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Integrated application of microbial inoculant and biostimulants enhances pea bioproductivity in semi-arid conditions of the Steppe zone

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Abstract. Pea crops are unable to realize their genetic potential, and yields are reduced due to unfavourable abiotic factors inherent in the semi-arid zone. The research aimed to determine the separate and integrated effects of biostimulants and microbial inoculant on the formation of root nodules, assimilation apparatus, and yield of pea of the Devyz variety in the semi-arid conditions of the Steppe of Ukraine. The biostimulants Stimpo (St), Regoplant (Rp), and microbial inoculant Rhyzohumin (Rhh) were used for pre-sowing treatment, and then biostimulants foliar treatment of pea crops was applied in the budding phase at concentrations described in the methodology. It was found that under the combined use of biostimulants with inoculant, the number of nodules and their weight increased by 33–40% and 9–17% respectively, compared with variants where preparations were used separately. The studied preparations increased the leaf area index of pea crops by up to 1.5 times (BBCH 12–17) and 1.6 times (BBCH 51–79). The combined use of Rhh+St and Rhh+Rp allowed the formation of a larger leaf surface area of pea crops. In the case of the integrated effect of Rhh+St and Rhh+Rp, the net productivity of photosynthesis of pea exceeded the indicator of the variants with separate application of the preparations on BBCH 61–79. Improvement of crop structural elements increased pea yield by 3.0%, 4.5%, 7.9%, 17.9%, and 13.9% compared with the control when using Rhh, St, Rp, Rhh+St, and Rhh+Rp, respectively. The obtained data indicate the presence of a combined interaction between biostimulants and microbial inoculant.

Key words: legumes, plant growth stimulator, microbial inoculant, root nodules, assimilation apparatus, yield.

INTRODUCTION

Crop production efficiency nowadays depends on combining intensification with sustainable agricultural technologies. Excessive application of mineral fertilizers and plant protection products causes soil dehumification, reduces the abundance and physiological activity of soil bacteria, and disrupts agrocenosis structure. At the same time, violations of agricultural practices, poor-quality seeds, limited agronomic background, unfavorable climatic conditions, and damage from diseases and pests contribute to yield reductions.

Due to the increasing global demand for protein-rich raw materials for both animal feed and human nutrition, interest in pulse crops has been revived. Pulses contain about 22.4% protein and are known for their high digestibility (Powers & Thavarajah, 2019). The planted area of this crop is approximately 12–15% of the total area of the earth and world (Tulbek et al., 2024). Among them, the field pea (*Pisum sativum* L.) is cultivated in Ukraine and is regarded as one of the world's oldest domesticated crops (Yermolaiev & Gamajunova, 2022). In Ukraine, peas are the most common crop and can yield fairly high, stable grain yields compared to other grain legumes. In the period from 2019 to 2025, the area sown to peas in Ukraine decreased to 270 thousand hectares, due to reduced sown areas during the war. The largest areas sown with peas were recorded in 2018 and amounted to 431 thousand hectares.

Pisum sativum L. culture is cold-resistant, early ripening, poor demanding to soils. At its roots, the pea plant forms root nodules, which harbor nitrogen-fixing microorganisms, thanks to which the pea plant provides itself with 2/3 of its nitrogen from the air. Pea is among the few legume crops that have shown notably positive responses to biofertilizer application through symbiotic association with *Rhizobium* bacteria (Abd-Alla et al., 2023).

Numerous studies demonstrate the biological effects of microbiological preparations on crops during pre-sowing seed treatment (Chen, 2019; Pal et al., 2021; Parihar et al., 2022; Glogoza et al., 2023; Singh & Kumar, 2024). The effect of preparations Rhizobophyte and Rhizohumin, containing active strains of microorganisms of the genus *Rhizobium*, on common chickpea has been shown, which improved nitrogen nutrition of plants, as a result of which they stimulated stem growth, the formation of leaves and beans on shoots, increased the fresh weight of above-ground organs, and affected the water-holding capacity of leaves (Gebremariam & Tesfay, 2021; Chernik et al., 2024). Another study showed that the combination of inoculation of pea seeds and chickling vetch seeds with the microbiological preparation Rhizohumin and mineral fertilizer at a dose of $N_{30}P_{45}K_{45} + N_{15}$ increased leaf area, fresh and dry weight of plants, number of beans and number of seeds per plant, weight of 1,000 seeds, yield by 26%, protein content and total protein yield per hectare (Kaminkyi et al., 2021; Yeremko et al., 2024). As reported (Khodanitska et al., 2024), pre-sowing inoculation with Rhizohumin and Optimize enhanced soybean root nodulation and improved seed productivity.

A review (Naik et al., 2020) demonstrated that when effective microbial formulations were applied, biomass accumulation, photosynthetic efficiency, and antioxidant responses to abiotic stress in rice, active nodule formation in beans, and increased drought tolerance in soybean were observed. Also, seed inoculation with strains of associative bacteria of the genus *Azotobacter*, as shown (Yousif & Yousif, 2020; Ibrahim & El-Sawah, 2022), stimulated nodules' plant growth processes, mineral nutrition, protected against a number of infections, and increased resistance to adverse conditions.

Bioregulators of growth mainly act on plant enzyme systems, accelerating biochemical reactions; as a result, they play a significant role in plant responses to environmental factors. As a result, growth bioregulators stimulate the formation of organic matter synthesized during photosynthesis. The latter leads to the expression of genetic characteristics of cultivated plants, stimulation of growth processes, nutrient recycling, improvement of grain quality, and increased productivity (Kumar et al., 2021).

There are a lot of evidence that demonstrates the effectiveness of pre-sowing or foliar using growth regulators in soybean (Tkachuk et al., 2024; Kolesnikov & Paschenko, 2024a), pea (Lemishko et al., 2022; Novytska & Ponomarenko, 2022; Kolesnikov et al., 2024b), bean (Pyda et al., 2021), sunflower (Onyshchenko et al., 2023), and vegetable crops (Bagale et al., 2022), which contribute to a significant increase in the activity of symbiotic and associative nitrogen fixation by forming a more powerful legume-rhizobial symbiosis and increase the quality indicators of seeds and plant growth processes, stimulated the accumulation of masses of vegetative and generative organs, contributed to the formation of the assimilation surface of leaves, activated metabolic processes, reduced ripening times, increased resistance to various diseases, and also had a positive effect on the formation of grain productivity of crops.

Biostimulants and microbiological preparations actively influence cellular respiration, nutrient recycling, photosynthetic apparatus function, chlorophyll synthesis in leaves, and carbohydrate accumulation, thereby allowing plants to adapt more quickly to stress conditions (Dubey et al., 2020). Pre-sowing seed treatment with biostimulants Stimpo and Regoplant affected the accumulation of oil in bean seeds and carbohydrates in legume leaves, activation of growth processes, and formation of the leaf apparatus in soybeans (Pyda et al., 2021). It was found that the aforementioned biostimulants had bioprotective properties, enhanced growth processes, improved crop salt tolerance, and contributed to a significant increase in nitrogen fixation by strengthening the legume-rhizobial symbiosis (Tsygankova et al., 2021).

It has been shown that when soybeans are treated with Stimpo, the mass and number of root nodules, as well as their nitrogen-fixing activity, increase. The bioregulator does not significantly affect soybean growth. However, using Stimpo to mitigate the negative effects of herbicides is not advisable (Rybachenko et al., 2021). Regoplant and Stimpo, separately and in combination with bacteria, stimulated the growth of radish and okra plants (plant height, fresh weight, and fruit weight) when irrigated with fresh or salt water (Al-Busaidia et al., 2020). Therefore, to improve plant mineral nutrition in crop rotation and stimulate growth, the use of nitrogen-fixing, growth-stimulating, and adaptogenic biological preparations is effective. Thanks to microbiological preparations based on active strains of nitrogen-fixing symbiotic or associative bacteria, it is possible to increase soil fertility, boost crop yields, and address protein resource deficiencies (Yermolaiev & Gamajunova, 2022; Tulbek et al., 2024). In the context of global climate change and the growing role of sustainable agriculture, the use of biological growth regulators requires a comprehensive study (Chen, 2019; Mazur & Didur, 2020; Singh & Kumar, 2024).

Despite numerous studies on microbial inoculants and plant biostimulants in legumes, the combined effects of specific inoculant–biostimulant complexes under semi-arid conditions remain insufficiently characterized. Therefore, the novelty of the present study lies in determining the stage-dependent effects of the integrated application of inoculant and biostimulants on symbiotic apparatus, photosynthetic activity, and yield formation of pea under the climatic conditions of the Southern Steppe of Ukraine. The study also clarifies whether the combined application provides advantages over the separate use of microbial inoculant and biostimulants. In agreement with the status, the objective of this study was to establish the features of the separate and integrated effects of microbial inoculant 'Rhizohumin' and biostimulants ('Stimpo', 'Rehoplant') on growth, and development of pea, photoassimilating apparatus, and processes of yield formation of peas Devyz variety in the Southern Steppe of Ukraine.

MATERIALS AND METHODS

Research sites

The study was conducted on an experimental field of Dmytro Motornyi Tavria State Agrotechnological University in the Zaporizhzhia region. Mid-season peas (*Pisum sativum* L.) of the Devyz variety with leafless morphology were sown in the experimental plots. The Devyz pea variety was included in the Register of Plant Varieties of Ukraine in 2007 and recommended for cultivation in the Steppe zone of Ukraine. The originator is the V.Y. Yuriev Institute of Plant Industry of the National Academy of Agrarian Sciences of Ukraine.

The territory of the Zaporizhzhia region is characterized by a temperate continental climate with high temperatures. According to data from the weather station, the average annual long-term air temperature in the region is 9.9 °C. In the year of the study, during the pea cultivation period, the average daily air temperature was 15.2 °C, exceeding the long-term average for the pea vegetation period (12.1 °C). During a characteristic precipitation deficit, high temperatures and low hydrothermal potential were observed in spring or summer. According to long-term observations, the region receives 475 mm of precipitation annually. During the year of research, the precipitation was 174 mm during the pea growing season, and it is approximated to the amount of precipitation according to the data of long-term observations (161 mm). Generally, spring and early summer climatic conditions favored the growth and development of legumes.

The soil of the experimental plots is a southern alluvial chernozem, characterised by the following indicators: humus content (according to Tyurin) – 2.6%, easily hydrolysable nitrogen (according to Kornfield) – 111.3 mg kg^{-1}, mobile phosphorus (according to Chirikov) – 153.7 mg kg^{-1} and exchangeable potassium (according to Chirikov) – 255 mg kg^{-1} of dry soil. This corresponds to a high potassium content, an increased phosphorus content, and a low nitrogen content. The soil solution was neutral (pH of water/salt 7.0/7.3). The soil profile was not saline, but the content of exchangeable sodium was 7% of CEC. Soil moisture during sowing was 70–80% of the lowest soil moisture capacity. The soil was of high quality, sufficiently saturated with nutrients, and had favorable physical, chemical, and agrophysical properties, making it suitable for growing pulses (DSTU 4362:2004, 2006).

Description of the preparations

Rhizohumin (Rhh) is a biofertilizer used to bacterize pea seeds, improving plant nitrogen nutrition and increasing crop productivity. The preparation contains a suspension of pea nodule bacteria *Rhizobium leguminosarum* 31, physiologically active substances of biological origin (auxins, cytokinins, amino acids, humic acids), trace elements in chelated form, and macroelement compounds at starting concentrations.

Biostimulants Stimpo (St) and Regoplant (Rp) were developed by the State Enterprise 'Agrobiotech' and are approved for use in organic production in accordance with the MAOS Standard for Organic Production and Processing, which is equivalent to EU Regulations No. 834/2007 and No. 889/2008, Regulation of the European Parliament and of the Council (EU) No. 2018/848, the National Standard of Canada (COR), the Law of Ukraine 'On the Basic Principles and Requirements for Organic Production, Circulation and Labeling of Organic Products'. Stimpo and Regoplant are composite multifunctional preparations, the bioprotective properties of which are due to the synergistic effect of the interaction of the products of vital activity in the in vitro culture of the micromycete fungus *Cylindrocarpon obtisiucuilum* 680, isolated from the root system of ginseng (saturated and unsaturated fatty acids (C_{14}–C_{28}), polysaccharides, 15 amino acids, analogues of phytohormones of cytokinin and auxin nature), a complex of biogenic trace elements – 1.75 g L^{-1}, potassium salt of alpha-naphthylacetic acid – 1.0 mg L^{-1} and aversectin C – a metabolic product of the soil streptomycete *Streptomyces avermitilis* (Ponomarenko et al., 2015).

Experimental design

The experimental design included six variants. The variants of experimental plots were arranged systematically (Randomized Complete Block Design) with four replications. The pea seeds of the control group (variant 1) were treated by spraying with Liposam solution (5 g L^{-1}) before sowing. Pea seeds of variant 2 were treated by spraying before sowing with the microbiological preparation Rhizohumin (0.5 L t^{-1}); variant 3 – Stimpo (25 mL t^{-1}) ; variant 4 – Regoplant (250 mL t^{-1}); variant 5 – Stimpo (25 mL t^{-1}) + Rhizohumin (0.5 L t^{-1}); variant 6 – Regoplant (250 mL t^{-1}) + Rhizohumin (0.5 L t^{-1}). Solutions of Stimpo and Regoplant were prepared on the basis of Liposam solution (5 g L^{-1}). After the seeds dried, sowing was carried out in well-prepared soil with a seeding rate of 1.1 million pcs ha^{-1}. The area of each plot was 10 m^2, and the row spacing was 15 cm. Foliar treatments were carried out at the 5–6 stipule phase and the budding phase using the recommended rates for Stimpo – 20 mL ha^{-1}, and Regoplant – 50 mL ha^{-1}. The working solution consumption was 300 L ha^{-1} during foliar treatment of pea crops. Pea crops were grown using the technology generally accepted for the region, without irrigation. The crops were treated with an insecticide based on thiamethoxam (0.11 L ha^{-1}) against a complex of sucking and partially leaf-gnawing pests. Harvesting was carried out manually.

Measurements and data collection

Plant samples were collected during the growing season of pea plants in 5 phases according to the BBCH code: 12–13 (2–3 pairs of stipules), 16–17 (6–7 pairs of stipules), 51–55 (budding), 61–65 (flowering), 75–79 (bean formation).

The beginning of each phase of growth and development of the pea was established after its onset in 10% of plants, complete phase in 75% of plants, and the Biologische Bundesanstalt, Bundessortenamt, and Chemical industry (BBCH) scale was used to describe the phenological development stages of plants (Meier, 2018). At the end of the germination phase (BBCH 09), the field germination of pea seeds was determined. The number of seedlings per 1 m^2 was counted, and field germination was calculated in relation to the number of seeds sown in a given area as described in Yeshchenko et al. (2014).

To check for nodulation, gently clear the soil from the root mass of 10 plants from each plot. Nodules were detached from the primary root and the lateral roots. Root nodules were counted and weighed.

The leaf surface area of pea plants was determined gravimetrically by the «cuts» method. The gravimetric method relates the weight of leaves from 10 plant samples to the weight of leaf cuttings. Incisions were made in the leaf area with a special key of a certain diameter (D=10 mm). Knowing the area of the leaf cuttings, the leaf surface area was calculated by an Eq. 1:

$$S = \frac{P \times S_1 \times n}{P_1}, \tag{1}$$

where S is the total leaf area, cm^2; S_1 is the area of one section, cm^2; P is the total leaf weight, g; n is the number of slices; and P_1 is the weight of the sections, g.

Once the leaf surface area of each plant was determined, the leaf area index (LAI) for each site was calculated. To do this, the average leaf surface area per plant was taken and multiplied by the number of plants per 1 m^2 of sown area. (Kaushik et al., 2021).

The total chlorophyll content was determined by the fluorometric method using an N-tester (Yara, Japan). The measurement point was chosen in the middle of the first developed leaf. Based on the results of 30 random measurements performed according to the usual 'W' scheme, the average value was obtained, and the results were presented in conventional units.

The net photosynthetic productivity (NPP) was calculated as the ratio of the growth of plant dry matter mass over a certain period to the half-sum of the leaf surface areas at the beginning and at the end of the period. The NPP was calculated using the following Eq. 2:

$$NPP = \frac{B_2 - B_1}{0.5(S_1 + S_2)n}, \tag{2}$$

where NPP is the net productivity of photosynthesis, g m^{-2} day^{-1}; B_1 and B_2 are the dry mass of plants at the beginning and end of the period, g; (B_2-B_1) is the growth of dry weight of plants for n number of days, g; S_1 and S_2 are the leaf surface area at the beginning and end of the period, m^2; 0.5 (S_1+S_2) is the average area of the leaf surface during the experiment; n is the period between two observations, days.

The dry mass of the substance was determined gravimetrically according to generally accepted methods.

The elements of yield structure were determined, namely: the average number of plants per 1 m^2, the average number of beans per 1 plant, the average number of seeds per bean, the weight of 1,000 seeds, seed moisture, and biological yield was calculated. The yield coefficient was calculated as the ratio of pea grain mass to total aboveground plant biomass in the plot, with a base moisture content of 14%. Harvesting was carried out from each plot separately at full maturity of pea seeds (BBCH 89). The biological yield of pea crops was calculated according to generally accepted methods in agrobiology.

The study adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

Statistical analysis

Data analysis was carried out using GraphPad Prism 10 and Agrostat software. All measurements represent the means and standard error (±*SE*). The data were analyzed using a 2-way ANOVA test, where factor A (growth phase) and factor B (treatment). Statistically significant differences between means were compared at the 0.05 probability level using Tukey's test. In the statistical analysis of crop elements and yield, the arithmetic means and least significant differences ($LSD_{0.95}$) were calculated (Yeshchenko et al., 2014).

RESULTS AND DISCUSSION

The experimental data demonstrate that the formation and dynamics of root nodules in the pea plants of the Deviz variety were substantially influenced by the application of biostimulators and microbial preparation Rhizohumin. At the early developmental stages (BBCH 12–13), only minor differences were detected between treatments. The average number of root nodules in the groups ranged from 15.9 to 18.5 pcs. per plant (Fig. 1).

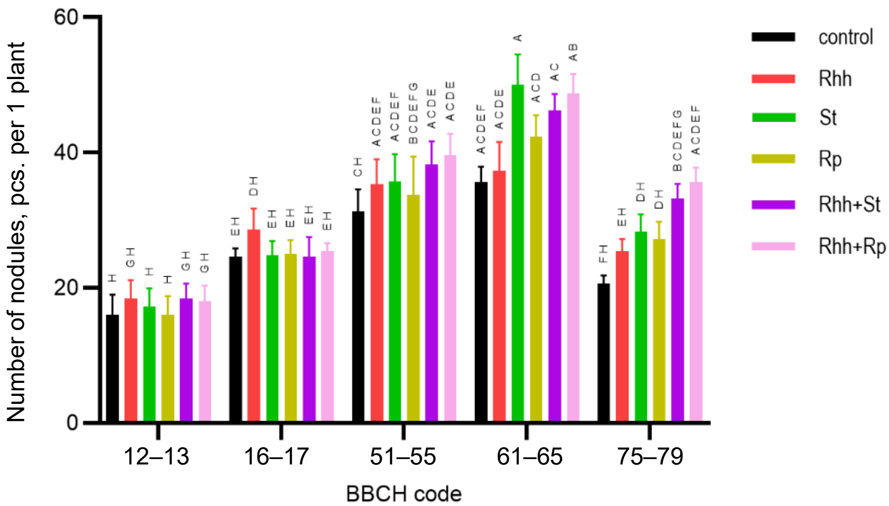

Figure 1. The number of nodules on the pea roots under the effect of separate and combined use of microbial inoculant and biostimulators. Vertical line – standard error of mean. (Here and in the following figures, values in columns with the same alphabets above do not differ significantly).

On the stage BBCH 51–55 onward, more distinct effects of integrated treatments became evident. A significant increase in the number of nodules under the conditions of combined use of Rhizohumin and biostimulants Stimpo and Regoplant by 11.7% and 15.8%, respectively, was recorded in the BBCH phase 51–55.

The most substantial differences were observed at BBCH 61-65, when nodule formation reached its peak. If the maximum number of nodules in the control variant was noted in the flowering phase and reached 35.7 pcs. per 1 plant, then when using biostimulants and a microbiological preparation, the maximum number of nodules in the specified phase was within 46–49 pcs. per 1 plant. In the flowering phase, a significantly increased number of root nodules was recorded both when the biostimulants Stimpo and Regoplant were used together with Rhizohumin, and in variants when they were used separately.

Thus, during the period of pea bean formation, a similar trend persists. The number of nodules on the roots of pea plants increased under the conditions of separate use of Rhizohumin, Stimpo, and Regoplant, respectively, by 22.7%, 36.7%, and 30.9% compared to the control. Stimpo and Regoplant, in combination with Rhizohumin, significantly increased the number of nodules by 33–40% during BBCH 75-79 compared with the variants in which these preparations were used separately.

Two-way ANOVA revealed that factors A and B are statistically significant in their effects on root nodule formation ($p < 0.0001$ for A and $p = 0.0008$ for B), while the interaction term was not significant ($p = 0.3583$). Both the growth phase and the biostimulators treatment independently influence nodule number, but no synergistic or antagonistic interactions are observed. The highest proportion of explained variance is attributed to factor A (62.51%), followed by factor B (5.18%).

The obtained data demonstrate that the weight of root nodules in pea plants varied significantly depending on the applied treatments with biostimulators and the microbial inoculant, both individually and in integrated application. The dynamics of nodule biomass formation throughout the growing season reflect the intensity of symbiotic development and nitrogen fixation activity in response to preparation influence. ANOVA showed that both factors A and B had a highly significant effect on nodule biomass ($p < 0.001$). The interaction between factors was also statistically significant ($p < 0.01$), indicating that the efficiency of the biostimulants and microbial inoculant treatments varied across pea plant phenological stages. Post-hoc comparisons using Tukey's HSD test confirmed the presence of statistically homogeneous groups, which are indicated in Fig 2 by different letters (A–J).

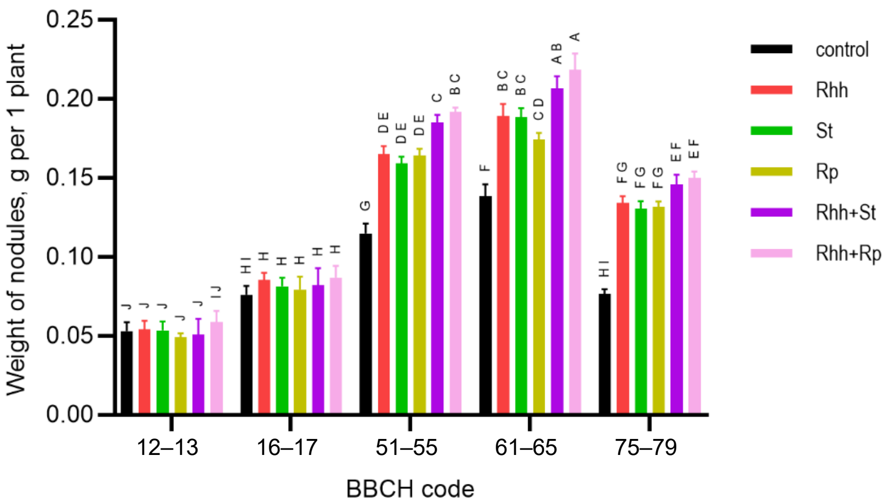

Figure 2. The weight of nodules on pea roots under the effect of separate and combined use of microbial inoculant and biostimulators. Vertical line – standard error of mean.

At the initial stages (BBCH 12–13 and 16–17), the differences among treatments were statistically insignificant ($p < 0.05$). The most pronounced differences occurred during the middle growth stages. At BBCH 51–55, the control plants produced 0.115 g of nodules per plant, whereas Rhizohumin, Stimpo, and Regoplant separately increased this value to 0.165, 0.159, and 0.164 g, respectively, representing 39–44% increases. Combined treatments demonstrated a stronger stimulatory effect, exceeding the control by 60–70% ($p < 0.05$). At the BBCH 61–65 stage, the nodule weight of control plants was 0.138 g, and it increased by 50% and 58% ($p < 0.01$) when biostimulators were combined with the microbial preparation.

A decline in nodule biomass was observed at the final stage, consistent with nodule senescence. Nevertheless, the integrated treatments retained significantly higher values than the control. So, when used together, biostimulators Stimpo and Regoplant with microbial inoculant approximately double the control value. These variants formed statistically separate homogeneous groups (F–H in Fig. 2).

The results presented in Table 1 demonstrate dynamic changes in the LAI of pea plants throughout the growing season under the action of biostimulators and the microbial inoculant, applied individually and in combination. At the beginning of vegetation (BBCH 12–13), LAI values were low (0.18–0.20 $m^2 m^{-2}$) and did not differ significantly among treatments. As growth progressed, the influence of treatments became more apparent.

Table 1. The leaf area index of pea crops under the effect of separate and combined use of microbial inoculant and biostimulators

BBCH code	Variants					
	Control	Rhh	St	Rp	Rhh+St	Rhh+Rp
12–13	0.19 ± 0.01^{m}	0.18 ± 0.02^{m}	0.20 ± 0.02^{m}	0.19 ± 0.01^{m}	0.20 ± 0.02^{m}	0.20 ± 0.02^{m}
16–17	0.86 ± 0.03^{l}	1.30 ± 0.05^{ik}	1.13 ± 0.05^{k}	1.31 ± 0.05^{ij}	1.44 ± 0.05^{gi}	1.53 ± 0.06^{gh}
51–55	1.21 ± 0.03^{jk}	1.38 ± 0.03^{hij}	1.30 ± 0.03^{ik}	1.42 ± 0.04^{hi}	1.95 ± 0.07^{e}	1.62 ± 0.07^{fg}
61–65	1.53 ± 0.06^{gh}	1.85 ± 0.05^{f}	2.41 ± 0.07^{b}	2.18 ± 0.09^{cd}	2.07 ± 0.07^{de}	2.39 ± 0.09^{b}
75–79	2.11 ± 0.07^{de}	2.32 ± 0.07^{bc}	2.81 ± 0.08^{a}	2.45 ± 0.09^{b}	2.84 ± 0.09^{a}	2.67 ± 0.10^{a}

Notes: Values represent the mean of four replications ± standard deviation (SD). Values in cells with the same alphabets do not differ significantly.

Pea seed treatment and foliar treatments with biostimulants Stimpo, Regoplant, and Rhizohumin separately during the growing season provided an increase in LAI by a maximum of 1.5 times during the vegetative growth period and 1.6 times during the generative development period of pea. Under the combined use of biological preparations, a more active formation of the leaf assimilation surface of pea plants was recorded than under separate use.

Thus, in the variants of pea crops with the integrated application of Rhh+St, the LAI exceeded the values in the variants where the separate action of the preparations was used by 10–27% in the phase of 5–6 stipules, by 41–50% in the budding phase, by 13% in the flowering phase, and by 22% in the bean formation phase. LAI of pea crops under the integrated effect of Rhh+Rp increased by 17% in the 5–6 stipules phase, by 16% in the budding phase, by 10–20% in the flowering phase, and by 9–16% in the bean formation phase, compared to those variants where the plants were treated with the preparations separately.

ANOVA analysis revealed that factors A and B are statistically significant in their effects on LAI ($p < 0.001$), and the interaction term was significant ($p = 0.002$). The final Tukey test for HSD showed that Rhh+St and Rhh+Rp were significantly different from the control at BBCH 51–55 and 61–65, confirming a significant combined effect on plant development.

The effect of biostimulants and the microbiological inoculant Rhizohumin on the chlorophyll content in pea stipules was indistinct (Table 2).

Table 2. The content of total chlorophyll in the stipules of pea plants under the effect of separate and combined use of microbial inoculant and biostimulators

BBCH	Variants					
code	Control	Rhh	St	Rp	Rhh+St	Rhh+Rp
12–13	560.3 ± 4.9 ^{k}	584.2 ± 5.8 ^{fk}	564.7 ± 4.9 ^{jk}	567.7 ± 4.4 ^{ik}	580.1 ± 5.8 ^{fk}	590.0 ± 4.6 ^{fk}
16–17	566.0 ± 5.8 ^{ik}	568.7 ± 5.3 ^{ik}	570.0 ± 5.3 ^{hk}	578.3 ± 6.4 ^{fk}	568.3 ± 5.8 ^{ik}	574.4 ± 5.8 ^{gk}
51–55	583.1 ± 5.8 ^{fk}	598.1 ± 7.0 ^{efghij}	590.1 ± 6.3 ^{fk}	595.0 ± 7.5 ^{ek}	602.0 ± 7.0 ^{efghi}	605.2 ± 6.4 ^{defgh}
61–65	609.7 ± 7.3 ^{defg}	640.3 ± 8.7 ^{cd}	626.7 ± 7.8 ^{ce}	613.4 ± 8.1 ^{def}	630.4 ± 7.1 ^{ce}	640.4 ± 6.9 ^{cd}
75–79	650.0 ± 8.1 ^{bc}	692.0 ± 7.0 ^{a}	685.0 ± 8.1 ^{ab}	693.5 ± 7.1 ^{a}	708.8 ± 7.2 ^{a}	710.6 ± 7.3 ^{a}

Notes: Values represent the mean of four replications ± standard deviation (SD). Values in cells with the same alphabets do not differ significantly.

During plant vegetative development, chlorophyll content in plant stipules gradually increased. During the experiment, an increase in chlorophyll content from 2% to 9% was observed with both the separate and combined use of the preparations. However, from the budding phase through the bean-formation phase, a probable exaggeration of chlorophyll content in pea stipules, with the integrated action of the preparations, was recorded at 2.6–3.4% compared to variants where separate treatments with preparations were applied. Overall, integrated applications tended to produce the greatest cumulative increases in chlorophyll content, with Rhh+Rp and Rhh+St showing the strongest effects at maturity (BBCH 61–79).

The 2-way ANOVA demonstrated significant effects of growth phase ($F_{(4.60)} = 1363.1$, $p < 0.001$), treatment ($F_{(5.60)} = 55.1$, $p < 0.001$), and their interaction ($F_{(20.60)} = 8.18$, $p < 0.001$) on chlorophyll content in pea stipules. The integrated application (Rhh+St and Rhh+Rp) produced the largest gains and formed a distinct homogeneous group. These results confirm the combined effect of microbial inoculant with biostimulants on maintaining higher pigment content and delaying senescence.

NPP gradually increased during the pea growing season. The maximum values of NPP in the interphase period of budding–flowering of peas were within 15–25 g cm^{-2} day^{-1} (Fig. 3). A decrease in the intensity of dry matter accumulation was noted during the following growing phase. The minimum NPP values were observed during the BBCH 12–17 phases, at about 5–6 g cm^{-2} day^{-1}. During this period, no statistical difference was found between the options with separate or combined use of preparations. One reason is the unfavorable temperature regime during the initial stages of pea leaf apparatus formation.

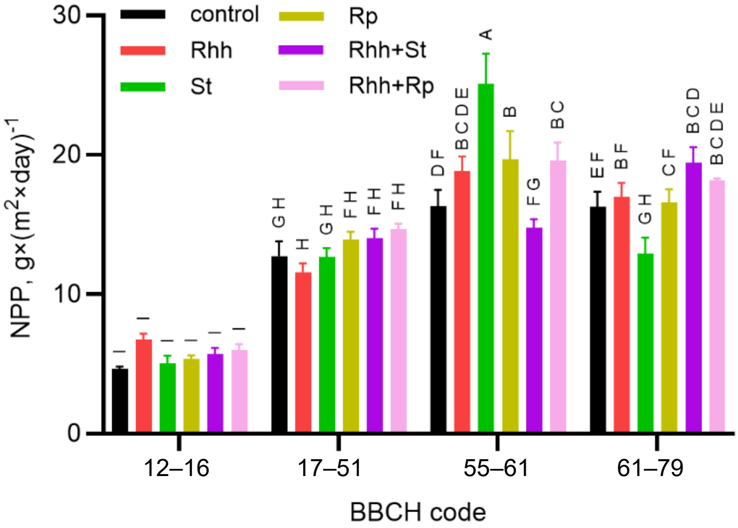

Figure 3. Changes in pea crops' NPP under the effects of separate and combined use of microbial inoculant and biostimulators. Vertical line – standard error of mean.

Treatments with biostimulators notably enhanced NPP, particularly in the middle and late phases of pea growth. For instance, during BBCH 55–61, Stimpo increased NPP by up to 61%, while Regoplant and Rhizohumin increased it by 25–30% relative to the control.

It was found that in the case of the combined effect of Rhh+St, the NPP exceeded by 21–27% between the phases of 5–6 stipules and budding, and exceeded by 7–14% the indicator of the best variant where the preparations were applied separately between the phases of flowering and bean formation.

The ANOVA demonstrated that the interaction between growth stage and treatment was highly significant ($F_{(15.48)} = 18.09$, $p < 0.0001$), as were the main effects of the stage ($F_{(3.48)} = 651.6$, $p < 0.0001$) and treatment ($F_{(5.48)} = 6.12$, $p = 0.0002$). Factor A accounted for the largest proportion of total variance (84.8%), indicating that developmental progression had the dominant influence on NPP, whereas factor B and the interaction AB accounted for 11.8% and 1.3% of total variance, respectively.

From the data presented in Table 3, it can be seen that the use of the preparations Rhizohumin, Stimpo, and Regoplant caused an increase in the number of beans on the plant by 22%, 4%, and 11%, respectively, compared to the control.

The combined interaction of biostimulants with Rhizohumin did not increase the number of beans on pea plants compared to using Rhizohumin separately. In this study, the integrated treatment of plants with biostimulants and the microbiological drug Rhizohumin did not cause significant changes in the number of seeds per bean, which ranged from 2.6 to 3.0 pcs per bean. It was also recorded that when pea plants were treated with Rhh+Rp, the weight of 1,000 seeds increased by 1.5–1.9% compared to the separate use of the preparations. The yield coefficient of control pea crops was 0.479, and it changed insignificantly under the action of Rhh, St, and Rp. Combining microbial inoculant with biostimulants increases the yield coefficient to 0.514 for Rhh+St and to 0.509 for Rhh+Rp.

Table 3. Influence of separate and combined use of biostimulants and microbial inoculant on the structure of the pea crop yield

Indicators	Variants						$SD_{0.5}$
	Control	Rhh	St	Rp	Rhh+St	Rhh+Rp	
Number of beans per 1 plant (pcs.)	2.7 ± 0.4	3.3 ± 0.4	2.8 ± 0.5	3.0 ± 0.3	3.3 ± 0.2	3.3 ± 0.4	0.3
Number of seeds per bean (pcs.)	2.8 ± 0.3	2.6 ± 0.2	2.8 ± 0.2	3.0 ± 0.2	2.9 ± 0.2	3.0 ± 0.1	0.2
Weight of 1,000 seeds (g)	238.1 ± 4.5	238.9 ± 2.4	245.7 ± 2.6	237.7 ± 5.1	240.3 ± 2.7	242.2 ± 1.8	2.1
Biological yield (c ha^{-1})	20.1 ± 2.6	20.7 ± 2.5	21.0 ± 2.8	21.7 ± 2.6	23.7 ± 1.9	22.9 ± 2.3	1.3
Yield coefficient	0.479 ± 0.082	0.492 ± 0.080	0.468 ± 0.075	0.476 ± 0.080	0.514 ± 0.091	0.509 ± 0.090	0.02

Notes: Values represent the mean of four replications ± standard deviation (SD). Differences between treatments were determined using the $LSD_{0.5}$ test.

The obtained biological yield of peas with separate use of the Rhizohumin, Stimpo, and Regoplant was 20.7, 21.0, and 21.7 c ha^{-1}, respectively, which exceeded the yield of control crops, which was 20.1 c ha^{-1}. While with the combined use of Rhh+St, the yield was 23.7 c ha^{-1} and Rhh+Rp – 22.9 c ha^{-1}, which in turn exceeded the yield of pea crops of all other options by 12–14% and 6–11%, respectively.

The biological fixation of atmospheric nitrogen by diazotrophs plays an important role in maintaining soil fertility and improving soil health (Chen, 2019; Pal et al., 2021; Singh & Kumar, 2024). The accumulated biological nitrogen, when interacting with plants, increases the protein content of leguminous products and yields of subsequent crops in crop rotation. This becomes important under the influence of adverse abiotic environmental factors (high temperatures, droughts, soil salinity, disturbances in the soil water regime, etc.), which are characteristic of the Southern Steppe zone of Ukraine. Thus, in our previous studies and other researchers, the effectiveness of biostimulants and their complexes with microbiological preparations in growing some crops in semi-arid climate conditions (Pyda et al., 2021; Glogoza et al., 2023; Khodanitska et al., 2024; Kolesnikov et al., 2024b) was shown, which confirms the results obtained in this study.

It should be noted that in the soil of the experimental plots, there were local races of nodule bacteria, which spontaneously inoculated the roots of the control plants. In turn, inoculation with Rhizohumin likely ensured the establishment of a more efficient and functionally active symbiosis, as reflected primarily in the greater number and, especially, the greater mass of nodules, as confirmed by other results (Pyda et al., 2021).

A possible explanation of the combined effect is that the phytohormonal and biologically active compounds contained in the biostimulants promoted root development, nutrient uptake, and metabolic activity, thereby creating more favorable conditions for rhizobial colonization and nodule growth. Similar mechanisms were discussed by Santos et al. (2019), who reported that the combined use of microbial inoculants and bioregulators may improve plant vigor through hormonal regulation and enhanced nutrient acquisition, or increase nitrogenase activity in diazotrophs (Bulgari et al., 2015). Our results also align with the concept proposed by Rouphael & Colla (2020), which holds that biostimulants can enhance plant–microbe interactions and partially mitigate stress effects under unfavorable environmental conditions. Under the semi-arid conditions of the Southern Steppe, these effects are likely to be especially important because drought and heat stress limit both photosynthetic activity and nitrogen fixation efficiency.

Statistical analysis revealed a strong positive correlation between the number of nodules and their total mass across all treatments and growth stages of pea plants. The Pearson correlation coefficient (r) ranged from 0.82 to 0.95 ($p < 0.01$) depending on the treatment, indicating a close linear relationship between nodule number and accumulated nodule biomass. The highest correlation ($r = 0.95$) was observed under the integrated treatment Rhh+St, followed closely by Rhh+Rp ($r = 0.93$). This affirms that integrated microbial and biostimulant applications enhance nodulation efficiency.

Optimization of nitrogen nutrition, due to the formation of additional nodules when using microbiological and biological preparations, primarily affected vegetative growth and the development of the assimilation surface of pea crops The stimulatory effect on leaf area development became more pronounced during the generative stages, when water deficit and elevated temperatures typical of the Southern Steppe can restrict leaf expansion and accelerate senescence. Under such conditions, biostimulants may help maintain physiological activity, allowing plants to sustain a larger assimilation surface. At the same time, the observed increase in LAI was not accompanied by proportionally large changes in chlorophyll content, indicating that the treatments affected canopy development more strongly than pigment accumulation. Previous studies on legumes have also shown that inoculants and biostimulants can stimulate leaf formation and biomass accumulation, although the magnitude of the response depends strongly on environmental conditions and cultivar characteristics (Gebremariam & Tesfay, 2021; Ibrahim & El-Sawah, 2022; Du et al., 2024). In our study, the integrated treatments mainly enhanced LAI during BBCH 51–79, suggesting that the positive effect was associated more with maintaining assimilating surface during reproductive development than with accelerated early vegetative growth, and this was confirmed in recent studies (Kour et al., 2021; Rathor et al., 2023; Kolesnikov et al., 2024b).

Within the framework of this study, it is impossible to assert the presence of a pronounced combined effect between biostimulants and the microbiological preparation on the processes of synthesis and accumulation of photosynthetic pigments. The changes in chlorophyll content were modest and mainly stage-dependent, which suggests that pigment synthesis was less sensitive to treatment than nodulation and leaf area development. Such a response may reflect the fact that biostimulants primarily influence physiological activity indirectly, through root development, membrane transport, and stress mitigation, rather than directly and uniformly stimulating chlorophyll accumulation (Kandil et al., 2023).

More accurate information about the course and features of the production process could be obtained by measuring plant dry matter accumulation during the growing season. The dynamics of net photosynthetic productivity were also variable across ontogenetic stages. However, a number of studies have indicated a positive effect of biostimulators and inoculants on photosynthetic productivity, emphasizing the role of biologically active compounds in enhancing photosynthetic efficiency and biomass accumulation in legumes via enhancing leaf gas exchange and nutrient uptake (Rouphael & Colla, 2020; Kumar et al., 2021; Parihar et al., 2022; Kandil et al., 2023).

Nevertheless, the tendency toward higher NPP under the combined treatments in some growth phases suggests a possible positive interaction between improved symbiotic functioning and the physiological action of the biostimulants. Similar stage-dependent responses have been reported for legumes under inoculation and biostimulant application, where the magnitude of the effect depended on cultivar, environmental stress, and developmental stage rather than on treatment alone (Batista & Singh, 2021; Kaminkyi et al., 2021; Ibrahim & El-Sawah, 2022; Khodanitska et al., 2024; Kolesnikov et al., 2024b).

A number of works highlight the complex use of microbiological and biostimulating preparations. The integrated action of two or more components at the concentrations recommended by the manufacturer often leads to leveling of the additive effect, which is probably explained by hormesis, the effects of an overdose of physiologically active compounds. Therefore, the combined use of micropreparations and growth stimulants remains debatable and requires consideration of the optimal provision of plants with physiologically active substances in these technologies (Castiglione et al., 2021; Rathor et al., 2023; Du et al., 2024).

The production process and yield formation of leguminous crops are determined by the coordination between assimilate production, nutrient availability, and reproductive development during ontogenesis (Yeremko et al., 2024). In the present study, the higher yield observed under the integrated treatments was mainly associated with an increased number of formed beans per plant, whereas changes in seed number per bean and 1,000-seed weight were comparatively small. This suggests that the treatments primarily affected reproductive establishment rather than seed filling processes. Enhanced vegetative activity during the reproductive stages could help maintain assimilate supply under semi-arid conditions, thereby supporting bean formation and retention.

In addition, the increase in yield index with the combined use of biostimulants and microbial inoculant indicates a possible switch of physiological processes to extend the flowering phase of peas, reducing the loss of flowers on the upper tiers of plants, and seed formation (Kour et al., 2021; Kumar et al., 2021). Similar relationships have been reported previously and explain the effects of biostimulants by their influence on hormonal status (ethylene) and on improving photoassimilate supply, and numerous confirmations have been found (Li et al., 2026), although the magnitude and physiological basis of the response depend strongly on environmental conditions and crop genotype. Our results indicate that under the conditions of the Southern Steppe of Ukraine, the integrated treatments had a greater effect on bean formation than on individual seed traits, which ultimately contributed to higher biological yield.

CONCLUSIONS

The application of microbial inoculant and biostimulants influenced the formation of the symbiotic and assimilation apparatus of pea plants under semi-arid conditions of the Southern Steppe of Ukraine. The integrated treatments Rhh+St and Rhh+Rp increased the number of root nodules by 33–40% and nodule biomass by 9–17% compared with the separate application of the preparations during the stages of maximum symbiotic activity (BBCH 61–79).

It was recorded that the combined application of Rhh+St and Rhh+Rp contributed to the LAI increase by up to 35–40% during the generative stages of pea development. The microbial inoculant and biostimulants increased NPP and chlorophyll content during the budding-bean formation stages, but the combined effect on these indicators was not significant at any stage of pea growth.

The biological yield slightly increased by 3.0%, 4.5%, and 7.9% compared with the control when Rhizohumin, Stimpo, and Regoplant were used separately. The integrated treatments Rhh+St and Rhh+Rp resulted in yield increases of 17.9% and 13.9%, respectively. Yield improvement was mainly associated with a greater number of formed beans per plant and enhanced development of the assimilation surface.

Overall, the obtained results indicate that the integrated application of microbial inoculant with biostimulants can improve nodulation, surface assimilation development. Therefore, we recommend that farmers use a combination of Rhh+St to ensure sustainable pea production in semi-arid conditions.

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