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The efficiency of combined application of mineral fertilizers, inoculants in soybean growing technology, and functioning of nitrogen-fixing symbiosis under increasing nitrogen rates

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Abstract. Soy is a valuable leguminous crop, whose productivity directly depends on many factors, among which nitrogen fertilizers are one of the most important. Nitrogen fertilizing of this crop is often given insufficient attention because the yield increase can be quite insignificant. **The purpose of the research** is to study the combined effect of biofertilizers and increasing rates of nitrogen mineral fertilizers on the growth, development, and yield of soybean varieties. The experiment is three-factorial: factor A - early ripening soybean varieties Annushka and Ustya, factor B – seeds inoculation by biofertilizer, and factor C - rates of mineral fertilizers application. **Results of research shows** the efficiency of nitrogen-fixing symbiosis during seed inoculation was established for nitrogen rates from N_0 to N_{60} against the background of $P_{60}K_{60}$ - the biomass of nodule bacteria is 418–675 mg plant^{-1}. The application of $N_{90}P_{60}K_{60}$ significantly reduces the number and mass of nodule bacteria - 207–241; $N_{120}P_{60}K_{60}$ - 32.0–42.0 mg plant^{-1}, and with the introduction of $P_{60}K_{60} + N_{150–180}$ nodule bacteria are not formed and the effectiveness of microbiological drugs is not recorded. Nitrogenase activity with $N_{0–90}$ application on the background of $P_{60}K_{60}$ and seed inoculation ranged from 3.25 to 7.76 µmol C_2H_4 per plant ha^{-1}. With a further increase in nitrogen levels, nitrogenase activity was not recorded. On typical chernozems of the Forest-Steppe of Ukraine, higher yields of early-ripening soybean varieties are formed by applying $N_{60}P_{60}K_{60}$ and pre-sowing seeds treatment with rhizohumin or rhizohumin/hetomics combination. $P_{60}K_{60}N_{150–180}$ showed a partial decrease in soybean yield due to the distress effect.

Key words: hetomic, mineral fertilizers, nodules, rhizohumin, soybean, variety, yield.

INTRODUCTION

Soybeans are a valuable legume that accumulates nitrogen in the soil, forming root nodules and accumulating protein in the seeds. Growing soybeans improves nitrogen circulation by approximately 3.6 times, which is accompanied by an increase in nitrogen-fixing bacteria in the soil (Matsumiya et al., 2013).

Soybean yield directly depends on many factors, among which one of the most important is nitrogen fertilizers in research at the Mississippi Agriculture and Forestry Experiment Station in Starkville, MS. (Adeli et al., 2005). Nitrogen is one of the most important nutrients affecting soybean yields (Liu et al., 2008; Dong et al., 2010; Wan, 2013). With fertilizer applying, the rate of phosphorus-potassium fertilizers to create a deficit-free balance in the soil can be calculated, but this can't be done for nitrogen, which has a much more complex cycle of transformations in nature. The nitrogen cycle is not limited to the plant-soil system but also covers the atmosphere due to the nitrogen-fixing and denitrifying ability of microorganisms. Therefore, the use of nitrogen fertilizers should be based on specific knowledge of the natural nitrogen cycle and on knowledge of the real use scale of biological nitrogen due to nitrogen fixation by microorganisms (Temrienko, 2018; Novytska et al., 2020).

Effective use of nitrogen fertilizers is crucial for soybean cultivation, in particular, optimization of chemical form, rate, time, and application of fertilizers for crop growing in different conditions. Muchow & Sinclair (1986) in studies of yield formation of early-maturing and late-maturing soybean varieties (*Glycine max* (L.) *Merr*.), which were grown on two different types of soil both under irrigation conditions and under conditions of limited water supply in a semi-arid tropical environment noted that the introduction of high levels of nitrogen was the 'main obstacle' to obtaining a high yield of soybeans. The researchers obtained the maximum grain yield at an average level of nitrogen application. Excessive or insufficient nitrogen fertilizers did not increase soybean yields, while intermediate levels of starting nitrogen fertilizers (N_{50}) increased crop yields. Similarly, an increase in soybean yields in response to moderate nitrogen levels was observed by Tian (2007). Studies conducted in the Sanjiang River Plain in China have also shown that nitrogen fertilizers at a rate of 50 kg ha^{-1} at sowing promote activation of the soybean root system, rate of leaf photosynthesis, and maximum grain yield (Gai et al., 2017).

However, Maw et al. (2011) at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand reported that four rates of nitrogen fertilizer application (0, 25, 50, and 75 kg N ha^{-1}) were applied as starting nitrogen, and the highest grain yield in both seasons was observed at N_{75}. Some researchers believe that a small amount of starting nitrogen fertilizer placed near the seeds can have a positive effect on the early growth and yield of soybean grain, especially in situations where soil fertility is low or there are adverse environmental conditions (Sij et al., 1979). However, the need to apply increased rates of nitrogen when growing soybeans is due to the high removal of nitrogen from the soil, which is 3–4 times greater than phosphorus.

Takahashi et al. (Takuji et al., 2017) investigated the effect of deep placement of coated urea (CU) on soybean seed yield in a rotating rice field in Niigata, Japan. They found that deep (20 cm) application of lime nitrogen or coated urea did not increase nitrate or ammonium concentrations in the upper layer, where nodules are primarily formed and promoted soybean growth and seed yield by promoting nitrogen fixation after the initial flowering stage. This method of feeding does not inhibit the formation of nodules and nitrogen fixation. The constant supply of nitrogen from the lower part of the soil contributes to the photosynthetic activity of the leaves until the seed ripening stage. Thus, a large amount of photosynthate can be transported to the nodules as well as to the seeds. A constant supply of N and C for seed growth increases seed yield and improves seed quality. The stimulating effect on the formation of nodules by the deep application of lime nitrogen was confirmed by an experiment with a rhizobox.

The application of nitrogen fertilizers at the beginning of soybean flowering helped to increase crop yields by increasing nitrogen fixation (Takuji et al., 2017). Other studies conducted in 23 northern parts of the Midwestern United States have shown that the application of mineral nitrogen fertilizers in the full flowering phase of soybean plants reduced the efficiency of biological nitrogen fixation by 16% and yield by 13 kg ha^{-1} (Tamagno et al., 2018).

Soybean yield depends on both the mineral nitrogen of the soil and its biological fixation. The interaction of these two nitrogen sources is biologically interesting and agronomically important (Kalenska et al., 2021). Studies in Brazil show, that seed inoculation increases nitrogen fixation efficiency by up to 85%, grain yield by up to 5%, and protein content in grain by up to 7%. Application of mineral nitrogen fertilizers at the rate of 30 kg ha^{-1} at sowing did not significantly affect the number and mass of nodules, while the application of 200 kg ha^{-1} of nitrogen dramatically reduced the number of nodules and up to 44% nitrogen fixation efficiency (Hungria et al., 2006). The results of the study by Caliskan et al. (2008), conducted in Turkey, demonstrated the positive effect of inoculation and nitrogen fertilizers on plant growth parameters and soybean productivity at nitrogen application rates up to 80 kg ha^{-1}. Further increases in nitrogen levels led to negative interactions and reduced crop yields.

The introduction of nitrogen fertilizers under legumes can be excluded only under optimal environmental conditions for the symbiotic activity of nodule bacteria. The high intensity of this process is achieved with optimal humidity, the reaction of the environment, the presence of a sufficient amount of phosphorus and potassium, the presence of active strains of nodule bacteria in the soil, or when plants are infected with virulent strains of specific races of nodule bacteria. If any of the factors are in suboptimal sizes, nitrogen fixation is weak or it is not assimilated at all. Unfortunately, in real agricultural production, the agrochemical properties of the soil (pH, the content of macro- and microelements), water and temperature regimes, or other environmental factors do not always contribute to symbiotic nitrogen fixation. Leguminous plants in this case experience nitrogen starvation, and switch to heterotrophic nitrogen nutrition, just like non-leguminous crops, and with mineral nitrogen deficiency, they give low yields. Therefore, under unfavorable conditions for nitrogen fixation, it is possible to increase the productivity of legumes only by using nitrogen fertilizers. At the same time, it is important to consider that the introduction of nitrogen fertilizers under leguminous crops increases the yield only in the absence of nitrogen fixation or when the symbiosis is weakened. The high efficiency of nitrogen fertilizers in leguminous crops indicates their low symbiotic activity (Kalenskiy et al., 2016; Novytska et al., 2020).

The presented research results show that when developing a soybean fertilization system and creating optimal crop nutrition conditions, the soil and climatic conditions of the growing region and the features of the symbiotic nitrogen fixation process should first of all be taken into account. The yield and quality of soybean seeds largely depend on the optimal combination of symbiotic and mineral nitrogen nutrition in the crop fertilization system. Therefore, the purpose of the research was to establish the effectiveness of the nitrogen-fixing symbiosis, the formation of yield, seed quality, structure, and yield index with the introduction of increasing nitrogen rates in combination with biofertilizers in soybean cultivation technology in the Right Bank Forest Steppe of Ukraine.

MATERIALS AND METHODS

Field research was conducted in 2006–2010 in a stationary field experiment at the 'Agronomic Research Station' of NULES of Ukraine in a 10-field field crop rotation and on the basis of educational and scientific laboratory 'Demonstration collection field of agricultural crops of the Plant Science Department of NULES of Ukraine. The soil is typical low-humus chernozem, medium-loamy in mechanical composition. The thickness of the humus horizon is 25–30 cm. The content of humus (Table 1) in the arable layer of the soil (0–30 cm) according to Tyurin is 5.3%, the pH of the salt extract is 7.8, the content of mobile phosphorus and exchangeable potassium according to Chirikov, respectively 35.7 and 90.19 mg kg^{-1} of soil, nitrogen - 213.2 mg kg^{-1} of soil, the reaction of the soil solution is close to neutral.

Table 1. Agrochemical indicators of the experimental field

INDEX	Test results	Accuracy
pH of salt extract, pH units	7.8	± 0.30
Humus substance (organic matter), %	5.3	± 0.53
Nitrogen (alkalin-hydrolized), mg kg^{-1}	213.2	± 26.65
Mass content of Potassium mg kg^{-1}	90.19	± 0.24
Mass content of Potassium %	0.00902	± 0.00002
Labile Phosphorus, mg kg^{-1}	35.7	± 5.35
Exchange Calcium, mmol per 100 g	8.7	± 0.65
Exchange Magnesium, mmol per 100 g	1.5	± 0.15
Carbonates, mmol per 100 g	0.1	± 0.001
Bicarbonates, mmol per 100 g	0.59	± 0.059
Mass content of Iron, mg kg^{-1}	0.067	± 0.09
Mass content of Iron, %	0.00007	± 0.00001
Mass content of Manganese, mg kg^{-1}	12.88	± 0.20
Mass content of Manganese, %	0.00129	± 0.00002
Mass content of Copper, mg kg^{-1}	0.141	± 0.001
Mass content of Copper, %	0.0000141	± 0.0000001
Mass content of Zink, mg kg^{-1}	0.38	± 0.01
Mass content of Zink, %	0.000038	± 0.000001
Particle size classification	Medium clay-loam soil, rough-dusty-muddy	
Sand 1–0.05 mm, %	9.0	
Rough dust 0.05–0.01 mm, %	49.85	
Dust 0.01–0.001 mm, %	18.0	
Silt < 0.001 mm, %	23.10	
Clay < 0.01 mm, %	34.80	

The soil of the experimental field has high natural fertility, is characterized by optimal parameters of agronomic properties, and has a slightly alkaline or neutral reaction of the soil solution. Soils of this type are well humus-rich, as a result of which they have a dark color and considerable depth, and are well structured. Such soils are rich in nutrients, and the physical and mechanical qualities of the soil are quite favorable for the cultivation of cultivated plants.

Climate conditions

The research area (Kyiv-Sviatoshynskyi district of Kyiv region) is a zone of sufficient humidity (HTC-1.2), with a warm, moderately humid climate. Winters are mild, cloudy with frequent thaws and only in some years with severe frosts, and summers are mostly warm, and moderately humid. Transitional periods (spring, autumn) are mostly long, and unstable, but on average warm springs prevail with sufficient (160–180 mm) reserves of productive moisture in a meter layer of soil.

Starting from 2006, the average temperature for the period April-October exceeded the average long-term indicator, and the dynamics of a gradual increase of this indicator were observed (Table 2).

Table 2. Weather conditions during the years of research

Year	Month							
	IV	V	VI	VII	VIII	IX	X	IV–X
	Monthly precipitation indicators, mm							
2006	30.2	120.4	44.0	25.3	130.4	43.0	15.4	408.7
2007	4.1	57.8	53.2	43.7	51.3	7.9	0.6	218.6
2008	53.6	9.8	14.7	43.9	38.2	132.4	22.0	314.6
2009	0.0	27.8	56.2	96.8	8.1	10.8	26.4	226.1
2010	42.1	46.8	53.9	110.2	33.6	29.7	26.7	343
Average multi-year	38.4	43.3	73.9	72.9	57.8	46.3	31	363.6
	Average monthly air temperature, °C							
2006	11.1	15.6	17.5	19.8	19.4	15.3	9.3	15.4
2007	9.8	18.6	21.3	21.9	21.8	15.0	8.9	16.8
2008	10.1	13.9	18.2	19.6	20.8	12.9	9.8	15.0
2009	9.4	14.2	20.0	21.3	18.6	16.4	8.8	15.5
2010	9.5	17.0	20.7	23.8	23.1	14.4	5.6	16.3
Average multi-year	8.9	15.3	18.4	20.1	19.3	14.0	8.2	15.5
	Hydrothermal coefficient (HTC)*							
2006	1.3	2.5	0.8	0.4	2.2	0.9	0.9	1.3
2007	0.2	1.1	0.8	0.6	0.8	0.2	0.0	0.7
2008	2.8	0.2	0.3	0.7	0.6	5.2	1.4	1.1
2009	0.0	0.7	0.9	1.5	0.1	0.2	1.7	0.7
2010	2.7	0.9	0.9	1.5	0.5	0.7	0.0	1.1
Average multi-year	1.8	1.0	1.1	1.4	1.0	0.8	1.6	1.2

*Note. HTC > 1.6 – excessive moisture; HTC 1.3–1.6 – wet conditions; HTC 1.0–1.3 – slightly arid conditions; HTC 0.7–1.0 – arid conditions; HTC 0.4–0.7 – very arid; HTC < 0.4 – dry conditions.

The average daily air temperature during the growing season of spring crops from April to October was on average 1.1 °C higher, compared to long-term data - 14.9 °C. In recent years, excessively high temperatures are increasingly observed in April, August, and September. In addition, the years of research significantly differed in humidity. Thus, the conditions of 2006 were generally typical, but the meteorological spring began earlier than usual, so April was atypical in terms of temperature as the average air temperature was 11.1 °C, which exceeded the long-term by 2, 2 °C. Accordingly, the sum of active temperatures above +5 and +10 °C was 80–82 °C higher than the long-term value, which accelerated the emergence of seedlings and the development of plants in the initial phases of ontogenesis. During the April-October period, 408.7 mm of precipitation fell, which is 45.1 mm more than the long-term value. The distribution during the growing season was uneven - the most precipitation fell in May (120.4 mm) and August (130.4 mm), which to some extent compensated for their lower amount in the following months. In particular, June of this year was characterized by dry conditions (Hydrothermal coefficient = 0.8), and July – severe drought (HTC = 0.5). In general, in terms of moisture supply and temperature, the year was typical, and dry periods had an insignificant effect on the productivity of most crops.

According to the coefficient of deviations significance of the temperature regime from the long-term value for the period April-October, the conditions of 2007 were rare with a significant excess. During this period, the average value of the air temperature was 16.8 °C, which is 1.9 °C higher than the long-term indicator. May, June, July, and August were the months with an atypical temperature regime (C_s 1–2), that is, during the period of active vegetation of spring crops, the sum of active temperatures also increased, which affected the duration of individual phenological phases. Regarding the wetting regime of the territory, only 218.6 mm of precipitation fell during the period from April to October, which is 145 mm lower than the long-term indicator. The main reason for this phenomenon was the low amount of precipitation in April, September, and October (0.6–7.9 mm per month). It should be noted that in months with atypically high air temperature, the amount of precipitation was within the range of the multi-year value, however, due to the inflow of thermal energy, the HTC decreased compared to typical years. In general, the year was characterized by dry conditions (HTC = 0.7), and very severe drought conditions were observed in April, September, and October. At the same time, June and August were dry (HTC = 0.8), and July was very dry (HTC = 0.5), which limited the productivity of spring crops, the critical periods of which fell on these months.

The temperature regime in 2008 corresponded to typical conditions, but in August it was atypically warmer (C_s = 1.1). The total amount of precipitation for the April-October period was 314.6 mm, which is 49 mm lower than the long-term norm, but its distribution during the growing season was uneven, which manifested itself in droughts at certain stages of crop development. In particular, after a wet April (HTC > 1.6) with 53.6 mm of precipitation, May and September experienced very severe drought (HTC = 0.2–0.3), and very dry conditions in July and August (HTC 0.6–0.7). The excessive amount of precipitation in September (132.4 mm) did not affect the formation of crops productivity, although in general the year was characterized by sufficient moisture (HTC 1.1), in critical periods there were severe droughts that limited productivity.

The year 2009 was typical in terms of temperature, although atypically hot conditions were observed in July and September (C_s 1–2), which negatively affected the productivity of spring crops. During this period, 226.1 mm of precipitation fell, which is by137.5 mm less than the long-term value, and its distribution was anomalous, since in June and July a total of 153 mm fell, and the remaining 73.1 mm was distributed over other months, while that there was no precipitation at all in April. According to the HTC, conditions in April, August, and September were characterized by very severe drought, while May and June were dry. The productivity of spring crops depended mostly on the reserves of productive moisture, given that the year was generally dry, especially in critical periods for moisture supply.

According to the temperature regime, the year 2010 belonged to the category of atypically warm (C_s 1.6), since the average temperature for the April-October period was 16.3 °C, which is by1.4 °C higher than the long-term value. It should be noted that some months were abnormally hot, while others were unusually cool. In particular, June of this year was atypically hot, and July and August were abnormally hot (C_s 3.0 and 2.9, respectively), which respectively affected the productivity of crops due to stressful conditions. At the same time, it was unusually cool in October, which indicated an earlier end of the growing season. The total amount of precipitation in April-October this year was 343 mm, which was only 20 mm less than the long-term value, and their distribution was relatively even. This is what made it possible to attribute the year to slightly arid conditions (HTC = 1.1), although in some months there was a moisture deficit. In particular, May, June, and October were dry, and a severe drought was in August. Due to the peculiarities of the October temperature regime, HTC was not calculated (the sum of active temperatures > 10 °C was 0 °C). In general, the year was hot, but the moisture regime allowed crops to form a competitive yield level.

Sampling and methods

The purpose of the research is to study the combined effect of biofertilizers and increasing rates of nitrogen fertilizers on the growth, development, and yield of soybean varieties. The experiment is three-factor (Table 3). Agricultural technology for growing soybeans is generally accepted for the Northern Forest Steppe. The seeds were sown at a soil temperature of 10–12 °C with a SON-4.2 vegetable planter. The total area of the elementary plot is 84 m^2, and the area of the accounting plot is 50 m^2 with four repetitions. The location of the plots is systematic (Ermantraut et al., 2014). Seeding sowing rate - 600 thousand similar seeds ha^{-1}.

Table 3. The scheme of the experiment

Factor A: variety	Factor B: seed inoculation	Factor C: fertilizing, kg ha^{-1} a.s.
Annushka	1. Without inoculation (control)	1. Without fertilizers (control)
Ustya	2. Rhizohumin	2. $P_{60}K_{60}$
	3. Hetomic	3. $N_{30}P_{60}K_{60}$
	4. Rhizohumin + Hetomic	4. $N_{60}P_{60}K_{60}$
		5. $N_{90}P_{60}K_{60}$
		6. $N_{120}P_{60}K_{60}$
		7. $N_{150}P_{60}K_{60}$
		8. $N_{180}P_{60}K_{60}$

Field research was carried out during 2006–2010 in the stationary and temporary experiments of the Plant Science Department at the PF of the National University of Life and Environmental Sciences of Ukraine 'Agronomic Research Station' (Pshenichne village, Vasylkiv district, Kyiv region) in the Right Bank Forest Steppe of Ukraine. Laboratory studies were carried out in the educational and scientific laboratories of the Plant Science Department 'Analytical research in plant science', 'Quality of seeds and planting material' of the National University of Life and Environmental Sciences of Ukraine and in the analytical biochemical scientific research laboratory 'Physiological bases of plant productivity' of the NSC 'Institute of Biology and Medicine' Taras Shevchenko Kyiv National University.

The studies used recommended soybean varieties for the Forest-Steppe zone: ultra-early Annushka (PE Scientific Breeding and Seed Company 'Soevyi vik', Kropyvnytskyi) and early-ripening Ustya (NSC 'Institute of Agriculture NAAS', Chabany); biofertilizers of the Institute of Agricultural Microbiology of NAAS (Melnyk et al., 2007): inoculant Rhizohumin (strain *Bradirizobium Japonicum* M-8), microbial biofungicide Hetomic (*Chaetomium cochliodes* 3250), application rate - 200 g $hectare^{-1}$ seeding rate.

In soybean varieties Annushka and Ustya, at 1–2 variants of factor B and 1–8 variants of factor C in the main phases of plant development - flowering and bean swelling, physiological and biochemical indicators of plant condition were determined: the content of photosynthetic pigments in leaves (chlorophyll a, b, carotenoids) and the amount of malondialdehyde (MDA) and grain quality (protein and fat content).The content of chlorophyll *'a'* and *'b'* in the leaves of plants was determined by biochemical analysis using a spectrophotometer, followed by calculation of pigments concentration according to the equation of Wetstein and Holm (Hrytsaenko et al., 2003). Nitrogenase activity of nodules of the soybean root system was determined by the acetylene-ethylene method (Makrushin et al., 2006). The intensity of lipid peroxidation processes was studied in soybean plant leaves for the content of TBA-active products (Platonova & Kostyshyn, 2000). Harvest accounting was performed separately by direct combining and 'test sheaf' methods. Grain quality was determined by infrared spectrometry on a NIP Scanner 4250 infrared analyzer with ADI DM 3114 computer software.

The productivity index was calculated as the coefficient of reduction of the level of average productivity of a separate agricultural crop relative to the level of average productivity in specific soil and climatic conditions of research.

Statistical data processing was performed using the Microsoft Excel program, the SAS 9.4 software package, and the 'Statistica 6' software package.

Fisher *LSD* was conducted for establishing significant difference between variants in yield structure (pods and seeds per plant, seed weight, and thousand seed weight, seed yield, MDA content, protein, and fat content). Analysis of variance was conducted by Statistica 13.3 for average seed yield. Means of yield structure were presented with standard error (*SE*).

RESULTS AND DISCUSSION

Adaptation and stability of plants largely depend on the functioning of their photosynthetic apparatus (Halliwel & Guteridge, 1984). Photosynthetic pigments change their quantitative indicators in response to the influence of various factors. It is known that lipid peroxidation (LPO) is one of the first consequences of oxidative damage to membrane systems, and the amount of malonic dialdehyde (MDA) - a stable product of this process, indicates the depth of this process and is used as an indicator of stress (Halliwel & Guteridge, 1984; Tuanjie et al., 2017). Intensification of lipid peroxidation (LPO) processes is the primary nonspecific response to a stress factor manifested in an increase in malonic aldehyde (MDA) in photosynthetic soybean tissues with nitrogen application (Platonova & Kostyshyn, 2000; Batsmanova et al., 2020). Therefore, the possible stressors of different rates of nitrogen fertilizers on soybean varieties were evaluated by the accumulation of MDA. In our research it was found that the processes of LPO were more actively developed in the phase of flowering and maximum growth - 55% in terms of control without fertilizers was achieved with the introduction of N_{180}; with pre-sowing seeds treatment by Rhizohumin and with the introduction of N_{30} the content of MDA in the leaves increased by only 12.5% (Table 4).

Table 4. The content of malonic dialdehyde in soybean plants leaves of different varieties depending on fertilizer (µm g^{-1} of raw material)

Fertilizing	Annushka		Ustya	
	flowering	beans swelling	flowering	beans swelling
	Control			
Control	50.86	55.44	53.95	88.20
$P_{60}K_{60}$ – background	48.73	56.70	54.77	88.83
background + N_{30}	48.40	65.37	73.59	112.68
background + N_{60}	49.88	76.69	79.80	108.78
background + N_{90}	54.03	87.63	81.71	107.42
background + N_{120}	60.09	97.67	92.80	109.01
background + N_{150}	58.93	97.09	92.02	110.51
background + N_{180}	61.60	101.07	94.88	109.97
	Rhizohumin			
Control	66.89	66.89	65.40	99.65
$P_{60}K_{60}$ – background	60.18	68.15	66.22	100.28
background + N_{30}	59.85	76.82	85.04	124.13
background + N_{60}	61.33	88.14	91.25	120.23
background + N_{90}	65.48	99.08	93.16	118.87
background + N_{120}	71.54	109.12	104.25	120.45
background + N_{150}	70.38	108.54	103.47	121.96
background + N_{180}	73.05	112.52	106.33	121.42
$LSD_{0.05}$	0.24	2.18	0.67	1.12

The biggest increase in the MDA content was observed in the phase of bean swelling with the use of high rates of nitrogen (180 kg ha^{-1}): in the Ustya variety the MDA content increased by 25% compared to the control; in the Annushka variety, the MDA content increased by 69% in the flowering phase. In the phase of bean swelling with pre-sowing seeds treatment by Rhizohumine and application of N_{30} and without its addition, the MDA content decreased by 16 and 18%, respectively, and with the application of N180 - increased by 17% relative to control.

Combined use of Rhizohumin + $N_{180}P_{60}K_{60}$ stimulated the doubling of MDA content compared to the control (without fertilizers + Rhizohumin) and higher by 35% compared to the absolute control, which may be due to the negative impact of high rates of mineral fertilizers on legume-rhizobial symbiosis formation.

According to the results of research, a rate-dependent increase in MDA content in the leaves of experimental varieties was established. In particular, the biggest increase in the MDA content was observed in the phase of swelling beans with the use of high rates of nitrogen (180 kg ha^{-1}): in the Ustya variety, the MDA content increased by 25% compared to the control. For the Annushka variety, the MDA content increased by 69% in the flowering phase, and already in the bean swelling phase, its values approached the control. The effect of the rate of 180 kg ha^{-1} was most negative for the Annushka variety in the flowering phase - the largest accumulation of its content was recorded compared to other experimental varieties. An increase in the amount of MDA in experimental soybean varieties may indicate a shift in the pro/antioxidant balance in the direction of enhanced ROS (reactive oxygen species) generation and their involvement in the oxidation of membrane lipids, which ultimately leads to pathological changes and growth inhibition.

Chlorophyll is vital for photosynthesis, allowing soybeans to absorb light energy (Tucker, 1979; Wan, 2013). The chlorophyll content is one of the most pronounced characteristics of the photosynthetic apparatus of plants' adaptation to environmental conditions (Zhang et al., 2001). The rate of photosynthesis of soybeans is negatively affected by stressful environmental conditions, in particular, during drought, respiratory conductivity decreases, significant over moistening causes the accumulation of starch in the leaves (Raymond et al., 2015). In the critical phase of plant development (flowering) there is a tendency for a rate-dependent increase in the content of photosynthetic pigments in plants without inoculation by Rhizohumine. Seed inoculation, depending on nitrogen rates, showed an increase in chlorophyll by 64% compared to control (without fertilizers), and a decrease of 30% with N_{180} and without seed inoculation - a manifestation of inhibitory action of excess nitrogen for effective nitrogen-fixing symbiosis as also noted by Shen et al. (2012). With applying N_{30} kg ha^{-1}, the quantitative content of pigment did not change. The content of chlorophyll *'b'* and carotenoids was at the control level.

In the phase of bean swelling there is a redistribution of assimilates with more intensive outflow to new attracting seed centers, which affected the reduction of photosynthetic pigments. In addition, the use of Rhizohumin showed a tendency to reduce the content of photosynthetic pigments, compared with the control without inoculation (Fig. 1).

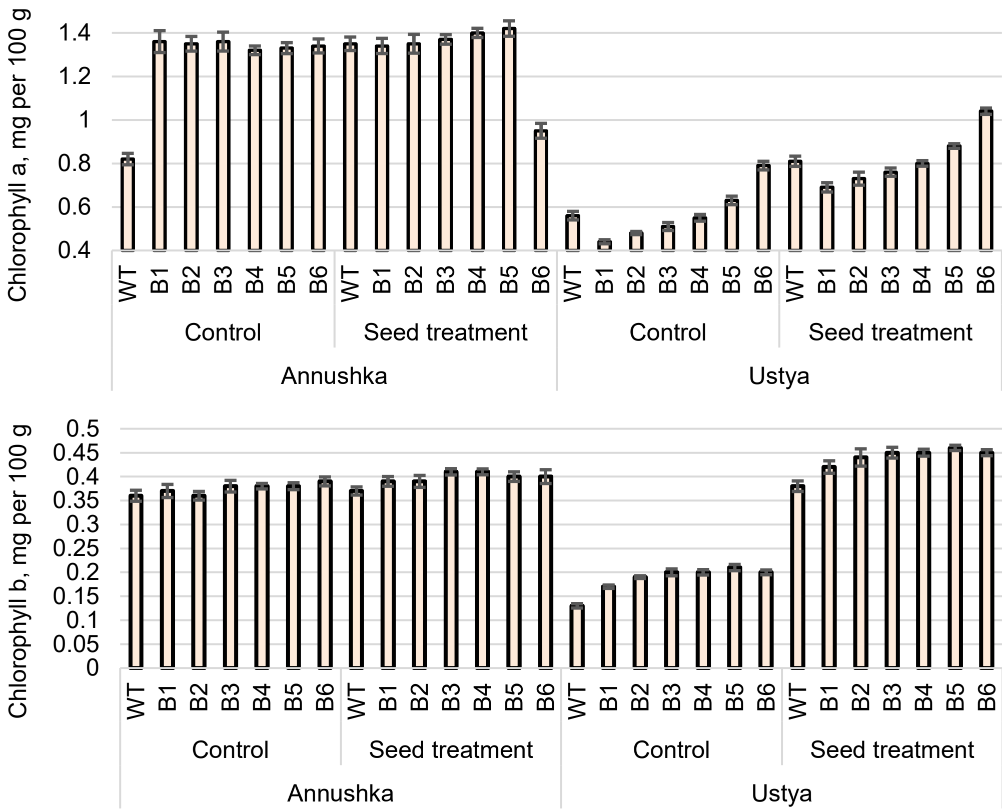
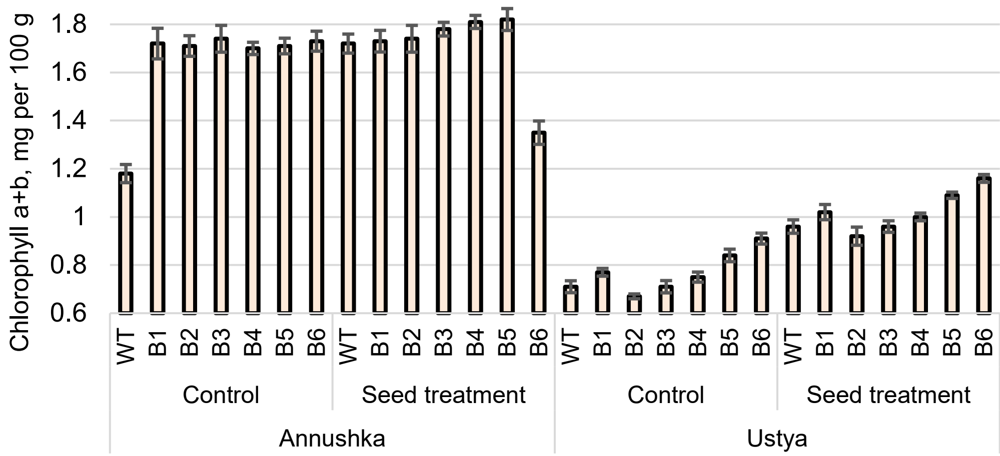
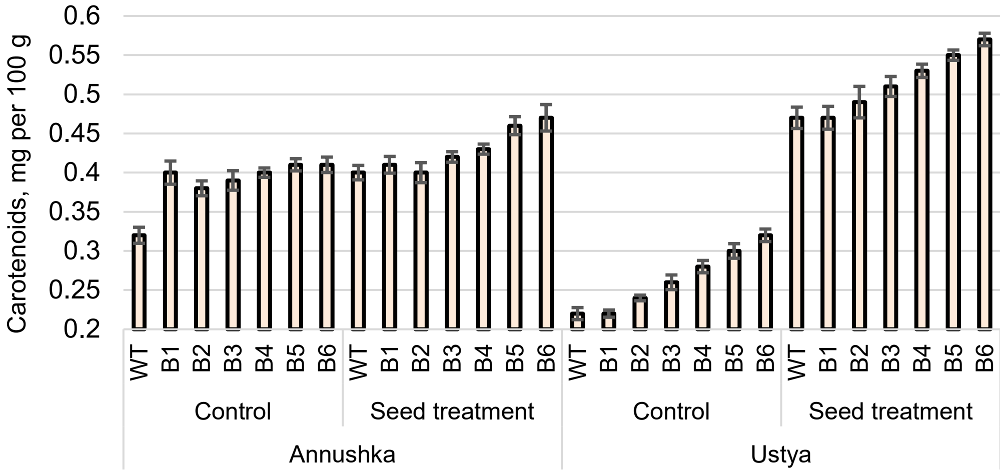
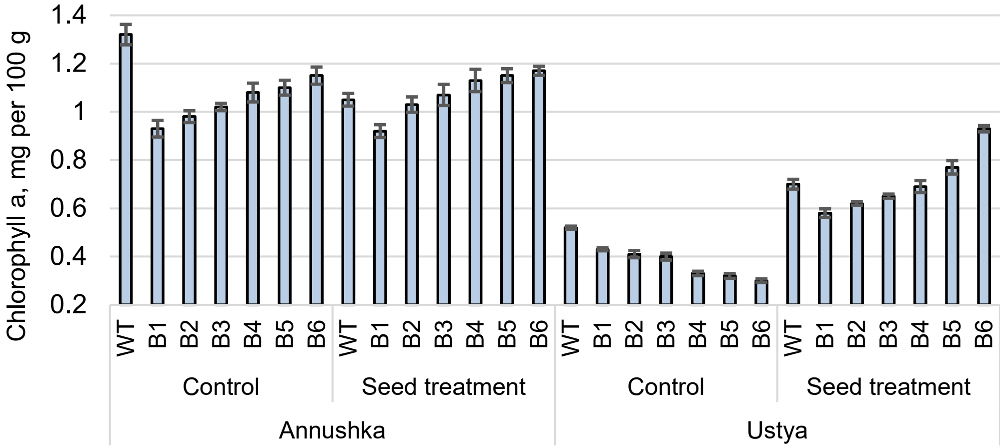


Figure 1 (continued)







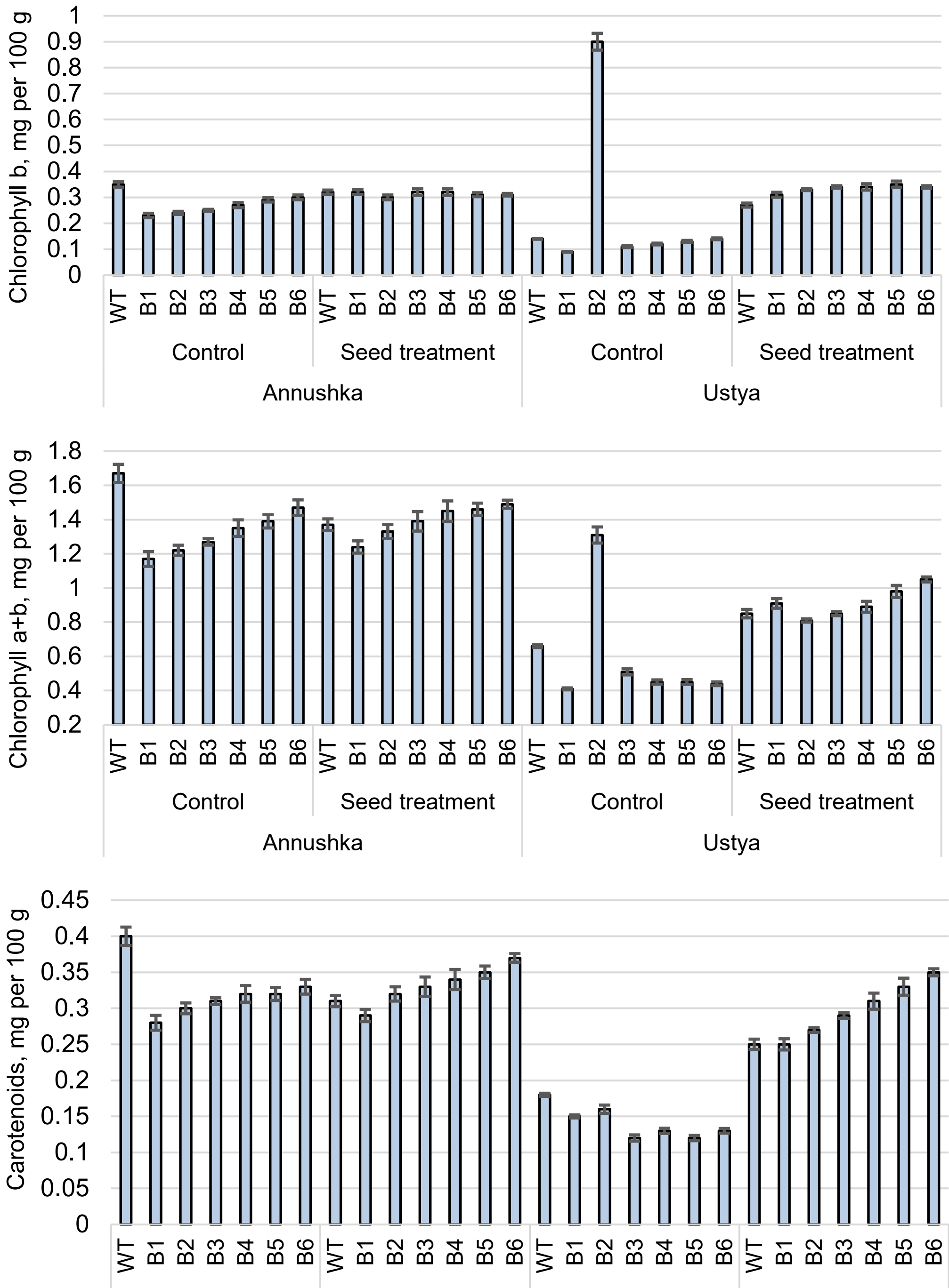


Figure 1. The content of photosynthetic pigments in the leaves of soybean plants depending on the rate of nitrogen fertilizers.

Note: yellow bars are the flowering phase, and blue bars are the iswelling of beans. Error bars describe the standard error of the mean (*SE*).

The results of our research are consistent with the data obtained by Zhang et al. (2001) at the Soybean Institute of Jilin Academy of Agricultural Sciences, Honchar et al. (2021) at the National University of Life and Environmental Sciences of Ukraine and Mutava et al. (2015) in the greenhouses of the Department of Plant Science, University of Missouri, Columbia show that the increase in the ratio of chlorophyll *'a'* and *'b'* with the introduction of nitrogen on the background of inoculation indicates the development of stress and reduced stability of the plant. In the flowering phase, there is an increase in this ratio with the combined use of nitrogen fertilizers with Rhizohumin and without it. The same trend persists in the bean swelling phase for almost all combinations of nitrogen and inoculation.

With the introduction of N_{30} and inoculation by Rhizohumin, in the phase of swelling beans, the ratio decreased by 30% compared to the control. This, in turn, indicates an adaptation aimed at improving the efficiency of photosynthesis. In soybean plants, this figure differed significantly in the experimental phases of development - flowering and swelling of beans. Thus, in the flowering phase in all experimental varieties, there was a tendency for a rate-dependent increase in chlorophyll *'a'*, while the content of other photosynthetic pigments (chlorophyll *'b'* and carotenoids) did not differ significantly from control values. In the bean swelling phase, which is characterized by an increase in the intensity of nitrogen use by non-photosynthetic tissues for seed formation, the expected decrease in pigment content in the studied soybean varieties was observed, depending on the rate of fertilizers. In the experimental varieties Annushka and Ustya, a significant decrease in the content of all pigments was recorded. In particular, the content of chlorophyll *'b'*, which is more sensitive to various anthropogenic influences, in the variety Annushka decreased by 33.5%, and in the variety Ustya by 27%.

The symbiotic activity of soybean varieties differed in such indicators as: the number (Table 5) and weight of nodules per plant, nitrogenase activity (Fig. 2). The increase in the number and weight of nodules occurred from the absolute control with seed inoculation and drugs to the introduction of $N_{60}P_{60}K_{60}$ and seed inoculation; from $N_{90}P_{60}K_{60}$ there is a sharp decrease in symbiotic activity; against the background of $N_{120}P_{60}K_{60}$ only traces of symbiotic activity are recorded, and when $N_{150}P_{60}K_{60}$ and $N_{180}P_{60}K_{60}$ are applied, symbiotic nitrogen fixation is absent.

Table 5. Number of active nodules, psc per plant

Marking	Rate of fertilizer, *factor C*	Variety, *factor A*							
		Annushka, A1				Annushka, A1			
		Seed treatment[1], *factor B*							
		C	H	R	R+H	C	H	R	R+H
		B1	B2	B3	B4	B1	B2	B3	B4
C1	Control	12.4	22.1	24.1	32.2	13.4	19.5	22.3	28.5
C2	$P_{60}K_{60}$ – background	17.2	40.6	42.4	50.4	22.1	36.4	38.8	42.8
C3	background + N_{30}	21.2	64.4	69.5	68.6	20.4	49.9	58.2	62.4
C4	background + N_{60}	26.6	84.9	85.1	88.3	28.6	64.4	76.1	78.2
C5	background + N_{90}	17.1	35.2	36.5	36.6	19.2	33.3	35.6	38.6
C6	background + N_{120}	9.1	11.2	12.4	16.6	10.8	9.2	10.6	12.1
C7	background + N_{150}	0	0	0	0	0	0	0	0
C8	background + N_{180}	0	0	0	0	0	0	0	0
$LSD_{0.05}$		2.1	8.8	11.4	9.5	1.7	12.1	10.6	7.6

The study by Osborne & Riedell (2006) in the unique environment of the northern Great Plains, USA and Wang & Han (2009) in China show that small amounts of nitrogen should be applied under soybeans, assuming that plants will be provided with it due to natural nitrogen fixation. However, under stress, not all plants or even crops form a nitrogen-fixing symbiosis, which significantly reduces yields. Our studies are consistent with the results of Matsumiya et al. (2013) conducted at Department of Biotechnology, College of Lifesciences, Ritsumeikan University, Japan, Gai et al. (2016, 2017), on the plains of the Sanjiang River in China, which confirm that the use of pre-sowing treatment of soybean seeds also had a positive effect on grain yield formation. Thus, in the variants with inoculation of soybean seeds by Rhizohumin and Hetomic, the yield of the crop was higher by 0.12–0.25 t ha^{-1} compared to the control. Among the varieties, the ultra-early variety Annushka turned out to be more productive when grown on typical chernozems.

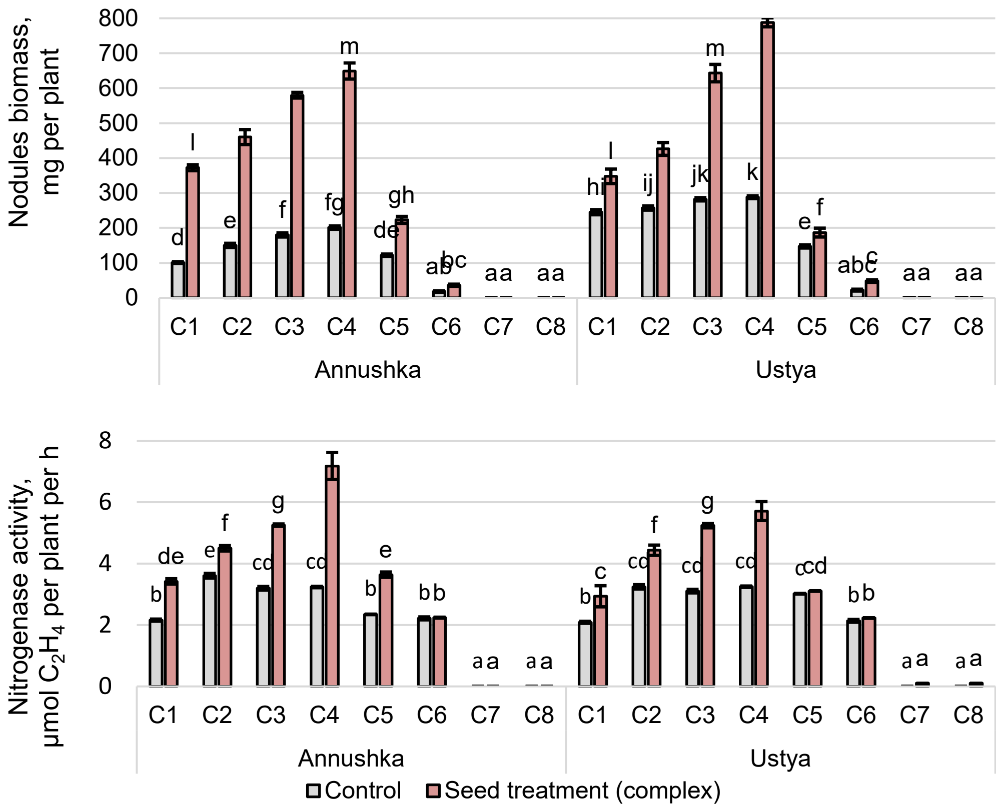


Figure 2. Nodules mass and soybean nitrogenase activity depending on the fertilizer and inoculation.

Note. Seed treatment: B1 – without inoculant (control); B2 – H, B3 – R, B4 – H + R; fertilizer option: 1 – control; 2 – $P_{60}K_{60}$ (Background); 3 – background + N_{30}; 4 – background + N_{60}; 5 – background + N_{90}; 6 – background + N_{120}; 7 – background + N_{150}; 8 – background + N_{180}.

Error bars describe the standard error of the mean (*SE*). The same letters indicate belonging to the same similarity group (no significant difference according to Fisher's LSD_{05}) within the factor. Columns without a letter are significantly different for Fisher's LSD_{05} from other variants.

Table 6. Soybean yield depending on fertilizer rates and pre-sowing seed treatment, t ha^{-1}

Marking	Rate of fertilizer, *factor C*	Variety, *factor A*									
		Annushka, A1					Ustya, A2				
		Seed treatment[1], *factor B*									
		C	H	R	R+H	+from	C	H	R	R+H	+from
		B1	B2	B3	B4	$B^{2–4}$	B1	B2	B3	B4	$B^{2–4}$
C1	Control	1.96	2.08	2.12	2.20	0.16	1.78	1.87	1.93	2.00	0.15
C2	$P_{60}K_{60}$ - background	2.10	2.24	2.28	2.36	0.19	1.94	2.03	2.09	2.16	0.20
C3	background + N_{30}	2.64	2.80	2.95	3.16	0.32	2.40	2.52	2.68	2.88	0.34
C4	background + N_{60}	3.84	4.07	4.15	4.18	0.29	3.49	3.66	3.76	3.80	0.25
C5	background + N_{90}	3.69	3.80	4.01	4.06	0.27	3.36	3.42	3.65	3.67	0.22
C6	background + N_{120}	3.61	3.63	3.64	3.63	0.16	3.27	3.23	3.24	3.28	0.03
C7	background + N_{150}	3.43	3.45	3.47	3.47	0.03	3.29	3.33	3.31	3.30	0.02
C8	background + N_{180}	3.29	3.33	3.33	3.36	0.05	2.71	2.72	2.71	2.72	0.05
$LSD_{0.05}$		0.13	0.14	0.15	0.14	-	0.13	0.14	0.13	0.12	-

Note: 1C – control; R – rhizohumin; R + H – rhizohumin + hetomic; H – hetomic; + from $B^{2–4}$ – average yield increase from seed treatment by inoculants.

The highest yield is formed by the application of $N_{60}P_{60}K_{60}$ and pre-sowing treatment of seeds with Rhizohumin or a combination of Rhizohumin and Hetomic (Table 6, 7).

Table 7. ANOVA of soybean yield data

Effect	SS	*df*	MS	Partial eta-square:
Variety (V)	23.672	1	23.672	0.984916
Fertilizing (F)	404.257	7	57.751	0.999104
Seed treatment (ST)	6.074	3	2.025	0.943676
Year (Y)	95.209	4	23.802	0.996207
V*F	4.732	7	0.676	0.928836
V*ST	0.047	3	0.016	0.114789
F*ST	4.143	21	0.197	0.919531
V*Y	10.219	4	2.555	0.965737
F*Y	157.956	28	5.641	0.997710
ST*Y	0.710	12	0.059	0.661860
V*F*ST	0.043	21	0.002	0.106712
V*F*Y	11.146	28	0.398	0.968497
V*ST*Y	0.120	12	0.010	0.248153
F*ST*Y	2.415	84	0.029	0.869458
V*F*ST*Y	0.694	84	0.008	0.656868
Error	0.363	640	0.001	

Note: All effects are significant at $p < 0.001$.

Note: Error bars describe the standard error of the mean (*SE*). The same letters indicate belonging to the same similarity group (without a significant difference according to Fisher's $LSD_{0.5}$) within the factor. Columns without a letter are significantly different for Fisher's $LSD_{0.5}$ from other variants.

With a further increase in nitrogen fertilizers to 180 kg ha^{-1} a.s. on background $P_{60}K_{60}$, there was a partial decrease in yield due to a significant increase in vegetative mass, which confirms the results of studies by Zhang et al. (2013). The leaf area of soybean crops Annushka variety at the end of flowering with the application of $N_{150}P_{60}K_{60}$ and $N_{180}P_{60}K_{60}$, regardless of seed treatment, exceeded 50.0 thousand m^2 ha^{-1}, Ustya variety - 49.1 thousand m^2 ha^{-1}.

The effectiveness of biofertilizer was manifested from N_0 to N_{90}, and the application of nitrogen fertilizers in the rate of N_{150} and N_{180} showed the absence of nodules on the root system of soybeans. Similar results were obtained by Tamagno et al (2018) and Mehmet (2008), who proved that soybean yields did not change depending on biofertilizer at high rates of nitrogen fertilizer application. Yield formation occurred only through the application of mineral fertilizers.

The studied factors differently affect the variation in yield of soybean varieties Ustya and Annushka (Fig. 3).

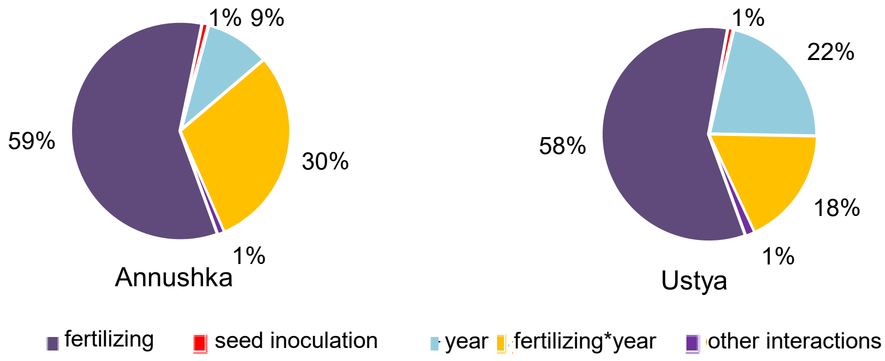


Figure 3. The share of factors in the yield formation of soybean varieties Annushka and Ustya.

In particular, what is common is that the studied fertilizer system accounted for 58–59% of the total yield variation, and seed inoculation only 1%. At the same time, the ratio of the influence of weather conditions (year) and the interaction of weather conditions with the fertilizer system differed. The influence of weather conditions on the yield of Annushka variety was 9 vs. 30% in the interaction of weather conditions with the fertilizer system, which indicates the sensitivity of this variety to nutrients and their utilization in the crop depending on weather conditions. At the same time, the influence of weather conditions on the yield of Ustya variety was 22%, and their interaction - 18%, which indicates greater inertia to the assimilation of nutrients under different conditions, but higher sensitivity to the various conditions of the year.

The yield index is parabolic depending on the rate of fertilizer within a particular inoculant - if in the control version the yield index was low due to lack of nutrients and differentiation of a small number of generative organs, then the introduction of $N_{150}P_{60}K_{60}$ and $N_{180}P_{60}K_{60}$ decreased by accumulating significant by-products (Fig. 4).

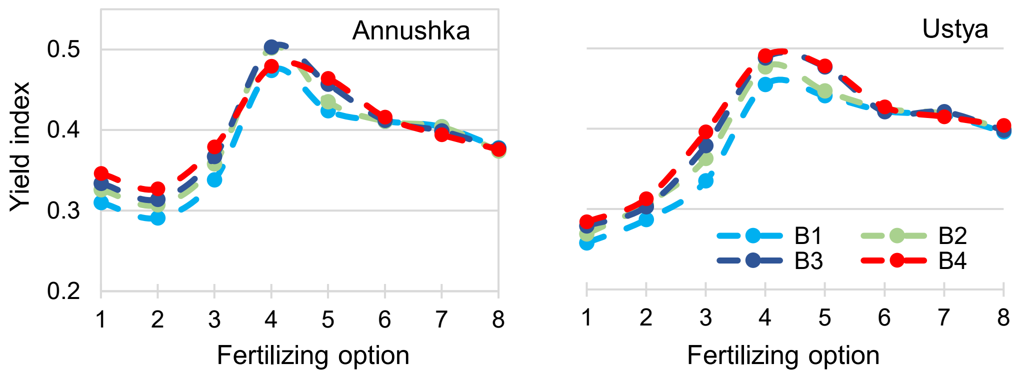


Figure 4. The soybean yield index depending on the fertilizer system and seed inoculation.

Note. Seed treatment: B1 – without inoculant (control); B2 – H; B3 – R; B4 – H + R; fertilizer option: 1 – control; 2 – $P_{60}K_{60}$ (Background); 3 – background + N_{30}; 4 – background + N_{60}; 5 – background + N_{90}; 6 – background + N_{120}; 7 – background + N_{150}; 8 – background + N_{180}.

Nitrogen is an inducer of the productive properties of plants, determining the yield and nutritional value of soybeans in terms of protein and fat content. The results of our studies confirm the data of Boroomandan et al. (2009) that the total protein content in control seeds without treatment by Rhizohumin increased depending on the rate of nitrogen fertilizers, and conversely, in soybean plants with pre-sowing treatment decreased significantly relative to control with Rhizohumin and without its use (Fig. 5).

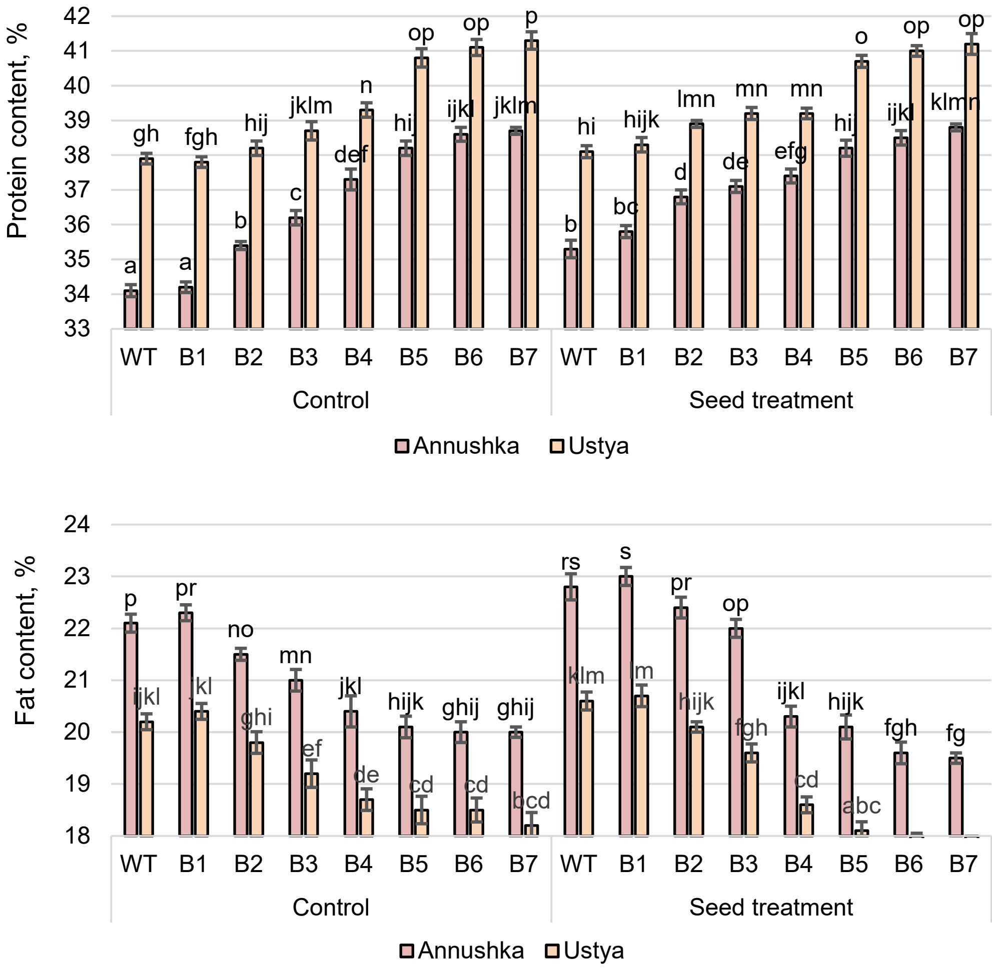


Figure 5. The protein and fat content in soybean seeds depending on the rate of nitrogen fertilizers and inoculation by Rhizohumine.

Note: Error bars describe the standard error of the mean (*SE*). The same letters indicate belonging to the same similarity group (no significant difference according to Fisher's LSD_{05}) within the factor. Columns without a letter are significantly different for Fisher's LSD_{05} from other variants.

This is due to the more efficient use of nitrogen in the metabolic reactions of plants formed from inoculated seeds (Dong et al. (2010)). Nitrogen fertilizers increased by 60%, and a significant increase in fat content of 60% was observed only with the application of N_{30}, compared with the absolute control.

CONCLUSIONS

Growing soybeans on typical low-humus chernozems of Ukraine, the balanced use of increasing rates of nitrogen fertilizers on the background of seed inoculation, in general, contributes to increasing yields, improving grain quality, and significantly reduces the chemical load on the plant due to its specific physiological response. The highest yield of soybeans on typical chernozems of the forest-steppe of Ukraine is formed by applying $N_{60}P_{60}K_{60}$ and pre-sowing seed treatment by rhizohumin or a combination of rhizohumin and hetomics. With a further increase in nitrogen rates and seed inoculation, a partial decrease in productivity is observed. The application of pre-sowing processing had a positive effect on grain yield formation. When soybean seeds were inoculated with rhizohumin and hetomik, the crop yield was higher by 0.12–0.25 t ha^{-1} compared to the control. The use of nitrogen fertilizers in the amount of 30 and 180 kg ha^{-1} is impractical for soybeans. This is evidenced by the adaptive changes in this variety to the application of these rates of nitrogen fertilizers, which are manifested in the increase in the amount of MDA and the parallel decrease in the content of photosynthetic pigments during the growing season, and as a result, the decrease in crop yield. The effectiveness of biofertilizer is manifested from N_0 to N_{90}, with the application of nitrogen fertilizers at the rate of N_{150} and N_{180}, the absence of nodules on the soybean root system was observed. At high rates of nitrogen fertilizer application, soybean productivity is formed only due to the application of mineral fertilizers, as the influence of biofertilizer is neutralized.

However, many issues of mineral nutrition have not yet been resolved. This is related to the biology of soybean plants, different requirements for nutrients during ontogenesis, and the presence of the process of biological assimilation of nitrogen. In particular, to investigate to what extent the excess mineral forms of nitrogen in the soil creates a precedent for nitrate pollution of the environment. In further research, it is also worth finding out how soil moisture deficiency affects both the level of symbiosis and the photosynthetic activity of soybean crops. It is extremely important to get strains of nodule bacteria resistant to drought. Scientific laboratories in the USA, China, and many European countries are working on this problem.

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Harrow with screw-type operating tools: optimisation of design and process parameters

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Abstract. A new design of the harrow with screw-type operating tools is presented in the paper. It describes the theoretical and experimental investigations carried out for the purpose of optimising the design and process parameters of the harrow with screw-type operating tools. Such optimisation will provide for improving the soil fertility, when burying chaff and other plant residues as organic fertilisers into the soil during its tillage. On the basis of the results obtained in the comprehensive experimental investigations, new regression relations have been generated. These relations provide for determining the proportion of the field surface, where the after harvesting residues have been completely buried in the soil with the harrow with screw-type operating tools. It has been established that the dominant effect on the relative amount of the field surface area S with completely worked-in plant residues is produced by the soil tillage depth h, then follows the factor of the screw-type operating device battery approach angle β. The pitch distance T of the screw-type operating device has the smallest effect. The results of the completed research prove that increasing the pitch distance T of the screw-type operating tool from 0.18 m to 0.26 m results in the decrease in the area S of the field surface with the plant residues buried in the soil by 1.4%. An increase in the approach angle β from 20° to 40° results in the increase in the field surface area S with the plant residues completely worked into the soil by 5.6%. Increasing the soil tillage depth from 0.08 m to 0.12 m results in the increase in the above-mentioned surface S by 7.1%. The simultaneous action of the factors of the tillage depth h and the approach angle β results in the percentage of the surface S rising from 72% to 82%.

Key words: diameter, disc angle, energy consumption, harrow, screw-type operating tool, soil tilling machine, tillage depth.

INTRODUCTION

One of the ponderable reserves in the improvement of the soil fertility is the utilisation of chaff and other plant residues in the quality of organic fertilisers, because the cattle-breeding industry currently does not produce a sufficient amount of manure (Aykas et al., 2005; Javadi & Hahiahmad, 2006; Damanauskas et al., 2019; Zubko et al., 2021). At the same time, mineral fertilisers are expensive and cannot provide the advantages achieved through the application of animal manure. These advantages include, first of all, the formation of organic matter and the improvement of the soil's mechanical properties manifested in the improved water absorbing and retaining power. In view of that, the field, where organic matter is regularly applied, will always be more promising in terms of the harvest, than the purely 'chemical' field. The utilisation of the residues from the harvested crop provides for the introduction into the soil of up to 60–70% of the organic matter that is effectively equivalent to classical farmyard manure. That said, the highest positive effect has been observed in rape, legume, maize and potato fields.

The soil tilling machines, in which process operations are performed by screw-type operating tools, feature such inherent qualities as simplicity in design, ease of operation, high process reliability and efficiency (Hevko et al., 2016; Boson et al., 2019). The existing methods of calculating their parameters are based on a number of recognised theoretical and experimental research works (Serrano et al., 2007, 2008; Salokhe et al., 2010; Ranjbar et al., 2013; Bulgakov et al., 2016; Kogut et al., 2016; Pylypaka et al., 2018; Zhuk & Sokht, 1018; Bulgakov et al., 2021). There is an established procedure of how to set and solve the problem of selecting the optimal parameters for screw-type operating devices in soil tilling machines with an aim to minimise their material (Hevko et al., 2016; Upadhyay & Raheman, 2018 and 2019) and energy intensity (Okyere et al., 2019; Balsari et al., 2021). However, the peculiarities associated with the performance of certain process operations impose a number of limitations.

The primary purpose of the screw-type operating tools in soil tilling machines is the high-quality performance of the process operation (soil pulverization quality, penetrability, shredding and working in of afterharvesting residues, process reliability, working width) coupled with the minimised energy and material intensity of the operating mechanism, i.e. the screw-type operating tool. The calculation of the screw-type operating tool parameters is specified by the physical and mechanical properties of the soil, the preceding crop, the harvesting technology, the technology of the soil preparation for the following crop, the initial agrotechnical requirements as well as the process schematic model of the harrows themselves.

The state of the art in the agricultural transport and process machinery stipulates looking for new ways in the improvement of the process and operation parameters of operating tools with an aim of raising the productivity and the quality of production processes and getting new operation capabilities.

The up-to-date development of transport and process machines and their operating tools has to be based on well-formed physical and mathematical models of the production processes and these models have to be operable with the use of available mathematical techniques.

The aim of this research was to improve the quality of shredding chaff and other plant residues and working them into the soil in the quality of organic fertilisers by means of optimazing the design and process parameters of the harrow with screw-type operating tools operated during the primary tillage.

MATERIALS AND METHODS

The design and process parameters of the harrow with screw-type operating tools have been optimised with an aim of raising the soil fertility with the use of chaff and other plant residues. The authors have developed a new design of the harrow, in which the operating tool is made in the form of a rod drum with helical coiling over the rods. The harrow (Fig. 1) comprises the frame 1 with the automatic hitch 2 installed in its fore end and the two batteries of screw-type operating tools 3. Each battery comprises the rod drum 4, to the outer surface of which the coils 5 of screw-type operating tools are attached, the guide 11 or 12, the clamps 13 with the fasteners 14 (the angle setting device), the battery frame 15 with the pivot pin 16. Each drum has a central axial rod, on which the framework of the operating tool is arranged. The framework comprises the two disks 6, to which the bearing assemblies 7 are attached together with the threaded axis 8 and the nuts 9, and the eight rods 10 that connect the discs 6 with each other. The rods 10 are placed symmetrically, at equal distances from each other, and attached to the circumferences of the discs 6.

The harrow with screw-type operating tools is mounted on the wheeled aggregating tractor with the use of the automatic hitch 2. The working width and approach angle of the harrow under consideration are adjusted as follows. The screw fasteners 14 on the clamps 13 are loosened. After that, the tilling battery 3 is restrained only by the pivot pin 16 in the guides 11 (as well as 12). Both the tilling batteries 3 are shifted along the guides 11 and 12 into the required positions. For example, when operating in the 'in trail' mode, the approach (attack) angle is set within the range of $0° < \beta < 40°$ (depending on the soil structure). Then the fastening elements 14 of the clamps 13 are tightened and the position of the tilling batteries 3 relative to the guides 11 and 12, which defines the harrow's approach angle β and its working width, becomes fixed.

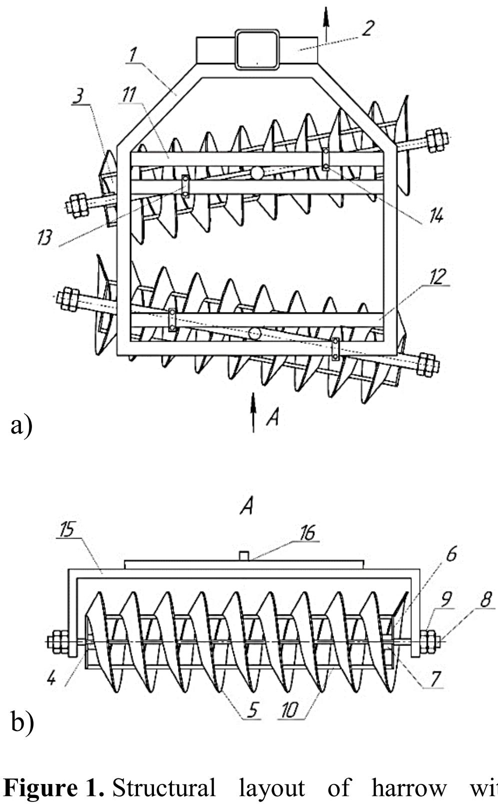

Figure 1. Structural layout of harrow with screw-type operating tools: a – top view; b – rear view along arrow *A*.

As a result of the interaction between the screw-type operating tool and the soil, a reaction force arises, which applies pressure to the working surface of the coil. One of the components of the force acts to shift the operating tool at right angle to its line of travel. In order to counterbalance this force component, it is advisable to lay out the unit with two operating tools acting in opposite phases. That is, the coils of the second operating tool have to be with the opposite winding direction and straight-line generator angle.

The described harrow with screw-type operating tools operates similarly to disk implements, which means that the profile of the tilled field features ridges and troughs. At the moment, when the blade contacts the field surface, the angles are present that are similar to the approach angle and the tilt angle of the conventional disk tools. The tilt angle can be changed by changing the straight-line surface generator angle relative to the axis of the surface. The developed new soil tilling operating tool has a simple design and a low steel intensity. As distinct from disk implements, it does not require individual bearing units for the installation of separate soil-tilling disks. The screw-type operating tools are mounted with the use of only two bearing units at the ends of each section.

The harrow with screw-type operating tools can be supplied in package with additional soil tilling batteries (for setting up combined harrowing tractor-implement units with the required working widths), which have to be with certain pre-set design parameter values. That is, the angle of tilt of the screw-type operating tool, its pitch of helix and the elevation of its coils above the rod drum have to be in accordance with the physical and mechanical properties of the soil, the depth of its tillage as well as the type and condition of the after-harvesting residues in the tilled field.

In order to optimise the design and process parameters of the new harrow with screw-type operating tools, it is necessary to set and solve an adequate optimisation problem. The problem of optimising the screw-type operating tool in a soil tillage machine is a multi-criterion problem with the following optimisation criteria: labour intensity of the auxiliary process operations F_{01}; energy consumption F_{02}; material intensity F_{03}. The sum of the cost equivalents of all the used resources can be assumed as the integrated criterion (Macmillan, 2002). That is:

$$F_0 = \varepsilon_1 \cdot F_{01} + \varepsilon_2 \cdot F_{02} + \varepsilon_3 \cdot F_{03}, \tag{1}$$

where ε_1, ε_2, ε_3 – weight coefficients of the components, which are determined essentially by the costs of the respective saved resources (labour intensity, energy consumption and material intensity).

In the process of optimising the design and process parameters of the screw-type operating tool in a soil tillage machine, the following parameters will be designated as the main optimisation parameters, i.e. the free variables: $x_1 = D$ – outer diameter of the operating tool; $x_2 = H$ – height of the helix in the screw-type operating device; $x_3 = \tan\alpha = \frac{T}{\pi D}$ – tangent of coil lead angle, defined by the pitch T of the helical spiral; $x_4 = \lambda$ – form factor of the helical surface, which takes into account the cut-outs of the helical spiral, their shapes, number and dimensions; $x_5 = \beta$ – approach angle of the operating tool; $x_6 = k_d = \frac{d}{D}$ – coefficient equal to the ratio of the internal diameter d to the external diameter D of the helical spiral; $x_7 = B$ – thickness of the helical spiral; $x_8 = L$ – length of the screw-type operating tool.

The above-listed factors have effect on the level of the criterion function F_o and its components, while their ranges of definition are determined by the constraint functions.

As an example of the process operation performance, one of the most commonly used operations can be used - the soil pulverisation and after-harvesting residue shredding. The quality of the working of the after-harvesting residues into the soil (specifically defined as the area S of the field surface with completely worked-in plant residues (%)) is designated as the optimisation criterion. It will be different for different designs and depend on the parameters x_i:

$$F_{01} = F_{01}(x_1, x_2, x_3, x_4, x_5, x_8) \rightarrow \min \tag{2}$$

In general, such process operations are rather difficult to describe formally. Therefore, the optimisation of the screw-type operating tool in a soil tillage machine by this criterion is usually carried out by implementing the mathematical design of the experiment (M. Klendii & O. Klendii, 2016; E. Priporov & I. Priporov, 2021).

In that case, the criterion function is represented by a quadratic polynomial:

$$F_{01} = b_0 + \sum b_i \cdot x_i + \sum b_{il} \cdot x_i \cdot x_l + \sum b_{ii} \cdot x_i^2 \tag{3}$$

The variation of the parameters x_i is bounded by the constraining relations $f_j = f_j(x_i)$, which are generally written in the form $f_i \leq 0$. When a mathematical design is developed, the upper and lower value limits are usually set for the factor variation, that is, $x_{\min} \leq x_i \leq x_{\max}$. *Hence:*

$$\begin{gathered} f_1 = x_{1\min} - x_1 \leq 0\,;\, f_2 = x_1 - x_{1\max} \leq 0\,;\, f_3 = x_{2\min} - x_2 \leq 0\,; \\ f_4 = x_2 - x_{2\max} \leq 0\,;\, f_5 = x_{3\min} - x_3 \leq 0\,;\, f_6 = x_3 - x_{3\max} \leq 0\,; \\ f_7 = x_{4\min} - x_4 \leq 0\,;\, f_8 = x_4 - x_{4\max} \leq 0\,;\, f_9 = x_{5\min} - x_5 \leq 0\,; \\ f_{10} = x_5 - x_{5\max} \leq 0\,;\, f_{11} = x_{6\min} - x_6 \leq 0\,;\, f_{12} = x_6 - x_{6\max} \leq 0\,. \end{gathered} \tag{4}$$

The above limitations define the range of definition that can be represented by the following generalised constraint function in implicit form:

$$f_o = \max f_j = u_j \sum_{j=1}^{2n} f_j \prod_{k=1;k\neq j}^{2n-1} [\mu_k \cdot (f_j - f_k)] \leq 0\,, \tag{5}$$

where u_j – undetermined Lagrange multiplier, $u_j \geq 0$; $u_k(f_j - f_k) = \frac{1+\mathrm{sgn}(f_j - f_k)}{2}$ – membership function, which is equal to $\mu_k = 1$, when $f_j > f_k$, and is equal to $\mu_k = 0$, when $f_j < f_k$.

The Lagrange function (Blinder, 2013) in accordance with each of the quality criteria is written in the form $\varphi(x,u) = F_0 + f_0$, the optimal parameters $x = \{x_1 \dots x_i \dots x_n\}$ can be found from the assumption $\frac{\partial \varphi(x,u)}{\partial x_i} = 0$. Consequently, the optimal parameters x_i^{opt} are determined by means of solving the linear equation system that comprises n equations, $i = (1 \dots n)$.

$$\frac{\partial \varphi(x,u)}{\partial x_i} = b_i + 2 \cdot b_{ii} + \sum_{l,l\neq i}^{n} b_{il} \cdot x_l + u_j \sum_{j=1}^{2n} \left(\frac{\partial f_j}{\partial x_i} \right) \prod_{k=1;k\neq j}^{2n-1} [\mu_k \cdot (f_j - f_k)] \tag{6}$$

The condition $\frac{\partial f_j}{\partial x_i} = \text{const}$ is applied to the linear constraint functions f_j. Accordingly, for the constraint function $f_1 = x_{1min} - x_{x1} \leq 0$ the condition $\frac{\partial f_j}{\partial x_i} = -1$ is applied, for $f_2 = x_1 - x_{1\max} \leq 0$ – $\frac{\partial f_j}{\partial x_i} = 1$. The component $\vartheta = \prod_{k=1;k\neq j}^{2n-1} [\mu_k \cdot (f_j - f_k)$, which is equal to 0 or 1, automatically selects the constraint function, at the limit of which the parameters can have optimal values. The use of (5) in the optimisation problem substantially simplifies the algorithms of its solving, which provides for the computerisation of the computation process.

In case the soil tillage process is optimised under the constraints (4), the system (6) appears as follows:

$$\left.\begin{aligned}
\frac{\partial\varphi(x,u)}{\partial x_1} &= b_1 + 2\cdot b_{11} + \sum_{l,l\neq 1}^{n} b_{1l}\cdot x_l - u_{1,} + u_2;\\
\frac{\partial\varphi(x,u)}{\partial x_2} &= b_2 + 2\cdot b_{22} + \sum_{l,l\neq 2}^{n} b_{2l}\cdot x_l - u_3 + u_4;\\
\frac{\partial\varphi(x,u)}{\partial x_3} &= b_3 + 2\cdot b_{33} + \sum_{l,l\neq 3}^{n} b_{3l}\cdot x_l - u_5 + u_6;\\
\frac{\partial\varphi(x,u)}{\partial x_4} &= b_4 + 2\cdot b_{44} + \sum_{l,l\neq 4}^{n} b_{4l}\cdot x_l - u_7 + u_8;\\
\frac{\partial\varphi(x,u)}{\partial x_5} &= b_5 + 2\cdot b_{55} + \sum_{l,l\neq 5}^{n} b_{5l}\cdot x_l - u_{9,} + u_{10};\\
\frac{\partial\varphi(x,u)}{\partial x_6} &= b_6 + 2\cdot b_{66} + \sum_{l,l\neq 4}^{n} b_{6l}\cdot x_l - u_{11} + u_{12}.
\end{aligned}\right\} \qquad (7)$$

When the optimal values of the tillage parameters are selected with the use of the developed mathematical design of experiment, the optimal parameters take on either values within the range of definition or the limiting values of the range. In case of the former option, $u_j = 0$ and the Lagrange function corresponds to the criterion function (2), therefore, the system of equations is significantly simplified.

In case $\frac{\partial F_0}{\partial x_i} > 0$, the parameter x_i takes on the minimum value $x_i = x_{\min}$ and one of the undetermined multipliers becomes $u_{2i} = 0$. On the other hand, when $\frac{\partial F_0}{\partial x_i} < 0$, the parameter takes on the value $x_i = x_{\max}$, and the other undetermined multiplier becomes $u_{2i-1} = 0$.

The results of solving the above problem provide the basis for determining the operating device parameters: the approach angle of the operating tool β; the tangent of coil lead angle $\tan\alpha$; the soil tillage depth h (height of the spiral of the screw-type operating tool) and the shape of the helical surface. The earlier research has proved that the use of the rational parameter value for the operating tool approach angle β in the process of working in the plant residues results in achieving the tilled field surface area

S with the plant residues completely worked into the soil that approaches a level of 90–100%. For the purpose of conducting experimental research, a harrow with screw-type operating tools (Fig. 1) has been engineered with the following initial parameters: length of the spiral $L = 1.3$ m; external diameter of the helical surface $D = 0.56$ m, its internal diameter $d = 0.32$ m; height of the spiral of the screw-type operating tool $H = 0.12$ m; pitch distance of the helical spiral $T = 0.25$ m. When the concentration of the key component was equal to 80%, the rational value of the operating tool approach angle was $\beta = 40°$.

When the process is optimised in terms of the criterion of the energy consumption during soil tillage, it is reasonable to designate the tractive resistance of the screw-type operating tool as the criterion function, which can be represented by the following relation (Nadykto et al., 2015):

$$P = k \cdot A \cdot n \cdot \sin\beta[1 + \tan(\gamma + \varphi)], \quad (8)$$

where k – specific resistance of the soil, $k = 20–130$ kN m^{-2}; A – area of contact between the front surface of the coil of the screw-type operating tool and the soil, m^2; β – approach (attack) angle of the screw-type operating tool; n – number of the screw-type operating tool coils simultaneously penetrating the soil; γ – angle between the front working surface of the screw-type operating tool coil and the furrow wall; φ – angle of repose of the soil on the working surface of the screw-type operating tool coil.

The analysis of the expression (8) has proved that the most efficient way of reducing the energy spent for the movement of the harrow, simultaneously ensuring the optimal operating speed conditions, is to use antifriction materials in the design of screw-type operating tools, that is, to reduce the coefficient of friction between the soil and the working surface of the screw-type operating tool coil. That said, the approach angle of the screw-type operating tool has to be maximal to ensure the sufficient quality of the performed soil tillage.

In terms of the material intensity of the operating tool, the criterion is the ratio of the unit length harrow's mass to the required productivity Q. The material intensity criterion is applied, when it is a decisive factor. In that case, the problem of minimising the material intensity (cost) of the harrow is set by the criterion:

$$F_{03} = \alpha_1 V_1 + \alpha_2 V_2 + \alpha_3 V_3 \rightarrow \min, \quad (9)$$

where V_1, V_2, V_3 – volumes of the harrow frame, the helical spiral and the central rod axis, respectively; α_1, α_2, α_3 – respective densities ρ_i (or production costs) of the materials, from which the helical spiral of the harrow is made.

The following process, design and operation constraints are applied, when determining the optimal parameters of the screw-type operating tool in a soil tillage machine and the soil tillage work process:

1. The process constraint of shaping the spiral from a flat work piece:

$$f_1 = -k_d + \frac{\sqrt{\pi^2 + 1 - c^2}}{\pi \cdot c} \leq 0, \quad (10)$$

where $f_1 = -k_d + \frac{\sqrt{\pi^2+1-c^2}}{\pi \cdot c} \leq 0,$ – permissible coefficient of metal elongation irregularity determined by the coefficient of elongation $c = (1 + 2 \cdot B)^2$.

2. The process constraint of ensuring the stability of the flat bar in the process of manufacturing the spiral:

$$f_2 = D \cdot (1 - k_d) - \frac{2 \cdot H}{B} \le 0, \qquad (11)$$

where B – allowable specific thickness of the metal work piece used for manufacturing the helical spiral, obtained by rolling – B = 0.02–0.03 mm; obtained by coiling – B = 0.05–0.70 mm.

3. The constraint of the minimal spiral thickness that is sufficient to resist the loss of the spatial stability by the coil:

$$f_3 = -B + B_{\min} \le 0 \qquad (12)$$

where $B_{\min}$ – minimal acceptable thickness of the helical spiral blank determined experimentally.

4. The constraint of ensuring the stability of the helical spiral in operation:

$$f_4 = \frac{K_{CT} \cdot B \cdot H^3 \cdot E}{\sqrt{1 + K_T}} - T \le 0 \qquad (13)$$

where K_{CT} – experimental coefficient; E – Young's modulus; k_T – helix pitch distance coefficient.

The following parameters are taken as independent ones in the optimisation of the operating tools: $x = (x_i) = (D, \lambda, \tan\alpha, \beta, L, k_d, H)$. At this stage, the following function is taken as the criterion function: $F_0 = F_{03}$.

Taking into account the above description of the criterion function, it can be written in the following form:

$$F_{03} = \pi x_1 \left[\alpha_1 S_K \left(1 + x_2 + \frac{S_K}{x_1} \right) + \alpha_2 x_7 \left(1 - x_6 \right) \sqrt{1 + \frac{1}{x_3}} + \pi \alpha_3 S_d x_1 \left(x_6 - \frac{S_d}{x_1} \right) \right], \qquad (14)$$

where S_k – thickness of the harrow frame; S_d – diameter of the central rod shaft.

It is necessary to determine the partial derivatives of the function. They appear as follows:

$$\frac{dF_{03}}{dx_1} = \alpha_1 \pi (S_k + x_2) + \alpha_3 \pi S_d x_6;$$

$$\frac{dF_{03}}{dx_2} = \alpha_1 \pi S_k x_1;$$

$$\frac{dF_{03}}{dx_3} = \frac{\alpha_1 x_7 (1 - x_6) + \alpha_3 \pi S_d x_6}{x_3^2 \sqrt{1 + \frac{1}{x_3}}}; \qquad (15)$$

$$\frac{dF_{03}}{dx_6} = \alpha_3 \pi S_d x_1;$$

$$\frac{dF_{03}}{dx_3} = \alpha_2 (1 - x_6) \sqrt{1 + \frac{1}{x_3}}.$$

The analysis of the partial derivatives of the function F_{03} has proved that it reaches its minimums at the extreme values of x_i, in particular, at the minimum values: $x_i = D$; $x_2 = \frac{2z}{D}$; $x_6 = k_d = \frac{d}{D}$; $x_7 = H$ and at the maximum value: $x_3 = \tan\alpha = \frac{T}{\pi D}$.

Accordingly, the possible solutions that will meet the Kuhn-Tucker conditions can be found from the systems of equations set up on the basis of the constraint functions.

The obtained relations provide for calculating with high accuracy the optimal design and process parameters of the screw-type operating tool. Such parameters will ensure the required quality of the soil tillage at a high efficiency of the process.

The experimental research into the efficiency of the process of working the plant residues into the soil (which implies determining the percentage of the field plot surface area S with the plant residues completely worked in, %) was carried out in field conditions during the cultivation of a wheat stubble field. The soil had the following properties: type of soil - grey podzolized, crushing strength value (hardness) - 87.4 kN m^{-2}, absolute moisture content - 18%, soil density - 1.6 kg m^{-3}. The harrow with screw-type operating tools during the field experiment investigations is shown in Fig. 2.

In the selected plot, the soil was tilled with the use of the harrow with screw-type operating devices. After the tillage of a certain area (approximately 100 m^2) was complete, the quality of the work was investigated, then the operating tools in the harrow were changed (operating devices of different designs and sizes had been prepared for the experiments) and their positions with respect to the line of travel were readjusted.

a) 

b) 

Figure 2. Harrow with screw-type operating tools: a) – general view; b) – side view.

The programme of experimental research included the experiments aimed at finding the relations between the proportion of the field plot surface area S with the plant residues completely worked into the soil and: 1) the positioning angle of the screw-type operating tool battery with respect to the travel line (approach angle β); 2) the screw-type operating tool pitch distance T and 3) the soil tillage depth h. The peak (maximum) values obtained during the experimental investigations were used to analyse the value of the investigated parameter.

The efficiency of the plant residue working in process was estimated using the method of spectral analysis. The information for the analysis was obtained from the series of photographic images shot from a height of 10 m (ensuring a longitudinal photograph overlap of at least 60% in accordance with the photogrammetry requirements) with the camera of a DJI Phantom 4 quadrocopter. The resulting images were processed in the computer programme Agisoft Photoscan and on their basis the cartographic materials were generated. For determining the percentage of the field surface area with the plant residues completely worked in, the geographic information system (GIS) QGis 3 was used. Within the system, controlled classification of the soil was carried out using the training sample method.

For each of the factors, the experiments were done with at least 3 replicates. Then the mean value was determined for each of the results and that value would be used for the further statistical analysis of the experimental results.

With an aim of determining how various design and process parameters of the screw-type operating tools in the harrow (i.e. the independent factors x_i) influence the proportion of the field plot surface area S with the plant residues completely worked into the soil, full factorial experiments were carried out. Basing on the results of the experiments, the relations between the above-mentioned indicator and the three main variable factors: the approach angle β (deg) of the operating tool, the pitch distance T (m) of the screw-type operating device and the soil tillage depth h (m), i.e. $S = f(\beta, T, h)$, were established. The respective data are presented in Table 1.

Table 1. Description of factors and their levels in experimental investigations for determining field surface area with plant residues completely worked into soil

Factor	Designation		Variation interval	Levels of variation, actual/coded		
	Coded	Actual				
Operating tool approach angle β, deg	X_1	x_1	10	20/-1	30/0	40/+1
Screw-type operating tool pitch distance T, m	X_2	x_2	0.04	0.18/-1	0.22/0	0.26/+1
Tillage depth h, m	X_3	x_3	0.02	0.08/-1	0.10/0	0.12/+1

After coding the factors, the design matrix was generated for the appropriate multifactorial experiment – a 3^3 full factorial experiment with the number of experiments $N = 3^3$.

The general regression equation for the percentage of the field surface area S with the plant residues completely worked into the soil as a function of the variable operating tool approach angle β, the screw-type operating tool pitch distance T and the soil tillage depth h, i.e. $S = f(\beta, T, h)$, appeared as follows:

$$S = 81.205 - 33.33T - 245.32h + 2.84\beta h + 1{,}639.61h^2. \quad (16)$$

The obtained regression equation (16) was used for determining the percentage of the field area S with the plant residues completely worked into the soil in relation to the following three main varying factors: operating tool approach angle β, screw-type operating tool pitch distance T and soil tillage depth h. The ranges of variation of the input factors were as follows: $20 \le \beta \le 40$ deg; $0.18 \le T \le 0.26$ m; $0.08 \le h \le 0.12$ m.

RESULTS AND DISCUSSION

The Statistica-6.0 software was used to plot the graphic relations of the transitional general regression models. They were plotted in the form of quadratic response surfaces for the percentage of the field surface area S with the plant residues completely worked in as a function of two variable factors $x_{i(1,2)}$ at a constant invariable level of the respective third factor $x_{i(3)}$ = const. The resulting graphic relations of the area S with buried plant residues, obtained with the use of the Statistica-6.0 software, are presented in Fig. 3.

The analysis of the obtained regression equation has proved that the factors x_3, x_1, (h, β) and the combinations of these factors have the greatest effect on the changes in the percentage of the field surface area with the plant residues completely worked in. Increasing the value of the factor $x_3(h)$ results in an increase of 7.4% in the percentage of the field surface area with the plant residues completely worked in. Overall, in order to increase the percentage of the field surface area with the plant residues completely worked in, it is necessary to increase the depth of tillage and the approach angle β and to decrease the pitch distance of the screw-type operating tool.

The analysis of the response surfaces shown in Fig. 3 makes it obvious that an increase in the tillage depth results in an increase in the proportion of the field surface area, where the plant residues have been worked in, the maximum percentage value being at 84%. The minimum proportion of the field surface area with the plant residues completely worked in is equal to 67% and it occurs at the minimum value of the approach angle β and the maximum value of the screw-type operating tool pitch distance T.

It has been established that increasing the screw-type operating tool pitch distance T from 0.18 m to 0.26 m results in the proportion of the field surface area S, where the plant residues have been worked in, decreasing by 1.4%. At the same time, increasing the approach angle β from 20 deg to 40 deg results in the percentage of the field surface area S with the plant residues completely worked into the soil increasing by 5.6%. An increase in the soil tillage depth h from 0.08 m to 0.12 m results in the field surface area S with the plant residues completely worked into the soil increasing by 7.1%. Simultaneous increases in the tillage depth h and approach angle β result in the field surface area S with the plant residues completely worked into the soil increasing from 72% to 82%.

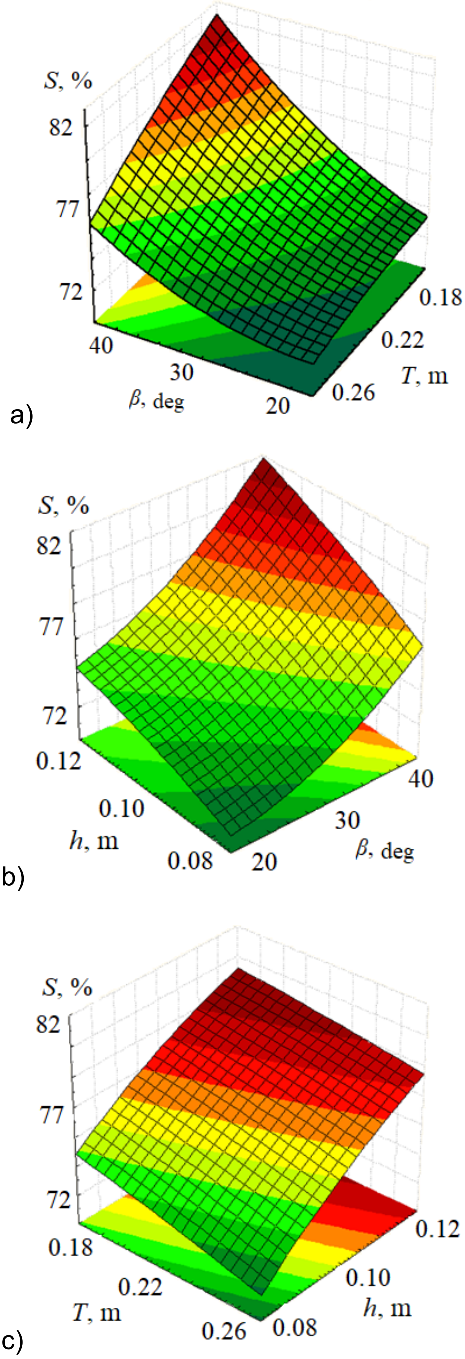

Figure 3. Response surfaces for variation of field plot surface area S with completely buried plant residues as a result of variation of soil tillage factors:
a – $S = f(\beta, T)$; b – $S = f(h, \beta)$; c – $S = f(T, h)$.

CONCLUSIONS

A new design of the harrow with screw-type operating tools has been developed and a pilot unit has been manufactured. The design and process parameters of the harrow with screw-type operating tools have been optimised with an aim of raising the soil fertility by way of working in chaff and other plant residues as organic fertilisers during soil tillage.

A set of experimental investigations has been carried out. On the basis of their results, the regression relations have been derived that provide for determining the percentage of the field plot surface area S, where the plant residues are completely worked in by the harrow with screw-type operating tools. It has been established that the soil tillage depth h has the dominant effect on the percentage of the field plot surface area S with the plant residues completely worked in, then follows the approach angle β of the screw-type operating tool battery. The pitch distance T of the screw-type operating tool has the smallest effect. The response surfaces that represent the relations between the percentage of the field plot surface area S, where the plant residues are completely worked in by the harrow with screw-type operating tools, and the main factors have been plotted. By analysing these surfaces, it has been established that increasing the soil tillage depth h results in the percentage of the field plot surface area S with the plant residues completely worked in rising, its highest value being equal to 84%. The minimum value of the percentage of the field plot surface area S with the plant residues completely worked in is equal to 67% and it occurs at the minimum approach angle β and the maximum pitch distance T of the screw-type operating tool.

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Comparative anatomical and morphological characteristics of two subspecies of *Melissa officinalis* L. (Lamiaceae)

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Abstract. The aim of the research is to comprehensively compare the anatomical and morphological structures of plants of *Melissa officinalis* subsp. *officinalis* (‘Krymchanka’ and ‘Lada’ varieties) and of *M. officinalis* subsp. *altissima* (Sm.) Arcang. These plants have pronounced differences in morphological features and production indexes. They are grown in the collection of aromatic plants of the Research Institute of Agriculture of the Crimea (Krymskaya Roza village, Belogorsky district of Crimea). These studies will allow us to supplement the botanical characteristics of both subspecies of *M. officinalis*, and also to evaluate their plasticity and possibilities of adaptation to growing conditions. For examination we collected plants in the flowering phase. Anatomical study was carried out using a fixed (alcohol: glycerin : water in a ratio of 1: 1: 1) and native preparation. A complex of mesomorphic and xeromorphic characters is established, that confirms the high plasticity and determines the adaptive capabilities of the species when introduced into various soil and climatic conditions. A variety of epidermal structures was revealed, which are represented by single and multicellular non-glandular trichomes and various types of glandular structures. Among them, trichomes with a multicellular pedicle and a unicellular head, which we found only in *M. officinalis* subsp*. officinalis*.

Key words: *Melissa officinalis*, anatomical structures, vegetative organs, morphology, pubescence.

INTRODUCTION

Melissa officinalis L. (Lamiaceae) is the valuable culture, widely utilized as spicy and medicinal plant both in healthcare and for food purposes. Its essential oil is used in the production of high-end perfumes (Denisova, 1989; Voitkevich, 1999; Ehsani et al., 2017; Calejaa et al., 2018; Abdel-Naime et al., 2019). *M. officinalis* subsp*. officinalis*, a type subspecies that has been well considered in terms of the content of a number of bioactive and aromatic compounds, is mentioned as cultivated (Chizzola et al., 2018). In the rank of the subspecies, M. *officinalis* subsp. *altissima* (Sm.) Arcang. is being considered

(World Checklist…, http://wcsp.science.kew.org/namedetail.do?name_id=124060). The synonym is *M. bicornis* Klokov (Mosyakin & Fedoronchuk, 1999), described on the Crimean preparation. However, such synonymizing with *M. officinalis* is not possible to assume as unequivocally accepted (Flora Yevropeiskoy chasti SSSR, 1978; World Flora.., http://www.worldfloraonline.org). *M. officinalis* subsp. *altissima* has a typical Mediterranean-Western Asian habitat, covering Spain, Italy, the Balkan countries, Turkey and the Caucasus region (The information resource…, http://www.emplantbase.org/home.html). Standard *M. officinalis* subsp. *officinalis* has the smaller habitat, which includes front Asia and Caucasian region, but more extensive artificial area, appeared, apparently, as a result of the introduction and subsequent naturalizing of plants, covering almost all of Europe and part of North Africa (The information resource…, http://www.emplantbase.org/home.html).

There were significant differences between the two subspecies in the study of the main indicators of productivity and biochemical parameters, including the component composition of essential oil (Nevkrytaya et al., 2020).

There is data concerning the inner structure of vegetative organs of *M. officinalis* without specifying the intraspecific membership (Birulova & Petrishina, 2014; Nikitina et al., 2018). The anatomical and morphological feature of M. officinalis subsp. *altissima* in the literature it is not revealed. In a number of sources there is some evidence of bioactive substances of the plants of this subspecies (Božović et al., 2018).

The aim of the research is to comprehensively compare the anatomical and morphological structures of plants of *M. officinalis* subsp. *officinalis* and *M. officinalis* subsp. *altissima* having pronounced differences in morphological features. The data obtained will complement the botanic feature of *M. officinalis*; to estimate its flexibility and adaptivity to the conditions of cultivation.

MATERIALS AND METHODS

The research was performed in 2018–2019 on plant preparation from the collection nursery of the department of essential oil and medicinal cultures of the Research Institute of Agriculture of Crimea. Experimental site is located in the foothill zone of the Crimea, in its eastern part (Krymskaya Roza village, Belogorsky district). Climate of this region is moderately continental. This territory belongs to one of the five agroclimatic regions - the upper foothill, warm, not humid enough; to the northern subarea with moderately mild winters (Savchuk, 2006).

Research material - plants of two subspecies *M. officinalis* subsp. *officinalis* (Krymchanka and Lada varieties) and *M. officinalis* subsp. *altissima* (Tavrida variety). Krymchanka and Tavrida are clonal varieties. Lada is a population variety. When laying the field plot, seedlings of all varieties derived by the method of propagation by herbaceous cuttings were used. For maximum coverage of the genetic diversity of Lada, the green cuttings were harvested from 100 plants. Samples were placed in three repetitions on the two-row plots. Plot length - 5 running meters, planting width - 0.6 m. Each row contains 17 plants. The experiment was conducted in the spring of 2017. The required processings and measurements were performed in accordance with the methodological guidelines (Essential oil…, 1977).

Plants of both subspecies of *M. officinalis* of the same age were used for the anatomical and morphological description. Anatomical studies were made in specimen prepared on fixed (alcohol: glycerin: water in a ratio of 1: 1: 1) and native material (Barykina et al., 2004). Anatomical and morphological description was carried out using generally accepted methods (Lotova, 2001; Serebryakova et al., 2006; Timonin, 2007; Evert, 2016). Epidermal leaf structures of subspecies *M. officinalis* were described on temporary preparations according to the methods of Aneli (1975) and Zakharevich (1954). The anatomical structures of vegetative organs were investigated in the permanent and temporary micro-preparations, obtained with the use of a microtome Rotmik 2- P and made by hand a dangerous razor. The study of the preparations was performed on Olympus CX31RTSF microscope with photographic recording of objects and with Olympus digital camera (Industrial Digital Camera TOUPCAMTM U3CMOS10000KPA). Magnification on the microscope: 4×10, 10×10, 20×10. The quantitative properties of individual anatomical and morphological elements (stomata, main cells of the epidermis, etc.) were specified in 30 replicates. Data obtained was statistically processed using Microsoft Office Excel 2010 software package (Lakin, 1980).

RESULTS AND DISCUSSION

The anatomical structure of the stem and root of *M. officinalis* was studied earlier (Birulova & Petrishina, 2014). A comparative analysis of the anatomical structure of the axial organs of the two subspecies *M. officinalis* showed that *M. officinalis* subsp. *officinalis* and *M. officinalis* subsp. *altissima* have similar anatomical structure of root and stem.

The most plastic organ of plants is the leaf. The peculiarities of its anatomical and morphological structure response the reaction of plants to environmental conditions (Serebryakova et al., 2006). This enables to supplement the ecological characteristics of the species and to indicate the degree of its adaptability to specific growing conditions. The anatomical structure of the leaf of both subspecies is consistent to the general plan of *M. officinalis* structure (Birulova & Petrishina, 2014). The differences between them were revealed in the dimensional parameters (linear and quantitative parameters of leaf cells). The leaves of the studied varieties are covered with one layer of epidermal cells. On the abaxial and adaxial sides of the leaf, the basic cells of epidermis in the paradermal plane have a flattened or elongated shape with more wavy anticlinal walls, obtuse or acute angles in adjacent boundaries (Fig. 1).

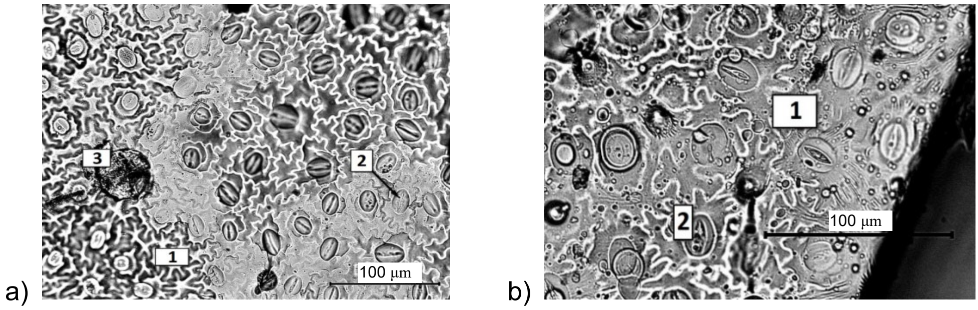


Figure 1. Lower epidermis of a leaf of *M. officinalis*: a – subsp. *altissima*; b – subsp. *officinalis*. (1 – main epidermal cells; 2 – stomata; 3 – glandular trichome).

The main epidermal cells on the abaxial side of the leaf are characterized by more wavy outlines. The size of the epidermal cells of the adaxial side along the long axis is in *M. officinalis* subsp. *altissima*, on average, 64.74 ± 1.61 μm, while subsp. *officinalis* - 38.65 ± 0.99 microns. The number of cells per 1 mm², on average, is 749.45 ± 9.09 and 1372.1 ± 19.26, respectively. The average size of epidermal cells on the abaxial side of the leaf along the long axis is in subsp. *altissima* 50.36 ± 2.09 μm, while subsp. *officinalis* – 46,53 ± 1.51 μm. The number of cells per 1 mm² in subspecies, on average, 1,125 ± 5.83 and 1,138.22 ± 9.71 pcs respectively. Therefore, the large sizes of cells of integumentary structures in subsp. *altissima* induce their smaller number per unit area.

A leaf of *M. officinalis* is hypostomatic (stomata are located only on the lower side of the leaf). The type of stomatal complex is diacytic. The number of stomata on the underside of the leaf is on average 436.67 ± 16.83 pcs mm^{-2} in subsp. *altissima* and 354.44 ± 18.79 pcs mm^{-2} in subsp. *officinalis*.

In the transversal section of the leaf, the epidermal cells of the adaxial and abaxial sides have a rounded or elongated shape along the leaf surface with uniformly thickened walls (Fig. 2).

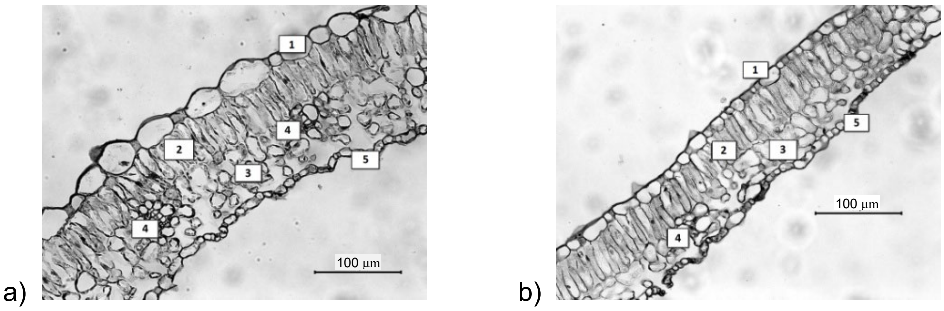


Figure 2. Anatomical structure of the leaf of *M. officinalis* (mesophyll): a – subsp. *altissima*; b – subsp. *officinalis*. (1 – upper epidermis; 2 – columnar mesophyll; 3 – spongy mesophyll; 4 – vascular bundle; 5 – lower epidermis).

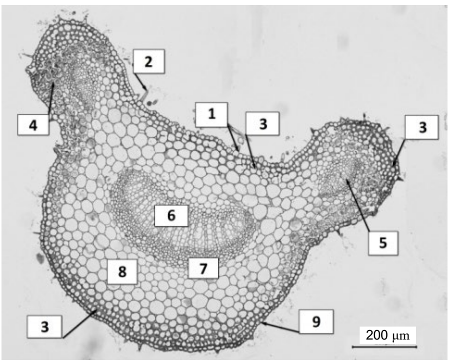
The cells of the upper epidermis are of unequal size; the external walls have a thin cuticle layer. The size of the tangent walls of the cells of the upper epidermis of *M. officinalis* subsp. *altissima* varies from 19.12 to 63.59 microns, and anticlinal - from 18.73 to 30.49 microns. Linear parameters of epidermal cells of *M. officinalis* subsp. *officinalis* are smaller. Tangent walls are 6.65–35.34 microns; anticlinal ones - 13.80–21.97 microns.

Lower epidermis of *M. officinalis* is small-celled. Tangent and anticlinal walls of cells in subsp. *altissima* are range from 12.99 to 29.72 μm and from 3.20 to 10.30 μm, correspondingly; as for subsp. *Officinalis* - it is substantially less: from 4.49 to 15.55 μm and from 5.05 to 14.71 μm, respectively. In this case, the dimensional parameters of the cells of the upper and lower epidermis of subsp. *officinalis* rarely exceed 10 microns.

The thickness of the leaf of *M. officinalis* subsp. *altissima* - is 140.98 ± 1.81 microns, which significantly exceeds the thickness of the subsp. *officinalis* - 101.06 ± 1.70 μm. The degree of development of palisade chlorenchyme is described by the palisade coefficient, which in subsp. *altissima* is 42% and in *officinalis* is 48%. It is an average indicator and confirmation of the belonging of this species to mesophytes.

The complex of mesomorphic qualities is native for both subspecies. The leaf is very thin, bifacial. It has a small number of mechanical elements. The leaf's system of intercellular spaces is developed. The stomata is on its lower side; the sheath of small bundles is poorly expressed and is represented by cells that do not differ from the main assimilation cells. Parenchymisation of tissues of axial organs is also may be seen. Additionally, there are a number of xeromorphic signs: a well-developed cuticle, thickened outer walls of the epidermis, the presence of pubescence, and the concentration of essential oil.

One of the areas in the anatomical methods of study is a petiolar anatomy (anatomical structure of petiole) (Kurkin et al., 2014; Gavrilenko & Novozhilova, 2015; Motorykina, 2015; Gavrilenko & Novozhilova, 2017). Literature contains only brief data concerning petiole structure of *M. officinalis* (Nikitina, 2018). Study of the anatomical structure of the cross section of the middle part of petiole of both the *M. officinalis* subspecies showed that it was covered with the small epidermal cells of rounded form with the thickened outer walls, which have the thin cuticle (Fig. 3). In the subepidermal zone of the central part of the petiole with its abaxial and adaxial sides you can find from 1 to 3 layers; in the corners - 4–5 layers of angular collenchyma. Nearer to the peripheral part of the petiole the assimilative tissue is located. It consists of 3–5 the layers of chlorenchima cells.

a) 

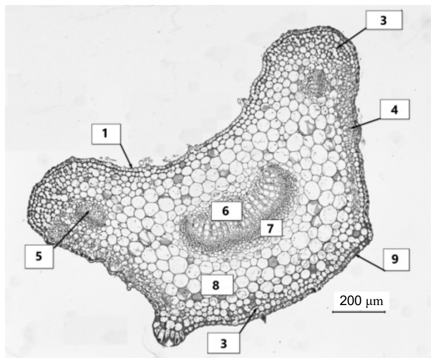
b) 

Figure 3. Anatomical structure of the petiole of *M. officinalis*: a – subsp. *altissima*; b – subsp. *officinalis*. (1 – upper epidermis; 2 – covering trichomes; 3 – angular collenchyma; 4 – chlorenchyma; 5 – vascular bundle; 6 – xylem; 7 – phloem; 8 – parenchyma cells; 9 – lower epidermis).

In the cross section the petiole has a round-sinuate shape. There are three vascular bundles. One is large, median (basic) and two are additional (lateral). The bundles are closed and collateral. They are represented by phloem and xylem. Median bundles are of the ring sector shape. They are inverted to the abaxial surface of the petiole. The pulps of the mechanical tissue were revealed from the side of the floem. The main parenchem in the petioles of the studied species is well developed and separates the lateral bundles from the central. In the cells of parenchyma, a small quantity of starch grains is contained.

The similarities of the anatomical structure of the petioles of the subspecies *M. officinalis* affirm data concerning that the petiolar anatomy is promising only for the development of diagnostic signs at the level of form and larger taxons (Kurkin et al., 2014; Gavrilenko & Novozhilova, 2015; Motorykina, 2015; Gavrilenko & Novozhilova, 2017).

The studied plants of both subspecies have a pubescence, presented by Among the small one- and two-cell non-glandular trichomes there are multicellular trichomes consisting of three to eight cells (Fig. 4).

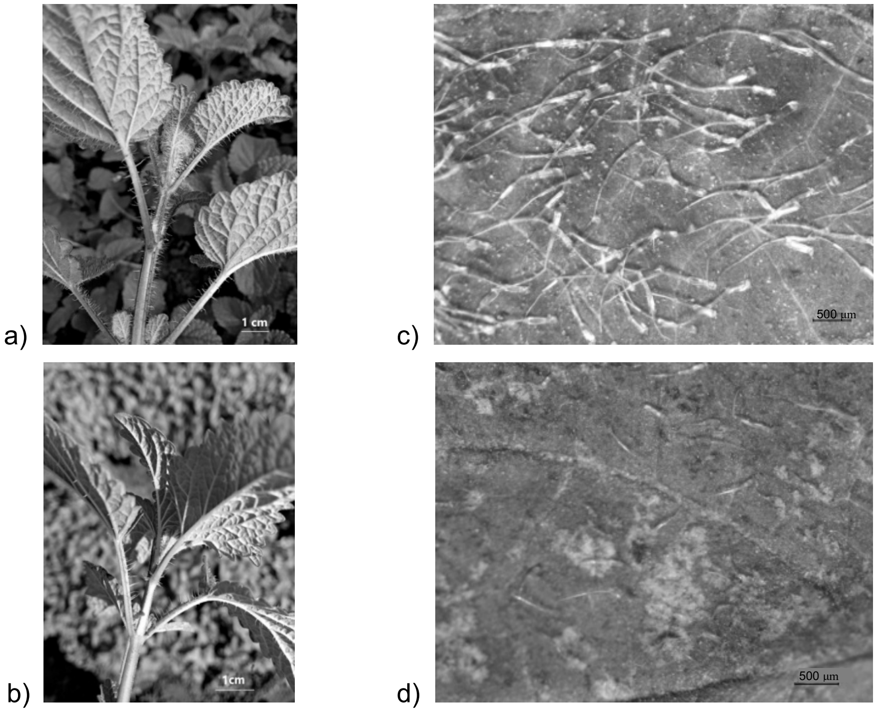


Figure 4. Multicellular trichomes of *M. officinalis*: subsp. *altissima*: a – fragment of the stem; c – adaxial side of the leaf; subsp. *officinalis*: b – fragment of the stem d – adaxial side of the leaf.

The number of multicellular non-glandular trichomes at the number of subsp. *altissima* for 1 mm is 4.66 ± 0.18 pcs; in subsp. they are single. The length of multicellular trichomes in subsp. *altissima* is on the average 1018.39 ± 49.11 µm with the diameter of the base of basal cell 95.53 ± 3.65 µm; in subsp. *officinalis* - 793.85 ± 45.04 µm and 33.45 ± 1.78 µm, respectively. Therefore, the multicellular trichomes, which are found in *M. officinalis* subsp. *officinalis* is considerably thinner in comparison with *M. officinalis* subsp. *altissima*.

According to the classification of terpenoid-containing structures of Denisova (1989), Werker (1993), on the organs of the studied *M. officinalis* subspecies were revealed: capitate glandular trichomes and peltate glandular trichomes. Capitate glandular trichomes with a short unicellular pedicel and the one or two-celled head (Fig. 5, a – d); glandular trichome with the long two-celled pedicel and the unicellular head (Fig. 5, e, f). Peltate glandular trichomes consists of a basal cell, a short pedicel and a multicellular head (four to eight cells) (Fig. 5, g, h).

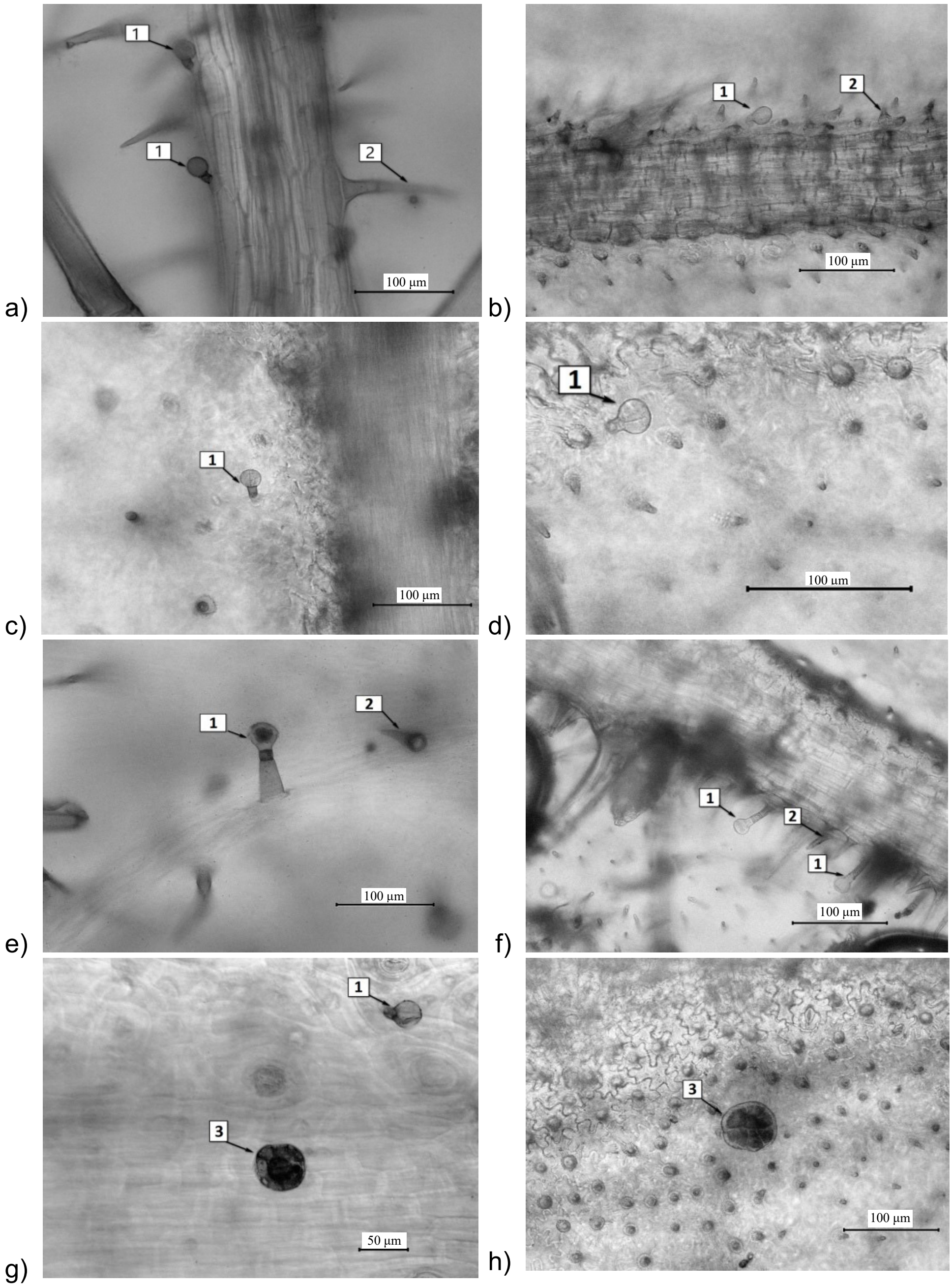


Figure 5. Glandular structures of the organs of *M. officinalis*: a, c, e, g – subsp. *altissima*; b, d, f, h – subsp. *officinalis*; a, b – glandular capitate trichome with a short 1–2 – cellular pedicel and unicellular head; c, d – glandular trichome with a short unicellular pedicel and unicellular head; e, f – glandular trichome with a long 2 – cellular pedicel and unicellular head; g, h – gland with multicellular head (1 – capitate glandular trichome; 2 – non-glandular trichome; 3 – peltate trichomes).

For *M. officinalis* subsp. *officinalis,* the presence of capitate glandular trichome with a multicellular pedicel (3–5 cell) and a unicellular head (Fig. 6) has also been revealed.

Additionally, to micromorphological differences, the samples studied were characterized by differences in the structure of the superius labium of calyx. Therefore, in subsp. *officinalis* the superius labium of calyx of flower has three well expressed triangular teeth, and in subsp. *altissima* they are reduced or missed. The superius labium of calyx is ended with two cusps. This corresponds to the description in the literature of *M. bicornis* 12, which we examine in our paper as the synonym to *M. officinalis* subsp. *altissima* (Fig. 7).

Figure 6. Glandular capitate trichomes with a multicellular (3–5 cell) pedicel and a unicellular head on the stem of *M. officinalis* subsp. *officinalis*.

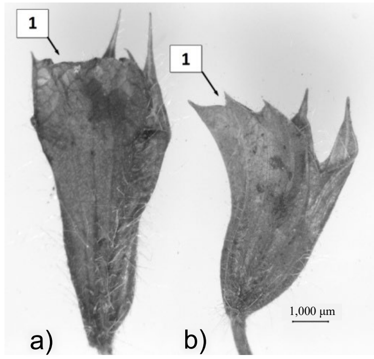


Figure 7. Structure of the upper flower calyx (1) of *M. officinalis:* a – subsp. *altissima;* b – subsp. *Officinalis*.

The habitus of subsp. *altissima* plants is characterized by a higher capacity compared to subsp. *officinalis*, due to a more developed shoot system. Height of subsp. *officinalis* plants (Krymchanka and Lada varieties) lagged appreciably to this index of the plants of the subspecies *altissima* (Tavrida variety). It is about 49.9 ± 2.0 cm; 55.4 ± 4.0 cm and 82.0 ± 5.6 respectively. The diameter of plants differs less significantly. It is about 65.7 ± 3.6 cm; 67.1 ± 4.6 cm and 73.4 ± 6.8 respectively.

Literary sources indicate that the chromosomal complement of subsp. *altissima* has 64 chromosomes; in subsp. *officinalis* - 32 chromosomes (Flora Yevropeiskoy…, 1978; Kittler et al., 2015). It is known that polyploidy causes an increase in plant size (Breslavec, 1963; Gosenova & Kolesova A, 2004; Fomin, 2009; Kittler et al., 2015). Apparently, this particular circumstance explains the above-indicated differences in the quantity indicators of the compared subspecies, including: habitus of plants, thickness of the leaf and compared microstructures.

CONCLUSIONS

Comparative anatomical and morphological study of *Melissa officinalis* subsp. *officinalis* and *M. officinalis* subsp. *altissima*. showed the similarity in anatomical and morphological structures of the vegetative organs of plants.

A complex of mesomorphic features has been established, confirming the belonging of subspecies to the ecological group of mesophytes, characterized by high flexibility. In addition to mesomorphic features, there is a presence of xeromorphic ones, providing their adaptive capabilities in introduction.

Epidermal structures, represented by uni- and multicellular non-glandular trichomes, as well as different types of glandular structures (capitate glandular trichomes and peltate glandular trichomes) have been found. Meanwhile, the glandular trichomes with a multicellular pedicel and unicellular head is noted only in *M. officinalis* subsp. *officinalis*.

An increase in the number of indicators: thickness of a leaf, the palisade ratio, high density of large multicellular covering trichome and habitus of the plants is noted in *M. officinalis* subsp. *altissima*. The explanation of this is in the literature. It is all because of different ploidy of the subtypes indicated: the chromosomal collection of subsp. *altissima* includes 64 chromosomes and subsp. *officinalis* - 32 chromosomes.

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Comparative analysis of oil flax varieties according to economically valuable traits in the Steppe zone of Ukraine

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Abstract. Interest in oil flax has been growing in recent decades. Linseed oil is a fast-drying oil and it is traditionally used in the production of linoleum, paints and coatings. Due to their content of essential polyunsaturated acids and vitamins, the growing use of flax seeds and oils in food and medicine has significantly increased the volume of its cultivation in different countries. This article presents the main research results for 2018−2020 of a large group of varieties of oil flax of Zaporizhzhia breeding for economically valuable traits. The purpose of research is to establish the genotypes that form the highest yields and oil content in arid conditions of the Steppe zone of Ukraine. It was found that the most productive year was 2020 due to more favorable temperatures and even distribution of precipitation during the growing season of oil flax. It was found that the yield of oil flax had a high correlation coefficient with weather conditions ($r = 0.67$) especially from precipitation in May-June ($r = 0.60$). A detailed study of the correlation between yield and habit traits and the main economically valuable traits revealed a close direct correlation between oil flax yield and seed weight per 1 plant ($r = 0.99$), yield and number of lateral stems per plant ($r = 0.93$), yield and number of bolls and seeds per 1 plant ($r = 0.77$), yield and weight of 1,000 seeds ($r = 0.73$), yield and duration of the growing season ($r = 0.65$). According to the results of three-year research, the highest yield was obtained from the variety Vodohrai 1.79 t ha^{-1}. The highest oil content and oil yield per hectare were formed by varieties Vodohrai 50.1% and 0.80 t ha^{-1}, Zolotystyi 49.7% and 0.76 t ha^{-1}, Aisberh 48.3% and 0.72 t ha^{-1}.

Key words: oil flax, variety, duration of the growing season, habit, element of productivity, yield, oil content, oil yield per hectare, correlation.

INTRODUCTION

Oil flax *Linum humile* Mill is an annual plant 40−60 cm tall, which is grown to obtain seeds with high oil content. Other than oil flax, cultivated flax also includes long flax. Long flax is grown for high quality fiber. Compared to the habitat of long flax, oil flax occupies a much larger area, which is due to its wider ecological potential and lower demand for soil moisture (Diakov, 2006).

Oil flax has been known to man as a cultivated plant since the ancient times and it has played an important role in the economic and social development of mankind (Zhuchenko Jr & Rozhmina, 2000; Krugla, 2002; Zohary et al., 2012; Zelentsov, 2017). In recent decades, interest in the cultivation of oil flax has grown significantly in different countries around the world, as evidenced by numerous publications, both with focus on genetic and plant breeding as well as with agronomic, botanical and biochemical focus (Özkutlu et al., 2007; Lafond et al., 2008; Desta & Bhagwan, 2014; Delesa & Choferie, 2015; Pecenka et al., 2016; Tretjakova et al., 2018; Kiryluk & Kostecka, 2020).

Linseed oil is categorized as a fast-drying oil and it is a necessary component of paints, inks, varnishes, linoleum, and anti-corrosion coatings. It is used in medicine, cosmetology and nutrition (Oomah, 2001; Kiralan et al., 2010; Peshuk & Nosenko, 2011; Kraevska et al., 2019; Lykhochvor et al., 2022). The main component of the oil - linolenic acid - is a main unsaturated acid, which determines its high biological activity and ability to dry quickly (Diakov, 2006; Peshuk & Nosenko, 2011).

In Ukraine, interest in this crop has also grown significantly in recent years, thanks to its drought resistance and profitability. Market dynamics indicate a steady increase in linseed oil demand and the rapid expansion of sown areas (Statistichniy byuleten, 2017–2021). Oil flax is successfully grown in all soil and climatic zones of Ukraine due to its biological properties and ecological adaptability (Makhova & Polyakov, 2012; Vishnivska, 2013; Voytovych & Shuvar, 2018). However, the main growing areas are located in the Steppe zone. Despite the fact that up to 20% of sown areas can be saturated with oil flax, this crop in Ukraine remains niche. In the general structure of oilseed crops it has a share of about 1%, and its share in the structure of oilseed production is at 0.3%. Oil flax occupies a small share in the domestic market segment of oilseed crops. It is grown for exporting seeds, which are then bought by EU member states, Egypt, China, Turkey and other countries (Linseed, mustard. Oilseed crops production, 2017).

Oil flax ripens well at a sum of temperatures of 1,600–1,850 °C during the growing season. Seedlings tolerate frost up to -2, -3 °C. The optimal sowing rate of flax seeds in the Steppe zone is 4–6 million units ha^{-1}. It is recommended to sow early so that the seeds ripen in warm weather. It is sown in the usual row method to a depth of 3–4 cm. Soils should have a neutral reaction. Growing season of oil flax is 90–130 days. It belongs to the group of crops with a short growing season. But during prolonged rains in ripening stage if oil flax does not dry out it might even 'grow back', so desiccation is required. Oil flax plants grown in drier areas have better developed lateral roots, which penetrate into the soil to greater depths. Researchers who grow oil flax agree on the high plasticity of the crop when growing it in different climatic, weather and soil conditions (Makhova & Polyakov, 2012; Nakamoto & Horimoto, 2016; Kalenska & Stolyarchuk, 2018; Voytovych & Shuvar, 2018).

To increase crop yields, it is necessary to create and implement high-yielding varieties. Total number of oil flax varieties in the State Register of Plant Varieties Suitable for Distribution in Ukraine is constantly increasing. About half of the Ukrainian varieties were created at the Institute of Oilseed Crops of the National Academy of Agrarian Sciences of Ukraine. At present, according to official statistics in Ukraine, 65–75% of sown areas are occupied by oil flax varieties of Zaporizhzhia breeding (State register of plant varieties, 2016–2020). To increase the efficiency of plant breeding in the Zaporizhzhia breeding school of oil flax, a parametric model was created, explaining the parameters of traits and properties of the variety that will create a genotype that meets modern production requirements (Poliakova, 2015).

Our research aimed to establish the genotypes of oil flax, which form the highest yields and oil content in arid conditions of the Steppe zone of Ukraine to increase the economic efficiency of cultivation.

MATERIALS AND METHODS

Research was conducted in 2018–2020 in the city of Zaporizhzhia (North-Western part of the Zaporizhzhia oblast) at the experimental site of the Department of Genetics and Plant Resources of Zaporizhzhia National University. Soil of the experimental site was ordinary chernozem, of medium strength and of low humus, with humus content in the arable layer of 0–30 cm - 3.4% (according to State standard of Ukraine 4362: 2004. Soil quality indicators of soil fertility), available nitrogen (N - by Cornfield) - 70–80, mobile phosphorus (P_2O_5 - by Chirikov) - 92–101, exchangeable potassium (K_2O - Chirikov) - 148–165 mg kg^{-1} of soil, soil pH - 6.5–7.0 (State standard of Ukraine 4362: 2004).

Setting up the experiments and conducting research was carried out according to generally accepted field experiment methods in agriculture and crop production (Littl & Hills, 1981; Dospekhov, 1989). Agricultural techniques in the experiments complied with the recommendations (Lyakh & Poliakova, 2008).

In the years of research, weather conditions for temperature and precipitation had some deviations from the long-term average, which allowed to fully establish their impact on the growth and development of flax plants and the realization of the appropriate amount of yield. Data on the amount of precipitation and temperature in the years of research compared to average multi-year indicators, is given in Figs 1 and 2.

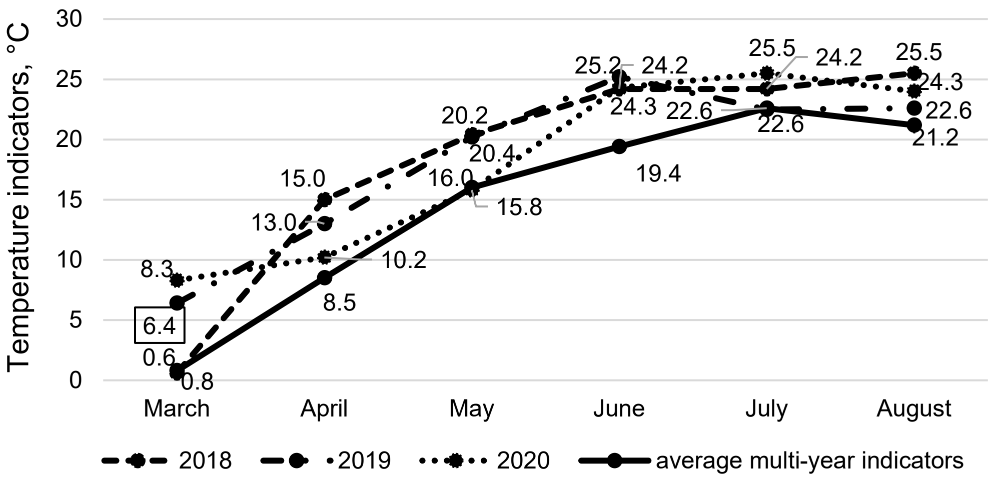

Figure 1. Temperature indicators during the growing season of oil flax (2018–2020).

Experiments used material from the collection of the Department of Genetics and Plant Resources of Zaporizhia National University - varieties Pivdenna Nich (year of registration 2000), Debiut (2000), Aisberh (2001), Orfei (2001), Zolotystyi (2005), Kivika (2007), Vodohrai (2010), Patrytsii (2018).

Plant description was written down according to generally accepted methods (Lyakh & Poliakova, 2008). Plot placement was randomized. Plot area constituted 25 m^2, flax predecessor was winter wheat and basic soil tillage consisted of plowing. Sowing was conducted in the first ten days of April. All measurements and observations were conducted three times.

To determine the characteristics of growth and development of flax plants a set of studies, calculations and phenological observations was conducted (Lyakh & Poliakova, 2008):

– length of vegetation period was determined between seedling and full maturity stages;

– phases of growth and development of flax were noted down: seedling stage, stem extension stage, budding stage, flowering stage, and full maturity. Beginning of the stages s was established when it occurred in 10% of plants, and stage completion for 75% of plants;

– the number of bolls per plant and the number of seeds per plant was counted in 25 plants;

– seed yield was determined continuously from each plot;

– weight of 1,000 seeds was calculated according to standard methods;

– oil content of seeds was determined according to DSTU 7577: 2014;

– statistical processing of the obtained data was carried out on the programs 'Microsoft Office Excel' and the modern statistics software package 'STATISTICA 10';

– obtained data was subject to mathematical analysis using variance and correlation calculations (Littl & Hills, 1981; Dospekhov, 1989).

RESULTS AND DISCUSSION

As can be seen from the figure, in terms of temperature indicators in all years of research, there was a surplus of them over the long-term average values (Fig. 1). The biggest deviations in temperature happened in 2018, especially in the first months of the growing season in the seedling and stem extension stages. That year, the temperature exceeded the averages by 6.5 °C in April and 4.4 °C in May. And in 2020, the temperature was closest to the long-term average and the deviations of its indicators were 1.7 °C in April and -0.2 °C in May. The most favorable temperature indicators during the 'budding-flowering-boll formation' period in June were also noted in 2020. The deviation in temperature from the average long-term values was 1.9 °C, while in 2018 it was 4.6 °C, and in 2019 it was 5.8 °C. Thus, 2020 turned out to be the most favorable year in terms of temperature indicators.

In 2018 there was also the most uneven distribution of precipitation throughout the growing season (Fig. 2). A significant surplus was noted only in March before sowing the crop and in July at the stage of seed ripening. And in April, May and June, the amount of precipitation was significantly less than the long-term average. Despite the fact that the total amount of precipitation in 2018 for the entire growing season of oil flax was 288 mm, which is more than in 2019 (238.6 mm) and in 2020 (211.2 mm), due to their uneven distribution and high air temperature it marked the lowest height of flax plants and their yield. It is important to emphasize that the biological peculiarity of oil flax is that it has slower growth at the beginning stages of development, a small leaf blade and a weakly spread root system, so the lack of moisture in the beginning stages of growth and development is very critical for this crop. Fig. 2 shows that in 2019 and 2020, precipitation during crop growth was closer to the long-term average and was more evenly distributed from March to August.

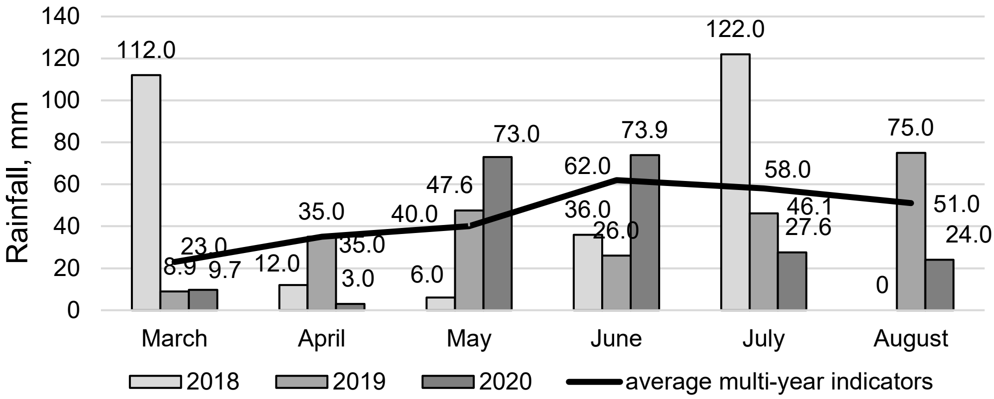

Figure 2. Amount of precipitation during the growing season of oil flax (2018–2020).

Studied weather conditions that happened during the research years significantly influenced the growth, development and formation of the productivity of oil flax plants.

Due to the increased temperature and insufficient amount of precipitation, the shortest growing season of oil flax plants was recorded in 2018, 79–97 days. In the most, favorable year 2020, this figure was 88–105 days, depending on the variety, and the average values for three years were 84–101 days. As can be seen from the data in Table 1 the studied varieties differed among themselves in the length of the growing season, both in terms of the average and across different years. But in general, it can be noted that the two varieties Kivika and Debiut belong to earlier varieties that ripen in 84 and 86 days, respectively, which is 8 and 6 days earlier than the standard variety Pivdenna Nich, while varieties Orfei and Zolotystyi have a growing season of 3 and 9 days longer than the standard variety. Longevity of the growing season, for any variety, is an important biological trait, that can be of great practical importance (Table 1).

Table 1. Length of the growing season of oil flax varieties in the conditions of the Steppe zone of Ukraine (2018−2020)

Varieties	Years			Average value
	2018	2019	2020	
Pivdenna Nich (standard)	88	92	95	92
Debiut	81	87	90	86
Aisberh	86	90	93	90
Orfei	91	95	98	95
Zolotystyi	97	102	105	101
Kivika	79	85	88	84
Vodohrai	92	93	97	94
Patrytsii	87	91	94	91
$LSD_{0.5}$	1.1	1.7	2.0	

Studied varieties also differed in other economically valuable traits (Table 2). We have studied the following traits: plant height, number of stems, number of bolls, number of seeds per 1 plant, weight of seeds from 1 plant, weight of 1,000 seeds.

Traits of plant habit are decisive in the breeding for high productivity. Habitual traits of flax plants include such basic external traits as the height of the plant and the number of lateral stems. In breeding studies, the length of the technical part of the stem is also taken into account in a more detailed description. Plant height is a trait that significantly depends on changes in weather and climatic conditions. In our research, the lowest rates for all varieties were obtained in 2018, and the highest in 2020. Most researchers agree that the formation of the height of flax plants is greatly influenced by external conditions that develop during the period of intensive plant growth. As can be seen from Table 2, the maximum value of plant height on average over the years of testing - 62.3 cm - was formed by the variety Vodohrai, and the minimum 49.7 cm by the variety Kivika. Average value of the plant height of the studied varieties for the years of research was 55.6 cm. Stem of oil flax is an important useful part of the plant because it contains valuable fiber. In addition to performing the main conductive and mechanical functions, flax stem to some extent performs the function of temporary storage of reserve carbohydrates and proteins, and it also contributes to the formation of products of photosynthesis (Diakov, 2006). Therefore, the height of flax plants is given a lot of attention when creating varieties. In our experiments, the varieties Vodohrai were significantly higher than the standard by 8.0 cm, Zolotystyi by 5.7 cm and Orfei by 4.1 cm.

Table 2. Economically valuable traits of oil flax varieties in the conditions of the Steppe zone of Ukraine (average value for 2018–2020)

Varieties	Traits					
	plant height, cm	number of stems, pcs	number of bolls, pcs	number of seeds from 1 plant, pcs	weight of seeds from 1 plant, g	weight of 1,000 seeds, g
Pivdenna Nich (standard)	54.3	2.7	6.9	51.9	0.40	7.7
Debiut	50.8	2.4	6.8	50.7	0.35	6.9
Aisberh	56.2	2.8	8.8	65.8	0.48	7.3
Orfei	58.4	2.6	7.4	55.7	0.39	7.0
Zolotystyi	60.0	3.1	8.3	62.0	0.49	7.9
Kivika	49.7	2.3	8.2	61.7	0.37	6.0
Vodohrai	62.3	3.1	8.5	63.8	0.51	8.0
Patrytsii	52.9	2.7	7.7	57.7	0.41	7.1
Average value	55.6	2.7	7.8	58.7	0.43	7.2
$LSD_{0.5}$	1.2–2.3	0.08–0.12	0.23–0.32	0.8–1.7	0.03–0.06	0.11–0.20

Number of stems per 1 plant averaged from 2.3 to 3.1, depending on the variety. At the same time, the standard variety Pivdenna Nich averaged 2.7 stems. Varieties Zolotystyi and Vodohrai exceeded the standard variety the most, by 0.4 stems, and the lowest numbers for this trait were in the varieties Debiut and Kivika. This trait is important because it directly correlates with indicators of the yield structure, such as the number of bolls and seeds per one plant. Thus, for the Zolotystyi variety, the number of bolls and seeds per plant was 8.3 and 62.0 pcs. And for the variety Vodohrai - 8.5 and 63.8 pcs. These varieties exceeded the Pivdenna Nich standard variety by 1.4 and 10.1 pcs. and 1.6 and 11.9 pcs, respectively. In addition, the Aisberh variety stood out by the formation of a large number of bolls (8.8 pcs) and seeds in them with (65.8 pcs) a relatively small number of side stems (2.8 pcs). Variety Debiut had the lowest indicators for the studied elements of the yield structure, namely: number of bolls - 6.8 pcs, number of seeds - 50.7 pcs, and number of stems - 2.4 pcs. And Orfei and Kivika varieties formed a lot of bolls and seeds in them, however, due to the low weight of

1,000 seeds, the indicator 'mass of seeds from 1 plant' was lower than that of the standard variety Pivdenna Nich by 0.01 g and 0.03 g.

'Weight of 1,000 seeds' indicator is important for flax since it is a small-seeded crop. In the presented range of flax varieties, it ranged from 6.0 to 8.0 g, and for standard variety it was 7.7 g. On average for three years of research, this figure was 7.2 g. It was found that the smallest seed belonged to Kivika variety (6.0 g). Vodohrai (8.0 g) and Zolotystyi (7.9 g) varieties had larger sized seeds. Therefore, they can be used in further breeding work as a source of large fruited seeds, because this trait is important when cleaning seeds from weeds.

An important summarizing indicator, which depends on the number of bolls and seeds, as well as their size, is the 'mass of seeds from 1 plant'. This indicator, on average over the years of research, ranged from 0.35 to 0.51 g, depending on the variety, and the average value was 0.43 g. The highest indicators were in the varieties Vodohrai (0.51 g), Zolotystyi (0.49 g) and Aisberh (0.48 g), which exceeded the standard variety Pivdenna Nich (0.40 g) by 0.11 g, 0.09 g and 0.08 g, respectively.

Yield is one of the most important traits of growing for any crop. The difference for this trait is the high degree of its integration. According to our data the average yield by variety over the years of research was 1.49 t ha^{-1}. The most productive year was 2020 with an average of 1.69 t ha^{-1}, then 2019 - 1.59 t ha^{-1} and the lowest yield in 2018 was 1.20 t ha^{-1}. This indicator directly correlates with the weather conditions described above, during the growing season. If we consider the varieties in comparison, the most productive in all years of research was variety Vodohrai (1.79 t ha^{-1}). Varieties Zolotystyi (1.72 t ha^{-1}) and Aisberh (1.68 t ha^{-1}) also significantly exceeded the variety-standard. Debiut and Kivika varieties produced lower yields than the standard Pivdenna Nich variety. Highest yield for the years of research was 2.13 t ha^{-1} for the variety Vodohrai in 2020 (Table 3).

Table 3. Yield of the oil flax in the conditions of the Steppe zone of Ukraine, t ha^{-1} (2018−2020)

Varieties	Years 2018	2019	2020	Average value
Pivdenna Nich (standard)	1.17	1.50	1.54	1.40
Debiut	0.97	1.31	1.38	1.22
Aisberh	1.28	1.81	1.94	1.68
Orfei	1.21	1.44	1.49	1.38
Zolotystyi	1.30	1.86	2.01	1.72
Kivika	1.12	1.39	1.43	1.31
Vodohrai	1.33	1.92	2.13	1.79
Patrytsii	1.24	1.52	1.59	1.45
Average value	1.20	1.59	1.69	1.49
$LSD_{0.5}$	0.07	0.10	0.11	

We experimentally proved that the yield of oil flax had a fairly high correlation coefficient with weather conditions in the years of research ($r = 0.67$). At the same time, the change in temperature indicators in comparison with the average long-term values did not play a significant role, both for the entire growing season and in individual months. Another trend that we noted happened in terms of rainfall and its distribution over the growing season. The amount of precipitation that happened during the entire growing season between April and August had a correlation coefficient $r = 0.52$. A closer correlation was observed between yield and the amount of precipitation that happened between May and June ($r = 0.60$). In our opinion, this period of growth and development of oil flax plants needs the most moisture and it is crucial for the formation of seed yield. Similar results were obtained by A. Shuvar (2021) only in the conditions of another natural zone, namely, the Western Forest-Steppe of Ukraine.

We analyzed the correlations between yield, habit traits, and productivity elements. A close direct correlation was established between oil flax yield and plant height ($r = 0.83$), number of side stems ($r = 0.93$), number of bolls and seeds per plant ($r = 0.77$), weight of a 1,000 seeds ($r = 0.73$). Closest relationship was found between seed yield and weight per plant ($r = 0.99$), and the average correlation with the length of the growing season ($r = 0.65$). According to our data, the length of the growing season is closely related to the height of plants ($r = 0.84$), the number of side stems ($r = 0.83$) and the weight of 1,000 seeds ($r = 0.77$), and the average relationship is between total weight of seeds from 1 plant and yield ($r = 0.65$). The existence of a close correlation was established between the habit traits, namely, the height and number of lateral stems, and the following elements of productivity: seed weight per 1 plant ($r = 0.83$) and ($r = 0.93$) and weight of 1,000 seeds = 0.79) and ($r = 0.91$), respectively. Estimation of the strength of the links between the elements of productivity revealed the existence of a linear close correlation between the number of bolls and the number of seeds ($r = 0.99$), the number of seeds per plant and seed weight per plant ($r = 0.75$) and between weight of the seeds from 1 plant and weight of a 1,000 seeds ($r = 0.75$). Our results are confirmed by the data of O. Kurach (2016) and O. Rudik (2019), who studied the relationship between yield and economically valuable traits of oil flax. In our research, we paid more attention to establishing the links between yield indicators with the length of the growing season, habit traits, and elements of productivity.

One of the main indicators of flax seed quality is its oil content. We found that the oil content in the seeds of varieties on average over the years of research was between 43.9 and 50.1%. Vodohrai (50.1%), Zolotystyi (49.7%) and Aisberh varieties (48.3%) had the highest oil content. They exceeded the standard variety by 5.3%, 4.9% and 3.5%, respectively. Highest oil content in the seeds was observed in 2020. In that year, all studied varieties had higher oil content than the average. In 2018, on the contrary, there was a much lower oil content in the seeds. As mentioned above, 2018 had extremely unfavorable dry weather conditions, which also affected the accumulation of oil in the seeds (Fig. 3).

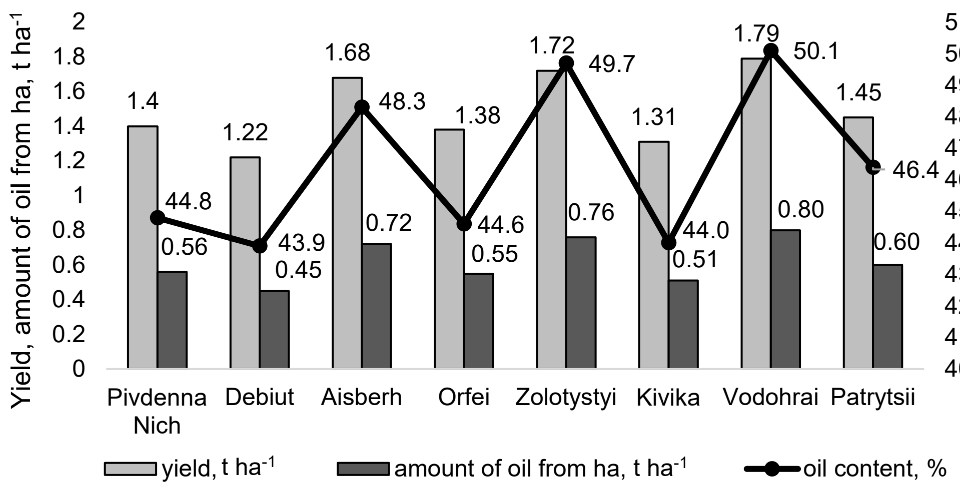

Figure 3. Yield, oil content and oil yield of different varieties of oil flax in the conditions of the Steppe zone of Ukraine (2018–2020).

The indicator ‘oil yield per hectare’ is very emblematic in the cultivation of oilseed crops is the indicator ‘oil yield per hectare’. It is derived from the yield and oil content and characterizes a particular variety's economic value. Varieties Vodohrai (0.80 t ha^{-1}), Zolotystyi (0.76 t ha^{-1}) and Aisberh (0.72 t ha^{-1}) had the highest oil yield per hectare (Fig. 3). Varieties Debiut and Kivika did not exceed the standard variety.

According to other researchers, oil flax varieties of Zaporizhzhia breeding in other natural and climatic zones of Ukraine also formed high oil content in seeds and oil yield per hectare (Kurach, 2016; Rudik, 2019; Shuvar, 2021).

High productivity sources are needed to increase flax yields and to increase oil yield per hectare. Oil flax breeding to create varieties with the highest possible level of productivity, takes into account a complex set of physiological, morphological, biological and other traits that determine the level of yield in specific growing conditions and use a variety model with defined parameters of different traits (Poliakova, 2015).

These results indicate that the varieties Vodohrai, Zolotystyi and Aisberh, had higher seed weight per 1 plant, plant height, number of side stems, over the standard variety and other varieties, which allowed them to form the highest yields. They are also characterized with high oil content. That is why, in our opinion, these three varieties are potential genetic sources of economically valuable traits for further breeding work.

CONCLUSION

Comprehensive analysis of eight commercial varieties of oil flax of Zaporizhzhya breeding was undertaken for the main economically valuable traits in contrasting weather conditions for three years in 2018, 2019, 2020. When conducting a correlation analysis, it was found that the yield of oil flax had a high correlation coefficient with weather conditions in the years of research; most significantly yield depended on precipitation. A close direct correlation was established between oil flax yield and plant height, number of side stems, number of bolls and seeds per plant, weight of 1,000 seeds, and the closest relationship was found between yield and weight of seeds from 1 plant.

Top indicators of economically valuable traits of the studied varieties were obtained in 2020. The highest yield, oil content and the highest oil yield per unit area was noted for varieties Aisberh, Zolotystyi and Vodohrai.

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Ecotoxicological assessment of mineralized stratum water as an environmentally friendly substitute for agrochemicals

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Abstract. As a result of military operations on the territory of Ukraine, sown areas are reduced, the cost of plant protection products and fertilizers increases which emphasizes the problem of obtaining maximum yields from a smaller area of farmland. Given that a shortage of food grains can cause a global food crisis, research on the use of MSW as an environmentally friendly substitute for agrochemicals is relevant today. The aim of the research was to assess the ecotoxicological properties of MSW as an environmentally friendly substitute for synthetic agrochemicals. The impact of MSW as a fertilizer on soil chemical properties and assessment of MSW phytotoxicity as an herbicide for weeds and productivity of winter wheat were studied under field conditions. Toxicological assessment of MSW under laboratory conditions was carried out according to the following parameters: acute oral toxicity and resorptive-toxic effect of MSW. As a result of the assessment of MSW impact on soil chemical properties, it was found that significant soil acidification occurs only when MSW dose of more than 2,400 L ha^{-1} is used. The content of nitrates and oil products did not increase and there was no soil salinity when the MSW was used in doses of 300–1,200 L ha^{-1}. It was determined that the greatest decrease in weed plant biomass (85.5%) was observed when 100% concentration of MSW was used in a dose of 350 L ha^{-1}. As a consequence, with reduced weed infestation, there was a 21.5% increase in winter wheat yield if 100% MSW was used and a 19.1% increase if 75% MSW was used. As a result of the toxicological assessment of MSW, it was found that it belongs to low-toxic compounds. These results of ecotoxicological investigation of MSW make it possible to assert that its use is safe in agriculture, in particular as an environmentally safe organomineral fertilizer and herbicide.

Key words: ecotoxicological assessment, mineralized stratum water, soil, phytotoxicity, winter wheat.

INTRODUCTION

During the extraction of oil and gas, large quantities of mineralized stratum water (MSW), which is a by-product, come to the surface. The problem of disposal of large quantities of this water is very significant, given that uncontrolled release of large quantities of stratum water on the ground, leads to salinization and deterioration of the agronomic structure of the soil, the destruction of biodiversity in natural ecosystems. Based on the research conducted by (Obire & Amusan, 2003; Reva, 2016) it was found that MSW contains a significant amount of mineral elements and inorganic compounds (about 60 different micro- and macroelements), in particular sulfates and chlorides, the total mineralization is in the range of 140–180 g dm^{-3}. However, the impact of MSW in different doses on the soil has not been studied sufficiently.

The previous research conducted by Markina (2019) established the possibility of using MSW as an environmentally friendly substitute for agrochemicals on the crops of cereals in order to increase their yields. As a result of warfare in Ukraine, sown areas are being reduced, which raises the issue of maximizing the yield from smaller areas of farmland, as well as the protection of crops from weeds by inexpensive and environmentally safe means. This issue is especially important nowadays, since the lack of food grains can cause a global food crisis. Therefore, it is necessary to search for new approaches of using environmentally friendly and cost-effective plant protection agents in agriculture. The use of mineralized stratum water, which is a by-product of oil production, can become one of such methods.

The possibility of using MSW in order to improve the technology of obtaining high-quality organic fertilizers was determined earlier, Pisarenko et al. (2022). The phytosanitary impact of MSW on the crops of cereals was studied as well (Pysarenko, 2021). The problems of application of MSW as a substitute for agrochemicals, in particular the determination of environmentally safe doses of MSW as a herbicide and the main fertilizer of cereals, require further research.

According to the assesment of Pisarenko (2019), mineralized stratum water of oil and gas fields in Poltava region was evaluated as mineral therapeutic bromine, iodine-bromine sodium chloride, calcium-sodium brines, promising for balneotherapy and safe for people. In accordance with the Decree The Cabinet of Ministers of Ukraine, (1996), before using any new plant protection agents, it is necessary to conduct their toxicological assessment and determine their class of hazard for the environment, warm-blooded animals and bees. Therefore, before using MSW as a substitute for agrochemicals, it is necessary to assess its toxicity by LD_{50} in accordance with the resolution of the World health organization (Executive Committee, 55^{th} session, 1974).

The problem under study is pressing both for the oil and gas industry and for agricultural production. The aim of the scientific paper was to assess the ecotoxicological properties of MSW as an environmentally friendly substitute for synthetic agrochemicals. This envisaged the study of the impact of different doses of MSW on the agrochemical soil properties, assessment of MSW phytotoxicity to weeds, determination of winter wheat productivity under these conditions, as well as assessment of MSW toxicity in order to determine its hazard class.

MATERIALS AND METHODS

For the purpose of maximum and comprehensive study of MSW for use as agrochemicals, field and laboratory experiments were set up. Under field conditions, the impact of MSW as a fertilizer on soil chemical properties, assessment of MSW phytotoxicity as an herbicide for weeds and productivity of winter wheat were studied. To use any solutions as agrochemicals, it is necessary to assess their toxicity to the environment, warm-blooded animals and bees (Decree The Cabinet of Ministers of Ukraine, 1996). Therefore, the toxicological assessment of MSW under laboratory conditions was performed according to the following parameters: acute oral toxicity and resorptive-toxic effect of MSW.

At the first stage, the impact of MSW on soil chemical properties was studied. This research was conducted for 5 years (2017–2021) in three-fold repetition in the experimental fields of PSAU (Poltava State Agrarian University). The plots with an area of 0.5 ha were made (Trybel, 2001) MSW concentrations of 300 L ha^{-1}, 600 L ha^{-1}, 900 L ha^{-1}, 1,200 L ha^{-1}, 2,400 L ha^{-1} and 4,800 L ha^{-1} were applied on these plots as a fertilizer during the basic tillage. The plots without MSW and the plots where full mineral fertilizer $N_{50}P_{50}K_{50}$ was applied were taken as control.

Cultivated crop is winter wheat. The soil of the experimental field was typical deep low-humus medium-loam soil of Haplic Luvisol type (according to WRB, 2014): humus content - 3.6%, total nitrogen - 0.32%; hydrolytic acidity - 2.39 mg eq per 100 g, easily hydrolyzed nitrogen (N) - 141 mg kg^{-1} soil, P_2O_5 - 269 mg kg^{-1} soil, K_2O - 87 mg kg^{-1} soil. MSW was characterized by: pH 8.7–8.9, Na+K 45.8–50.2 g dm^{-3}, Ca^{2+} 10.9–11.1 g dm^{-3}, Mg^{2+} 0.9–1.0 g dm^{-3}, Cl^- 95.6–105.2 g dm^{-3}, SO_4^{2-} 6.8–7.0 g dm^{-3}, HCO_3^- 0.82–1.15 g dm^{-3}, the oil-hydrocarbon content - 3–5%.

Soil chemical properties were determined 30 days after MSW application by the following methods: actual soil acidity by measuring H^+ concentration in the solution by potentiometry method using ion-selective electrodes on a potentiometer of pH-150 M (ISO 10390:2021); the content of exchangeable sodium, macro- and microelements: potassium, calcium, magnesium in the aqueous extract by using a flame photometer with a wavelength of 589 and 569.9 nm (ISO 11047:1998); hydrocarbonates, carbonates (ISO 10693:1995), sulfates (ISO 11048:1995), chlorides, iodine, bromine and others were determined according to ISO 11260:2018. Petroleum products were determined by gravimetric method ISO 11504:2017.

At the second stage, the phytotoxicity of MSW to weeds in winter wheat crops was assessed by weight method under field conditions. For this purpose, the plots with an area of 0.5 ha were set up, where spraying was carried out during the period of winter wheat tillering. Consumption rate of MSW was 350 L ha^{-1}, when it was used as a herbicide (Trybel, 2001). The following experimental plots with a concentration of 100% MSW; 75% MSW; 50% MSW were examined. The comparison was made with the herbicide Desormone 600 (the main active ingredient - 2.4 dichlorophenoxyacetic acid in the form of dimethylamine salt, 600 g L^{-1}). On day 30 after treatment, soil weed control was carried out. All weeds were counted, uprooted, air-dried and weighed (Trybel, 2001). Part of the damaged surface was separated, weighed, and the percentage of leaf surface damage was measured. The experiment was conducted in three-fold repetition for 5 years.

On these experimental plots, where MSW was used as a herbicide, winter wheat yields were tested for 5 years. Winter wheat yield accounting was carried out by sheep sampling in 3-fold repetition on the accounting plots in the phase of full grain ripeness (Trybel, 2001).

In accordance with the Decree The Cabinet of Ministers of Ukraine (1996) before the beginning of state trials of new chemical plant protection agents, it is necessary to carry out their toxicological assessment, which will determine the class of hazard and develop measures for the safe use of the preparation.

Toxicological studies used white rats and white mice that have taken the 14-day quarantine in the vivarium of Poltava State Agrarian University according to the method of Menshikova (1987). Acute oral toxicity of MSW was studied on white rats of *Wistar* line and nonlinear white mice. The main criterion for the toxic effect was the dose that caused the death of 50% of the animals (LD_{50}). Six *Wistar* rats and 10 white mice were used to determine the mean lethal dose. Adult rats weighing 225–240 g were administered MSW at a rate of 21,300 mg kg^{-1} body weight. The preparation was administered in the native state. The fluid intake was 5 ml per rat. Adult white mice weighing 20–30 g were administered MSW at rates of 24,000 mg kg^{-1}, 34,000 and 36,000 mg kg^{-1}. The technique of administration of the preparation to the stomach was followed and the data on the amount of liquid allowed to the animals depending on the method of administration, their species and weight were taken into account (Sidorov, 1976) Within 14 days we performed clinical examination and determined the dynamics of animal body weight. The mean lethal dose of MSW was determined by probit analysis of lethality curves (Prozorovskiy, 1961). f it was impossible to calculate LD_{50}, the injected amount of the preparation, which did not cause animal death, was fixed.

The resorptive-toxic effect of MSW was studied on 6 white *Wistar* rats weighing 250 g (Kundiev, 1964). The criterion was the presence or absence of lethal cases, time and severity of intoxication manifestations. The preparation in native form was carefully applied to the clipped skin areas of rats at a rate of 8,000 mg kg^{-1}.

Statistical Analysis

MS Excel and the software Statistics, version 7.0, were used for the data analysis. The research results of acute oral toxicity of MSW, given as the mean ± standard error (*SE*). Significance was tested by applying the *Student t-test*. Values of less than 0.05 ($p < 0.05$) were considered significant.

RESULTS AND DISCUSSION

At the first stage of the research, the impact of MSW on soil chemical properties was determined. The main indicators of soil system stability were studied in the scientific chemical-analytical laboratory of Agroecological Monitoring of the Poltava State Agrarian University conducted for 5 years (2017–2021), in particular, the reaction of the soil solution, the content of nitrates, chlorides, mobile sulfur, heavy metals, and petroleum products (Table 1).This research is due to the fact that MSW, in addition to a variety of chemical elements, also contains heavy metals and residual amounts of petroleum products. The plots without MSW and plots which used the full mineral fertilizer $N_{50}P_{50}K_{50}$ were taken as control.

Table 1. Changes in soil chemical parameters when using MSW as the main fertilizer (average for 2017–2021)

Variants of the experiment	pH	Anions, mg kg^{-1}			Petroleum products, mg kg^{-1}	Heavy metals, mg kg^{-1}				Minera-lization, %
		NO_3^-	Cl^-	SO_4^{2-}		Hg	Cu	Pb	Zn	
1. Control	7.6	9.8	131	42.0	330	0.091	0.6	2	28	0.23
2. MSW, 300 L ha^{-1}	6.8	9.8	131	42.0	175	-	0.7	3	17	0.28
3. MSW, 600 L ha^{-1}	6.8	4.9	93	10.2	195	0.065	0.7	3	22	0.24
4. MSW, 900 L ha^{-1}	6.4	8.7	149	40.2	200	0.065	1.0	4	22	0.3
5. MSW, 1,200 L ha^{-1}	6.4	8.7	149	42.8	200	0.052	0.7	4	14	0.21
6. MSW, 2,400 L ha^{-1}	6.2	8.7	149	58.6	200	0.052	0.7	6	16	0.24
7. MSW, 4,800 L ha^{-1}	**5.2**	19.5	224	64.6	320	0.046	0.8	7	24	0.21
8. $N_{50}P_{50}K_{50}$	6.4	30.5	149	34.4	340	0.090	0.8	6	23	0.24

It was found that significant soil acidification occurs only when a dose of MSW higher than 2,400 L ha^{-1} is used. Thus, at the maximum dose of MSW 4,800 L ha^{-1}, pH of the soil solution was 5.2. It should be noted that the use of MSW in the soil solution does not increase the content of nitrates, but rather reduces them, although they are part of its composition. Also the use of MSW in doses of 300–2,400 L ha^{-1} does not contribute to the accumulation of petroleum products and heavy metals in the soil and there is no salinization of the soil. The previous studies (Pysarenko et al., 2021) found that the use of MSW as a herbicide is expedient only in the fields of cereal crops. Therefore, at the second stage, we studied the phytotoxicity of MSW to weeds on winter wheat crops (Table 2).

Table 2. Phytotoxicity of MSW to weeds on winter wheat crops (average for 2017–2021)

Plant	MSW concentration, %			Herbicide Desormone 600 (2.5 kg ha^{-1})
	100	75	50	
	Damage of leaf surface, %			
Caseweed	90.8	89.8	53.7	97.8
Field pennycress	88.9	83.2	38.6	98.0
Winter cress	94.2	86.9	40.4	98.5
Canada thistle	71.5	55.7	30.5	96.9

It was found that after treatment of winter wheat crops in the phase of spring tillering - the beginning of stem elongation, when wheat plants are not sensitive to MSW (Pysarenko et al., 2021), the damage to the leaf surface of weeds is significant. The highest loss of leaf surface was recorded after MSW treatment with a concentration of 100–75%. Burns of the leaf surface of the mentioned weed plants resulted in their death.

Table 3. Weediness of winter wheat crops after MSW treatment (average for 2017–2021)

Variants of the experiment	Biomass of weeds, g		Decrease of dry biomass, %
	Wet weight, g	Air-dry weight, g	
Control (without treatment)	49.2	8.3	-
MSW, 100% concentration	9.6	1.1	85.5
MSW, 75% concentration	11.9	1.9	84.1
MSW, 50% concentration	13.2	2.1	84.1

The data in Table 3 show that the number of weeds is higher on the control than after treatment with different concentrations of MSW. Weed biomass reduction (85.5%) was the largest when MSW concentration of 100% was used. Reducing the number of weeds on the crops leads to increased competitiveness of cultivated plants and improved harvesting conditions. Reduction of weeds on the crops was followed by the increase of winter wheat yields (Table 3).

At the third stage, we studied the effectiveness of MSW on winter wheat crops as a herbicide for 5 years (Table 4).

Table 4. Effects of treatment with different concentrations of MSW on productivity of winter wheat, t ha^{-1}

Variants of the experiment	2017	2018	2019	2020	2021	Average
1. Control (without treatment)	4.11	3.55	2.34	3.49	3.54	3.40
2. Herbicide Desormone, 2.5 kg ha^{-1}	4.74	4.51	3.22	4.42	4.56	4.29
3. MSW, 100% concentration	4,76	4.55	3.29	4.56	4.48	4.33
4. MSW, 75% concentration	4.69	4.49	3.15	4.42	4.27	4.20
5 MSW, 50% concentration	4.10	4.15	2.74	4.29	3.78	3.81

It was established that the average yield of winter wheat over five years was: on control - 3.40 t ha^{-1}, after treatment with herbicide Desormone (2.5 kg ha^{-1}) - 4.29 t ha^{-1}, which is 0.89 t ha^{-1} more than on the control. Yield after treatment with MSW (100% concentration) averaged 4.33 t ha^{-1}, which is 0.93 t ha^{-1} more than control. Yield after MSW treatment (75% concentration) averaged 4.20 t ha^{-1}, which is 0.80 t ha^{-1} more than control. Therefore, the application of 100% and 75% concentration of MSW as a herbicide on winter wheat crops increased the yield by 21.5% and 19.1%, respectively, compared with control.

In order to use any solutions as agrochemicals, it is necessary to carry out their assessment for the environment, warm-blooded animals and bees (Decree The Cabinet of Ministers of Ukraine, 1996). In accordance with World health organization (Executive Committee, 55th session, 1974), it is necessary to carry out their toxicity assessment by LD_{50} in advance. Thus, at the fourth stage, toxicological assessment of MSW was performed according to the following parameters: acute oral toxicity (low-hazard compounds more than 2,000 mg kg^{-1}), resorptive - toxic effect of MSW (low-hazard compounds more than 4,000 mg kg^{-1} (Executive Committee, 55th session, 1974).

Study of acute oral toxicity of MSW. The body weight dynamics of the experimental rats did not differ from the control ($p > 0.05$) (Table 4). It was found that the LD_{50} of MSW for rats in case of oral administration was more than 21,300 mg kg^{-1}.

During the first 1–2 hours after administration of the preparation, the mice showed symptoms of intoxication in the form of immobility, depression, rapid breathing, and movement coordination disorders. The general condition of the survived mice was satisfactory. On day 7, a 10% decrease in body weight gain was observed ($p < 0.05$) (Table 5). It was found that the LD_{50} of MSW for mice when administered orally exceeds 31,000 mg kg^{-1}. According to the parameters of acute oral toxicity for rats and mice, MSW belongs to low-toxic substances according to Executive Committee, 55th session, 1974 (for acute oral toxicity, substances over 2,000 mg kg^{-1} are low-hazard). Variability in species and sex sensitivity to the preparation is insignificant.

Table 5. The research results of acute oral toxicity of MSW

Group of animals	Research period, days		
	0	7	14
	The average weight of rats with acute oral effect of MSW		
Control	229.2 ± 6.2	238.3 ± 6.2	246.7 ± 5.3
Experimental (21,300 mg kg^{-1})	235.0 ± 2.6	240.8 ± 2.6*	250.0 ± 4.4
	Dynamics of body weight of mice under acute oral effect of MSW		
Control	22.6 ± 0.31	26.9 ± 0.92	24.8 ± 0.22
24,000 mg kg^{-1}	21.7 ± 0.25	26.0 ± 0.56*	23.7 ± 0.57
34,000 mg kg^{-1}	22.1 ± 1.30	26.4 ± 0.22*	24.1 ± 0.63
36,000 mg kg^{-1}	21.9 ± 0.54	26.0 ± 0.32*	23.9 ± 1.02

* $p < 0.05$ comparing with control.

The study of the resorptive-toxic effect of MSW. The symptoms of intoxication and death of animals during application of the preparation and throughout the 14-day period were not noted. General condition of the animals and their behavior did not differ from that of the control animals. There was no irritation on the site of MSW application. Thus, MSW had no resorptive-toxic impact on the body of animals when it was put on the skin. The LD_{50} of MSW for rats when applied to the skin is more than 8,000 mg kg^{-1} body weight. According to Executive Committee, 55th session, 1974 (under the resorptive-toxic effect low-hazard substances make more than 4,000 mg kg^{-1}). Therefore, it was determined that MSW belongs to hazard class IV - low-hazard compounds.

CONCLUSIONS

The safety of MSW for the environment in the recommended doses was established as a result of the research. Assessment of MSW impact on soil chemical properties showed that significant soil acidification occurs only when MSW doses higher than 2,400 L ha^{-1} are used. The content of nitrates and petroleum products did not increase and there was no soil salinization when MSW was used in doses of 300–1,200 L ha^{-1}.

The study of MSW phytotoxicity to weeds found that the highest loss of leaf surface was observed after treatment with MSW of 100–75% concentration. The usage of 100% concentration of MSW in a dose of 350 L ha^{-1} resulted in the largest reduction of weed biomass (85.5%). Consequently, winter wheat yield increased by 21.5% when using 100% MSW and by 19.1% when using 75% MSW, while the weediness of crops decreased. As a result of the toxicological assessment of MSW it was found that it belongs to low-toxic compounds. When administered orally, the LD_{50} of white rats is more than 21,000 mg kg^{-1}, of mice - 31,000 mg kg^{-1}; when applied to the skin of rats - more than 8,000 mg kg^{-1}. The value of LD_{50} for female rats is more than 5,000 mg kg^{-1}. The results of the ecotoxicological research of MSW allow us to assert that its application is safe in agriculture, in particular, as an environmentally friendly organomineral fertilizer and herbicide.

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Integrative effects of biostimulants and salinity on vegetables: Contribution of bioumik and Lithovit®-urea50 to improve salt-tolerance of tomato

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Abstract. The separate and combined effect of lithovit-urea50 and bioumik was tested on salt-stressed tomato crops. Salinity was induced using three different NaCl solutions (2, 4 and 8 dS m^{-1}). Under the salinity effect, all aspects of plant growth were inhibited. Total chlorophyll and carotenoids reduced from mg g^{-1} FW and 1.1 mg g^{-1} FW at 2 dS m^{-1} to reach 1.01 mg g^{-1} FW and 0.66 mg g^{-1} FW at 8 dS m^{-1} in control plants. Plants treated by the combination of both products had the highest chlorophyll and carotenoids content with 2.24 mg g^{-1} FW and 1.34 mg g^{-1} FW, 1.88 mg g^{-1} FW and 1.05 mg g^{-1} FW, and 1.39 mg g^{-1} FW and 0.86 mg g^{-1} FW respectively at 2, 4 and 8 dS m^{-1}. Treating plants by this combination maximized flower number, fruit weight, yield and fruit diameter at 2 dS m^{-1} (17 flowers, 47.93 g, 431.1 g $plant^{-1}$ and 3.23 cm respectively) and 4 dS m^{-1} (15flowers, 36.45 g, 291.85 g $plant^{-1}$ and 2.8 cm respectively). The separate application of bioumik minimized cell electrolyte leakage at 2 dS m^{-1} (8.82%) compared to control (11.43%). Additionally, plants treated by lithovit-urea and bioumik had the highest relative water content with 107.3%, 96.5% and 91.2% respectively at 2, 4 and 8 dS m^{-1}. N, Ca and Mg in roots were significantly the highest at 2 dS m^{-1} (4.5%, 2.6% and 0.5% respectively), at 4 dS m^{-1} (3.74%, 2.49% and 0.48% respectively) and at 8 dS m^{-1} (3.21%, 2.61% and 0.32% respectively). K content in roots was maximized following the separate application of bioumik with 3.21% at 2 dS m^{-1} and 2.55% at 8 dS m^{-1}. Conclusively, lithovit-urea and bioumik helped plants in tolerating salt-stress with an optimal effect obtained after their combination.

Key words: biostimulants, growth, physiology, salinity, small scale fertilizers, tomato.

INTRODUCTION

Tomato crop is one of the most important horticultural crops with a total production volume of 180 million tons and a cultivated area of 5 million ha. China, USA and India are the top producers worldwide with 36.6, 12.8 and 11.7 million tons produced annually, respectively (FAOSTAT, 2021). This crop is considered as moderately sensitive to salinity tolerating a nutrient solution not exceeding 2.4 to 4 dS m^{-1}. Above this level, all aspects of plant growth and production is affected (Cuartero & Fernández-Muñoz, 1998; Bustomi et al., 2014). Salinity inhibits the development of roots and aerial parts and causes a drastic reduction in the yielding capacity of stressed tomato plants. The primary adverse effect caused by salinity is the osmotic stress due to the hyperaccumulation of sodium in the root zone and plant parts. This stress inhibits water and vital nutrient movement in the plant and causes subsequent alteration in physiological and metabolic processes including photosynthesis and cell division (Shimul et al., 2014; Zhang et al., 2016; Zhang et al., 2017). Plants have developed various means to withstand salinity such as the endogenous accumulation of organic solutes including sucrose, sorbitol, and mannitol (Flowers & Colmer, 2015) and, enzymatic (ascorbate peroxidase, peroxidase, catalase) and non-enzymatic (glutathione and glutathione reductase) antioxidants (Blokhina et al., 2003). In the same context, researchers have tried to apply exogenously different compounds, similar to those accumulated naturally in stressed-plants, as a method to improve the salt-tolerance of the crop. In previous studies, foliar spraying of osmoprotectans (glycine betaine), auxin-like substances (acetyl salicylic acid), nutrient-rich fertilizers (monopotassium-phosphate) and sugar alcohols was highly efficient (Gul et al., 2017; Sajyan et al., 2018, 2019a, 2019b, 2019c; Issa et al., 2020). Such implementation counteracted the negative impacts of salinity through an improvement in nutrient content, photosynthetic pigments and plant physiology. Consequently, the use of these components maximized the vegetative growth and yielding capacity of stressed plants compared to the non-treated ones.

Other methods adopted included the use of biostimulants as priming material or through direct application on plants. In the agricultural industry, the manufacturing of biostimulants is a witnessing a rapid growth as an efficient tool of yield promoters as well as pre-stress conditioners (Yakhin et al., 2017). The application of plant derived protein hydrolysate increased vegetative performance and boosted the photosynthetic apparatus of salt-stress lettuce crops (Lucini et al., 2015). Similar ameliorative effects were reported following the use of licorice root extract on *Phaseolus vulgaris* under salinity stress (Rady et al., 2019). Furthermore, the use of *Moringa oleifera* extracts enhanced hormones and nutrient content in stressed sorghum, and promoted enzymatic and non-enzymatic antioxidants (Desoky et al., 2018).

Other groups of biostimulants including small sized-biofertilizers are bein g Lately tested on crops subjected to salinity. Such components include lithovit-standard and lithovit-guano 25 which were previously sprayed on tomato, eggplant and pepper under saline conditions, and alleviated the negative effect induced by this abiotic stress (Sajyan et al., 2019d, 2019e; Issa et al., 2020; Sajyan et al., 2020). Products such as lithovit-urea50, a nitrogenous rich fertilizer, were not previously tested under environmental stress. However, previous studies implemented this product in mushroom production mainly *Pleurotus ostreatus.* As a result, lithovit-urea50 applied at different timing maximized production and qualitative attributes of the produced mushroom (Naim et al., 2020; Sassine et al., 2021). Bioumik is another similar product that has been less applied on vegetables under abiotic stress. Bioumik is a balanced biological method combining microorganisms and micronutrients (iron, zinc, manganese, magnesium, molybdenum, and calcium) of humic and folic acid compounds with amino acids. Accordingly, soil fertility is enhanced and the ability of the plant to absorb vital elements is ameliorated with this product (Biozar, 2021).

Based on their composition, lithovit-urea50 and bioumik seemed to be highly beneficial when applied to tomato crops irrigated with saline solutions. The current trial aimed to study for the first time the separate and combined effect of lithovit-urea50 and bioumik on the performance of salt-stressed tomato crop.

MATERIALS AND METHODS

Treatments and measurements

Tomato seeds (var. Sila) were sterilized in 0.1% sodium hypochlorite for 30 min, washed with distilled water and sown in plastic trays. After 30 days, uniform seedlings having 3 to 4 true leaves were transplanted in pots containing a mixture of peat and soil. Plants were kept in open-field conditions with a temperature 20 ± 5 °C and a relative humidity of 70% during the growing period which lasted 100 days (from May to August/ 2020). Two products were applied through foliar spraying: lithovit-urea (5 g L^{-1}) and bioumik (5 g L^{-1}). These products were applied in a separate or a combined form on salt-stressed tomato irrigated by three different NaCl solutions (2, 4 and 8 dS m^{-1}). Lithovit-urea is manufactured by Tribodyn/Germany and has the following composition: 33% $CaCO_3$, 21% N total nitrogen, 18.5% CaO, 6.5% SiO_2, 1.2% MgO, 0.5% Fe and 0.01% Mn. Bioumik is manufactured by Biozar/Iran and has the following composition: 5% Fe, 3% Zn, 0.36% Ca, 2% Mn, 0.36% Mg, 3% K, 0.1% Mo, 10% humic acids. Products were applied three times during plant growth cycle at 15, 30 and 45 days after transplantation (DAT). Salinity was induced using three different NaCl solutions with different EC levels; 2, 4 and 8 dS m^{-1}. Saline irrigation started at 20 DAT with an interval of 2 days. The EC of the saline solution was continuously monitored. At each salinity level, enough drainage was allowed until obtaining an $EC_{water\ drainage} = EC_{irrigation\ solution}$. Adjustment of $EC_{water\ drainage}$ was done using the corresponding saline solution. The experiment was arranged as randomized completely block design with three replications. Two experimental factors with different levels were studied; 'Salinity' including three levels (three NaCl solutions: 2, 4 and 8 dS m^{-1}) and product application including 4 levels (control: no application, lithovit-urea, bioumik and lithovit-urea + bioumik).

During the growth cycle, several measurements were taken to study the effect of salinity and treatments. Vegetative traits included, plant height, leaf number, stem diameter and weight of plants parts. For the determination of fresh weights plants were removed from pots, washed to removed adherent soil, then, separated into roots, stems and leaves. First, fresh weight of different part was measured using a digital balance. Afterwards, plant parts were oven dried at 70 °C until a constant weight was obtained. Additionally, photosynthetic pigments including chlorophyll content and carotenoids were also determined in leaves as described by Sassine et al. (2020). Cell electrolyte leakage and relative water content were measured also on leaves as described by Sajyan et al. (2020). Nutrient content in roots and shoots were measured on ash of roots and shoots ground, heated to 550 °C and dissolved in diluted HCl with a few drops of nitric acid as described by Cottenie et al. (1982). Relative water content in leaves was measured as described by Mata & Lamattina (2001). Fruit traits included flower number, cluster number, fruit number, fruit fresh weight, yield and fruit diameter.

Statistical analysis

Data was subjected to analysis of variance using Statistical Package for Social Sciences (SPSS) software version 25® software. Means were compared by Duncan's multiple range tests at $P \leq 0.05$.

RESULTS

Vegetative traits

Vegetative growth on stressed tomato was inhibited under the effect of salinity. Plant height, leaf number and stem diameter were the lowest at 8 dS m^{-1} in all plants (Table 1). Treating plants with lithovit-urea, bioumik and their combination helped in reducing the adverse effect of salinity. In specific, spraying plants with a combination of both products maximized plant height and stem diameter with 70.48 cm and 1.41 cm, 48.63 cm and 1.2 cm respectively at 2 and 4 dS m^{-1}. A separate application of bioumik maximized leaf number under all EC levels.

Table 1. Vegetative traits of tomato as affected by salinity and treatments

	PH (cm)	LN	SD (cm)
2 dS m^{-1}/ Control	48.10de	10.00d	1.00c
2 dS m^{-1}/ lithovit-urea	56.90c	11.75b	1.30ab
2 dS m^{-1}/ bioumik	61.53b	15.25a	1.20abc
2 dS m^{-1}/lithovit-urea+bioumik	70.48a	12.00b	1.41a
4 dS m^{-1}/ Control	40.15h	10.00d	1.07c
4 dS m^{-1}/ lithovit-urea	47.65de	11.25bc	1.10bc
4 dS m^{-1}/ bioumik	44.55fg	11.75b	1.20abc
4 dS m^{-1}/lithovit-urea+bioumik	48.63d	10.00d	1.20abc
8 dS m^{-1}/ Control	32.50i	8.75e	0.80d
8 dS m^{-1}/ lithovit-urea	46.40ef	10.00d	1.10bc
8 dS m^{-1}/ bioumik	44.00g	10.25cd	1.10bc
8 dS m^{-1}/lithovit-urea+bioumik	43.25g	9.75de	1.20abc

Means ($n = 9$) followed by the same letter within each column are not significantly different according to Duncan's multiple range tests. PH – plant height; LN – leaf number; SD – stem diameter.

Fresh and dry weights of plants parts (Table 2) was similarly inhibited with salinity and improved by different treatments. It was observed that spraying tomato with a combination of bioumik and lithovit-urea maximized fresh and dry weight of roots, stems and leaves almost under all EC levels. For instance, fresh weights of roots of plants treated by the combination significantly higher significantly compared to control at all EC levels. This ameliorative effect was similarly observed with a less extent in the remaining treatments compared to control at all EC levels. For instance, spraying plants by both products separately or in combination doubled fresh weight of leaves compared to control at 4 and 8 dS m^{-1}.

Table 2. Vegetative traits of tomato as affected by salinity and treatments

	FWR (g)	DWR (g)	FWS (g)	DWS (g)	FWL (g)	DWL (g)
2 dS m^{-1}/ Control	6.83de	1.70de	27.47c	6.62bcd	57.33f	12.30d
2 dS m^{-1}/ lithovit-urea	8.00bc	2.00bc	34.37a	7.62a	95.67ab	13.67cd
2 dS m^{-1}/ bioumik	8.65b	2.15ab	33.53a	7.52ab	96.30ab	14.30bc
2 dS m^{-1}/lithovit-urea+bioumik	10.23a	2.30a	36.00a	6.76abcd	100.33a	16.39a
4 dS m^{-1}/ Control	6.00fg	1.66de	20.87ef	5.79d	40.47g	10.10e
4 dS m^{-1}/ lithovit-urea	7.06de	1.83cd	24.20d	6.48cd	84.33de	13.33cd
4 dS m^{-1}/ bioumik	7.45cd	1.89cd	24.33d	6.01cd	93.33b	14.30bc
4 dS m^{-1}/lithovit-urea+bioumik	8.27b	2.20ab	30.63b	6.77abc	91.67bc	15.73ab
8 dS m^{-1}/ Control	5.40g	1.70de	19.50f	5.88cd	40.00g	10.07e
8 dS m^{-1}/ lithovit-urea	6.00fg	1.66de	24.33d	6.35cd	80.20e	14.00bcd
8 dS m^{-1}/ bioumik	6.06fg	1.58e	23.20de	6.08cd	87.17cd	13.40cd
8 dS m^{-1}/lithovit-urea+bioumik	6.60ef	1.76de	28.60bc	6.57cd	85.67d	15.00abc

Means (n = 9) followed by the same letter within each column are not significantly different according to Duncan's multiple range tests. FWR – fresh weight of roots; DWR – dry weight of roots; FWS – fresh weight of stems; DWS – dry weight of stems; FWL – fresh weight of leaves; DWL – dry weight of leaves.

Photosynthetic pigments, cell electrolyte leakage and relative water content

Under salinity effect, photosynthetic pigments including chlorophyll a, b and carotenoids were significantly reduced (Table 3). In control plants, total chlorophyll and carotenoids reduced from 1.6 mg g^{-1} FW and 1.1 mg g^{-1} FW, respectively at 2 dS m^{-1} to a reach minimum of 1.01 mg g^{-1} FW and 0.66 mg g^{-1} FW, respectively at 8 dS m^{-1}.

Table 3. Photosynthetic pigments of tomato as affected by salinity and treatments

	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	T Chl (mg g^{-1} FW)	Car (mg g^{-1} FW)
2 dS m^{-1}/ Control	0.98de	0.62c	1.60c	1.10b
2 dS m^{-1}/ lithovit-urea	1.21bc	0.71b	1.92b	1.11b
2 dS m^{-1}/ bioumik	1.31ab	0.66bc	1.97b	1.22ab
2 dS m^{-1}/lithovit-urea+bioumik	1.42a	0.82a	2.24a	1.34a
4 dS m^{-1}/ Control	0.87ef	0.41e	1.28de	0.80de
4 dS m^{-1}/ lithovit-urea	1.15bcd	0.52d	1.67c	0.85d
4 dS m^{-1}/ bioumik	1.11cd	0.51d	1.62c	0.90cd
4 dS m^{-1}/lithovit-urea+bioumik	1.22bc	0.66bc	1.88b	1.05bc
8 dS m^{-1}/ Control	0.69f	0.32f	1.01f	0.66e
8 dS m^{-1}/ lithovit-urea	0.90e	0.43e	1.33de	0.80de
8 dS m^{-1}/ bioumik	0.85ef	0.32f	1.17ef	0.85d
8 dS m^{-1}/lithovit-urea+bioumik	0.86ef	0.53d	1.39d	0.86d

Means (n = 3) followed by the same letter within each column are not significantly different according to Duncan's tests. Chl a – chlorophyll a; Chl b – chlorophyll b; T Chl – total chlorophyll; Car – carotenoids.

Reductions caused by salinity were lowered following the application of different products in separate or combined form. These treatments increased significantly the content in photosynthetic pigments of stressed tomato compared to control at all EC levels. A maximum ameliorative effect was detected after spraying bioumik and lithovit-urea in combination. Plants treated by this combination had significantly higher total chlorophyll and carotenoids content compared to all the remaining treatments including control at all EC levels with 2.24 mg g^{-1} FW and 1.34 mg g^{-1} FW, 1.88 mg g^{-1} FW and 1.05 mg g^{-1} FW, and 1.39 mg g^{-1} FW and 0.86 mg g^{-1} FW respectively at 2, 4 and 8 dS m^{-1}. On the contrary, cell electrolyte leakage was improved by increasing in salinity peaking in control plants at 8 dS m^{-1} (35.43%) (Table 4). The separate application of bioumik minimized cell electrolyte leakage at 2 dS m^{-1} (8.82%) compared to control (11.43%). Additionally, the application of bioumik and lithovit-urea in combination minimized this trait at 4 and 8 dS m^{-1} with 13.4% and 26.6% respectively. Finally, although relative water content was lowered with salinity, however, treating plants by both products improved significantly such trait. When comparing between treatments, it was obvious that the combination of both products was optimal on this trait at all EC levels. Plants treated by lithovit-urea and bioumik had the highest relative water content with 107.3%, 96.5% and 91.2% respectively at 2, 4 and 8 dS m^{-1}. In general, all treatments induced a significant improvement in relative water content except at 8 dS m^{-1}.

Table 4. Cell electrolyte leakage and relative water content of tomato as affected by salinity and treatments

	CEL (%)	RWC (%)
2 dS m^{-1}/ Control	11.43fg	91.97cd
2 dS m^{-1}/ lithovit-urea	9.49g	101.57b
2 dS m^{-1}/ bioumik	8.82g	100.53b
2 dS m^{-1}/lithovit-urea+bioumik	8.89g	107.30a
4 dS m^{-1}/ Control	19.43c	80.87g
4 dS m^{-1}/ lithovit-urea	15.23de	89.47de
4 dS m^{-1}/ bioumik	17.47cd	87.43def
4 dS m^{-1}/lithovit-urea+bioumik	13.40ef	96.50bc
8 dS m^{-1}/ Control	35.43a	82.60fg
8 dS m^{-1}/ lithovit-urea	28.40b	84.40efg
8 dS m^{-1}/ bioumik	26.67b	88.23def
8 dS m^{-1}/lithovit-urea+bioumik	26.60b	91.20cd

Means (n = 3) followed by the same letter within each column are not significantly different according to Duncan's multiple range tests. CEL – cell electrolyte leakage; RWC – relative water content.

Nutrient content in roots and shoots

As shown in Table 5, the increase in salt-stress from 2 to 8 dS m^{-1} caused a significant reduction in nutrient content including N, P, K, Ca and Mg in both treated and non-treated plants. On the other hand, a significant increase almost in all macronutrients content was observed following the application of different treatments. The increase peaked following foliar spraying of the combination of lithovit-urea and bioumik. For instance, N, Ca and Mg in roots were significantly the highest at 2 dS m^{-1} (4.5%, 2.6% and 0.5% respectively), at 4 dS m^{-1} (3.74%, 2.49% and 0.48% respectively) and at 8 dS m^{-1} (3.21%, 2.61% and 0.32% respectively). Additionally, K content in roots was maximized following the separate application of bioumik with 3.21% and 2.55% respectively at 2 and 8 dS m^{-1}. P content in roots was significantly improved by product application only at 8 dS m^{-1} following the application of bioumik alone or in combination with lithovit-urea. When comparing between both products applied in a separate form, it was observed that application of bioumik was slightly better than lithovit-urea. N, K, Ca and Mg content in roots of plants treated by bioumik were higher than those of plants treated by lithovit-urea. The application of different treatments reduced significantly Na accumulation in roots. Na content was minimized following bioumik application at 2 (0.28%) and 4 dS m^{-1} (0.31%), and lithovit-urea application at 8 dS m^{-1} (0.45%).

Table 5. Nutrient content in roots of tomato as affected by salinity and treatments

	N (%)	P (%)	K (%)	Ca (%)	Na (%)	Mg (%)
2 dS m^{-1}/ Control	3.02d	0.63ab	3.04ab	2.21de	0.61c	0.22de
2 dS m^{-1}/ lithovit-urea	4.00b	0.66a	3.07ab	2.42abcd	0.32g	0.44abc
2 dS m^{-1}/ bioumik	4.42a	0.59abcd	3.21a	2.45abc	0.28g	0.46ab
2 dS m^{-1}/lithovit-urea+bioumik	4.50a	0.50def	2.98bc	2.60a	0.30g	0.50a
4 dS m^{-1}/ Control	2.55e	0.51cdef	2.59ef	2.11e	0.82b	0.16e
4 dS m^{-1}/ lithovit-urea	3.22d	0.50def	2.57ef	2.37bcd	0.51def	0.32cd
4 dS m^{-1}/ bioumik	3.66c	0.56bcde	2.75de	2.45abc	0.31g	0.34bcd
4 dS m^{-1}/lithovit-urea+bioumik	3.74c	0.51cdef	2.81cd	2.49ab	0.44f	0.48a
8 dS m^{-1}/ Control	1.70g	0.45f	2.50f	1.60f	0.96a	0.16e
8 dS m^{-1}/ lithovit-urea	2.20f	0.49ef	2.52f	2.03e	0.45ef	0.23de
8 dS m^{-1}/ bioumik	3.00d	0.60abc	2.55ef	2.24cde	0.60cd	0.29de
8 dS m^{-1}/lithovit-urea+bioumik	3.21d	0.55bcde	2.47f	2.61a	0.54cde	0.32cd

Means (n = 3) followed by the same letter within each column are not significantly different according to Duncan's multiple range tests.

The content of nutrient in shoots (Table 6) was similarly influenced by salinity and product application. N and Ca in shoots were significantly the highest after the application of a combination of lithovit-urea and bioumik at 2 dS m^{-1} (4.5% and 3.1% respectively) and at 4 dS m^{-1} (4.31% and 2.8% respectively). P content did not significantly improve following the application of any treatment at all EC levels.

Table 6. Nutrient content in shoots of tomato as affected by salinity and treatments

	N (%)	P (%)	K (%)	Ca (%)	Na (%)	Mg (%)
2 dS m^{-1}/ Control	3.44d	0.88a	4.72bc	2.50de	0.41c	0.40cd
2 dS m^{-1}/ lithovit-urea	4.25bc	0.81a	4.74bc	3.00ab	0.31de	0.58b
2 dS m^{-1}/ bioumik	4.40ab	0.91a	4.85ab	2.95ab	0.25e	0.60ab
2 dS m^{-1}/lithovit-urea+bioumik	4.54a	0.79ab	5.00a	3.10a	0.26e	0.70a
4 dS m^{-1}/ Control	3.01e	0.66bc	4.50c	2.40e	0.56b	0.32de
4 dS m^{-1}/ lithovit-urea	4.00c	0.79ab	4.57c	2.66cd	0.35cd	0.43cd
4 dS m^{-1}/ bioumik	4.25bc	0.79ab	4.70bc	2.77bc	0.40c	0.50bc
4 dS m^{-1}/lithovit-urea+bioumik	4.31ab	0.80ab	4.60bc	2.80bc	0.30de	0.50bc
8 dS m^{-1}/ Control	2.00f	0.55c	3.37e	1.90f	0.66a	0.28e
8 dS m^{-1}/ lithovit-urea	3.19de	0.59c	3.72d	2.30e	0.52b	0.40cd
8 dS m^{-1}/ bioumik	3.43d	0.61c	3.80d	2.40e	0.50b	0.45c
8 dS m^{-1}/lithovit-urea+bioumik	3.40d	0.57c	3.60d	2.43e	0.53b	0.41cd

Means (n = 3) followed by the same letter within each column are not significantly different according to Duncan's multiple range tests.

K content responded differently according to EC level; it was maximized by the combination of both products at 2 dS m^{-1} (5%) and by the separate application of bioumik at 4 (4.7%) and 8 dS m^{-1} (3.8%). Mg was affected similarly by product application. Finally, Na content was significantly reduced compared to control following the application of all treatments. It was the lowest in plants sprayed by bioumik at 2 (0.25%) and 8 dS m^{-1} (0.5%) and by the combination at 4 dS m^{-1} (0.3%).

Fruit traits

As shown in Table 7, salinity caused a significant reduction in fruit traits. In control plants, number of flowers, number of fruits, fruit weight, yield and fruit diameter were reduced from 11 flowers, 6fruits, 29.37 g, 176.12 g plant^{-1} and 2.21 cm, respectively at 2 dS m^{-1} to reach a minimum of 9 flowers, 4.25 clusters, 25.38 g, 107.95 g plant^{-1} and 2.02 cm respectively at 8 dS m^{-1}. All treatments applied helped in reducing the adverse effect caused by salinity on these traits. For instance, the number of clusters was increased by 1 to 2 clusters in treated plants compared to control at all EC levels. Additionally, the application of lithovit-urea and bioumik in combination maximized flower number, fruit weight, yield and fruit diameter at 2 dS m^{-1} (Table 7). Fruit number was maximized by the application of bioumik at 2 (8.25 fruits), 4 (8 fruits) and 8 dS m^{-1} (7.25fruits).

Table 7. Fruit traits of tomato as affected by salinity and treatments

	CN	Fl N	Fr N	FW (g)	Yield (g plant^{-1})	FD (cm)
2 dS m^{-1}/ Control	3.00bc	11.00fg	6.00de	29.37ef	176.12ef	2.21g
2 dS m^{-1}/ lithovit-urea	5.00a	14.00cd	7.00cd	40.48b	284.10c	2.70bc
2 dS m^{-1}/ bioumik	4.75a	16.00ab	8.25ab	41.95b	345.25b	3.08a
2 dS m^{-1}/lithovit-urea+bioumik	5.00a	17.00a	9.00a	47.93a	431.10a	3.23a
4 dS m^{-1}/ Control	3.25bc	10.00gh	5.00ef	28.40f	141.85fg	2.30fg
4 dS m^{-1}/ lithovit-urea	4.00ab	12.00ef	6.00de	31.00e	186.00e	2.60cd
4 dS m^{-1}/ bioumik	4.00ab	14.00cd	8.00abc	35.00cd	280.13c	2.50de
4 dS m^{-1}/lithovit-urea+bioumik	4.75a	15.00bc	8.00abc	36.45c	291.85c	2.80b
8 dS m^{-1}/ Control	2.50c	9.00h	4.25f	25.38g	107.95g	2.02h
8 dS m^{-1}/ lithovit-urea	4.00ab	12.00ef	7.00cd	30.63e	214.53de	2.31fg
8 dS m^{-1}/ bioumik	4.00ab	12.00ef	7.25bc	33.48d	241.35d	2.50de
8 dS m^{-1}/lithovit-urea+bioumik	4.00ab	13.00de	6.00de	34.23d	205.93de	2.40ef

Means (n = 9) followed by the same letter within each column are not significantly different according to Duncan's multiple range tests. CN – cluster number; Fl N – flower number; Fr N – fruit number; FW – fruit weight; FD – fruit diameter.

DISCUSSION

Increasing in salinity had inhibitory effects on all traits. It caused a significant reduction in plant height, leaf number and stem diameter. In previous studies, similar inhibitory effects caused by saline conditions were reported (Sajyan et al., 2018; Mahmoud et al. 2020). As mentioned, such effect is due primarily to an osmotic stress upcoming from salt accumulation in the roots, reducing shoot growth rate and inhibiting cell division and expansion (Liang et al., 1996). High Na+ content in plants reduces the absorption of fundamental nutrient including K, Ca, Mg and others. On pepper crop, an increase in salinity up to 6 dS m^{-1} caused a reduction in nutrient uptake and, an accumulation in Na content up to two-fold (Sajyan et al., 2020). Abdeldym et al. (2020) reported an inhibition in water movement reducing fresh weights of plants and increasing dry matter. In the current study, comparable negative results were observed including reduction, in fresh weights of plant parts and in macronutrients accumulation (Table 2, 5 and 6).

On the other hand, lithovit-urea50 and bioumik applied separately or in combination induced tolerance of tomato to salinity especially when comparing to non-treated plants. Regarding the separate effect of these products, it was observed that bioumik was better than lithovit-urea. Plants treated with the former product had more or less higher values for almost all the studied traits with some exceptions. In fact, both products include in their composition many nutrients such as nitrogen and others. However, bioumik contains humic, folic and amino acids which are not found in lithovit-urea50.

In fact, bioumik was less applied previously on crops. Therefore, the exact mechanism behind the ameliorative effect is still poorly understood. However, based on the current results, it was observed that the separate use of this product rich in humic, fulvic and amino acids and in nutrients was significantly efficient. Foliar spray of humic acid to leaves promoted growth in cucumber (Canellas et al., 2015; De Hita et al., 2020). According to these reports, an increase in hormonal concentration mainly auxins in roots and shoots was associated with the application of humic substances which might enhanced the rate of growth in all plant parts including roots, leaves and stems. These results were translated in the current study following the application of bioumik especially when observing fresh and dry weights of plant parts. Additionally, increasing in humic substances in plant parts was associated with a decrease in abscisic acid (ABA) contributing also in the stimulation of plant growth. ABA is well known to be associated with leaf senescence and inhibition in shoot growth (Ghanem et al., 2008; Vysotskaya et al., 2018). Other reports also coupled the application of humic substances with an increase in nutrient content (N, K, Ca, Mg, and P absorption) and a decrease in toxic elements including Na in maize and garden cress under salt stress (Elmongy et al., 2018; Kaya et al., 2018). Bioumik containing both organic substances (fulvic and humic acids) and nutrients maximized all nutrient content in roots and shoots of tomato crop subjected to salinity (Table 5 and 6) especially when combined to lithovit-urea50.

Furthermore, the application of bioumik caused a significant increment in photosynthetic pigments which were inhibited by salinity. The possible reason behind this effect is the combination of organic acids with nutrient in plant foliage and roots. This combination was maximized when bioumik and lithovit-urea50 were combined. In other terms, organic acids found in bioumik might have cause reduction in pH, and a release for cationic element such as Fe required for photosynthesis (Latif & Mohamed, 2016). Similar effects were observed in many previous studies (Akladious & Mohamed, 2018; Kaya et al., 2018). In this way, the enrichment in organic acids increased salt-tolerance of crops through an increase in rubisco enzyme (Latif & Mohamed, 2016). The already discussed effect was boosted after the addition of lithovit-urea50 containing vital elements required for chlorophyll formation such as Fe and Mg. The combination of bioumik with lithovit-urea50 boosted the immunity system of tomato plants by minimizing sodium accumulation starting from roots to shoots, and by maximizing nutrient uptake. This combination also boosted relative water content in leaves reflecting a better water movement in the plant under salt-stress compared to non-treated plants. This stimulatory effect was translated by an increase in weight, size and number of tomato fruits. Consequently, yielding capacity of tomato crop was maximized. Basically, as mentioned in previous sections, the presence of organic acids and nutrients (found in both products) promoted fruit set and growth through a decrease in ABA which cause fruit abscission under salinity. The presence of vital elements in both products such as calcium and phosphorus ensured good conditions for fruit set and for fruit growth. Similar stimulatory effects were observed following the implementation of lithovit-urea50 in mushroom production at different timings; yields produced were maximized at all flushes. Additionally, the accumulation of nutrient and amino acids was promoted after the use of this product (Naim et al., 2020; Sassine et al., 2021).

The implementation of these products helped plants in withstanding salinity by ensuring a balanced nutritional status coupled with a protected cell membrane reducing electrolyte leakage from cells. In this way, Na uptake was inhibited under saline conditions, and photosynthetic machinery of the plants was re-established.

CONCLUSIONS

From the current study, it was revealed the combination of bioumik with lithovit-urea50 maximized almost all the studied traits including vegetative, physiological and reproductive attributes of tomato crops compared to non-treated plants. Additionally, in this combination an adjuvant rather than antagonistic effect was evidenced between lithovit-urea50 and bioumik.

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Using data of optic sensors and pigment content in leaves for efficient diagnostics of nitrogen nutrition

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Abstract. Opportune monitoring and diagnostics of a condition of crops permit to make prompt and proper activities on dressing nitrogen fertilizers. This will allow the plants to use the nitrogen applied efficiently, and therefore reduce their use in field. Since nitrogen that has not been utilized by plants is able to escape into the atmosphere or be washed out of the soil with water. The most accurate diagnostic method is to determine the chemical composition of plants, but it takes quite a long time and requires laboratory conditions, which is not always possible in the field. One of the promising methods is photometric diagnostics of crops using optical instruments. Experiment is carried out in contrasting weather conditions, on soddy-podzolic soil with spring barley and spring rapeseed being investigated. Results of research show the efficiency of using optic sensors (N-testers) for efficient diagnostics of nitrogen nutrition of plants. The readings of the device (N-tester) were compared with the concentration of *a* and *b* chlorophyll, determined by a chemical method. Results of diagnostics with portable photometric device 'Yara' are correlating with concentrations of chlorophylls *a* ($r = 0.96$) and *b* ($r = 0.91$) in spring rapeseed. Moreover, correlation of rapeseed yield and concentrations of chlorophylls *a* and *b* has quantity and inverse relation similar to device indication ($r = -0.81$ and $r = -0.70$ respectively). Results of diagnostics with N-tester 'Spectroluxe' are strongly correlating with chlorophyll concentration. Device indication correlates stronger with chlorophyll *b* concentration in spring barley and chlorophyll *a* concentration in spring rapeseed (rapeseed was investigated in dryer conditions). Thus, such a modern optical device as N-tester, whose action is based on measuring the concentration of leafy chlorophyll, can replace chemical methods and increase the efficiency of nitrogen fertilization, which means increasing the productivity of plants and reducing the negative impact of unreasonable use of nitrogen fertilizers.

Key words: chlorophyll fluorescence, fertilizers, optical sensors, nitrogen status, ammonium nitrate, spring barley, spring rape, plant diagnostics.

INTRODUCTION

An increasing the crop yields and the efficiency of fertilizers applied were the main goals of scientists at all times. Often, high doses of nitrogen fertilizers are used in tillages that are an insurance against ignorance of the soil fertility degree. This practice leads to a decrease in the efficiency of nitrogen utilization by plants, an excess nitrogen residue in soil and in crop products (Ferguson et al., 2002; Hashimoto et al., 2007; Tremblay, 2012; Hatamian, 2020). Determination of the nitrogen concentration in soil does not always give the desired result, since nitrogen is quite labile in soil (Gamzikov, 2018). At the same time, plants are a fairly accurate indicator in determining their availability of nitrogen. Various types of checking are used to diagnose plants and quickly make decisions regarding dressing of nitrogen fertilizers. The simplest is visual diagnostics. When an agronomist assesses the condition of crops according to his practical experience, which is not always correct. Chemical diagnostics of plants began to be carried out using a field portable laboratory. Such a diagnostic involves the analysis of fresh plant samples without ashing to determine the content of inorganic forms of elements in them (Shchuklina et al., 2021). To obtain the result, one drop of a 1% solution of diphenylamine dissolved in H_2SO_4 is applied to a cut of stem taken from the upper tier of plant. The results are evaluated in points: 1 point - the drop is colorless or pale blue (severe lack of nitrogen); 2 points - the drop turns blue (the average need of plants in nitrogen); 3 points - the drop is evenly colored in a thick blue-violet color (plants have little need for nitrogen or sufficient nitrogen supply). This method accurately determines the condition of plants, but requires skills in working with acids and is limited both by weather conditions and the agronomist's time. Since these two methods have their drawbacks, another method was developed for determining the supply of plants with nitrogen, using photometric instruments. The method of estimating of nitrogen level in plants with the help of photometric devices is based on measuring of chlorophyll concentration. Concentration measurement is based on intensity of chlorophyll fluorescence and on transparency of leaf plate (Avagyan, 2010).

Monitoring of nitrogen utilization in plants using of optic devices is performed in many countries (Lunagaria et al., 2015, Shchuklina et al., 2021). The process of biosynthesis of organic matter in plant cells and tissues depends on the chlorophyll content, a pigment that determines the functioning of photosynthesis. Both of these factors depend on the amount of chlorophyll in leaf cells. Currently, there are five chlorophyll forms discovered: *a*, *b*, *c*, *d*, *f* (Ke et al., 2021). These forms are found in plants, algae and cyanobacteria. Chlorophyll *a* are detected in plants, chlorophyll *c* is found only in some algae, and chlorophylls *d* and *f* are only in some cyanobacteria (Li & Chen 2015). Chlorophyll is cholorophyllinic acid ester with methyl (for chlorophyll a) or aldehyde (for chlorophyll b) group. Chlorophylls *a* ($C_{55}H_{72}O_5N_4Mg$) and *b* ($C_{55}H_{70}O_6N_4Mg$) both contain four nitrogen atoms, so chlorophyll concentration in plants depends on the amount of nitrogen in plants (Eroshenko et al., 2019). Moreover, concentration of chlorophyll in cells is related to water conditions and other abiotic factors (Afanasiev et al., 2013).

According to the implementators, recently developed commercial optical devices are able to determine the chlorophyll content in leaves regardless of weather conditions, soil pollution levels, and biomass conditions (Samborski et al., 2009; Tremblay, 2010). However, usually the norms of applying of nitrogen fertilizers for a particular crop are advisory in nature. Rationale of the application of optical instruments in agriculture will greatly facilitate the demand of the agronomist in monitoring the agricultural plant condition and diagnosing deviations. This will lead to a more rational use of nitrogen fertilizers, reducing the risk of environmental pollution with residual nitrogen and the unwanted accumulation of pesticides in crop products.

The objective of research was to justify the usage of photometric devices for the analysis of nitrogen level in plants and to investigate the relation of pigment content in leaves to nitrogen nutrition of plants.

MATERIALS AND METHODS

The experiment was carried out in Field experimental station of Russian State Agrarian University - Moscow Timiryazev Agricultural Academy (Moscow, Russian Federation). Soil was loam, soddy-podzolic (Table 1). Soil had high content of available phosphorus (P_2O_5), average content of soluble potassium (K_2O) and low content of humus (1.9%).

Table 1. Soil agrochemical characteristics (0–0.2 m)

Year	pH_{KCL}	Humus, %	N_{EH}	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	P_2O_5	K_2O
			mg kg^{-1} of soil				
2008[1]	4.8	1.9	-	-	-	283	134
2011[1]	5.5	1.7	67.2	4.2	8.7	304	83

[1] Data from Afanasiev R.A. (Afanasiev, 2008; Afanasiev, 2013).

Spring rapeseed (*Brassica napus* L.) cultivar 'Vikros' (2010) and spring barley (*Hordeum vulgare* L.) cultivar 'Mikhailovsky' (2012) were used in the study. The scheme of experiment included spring soil dressing with increasing doses of NH_4NO_3 from 30 kg of active component per hectare to 150 kg of active component per hectare. There were four replications in test and randomized arrangement of variants. Control groups for every cultivar were variants without soil dressing. Crop management practice, accepted for Central region of Non-chernozem zone (Russian Federation). Optic instrument for measurement of chlorophyll concentration in leaves 'Yara' (Konica Minolta, Japan) and experimental optic N-tester 'Spectroluxe' (SPA 'Spectroluxe', Russia) were used. To determine the amount of chlorophyll on the day of measurement with optical sensors, 10 flag leaves from individual plants were selected diagonally from each experimental plot. One medium sample was taken from the crushed mass of one flag leaf. The amount of chlorophyll in leaves was estimated by method, developed by Department of Plant Physiology of Russian State Agrarian University (Tretyakov et al., 1990). Pigment extraction from plants involved the usage of ethanol. Chlorophyll concentration was estimated by spectrophotometer 'Helios Omega' (Termo Scientific Spectronic, the USA) in the laboratory of Pryanishnikov institute of agrochemistry.

Agrometeorological conditions during vegetative period in 2010 were one of the driest for the last 70 years in Central region of Russian Federation. Air drought lasted longer than 50 days (middle June - middle August). During this period precipitation was about 51.6 mm (Fig. 1). According to average annual recordings, 158 mm of precipitation falls during the same period of time (Belolyubtsev & Sukhoveeva, 2012). Drought was accompanied by the temperatures higher than the average annual recordings say. In the third decade of July the temperature was 10.4 °C higher than the average value, and in the third decade of August the temperature was 11.7 °C higher than the average temperature. All these weather conditions result in decrease in spring rapeseed yield.

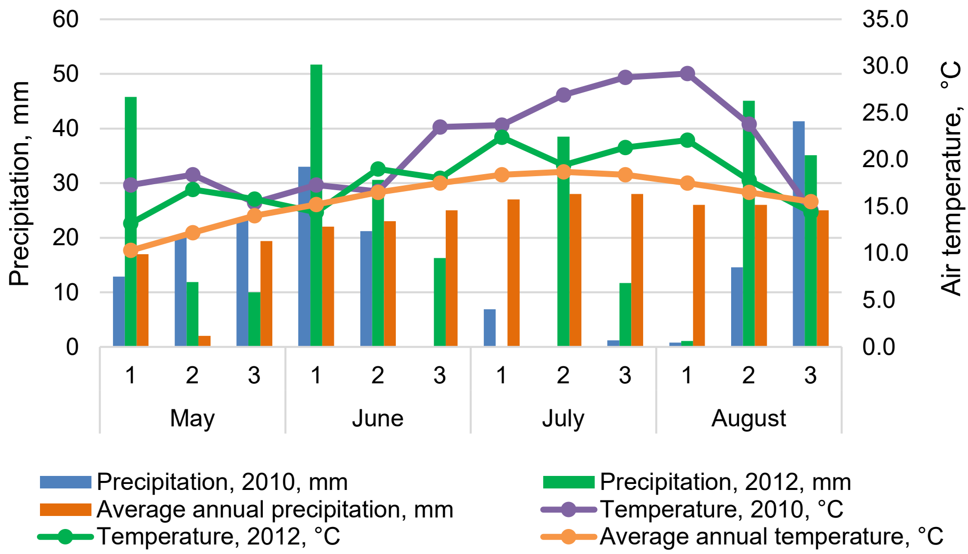

Figure 1. Meteorological conditions of spring rape and spring barley (2010, 2012).

Meteorological conditions in 2012 were favorable for growth and development of spring barley. However, there were periods, lacking in precipitation (first decades of July and August), and periods with the amount of precipitation almost two times higher than the average annual value (first decade of June, second decade of August). Precipitation that fell during the milky stage of spring barley (code BBCH 73-75) led to partial lodging of cereals, which influenced the quality of harvest and the crop yield.

RESULTS AND DISCUSSION

Agrometeorological conditions such as amount of precipitation and air temperature, optimal for growth and development, highly influence crop yield (Cosentino et al., 2012). Other factors such as cultivar and dressing have strong influence on crop yield as well (Turko et al., 2018). In 2010, conditions were abnormal for rapeseed growth not only because higher air temperature, which exceeded the average annual value by 3–12 °C, but because of lack of precipitation as well. Lack of humidity is the main factor that prevents the realization of plants genetic potential (Boyer, 1982). Moreover, the drought stress leads to reducing in assimilation and changes in the process of respiration (Yin et al., 2006). Drought or water stress can significantly reduce plant growth the yield (Shooshtari et al., 2020). At the same time, optimal water conditions are detrimental for efficient usage of nitrogen fertilizers (Belousova et al., 2015).

Weather conditions led to noticeable decrease in rapeseed yield in general. The crop yield equaled to 0.96–1.40 kg ha^{-1} (Fig. 2). Yield of rapeseed, grown without nitrogen soil dressing, was 0.28–0.44 kg ha^{-1} lower than yield of rapeseed, grown with nitrogen fertilizers.

Estimation of chlorophyll concentration in spring rapeseed leaves was carried out during flowering stage (BBCH 57). There was no precipitation three weeks before the estimation. There was 0 mm of precipitation in the third decade of June and 6.9 mm in the first decade of July, which is three times lower the average annual value. However, there was enough soil moisture for normal vital functions of plants and uptake of nitrogen fertilizers. The chlorophyll concentration sequentially increased as the dose of nitrogen fertilizers increased (Fig. 1). Correlation coefficient for chlorophyll *a* equalled 0.94. The increase in chlorophyll *b* concentration occurred in steps. In control group and the group, which obtained the lowest dose of nitrogen (N_{30}), chlorophyll *b* concentration was 0.32–0.37 mg per g, and chlorophyll *b* concentration was 0.49–0.52 under the dose of nitrogen N_{60}. Correlation coefficient for chlorophyll *b* was 0.71.

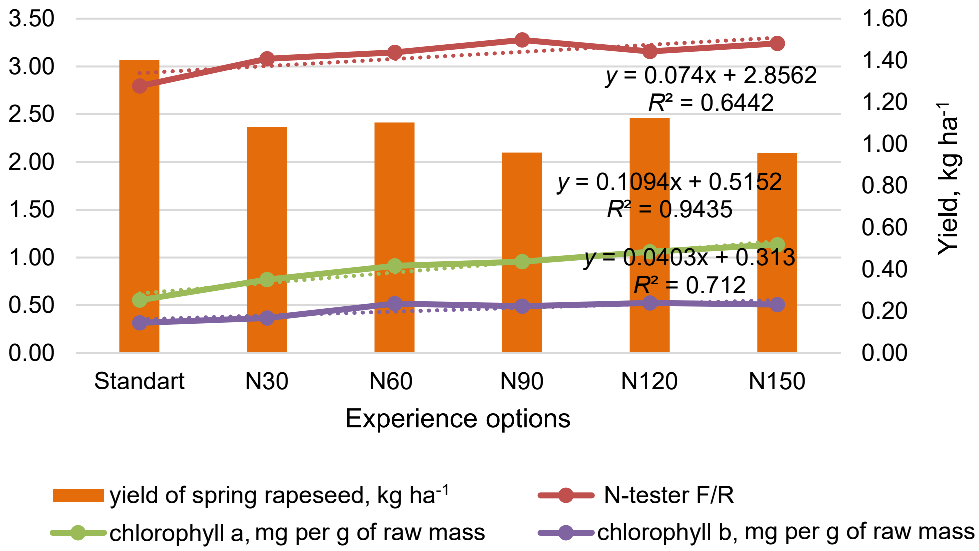

Figure 2. The dependence of the chlorophyll *a* and *b* content and the readings of the Spectrolux N-tester on nitrogen doses on spring rapeseed (2010).

The nitrogen level was measured as well. Measurement was carried out with N-testers 'Spectroluxe' and 'Yara'. The working principle of these instruments is based on the measurement of chlorophyll fluorescence, which represents the amount of chlorophyll in leaves indirectly (Goltsev et al., 2014). The usage of fluorometric methods allows evaluate plants reactions under different stresses: high and low temperatures, drought, saltiness etc. (Fracheboud & Leipner, 2003; Kalaji & Pietkiewicz, 2004; Massacci, 2008). According to results, collected with 'Spectroluxe' in the beginning of July, chlorophyll fluorescence sequentially increased from 2.79 (control group) to 3.28 (N_{120}) with the increase of dose of nitrogen fertilizers. There is a small decrease in fluorescence in groups that consumed higher amounts of nitrogen. N-tester 'Spectroluxe' data strongly correlated with chlorophylls *a* and *b* concentration values (0.89 and 0.84 respectively, Table 2). Moreover, correlation of 'Spectroluxe' data and crop yield was even stronger, but inverse (-0.97), since the rapeseed yield decreased as the dose of nitrogen fertilizers increased. Data from all of the devices used strongly correlated with chlorophyll concentrations. The strongest correlation between chlorophyll *a* concentration and N-tester 'Yara' was shown. The chlorophyll *a* concentration correlated with testers' data as strongly as the sum of *a* and *b* chlorophyll concentrations does. This is because chlorophyll *a* is more present in chloroplasts and has stronger fluorescence, which can be registered by fluorometers (N-testers) (Goltsev et al., 2014).

Table 2. Correlation of N-testers' data and crop yield with the chlorophyll content in spring rapeseed leaves (*: $p < 0.05$)

Parameter	Chlorophyll *a*	Chlorophyll *b*	Sum of the chlorophylls *a* and *b*
Crop yield	-0.81	-0.70	-0.79
N-tester 'Spectroluxe'	0.89	0.84	0.89
N-tester 'Yara'	0.96	0.91	0.96

The water status of spring barley was much better than the water status of spring rapeseed, which was favorable for the crop yield of the barley. However, abundant precipitation, which fell in the beginning of maturing, led to the lodging of the cereals in the groups that received nitrogen doses higher than 90 kg per hectare, which is characteristic for barley (Chen et al., 2014). The lodging of the cereals in some experimental plots was as high as 75%. As a result, the highest crop yield (4.41 kg ha^{-1}) was in experimental plots, where the nitrogen concentration in fertilizer was 60 kg per hectare (Fig. 3), since the lodging of the cereals was not as severe.

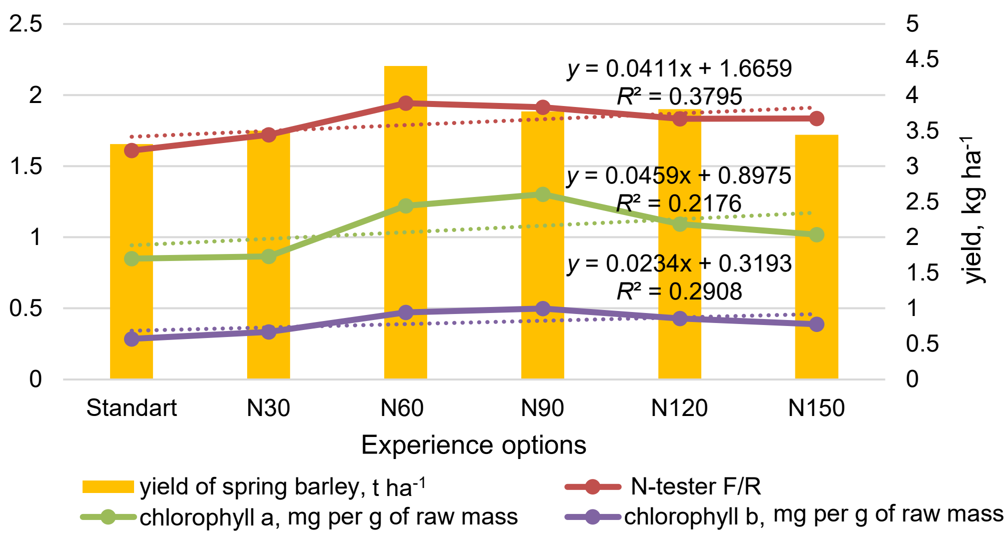

Figure 3. Dependence of chlorophyll a and b content and the readings of the Spectrolux N-tester on nitrogen doses on spring barley (2012).

The measurement of chlorophyll fluorescence and pigments content in spring barley appeared to be useful in the estimation of drought tolerance of new cultivars (Li, 2006). Results, obtained with N-tester 'Spectroluxe' in earing phase (code BBCH 51-52) correlated highly with the chlorophyll concentration in barley leaves. But this correlation was higher in the case of chlorophyll *b*, compared to chlorophyll *a* (Table 3). Data, received from N-tester 'Yara', poorly correlated with chlorophyll concentration. This correlation was higher in the case of chlorophyll *b* ($r = 0.29$). Despite the influence of the lodging of the cereals on the crop yield, strong correlation of the crop yield of barley with the amount of chlorophyll was noted. In the case of chlorophyll *a*, the correlation coefficient equaled 0.73, and for the chlorophyll *b* the correlation coefficient was 0.75.

Table 3. Correlation of N-testers data and crop yield with the chlorophyll content in spring barley leaves (*: $p < 0.05$)

Parameter	Chlorophyll *a*	Chlorophyll *b*	The sum of chlorophylls *a* and *b*
Crop yield	0.73	0.75	0.74
N-tester 'Spectroluxe'	0.92	0.96	0.94
N-tester 'Yara'	0.16	0.29	0.20

The number of green pigments in barley leaves weakly correlated with increasing doses of nitrogen. However, high correlation between chlorophyll *b* concentration and increasing doses of nitrogen was noted ($r = 0.29$).

CONCLUSIONS

Chemical diagnostics (the concentrations of chlorophyll and nitrogen in leaves) is the most precise method of estimation of nitrogen status of plants. However, these methods require a lot of time, laboratory conditions, availability of special devices and reagents, including acids, and skills of working with these reagents (Hatamian et al., 2018; Souri et al., 2018). Modern optical instruments, which working principle is based on chlorophyll concentration measurement, can substitute chemical methods. In the present study results of diagnostics with portable photometric instrument 'Yara' strongly correlate with the results of measurement of green pigments concentration in rapeseed leaves by chemical methods. The correlation coefficient for chlorophyll *a* and the sum of chlorophylls *a* and *b* reached -0.96, and the correlation coefficient for chlorophyll *b* was -0.91. Moreover, the data from 'Yara' weakly correlated with the number of pigments in barley leaves. Results of diagnostics, received due to the new device 'Spectroluxe', highly correlate with pigment content in rapeseed and barley leaves. However, it is necessary to conduct research, evaluating the cultivars in certain conditions, to determine the dose of nitrogen fertilizers applied.

Our study confirms the results obtained earlier by other researches and will contribute to the development of algorithms for converting the readings of optical sensors into real recommendations for the application of nitrogen fertilizers to various crops grown in different soils and climatic conditions.

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Yield and content of biologically active substances in blue honeysuckle fruit (*Lonicera caerulea* L.) grown in the Forest Steppe of Ukraine

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Abstract. The blue honeysuckle (*Lonicera caerulea* L.) is a relatively new crop in Ukraine, its industrial cultivation is only 100 hectares. The main constraints are the lack of varieties with high yield and nutritional value of berries. Therefore, a study of the yield and quality of introduced varieties is necessary and relevant, both for producers and breeders. With our research, we determined the potential of the early stage of blue honeysuckle berries under the conditions of their cultivation in the Forest Steppe of Ukraine and the weather conditions of the year of the specified region. We assessed how early we can get a crop and set what quality and what it will be. To clearly understand the quality of the grown fruits, their average weight, size and uniformity were studied. From nutritional indicators of fruit quality, the content of dry matter, soluble solids, sugars and titrated acids was studied, from biologically active substances, the content of vitamin C and total phenolic was determined. It was found that in the zone of the Forest Steppe of Ukraine from the studied group of varieties, the highest yield potential, 3.13 kg from a bush for the second year of fruiting and fruit mass 2.4 g, had a variety of Canadian breeding 'Boreal Beauty'. The fruits of the cultivars 'Duet' (4.3) and 'Boreal Blizzard' (4.5) were distinguished by the balance of taste according to the sugar-acid index, and the maximum amount of total phenolics for the studied group of varieties was accumulated by the fruits of 'Boreal Beast' (1,000 mg per 100 g).

Key words: *Lonicera caerulea* L., mass, fruit size, sugars, vitamin C, total phenolic content.

INTRODUCTION

Consumption of foods rich in nutrients, including biologically active foods with an increased content of antioxidants, is not only the trend of today, but also a common concern for health. In view of this, there is a search for new sources of the supply of useful substances to the human body. The first place in this list belongs to the so-called 'superfruits' - fruits of strawberries, raspberries, black currants, blueberries and others

(Chmiel et al., 2014; Chang et al., 2018). Since recent times, the fruits of blue honeysuckle have been included in this list.

In ethnic medicine, blue honeysuckle berries have long been known as medicines (Lefol, 2007). Confirmation of the usefulness of *L. caerulea* L. fruits is numerous results of studies of modern scientists who emphasize their antioxidant and immunological activity (Cory et al., 2018). They have a protective effect against cardiovascular and neurodegenerative diseases, osteoporosis, type 2 diabetes, as well as anti-carcinogenic and anti-inflammatory activity (Cory et al., 2018). The health-improving properties of blue honeysuckle berries are based on a significant content of natural antioxidants such as ascorbic acid and polyphenolic compounds. It is the high content of the latter of the named substances that makes blue honeysuckle berries an excellent antimicrobial, antiviral and antitumour agent (Chaovanalikit et al., 2004; Farcasanu et al., 2006; Palikova et al., 2008; Gruia et al., 2009). The health benefits of polyphenols attract researchers and breeders to identify and create new varieties with enhanced functional properties (Kaushik et al., 2015; Sytar, 2015). The undeniable consumer, preventive and therapeutic value of *L. caerulea* L. berries and their official recognition in world markets will definitely be an impetus for increasing the world's areas of their production. Many varieties of different maturation terms are commercially grown in European countries such as the Czech Republic, Poland, Slovakia, Lithuania and Romania, as well as in Japan and Canada (Kucharska et al., 2017).

Therefore, the study of the quality indicators of introduced varieties for growing conditions in the Forest Steppe of Ukraine is a prerequisite for the selection of the best, with the purpose of creating industrial plantations, the fruits of which will be able to satisfy the wishes of the most demanding consumers of this type of product. For the same breeding, an important feature of the variety is the homeostaticity of the commercial and consumer qualities of the fruit (Shevchuk et al., 2021). Therefore, the assessment of the stability of yield, marketability, consumer and bioactive quality indicators of introduced blue honeysuckle varieties under the influence of weather conditions of the year of cultivation will be useful for breeding scientists when selecting parent couples to conduct the breeding process for the creation of new blue honeysuckle varieties.

METHODS AND MATERIALS

Field studies

The blue honeysuckle (*L. caerulea* L.) grown on the research sites of the Podilskyi Research Station of the Institute of Horticulture (Podilskyi DSS IH) was subject to research. The location of the natural and climatic zone is the Forest Steppe of Ukraine (Podillya), the height above sea level is 276 m, (49°11`08``N, 28°18`40``W), the distance to the regional center of Vinnytsia is 12 km.

Planting year of blue honeysuckle is 2017, planting pattern is one meter between plants in a row and 3 m in between rows, 2020 is the first year of fruiting, 2021 is the second. The ground of the study area is medium-loam alfisol podzolized soil. The system of soil retention in a row mulching with sawdust, in between rows turfing, the experiment was laid with irrigation, care for plantations is recommended for the Forest-Steppe zone of Ukraine.

The climate of the Podilska Horticultural Research Station of Institute of Horticulture (Podilska HRS IH) location region is temperate-continental. According to long-term data, the average annual air temperature is 7.0 °C, the maximum +36 °C and the minimum -25 °C, the annual sum of active temperatures of 10 °C and above - 2,700 °C, and precipitation - 525 mm.

In the first year of research (2020), spring came earlier, March was marked by average annual air temperatures at +5.5 °C, in 2021, in the same month, the temperatures were +1.7 °C, the average annual indicator (average for 2012–2021) - 3.2 °C (Table 1).

The objects of research were plantations and fruits of blue honeysuckle of 4 varieties of Canadian ('Boreal Blizzard', 'Boreal Beast', 'Boreal Beauty', 'Aurora') and 2 Polish breeding ('Duet', 'Karina'). To determine the yield, all berries harvested from 5 bushes of the same variety were weighed by the dates of harvest and summed, then the average yield from the bush was found. The average mass of the berries was determined by weighing 50 berries on a laboratory scale with an accuracy to the first sign. The diameter and length of the berry were determined using a circular barbell. For measurements, 50 berries from different bushes were chaotically selected. The studies were performed in triplicate.

Table 1. Temperature indicators and amount of precipitation of the period of growth and development of fruits, Podilska HRS IH, Forest-steppe of Ukraine

Month	Decade	Average daily t °C		Amount of precipitation, mm	
		2020	2021	2020	2021
March	I	6.8	0.2	4.0	6.2
	II	6.0	1.6	3.6	16.0
	III	2.9	3.2	3.0	18.2
per month		5.5	1.7	10.6	40.4
average 2012–2021		3.2		30.8	
April	I	8.0	5.5	0.0	2.8
	II	8.3	7.2	5.2	2.8
	III	11.8	7.9.	19.0	16.5
per month		9.4	6.9	24.2	22.1
average 2012–2021		10.5		37.9	
May	I	11.9	11.6	14.0	15.0
	II	13.1	14.2	13.2.	64.6
	III	10.6	14.7	32.4	75.6
per month		11.8	13.6	59.6	155.2
average 2012–2021		15.6		59.5	
June	I	17.8	16.3	10.2	15.1
	II	28.8	19.5	17.6.	52.2
	III	21.4	23.4	68.4	7.2
per month		20.6	19.7	96.2	74.4
average 2012–2021		20.3		102.7	

Laboratory investigation

The determination of the biochemical components of blue honeysuckle fruit was performed in 2020 and 2021 in the laboratory of post-harvest quality of fruit and berry products of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine (IH NAAS of Ukraine). Laboratory studies were subject to the blue honeysuckle fruits in the stage of consumer ripeness with the shape and color characteristic of the variety, collected 10 days after ripening the first berries. The mass of the sample for determining the biochemical components was equal to one kilogram, according to the 'Methods for assessing the quality of fruit and berry products' (Kondratenko et al., 2008). On the day of harvesting the fruits was determined the content of dry solid, soluble solids, sugars, titrated acids, ascorbic acid and total phenolic content. All analytical studies were performed in triplicate.

Dry matter (DM)

To determine the content of dry matter (moisture), was used the method of drying the sample. For this purpose, sliced fruit particles in the amount of 3–4 g were put in a box with previously prepared weighed river sand. Drying to a constant weight of the prepared sample was carried out in a drying cabinet SNOL 58/350 at a temperature of 98–100 °C, the data were expressed as a percentage.

Soluble solids (SS) was determined using an ATAGO PAL-1 portable refractometer (Japan). To prepare an analytical sample, fruits in the amount of 15–20 pieces were crushed using a homogenizer, then a drop of juice was squeezed through the tissue on the refractometer glass, while recording the data, the error on the temperature was taken into account. The data were denoted as a percentage per raw mass.

Organic titrated acids (TA)

For the extraction of acids, 25 g of the crushed sample was transferred without loss, by washing with hot distilled water, with a volume of not more than 150 mL, into a volumetric flask with a capacity of 250 mL. The flask was held in a water bath for 30 min at a temperature of 80 °C, cooled. The content of the flask was adjusted to the mark with distilled water and filtered through a filter into a conical flask with a capacity of 250 mL. 20 mL of the extract was pipetted into a 250 mL conical flask, 3–4 drops of phenolphthalein were added and 0.1 N sodium hydroxide was titrated until a pink coloration corresponding to pH 7.0 appeared. The content of titrated acids in the sample was calculated according to the formula, using a titer index of 0.1 N sodium hydroxide and a conversion factor to citric acid. The data were denoted as a percentage per raw mass.

Sugars

Extraction of sugars from blue honeysuckle was carried out with hot distilled water. The extract obtained was purified from proteins and pigments obtained by precipitation of the latter with acetic lead. Sucrose was subjected to hydrolysis to glucose and fructose when heated in the presence of 10% hydrochloric acid. The hydrolysis products were oxidized with Fehling's solution. The optical density of the resulting solutions was determined on a ULAB 102UV spectrophotometer at a wavelength of 640 nm. The content of sugars in the sample was calculated by the formula, using the indicator of the graduated graph. Standard solutions of glucose with different concentrations were used to construct a graduated curve of the dependence of optical density (unit of optical density) on the glucose concentration (mg mL^{-1}). The data were denoted as a percentage per raw mass.

Sugar Acid Index (SAI) was defined as the ratio of the total amount of sugars to the amount of titrated acids.

Ascorbic acid

For the extraction of ascorbic acid, the sample was mashed in a porcelain mortar with the addition of broken glass and a mixture of 2% oxalic and 1% hydrochloric acids (80+20, vol+vol), transferred to a volumetric flask with a capacity of 100 mL. The content of the flask was adjusted to the mark with a mixture of 2% oxalic acid and 1% hydrochloric acid (80+20, vol+vol) and filtered. The resulting extract was titrated with a solution of 2,6-dichlorophenolindophenol (Tilmans paint). The content of ascorbic acid in the sample was calculated by the formula using the Tilmans paint titer. The data was expressed as 1 mg per 100 g of raw mass.

Total phenolic content (TPC)

For the extraction of total phenolic content, the sample was mashed in a porcelain mortar with a small amount of ethyl alcohol and filtered using a vacuum on the Büchner funnel through a blue ribbon paper filter into a Bunsen flask. The residue on the filter is washed with small amounts of ethyl alcohol until the sample is completely discolored. The volume of used alcohol (ml) was traced. 7.9 mL of distilled water, 0.1 mL of extract, 1 mL of Folin-Denis reagent were added to the tube, stirred and after 3 minutes 1 mL of saturated sodium carbonate solution was added and stirred again. For an hour, the optical density of the contents of the tubes was recorded on a ULAB 102UV spectrophotometer at a wavelength of 640 nm. As a control, a mixture prepared as follows was used: 8 mL of distilled water, 1 mL of Folin-Denis reagent were poured into a tube, stirred, after 3 minutes 1 mL of saturated sodium carbonate solution was added and stirred again. At least 3 parallel measurements were carried out and the average value of the optical density index was found. The total phenolic content in the sample was calculated according to the formula, using the indicators of the graduated graph. Standard solutions of chlorogenic acid with different concentrations were used to construct a graduated graph of the dependence of optical density (unit of optical density) on the concentration of chlorogenic acid (μg mL^{-1}). The data were expressed as a mg per 100 g of raw mass.

Statistical analysis of the study data was performed using STATISTICA 13/1 software (StatSoft, Inc., USA). The results are presented as mean values with their standard errors (x ± SE). The Shapiro-Wilk test was used to evaluate the assumptions of normality and homogeneity of dispersions. Significant differences between mean values were determined using one-way ANOVA analysis. The results were presented at a confidence level of $P < 0.05$.

RESULTS AND DISCUSSION

Ripening period, yield, weight and dimensions

The peak of flowering of blue honeysuckle varieties in 2020 was in the middle of the second decade of April. In 2021, the studied varieties flowered intensively two weeks earlier than in 2020, namely on the first of May. The amount of days of the period of growth and development of blue honeysuckle in 2020 was 71 days, and in 2021 in this period was less by 11 days.

The beginning of ripening of the studied honeysuckle varieties in 2020 was on 16, and in 2021 - on May 19. This is a little later than in the warm climate of South Moravia, where the first berries can be obtained on May 15 (Řezniček, 2007) but earlier than in the northern part of the United States, where the beginning of their ripening falls on June 18–28 (Hummer, 2006). In the Wellington County of Canada, the early blue honeysuckle fruits begin to ripe on June 26–July 6, and late July 17–27 (MacKenzie et al., 2018). In the conditions of the Forest Steppe of Ukraine, the fruits of late varieties of blue honeysuckle ripe massively on June 26–29, which is 21–28 days earlier than in Canada.

Polish researchers have established the fact of the influence of the conditions of the year on the duration of the period of growth and development of fruits, they argue that the amount of days, from the beginning of flowering to the first harvest, is almost the same for the years of research and only under the influence of the weather the timing of their beginning changes (Małodobry et al., 2010), and which we confirmed with our research.

Yields of blue honeysuckle in the first year of fruiting (2020) ranged from 0.57 'Boreal Beast' variety to 1.91 kg from the 'Boreal Beauty' bush. Above average, in addition to the mentioned variety, 'Aurora' had a high yield (1.25 kg per bush). In the rest of the studied varieties in 2020, it did not exceed one kilogram from the bush (Table 2). In the second year of fruiting (2021), higher than average yields were in 'Boreal Blizzard' (2.58) and 'Boreal Beauty' (3.13 kg per bush) varieties. Less than 2.0 kg per bush were harvested from 'Karina' berries (1.45 kg per bush), 'Duet' berries (1.67 kg per bush) and 'Boreal Beast' berries (1.96 kg per bush). Under Canadian conditions, the yield of blue honeysuckle is 0.5–0.75 kg from the bush (Bors et al., 2012), although it was slightly higher in Polish breeding varieties grown in Poland (0.895–0.940 kg per bush) (Ochmian et al., 2010). Scientists from the University of Warsaw in the first year of fruiting harvested 1.04–1.35 kg from the bush, and in the second year 2.31–2.87 kg (Małodobry et al., 2010). Slightly lower than the yield of Polish varieties, but higher than the yield of Canadian honeysuckle varieties grown in the Forest Steppe of Ukraine, in the first year of fruiting, according to the average intercultural indicator, it was 1.04 kg per bush, and in the second - 2.1 kg per bush (Table 2).

Given the young age of plantations, we did not establish the dependence of the load of bushes on the harvest on the weather conditions of the year of cultivation, but the inter-variety was quite significant, the corresponding coefficients in both years of research were 46.4 in 2020 and 39.2% in 2021 (Table 2).

Table 2. Yield and weight of fruits of different varieties of blue honeysuckle, 2020–2021 ($n = 5$)

Variety	Yield from the bush, kg			Fruit weight, g		
	2020	2021	average	2020	2021	average
'Boreal Blizzard'	0.77 ± 0.27	2.58 ± 0.46	1.68 ± 0.83	1.7 ± 0.3	1.8 ± 0.2	1.8 ± 0.2
'Boreal Beast'	0.57 ± 0.18^{a}	1.96 ± 0.49	1.26 ± 0.65	1.7 ± 0.2	1.8 ± 0.2	1.7 ± 0.1
'Boreal Beauty'	1.91 ± 0.24^{b}	3.13 ± 0.38^{b}	2.52 ± 0.57^{b}	2.1 ± 0.3	2.4 ± 0.3	2.2 ± 0.2
'Aurora'	1.25 ± 0.59	1.87 ± 0.30	1.56 ± 0.40	1.9 ± 0.2	2.3 ± 0.4	2.1 ± 0.2*
'Karina'	0.85 ± 0.20	1.67 ± 0.33^{a}	1.26 ± 0.40	1.6 ± 0.3	1.9 ± 0.3	1.8 ± 0.2
'Duet'	0.88 ± 0.33	1.45 ± 0.22^{a}	1.16 ± 0.31	1.3 ± 0.3	1.5 ± 0.2*	1.4 ± 0.2*
Average ± *SE***	**1.04 ± 0.15**	**2.11 ± 0.15**	**1.57 ± 0.48**	**1.7 ± 0.1**	**2.0 ± 0.3**	**1.8 ± 0.2**
*CV****, %	46.4	39.2	32.1	16.8	16.2	16.2

*The upper indices (a and b) in the rows near the indicators indicate significantly different values of yield and weight of blue honeysuckle fruit relative to the average indicator (x) for the study group at $p < 0.05$; ***SE* – standard error of the average value; ****CV* – coefficient of variation.

Fruit weight and dimensions are the main indicators for assessing the benefits of the variety for consumers. Under the conditions of cultivation in the Republic of Kazakhstan, the blue honeysuckle fruit had a fruit weight of 0.74 to 0.88 g (Řezniček, 2007). The berries were grown in Estonia with a weight of 0.5–2.4 g (Arus & Kask, 2007), their average weight in Poland was 0.73–1.11 g, in particular, the 'Aurora' variety had it at the level of 1.11 g (Dziedzic et al., 2020). The mass of fruits of introduced blue honeysuckle varieties grown in the Podilsky region of Ukraine was at the level and above the large-fruited ones studied by Estonian scientists, and some of them, including 'Boreal

Blizzard' and 'Aurora', in 2021 accumulated mass at the level of Japanese breeding varieties grown in the Baltic States. The range of variation in fruit weight in the varieties we studied was in the range from 1.4 g ('Karina') to 2.2 g ('Boreal Beauty'), more than 2.0 g of berry weight was 'Aurora' (2.1 g). We did not find a large difference in the weight of fruits in the years of fruiting, although almost all of them, despite a significant increase in the harvest in 2021, accumulated the weight of the fruit by 0.1–0.4 g more than in 2020 (Table 2).

Another confirmation of the fact that the blue honeysuckle under the condition of growing on the Forest-Steppe of Ukraine is able to form highly marketable fruits is that their size during the period of mass harvesting was greater than that of the blue honeysuckle at the end of the harvest. After all, as claim Polish researchers (Skupień et al., 2009) that the largest size of berries of this crop are at the date of the last harvest, they were 18.8–22.1 mm long and 10.4–13.5 mm in diameter, while we had 11–27 and 13–17 mm, respectively. The largest diameter of the fruit, among the varieties we studied, was 'Boreal Beauty' (15 mm), and the smallest 'Boreal Blizzard' (12 mm), the average value was 13 mm. In 2021, when rainfall in May-June was higher than in 2020, honeysuckle fruits were larger in both transverse diameter and length, which was reflected naturally on their mass. The length of the fruit of the studied varieties varied from 11 mm ('Karina') to 27 mm, the 'Boreal Blizzard' varieties, the average length was 20 mm (Table 3).

Table 3. Dimensions of fruits of different varieties of blue honeysuckle, 2020–2021 ($n = 5$)

Variety	Fruit diameter, mm			Fetal length, mm		
	2020	2021	average	2020	2021	average
'Boreal Blizzard'	11 ± 1.6	13 ± 1.1	12 ± 1.0	26 ± 3.9^{b}	29 ± 3.1^{b}	27 ± 2.6^{b}
'Boreal Beast'	11 ± 1.4	14 ± 1.6	13 ± 1.4	19 ± 2.1	25 ± 3.1^{b}	22 ± 2.6
'Boreal Beauty'	13 ± 2.6	17 ± 2.1^{b}	15 ± 2.0	26 ± 5.4	23 ± 2.3	25 ± 2.9^{b}
'Aurora'	12 ± 2.1	14 ± 1.3	13 ± 1.3	19 ± 3.9	22 ± 3.5	20 ± 2.8
'Karina'	14 ± 1.2	13 ± 1.2	14 ± 1.0	14 ± 3.6	16 ± 3.2a	15 ± 2.4^{a}
'Duet'	14 ± 1.2	13 ± 1.0	13 ± 0.7	10 ± 1.8	13 ± 2.3^{a}	11 ± 1.7^{a}
Average ± *SE***	**13 ± 0.3**	**14 ± 1.4**	**13 ± 1.25**	**19 ± 1.7**	**21 ± 0.7**	**20 ± 1.2**
*CV****, %	10.3	11.8	6.9	34.4	27.7	30.1

*The upper indices (a and b) in the rows near the indicators indicate significantly different values of yield and weight of blue honeysuckle fruit relative to the average indicator (x) for the study group at $p < 0.05$; ***SE* – standard error of the average value; ****CV* – coefficient of variation.

Consumer qualities of blue honeysuckle fruit

Biochemical components are a characteristic of the consumer value of blue honeysuckle fruit, and the dry solid content is an indicator of their hardness and transportability. The amount of dry matter in the fruit that we studied was 18.3% with a minimum of 17.2 and a maximum of 19.5% (Table 4), which is more than the amount (14–15%) that fruit had grown in Switzerland (Auzanneau et al., 2018). Although the 'Boreal Blizzard' and 'Duet' varieties accumulated a level of dry matter that was found in berries grown in the western part of Slovakia - 17.39% (Pokorna-Jurikova & Matuskovic, 2007), and the 'Karina' variety contained as many as in the Slovak Nitra (14.15–18.24%) (Jurikova et al., 2014).

Soluble solids in most fruits are a commercially controlled component that characterizes the quality of the product. Their amount in the fruit is significantly adjusted by the conditions of the region and the year of cultivation. Thus, berries grown in the city of Elora (Canada), depending on the year of cultivation, accumulated from 13.8% to 17.1% (°Brix) of soluble solids, the same varieties, but collected in the city of location of Research Station Simcoe had them from 12.7–16.1% (MacKenzie et al., 2018). In the varieties studied by Polish scientists, the content of soluble solids (SS) ranged from 10.1–15.8% (Wojdyło et al., 2013), other researchers from this country found that it varied from 12.47 to 14.43%, and in Aurora it was 13.4% (Dziedzic, 2020). In the same variety, but grown in the conditions of the Forest Steppe of Ukraine, the amount of soluble solids was at the same level, and in general for the studied varieties ranged from 11.2 to 13.3%, which is less than the minimum and maximum value for Canada, but approximately at the same level as the indicators obtained by Polish colleagues (Table 4).

Table 4. The content of dry matter and soluble solids in blue honeysuckle fruit, % per raw weight (2020–2021) ($n = 5$)

Variety	Dry matter (DM)				Soluble solids (SS)			
	2020	2021	average	*CV*, %	2020	2021	average	*CV*, %
'Boreal Blizzard'	16.8 ± 1.6	18.1 ± 2.1	17.5 ± 1.3	9.3	12.9 ± 0.4	10.6 ± 0.4	11.8 ± 1.1	11
'Boreal Beast'	19.4 ± 0.6	19.7 ± 1.3	19.5 ± 0.7[b]	4.2	13.6 ± 0.4	11.3 ± 0.4	12.4 ± 1.0	10
'Boreal Beauty'	18.9 ± 3.2	19.1 ± 0.6	19.0 ± 1.4	9.5.	10.8 ± 1.0	11.5 ± 0.9	11.2 ± 0.7[a]	8
'Aurora'	19.0 ± 2.5	18.8 ± 1.3	18.9 ± 1.3	8.4	13.0 ± 0.5	13.5 ± 0.9	13.3 ± 0.5[b]	5
'Karina'	18.6 ± 2.4	17.5 ± 1.3	18.1 ± 1.3	9.2	10.7 ± 0.3	11.7 ± 0.2	11.2 ± 0.5[a]	5
'Duet'	17.6 ± 0.8	16.7 ± 0.4	17.2 ± 0.5[a]	4.0	12.8 ± 0.3	13.2 ± 0.3	13.0 ± 0.3[b]	3
Average ± *SE***	**18.4 ± 0.4**	**18.3 ± 0.3**	**18.3 ± 0.23**		**12.3 ± 0.4**	**12.0**	**12.1 ± 0.2**	
*CV****,%	5.3	5.9.	5.1		11.9	14.1	9.4	

*The upper indices (a and b) in the rows near the indicators indicate significantly different values of yield and weight of blue honeysuckle fruit relative to the average indicator (x) for the study group at $p < 0.05$; ***SE* – standard error of the average value; ****CV* – coefficient of variation.

The taste qualities of the fruits are decisive in the direction of their consumption, and the sugars contained in them are largely taste-forming. Total sugar content in blue honeysuckle berries according to Senica et al. (2018) ranged from 15.00 to 25.85 mg per 100 g. Their amount in fruits from Slovakia varied from 3.30 to 9.50% (Jurikova et al., 2014), according to other Slovak scientists, the content of sugars in honeysuckle berries was 8.26% (Pokorna-Jurikova & Matuskovic, 2007). We also obtained similar data to the Slovak scientists, the berries of honeysuckle studied by us accumulated sugars from 7.2 to 9.2%, the average is 8.2% on a raw basis (Table 5).

In 2020, ten days before harvesting, average air temperatures were 9.3°C higher than in the same period in 2021. The amount of precipitation was 34.6 mm less in 2020. Under such conditions, in 2020 the sugar content in the studied varieties was 9.0%, in 2021 - 10.1%. The amount of titrated acids did not differ significantly by the years of research. The trend of higher content of sugars and lower content of titrated acids in 2020 compared to 2021 preserved practically all studied varieties, which is evidence of the dependence of the process of synthesis of these substances on the weather of the growth period and development of blue honeysuckle fruits. According to the data obtained for two years of research, the largest content of sugars accumulated the fruits of the 'Boreal Beast' variety (9.2%), while the stability of the specified content was high, the coefficient of variation was 7%. The sugar content of the fruit of the 'Boreal Beauty' variety turned out to be significantly low 7.2%. In the rest of the studied varieties, the content of sugars varied from 8.0 to 8.5% (Table 5).

Table 5. Content of sugars and titrated acids in blue honeysuckle fruit, % per raw weight (2020–2021) (n = 5)

Variety	Sugars				Titrated acids			
	2020	2021	average	*CV*, %	2020	2021	average	*CV*, %
'Boreal Blizzard'	9.6 ± 0.1	6.5 ± 0.2	8.0 ± 1.4	21	1.7 ± 0.1[a]	1.9 ± 0.1[a]	1.8 ± 0.1[a]	8
'Boreal Beast'	9.7 ± 0.1	8.6 ± 0.2	9.2 ± 0.5[b]	7	2.8 ± 0.2[b]	3.1 ± 0.1	3.0 ± 0.2[b]	7
'Boreal Beauty'	6.6 ± 0.3	7.9 ± 0.2	7.2 ± 0.6[a]	10	2.7 ± 0.2[b]	3.2 ± 0.1[b]	2.9 ± 0.2	9
'Aurora'	8.6 ± 0.1	8.5 ± 0.1	8.5 ± 0.1[b]	1	2.4 ± 0.1	2.3 ± 0.1[a]	2.3 ± 0.1[a]	4
'Karina'	9.2 ± 0.2	8.1 ± 0.1	8.2 ± 0.5	4	1.8 ± 0.1	2.1 ± 0.1[b]	2.0 ± 0.2[a]	10
'Duet'	10.4 ± 0.2	8.1 ± 0.2	8.2 ± 1.4	23	2.4 ± 0.2[a]	2.7 ± 0.2[b]	2.6 ± 0.2[b]	12
Average ± *SE***	**9.0 ± 0.1**	**7.9 ± 0.1**	**8.2 ± 0.1**		**2.3 ± 0.1**	**2.6 ± 0.1**	**2.4 ± 0.1**	
*CV****,%	14.8	9.7	12		19.0	21.0	24	

*The upper indices (a and b) in the rows near the indicators indicate significantly different values of yield and weight of blue honeysuckle fruit relative to the average indicator (x) for the study group at $p < 0.05$; ***SE* – standard error of the average value; ****CV* – coefficient of variation.

Other substances that, along with sugars, shape the taste are titrated acids, namely their concentration in the cellular juice of the fruit. The amount of these substances in the berries of blue honeysuckle of the studied varieties varied from 1.8 ('Boreal Blizzard') to 3.0% ('Boreal Beast') with an average value of 2.4% (Table 5) on the raw weight, which is less than in Slovakia, where there were 4.04% (Pokorna-Jurikova & Matuskovic, 2007), but almost at the level of the content found in Polish berries (1.97–3.09%) (Dziedzic et al., 2020).

The low content of titrated acids (1.8%) and high sugars in 'Boreal Blizzard' and 'Karina' provided their sugar-acid index (SAI) at 4.5 and 4.3, respectively, the lowest being in 'Boreal Beauty' and 'Duet' berries (2.5).

Honeysuckle berries are endowed with a high content of primary and secondary metabolites (Senica et al., 2018b). One of these is ascorbic acid, which is a very important vitamin for the normal functioning of the human body. Its content in the fruits of one crop can vary depending on the climate and growing conditions, genotype, stage of ripeness and time of harvesting (Jurikova et al., 2009; Ochmian et al., 2012) and varies significantly within different varieties. One of the crops, the fruits of which are able to accumulate about the same amount of vitamin C as the blue honeysuckle, is highbush blueberries, which contain this vitamin from 17 to 20 mg per 100 g (Shevchuk et al., 2021). The study was conducted by Jurikova et al. (2012) have proven that the ascorbic acid content of some varieties of honeysuckle berries may even exceed the values of other fruits, which are usually considered to be rich in vitamin C. In particular, Jurikova et al. (2012) have found that some varieties of *Lonicera caerulea L.* are able to accumulate up to 135.11 mg per 100 g of ascorbic acid. Other studies indicate vitamin C values ranging from 17 to 25 mg per 100 g (Bors et al., 2012). The content of this vitamin in blue honeysuckle berries according to the results of studies of Czech scientists was 20.83–24.02 mg per 100 g and depended on the growing conditions. According to their data, in the region with more light, the content of vitamin C in honeysuckle berries was higher (Orsavová et al., 2022). Similar data were obtained in Switzerland, where the amount of vitamin C in honeysuckle berries was 17.8–42.1 mg per 100 g (Auzanneau et al., 2018). The average value of vitamin C content in the berries studied by Slovak scientists was 55.71 mg per 100 g (Pokorna-Jurikova & Matuskovic, 2007). Blue honeysuckle varieties of Canadian and Polish breeding grown in the Forest Steppe of Ukraine accumulated from 24.5 to 29.6 mg per 100 g of ascorbic acid, which is higher than the values obtained by Canadian researchers, and the lowest value exceeds the minimum that was found in berries from Switzerland.

At the same time, the stability of these indicators was high, the coefficients of variation being 4 and 8%, respectively. Of the varieties we studied, a significant amount of this vitamin was found in the fruits of 'Boreal Blizzard', although its dependence on the weather conditions of the year of cultivation was high, the coefficient of variation was 27%. The mean interclass content of vitamin C, for the group of varieties studied, was 26.5 mg per 100 g (Table 6).

Table 6. Content of biologically active substances in blue honeysuckle fruit (2020–2021) (n = 5)

Variety	Ascorbic acid, mg per 100 g				Polyphenolic substances, mg per 100 g			
	2020	2021	average	*CV*, %	2020	2021	average	*CV*, %
'Boreal Blizzard'	35.6 ± 0.7^{b}	21.6 ± 0.7^{a}	28.6 ± 6.2	27	1022 ± 26^{b}	908 ± 57b	965 ± 57	7
'Boreal Beast'	31.7 ± 0.8^{b}	27.5 ± 0.7^{a}	29.6 ± 1.9	8	1151 ± 22^{b}	849 ± 27^{a}	1,000 ± 134^{b}	17
'Boreal Beauty'	29.6 ± 0.7^{b}	24.6 ± 0.7	27.1 ± 2.3	10	938 ± 89	813 ± 20^{a}	876 ± 68	10
'Aurora'	25.6 ± 0.6^{a}	29.5 ± 0.7^{b}	27.5 ± 1.8	8	963 ± 37	902 ± 21^{b}	933 ± 33	4
'Karina'	24.6 ± 0.8^{b}	26.6 ± 0.7	24.5 ± 0.9^{a}	4	577 ± 24^{a}	444 ± 21a	511 ± 60^{b}	15
'Duet'	17.7 ± 0.6^{b}	22.7 ± 0.9	26.5 ± 1.9	9	927 ± 20	560 ± 55^{a}	744 ± 163	27
Average ± *SE***	**27.5 ± 0.2**	**25.4 ± 0.7**	**27.2 ± 1.6**		**930 ± 27**	**746 ± 27**	**838 ± 82**	
*CV****,%	22.8	11.8	7		20.6	26.2	22	

*The upper indices (a and b) in the rows near the indicators indicate significantly different values of yield and weight of blue honeysuckle fruit relative to the average indicator (x) for the study group at $p < 0.05$; ***SE* – standard error of the average value; ****CV* – coefficient of variation.

A group of Polish researchers from the University of Natural Sciences established that the fruits of the 'Aurora' variety are capable of accumulating 15.6 mg per 100 g of ascorbic acid (Gawro´nski et al., 2020). The same variety, but grown in Ukraine, contains 11.9 mg per 100 g more of this vitamin, namely 27.5 mg per 100 g (Table 6).

Blue honeysuckle fruits are able to accumulate significant amounts of total phenolic, which are among the strongest antioxidant compounds (Szajdek & Borowska, 2008; Skinner & Hunter, 2013). The total phenolic content in blue honeysuckle grown under the conditions of the Czech Republic ranged from 575 to 903 mg per 100 g (Rop et al., 2011). Skupien et al. (2009) reported lower total phenolic content (166–319 mg per 100 g), Ochmian et al. (2012) according to their data, the content of total phenolic was 149 mg per 100 g. According to the results of studies by Rupasinghe et al. (2012), the total phenolic content of in the honeysuckle of the 'Borealis' variety was 699 mg per 100 g. The high instability of total phenolic content in blue honeysuckle berries caused by growing conditions and peculiarities of the variety is evidenced by the data of studies of many other researchers, in particular, according to Senica et al. (2018a) their amount varied from 173.5 to 865.9 mg per 100 g (Lefol, 2007; Sochor et al., 2014). The coefficients of variation for the varieties which we studied were 22%, which indicates the average variability in the total phenolic content. Blue honeysuckle grown in Western Canada accumulates 1,111 mg per 100 g of total phenolic content, the largest among all the small berries studied (Bakowska-Barczak et al., 2007). Honeysuckle berries of blue varieties of Canadian breeding, if grown in the Forest Steppe of Ukraine, can contain from 876 ('Boreal Beauty') to 1,000 mg per 100 g ('Boreal Beast') of total phenolic content with intermediate values of 933 ('Aurora') and 965 mg per 100 g ('Boreal Blizzard'), and Polish 511 ('Karina') and 744 mg per 100 g ('Duet'), which is significantly higher than the data obtained by Czech colleagues, but almost at the level of data obtained by other researchers.

It was found that the less humid period of growth and development of blue honeysuckle fruit in 2020 was more favorable for their synthesis of total phenolic content. Under the condition that in 2020 precipitation fell 101.5 mm less than in the next year, and the average daily air temperatures were almost the same, the amount of total phenolic varied from 577 ('Karina') to 1,151 mg per 100 g ('Boreal Beast'). In 2021, the minimum content was at 444 ('Karina'), and the maximum was 908 mg per 100 g ('Boreal Blizzard'). The most variable and dependent on the year of cultivation was the total phenolic content in the 'Duet' variety, a coefficient of 27%, and the least dependent it was in the 'Aurora' and 'Boreal Blizzard' varieties, the corresponding coefficients of 4 and 7% (Table 6).

CONCLUSION

According to the results of research, 4 Canadian varieties of blue honeysuckle and 2 of Polish breeding are suitable for wide cultivation in the Forest Steppe of Ukraine. The climatic and weather conditions of this climatic zone of Ukraine ensure high productivity of plantations and contribute to the formation of crops with excellent commercial and consumer quality indicators. 'Boreal Beauty' and 'Aurora' varieties can be donors of yield and marketability for breeding work.

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Optimum ratio of complex biological product and fertilize (NPK) and the contribution of fungi and bacteria to the general decomposition and mulching of coniferous wood waste

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Abstract. The use of a complex biological product (CBP) based on native microbiological consortiums of coniferous forest litter accelerated the composting process of coniferous wood waste. The contribution of micromycetes and bacteria to the activation of coniferous wood waste composting processes using the different fertilizers rates was studied. A fractal analysis has confirmed the formation of a micromycetes-bacterial system in the treatment with optimal rates of NPK and CBP. In this case the better decomposition of wood waste was observed. It was noted that micromycetes of the genus *Penicillium* dominated in the composts obtained with CBP addition. This compost was not phytotoxic. Thus, for coniferous sawdust decomposition and its further humification, it is necessary to use both micromycetes and bacteria. The use of organic material resulting from wood waste decomposition with CBP and optimal rates of NPK is an effective way to increase the content of organic substances in soils and their potential fertility.

Key words: humification, sawdust, microorganisms, fractal portrait.

Abbreviations: CBP – complex biological product; GMC – graph of maximum correlations; FGM – fractal group of microorganisms.

INTRODUCTION

The bark and wood of coniferous trees decompose to form humus substances in the forest floor as a result of natural processes of microbiological destruction (Kononova & Bel'chikova, 1961). Coniferous wood contains about 50% of hard-to-decompose lignin, up to 40–55% of cellulose and 0.2% of nitrogen, which microorganisms can transform into humus forms of substances (Orlov, 1990; Nenajdenko & Mazirov, 2002; Zavarzin, 2004).

For a long time, a self-organizing biological system of micromycetes and bacteria with effective transformation of organic substrates has been formed in litter of coniferous forests, which most effectively transforms complex organic substrates (Young & Crawford, 2004; Crawford et al., 2012). The use of these biological systems in the manufacture of biological products accelerate the processing of waste on woodworking enterprises into organic fertilizers (Lozanovskaya et al., 1987; Six et al., 2006; Baldock, 2007; Fageria, 2012; Singh & Nain, 2014; Hatfield & Walthall, 2015). That improves the environmental situation around these enterprises, as well as gets some economic effect from the introduction of fertilizers in agricultural production. Otherwise, deposits of wood waste will release toxic phenolic compounds into the environment, worsening the environmental situation around them.

One way to enhance the decomposition of crop residues can be obtained by processing of microbiological inoculants containing high efficiency of microorganisms-destructors (Rusakova, 2016; Wu et al., 2017). Meanwhile, for the decomposition of organic substrates and further humification, it is necessary to use a micromycetes and bacteria (Kaszab et al., 2011; Sviridova et al., 2016; Fan et al., 2017; Leow et al., 2018). In the content of organic fertilizers obtained from wood waste humus substances are present (Vallini et al., 1997). Enzymatic systems of micromycetes of the genera *Trichoderma, Aspergillus, Penicillium* and cellulolytic bacteria can participate in the biosystem transformation of wood wastes into humus substances (Imsheneczkij, 1953; Nannipieri et al., 2003; Emcev & Mishustin, 2006).

Destruction of lignin during decomposition of wood wastes is carried out in the presence of oxygen by multi-stage biochemical transformation of organic wood molecules into low-molecular compounds by enzyme systems of fungi and bacteria. In this case, the contribution of micromycetes to decomposition of wood wastes is of particular importance, since their mycelium is able to penetrate into the cell structures of wood and provide direct contact of bacteria and their enzymes with organic molecules of plant cells from the inside (Skryabin et al., 1986; Vedenyapina et al., 2010). Thus, only in the self-organizing biological system (Young & Crawford, 2004; Crawford, 2012) when bacteria and micromycetes act together, the most rapid and organized transformation of wood wastes into humus substances will occur (Kumar & Shweta, 2011; Vorobyov et al., 2011).

In addition, there was an evidence that lignin degradation into phenols and other humic acid precursor substances can promote the formation of humic acid (Wu et al., 2017).

To decompose the bark of coniferous trees from the forest floor of coniferous forests, micromycetes and degrading bacteria were isolated, which served as the basis for a complex biological product (Patent No. 1792974 'Method of decomposition of wood') (Sviridova et al., 1993). To develop the application technology for CBP, a multi - sided study of the mechanisms of functioning of isolated lignin destructors when converting wood waste into humus substances and the principles of organizing micromycetes-bacterial biosystems in which biochemical processes must be supported by effective and coordinated actions of biosystem components was required.

The goal of these studies was to determine the optimal rates of CBP and fertilizers (NPK) and investigate the contribution of micromycetes and bacteria to joint decomposition and humification of coniferous wood waste.

MATERIALS AND METHODS

Content of CBP

The CBP contains lignin- degrading microorganisms isolated from decaying wood located on the soil of forest litter (Sviridova et al., 2001). The total number of microorganisms of CBP is about 10^7 CFU mL^{-1}, of which 10^3 CFU mL^{-1} of micromycetes, 5.0×10^6 CFU mL^{-1} of proteolytic bacteria, 4.0×10^6 CFU mL^{-1} of amylolytic bacteria, 1.0×10^6 CFU mL^{-1} of oligotrophs, and 9.0×10^4 CFU mL^{-1} of cellulolytic bacteria. Dominant cultures of CBP were identified and deposited in ARRIAM collection (RCAM): *Penicillium chrysogenum* (Thom 1910) OH 4 RCAM 00741 and *Pseudomonas fluorescens* 7 RCAM 00537. Sequences was obtained and deposited in GenBank, (GenBank Accession Numbers: MT156331 for *Penicillium chrysogenum* and MT156340 for *Pseudomonas fluorescens*). The dominant micromycetes *Penicillium chrysogenum* (Thom 1910) OH 4 RCAM 00741 and bacteria *Pseudomonas fluorescens* 7 RCAM 00537 possessed of high catalase, oxidase, cellulase, proteolytic activities and were aerobes.

Identification of dominant cultures

Identification of dominant cultures was performed by PCR-amplification and sequencing of 16s rRNA fragment (for bacteria) and internal transcribed spacer (ITS) region (for micromycetes). The 16S rDNA gene was amplified using primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCC-3’). The ITS region of the nuclear ribosomal RNA gene was amplified with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'–TCCTCCGCTTATTGATATGC-3’) (White et al., 1990). PCR-fragments were extracted from 1% agarose gel (Onishchuk et al., 2015) and sequenced using the ABI PRISM 3500xl (Applied Biosystems, Waltham, MA, USA) according to the manufacturer’s instructions. Homology searches were carried out using the BLASTn program (Altschul et al., 1990) in NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The dominant microorganisms were identified also with using a morphological and biochemical characteristics by standard methods (Raper et al., 1949; Holt, 1980; Bilaj et al., 1982; Tepper et al., 1993).

Experimental design

The model composting experiments with coniferous sawdust of and different NPK rates were carried out during 20 days in aerobic conditions with daily mixing. The sawdust of coniferous trees from sawmill (Leningrad region, Russia) was used. 15 g of air-dry mass sawdust was placed into plastic container and was distributed in triplicate The humidity of compostable sawdust was maintained to 60%, average temperature of the composting was +20 °C. The NPK rate (per 100 g of air-dry mass of sawdust) was corresponded to: NH_4NO_3 - 5.7 g; KH_2PO_4 - 2.6 g; $CaCO_3$ - 0.15 g and CBP rate is 10 mL per 100 g of air-dry mass of sawdust. Sawdust was treated with NPK and CBP.

The scheme for composting included the following experimental treatments: 1) NPK; 2) 1/2 NPK; 3) NPK + CBP; 4) NPK + 1 / 2CBP; 5) 1/2 NPK + CBP; 6) 1/2 NPK + 1 / 2 CBP.

Analytical methods

Microbiological and chemical analyzes of the composted mass of sawdust were carried out at the end of the experiment, on 20 days of composting. A decrease in C_{org} may be explained by the decomposition of organic matter. Therefore, the degree of decomposition of composted sawdust was evaluated by the loss of organic C. The degree of humification was evaluated by the amount of humic substances formed (fulvic acids and humic acids), as well as by the change in the physical characteristics of composted sawdust (by darkening, compost friability and degree of sawdust destruction).

Assay the composts toxicity

We used a biological test method to assay the toxicity of the composts. The radishes were grown for 3 days in Petri dishes with an extract from the finished compost. 1 g of a compost sample was taken and placed in a flask with 50 mL of water, mixed thoroughly, and then radish seeds were soaked in the resulting filtered solution for one day. After that, radish seeds at the rate of 25 pieces were placed on Petri dishes (4 replicates in each treatment) with filter paper humidified with 10 mL of water.

Microbiological tests

Accounting for various groups of microorganisms in samples of compost treatments was carried out according to the formation of colonies on standard media (Netrusov, 2005). The numbers of colonies of microorganisms were counted on: micromycetes - on Chapek medium with lactic acid, proteolytic bacteria - on meat - peptone agar; amylolytic bacteria - on starch-ammonia agar; oligocarbofiles - on starvation agar and cellulolytic bacteria - on a liquid Hetchinson's medium with filter paper (Tepper et al., 1993). 10 g of each compost samples were placed into flasks with 100 mL distilled water and shaken on for 30 minutes. Serial dilutions from 10^{-2} till 10^{-6} for each sample were prepared 1 mL dilutions were plated to the Petri dishes. Deep passages were made. Plates were incubated at +25 °C during six day and for cellulolytic bacteria - during twenty one days, and colony forming units (CFUs) were counted.

Assay of chemical parameters of composts

The chemical parameters of composts were determined by the following methods: Total nitrogen content (Ntotal) in compost was determined by digesting the samples in sulfuric acid (H_2SO_4) followed by Kjeldahl analysis on Kjeltek apparatus (Tekator, Sweden) (Bremner & Mulvaney, 1982). Nitrates content ($N\text{-}NO_3$) was assayed by photometric method with the phenoldisulfonic acid on KFK-2 electro photocolorimeter (Peterburgskij, 1975). CO_2 content was assayed on a gas chromatograph ('Tsvet-110', Russia), katharometer was a detector, helium was a carrier gas.

Humus fractionation

Humus fractionation was performed and the content of humus compounds was determined by the modifications method (Canellas & Façanha, 2004). Samples were treated with 2 mol L^{-1} o-phosphoric acid to separate the free fulvic acids fraction. Humic fraction was extracted by adding 100 mL of 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ to 10 g sample and shaken for 16 hours. The dark-colored supernatant solution was separated by centrifugation at 3,000 g for 30 min. The residue was resuspended in 50 mL of 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ then shaken for 4 hours. The solution was centrifuged again and the supernatant was added to the supernatant collected previously. The extracted alkaline solution containing dissolved humic and fulvic acids was acidified to pH 1.0–1.5 with H_2SO_4. After 12 hours at 8 °C the extract was separated into soluble and insoluble parts by centrifugation. After fractionation the content of organic carbon in the soluble humified organic matter was determined.

Content of organic carbon (C_{org}) - by oxidation with potassium dichromate by Tyurin method (Orlov & al., 1995). Compost samples were dichromate digested with 10 mL 0.4 N oxidizing solution ($K_2Cr_2O_4$:H2SO4=1:1) by applying in external heat of 150 °C for 20 min in presence of catalyzer Ag_2SO_4; the using oxidizing agent was determined by titration with $Fe(NH_4)_2(SO_4)\cdot 6H_2O$ using phenylanthranilic acid as an indicator.

Data analysis

The experimental data were analyzed using variance, correlation and fractal statistical analysis (Rajzin, 1980; Lakin, 1990; Kronover, 2006). Cluster analysis was used to determine the main factor in grouping experiment treatments into clusters. Euclidean distance was used for data points. The distance between the points and the clusters was estimated using the distant neighbor algorithmic method (Sneath & Sokal, 1973). Correlation analysis was used to calculate the values of the matrix of pair correlations between the measured chemical and microbiological characteristics of composts, using vectors of variation of the values of these characteristics in six experimental treatments. The maximum correlation graph was constructed based on the lower limit of the module of the values of the correlation coefficient is equal to 0.5 (Vorobyov et al., 2006). Fractal analysis modified was used to assess the participation of various physiological groups of microorganisms in the joint transformation of organic substrates.

When three physiological groups of microorganisms arrange on a straight line they are selected as fractal object and named as fractal group of microorganisms (FGM). The frequencies of microorganisms in FGM are the numerical power series (for example, 0.1; 0.01; 0.001). We assume that FGM is an indicator of the biological system organization of microorganisms. When the FGM arrive, three points into fractal portrait arrange on a straight line. Microorganisms of different physiological groups are represented by points on the fractal portraits. The coordinates of these points depend on the logarithms of their frequencies (Vorobyov et al., 2019). This method allows determining of groups microorganisms involved in biological system processes.

The index of microbial bioconsolidation was calculated on the basis of a topological analysis of the location of groups of microorganisms in fractal portraits (Fig. 3). The total number of groups of microorganisms ($N0 = 4$) represented in the portraits was taken into account, excluding the point located at the origin ($X = 0$, $Y = 0$). The number of groups of microorganisms (NF) represented in the portraits and on the dotted lines was taken into account, excluding the point located at the origin ($X = 0$, $Y = 0$). The Index of Microbial Bioconsolidation (IMB) was calculated using the following formula:

$$IMB = NF / N0$$

The IMB values for the treatments of the experiment are presented in Table 1. In the same table, the correlation coefficients of IMB with the measured characteristics of the compost are presented.

Table 1. Data of microbiological and chemical analyzes of composts of sawdust conifers with using NPK and CBP*, and ANOVA data, and correlation coefficients with the Index of Microbial Bioconsolidation

No.	Indicators	Experimental treatments						±SE	One-way ANOVA data	Correlation coefficient with IMB
		NPK	1/2 NPK	NPK + CBP	NPK + 1/2 CBP	1/2 NPK + CBP	1/2 NPK +			
		1	2	3	4	5	6			
1	Micromycetes, 10^3 CFU g^{-1}	130c**	77ab	27a	26a	28a	37a	25	$p < 0.002$	-0.61
2	Proteolytic bacteria, 10^3 CFU g^{-1}	2,260c	270a	600ab	500a	830b	4,470d	150	$p < 0.0001$	0.28
3	Amylolytic bacteria, 10^3 CFU CFU g^{-1}	110b	27a	20a	170c	50a	87b	20	$p < 0.0001$	-0.58
4	Oligocarbofiles, 10^3 CFU g^{-1}	3.7a	3.3a	4.0a	6.0a	12.7b	21.0c	2.0	$p < 0.0001$	0.62
5	Cellulolytic bacteria, 10^3 CFU g^{-1}	450c	45a	95ab	45a	45a	8a	30	$p < 0.0001$	-0.49
6	Nitrogen total, %	1.8b	1.0a	1.9b	1.6b	1.1a	0.9a	0.2	$p < 0.0001$	-0.29
7	N-NO_3, g per 100 g compost	1.7a	4.4c	4.3c	4.0bc	3.0b	3.5b	0.4	$p < 0.0001$	0.20
8	Corg of compost, %	42.0a	43.5a	42.1a	42.2a	42.3a	42.1a	1.0	$p < 0.48$	-0.26
9	Compost readiness, points (maximal 10)	5	3	10	7	7	8	-	-	0.68
10	C-CO_2, % (per 1 g of compost and per	0.29a	0.35ab	0.45c	0.33a	0.30a	0.30a	0.03	$p < 0.0003$	0.28
	Index of Microbial Bioconsolidation	0.6	0.6	1.0	0.5	1.0	1.0			

*Notes: 20 days of composting.

**Letters next to numbers indicate differences at $p < 0.05$ and according to the Dunkan test.

Microbiological and chemical data of the experiments

The microbiological and chemical data of the experiments on the humification of wood waste are shown in Table 1. The content of microorganisms in composts content was about 10^6 CFU g^{-1}. The participation of different groups of micromycetes and bacteria in the transformation of coniferous sawdust was revealed. In treatments No. 3–6 (with addition of CBP) the most effective composting of sawdust was noted. In these treatments, the dominant microorganisms were micromycetes *Penicillium chrysogenum* (Thom, 1910), which reached up to 80–90%. The number of *Trichoderma viride* (Pers, 1794) amounted 10%. Among bacteria *Pseudomonas fluorescens* was dominated. The increased abundance of *Penicillium chrysogenum* (Thom, 1910) and *Pseudomonas fluorescens* in the microbial community of composted material indicated their joint action in sawdust decomposition. These bacteria also were dominant in microbial community of CBP.

It was noted that the application of NPK increased the total number of microorganisms due to increasing of micromycetes, proteolytic bacteria, and cellulolytic bacteria. In treatments No. 1 and No. 2 (without CBP) the number of phytopathogenic micromycetes *Fusarium oxysporum* (Schltdl 1824) and *Fusarium culmorum* (Wm.G. Sm., Sacc. 1892) reached to 50%. Whereas as the number of micromycetes of other genera consisted: for *Penicillium* - 20%, for *Trichoderma* (*T. viride* Pers., 1794) - 10–20%, for *Aspergillus* (*A. niger* Tiegh., 1867) - 10%, for *Mucor* - 1–5%. In that case a dominant bacteria were *Bacillus.* Thus, in treatments without CBP the micromycetes of the genus *Fusarium* prevailed.

The decrease of C_{org} content in composts average by 6% (from 48 to 42%) in comparison with the sawdust was revealed, that in all composts there was a decrease in C_{org} from 48% (initial sawdust) to 42% (experience). It confirms the active decomposition of sawdust when using of NPK. The experimental treatment with addition of NPK (in maximal rate) and CBP had the best indicators for substrate decomposition, the destruction of sawdust was better and compost has high degree of destruction.

A small content of fulvic acids was detected in all treatments. In the treatment No. 1 (NPK) the content of fulvic acids was 0.03%. In treatment No. 2 (1/2 NPK) the content of fulvic acids was 0.01%, but humic acids were not found. However, in the treatments with addition of CBP the presence of humic acids was detected. In treatment No. 3 (CBP + NPK) the content of fulvic acid was 0.12% and the content of humic acid was 0.02%. In treatments No. 4–6 the content of fulvic acids was 0.07% and humic acids was 0.01%.

The composts obtained in experimental treatments with CBP stimulated biomass of radish seedlings up to 10% and radish seeds germination was 100%. In the same time in treatments No. 1 and 2 (without CBP) radish seeds germination was only 95–97%.

It was noted that the intensity of CO_2 emission during composting was lower in the treatment without the use of CBP and with a full dose of nitrogen (No. 1) - at the level of 0.20–0.29%. This may be due to denitrification processes, which can occur at low respiration rates. This is also indicated by a decrease in the content of $N\text{-}NO_3$ in the compost of this treatment relative to others. In the treatment with a half dose of nitrogen (No. 2), the respiration rate gradually increased to 0.35%, which may be due to longer processes of microbiological decomposition of the sawdust.

With a decrease of the application rate of CBP (1/2 CBP), the respiratory rate also decreased. The increased activity of CO_2 emission in the early stages of composting in treatments with CBP indicate an intensification of the decomposition of sawdust by the CBP community of micromycetes and bacteria initiated by CBP.

It was noted that in compost of treatment No. 1 (NPK) was a sufficiently high contents of micromycetes, proteolytic bacteria and cellulolytic microorganisms, but the degree of humification of sawdust was lower than in treatments with CBP. In treatment No. 6 (1/2 NPK + 1/2 CBP) with a good degree of humification of the substrate, a higher content of micromycetes was noted than in other treatments with addition of CBP, but the number of cellulolytic microorganisms was lower than in treatment No. 3 (CBP and NPK), i.e. degrading processes slowed down.

Meanwhile, in the optimal treatment No. 3 (NPK + CBP), the number of proteolytic and amylolytic microorganisms was average, but the content of cellulolytic microorganisms was twice higher compared to treatments No. 2, No. 4, No. 5 and No. 6 with a fairly high number of micromycetes.

Probably, a biosystem of microorganisms formed in this ratio could be optimal for the processes of humification. This may indicate the influence of the micromycete-bacterial complex of CBP (contains a biosystem community of lignin destructors) on the activation of the processes of transformation of lignocellulosic substrates into humic substances.

RESULTS OF DATA ANALYSIS

As a result, of the cluster analysis of the characteristics of the experimental treatments, a dendrogram was constructed (Fig. 1) and the factors uniting the experimental treatments into clusters were determined. It noted that the treatments with NPK (No. 1and No. 2) and without CBP did not form a cluster, two clusters formed when CBP was add. Treatments No. 3 and No. 4 (with full dose of NPK) united into the first cluster. Treatments No. 5 and No. 6 (with 1/2 NPK) conform the second cluster. Thus, the main factor in the clustering was NPK rates, which unite these treatments into clusters.

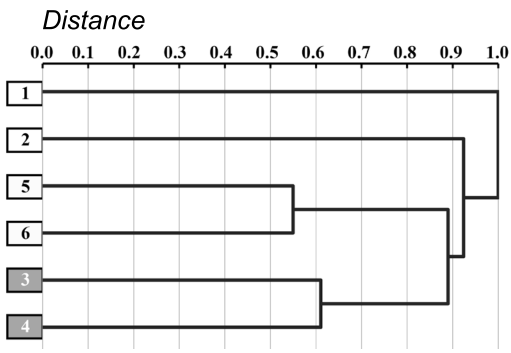

Figure 1. A dendrogram of the proximity of experimental options, built on their microbiological and chemical characteristics: 1) NPK; 2) 1/2NPK; 3) NPK+CBP; 4) NPK+1/2BP; 5) 1/2NPK +CBP; 6) 1/2NPK +1/2CBP (distance in the Euclidean space according to the principle of ‘distant neighbor’).

The analysis of pair correlations of the compost’s characteristics and microbial community data (Table 1) made it possible to single out the highest absolute coefficients of correlations and construct a nondirected graph of maximum correlations (GMC, Fig. 2).

It was taken into account the absolute value of the correlation coefficients should exceed 0.5. GMC demonstrates the relationship ($r = 0.89$) of micromycetes (1) with cellulolytic bacteria (5), which indicates their joint participation in the decomposition of lignocellulose from sawdust. The absence of a connection between amylolytic bacteria (3) and other components of the biosystem means that this group of microorganisms does not take an active part in biosystem processes of composting. Their activity requires readily available carbohydrates that have not formed in the right amount. GMC demonstrates that proteolytic bacteria (2) and oligocarbofilic bacteria (4) act in a close correlation ligament (r = 0.76), actively participating in the processes of transformation of the organic substrate.

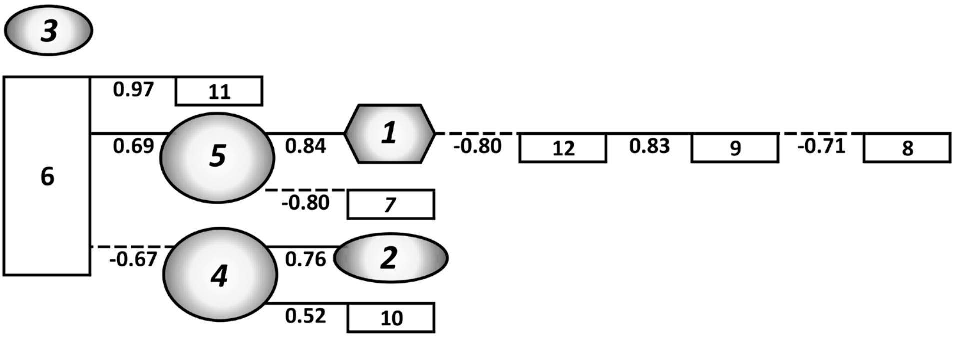

Figure 2. A graph of the maximum correlation coefficients between the chemical and microbiological characteristics of compost during the humification of wood waste: 1 – micromycetes; 2 – proteolytic bacteria; 3 – amylolytic bacteria; 4 – oligotrophs; 5 – cellulolytic bacteria; 6 – N_{total}; 7 – N-NO_3; 8 – Corg. of compost; 9 – the degree of compost readiness; 10 – C-CO_2; 11 – dose of N; 12 – the rate of application of the biological product.

Oligocarbofilic bacteria (4) with active oxidoreductases participate in the decomposition of lignin together with proteolytic bacteria (2) (r = 0.76) and decrease the N_{total} (6) (r = -0.67). Micromycetes (1) and cellulolytic bacteria (5) also act in conjunction (r = 0.84) destroying polymer organic molecules in compost. In this case N-NO_3 (7) is consumed and the connection with N_{total} (6) (r = 0.69) is strengthened with an increase in its dose. Thus, two groups of microorganisms (2)–(4) and (1)–(5) perform the main function of the destructive biosystem. The use of CBP (12) accelerates composting processes, which is reflected in an increase in the level of humification of compost (9) (r = 0.83). A decrease in the total number of micromycetes (1) when using CBP (12) indicates that micromycetes function in the composition of biosystems with a smaller number (r = -0.81), but with a greater efficiency of further biochemical transformations of organic substrates and with reducing of consumption of energy and nutrient resources.

To assess the extent of participation of the functional groups of microorganisms in biosystem processes, we used a fractal analysis of microorganism's amount. For this, the fractal portraits of microbial communities were constructed (Fig. 3). In fractal portrait points represent the groups of microorganisms with coordinates depending on the frequency of occurrence of the corresponding microorganisms. The topological analysis of the arrangement of points in a fractal portrait showed that some groups of three points (triplets) are located on straight lines. We assume that this arrangement of points is a nonrandom event, and it indicates the formation of a biosystem by the corresponding groups of microorganisms. The absence of (1)–(5) or (2)–(4) microorganism's groups in triplets in treatments No. 1 and No. 2 (without CBP, Fig. 3, a and 3, b) demonstrated the absence of CBP influence on biosystem processes. The location of micromycetes (1) in the upper part of the fractal portrait demonstrate low efficiency of waste humification the in treatments No. 1 and No. 2 (Fig. 3, a and 3, b). In treatments No. 3 (CBP + NPK, Fig. 3, c) and No. 6 (1/2CBP + 1/2NPK) (Fig. 3, f), all points depicting trophic groups of microorganisms are covered by straight dashed lines, i.e. these microorganisms form common biosystems. We believe that in these treatments, the biosystems are formed and micromycetes (1) take part in biosystem's processes in the first positions, at the very beginning of composting, since they are located in the lower part of the fractal portraits.

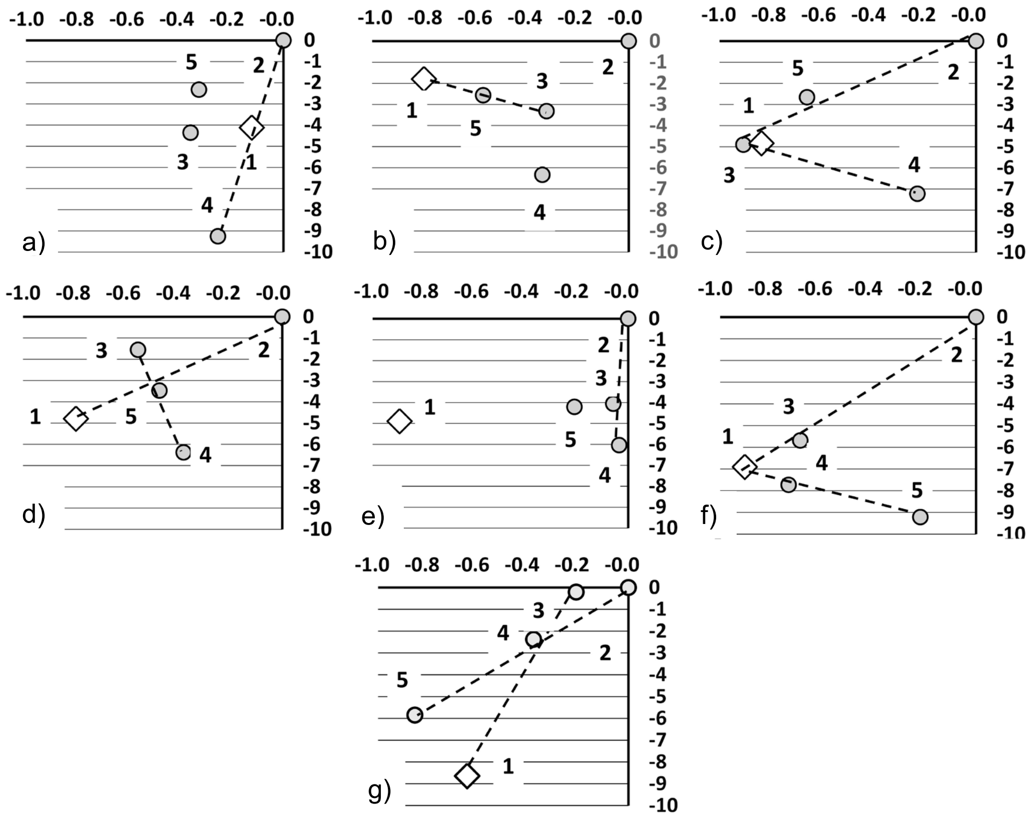

Figure 3. Fractal portraits of trophic groups of microorganisms participating in the processes of humification of wood waste according to experimental options.
Treatments of experience: a – NPK; b – 1/2 NPK; c – NPK +CBP; d – NPK + 1/2 CBP; e – 1/2 NPK +CBP; f – 1/2 NPK +1/2 CBP; g – Complex Biological Product (CBP).
Groups of microorganisms: 1) micromycetes, 2) proteolytic bacteria, 3) amylolytic bacteria, 4) oligocarbofiles, 5) cellulolytic bacteria.

Notes: The numbering of the points in the portrait corresponds to the numbering of microorganisms in Table 1. On the X-axis graphs, the fractional part of the $log_2(p_i / p_{max})$ value is plotted, and along the Y-axis, the full $log_2(p_i / p_{max})$ value is plotted, where pi and $p_{max} = p_2$ are the frequency of occurrence of microorganisms in compost with the number (i = 1, 2, 3, 4, 5) and the frequency of occurrence of dominant microorganisms, which are proteolytic bacteria - (i = 2).

DISCUSSION

The microbiological transformation of wood wastes from the woodworking industry into substances makes it possible to solve ecological problem and agricultural fertilizing. To increase the efficiency of microbiological transformation of wood waste into humic substances, it is necessary that the microbial community forms an active destructive biosystem. Microorganisms in the composition of sawdust destructive biosystems transform organic substrates in an organized and orderly manner, evenly distributing the enzymatic load among the participants of a conversion processes. Since microorganisms in the composition of biosystems transform organic substrates in an organized and orderly manner, evenly distributing the enzymatic load among the participants in the conversion process. For example, in consortium of cellulolytic and oligocarbofilic bacteria in soils, bacteria are consistently responsible for the hydrolytic degradation of complex high-molecular nitrogen-free organic substances (cellulolitic), as well as for the redox processes of transformation of residual products into humus links (oligocarbofiles). Such active destructive biosystem was formed in sawdust composts obtained with addition of CBP (Kozlov et al., 2017). For fast humification of composts the optimal ratio between the numbers of different groups of microorganisms should be set. The active destructive CBP biosystem (which containse a micromycetes, proteolytic, cellulolitic and oligocarbofilic bacteria) activated the processes of transformation of lignocellulosic substrates into humic substances. The increasing of activity of CO_2 emission in the early stages of experiment in treatments with addition of CBP indicated the increasing of decomposition of sawdust.

Before composting experiments, a long cultivation of the microbial community was carried out, and the micromycetes-bacterial biosystem was selected for CBP. The fractal portrait (Fig. 3, g) confirmed the systemic consolidation of microorganisms in the CBP.

It is noted, that the compost from other treatments (without addition of CBP) decreased the total mass of radish seedlings below the control on 5%. Because in these composts dominated the phytopathogenic micromycetes of the genus *Fusarium*. Whereas the toxicity of composts obtained with addition of CBP was eliminated. Because in biosystems of these composts *Penicillium chrysogenum* (Thom, 1910) and *Pseudomonas fluorescens* were dominated. The possibility of suppressing soil plant pathogens using compost has been widely studied (Noble & Coventry, 2005: Pugliese et al., 2010). Compost quality is also related to the concentration of humic substances in the final product; thus, determining humification progress is essential for evaluating composting process (Kulikowska & Sindrewicz, 2018). Composts obtained with addition of CBP had a high degree decomposition and small content humic acids, which depend on the composting time. Humic substances increase a soil fertility (Vergnoux et al., 2011).

The dendrogram (Fig. 1) demonstrates the dependence of sawdust composting and transformation into humic substances on rates of NPK and 1/2 NPK, because two different cluster are formed with these rates. When using CBP, the NPK and 1/2NPK rate determines the biochemical characteristics of the destructive processes and the number of microorganisms involved in these processes. Apparently, the low dose of NPK is necessary to start of destruction and to grow of microorganisms in the required quantitative ratio.

The graph of maximum correlations (Fig. 2) shows the formation of two binary microbial complexes in a destructive biosystem: (1)–(5) - micromycetes and cellulolytic bacteria, (2)–(4) - proteolytic bacteria and oligocarbofiles. The greatest emission of CO_2 and decomposition of the composted mass were recorded in experimental treatment No. 3. Based on this data it can be argued that in experimental treatment No. 3 the (2)–(4) microbial complex acts maximal effectively, which determines the highest intensity of the processes of transformation of the organic substrate in humic substances. At the same time, the (1)–(5) microbial complex increases the nitrogen content in humic organic mass that increases its fertilizing properties.

Fractal portraits of microbial communities of compost masses showed that in the experimental treatments No. 3, 4, and 6 (Fig. 3, c; 3, d; 3, f) biosystems similar to CBP biosystem (Fig. 3, g), were formed. But, in the experimental treatments No. 1, 2 (Fig. 3, a; 3, b) this biosystems were not formed. This indicates that the addition of CBP stimulated the formation of microbial biosystems in composts.

This confirmed also by emitting of a greater amount of CO_2 (45, 33, 30% per g of compost and per day) in these experimental treatments.

The correlation of IMB with the compost decomposition scores (feature 9, $r = 0.68$) indicates an increased importance of the biosystem organization of the microbial community for accelerated composting of wood waste.

CONCLUSION

The micromycetes closely interacted with cellulolytic, oligocarbofiles and proteolytic bacteria during composting of sawdust of coniferous trees. The micromycetes closely interacted with cellulolytic, oligocarbofiles and proteolytic bacteria during the process of coniferous sawdust decomposition. As a result, fulvic acids and humic acids were formed.

The addition of CBP during coniferous sawdust composting resulted in production of a high-quality non-toxic compost and in micromycetes-bacterial biosystem (similar to the biosystem in CBP) formation. The optimal rates of CBP and fertilizers (NPK) for composting were determined. The fractal portrait demonstrated the formation of a micromycetes-bacterial system when NPK and CBP were applied.

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The influence of agroecological and agrotechnological factors on the generative development of oilseed radish (*Raphanus sativus* var. oleifera Metzg.)

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Abstract. During the eight-year research period, we determined the peculiarities and regularities of morphological (length and diameter) and anatomical (stem thickness) features of oilseed radish pods considering their location within the generative part of plants for different types of spatial structure of the inflorescence generated in agrocenosises of different densities. We carried out the analysis and statistical grouping of morphological features of oil radish pod in the full range of possible technological options of pre-sowing construction of its agrocenosises, as well as within the selected three zones (tiers) of inflorescence by the nature of variation and variability of morphoparameters pod, namely, lower, middle and upper. We described in detail the stages of pod formation (microstages BBCH 69-87) considering features of its linear and radial growth, peculiarities of formation of general internal anatomical structure with analysis of mathematical and statistical regularities of changes in these parameters in accordance as per order of its placement within an inflorescence (separately main axis and system of lateral branches). We determined the optimum technological intervals for the construction of oilseed radish agrocenosises, which ensure the combination of appropriate levels of morphometry formation of its fruit elements with the predicted level of reproductive effort and seed productivity. We made a general assessment of the peculiarities of formation of pod technological effectiveness in terms of ease of threshing and possible losses of seeds depending on the complex of factors under study.

Key words: inflorescence, layering, morphological characteristics, *Raphanus sativus* var. *oleifera* Metzg., pod, variability, variation.

INTRODUCTION

Productive flowering, fruit formation and ripening for different plant species is a common biological basis for successful tactics of reproduction, seed dissemination and dominance in certain areas (Forlani et al., 2019). Aspects of variability of the generative part of plants are crucial in assessing the formation and realization of seed potential of plants (Ballester & Ferrendiz, 2017). The peculiarities of the formation of such variability are determined by many factors depending on genetically determined values, taking into account the species specificity of plant response to abiotic environmental parameters, especially the ratio of temperature and atmospheric humidity from the beginning to the end of flowering and seed maturation (Parvaiz, 2017; Van der Knaap & Ostergaard, 2018; Dong & Østergaard, 2019; Chai et al., 2020; Chen et al., 2021; Ahmad et al., 2021).

For cruciferous plant species, morphological variability of pods is decisive for predicting yield levels, ensuring crop planning with minimal losses and ensuring the high technological quality of harvested seeds (Gulden et al., 2017; Nikolov, 2019; Shafighi et al., 2020). In general, the pronounced variability of morphometry of cruciferous pods is due to a number of factors. The main ones are related to the growth processes of the inflorescence itself, which is usually characterized by an insert type of growth with the formation of flowers from its base to the apex (Qing et al., 2021). The process of flower formation in cruciferous species is quite stretched in time. Due to this, in the generative part of the plants there are both fully formed pods and flowers at the stage of flowering (Ågren et al., 2017; Tsytsiura, 2019). This ultimately determines the variability of future morphometry of pods (on such features as pod length, pod diameter, thickness of pod walls, intensity of its pubescence, thickness of intrafertile histological elements) in the spatial structure of inflorescences with differentiation of pods in the direction from inflorescence base to its apex (Łangowski et al., 2016; Yang et al., 2016a, 2016b, 2017; Dong et al., 2018; Li et al., 2019; He et al., 2020; Li et al., 2020; Sun et al., 2021).

The spatial structure of both the inflorescence and the subsequent placement of pods is complicated by the presence of lateral branches of different order (Lu et al., 2017; Strelin & Aizen, 2018; Dogra & Dani, 2019; Tsytsiura, 2019; Li et al., 2020). Changes in the spatial structure of the reproductive part of both cruciferous and other plant species are based on the interaction of genotypic characteristics, the level of modifying variability (ecological, matrix), environmental factors and agrotechnological parameters of agrocenosis and fertilization (Inger et al., 2019; Gianella et al., 2021; Li et al., 2021). Thus, the level of adaptability of the applied agro-technological parameters of pre-sowing construction of agrocenosis can potentially be assessed by the nature of the architecture of the reproductive part of plants. It should be noted that for many groups of crops, aspects of these factors are insufficiently studied (Labraa et al., 2017; Kayaçetin et al., 2018; Rauf & Rahim, 2018; Tsytsiura, 2021).

On the other hand, studies conducted on spring and winter rape, white mustard and a number of other cruciferous crops have shown that the assessment of generative part formation is one of the basic requirements for predictive modeling of seed productivity (Liao et al., 2009; Neuffer & Paetsch, 2013; Salisbury et al., 2017). It is important to form the initial system of indicators that clearly regulate the sequence of inflorescence formation and features of its morphometry depending on agrotechnological options for constructing agrocenosis (Classen-Bockhoff & Bull-Herenu, 2013; Walker et al., 2021). Insufficient study of the peculiarities of the formation of the spatial structure of the inflorescence, the dynamics of pod development and the impact of these factors on the structure of individual biological seed productivity of plants (Harder & Prusinkiewicz, 2013; Matar et al., 2021). According to Menendez et al. (2019) this approach is the main one in view of current trends in assessing the effectiveness of various technological methods and allows to separate the formation of vegetative mass and seeds in a staged format with the identification of the main factors influencing it. For cruciferous crops, the analysis of the ratio of vegetative and reproductive parts is a basic aspect of the analysis of yield and adaptability (Wang et al., 2018).

It should be noted that the assessment of the peculiarities of inflorescence formation will allow to detail the processes of seed loss during its maturation and harvesting (Raboanatahiry et al., 2021). In particular, for spring and winter rape, these issues are included in the program of breeding research and according to Shahzadi et al. (2015) need to re-evaluate and apply new approaches. Given that the pods of oilseed radish are undiscovered with certain features of the anatomy (Tsytsiura, 2020, 2021) it would be important to study the dynamics of anatomical changes in the its formation. Such studies will allow to apply the identified features in the selection improvement of traditional cruciferous crops, the fruits of which are revealed during the ripening of seeds.

It is established that the problems of successful mechanized threshing of oilseed radish due to the anatomical features of its pod limit the prevalence of this culture from the standpoint of successful seed production (Chammoun, 2009). In view of this, the study of biological components of the formation of oilseed radish pods on the background of the full range of applied agro-technological parameters of pre-sowing design of its agrocenoses will identify specific solutions to this pressing problem.

Most research in this area is related to cruciferous crops (Weberling, 1992; Alvarez-Buylla et al., 2010; Penin & Logacheva, 2011; Harder & Prusinkiewicz, 2013; Brunel-Muguet et al., 2015; Wang et al., 2018; Siles et al., 2021) cover the assessment of the peculiarities of inflorescence formation, reproductive effort and don’t cover the full cycle from the stage of inflorescence formation to pods formation in the spatial structure of its branches. Given this fact, the combination of a comprehensive study of the impact of agroecological factors (in a complex combination of agrotechnological parameters of agrocenosis and hydrothermal regimes) on the stages, dynamism and spatial structure of cruciferous elements from flowering to pod ripening is an important task. This area of research has all the hallmarks of innovation and scientific novelty and will be useful for modern agrobiological and breeding practice of cruciferous crops. The results obtained due to such an integrated approach on the example of oilseed radish will allow to use them as indicators for optimizing the technological formation of cruciferous agrocenoses.

MATERIALS AND METHODS

The research was conducted during 2013–2020 on the experimental field of the Vinnytsia National Agrarian University (49°11′ N, 28°22′ E) on dark gray forest soils (Luvic Greyic Phaeozem soils (WRB, 2015)). During the oilseed radish growing season of April-September (178 days). Height above sea level: 325 m. The general agrochemical characteristics of this soil type are as follows: humus content: 2.02–3.20%, lightly hydrolyzed nitrogen 67–92, mobile phosphorus 149–220, exchangeable potassium 92–126 mg kg^{-1} of soil at pHкcl 5.5–6.0. Technological parameters for the formation of oilseed radish agrocenosises were carried out in the interval of recommended variants in terms of common row (row spacing of 15 cm) and wide-row (row spacing of 30 cm) sowing methods (Table 1).

The study was conducted on basic area-specific oilseed radish genotypes, namely ‘Zhuravka’, ‘Lybid’ and ‘Raiduha’. Given the similarity of the identified regularities and peculiarities, the materials presented in this paper relate to the ‘Zhuravka’ variety with relevant practical conclusions regarding the general species of oilseed radish (Raphanus sativus var. oleifera Pers.). The sowing period for all research variants was in the range of April 8–12.

The analysis of pod morphology was carried out both within the generalized typical zones of the inflorescence and in its dominant middle zone, considering such features as pod length (l, cm), diameter (d, mm), wall thickness (hw, mm). The specified indicators were considered on 10 typical plants in incompatible replications for each technological variant of agrocenosis construction. The plant typicality and determination of the inflorescence zones were carried out according to the systems of studying the formation of the tiered spatial structure of plants according to the basic principles of phytocenology for technologically formed artificial agrocenoses (Ramensky, 1971; Rabotnov, 1978), and according to the methods of similar estimations applied to white mustard (Kumar et al., 1996) and spring rape (Khmelyanchyshyn, 2005). We assessed the variation in the morphological parameters of the fruit by sampling 125 pods for each repetition in the section of the selected inflorescence zones (a total sample of 500 pods from each inflorescence zone). The total number of replicates of each variant is 4. The plant analysis involved evaluating a group of 5 typical plants according to row length stochastically by plot width with a shift in row horizontally from the beginning of flowering of the plants ((Biologische Bundesanstalt, Bundessortenamt and Chemical industry (BBCH) 62 (Meier, 2001)) to the phase when all pods have reached the variety and typic size (BBCH 87). For dynamic observation of the intensity of linear and lateral growth of the pod, we marked the indicator plants using coloured markers with the corresponding numbers.

Table 1. The range of acceptable common options for the formation of oilseed radish agrocenosis at the location of the study

Planting method and seeding rates (million germinable seeds·ha^{-1})		Fertilization (of the active substance), kg·ha^{-1}
Row method (15 cm)	Wide-row method (30 cm)	
1.0	0.5	$N_0P_0K_0$
2.0	1.0	$N_{30}P_{30}K_{30}$
3.0	1.5	$N_{60}P_{60}K_{60}$
4.0	2.0	$N_{90}P_{90}K_{90}$

To determine the morphometric characteristics of the pod a Topex 31C625 digital caliper (± 0.01 mm) and a Digital Caliper electronic micrometer (± 0.01 mm) was used. We used the USB microscopy method for microscopic examination of the pod slices (Sigeta MCMOS 5100 5.1 MP USB 2.0 (x10 and x40 optical zoom formats) and Ootdty DM-1600, 2 Megapixel + Image J software package v1.52. Photo images were taken using Canon EOS 750D Kit with Canon EF 100 mm f/2.8L USM and Canon MP-E 65 mm f/2.8 1-5x Macro.

The general research methodology, the recording of the macrostages of the phenological phases of the oilseed radish fruiting period and other related observations and records were conducted in accordance with the basic research recommendations for cruciferous crops (Sayko, 2011) and the methodological descriptive recommendations of the classification ranking tables (Test Guidelines…, 2017) using experimental statistical approaches (Dunstone & Yager, 2009) in the format of four-factor dispersion analysis (Multivariate Analysis of Variance (MANOVA) and pack of statistical programs Statistica 10, Exel 2013. We performed simulations with the selection of appropriate functional dependencies using the CurveExpert Professional 2.7.3 software package.

The climate of the region is moderately continental (Dfb according to the Köppen-Geiger climate classification (Pivoshenko, 1997). During the study period, the maximum and minimum average monthly temperature were 18.3 °C in July and 15.8 °C in May. Mean annual relative humidity was 77% and mean annual precipitation was 480–596 mm.

The hydrothermal parameters of the oilseed radish vegetation period varied, having formed certain typological features of the research years (Fig. 1, Table 2).

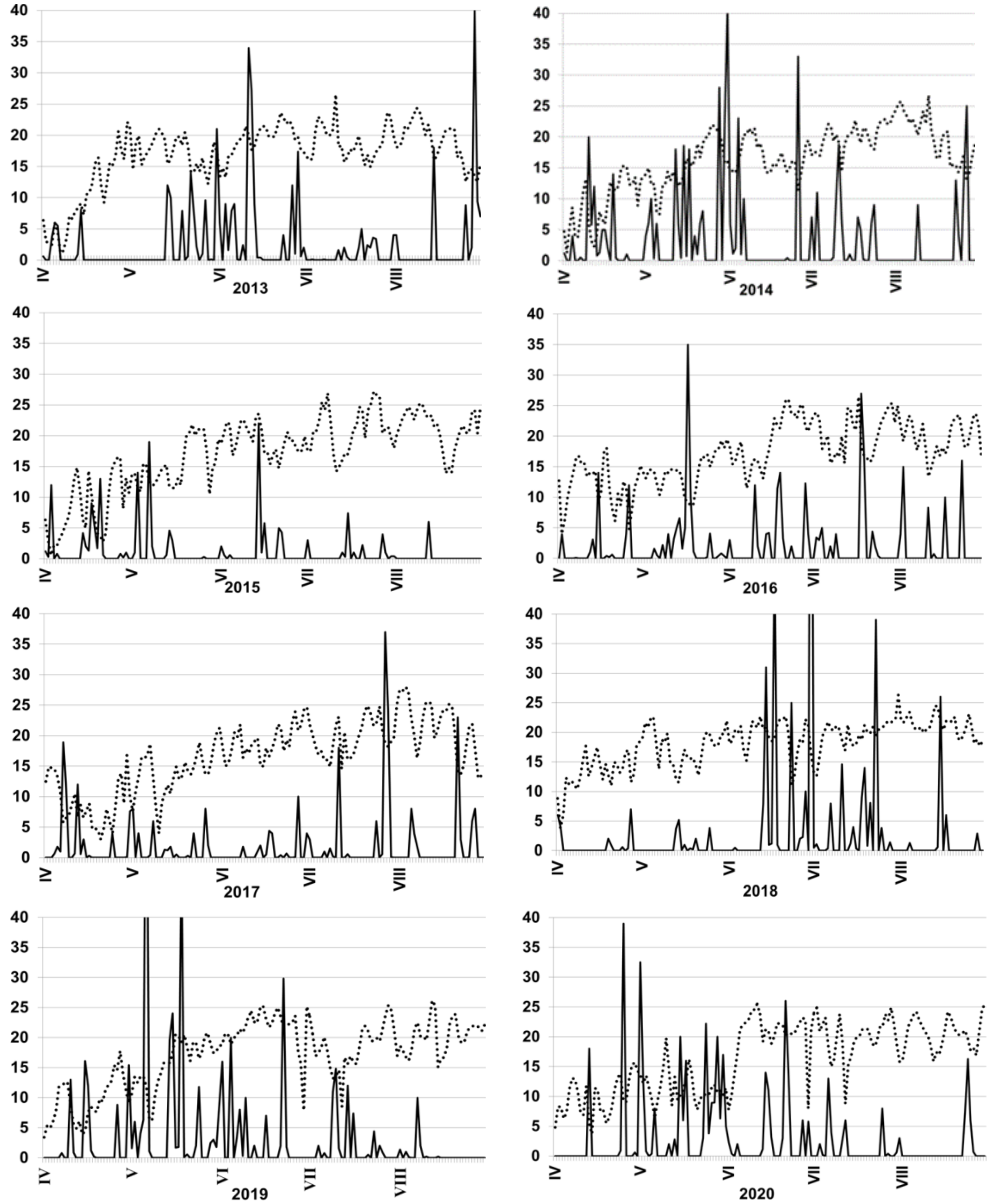


Figure 1. The hydrothermal conditions for April-August (2013–2020) (dashed line – average daily temperature, °C, solid line – rainfall, mm).

The conditions of 2013 and especially of 2014 can be referred to as the most optimal for the growth processes of the oilseed radish due to the combination of slow rates of increase in average daily temperatures and equal precipitation from the end of May to mid-June. This corresponds phenologically to the active vegetation in the study area, and the rare vegetation coincides with the interphase of the stem-flowering phenological period (BBCH 30-65). For the period of research, we must classify the conditions of 2015 and 2018 as stressful for the physiological and growth processes of oilseed radish plants according to the ratio of precipitation equality and the nature of the average daily temperature curve. For instance, the precipitation distribution in 2015 was uneven with the total absence during the period of the second decade of May - the second decade of June due to the intense and rapid increase of average daily temperatures during the same period at the high amplitude of values. This created a double effect of the overall stress of the environmental factor in the inter-phase start of budding-flowering (BBCH 38-64) with respect to the oilseed radish plants and made it possible to evaluate the studied indicators in the environmental-trait system effectively.

Table 2. Monthly average hydrothermal coefficient* over the growing season of oilseed radish, 2013–2020

Year of research	Months					Average value for the period of pod formation**	Average for the years of vegetation
	V	VI	VII	VIII	IX		
2013	1.305	2.202	0.377	1.047	3.441	1.614	1.527
2014	2.783	1.078	1.137	0.750	0.736	1.618	1.269
2015	0.719	0.613	0.230	0.061	0.684	0.535	0.430
2016	1.227	0.893	0.682	0.486	0.063	0.987	0.663
2017	0.645	0.349	0.806	0.563	1.983	0.562	0.824
2018	0.258	3.124	1.349	0.349	0.680	2.628	1.179
2019	4.710	1.555	1.003	0.235	0.945	1.569	1.690
2020	5.489	1.474	0.649	0.474	1.208	1.331	1.859

* – $HTC = \frac{\sum R}{0.1 \times \sum t_{>10}}$ (Selyaninov, 1928), where the amount of rainfall (ΣR) in mm over a period with temperatures above 10 °C, the sum of effective temperatures (Σt > 10) over the same period, decreased by a factor of 10. Ranking of values HTC (Selyaninov, 1928; Evarte-Bundere & Evarts-Bunders, 2012): HTC > 1.6 – excessive humidity, HTC 1.3–1.6 – humid conditions, HTC 1.0–1.3 – slightly arid conditions, HTC 0.7–1.0 – arid conditions, HTC 0.4–0.7 – very arid conditions. ** – Period of pod formation (perennial variable interval): III decade of May – II decade of July.

We observed a prolonged atmospheric and soil drought with a slight humidity until the second decade of June for the conditions of 2018 against the background of low average daily temperatures, which, unlike the conditions of 2015, affected the magnitude of the architecture of oil radish plants from the stage of rosette formation and its further stalking (BBCH 19-38). For these reasons, the stressful year 2018 is the most illustrative in the assessment of stress. We must attribute the 2016 and 2017 years of research to the intermediate ones by hydrothermal parameters in the six-year study cycle with a similar dynamic regime of average daily temperatures and uneven atmospheric humidity. In this case, the conditions of 2016 are close to 2013–2014, and the conditions of 2017 are similar to those of 2015. The 2019 growing season was characterised by a cool period from sowing to the rosette phase against a background of intense atmospheric moisture over the period from May to June, which was significantly higher than the multi-year average. An intense rise in temperatures was recorded from mid-June with steady atmospheric moisture until mid-July. The 2020 hydrothermal conditions were marked by the temperature conditions as abnormally low between April and May, in line with the biological optimum of oilseed radish growth and development. The amplitude of temperature fluctuations for this year of research was one of the largest.

As a result, the increase in the overall favourable hydrothermal regimes of oilseed radish vegetation in the direction of reducing the weather risks of impact on the reproductive part of the plants should be placed in the following order: 2018–2015–2017–2016–2013–2020–2019–2014.

RESULTS AND DISCUSSION

Oilseed radish is characterized by a certain response in plant morphogenesis to changes in such technological parameters as the density of plants and the width of row spacing against the background of various options of using mineral fertilizers (Tsytsiura, 2020). This reaction is determined by a certain morphotypes of oilseed radish plants in terms of the spatial structure of their inflorescence (Figs 2–3).

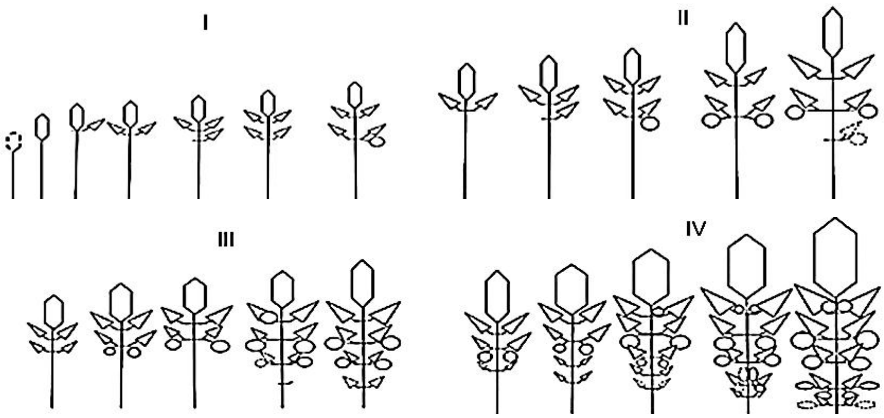


Figure 2. Types of spatial structure of the inflorescence of oilseed radish plants of the 'Zhuravka' variety of different tiers of agrocenosis at different variants of its technological construction (I – at the sowing rate of 4.0 million pcs. ha^{-1} of germinable seeds on a nonfertilized ground; II – at the sowing rate of 4.0 million pcs. ha^{-1} of germinable seeds with fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1}; III – at the sowing rate of 0.5 million pcs. ha^{-1} of germinable seeds on a nonfertilized rate; IV – at the sowing rate of 0.5 million pcs. ha^{-1} of germinable seeds with fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1} based on the results of multi-year assessments for the 2013–2020 period (conventional symbols: ⬭ – main stem inflorescence; ▻ – inflorescences of lateral branches of the first tier; ⬭ – inflorescences of branches of the second tier; ○ – inflorescences of branches of the second tier).

According to some research (Rabotnov, 1978; Al-Doori & Hasan, 2010; Bhushan et al., 2013; Chang et al., 2020) such changes in the architectonics of the reproductive part indicate an appropriate level of compensatory capacity of plants and an appropriate level of adaptation to growing conditions. The studies found (Yang et al., 2016b; Labraa et al., 2017; Kirkegaard et al., 2018; Han et al., 2021), that according to the character of changes in the inflorescence structure in terms of potential productivity (number of flowers, number of inflorescence branches, mass fraction of inflorescence in relation to the weight of the above-ground plant part), we can draw conclusions about the compensatory adactive biological potential of plants. Studies on the variability of the number of lateral branches of the inflorescence with changes in seeding rate and nutrition area found the value of this indicator for winter and spring rape in the range from 2 to 15 branches (Gomez-Campo, 1999; Koenig et al., 2011; Brunel-Muguet et al., 2015; Pinet et al., 2015; Li et al., 2016; Halevy, 2019; Haliloglu & Vedat, 2019; Adhikari et al., 2021) and for white mustard from 5 to 17 branches (Chaniyara et al., 2002; Zając et al., 2011; Zhuikov, 2014; Jat et al., 2017; Rauf & Rahim, 2018). According to these values, the potential for branching of oilseed radish inflorescences in the comparison of options 4.0 and 0.5 million pcs. ha^{-1} of germinable seeds in the range from 1 to 21 branches indicated a higher level of its compensatory response than in other traditional cruciferous crops. Levels of lateral branching of inflorescences for limiting technological variants of sowing and fertilizer rates had significant differences (Fig. 2). It was noted over a long period of study of 1–5 lateral branches for the variant of 4.0 million pcs. ha^{-1} of germinable seeds without fertilizers and 9–21 for the variant of 0.5 million pcs. ha^{-1} of germinable seeds with fertilization rate of of 90 kg N ha^{-1}, and 90 kg K ha^{-1}. Supplementary mineral nutrition ensures quantitative optimisation of the reproductive organs of oilseed radish plants especially as regards both the total number of branches and indicators such as their length, the number of flowers on each branch, etc.

At the same time, we determined the sequence of quantitative increase in the general indicators of development of the reproductive part of oilseed radish plants when decreasing the seeding rate, increasing the area of plant nutrition, and optimizing their mineral nutrition (Fig. 3, Table 3). We found that with a high level of variation in the number of inflorescence branches within the studied technological variants, the increasing trend is stable with optimization of mineral nutrition and increase in nutrition area. So, for the variant of maximum density of 4.0 million pcs. ha^{-1} of germinable seeds the interval range of the indicator was 1.0–8.8 of lateral branches, and for the variant of 0.5 million pcs. ha^{-1} of germinable seeds it was 5.5–20.5.

Figure 3. Morphotype of oilseed radish plants of the 'Zhuravka' variety with fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1} (from left to the right the first three positions: 0.5 million pcs. ha^{-1} of germinable seeds (wide-row sowing); the next three positions – 4.0 million pcs. ha^{-1} of germinable seeds), 2020.

Table 3. Summary morphological individual parameters of oilseed radish pods for the middle zone of the inflorescence at the yelow pod phase (BBCH 79-81) depending on the technological variation of agrocenosis construction, 2013–2020

Options of the experiment	Fertilizer	Average for 3 varieties ('Zhuravka', 'Raiduha' and 'Lybid')									
		Range of				Average values			V_R*		
		Inflores-cence branches (ib)	Length of the pod (l), cm	Diameter of the pod (d), mm	Thickness of walls' pod (hw), mm	l, cm	d, mm	hw, mm	l	d	hw
4.0 million, row	$N_0P_0K_0$	1.0–6.3	2.17–6.09	4.14–9.06	0.33–1.42	4.36	6.78	1.03	0.90	0.73	1.06
	$N_{30}P_{30}K_{30}$	1.5–7.5	2.35–6.84	4.26–9.62	0.45–1.64	4.96	7.24	1.08	0.91	0.74	1.10
	$N_{60}P_{60}K_{60}$	2.1–8.3	2.62–7.02	4.37–9.79	0.49–1.72	4.91	7.21	1.1	0.90	0.75	1.12
	$N_{90}P_{90}K_{90}$	2.8–8.8	2.26–6.94	3.95–9.84	0.41–1.78	4.85	7.18	0.98	0.96	0.82	1.40
3.0 million, row	$N_0P_0K_0$	1.5–7.1	3.08–6.22	4.19–9.03	0.42–1.55	4.72	7.31	0.98	0.67	0.66	1.15
	$N_{30}P_{30}K_{30}$	1.8–8.1	3.24–6.96	4.29–9.78	0.45–1.67	5.32	7.92	1.21	0.70	0.69	1.01
	$N_{60}P_{60}K_{60}$	2.5–8.9	3.51–7.15	4.45–9.87	0.49–1.85	5.26	7.84	1.18	0.69	0.69	1.15
	$N_{90}P_{90}K_{90}$	2.8–9.4	3.36–7.21	4.29–9.75	0.40–1.80	5.26	7.79	1.09	0.73	0.7	1.28
2.0 million, row	$N_0P_0K_0$	2.2–8.4	3.41–6.48	4.26–9.31	0.45–1.41	4.95	8.09	1.02	0.62	0.62	0.94
	$N_{30}P_{30}K_{30}$	2.7–9.2	3.54–7.23	4.39–10.04	0.52–1.77	5.59	8.76	1.26	0.66	0.64	0.99
	$N_{60}P_{60}K_{60}$	3.2–10.5	3.62–7.35	4.48–10.26	0.58–1.95	5.65	8.91	1.22	0.66	0.65	1.12
	$N_{90}P_{90}K_{90}$	3.5–11.1	3.58–7.25	4.41–10.08	0.55–1.82	5.61	8.82	1.17	0.65	0.64	1.09
1.0 million, row	$N_0P_0K_0$	2.9–11.7	3.68–7.12	4.39–9.34	0.58–1.51	5.22	8.51	1.34	0.66	0.58	0.69
	$N_{30}P_{30}K_{30}$	3.5–13.9	3.79–7.81	4.51–10.26	0.65–1.94	5.78	8.99	1.44	0.70	0.64	0.90
	$N_{60}P_{60}K_{60}$	3.9–14.4	3.88–7.94	4.67–10.34	0.69–2.06	5.87	9.08	1.56	0.69	0.62	0.88
	$N_{90}P_{90}K_{90}$	4.5–17.3	3.91–8.05	4.68–10.47	0.71–2.13	6.02	9.32	1.62	0.69	0.62	0.88
2.0 million, wide-row	$N_0P_0K_0$	2.1–7.5	3.16–6.88	4.35–9.21	0.44–1.32	5.11	9.12	1.31	0.73	0.53	0.67
	$N_{30}P_{30}K_{30}$	2.6–8.4	3.21–7.44	4.49–9.96	0.56–1.64	5.64	9.64	1.35	0.75	0.57	0.80
	$N_{60}P_{60}K_{60}$	3.2–9.6	3.36–7.59	4.55–10.08	0.62–1.75	5.78	9.74	1.47	0.73	0.57	0.77
	$N_{90}P_{90}K_{90}$	3.6–10.3	3.39–7.64	4.59–10.14	0.59–1.79	5.81	9.72	1.58	0.73	0.57	0.76
1.5 million, wide-row	$N_0P_0K_0$	2.8–8.4	3.74–7.25	4.49–9.38	0.52–1.25	5.24	9.47	1.44	0.67	0.52	0.51
	$N_{30}P_{30}K_{30}$	3.2–9.2	3.92–8.22	4.61–10.59	0.62–1.71	5.97	9.99	1.58	0.72	0.6	0.69
	$N_{60}P_{60}K_{60}$	3.7–11.1	4.15–8.41	4.72–10.65	0.69–1.79	6.09	10.16	1.69	0.70	0.58	0.65
	$N_{90}P_{90}K_{90}$	4.2–12.4	4.25–8.57	4.84–10.72	0.71–1.82	6.12	10.28	1.78	0.71	0.57	0.62

Table 3 (continued)

1.0 million, wide-row	$N_0P_0K_0$	4.4–10.5	3.11–7.55	4.14–9.68	0.59–1.57	5.54	10.05	1.59	0.80	0.55	0.62
	$N_{30}P_{30}K_{30}$	4.8–11.3	3.23–8.91	4.27–10.92	0.69–1.86	6.08	10.51	1.77	0.93	0.63	0.66
	$N_{60}P_{60}K_{60}$	5.2–13.6	3.42–9.17	4.34–11.24	0.78–2.03	6.12	10.64	1.87	0.94	0.65	0.67
	$N_{90}P_{90}K_{90}$	6.1–15.7	3.54–9.27	4.51–11.39	0.75–2.08	6.26	10.76	1.94	0.92	0.64	0.68
0.5 million, wide-row	$N_0P_0K_0$	4.7–14.2	3.45–9.08	5.11–11.87	0.71–2.14	6.08	10.87	1.83	0.93	0.62	0.78
	$N_{30}P_{30}K_{30}$	5.5–15.1	3.14–9.32	5.13–12.69	0.78–2.33	6.59	11.41	1.95	0.94	0.66	0.79
	$N_{60}P_{60}K_{60}$	6.7–17.3	3.19–9.84	5.16–12.89	0.79–2.44	6.91	11.44	2.04	0.96	0.68	0.81
	$N_{90}P_{90}K_{90}$	8.6–20.5	3.23–10.07	5.24–13.07	0.81–2.52	6.98	11.85	2.11	0.97	0.68	0.86

$LSD_{0.5}$	ib	l	d	hw	The share of influence				
						ib	l	d	hw
$LSD_{0.5}$ factor A (year)	0.11	0.04	0.05	0.006	A	11.81	32.32	26.40	22.69
$LSD_{0.5}$ factor B (sowing method)	0.05	0.02	0.02	0.003	B	16.27	13.17	26.01	29.13
$LSD_{0.5}$ factor C (sowing rate)	0.08	0.03	0.03	0.004	C	38.41	29.12	26.72	31.81
$LSD_{0.5}$ factor D (fertilizer)	0.08	0.03	0.03	0.004	D	26.41	12.15	10.50	7.86
$LSD_{0.5}$ interaction AB	0.15	0.06	0.07	0.009	AB	0.38	1.90	1.52	1.14
$LSD_{0.5}$ interaction AC	0.21	0.09	0.10	0.013	AC	2.69	6.35	5.10	2.22
$LSD_{0.5}$ interaction AD	0.21	0.09	0.10	0.013	AD	0.68	0.05	3.04	2.05
$LSD_{0.5}$ interaction BC	0.11	0.04	0.05	0.006	BC	0.78	0.36	0.41	0.68

* – oscillation coefficient by Gumbel (1947).

This means that the inflorescence branching growth index was proportional to the reduction in standing density. The high variability of the interval given the studies (Kirkegaard et al., 2018; Pontes et al., 2018; Nabloussi et al., 2019; Shafighi et al., 2020) indicates the specific nature of the stress-tolerant response of oilseed radish plants, since the nutrition area factor is considered as a stress in current scientific practice, especially with the intensive formation of phytomass of the plant (Roques & Berry, 2016; Vann et al., 2016; Koscielny et al., 2018; Tariq et al., 2020; Adhikari et al., 2021). For these reasons, the nutrition area is the determining criterion in the technology of oilseed radish cultivation. It is interesting to note that for winter rape, additional branching of the generative part during the growth of the nutrition area of plants is formed mainly in the zone of the main axes of the inflorescence. At the same time, for oilseed radish, such branching containing pods may also are located in the lower lateral tiers removed in height of the stem from the main axis of the inflorescence and lateral branches of 2–7 orders. Such a system of formation of the reproductive part of the plants is clearly evident for the variants in the range of 1.0–2.0 million pcs. ha^{-1} of germinable seeds for row sowing and 0.5–1.0 million pcs. ha^{-1} of germinable seeds for the wide-row sowing.

In contrast to the statement (Kumar et al., 1996; Weiner, 2009; Devi & Sharma, 2017; Li et al., 2017; Mitrovic et al., 2020; Wynne et al., 2020; Schwabe et al., 2021) where the main aspect of the cenosis construction of spring rape, white mustard is the total stand density. Our research proves that it is necessary to consider the combination of row spacing and stand densities in the row. While for optimal combination of branching level and seed productivity of plants we should give preference to those variants for cruciferous species, where the factor of standing density is compensating for the total yield of biomass of plants and seeds and causes a narrower rate of response of changes in a certain morphological and productive indicator.

Studies also proved that it is necessary to consider the combination of the width of inter-row spacing and density of plants in a row, and for an optimal combination of branching level and seed productivity of plants we should give preference to those variants where the factor of standing density is compensating for the total yield of biomass of plants and seeds and causes a narrower response rate of changes in a particular morphological and productive indicator for cruciferous species. This is proved by the conducted evaluation of the share of the influence of factors in the system of dispersion analysis of the variant part of the research. Thus, the maximum value of influence in the dispersion system of branching indicator was obtained for the factor seeding rate - 38.41% and fertilizer - 26.41%.

The value of the seeding method factor in this system of analysis indicates the specificity of the response of oilseed radish plants for different intervals of seeding rate with a row and wide-row sowing. According to the inflorescence branching index, the optimum for this combination in the row sowing is observed at the rate of seeding 2.0 million pcs. ha of germinable seeds, where the difference between the boundary options branching is 5.8–6.7 depending on the fertilizer. As for the wide-row sowing, this optimum is noted for the technological variant of 1.5 million pcs. ha^{-1}. This is proved in view of the conducted evaluation of the share of the influence of factors in the system of dispersion analysis of the variant part of the research. Thus, we obtained the maximum value of influence in the dispersion system of branching indicator was obtained for the factor seeding rate - 38.41% and fertilizer - 26.41%. of germinable seeds with the difference between the limit branching options of 6.0–8.2. The technological parameters in the variant of row sowing with the highest seeding rate form a significantly smaller branching interval, which ultimately provides lower levels of reproductive effort of plants. In the variants of wide-row sowing the character of the formation of inflorescence branching was more complicated with the maximum range in the variant 0.5 million pcs. ha^{-1} of germinable seeds at the level of 9.6–11.9 depending on the fertilizer, which at the highest value in the variant system of reproductive effort potentially formed lower levels of both the biomass yield and the seed yield, based on the functional product of standing density on the individual bioproductivity of plants. In our opinion, the value of the compensatory index of plants, which is indicated by the total branching of inflorescence, should be used in determining the optimal technological variants of pre-sowing construction of cruciferous crops agrocenosis. This is clearly confirmed by the results presented and indicated in a number of several similar studies (Nandaa et al., 1996; Chaniyara et al., 2002; Shahin & Valiollah, 2009; Shekhawat et al., 2012; Pandey et al., 2015; Bennett et al., 2017; Kayaçetin et al., 2018; Chang et al., 2020; Adhikari et al., 2021). On the other hand, in several studies the emphasis in determining the technological parameters of pre-sowing formation of cruciferous crops agrocenosis is reduced to the analysis of the effectiveness of their recommended levels and the analysis of additional branching is done in terms of its regulation using mineral fertilizers and sowing timing (Bhajan et al., 2015; Lääniste et al., 2016; Ahmad, 2017; Ahmad et al., 2021). In our opinion, this approach does not allow us to develop a full-fledged adaptive strategy for the formation of the cenosis of this group of crops, based on the determined specifics of interaction between the area of plant nutrition and the level of their mineral nutrition for the oilseed radish.

The formation of morphological parameters of the pod and features of its anatomy depended on the technological factors under study. We noted a constant dynamic of consistent increase in pod length both in the dynamic row both in the section of row sowing and of wide-row sowing. The range of pod length values within the studied variants indicates a high variation component of pod morphometry in oilseed radish. At the same time, we found that the variability increases with the growth of the nutrition area of plants in combination with additional mineral nutrition. For example, the growth of pod length in comparison with unfertilized variants 0.5 and 4.0 million pcs. ha^{-1} of germinable seeds averaged 36.43% for the period of research while for fertilized variants with the fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1} averaged 43.9%. The variability of pod length in the system of variants of row sowing in general is 7.82–10.35% lower than the variability in the system of variants of wide-row sowing. This once again emphasizes the character of oilseed radish stress-strategy due to the inherent features of additional branching and tier formation of additional generative shoots, confirming those noted in several studies (Abley et al., 2016; Li et al., 2016; Parvaiz, 2017; Fujikura et al., 2018; Ahmadzadeh rt al., 2019; Abdo Bakri & Al-dhubibi, 2021). This means that an increase in the area of plant nutrition with additional mineral nutrition provides in oilseed radish both growth of the total length of the pod and variation of its linear size in oilseed radish. At the same time, according to the value of the oscillation coefficient, the total share of variability of both pod diameter and thickness of its walls in comparison to their average value for each separately taken variant at maximum density of oilseed radish plants by row sowing method is significantly higher than for variants of lower technological gradations of the same sowing method. For the wide-row sowing method, the growth tendency of the oscillation coefficient is reversed with an increase in the area of plant nutrition and fertilizer. This confirms the earlier conclusions concerning the general stressfulness of technological gradations of oilseed radish agrocenosis formation and the specific compensatory properties of the plant itself when optimizing the area of plant nutrition with increasing fertilizer. At the minimum plant nutrition area mineral fertilizers are an additional stress factor for standing density at the level of 3.0–4.0 million pcs. ha^{-1} of germinable seeds. Similar conclusions for several variants of seeding rates are made in studies of white mustard (Akbar et al., 2007; Vovchenko & Fursova, 2012; Shekhawat et al., 2012) and winter rape (Khan et al., 2018; Abdo Bakri & Al-dhubibi, 2021; Hashim & Mahmood, 2021).

We also noted a certain specificity of the formation of anatomical features of the oilseed radish pod depending on the system of oilseed radish cenosis construction. With a generally stable growth of both the pod diameter and its wall thickness, the growth rates of these indicators were significantly higher than for the pod length indicator. So, for limit values of technological options 4.0 million pcs. ha^{-1} of germinable seeds without fertilizers and 0.5 million pcs. ha^{-1} of germinable seeds with fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1}, the increase in pod diameter was 60.34%, in pod wall thickness was 85.41%. This means that the improvement of nutritional conditions both with additional mineral nutrition and optimization of soil nutrition contribute to the activation of growth rates of anotomic morphometry of the oilseed radish pod. These rates are determined for oilseed radish higher than similar rates for several cruciferous crops noted in the some studies (McGregor, 1981; Habekotté, 1993; Child et al., 2003; Kuchtová & Vašák, 2004; Miri, 2007; Bennett et al., 2011; Kuai et al., 2016). It is also noted that morphological and anatomical changes of pods are characteristic for winter rape with optimization of plant conditions, including edaphic nature, which leads to the formation of plants of another productive morphotype (Tayo & Morgan, 1979; Tan et al., 2006; Zhuikov, 2014; Li et al., 2017). Our data also confirm this fact. Given the levels of development of the generative part and the potential of seed productivity, the oilseed radish agrocenosis formed by the seeding rates of 2.0–4.0 million pcs. ha^{-1} of germinable seeds, should be attributed to forage use, where a high leafy mass with a low proportion of seeds is formed. Agrocenoses formed in the rate interval of 1.0 million pcs. ha^{-1} of germinable seeds in a row and 0.5–1.5 million pcs. ha^{-1} of germinable seeds at wide-row sowing provide formation of seed type plants. Given the fact that the thickness of the fruit walls of cruciferous crops is a limiting argument regarding the ergonomics of threshing (Li et al., 2012) and control of seed loss during harvesting (Luo et al., 2015), i and has a desirable interval for many cruciferous crops (Tan et al., 2006; Davies & Bruce, 2007; Pu et al., 2013; Yu et al., 2020; Qing et al., 2021), we identified certain technological limitations on ensuring seed production of oilseed radish. Given the specificity of threshing pods of other cruciferous crops with pods that do not open during ripening (Qing et al., 2021), for oilseed radish the diameter of the pod is desirable in the range of 9.0–10.0 mm with a thickness of the pod walls of 1.3–1.8 mm. Based on these positions, the optimum of seed crops of oilseed radish is formed in the version of 1.0 million pieces ha^{-1} of germinable seeds of row sowing - 1.5 million pieces ha^{-1} of germinable seeds of wide-row sowing.

We should note that in accordance with the conducted analysis of the influence of factors in the overall scheme of dispersion analysis (Dunstone & Yager, 2009), we found that the hydrothermal conditions of the growing season with a level of determination of 32.32% are the most determinative in the formation of the length of the oilseed radish pod and the least determinative in the formation of the number of branches of the generative part of plants (11.81%). At the same time, the leading role of seeding rate - 26–31% and seeding method 26–29% in the formation of morphological and anatomical features of the pod was noted. From the position of the formation of morphology and anatomy pods mineral fertilizers had a compensatory nature with the share of influence at the level of 8–12%. However, their influence on branching and formation of the corresponding plant morphotype was significant and amounted to 26.41%. The data obtained prove the high adaptive potential of oilseed radish concerning the resistance to high temperatures and drought on the intensity and effectiveness of the process of formation of the reproductive organs of plants. Especially if we compare similar data for spring and winter rape (Weymann et al., 2015; Nowosad et al., 2016; Chen et al., 2020), white mustard (Bose, 1973; Vovchenko & Fursova, 2012; Pandey et al., 2015) and other cruciferous species (Annisa et al., 2013; Bhajan et al., 2015; Hasanuzzaman, 2020).

Deeper analysis of the factor system of interaction of experience factors against the background of hydrothermal conditions of the period of pod formation can be carried out by analyzing the regression graphical material in the form of reaction surfaces (Fig. 4). According to this, the pod length of oil radish (Fig. 4 (a, b)) had a complex differential nature depending on hydrothermal coefficient (HTC) of pod formation period and sowing rate with maximum of 1.2–1.6, sowing rate in the range 0.5–1.5 million pcs. ha^{-1} of germinable seeds with fertilizer 1.5–3.0 expressed in index form in vriants without fertilizes. The pod diameter (Fig. 4 (c, d)) reached its maximum value at the HTC value of 1.2–1.4 at the seeding rate of 0.5–1.5 million pcs. ha^{-1} of germinable seeds. The effect of mineral fertilizers on the formation of pod diameter was determined by the interaction of HTC and fertilizer dose. At HTC 1.5–1.6 the growth of stem diameter had an intensive growth nature with the minimum index fertilizer expression at 0.5. For HTC 1.0–1.4 the intensity of increase of index was marked by index fertilizer at value 1.0. The HTC level < 0.8 canceled the positive effect of mineral fertilizers.

Pod wall thickness (Fig. 4 (e, f)) had a similar pattern of formation in the HTC system and seeding rate, but the response of recall had a tighter technological regulation. Thus, the maximum values of pod wall thickness were noted at sowing rate in the range of 0.5–1.0 million pcs. ha^{-1} of germinable seeds at HTC between 1.2–1.4 and index fertilizer in the range of 2.0–3.0.

Thus, the maximum reproductive architectonics of the oil radish plants is formed under moderate and sufficient moistening for the pod formation stage with wide-row sowing at a sowing rate in the range from 0.5 to 1.5 million pcs. ha of germinable seeds. At the same time, the agrocenotic productivity of 1 m^2 of sowing at the achievable level of the technological variant of pre-sowing construction is 1.5 million pcs. ha^{-1} of germinable seeds. The full positive effect of mineral fertilizers will be observed at HTC of 1.0 or higher. Fertilizer effect by HTC in the range 0.4–0.8 will be found reasonable by seeding rates in the range of 0.5–1.0 million pcs. ha^{-1} of germinable seeds using a wide-row sowing method. In technological variants with the rate of seeding 3.0–4.0 million pcs. ha^{-1} of germinable seeds, positive effect of fertilizers will be predicted by the effect on the reproductive part of oilseed radish plants at the level of HTC in the range of 1.6–2.0. The obtained results showed a less identical dependence on the hydrothermal conditions of the fruiting period at the stage of the beginning of pod formation established for other cruciferous crops (Angadi et al., 2000; Robertson et al., 2002; Morrison & Stewart, 2002; Sabaghnia et al., 2010; Kirkegaard et al., 2018;

Hasanuzzaman, 2020). However, at later stages of pod formation known as the 'yellow-green pod phase', the dependence decreases and changes its nature of influence on the formation of its anatomical features, in particular wall thickness.

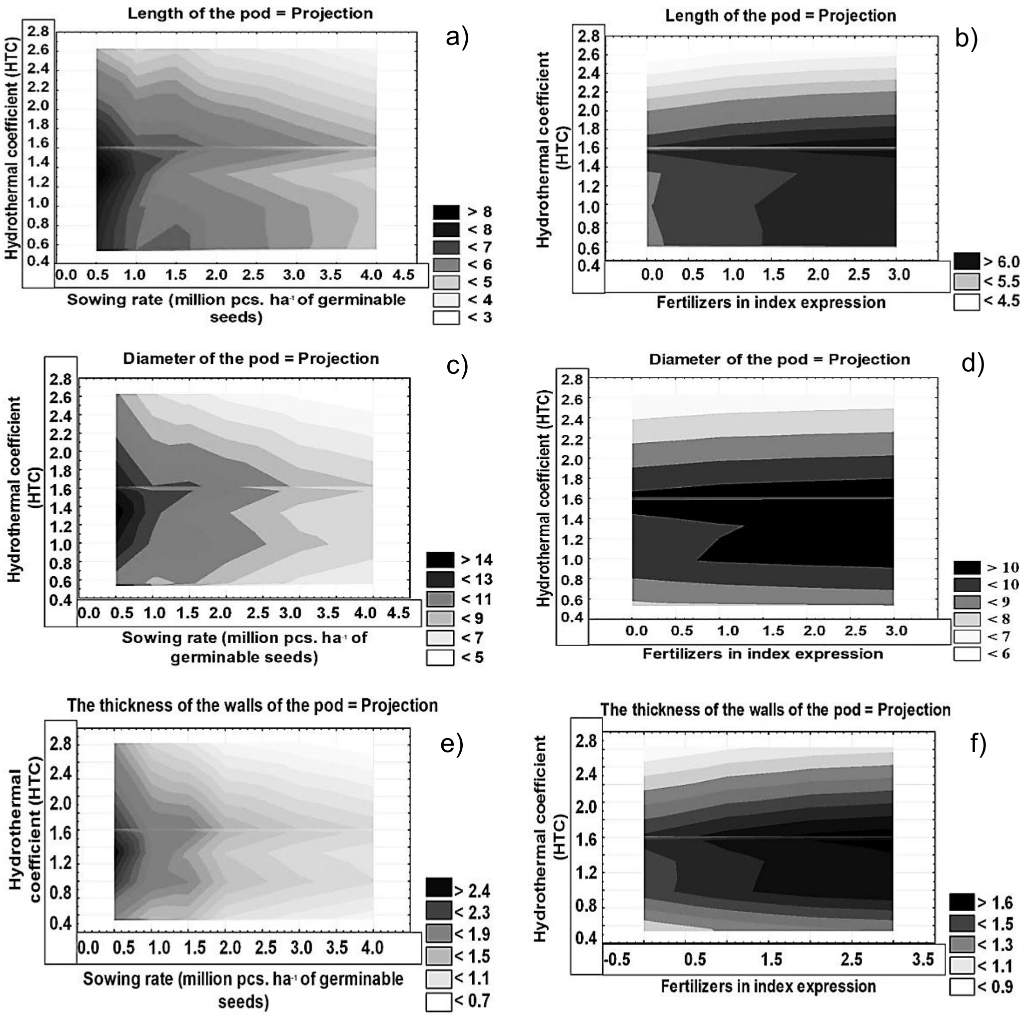
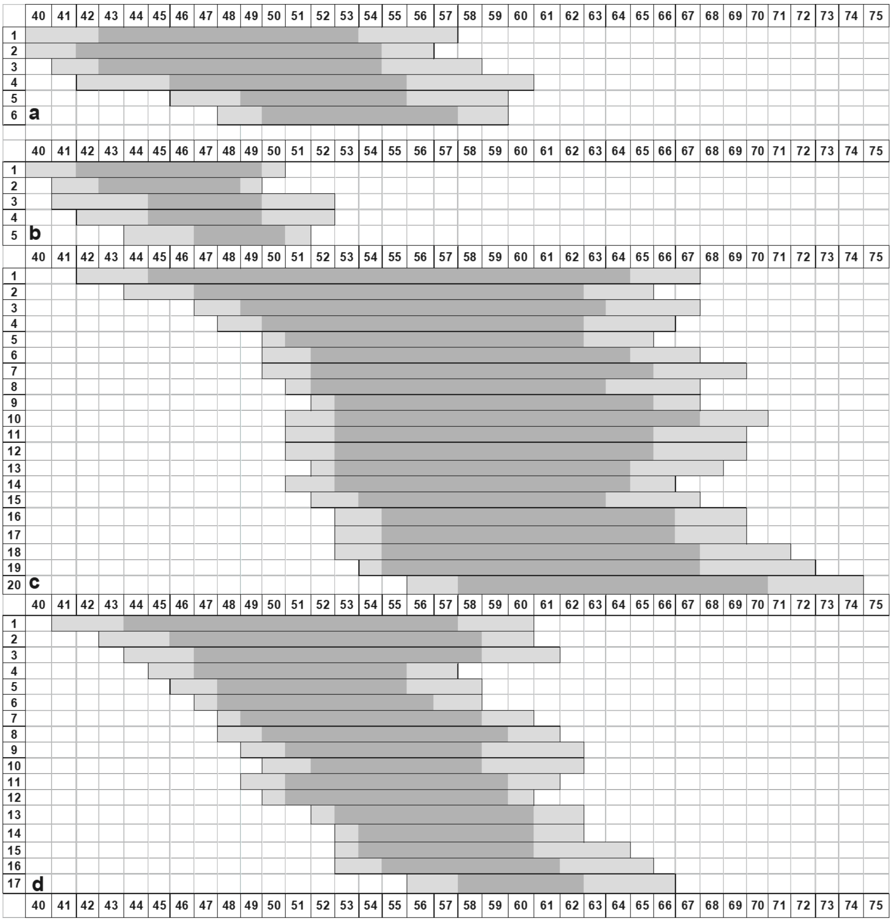


Figure 4. Graphs of projections of morphological features of the oilseed radish plant pod at the yelow pod phase (BBCH 79–81) depending on hydrothermal conditions of vegetation and technological parameters of its agrocenosis construction (averaged for three varieties), 2013–2020. (Fertilizers in index expression: $N_0P_0K_0$ – 0; $N_{30}P_{30}K_{30}$ – 1; $N_{60}P_{60}K_{60}$ – 2; $N_{90}P_{90}K_{90}$ – 3).

Our long-term studies have shown that the averaged nature of the consideration of morphometric parameters of oilseed radish pods does not allow us to fully characterize the patterns of their formation, especially given the high levels of heterocarpy that is typical for cruciferous crops (Dorofeev, 2004; Gangapur et al., 2009; Hasanuzzaman, 2020; Khan et al., 2022). The long-term records of the pod morphometry of the oilseed radish varieties under study during the phase of their yellow-green ripeness allowed us to evaluate the spatial and temporal heterogeneity in the formation of both the inflorescence as a whole and its particular lateral branches. This leads to morphological heterogeneity in the structure of the fruit of oil radish plants due to certain principles of heterocarpy by distancing from the main axis of the inflorescence, features of growth in a certain direction from the base of the main axis of the inflorescence or the corresponding lateral branches to the top of these structures. Differences in the onset of phenological stages of flowering in the indicated acropetal direction, which against the background of successive lengthening of both the head and lateral axes of the inflorescence provides differentiation in flowering depending on their spatial location by height position on the corresponding branching. This creates different conditions for the formation of pods due to changes in hydrothermal conditions during their formation and growth, as well as due to different duration of their morphogenesis. Unlike rape and white mustard, which have a similar pattern of flowering and fruiting (Habekotté, 1993; Wright et al., 1995; Pinet et al., 2015; Hunter et al., 2017; Halevy et al., 2019) the possibility of simultaneous flowering of close tiers of flowers in height has been established for oil radish, which provides a sharp reduction in the duration of flowering of the corresponding axes against the background of an active increase in high average daily temperatures and a deficit of moisture supply. In fact, each inflorescence axis may show a certain specificity in the total duration of its flowering depending on the nature of weather conditions with a certain general reduction in the duration of flowering of the lateral branches of the inflorescence of the corresponding orders in comparison with its main axis (Fig. 5).

Figure 5. Flowering pattern and formation of oilseed radish pods within the main axis of the inflorescence (1 – beginning of flowering at the base of the main axis; 2 – formed pods at the base of the main axis of the inflorescence; 3, 4 – middle zone of the main axis of the inflorescence with consecutive decrease in age of formed fruit elements toward the apex; 5, 6 – apical part of the main axis of the inflorescence with apical placement of flowers in the flowering stage), 2020.

We can confirm these conclusions by the chart of periodization of flowering of oilseed radish plants within two technological limits of its agrocenosis construction of 0.5 and 4.0 million pcs. ha^{-1} of germinable seeds with application of fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1} (Fig. 6) in the comparison of cardinally opposite years in terms of the HTC period of pod formation. The variant with the maximum rate of fertilization was chosen considering the methodology of studying the maximum amplitude of variability of plant morphological signs in accordance with several recommendations (Akbar et al., 2007; Bennett et al., 2017). These charts allow us to assert that the oilseed radish plants are characterized by a variegated system of the duration of flowering of the lateral branches of the inflorescence in comparison with the main axis.



*The variation component from the average is presented in the form of light gray columns for each branch of the inflorescence

Figure 6. Days to flower and duration of flowering of individual inflorescences from plants with fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1} (vertical axis – number of the branch of inflorescence; horizontal axis – days after seeding; a – 4.0 million pcs. ha^{-1} of germinable seeds (row sowing), 2020; b – 4.0 million pcs. ha^{-1} of germinable seeds (row sowing), 2015; c – 0.5 million pcs. ha^{-1} of germinable seeds (wide-row sowing), 2020; d – 0.5 million pcs. ha^{-1} of germinable seeds (wide-row sowing), 2015.

At the same time, hydrothermal conditions during flowering determine the total duration of flowering of each axis and the total duration of flowering. Thus, under the 2015 conditions with the HTC value for the pod formation period of 0.535, the period of both plants with the maximum morphological variability (Fig. 1) at the seeding rate of 4.0 million pcs. ha^{-1} of germinable seeds and plants with the maximum morphological variability at the seeding rate of 0.5 million pcs. ha^{-1} of germinable seeds was 8 and 5 days shorter than for the 2020 conditions with the corresponding HTC value of 1.331. We also found that improvement of hydrothermal conditions of pod formation period differently affects both the number of branches for both extremely distant variants of studies in their overall scheme, and the variation component of the average values of flowering duration. At the lowest value of HTC, there is a total reduction in the number of branches and an increase in the spread of the duration of flowering of each branch (its variation component). Similar results have been observed in a number of cruciferous crops in other studies (Habekotté, 1993; Sabaghnia et al., 2010; Zhuikov, 2014; Hasanuzzaman, 2020).

In addition, stressful conditions during the period of pod formation provide a shift in the start date of flowering of different branches, increasing the differentiation of phenological stages of flowering within the whole inflorescence and reducing the overall phenotype of flowering periodization in the aggregate for all branches. According to a number of studies, such nature of changes in phenological stages of flowering in cruciferous crops is due primarily to the level of abortion of flowers on each branching, especially those that fall under the peak values of high temperatures at the low moisture observed and in our studies. However, in our opinion, this does not fully explain the process of differences in flowering for different types of plant inflorescence structure. Based on our studies, we believe that the influence of stressful conditions is caused by three factors. The first of them concerns already mentioned hydrothermal maximums or minimums in the period of pod formation, the second is associated with the density of plant placement per unit area, the third is related to the features of flowering within each branching. Regarding the density of plant placement, as indicated earlier, in oilseed radish it affects the overall architectonics of plants with formation of a regular morphological series of different types of plant inflorescence structure within each technological variant under study. On this basis, the general scheme of the spatial structure of the main axis and lateral branches of the inflorescence, as well as their altitudinal placement, changes significantly. In particular, the sowing rate of 4.0 million pcs. ha^{-1} of germinable seeds (Fig. 2) is characterized by formation of an average of 5–6 common branches of inflorescence with their apical placement (compact type of generative part), and the variant with the sowing rate of 0.5 million pcs. ha^{-1} of germinable seeds forms 14–20 branches with a wide range of their height placement (spatial type of generative part). As a result, we saw high variability in the dates of the beginning and duration of flowering of each of the axes with a subsequent decrease in the density of standing plants, especially against the background of high levels of additional mineral nutrition. In the overall result, branch axes of 6–20 order (depending on the variant of agrocenosis construction), forming a smaller number of flowers against the background of general reduction of stages of their development, have a significantly lower average total duration of flowering.

At the same time for oilseed radish, we determined the feature of one-stage flowering of several axes close in order (in the graph these are the chart bars with the same coordinate beginning), which is inherent in plants with lower density per unit area. According to several studies (Bose, 1973; Masierowska et al., 2003; Osborn & Lukens, 2003; Wang et al., 2011; Raman et al., 2013; Shah et al., 2018; Andrimont et al., 2020; Sun et al., 2021; Zhang et al., 2021), the difference in the timing of flowering on different axes and the long flowering period of an individual plant contribute to the intensive influence of weather conditions on all the processes of fruit formation. This is confirmed by the results of factor analysis in the scheme of variance processing of research results (Table 3), where the share of year conditions in terms of their influence on the morphology and anatomy of pods is 26–32%. In fact, analyzing each axis by the flowering pattern, pod development, the level of their abortiveness (in the presence of the stem without a pod (Xiujuan, 2011)) in cruciferous plants, we can conclude about the intensity of stress factors in the cycle of phenological development of oilseed radish. The maximum levels of abortion of pod rudiments under unfavorable conditions are observed at the microstage BBCH 67-71 during the period from complete petal fall to the stage when 10% of pods reach the final size. In view of the above arguments, at different stages of flower and fruit formation, the hierarchical structure of the oil radish inflorescence has corresponding tier features, which are expressed both in the difference in stages of pod and seed formation and in the morphological parameters of the latter gradation from the inflorescence base to its apex. Even during the brown pod phase (BBCH 83-87), the presence of flowers on the apex part of the generative part of oilseed radish plants is noted.

The above-mentioned studied features in the stages of flowering within the inflorescence of oil radish allow us to make a statement about the different stages of morphological development of the pods within the spatial structure of the inflorescence itself. The above multistage is based on the already mentioned biological features of flowering stages within the lateral branches of the inflorescence of oilseed radish plants depending on its inflorescence structure. Primarily, this is related to the peculiarities of inflorescence formation due to elongation of its main axis and gradual formation of new flowers in the direction from the base of the pedicel to its apex, which is traced both on the main axis of the inflorescence and on its lateral branches and inflorescences of lateral shoots. In addition, the above features of the formation of the spatial structure of inflorescence cause a long period of fruit formation and seed ripening and a significant difference in the time of pod formation and duration of seed ripening depending on its placement in the inflorescence: fruit elements of the lower placement have respectively a longer formation period and, consequently, higher values of morphological development than fruit elements of the middle and especially the upper tier. These processes of pod formation are evident already at the stages of the beginning of oilseed radish fruit formation and are completed in stages with different degrees of heterocarpy within different orders of inflorescence, which is clearly confirmed by the data shown in the Fig. 7.

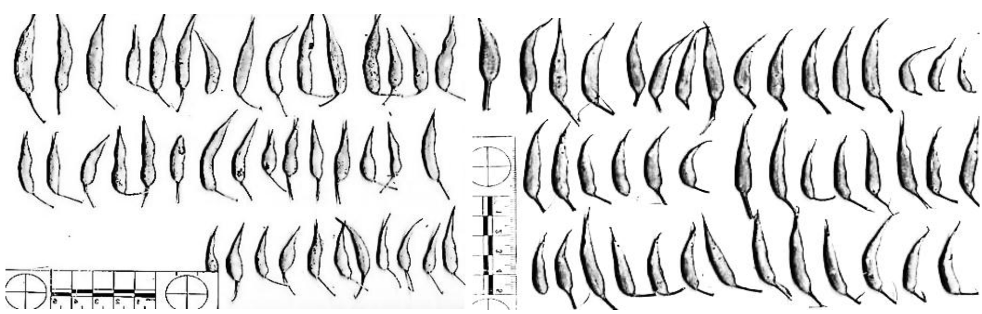


Figure 7. Dynamic rows of pods within the first three branches of the inflorescence of oilseed radish plant (variety 'Zhuravka') during the green pod phase BBCH (75–77) for the variant 0.5 million pcs. ha^{-1} of germinable seeds with fertilization rate of 90 kg N ha^{-1}, 90 kg P ha^{-1}, and 90 kg K ha^{-1} (left position – 2015, right position – 2020, (each row of pods corresponds to one of the branches, in the direction from the base to the top of the branch)).

Table 4. Regression model for estimating oilseed radish pod length depending on its placement on the inflorescence axis in the order of its botanical tropation, 2013–2020

Model no.	Form of model tested	Fitted coefficients and costant				Test of the models			
		a	b	c	d	r	R^2	Sr	RMSE
a	$y = (a+bx)/(1+cx+dx^2)$	8.901E-003	9.221E+000	7.326E-001	1.420E-001	0.999	0.998	0.144	0.321
b	$y = a\exp((-(b-x)^2)/(2c^2))$	6.249E+000	3.503E+000	7.832E+000		0.968	0.936	0.179	0.809
c	$y = (a+bx)/(1+cx+dx^2)$	1.097E-002	1.005E+001	7.624E-001	1.745E-001	0.986	0.972	0.407	0.851
d	$y = a\exp((-(b-x)^2)/(2c^2))$	6.107E+000	3.427E+000	4.918E+000		0.949	0.902	0.289	1.112
e	$y = (a+bx)/(1+cx+dx^2))$	9.762E-004	1.579E+001	1.635E+000	1.592E-001	0.991	0.982	0.278	0.453
f	$y = a\exp((-(b-x)^2)/(2c^2))$	8.256E+000	2.161E+000	1.989E+001		0.985	0.970	0.272	0.917
g	$y = (a+bx)/(1+cx+dx^2)$	5.421E-002	1.545E+001	1.014E+000	1.314E-001	0.960	0.922	0.677	1.133
h	$y = a\exp((-(b-x)^2)/(2c^2))$	8.233E+000	2.568E+000	1.20E+001		0.956	0.914	0.695	1.296

Models: a, b – respectively Rational Function and Gaussian Model for the main axis of the inflorescence in the technological variant of 4.0 million pcs. ha^{-1} of germinable seeds without fertilizers; c, d – respectively Rational Function and Gaussian Model averaged for 2nd–6th order lateral branches in the technological variant of 4.0 million pcs. ha^{-1} of germinable seeds without fertilizers; e, f – respectively Rational Function and Gaussian Model for the main axis of the inflorescence the technological variant of 0.5 million pcs. ha^{-1} of germinable seeds without fertilizers; g, h – respectively Rational Function and Gaussian Model averaged for 2nd–20th order lateral branches in the technological variant of 0.5 million pcs. ha^{-1} of germinable seeds without fertilizers (The X axis is the sequence number of the pod from the base to the apex of the corresponding axis of inflorescence branching, the Y axis is the pod length (cm)).

We confirmed our earlier conclusions about the peculiarities of pod morphometry within the inflorescence axes and analysis with the selection of the functional addiction of pod length depending on its placement in the inflorescence, the results of which are presented in Table 4 and Fig. 8. According to the conducted functional selection by R^2 and RMSE criteria, we found that the nature of pod length formation is most fully described by two types of power functions, namely, Rational Function and Gaussian Model.

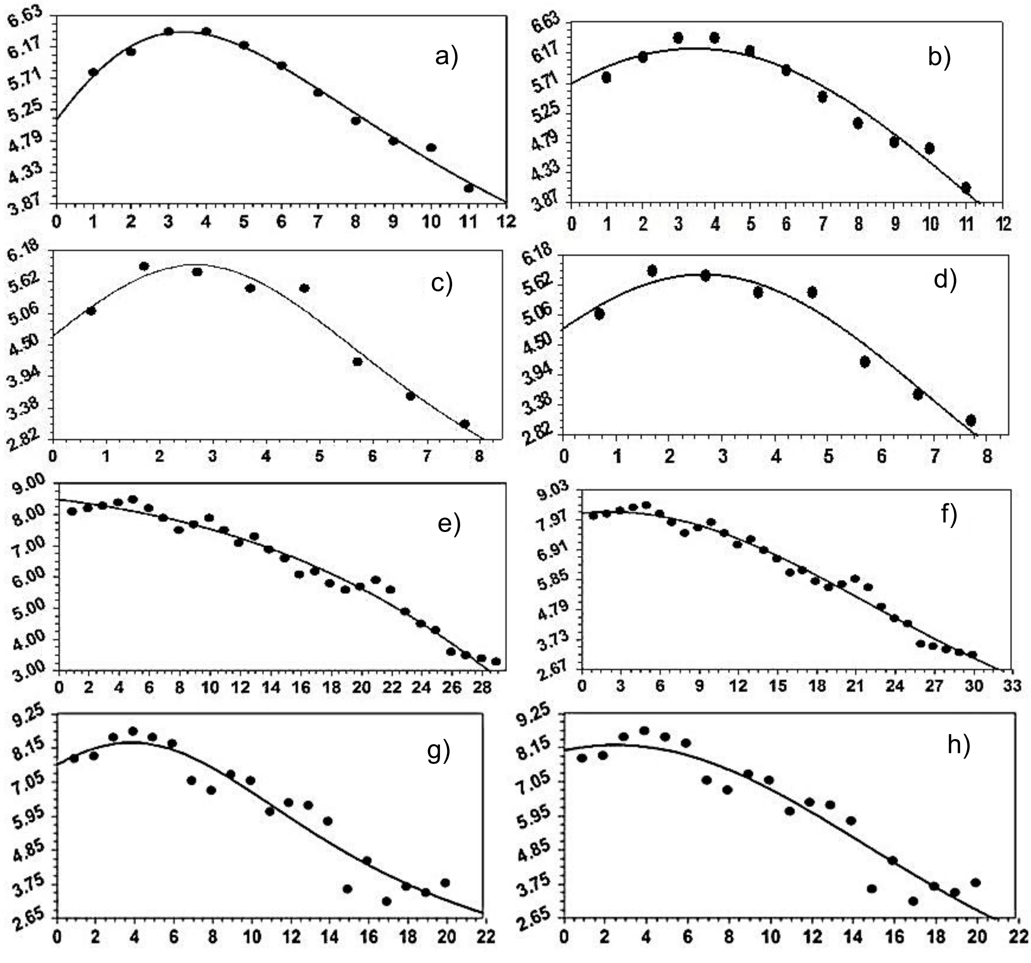


Figure 8. The graphs of mathematical models of changes in pod length on the corresponding branches of the inflorescence in order from the base to the top of the inflorescence are averaged for the three studied varieties of oilseed radish at the phase of yellow-brown pod ripeness (BBCH 86–87) for the period between 2013–2020 (mathematical interpretation of the e–h models which is shown in Table 4).

Application of other function variants had significant values of correlation dependence of factors with significantly higher indices of standard error and RMSE criterion. The indicated dependences indicate a consistent constant decrease in the linear size of the oilseed radish pod from the base to the apex of both the main axis and the lateral branches of the inflorescence. Fewer points of determination in the plots of lateral branches (Fig. 8 c, d, g, h) and higher levels of dispersion of points relative to the predictive model curve indicate both a smaller number of pods on lateral branches in comparison to the main axis of the inflorescence, and an increase in variation of linear pod size within these branches especially in low plant density variants at maximum mineral fertilization. This creates a complex spatial structure of oilseed radish pod morphometry both in the acropetal direction from the base to the apex of each inflorescence axis and in the radial direction from the main inflorescence axis to the corresponding lateral branches. The growth of variation in morphological size of the pod increases during formation of plant morphotypes in technological variants of low density at high levels of fertilization.

The complex system of dependence of the second stage indicates the possibility of formation within the main axis and lateral branches of intervals with insignificantly different parameters of pod length (belts of the same morphometry) as well as the oscillatory nature of the appearance of significantly different morphological pods within a similar morphometric interval. Such features are indicated in several studies (Cao et al., 2006; Skriabin et al., 2006; Jullien et al., 2011; Bennett et al., 2017; Zhang et al., 2018, 2020). However, in contrast to these studies, it relate to revealing types of pods and are based on empirical assumptions. Our calculations are based on long-term estimates of the morphometry of pods from the view of the spatial structure of the inflorescence. This allows to accurately predict the morphological parameters of oilseed radish pods depending on their spatial location in the inflorescence, given the applied agro-technological variants for constructing its agrocenosis.

Such features are most noticeable at low values of HTC for variant with a high sowing rates 3.0–4.0 million pcs. ha^{-1} of germinable seeds of row sowing or, on the contrary, at high values of HTC for variant with a low sowing rates 0.5–1.0 million pcs. ha^{-1} of germinable seeds. This is clearly demonstrated by comparing models a–b and e–f (Fig. 8). Based on a detailed analysis of such intervals of variation in morphological parameters of the oilseed radish pod, we identified three tiers in the spatial structure of the inflorescence of oil radish, which statistics for two limiting technological options for the construction of its cenosis (Table 5) allows us to assess the specific patterns of formation of fruit elements of oilseed radish. Regarding the formation of the inflorescence layer of cruciferous crops was noted in studies McGregor (1981), Habekotté (1993), Bowman et al. (1999), Chub & Penin (2004), Zhang et al. (2018). Despite the fact that there is a certain layering in cruciferous inflorescences, this issue was considered in these publications from general approaches to varying the morphology of pods in the overall structure of the inflorescence without assessing the development of certain zones, their percentage concentration in the inflorescence structure and statistical assessment of morphological parameters. The question of the factors that determine the formation of inflorescence stratification by morphological development of pods is poorly studied. Attempts to develop and explore these important issues have been made in research of Khmelyanchyshyn (2005), Xiujuan (2011), Oleksy et al. (2018), Matar et al. (2021).

Table 5. Statistical evaluation of variability of morphological development parameters of oilseed radish pods of 'Zhuravka' variety at brown pod phase (BBCH 88-90) within reproductive branching zones (average for 2013–2020 for the annual sample n = 500 with the general totality of observations N = 4,000)

Inflorescence zones	Pod length (l), cm			Pod diameter (d), mm			Pod wall thickness (hw), mm		
	R, cm	V, %	X_{av}, cm	*R*, mm	V, %	X_{av}, mm	*R*, mm	V, %	X_{av}, mm
Row sowing with a sowing rate of 4.0 million pcs. ha of germinable seeds $N_0P_0K_0$									
Lower	3.98–7.52	10.61	5.15 ± 1.18*	4.80–8.50	9.78	7.55 ± 1.27	0.52–1.77	18.29	1.33 ± 0.27
Middle	2.17–6.09	11.15	4.36^a ± 1.21	4.14–9.06	11.25	6.78^b ± 1.41	0.33–1.42	18.50	1.03^c ± 0.25
Upper	1.97–5.33	13.52	4.12^a ± 1.59	3.57–7.58	10.80	5.63^a ± 1.37	0.68–1.97	18.68	0.91^b ± 0.33
Row sowing with a sowing rate of 4.0 million pcs. ha of germinable seeds with $N_{90}P_{90}K_{90}$									
Lower	3.19–7.84	12.35	5.29 ± 1.56	4.53–8.72	11.44	7.82 ± 1.44	0.52–1.95	16.74	1.12 ± 0.27
Middle	2.26–6.94	11.63	4.85^a ± 1.37	3.95–9.84	11.75	7.18^a ± 1.39	0.41–1.78	17.53	0.98 ± 0.34
Upper	2.02–5.62	12.97	4.43^a ± 1.51	4.02–6.75	13.25	5.23^a ± 2.05	0.38–1.99	20.56	0.75^b ± 0.51
Wide-row sowing with a sowing rate of 0.5 million pcs. ha of germinable seeds $N_0P_0K_0$									
Lower	3.50–10.40	14.28	6.89 ± 1.67	5.70–13.20	12.37	12.65 ± 1.88	0.75–2.38	16.89	2.03 ± 0.28
Middle	3.45–9.08	14.49	6.08^b ± 1.73	5.11–11.87	11.55	10.87^b ± 1.89	0.71–2.14	17.82	$1,83^b$ ± 0.37
Upper	2.54–7.71	15.08	5.69^a ± 1.89	3.89–10.60	16.37	8.81^a ± 2.10	0.63–2.32	18.29	1.62^a ± 0.53
Wide-row sowing with a sowing rate of 0.5 million pcs. ha of germinable seeds with $N_{90}P_{90}K_{90}$									
Lower	4.17–10.25	15.87	7.63 ± 1.79	6.15–13.84	12.55	12.85 ± 1.73	0.85–2.88	18.83	2.39 ± 0.34
Middle	3.23–10.07	15.12	6.98^a ± 1.58	5.24–13.07	14.76	11.85^a ± 2.28	0.81–2.52	19.08	2.11^b ± 0.45
Upper	3.23–7.89	19.72	5.82^a ± 2.09	4.18–11.19	17.20	9.37^a ± 2.54	0.71–2.57	19.55	1.80^a ± 0.54

Significance levels of the middle and upper zone data versus the lower zone: *a* – 0.1%; *b* –1%; c– 5%.

* – arithmetic mean error for $a \leq 0.05$.

The presented results allow us to conclude about the tiered heterocarpy in oilseed radish, which leads to differentiation of pods by the main parameters of morphological development both in variants of the highest technologically applicable density of its agrocenosis of 4.0 million pcs. ha^{-1} of germinable seeds and in variants of maximum allowable technological liquefaction by sowing rate of 0.5 million pcs. ha^{-1} of germinable seeds. The most variable trait was pod wall thickness in the middle zone with a range from 0.41 to 1.49 mm, which corresponds to an average gradation of variation of 18–20%. The variability of pod diameter was the lowest with a coefficient of variation within the studied variants of 9.34–14.5%. It should be noted that several studies reported signs of fruit heterocarpy in cruciferous plants and its tiered expression in the inflorescence (Khmelyanchyshyn, 2005; Naomab, 2008; Lu et al., 2010; Xiujuan, 2011; Li et al., 2016, 2020; Zhang et al., 2018). In evaluation of morphometry of formation of features behind the tiers of inflorescence in the direction from base to apex the following regularities should be noted: reduction of pod length when its shape changes in the interval by 15.8–24.6% in comparison of the upper tier to the lower tier, reduction of pod diameter in the interval by 6.5–11.8% in comparison of the upper tier to the lower tier, increase of pod wall thickness in the middle part in the interval by 8.3–9.6% for the same level of comparison.

We found that the use of mineral fertilizers contributes to the increase in the manifestation of heterocarpy in oilseed radish by increasing both the actual linear size of the pod and the range of values of the indicators. The maximum effect of fertilizers in terms of variability of fruits is noted precisely in the variants with a lower density of cenosis. In cenoses with maximum plant density due to cenotic pressure, the variability of morphological parameters was lower in the value of the coefficient of variation and, accordingly, the significance of morphological differences in the fruit within the inflorescence was less evident. The evaluation of the intensity of development of these tiers of different morphometric pods in oilseed radish varieties in the interval of years of research also confirms the earlier conclusions (Fig. 9). According to this graph, the expression of the formation of individual zones of the generative part of oilseed radish plants depended significantly on the technological variant of pre-sowing formation of its agrocenosis. For the variant 4.0 million pcs. ha^{-1} of germinable seeds without fertilizer application in comparison with the data of the variant 0.5 million pcs. ha^{-1} of germinable seeds against the background of $N_{90}P_{90}K_{90}$ application, the share of the lower zone was 1.2–1.6 times less, the share of the upper zone was also less in 1.1–1.3 times, and the share of the middle zone was 1.1–1.3 times more, depending on the year of research.

The fluctuating nature of the ratio of zones was more pronounced at lower density of standing on the background of additional mineral nutrition. Thus, the sowing rate of 4.0 million pcs. ha^{-1} of germinable seeds on unfertilized background interval of the proportion of the lower zone in the interval of years of study was 14.2–26.3%, the middle zone 58.9–69.7%, the upper zone 8.6–19.1%. A similar interval for the variant 0.5 million pcs. ha^{-1} of germinable seeds against the background of $N_{90}P_{90}K_{90}$ application was 21.8–36.7%, 48.6–56.4, 12.6–28.7% respectively. This confirms the earlier conclusions about the effect of sowing rate and sowing method on the variability of linear size of the pod given the established range of values and oscillation coefficient, which increase both when optimizing the nutrition area and the fertilization of oilseed radish plants (Table 3). It should be noted that the development of the lower and especially the upper zones of the generative part of oil radish plants allows us to conclude about the general stressful weather conditions of the year.

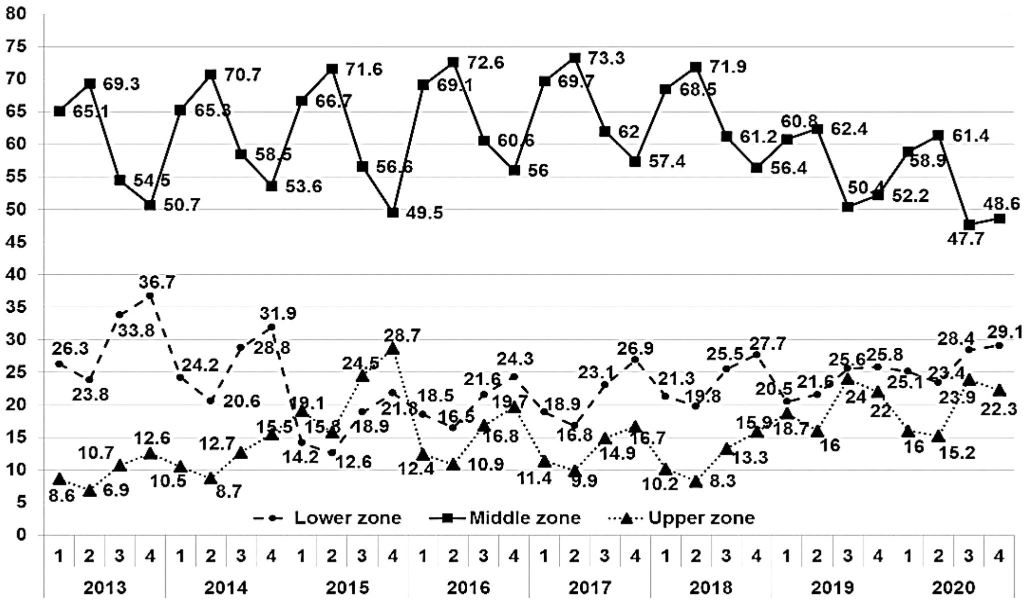


Figure 9. Share of inflorescence zones of oilseed radish plants by indicators of morphological development of pods, depending on technological options for the design of its agrocenosis, 2013–2020 (research variants: 1 – 4.0 million, row $N_0P_0K_0$; 2 – 4.0 million, row + $N_{90}P_{90}K_{90}$; 3 – 0.5 million, wide-row $N_0P_0K_0$; 4 – 0.5 million, wide-row + $N_{90}P_{90}K_{90}$).

Thus, for the conditions of 2015, as the most stressful, the average ratio of inflorescence zones in oil radish was 16.9:61.1:22.0%, and for the conditions of 2014, as the most favorable in the formation of fruiting elements it was 26.4:62.0:11.9%. That is, optimization of the period of pod formation by improving the hydrothermal conditions contributes to a significant decrease in the proportion of fruits in the upper inflorescence zone with a similar increase in the proportion of its lower zone. We can trace this pattern in the context of all years of observations and gives grounds to adjust (for regions with different nature of the HTC indicator) the formation of reproductive effort of the oilseed radish plants with the selection of the most appropriate variant of pre-sowing construction of its agrocenoses. The interval grouping of the analyzed features of the pod is a confirmation of a certain zoning of the generative part according to the morphological features of the pod (Fig. 10, a; 10, b). Such an analysis, based on a pooled general population, makes it possible to analyze the overall dynamism of the distribution over the entire observation period. Such approaches proved to be successful in other similar studies as well (Weiner et al., 2009; Zając et al., 2011; Xiujuan, 2011; Vovchenko & Fursova, 2012; Tariq et al., 2020). However, in contrast to them, we used grouping by several morphological features of the pod and for different zones of the inflorescence.

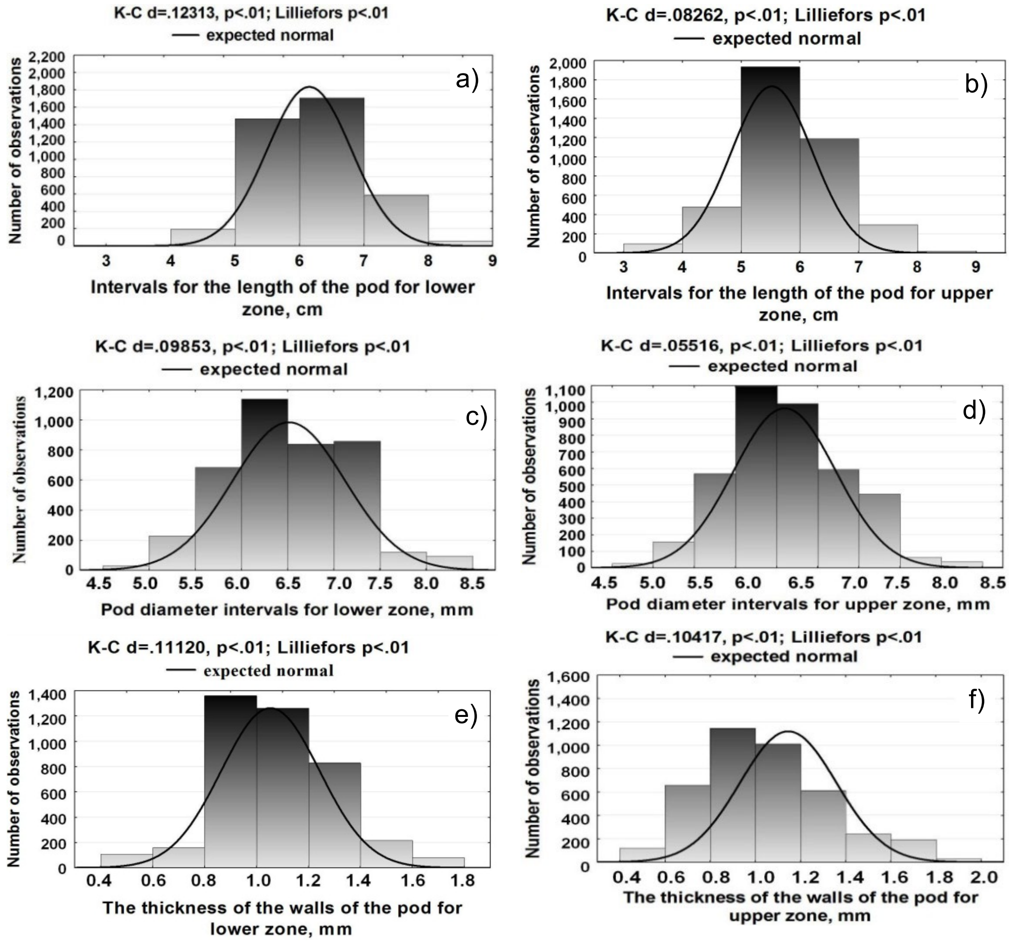


Figure 10, a. Distribution histogram of interval values of morphological features of oilseed radish pod in the section of the lower and upper zones of inflorescence at the rate of seeding 4.0 million pcs. ha^{-1} of germinable seeds on a variant without fertilizes (average of the three varieties for the general totality, 2013–2020).

This allowed to determine the peculiarities of the variation component of pod morphology within the general interval of each zone and additionally analyze the influence of technological options of pre-sowing design of the oilseed radish agrocenosis on the degree of variability of fruit elements. Thus, regarding the pod length at the sowing rate 4.0 million pcs. ha^{-1} of germinable seeds for the pods of the lower zone (Fig. 10, a (a–b)) we determined 5 intervals, and for the upper zone of the inflorescence we determined 6 intervals. Under the same conditions, pod length in the interval of 5–7 cm resulted in 86.8% of the considered pods in the lower inflorescence zone and 77.7% in the upper zone for an increase in the pod length interval of 4–5 cm from 3.3% for the lower inflorescence zone to 20% for the upper zone. When the density of oilseed radish agrocenosis decreases to 0.5 million pcs. ha of such seeds (Fig. 10 b, (a–b)), the total number of interval groups by pod length increases, and the dominant interval of 6–7 and 7–8 cm is 41.9% and 34.7% for the lower inflorescence zone, which is 25.2 and

30.9% less than for similar intervals of the upper inflorescence zone. According to the peculiarities of formation of pod diameter (Fig. 10 a, 10 b, (c–d)), similar patterns were determined in comparing the lower and upper zones: the variant of higher density of oilseed radish agrocenosis has a greater interval range of the indicator due to expansion of the lower limit of the range than the variant of 0.5 million pcs. ha^{-1} of germinable seeds. For the lower zone of the inflorescence, a significant increase in diameters above 6.0 mm for sowing rate of 4.0 million pcs. ha^{-1} of germinable seeds and above 8.0 mm for sowing rate of 0.5 million pcs. ha^{-1} of germinable seeds was established for both sowing rates. In particular, at the sowing rate of 0.5 million pcs. ha^{-1} of germinable seeds, the number of pods with a diameter of more than 8 mm was 35.9% for the lower zone and 12.7% for the upper zone.

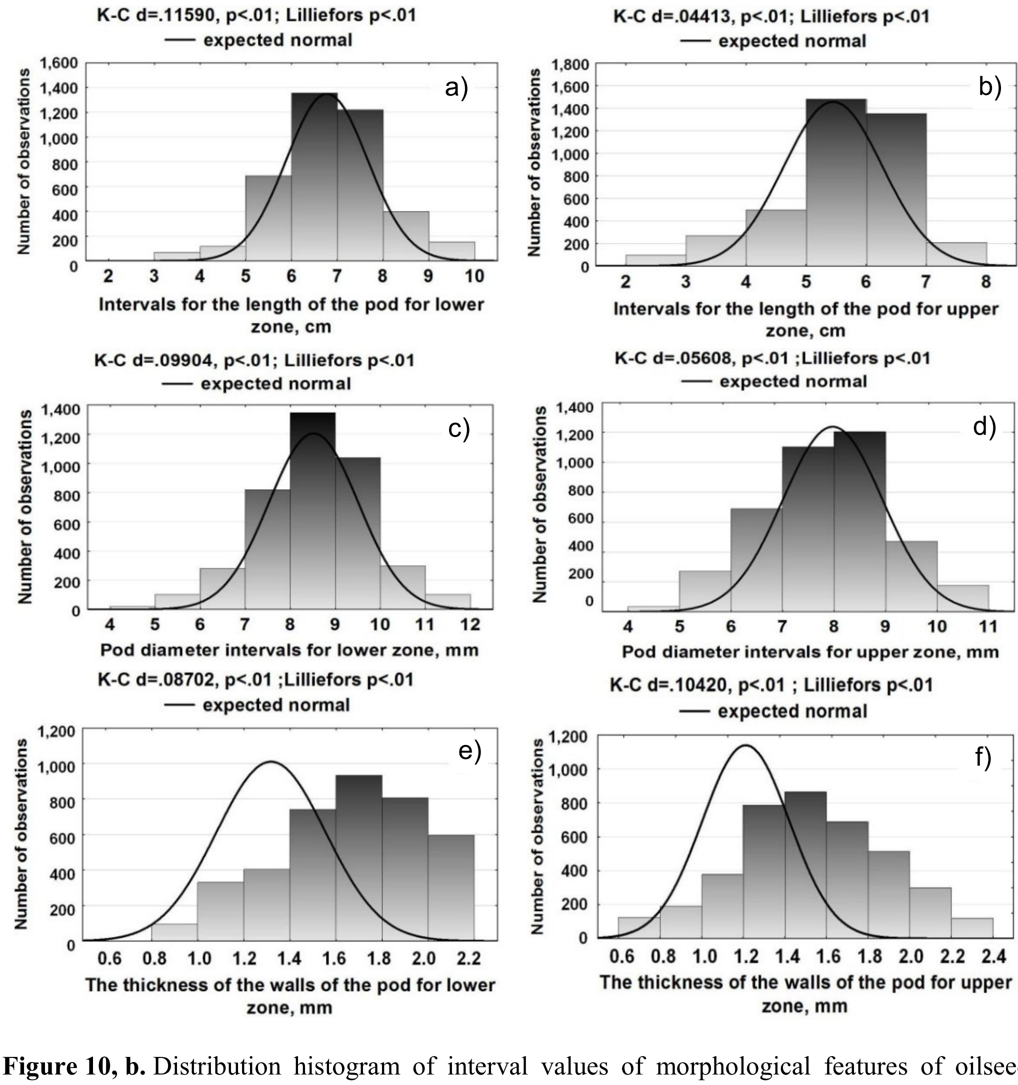
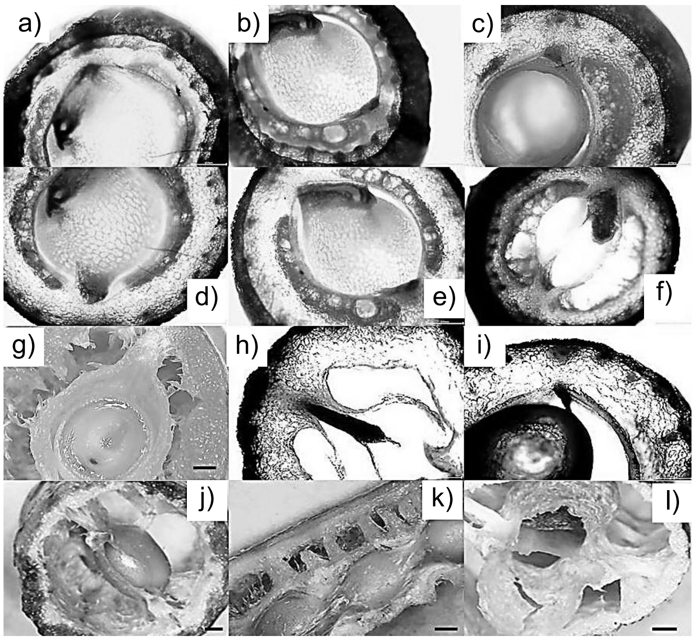


Figure 10, b. Distribution histogram of interval values of morphological features of oilseed radish pod in the section of the lower and upper zones of inflorescence at the rate of seeding 0.5 million pcs. ha^{-1} of germinable seeds on a variant without fertilizes (average of the three varieties for the general totality, 2013–2020).

We determined the specificity of pod wall thickness formation when its altitude tropation changes within the axes of inflorescence. In comparison with the lower zone, the index range increases with the appearance of limiting intervals, which were not considered. Thus, for the variant 4.0 million pcs. ha^{-1} of germinable seeds this interval range is 1.8–2.0 mm, and for the variant 0.5 million pcs. ha^{-1} of germinable seeds this interval range is 2.2–2.4 mm. At the same time, the change in the value of the interval for the upper zone has a greater amplitude of fluctuations within gradations, which confirms the earlier conclusions regarding the growth of morphological features of the pod in the direction from the base of the inflorescence axis to its apex. For the variant 0.5 million pcs. ha^{-1} of germinable seeds, a certain asymmetry in the value of filling intervals with a close indication in the interval of 1.6–2.0 mm is quite noticeable.

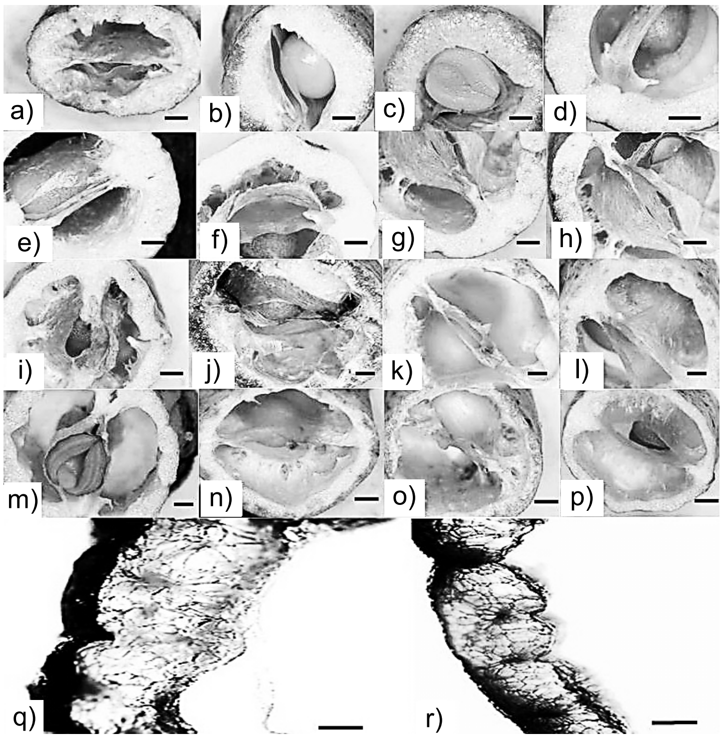


* the dimension scale line at the bottom of each image 1 mm long

Figure 11. Morphological stages of formation of the internal anatomical structure of the oilseed radish pod from the middle zone of the inflorescence (consistently: a – phenological stage BBCH 71; b – BBCH 73; c – BBCH 74; d, e – BBCH 75-76; f, g – BBCH 77-78; h–l – BBCH 77-85), 2020.

In our opinion, the appearance of pods with thicker walls in the upper inflorescence zone is determined by the peculiarities of pod formation stages and peculiarities of this process depending on pod placement in the inflorescences by height. In the initial stages of its formation, the endocarp is represented by parenchymal tissue adjacent to the seminal chamber (Fig. 11, a–b) (stage BBCH 69-71). Consecutively in the process of seed maturation, the filling parenchyma of the mesocarp is transformed with the formation of the space between the septum and the walls of the pod. It is formed a central longitudinal membrane of the pod, which creates a kind of capsule around the seeds, anatomically attached to the walls of the pod. At the same time the pods have a septum semitric to the seed chamber and a developed endocarpic multiple placental-type replum to the endocarp walls (Fig. 11, j–l) (stage BBCH 75-82).

According to Habekotté (1993), Bennett et al. (2011), Xiujuan (2011), Zhang et al. (2018) and Hasanuzzaman (2020) the nature of the formation of the internal anatomical structure of the oilseed radish pod guarantees protection against seed shedding and natural cracking of the pod walls. On the other hand, the staged transformation of the mesocarpic structure of the pod walls on the one hand is additional protection of seeds at the stage of its formation and maturation and on the other hand increases the dependence of the anatomical structure of the pod in the spatial structure of the inflorescence depending on environmental conditions. This has been emphasized in research Menendez et al. (2019). In our research this is confirmed both by the previously presented data of groupings (Fig. 10, a, b) and by microscopic study of the anatomical structure of the walls of the pods taken from different tiers of the inflorescence of oilseed radish. During the formation and maturation of seeds, the pod wall thickness, according to our surveys, was in the range 1.820–2.968 mm. During the microstage BBCH 76-83, the process of formation of the membranous sulcus with fetal seeds continues with a decrease in wall thickness to 1.156–1.698 mm. During the microstage BBCH 84-88, the wall thickness interval is set at 0.659–1.368. Under the same conditions, linear and radial growth of pods in different tiers of inflorescence differ (Fig. 12, q, r).



* indicator black line – a segment of the linear dimension of the image with a length of 1 mm.

Figure 12. Variation aspects of variants of pod wall thickness formation ((a–f) – pods of upper inflorescence zone with thickened walls; (g–k) – pods of middle inflorescence zone; (l–p) – pods of upper inflorescence zone with thin walls; q – cross section of the pod wall of the upper zone and r – cross section of the pod wall of the lower zone of the inflorescence at the phenological phase BBCH 87) in variant of sowing rate 0.5 million pcs. ha^{-1} of germinable seeds on a variant without fertilizers, 2020.

For the pods of the lower tier, which are formed first and accordingly to their morphological development in relation to the pods of the upper part shifted by 12–20 days, the rates of linear and radial growth are comparable in intensity. For the pods of the upper tier, due to the general weakening of physiological growth processes and a stage shift from the time of formation of the main part of the inflorescence, the specified growth rates are disproportionate.

CONCLUSIONS

Technological variants of pre-sowing construction of oilseed radish agrocenosis are determinative in the formation of pod morphometry and variability of its main linear and anatomical parameters due to changes in reproductive architectonics of plants, implementation of their compensatory ability and changes in the stages of flowering and spatial fruit formation. We proved that they provide the formation of morphologically different in the spatial structure of the generative part of the plant within each technological variant used in the study. Increasing the nutrition area with appropriate combinations of sowing rate and sowing method from 4.0 million pcs. ha^{-1} of germinable seeds to 0.5 million pcs. ha^{-1} of germinable seeds provides an overall increase in pod length by 38.5%, pod diameter by 60.4%, wall thickness by 92.5%. The application of additional mineral nutrition in the range from 30 to 90 kg ha^{-1} of the active substance provides an increase in these morphological parameters in comparison with the unfertilized control by 9.6–23.6% with a dominant positive effect on the level of general morphological development of plants and the formation of plants with intensive branching reproductive part. When reducing the nutrition area of oilseed radish, mineral fertilizers contribute to the range of variation of linear size of the pod on average in the studied variants in the range 12.5–24.7%. Variation of the nutrition area of oilseed radish plants determines the variability of morphological features of the pod within their vertical placement within the main axis and lateral branches of the inflorescence forming three distinct zones of such variability, namely, lower, middle and upper. The share of the middle zone for the studied technological variants was in the interval of 48.6–73.3% depending on the year of research, the lower was in the interval of 12.6–36.7%, the upper was in the interval of 6.9–23.9%. The growth of nutrition area and sowing method comparing the limiting technological variants 4.0 and 5.0 million pcs. ha^{-1} of germinable seeds provides, depending on hydrothermal conditions of pod formation period, an increase in the share of the upper zone by 4.1–6.7%, and the lower by 4.8–10.3%. At the same time, the nature of changes in pod length within the main axis and lateral branches has certain mathematical regularities of formation of a power function of the second order, most fully described by the equations in the Rational Function and Gaussian Model system. Determined features in the formation of pods depending on their altitudinal location in the direction from the base of the inflorescence axes to their apex and the system of grouping of morphometric parameters of oil radish pods within certain zones of variability allowed us to determine the patterns of formation of fruit elements in the spatial structure of the generative part of plants and compare their general morphological development with anatomical changes in the general pattern of microstages of pod formation, particularly pod wall thickness and the predicted effect of its ease of threshing for different inflorescence zones.

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Influence of agroecological factors on biologically active compounds in globe artichoke heads

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Abstract. The composition of biologically active compounds in plants depends on the climate and growing conditions, cultivar properties, plant development stage, harvesting time and other factors. The research aimed to evaluate the effect of agroecological factors on the composition of biologically active compounds in globe artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori) heads. The experiment was carried out under open field conditions in the Institute of Horticulture, in Pūre Research centre during the vegetation period of 2015 and 2016. The experiment was arranged in two different soils: brown soil with residual carbonates and the soil strongly altered by cultivation and used two types of seed treatment (without vernalisation, with vernalisation). The quality was evaluated at each harvest time during the all vegetation period. Significant influence of the tested factors on the biochemical content of heads was stated on the content of phenols (73–213 mg GAE per 100 g fresh weight) and vitamin C in artichoke heads (5–20 mg per 100 g fresh weight). Pigments, flavonoids and antiradical activity was not significantly influenced by the tested factors. A slight tendency on producing a higher content of biologically active compounds in more harsh conditions was observed, particularly for phenols and flavonoids.

Key words: *Cynara cardunculus*, flavonoids, pigments, soil.

INTRODUCTION

The globe artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori), (Fam. *Asteraceae*) is widely distributed all over the world and especially in South Europe, the Middle East, North Africa, South America, the United States and China (Pandino et al., 2013). Artichokes have a long history as a medicinal plant, used in folk medicine since Roman times. All parts of the plant can be used for medicinal purposes (Christaki et al., 2012). The medicinal uses of artichokes are very wide. The edible part of the plant is the immature inflorescence, commonly called capitulum or head. Artichoke heads are of low protein and fat content, and a high content of minerals, vitamins, carbohydrates, inulin and polyphenolic compounds (Kolodziej & Winiarska, 2010; Pandino et al., 2013).

Artichokes have a higher polyphenol content compared to other vegetables. They have beneficial effects on consumer health (Turkiewicz et al., 2019). Polyphenolic compounds have strong antioxidant properties, although their content varies between different artichoke varieties and plant parts (Ciancolini et al., 2013). Phenols are characterised by a 90% higher antioxidant activity compared to, for example, vitamin C and carotenoids (Correa et al., 2015). Although the description of artichoke cultivation can be found in the oldest books on horticulture dating back to the 1st half of the 20th century (Gailītis, 1946), nowadays they are not widely grown in Latvia - only in home gardens for experimental and/or ornamental purposes.

Globe artichoke is a perennial herbaceous plant (Cult et al., 2002). In Latvia, there are hard wintering conditions for artichoke to overcome winter periods characterized by fluctuating and often too low soil and air temperature damaging plants. Long periods without snow or often thaws are typical in the period under changing climate conditions. It has a negative influence on the plant's overwintering ability (Bratch, 2014). Therefore, artichokes are considered as an annual plant in Latvia conditions. Also in Poland artichokes are grown as an annual crop (Salata et al., 2012). For the first year of production, vernalized seeds are used or the plants are planted before frost to ensure a 10-day cold period below 10 °C (Fernandez & Curt, 2005). Artichokes are characterised by high genetic variability, resulting in inhomogeneous cultivar material. It is referred that even 15–25% of the plants may be unproductive in the first year, even if they have received the necessary cold conditions (Bratsch, 2014).

Seed germination is strongly influenced by temperature and substrate. Different substrates and materials can be used for seed germination: filter paper, sand, soil, compost, peat (Lekič et al., 2011). Artichokes are well adapted to different soil conditions. Soils with good water drainability, fertile, high nutrient content and deep tilled are the most suitable for artichoke cultivation, as they have a deep root system. Heavy clay soils and light sandy soils are not preferred for artichoke cultivation (Bratsch, 2014; Colla et al., 2012). The optimal temperature for artichokes growth and development is day temperatures between 20–25 °C and night temperatures between 12–14 °C for good quality and high yields (Cianciloni, 2012; Bratsch, 2014). The plants are also tolerant to higher temperatures, but the quality of the heads decreases at 30 °C (Smith et al., 2008). Drought is one of the main abiotic stress factors affecting plant growth and development. It is reported that at least 500 mm of total water is required (Shinohara et al., 2011). Limited water supply is one of the main factors that also affect plant physiological and metabolic processes. Water stress can significantly reduce plant height, shoot and root dry mass. Abiotic stress has a significant effect on the biochemical content of the plant itself and on the artichoke heads. During the long period of drought stress, photosynthetic activity in the plant is reduced, as well as antioxidant activity and vitamin C content are negatively influenced (Tanha et al., 2014).

The influence of agroecological conditions on the growth and biochemical composition of globe artichoke in Latvia is not investigated until now. The research aimed to evaluate the effect of agroecological factors on the content of biologically active compounds of globe artichoke in Latvia. Research hypothesis - ensuring suitable agroecological factors can increase the content of biologically active compounds in globe artichoke heads.

MATERIALS AND METHODS

The investigation was carried out in Pūre (Tukums district, Latvia 57°2′9″N 22°54′25″E) in the vegetation seasons of 2015 and 2016. The trial was set up in a two-factorial design, with four replications, where factor A - soil (strongly altered by cultivation soil - A_1, brown soil with residual carbonates - A_2) and factor B - the type of treatment of germinating seeds (without vernalisation - B_1, with vernalisation - B_2). In the beginning at March, the seeds of artichokes variety 'Green Globe' were germinated in Petri dishes on filter paper saturated with water. After 10 days, half of the sprouts were planted in trays and half placed in the refrigerator at 2–3 °C and after two weeks transplanted in 12 cm diameter plastic pots. Two different soils were compared for artichoke growing: strongly altered by cultivation soil (Ant), with high content of nitrogen and phosphorus, low content of potassium (total N 0.21%, P_2O_5 - 352.1 mg kg^{-1}, K_2O - 133.5 mg kg^{-1} and organic matter 5.4%) and brown soil with residual carbonates (BRk), with optimal content of nitrogen, phosphorus and potassium (total N 0.10%, P_2O_5 - 190.4 mg kg^{-1}, K_2O - 191.8 mg kg^{-1} and organic matter 2.8%). In the middle of May, the plants were planted on the field, in 4 replicates, with a planting scheme of 0.7×0.9 m. The first artichoke heads were harvested at the beginning of August. As the artichoke plant develops heads continuously through the vegetation period, and they mature gradually, all heads were harvested from each plot continuously every week until October for both years.

Meteorological conditions during the investigation period (precipitation and average air temperature) were collected by an automatic meteorological station 'Lufft' located at Pūre (Fig. 1). Overall the years 2015 and 2016 were suitable for artichokes yield production, although they were not optimal in terms of humidity and temperature, especially 2015 was too dry for good artichoke yield production. In 2015 rainfall was only 177 mm and in 2016 - 314 mm.

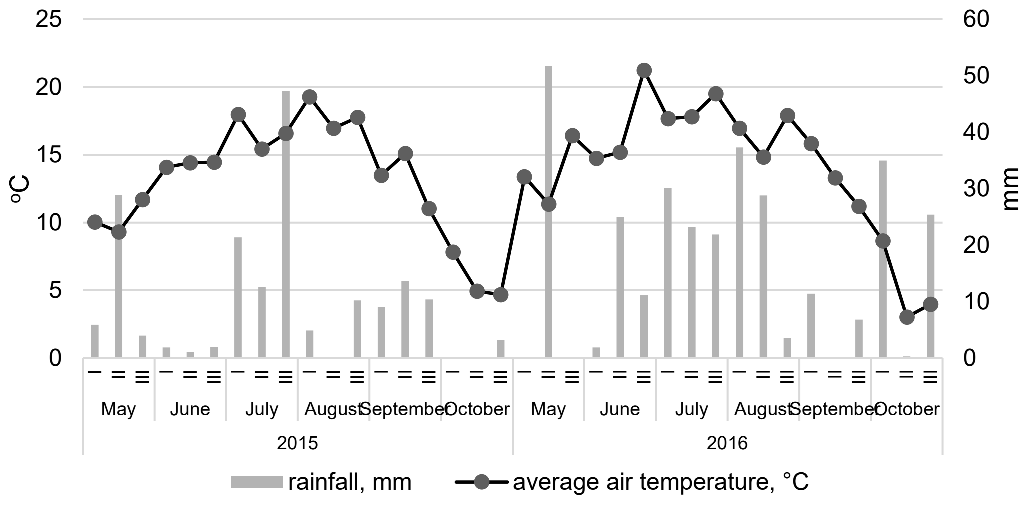

Figure 1. Meteorological conditions of vegetation seasons of 2015 and 2016, in 10-day periods (I, II, III).

Biochemical content analyses for artichoke heads were performed at the Latvia University of Life Sciences and Technologies, Institute of Soil and Plant Sciences. All biochemical parameters were determined for each harvest time for the average sample from the variant in three replications. The data were analysed by averaging over the vegetation period.

The chlorophyll and carotenoid contents were determined spectrophotometrically in an ethanol solution, the light absorbance of the solution was read at 665 nm, 649 nm and 440 nm wavelengths. The content of chlorophyll and carotenoid in the plant material was expressed as mg per 100 g fresh weight (Linchtenthaler, Buschmann, 2001). The ascorbic acid content was determined by titration of filtrate of the acidified (HCl+ HPO_3) extract with 0.001 M iodine. The content of vitamin C (ascorbic acid) in the plant material was expressed as mg per 100 g fresh weight (Moor et al., 2005). The anthocyanin content was determined spectrophotometrically and the light absorbance of the solution was read at 535 nm. The content of anthocyanin in the plant material was expressed as mg per 100 g fresh weight (Moor et al., 2005). The phenolic content was determined spectrophotometrically in acidified ethanol solution; the light absorbance of the solution was read at 320 nm. The content of phenols in the plant material was expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight (Singleton et al., 1999). Flavonoids were determined spectrophotometrically in ethanol solution; the light absorbance of the solution was read at 415 nm. Flavonoid content in the plant material was expressed as mg quercetin equivalent (QE) per 100 g fresh weight (Kim et al., 2003). The antiradical activity was determined spectrophotometrically in methanol extract using 1.1. - diphenyl - 2 picrylhydrazyl radical (DPPH*) the light absorbance of the solution was read at 517 nm. The antiradical activity was calculated as a percentage (%) of DPPH discoloring (Barros et al., 2007). The spectrophotometer used for the analyses was a 'SHIMAZU UV-1800'. The yield from each plot was weighted by using the weighting scales KERN at each harvest time.

The results were analysed using Microsoft Excel 2016 and STATISTICA TM, at the significance level of $\alpha = 0.05$. The data were processed using ANOVA. The data were processed using dispersion analysis. A significant difference (*LSD*) between individual factor values is indicated in the graphs. A 95% confidence level was used to determine the significance of the difference between the variables.

RESULTS AND DISCUSSION

The chlorophyll content is often measured to assess plant growth intensity, as it is closely related to photosynthetic rate. Differences in biochemical content of plants between all variants were evaluated in both years of harvest. The average chlorophyll content of artichoke heads significantly differed between years ($p = 0.000$) and between seeds treatments ($p = 0.000$) when evaluated several times per season. It was not significantly affected by the soil ($p = 0.37$). In both years, the average chlorophyll content in all experimental variants ranged from 41 to 114 mg per 100 g fresh weight (Fig. 2). In the first year, the higher chlorophyll content was determined in plants grown in the strongly altered by cultivation soil, while in the second year the opposite tendency was observed: the higher chlorophyll content was in the artichoke heads grown in the brown soil with residual carbonates, but it should be stressed that the differences were not statistically significant.

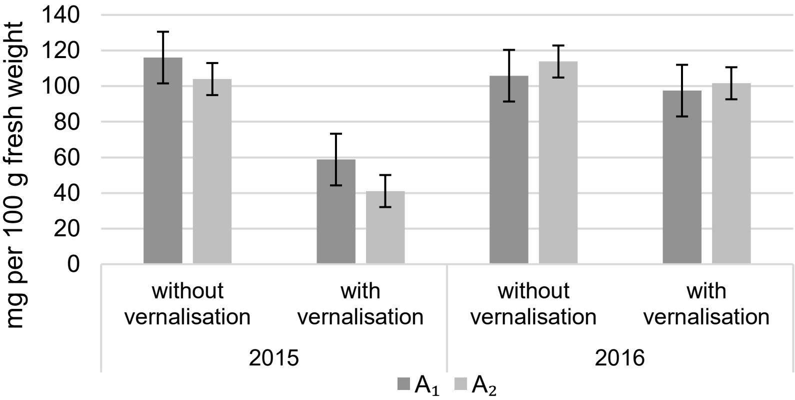

Figure 2. Chlorophylls content in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 29, A_2 – 18).

In both years, the chlorophyll content was higher in the variants where the seeds germinated without vernalisation, but this difference was significant only in 2015. A notable difference between years in agroecological conditions was observed - the lower temperature and precipitation in June and July of 2015 in comparison to 2016. This leads to an assumption that plants grown from vernalised seeds are more responsive to unfavourable conditions during the period of intensive plant growth and heads formation (June - July). In Spain, the evaluation of artichoke heads of two varieties and three hybrids showed chlorophyll contents ranging from 16–55 mg per 100 g fresh weight (Turkiewicz et al., 2019). In comparison to this reference, our plants had higher chlorophyll content.

The average carotenoid content of artichoke heads over the growing season was significantly different between years ($p = 0.02$) and between soils (p = 0.01), but was not significantly affected by the type of seed treatment ($p = 0.13$). In both years, the carotenoid content on average in all experimental variants ranged from 8.3 to 12.3 mg per 100 g fresh weight (Fig. 3).

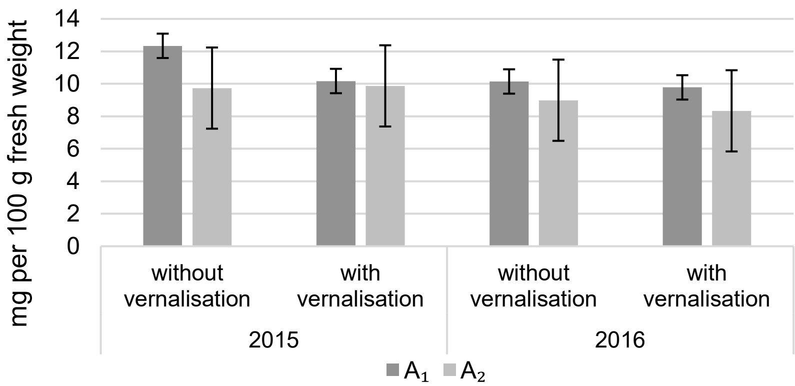

Figure 3. Carotenoid content in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 1.5, A_2 – 5).

In both years, there was observed a common tendency for the carotenoid content to be insignificantly higher in the cultivated soil, except in 2015 for the variant without vernalization treatment, when there were observed significant differences between the soils. This leads us to assume that in rich soil more carotenoids are produced by the plant in comparison to less fertile soil. The carotenoid content of artichoke heads in the cultivars and hybrids evaluated in Spain was similar to the values obtained in the described trial, ranging from 3.5 to 8.5 mg per 100 g fresh weight (Turkiewicz et al., 2019).

The high genetic variability of the artichoke varieties resulted in visual differences in the intensity of the blue colour hue produced by the anthocyanins (Bekheet & Sota, 2019). When compared statistically, only the seed treatment had a significant effect on the anthocyanin content in the artichoke heads throughout the growing season ($p = 0.000$), although a clear tendency for one particular investigated factor influence on the anthocyanin content in the plants was not found. In both years, the anthocyanin content averaged over all experimental variants ranged from 0.6 to 5.1 mg per 100 g fresh weight (Fig. 4), which is similar to plants grown in Tunisia, where anthocyanin content varied between 2.5 and 3.7 mg per 100 g fresh weight, depending on the variety (Dabbou et al., 2017).

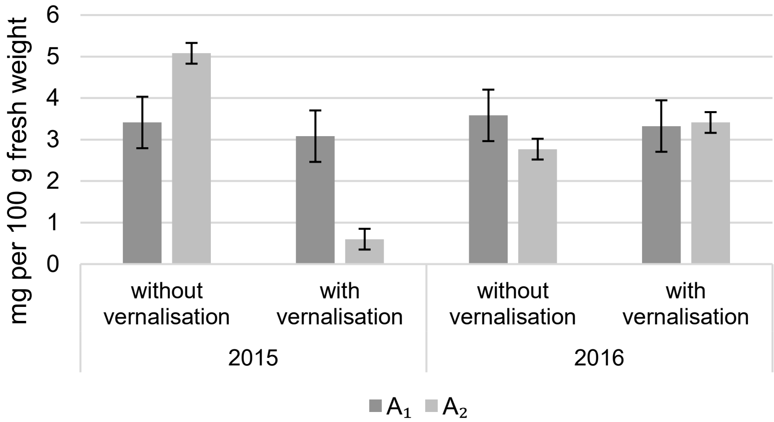

Figure 4. Anthocyanin content in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 1.1, A_2 – 0.5).

The two main phenolic compounds found in artichoke heads are chlorogenic acid and cynarin. They have strong antioxidant activity. The phenolic content and their activity in artichokes may vary depending on the plant part and variety, the ripeness of the head, storage and processing (Shinohara et al., 2011). In both years of the trial, the phenolic content varied from 73 to 213 mg GAE per 100 g fresh weight (Fig. 5). All factors in the trial had a significant influence ($p = 0.00$) on the phenolic content of artichoke heads. In both harvest years, there was a common tendency when more phenolics were synthesised in plants grown on less fertile brown soil with residual carbonates (differences were not significant). In 2015, regarding the phenolic content, there were stated significantly higher values in the heads of plants grown by vernalisation treatment.

A study in Texas has shown that under drought stress, more phenolic compounds are synthesised (Shinohara et al., 2011). The data of 2015 somehow is in agreement with the finding of Shinohora (2011), that less favourable conditions promote the synthesis of phenols. Especially it was observed for vernalised plants, which are assumed as being in light stress in 2015 also in accordance with chlorophyll data.

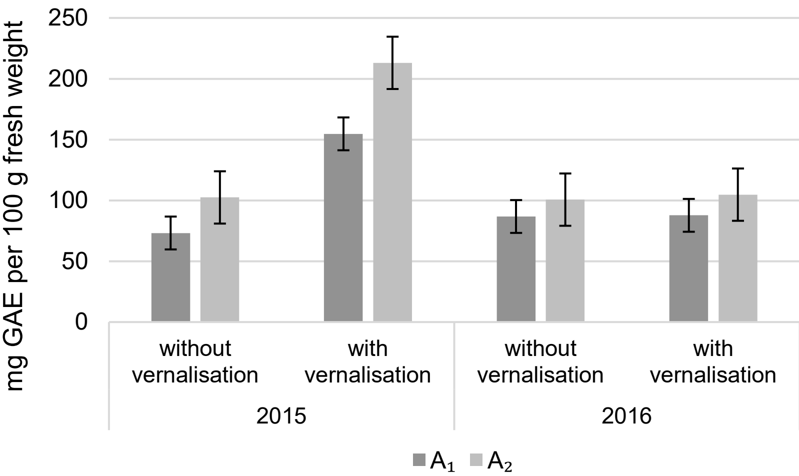

Figure 5. Phenols content in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 27, A_2 – 43).

The flavonoids in the plant affect its colour, taste and aroma (Sergejeva et al., 2018). Flavonoids are the largest group of polyphenols and their content is also influenced by various factors such as genotype, environmental conditions (temperature, light, soil), cultivation system, and storage. Several methods are used to determine polyphenols in plants, which play an important role in data interpretation. Consequently, it is often difficult to compare data from different studies. Genotype and analysed plant parts (head, stem, leaves) are the main factors influencing the content of polyphenol compounds in the sample. In a study carried out in Italy, evaluating 17 globe artichoke cultivars, their total polyphenolic content varied from 12 to 59 mg quercetin equivalent (QE) g^{-1} fresh weight (Pandino et al., 2013). The values of flavonoid content obtained in the described trial varied from 13 to 28 mg QE per g fresh weight (Fig. 6).

Statistically significant differences in the flavonoid content of artichoke heads were observed between trial years as well as between seeds treatment ($p = 0.000$). The soil type did not have a significant effect. A common tendency was observed in both crop years: plants grown without vernalization had a higher flavonoid content in brown soil with residual carbonates (significant only in 2015), while in the variant with vernalisation more flavonoids were synthesised in the plants grown in strongly altered by cultivation soil (significantly only in 2015). Comparing the flavonoid content of the plants by year, the highest flavonoid content in artichoke heads was found in 2015. Which supports previous statements in our research and also refers to others about the plants defence mechanisms in unfavourable conditions by increased production of physiologically active compounds (Kolodziej & Winiarska, 2010).

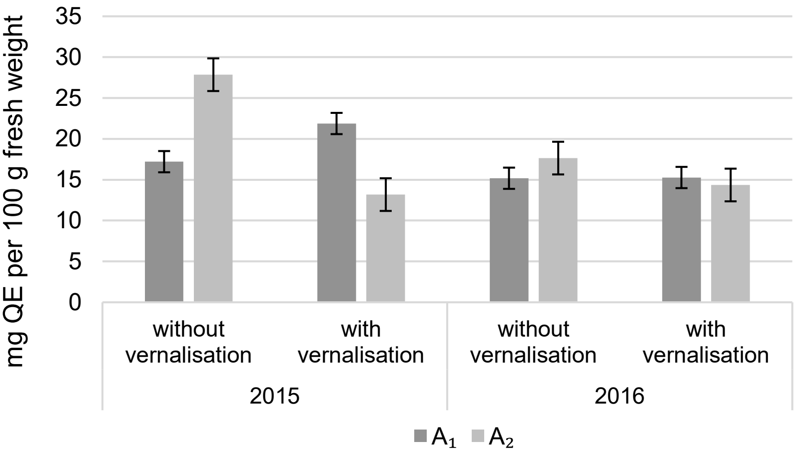

Figure 6. Flavonoid content in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 2.6, A_2 – 4).

The differences in the content of vitamin C in artichoke heads were significant between all tested factors ($p < 0.05$). From all harvests over the two years, the average vitamin C content of the heads varied between 5 and 20 mg per 100 g fresh weight per variant (Fig. 7). In 2015, the content of vitamin C was significantly higher in non-vernalised plants in both soils. However, no significant differences were observed in 2016.

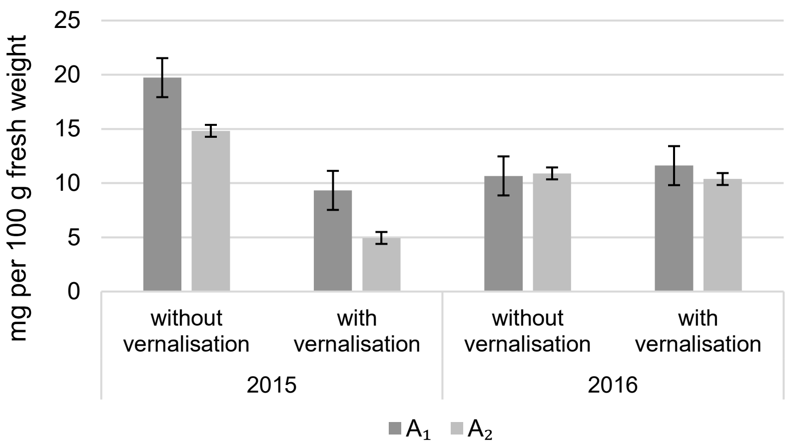

Figure 7. Vitamin C content in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 3.6, A_2 – 1.1).

Two varieties of globe artichoke were grown in Poland over for three years and the vitamin C content of the petals and the central part of the heads was determined. One of the varieties ('Symphony') had an average vitamin C content of 14.5 mg in the central part of the heads and 11.3 mg the outer petals per per 100 g fresh weight over the three years. The other variety ('Madrigal') showed a significant difference between the vitamin C content in the central part of the head and in the outer petals, 15.8 and 6.5 mg per 100 g fresh weight respectively (Salata et al., 2012).

From the results over the two trial years, it is not possible surely determine which conditions promote the synthesis of vitamin C. Also in southern Italy, differences were found between genotypes and harvest times. The four genotypes were evaluated for vitamin C content between 3 and 6 mg per 100 g fresh weight and were higher at later harvest times, but the meteorological conditions were not specified in the description of the experiment to compare with the results of the designed study (Melilli et al., 2013). In another study in Italy, the content of vitamin C in artichoke heads was 14 mg per 100 g fresh weight (Dosi et al., 2013).

The antiradical activity differs from the colorimetric method used (Dabbou et al., 2017; Mabeau et al., 2007). In Poland, it was found that, depending on the method, the antiradical activity of an extract obtained from globe artichoke leaves of the cultivar 'Green Globe' ranged from 44% (DDPH method) to 80% (ABTS method) (Biel et al., 2019). In the other trial in Tunisia, when two cultivars were grown and analysed by the DPPH method, antiradical activity was determined to be 63 and 70% (Dabbou et al., 2017). In the established trial, the antiradical activity (as determined by the DPPH method) varied on average between 41 and 68% over the two years (Fig. 8). However, the results are not exactly comparable as different stock solutions and reagent volumes were used in each trial, resulting in different dilutions.

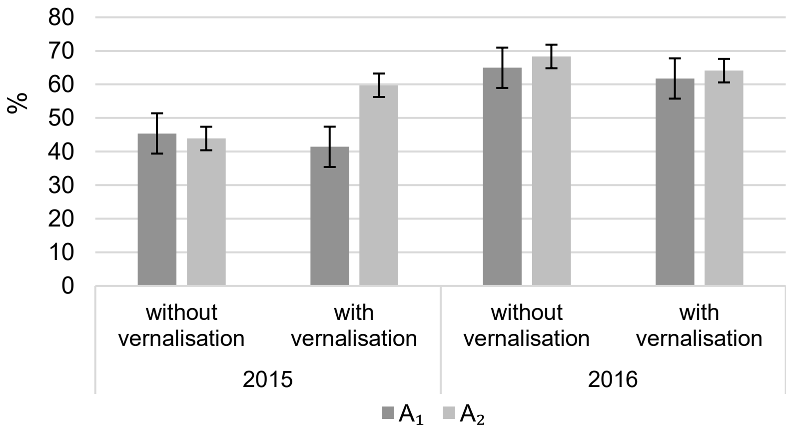

Figure 8. Antiradical activity in the analysed artichoke heads: A_1 – strongly altered by cultivation soil; A_2 – brown soil with residual carbonates, error bars represent *LSD* for particular soils (A_1 – 12, A_2 – 7).

There were stated significant differences between years ($p = 0.000$) and between soils ($p = 0.01$), while the type of seed treatment did not have a statistically significant influence on the antiradical activity ($p = 0.57$). In general, there was an observed tendency for having higher antiradical activity in plants grown on less fertile brown soil with residual carbonates, but overall the differences were not significant. In 2016, there were not found significant differences between any factors. After two years of the trial, it seems that in less favourable meteorological conditions (2015) antiradical activity of plants is lower. Further trials are necessary to certainly approve the observed tendencies.

CONCLUSIONS

The content of phenols and vitamin C in artichoke heads was significantly influenced by all the factors evaluated in the trial. The common influence was stated for the vegetation season - both components were higher in the harshest year - 2015. The chlorophyll and flavonoid contents were significantly affected by the conditions of the year and type of seed treatment, when chlorophyll was higher in better meteorological conditions in 2016, but flavonoids were higher in not so favourable conditions - in drier 2015 and less fertile soil. Both parameters were negatively influenced by the seed treatment - vernalisation. The carotenoid content and antiradical activity were significantly affected by the differences in the years and soils - higher carotenoid content was stated in the dry vegetation period of 2015 and more fertile soil, but ARA was higher in 2016 in less fertile soil. Only the vernalisation had a significant effect on the anthocyanin content. Often no significant effect was found for a single factor, but the effect was significant in combination with other factors. There was no observed common tendency for particular conditions to ensure a higher level of all bioactive compounds in artichokes. A slight tendency on producing a higher content of biologically active compounds in more harsh conditions was observed, particularly for phenols and flavonoids. Further trials are necessary in a longer period time to cover more different agroclimatic conditions to get a clear understanding of the influence of different factors on the content of biochemically active compounds in artichoke plants.

ACKNOWLEDGEMENTS. This study was supported by Latvia Council of Science, project 519/2012 'Methods of increasing physiologically active compounds in vegetables cultivated in Latvia in changing climate conditions' and grant 'LLU Transition to a new funding model of doctoral studies', LLU-8.2.2.0/20/I/001.

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