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Application of biologically active substances in agriculture preparations

Abstract. High-quality, naturally protected seeds prior to sowing, along with growth activation of seedlings, represent a promising approach to stabilising crop yield and quality. Enhancing plant resistance to dynamic environmental stresses, including harmful organisms, is one of the strategies for realising the biological potential of crop yields in breeding and seed production. This research aimed to experimentally evaluate a preparation based on humic substances, film formers, a nanocomposite, succinic acid, and microbiological carotene. Experiments were conducted using spring barley and wheat seeds. A seed encrustation technology employing a functional preparation

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was applied. Laboratory and field experiments were conducted at V. Dokuchaev Kharkiv National Agrarian University, Department of Plant Growing, over two years. The experimental design and economic efficiency assessment of the functional preparation in enhancing yield was carried out according to established methodologies. Pre-sowing seed treatment with the preparation resulted in improved field germination, synchronised seedling emergence, and increased yield. Comprehensive studies revealed that the preparation was compatible with fungicides, demonstrating a synergistic effect of their joint protective effect. Experimental results confirmed that seed incrustation with protective and stimulating formulations based on water-soluble polymers is an effective method for protecting plants from seed- and soil-borne infections while reducing the level of environmental pollution. The extended and enhanced fungicidal activity of film-forming protective and stimulating compositions was also demonstrated. Agricultural production tests indicated that the developed preparation was user-friendly, environmentally safe, and economically efficient, contributing to increased crop yields. The positive test results support practical recommendations for its application in both seed encrustation and grain crop spraying during the tillering and milky-wax ripeness phases

Keywords: growth stimulators; film-forming agents; fungicides; root rot disease; grain crop yield

INTRODUCTION

The development of biologically active preparations for plant growth is of both great scientific and practical significance. According to numerous studies, the use of plant growth regulators or biostimulants is a key component of environmentally sustainable, resource-efficient technologies for cultivating various agricultural crops. These technologies contribute to increased yield and improved nutritional quality of the resulting products (Bezpalko *et al.*, 2020; Slobodianyuk *et al.*, 2022). As noted by D. Volhin & V. Havii (2023), modern agricultural science prioritises the identification of novel methods for enhancing crop production and quality. This research focuses on strategies that minimise reliance on fertilisers and other agricultural chemicals. Simply intensifying farming by increasing fertiliser application has not resulted in proportional yield improvements. Furthermore, pesticide use can degrade soil quality and adversely affect the chemical composition of crops, including levels of vitamins, enzymes, and other essential components (Nepran *et al.*, 2021). Consequently, the search for alternative compounds capable of improving crop yields and productivity in food production remains highly relevant.

M. Matkovska (2020) presented findings from a comparative study on winter barley yield under different fungicidal protection methods across three mineral nutrition regimes

($N_{40}P_{30}K_{40}$, $N_{80}P_{60}K_{80}$, $N_{120}P_{90}K_{120}$). The impact of mineral nutrition on disease development and the effectiveness of fungicide application in controlling net blotch, powdery mildew, and dark brown blotch in the Vintmalt winter barley variety was examined. The study concluded that fungicidal protection was beneficial across all nutrition levels: however, the greatest yield increase from fungicides was observed in the $N_{120}P_{90}K_{120}$ treatment.

A proven approach for improving the sowing quality of seeds is pre-sowing treatment with growth stimulators, particularly those derived from humic substances and biologically active additives. For instance, I. Nepran *et al.* (2021) and A. Bahan & S. Nevodnychi (2023) investigated the effects of various growth stimulators on the sowing qualities of common chickpea varieties. The impact of the studied preparations on germination energy, laboratory germination, and fungicidal activity was analysed. The effect of a growth stimulant with antifungal properties on the seed quality was also examined. A minor influence of the growth stimulators on the germination energy, laboratory germination, and uniformity of germination in common chickpea varieties was observed.

Currently, there is considerable scientific interest in plant growth stimulators, as evidenced by numerous publications. These substances

are being explored both as standalone treatments and in combination with traditional organo-mineral fertilisers as bioadditives. Research also investigates their potential synergy with fungicides (Siroshstan & Kavunets, 2021). Among the most promising approaches is the development of preparations based on plant growth regulators, including biostimulants obtained through microbiological processes or chemical synthesis (Zhang *et al.*, 2024). However, the feasibility of applying microorganisms as biostimulants and biofertilisers remains uncertain due to potential risks to human health. Developing effective and safe biological agents that promote plant growth presents a significant scientific and technical challenge.

L. Krychkovska *et al.* (2024) developed and tested experimental batches of microencapsulated barley and wheat seeds under laboratory and field conditions following a formulated composition that included natural components – hydrated fullerenes, sodium humate, microbiological carotene, and succinic acid – in a specific ratio. The preparation also contained an adhesive additive, which enhanced seed adherence and plant contact, thereby improving the penetration efficiency of the preparation into the seed coat. This formulation was conditionally named Humir.

This research aimed to experimentally and practically evaluate a growth-stimulating preparation with fungicidal properties developed by the authors for application to spring barley and spring wheat using encrusted seeds. Seed encrustation with the developed preparation enhanced the uptake of biogenic nutrients by plants during the growing season. Bioadditives were continuously released from the multilayered seed coating. The primary factor contributing to growth intensification was the bioadditive, which contained plant growth stimulants and exhibited fungicidal and bioprotective properties.

LITERATURE REVIEW

According to an analysis of cereal cultivation data, particularly for spring barley, the cultivated area of this grain crop in Ukraine has declined by 56% over the past 15 years (Kasatkina & Gamayunova, 2018). As a result, interest among

commercial producers in barley cultivation has diminished in recent years. To enhance cereal yield and improve plant quality, a study was conducted to examine the impact of a formulation developed using humates, fullerene, succinates, and microbiological carotene on the yield and quality of spring barley and spring wheat. In addition, the use of film-forming compositions to ensure more stable retention of bioadditives on seeds has been justified. In the review by O. Matyshevska *et al.* (2010), the high biological efficacy and antioxidant properties of fullerene – which was incorporated as an antioxidant in the aforementioned composition – were discussed under the literature data.

In the study by T. Kasatkina & V. Gamayunova (2018), the current state and trends in the cultivation of spring barley – a valuable food, feed, and industrial crop – in southern Ukraine were analysed. The factors influencing the realisation of the crop's biological potential through the implementation of modern, competitive cultivation technologies were highlighted. Such technologies should prioritise the selection of high-yielding varieties adapted to the conditions of southern Ukraine, along with optimisation of nutrition through modern growth regulators, biological agents, and organo-mineral fertilisers. The study also described various methods of fertiliser application within traditional barley cultivation technologies. It was concluded that the use of plant growth regulators represents a modern strategy to increase barley grain yield. M. Marenych & S. Yurchenko (2016) noted that humic substances positively influence cell membrane permeability, activate enzymes involved in protein and carbohydrate synthesis, and enhance respiration and water exchange processes.

According to a study conducted by T. Kasatkina & V. Gamayunova (2018), the most effective and economically advantageous methods of applying growth regulators were seed treatment and foliar feeding of vegetative plants. Upon contact with the leaf surface, growth regulators penetrate plant tissues and become involved in the biochemical reactions of metabolism. Overall, the use of biostimulants in seed production – an advanced branch of plant breeding – and their impact on seed quality and yield

characteristics of major grain crops require further investigation. In particular, the application of biostimulants compared to pesticides, which have the potential to enhance seed germination and emergence while exhibiting as fungicidal and bactericidal properties, warrants detailed research and development.

The use of nano-formulations in crop production, which contain components with nano-sized particles, is an effective approach to enhancing the yield of major crops (Rouphael & Colla, 2018). Nanoparticles enable more effective delivery of micronutrients to plants, increasing their absorption and impact on plant health while significantly reducing fertiliser costs and minimising the environmental impact of plant protection chemicals. Research into the use of nanotechnologies in agriculture is continuously expanding (Tarazona *et al.*, 2019; Slobodianyuk *et al.*, 2022). According to O. Matyshevska *et al.* (2010), nano-sized carbon materials, particularly hydrated fullerenes, are increasingly utilised in agriculture, cosmetics, and the food industry due to their high biological activity and antioxidant, antiviral, and antimicrobial effects.

Organic non-microbial plant biostimulants, which include humic substances and seaweed extracts, continue to play a leading role in crop production, where they are used to stimulate growth, increase plant stress resistance, and improve the nutritional value of agricultural products (Marenych & Yurchenko, 2016; Godara & Bakshi, 2021). According to Z. Chen *et al.* (2023), algae serve as an important source of biologically active substances, chlorophyll, carotene, and other natural pigments. Phytohormones, which are carotene derivatives – particularly abscisic acid – function as components of plant growth regulators, providing a protective effect and enhancing plant resistance to various types of stress, as noted by T. Sun *et al.* (2022). According to H. Slobodianyuk *et al.* (2022), succinic acid acts as a universal plant growth stimulant that significantly enhances seed germination promotes root formation, and boosts yield. Pre-sowing treatment of soybean seeds with formulations containing succinic acid complexes with specific elemental ions exhibited both growth-stimulating and fungicidal effects.

MATERIALS AND METHODS

Studies on the effects of a specific preparation on spring barley and wheat growth and development were conducted at the Department of Plant Growing at V. Dokuchaev Kharkiv National Agrarian University during 2020-2021. These studies included both laboratory and field experiments. Laboratory germination involved placing seeds on a gauze-covered cotton wool pad in Petri dishes and rolled paper strips. Nutrient solutions (90 mL) were added beneath the pad. During the initial stage of seed germination, Petri dishes were covered with lids, each with a small hole to allow air penetration. Once the first seedlings emerged, the lids were removed. In the test variants, a 0.01% solution of each component under study was applied to the growing plants (the optimal concentration was determined through preliminary experiments). Tap water served as the control. Throughout the study period, the ambient temperature was regulated between 21-23°C during daylight hours and 17-18°C overnight. Plant growth was monitored daily, with measurements taken of seedling count and length. At the conclusion of the study, yield was evaluated by harvesting and weighing the plants' fresh biomass.

Field experiments were conducted following the variety testing method. Spring wheat of the Kharkivska 26 variety (super elite) and spring barley of the Zvershennia variety (super elite) were sown at optimal times using a continuous row method, with a seeding rate of 5.5 million and 5 million viable seeds per 1 hectare, respectively, using an SSFK-7 seeder (Ukraine). The plot area was 10 m², with three replications. Seed encrustation with a fungicide in a film-forming solution containing the growth regulator Humir (2% solution) was performed in the laboratory using specialised equipment. The working solution was prepared with varying concentrations of the encrusting mixture components. Humir is a complex formulation containing biologically active substances of natural origin and water-soluble polymers with a mild mode of action. It includes polyethylene glycol (PEG; polyethylene oxide) with a molecular weight of 500 (230 g/L) and PEG-1500 (540 g/L), as well as sodium humate (35 g/L), fullerene, and microbiological carotene.

The harvest was recorded continuously and systematically. The yield data were processed using the analysis of variance method. Seed quality before and after treatment was determined in the laboratory according to DSTU 4138-2002 "Seeds of Agricultural Crops. Methods for Determining Quality" (2004). Monitoring of insect population dynamics and plant damage across ontogenetic phases was conducted through weekly observations, where the incidence of diseases and insects by crop type, dominant species, and their role in plant damage were established according to the methodology of the Institute of Plant Protection (Omeliuta, 1986).

During the growing season, phytosanitary inspections of crops, as well as assessments of sooty mould, Fusarium ear rot, and Fusarium root rot, were conducted following the "Methodology for Conducting Phytopathological Studies under Artificial Infection of Plants" (2016). The experiments were conducted in the microbiology laboratory of the Yuryev Plant Production Institute of NAAS of Ukraine.

The degree of crop resistance to common diseases was determined following the scale presented in the manual by L. Babayants *et al.* (1988) "Methods of Selection and Assessment of Resistance of Wheat and Barley in the CMEA Member Countries" (Table 1).

Table 1. The rating scale for crop resistance to common diseases

Resistance, point	Disease incidence, %
9	0
8	0.1-5.0
7	5.1-10.0
6	10.1-15.0
5	15.1-25.0
4	25.1-40.0
3	40.1-65.0
2	65.1-90.0
1	91.0-100

Source: compiled by the authors

Field experiments were conducted under the climatic conditions of the moderate continental zone. The average annual air temperature ranged from 6 to 10°C. Annual rainfall totalled 450-460 mm, with the majority (320-340 mm) occurring between June and October. Meteorological conditions during the 2020-2021 growing season, compared with long-term averages, were characterised by an increase in air temperature of 1-3°C.

The soil in the experimental fields was a strongly developed, slightly leached black soil on siltyloamy loess, characterised by an agronomically valuable granular-lumpy structure with a total humus content of 5.89-6.15%. The arable soil layer contained 1.9-2.0% potassium, 0.28-0.29% nitrogen, 0.17-0.18% phosphorus, 12 mg/kg of mobile zinc, 20 mg/kg of manganese, and 0.17 mg/kg of molybdenum. Soil pH ranged

from 6.8 to 7.5. The microelement content was measured using an X-ray energy spectrometer, SER-01 "ElvaX ProShector LE" model (Ukraine). The research was conducted in compliance with national laws and regulations, following ethical guidelines for plant-related experiments (Convention on International Trade in Endangered Species of Wild Fauna and Flora, 1973; Convention on Biological Diversity, 1992).

RESULTS AND DISCUSSION

The phytosanitary situation in grain crop agrocenoses is worsening each year. The increasingly contrasting weather conditions in recent years have contributed to a decline in plant resistance to environmental stresses, including diseases and insect pests. This has increased the prevalence and aggressiveness of many harmful organisms. The greatest impact of the

phytophagous complex on plants was observed in low agrotechnical conditions, with disruptions to agricultural practices, as well as delays in sowing. During 2020-2021, the phytosanitary

condition of spring barley and spring wheat crops in crop rotations was monitored. Diseases and pests were identified at different stages of plant ontogenesis (Table 2).

Table 2. Phytosanitary condition of spring barley and spring wheat crops in 2020-2021

No.	Type of pathogen	Disease	Spring barley		Spring wheat	
			Prevalence rate of disease			
			%	point	%	point
1	<i>Fusarium culmorum</i> Sacc.	Fusarium root rot	25	5	20	5
2	<i>Helminthosporium sativum</i> P.	Common root rot	30	4	25	5
3	<i>Erysiphe graminis</i> (DC.)	Powdery mildew	20	5	20	5
4	<i>Drechslera graminea</i> Ito.	Leaf stripe	20	5	–	–
5	<i>Rhynchosporium graminicola</i> Heinsen	Rhynchosporiosis	10	7	–	–
6	<i>Ustilago nuda</i> Kell. et Sw.	Loose smut	0.01	0	–	–
7	<i>Ustilago tritici</i> Jens.	Loose smut	–	–	0.03	0
8	<i>Tilletia caries</i> Tul.	Hard wheat smut	–	–	0.1	8
9	<i>Ustilago hordei</i> Kell. et Sw.	Covered smut	0.1	8	–	–
10	<i>Puccinia graminis</i> Pers.	Stem rust	15	5	20	5

Source: compiled by the authors

The data in Table 2 indicate that, in 2020-2021, root rots were the predominant disease in barley and wheat crops. Fusarium root rot leads to seedling death, resulting in crop thinning, reduced grain mass, decreased 1,000-grain weight, empty ears, lodging, and deterioration of grain quality. Infection occurs at temperatures between 3 and 35°C, with an optimal range of 15-22°C, and in soils with moisture levels exceeding 40%. The pathogen persists on grain, plant residues, and in the soil. The unfavourable conditions for plant growth and development in 2020-2021 facilitated disease proliferation. In this context, plant protection and growth stimulation measures are crucial, which could be achieved using film-forming growth regulators such as Humir, which combines the properties of a growth regulator, adaptogen, cryoprotectant, fungicide, bactericide, and film-forming agent.

Polyethylene glycols (PEG-500 and PEG-1500) were selected as film-forming agents due to their low toxicity to humans and their ability to mitigate plant stress under adverse environmental conditions. High molecular weight PEGs administered orally to living organisms are

practically nontoxic. For PEGs with a molecular weight up to 2,000, LD50 values range from 14 to 50 g/kg when taken orally. The toxicity of PEG decreases with increasing molecular weight (Hartwig *et al.*, 2020). According to a study by L. Ma *et al.* (2024), pre-sowing seed treatment with polyethylene oxides increased osmotic pressure and cell membrane permeability, resulting in positive changes in plant metabolism. Consequently, plant resistance to stress was enhanced, and grain quality improved, particularly with an increase in protein content in grain crops.

According to the studies, the preparation had no adverse effect on seed germination, so it can be used as a film-forming agent (Table 3).

As illustrated in Table 3, seed germination increased in all treatments involving PEG500 + PEG1500 mixtures as film formers, which was attributed to the enhanced retention of the functional compound on the seeds. Furthermore, the inclusion of hydrated fullerenes and succinic acid in the formulation of the functional compound reduced the level of root rot development in germinating seeds. This finding suggests the potential of these components for use in growthstimulating formulations.

Table 3. The impact of film formers, fullerenes and succinic acid in the composition of Humir on seed germination and effectiveness against root rot for spring wheat

Film former	Film former consumption rate ¹	Degree of retention, %	Germination %	Development of root rot disease <i>Fusarium ssp.</i> , % ²
Control: sodium humates (2.0 kg/t)	–	85.6	88.2 ± 5.3	33.3 ± 3.5
Humates + PEG-500	0.2 kg/t	94.4	93.3 ± 3.0	30.4 ± 3.4
Humates + PEG-500 + PEG-1500	0.1 kg/t	94.7	96.0 ± 2.8	28.7 ± 3.2
Humates + PEG-500 + PEG-1500 + fullerenes + succinic acid	0.1 kg/t	92.3	97.0 ± 1.9	21.0 ± 2.1

Note: laboratory experiment, average values over two years, 2020-2021; ¹ – the volume of working fluid was 20 L/t; ² – root rot development was assessed in seedlings at 20 days of age

Source: compiled by the authors

In preliminary laboratory experiments with wheat and barley seeds, the optimal concentration of the preparation for pre-sowing seed treatment was determined to be within the range of 1.5-2.5%. At this concentration, the seeds retained their flowability, and the added fungicide (with filler) remained intact during subsequent planting. Field and laboratory studies on seed germination concerning preparation concentration, as well as the vigour of early growth, demonstrated that seed treatment with Humir at concentrations ranging from 0.1 to 4.0% did not exert an inhibitory effect on these parameters. However, further increases in concentration led to reductions in both laboratory and field germination, as well as growth vigour. From a physiological perspective, the positive effect was attributed to the

activation of metabolic processes. As a result, the seeds progressed through the germination stages more efficiently, which was positively reflected in subsequent plant growth and development, resulting in increased yield and improved quality.

In the studies conducted in 2020-2021, the potential for combining Humir with fungicides for seed encrustation was investigated. Field experiments demonstrated that the combination of a fungicide with Humir for the seed treatment of spring barley and spring wheat significantly increased grain yield compared to conventional fungicide treatment. The effectiveness of the growth-stimulating compound Humir was also confirmed for both pre-sowing seed treatment and foliar application in spring barley and spring wheat (Table 4).

Table 4. Phytosanitary condition of crops, yield and nutritional quality of spring barley seeds *Zvershennia* depending on the application of the Vitavax fungicide and Humir growth stimulants, 2020-2021

Variant	Root rot infection in the MWR phase ¹		Infection by loose smut	Crop yield, c/ha	Protein content in grain, %	Starch content in grain, %
	damage, %	infection rate, %				
Control (seeds and seedlings untreated)	29.0	9.0	0.1	24.0	12.75	58.50
Standard (seeds treated with Vitavax (2.5 L/t))	18.0	6.0	–	25.7	11.28	59.43
Seeds treated with Vitavax (2.5 L/t) + Humir (2.0 kg/t)	6.0	2.0	–	27.5	12.24	59.25

Table 4, Continued

Variant	Root rot infection in the MWR phase ¹		Infection by loose smut	Crop yield, c/ha	Protein content in grain, %	Starch content in grain, %
	damage, %	infection rate, %				
Same as above + spraying with Humir during tillering (250 g/ha)	6.0	2.0	–	28.0	12.25	59.30
Same as above + spraying during tillering (250 g/ha) and MWR phases (250 g/ha)	6.0	2.0	–	28.3	12.50	60.53

Note: ¹ – milky-wax ripeness (MWR) phase

Source: compiled by the authors

According to the data presented in Table 4, the maximum barley yield obtained in the seed encrustation treatments was 27.5 c/ha (+ 3.5 c/ha, or 15% compared to the control, and +1.7 c/ha, or 7% compared to conventional seed treatment). Subsequent spraying of barley seedlings with Humir led to an additional increase in grain yield of 2.6 c/ha (+10%) compared to the standard treatment. Furthermore, it was found that conventional fungicide treatment of barley seeds resulted in a 1.47% decrease in protein content compared to the control. In contrast, in the Humir treatment variant, the protein content returned to near control levels, reaching 12.24%. Additionally, subsequent spraying of barley seedlings with Humir further improved grain quality. For instance, in the variant where plants were sprayed during the tillering phase and again at the MWR phase, the protein content in barley grain was 12.5%, while starch content

increased by 2.03% compared to the control and by 1.1% compared to the standard.

In all seed encrustation variants, no deviations were observed in the progression of phenological phases compared to the control. However, significant trends and differences were noted for other indicators influencing crop formation. In 2021, the field germination rate of the studied crops in the pre-sowing seed treatment variants with Humir, a plant growth stimulator, generally exceeded the control values by an average of 3-4%. As can be seen from Tables 4 and 5, conventional seed treatment with only a fungicide significantly reduced plant damage caused by root rot and the overall infection rate, while also completely disinfecting seeds from loose smut. In contrast, seed treatment using the encrustation method with Humir, a film-forming growth stimulator, more than doubled the protection of spring wheat against root rot, which positively influenced crop yield (Table 5).

Table 5. Phytosanitary condition of crops and grain yield of spring wheat Kharkivska 26 depending on the application of the Vitavax fungicide and Humir preparation, 2020-2021

Variant	Root rot infection in the MWR phase		Infection by loose smut	Crop yield, c/ha
	damage, %	infection rate, %		
Control (seeds and seedlings untreated)	25.0	12.0	0.2	33.2
Standard (seeds treated with Vitavax (2.5 L/t))	15.0	7.0	–	35.9
Seeds treated with Vitavax (2.5 L/t) + Humir (2.0 kg/t)	7.0	2.0	–	37.2
Same as above + spraying with Humir during the tillering phase (250 g/ha)	7.0	2.0	-	38.1
Same as above + spraying during tillering (250 g/ha) and MWR phases (250 g/ha)	7.0	2.0	-	38.9

Source: compiled by the authors

As shown in Table 5, the maximum wheat yield obtained through seed encrustation treatments was 37.2 c/ha (+ 4 c/ha, or 12% compared to the control, and + 2.7 c/ha, or 8% compared to conventional seed treatment). Subsequent spraying of wheat seedlings with Humir led to an additional grain yield increase of 3.0 c/ha (+ 8%) compared to the standard treatment. It was assumed that the positive effect of spraying plants with the Humir growth stimulator was largely attributable to its high wettability and film-forming properties. After application to the leaf surface, a thin film of the compound was observed, and its active components were gradually absorbed by the plant over several days. As a result, Humir was effective in combating diseases such as powdery mildew, late blight, and bacterial infections. When infection foci appeared on the leaves and stems, careful treatment with the Humir film-forming preparation facilitated the fixation of fungal spores, effectively blocking the spread of infectious plant diseases.

Satisfying the growing food demands of the population has increased the environmental impact of chemical use and requires alternative methods to produce more food (Barros-Rodríguez et al., 2020). The use of biostimulants and biofertilisers deserves attention as an alternative solution to this problem. In this regard, plant biostimulants appear to be among the most appropriate solutions due to their natural origin and potential to replace traditional agricultural methods. According to M. Baltazar et al. (2021), humic substances, seaweed extracts, and microorganisms have demonstrated the potential to accelerate plant growth, increase crop yield and quality, and mitigate the effects of stress. Thus, humate- and bioadditive-based formulations are particularly relevant for increasing cereal yields.

To protect seeds from mould fungi, fullerenes and succinic acid were included in the developed encrusting preparation as bactericidal agents. This is especially important for pre-sowing seed treatment. Fullerenes were studied for their impact on the microbiological stability of solutions containing biologically active substances and compared with carbon-containing water infused with shungites crushed to nanosize. It was experimentally proven by

L. Krychkovska et al. (2024) that the inclusion of hydrated fullerene C60 (C60FWS, nanoparticle size 1.6-1.8 nm) in the composition of the functional preparation Humir resulted in high resistance to microbiological contamination.

The development of new complex formulations containing succinic acid is of particular interest in the creation of a functional preparation with multifaceted biological properties, including antimicrobial, growth-stimulating, and antifungal activity. Succinic acid, as a natural analogue of salicylic acid, has shown potential for increasing plant resistance to pathogens when combined with antimicrobial and growth-stimulating components. This approach enhances growth, induces antimicrobial properties, and ultimately leads to increased yields. N. Levchyk et al. (2017) investigated the effect of succinic acid solutions using biotesting methods under field conditions and *in vitro* culture. The test object was cucumber seedlings (*Cucumis sativus* L.). The study demonstrated that aqueous solutions of succinic acid can be effectively used for growing agricultural plants both *in vivo* and *in vitro*.

In the research by S. Kabdrakhmanova et al. (2023), the biological effectiveness of succinates in soybeans was investigated, with the aim of increasing the germination rate while reducing the presence of phytopathogens and dwarfism. Complexes of succinic acid with copper, silver, and boron ions exhibited a distinct morphology with a monoclinic crystal structure. These complexes demonstrated a dual effect – both stimulating and fungicidal – significantly increasing the germination rate by 19.7-24.4 times and reducing susceptibility to phytopathogenic organisms such as *Fusarium* and *Alternaria tenuis* Nees by 2.75-8.66 times compared to the control sample.

In the study by L. Krychkovska et al. (2024), it was found that the treatment of spring wheat seeds with the carotene-containing Humir preparation resulted in an increase in the number of plants and spikelets, as well as the dry weight of plants, compared to the same preparation without carotene. Thus, the composition and effects of the developed preparation on seed germination align with the general trend in the combined application of its components, as demonstrated by multiple studies cited above.

PEG enhances osmotic regulation and antioxidant capacity, succinic acid and fullerenes exhibit antifungal activity, and their synergistic action contributes to increased crop yields.

The method of seed encrustation using water-soluble polymers has been identified as the most successful form of pre-sowing seed treatment, as demonstrated in the overview by M. Baltazar *et al.* (2021). This method improves the adhesion of preparations, does not impede seed respiration and moisture absorption, and increases resistance to drought and low temperatures. Ultimately, seed encrustation with biologically active substances enhances crop yields in plant growth technologies. Recently, the number of studies on the development of film-forming compositions has increased. These formulations include, in addition to pesticides and polymers, organic and mineral nutrients, growth stimulants, and surfactants. Their application strengthens and expands the fungicidal and bactericidal effects on phytopathogenic and mould fungi. Increased adhesion of preparations and the creation of a nutrient reserve on the seed surface enhances germination and improve the physiological status of plants.

Enhancing grain yield through the use of biostimulants has shown a positive trend in response to changing climatic conditions in Ukraine. However, sustainable grain production under sharp fluctuations in weather conditions is only possible through improvements in agrotechnological schemes. Therefore, testing the developed preparation, as conducted in this research, is relevant for increasing crop yields and antifungal activity. It has been demonstrated that the seed treatment method involving the encrustation of grain seeds with plant growth regulators is ecologically safe and increases crop yields by up to 20%.

CONCLUSIONS

A complex preparation for crop production, named Humir, containing biologically active substances of natural origin and water-soluble polymers with mild effects, has been tested for its properties as a growth regulator, adaptogen, cryoprotectant, fungicide, bactericide,

and film-forming agent. Presowing treatment of grain seeds with Humir led to increased field germination, synchronised seedling emergence, and higher yield. Comprehensive studies have revealed that Humir is compatible with fungicides, demonstrating a synergistic protective effect when used together. Experiments have shown that encrusting seeds with protective and stimulating formulations based on water-soluble polymers is an effective method for protecting plants from seed- and soil-borne infections and contributes to reducing environmental pollution. A range of film formers based on water-soluble polymers, suitable for the development of protective and growth-stimulating formulations for presowing seed treatment, has been identified. The use of two-component polymer systems has been shown to enhance the adhesion of preparations to seeds and increase the biological activity of protective and stimulating formulations. The effectiveness of film-forming protective and stimulating compositions in enhancing and prolonging the fungicidal action of treatments has been demonstrated. The prospects of seed encrustation with protective and stimulating formulations based on watersoluble polymers have been confirmed for growing healthy plants and reducing environmental pollution.

Agricultural production tests have shown that the Humir preparation is convenient to use, environmentally safe, and economically beneficial, as evidenced by increased crop yields. The positive test results provide a basis for practical recommendations for its use in agricultural production, both for seed encrustation and for spraying grain crops during the tillering and milkywax ripeness phases. Further research on this topic will explore synergistic biostimulant effects and contribute to the development of next-generation plant biostimulants for sustainable agriculture.

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CONFLICT OF INTEREST

None.

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Застосування біологічно активних речовин у препаратах для сільського господарства

Анотація. Високоякісне, природно захищене в посіві насіння та роста активація сходів є перспективним напрямом стабілізації величини і якості урожаю. Одним із шляхів реалізації біологічного потенціалу урожайності сільськогосподарських культур в селекції і насінництві є підвищення стійкості рослин до динамічних екологічних стресів, в тому числі і до шкідливих організмів. Метою дослідження було експериментальне тестування препарату на основі гумінових речовин, плівкоутворювачів, нанокompозиту, янтарної кислоти та каротину мікробіологічного. Експерименти проведено на насінні ярого ячменю та ярої пшениці. Використано технологію інкрустування насіння функціональним препаратом. Лабораторні та польові експерименти проводили на базі Харківського національного аграрного університету ім. В.В. Докучаєва на кафедрі рослинництва протягом двох років. Постановку експериментів та оцінку економічної ефективності функціонального препарату у підвищенні врожаю проводили за встановленими методиками. Передпосівна обробка насіння зернових культур препаратом забезпечувала зростання польової схожості, синхронності сходів і урожайності. Різносторонні випробування довели, що препарат сумісний з протруювачами насіння, при цьому проявляється синергізм їх спільної дії, а саме підвищення фунгіцидного ефекту. Результати експериментів показали, що інкрустація насіння захисно-стимулюючими препаратами на основі водорозчинних полімерів є ефективним методом захисту рослин від насінневих та ґрунтових інфекцій і способом

зниження рівня забруднення навколишнього середовища. Доведено посилення та пролонгація дії фунгіциду при застосуванні в плівкоутворюючих захисно-стимулюючих композиціях. Виробничі випробування показали, що препарат зручний при використанні, екологічно безпечний, а господарська ефективність препарату проявляється у збільшенні урожайності. Позитивні результати випробування дали підстави для рекомендацій щодо його застосування у виробничих умовах як для інкрустації насіння, так і для обприскування рослин зернових колосових культур у фазу куцнення та молочно-воскової стиглості

Ключові слова: стимулятори росту; плівкоутворювачі; фунгіциди; кореневі гнилі; урожайність зернових культур

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Effects of seed treatment and foliar fertilisation by chelated fertilisers on the productivity of sugar beets (*Beta vulgaris* L.)

Abstract. Sugar beets are a highly productive crop that requires a significant amount of micronutrients and trace elements. They are sensitive to a lack of micronutrients in the early stages of development, which can affect the morphological changes in the root crop and, consequently, reduce yield. The purpose of this study was to determine the effects of seed treatment with a complex of chelated fertilisers and the effects of foliar feeding on the biometric indicators of sugar beets, photosynthetic productivity, and technological qualities. The study was conducted on typical low-humus chernozems in the Right-Bank Forest-Steppe zone of Ukraine. To determine the mass of roots and leaves, leaf area, and photosynthetic productivity, samples were taken during the row closure phase, the mid-phase of intensive root growth, and the row reopening phase. Technological

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qualities, root yield, and sugar content were determined in plants selected at the physiological maturity phase. The study found that foliar feeding significantly increased root mass during the row reopening phase and physiological maturity. The highest root mass was formed in the seed treatment variant with a high content of trace elements and foliar feeding. The root mass reached 692 g with a yield of 59.6 t/ha and a sugar content of 16.6%. Technological indicators, particularly sugar yield for this variant, were also maximal at 9.86 t/ha. Nanofertilisers for foliar feeding enabled an increase in leaf area until the row reopening phase without reducing net photosynthetic productivity, which allowed the crops to accumulate more dry matter. Seed treatment with chelated fertilisers increased the accumulation of dry mass in the early stages, leading to a larger photosynthetic apparatus, which collectively resulted in increased productivity

Keywords: nanofertilisers; net photosynthesis; leaf area; dry matter; mass of the root crop; productivity; sugar content

INTRODUCTION

The creation and implementation of new environmentally safe and technological preparations designed to increase the efficiency of plant nutrient use of mineral fertilisers and soil is relevant for the global agricultural sector. According to D. Kour *et al.* (2020), biofertilisers, consisting of beneficial microorganisms, offer a sustainable and eco-friendly alternative to chemical fertilisers by enhancing soil nutrient availability and promoting plant growth through various direct and indirect mechanisms, ultimately improving crop yields without harming the environment. In their research on soybeans, S. Kalenska *et al.* (2022) highlighted the potential for advanced fertiliser technologies, including nanofertilisers, to offer targeted nutrient delivery, minimising environmental impact while maximising crop yield. As mentioned by N. Ahirwar *et al.* (2020), this helps to increase crop yields and product quality while increasing the overall efficiency of crop cultivation. The development of innovative technologies for growing plants in crops, such as nanofertilisers, growth-stimulating complexes, etc., are starting to play a significant role in increasing the efficiencies and quality of crops (Marchiol *et al.*, 2020). T. Farooq *et al.* (2023) highlighted the shift from conventional chemical fertilisers, which have a negative environmental impact, towards the use of nanofertilisers in sustainable agriculture. Nanofertilisers, utilising nanomaterials, offer controlled nutrient release and enhanced plant growth while minimising ecological harm.

The long-term availability of all nutrients to the plant during the full growing period is crucial to promote germination, growth, flowering, and fruiting (Koch *et al.*, 2020). The undoubted prospects for the development and widespread use of nanotechnology are that nanoparticles of biogenic metals have extremely high activity and sizes corresponding to the size of living cells, contribute to an increase in yields, gross harvests of crops, and product quality; increase the efficiency of nutrient use and their intended use, and reduce the toxicological burden on the environment due to a significant reduction in the gross volume of fertiliser and pesticide application (Gowda *et al.*, 2022).

According to J. Wang *et al.* (2022), the controlled release of nanofertilisers (nanoparticles) enhances plant growth and development while contributing to increased yields and overall productivity. The targeted delivery of nutrients using nanoparticles is employed to optimise the production process by directing their movement through specific areas of functional efficiency (Helal *et al.*, 2023). N. Konappa *et al.* (2021) explored how nanotechnology, specifically through nanopesticides and nanofertilisers, can revolutionise agriculture in developing countries by providing controlled release of agrochemicals, enhancing nutrient efficiency, and minimising environmental risks associated with conventional farming practices. The encapsulation of fertilisers and pesticides within nanoparticles, enabling a “slow release” mechanism, offers the potential

for precise dosage delivery to plants. This not only significantly reduces chemical waste and environmental contamination but also optimises nutrient uptake, thereby improving crop yields and promoting sustainable agricultural practices. A. Elnahal *et al.* (2022) highlighted that incorporating nanomaterials into crop cultivation technologies ensures consistently high yields while reducing the environmental impact of agricultural practices. Specifically, M. Rizwan *et al.* (2019) found that the effects of these changes depend on varietal characteristics, the phase of plant development, and the intensity and duration of stress factors. The researchers found that foliar applications of titanium dioxide and silicon nanoparticles can effectively mitigate cadmium toxicity in rice plants, enhancing photosynthesis, and improving the antioxidant defence system.

The purpose of the present study was to determine the effect of chelate nanofertilisers for seed treatment and foliar fertilisation on the formation of photosynthetic parameters (leaf area, dry biomass accumulation, net productivity of photosynthesis), plant biometric indicators, and the productivity of yield and sugar content. Since microelements improve the course of physiological cycles and help counteract stress, they assist in realising the yield potential under existing conditions. Therefore, their effectiveness in field conditions should be considered.

MATERIALS AND METHODS

The field study was conducted at the farm enterprise "Rasavske", located in the Right-Bank Forest-Steppe zone of Ukraine (49°46' N, 30°44' E) in 2021-2022. The weather conditions are presented in Table 1.

Table 1. Weather conditions in 2021-2022 in the Right-Bank Forest-Steppe zone of Ukraine

Month	Air temperature, °C			Precipitation, mm		
	2021	2022	MA	2021	2022	MA
IV	10.5	13.3	8.6	55.7	23.1	45
V	15.4	18.4	15.0	38.0	42.1	44
VI	20.6	20.2	18.0	27.7	162.6	77
VII	20.9	21.1	19.4	18.5	103.8	88
VIII	22.4	22.0	18.7	22.7	21.2	61
IX	17	16.7	14.2	18.5	123.5	41
Average/sum	17.8	18.6	15.7	291.1	476.3	356

Note: MA – multi-annual average/sum based on data from 1990 to 2010

Source: developed by the authors

The weather conditions during the years of the study varied significantly from each other and from the long-term average. Notably, the beet growing season (late April to late September) in 2021 was characterised by high temperatures (+17.8°C) and lower moisture availability compared to the long-term average. The total precipitation was 291.1 mm, with a small amount in the summer months. In contrast, the conditions in 2022 were more favourable, with higher precipitation and an average temperature of +18.6°C. The total precipitation for that year was 476.3 mm during the beet growing season, with a peak in June (162.6 mm). These conditions are conducive to the development of a robust leaf apparatus, and consequently, increased root biomass and sugar content. Considering the contrasting weather

conditions of the years under study, the research on the effects of foliar application and seed treatment was comprehensive and addressed abiotic stresses caused by weather factors.

Soil of research plot was a typical low-humic chernozem, with the arable layer characterised by the following agrochemical and agrophysical indices: pH_{KOH} – 6.8; humus content – 4.9%; nitrogen content (alkaline-hydrolysed) – 145.5 mg per 1 kg of soil; labile phosphorus – 38.5 mg per 1 kg of soil; exchangeable potassium – 117.5 mg per 1 kg of soil. Micronutrient content of soil was as follows: Fe – 0.26 mg kg⁻¹; Mn – 5.21 mg kg⁻¹; Cu – 0.08 mg kg⁻¹; Zn – 0.11 mg kg⁻¹. The research design for investigating the effects of nanofertilisers on sugar beets of cultivar Nastya is presented in Table 2.

Table 2. Research design

Factor A: seed-treatment composition, g L ⁻¹ of solution				Factor B: Fertilisation system
Option	NCF SMP	NCF Zn	NCF P	
Control	–	–	–	B1. N ₁₂₀ P ₈₀ K ₂₀₀ + foliar fertilisation with Boron
T1	7	11	13	
T2	9	8	8	B2. N ₁₂₀ P ₈₀ K ₂₀₀ + foliar fertilisation with NCF
T3	8	12	7	

Note: NCF SMP – Nano Chelate Fertiliser Super Micro plus

Source: developed by the authors

The fertilisation system factor included two options: a background with spraying of Boron (500 g of Boron acid) and foliar fertilisation. The baseline option involved the application of NPK in autumn at 120:80:200 kg/ha. The alternative option involved the application of preparations according to the scheme

presented in Table 3 (Boron was applied at same stages). Aqueous solutions of fertilisers were prepared before spraying, which was conducted in the evening after 18 hours with a sprayer with a working fluid consumption rate of 300 l/ha. Sprayer OP-2000, volume 2000 l, working solution rate – 300 l/ha.

Table 3. Scheme of foliar fertilisation with nanochelate fertilisers

#	Application stage	Fertilisation	Rate, kg ha ⁻¹
1	8-10 leaves	Nano Chelate fertiliser Potassium 23% (P)	1.0
		Nano Chelate fertiliser Nitrogen 20%	0.2
		Nano Chelate enriched fertiliser Iron 10%	1.0
2	12-14 leaves	Nano Chelate Fertiliser Super Micro plus (SMP)	1.0
		Nano Chelate fertiliser Magnesium 25%	1.0
3	16-18 leaves	Nano Chelate fertiliser Potassium 23% (P)	2.0
		Nano Chelate fertiliser Calcium 25%	0.5

Note: fertiliser developer information

Source: developed by the authors

Nanopreparations for seed treatment contain micronutrients and macronutrients in a rapidly accessible form. Since certain micronutrients can exhibit phytotoxicity on seedlings,

their concentration in the treatment solution is maintained within permissible ranges. Nutrient content of applied fertilisers upon seed treatment is presented in Table 4.

Table 4. Nanofertiliser characteristics

Variant	Nutrient content in fertiliser, %											
	Fe	Zn	Mn	K	Mg	Cu	N	P	P ₂ O ₅	Mo	Ca	B
SMP	4.5	8	0.8	3	6	0.65	5	3	7.8	0.1	6	0.65
Zn		20					5					
P								25	65			
Nutrient content in seed treatment composition, mg L ⁻¹ of solution												
T1	315	2,760	56	210	420	45.5	900	3,460	9,000	7	420	45.5
T2	405	2,320	72	270	540	58.5	850	2,270	5,900	9	540	58.5
T3	360	3,040	64	240	480	52	1,000	1,990	5,170	8	450	52.0

Note: fertiliser developer information

Source: developed by the authors

The area of the individual plot was 40 m² (2.7 m × 15 m, 8 rows). The row spacing was 45 cm. Sowing was carried out in the third decade of April (22 April 2021 and 25 April 2022). The repetition of the experiment was threefold. Seed rate was 100 thsd seeds/ha. Field seed germination was 89.3-90.4%. The study was conducted using bioadaptive technology of sugar beet cultivation. The main soil tillage was semi-steam tillage. It included the following operations: stubble was peeled 5-6 days after harvesting the predecessor (winter wheat) with a universal disk stubble harvester UDA-2.4-20, which was aggregated with a tractor MTZ-82 to a depth of 8-10 cm. Under the predecessor (winter wheat), the following fertilisers were applied: potassium chloride – 120 kg/ha (potassium content 60%), phosphorus fertilisers – 80 kg/ha (ammonium phosphate with a phosphorus content of 50% and nitrogen content of 12%), nitrogen fertilisers – 200 kg/ha (ammonium phosphate in autumn and urea with a nitrogen content of 34% in spring).

Ploughing was carried out until August 15 after applying mineral fertilisers to a depth of 30-32 cm with a Lemken EuroDiamant 100-F 7 plow in combination with a CLAAS-Axion 840 tractor. In the first decade of October, the area was levelled, and weeds were destroyed with a USMK-5.4 cultivator, the working bodies of which were arranged for continuous tillage. In spring, the moisture was closed and pre-sowing tillage was conducted with the USMK-5.4 working razors for continuous tillage to a depth of 4 cm. Sugar beet was sown with a Kleine Multicorn precision beet seeder to a seeding depth of 4 cm. The seeds were treated with Cruiser, Force, Tachigaren, TMTD with a fraction of 3.5-4.5 mm. Sowing was carried out with KZK-6P rollers, which were aggregated with an MTZ-82 tractor.

The crop management system was aimed at controlling the number of weeds – spraying (first application) was performed when the weeds were in the cotyledon to two-leaf stage with Betanal Expert herbicides at 1 L/ha, Kariibu – 30 g/ha with Trend 90 adhesive at 0.3 l/ha, the second application was performed when new weed shoots appeared, from the cotyledon to the stage of 2 leaves, according to the same scheme. Against annual cereals in the phase of 2-4 leaves in weeds, the herbicide Fusilade

Forte 150 ES, e.e. was used at 1-2 l/ha. To protect plants from pests, the insecticide Nurel-D was used at 0.8 l/ha. Herbicides and insecticides were applied with an OP-2000 sprayer, which was aggregated with an MTZ-82 tractor. To protect against diseases of the leaf apparatus, if necessary, the fungicide Alto-Super 330 ES, c.e. with a consumption rate of 0.5 l/ha was used, spraying was performed with a knapsack sprayer. Sugar beet was harvested in late terms (1 October).

The research programme included the selection of plant samples across three stages of development: Stage 1 – leaf canopy cover interrows (Date 10.07), Stage 2 – intensive root growth (Date 10.08), and Stage 3 – pre-harvest (leaves discovered interrows, 10 September). At each growth phase, a minimum of 10 plants were sampled to determine the mass of roots and leaves. Leaf area was assessed by scanning the leaf surface of each plant, calculating the average and extrapolating to density per hectare.

The dry matter content was determined using a thermogravimetric method in a two-phase scheme. Samples were dried at 60°C for 12 hours, followed by further drying at 105°C until a stable weight was achieved. Accumulation of dry mass was calculated as the difference between the actual weight and the weight in the previous phase. Additional samples were taken during the Emergency phase (“E” in the table) to determine dry matter content and leaf area.

Net photosynthetic productivity (NPP) was calculated using the following formula (1):

$$NPP = \frac{DMA_n - DMA_{n-1}}{LA \times T_v}, \quad (1)$$

where *DMA* is the accumulation of dry biomass (*n* is a current stage, *n-1* is a previous stage), g/m²; *LA* is the average leaf area between two stages, m²/m²; *T_v* is the duration of the recording period, days.

Root mass for yield determination, sugar content, and sugar yield were assessed from samples obtained during the technological ripeness phase of beetroot as of 30 September. Plant density per hectare for individual plant metrics was calculated separately for each variant. Sugar content was determined by digesting the juice in a saccharimeter. Statistical calculations

were performed using Statistica 10.0. Two-way ANOVA and post-hoc analysis based on Tukey's HSD were conducted to determine differences between variants. The study followed the ethical standards outlined in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973) and the Convention on Biological Diversity (1992).

RESULTS

The positive effects of using nanofertilisers for seed treatment are manifested in an increase

in root mass during the phase of row closure (Table 5, Stage 1). The study found that foliar application significantly increased root mass in the row closure phase compared to similar seed treatment options without foliar application. Seed treatment with the T3 formulation led to a significant increase in root mass even without foliar application (182 g), and therefore the gain from foliar application was negligible. This fact indicates the effectiveness of foliar fertilisation, as on average, root weight gains were greater than the effect of this factor at this stage.

Table 5. Root, shoot, and plant weight of sugar beet during vegetation (average in 2021-2022), g

Factors		Stage 1			Stage 2			Stage 3		
F1	F2	R	L	Plant	R	L	Plant	R	L	Plant
C	B	171 ^a	276 ^{bc}	446 ^a	319 ^a	289 ^a	608 ^a	479 ^a	211 ^a	690 ^a
	B+FF	187 ^c	289 ^d	476 ^b	347 ^c	309 ^{ab}	656 ^c	506 ^c	220 ^{abc}	726 ^{cde}
T1	B	175 ^{ab}	266 ^{ab}	442 ^a	328 ^a	304 ^{ab}	632 ^{abc}	502 ^{bc}	215 ^{ab}	717 ^{bcd}
	B+FF	190 ^c	282 ^{cd}	471 ^b	341 ^{bc}	310 ^b	651 ^{bc}	518 ^c	230 ^c	747 ^e
T2	B	176 ^{ab}	264 ^a	440 ^a	327 ^a	308 ^{ab}	635 ^{abc}	487 ^{ab}	221 ^{abc}	708 ^{abc}
	B+FF	185 ^c	280 ^{cd}	465 ^b	341 ^{bc}	296 ^{ab}	636 ^{abc}	509 ^c	227 ^{bc}	736 ^{de}
T3	B	182 ^{bc}	266 ^{ab}	448 ^a	330 ^{ab}	298 ^{ab}	627 ^{ab}	479 ^a	221 ^{abc}	697 ^{ab}
	B+FF	185 ^c	282 ^{cd}	467 ^b	341 ^{bc}	303 ^{ab}	643 ^{bc}	513 ^c	226 ^{bc}	735 ^{de}
Average		181.3	275.5	456.8	334.0	302.2	636.2	498.8	221.3	720
CV, %		4.2	4.0	3.5	8.5	4.3	5.9	7.1	4.1	5.8

Note: R – root weight, g; L – leaves weight, g; Stage 1 – leaf canopy cover interrow; Stage 2 – intensive root growth; Stage 3 – pre-harvest (leaves discovered interrows); B – background; B+FF – background + foliar fertilisation. Same letters in column show no significant differences between variants by Tukey's HSD

Source: developed by the authors

The weight of the leaf surface in Stage 1 was the highest in the control variants of treated seeds (276 g and 289 g, respectively, in the system without and with foliar fertilisation), but was significantly lower in the nanopreparation treatment. At the same time, the total mass of the plant indicated that as early as at this stage, the processes of assimilation of dry matter and biomass in the root were dominant. The largest weight was formed by variants with foliar fertilisation (465-476 g), without a significant difference in the factor "seed treatment", while the plant weight was within 440-448 g without foliar fertilisation.

The root weight in Stage 2 repeated the trend of the previous stage, as foliar treatments gave significant increases compared to options without foliar application, considering the same seed treatments. Notably, the greatest effect of foliar

application was observed in the control variant, which resulted in a 28 g increase (+8.8%), while the others had a lower effect. Leaf mass in this stage varied little among the treatments and averaged 302.2 g. Most variants did not differ significantly from each other, with only the "T1 with foliar fertilisation" combination showing a significant difference from the untreated control. Foliar application significantly increased plant mass compared to the variant without its use, but for the T2 seed treatment, the difference was negligible (including the control variant).

In Stage 3, the root weight increased to 479-518 g, while the stem and leaves decreased to 211-230 g. The fertiliser system factor (presence or absence of foliar fertilisation) significantly influenced any variant of seed treatment with nanopreparations. The effectiveness of nanopreparations for seed treatment was manifested

only in the formulation T1, which significantly increased the root weight compared to the control and other variants – up to 502 g without foliar fertilisation and up to 418 g with their application. The total weight of the plant when applying nanopreparations for seed treatment was significantly greater both in the fertiliser system with and without foliar application, so seed treatment can be considered as a factor in increasing crop productivity with a long-term effect. Notably, the T3 seed treatment formulation without foliar application did not provide a significant increase compared to the control in terms of root growth and overall plant growth.

Trace elements in nanofertilisers play a vital role in the functioning of the enzymatic and photosynthetic systems of plants, and therefore their effects on dry matter accumulation are indirect. Since an increase in the concentration of

trace elements in a plant does not directly affect the dry matter growth, as with macrofertilisers, only the indirect effects on biometric parameters can be assessed or a nutrient deficiency can be stated. The study found that the investigated factors significantly affected the leaf area index in all the studied periods, but with different effects in the combination of factors (see Table 6). On the control variant without seed treatment in stage 1, the crops formed 27.6 thsd m²/ha of leaves in the variant without foliar fertilisation and 29.6 thsd m²/ha with their application (this variant also significantly differed from all others). The use of nanopreparations for seed treatment positively influenced the leaf area in the variants T2 and T3 in the system with foliar fertilisation, but when they were applied, it had the opposite effect – the leaf area was insignificantly lower than without seed treatment.

Table 6. Leaf area and photosynthetic parameters of sugar beet

Factors		LA, thou. m ² ha ⁻¹			DM accumulation, g m ⁻²			NPP, g DM per m ² ×day		
F1	F2	S1	S2	S3	E-S1	S1-S2	S2-S3	E-S1	S1-S2	S2-S3
C	B	27.6 ^a	37.7 ^a	27.6 ^a	469 ^a	312 ^a	180 ^{ab}	7.5 ^a	3.2 ^a	1.8 ^{bc}
	B+FF	29.6 ^d	41.4 ^{cd}	30.8	519 ^c	318 ^a	165 ^a	7.7 ^{ab}	3.0 ^a	1.5 ^a
T1	B	27.8 ^{ab}	40.7 ^{bcd}	28.6 ^b	480 ^{ab}	330 ^a	190 ^{bc}	7.6 ^{ab}	3.2 ^a	1.8 ^{bc}
	B+FF	28.8 ^{bcd}	42.5 ^d	32.2 ^{cd}	511 ^c	318 ^a	208 ^{cd}	7.8 ^b	3.0 ^a	1.8 ^{bc}
T2	B	28.2 ^{abc}	41.6 ^{cd}	28.2 ^{ab}	480 ^{ab}	338 ^a	166 ^a	7.5 ^a	3.2 ^a	1.6 ^{ab}
	B+FF	28.9 ^{cd}	39.7 ^{abc}	31.7 ^c	507 ^c	305 ^a	216 ^d	7.7 ^{ab}	3.0 ^a	1.9 ^c
T3	B	28.4 ^{abc}	38.2 ^a	28.8 ^b	489 ^b	314 ^a	164 ^a	7.6 ^{ab}	3.1 ^a	1.6 ^{ab}
	B+FF	29.3 ^{cd}	38.6 ^{ab}	32.7 ^d	509 ^c	316 ^a	208 ^{cd}	7.6 ^{ab}	3.1 ^a	1.9 ^c
Average		28.6	40.1	30.1	495.5	319.0	187.2	7.6	3.1	1.7
CV, %		3.4	5.2	6.6	5.4	13.6	13.2	2.6	11.9	12.2

Note: Stage 1 – leaf canopy cover interrow; Stage 2 – intensive root growth; Stage 3 – pre-harvest (leaves discovered interrows); B – background; B+FF – background + foliar fertilisation. Same letters in column show no significant differences between variants by Tukey's HSD

Source: developed by the authors

An analogous trend was observed in the next period is the maximum growth of the leaf surface. In the variant without seed treatment with nanopreparations, the leaf area was 37.7 thsd m²/ha under the fertiliser system without foliar fertilisation and increased to 41.4 thsd m²/ha with their implementation. At the same time, the T1 formulation was effective in both systems, as the leaf area increased by 3.0 thsd m²/ha (8.0%) and 1.1 thsd m²/ha (2.7%), respectively. When treating the seeds with the T2 formulation, the effect was different: the leaf area increased

significantly in the system without foliar fertilisation by +3.9 thsd m²/ha (10.3%), and in the system with foliar fertilisation, conversely, it decreased by 1.7 thsd m²/ha (-4.1%). Formulation of T3 was generally ineffective in the variant without foliar fertilisation and led to a decrease in leaf area when they were performed.

Since the activity of the photosynthetic apparatus depends on the leaf area and its viability during the growing season, it is advisable to evaluate this indicator at the time of harvesting (Stage 3). The study found that the effect of seed

treatment and foliar fertilisation was positive in terms of preserving a viable leaf surface. In the control variant of seed treatment (no treatment) under the fertiliser system without foliar application, the leaf area was 27.6 thsd m²/ha, while with fertilisation it was 30.8 thsd m²/ha. Under the fertilisation system without fertilisation, seed treatment allowed preserving more leaf surface – 28.6 thsd m²/ha and 28.8 thousand m²/ha (T1 and T3, respectively), which is significantly more than in the control. During foliar fertilisation, different formulations of nanopreparations for seed treatment showed differing effects. The most effective was the T3 formulation, where the leaf area in Stage 3 was 32.7 thsd m²/ha, which is 1.9 thsd m²/ha (+6.2%) more than in the control variant, while in T1 and T2 the increase was 1.4 thsd m²/ha (4.5%) and 0.9 thsd m²/ha (2.9%), respectively.

The net photosynthesis productivity (NPP) in the period from emergence to closure of rows varied insignificantly within the fertilisation system, i.e., did not depend on seed treatment. NPP during this period averaged 7.6 g DM/m² of leaves per day, with no significant difference between treatments except for the “T1 with foliar fertilisation” combination (7.8 g DM/m² × day). The dry mass accumulated by the crops varied more significantly. Under the basic fertilisation system, the crops accumulated 469 g/m² of dry matter, and when the seeds were treated with the T1 and T2 formulation, this indicator increased slightly (+11 g/m²), while T3 had a more significant effect (+20 g; +4.3%). Seed treatment with nanopreparations with foliar application increased biomass significantly compared to variant without foliar treatment, but in context of seed treatment difference between variant is absent). In the period from row closure to intensive root growth, the NPP did not differ significantly depending on the fertiliser or seed treatment and amounted to 3.0-3.2 g DM/m² per day. This is

explained by the variation in the accumulation of dry biomass during this period (CV 13.6%), and therefore the variants did not differ significantly from each other, although this indicator increased with foliar treatment. In the future (from intensive growth to the opening of the rows), the NPP decreased and ranged within 1.5-1.9 g of DM/m² per day. In the variant with foliar fertilisation without seed treatment, a significant decrease in NPP was observed, whereas in the other variants, this indicator did not change significantly. At the same time, foliar fertilisation positively influenced the accumulation of dry matter. When treated with the T1 and T3 formulation, the amount of dry matter increased by 15.6% (208 g), while in T2 it increased by 20.0%.

Since after the phase of intensive growth of root crops and opening of rows the redistribution of dry matter between the root and leaves occurs and trace elements play a vital role in these processes, the direct influence of these factors on the yield of root crops, their sugar content, and sugar yield per area should be considered. Indicators of root weight and root yield in the authors' studies have an almost linear relationship because the plantation density in all plots was within the same range with minor fluctuations in some variants. The study found that foliar application of fertilisers significantly increased root mass in all variants compared to control without foliar fertilisation (Table 7). The largest root mass was observed in the “T2-foliar fertilisation” combination at 692 g, while in T2 and T3 variants this value was slightly lower (672-684 g). This allowed achieving a yield level of 59.6 t/ha in the best variant, which is 14.4% greater than the control variant. The average sugar content in the study was 16.2%. There was a significant difference in sugar content between the control variant without foliar treatment (15.8%) and the T2 and T3 seed treatments with foliar fertilisation (16.6%).

Table 7. Root weight, root yield, and sugar yield as of 30 September

Factors		Root weight, g	Root yield, t ha ⁻¹	Sugar content, %	Sugar yield, t ha ⁻¹
F1	F2				
C	B	608	52.1	15.8 ^a	8.25 ^a
	B+FF	652 ^{ab}	55.9 ^{ab}	16.2 ^{ab}	9.06 ^{bc}
T1	B	637 ^a	54.7 ^a	16.0 ^{ab}	8.76 ^{ab}
	B+FF	644 ^a	55.1 ^a	16.4 ^{ab}	9.05 ^{bc}

Table 7, Continued

Factors		Root weight, g	Root yield, t ha ⁻¹	Sugar content, %	Sugar yield, t ha ⁻¹
F1	F2				
T2	B	672 ^{bc}	57.6 ^{bc}	16.2 ^{ab}	9.29 ^{cd}
	B+FF	692 ^c	59.6 ^c	16.6 ^b	9.86 ^e
T3	B	677 ^{bc}	58.1 ^{bc}	16.2 ^{ab}	9.36 ^{cde}
	B+FF	684 ^c	58.8 ^c	16.6 ^b	9.74 ^{de}
Average		658.2	56.5	16.2	9.17
CV, %		6.8	13.9	2.5	9.3

Note: B – background; B+FF – background + foliar fertilisation. Same letters in column show no significant differences between variants by Tukey's HSD

Source: developed by the authors

All research variants significantly increased sugar yield compared to the absolute control (without seed treatment and foliar fertilisation). The highest sugar yield (9.86 t/ha) was observed in variant T2 with seed treatment and foliar fertilisation. However, in variants with treatment T3, there was only a non-significant decrease in this indicator (9.36-9.74 t/ha).

The sugar content and yield correlate with the factory sugar yield, but there may be differences due to sugar losses through excess molasses formation. The main elements contributing to molasses formation are potassium and sodium, which are present in the soil and additionally supplied through fertilisers. Additionally, alpha-amino nitrogen, an intermediate product in plant nitrogen metabolism, can also contribute to molasses formation. Managing nitrogen nutrition is crucial from this perspective because increasing yields through nitrogen fertilisation can potentially reduce sugar yield. Therefore, microelements influencing metabolic cycles can mitigate high sugar content.

DISCUSSION

The effects of chelated fertilisers on plant growth and development are stimulating, as the gross indicators of macroelements they contain are insufficient for a significant biomass increase (Honchar et al., 2021). Certain micronutrients help overcome abiotic stresses, complicating the isolation of their real impact on growth factors. Root crops accumulate significantly more microelements in the leaf mass than in the roots, making foliar fertilisation an effective means to prevent or overcome

deficiencies (Trembitska & Bohdan, 2023). There is sufficient research on sugar beets that examines the effects of individual elements on yield formation, but the application of complex fertilisers is still difficult to predict (Kalenska et al., 2024). According to E. Kandil et al. (2020), the combination of nanopreparations with boron increases plant biomass and root mass, but without a clear dependency.

Complex chelated fertilisers help maintain the necessary concentration of micronutrients in the plant, enabling the full utilisation of available resources. To reduce macro- and micronutrient deficiencies and address the problem of eutrophication, nanofertilisers are effective in releasing nutrients in a controlled manner according to plant needs (Shukla et al., 2019; Bielashov et al., 2022). Unlike conventional nitrogen fertilisers, where losses due to evaporation or leaching can reach 50-70% (Ramos-Ulate et al., 2022; Firmanda et al., 2023). Nano-formulations of nitrogen fertilisers synchronise the release of nitrogen with crop needs, minimising nutrient losses and reducing nutrient interactions with soil, water, air, and micro-organisms (Kalia & Sharma, 2019; Mejias et al., 2021; Pavlichenko et al., 2023). The use of porous nanomaterials such as zeolites, clay, or chitosan helps to regulate release on demand and increase nitrogen uptake by plants (Dimkpa et al., 2020; Thambiliyagodage et al., 2023). According to S. Jafarzadeh et al. (2023) and A. Yadav et al. (2023), ammonium zeolites can also increase the solubility of phosphate minerals.

The best options for seed treatment had higher contents of most micronutrients

compared to alternative options, which, even at relatively low rates, increased the plants' biometric indicators. According to H. Aktaş *et al.* (2006), the main micronutrients with such effects are zinc and boron, which are effective even in small doses. R. Zewail *et al.* (2020) indicated that doubling the concentration resulted in an increase in root dry mass from 8 to 35%, depending on the year's conditions. Increasing the fertiliser rate also positively affected the leaf area of individual plants and the leaf area index. These elements also increased photosynthetic productivity, pigment content in leaves, overall sugar content, and reducing sugar content. The overall increase in photosynthetic productivity may be related to the increase in zinc (Abdel-Motagally & Attia, 2009). The complex application of nanopreparations in several treatments positively affects leaf growth at distinct levels and the photosynthetic parameters of beets, including the efficiency of various photosystems (Artyszak *et al.*, 2018).

According to A. Artyszak & D. Gozdowski (2021), the use of preparations containing silicon and iron also affects root weight, the content of molasses-forming substances, and sugar loss during processing. However, unlike boron and zinc, it almost does not affect leaf mass growth. Pre-sowing application of preparations containing copper, manganese, and iron also enables a significant increase in yield and sugar content (Prośba-Białczyk *et al.*, 2017). Increasing the concentration during treatment also leads to an increase in ash elements and nitrogen in the roots. The results show some differences, manifested in a weaker change in potassium content in the roots compared to previous studies (Zewail *et al.*, 2020). Boron, which is present in the preparations for foliar feeding, may balance the content of alpha-amino nitrogen and potassium and sodium content (Nemeat-Alla *et al.*, 2021). The results on sugar losses in molasses indicate that balanced micronutrient application does not lead to negative consequences, as is the case with the unilateral application of individual micronutrients (Zewail *et al.*, 2020). The application of individual micronutrients in combination with NPK can increase root yield without deteriorating quality across a wide range of conditions (Draycott & Christenson, 2003; Varga *et al.*, 2022).

Nanopreparations, due to their high bio-availability, enhance sugar beet productivity by supplying young plants with a balanced complex of essential micro- and macronutrients. This supports the process of root moulting and positively influences the productivity potential of the root. Foliar fertilisation plays a stimulating role during growth stages when nutrient uptake is high, but the demand for microelements stays at a level that can be effectively supplemented. These measures can increase the productivity of sugar beets and sugar yield while maintaining the same nutrient supply level in the crop.

CONCLUSIONS

The analysis of the photosynthetic activity of the crop and the features of sugar accumulation and extraction at factories suggested the significant effectiveness of nanopreparations for seed treatment and foliar spraying. The application of these preparations enabled a reliable increase in plant mass as early as in the intensive root growth phase, which was reflected in the root mass at early harvesting maturity (10 September, Stage 3) and technological maturity of the tubers (30 September). Root mass was primarily influenced by seed treatment, leading to an increase of 9.5% compared to the control with the T2 formulation and 10.2% with the T3 formulation at the technological maturity phase. Root weight increased by 64-69 g in untreated plots, but foliar fertilisation resulted in a smaller increase in root mass (5.8% and 4.7%, respectively).

The net photosynthesis productivity significantly increased at the beginning of vegetation with foliar treatments, while in the subsequent stages after row closure, further growth did not significantly differ. On the other hand, foliar treatments helped maintain a larger leaf area until harvest (30.8-32.7 thsd m²/ha vs. 27.6-28.8 thsd m²/ha in plots without foliar treatment), increasing the overall productivity of the crop. The sugar content under the proposed treatment schemes did not differ significantly from the control (15.8%) in most cases. However, the T2 and T3 seed treatment variants in combination with foliar fertilisation significantly increased the sugar content in the root to 16.6%. This is promising in the context of increasing sugar yield per unit area, while also positively

influencing other yield parameters. The application of T2 and T3 schemes for seed treatment with high microelement content (compared to T1) enabled the formation of a more robust plant, which, combined with foliar fertilisation, resulted in a beetroot yield of 57.6-59.6 t/ha with a potential sugar yield of 9.29-9.86 t/ha.

The application of nanoproducts for seed treatment and the selection of optimised complexes for foliar application enabled the fulfilment of genetic potential under limited resource

conditions. Future studies should explore the long-term effects of these treatments on soil health and the sustainability of sugar beet production in varying agroecological zones.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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Вплив обробки насіння та позакореневого підживлення хелатними добривами на продуктивність буряків цукрових (*Beta vulgaris* L.)

Анотація. Буряки цукрові є високопродуктивною культурою, яка потребує значної кількості мікроелементів та поживних речовин. Вони чутливі до нестачі мікроелементів на ранніх етапах розвитку, що може вплинути на морфологічні зміни коренеплоду і, як наслідок, знизити врожайність. Метою дослідження було визначення впливу обробки насіння комплексом хелатних добрив та позакореневого підживлення на біометричні показники буряків цукрових, їх фотосинтетичну продуктивність та технологічні якості. Дослідження проводили на чорноземах типових малогумусних Правобережного Лісостепу України. Для визначення маси коренеплодів і листків, площі листової поверхні та фотосинтетичної продуктивності відбирали зразки у фазі змикання рядків, у середині фази інтенсивного росту коренеплодів та у фазі розмикання листків у міжряддях. У рослин, відібраних у фазі фізіологічної стиглості, визначали технологічні якості, урожайність коренів і цукристість. Встановлено, що позакореневе підживлення значно збільшувало масу коренів у фазі розкриття рядків і фізіологічної стиглості. Найбільша коренева маса формувалася у варіанті з обробкою насіння з високим вмістом мікроелементів і позакореневим підживленням. Маса коренеплоду досягала 692 г, урожайність – 59,6 т/га, цукристість – 16,6 %. Технологічні показники, зокрема урожайність цукру, для цього варіанту також були максимальні – 9,86 т/га. Нанодобрива для позакореневого підживлення дозволила збільшити площу листя до фази розкриття рядків без зниження чистої продуктивності фотосинтезу, що, у свою чергу, дозволило посівам накопичувати більше сухої речовини. Обробка насіння хелатними добривами збільшила накопичення сухої маси на ранніх стадіях, сприяла збільшенню фотосинтетичного апарату, що в сукупності призвело до підвищення продуктивності

Ключові слова: нанодобрива; чиста продуктивність фотосинтезу; площа листової поверхні; суха речовина; маса коренеплоду; урожайність; цукристість

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Phenological growth and development stages of asparagus pea (*Tetragonolobus purpureus* Moench.) under different sowing patterns in the conditions of the Right-Bank Forest-Steppe of Ukraine

Abstract. The study synthesised data on the application of phenological models to develop adaptive production technologies for asparagus pea, enabling the regulation of phenological phase progression while considering the unique characteristics of the cultivar and regional conditions. This approach is crucial for improving both yield and quality across various ecological conditions. The study aimed to determine the rate of key growth and development phases of asparagus pea and to establish the dependence of these processes on specific cultivation technology elements, particularly sowing patterns, in the Right-Bank Forest-Steppe of Ukraine. A comprehensive

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approach was employed, integrating field research on cultivation practices, visual observations of plant development dynamics, and statistical analysis to quantify the impact of various factors. The findings revealed a correlation between sowing patterns and the duration of phenological phases. In the 45×10 cm and 45×15 cm (control) variants, emergence occurred on 11 May, 11 days after sowing, under a cumulative temperature above 10°C of 59.4°C and 45 mm of precipitation. In the 45×20 cm and 45×25 cm variants, emergence was recorded on 17 May, 13 days after sowing, with a cumulative temperature of 71.2°C and 45.7 mm of precipitation. The onset of flowering was recorded between 16 and 23 June, depending on plant density. The shortest “emergence-flowering” period was observed in the 45×10 cm variant (32 days), with a cumulative temperature of 252.9°C and 84.5 mm of precipitation, while the longest was in the 45×25 cm variant (37 days), with a cumulative temperature of 328.5°C and 92.7 mm of precipitation. The onset of the technical maturity stage was noted between 24 June and 5 July, with the “flowering-technical maturity” period ranging from 8 to 12 days, depending on plant density. Biological maturity occurred between 10 and 18 July, with the “technical-biological maturity” phase lasting 12-16 days. The growing season lasted 56-62 days, with a cumulative temperature of 534-619.9°C and precipitation levels of 156-169.7 mm. A strong inverse correlation was established between plant density and the duration of the interphase periods from sowing to technical maturity ($r = -0.84$ to -0.98), while a strong direct correlation was observed between density and the “technical-biological maturity” period ($r = 0.92$). Increasing plant density by 5,000 plants per hectare shortened phenological periods by 0.8-2 days. A direct correlation was also identified between precipitation ($r = 0.86$ to 1.0), temperature ($r = 0.97$ to 1.0), and phase duration. A temperature increase of 10°C extended the phases by 0.6-1.7 days. The findings provide a basis for optimising sowing patterns to enhance plant growth and development, thereby improving asparagus pea productivity

Keywords: BBCH; emergence; flowering; growing season; critical phases; cumulative temperature and precipitation

INTRODUCTION

Food security has become one of the most pressing global challenges due to the rapidly increasing world population. This situation is further complicated by climate change, which leads to a rise in both environmental and biological stresses. A lack of diversity is another significant issue within the current food system. Globally, humans rely on a limited range of food sources, with 75% of the world's food supply coming from just 12 plant and 5 animal species (Colgrave *et al.*, 2021). As highlighted by R. Ambikapathi *et al.* (2022), a lack of dietary diversity may have negative health implications. Research into underutilised and overlooked agricultural species is crucial for addressing global food security. These crops, often rich in nutrients and resilient to climate change, are frequently neglected due to their low commercial value. However, they are key to reducing malnutrition and enhancing food security, particularly in vulnerable regions (Aboltins *et al.*, 2024). For example, F. Mudau *et al.* (2022)

point out that Southern Africa has substantial potential in utilising underutilised indigenous crops, but these remain underdeveloped due to insufficient attention from researchers and policymakers. The authors stress the need to accelerate research and develop value chains for these crops, and advocate for a transdisciplinary approach to successfully integrate them into modern food and medical systems.

One promising crop for addressing food security is asparagus pea (*Tetragonolobus purpureus* Moench.), a tropical legume with high protein content in its seeds, often referred to as “the soybean for the tropics” (Ho *et al.*, 2024). R. Bepary *et al.* (2023) describe this vegetable crop as a “single species supermarket” or “one stalk supermarket” because all parts of the plant, including pods, young seeds, flowers, leaves, tubers, and mature seeds, are consumable. It is worth noting that the plant also has a high nutritional value. Specifically, as noted by H. Bassal *et al.* (2021), it is an important source of vitamins

(A and C), minerals (calcium and iron), as well as erucic acid, polyunsaturated fatty acids, and proteins (30-45% of which are lectins).

The yield of modern asparagus pea varieties ranges from 5 to 10 tonnes per hectare of fresh pods, while seed yield ranges from 1 to 1.5 tonnes per hectare. Therefore, P. Singh *et al.* (2022) identify overcoming the “yield gap” as a key task for expanding production and consumption. According to S. Klutse *et al.* (2025), optimising plant density is a crucial factor not only for achieving maximum yield but also for improving its quality. A high density of plant cover increases competition between plants for resources, leading to the depletion of limited resources (Azmat *et al.*, 2024). Practical observations by M. Haque & S. Sakimin (2022) indicate that high plant density leads to many adverse effects, including disease susceptibility, fruit drop, reduced fruit size, delayed ripening, decreased individual plant growth, and light interception. Through better planting structure, an optimal leaf area index can be achieved, which promotes increased photosynthetic capacity of plants through efficient absorption of solar radiation (Li *et al.*, 2021). However, the optimal plant density, according to I. Bobos *et al.* (2024), vary depending on the biological characteristics of the crop, including varietal differences in growth vigour, height, degree of branching, as well as sowing dates and weather conditions during the growing season.

Due to variations in soil type and other environmental conditions, the optimal plant density for a particular crop may not be suitable for other locations. This necessitates the development of region-specific recommendations to ensure effective and sustainable management of agricultural resources. Thus, this study aimed to determine the rate of progression of the main growth and development phases of asparagus pea and to establish the dependence of these processes on specific elements of cultivation technology, in particular sowing patterns, under the conditions of the RightBank Forest-Steppe region of Ukraine.

MATERIALS AND METHODS

The experimental study was conducted over three years (2016-2018) at the National University

of Life and Environmental Sciences of Ukraine (NULES of Ukraine). The soil of the experimental site is classified as dark grey, medium podzolic, and light loamy, with a soil pH of 6.1. The humus horizon was 24-28 cm. The experimental site was characterised by a low humus content, ranging from 1.5 to 2.2%, a medium nitrogen content of 26 to 38 mg/kg, phosphorus content of 43 to 61 mg/kg, and potassium content of 28 to 34 mg/kg.

In December, a gradual decrease in temperature was observed, with minor peaks in individual years and an average ten-day temperature ranging from 2.2°C to -2.8°C, while precipitation ranged from 9.5 to 73.0 mm per ten-day period (Fig. 1). In January and February, the average temperature continued to decrease, reaching -9.6°C in the coldest ten-day period. Precipitation during this time was relatively low (0.4-57.1 mm), typical of the winter period when snowfall and periods of relative dryness prevail. Some instability was noted: in the third ten-day period of February 2016, precipitation significantly exceeded the corresponding values in other years.

The onset of spring was marked by a gradual warming, increasing on average by 2-3°C per ten-day period. March 2018 saw relatively low air temperatures, fluctuating between -4.3 and 0.7°C. This was accompanied by increased precipitation, particularly in the first ten-day period of March (2018), when values reached 58.1 mm. In April, temperatures continued to rise (to 7.4-15.6°C), while precipitation became uneven, with both dry ten-day periods and periods of intense rainfall (up to 90.2 mm in the second ten-day period of 2016). In May, temperatures rose to 13.3-20.8°C, maintaining a trend of gradual increase. Precipitation reached maximum values, especially in the third ten-day period of May 2016, when significant downpours occurred with a maximum value of 89.5 mm per ten-day period.

The summer months were characterised by the highest temperatures: in June, they ranged from 17.0 to 24.8°C per ten-day period, in July they reached values from 19.3 to 23.5°C, and in August they remained at 17.1 to 25.6°C. Precipitation during this period showed significant variability: June saw periods of heavy rain (up to 113.1 mm in the third ten-day period of 2018), while July proved to be drier in some years. August was characterised by

alternating dry and rainy ten-day periods – in 2016 in the first ten-day period and in 2017 in the second ten-day period, minimal precipitation

was recorded, while in the third ten-day period of 2017, a sharp increase in precipitation was observed (up to 55.2 mm).

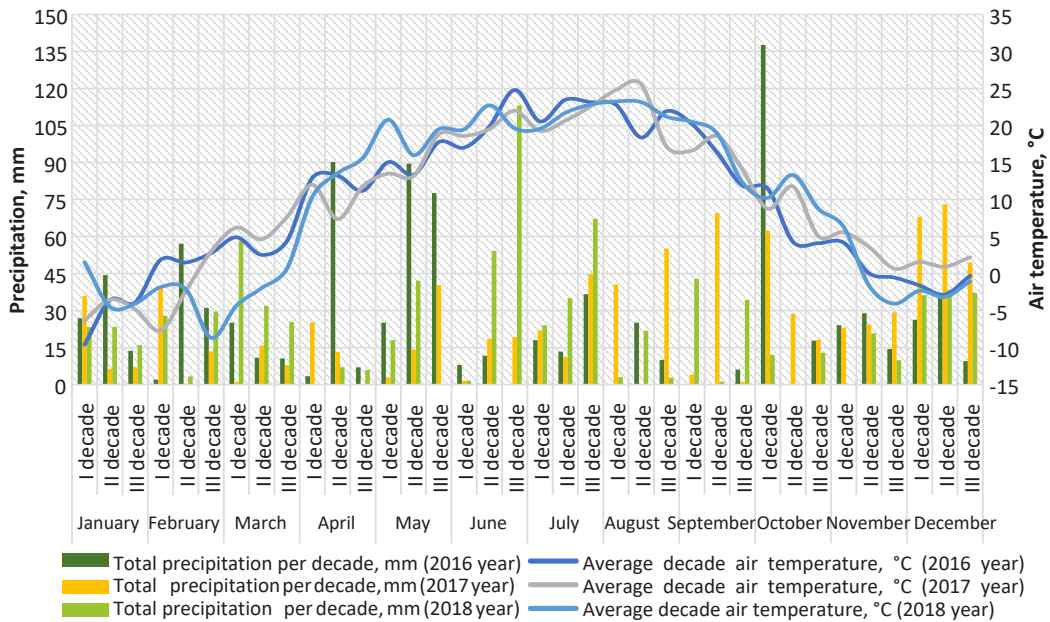


Figure 1. Analysis of air temperature and precipitation dynamics for 2016-2018

Source: developed by the authors based on the conducted study

In September, temperatures gradually decreased (from 20.2°C in the first ten-day period to 11.9°C in the third ten-day period), and precipitation was unevenly distributed: heavy rains were observed in the second ten-day period, while the first and third ten-day periods were relatively dry. October showed a steady decrease in temperature (to 4.1-8.8°C in the third ten-day period). An unusually high amount of precipitation was recorded in the first ten-day period of 2016 – 137.6 mm. November continued the trend of decreasing temperature (from 6.3°C at the beginning of the month to 4.1°C at the end), and precipitation was unevenly distributed across the years (from 0.0 mm in the first ten-day period of 2018 to 29.3 mm in the third ten-day period of 2017).

The Department of Vegetable Crops at NULES of Ukraine studied four sowing patterns of asparagus pea during 2016-2018: A) 45×10 cm; B) 45×15 cm; C) 45×20 cm; D) 45×25 cm.

The control was the distance between plants in the row of 15 cm. Seeds were sown on 4 May at a depth of 2-3 cm. The area of each plot was 5 m². The phenological development of asparagus pea plants was determined using the BBCH scale (Meier *et al.*, 2009). According to the BBCH scale, the life cycle of asparagus pea includes nine developmental stages, each with characteristic duration and distinct features, where: 0 – germination (00: dry seed); 1 – leaf development (10: cotyledons fully unfolded; 13: 3rd true leaf (first trifoliolate) unfolded); 2 – development of side shoots (29: 9 or more side shoots visible); 5 – inflorescence emergence (59: first petals visible, flowers still closed); 6 – flowering (65: stage reached when 50% of flowers are open); 7 – fruit development (75: pods have reached typical length in approximately 50% of cases, with pods beginning to fill); 8 – fruit and seed ripening (89: pods fully ripe, showing hardened state, indicating complete maturity) (Fig. 2).

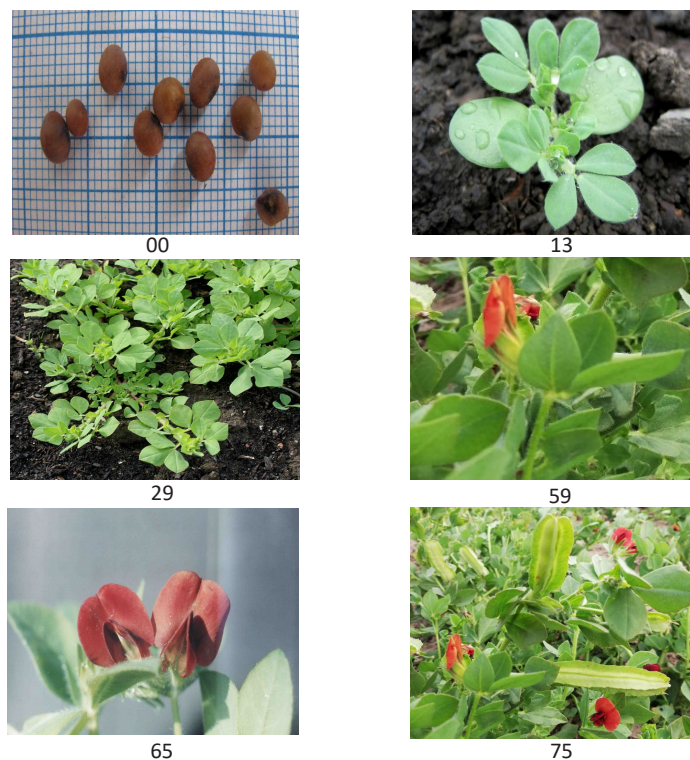


Figure 2. Main and secondary phenological stages of asparagus pea (*P. tetragonolobus*) according to the extended BBCH scale

Note: 0 – germination (00: dry seed); 1 – leaf development (13: 3rd true leaf (first trifoliolate) unfolded); 2 – development of side shoots (29: 9 or more side shoots visible); 5 – inflorescence emergence (59: first petals visible, flowers still closed); 6 – flowering (65: stage reached when 50% of flowers are open); 7 – fruit development (75: pods have reached typical length in approximately 50% of cases, with pods beginning to fill)

Source: developed by the authors based on the conducted study

The growth and development stages of asparagus pea were determined visually for the entire experiment simultaneously. The onset of a stage was recorded when it was observed in 10% of the plants in the plot, and mass onset was recorded upon reaching 75%. The sum of effective air temperatures was calculated using formula (1):

$$\Sigma t_{eff} = (t_{avg} - B) * n, \quad (1)$$

where Σt_{eff} is the sum of effective air temperatures for the period, °C; t_{avg} is the average active air temperature for the period, °C; B is the biological minimum, which was taken as 10°C in this study; n is the number of days in the period.

The research results were processed using Statistica 13.1 software (StatSoft, Inc., Tulsa, OK, USA). To determine the direction and degree of correlation between the studied indicators, the correlation coefficient was calculated (Nageswara Rao, 2021). During the study, the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973) were adhered to.

RESULTS AND DISCUSSION

The analysis results show a clear relationship between the sowing pattern and the duration of the asparagus pea germination period. In the variants with a row spacing of 45 cm and

a plant spacing of 10 cm and 15 cm (control), seedlings emerged on 11 May, which corresponds to 11 days from the time of sowing (Figs. 3, 4). The characteristic conditions for the “sowing-emergence” phenological phase were a total temperature (above 10°C) of 59.4°C and a precipitation amount of 45 mm (Fig. 5). In the variants with a row spacing of 45 cm and

a plant spacing of 20 and 25 cm, seed germination occurred somewhat slower, and seedlings appeared on 17 May. The duration of the “sowing-emergence” phenological phase in these variants was 13 days, which is 2 days later than the control, and was accompanied by a total temperature (above 10°C) of 71.2°C and a precipitation amount of 45.7 mm.

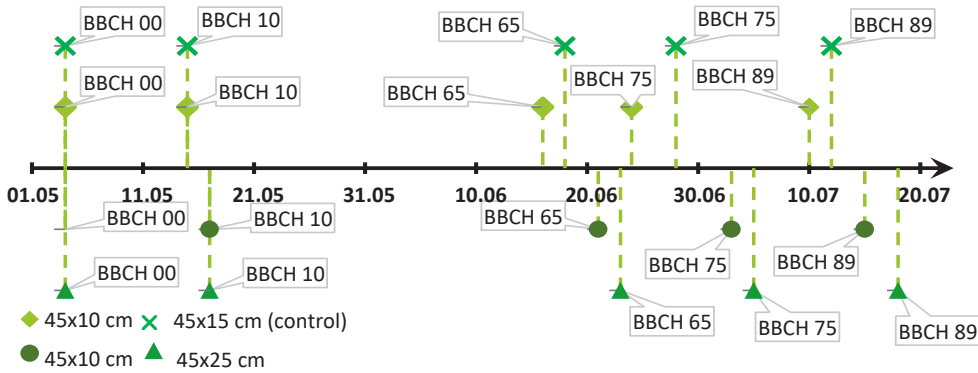


Figure 3. Results of phenological observations of asparagus pea plant growth and development under different sowing patterns (average for 2016-2018)

Note: phenological development of asparagus pea plants: sowing (BBCH 00) – dry seed; emergence (BBCH 10) – cotyledons fully unfolded; start of flowering (BBCH 65) – stage reached when 50% of flowers are open; technical maturity (immature (green) pods) (BBCH 75) – pods have reached typical length in approximately 50% of cases, with pods beginning to fill; biological seed maturity (BBCH 89) – pods fully ripe, showing hardened state, indicating complete maturity

Source: developed by the authors based on the conducted study

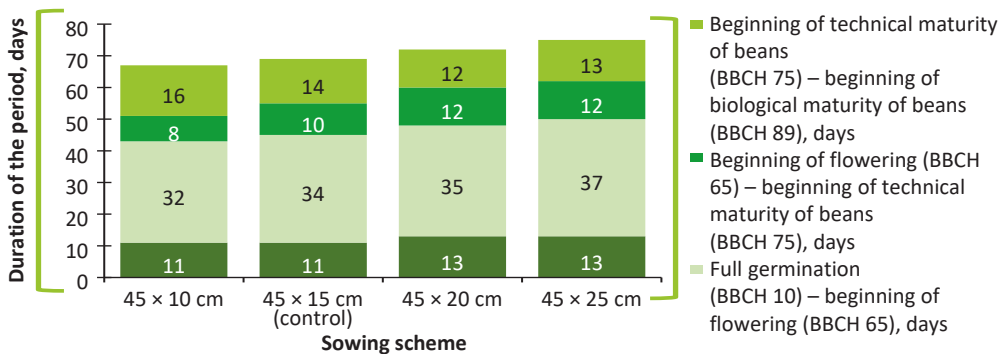


Figure 4. Duration of asparagus pea plant growth and development phenological stages depending on plant density (average for 2016-2018)

Note: phenological development of asparagus pea plants: sowing (BBCH 00) – dry seed; emergence (BBCH 10) – cotyledons fully unfolded; start of flowering (BBCH 65) – stage reached when 50% of flowers are open; technical maturity (immature (green) pods) (BBCH 75) – pods have reached typical length in approximately 50% of cases, with pods beginning to fill; biological seed maturity (BBCH 89) – pods fully ripe, showing hardened state, indicating complete maturity

Source: developed by the authors based on the conducted study

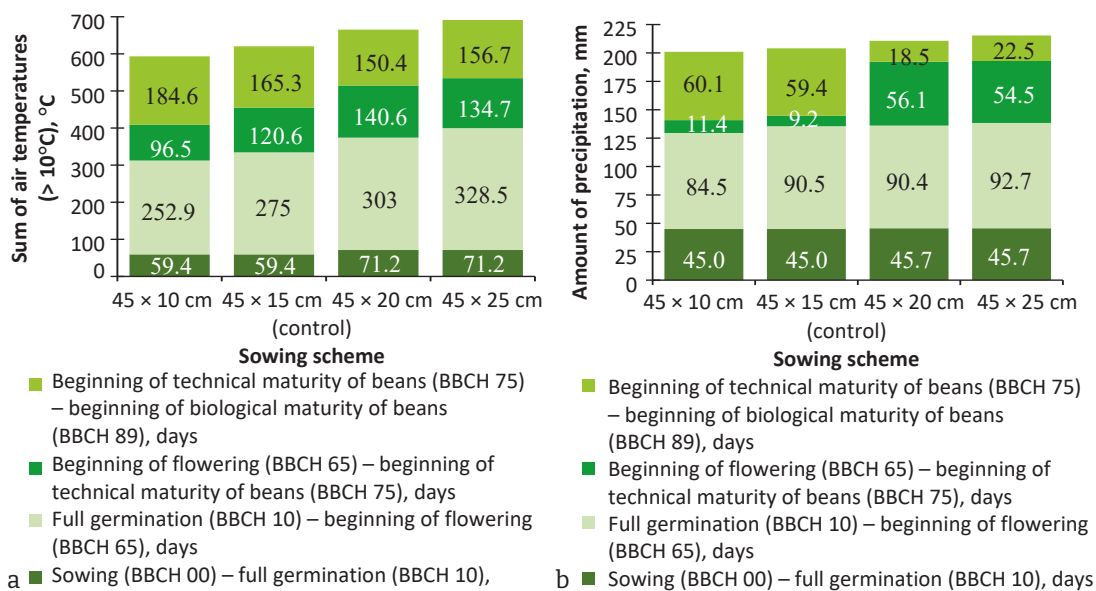


Figure 5. Analysis of the dynamics of the sum of effective temperatures (>10°C) (a)

and precipitation (b) during the asparagus pea growing season (average for 2016-2018)

Note: phenological development of asparagus pea plants: sowing (BBCH 00) – dry seed; emergence (BBCH 10) – cotyledons fully unfolded; start of flowering (BBCH 65) – stage reached when 50% of flowers are open; technical maturity (immature (green) pods) (BBCH 75) – pods have reached typical length in approximately 50% of cases, with pods beginning to fill; biological seed maturity (BBCH 89) – pods fully ripe, showing hardened state, indicating complete maturity

Source: developed by the authors based on the conducted study

The studies noted the influence of row spacing on the onset of flowering. The earliest flowering was observed on 16 June in the 45 × 10 cm variant, while in the control (45 × 15 cm) it occurred on 18 June, and in the 45 × 20 cm and 45 × 25 cm variants, it occurred even later, on 21 and 23 June. The shortest period from emergence to flowering was 32 days in the 45 × 10 cm variant, which is 2 days less than the control. The average precipitation was 84.5 mm, and the sum of air temperatures (above 10°C) during this interphase period was 252.9°C. The longest “emergence-flowering” interphase period was characterised by the 45 × 25 cm variant – 37 days, which is 3 days longer than the control, and was accompanied by a total temperature of 328.5°C and a precipitation amount of 92.7 mm. With a sowing pattern of 45 × 20 cm, the duration of the “emergence-flowering” period was 35 days, which is 1 day longer than the control. In this case, the average sum of temperatures (above 10°C) was 303°C, and the precipitation amount was 90.4 mm.

Meanwhile, in the 45 × 15 cm control, the period from emergence to flowering lasted 34 days with an average sum of temperatures (above 10°C) of 275°C and a precipitation amount of 90.4 mm.

The onset of technical maturity of the pods in the experimental plants was observed from 24 June to 5 July. The longest period from the start of flowering to the start of technical maturity of the pods was recorded in the variants with sowing patterns of 45 × 20 cm and 45 × 25 cm (12 days), which is 2 days longer than the control. The sum of temperatures (above 10°C) during this phenological period was 134.7-140.6°C, and the average precipitation was 54.5-56.1 mm. With a sowing pattern of 45 × 10 cm, the shortest period “start of flowering–start of technical maturity of pods” was observed (8 days), which is 2 days less than the control, and was accompanied by a sum of temperatures (above 10°C) of 96.5°C and a precipitation amount of 11.4 mm. In the variant with a plant spacing of 45 × 15 cm (control), the onset of technical maturity of the pods occurred

10 days after the start of flowering and was marked by a sum of temperatures (above 10°C) of 120.6°C and a precipitation amount of 9.2 mm.

The onset of biological maturity of the pods was reached earliest in the 45×10 cm variant (10 July), while in the control (45×15 cm) it occurred on 12 July, and in the 45×20 cm and 45×25 cm variants, it occurred on 15 and 18 July, respectively. The period from technical to biological maturity shortened with increasing distance between plants in the row: it was longest in the planting with a plant spacing of 10 cm and lasted 16 days, which is 2 days longer than the control, in the plantings with a spacing of 20 cm and 25 cm – 12 and 13 days respectively, or 1-2 days less than the control, and the control variant (15 cm) – 14 days. The duration of the “technical maturity-biological maturity” phenological phase was accompanied by the accumulation of a temperature sum (above 10°C) from 184.6 to 165.3°C and a precipitation sum from 18.5 to 60.1 mm. This indicates that denser plant spacing leads to slower pod development, which may be due to increased mutual shading. For asparagus pea, the growing season, depending on the sowing pattern, ranged from 56 to 62 days. Plant growth and development from emergence to the onset of biological maturity of the pods was directly accompanied by a total temperature (above 10°C) from 534 to 619.9°C and a precipitation amount of 156 to 169.7 mm.

It was established that there is a strong inverse relationship between the density of asparagus pea plants and the duration of the interphase period “sowing-emergence” ($r = -0.84$), “emergence-start of flowering” ($r = -0.96$), “start of flowering-start of technical maturity of pods” ($r = -0.98$), and the growing season ($r = -0.92$). A strong direct relationship was found between the density of asparagus pea plants and the duration of the interphase period “start of technical maturity of pods-start of biological maturity of pods” ($r = 0.92$) (Fig. 6). Statistical analysis of the experimental data and their graphical representation revealed that an increase in plant density by 5,000 plants/ha led to a reduction in the interphase periods: “sowing-emergence” by 0.8 days, “emergence-start of flowering” by 1.7 days, “start of flowering-start of technical maturity of pods” by 1.6 days, and the growing season by 2 days. At the same time, an increase in the period “start of technical maturity

of pods-start of biological maturity of pods” by 1.3 days was observed.

The research results demonstrated a direct correlation between the amount of precipitation and the duration of asparagus pea interphase periods, specifically the periods “sowing-emergence” ($r = 1.0$); “emergence-start of flowering” ($r = 0.93$); “start of flowering-start of technical maturity of pods” ($r = 0.88$); “start of technical maturity of pods-start of biological maturity of pods” ($r = 0.86$); and the growing season ($r = 0.97$). A direct correlation was also found between the total air temperature (above 10°C) and the duration of asparagus pea interphase periods, namely the periods “sowing-emergence” ($r = 1.0$); “emergence-start of flowering” ($r = 0.98$); “start of flowering-start of technical maturity of pods” ($r = 0.98$); “start of technical maturity of pods-start of biological maturity of pods” ($r = 0.99$); and the growing season ($r = 0.97$) (Fig. 7). Based on the regression equations, it was established that an increase in the sum of air temperatures (above 10°C) by 10°C led to an increase in the interphase periods: “sowing-emergence” by 1.7 days, “emergence-start of flowering” by 0.6 days, “start of flowering-start of technical maturity of pods” by 1.0 day, “start of technical maturity of pods-start of biological maturity of pods” by 1.1 days, and the growing season by 0.6 days.

The development of plant phenological processes is determined by weather conditions (Katal *et al.*, 2022). Prolonged exposure to high temperatures causes stress in plants, which manifests as a decrease in photosynthetic efficiency. As a result, structural and functional changes occur in the photosynthetic apparatus (Ji *et al.*, 2022). The results of research by R. Reed *et al.* (2022) show that thermal stress caused by high temperatures leads to several problems, namely: it negatively affects the process of seed formation and crop volume, and also significantly reduces the viability of already harvested seeds. At the same time, the amount and distribution of precipitation play a key role in plant growth and development, affecting the water balance and nutrient availability in the soil (Wang *et al.*, 2022). Moisture deficit can lead to a slowdown in metabolic processes and a decrease in yield, while excessive precipitation can cause leaching of nutrients and the development of root diseases (Bhattacharya, 2021; Yanagi, 2021).

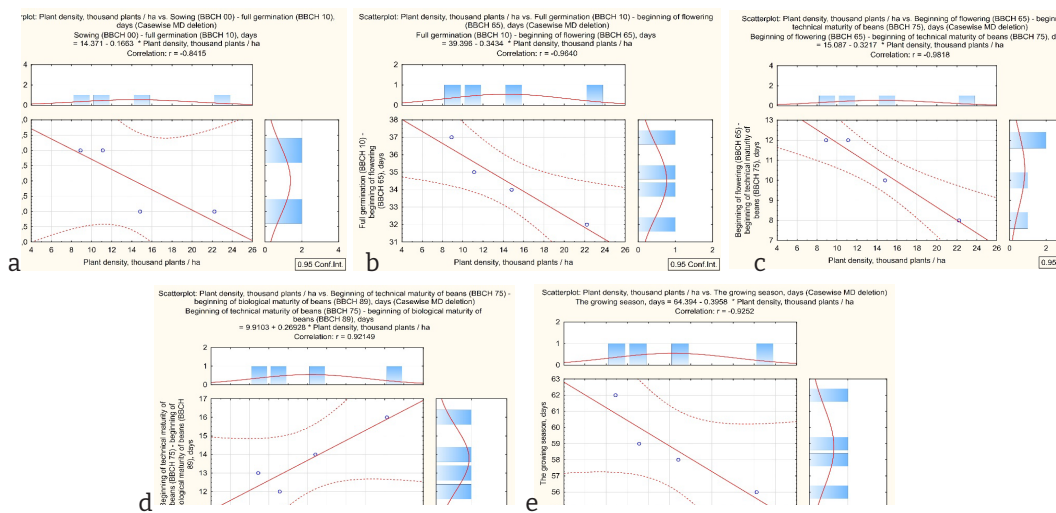


Figure 6. Influence of plant density on the duration of asparagus pea interphase periods (average for 2016-2018)

Note: phenological development of asparagus pea plants: sowing (BBCH 00) – dry seed; emergence (BBCH 10) – cotyledons fully unfolded; start of flowering (BBCH 65) – stage reached when 50% of flowers are open; technical maturity (immature (green) pods) (BBCH 75) – pods have reached typical length in approximately 50% of cases, with pods beginning to fill; biological seed maturity (BBCH 89) – pods fully ripe, showing hardened state, indicating complete maturity a) “sowing-emergence”; b) “emergence-start of flowering”; c) “start of flowering-start of technical maturity of pods”; d) “start of technical maturity of pods-start of biological maturity of pods”; e) growing season
Source: developed by the authors based on the conducted study

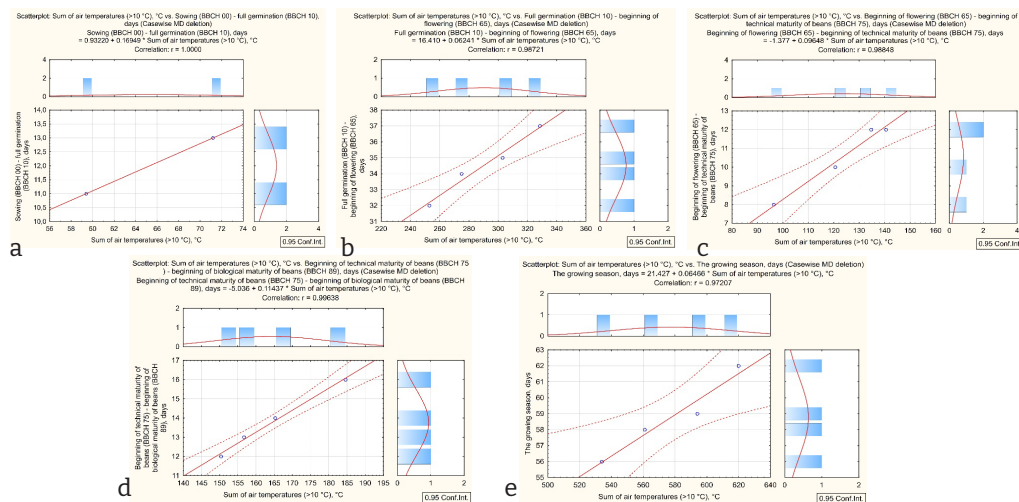


Figure 6. Influence of total air temperature (> 10°C) on the duration of asparagus pea interphase periods (average for 2016-2018)

Note: phenological development of asparagus pea plants: sowing (BBCH 00) – dry seed; emergence (BBCH 10) – cotyledons fully unfolded; start of flowering (BBCH 65) – stage reached when 50% of flowers are open; technical maturity (immature (green) pods) (BBCH 75) – pods have reached typical length in approximately 50% of cases, with pods beginning to fill; biological seed maturity (BBCH 89) – pods fully ripe, showing hardened state, indicating complete maturity a) “sowing-emergence”; b) “emergence-start of flowering”; c) “start of flowering-start of technical maturity of pods”; d) “start of technical maturity of pods-start of biological maturity of pods”; e) growing season
Source: developed by the authors based on the conducted study

J. Dhillon *et al.* (2020) note that insufficient understanding of morphological scales of plant development complicates decision-making, in particular regarding the optimal timing of sowing and harvesting. In recent years, numerous models of agricultural crop growth have been developed, which focus on predicting key stages of development, such as flowering or ripening, or individual periods, for example, from sowing to flowering (Schieler *et al.*, 2023).

The growing degree-day indicator is often used to characterise crop cultivation processes. It reflects the accumulated sum of temperatures exceeding a defined threshold, specific to each crop. It is believed that using this parameter instead of conventional time counting helps to make the growth process more predictable and proportional to accumulated heat (Tschurr *et al.*, 2023). In particular, E. Pinzón-Sandoval *et al.* (2024) in their study highlight the influence of temperature regime on the processes of forming total and organ-specific mass in beans. It allows for a deeper understanding of the patterns of phenological development, features of growth and accumulation of degree-days in different periods – from the vegetative to the reproductive phase. The authors of this study propose to use phenological analysis with the use of such assessments as the sum of effective temperatures and the amount of precipitation, which allows for building more accurate growth models adapted to the real conditions of asparagus pea. This will allow not only to more accurately describe the development of the crop, but also to minimise errors associated with subjective interpretation of morphological scales in different regions.

It was observed that elevated temperatures significantly shortened the pod development period of asparagus pea, thereby reducing the time available for pod filling and assimilate partitioning, and consequently, the yield was reduced. In legumes, heat stress during flowering causes a decrease in male fertility, and also negatively affects the structure of the female reproductive system (Sher *et al.*, 2024). The results of research by F. Angelotti *et al.* (2020) demonstrate that genotypes in which flowering begins before the onset of extreme temperature conditions may be able to avoid the negative impact of such high temperatures. A. Lamichaney *et al.* (2021) found

a significant negative correlation ($p < 0.001$) between pea seed germination and maximum temperature during flowering and the reproductive period. In addition, the number of accumulated growing degree-days during the growing season had a positive correlation with seed germination ($p < 0.001$). Experiments on asparagus pea plants with different sowing patterns showed that with an increase in the sum of air temperatures, the development stages took much longer.

Research in Australia, conducted with a sowing pattern of 100×75 cm and encompassing three sowing dates and three asparagus pea samples, showed that the average duration of the period from sowing to the opening of the first flower ranged from 68 to 167 days (Eagleton, 2022). Another study conducted in India with a sowing pattern of 100 × 60 cm revealed a minimum duration of this period in the VRWB-84 genotype (68.66 days), and the longest period was recorded in the VRWB23 genotype (83.3 days) (Hansda *et al.*, 2023). Based on the observations of the authors of this study, in Ukraine this period lasted from 51 to 62 days. Significant correlations showed that lengthening the periods of interphase events to 50% flowering and ripening negatively affects seed yield. In addition, the presence of a strong correlation between these periods indicates their synchronicity, where earlier flowering ensures earlier ripening and, accordingly, increased yield (Bhadmus *et al.*, 2023). A genetic correlation coefficient ≥ 0.95 between the average duration of the period to flower opening and the number of days to the appearance of the first pod and 50% of the pods is biologically significant (Adebayo & Shonde, 2024).

This research demonstrates the significant impact of sowing patterns on the development of asparagus pea, a key factor for improving agricultural technologies. The results indicate that increasing the distance between plants in a row, thus reducing their overall density, can slow down certain stages of development, including germination, the start of flowering, and the achievement of technical maturity. At the same time, during the pod ripening stage, there is a tendency for slower ripening with less distance between plants. This will allow for better adaptation of technologies to the growing conditions of the crop.

CONCLUSIONS

Based on the conducted phenological observations, it was established that the growth and development of asparagus pea plants (*Tetragonolobus purpureus* Moench.) in the Right-Bank Forest-Steppe region of Ukraine is clearly dependent on the sowing pattern and plant density. The optimal conditions for rapid seedling emergence were found to be in the variants with a row spacing of 45 cm and a plant spacing of 10-15 cm, where seedlings appeared 11 days after sowing. Increasing the distance between plants to 20-25 cm slowed down the germination process by 2 days. It was established that plant density significantly affected the duration of interphase periods. The shortest period from emergence to the start of flowering (32 days) was observed with the 45 × 10 cm pattern, while in the 45 × 25 cm variant, this period lasted the longest – 37 days. Similar trends were observed for other phenological phases: shortening of interphase periods was observed with higher plant density, which is due to more intense competition for resources.

The period from the start of flowering to technical maturity of the pods lasted from 8 to 12 days, depending on the planting density, and the transition from technical to biological maturity occurred within 12-16 days. The earliest date for achieving biological maturity of the pods was 10 July (with the 45 × 10 cm pattern),

and the latest was 18 July (with the 45 × 25 cm pattern). It has been proven that increasing plant density by 5,000 plants per hectare leads to a reduction in the overall growing season by 2 days. A strong correlation was found between plant density and the duration of phenological phases (r =from -0.84 to -0.98; 0.98), which confirms the significant impact of the sowing pattern on crop growth and development. Analysis of meteorological conditions showed that the duration of interphase periods positively correlates with the total air temperature ($>10^{\circ}\text{C}$) ($r=0.97-1.0$) and the amount of precipitation ($r=0.86-1.0$). In particular, an increase in the sum of air temperatures by 10°C caused an extension of the interphase periods by an average of 0.6-1.7 days.

Future research will focus on an in-depth analysis of the impact of mineral nutrition, water regime, and varietal characteristics on the growth, development, and productivity of asparagus pea, taking into account predicted climatic conditions, which will contribute to the development of an adapted cultivation technology to increase the yield and stability of this crop in the region.

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CONFLICT OF INTEREST

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Проходження фенологічних фаз росту та розвитку тетрагонолобуса (*Tetragonolobus purpureus* Moench.) залежно від різних схем сівби в умовах Правобережного Лісостепу України

Анотація. Узагальнено отримані дані з використання фенологічних моделей для створення адаптивних технологій виробництва тетрагонолобуса для забезпечення регулювання проходження фенологічних фаз з урахуванням індивідуальних особливостей сортозразку та умов регіону, що є критично важливим для підвищення врожайності та якості культури в різних екологічних умовах. Метою дослідження було визначення швидкості проходження основних фаз росту і розвитку тетрагонолобуса та встановлення залежності цих процесів від окремих елементів технології вирощування, зокрема схеми сівби, в умовах Правобережного Лісостепу України. У дослідженні застосовано комплексний підхід, що поєднує польові дослідження технологічних аспектів вирощування, візуальні спостереження за динамікою розвитку рослин та статистичний аналіз для кількісної оцінки впливу різноманітних факторів. Дослідження встановило залежність між схемами сівби тетрагонолобуса та тривалістю фенологічних фаз. У варіантах 45 × 10 см і 45 × 15 см (контроль) сходи з'явилися 11 травня, через 11 діб після сівби, за сумарної температури понад 10 °C 59,4 °C та опадів 45 мм. У варіантах 45 × 20 см і 45 × 25 см сходи з'явилися 17 травня, через 13 діб, при сумарній температурі 71,2 °C і опадах 45,7 мм. Початок цвітіння фіксували 16-23 червня залежно від густоти. Найкоротший період «сходи-цвітіння» спостерігався у варіанті 45 × 10 см (32 доби), за сумарної температури 252,9 °C і опадів 84,5 мм, а найдовший – у варіанті 45 × 25 см (37 діб), при температурі 328,5 °C і опадах 92,7 мм. Початок технічної стиглості відзначали 24 червня – 5 липня, тривалість періоду «цвітіння-технічна стиглість» варіювала від

8 до 12 діб, залежно від густоти. Біологічна стиглість настала 10-18 липня, а тривалість фази «технічна-біологічна стиглість» складала 12-16 діб. Вегетаційний період тривав 56-62 доби за сумарної температури 534-619,9 °C й опадах 156-169,7 мм. Встановлено сильний обернений зв'язок між густиною рослин і тривалістю міжфазних періодів від сівби до технічної стиглості ($r = -0,84 \dots -0,98$) та прямий сильний зв'язок між густиною і періодом «технічна-біологічна стиглість» ($r = 0,92$). Підвищення густоти на 5 тис./га скорочувало фенологічні періоди на 0,8-2 доби. Виявлено пряму кореляцію між опадами ($r = 0,86 \dots 1,0$), температурою ($r = 0,97 \dots 1,0$) і тривалістю фаз. Збільшення температури на 10 °C подовжувало періоди на 0,6-1,7 доби. Отримані результати дозволяють оптимізувати схему сівби для покращення росту і розвитку рослин для підвищення продуктивності тетрагонолобуса

Ключові слова: ВВСН; сходи; цвітіння; вегетаційний період; критичні фази; сума температур та опадів

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Biological control of *Alternaria* and late blight of potatoes

Abstract. Infectious diseases, such as *Alternaria* and late blight, caused by the microorganisms *Alternaria solani* and *Phytophthora infestans*, often reduce potato yields and quality. Disease control involves the intensive use of chemical pesticides, which leads to pathogen resistance and various environmental challenges. Therefore, it is vital to investigate safe methods of plant protection. The purpose of the present study was to evaluate the effectiveness of various combinations of biological products against *Alternaria* and late blight of potatoes. The experiments were conducted at the Ukrainian Research Station of Plant Quarantine of the Institute of Plant Protection of the National Academy of Agrarian Sciences. Biofungicides based on micromycetes of the genera *Gliocladium* and *Trichoderma* and bacteria of the genera *Pseudomonas* and *Bacillus* were used for the study. The use of combinations of different products included pre-planting treatment of tubers and three sprayings of vegetative plants at different stages of their growth and development. The use of biological products on potatoes reduced the spread of *Alternaria* by 38.7-51.2% and reduced the intensity of the disease development by 19.1-24.2% compared to the control. The technical efficiency ranged within 70.0-88.6%. The most effective combinations for disease control were Biospectrum BT + Vitastim BT, Bactofit BT + BioHibervit BT, Bactofit BT + Vitastim BT, which provided technical efficiency of 82.0-88.6%. The studied biological products also reduced the damage to plants by the pathogen of late blight. Technical efficiency under different variants of application of the preparations ranged from 61.6% (Trichopsin BT + BioHibervit BT, Fluorescin BT + BioHibervit BT) to 84.9% (Bactofit BT + Vitastim BT). The biological products had a positive effect on plant growth, yield formation, and overall productivity. The highest yield increase was observed in the variants with the use of Bactofit BT + BioHibervit BT, Trichopsin BT + Vitastim in Biospectrum BT + Vitastim BT. The studies confirmed the potential of biological products in the management of potato diseases

Keywords: biological products; fungal diseases; microorganisms-antagonists; efficiency; tubers; yield

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INTRODUCTION

Potatoes are one of the main crops and are essential for food security. Infectious diseases are among the factors that lead to lower crop yields and poorer product quality. Fungi often cause the most harmful plant pathologies. Specifically, potato plants are parasitised by the fungus *Alternaria solani* Sorauer and the oomycete *Phytophthora infestans* (Mont.) de Bary, which cause early dry spot or *Alternaria* and late blight, respectively (Hjelkrem *et al.*, 2021). M. Riolo *et al.* (2020) emphasised the great harmfulness of late blight worldwide, while K. Schiffer-Forsyth *et al.* (2023) estimated annual global potato yield losses and costs of late blight control at USD 3-10 billion.

R. Quds *et al.* (2023) pointed out that in potato cultivation technologies, pesticides must be used repeatedly during the growing season to control *Alternaria* and late blight. At the same time, according to C. Davies *et al.* (2021), the widespread use of numerous synthetic fungicides has led to the development of pathogen resistance to their active ingredients and a decrease in the effectiveness of the preparations. S. Juby *et al.* (2023) noted that to address a series of negative phenomena, the world is researching environmentally sustainable approaches to plant disease control based on biological agents. Specifically, the use of fungi of the genera *Trichoderma*, *Chaetomium*, *Pythium*, micro-mycetes *Gliocladium virens*, and *Coniothyrium minitans*, as well as yeast and other organisms is promising. The protective effect of biocontrol agents lies in direct antagonistic interaction or indirect interaction through induced changes in plant structure and physiology.

The findings of U. Alshimaysawe *et al.* (2024) suggest that the strains of micromycetes *T. hamatum* T8 and *T. harzianum* T5 have great potential as biocontrol agents for inhibiting the growth of pathogens in dual culture and culture filtrate methods, specifically, potato pathologies caused by *R. solani*. J. Hao *et al.* (2024) found an inhibitory effect of the fermentation broth of bacteria *Bacillus subtilis* ZWZ-19 (B) and fungus *Trichoderma asperellum* PT-29 (T) on the pathogen *Fusarium oxysporum*. The effect was better when co-cultivated with a 1:1 inoculation ratio (B1T1). M. Shuang *et al.* (2022) established

the ability of the *Bacillus* sp. strain K-9 to inhibit *in vitro* the common potato scab pathogen (*Streptomyces scabies*). In addition, the studied strain K-9 inhibited the growth of the pathogen of black scab of potatoes at 70.39%. In field trials, the protective effect of the K-9 strain against potato scab did not differ significantly from the effectiveness of a mixture of bacteria or chemicals, but the disease index under K-9 treatment was significantly lower than in the control. The potato yield under the treatment with *Bacillus* sp. K-9 was 12.44% greater than in the control. H. Jabnoun-Khiareddine *et al.* (2023) found antifungal activity against a series of potato pathogens (*Fusarium oxysporum* f. sp. *tuberos*, *Rhizoctonia solani*, *Colletotrichum coccodes*) of strains SV39 and SV104 (*B. tequilensis*), SV41 (*B. subtilis*), SV44 (*B. methylophilus*) and SV65 (*B. amyloliquefaciens* subsp. *plantarum*). Field studies showed that soil treatment with *Bacillus* spp. strains provided substantial control of fungal diseases and improved potato growth and productivity parameters.

F. Fathi *et al.* (2021) investigated the ability of the bacterium *Pseudomonas fluorescens* VUPF506 to effectively suppress the causative agent of potato black scab, the fungus *Rhizoctonia solani*. X. Shi *et al.* (2024) established the growth stimulation and biological control of fungal diseases of another solanaceous crop, tomatoes, using the rhizobacterium *Pseudomonas chlororaphis* subsp. *aureofaciens* SPS-41. Specifically, the antifungal properties were manifested against the phytopathogenic fungi *Fusarium oxysporum* and *Botrytis cinerea*. S. Feng *et al.* (2021) identified a rare strain of the actinomycete *Saccharothrix texasensis* 6-C, which was isolated from the potato rhizosphere and proved its ability to inhibit *P. infestans*. L. Zhang *et al.* (2024) observed a reduction in *Alternaria* leaf spot index from 78.36 to 37.03% in potato plants treated with *Talaromyces muroii* strain SD1-4, along with significantly improved growth characteristics, including plant height, root length, fresh weight, dry weight, chlorophyll content, and photosynthetic rate.

Thus, considering the significance of potatoes as a valuable food crop in the world and in Ukraine and the harmfulness of *Alternaria* and

late blight, it is vital to investigate the effectiveness of plant protection products based on biological control agents. The purpose of the present study was to investigate the effectiveness of various combination schemes of biological products against the main diseases of potatoes – *Alternaria* and late blight.

MATERIALS AND METHODS

Field studies were conducted during 2020–2021 at the Ukrainian Research Station of Plant Quarantine of the Institute of Plant Protection of the National Academy of Agrarian Sciences. The soil of the experimental plot was grey forest heavy loam. To protect potatoes from *Alternaria* and late blight, the technical effectiveness of a series of biological products was investigated. Gliocladin BT is a biological product based on mycelium and spores of a micromycete from the genus *Gliocladium* with a titre of at least 1.5×10^9 CFU/cm³; biologically active substances. Trichopsin BT contains mycelium and spores of micromycetes of the genus *Trichoderma*; rhizosphere bacteria of the genus *Pseudomonas* with a titre of at least 2.0×10^{10} CFU/cm³, biologically active substances. Fluorescin BT is a preparation based on a bacterium of the genus *Pseudomonas* with a titre of at least 5.0×10^9 CFU/cm³; biologically active substances (phenazine-carboxylic acids, siderophores, cytokinins). Biospectrum BT consists of

rhizosphere bacteria of the genus *Pseudomonas* with a titre of at least 5.0×10^9 CFU/cm³, biologically active substances (acids of the genus phenazine-carboxylic, a complex of active pigments). Bactofit BT contains live cells and spores of bacteria of the genus *Bacillus* with a titre of at least 2.0×10^9 CFU/cm³. BioHibervit BT consists of chlamydo spores, mycelium, and conidia of *Trichoderma* micromycetes, as well as their metabolites. Vitastim BT is a preparation based on the joint deep cultivation of three strains of fungi and bacteria of the genera *Trichoderma* and *Pseudomonas* in liquid nutrient medium.

Field studies, disease diagnostics, evaluation of the technical effectiveness of biological preparations, and structural analysis of the crop were conducted according to generally accepted methods (Trybel *et al.*, 2001; Kyryk *et al.*, 2012). The potato variety Podolyanka was cultivated. The experimental design included the variants presented in Table 1. Tubers were treated before planting potatoes at the rate of 20 litres of working solution per 1 tonne. The vegetative plants were treated with a battery-powered backpack sprayer FORTE KF-16 during the following periods of plant growth and development: at the beginning of plant closure in the row; in the budding phase; in the flowering phase. Combinations of preparations were applied at the rate of 200 litres of working solution per 1 ha.

Table 1. Scheme of the experiment to investigate the effectiveness of combinations of biological products against potato diseases

No. of experiment variant	Treatment of tubers before planting	Spraying of plants during the growing season (3 times)
1	Control (without preparations)	
2	Gliocladin BT, 1.5 l/t + BioHibervit BT, 1.5 l/t	Gliocladin BT, 3 l/ha + BioHibervit BT, 3 l/ha
3	Trichopsin BT, 1.5 l/t + BioHibervit BT, 1.5 l/t	Trichopsin BT, 3 l/ha + BioHibervit BT, 3 l/ha
4	Fluorescin BT, 1.5 l/t + BioHibervit BT, 1.5 l/t	Fluorescin BT, 3 l/ha + BioHibervit BT, 3 l/ha
5	Biospectrum BT, 1.5 l/t + BioHibervit BT, 1.5 l/t	Biospectrum BT, 3 l/ha + BioHibervit BT, 3 l/ha
6	Bactofit BT, 1.5 l/t + BioHibervit BT, 1.5 l/t	Bactofit BT, 3 l/ha + BioHibervit BT, 3 l/ha
7	Gliocladin BT, 1.5 l/t + Vitastim BT, 1.5 l/t	Gliocladin BT, 3 l/ha + Vitastim BT, 3 l/ha
8	Trichopsin BT, 1.5 l/t + Vitastim BT, 1.5 l/t	Trichopsin BT, 3 l/ha + Vitastim BT, 3 l/ha
9	Fluorescin BT, 1.5 l/t + Vitastim BT, 1.5 l/t	Fluorescin BT, 3 l/ha + Vitastim BT, 3 l/ha
10	Biospectrum BT, 1.5 l/t + Vitastim BT, 1.5 l/t	Biospectrum BT, 3 l/ha + Vitastim BT, 3 l/ha
11	Bactofit BT, 1.5 l/t + Vitastim BT, 1.5 l/t	Bactofit BT, 3 l/ha + Vitastim BT, 3 l/ha

Source: developed by the authors based on the findings

Statistical processing of experimental data was performed using Microsoft Office® for Microsoft Windows®. The study followed the ethical standards specified in the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

One of the ways to protect potato plants from pests in an environmentally friendly way is to use biological products. Thus, during 2021-2022, the study investigated various schemes of combinations of biological products based on microorganisms, their metabolites, and biologically active substances against the most widespread potato diseases during the growing season – *Alternaria* and late blight. The treatment of potato tubers before planting and threefold spraying of plants led to a decrease in the spread and development of *Alternaria* on the aboveground organs in all variants of the experiment (Fig. 1). Specifically, the number of diseased plants was less by 38.7-51.2%, and the degree of damage decreased within 19.1-24.2% compared to the control, where these indicators were 65.8% and 27.3%, respectively. Preparations based on fungi of the genera *Gliocladium* and *Trichoderma* and bacteria of the genera *Pseudomonas* and *Bacillus* provided technical efficiency against *Alternaria* within 70.0-88.6%. Mixtures of Biospectrum BT + Vitastim BT, Bactofit BT + BioHibervit BT and Bactofit BT + Vitastim BT had the greatest effect on reducing the damage to potato plants by the pathogen (technical efficiency – 82.0-88.6%).

The studied biological products also had a fungicidal effect on potato late blight, which was

manifested in a decrease in the spread of the disease by 23.3-37.2% and a decrease in its development within 11.4-15.7% (Fig. 2). In the variants with the use of mixtures of Bactophyte BT (*Bacillus* spp.) + Vitastim BT (*Trichoderma* spp., *Pseudomonas* spp.), Biospectrum BT (*Pseudomonas* spp.) + Vitastim BT (*Trichoderma* spp., *Pseudomonas* spp.), Trichopsin BT (*Trichoderma* spp., *Pseudomonas* spp.) + Vitastim BT (*Trichoderma* spp., *Pseudomonas* spp.) and Bactophyt BT (*Bacillus* spp.) + BioHibervit BT (*Trichoderma* spp.) showed the maximum effect on reducing the intensity of late blight development. Technical efficiency under different variants of application of the preparations ranged from 61.6% (Trichopsin BT + BioHibervit BT, Fluorescin BT + BioHibervit BT) to 84.9% (Bactofit BT + Vitastim BT).

The use of biological products in the treatment of potato tubers and spraying of plants during the growing season positively influenced plant growth, yield formation, and generally increased crop productivity (Fig. 3). Depending on the biological products used, potato growth was stimulated. Specifically, compared to the control, the height of plants in different variants was 1.9-6.7 cm higher. Under the influence of the studied biological preparations, the total number of tubers increased by 0.4-8.5 per plant. Therewith, the marketable fraction increased, depending on the variant, from 1.3 to 4.5 pieces per plant. The greatest increase in potato yield was observed in the variants Bactophyt BT + BioHibervit BT – 14.0 t/ha, Trichopsin BT + Vitastim BT – 14.7 t/ha, and Biospectrum BT + Vitastim BT – 15.0 t/ha. The increase in potato yield in other variants with the use of biological products ranged within 4.4-12.8 t/ha.

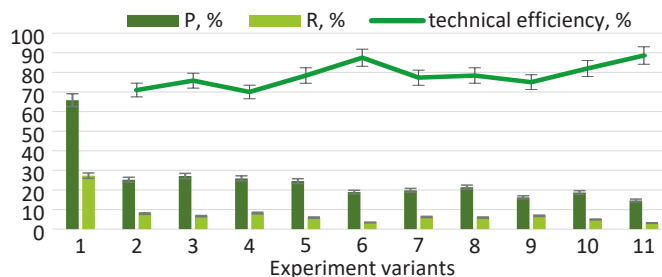


Figure 1. Efficiency of biological products against potato *Alternaria* (average for 2021-2022)

Note: P – disease spread; R – disease development

Source: developed by the authors based on the findings

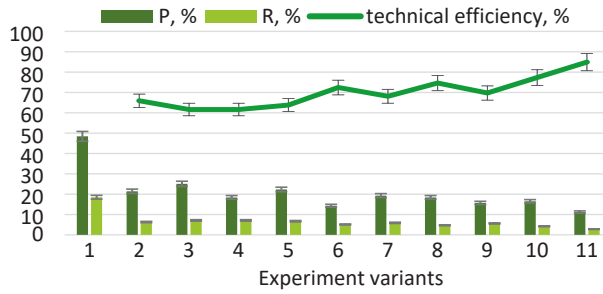


Figure 2. Efficiency of biological products against potato late blight (Podolyanka variety, average for 2021-2022)

Note: P – disease spread; R – disease development

Source: developed by the authors based on the findings

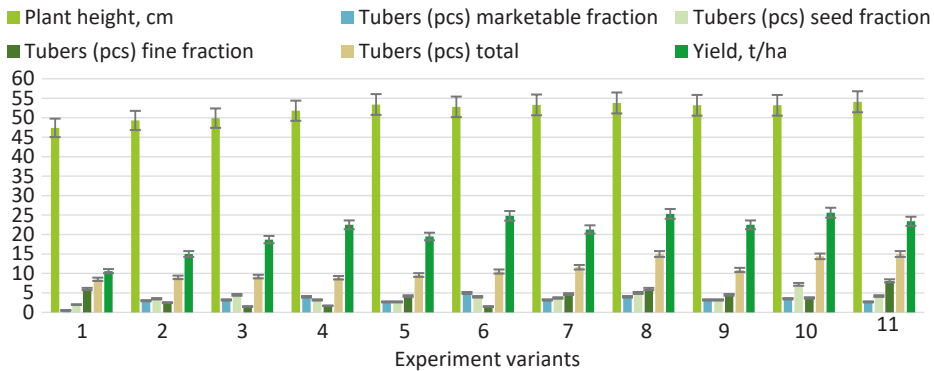


Figure 3. Influence of fungicidal and stimulating biological preparations on the indicators of structure elements and yield of potato tubers (average for 2021-2022)

Source: developed by the authors based on the findings

The conducted studies confirmed the results obtained in different regions of potato cultivation using biological products and antagonist microorganisms (Tleuova *et al.*, 2020). N. Rokaya *et al.* (2023) tested isolates of *Trichoderma* spp. against the potato late blight pathogen. Their effectiveness was manifested in a decrease in the size of the pathogen lesion area by 19-46% compared to the control. It was found that isolates of *Trichoderma* spp. together with the commercial product Sanjeevni (based on *T. viride*), in field experiments on a natural infection background stimulated plant growth, tuber yield, and reduced the development of late blight. Specifically, *Trichoderma* isolates TL1-2A, TL4-81A, and TL5-21A reduced the intensity of the disease by 37.3%, 37.2%, and 30.4%, respectively. According to the findings of two-year

studies, isolate TL1-2A increased tuber yield by 46.4%. S. Kumar *et al.* (2023) found that biopriming of potato tubers by a microbial consortium (*T. viride*, *B. subtilis*, *P. fluorescens*) improved plant growth and induced defence responses against *A. solani*, which was conditioned by the induction of systemic resistance.

In laboratory experiments conducted by M. Sulaiman *et al.* (2020), the inhibition of the pathogen of soft rot of potato tubers – *Erwinia carotovora*, subsp. *carotovora* – by *Trichoderma* spp. isolates was proved. A decrease was also found in the damage of tubers treated with *T. viride* (TV3) and *T. harzianum* (TH2), respectively, by 20.27% and 16.47%, compared to the control. Yu. Kolomyets & L. Butsenko (2023) emphasised that soft rot pathogens are the primary reason for limiting potato production in

many regions around the world. J. Leiminger & H. Hausladen (2012) noted that parasitism of potato plants by *A. solani* can lead to a 50% yield loss. M. Solomiychuk *et al.* (2024) used the biological product PhytoDoctor (*Bacillus subtilis*), 2 l/ha in combination with Humat Ultra, 60 g/ha in the conditions of Ukraine and noted a decrease in the development of *Alternaria* by 54.9% compared to the control.

I. Debez *et al.* (2024) found endophytes of *Bacillus* (*B. halotolerans* SpS5, *B. amyloliquefaciens* LiR9, *B. haynesii* ReR10, *B. velezensis* KnL15, *B. aryabhatai* FaR1) to have an antagonistic effect against the fungus *R. solani*, the causative agent of potato black scab. The vegetative and spore-forming strain SpS5, as well as 5 other *Bacillus* and their SpS5-based consortia were effective in reducing disease development and increasing the yield of healthy, high-quality potato tubers. The results of the study of the effectiveness of various biological products suggest the possibility of their use for the protection of potatoes against *Alternaria* and late blight. The studied biological agents had a protective effect on potato plants, which was manifested in a decrease in their damage by pathogens compared to the control. In addition, the mechanisms of plant growth and development were activated, and the yield increased.

CONCLUSIONS

As a result of the study, the use of combinations of various biological preparations based on microorganisms, their metabolites, and biologically active substances was found to decrease the spread and development of potato diseases on the aboveground organs of plants in all variants of the experiment. Treatment of potato tubers before planting and threefold spraying of plants during the growing season reduced the spread of *Alternaria* by 38.7-51.2% and reduced the

intensity of plant damage within 19.1-24.2% compared to the control. Biological products provided technical efficiency against *Alternaria* in the range of 70.0-88.6%.

The investigated biological products reduced the spread of late blight within 23.3-37.2% and limited the intensity of the disease by 11.4-15.7%, while their technical efficiency ranged within 61.6-84.9%. The use of biological products positively influenced the growth of potato plants, the formation of the crop and, as a result, the overall productivity. Specifically, the height of plants in different variants was 1.9-6.7 cm higher compared to the control. Under the influence of the studied biological preparations, the total number of tubers increased by 0.4-8.5 per plant. The marketable fraction increased, depending on the experimental variant, from 1.3 to 4.5 pieces per plant. The highest increase in tuber yield was observed in the variants with the introduction of biological products Bactophyt BT + BioHibervit BT – 14.0 t/ha, Trichopsin BT + Vitastim BT – 14.7 t/ha and Biospectrum BT + Vitastim BT – 15.0 t/ha.

In further research, it will be significant to assess the technical effectiveness of biological products under different meteorological conditions, as well as on potato varieties with different resistance, which will allow establishing the best combinations of biological control agents and improve the scheme of their application. Overall, the findings of the study confirmed the ability of biological products to control *Alternaria* and late blight of potatoes, which is relevant in modern agrarian production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Біологічний контроль альтернаріозу та фітофторозу картоплі

Анотація. Інфекційні хвороби – альтернаріоз і фітофтороз, викликані мікроорганізмами *Alternaria solani* і *Phytophthora infestans*, – часто знижують урожайність і якість картоплі. Контроль захворювань передбачає інтенсивне використання хімічних пестицидів, що призводить до виникнення резистентності патогенів і різних екологічних викликів. Тому актуальним є вивчення безпечних методів захисту рослин. Метою дослідження була оцінка ефективності різних комбінацій біологічних препаратів проти альтернаріозу та фітофторозу картоплі. Експерименти проводили в умовах Української науково-дослідної станції карантинурослин Інституту захисту рослин Національної академії аграрних наук. Для досліджень використовували біофунгіциди на основі мікроміцетів родів *Gliocladium* і *Trichoderma* та бактерій родів *Pseudomonas* і *Bacillus*. Застосування комбінацій різних препаратів включало передпосадкову обробку бульб і три обприскування вегетуючих рослин на різних етапах їх росту та розвитку. Використання біопрепаратів на картоплі зменшувало поширення альтернаріозу на 38,7-51,2 % та знижувало інтенсивність розвитку хвороби в межах 19,1-24,2 % порівняно з контролем. При цьому технічна ефективність становила від 70,0 до 88,6 %. Найбільш дієвими комбінаціями для обмеження хвороби були Біоспектр БТ + Вітастим БТ, Бактофіт БТ + БіоГібервіт БТ, Бактофіт БТ + Вітастим БТ, які забезпечували технічну ефективність 82,0-88,6 %. Досліджувані біопрепарати також зменшували ураження рослин збудником фітофторозу. Технічна ефективність за різних варіантів застосування препаратів становила від 61,6 % (Трихопсин БТ + БіоГібервіт БТ, Флуоресцин БТ + БіоГібервіт БТ) до 84,9 % (Бактофіт БТ + Вітастим БТ). Біологічні препарати позитивно впливали на ріст рослин, формування врожаю та загальну продуктивність. Найбільший приріст урожаю спостерігали у варіантах із застосуванням Бактофіт БТ + БіоГібервіт БТ, Трихопсин БТ + Вітастим БТ, Біоспектр БТ + Вітастим БТ. Проведені дослідження підтвердили потенціал біопрепаратів в управлінні хворобами картоплі

Ключові слова: біопрепарати; грибні хвороби; мікроорганізми-антагоністи; ефективність; бульби; урожайність

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Productivity of binary crops under the application of different cultivation technology elements

Abstract. Enhancing cultivation technologies adapted to climate change while maintaining soil fertility is a key objective in the agricultural sector. Particular attention is given to implementing intercropping systems and selecting the most effective technological elements to maximise agroecosystem productivity. This study aimed to examine the effects of various biopreparations on the productivity of a barley-pea binary crop and the mineral nitrogen content in the soil. The research was conducted in 2023-2024 at the experimental field of the Separate Subdivision of the National University of Life and Environmental Sciences of Ukraine, "Agronomic Research Station" in the Kyiv Region. The experiment comprised five treatments: control, biochar, humus extract, EM-5 preparation, and LF 20 humate. Plant samples for biomass assessment were collected on the 30th, 60th, and 90th days after emergence. Soil samples from the 0-10 cm, 10-20 cm, and 20-30 cm layers of typical medium loam chernozem were analysed for mineral (nitrate and ammonium)

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nitrogen content following DSTU 4729:2007. It was established that the best seedling emergence in the pea-barley intercropping system was observed with the application of biochar, reaching 64 and 140 plants per m², respectively. However, by the 30th day, biochar application suppressed plant growth, whereas the highest values were recorded in the humus extract treatment, followed by the EM-5 treatment. A similar trend was observed on the 60th day after emergence. Pea yield in the LF 20 humate treatment was 1.56 t/ha, while in the humus extract treatment, it was 1.52 t/ha. The corresponding barley yields were 4.52 t/ha and 5.19 t/ha. Biochar application was the least effective in enhancing crop productivity in intercropping; the pea yield increase relative to the control was negligible, while barley yield decreased by 0.23 t/ha. This decline was attributed to the deterioration of the soil nitrogen regime, as mineral nitrogen content in this treatment was the lowest on the 60th day after crop emergence, ranging from 31.1 to 34.8 mg/100 g of soil. The findings hold practical significance for improving organic fertilisation systems in binary cropping and enhancing agroecosystem resilience. They may be utilised by agricultural producers across various ownership structures

Keywords: intercropping; pea; barley; biochar; humates; biopreparations

INTRODUCTION

In contemporary agricultural production, ensuring food security requires producers to continuously improve crop cultivation technologies, adapt them to changing climatic conditions, and preserve soil fertility. One key element of this improvement is the utilisation of intercropping systems, including binary crop systems, and the selection of optimal technological components to maximise productivity with appropriate nitrogen nutrition. Binary cropping, which involves growing two different crops in the same field, is becoming increasingly prevalent as it enables greater efficiency and reduces the risks associated with monoculture. However, the choice of specific crops for binary cropping, as well as the correct combination of cultivation technology elements – such as soil tillage, fertiliser systems, and plant protection – are of paramount importance for achieving maximum productivity and maintaining soil fertility.

R.-P. Yu *et al.* (2025) note that intensive crop cultivation technologies, which have met the significant demand for agricultural products, are based on monoculture and the use of mineral fertilisers and pesticides, leading to the loss of biodiversity in agricultural lands and a negative impact on the environment. In recent years, scientists' attention has been focused on intercropping and mixed cropping systems. A. Scavo *et al.* (2022) conclude that long-term intercropping research is warranted, particularly regarding

its impact on soil fertility indicators. Y. Fan *et al.* (2020) highlight the higher economic efficiency and productivity of maize and soybean intercropping compared to monocultures of these crops. As this practice is effective in influencing land productivity, specifically, the highest Land Equivalent Ratio (LER), System Productivity Index (SPI), and Monetary Advantage Index (MAI) were obtained when mixing barley at 25% with a grass pea sowing rate of 75%. Intercropping barley with peas has significant potential to improve forage productivity with high land-use efficiency (Jaskulska *et al.*, 2022). R. Cowden *et al.* (2020) state that growing cereals and legumes together increases nitrogen input through biological nitrogen fixation. A. Oberson *et al.* (2024) point to the improved utilisation of both fertilisers and soil nitrogen, which often leads to increased grain nitrogen content and higher productivity per unit area compared to monocultures. Research results from V. Vakhnyak *et al.* (2025) have shown that over 40% of Ukraine's arable land is degraded, resulting in a 20-30% yield reduction and economic losses reaching 15 billion UAH annually. However, the application of biochar in areas with organic matter deficiency has increased humus content by up to 12%, and innovative restoration approaches generally provide a 15-25% yield increase in the first five years.

Biopreparations have a positive impact on crop productivity indicators. The research by

Y. Ma et al. (2024) has established the positive effect of humic acid preparations on agricultural crop productivity and nitrogen utilisation, thereby ensuring the sustainability of agricultural landscapes by reducing the excessive use of synthetic fertilisers. The use of 50% compost + 50% mineral nitrogen fertilisers together with 1.5 L ha⁻¹ of biostimulants (VIUSID® agro) increased the yield of both wheat varieties (Abbas et al., 2022). A 100% compost application significantly increased protein, crude fibre, total sugars, Mg, and Mn content in the grain, while a 100% mineral nitrogen fertiliser treatment significantly increased ash, total phenols, P, and Ca content in the grain. Replacing nitrogen fertilisers with compost significantly increased ether extract and carbohydrate content in the Nigerian variety's grain, while N, K, and Fe content increased in the grain of both studied varieties. Foliar feeding with biostimulants at various levels significantly increased protein, carbohydrate, total sugar, P, K, Ca, Cu, and Zn content. Replacing mineral nitrogen fertilisers with a combination of compost and mineral nitrogen (50% compost + 50% mineral nitrogen) together with 1.5 L ha⁻¹ of biostimulants is recommended to enhance yield and grain quality. The biologisation of the

fertiliser system in the Right-Bank Forest-Steppe of Ukraine contributed to improving the humus status of typical chernozem and enhancing its physicochemical properties (Balayev et al., 2020).

This research aimed to determine the impact of biostimulants on the biomass accumulation of barley-pea intercropping and the dynamics of mineral nitrogen consumption in typical chernozem. This will enhance the productivity and stability of agricultural production by improving the understanding of the effects of soil nitrogen consumption and cultivation technology elements in binary cropping systems.

MATERIALS AND METHODS

The research was conducted in 2023-2024 at the Separate Subdivision of the National University of Life and Environmental Sciences of Ukraine, "Agronomic Research Station" in the village of Pshenychne, Fastiv District, Kyiv Region, Ukraine (located in the northeastern part of the RightBank Forest-Steppe, 49°46' N, 30°44' E). In the field experiment, the size of the experimental plots was 15 m² with three replications of each treatment. The experimental design included the following treatments involving biopreparations and organic fertilisers (Table 1).

Table 1. Experimental treatments at the Separate Subdivision of NULES of Ukraine "Agronomic Research Station"

Treatment name	Application rates of organic fertilisers and biopreparations
T.1	Control – no fertilisers or biopreparations applied
T.2	Biochar – 250 kg/ha applied during primary soil tillage
T.3	Humus extract – 50 L/ha at 30 days after emergence + 50 L/ha at 60 days after emergence
T.4	EM-5 – 5 L/ha at 30 days after emergence + 5 L/ha at 60 days after emergence
T.5	Humate LF 20 – 0.4 L/ha at 30 days after emergence + 0.4 L/ha at 60 days after emergence

Source: developed by the authors

Biochar is a charcoal produced by the thermal decomposition of biological materials under limited oxygen conditions. Biochar IDEALE is a highly active soil amendment composed of 92-96% carbon accumulated in biomass, processed via hydrothermal carbonisation (Biochar, n.d.). Humus extract is an organic product derived from the processing of humus waste.

EM-5 is a complex of organically fermented ingredients with Effective Microorganisms® (EM®), which enhance plant resistance to conditionally pathogenic and pathogenic microorganisms. This preparation is used as a preventative measure to avoid plant diseases and as a pest control agent (EM-Ukraine, n.d.). Humate LF 20 is a concentrated organo-mineral

fertiliser, plant growth stimulant, and soil conditioner based on humic acids derived from leonardite. It contains 180 g/L of humic acids, 20 g/L of fulvic acids, and 30 g/L of potassium, has a pH of 9-11, and contains no less than 5 g/L of microelements (AgroPlant, n.d.).

Crops were sown using a row method with a 12.5 cm inter-row spacing at a soil temperature of 4-6°C at seed placement depth, utilising a Klen-1.5 seeder for binary cropping of peas and barley under various cultivation technologies. The soil of the experimental plot was a typical medium loam chernozem developed on carbonate loess. The organic carbon content ranged from 2.55-2.61% in the 0-30 cm layer, pH was 8.2-8.4, and the cation exchange capacity was 31.7-32.2 meq per 100 g of soil.

The 2023 season was characterised by a stable increase in temperature during spring, while 2024 experienced high temperatures throughout May-August and very low precipitation. Sowing in 2023 was conducted at the end of March, followed by the total monthly precipitation. A significant amount of April precipitation occurred before crop emergence, resulting in soil moisture levels close to full field capacity. Temperature increases were moderate until June, which somewhat slowed crop development. Soil moisture reserves were sufficient for normal crop development despite low May precipitation. In June, the average monthly temperature exceeded 20°C, but precipitation was adequate for normal crop development. After the previous crop was harvested, ploughing was carried out to a depth of 20-22 cm. In spring, moisture was retained using tine harrows, and pre-sowing cultivation was performed to a depth of 6-8 cm. The application of mineral fertilisers and plant protection products was not included in the experimental design.

Identical varieties for production systems were used in the experiment: pea ORCHESTRA and barley PROSPECT. On the day of sowing, pea seeds were inoculated with Rizoactive Legumes at a rate of 2 L/tonne of seeds. Seeds were sown without seed dressing. The sowing depth for peas was 6-8 cm, and for barley 4-6 cm. In intercropping, peas were sown first at a depth of

6-8 cm, and then barley was sown in the same rows at a depth of 4-6 cm.

Plant samples for biomass determination were collected on the 30th, 60th, and 90th days after emergence. For barley, the density of standing ears, the number and weight of seeds per ear, and the 1,000-seed weight were determined. For peas, the number and weight of seeds per plant, the number of plants, and the 1,000-seed weight were recorded. Yield and weight elements of yield structure are presented in terms of 14% moisture content. Soil samples were collected from soil layers 0-10, 10-20, and 20-30 cm following DSTU 4287:2004 (2005), and preparation for analysis and preliminary processing of soil samples for physicochemical analysis were performed according to DSTU ISO 11464:2007 (2009). The content of mineral (nitrate and ammonium) nitrogen was determined in soil samples according to DSTU 4729:2007 (2008). Dispersion analysis was performed using the STATISTICA 13.0 package (©TIBCO). Fisher's LSD-post hoc test ($\alpha = 0.05$) was used to assess differences between treatments. The research complied with the ethical standards set out in the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

The research revealed that the germination rates of peas and barley, sown at the same seeding rate, varied across the different treatments, with the best results observed in the biochar treatment – 64 pea plants/m² and 140 barley plants/m². The control treatment yielded 54 pea plants/m² and 148 barley plants/m², indicating a positive effect of biochar on the germination of these crops (Table 2). The differences in pea plant numbers during the germination phase due to the influence of different biostimulants were not statistically significant.

The results of the study on the weight of peas and barley in green biomass under the influence of various biostimulants on the 30th day of the growing season (PS30 – plant weight) are presented in Figure 1.

Table 2. Germination rates of peas and barley under different biostimulants treatments (average for 2023-2024), plants/m²

Experimental variant	Peas	Barley
Control	54	148
Biochar	64	140
Humus Extract	64	124
Humate LF 20	62	112
EM-5	62	127

Source: developed by the authors based on research

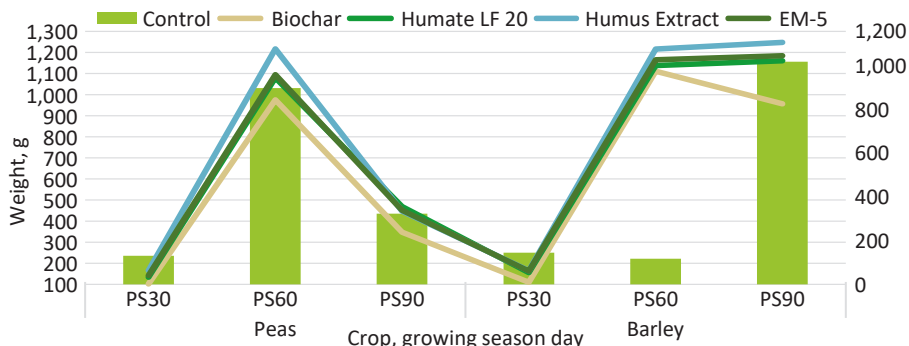


Figure 1. Dynamics of green biomass accumulation in peas and barley on the 30th, 60th, and 90th days of the growing season for mixed cultivation of peas and barley, g/m² (average for 2023-2024)

Source: developed by the authors based on research

In the control treatment without the application of biopreparations, the green biomass weight was 130 g for peas and 144 g for barley. The use of biochar led to a decrease in weight for both crops by 27% and 31%, respectively, while the application of humus extract resulted in an increase of 27% and 15% compared to the control. The application of Humate LF 20 and EM-5 did not lead to significant changes in the indi-

cators relative to the control. Corresponding changes on the 30th day of the growing season were observed in dry matter (Fig. 2). The decrease in weight with the application of biochar was 19% for peas and 20% for barley. The increase in pea weight with the use of humus extract was 20%, while the other biostimulants did not have a significant effect on the dry matter of peas and barley.

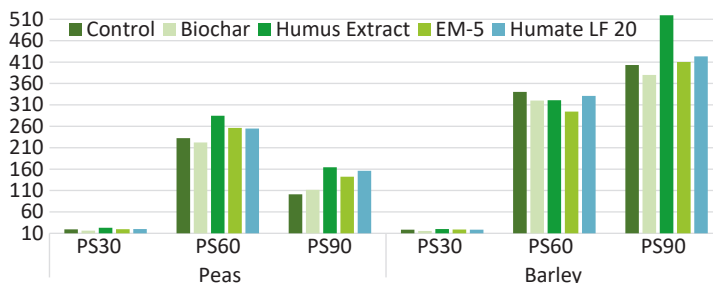


Figure 2. Dynamics of dry mass accumulation in peas and barley on the 30th, 60th, and 90th days of the growing season for mixed cultivation of peas and barley, g/m² (average for 2023-2024)

Source: developed by the authors based on research

At 60 days of growing season, the control treatment showed a pea weight of 894 grams in green biomass and 232.4 grams in dry matter, while barley weight was 1,170 grams in green biomass and 360.4 grams in dry matter. The biochar treatment showed a slight increase in pea weight and a decrease in barley weight at 60 days of growing season compared to the control. Peas reached 975 grams in green biomass and 222.3 grams in dry matter, while barley reached 1,111 grams in green biomass and 320.0 grams in dry matter. The use of Humate LF 20 also contributed to an increase in pea weight but decreased barley weight at this stage of the growing season. Peas reached 1,080 grams in green biomass and 254.9 grams in dry matter, while barley reached 1,138 grams in green biomass and 320.9 grams in dry matter. The highest results were observed in the humus extract treatment. Peas reached 1,217 grams in green biomass and 284.8 grams in dry matter, and barley reached 1,216 grams in green biomass and 294.3 grams in dry matter.

At 90 days of growing season, the control treatment showed a pea weight of 322.2 grams in green biomass, with a seed weight of 101.3 grams per square meter. Barley weight was 1,014.0 grams in green biomass and 403.0 grams per square meter. The use of biochar increased

pea weight compared to the control, reaching 347.7 grams in green biomass, and a seed weight of 111.7 grams per square meter, while barley decreased relative to the control to 956.6 grams in green biomass and 380.1 grams per square meter. Humate LF 20 significantly increased the weight of both peas and barley at this stage of the growing season, reaching 469.1 grams in green biomass, with a seed weight of 156.1 grams per square meter, while barley reached 1,160.6 grams in green biomass and 451.6 grams per square meter.

The highest results were obtained in the humus extract treatment, with 446.9 grams of green biomass and a seed weight of 152.1 grams per square meter for peas, and 1,247.8 grams of green biomass and 519.3 grams per square meter for barley. EM-5 also showed high results, while biochar had a lesser impact on the yield of these crops at this stage. Biopreparations have different mechanisms of action on legumes. In studies by M. Solomiychuk & M. Pikovskiy (2021), the application of biological preparations for seed treatment and vegetating soybean plants increased yield and reduced the manifestation of the soybean mycotic complex. As a result, biostimulants had a positive effect on the yield of peas and barley, except biochar, the use of which led to a slight decrease in yield (Table 3).

Table 3. Effect of biostimulants on pea and barley yield in intercropping, t/ha (average for 2023-2024)

Experimental variant	Peas		Barley	
	t/ha	±	t/ha	±
Control	1.01	-	4.03	-
Biochar	1.12	0.11	3.80	-0.23
Humate LF 20	1.56	0.55	4.52	0.49
Humus Extract	1.52	0.51	5.19	1.16
EM-5	1.42	0.41	4.17	0.14
LSD _{0.95}	0.16		0.24	

Source: developed by the authors based on research

Significant yield increases in peas were achieved with the use of humates and the EM-5 preparation. The highest yield increases were obtained with the use of humus extract, amounting to 0.51 t/ha for peas and 1.16 t/ha for barley. The use of Humate LF 20 resulted in an increase in pea yield of 0.55 t/ha and barley yield of 0.49 t/ha compared to the control, indicating

its high effectiveness in intercropping. The high effectiveness of applying a mixture of bioactive humic molecules together with microbial consortia to improve crop productivity in binary crops is also evidenced by the results of other researchers (Atero-Calvo *et al.*, 2024).

Regarding humus extract, it also contributed to an increase in yield for both peas (by 0.51 t/ha)

relative to the control and barley (1.16 t/ha). In the biochar treatment, pea yield increased by 0.11 t/ha compared to the control, while barley yield decreased by 0.23 t/ha compared to the control. However, the use of biochar in monoculture had a positive effect and improved soil fertility indicators, as well as increased grain yield of barley by 2.84-19.88% and maize by 12.27-16.74% (Tang *et al.*, 2023). Intercropping advantages only arise when each species has sufficient time and space to maximise cooperation and minimise competition between them. Research on binary cropping of other crops was conducted by F. Han *et al.* (2023), who studied the impact of maize and soybean binary cropping systems on the uptake and utilisation of nitrogen, phosphorus, and potassium. They found that compared to maize monoculture, biological yield and economic efficiency increased. In other studies, it was established that the application of a mixture (1:1) of chemical and organic nitrogen fertilisers increased maize seed yield and quality, and soybean yield in studies conducted in China (Lin *et al.*, 2022). It is important to compare not only fertilisation treatments but also spatial variations in the arrangement of a barley-pea 50:50 mixture. In all cases of spatial variations, the dry matter content in companion crops was higher than in monocultures. Intercropping also improves soil physical properties, including microaggregation, porosity, and infiltration. It also leads to the accumulation of soil organic carbon (Walker *et al.*, 2011). Research by J. Abdollah *et al.* (2014) demonstrated that intercropping barley (*Hordeum vulgare* L.), vetch (*Vicia villosa*), and grass pea (*Lathyrus sativus* L.) in barley:legume ratios of 75:25, 50:50, and 25:75 reduced the dry matter yield of the three component plants compared to their respective monocultures. Intercropped barley had a higher crowding coefficient ($K = 1.64$) than intercropped legumes ($K = 1.20$), indicating that barley was more competitive

than legumes in the mixtures. Furthermore, grass pea was more competitive than vetch in mixtures with barley. Studies by T. Darch *et al.* (2018) have shown that growing intercrops of barley and legumes accumulate 10-70% more phosphorus compounds and up to 40% more plant biomass than monocultures. Moreover, the greatest increase occurred at or below the sub-critical phosphorus requirement for barley.

The cultivation of binary crops of pea and barley led to an increase in total accumulated nitrogen compared to monocultures. In the barley field, more mineral nitrogen was consumed, while peas relied on nitrogen fixation. Regardless of the location and pattern of the binary crop, a higher contribution of nitrogen fixation to the total nitrogen in pea plants was observed when grown in intercropping with barley. An increase in the accumulation of phosphorus (P), potassium (K), and sulphur (S) was also observed in the binary crop, which can influence overall yield and competitiveness for other resources. Overall, the binary sowing of pea and barley in an organic cultivation system proved to be an effective way to enhance nitrogen fixation (Hauggaard-Nielsen *et al.*, 2009). Studies by T. Sahota & S. Malhi (2012) found that growing a mixed crop of barley and peas on grey-clay soil in Canada improved barley yield by 420-488 kg/ha and reduced land use in production by 7-17%. The net profit from mixed cropping without nitrogen application increased to 854-939 USD per hectare, which is more than for monoculture barley with 80 kg N/ha (628 USD per hectare). It was also shown that the protein concentration in barley grain increased with nitrogen application, and mixed cropping with peas also led to an increase in this indicator.

Table 4 presents data on the mineral nitrogen content in the 0-30 cm soil layer for different treatments in the barley-pea intercropping experiment.

Table 4. Effect of biostimulants on mineral nitrogen dynamics in typical chernozem under barley:pea intercropping, mg/100 g soil (average for 2023-2024)

Experimental variant	Depth, cm	Mineral nitrogen content, mg/100 g of soil		
		30 days after germination	60 days after germination	90 days after germination
Control	0-10	21.2	34.2	21.3
	10-20	23.3	33.3	23.5
	20-30	23.7	31.7	19.2

Table 3, Continued

Experimental variant	Depth, cm	Mineral nitrogen content, mg/100 g of soil		
		30 days after germination	60 days after germination	90 days after germination
Biochar	0-10	23.1	31.1	24.6
	10-20	23.5	33.5	24.9
	20-30	23.8	34.8	23.7
Humate LF 20	0-10	27.6	38.6	25.8
	10-20	27.4	37.4	25.2
	20-30	27.8	37.8	22.3
Humus Extract	0-10	26.4	38.6	29.2
	10-20	27.1	34.4	30.1
	20-30	26.2	31.8	28.6
EM -5	0-10	26.9	36.9	24.2
	10-20	26.6	36.6	21.7
	20-30	24.1	34.1	23.0
LSD _{0.95}		1.74	2.04	1.45

Source: developed by the authors based on research

A seasonal dynamic in nitrogen content was observed, with the highest values occurring on the 60th day of intercropping. T. Chapagain & A. Riseman (2014) indicate that the decrease in land productivity caused by a reduction in soil organic carbon and nitrogen content is a serious problem in barley monoculture. Intercropping barley and peas can increase field productivity. In their study conducted in Vancouver, it was established that barley and pea intercropping is an effective strategy for increasing land productivity, yield, biomass, and grain quality, as well as nitrogen and carbon content, at a barley:pea ratio of 2:1 in the intercrop. In the control treatment, the nitrogen content at the beginning of the growing season was 21.2 mg/100 g. On the 60th day, as in the other treatments, it was the highest at 34.2 mg/100 g and then decreased by the 90th day after emergence to almost the initial level. The use of biochar had little effect on the change in mineral nitrogen content, indicating the suppression of nitrification processes and the binding of nitrogen compounds. Therefore, when using this biopreparation, special attention should be paid to plant nitrogen nutrition. It is also important to study not only the content of mineral nitrogen in the soil but also its ratio to carbon, since the critical C:N ratio in fertilisers is 10:1, and below this level, significant nitrogen losses can occur, negatively affecting the soil, plants, and the environment. A high C:N ratio leads to the loss of a significant amount of carbon and an enhanced greenhouse effect (Balayev *et al.*, 2023).

Scientists Z. Ahmad *et al.* (2021) established improved nitrogen mineralisation in soil based on laboratory studies, which, in their opinion, complicates the understanding of nitrogen transformation processes in soil with nitrogen application. Therefore, they warn that when applying biochar, soil nitrogen content should be monitored. C. Xiao *et al.* (2023) note that enzymes involved in the nitrogen cycle are extremely important in the biological catabolism of organic and mineral soil components in agroecosystems.

Studies on the use of Humate LF 20, humus extract, and EM-5 showed a statistically significant increase in mineral nitrogen compound content in the 0-10 and 10-20 cm soil layers by 10-30.2% relative to the control throughout the growing season. The greatest increase in nitrogen content was observed with the use of Humate LF 20 in the 0-10 cm layer, amounting to 30.2% on the 30th day, 12.3% on the 60th day, and 21.5% on the 90th day. Thus, the use of humates promoted pea nitrogen fixation. Similar conclusions were established by S. Malhi (2012), who noted that the use of nitrogen fertilisers improved the yield of barley and canola intercrops. The LER for intercropping were always greater than 1, indicating a lower need for land resources in intercropping compared to monocultures for the same yield. The general conclusion is that intercropping improves yield, nitrogen uptake, and economic outcomes, highlighting its potential in organic farming systems. Thus, a positive effect of biopreparations in companion crops of barley

and peas on productivity and the nitrogen pool of typical chernozem was established.

CONCLUSIONS

Biopreparations and organic fertilisers had a significant impact on the productivity indicators of peas and barley in binary crops under the conditions of the Kyiv region in the Right-Bank ForestSteppe of Ukraine on typical medium loam chernozems. The greatest impact of biopreparations in the experiment was established in terms of crop yield in intercropping. Biostimulants had a positive effect on the growth, development, and yield of peas and barley, except for the biochar treatment. The application of humus extract led to a significant increase in pea and barley yield, with the largest yield increase recorded in peas at 0.51 t/ha, while the barley yield increase was the highest at 1.16 t/ha. Humus extract and EM-5 preparation showed the greatest impact on plant weight and seed weight indicators at various stages of the growing season compared to other treatments. The application of biochar led to a decrease in plant weight for both crops by 27% and 31% compared to the control, while humus extract increased these indicators by 27% and 15%. The application of humates and EM-5 preparations noticeably increased the mineral nitrogen content in the

soil layers, indicating an improvement in the nitrogen regime due to symbiotic nitrogen fixation during pea cultivation.

The application of biochar caused a decrease in barley yield in the intercrop by 0.23 t/ha relative to the control. These results correlate with the mineral nitrogen content, which was the lowest in this treatment, especially on the 60th day after crop emergence, ranging from 31.1-34.8 mg/100 g of soil, indicating some immobilisation of nitrogen compounds by biochar. Thus, the issue of studying the effect of biochar in companion crops on the soil nitrogen regime is promising and requires detailed study in the context of researching the processes of organic and mineral compound transformations in soils.

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CONFLICT OF INTEREST

None.

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Продуктивність бінарних посівів за застосування різних елементів технологій вирощування

Анотація. Удосконалення технологій вирощування сільськогосподарських культур, що адаптовані до змін клімату і забезпечують збереження родючості ґрунтів, є одним із важливих завдань аграрного сектору. Особливу увагу приділяють впровадженню сумісних посівів і вибору найбільш ефективних технологічних елементів для забезпечення максимальної продуктивності агроландшафтів. Метою роботи було дослідження впливу різних біопрепаратів на продуктивність бінарного посіву ячменю та гороху, а також вміст мінерального азоту у ґрунті. Дослідження проводили протягом 2023-2024 рр. на дослідному полі у Відокремленому підрозділі Національного університету біоресурсів і природокористування України «Агрономічна дослідна станція» Київської області. Дослід включав п'ять варіантів: контроль, біочар, гумус екстракт, препарат ЕМ-5, гумат LF 20. Проби рослин для визначення біомаси відбирали на 30-й, 60-й та 90-й день після появи сходів. У зразках ґрунту, відібраних з 0-10, 10-20 та 20-30 см шару чорнозему типового середньосуглинкового, визначали вміст мінерального (нітратного та амонійного) азоту за ДСТУ 4729:2007. Встановлено, що найкраще проростання сходів сумісних посівів гороху та ячменю було за внесення біочару і складало 64 і 140 рослин на 1 м² відповідно. Водночас вже на 30-й день внесення біочару пригнічувало ріст рослин, а найвищі значення були на варіанті гумус екстракту, дещо поступався варіант ЕМ-5. Аналогічна тенденція була на 60-й день після появи сходів. Показники врожайності гороху на варіанті гумат LF 20 склали 1,56 т/га, гумус екстракт – 1,52 т/га, ячменю – відповідно 4,52 і 5,19 т/га. Внесення біочару мало найменшу ефективність за впливом на продуктивність культур у сумісному посіві: приріст врожаю гороху відносно контролю був несуттєвим, а урожайність ячменю знизилась на 0,23 т/га. Таке зниження пояснювалось погіршенням азотного

режиму ґрунту, адже вміст мінерального азоту за цього варіанту був найнижчим на 60-й день після сходів культур і складав 31,1-34,8 мг/100 г ґрунту. Отримані результати мають практичну цінність у вдосконаленні органічних систем удобрення бінарних посівів культур та забезпечення стійкості агроєкосистем і можуть бути використані виробниками сільськогосподарської продукції різних форм власності

Ключові слова: сумісні посіви культур; горох; ячмінь; біочар; гумати; біопрепарати

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