uses, contamination of natural water resources and released greenhouse gases harm the environment in a way that will weaken future food production. Moreover, deforestation for agricultural production, extracting water from lakes and rivers greatly damage biodiversity (Godfray et al., 2014).

From this point of view, the solution is not only to increase food production, but also to supply sustainability by increasing agricultural production in harmony with the environment (Godfray et al., 2014).

Agronomists and other disciplines have a great responsibility to about ensuring the sustainability, to use resources most efficiently and to use new methods and technologies to protect the ecosystem (Germer et al., 2011). In this context, adopting new technologies and using them in agricultural activities will be the key solution to ensuring a sustainable production (Hakkim et al., 2016).

Undoubtedly, a single technological strategy will not be enough to solve the ever increasing food supply, but it is an important step to be taken in this regard. Considering the changing climate, the restriction of other natural resources, especially water, and the harm of fossil fuels, the need for a rapid transition is rather than behaving optimistically. At this point, vertical farming is one of the most promising solution to adapt new technologies to agricultural system with its numerous opportunities (Godfray et al., 2010).

Opportunities and Curiosities Offered by Vertical Farming

Vertical farming provides maximum yield per square meter, and year-round production without being affected by climatic conditions (Despommier, 2013). Considering that the economy of developing countries was damaged by crop and livestock production worth 96 billion dollars due to the natural disasters experienced between 2005 and 2015 with the effect of changing climate conditions (FAO, 2018), importance of an isolated production system from the environmental conditions could be better understood. However, unlike growing conditions in field, a well-designed

vertical farming systems can minimize or even eliminate the risk of various harmful pests and weeds beside other naturel disasters (Despommier, 2009).

On the other hand nitrogen and phosphorus which are excessively used in crop production do not leak into natural water sources and environmental burden is reduced, as forest areas will not need to be converted into agricultural land (Germer et al., 2011).

Furthermore, vertical farming systems save up to 95% water use compared to the traditional agriculture systems. In addition, storage risks and costs of agricultural products are limited in this system since it provides continuous production.

Besides to these advantages of vertical farming, many uncertainties are concerned about this new production system (Epting, 2016). For instance, installation costs and energy requirement (lighting, heating, cooling etc.) of the system are still under discussion. For this purpose, an economic analysis performed with simulated 37-storey vertical agriculture centers (Banerjee & Adenauer, 2014). The authors suggested that vertical agricultural production systems are still profitable as all the costs are considered. However they also stated that the real systems should be evaluated instead of the simulations to conclude. Although limited researches have been published on economic analysis of vertical farming systems, at least many of processes and productivity of vertical farming system are still open to improvement from the economical point of view.

Another discussion point on vertical farming systems is its effects on environment. Al-Chalabi (2015) found that lettuce grown in vertical agriculture system has higher carbon footprint than lettuce produced in traditional system. However, he also stated that using renewable energy sources in the system could be changed the results other way around. Although several advantages of the system are predicted, further studies have to be performed from the technical, environmental and economic aspects.

Barley fodder in vertical farming system

Animal feed production is one of the important agricultural activities that affect food safety and environment in the face of changing climate

and increasing population. Totally 33 million cattle were raised in the USA in 2003 and millions of hectares of forest were damaged to produce cereals to feed these animals (Despommier & Ellingsen, 2008). On the other hand approximately 43% of the wheat and maize products in the world are used for animal feed. Therefore, decreasing feed demand has an remarkable importance to struggle with food safety problem (Germer et al., 2011). From this point of view, alternative high quality forage sources have great importance to reduce the constraint on products such as wheat and maize which are mainly needed for human nutrition.

Taking into account that livestock is an important source as income of rural community especially in developing countries, economic profitability and quality of forage sources both play crucial role in decision mechanism (Godfray & Garnett, 2014). For these regions, providing forage, especially in winter period is one of the most important restricting factor for livestock industry beside negative effects of changing climatic conditions. Because of these limiting factors, poor quality feed sources such as straw are used (Kılıç, 2016). Consequently, increasing proportion of low quality feeds in animal diet leads several health problem and reduces productivity (Godfray & Garnett, 2014). From this point of view, using barley fodder as forage is an important topic of argument.

The plant groups that are widely grown with vertical farming system today are mostly carrots, radishes, potatoes, tomatoes, peppers, peas, cabbage, spinach, lettuce and strawberries (Şahin & Kendirli, 2016). On the other hand producing grain crops in such systems currently seems not economic (Hughes, 2018). However, considering deficit in livestock feed (30 million tons in Turkey according to do Özkan & Demirbağ, 2016) and environmental restrictions, using vertical systems have also become a remarkable option for this purpose. Beside other forage crops, barley fodder production in vertical systems has specific advantages because of vigor of barley seeds, content of the product and environmental requirements of the plant.

Several studies have been reported on growth and quality of barley in fodder production

systems. Most of the studies indicated that barley fodder reach highest nutrition value in 7 days after seeding (Akbağ et al., 2015). After the first week of the growth, decreasing energy and organic matter contents of the product were observed (Fazaeli et al., 2012). Considering several studies, fresh barley fodder weight increases to 20-30 kg/m² until 7 days after seeding under controlled environment (Al-Karaki & Al-Hashimi, 2012; Del Castillo et al., 2013; Dung et al., 2010; Fazaeli et al., 2012). According to these findings, production amount of barley fodder in a 0.1 ha vertical system consisting of 10 layers (52 growth period) reaches 10.400-15.600 ton fresh fodder per year. Considering maize silage production in a same size of land and period [approx. 5 tons in Turkey (TUIK, 2018)], these results are perceived quite tempting although several points such as quality, water content, energy content etc. of end products and inputs of the system are still under discussion.

However, environmental benefits of the vertical system are better revealed. Beside no-chemical use in vertical barley fodder production, 95-97% of water saves if irrigation water regain by suitable drainage system (Ajmi et al., 2009; Karaşahin, 2014).

CONCLUSIONS

In general, a vertical farming facility to be established is expected to be affordable, operable, sustainable and reliable (Kalantari et al., 2017). In this context, when the researches, studies and inferences made in the literature are evaluated. Barley fodder, which will be produced as forage in the vertical farming system, has an significant potential in reducing the negative impact of agriculture on environment and ensuring food safety by using resources such as water and soil in the most efficient way. On the other hand, the efficiency and cost level of the system to be installed is directly related to the optimization of growing conditions such as temperature, humidity, seed frequency, water and energy requirements of barley fodder. Consequently, further studies on technical, environmental and economic aspects of the issue should be conducted.

REFERENCES

- Acosta Aragón, Y., Jatkauskas, J. & Vrotniakienė, V. (2012). The effect of a silage inoculant on silage quality, aerobic stability, and meat production on farm scale. *ISRN Veterinary Science*, 1–6.
- Ajmi, A.A., Salih, A.A., Kadim, I. & Othman, Y. (2009). Yield and water use efficiency of barley fodder produced under hydroponic system in GCC countries using tertiary treated sewage effluents. *Journal of Phytology*, 1(5), 342–348.
- Akbağ, H.I., Türkmen, O.S., Baytekin, H. & Yurtman, İ.Y. (2015). Effects of harvesting time on nutritional value of hydroponic barley production. *Türk Tarım ve Doğa Bilimleri Dergisi*, 1(Özel Sayı-2), 1761–1765.
- Al-Chalabi, M. (2015). Vertical farming: Skyscraper sustainability? *Sustainable Cities and Society*, 18, 74–77. <https://doi.org/10.1016/j.scs.2015.06.003>.
- Al-Karaki, G.N. & Al-Hashimi, M. (2012). Green Fodder Production and Water Use Efficiency of Some Forage Crops under Hydroponic Conditions. *ISRN Agronomy*, 1–5.
- Al-Kodmany, K. (2018). The vertical farm: A review of developments and implications for the vertical city. *Buildings*, 8(2), 24.
- Askew, K., (2019). Vertical farming: A sustainable solution to food insecurity? Retrieved February 22, 2020 from <https://www.foodnavigator.com/Article/2019/01/28/Vertical-farming-A-sustainable-solution-to-food-insecurity>.
- Banerjee, C. & Adenauer, L. (2014). Up, Up and Away! The Economics of Vertical Farming. *Journal of Agricultural Studies*, 2(1), 40.
- Besthorn, F.H. (2013). Vertical farming: social work and sustainable urban agriculture in an age of global food crises. *Australian Social Work*, 66(2), 187–203.
- Del Castillo, F.S., Del Carmen Moreno Pérez, E., Magaña, E.C. & Gómez, J.M. (2013). Producción de forraje hidropónico de trigo y cebada y su efecto en la ganancia de peso en borregos. *Revista Chapingo, Serie Horticultura*, 19(4 SPEC.), 35–43.
- Despommier, D. (2009). The rise of vertical farms. *Scientific American, a Division of Nature America, Inc.*, 301(5), 80–87.
- Despommier, D. (2013). Farming up the city: the rise of urban vertical farms. *Trends in Biotechnology*, 31(7), 388–389.
- Despommier, D. (2014). Vertical Farms in Horticulture. In P.B. Thompson & D.M. Kaplan (Ed.), *Encyclopedia of Food and Agricultural Ethics* (1791–1799). Springer, Netherlands.
- Despommier, D., Ellingsen, E. (2008). The vertical farm: The sky-scraper as vehicle for a sustainable urban agriculture. *CTBUH 2008, 8th World Congress - Tall and Green: Typology for a Sustainable Urban Future, Congress Proceedings*, (December), 311–318.
- Dung, D.D., Godwin, I.R., Nolan, J.V. (2010). Nutrient content and in sacco degradation of hydroponic barley sprouts grown using nutrient solution or tap water. *Journal of Animal and Veterinary Advances*, 9(18), 2432–2436.
- Epting, S. (2016). Participatory budgeting and vertical agriculture: A thought experiment in food system reform. *Journal of Agricultural and Environmental Ethics*, 29(5), 737–748.
- Fazaeli, H., Golmohammadi, H.A., Tabatabayee, S.N. & Asghari-Tabrizi, M. (2012). Productivity and nutritive value of barley green fodder yield in hydroponic system. *World Applied Sciences Journal*, 16(4), 531–539.
- Gebremedhin, W.K., Deasi, B.G. & Mayekar, A.J. (2015). Nutritional evaluation of hydroponically grown barley fodder. *Journal of Agricultural Engineering and Food Technology*, 2(2), 86–89.
- Germer, J., Sauerborn, J., Asch, F., de Boer, J., Schreiber, J., Weber, G. & Müller, J. (2011). Skyfarming an ecological innovation to enhance global food security. *Journal Für Verbraucherschutz Und Lebensmittelsicherheit*, 6(2), 237–251.
- Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., ... Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, 327(5967), 812–818.
- Godfray, H., Charles J. & Garnett, T. (2014). Food security and sustainable intensification. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1639), 20120273.
- Hakkim, V., Joseph, E., Gokul, A. & Mufeedha, K. (2016). Precision farming: The Future of indian agriculture. *Journal of Applied Biology and Biotechnology*, 4(06), 068–072.
- Hughes, B.B. (2018). *International futures*. Boulder, RO: Westview Press.
- Jia, H. (2019). Agriculture: science and technology safeguard sustainability. *National Science Review*, 6(3), 595–600.
- Kalantari, F., Mohd Tahir, O., Mahmoudi Lahijani, A. & Kalantari, S. (2017). A review of vertical farming technology: A guide for implementation of building integrated agriculture in cities. *Advanced Engineering Forum*, 24, 76–91.
- Karavaşin, M. (2014). Kaba yem kaynağı olarak hidroponik arpa çimi üretiminde kuru madde ve ham protein verimleri üzerine farklı uygulamaların etkileri. *SDÜ Ziraat Fakültesi Dergisi*, 3(1), 27–33.
- Kılıç, Ü. (2016). Türk Tarım - Gıda Bilim ve Teknoloji Dergisi. *Türk Tarım-Gıda Bilim ve Teknoloji Dergisi*, 4(9), 793–7999.
- Özkan, U. & Demirbağ, N.Ş. (2016). Türkiye'de kaliteli kaba yem kaynaklarının mevcut durumu türkiyede kaliteli kaba yem kaynaklarını mevcut durumu. *Türk Bilimsel Derlemeler Dergisi*, 9(1), 23–27.
- Rajesh Kumar, Manisha Mathur, Monika Karnani, S.D.C. and D.J. (2018). Hydroponics: An alternative to cultivated green fodder: A review. *Journal of Entomology and Zoology Studies*, 6(6), 791–795.
- Şahin, G. & Kendirli, B. (2016). Yeni Bir zirai İşletme modeli: Dikey çiftlikler. *TÜCAUM Uluslararası Coğrafya Sempozyumu*, (October), 682–695.

Tezel, M. (2018). Türkiye’de silajlık mısır üretimi ve hayvan beslemede yeri. *TÜRKTOB Dergisi*, 25, 17–19.

***Food and Agriculture Organization of the United Nations (2018). Disasters causing billions in agricultural losses, with drought leading the way. Retrieved February 22, 2020 from

<http://www.fao.org/news/story/en/item/1106977/icode/>.

***United Nations (2017). World population to hit 9.8 billion by 2050, despite nearly universal lower fertility rates (Online). Retrieved December 18, 2019 from <http://www.un.org/apps/news/story.asp?NewsID=57028>.



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