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Labile organic matter and fertility of chernozems

Abstract. Soil organic matter can be divided into stable and labile, with the latter directly supporting plant growth and development during the growing season. To restore soil fertility and ensure high productivity of agrocenoses, a certain ratio between the labile and stable parts of humus must be maintained. The purpose of this study was to determine the content of labile organic matter under different tillage and fertilisation options and to establish the optimal ratio between their content and the content of total carbon, based on which to develop measures to restore and improve the fertility of chernozem soils. In stationary experiments, the study investigated the impact of the intensity of chernozem use, its cultivation and fertilisation systems on the humus state of the amount of labile organic matter and its ratio to humus carbon. Field research, laboratory method of soil sample analysis, systematisation, analysis, and generalisation were used in the study. The soil

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samples were analysed for humus content according to DSTU 4289:2004 and labile organic matter content according to DSTU 4732:2007. When chernozems are ploughed up, labile organic matter is mineralised the most and their share in humus decreases, while when arable land is converted to fallow land, it is restored the fastest. Minimising tillage systems and applying organic and mineral fertilisers increases the amount of labile organic matter in the humus of chernozems. The use of no-till for sixteen years resulted in a 0.13% increase in humus content, and a 0.53% increase over forty-two years compared to ploughing. The application of organo-mineral fertiliser increased the ratio of labile organic matter carbon content to total carbon content compared to the unfertilised variants. A strong correlation was found between the content of labile organic matter in chernozems and the yield of winter wheat and maize. The practical significance of the research results is to minimise the fertiliser rate and the C:N ratio to ensure gross crop yields and restore soil fertility

Keywords: humus; mineralisation; humification; winter wheat; maize

INTRODUCTION

Understanding the essence of fertility and the factors that have the greatest impact on crop yields and cenosis productivity will help determine land quality more accurately and allow developing measures to restore and improve fertility. One of these factors will be the content and reserves of humus in the root or half-metre layer of soil, which provides 80-85% of crop yields (Zabaluev *et al*., 2020). Humus is called an integral indicator of fertility because it affects most soil properties, especially chernozems, regulates and coordinates the processes on which their fertility depends, and determines the level of crop productivity for a long period (Volkogon *et al*., 2019; Oldfield *et al*., 2019; Rattan, 2020).

Many authors note the ongoing dehumification of agricultural land and provide evidence of a decline in the humus content of Ukrainian soils, especially chernozem (Litvinova *et al*., 2019; Balyuk *et al*., 2023). According to S. Balyuk *et al.* (2023), humus losses are observed on 43% of arable land at an average rate of 620 kg/ha, and this process has been ongoing for decades. M. Mayer *et al.* (2022) indicate that agricultural use has led to substantial losses of soil organic matter, accompanied by increased CO_2 emissions and reduced soil fertility. That is why, in view of global warming, it is necessary to ensure the sustainable development of agroecosystems.

Humus is a multicomponent organic substance that includes dozens of soil-specific and non-specific compounds that have different transformation capacities during the growing season, including resistance to mineralisation. Humus substances are divided into stable and labile on this basis. Some authors (Volkogon *et al*., 2018) believe that labile organic matter is associated with the microbiological regime of the soil and due to their mineralisation, plants can consume macro- and microelements released from them. Furthermore, they contain enzymes and plant growth stimulants, which also contribute to crop productivity.

Labile organic matter acts as the closest reserve of micronutrient nutrition, determining the content and dynamics of mobile forms of trace elements. Co, Cu, Fe can be actively released from them and transferred to the soil solution, while Mn and Zn are subject to fixation. The positive effect of fertilisation on the humus state of organic systems is reported in M. Chen *et al.* (2022). The issues of regulating the content of labile organic matter and humus have been investigated and discussed in scientific sources by Ukrainian and foreign researchers (Volkogon *et al*., 2019; Yang *et al*., 2019).

Stable organic matter provides soils with such characteristics as structural and water-physical properties, indicators of the soil-absorbing complex, buffering capacity, humus group composition, carbon, nitrogen, phosphorus, and sulphur reserves (Demydenko, 2019; Rattan, 2020). Therewith, J. Zhou *et al.* (2021) point out that the supply of labile carbon to the soil can accelerate the decomposition of soil organic matter, as well as cause changes in the dynamics of soil organic matter, changing the direction and intensity of humus formation. Therefore, the carbon balance depends on both the amount and frequency of labile carbon input. They conclude that the supply of labile organic matter in large quantities can lead to intensive decomposition of soil organic matter.

Potentially mineralised C represented the largest labile C pool, while microbial N biomass was the largest labile N pool. As for the rate of carbon and nitrogen mineralisation, they were oppositely distributed over the seasons. The authors also conclude that it is important to achieve zero soil degradation based on a detailed investigation of organic matter (Koković *et al*., 2021)

The purpose of this study was to investigate labile organic substances, establish their relationship and optimal correlation with total soil carbon and crop productivity, and develop methods for regulating their amount in the soil to restore and improve the fertility of chernozems.

MATERIALS AND METHODS

The study was conducted in long-term stationary experiments in 2017-2022, which investigated the impact of the intensity of use of chernozem soils, different tillage systems and fertilisation on the content of humus and labile organic matter, their correlation and relationship with crop productivity. The virgin chernozem of the Mykhailivska Tsilyna Reserve was chosen as a reference, where organic matter transformation processes contribute to maintaining a high level of soil humus with an optimal content of labile organic matter. The amount of labile substances and their share in chernozem humus were investigated on virgin plots and fallow land, which served as models of natural self-regulation of humus formation and humus accumulation.

In a stationary experiment at the Separate Subdivision of the National University of Life and Environmental Sciences of Ukraine "O.V. Muzychenko Velykosnitynske Educational and Research Farm" in Fastiv district, Kyiv region, the study investigated the impact of 16 years of use of different tillage and fertilisation systems on the humus and labile organic matter content, their correlation and relationship with crop productivity at the Drabivske Experimental Field of the Cherkasy Experimental Station of the Educational and Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences" over 42 years of use of different tillage and fertilisation systems. The fallow land options were taken as natural models of organic matter transformation and restoration of chernozem fertility. The experimental variants were replicated three times and randomised across replications. Short rotation crop rotations were used in the experiments.

A 16-year experiment in the Kyiv region at the Separate Subdivision of the National University of Life and Environmental Sciences of Ukraine "O.V. Muzychenko Velykosnitynske Educational and Research Farm" had the following crop rotation: soybeans, winter wheat, grain maize, barley, and at the Drabivske experimental field of the Cherkasy Experimental Station of the Educational and Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences" – perennial grasses, winter wheat, sugar beet, grain maize, barley with grasses.

Soil of the stationary experiment at the SS of the NULES of Ukraine "O.V. Muzychenko Velykosnitynske ERF" – a typical coarse-grained and light-loamy in the forest – was characterised by the following indicators: soil sericulture reaction was neutral, the amount of extracted cations was 35.4 mg-eq/100 g of soil, and the degree of saturation of cations was 93.4%.

In the multifactorial stationary experiment of the Cherkasy State Research Station of the ERC "Institute of Agriculture of NAAS" at the Drabivske experimental field, the soil of the experimental plot was typical low-humus light loamy chernozem.

Laboratory tests were carried out according to standardised methods. The humus content was determined according to DSTU 4289:2004 "Soil quality. Methods for determining organic matter" (2005); the content of labile organic matter (LOM) was determined according to DSTU 4732:2007 "Soil quality. Methods of determining available (labile) organic matter" (2008). The study was conducted per the requirements of the International Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Soil functioning and fertility depend on environmental conditions, soil use, and crop cultivation techniques. They determine the intensity of soil formation processes, their focus on preserving and restoring soil fertility or on increasing degradation processes and loss of fertility. These factors also affect the integral indicator of chernozem fertility – the humus content of its upper layers. According to researchers (Dmytruk & Demid, 2019), it is the top 0-50 cm layer of soil that is crucial for the productive function of chernozems. This layer accounted for 80-85% of crop yields in the author's research. Bagherifam *et al*. (2023) emphasise that soil organic matter plays a key role in the environmental chemistry of macro- and micronutrients.

Table 1 shows the results of a study of the impact of different uses of typical chernozem in the Mykhailivska Tsilyna Reserve on the indicators of their humus state, namely the content of humus and labile organic matter.

Table 1. Content of humus and labile organic matter in typical chernozem under different uses in the Mykhailivska Tsilyna Reserve, %

Land	Layers of soil, cm	Organic substances					C_{lom}:C_{hum}
		Humus	C_{humus}	%, of absolute virgin soil	C_{los}	%, of absolute virgin soil	
Absolute virgin soil	0-20	8.79	5.36	100	0.63	100	11.7
	20-40	6.25	3.62	100	0.34	100	9.4
Mown virgin soil	0-20	7.79	4.51	84.1	0.51	81.0	11.3
	20-40	5.33	3.09	85.4	0.21	67.7	6.7
Fallow	0-20	7.29	4.23	79.0	0.33	52.4	7.8
	20-40	5.79	3.36	92.8	0.20	64.5	5.9
Arable land	0-20	5.62	3.25	60.6	0.15	17.5	4.6
	20-40	5.40	3.13	86.5	0.11	25.8	3.5

Note: C_{humus} – carbon content of humus, %; C_{lom} – carbon content of labile organic matter
Source: compiled by the authors of this study

The table shows that the more intensively the soil is used, the more humus mineralisation processes are manifested. Therewith, the components of humus that are more easily accessible to microorganisms mineralise faster. Thus, while the humus content of mown virgin soil, fallow land, and arable land compared to absolute virgin soil in the upper 0-20 cm soil layer decreased by 15.9%, 21.0%, 39.4%, respectively, the labile organic matter content decreased by 19.0%, 47.6%, and 82.5%, respectively. Given that these compounds can be an affordable source of macro- and microelements for plant growth and development during the growing season, the findings suggest a substantial decrease in potential soil fertility and, consequently, crop productivity. On all variants with natural vegetation, they are much more numerous in the upper 0-20 cm layer, which is saturated with plant roots and their secretions are part of labile organic matter. Thus, while in the variant of absolute virgin soil in the 0-40 cm layer their share was on average 10.6, in mown virgin soil, fallow land, and arable land, respectively, it was 8.5, 6.9, 4.1. On arable land their number in the upper layer is 4.2 times less than in absolute virgin soil. The high ratio (S_{lom}:C_{hum})/100 against the background of significant humus content of the upper layers of virgin chernozem indicates the stability of the humus state of this soil and its large reserves of available macro- and microelements for microorganisms and plants, and thus high potential productivity. A decrease in this ratio indicates a deterioration in the conditions for humus formation and a possible deficit in the balance of organic matter in the soil and a decrease in its potential fertility.

The natural humus-accumulative process takes place in chernozems under natural vegetation on fallow land, which helps restore the content of humus and labile organic matter. Therewith, comparing arable land and fallow land, the share of labile organic matter in humus increases faster on the latter. Thus, their content on fallow land increased from 17.5% to 52.4%, i.e., by 34.9%, and humus by only 18.4%, from 60.6% to 79.0%. Notably, both on virgin soil and on fallow land, relative changes in the amount of LOM are 2 times faster than humus.

In the modern period, there are three primary areas of achieving a neutral level of degradation while restoring the fertility of chernozems, including minimising tillage, biologising fertiliser systems, and ensuring a deficit-free balance of basic nutrients in crop rotation (Balyuk *et al.*, 2023). These factors were studied in a stationary experiment at the SS of the NULES of Ukraine "O.V. Muzychenko Velykosnitynske ERF", which investigated the effect of different tillage and fertilisation systems on the humus status of typical chernozem (Table 2). The table data shows that minimising tillage helped preserve the organic matter of chernozems and, when organic and mineral fertiliser was applied, increased the humus content.

Table 2. Humus and labile organic matter content and their ratio for 16 years of using different tillage and fertilisation systems in typical chernozem in the SS of the NULES of Ukraine "O.V. Muzychenko Velykosnitynske ERF", %

Soil cultivation system	Layers of soil, cm	Fertilizer variants								
		Control (no fertiliser)			Straw 1.2t/ha+ N_{12}+$N_{78}P_{68}K_{68}$			Straw 1.2t/ha+ green manure+ N_{12}+$N_{78}P_{68}K_{68}$		
		humus / C_{humus}	C_{los}	C_{los}:C_{hum}	humus / C_{humus}	C_{los}	C_{los}:C_{hum}	humus / C_{humus}	C_{los}	C_{los}:C_{hum}
Different depth ploughing	0-20	3.18 / 1.84	0.09	4.8	3.58 / 2.07	0.14	6.7	3.72 / 2.16	0.20	9.2
	20-40	3.05 / 1.77	0.11	6.2	3.48 / 2.02	0.16	7.9	3.69 / 2.14	0.23	10.7
Different depth non-ploughing tillage	0-20	3.26 / 1.89	0.12	6.3	3.64 / 2.11	0.16	7.6	3.85 / 2.23	0.28	12.5
	20-40	3.01 / 1.75	0.10	5.7	3.53 / 2.05	0.18	8.7	3.68 / 2.14	0.23	10.7
Mimimum non-ploughing tillage	0-20	3.28 / 1.90	0.11	5.8	3.59 / 2.08	0.16	7.7	3.79 / 2.19	0.24	10.9
	20-40	2.97 / 1.72	0.09	5.2	3.50 / 2.03	0.11	5.4	3.58 / 2.02	0.19	9.3
Fallow	0-20	3.98 / 2.31	0.30	12.9						
	20-40	3.92 / 2.27	0.24	10.5						

Source: compiled by the authors of this study

Without fertilisation, there was a tendency to increase the humus content only in the top 0-20 cm of the soil layer. The same pattern was observed in the content of labile organic matter, where in the non-ploughing variant their content was higher than in ploughing.

Fertiliser application contributed to the enhancement of the humus-accumulative process, the accumulation of humus and labile organic matter in both soil layers under study. The growth in the upper layer of 0-20 cm was 0.40-0.53% in the variants with straw and mineral fertilisers and increased to 0.54-0.59% when green manure was also used. In the lower layer of 20-40 cm, the growth from fertilisation and green manure use was significant and amounted to 0.43-0.67%. The effect of fertilisers on labile organic matter was even more significant, with the content of LOM increasing by 1.5-2.0 times or more compared to the unfertilised variant. The best results, especially in the topsoil, were observed in the variants with non-ploughing and amounted to 0.24-0.28%. The best indicators of the humus state of typical chernozem were observed on the fallow land variant, which served as a benchmark for comparing the effectiveness of soil fertility restoration measures, and where the humus content was 3.98% and labile organic matter was 0.30%.

Comparable patterns were observed in the typical chernozem of the Drabivske experimental field, where the content of humus and labile organic matter in the soil decreased without fertilisation (Table 3). Minimal tillage, even without fertilisers, substantially reduces humus mineralisation, as its content is much higher in the variant of different depth non-ploughing tillage than in different depth ploughing.

Table 3. Humus and labile organic matter content and their ratio under different tillage and fertilisation systems in typical chernozem at the Drabivska Experimental Station, %, average for 2017-2019

Soil cultivation system	Layers of soil, cm	Fertilizer variants					
		Control (no fertiliser)			$N_{130}P_{110}K_{110}$/ha of crop rotation		
		humus / C_{humus}	C_{los}	C_{los}: C_{hum}	humus / C_{humus}	C_{los}	C_{los}: C_{hum}
Different depth ploughing	0-20	2.97 / 1.73	0.041	2.36	3.02 / 1.75	0.142	8.11
	20-40	2.54 / 1.47	0.035	2.38	2.95 / 1.71	0.085	4.80
Different depth non-ploughing tillage	0-20	3.29 / 1.90	0.152	8.08	3.55 / 2.06	0.211	10.3
	20-40	3.00 / 1.80	0.079	4.38	3.14 / 1.82	0.078	4.29
Fallow	0-20	4.02 / 2.33	0.113	4.85			
	20-40	3.22 / 1.87	0.061	3.26			

Source: compiled by the authors of this study

The content of labile organic matter varied in proportion to the total humus. Notably, it was high – 0.152% in the upper 0-20 cm layer in the variant with different depths of non-ploughing tillage, which may be caused by more than 40 years of incorporating plant residues in this layer. In addition, very low values of the C_{lom}:C_{hum} ratio (2.36-2.38) were observed in the ploughed variant, indicating an unfavourable humus regime, namely, increased mineralisation of organic matter caused by intensive tillage and insignificant new formation of humus substances due to the lack of fresh organic matter. On the fallow land variant, the humus content was the highest, and an increased amount of labile organic matter was observed in the upper 0-20 cm sod layer saturated with plant roots. The absence of tillage and excessive loosening contributes to the formation of more formed and stable humus substances, which increase humus accumulation.

Figure 1 shows the dependence of winter wheat and grain maize yields on the content of LOM in typical chernozem.

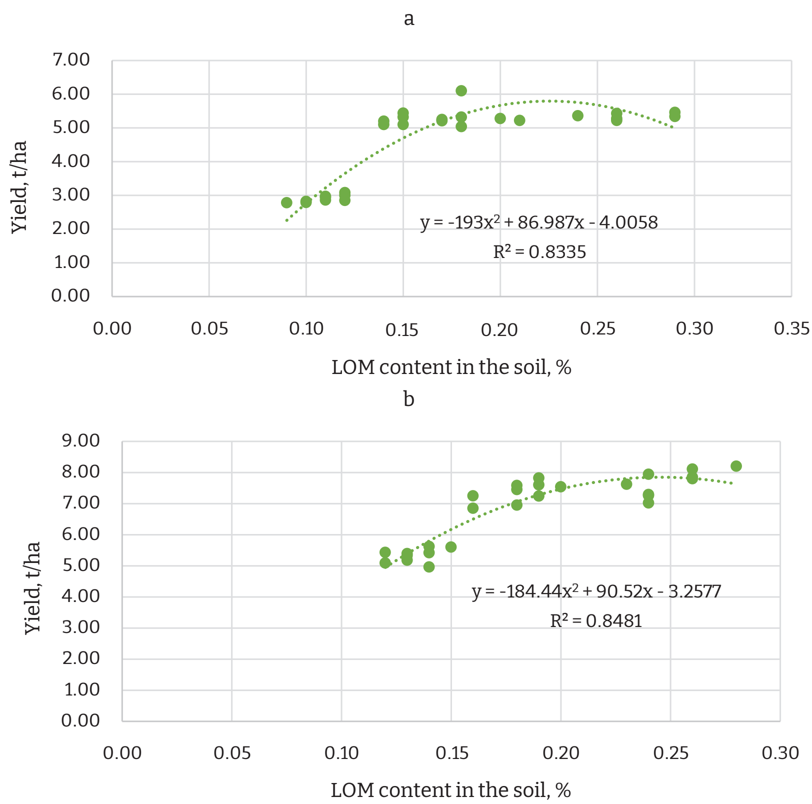

Figure 1. Influence of LOM content on the yield of winter wheat and grain maize (polynomial dependence)

Note: a – winter wheat, b – grain maize

The lowest yields of winter wheat and grain maize are dependent on the content of LOM in typical chernozem. The lowest crop yields and LOM content were recorded without fertilisation and amounted to 2.78-3.08 t/ha and 0.09-0.12% for winter wheat, respectively, and 5.43-5.62 t/ha and 0.12-0.15% for grain maize. When organo-mineral fertilisers were applied, the yields increased for winter wheat by 5.10-5.44 t/ha and 0.14-0.18%, and for grain maize by 6.85-7.82 t/ha and 0.16-0.19%. When green manure was used in this fertilisation system, the content of LOM and the yield of grain maize increased to 0.20-0.29% and 7.29-8.20 t/ha and were the highest, while the productivity of winter wheat stayed at the same level.

The intensification of mineralisation processes in chernozems under agricultural use reduces the proportion of labile organic matter in the fractional composition of humus. The application of fresh organic matter to the soil in combination with mineral fertilisers restores soil humus content, increases the amount of labile organic matter, and helps optimise the formation of soil microorganisms, enhances biological processes in the soil, which has a positive impact on potato yields. From an environmental standpoint, it is undesirable to use mineral fertilisers when there is a deficit of organic matter in the soil, as this increases gaseous nitrogen losses and intensifies mineralisation processes (Volkogon *et al.*, 2018). According to the authors (Balyuk *et al.*, 2016), a decrease in the humus content in the 0-20 cm layer of typical chernozem from 4.70% in the virgin variant to 3.60-4.00% under different fertilisation systems led to a significant reduction in labile humus substances. Therewith, in the organic-mineral fertilisation system, they accounted for 0.38%, 0.22% – upon mineral fertilisation, 0.15% – upon organic fertilisation, and 0.14% – no fertilisation.

It is safe to say that there is a connection between total soil carbon and labile organic matter, and it is logical to assume a correlation between them and plant productivity. This is noted in the studies of several authors (Balaev *et al.*, 2019). Land productivity is associated with labile organic matter as an easily accessible and balanced source of macro- and microelements for agricultural plants and microorganisms, which are released during its mineralisation, and it also contributes to the release of hard-to-reach compounds into solution. The authors (Rattan, 2020) note a prominent correlation between the content of LOM and the yield, and a closer correlation between the yield and the content of total carbon in the soil.

In modern conditions, it is necessary to consider not only the crops grown and soil fertility indicators, but climate aridisation. Studies by G. Wu *et al.* (2023) revealed the impact of global warming on changes in soil organic matter. The multifunctionality of the soil, caused by enzyme activity, was lower when warming due to lower soil moisture, nutrient availability and the supply of labile organic matter. The close relationship between microbial diversity and functional genes and soil multifunctionality has highlighted the importance of soil biodiversity in maintaining the functioning of agroecosystems, which is linked to nutrient cycling. With increasing soil and air temperatures, leaving the straw on the field resulted in higher bacterial diversity (by 3.6%) and the number of microbial functional genes of the C, N, and P cycle compared to removing the straw from the field. The straw reduced the amplitude of soil temperature fluctuations. Overall, the return of straw created a favourable habitat for microorganisms and thus mitigated the adverse effects of warming on microbial communities and soil functionality. Comparable results were obtained from the study of the microbiological state of meadow chernozem soil (Motruk *et al.*, 2021)

In soils where the humus-accumulative process is manifested and soil fertility is restored, the proportion of labile organic matter in the humus composition increases. In the experiment at the SS of the NULES of Ukraine "O.V. Muzychenko Velykosnitynske ERF", the ratio of C_{lom}:C_{hum} was the lowest in the variants without fertilisation and increased with the improvement of the humus state of the chernozems, which is typical for the application of different fertiliser rates or the use of minimised tillage. The highest indicators were observed in multi-depth no-till cultivation and on fallow land, where they were 10.7-12.5 and 10.5-12.9, respectively. Studies by E. Litvinova *et al.* (2019) found that the use of no-till variant created conditions for increasing the content of labile organic matter in the arable layer of typical chernozem by 4-7% compared to ploughing.

Notably, there is a direct correlation between stable and labile organic substances and a certain ratio between them should be maintained for the soil to effectively perform its productive function over a long period of time, as well as its ecological function in the biosphere (Lukas & Weil, 2012; Gamkalo & Bedernyk, 2014; Balyuk *et al.*, 2016).

Due to the high humus content, the C_{lom}:C_{hum} ratio averaged 4.85-3.26, and was highest in the top layer upon multi-depth no-till cultivation – 8.08.

Correlation analysis of the data for the 0-20 cm soil layer showed that soil organic matter stock was positively related to labile compounds, the proportion of large macroaggregates, and negatively related to small macroaggregates, aromatic compounds, and the aromaticity index. The accumulation of soil organic matter in the topsoil under no-till was influenced by the accumulation of labile organic compounds (Briedis *et al.*, 2023).

S.T. Lukas & R.R. Weil (2012) found that the content of labile organic matter is closely related to total organic carbon in the soil (r=94). The results of the study show that labile organic matter can serve as a test in soils where agronomic practices are used to increase their productivity.

When fertilising the Drabivske experimental field, an increase in the humus content of the soil layers was observed under both tillage systems. The highest results were observed in the variant of different depth non-ploughing tillage, where the humus content in the layers 0-20 and 20-40 cm was 3.55% and 3.14%, which is 0.25% and 0.14% higher than upon ploughing, respectively. The content of labile organic matter was also better here, especially in the top 0-20 cm of soil. Fertiliser application increased their share in the humus composition, indicating favourable conditions for the formation of humus substances, the predominance of humification over humus mineralisation and the focus of soil processes on restoring the fertility of typical chernozems.

In the studies of V.V. Volkogon *et al.* (2018), the authors pointed out that it is not possible to improve the microbiological and nutrient regimes of the soil under the influence of LOM, although there is little evidence of a direct link between their content and crop yields. The paper by T. Yang *et al.* (2019) notes that grain yields are correlated with both LOM and soil organic matter content. The maximum yield was achieved at an organic matter content of about 4.0% and a high LOM value. J. Shen *et al.* (2023) found that water-soluble organic matter stability is not an intrinsic trait during initial pedogenesis: stable water-soluble organic matter compounds stay after bacteria rapidly utilise its labile compounds, while fungi slowly decompose the remaining pools.

The dependence of winter wheat and grain maize yields on the content of LOM was strong, with correlation coefficients of r=0.83 and r=0.85, respectively. E. Oldfield *et al.* (2019), using data from 240 experiments from 29 countries at the global level, established a link between organic carbon content from 0.1% to 2.0% and maize and wheat yields. Thus, the yield of these crops was 1.2 times higher with an organic carbon content of 1.0% compared to 0.5%. The yield increase stabilises at a concentration of about 2.0%, and this value is proposed as a critical limit for temperate soils. An analogous opinion is shared by L. Rattan (2020), who suggests that the critical threshold for reducing the organic carbon content in temperate soils is 2.0%, and for tropical soils 1.1%. The author notes the relationship between the content of organic carbon in the soil and crop yields due to its ability to improve the water regime of the soil, especially in arid conditions, and provide plants with nutrients, primarily nitrogen, phosphorus, and sulphur.

Thus, the content of LOM can be considered not only as an indicator of the humus state of the soil and a favourable regime of humus formation in chernozems, but as an indicator of potential soil fertility and crop productivity.

CONCLUSIONS

The intensity of mineralisation of the organic matter of chernozems depends on the methods and period of their agricultural use. Increased anthropogenic pressure, intensification of soil cultivation, and a decrease in the supply of fresh organic matter to the soil under arable conditions lead to losses in the content of humus reserves. When virgin chernozems are ploughed up, labile organic matter in the humus is mineralised more rapidly, and when arable land is converted to fallow, it is restored more quickly. Relative changes in the amount of labile organic matter under the influence of agricultural practices are twice as fast as humus. Thus, labile organic matter can be an indicator of changes in the humus state of chernozems under the influence of agricultural practices and crop cultivation technologies.

Minimising soil tillage and applying organic and mineral fertilisers enhances the humus-accumulative process, increases the amount of humus and LOM, and increases their ratio in typical chernozem. The long-term use of non-ploughing tillage and fertilisation significantly increases the humus content, especially in the top layer, compared to ploughing. During the 16-year period of their use, the increase in humus was 0.13%, and during the 42-year period – 0.53%. Without fertilisation, the ratio of labile organic matter carbon to humus carbon was 2.36-6.3, with fertilisation it was 4.8-12.5. The highest ratio was in the case of non-ploughing tillage and fertilisation and was 10.9-12.5.

The content of labile organic matter and the ratio of labile organic matter carbon to humus carbon in chernozems can indicate potential soil fertility and favourable conditions for the growth and development of crops during the growing season. The prominent ratio of LOM to humus carbon indicates a favourable humus condition and the predominance of humification processes over the mineralisation of organic matter. A strong correlation was found between the content of LOM in typical chernozems and the yield of winter wheat and grain maize with correlation coefficients of 0.83 and 0.85, respectively. Studies on changes in the content of LOM in other soil types are considered promising.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Лабільні органічні речовини і родючість чорноземів

Анотація. Органічні речовини ґрунту умовно можна розділити на стабільні і лабільні, причому останні безпосередньо забезпечують ріст і розвиток рослин впродовж вегетаційного періоду. Для відновлення родючості ґрунтів і високої продуктивності агроценозів у них повинно витримуватись певне співвідношення між лабільною і стабільною частиною гумусу. Мета дослідження – визначити вміст лабільних органічних речовин за різних варіантів обробітку ґрунту та удобрення, а також встановити оптимальне співвідношення між їх вмістом та вмістом загального вуглецю, на основі якого розробити заходи з відновлення та підвищення родючості чорноземних ґрунтів. У стаціонарних дослідах вивчався вплив інтенсивності використання чорноземів, їх систем обробітку і удобрення на гумусний стан кількості лабільних органічних речовин і їх співвідношення з вуглецем гумусу. У роботі використовували польові дослідження, лабораторний метод аналізу зразків ґрунту, систематизацію, аналіз та узагальнення. У зразках ґрунту визначали вміст гумусу згідно ДСТУ 4289:2004 і вміст лабільних органічних речовин за ДСТУ 4732:2007. За розорювання чорноземів найбільше мінералізуються лабільні органічні речовини і їх частка в складі гумусу зменшується, а за переведення ріллі в перелоги вони відновлюються найшвидше. Мінімізація систем обробітку ґрунту і внесення органо-мінерального удобрення збільшує кількість лабільних органічних речовин в складі гумусу чорноземів. Застосування безполицевого обробітку ґрунту протягом шістнадцяти років зумовила підвищення вмісту гумусу на 0,13 %, протягом сорока двох років – на 0,53 % відносно оранки. Внесення органо-мінерального удобрення підвищувало співвідношення вмісту вуглецю лабільних органічних речовин до вмісту загального вуглецю порівняно з варіантами без удобрення. Встановлена сильна кореляційна залежність між вмістом лабільних органічних речовин в чорноземах і врожайністю озимої пшениці і кукурудзи на зерно. Практичне значення результатів досліджень полягає в мінімізації норми удобрення та співвідношення в ній C:N для забезпечення валової врожайності культур і відновлення родючості ґрунтів

Ключові слова: гумус; мінералізація; гуміфікація; пшениця озима; кукурудза

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Assessment of sweet cherry fruit quality according to the requirements of the modern market

Abstract. Assessment of commercial properties of sweet cherry fruit determines the competitiveness of fresh fruit in the modern market. The selection of cultivars with the highest number of high-quality fruits to meet the preferences of all stakeholders is relevant. The purpose of this study was to substantiate the commercial indicators for assessing the quality of sweet

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cherry fruit of three ripening periods and to select the most competitive cultivars for sustainable supply of fresh fruit to the modern market. The methods used in the study were field, laboratory, and comparative. The commercial properties of 33 sweet cherry cultivars of early, middle, and late ripening were determined. The sweet cherry fruits of the cultivars under study were divided into two commercial classes according to fruit size and defects. The fruits were analysed for the following defects: cracked, scarred, and browned skin. The amount of non-standard sweet cherry fruit production for each cultivar was determined. For each ripening period of sweet cherry varieties, the average value of the volume of fruits of classes I-II and non-standard products was established. A general trend towards an increase in the amount of high-quality fruit raw materials for late-ripening cultivars was identified. The most valuable cultivars of early (Skazka and Zabuta), medium (Uliublenytsia Turovtseva and Temp) and late (Anons) ripening were identified, which provided the maximum number of class I fruits. The maximum production was determined in the Merchant (early ripening), Cordia (medium ripening) and Karina (late ripening) cultivars. The lowest volume of non-standard products was found in cultivars of different ripening periods – Skazka, Prostir, and Anons. The fruit classification into commercial classes can be used to evaluate the fruit of sweet cherry cultivars and determine the possibility of using them fresh, for long-term chilled storage and for various further processing methods

Keywords: *Prunus avium* L.; cracking; fruit diameter; cultivars; commercial classes; fruit browning; non-standard products

INTRODUCTION

The sustainable development of horticulture is essential for ensuring food security in Ukraine, as fruit is an indispensable component of a healthy diet (Dogbe & Revoredo-Giha, 2021; Janda *et al*., 2022). Growing sweet cherries helps diversify the diet and extend the period of fresh fruit consumption due to the early ripening of the fruit. Sweet cherries are characterised by a pleasant taste, juicy pulp, and a dark red colour. The study of fruit quality is becoming increasingly important due to the value of sweet cherry fruit as a food product and as a raw material for the processing industry.

Sweet cherry (*Prunus avium* L.) is the leader in the cultivation of fruit plantations, which is a priority in many countries (Ivanova *et al*., 2023). This culture is of tourist importance for the regions where it is recognised as a geographical brand (Trusova *et al*., 2020). According to research by scientists, sweet cherry fruits are rich in natural antioxidants (vitamins and polyphenols), which has a beneficial effect on human health, as they neutralise harmful radicals and reduce the risk of some diseases. The sweet cherry fruits are characterised by the presence of an increased amount of monosaccharides and have a low calorie content. The content of organic acids, vitamins, minerals, and biologically active substances enhances the nutritional, medicinal, and dietary value of sweet cherries (Vignati *et al*., 2022).

The demand for sweet cherry consumption leads to an increase in fruit production and the study of commercial parameters of fruits of varieties of different ripening periods. This stimulates researchers to analyse fruit raw materials on the world market to divide them into commercial classes. The quality parameters of sweet cherries are determined by the preferences of consumers who are willing to pay high prices for fruit with the desired characteristics. To meet the needs of consumers, researchers have focused on identifying the most important fruit quality parameters (López *et al*., 2023).

Scientists in many countries have conducted online surveys to identify the most attractive external quality indicators for consumers. The survey determined the respondents' attitudes towards the size and hardness of the fruit, and the presence of a stalk, depending on their place of residence. The following dominant characteristics of sweet cherry fruit have been identified: fruit size and shape, fruit damage, and stalk length (Paunović *et al*., 2022). Consumers prefer sweet cherry fruit without external defects, not damaged by pests and not affected by diseases from pests and diseases.

According to scientists, it has been found that the weight of the fruit and its size, as well as the degree of fruit cracking, depends on the cultivar (Pereira *et al*., 2020). In sweet cherry production, fruit cracking is one of the priority issues, depending on the amount of precipitation during the ripening period. The fruit cracking defect causes high economic losses. Therefore, such fruits were inspected depending on the presence of cracks in the fruit. The emphasis was on damage in the cheeks of the fruit (lateral cracks), the lateral scar area or the stem cavity. Cracking of juicy fruits such as sweet cherries poses a genuine issue in production and is associated with massive commercial losses worldwide.

A study of nine cultivars of sweet cherries over four years of research revealed the dependence of the type of cracks on the fruit on varietal characteristics. The cause of deep cracks in the fruit was determined. According to scientists, the degree and type of fruit cracking depends on the cultivar, season, and load on the fruit tree. Diurnal rhythms and environmental conditions play a role in the development of cracking (Grimm *et al*., 2019).

The study of the degree of cherry fruit cracking is important because it predicts the depth of tissue destruction of fruit crops. Microcracks on cherry fruits lead to destruction at the cuticle level. Macrocracks destroy the skin, cuticle, and inner cell layers of the fruit (Knoche & Lang, 2017). Research has shown that an important cause of cracking is the rapid absorption of water by the fruit's skin or the plant's vascular system (Brinkmann *et al*., 2022). In addition to the elements of cultivation technology (variety/rootstock combination, tree spacing, irrigation and pruning), fruit characteristics such as size, firmness, and maturity are also factors that can increase fruit cracking. S. Correia *et al*. (2018) found a low correlation between cracking and fruit size, shape, and firmness.

The market season for fresh sweet cherries is short. The fruit is perishable and has a short shelf life (Wu *et al*., 2018). Therefore, the study of sweet cherry fruits of different cultivars according to their commercial properties will enable the distribution of raw materials for fresh consumption, selection of samples for long-term storage and identify cultivars that are more suitable for different types of processing.

The purpose of this study was to assess the quality of fruits according to commercial parameters of early, medium, and late ripening sweet cherry cultivars and to identify the most valuable ones for sustainable supply of fresh fruit to the modern market. The objectives of this study were as follows: to describe in detail the methodology used to assess commercial quality parameters of sweet cherry fruit according to current market requirements; to assess the feasibility of effectively distinguishing sweet cherry fruit of different ripening periods into commercial classes; to identify the best sweet cherry cultivars in each ripening group according to selected commercial fruit quality parameters to meet the preferences of all stakeholders and promote food security.

MATERIALS AND METHODS

The study was conducted in 2008-2019 in the laboratories of the Research Institute of Agrotechnology and Ecology of the Dmytro Motornyi Tavria State Agrotechnological University. Cultivars of three ripening periods were selected for inspection of sweet cherry fruits and division into classes I-II and non-standard products according to commercial indicators: 1st (early) – Svit Erliz, Merchant, Bigaro Burlat, Rubinova early, Valerii Chkalov, Skazka, Zabuta; 2nd (medium) – Cordia, Octavia, Vynka, Pervistok, Temp, Uliublenytsia Turovtseva, Talisman, Dilemma, Melitopolska black, Orion, Chervneva early, Dachnytsia, Prostir; 3rd – late – Karina, Regina, Mirage, Krupnoplidna, Udivitelna, Zodiac, Surprise, Kolkhoznytsia, Kosmichna, Prazdnychna, Anons, Temporion, Meotida.

For the study, fruits were selected from typical trees for each pomological cultivar of the same age with an average fruiting intensity. Commercial evaluation of sweet cherries was performed during the period of consumer ripeness. During this period, the fruit flesh was still quite dense, with a taste and colour inherent in the pomological cultivar under study. To determine the diameter of one sweet cherry fruit, a sample of 100 fruits was taken from 3-5 typical trees during the period of consumer ripeness. The repetition of the experiment was threefold. (Serdiuk *et al*., 2020). The fruit should be selected in such a way that the sample is characteristic of the quality of the harvest of the given season. The fruits from the trees of the cultivars under study were removed completely. If the fruit ripened unevenly, they were partially removed in proportion to the location of the crop on the tree.

Fruit quality was assessed in the sorting room. All selected fruits were calibrated by diameter, and then the diameter of one fruit was determined by dividing the total weight by their number (100 pcs). Fruit samples were taken in fourfold repetition. One pack unit was taken as a repetition. To evaluate the quality of the fruit according to the indicators of classes I and II, an average sample of 5% of each package was taken. The weight of the package was 12 kg. The commercial properties of sweet cherry fruits were determined per the current regulatory and technical document DSTU 8153:2015 "Fresh sweet cherry. Technical specifications" (2017). Commercial characterisation and division of fresh sweet cherry fruits into classes I and II was performed according to the following quality indicators: fruit size, number of cracked fruits, number of fruits with fresh and scarred mechanical damage, number of fruits with browning of the skin in the form of spots (Table 1).

Table 1. Commercial characteristics of fresh cherry fruit class and permissible deviations of quality indicators

Indicator	Class characteristics	
	I	II
Fruit size according to largest transverse diameter, mm not less than	20	17
Permissible quality deviations, % not more than		
Cracked fruit	2	4
Fruits with mechanical damage:		
- scarred	4	10
- fresh	2	4
Fruits with browned skin in the form of spots with an area of 0.2 cm^2, no more than	4	8

Source: compiled by the authors of this study

The distribution of the sample of fresh sweet cherry fruit into classes was determined in percentage. Fruit diameter yield was determined as a percentage of the total number of fruits.

RESULTS AND DISCUSSION

The competitiveness of fruit products in the modern European market is determined according to a number of important commercial properties of fruit. Among the most important parameters of cherry fruit quality are the size of the fruit and its cracking. Fruit cracking leads to a deterioration in the quality of sweet cherry fruit and causes large economic losses for producers (Santos *et al*., 2023). Cracking of sweet cherry fruit depends on the cultivar, growing conditions, rootstock, size, amount of free and bound water in the cells, elasticity of the skin cuticle and ripening stage (Pereira *et al*., 2020).

According to the size of the fruit, sweet cherry varieties of three ripening periods were divided into commercial classes I and II and non-standard products. The inspection of fruits for commercial properties allowed to determine the percentage of fresh sweet cherry fruits of I and II classes for the early cultivars under study 92.8-94.8% (Table 2).

Table 2. Commercial characteristics of early ripening sweet cherry fruits of classes I and II, non-standard products (2008-2019)

Cultivar	Fruit size by largest transverse diameter, mm not less than:										Classes I and II total, %	Non-standard products, % by fruit size
	Class I, %					Class II, %						
	fruit size (20 mm and larger)	cracked fruits, up to 2%	scarred fruits, up to 4%	fruits with browning of the skin, up to 4%	total, %	fruit size (17 mm and smaller)	cracked fruits, 2-4%	scarred fruits, up to 10%	fruits with browning of the skin, up to 8%	total, %		
Rubinova early	83.5	0.8	1.0	0.8	86.1	3.9	2.4	1.1	0.4	7.8	93.9	6.1

Table 2. Continued

Cultivar	Fruit size by largest transverse diameter, mm not less than: Class I, % fruit size (20 mm and larger)	cracked fruits, up to 2%	scarred fruits, up to 4%	fruits with browning of the skin, up to 4%	total, %	Class II, % fruit size (17 mm and smaller)	cracked fruits, 2-4%	scarred fruits, up to 10%	fruits with browning of the skin, up to 8%	total, %	Classes I and II total, %	Non-standard products, % by fruit size
Valerii Chkalov	86.5	0.5	0.4	0.2	87.6	2.4	2.1	2.0	0.0	6.5	94.1	5.9
Svit Erliz	82.9	0.5	0.3	0.2	83.9	2.4	2.4	4.1	0.0	8.9	92.8	7.2
Merchant	82.5	0.7	0.4	0.3	83.9	7.3	2.0	0.0	0.8	10.1	94.0	6.0
Kazka	88.4	0.6	0.3	0.1	89.4	3.3	2.0	0.0	0.1	5.4	94.8	5.2
Bigaro Burlat	86.8	0.6	0.5	0.3	88.2	0.0	4.0	0.0	1.1	5.1	93.3	6.7
Zabuta	88.2	0.6	0.4	0.2	89.4	2.9	2.0	0.0	0.0	4.9	94.3	5.7
Average value	**85.5**	**0.6**	**0.5**	**0.3**	**86.9**	**3.2**	**2.4**	**1.0**	**0.3**	**7.0**	**93.9**	**6.1**

Source: compiled by the authors of this study

The largest number of fruits with a size of more than 20 mm in transverse diameter was determined in samples of early-ripening cultivars Zabuta (88.2%) and Kazka (88.4%). The minimum percentage of fruits with a size of more than 20 mm in transverse diameter was determined in the sample of Merchant (82.5%) and Svit Erliz (82.9%) cultivars. Variations in fruit size depending on varietal characteristics have been investigated previously. Fruit size is a variety trait that can vary depending on climatic conditions and agricultural practices (Lanauskas *et al*., 2023). Furthermore, the size of the fruit plays a vital role in assessing the quality of the fruit and influences consumer perception. According to A. Wojdyło *et al*. (2014), most of the sweet cherry cultivars studied in Poland formed fruits with a diameter ranging from 13.71 (Fortuna) to 25.96 mm (Stevensbaer). The average fruit diameter of the 33 cherry cultivars under study was 18.2 mm. Among them, 8 cultivars had an average fruit diameter of more than 20 mm – Lucyna (20.07 mm), Morina (21.98 mm), Nana (21.31 mm), Wilena (21.33 mm), Wilga (20.37 mm), Wisok (20.69 mm), Stevensbaer (25.96 mm), Wołyńska (20.88 mm).

It was found that the maximum amount of class I products for early ripening cultivars was produced by the fruits of the Kazka and Zabuta sweet cherry cultivars – 89.4%. This indicator was 1.5% higher than the average value (86.9%) of the number of fruits of the first class of early ripening cultivars. The lowest number of class I products was found in the sample of Merchant and Svit Erliz cultivars – 83.9%, which is 3% less than the average value in the group of early ripening cultivars. The largest amount of class II products was found in the sample of sweet cherry fruit of the Merchant cultivar (10.1%), which is 3.1% more than the average value (7.0%). In the sample of sweet cherries of the Zabuta cultivar, the lowest number of class II fruits was found (4.9%), which is 2.1% less than the average in the group of early ripe cultivars. The yield of non-standard products in terms of fruit size was highest in the Svit Erliz cultivar (7.2%), which is 1.1% higher than the average (6.1%). The lowest number of non-standard products was observed in the Kazka variety (5.2%), which is 0.9% lower than the average value of the early ripening group.

In the study, one of the parameters that increases the yield of non-standard products is fruit cracking. The publication by G. Fischer *et al*. (2021) highlighted that fruit cracking is caused by external (agronomic and environmental) and internal factors. According to the authors, the frequency of cracking varies substantially depending on climatic conditions during fruit development, different types and cultivars of fruit, place of cultivation and crop care. This physiological defect is exacerbated by an increase in the intensity of precipitation, especially after the dry season or in regions with hot temperatures. The publication of M. Corneanu *et al.* (2020) presents the results of the evaluation of sweet cherry cultivars according to resistance to cracking. Eight varieties with low values of fruit cracking (1.3-9.3%) were identified. A high resistance to cracking was observed in the fruits of the cultivars "Iaşirom" (1.3%) and "Paulică" (3.3%) compared to the cultivars "Van" (46.8%) and "Stella" (74%).

The presence of physiological disorders, especially fruit cracking, severely limits fruit production and quality (Ramteke *et al.*, 2017). This defect affects the appearance of the fruit, reduces shelf life, and limits uses, causing considerable commercial losses for producers (Ginzberg & Stern, 2016; Yu *et al.*, 2020). The cracking resistance of the fruit peel, transportation, storage, and quality of fruit products during storage play a crucial role (Wang *et al.*, 2021).

In the studies conducted, the places where fruit cracks were detected was not detailed. In M. Ur. Rehman *et al.* (2015), three forms of cherry fruit cracks are described: cuticular cracks at the stem ends, cracking at the apical (cone) end of the fruit, and lateral cracks at the cheek seam. According to scientists, the first two forms are shallow cracks that appear at both ends of the cherry fruit and are called "stem" and "nose or apical" cracks, respectively. These small cracks form at an early stage of fruit development, leading to a decrease in the market price of fruit. The third type starts from the cheeks and spreads over the bulk of the fruit, causing the greatest physical and economic damage. As a result of commercial fruit evaluation, the percentage of fresh sweet cherry fruits of classes I and II for the cultivars under study with an average ripening period of 93.5-94.5% was determined (Table 3).

Table 3. Commercial characteristics of medium-ripening sweet cherry fruits of classes I and II, non-standard products (2008-2019)

Cultivar	Fruit size by largest transverse diameter, mm not less than:										Classes I and II total, %	Non-standard products, % by fruit size
	Class I, %					Class II, %						
	fruit size (20 mm and larger)	cracked fruits, up to 2%	scarred fruits, up to 4%	fruits with browning of the skin, up to 4%	total, %	fruit size (17 mm and smaller)	cracked fruits, 2-4%	scarred fruits, up to 10%	fruits with browning of the skin, up to 8%	total, %		
Vynka	88.4	0.5	0.4	0.1	89.4	4.4	0.0	0.0	0.3	4.7	94.1	5.9
Pervystok	87.3	0.4	0.6	0.7	89.0	4.6	0.0	0.0	0.6	5.2	94.2	5.8
Temp	89.6	0.3	0.2	0.4	90.5	3.5	0.0	0.0	0.4	3.9	94.4	5.6
Uliublenytsia Turovtseva	88.8	0.4	0.3	0.3	89.8	2.0	2.1	0.0	0.2	4.3	94.1	5.9
Talisman	87.1	0.3	0.4	0.2	88.0	1.9	0.0	4.0	0.0	5.9	93.9	6.1
Dylema	87.7	0.5	0.3	0.2	88.3	5.2	0.0	0.0	0.1	5.3	94.0	6.0
Melitopolska black	87.5	0.3	0.1	0.1	88.0	6.1	0.0	0.0	0.0	6.1	94.1	5.9
Cordia	82.9	0.6	0.3	0.6	84.4	8.6	0.0	0.0	0.5	9.1	93.5	6.5
Octavia	85.8	0.4	0.2	0.2	86.6	5.4	0.0	2.1	0.0	7.5	94.1	5.9
Orion	88.4	0.3	0.3	0.4	89.4	4.8	0.0	0.0	0.1	4.9	94.3	5.7
Chervneva early	86.1	0.7	0.4	0.7	87.9	5.3	0.0	0.0	0.8	6.1	94.0	6.0
Dachnytsia	88.1	0.6	0.3	0.3	87.5		4.0	0.8	0.2	6.8	94.3	5.7
Prostir	87.9	0.5	0.4	0.3	89.1	3.4	2.0	0.0	0.0	5.4	94.5	5.5
Average value	**87.4**	**0.4**	**0.3**	**0.3**	**88.3**	**4.2**	**0.6**	**0.5**	**0.2**	**5.8**	**94.1**	**5.9**

Source: compiled by the authors of this study

The maximum number of fruits with a size of more than 20 mm in transverse diameter was determined in samples of the mid-season cultivar Temp (89.6%), which is 2.2% more than the average value of the group (87.4%). The minimum percentage of class I fruits with a size of more than 20 mm in transverse diameter was determined in the sample of cultivars Cordia (82.9%) and Octavia (85.8%), which is 1.6 and 4.5% less than the average value of the medium-ripening group. The results of the study suggest that the sweet cherry cultivars under study differ in fruit size. This conclusion is confirmed by the data obtained by other scientists. In the study by M. Corneanu *et al*. (2020), the sweet cherry cultivars with the largest fruit sizes were noted – "Andreiaş", "Bucium", "Ştefan", "Paulică", "Golia", "Van", and "Stella". The average fruit diameter ranges from 22.4 mm (George) to 25.8 mm (Paulică). The largest amount of class I products was found in samples of mid-season cultivars Uliublenytsia Turovtseva (89.8%) and Temp (90.5%). This figure was 1.5% and 2.2% higher than the average value (88.3%) of the number of fruits of the first class of varieties of medium-ripening period.

The lowest number of class I fruits was found in the sample of sweet cherry cultivars Cordia (84.4%) and Octavia (86.6%), which is 1.7 and 3.9% less than the group average. The largest amount of class II products was found in the sample of cherry fruit of the Cordia cultivar (9.1%), which is 3.3% more than the average value (5.8%).

In the sample of sweet cherries of the Temp cultivar, the lowest number of class II fruits was found (3.9%), which is 1.9% less than the average in the group. The number of non-standard products in terms of fruit size was highest in the Talisman cultivar (6.1%). The minimum amount of non-standard products is set for the Prostir cultivar (5.5%). As a result of the analysis of commercial indicators of fruits in samples of late-ripening cultivars, it was found that the amount of fresh sweet cherry fruits of I and II classes was 94.1-96.2% (Table 4).

Table 4. Commercial characteristics of late-ripening sweet cherry fruits of classes I and II, non-standard products (2008-2019)

Cultivar	**Fruit size by largest transverse diameter, mm not less than:**										Classes I and II total, %	Non-standard products, % by fruit size
	Class I, %					Class II, %						
	fruit size (20 mm and larger)	cracked fruits, up to 2%	scarred fruits, up to 4%	fruits with browning of the skin, up to 4%	total, %	fruit size (17 mm and smaller)	cracked fruits, 2-4%	scarred fruits, up to 10%	fruits with browning of the skin, up to 8%	total, %		
Krupnoplidna	91.3	0.3	0.1	0.2	91.9	1.9	2.0	0.0	0.0	3.9	95.8	4.2
Karina	85.9	0.4	0.3	0.2	86.8	7.2	0.0	0.0	0.1	7.3	94.1	5.9
Regina	87.7	0.2	0.2	0.2	88.3	5.5	0.0	0.7	0.0	6.2	94.5	5.5
Mirage	90.3	0.2	0.3	0.2	91.0	4.1	0.0	0.0	0.1	4.2	95.2	4.8
Udivitelna	90.4	0.4	0.2	0.1	91.1	3.8	0.0	0.0	0.2	4.0	95.1	4.9
Zodiak	90.9	0.5	0.3	0.2	91.9	3.3	0.0	0.5	0.0	3.8	95.7	4.3
Surprise	89.4	0.4	0.3	0.8	90.9	4.4	0.0	0.0	0.6	5.0	95.9	4.1
Kolkhoznytsia	89.9	0.4	0.2	0.7	91.2	4.1	0.0	0.0	0.7	4.8	96.0	4.0
Kosmichna	89.7	0.3	0.2	0.8	91.0	4.3	0.0	0.0	0.6	4.9	95.9	4.1
Prazdnichna	90.7	0.4	0.3	0.1	91.5	4.1	0.0	0.4	0.0	4.5	96.0	4.0
Anons	92.4	0.2	0.2	0.2	93.0	3.2	0.0	0.0	0.0	3.2	96.2	3.8
Temporion	92.0	0.2	0.3	0.1	92.6	3.2	0.0	0.3	0.0	3.5	96.1	3.9
Meotida	90.7	0.4	0.4	0.1	91.6	3.7	0.0	0.0	0.0	3.7	95.3	4.7
Average value	**90.1**	**0.3**	**0.3**	**0.3**	**91.0**	**4.1**	**0.2**	**0.1**	**0.2**	**4.5**	**95.5**	**4.5**

Source: compiled by the authors of this study

The maximum number of fruits with a size of more than 20 mm in transverse diameter was determined in samples of late-ripening cultivars Temporion (92.0%) and Anons (92.4%). Samples of cultivars Karina (85.9%) and Regina (87.7%) had a minimum number of fruits with a size of more than 20 mm in cross diameter. The dependence of sweet cherry fruit diameter on the genetic characteristics of the cultivar is also confirmed by studies by other authors (Lanauskas *et al*., 2023). As a result of studies of 45 cultivars of sweet cherry by A. Khadivi *et al*. (2019) in Iran, it was found that the diameter of sweet cherry fruit ranged from 18.88 to 28.45 mm. According to E. Iurea *et al*. (2019), the evaluation of sweet cherry fruits by size revealed that the fruit diameter of the cultivars under study ranged from 21.8 (Cetăţuia) to 24.0 mm (Cătălina).

For late-ripening cultivars, the largest amount of Class I products was produced by the Anons sweet cherry cultivar – 93%. This figure is 2% higher than the average number of class I fruits in the group of late-ripening cultivars (91%). The minimum amount of class I products was determined in the sample of sweet cherry cultivars Karina (86.8%) and Regina (88.3%), which is 2.7% and 4.2% less than the average value in the group of late-ripening cultivars. The largest amount of class II products was found in the sample of sweet cherry fruit of the Karina cultivar (7.3%), which is 2.8% higher than the average value (4.5%). In the sample of sweet cherries of the Anons cultivar, the lowest number of class II fruits was found (3.2%), which is 1.3% less than the average in the group of late-ripening cultivars. The number of non-standard products in terms of fruit size was highest in the Karina variety (5.9%), which is 1.4% higher than the average (4.5%). The lowest number of non-standard products was observed in the Anons cultivar (3.8%).

The number of the class I fruits for early, medium-, and late-ripening varieties is 86.9%, 88.3%, 91.0%, respectively. A comparative analysis of the quantity of class I fruit products revealed a tendency to increase the quality of raw materials depending on the ripening period. The volume of class II products for early, medium, and late ripening varieties is 7.0%, 5.8%, and 4.5%, respectively. The number of non-standard products in terms of fruit size for early, medium, and late ripening varieties is 6.1%, 5.9%, and 4.5%, respectively. There is a gradual increase in the size of class I fruits and, conversely, a decrease in the volume of non-standard products from early to late ripening cultivars.

A comparable tendency to reduce the number of cracked fruits is observed in late-ripening cultivars compared to medium- and early-ripening cultivars. According to scientists, cultivars with higher skin tensile strength and elasticity are more resistant to fruit cracking (Brüggenwirth & Knoche, 2017).

Late-ripening, mid-ripening and early-ripening cultivars in class I products had average values of cracked fruit – 0.3%, 0.4%, and 0.6%, respectively. The sweet cherry cultivars Valerii Chkalov and Svit Erliz (early-ripening), Talisman, Orion, Temp, and Melitopolska black (medium-ripening), Mirage and Regina (late-ripening) had the lowest cracking levels. In class II products, the average values of cracked fruits in the groups of late-ripening, medium-ripening, and early-ripening cultivars were 0.2%, 0.6%, and 2.4%, respectively. The dependence of sweet cherry fruit cracking on varietal characteristics is also confirmed by the studies of other authors (Measham *et al*., 2014). According to their data, over 4 years of research, as a result of monitoring the cracking of sweet cherry fruits of different varieties, it was found that the lowest level of crack development was demonstrated by the varieties 'Sylvia', 'Summit', and 'Van'. Cracked fruit loses its commercial value for the fresh market and can only be sold locally or for processing. Cherry fruit cracking is an unpredictable and economically important problem for growers worldwide.

The publication by A. Khadivi-Khub (2015) highlights that the most resistant cultivars to cracking and with better fruit quality can be recommended for further cultivation and obtaining a sustainable economic effect. These findings are important for the development of future management strategies for sweet cherry fruit cracking. This reduces economic risks for regions where sweet cherries are a priority fruit crop (Trusova *et al*., 2018).

It is known that early-ripening fruits have a more delicate flesh texture and can deteriorate more than fruits of medium and late ripening cultivars. Late-ripening bigaro cultivars with a dense texture and harder skin are less damaged during fruit formation (Ivanova *et al*., 2023). This trend may be due to the influence of weather conditions on the quality of raw materials when early-, medium-, and late-ripening sweet cherry cultivars ripen. This trend may also be influenced by the genetic characteristics of cultivars of these ripening periods.

The evaluation of early-, medium-, and late-ripening sweet cherry cultivars by commercial classes will allow the fruit to be divided into categories, which will enable the selection of raw materials for further use in fresh form, long-term storage, freezing, and manufacturing of processed products by heat sterilisation. Forecasting the volume of fruit products by commercial classes, having information on the suitability of raw materials for table and technical use, and the optimal choice of fruit processing methods will help processing companies coordinate their work according to a rational technological scheme with maximum economic effect.

CONCLUSIONS

The results of twelve years of research suggest that the average stone weight of sweet cherries grown in the southern steppe zone of Ukraine was 8.41 grams, with an average stone weight of about 0.56 grams. The sweet cherry cultivars of three ripening periods were analysed and divided into commercial classes according to fruit size and degree of external damage. The maximum amount of class I products for the group of early ripening cultivars was produced by the fruits of the Kazka and Zabuta sweet cherry cultivars – 89.4%. The largest amount of products of the second class of early ripe cultivars was determined in the sample of sweet cherry fruit of the Merchant cultivar (10.1%), and the smallest – of the Zabuta cultivar (4.9%). The percentage of non-standard products in terms of fruit size was highest in the Svit Erliz cultivar (7.2%), and the lowest – in the Kazka cultivar (5.2%).

In the samples of medium-ripening cultivars Uliublenytsia Turovtseva (89.8%) and Temp (90.5%), the largest number of class I fruits was determined. The medium-ripening sweet cherry cultivars Cordia (84.4%) and Octavia (86.6%) had the smallest volume of class I fruit. The maximum percentage of class I fruits with a diameter of more than 20 mm was observed in the medium-ripening cultivar Temp (89.6%). The largest amount of class II products in medium-ripening cultivars was determined in the sample of cherry fruit of Cordia (9.1%), and the smallest – in the Temp variety (3.9%). The percentage of non-standard products in terms of fruit size was highest in the Talisman cultivar (6.1%), and lowest – in the Prostir cultivar (5.5%). The maximum percentage of class I fruits was determined in the late-ripening cultivar Anons – 93%. The largest amount of class II products was found in the sample of the Karina cultivar (7.3%), and the smallest amount – in the Anons cultivar (3.2%). The number of non-standard products in terms of fruit size was highest in the Karina cultivar (5.9%), and the lowest – in the Anons cultivar (3.8%). As a result of the division of fresh sweet cherries of early, medium, and late ripening into classes I and II and non-standard products by commercial properties, a general trend towards an increase in the number of high-quality fruits in late-ripening cultivars was established.

The prospect of further research is to evaluate the suitability of sweet cherries of early, medium, and late ripening for long-term storage in a chilled state and their suitability for the manufacture of processed products.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Оцінювання якості плодів черешні відповідно до вимог сучасного ринку

Анотація. Оцінка комерційних властивостей плодів черешні визначає конкурентоспроможність свіжих фруктів на сучасному ринку. Виділення сортів з найвищою кількістю плодів високої якості для задоволення уподобань усіх зацікавлених сторін (стейкхолдерів) є актуальним. Метою дослідження було обґрунтування комерційних показників для оцінки якості плодів черешні трьох строків достигання та вибору найбільш конкурентоспроможних сортів для стійкого забезпечення свіжими фруктами сучасного ринку. У ході дослідження використано методи: польовий, лабораторний і порівняльний. Проведено визначення комерційних властивостей 33 сортів черешні раннього, середнього і пізнього строків достигання. Плоди черешні досліджуваних сортів за показниками розміром плоду і наявністю дефектів було поділено на два комерційні класи. Проведено аналіз плодів на наявність таких дефектів: розтріснуті, з рубцями, з побурінням шкірочки. Визначено кількість нестандартної продукції плодів черешні для кожного сорту. Для кожного строку достигання сортів черешні встановлено середнє значення обсягу плодів I-II класів і нестандартної продукції. Виявлена загальна тенденція щодо збільшення кількості якісної плодової сировини для сортів пізнього строку достигання. Було виділено найбільш цінні сорти раннього (Казка і Забута), середнього (Улюблениця Туровцева і Темп) і пізнього (Анонс) строків достигання, які забезпечили максимальну кількість плодів I класу. Максимальна кількість продукції була визначена у сортах Мерчант (ранній строк достигання), Кордія (середній строк достигання) і Каріна (пізній строк достигання). Найменший обсяг нестандартної продукції було виділено у сортах різних строків достигання – Казка, Простір і Анонс. Проведений розподіл фруктів на комерційні класи може бути використаний для проведення оцінки плодів сортів черешні та визначення можливості їх використання у свіжому вигляді, для тривалого зберігання в охолодженому стані і різних способів подальшої переробки

Ключові слова: *Prunus avium* L.; розтріскуваність; діаметр плоду; сорти; комерційні класи; побуріння плодів; нестандартна продукція

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Iodine-containing preparations in grain growing technologies

Abstract. The urgency of researching and solving the problem of open and masked iodine deficiency, which manifests itself through several diseases, is now global. Many studies and programmes have been initiated on alternative methods of introducing iodine into human nutrition, and one of them is biofortification of crops. Biofortification of crops through fertilisers and foliar iodine fertilisation

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provides the best bioavailability of water-soluble forms of iodine for plants. At the same time, the functional role of iodine as a plant nutrient is understudied. The purpose of this study was to establish and substantiate the effectiveness of iodine-containing preparations in grain crops cultivation technologies. To fulfil this purpose, field and laboratory studies were carried out. The field multifactorial experiment was conducted on podzolic chernozems in a stationary experiment of the Department of Plant Science of the National University of Life and Environmental Sciences of Ukraine. Mathematical processing of the results was carried out using SAS 9.4 and Agrostat 6 software. The anti-stress effect of preparations with biologically active iodine under combined pre-sowing seed treatment and plant treatment during the growing season was identified – significant yield increments were obtained, even under conditions of severe air and soil drought during the growing season of grain crops. The application of background doses of fertiliser and the use of biologically active iodine products ensured a significant increase in yields. The average yield of winter wheat ranged from 2.96 to 6.46 t/ha; oats – from 2.79 to 5.41 t/ha, depending on the background of nutrition, disinfectant and preparations with biologically active iodine. Iodine-containing preparations are effective in the system of preventive protection during the treatment of seeds and plants during the growing season, which can substantially reduce the damage to wheat and oat plants by diseases. Treatment of crops during the growing season with iodine-containing preparations, especially in the late microstages, reduces the level of secondary disease infection and prevents infection of seeds that form on the mother plant and are subsequently the main source of pathogenic infection that develops after sowing seeds into the soil. The developed regulations will promote the use of iodine-containing preparations in crop cultivation technologies, which are particularly effective in regions with iodine deficiency in the environment

Keywords: biologically active iodine; winter wheat; oats; disease damage; yield

INTRODUCTION

An important trend in crop production is the reduction of harmful chemicals in fertilisers and the transition to the use of microfertilisers, which will help increase crop yields without adversely affecting product quality. Halogens are the least represented chemical group of trace elements in plant nutrition. Chlorine is the only trace element currently recognised in plant physiology for its regulatory effect in proton transfer reactions in photosystem II. There is a lack of research on the effect of different concentrations and forms of iodine on agricultural growth, and the results suggest that iodine is an essential micronutrient for plant nutrition (Kiferle *et al.*, 2021; Brown *et al.*, 2021). It is believed that the use of iodine will allow obtaining biologically valuable crop products enriched with this element for medicinal and dietary nutrition (Kiferle *et al.*, 2021; Zaremba *et al.*, 2023). For a long time, the use of active iodine in agriculture was out of the scope of scientists' attention due to the initial high cost of production and its small volumes, which led to preference for more profitable areas.

Iodine is an essential element for the proper development and functioning of the human and animal body (Brown *et al.*, 2021). About 2 million people suffer from iodine deficiency in the diet – a hidden hunger for this micronutrient – and 50 million have diseases caused by iodine deficiency (Cakmak *et al.*, 2020; Zaremba *et al.*, 2023). Iodine deficiency is usually manifested in regions where soils are poor in this element, leading to low levels in food and, consequently, insufficient human consumption (Lyons, 2018).

Currently, many studies and programmes have been initiated on alternative methods of iodine intake in human nutrition, and one of them is biofortification of food or crops (Germ *et al.*, 2019, Prom-u-thai *et al.*, 2020; Zaremba *et al*, 2022). Biofortification of crops using fertilisers, foliar iodine fertilisation, and irrigation with iodine added to water provide the best bioavailability of water-soluble forms of iodine for plants (Dobosy *et al.*, 2020b; Cakmak *et al.*, 2020). The effect of iodine on plant growth and the benefits of increasing iodine content in plants as a bio-enrichment for human and animal health have been reported (Germ *et al*., 2019; Kiferle *et al*., 2019; Acar *et al*., 2020). According to the results of research conducted by scientists in India, iodine has been shown to have an effective effect on basmati rice varieties by foliar application. Higher yields in rice varieties and increased iodine concentrations in grain were noted. The results also suggested the presence of genotypic variation in response to foliar spraying of micronutrients and demonstrated that foliar application of a mixture of I, Se, and Zn can be an effective simultaneous strategy to bioenrich rice grain with these micronutrients to address the hidden problem of iodine deficiency among Asian populations (Naeem *et al*., 2021).

The iodine content in plant tissues usually increases after its exogenous administration. However, the presence of iodine as a "trace element" or "contaminant" in soil and atmosphere makes it difficult to easily identify effects associated with the presence or absence of this element (Koening *et al*., 2020). Studies on the use of iodine as a source of trace elements in low concentrations (micromolar range) have shown a difference in plant growth between control and iodine-treated plants. This can be perceived as a positive effect of adding this element to the nutrition and can also be interpreted as a negative effect of removing iodine from plant nutrition. An increase in biomass and seed growth, together with a clear acceleration of flowering, was observed when plants were fed with KIO_3 or KI at a dose of 0.2 and/or 10 µM. However, the positive effect of iodine on growth was lost at a concentration of 30 µM, which may indicate that the concentration of 30 µM applied as I–, may be above the toxicity threshold (Kiferle *et al*., 2021). A significant increase in the concentration of iodine in cereals can help prevent iodine deficiency in populations with low dietary intake. The possibility of protein iodination was confirmed in studies with several types of crops (Szymandera-Buszka *et al*., 2021; Kutlu *et al*., 2023).

Iodine is involved in physiological and biochemical processes in plants. Plants can absorb iodine through the root system or through aboveground organs and their individual structures (stomata and cuticular waxes) (Humphrey *et al*., 2019; Smolen, 2020). The organisation and functional role of iodine in proteins was also demonstrated. The iodinated proteins found in shoots are mainly associated with the chloroplast and are functionally involved in photosynthetic processes, while iodinated proteins in roots are mainly associated with the action of various peroxidases (Kiferle *et al*., 2021).

Low iodine concentrations have been proved to have a positive effect on plant growth, productivity, and stress resistance (Medrano-Macías *et al*., 2021). While toxic effects are observed when iodine is applied in high concentrations, especially in the I– form, which is more phytotoxic than iodate (IO^{3-}) (Incrocci *et al*., 2019; Kiferle *et al*., 2021). The concentrations of iodine added to nutrient solutions that are associated with positive effects on plants (approximately 102-104 nM) (Medrano-Macías *et al*., 2021) are comparable to those of other elements described as micronutrients in plant nutrition (Smolenet *et al*., 2020 Brown *et al*., 2021), suggesting that iodine may play an analogous role in plant nutrition.

The purpose of this study was to substantiate and establish the effectiveness of iodine-containing preparations in the technologies of growing grain crops in their diverse applications.

MATERIALS AND METHODS

To establish the effectiveness of the use of iodine-containing preparations for growing grain crops, in 2021-2022, field studies were conducted in a stationary field experiment of the Department of Plant Growing at the Separate Subdivision "Agronomic Research Station" of the National University of Life Sciences of Ukraine, Pshenychne village, Vasylkiv district, Kyiv region. The experimental station is in the north-eastern part of the Right Bank Forest-Steppe. Laboratory studies were carried out at the Seed and Planting Material Quality Laboratory of the Department of Plant Industry at the National University of Life and Environmental Sciences of Ukraine.

Field experiments were conducted on typical low-humus coarse-dusty chernozems with light loamy texture per the developed scheme (Table 1). To establish the effectiveness of the preparations and, per the requirements for the establishment of field experiments, the experimental scheme included three control variants: absolute control (AC) – no seeds were treated before sowing and no fertilisers were applied; control 1 (CF) – no seeds were treated before sowing, but mineral fertilisers were applied (the rate of nutrients is specified in the research methodology for each crop separately), which were the general background for all variants; control 2 (CF+P) – seeds were treated with a protectant and background fertilisers were applied before sowing. The vegetative plants were treated according to the microstages of plant development, which are indicated in the relevant schemes for each crop.

Table 1. Scheme of the field experiment "Efficiency of iodine-containing preparations in technologies of grain crops cultivation"

No.	Variant [1]	Variant designation	Processing Seeds	Processing during the growing season		
				BBCH microphase		
				31-32	51-52	71-72
1	Control	AC	Absolute control – no fertiliser or seed treatment			
2	Control 1	CF	Control – background with fertilisation			
3	Control 2	CF+P	Background fertiliser + protectant			
4	BAI n	BAIn	+	-	-	-
5	BAI n	BAIn+1	+	+	-	-
6	BAI n	BAIn+2	+	+	+	-
7	BAI n	BAIn+3	+	+	+	+
8	P1n	P1n	+	-	-	-
9	P1 n	P1n+1	+	+	-	-
10	P1 n	P1n+2	+	+	+	-
11	P1 n	P1n+3	+	+	+	+
12	P2 n	P2n	+	-	-	-
13	P2 n	P2n+1	+	+	-	-
14	P2 n	P2n+2	+	+	+	-
15	P2 n	P2n+3	+	+	+	+

Note: variants 4-15 – background fertiliser has been applied
Source: compiled by the authors of this study

Biologically active iodine *(BAI)* and two iodine-containing preparations (P1 and P2) were used for pre-sowing seed treatment and twice for vegetative plants. The concentration of the initial solution of biologically active fertiliser was 80 mg/dm^3. To treat seeds and plants during the growing season, a solution diluted in a ratio of 1:50 was used.

The research was conducted with two grain crops – winter wheat and oats. Crops differ in the length of the growing season, which is important in studies on the effectiveness of iodine in shaping grain yields. At the same time, both crops are quite severely affected by diseases, especially under specific weather conditions. The area of the experimental plot is 25m^2 with fourfold repetition (Rozhkov, 2016).

The soft winter wheat variety MIP Valencia (erythrospermum variety) was sown, medium-early maturing. Before sowing, phosphorus and potash fertilisers were applied at a rate of 90 kg/ha of each active ingredient. During the growing season, nitrogen fertilisation was carried out. Winter wheat seeds in the variant "Control 2" were treated with the protectant Lamardor® Pro 180 FS, TN. Lamardor is a combination of three systemic highly active ingredients with increased effectiveness against Pinicilium, Alternarium, Ramularium, and Helminthosporium. Lamardor® Pro controls a wide range of root rot and is highly effective against smut and other seed and soil-borne diseases. Winter wheat seeds were sown in the second decade of September using a Great Plains seeder with a working width of 1.4 m. Seeding rate – 5 million germinating seeds per 1 ha. The predecessor of winter wheat was soybeans.

In the research with oats, the Ivory variety was grown. All variants in the studies, with the exception of variant 1 "absolute control", were laid on the background of nutrients introduced for pre-sowing cultivation at the rate of $N_{65}P_{60}K_{60}$ + S 22.5. Fertilisers were applied in the form of ammonium nitrate and Polifoska, calculated based on active ingredient (a.i.): NH_4NO_3 45.41 + Polifoska $N_{20}P_{60}K_{60}$ + $S_{22.5}$. Yields of main and by-products were determined on a plot-by-plot basis, using the continuous accounting method. The resulting weight of winter wheat and oats was converted to a yield per hectare, considering weediness and moisture content in terms of 14% moisture.

Weather conditions in the years of research. The winter wheat sowing took place in warm weather with insufficient rainfall. The average daily air temperature in September-October was at the level of the long-term average – 13.7°C and 8.7°C, and the amount of precipitation was critical and amounted to merely 13 and 18 mm, respectively (Table 2). However, November turned out to be cold, with the temperature at 2.8°C, and in the second decade it averaged -0.6°C. Therewith, the amount of precipitation was insufficient and amounted to only 18 mm.

Table 2. Average daily air temperatures, °C

Year	Decade	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2021	1	1.3	-6.0	0.1	6.5	11.3	17.8	24.2	22.7	14.8	9.3	6.8	0.2
	2	-9.3	-9.0	2.3	9.5	15.1	22	26.3	22.1	16.7	8.4	0.6	-2.4
	3	0	2.9	5.7	8.8	16.1	25.3	23.8	19.4	9.6	8.5	1.1	-6.8
	∑	-2.7	-4.0	2.70	8.27	14.1	21.7	24.8	21.4	13.7	8.73	2.83	-3.0
2022	1	1.3	-1.3	-0.5	6.4	12.4	20.4	22	21.4	13.9	11.5	5.5	-1.7
	2	-3.3	2.3	0.1	6.5	14.5	20.9	18.1	22.8	12.8	6.7	3.4	-3.3
	3	-2.8	2.1	7	10.7	15.1	22	22.2	24	12.5	6.7	-	-
	∑	-1.6	1.0	2.2	7.87	14	21.1	20.8	22.7	13.1	8.30		
Multi-year data for a month		-5.9	-4.4	0.3	8.4	14.9	17.8	19.0	18.4	13.8	7.8	2.0	-2.1

Source: compiled by the authors of this study

The winter period in 2021 was substantially different from previous years of research, with low temperatures ranging from the second decade of December – minus 2.4°C to the first decade of February – minus 1.3°C. This period was characterised by insufficient precipitation (13.8 mm) and almost no precipitation in February (4.6 mm).

The weather conditions in 2021 and 2022 were characterised by rather low rainfall in the spring. The spring of 2022 was also characterised by low temperatures and was prolonged, with a late resumption of the spring vegetation of winter wheat and oat sowing. The average daily temperature in March was slightly higher than the long-term average by 1.9°C, while in April and May the average daily temperatures were lower than the long-term average. The average daily air temperatures during the active growing season (May-August) were substantially higher than the long-term average and the conditions were quite dry. The 2021/2022 growing season was extremely dry. For winter wheat and oats, there were critical conditions for moisture supply before sowing and throughout the growing season (Table 3). Precipitation fell only in December – at the level of long-term data – at 49 mm, and then the amount of precipitation was significantly lower compared to long-term data. Thus, in early March and May there was no precipitation, and in the third decade of May only 1.2 mm fell, which substantially affected the growth and development of wheat plants. June was warm and dry with an average daily temperature of 22°C, which was 3.2°C above the long-term average, while precipitation was only 45.8 mm. July also exceeded the long-term average by 4.3°C, while precipitation was even lower at 33.7 mm.

Table 3. Total precipitation, mm

Year	Decade	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2021	1	26	52	4	13	29	17	16	13	0	1	11	17
	2	5	40	14	21	21	28	53	9	4	8	3	14

Table 3. Continued

Year	Decade	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	3	33	7	8	11	66	15	11	5	9	9	4	18
	∑	64	99	26	45	116	60	80	27	13	18	18	49
2022	1	5	2.4	3	9.8	0	15.6	5.2	62	22	12.2	-	-
	2	4.2	2	0	3.2	1.2	8.2	27.6	17.6	35.2	1.4	-	-
	3	4.6	0.2	2.2	13.2	18.6	22	0.9	0	5.2	24	-	-
	∑	13.8	4.6	5.2	26.2	19.8	45.8	33.7	79.6	62.4	37.6		
Multi-year data for a month		30.0	33.0	29.0	38.4	43.3	73.9	72.9	57.8	46.3	31.0	41	47

Source: compiled by the authors of this study

This lack of moisture had a negative impact not only on the development of winter wheat, but on the spring grain crop, oats.

Statistical analysis of the data was performed using the SAS 9.4 software package. The study was conducted per the requirements of the International Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Crop yields. The role of iodine in plant nutrition requires research using various experimental approaches, as there are many questions regarding its necessity for crop growth and development. The results of studies by P. Dobosy *et al.* (2020a), C. Kiferle *et al.* (2021) indicate a functional role of iodine in plant nutrition. The first published results on the importance of iodine in plant nutrition were made by J. Lehr *et al.* (1958). Iodine deficiency and iodine supply to plants is largely related to soil type (Hunchak *et al.*, 2016). Iodine in a clearly defined concentration range has a positive effect on plant phenotype (Golob *et al.*, 2020). Experiments have shown that the removal of iodine from the nutrient solution harms plant growth, while its introduction in micromolar concentrations promotes biomass accumulation and leads to earlier flowering of plants (Gonzali *et al.*, 2017). The results of the studies also agree with the opinion that iodine has a substantial effect on the phenotype of plants, which is manifested in more intensive plant growth rates – an increase in linear plant size and mass. C. Kiferle *et al.* (2021) were the first to prove the possibility of protein iodination. These results strongly suggest the role of iodine as a plant nutrient (Voogt *et al.*, 2010). The weather conditions of the growing seasons had a significant impact on grain yields (Table 4; Table 5). The average yield in the control variants was extremely low – 2.96 t/ha and 2.79 t/ha in winter wheat and oats, respectively. The application of background doses of fertiliser and the use of biologically active iodine products ensured a significant increase in yields. The average yield of winter wheat ranged from 2.96 t/ha to 6.46 t/ha, depending on the nutrition background, the protectant and preparations with biologically active iodine. The results of studies on the effectiveness of iodine in the cultivation of grain crops, depending on the soil and other environmental factors, indicate an important role of this nutrient in the formation of field crop productivity (Tsukada *et al.*, 2008; Zia *et al.*, 2015)

The effectiveness of iodine-containing preparations varied over the years. The yield of winter wheat in 2020/2021 was considerably higher compared to the yield in 2021/2022, which was formed under drought conditions, but even under such weather conditions a significant level of yield was obtained, which may indicate an increase in plant resistance to stress. Increasing plant stress tolerance was also noted, as wheat grain yields in the 2021/2022 growing season were quite low, due to extremely dry conditions throughout the growing season. In 2020/2021, the range of yield changes ranged from 3.56 t/ha (AC) to 7.06 t/ha (BAI n+3); in 2021/2022 – from 2.35 t/ha (AC) to 5.85 t/ha (BAI n+3) with threefold plant treatment during the growing season with BAI (Table 4). The highest yields in both growing years were formed with the integrated use of biologically active iodine – for pre-sowing seed treatment and threefold application to vegetating plants.

According to a study by Polish scientists (Grzanka *et al.*, 2022), iodine application helps accelerate the emergence and increase the uniformity of crops, which was also confirmed by the present study on wheat and oats.

The increase in winter wheat yields with the use of iodine-containing preparations compared to the yield in the absolute control was 1.76-3.50 t/ha; to the background control (CF) – 1.11-2.83 t/ha; to the CF+P control – from -0.01 t/ha to +1.71 t/ha. The maximum yield was formed under the combined pre-sowing seed treatment and vegetation treatment in three doses with BAI – 5.85 t/ha. The formation of such a yield was possible due to morphological changes in the plant, the formation of more productive stems and the differentiation of more synchronously developed flowers, which manifested in the high grain/ear, which is also confirmed by A. Golob *et al.*, (2020) When seeds were treated only with iodine-containing preparations, the yield was at the level of the variant where the seeds were treated with Lamardor protectant before sowing.

Table 4. Winter wheat yield depending on the use of iodine-containing preparations, 2021-2022

Variant	Processing during the growing season			Yield, t/ha			Increment, t/ha from		
	BBCH microphase								
	31-32	51-52	71-72	2021	2022	mean	AC	CF	CF+P
AC	-	-	-	3.56^{a}	2.35^{a}	2.96^{a}	-	-	-
CF	-	-	-	5.87^{b}	3.02^{b}	4.45^{b}	1.49	-	-
CF+P	-	-	-	6.98^{d}	4.14^{c}	5.56^{d}	2.60	1.87	-
BAI n	-	-	-	6.44^{c}	4.34^{d}	5.39^{d}	2.43	1.70	-0.20
BAI n+1	+	-	-	6.76^{d}	4.54^{de}	5.65^{e}	2.69	1.96	0.05
BAI n+2	+	+	-	6.84^{d}	4.96^{e}	5.90^{e}	2.94	2.21	0.30
BAI n+3	+	+	+	7.06^{f}	5.85^{f}	6.46^{f}	3.50	2.77	0.86
P1n	-	-	-	6.02^{b}	4.16^{c}	5.09^{c}	2.13	1.40	-0.51
P1 n+1	+	-	-	6.34^{c}	4.31^{d}	5.33^{c}	2.37	1.64	-0.28
P1 n+2	+	+	-	6.47^{c}	4.39^{d}	5.43^{d}	2.58	1.85	-0.17
P1 n+3	+	+	+	6.68^{cd}	4.54^{de}	5.61^{de}	2.65	1.92	0.01
P2 n	-	-	-	6.22^{bc}	4.11^{c}	5.17^{c}	2.21	1.48	-0.44
P2 n+1	+	-	-	6.48^{c}	4.25^{d}	5.37^{c}	2.41	1.68	-0.23
P2 n+2	+	+	-	6.68^{d}	4.33^{d}	5.51^{de}	2.55	1.82	-0.09
P2 n+3	+	+	+	6.77^{d}	4.42^{d}	5.60^{e}	2.64	1.91	0.01
$LSD_{0.95}$				0.21	0.18	0.2			

Source: compiled by the authors of this study

In terms of yield, spring grain crops are inferior to winter grain crops. Given that oats are a wet crop, the average yield of this crop, which was formed in the dry conditions of 2021 and 2022, was lower compared to winter wheat and ranged from 2.79 t/ha to 5.41 t/ha (Table 5). Yield increases were 0.1-2.15 t/ha compared to the experimental controls. In the variants with the use of P1 and P2 for seed treatment, the yield was at the level of the control protectant.

Table 5. Oat yield depending on the use of iodine-containing preparations, 2021-2022

Variant	Processing during the growing season			Yield, t/ha			Increment, t/ha from		
	BBCH microphase								
	31-32	51-52	71-72	2021	2022	mean	AC	CF	CF+P
AC	-	-	-	3.14^{a}	2.44^{a}	2.79^{a}	-	-	-
CF	-	-	-	5.02^{b}	2.98^{b}	4.00^{b}	1.21	-	-
CF+P	-	-	-	5.24^{b}	3.78^{c}	4.51^{c}	1.72	0.51	-
BAI n	-	-	-	5.26^{b}	3.96^{c}	4.61^{c}	1.82	0.61	0.10
BAI n+1	+	-	-	5.48^{c}	4.24^{d}	4.86^{d}	2.07	0.86	0.25

Table 5. Continued

Variant	Processing during the growing season BBCH microphase			Yield, t/ha			Increment, t/ha from		
	31-32	51-52	71-72	2021	2022	mean	AC	CF	CF+P
BAI n+2	+	+	-	5.69c	4.56e	5.13df	2.34	1.13	0.27
BAI n+3	+	+	+	5.94d	4.88f	5.41f	2.62	1.41	0.29
P1n	-	-	-	5.18b	3.70c	4.44c	1.65	0.44	-0.97
P1 n+1	+	-	-	5.34b	3.94c	4.64c	1.85	0.64	0.20
P1 n+2	+	+	-	5.44c	4.08c	4.76d	1.97	0.76	0.12
P1 n+3	+	+	+	5.50c	4.34d	4.92d	2.13	0.92	0.16
P2 n	-	-	-	5.16b	3.71c	4.44c	1.65	0.44	-0.48
P2 n+1	+	-	-	5.26b	3.98c	4.62c	1.83	0.62	0.19
P2 n+2	+	+	-	5.38c	4.21d	4.80d	2.01	0.80	0.18
P2 n+3	+	+	+	5.49c	4.39d	4.94d	2.15	0.94	0.15
$LSD_{0.95}$				0.21	0.18	0.21			

Source: compiled by the authors of this study

Protective effect of iodine. An important task of the study was to determine the effectiveness of iodine-containing preparations in comparison with one of the most effective protectants – Lamardor® Pro 180 FS, TN.

The examination of wheat and oat crops at early microstages of crop development helped obtain the results confirming the protective properties of iodine-containing preparations (Table 6; Table 7).

Table 6. Disease incidence (spread) of winter wheat in autumn, %

Preparation	Septoria blight		Powdery mildew		Root rot	
	2020	2021	2020	2021	2020	2021
Control 0	12.4	8.74	26.6	20.4	22.4	12.0
Control 1	10.2	5.61	20.4	16.7	12.6	7.04
Control 2	1.08	0.41	1.66	1.04	2.42	0.82
BAI n	0.98	0.45	1.89	2.09	2.81	1.99
P1n	1.12	0.88	2.98	3.66	2.96	2.09
P2 n	1.13	0.88	2.96	3.68	2.98	2.09

Source: compiled by the authors of this study

When seeds were treated with P1 and P2, the protective effect of the preparations was also noted – the level of disease damage was higher compared to Lamardor treatment, but significantly lower compared to the variants where the seeds were not treated with the preparations.

In the dry autumn of 2020 and 2021, the level of disease damage to winter wheat plants was low. Winter wheat plants were most affected in the absolute control variant, wherein no seed was treated before sowing with either a protectant or iodine-containing preparations and no fertiliser was applied: powdery mildew – 26.6% and 20.4%; Septoria blight – up to 12.4% and 8.74%; root rot – 22.4% and 12% in 2020 and 2021, respectively. When seeds were treated with BAI, the protective function was close to the effect of the Lamardor protectant – the level of disease damage was almost the same (Table 6). A comparable pattern was found for preparations P1 and P2.

Considerable shortfalls in the oat harvest, poor grain and green mass quality cause numerous diseases. The use of iodine to protect plants from diseases regulates the expression of several genes that are mainly involved in the plant defence response, indicating that iodine contributes to the increase in plant resistance to both biotic and abiotic stress. The damage to oat plants by Septoria blight (*Septoria avenae*) in the control variant (AC) was the highest – 8.74%: with the introduction of background fertiliser (CF) it slightly decreased – 5.61; with pre-sowing seed treatment with a protectant (CF+P) the level of damage decreased to 0.41% (Table 7). BAI seed treatment provided protection of plants against septoria blight at the level of the protectant – 0.45%. A substantial decrease in the level of septoria blight infection was also observed upon pre-sowing treatment of seeds with solutions P1 and P2 – 0.88%. Young oat plants are infected with powdery mildew by conidia formed on carrion and by ascospores at temperatures ranging from 3°C to 31°C (optimum 15-20°C) and relative humidity of 50-100%. Conditions for the development of the disease are particularly favourable at air temperatures of 18-22°C and with frequent alternations of warm and humid days. Powdery mildew epiphytoties are most common in years with good moisture. Due to low moisture supply in the spring, powdery mildew was only present at the initial microstages of development, at a rather low level, and the disease did not develop further. The level of oat damage at the early stages of disease development in 2021 and 2022 was slightly different, but the effectiveness of iodine-containing preparations for plant protection against disease was found to be reliable (Table 7).

Table 7. Disease damage to oats, BBCH microstage 24-26[1], %

Preparation	Septoria blight		Powdery mildew		Root rot	
	2021	2022	2021	2022	2021	2022
Control 0	14.1	8.74	34.8	20.4	26.0	12.0
Control 1	15.2	5.61	38.8	16.7	26.8	7.04
Control 2	2.34	0.41	6.21	1.04	5.21	0.82
BAI n	3.45	0.45	6.43	2.09	6.04	1.99
P1n	6.44	*0.88*	8.21	*3.66*	6.87	*2.09*
P2 n	6.98	*0.88*	8.99	*3.68*	7.11	*2.09*

Note: [1]BBCH microstage 24-26 corresponds to the tillering phase of oats
Source: compiled by the authors of this study

Iodine-containing preparations had a protective effect that was slightly inferior to the protective effect of the protectant. Treatment of crops during the growing season with iodine-containing preparations, especially in the late microstages, reduces the level of secondary disease damage and, what is extremely important, prevents infection of seeds that are formed on the mother plant. Seeds are the main source of pathogenic infection that develops after the seeds are sown in the soil.

CONCLUSIONS

Based on the findings of this study, it is possible to draw conclusions about the effectiveness of the use of iodine-containing preparations for growing grain crops. The yield of winter wheat ranged from 2.96 t/ha to 6.46 t/ha, depending on the nutrition background, the protectant and preparations with biologically active iodine. The increment in the use of iodine-containing preparations to the yield in the absolute control was 1.76-3.50 t/ha; to the background control, fertilisation – 1.11-2.83 t/ha; to the control with a protectant – up to 1.71 t/ha. The maximum yield was formed under the combined pre-sowing seed treatment and vegetation treatment in three doses with BAI – 5.85 t/ha. Increments in oat yields were 0.1-2.15 t/ha compared to the experimental controls or were at the control level, with average oat yields of 2.79-5.41 t/ha.

The anti-stress effect of preparations with biologically active iodine under combined pre-sowing seed treatment and plant treatment during the growing season was identified – significant yield increments were obtained, under conditions of severe air and soil drought during the growing season of grain crops. Iodine-containing preparations are effective in the system of preventive protection during the treatment of seeds and plants during the growing season, which substantially reduces the damage to wheat and oat plants by diseases. The damage to oats in the early stages of development by diseases in different weather conditions varied, but the effectiveness of iodine-containing preparations for plant protection against disease was established.

Further research has prospects for developing regulations for the use of iodine-containing preparations for iodine bioenrichment of grain used for food and feed production.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Йодовмісні препарати в технологіях вирощування зернових культур

Анотація. Актуальність дослідження та вирішення проблеми відкритого та замаскованого йодного голодування людей, що проявляється через низку хвороб, є нині глобальною в світі. Ініційовано багато досліджень та програм щодо альтернативних методів введення йоду в харчування людини і один із них – біофортифікація посівів. Біофортифікація посівів шляхом застосування добрив, позакореневого підживлення йодом – забезпечують найкращу біологічну доступність для рослин водорозчинних форм йоду. В той же час функціональна роль йоду, як елемента живлення для рослин, є досить слабо дослідженою. Метою дослідження є встановлення та обґрунтування ефективності йодовмісних препаратів в технологіях вирощування зернових культур. Для досягнення поставленої мети були проведені польові та лабораторні дослідження. Польовий багатофакторний дослід закладався на чорноземах опідзолених в стаціонарному досліді кафедри рослинництва НУБІП України. Математичну обробку результатів проводили з використанням програм SAS 9.4 та «Агростат 6». Ідентифіковано антистресову дію препаратів з біологічно активним йодом за комбінованої передпосівної обробки насіння та обробки рослин по вегетації – отримано суттєві прирости урожайності, навіть за умов сильної повітряної та ґрунтової посухи впродовж вегетації зернових культур. Внесення фонових доз добрив та застосування препаратів з біологічно активним йодом забезпечили суттєве зростання урожайності. Середня урожайність пшениці озимої коливалася від 2,96 до 6,46 т/га; вівса – від 2,79 до 5,41 т/га залежно від фону живлення, протруйника та препаратів з біологічно активним йодом. Йодовмісні препарати є ефективними в системі профілактичного захисту за обробки насіння і рослин по вегетації , що дозволяє суттєво зменшити ураження рослин пшениці та вівса хворобами. Обробка посівів по вегетації йодовмісними препаратами, особливо на пізніх мікростадіях дозволяє знизити рівень вторинного ураження хворобами та попереджає інфікування насіння, що формується на материнській рослині і в подальшому є основним джерелом патогенної інфекції, яка розвивається після висіву насіння в ґрунт. Розроблені регламенти будуть сприяти поширенню використання йодовмісних препаратів в технологіях вирощування сільськогосподарських культур, що є особливо ефективними для регіонів із дефіцитом йоду в довкіллі

Ключові слова: біологічно активний йод; пшениця озима; овес; ураження хворобами; урожайність

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Formation of productivity of giant miscanthus (*Miscanthus × giganteus*) under symbiosis of its root system with fungi and bacteria

Abstract. The relevance of the subject under study is to increase the yield of dry biomass of giant miscanthus plants by using fungi and bacteria in its cultivation technology. The purpose of this study was to determine the effect of mycorrhization of the root system of this crop by fungi and symbiosis with bacteria on the growth and development of plants and the formation of their productivity. Field studies were conducted at the Veselopodilska Experimental Breeding Station of the Institute of Bioenergy Crops and Sugar Beet of the National Academy of Agrarian Sciences of Ukraine (Poltava region) during 2017-2019 on saline and slightly saline black soil. The significance of the deviation between the experimental variants was determined by the P-level calculated according to the Student's test. The use of symbiosis of fungi and bacteria with the root system of giant miscanthus contributed to positive results in improving plant growth, development and dry biomass yield. The use of biological products Mycofriend, Mikovital, and Florobacillin (fungi *Glomus VS*. and *Trichoderma harzianum* Rifa, *Tuber melanosporum* Vittad and bacteria *Bacillus subtilis* Cohn*)* significantly increased the area of the leaf apparatus (by 6.9-19.0%), the weight of the root system (by 4.1-16.3%), increased plant height (by 3.7-13.6%), and the number of stems (by 5.7-15.1%) compared to the control.

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At the same time, there was an improvement in the soil's moisture-holding capacity by 10.3-23.7% and its structural and aggregate state – the proportion of clods 0.25-10.0 mm in size was 3.2-5.7% higher than in the control. The use of mycorrhizal fungi and nitrogen-fixing bacteria contributes to an increase in the yield of dry plant biomass by 1.82-6.11 t/ha compared to the control, which is of practical importance in increasing the raw materials for biofuel production

Keywords: root system weight; leaf area; leaf weight; moisture retention capacity; structural aggregate state; yield

INTRODUCTION

The global energy crisis is one of the world's challenges of the 21st century, and therefore research that complies with international safety guidelines aimed at improving the environmental safety of agricultural production, increasing efficiency and reducing losses is becoming increasingly important (Polovyi *et al.,* 2021).

Giant miscanthus is a perennial crop for agricultural production and, under optimal growing conditions, is suitable for use for a long time (Lopushniak *et al.*, 2021a; Pidlisnyuk *et al.*, 2022). It is known that crop yields increase substantially with the use of fertilisers, and in recent years there has been a growing trend towards local application and pre-sowing seed treatment, which increases the efficiency of their use (Bielashov *et al*, 2022; Kalenska *et al*, 2022). To improve the efficiency of crop growth and development, microorganisms are used to enhance their nutrition (Dymytrov *et al.*, 2021; Prysiazhniuk & Penkova, 2022). This is evidenced by long-term studies with miscanthus conducted in the United States, which show that microbial communities are formed in the endophytic zone of the soil, which have a close correlation with the total number of bacteria and diazotrophs in the rhizosphere of the root system (Ivanina *et al.*, 2021). The results of studies conducted in Germany (Semenchuk *et al.,* 2021) proved the existence of specific N-fixing bacterial species in miscanthus rhizomes, which can fix nitrogen in organ-specific bacterial communities formed in their zone.

According to literary sources on plants (Katelevskij, 2020; Gupta & Abbott, 2021; Nedilska, 2021), microorganisms have a significant role in these communities, enhancing the root system's absorptive capacity, optimizing plant nourishment, and mitigating the adverse effects of drought and soil salinity. The instability of the water balance of plants caused by the instability of several factors negatively affects the physiological processes that underlie the formation of the crop and its quality. Plants need water for all the biochemical reactions that take place in their bodies. The decrease in water leads primarily to a deterioration in photosynthesis and some other biochemical reactions in the plant.

Some authors (Sabluk *et al.,* 2021; Kurhak & Sliusar, 2021; Honcharuk & Prysiazhniuk, 2022) have pointed out that water shortages result in a decrease in chlorophyll biosynthesis, and drought causes its destruction. The plant consumes the most water at the beginning of the growing season and almost all of it is used for transpiration. Droughts and poor soil aeration lead to a decrease in the water content of plant tissues, which adversely affects their growth and development. Under this condition, there is a massive death of roots and a decrease in growth. At the same time, the use of mycorrhizal fungi and nitrogen-fixing bacteria significantly improves the moisture supply and nutrition of plants. This is evidenced by the results of these studies, which for the first time in the Forest-Steppe conditions of Ukraine established the effectiveness of using fungi and bacteria for their symbiosis with the root system of giant miscanthus plants, which contributes to increasing its productivity and is important for biofuel production.

The purpose of this study was to determine the effect of mycorrhization of the root system of the Miscanthus giganteus by fungi and its symbiosis with bacteria on the growth and development of plants and the formation of their productivity.

MATERIALS AND METHODS

Studies to determine the effectiveness of biological products for improving the growth and development of giant miscanthus plants were conducted considering the requirements of the International Convention on Biological Diversity (1992), which make provision for the exclusion of their negative impact on plants, the microbiological state of the soil and the environment, as it concerns the beneficial interaction of fungi and bacteria with the root system of this crop.

Field studies were conducted in 2017-2019 at the Veselopodilska Experimental Breeding Station (VEBS) of the Institute of Bioenergy Crops and Sugar Beet of the National Academy of Agrarian Sciences of Ukraine, located on the left bank of the Dnipro River in the typical Forest-Steppe zone. Soil cover is characterised by a variety of soils, with predominantly saline and slightly saline black soils. The biological products used in the study were Mycovital, Mycofriend, and Florobacillin, which were applied to the soil with a special device in the root zone.

The experiments were carried out in 4 replications, with an area of 25 m^2. According to the programme, the study investigated the effect of these microorganisms on leaf water content and root mass, stem formation, and plant height at different periods of organogenesis (30, 60, 90, and 120 days), as well as the moisture-holding capacity and structural and aggregate state of the soil and dry biomass yield.

To determine the weight of miscanthus leaves, they were selected on the indicated days of the plant vegetation, 25 pcs. from each replicate and weighed on a laboratory balance. The data were processed using statistical methods. The significance of the deviation between variants was determined by the P-level calculated according to the Student's test.

The mass of the root system of plants of this crop was determined at the specified time. For this, 10 plants from each replication were dug up to a depth of 30 cm and cut to the root collar, cleaned of soil, washed with water, dried for 1 hour, and weighed on a laboratory balance.

The area of 100 miscanthus leaves per variant was determined on days 30, 60, 90, 120 of the plant vegetation by scanning them using the Pitiole mobile application software. The height of the plants was measured with a measuring ruler and the number of stems per bush was determined simultaneously. The water-holding capacity of the soil was determined by the difference in the mass of the soil before and after drying. For this, 150 g of soil was taken from each replicate at a depth of 30 cm, weighed, and placed in a drying oven for 5 minutes at 100°C.

To establish the structural and aggregate state of the soil, 100-150 g were taken from each replicate, weighed, sieved on sieves with a mesh diameter of 0.25 and 10.0 mm, and the proportion of lumps of 0.25-10.0 mm in its total mass was determined.

RESULTS AND DISCUSSION

In the symbiosis of fungi with the root system of plants, they colonise their organs internally or externally, and bacteria produce metabolites that suppress the development of pathogens and increase plant immunity. Furthermore, phosphorus-mobilising bacteria can convert hardly soluble phosphates from the soil into mineral phosphoric acid salts available to the plant and assimilate both organic and mineral nitrogen. Accordingly, these microorganisms contribute to a substantial improvement in the mineral nutrition of plants, and thus to an increase in their productivity. This is evidenced by studies on the use of biological products with fungi and bacteria for their root application in giant miscanthus plantings (Kalenska *et al.*, 2022).

It was established that the use of mycorrhizal fungi and nitrogen-fixing bacteria improves all elements of growth and development of miscanthus plants and increases their productivity. One of the essential elements of this process is the water content of the leaves, which characterises the level of moisture supply, as this factor largely determines the productivity of photosynthesis and, consequently, the productivity of the crop.

In the experiments conducted, the water content of the leaves of the crop by determining their wet weight in all variants using biological products at different periods of plant vegetation was higher than in the control. At the same time, it was noted that the greatest impact of fungi and bacteria on this indicator is manifested in the early stages of plant growth. Thus, on the 30th day of organogenesis, the water content of miscanthus leaves in the experimental variants was significantly higher than in the control, while on the 90th and 120th day of plant vegetation using all preparations, these indicators were slightly lower, indicating a more intense saturation of leaf cells with water in the younger state (Table 1).

Table 1. Leaf mass of giant miscanthus depending on the symbiosis of fungi and bacteria with its root system (VEBS, 2017-2019)

Plant growing season, days	Weight of 100 leaves												
	Control, g	Mycofriend				Florabacillin				Mycovital			
		g	± to control		P-level	g	± to control		P-level	g	± to control		P-level
			g	%			g	%			g	%	
30	44.0	56.5	12.6	28.6	0.02	48.8	4.9	11.0	0.04	52.4	8.4	19.2	0.03
60	66.2	80.9	14.7	22.2	0.02	72.4	6.3	9.4	0.05	77.3	11.2	16.9	0.03
90	98.1	117.1	19.0	19.4	0.02	106.1	7.9	8.1	0.05	112.0	13.9	14.1	0.03
120	118.8	143.4	24.7	20.8	0.02	129.0	10.2	8.6	0.04	136.8	18.0	15.2	0.03

Source: developed by the authors

Furthermore, this indicator is influenced by other factors, one of which is temperature. The optimum temperature for their development is 15-25°C, and deviations from this temperature in a certain direction negatively affect their condition. The type of microorganism on which the biological product is based is also important. Thus, using the biological product Mycofriend, which is based on the fungi *Glomus* VS and *Trichoderma harzianum* Rifai, the highest leaf water content was obtained, which was higher than the control at different stages of organogenesis.

In the variants with the use of biological preparations Mycovital and Florobacillin, based on the fungus *Tuber melanosporum* Vittad. and the bacteria *Bacillus subtilis* Cohn, the increase in leaf water content compared to the control was slightly less than with the use of the biological preparation Mycofriend.

Root formation is also an essential element of plant growth and development. This process is influenced by many factors, the main ones being soil quality, soil preparation, humidity, and temperature conditions, etc. As for the effect of microorganisms on improving the accumulation of underground plant parts and increasing their mass, there are many examples of their effective use in the world practice. At the same time, much attention is paid to the use of mycorrhizal fungi from different families, as well as many types of bacteria. Notably, in the mycorrhizal interaction between a plant and a fungus, its mycelium colonises the root system and actually becomes its extension, forming densely growing hyphae, and bacteria contribute to the improvement of metabolism and thus have a positive effect on the formation of the root system of plants and increase its mass.

The conducted research confirms the fact of a significant increase in the mass of the root system of miscanthus plants under its mycorrhization by fungi and symbiosis with bacteria in different periods of their vegetation, and this indicator largely depends on the type of biological preparation. This process is particularly affected by Mycofriend (fungi *Glomus* VS and *Trichoderma harzianum* Rifai). In this variant, the weight of the root system increased the most compared to the control. The growth of the root system of plants of this crop is slightly less than that of the variant with Mycofriend, when using Mycovital (fungus *Tuber melanosporum* Vittad.) and Florabacillin (bacteria *Bacillus subtilis* Cohn.).

Notably, in the first period of vegetation of giant miscanthus plants (30-60 days) and in the later periods of its organogenesis (90-120 days) the difference in root weight compared to the control was insignificant, indicating that this factor did not affect their formation (Table 2).

Table 2. The influence of symbiosis of fungi and bacteria with the root system of Miscanthus giganteus plants on its mass (VEBS, 2017-2019)

Plant growing season, days	Root system weight of 10 plants												
	Control, g	Mycofriend				Florabacillin				Mycovital			
		g	± to control		P-level	g	± to control		P-level	g	± to control		P-level
			g	%			g	%			g	%	
30	122	142	19.8	16.3	0.02	129	7.6	6.3	0.05	135	13.6	11.1	0.04

Table 2. Continued

Plant growing season, days	Root system weight of 10 plants												
	Control, g	Mycofriend				Florabacillin				Mycovital			
		g	± to control		P-level	g	± to control		P-level	g	± to control		P-level
			g	%			g	%			g	%	
60	138	157	19.6	14.2	0.02	145	6.9	5.0	0.05	151	12.9	9.4	0.04
90	150	170	20.5	13.7	0.02	156	6.2	4.1	0.05	164	14.1	9.4	0.04
120	174	197	23.6	13.6	0.02	183	9.6	5.5	0.05	190	16.6	9.6	0.04

Source: developed by the authors

The formation of a giant miscanthus harvest is a complex process caused by environmental factors and biological characteristics of the growth and development of plants of this crop. The leaf area is of immense importance. It is directly related to the overall development of the plant's ground mass, since most of it is made up of leaves. The leaf surface plays a significant role in absorbing CO_2 and producing photosynthesis. The current study found that the microorganisms based on which the biological products were created contribute significantly to the formation of the leaf surface of giant miscanthus plants. Under the influence of fungi and bacteria, the leaf area of plants of this crop during the growing season was significantly larger than the control (Table 3).

Table 3. Leaf surface area of giant miscanthus plants under symbiosis of its root system with fungi and bacteria (VEBS, 2017-2019)

Plant growing season, days	Leaf surface area												
	Control, thous. m²/ha	Mycofriend				Florabacillin				Mycovital			
		thous. m²/ha	± to control		P-level	thous. m²/ha	± to control		P-level	thous. m²/ha	± to control		P-level
			thous. m²/ha	%			thous. m²/ha	%			thous. m²/ha	%	
30	17.2	20.4	3.3	19.0	0.01	18.7	1.5	8.9	0.04	19.3	2.1	12.3	0.03
60	25.6	30.3	4.7	18.3	0.01	27.4	1.8	6.9	0.04	28.7	3.1	12.0	0.03
90	680.0	789.1	109.1	16.0	0.02	729.0	49.0	7.2	0.04	758.9	78.8	11.6	0.03
120	1072.1	1240.3	168.2	15.7	0.02	1154.1	82.0	7.7	0.04	1189.9	117.8	11.0	0.03

Source: developed by the authors

The greatest difference in this indicator compared to the control was obtained on day 120 of organogenesis. A particularly noticeable effect on its growth was noted in the variant with the biological preparation Mycofriend for its root application to the soil, while in the variants with other preparations, a slightly smaller increase in leaf surface was obtained compared to Mycofriend. When using the biological products Mycovital and Florobacillin, the growth of this indicator was 1.08-1.11 times higher than in the control, while in the variants with the biological preparation Mycofriend it was 1.18 times higher.

Plant height is a crucial factor in the productivity of miscanthus giganteus, similar to other aspects of plant growth and development. The higher the stem height, the higher the dry biomass yield, which is an important bioenergy indicator. Mycorrhizal-forming fungi and nitrogen-fixing bacteria play a significant role in determining the height of giant miscanthus plants when introduced at the root.

It was found that in the variants with the use of biological preparations, the plant height was greater than in the control at different periods of plant vegetation (Table 4). Among the biological products, the greatest impact on this indicator was found, as in the previous results, with the use of the biological preparation Mycofriend, and with the use of other products, the increase in plant height compared to the control was slightly inferior to the performance of the Mycofriend preparation.

Table 4. Plant height of giant miscanthus depending on the symbiosis of its root system with fungi and bacteria (VEBS, 2017-2021)

Plant growing season, days	Plant height									
	Control, cm	Mycofriend			Florabacillin			Mycovital		
		cm	± to control		cm	± to control		cm	± to control	
			cm	%		cm	%		cm	%
30	54	62	7	13.6	57	3	6.0	60	6	10.2
60	112	127	15	13.4	119	7	6.3	122	10	8.6
90	188	209	21	11.2	195	7	3.7	201	14	7.2
120	218	244	26	11.7	227	9	4.1	235	17	7.9

Source: developed by the authors

The results obtained on the increase in the height of Miscanthus giganteus plants in variants with mycorrhiza and symbiosis with bacteria indicate a significant influence of this factor on this element of plant growth and development. The structure of the crop is a crucial element of miscanthus productivity, the components of which determine not only the yield of dry biomass, but also its quality. One of the main indicators in the structure of the crop yield is the number of stems per bush. It was found that in the variants with the biological product Mycofriend (fungi *Glomus* VS and *Trichoderma harzianum* Rifai) the number of stems per bush was higher than in the control. A significant increase in this indicator was also observed with Mycovital (fungus *Tuber melanosporum* Vittad.) and Florobacillin (bacteria *Bacillus subtilis* Cohn.) compared to the control (Table 5).

Table 5. Number of stems of giant miscanthus in one bush depending on the symbiosis of its root system with fungi and bacteria (VEBS, 2017-2019)

Indicator	Biological preparations									
	Control, pcs.	Mycofriend			Florabacillin			Mycovital		
		pcs.	± to control		pcs.	± to control		pcs.	± to control	
			pcs.	%		pcs.	%		pcs.	%
Number of stems in 1 bush	66	76	10	15.1	69	4	5.7	72	6	9.4

Source: developed by the authors

The greatest number of stems in the bush, compared to the control, was observed in the variants with the biological preparation Mycofriend. Thus, the root application of biological products helps to increase the yield structure of giant miscanthus due to the improvement of plant nutrition and water balance.

The importance of this research is pointed out in a number of scientific papers (Katelevskij, 2020; Shuvar *et al.,* 2021), which state that the use of these microorganisms helps to improve the moisture supply of plants and has a positive effect on soil condition. Specifically, it is pointed out that under dry conditions, which are often encountered during the growing season, fungi and bacteria have a positive effect on this process. The scientific works of the above-mentioned authors note that in variants with the use of mycorrhizal fungi, the soil moisture holding capacity increased by 8-10% compared to the control, which is an important indicator in dry periods of plant vegetation. Similarly, the works of several authors (Wang *et al.*, 2021; Lopushniak *et al.,* 2021b) note the significant role of microorganisms in the formation of the structural and aggregate state of the soil.

Thanks to the secretions produced as a result of their vital activity, lumps of soil are formed from its dusty fraction. The present study confirms these conclusions. It was found that in the variants with fungi, the moisture retention capacity of the soil was higher compared to the control due to the penetration of their filamentous hyphae into the finest pores of the soil. As in the previous experiments, the best indicators of soil moisture retention capacity were obtained in the variants with the fungi *Glomus* VS and *Trichoderma harzianum* Rifai (Mycofriend) in the initial period of plant vegetation (on day 30 of organogenesis) – 7 mm more than in the control. With the use of the fungus *Tuber melanosporum* Vittad. (Mycovital preparation), this figure was 5 mm, which is also positive for improving the conditions of growth and development of plants of this crop (Fig. 1).

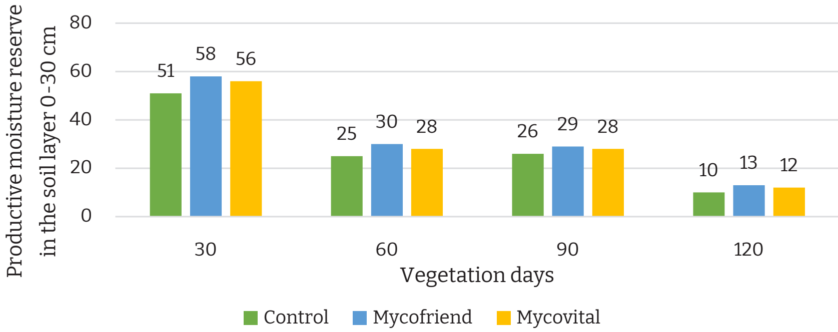

Figure 1. Soil moisture retention capacity depending on the interaction of mycorrhizal fungi with the root system of Miscanthus giganteus plants, VEBS, 2017-2019

As for the impact of fungi on improving the structural and aggregate state of the soil, it was found that it is substantially improved by creating a mycelial network and forming an adhesive component of the glycoprotein-glomatin, which glues part of the fine fraction of the soil, which coincides with the findings of several studies (Yu *et al.*, 2018; Moyano *et al.*, 2020). The proportion of lumps 0.25-10.0 mm in size in its total weight was 3.2-5.7% higher than in the control (Fig. 2).

This is also evidenced by the results of studies on the growth of the root system weight in variants using biological products. According to Table 4, in these variants, the mass of roots is significantly higher than in the control, i.e., due to the growth of fungal hyphae and the formation of arbuscules and vesicles, a mycelial network is created in the soil, which changes the soil structure, primarily affecting the reduction of its dusty fraction. That is, the mycelium of the fungus, developing intensively, forms a network that binds soil particles together. Furthermore, fungi, settling on the root system and entering into a symbiotic relationship with it, can accumulate the adhesive component glycoprotein-glomatin in the soil, which causes parts of its smallest fractions to stick together, forming lumps of various sizes and thus improving its structure.

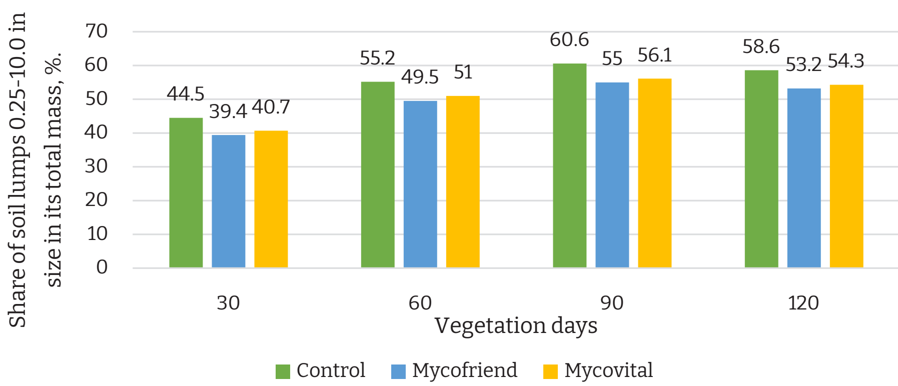

Figure 2. Proportion of lumps 0.25-10.0 mm in size in the symbiosis of mycorrhizal fungi with the root system of giant miscanthus plants, VEBS, 2017-2019

As a result of the positive impact of microorganisms on plant growth and development and soil quality, the use of mycorrhizal fungi and nitrogen-fixing bacteria contributes to a significant increase in the productivity of giant miscanthus. In the experiments conducted, the yield of dry biomass of this crop in variants with the use of biological products based on fungi *Glomus* VS, *Trichoderma harzianum* Rifai and *Tuber melanosporum* Vittad. (Mycofriend and Mikovital) and bacteria Bacillus subtilis Cohn. (*Florobacillin*) was significantly higher than the control (Fig. 3).

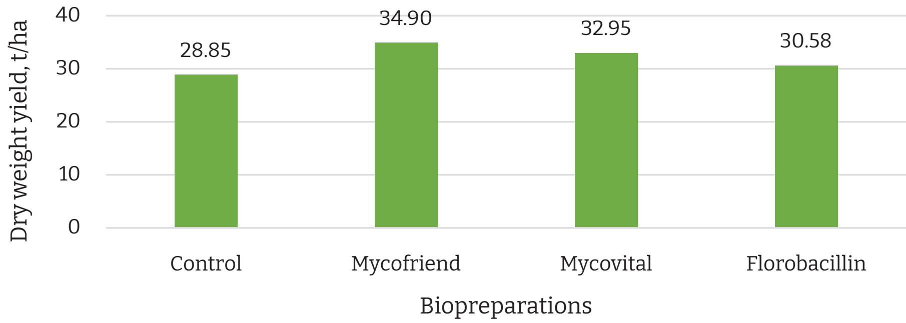

Figure 3. Yield of dry biomass of Miscanthus giganteus plants under symbiosis of its root system with fungi and bacteria, VEBS, 2017-2019

The type of microorganism plays a major part. In the variants with Mycofriend (fungi *Glomus* VS and *Trichoderma harzianum* Rifai), the yield of dry biomass of miscanthus was the highest compared to other preparations and control. The latter indicates that this biopreparation has the greatest positive effect on all elements of growth and development of miscanthus giganteus plants and, accordingly, contributes to the highest yield of dry biomass of this crop.

CONCLUSIONS

Mycorrhization of the root system of miscanthus giganteus by fungi *Glomus* VS and *Trichoderma harzianum* Rifai (Mycofriend), *Tuber melanosporum* Vittad (Mikovital) and its symbiosis with bacteria *Bacillus subtilis* Cohn (Florobacillin) contribute to the improvement of plant growth and development and increase their productivity.

Under this condition, by determining the dry mass of the leaves, their water content increased by 8.1-28.6% compared to the control, which indicates the ability of fungi to form many branches of filamentous hyphae, which significantly increase the area of water absorption by the root system from the soil, penetrating into its thinnest pores. The mass of the root system of Miscanthus giganteus in the variants with root application of fungi and bacteria to the soil was 4.1-16.3% higher than in the control due to the formation of mycelial network around the root hairs and the formation of arbuscules and vesicles by fungi. The role of bacteria in this is to help improve metabolism in the root system, which in turn affects its weight.

The height of miscanthus giganteus plants in the variants with fungi and bacteria was 3.1-13.6% higher than in the control, which indicates more favourable conditions for the growth and development of plants of this crop in the variants using these microorganisms. In these variants, the number of stems per bush also increased by 5.7-15.0% compared to the control, which is an important component in the formation of crop productivity.

The leaf surface area of Miscanthus giganteus plants in experiments with fungi and bacteria at different growing seasons was 6.9-19.0% larger than the control, which underlies efficient solar energy absorption and creation of organic products.

Apart from the positive effect of fungi on plant growth and development, the use of these microorganisms helps improve the soil moisture retention capacity and its structural and aggregate state. In the variants with mycorrhizal fungi *Glomus* VS and *Trichoderma harzianum* Rifai and *Tuber melanosporum* Vittad, the soil moisture retention capacity was 10.0-25.7% higher, and the proportion of lumps 0.25-10.0 mm in size exceeded the control by 3.2-5.7%. The yield of dry biomass of giant miscanthus in variants with biological products was higher than the control by 1.82-6.11 t/ha, which had a positive effect on the supply of biofuel raw materials to the market. This area of research has the potential for further development in other highly productive crops used as feedstocks to produce diverse types of biofuels.

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None.

CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Формування продуктивності міскантуса гігантського (*Miscanthus × giganteus*) за симбіозу його кореневої системи з грибами та бактеріями

Анотація. Актуальність обраної теми полягає у підвищенні врожайності сухої біомаси рослин міскантуса гігантського за використання грибів та бактерій у технології його вирощування. Метою дослідження є встановлення впливу мікоризації кореневої системи цієї культури грибами та симбіозу з бактеріями на ріст і розвиток рослин та формування їх продуктивності. Польові дослідження проводили в умовах Веселоподільської дослідно-селекційної станції Інституту біоенергетичних культур і цукрових буряків Національної академії аграрних наук України (Полтавська область) упродовж 2017-2019 рр. на чорноземі солонцюватому та слабо солонцюватому. Суттєвість відхилення між варіантами дослідів визначали за показником P-level, розрахованим за критерієм Стьюдента. Використання симбіозу грибів і бактерій з кореневою системою міскантуса гігантського сприяло отриманню позитивних результатів у покращенні росту, розвитку рослин та підвищенні врожайності сухої біомаси. Зокрема, за використання біопрепаратів Мікрофренд, Міковітал, та Флоробацилін (гриби *Glomus VS.* і *Trichoderma harzianum* Rifa, *Tuber melanosporum* Vittad та бактерії *Bacillus subtilis* Cohn*)* істотно зростала площа листкового апарату (на 6,9-19,0 %), маса кореневої системи (на 4,1-16,3 %), збільшилась висота рослин (на 3,7-13,6 %) та кількість стебел (на 5,7-15,1 %) порівняно з контролем. Водночас, відмічається покращення вологоутримуючої здатності ґрунту на 10,3-23,7 % і його структурно-агрегатного стану – частка грудочок розміром 0,25-10,0 мм була більшою від контрольних на 3,2-5,7 %. Використання мікоризоутворюючих грибів і азотофіксуючих бактерій сприяє зростанню порівняно з контролем врожайності сухої біомаси рослин на 1,82-6,11 т/га., що має практичне значення у збільшенні сировини для виробництва біопалива

Ключові слова: маса кореневої системи; площа листків, маса листків; вологоутримуюча здатність; структурно агрегатний стан; врожайність

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Impact of nutrient supply on growth and synthesis of metabolites of *in vitro* shoot cultures of *S. rebaudiana*

Abstract. *Stevia rebaudiana* plants produce sweet tasting compounds, steviosides, which are 300 times sweeter as sugar and can be used as natural sweeteners. Moreover, stevia leaves contain high amount of antioxidants. The use of plant compounds as components of functional nutrition and nutraceuticals is extremely relevant today. The purpose of this study was to investigate the

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effect of increased nitrogen and saccharose supply on growth and accumulation of steviosides and anthocyanins as well as antioxidative activity in fast-growing shoot cultures of *S. rebaudiana*. The morphological changes of the leaves, the accumulation of steviosides and anthocyanins, and the antioxidant properties of the extracts were determined. It was shown that during three weeks of *in vitro* cultivation of shoots on MS medium with double concentration of nitrogen, the biomass increased by 71.0%, after application of double concentration of saccharose – by 133%, and after increasing nitrogen in combination with saccharose supply – by 162.0% compared to the control. Sucrose stimulated the accumulation of biomass. Anti-oxidative potential after nitrogen application was 2.4 times higher than the control, and with saccharose supply it increased 2.7 times. Shoots treated with increased nitrogen and saccharose concentration contained 50.7 and 57.8 mg/g^{-1} steviosides, respectively. However, the combination of nitrogen and saccharose led to accumulation of 73.4 1 mg/g^{-1} steviosides. Shoots grown on MS medium culture had 26.0 mg/g^{-1} of steviosides. The content of anthocyanins was 1.7 times greater under added nitrogen supply, and 2.3 times greater after the application of nitrogen and saccharose. Optimum cultivation media developed individually for each *in vitro* culture increase the production of valuable plant secondary metabolites up to 3 times

Keywords: plant biomass; cultivation; nutrient medium; secondary metabolites; steviosides; anthocyanins; antioxidative activity

INTRODUCTION

Many health issues and complications – from cardiovascular diseases, bowel, and dental issues to cancer – have been associated with obesity and excessive consumption of free sugars. According to WHO recommendations, consumers must reduce daily free sugars intake to less than 10% of their total calorie intake (WHO Guideline, 2023). As consumers become more aware of the health benefits of low-calorie foods, the market for these products is expected to grow rapidly (Mordor Intelligence, 2022). Artificial sweeteners are the predominant sugar substitutes in the market to produce low-energy products. In addition, due to the COVID-19 pandemic, people started to show more interest in natural non-caloric sweeteners, such as stevia (Emergen Research, 2022). *Stevia rebaudiana* plant, commonly known as stevia, has been used by South Americans for thousands of years.

Steviol glycosides are sweetening compounds from the leaves of the *S. rebaudiana* plant. However, some steviosides are characterized with bitter aftertaste (Nguyen & Tan, 2021). The technological advantage of steviosides is the low-cost extraction technology which reduces the production costs (Al-Taweel *et al.,* 2021). Steviosides are not only non-caloric sweeteners of natural origin but also are suitable to use in the food industry due to their technological characteristics. The attributes of being heat and pH stable, water and alcohol soluble provides a technological advantage (Raspe & Silvio, 2022).

S. rebaudiana is the sweetest species of the genus *Stevia* which involves about 150 species of herbs. They belong to the *Asteraceae* family also known as daisy or sunflower family (Nguyen *et al.,* 2019). *S. rebaudiana* is an herbaceous, semi-bushy, perennial shrub which grows in humid environments at an average temperature of 24°C. The plant is native to Paraguay and some parts of Brazil. For at least 1,500 years, stevia leaves have been used in the cuisine of Guarani tribes on a daily basis. Dr. Bertoni rediscovered this plant by the end of 19th century.

Steviol glycosides are mainly found in plants, including stevia. The molecular structure of steviol glycosides is described as a sugar molecule bound to a non-carbohydrate moiety. The chemical names of individual sweet tasting substances from stevia plants depend on the type of sugar they contain, e.g., glucosides (glucose), pentosides (pentose), fructosides (fructose), etc. The substances can be converted into sugar and non-sugar compounds, the so-called aglycones, by hydrolytic cleavage (Jawad *et al.,* 2022). Steviol occurs in the plant as steviol glycosides, which are sweet compounds.

Moreover, stevia plants hold a high content of polyphenols, including anthocyanins. Polyphenols are the secondary metabolites that can act as free radical scavengers. However, the seeds of this plant have extremely low germination rate (Shevchenko *et al.,* 2010). A promising method of obtaining *S. rebaudiana* biomass with a high and stable content of steviosides and phenolic compounds, free from plant protection agents, from mycotoxins that can be synthesized during unfavourable plant storage conditions, is the application of *in vitro* techniques. Additionally, synthesis of metabolites in *in vitro* cultures can be regulated through nutrient supply. Due to the slow growth often encountered in plant cell cultures, inducing *in vitro* shoot culture of *S. rebaudiana* has been proposed.

The hypothesis of the study is that application of certain nutrients as nitrogen and saccharose can induce biomass accumulation of *in vitro* shoot culture and influence synthesis of valuable secondary metabolites. The purpose of this study was to identify the role of nitrogen and saccharose on growth and biochemical composition of *in vitro* shoot cultures of *S. rebaudiana*.

LITERATURE REVIEW

Numerous protocols for micropropagation of *Stevia rebaudiana* have been established (Ramírez-Mosqueda *et al.,* 2016). Nonetheless, for the past few decades, the high expenses incurred in micropropagation compared to that seen in traditional field cultivation have been significant limiting factors (Amien *et al.,* 2022). The latest developments in plant *in vitro* techniques have led to a successful transition from the experimental stage to industrial-scale production. Cultivation of plant differentiated *in vitro* systems from last year is believed to be an effective method for generating valuable natural compounds (Hassanein, 2022). Numerous research studies have investigated the effect of nitrogen concentration in cultivation media on the growth and morphogenesis of tissue cultures. According to J. Wang *et al*. (2020), the concentration and chemical form of nitrogen were deemed as highly significant.

In vitro cultures typically consider nitrate as the most essential form of nitrogen. Yet, in many instances, the application of nitrate only as a nitrogen source has not resulted in success. Tissue cultures require ammonium as a source of nitrate ion, according to P. Olgunsoy *et al*. (2017) findings on the *Rosa sp*. callus culture cultivation. Research conducted by T. Wu *et al*. (2021) has demonstrated that cell cultures of *Nicotiana tabacum* can flourish on cultivation medium containing solely ammonium. However, the resulting accumulation of biomass was only half as much as that with a combination of ammonium and nitrate sources. Meanwhile, S. Alvarez & J.R. Motos (2023) noted that dicarboxylic acid must also be included to promote *in vitro* cultures' growth if the cultivation medium solely depends upon ammonium as a nitrogen source. M.M. El-Dawayati & Z.E. Zayed (2017) hypothesized that the suitable concentrations of ammonium and nitrate ions in culture media for *in vitro* cultures should be selected at 10:15 (825:1425 mg/L) of the MS culture. In experiments on hairy root cultures of *Psoralea corylifolia*, M. Zaheer *et al*. (2016) demonstrated that the uptake of ammonium and nitrate was dependent on the pH of the cultivation medium. Under acidic pH, nitrate was utilized more intensively, leading to an increase in the alkalinity of the cultivation medium. The uptake of ammonium led to a shift in the pH of the medium towards acidity, which further inhibited the uptake of ammonium by the cultures.

Plant *in vitro* cultures are mostly heterotrophic, meaning that they use monosaccharides or disaccharides as a carbon source. According to T. Fidemann *et al*. (2018), the concentration of saccharides in culture media essentially influences both the growth of biomass and the synthesis of secondary metabolites in *in vitro* cultures. To provide a source of carbohydrates, sucrose or glucose is usually added as a carbon source in concentrations ranging from 2 to 4%. Certain cultures require fructose or maltose as a source of sugar. The selection of the most efficient source of carbon and its concentration depends on the species of the plant, as well as the form of its *in vitro* cultivation and the objective of the cultivation, such as the synthesis of alkaloids or phenolic compounds. K. Ibrahim & H. Musbah (2018) discovered that *Coleus blumei* cell cultures, when subjected to a saccharose concentration of 2.5% in the cultivation medium, produced 0.8 g/L^{-1} of rosmarinic acid, but after increasing the saccharose concentration to 7.5%, the yield of rosmarinic acid reached 3.3 g/L^{-1}.

Currently, many research groups worldwide are focusing on cultivating differentiated shoot *in vitro* cultures. Shoot cultures are characterized by intensive growth and metabolite synthesis due to their tissue differentiation. According to M. Kikowska *et al.* (2020), the biomass accumulation in the *in vitro* shoot culture of *Eryngium alpinum* L. was 12.5 times higher than the biomass growth in callus cultures. A. Król *et al.* (2021) have also demonstrated the feasibility of effectively utilizing *in vitro* shoot cultures from various plants for producing phenolic compounds and antioxidants. *In vitro* shoot cultures are particularly valuable due to their capability of plant tissue differentiation, which contrasts with undifferentiated cell and root *in vitro* cultures, making them capable of producing metabolites that are not present in the latter. Equally important, the growth of shoot cultures is often more intensive when compared to other *in vitro* cultures and, in many instances, even compared to their native counterparts.

MATERIALS AND METHODS

Introduction of *in vitro* shoot cultures. The surface of *S. rebaudiana* seeds was sterilised for 5 minutes using 70% ethanol, followed by 10% sodium hypochlorite for 10 minutes and rinsed three times with distilled water. The explants (leaf, nodal, and inter-nodal segments) were cut into small pieces of approximately 0.1 cm in length, one week after germination. The pieces were transferred to 250 mL Erlenmeyer flasks containing 50 mL of liquid MS medium and 0.4 mg/L kinetin. The cultures were grown at a temperature of 25°C on a rotary shaker (75 rpm), under illumination of 18:6 (3000 lx). An autoclave, a sterile cabinet, and an incubator (VWR, Germany) were utilized for the *in vitro* cultivation process. To prepare the material for chemical analysis, the following devices were used: ALPHA 1-4 lyophilic dryer (Christ, Germany), Memmert thermal cabinet, Eppendorf centrifuges (without and with cooling), vacuum concentrator Speed Vac SC110, ultrasonic bath, and ultrasonic homogenizer.

Propagation medium. The growth and development of stevia cultures were tested for their susceptibility to four different propagation medium compositions. A standard nutrient MS medium, containing 1.90 g/L^{-1} KNO_3, 1.65 g/L^{-1} NH_4NO_3, and 30 g/L^{-1} saccharose, with 2% agar, was used as a control. The experiment tested modifications of the MS medium with double the concentration of nitrogen (3.80 g/L^{-1} KNO_3 and 3.30 g/L^{-1} NH_4NO_3), 60 g/L^{-1} saccharose, and twice the concentration of nitrogen and saccharose (3.80 g/L^{-1} KNO_3, 3.30 g/L^{-1} NH_4NO_3, and 60 g/L^{-1} saccharose). Cultivation media was autoclaved at 121°C. Vitamins were added after autoclaving, using microfiltration through sterile 0.25 μm disc membrane filters (Membrane Solution, USA).

The experiments were conducted as randomized controlled factorial trials using *S. rebaudiana* seeds. The measurement results were analysed using the ANOVA method – a statistical technique for variance analysis.

Measurement of anthocyanins. To the *S. rebaudiana*, 100 mg was added, and subsequently 750μl of CH_3COOH/EtOH was also introduced. For a period of 20 minutes at 85°C, the sample was placed on the block heater. Subsequently, the mixture was centrifuged for 5 minutes at 12,000 rpm and the supernatant was collected. The given extraction procedure was repeated three times, and all the supernatants were subsequently pooled together. To each sample, 50 μl 37% HCl was added and vibrated on the vortex for 30 seconds. λ=535 nm was used for photometric determination of anthocyanin.

Analysis of steviosides. Stevioside content measurements were made according to Y. Shevchenko *et al.* (2010), with some modifications. One gram of *S. rebaudiana* biomass was ground to a powder in a ball mill. Subsequently, 25 mL of distilled water was added. The samples were heated to 95°C in a microwave oven for 30 minutes, then cooled to 25°C and filtered using filter paper. This process was performed three times, and the extracts were combined in a graduated flask. The total volume was then adjusted to 100 mL using distilled water. To clean 0.5 ml of the extract, a solid-phase-extraction column was used. The extract was washed with 3 ml of methanol followed by 3 mL of distilled water. The final elution was achieved by adding a mixture of methanol and acetonitrile in a 50:50 (v/v) ratio to the SPE column using 1 mL of the said mixture. Subsequently, the eluted solution was analysed using HPLC, following the methodology of Y. Shevchenko *et al.* (2010).

Determination of antioxidant activity. The DPPH radical scavenging activity assay was conducted following A.A. Mohdaly *et al.* (2011) method. To obtain an absorbance value of 0.7±0.02 at 515 nm, 100 mL of methanol was mixed with 6 mL of the stock solution to prepare DPPH solution (6×10^{-5} mol/L^{-1}). 0.02 g of the sample was mixed with 1 mL of distilled water and heated at 95°C for 15 minutes. Afterward, the sample was centrifuged at 12000 rpm for 5 minutes. 5 µL supernatant with 2 mL DPPH was vortexed for 30 s and allowed to react for 30 min. The absorbance at 515 nm was measured using the UV-Vis Spectrophotometer Jena-Specol 2100. The scavenging activity was calculated using the following formula:

$$DPPH\,(\%)=[(Abs.control - Abs.sample)/Abs.control] \times 100, \quad (1)$$

where Abs – is the absorbance at 515 nm.

The study complied with the requirements of the Convention on Biological Diversity (1992).

RESULTS

In vitro shoot cultures of *S. rebaudiana* were established from seeds as described above. After germination, the shoots were transferred into a sterile medium and cultivated for 21 days. The development of shoots cultivated on the MS medium is presented in Figure 1.

The effect of nutrient composition in cultivation media on the dynamic biomass growth of sprout cultures was investigated.

in vitro plants　1st day　*in vitro* shoot culture 14th day　21st day

Figure 1. *In vitro* shoot culture growth of *S. rebaudiana*

In vitro shoots of *S. rebaudiana* demonstrated a significant positive response to nitrogen and saccharose application in terms of biomass accumulation. The initial weight of the shoots grown on MS medium at the time of transformation was 20 g/$flack^{-1}$, and after three weeks of cultivation, it increased to 122.3 g/$flack^{-1}$ (see Fig. 2a).

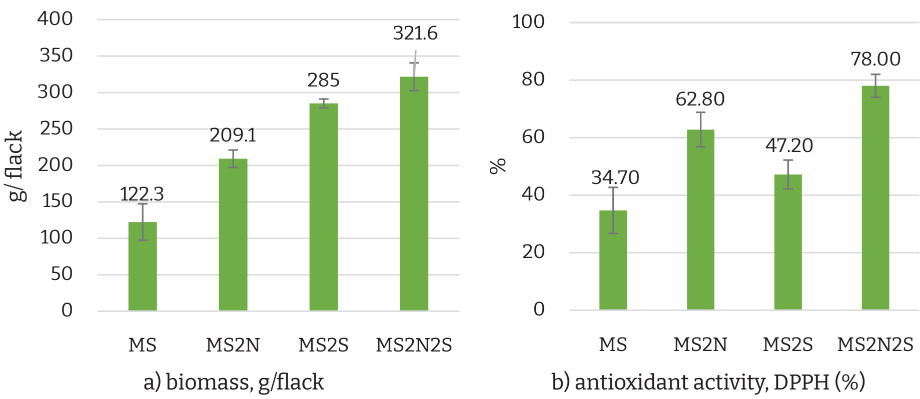


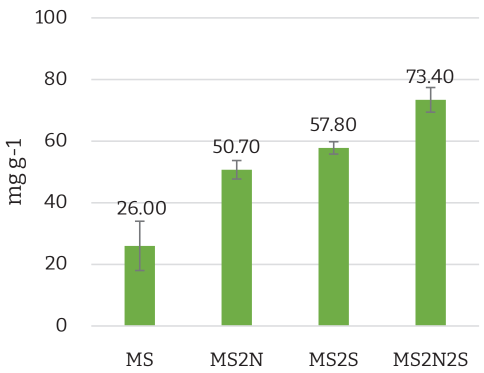
Figure 2. Biomass growth (a) and antioxidant activity (b) of *in vitro* cultures of *S. rebaudiana* on the 21st day of cultivation

Note: MS is the standard medium, MS2N is the MS medium with twice the concentration of nitrogen, MS2S is the MS medium with twice the concentration of saccharose, MS2NS is the MS medium with twice the concentration of nitrogen and saccharose

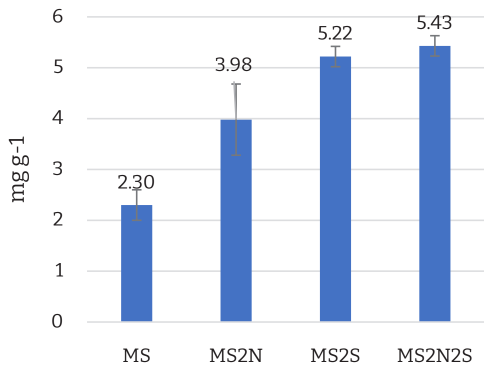
Raising the KNO_3 and NH_4NO_3 concentrations in the MS medium from 1.90 g/L^{-1} and 1.65 g/L^{-1} to 3.80 g/L^{-1} and 3.30 g/L^{-1}, respectively, stimulated a significant accumulation of biomass in the shoot cultures. After 21 days of cultivation, the sprout biomass reached 209.1 g/flack^{-1}, a 71% increase compared to the control group. Raising the saccharose concentration in the MS medium from 30 g/L^{-1} to 60 g/L^{-1} resulted in the formation of 285.0 g/flack^{-1} of shoot biomass, which was 133% higher than the biomass in the control group. After applying additional nitrogen and carbohydrate sources, the fresh weight of the sprout cultures reached 321.6 g/flack^{-1}, which was 199.3 g more than the biomass of shoot cultures grown on MS medium. Four different media showed varying results concerning the growth parameters of the stevia sprout cultures. For the growth of *S. rebaudiana*, MS medium was the least suitable. This conclusion can be drawn from the results obtained 21 days after *in vitro* sprout cultivation, which are represented in Figures 1 and 2. These data showed statistically significant differences for all other experiment variants.

Y. Shevchenko *et al.* (2010) showed that the DPPH-free radical scavenging capacity of the shoot culture of stevia grown on the MS medium was 34.07% on the 21st day of cultivation (Fig. 1b). Increasing the nitrogen supply led to an enhancement of DPPH to 62.8%, which was 2.5 times higher than in the tissues of stevia cultivated on MS medium. The treatment with 60g/L^{-1} of saccharose rather than 30g/L^{-1} did not affect the DPPH significantly compared to the additional application of nitrogen. However, it captured 88% prevalence of the control plot. The combined modification of MS medium, with increased nitrogen and carbohydrate content, resulted in the formation of 78% DHHP activity. Afterward, it was concluded that it could be attributed to the higher concentrations of steviosides and anthocyanins present in the plant biomass.

The application of additional KNO_3 and NH_4NO_3 caused an accumulation of 50.70 mg of steviosides per 1 g of fresh weight of biomass in the shoots after 21 days of cultivation. This amount was 95% more than that in cultures grown on MS medium (Fig. 3a).



a) steviosides, mg g^{-1}



b) anthocyanins, mg g^{-1}

Figure 3. Content of steviosides and anthocyanins in *in vitro* cultures of *S. rebaudiana* on the 21st day of cultivation

Note: MS is the standard medium. MS2N is the MS medium with twice the concentration of nitrogen compared to the standard medium, MS2S is the MS medium with doubled saccharose concentration, MS2NS is the MS medium with doubled concentration of nitrogen and saccharose

The culture's stevioside content increased to 57.8 mg/g^{-1} after a 21-day cultivation period, with the additional carbohydrate source resulting in a 7.1 mg/g^{-1} increase compared to the nitrogen treatment. The plot with *in vitro* cultures supplied with additional nitrogen and saccharose had the highest stevioside content, exceeding the control by 2.8 times and reaching

73.40 mg/g^{-1}. The content was 2.8 times higher than the control, reaching 73.40 mg/g^{-1}.

The anthocyanin content in the shoot culture of *S. rebaudiana* reached 2.3 mg/g^{-1} of fresh biomass after being cultivated for 21 days on MS medium. Synthesis of 3.98 mg/g^{-1} of anthocyanins occurred after supplementing with a double concentration of KNO_3 and NH_4NO_3 compared to MS medium typical concentration. Application of 60 g/L^{-1} saccharose, instead of 30 g/L^{-1}, caused a two-fold increase in anthocyanin quantities. The substance content of this plot reached 5.22 mg/g^{-1}, which was similar to that after the treatment of sprout culture with increased nitrogen and saccharose concentrations. The combination of nitrogen and saccharose did not have as significant an effect as the single application of saccharose alone.

MS is among the most commonly used media for *in vitro* plant cultures. Additionally, B5 medium (Gamborg), LS (Linsmaier & Skoog), Velicky and Martin, and Blaydes and Heller medium compositions are widely used. The selection of the cultivation medium depends on the requirements of the initial plant and cultivation form, such as callus, suspension, hairy root, or shoot cultures. The MS culture is commonly employed for *in vitro* cell cultures, whereas B5 is used for hairy root cultures. On the other hand, LS medium is known to promote intensive biomass production in many cases. M.H. Zenk *et al.* (1978) conducted several tests to produce the indole alkaloid serpentine using *Catharanthus roseus* suspension cell cultures. The alkaloid content in cell cultures was dependent on the nutrient medium's composition. It was discovered that LS medium was optimal for the production of alkaloids, whereas MS medium was optimum for cell growth.

J.J. Zhong (2001) demonstrated that the intensity of alkaloid synthesis in cell suspension cultures of *Holarrhena antidysenterica* varies depending on the form of nitrogen in the cultivation media. Adding nitrogen to the MS nutrient solution stimulated anthocyanin formation in cell suspensions of *Vitis vinifera*. D.J. Kim & H.N. Chang (1990) reported the increased production of shikonin in *Lithospermum erythrorhizon* cell cultures under the influence of increased nitrogen concentration. However, K. Sakamoto *et al.* (1994) reported that a reduced level of total nitrogen in the cultivation media improved the production of capsaicin in *Capsicum frutescens* and anthraquinones in *Morinda citrifolia*. It is possible to conclude that the response of *in vitro* culture to increased nitrogen supply may differ, depending on the physiological and biochemical status of native plants.

J.J. Zhong's (2001) research revealed that the accumulation of alkaloids in *Holarrhena antidysenterica* and shikonin in *Lithospermum erythrorhizon* suspension cell cultures were significantly affected by the carbon source. In 1980, Knobloch and Berlin examined the impact of saccharose concentration on the synthesis of indole alkaloids in *Catharanthus roseus* cell cultures, ranging between 4-12%. It was found that the optimal concentration of sucrose was 8%. Moreover, by increasing the sucrose concentration to 8%, the yield of benzophenanthridine alkaloids in *Eschscholtzia californica* cell suspension culture increased tenfold and reached 150 mg/L^{-1} (Berlin *et al.*, 1981). However, Do and Cormier (1990) found that greater amounts of sucrose may induce osmotic stress in cells of suspension cultures, resulting in reduced biomass accumulation, but also increased anthocyanin production in *Vitis vinifera* cell cultures.

N. Sae-Lee *et al.* (2014) added NH_4NO_3 in quantities of 0.5, 1.0, 5.0, and 10.0 mg/L to the MS medium as a nitrogen source to stimulate *Vitis vinifera* cv. Pok Dum cell culture growth and induce secondary metabolite synthesis. The optimum result was achieved using MS medium enriched with 0.5 mg/L^{-1} NH_4NO_3. On the 14th day of the experiment, the biomass of the cell cultures was 27.8 g/L^{-1}, representing a 5.6-fold increase compared to the control group.

F. Akbari *et al.* (2018) showed an increase in shoot length, seedling dry weight, and leaf fresh weight of stevia when NH_4NO_3 was applied along with KNO_3.

In this experiment, increasing the concentration of KNO_3 and NH_4NO_3 in MS medium stimulated intensive biomass accumulation of shoot cultures. After a 21-day cultivation period, the biomass of sprouts was 71% higher than the biomass of shoots grown on the control plot on MS medium. The increase in saccharose concentration in MS medium elicited 2.3-fold growth stimulation of biomass. The combination of extra nitrogen and carbohydrates was found to be the most effective. This methodology stimulated biomass accumulation, which exceeded the control plot by 2.6 times. The stevia sprout cultures' growth parameters varied across four distinct media. The MS medium was found to be the least suitable for the growth of *S. rebaudiana*. This conclusion was drawn based on the results obtained after 21 days of *in vitro* sprouts cultivation, as presented in Figures 1 and 2. These data show significant statistical differences when compared to all other experimental variants.

Evaluating the antioxidant potential is important for determining the stability of a product during storage, and this value has a direct relationship with the concentration of substances with antioxidative properties. Substances with antioxidative properties can reduce the number of free radicals in foods and are therefore important components of a healthy diet (Nshimiyimana & He, 2010). Plant secondary metabolites, including polyphenolics such as anthocyanins, are known for their high antioxidant potential.

Prior research into the antioxidant potential of phenolic compounds has shown that their mechanism of action involves directly capturing free radicals. Furthermore, free radicals (ROO•) oxidise phenolic compounds and inactivate them, causing the fragmentation of less reactive substances (Smetanska *et al*., 2021). The phenolic compound radical (O•) formed during the reaction is stable. The unpaired electron can delocalise within the aromatic ring and can cause dimerisation, dismutation, recombination with other radicals, oxidation of quinones, producing semiquinones. Semiquinones can react with other radicals, forming stable quinones (Mohdaly *et al*., 2010). Antioxidants can prevent or inhibit substrate oxidation, thus eliminating or decreasing the oxidative damage caused to target molecules (Fig. 4).

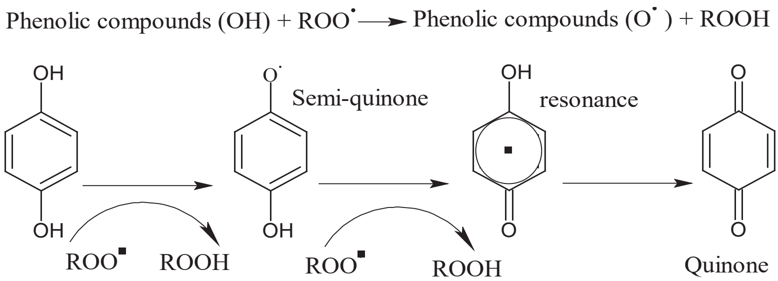


Figure 4. Mechanism of neutralization of reactive radicals by phenolic compounds
Source: A. Mohdaly *et al*. (2010)

Y. Shevchenko *et al*. (2010) demonstrated that the DPPH of the shoot culture of stevia increased by 150% after the introduction of additional nitrogen supply. However, the application of saccharose caused a slight increase in DPPH compared to the control. On the other hand, the combined application of additional nitrogen and saccharose positively influenced the increase of DPPH in plant tissues. There has been a recent rise in demand for industrial biotechnologies and natural antioxidants, aimed at replacing synthetic analogues. Free radicals are responsible for lipid peroxidation, which in turn can lead to many chronic illnesses in humans, including cancer and cardiovascular disorders; thus, they play a crucial role in their development.

N. Sharma *et al*. (2015) also reported that using a nutrient MS medium with twice the concentration of NH_4NO_3 and 4.5% sucrose increased the growth of *S. rebaudiana* cell cultures and the synthesis of steviol glycosides. Yet, the steviol glycoside amount in cell cultures was 2.99 mg/g^{-1}, whereas in the leaves of the original plants, it was 40.0 mg/g^{-1}.

These studies indicate that nitrogen positively affects the content of steviosides and anthocyanins, while sucrose has an even stronger impact. The concentration of steviosides in *in vitro* culture was 2.7 times higher after the treatment with nitrogen and sugar as in MS-cultivated *in vitro S. rebaudiana*. Anthocyanins are secondary metabolites found in plants that respond to stressors from unfavourable biotic and abiotic conditions, such as a lack or an excess of minerals. According to N. Sae-Lee *et al*. (2014), there was an increase in the accumulation of anthocyanins in grape (*Vitis vinifera* L.) cell suspension cultures with increased nitrogen supply.

The effect of various concentrations of ammonium nitrogen on anthocyanin synthesis was investigated in *in vitro* cell cultures of *Rosa hybrida* by P. Olgunsoy *et al.* (2017). The induction of rapidly growing callus was observed during cultivation on MS solid medium with 4.0 mg/L^{-1} 2,4-dichlorophenoxyacetic acid, but without additional nitrogen supply. According to J.J. Zhong (2001), raising the nitrogen content of the nutrient medium augments the anthocyanin composition in suspension cultures of *Vitis vinifera*. T.P. Magangana *et al.* (2018) demonstrated that the availability of nitrogen directly affects steviol concentration in microplants. Microplants subjected to a medium containing half the phosphate concentration were associated with the highest levels of stevioside (740 mg g/L).

Suspension cultures of *Nicotiana tabacum* have been found to accumulate more nicotine when exposed to increased saccharose concentration, resulting in osmotic stress (Shibasaki *et al.*, 2007). *Aralia cordata* cell suspension cultures showed decreased anthocyanin synthesis with a higher concentration of sucrose at 5%, whereas a concentration of 3% sucrose promoted anthocyanin accumulation. The osmotic pressure was also increased by adding mannitol to the medium, and it was discovered that after the addition of 3 g/L^{-1} of this substance, the level of anthocyanins accumulated in the *Vitis vinifera* culture was elevated by up to 1.5 times, reaching 55 µg/cell^{-1} (Dai *et al.*, 2014). An additional boost in anthocyanin content was observed in the callus cultures of *Euphorbia milli* grown in MS medium plus 7% sucrose. This indicates that, in numerous instances, growth-promoting environments are distinct from those required for synthesising secondary metabolites in cultures.

CONCLUSIONS

It was established that various breeding environments had diverse effects on *in vitro* development of stevia culture. The four chosen media demonstrated distinct outcomes in terms of the growth parameters and synthetic potential of stevia cultures.

The MS medium was the least suitable for *S. rebaudiana* growth. The biomass increased twice as much as the control plot, after nitrogen and sucrose content was increased. The best medium for obtaining steviosides was MS with twice the nitrogen concentration. The stevioside content exceeded the control by 182.0%. However, this environment was less suitable for accumulating biomass. Anthocyanin accumulation increased by 1.5-fold with an additional nitrogen supply and 2.0-fold was associated with an increased sucrose concentration. This could be due to the role of carbohydrates in the anthocyanin biosynthetic pathway.

The findings strongly suggest that it is possible to modify the nutrient solution according to various tasks, such as increasing the biomass, antioxidants or certain valuable metabolites like steviosides and anthocyanins. In this particular case, the MS medium with a doubled nitrogen and sucrose supply can be used for high biomass production of stevia hoot culture and for obtaining antioxidants. To increase biomass for stevioside production, it is advantageous to increase nitrogen supply and possibly extend the cultivation period. For anthocyanin production, increasing the saccharose content in the cultivation media would be meaningful.

Achieving the optimal nutritional environment using MS is an important step in the innovative biotechnology of obtaining valuable secondary metabolites from plants, considering the biological features of each *in vitro* culture.

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None.

CONFLICT OF INTEREST

The author of this study declares no conflict of interest.

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Вплив поживних речовин на ріст і синтез метаболітів у культурах пагонів *in vitro S. rebaudiana*

Анотація. Рослини стевії ребаудіани виробляють солодкі на смак сполуки – стевіозиди, які в 300 разів солодші за цукор і можуть використовуватися як натуральні підсолоджувачі. Крім того, листя стевії містить велику кількість антиоксидантів. Використання рослинних сполук як компонентів функціонального харчування та нутрицевтиків є надзвичайно актуальним на сьогоднішній день. Метою даної роботи було дослідити вплив підвищеного азотного живлення та сахарози на ріст і накопичення стевіозидів та антоціанів, а також антиоксидантну активність у швидкорослих культурах пагонів *S. rebaudiana*. Визначено морфологічні зміни листків, накопичення стевіозидів і антоціанів та антиоксидантні властивості екстрактів. Показано, що протягом трьох тижнів культивування пагонів *in vitro* на середовищі MS з подвійною концентрацією азоту біомаса збільшилася на 71,0 %, після застосування подвійної концентрації сахарози – на 133 %, а після збільшення азоту в поєднанні з подачею сахарози – на 162,0 % порівняно з контролем. Сахароза стимулювала накопичення біомаси. Антиоксидантний потенціал після внесення азоту був у 2,4 рази вищим за контроль, а при внесенні сахарози – у 2,7 рази. Пагони, оброблені підвищеною концентрацією азоту та сахарози, містили 50,7 та 57,8 мг/г$^{-1}$ стевіозидів відповідно. Однак поєднання азоту та сахарози призводило до накопичення 73,4^{-1} мг/г$^{-1}$ стевіозидів. Пагони, вирощені на середовищі MS, містили 26,0 мг/г$^{-1}$ стевіозидів. Вміст антоціанів був в 1,7 рази більшим за умови внесення азоту та в 2,3 рази більшим за умови внесення азоту та сахарози. Оптимальні живильні середовища, розроблені індивідуально для кожної культури *in vitro*, збільшують продукцію цінних вторинних метаболітів рослин до 3 разів

Ключові слова: рослинна біомаса; культивування; поживне середовище; вторинні метаболіти; стевіозиди; антоціани; антиоксидантна активність

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Methodology for determining the residual content of active ingredients of agrochemicals in surface waters

Abstract. Agricultural water bodies are multifunctional objects in the agricultural production cycle. Residual amounts of the active ingredients of pesticide agrochemicals can enter and contaminate a water body during some agro-technological tasks. The purpose of this study was to investigate the conditions for the extraction of residual amounts of xenobiotics from surface water containing suspended particles and to measure their content by chromatographic methods. To determine the optimal conditions for the extraction of target xenobiotics, the values of the lipophilicity parameters of their molecules were considered. The methodology for determining the content of lipophilic xenobiotics by chromatographic methods with mass-selective detection in surface water samples includes a step of separating suspended particles, the content of which in the samples under study was controlled by gravimetric analysis and varied within 135-1500 mg/m^3. The target compounds were extracted using n-hexane and acetonitrile. The analytes in the acetonitrile concentrate were

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determined according to high-performance liquid and gas chromatography with mass-selective detectors (HPLC/MS/MS and GC/MS). The achieved limit of detection of xenobiotics was 0.02 µg/m^3, the limit of quantification of xenobiotics was 0.10 µg/m^3. To substantiate the possibility of applying the proposed methodology, the following indicators were investigated: linearity of analytical signals with the amount of analytes in the solution, correctness, convergence, and accuracy of measurement results. The linear concentration range of the method for the determination of xenobiotics of diverse groups is 0.10-1.00 µg/m^3, characterised by a regression coefficient of the linear dependence of the measurement of individual compounds (R^2) exceeding 0.99. The degree of analytes recovery (percentage of recovery *r*, %) was within 85-120%, which indicates the acceptability of the proposed xenobiotic extraction procedure. The error of the measurement results was calculated as the standard deviation (S_r, %), which did not exceed 6%. The findings of this study suggested that the developed methodology is suitable for monitoring the residual content of active ingredients of agrochemicals in surface waters and predicting the level of water pollution

Keywords: lipophilic xenobiotics; extraction; water bodies; surface water; suspended solids; chromatography

INTRODUCTION

The anthropogenic load of pesticides on the environment, specifically on water bodies located in close proximity to the area where plant protection products are used, is an undeniable fact. Crop cultivation technologies involve the use of plant protection products and require further control of their residual amounts in products and in the environment, including water.

It was proved (Kurbatova *et al*., 2022) that xenobiotics of different chemical nature and mechanism of action, when released into natural water bodies with wastewater from industrial enterprises, municipal and agricultural facilities, adversely affect metabolic processes in the tissues of aquatic organisms. The impact of xenobiotic substances on aquatic organisms poses not only a constant threat but entails an urgent need to monitor the threats they pose and develop modern methods for restoring polluted ecosystems (Piwowarska & Kiedrzyńska, 2022).

To assess xenobiotic contamination of a water body, the content of individual xenobiotics is measured and compared with their maximum permissible concentration (MPC) or assessed using multiplicative models using various parameters of anthropogenic load (DSanPiN 8.8.1.2.3.4.-000-2001, 2001; González-Gaya *et al*., 2021). The results of monitoring the content of pesticides of different chemical groups in water correlate with the amount of precipitation that washes away and transfers xenobiotics directly with runoff to surface water bodies or ensures their movement to different depths up to groundwater layers. The presence of long-term effects and remote occurrence of some pollutants from diverse groups of pesticides in water bodies is associated with the physicochemical properties of their molecules, the specific features of the processes of degradation, migration and biotransformation of molecules in the soil (Baran *et al*., 2021). The duration of migration in soil layers ranges from one year to decades, especially for organochlorine pesticides. Atrazine, the active ingredient of herbicides, has an MPC in water of reservoirs of 0.001 mg/m^3 (DSanPiN 8.8.1.2.3.4.-000-2001, 2001). Residual amounts of atrazine are found in water bodies almost after several months of the last application in agricultural areas close to water (Prukjareonchook *et al*., 2023). Scientists use different methods to determine atrazine in water bodies (Hassan *et al*., 2023, Huang *et al*., 2023). Since 2004, according to the Stockholm Convention on Persistent Organic Pollutants, nearly 170 countries have banned the use of DDT insecticides for agricultural purposes. The MPC for this persistent pollutant in water is 0.002 mg/m^3. It is in the focus of modern monitoring studies of water resources around the world (Sackaria & Elango, 2020; Linlin *et al*., 2020).

Considering the nature of the samples and detection methods, researchers managed to detect even small concentrations of xenobiotics in water bodies, their accumulation processes and dispersed-phase distribution (Štefanac *et al*., 2021; Milyukin & Gorban, 2021). Atrazine and DDT are lipophilic xenobiotics that are absorbed by aquatic organisms in aquatic ecosystems and adsorbed to suspended particles of sand, clay, silt, plankton, and plant decomposition products. The component composition of not only the extracts obtained from water, but the sediment removed during the preparation of a laboratory water sample is also under investigation (Tereshchenko *et al*., 2020).

The purpose of this study was to investigate the conditions of extraction from surface water and to measure the content of residual amounts of active ingredients of agrochemicals by chromatographic methods.

MATERIALS AND METHODS

The solvents and reagents used in the study corresponded to the qualification "for chromatography" and "purity for analysis": acetonitrile, isopropanol, acetone, deionised water, n-hexane, sodium chloride, sodium citrate, magnesium sulphate. Water samples from non-flowing water bodies and from adjacent rivers were collected per (DSTU ISO 5667-4:2003, 2003; DSTU ISO 5667-6:2009, 2012), and a series of laboratory samples were prepared. These samples contained suspended compounds. Model systems (based on deionised water and on laboratory samples of surface water) were created to find optimal conditions for sample preparation for extraction of xenobiotics. The model systems contained lipophilic and hydrophilic xenobiotics introduced by dissolving the respective analytical standards produced by Sigma-Aldrich. Reference data on the physical and chemical properties of these xenobiotic molecules were collected from the ChemDraw library. Suspended solids were extracted from water samples according to the vacuum filtration method developed and tested in our previous studies using nylon membrane filters (Tereshchenko *et al*., 2020). The gravimetric analysis was performed using a KERN analytical balance of the first accuracy class (division value of 0.0001 g). The dry residue (D_r) was quantified by mass concentration, calculated according to the following formula:

$$D_r = m/V, \quad (1)$$

where D_r is the mass concentration of suspended solids in the water sample, mg/m^3; m is the mass of suspended solids removed from the water sample on the filter, mg; V is the volume of the water sample, m^3.

The dry residue was quantitatively transferred to an extractor and extracted in two steps with n-hexane and acetonitrile. The aqueous filtrate was divided into two parallel laboratory samples and subjected to extraction with n-hexane, buffering of the solution, followed by extraction with acetonitrile. The obtained extract was evaporated in portions of 10 cm^3 and dried to dryness in a flask of a rotary evaporator RV05 basic 2-B IKA, the concentration ratio was 30:1. The content of chemical compounds in the obtained concentrate was investigated according to chromatographic methods. The xenobiotics content was measured by high-performance liquid and gas chromatography with mass-selective detectors (HPLC/MS/MS and GC/MS) using HPLC UltiMate 3000-MSD 3200 Q TRAP and Agilent Technologies 7890-MSD 5975C instruments. Statistical processing of the experimental data was performed using the Microsoft Excel software package, the measurement error was calculated as the standard deviation (S_r, %), and the degree of xenobiotic extraction from artificially enriched laboratory samples was estimated as a percentage (r, %). The proper functioning of the measuring equipment was checked using test mixtures, and the test results were recorded and analysed using Schuchart's control cards (DSTU ISO 7870-2:2016, 2016).

RESULTS AND DISCUSSION

To perform laboratory control of the content of residual amounts of pesticide formulations in surface water, standardised or proprietary methods that have passed the validation procedure are used. However, the variability of the component composition of agrochemicals, the complexity and high cost of xenobiotic determination studies prompts researchers to further search for optimal conditions for the extraction and determination of pollutants in surface waters. The methodology proposed in this paper differs from the existing ones (Lopez *et al*., 2015; Milyukin & Gorban, 2021) by using organic solvents unified for chromatographic analysis and extraction of target substances, a lower temperature regime at the stage of evaporation of the extractant, and a new concentration interval that allows working with water samples contaminated with pesticides of different chemical groups. Validation studies of the developed model of the methodology for investigating safety indicators allow applying the methodology for monitoring surface waters of Ukraine. The search for a methodology for effective monitoring and detection of the effects of mixing surface water with various sources of pollution is described in the studies of other authors (Linlin *et al*., 2020; Pinasseau *et al*., 2023), which do not consider the content of suspended particles and their ability to absorb pesticides, but assume the presence of xenobiotics of various chemical groups in water.

At the preparatory stage of creating a new laboratory control methodology, it is necessary to assess the possibility of extracting target analytes from the sample (Tereshchenko *et al*., 2020), and, considering potential sources of contamination and the results of literature research, to create an expanded list of contaminants (Lopez *et al*., 2015; Pinasseau *et al*., 2023). The study of lipophilic xenobiotics in natural water samples is complicated by the presence of suspended particles that can be adsorbed by analytes (Milyukin & Gorban, 2021). Sample preparation for testing should include the extraction of xenobiotics from the dispersed medium (water) and their desorption from the dispersed phase (suspended particles). The main parameters that allow predicting the course of the extraction process of each xenobiotic are the value of the dipole moment of its molecule, the analyte distribution constant in the octanol-water system (C_{ow}) and the logarithm of the distribution coefficient in the octanol-water system (log*D*) (Sangster, 1997). These parameters are widely used in numerous theoretical and experimental models and studies of the distribution of differently functional chemical compounds in a system of two immiscible solvents (Ferrari *et al*., 2018; Milyukin & Gorban, 2021). Given the changes in the component composition of agrochemicals, the persistence of xenobiotics in the environment and the prevalence of the use of a certain list of pesticides (Pinasseau *et al*., 2023), the search for optimal conditions for the methodology of extraction of target xenobiotics began with the consideration of the values of lipophilicity parameters (log*D*) of the molecules of active ingredients of various pesticide formulations (Table 1), which are allowed for use in Ukraine and are subject to laboratory control in products and environmental objects (DSanPiN 8.8.1.2.3.4.-000-2001, 2001).

Table 1. log*D* parameter and pesticidal effect of xenobiotic molecules

Pesticide	log*D*	Pesticidal effect
Atrazine	2.58	Herbicide
Acetochlorine	4.14	
Bifenox	4.37	
Glyphosate	-3.12	
Dicamba	-1.88	
Paraquat	-4.50	
Acephate	-0.85	Insecticide
DDT	6.86	
Deltamethrin	6.21	
Imidocloprid	1.19	
Oxadixyl	1.38	Fungicide
Propamocarb	0.84	
Ciproconazole	3.09	
Fosetyl	-0.70	
Ethephone	-1.89	Growth regulator

Source: compiled by the authors of this study

Table 1 shows that the following pesticides cannot be extracted with octanol in the model laboratory methodology for the study of the list of pesticides: acephate, glyphosate, dicamba, paraquat, fosetyl, ethephon, since they are lipophobic (hydrophilic) compounds. Therewith, atrazine, acetochlor, biphenox, DDT, deltamethrin, imidocloprid, oxadixyl, propamocarb, and ciproconazole are lipophilic and are subject to redistribution to the organic layer. To extract xenobiotics from the samples considered in Table 1, the extraction conditions proposed in this study were applied, which differ from the extraction conditions proposed by other authors, specifically, at the first stage by gravimetric accounting of suspended particles and subsequent extraction of xenobiotics (Sackaria & Elango, 2020; Milyukin & Gorban, 2021; Milyukin & Gorban, 2022).

In general, the scheme of sample preparation according to the proposed methodology is presented in Figure 1, aimed at the extraction of target xenobiotics molecules characterised by a wide range of lipophilicity parameters (Table 1).

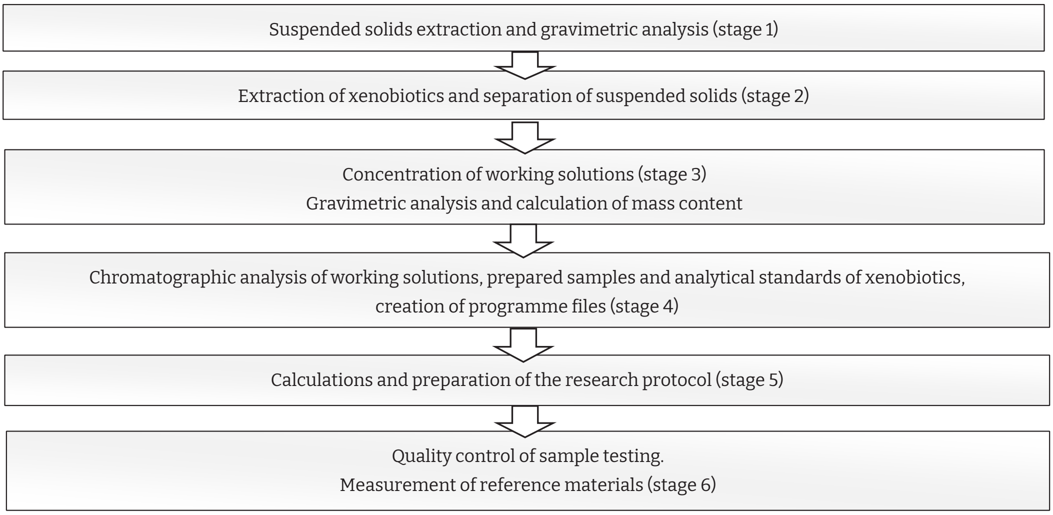

Figure 1. Schematic of the methodology for laboratory control of xenobiotics in water samples with suspended solids

Source: compiled by the authors of this study

The proposed methodology is interesting because different molecules with appropriate log*D* parameters (Table 1) can be studied in the extract, and the methodology is not overloaded with the extraction and measurement of pesticide degradation products, specifically atrazine, which was investigated by other authors (Lopez *et al*., 2015) in surface water extracts.

Since mass spectrometric methods of research allow identifying chemical compounds according to their individual characteristics, namely: retention time on the chromatographic column (*t*, min), the ratio of the ion mass to its charge (*m*/*z*). The results are used to obtain calibration dependencies (Fig. 2) and further quantitative analysis.

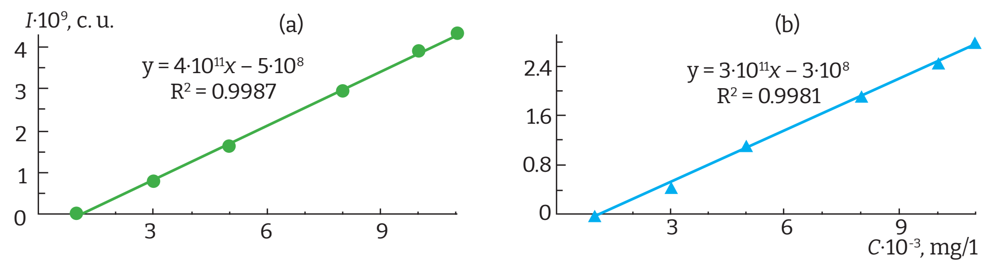

Figure 2. Scaling dependence of the intensity of the analytical signal of DDT (*a*) and imidacloprid (*b*) on their content in the calibration solution: study by GC/MS (*a*) and HPLC/MS/MS (*b*)

Source: compiled by the authors of this study

To establish the specificity of the methodology and the efficiency of the extraction of target components, mixtures of xenobiotics characterised by different log*D* values were used in the study (Table 1). Due to their physicochemical properties, hydrophilic xenobiotics are not extracted by organic extractants and do not interfere with the chromatographic control of target substances. The difference in log*D* values of lipophilic analytes is noteworthy. Based on the analysis of log*D* values, it can be expected that DDT will be extracted into the organic layer most rapidly and in larger quantities, while imidocloprid will be extracted most slowly and with concentration losses. The efficiency of lipophilic xenobiotics extraction into the organic layer can be amplified by buffering the aqueous layer with salt mixtures (Hrybova *et al*., 2019), the ions of which inhibit the ionisation of functional groups of xenobiotic molecules and shift the equilibrium towards the formation of target organic molecules, which ensures reproducibility of the results of the study of model solutions and is a more affordable way to extract pesticides compared to the method of their solid-phase extraction from water and water-containing objects (Harshit *et al*., 2017). Quantitative control of the analytes content was performed using the methods of chromatographic separation and mass spectrometric measurement described above in the experimental procedure.

Fixing the noise value and calculating the ratio of the noise value on the chromatogram to the analytical signal value of each individual xenobiotic allowed determining the limit of identification (qualitative detection) and the limit of quantification of the lipophilic substances mentioned in the study. The limit of quantification of a xenobiotic is set as the concentration of a substance that produces an analytical signal (peak) on a chromatogram, the intensity of which is 10 times higher than the intensity of system noise (signal: noise ratio ≥ 10). Considering the preliminary concentration (30 times), the limit of detection of xenobiotics is 0.02 μg/m^3, the limit of quantification of xenobiotics is 0.10 μg/m^3. The linearity of the analytes' signals on the chromatograms with their content in the samples is within 0.10 μg/m^3 to 1.00 μg/m^3. The analysis of the experimental observations and the obtained equations of calibration dependencies, together with the approximation values, indicate the high sensitivity of the mass spectrometric detectors used in this study (Fig. 2). The water samples, with a content of 135±20 to 1,500±100 mg/m^3 of suspended solids, required a stepwise extraction of the dispersion medium and dispersed phase analytes. The resulting acetonitrile extracts were combined for chromatographic control. For the metrological certification of the multi-stage methodology for measuring the xenobiotic content, a study was conducted to determine the convergence, accuracy, and reproducibility of the measurement results. Given that the amount of data required for statistical analysis can vary, four parallel water samples were prepared in each series of studies. One sample from the created parallel of samples was studied as a blank sample of surface water (without xenobiotics), which helped control matrix signals and identify non-target co-extractive substances. Three samples were studied in parallel after artificial introduction of xenobiotics in the amount of: 0.10, 0.50, and 1.00 μg/m^3. The measurements of each sample were repeated ten times, which helped obtain a sample of data for estimating the standard deviation (S_r, %). The results of the chromatographic control of artificially contaminated samples were compared with the expected xenobiotic content. The parameter that characterised the accuracy of the measurement results was the degree of xenobiotic extraction after artificial contamination, or the percentage of return (r, %) of the xenobiotic added to the sample. Quantitative parameters for assessing the convergence and accuracy of measurements are presented in Table 2.

Table 2. Results of the evaluation of xenobiotics measurement in surface water samples under convergence conditions (n=10, P=0.95)

Xenobiotic	Added to the sample	Defined	r	S_r
	μg/m^3		%	
Atrazine	0.10	0.12	120.0	5.5
	0.50	0.49	98.0	2.9
	1.00	1.00	100.0	3.1

Table 2. Continued

Xenobiotic	Added to the sample	Defined	r	S_r
	μg/m³		%	
Acetochlorine	0.10	0.10	100.0	4.1
	0.50	0.51	102.0	2.9
	1.00	1.00	100.0	2.7
Bifenox	0.10	0.11	110.0	5.4
	0.50	0.48	96.0	5.1
	1.00	1.00	100.0	3.1
DDT	0.10	0.11	110.0	2.7
	0.50	0.50	100.0	1.9
	1.00	1.00	100.0	1.5
Deltamethrin	0.10	0.10	100.0	4.8
	0.50	0.50	100.0	3.5
	1.00	1.00	100.0	1.9
Imidocloprid	0.10	0.12	120.0	5.1
	0.50	0.50	100.0	4.1
	1.00	1.00	100.0	2.9
Oxadixyl	0.10	0.11	110.0	4.7
	0.50	0.48	96.0	4.5
	1.00	1.00	100.0	2.9
Propamocarb	0.10	0.11	110.0	5.9
	0.50	0.45	90.0	3.9
	1.00	1.00	100.0	5.4
Ciproconazole	0.10	0.10	100.0	4.9
	0.50	0.50	100.0	2.5
	1.00	1.00	100.0	1.7

Source: compiled by the authors of this study

Comparison of the amounts of xenobiotics introduced into the sample with the determined amounts showed that under the optimal conditions of the proposed method, both a decrease (loss) of analyte content and an increase in analyte content occurred. As the amount of analyte added to the sample increases, the recovery rate of this substance approaches 100%. The lowest concentrations of administered xenobiotics are characterised by results that exceed the corresponding expected value. By examining the degree of extraction of substances that were added to the sample in an amount according to the values of the middle of the linear range, one can see a decrease in the parameter (90-100%). This is conditioned upon the integration of analytical signals on the chromatogram with random noise, which has the greatest impact on the signals generated by the lowest concentrations of analytes. Analysing the obtained values and considering the methodological recommendations for acceptance of measurement results (Document SANCO/12571/2013, 2013), the results obtained can be considered satisfactory, as they do not exceed the permissible deviation of the expected content (80-120%) and indicate the suitability of the methodology for measurement. In turn, the value of the standard deviation (S_r, %) can be used to assess the convergence of measurement results, as it is obtained as a result of experimental work carried out under the same conditions using the same equipment, by the same researcher, in a short period of time, i.e., under convergence conditions. The obtained standard deviation value does not exceed 6.0%, which shows the proximity of the results obtained, the result being within a fairly narrow range within the method error.

To verify the possibility of performing measurements according to the optimal conditions of the methodology by independent performers using different batches of reagents and at different times of measurement, the conditions were created, and the metrological parameters of intra-laboratory accuracy were calculated. Four parallel water samples were prepared: a blank sample and three samples with artificially added xenobiotics, µg/m³: 0.20, 0.40, 0.60. The quantitative parameters of the definition are presented in Table 3.

Table 3. Results of evaluation of xenobiotics determination in surface water samples under precision conditions (n=10, P=0.95)

Xenobiotic	Added to the sample	Defined	r	S_r
	µg/m³		%	
Atrazine	0.20	0.18	90.0	3.7
	0.40	0.39	97.5	4.3
	0.60	0.59	98.3	2.7
Acetochlorine	0.20	0.20	100.0	3.5
	0.40	0.38	95.0	2.9
	0.60	0.62	103.3	2.7
Bifenox	0.20	0.19	95.0	4.9
	0.40	0.37	92.5	4.7
	0.60	0.57	95.0	4.4
DDT	0.20	0.20	100.0	2.5
	0.40	0.39	97.5	1.9
	0.60	0.61	101.7	1.6
Deltamethrin	0.20	0.20	100.0	2.3
	0.40	0.39	97.5	3.4
	0.60	0.58	96.7	2.8
Imidocloprid	0.20	0.18	90.0	4.1
	0.40	0.41	102.5	4.0
	0.60	0.60	100.0	3.9
Oxadixyl	0.20	0.18	90.0	4.5
	0.40	0.42	105.0	4.1
	0.60	0.58	96.7	3.8
Propamocarb	0.20	0.17	85.0	5.7
	0.40	0.36	90.0	4.9
	0.60	0.61	101.7	5.0
Ciproconazole	0.20	0.20	100.0	2.5
	0.40	0.39	97.5	2.3
	0.60	0.62	103.3	2.7

Source: compiled by the authors of this study

As a result of the studies, it was found that the standard deviation of the results of determining the content of xenobiotics in water samples obtained under conditions of intra-laboratory precision ranges from 1.6 to 5.7%, which indicates the absence of systematic errors in the implementation of laboratory control according to the conditions of the proposed methodology.

CONCLUSIONS

Thus, the laboratory control of xenobiotics in surface waters with suspended solids content from 135±20 to 1500±100 mg/m³ has several features. The proposed method for determining the content of lipophilic xenobiotics in surface water samples includes several stages: separation of suspended particles from water samples by vacuum filtration using membrane filters; extraction of target compounds from the dry residue using n-hexane and acetonitrile.

The treatment of the aqueous filtrate between the n-hexane and acetonitrile extractions includes a stage of buffering the solution with salt mixtures to enhance the efficiency of xenobiotic extraction into the organic layer. Hydrophilic xenobiotics are not extracted with an organic extractant and do not interfere with the chromatographic control of target substances. The resulting extracts are concentrated thirty times in a rotary evaporator at 40°C to a volume of 1 ml. Laboratory control of xenobiotics is conducted according to high-performance liquid and gas chromatography with mass-selective detectors (HPLC/MS/MS and GC/MS).

The linearity of the analytical signal on the chromatograms was recorded in the study of xenobiotics of diverse groups in the concentration range of 0.10-1.00 μg/m^3, the regression equations are characterised by correlation coefficients (R^2) greater than 0.99. The limit of detection for xenobiotics is 0.02 μg/m^3, and the limit of quantification is 0.10 μg/m^3. The degree of analytes extraction ranges from 85 to 120%, which indicates the acceptability of the proposed xenobiotic extraction procedure.

The results of measuring the content of xenobiotics in surface water analysed in this study, obtained under conditions of convergence and intra-laboratory reproducibility, give grounds to assert that the developed method is suitable for use in other laboratories equipped pursuant to the requirements of the method. The proposed methodology will allow monitoring the content of xenobiotics in the surface waters of Ukraine. Further research in the context of this issue may expand the list of xenobiotics that can be identified by the developed methodology in surface waters.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Методика визначення залишкового вмісту активних інгредієнтів агрохімічних препаратів у поверхневих водах

Анотація. Водойми сільськогосподарського призначення є багатофункціональними об'єктами у циклі виробництва сільськогосподарської продукції. Залишкові кількості активних інгредієнтів агрохімікатів групи пестицидів можуть потрапляти і забруднювати водоймище під час виконання низки агротехнологічних завдань. Метою даної роботи стало вивчення умов вилучення з поверхневої води, що містила завислі частинки, залишкових кількостей ксенобіотиків та вимірювання їх вмісту хроматографічними методами. Для визначення оптимальних умов екстракції цільових ксенобіотиків розглянуто величини параметрів ліпофільності їх молекул. Методика визначення вмісту ліпофільних ксенобіотиків хроматографічними методами з мас-селективним детектуванням у зразках поверхневої води містить етап відокремлення завислих частинок, вміст котрих в досліджених зразках контролювався гравіметричним аналізом та варіювався в діапазоні 135-1500 мг/м^3. Екстракцію цільових сполук здійснено за допомогою н-гексану та ацетонітрилу. В ацетонітрильному концентраті визначали аналіти безпосередньо методами високоефективної рідинної та газової хроматографії з мас-селективними детекторами (ВЕРХ/МС/МС та ГХ/МС). Досягнута межа виявлення ксенобіотиків становила 0,02 мкг/м^3, межа кількісного визначення ксенобіотиків становить 0,10 мкг/м^3. Для обґрунтування можливості застосування запропонованої методики проведено дослідження показників: лінійності величин аналітичних сигналів від кількості аналітів в розчині, правильності, збіжності, прецизійності результатів вимірювань. Лінійний діапазон концентрацій застосування методики для визначення ксенобіотиків різних груп становить 0,10-1,00 мкг/м^3, характеризується величиною коефіцієнта регресії лінійної залежності вимірювання індивідуальних сполук (R^2), що перевищує 0,99. Ступінь вилучення аналітів (відсоток повернення *r*, %) знаходиться в межах 85-120 %, що вказує на прийнятність запропонованої процедури екстракції ксенобіотиків. Похибка результатів вимірювання розраховано через середньоквадратичне відхилення (S_r, %), не перевищувала 6 %. Результати дослідження показують, що розроблена методика є придатною для моніторингових досліджень залишкового вмісту активних інгредієнтів агрохімічних препаратів у поверхневих водах та прогнозування рівня забруднення водойм

Ключові слова: ліпофільні ксенобіотики; екстракція; водні об'єкти; поверхнева вода; завислі частинки; хроматографія

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