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IMPACT OF BIOLOGICAL FUNGICIDES ON THE FORMATION AND FUNCTIONING OF SYMBIOTIC SYSTEMS SOYBEAN–*BRADYRHIZOBIUM JAPONICUM*

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Background. In order to reduce the negative impact of mineral fertilizers and pesticides on agroecosystems, environmentally friendly plant protection systems are increasingly being utilized in soybean cultivation technologies. These systems aim to provide essential nutrients to plants while minimizing ecological harm.

Materials and Methods. In vegetative studies, the processes of formation and functioning of symbiotic systems in soybeans with active strains of nodulating bacteria *Bradyrhizobium japonicum* (PC07, PC09, B78, B144) were investigated under the influence of pre-sowing seed treatment with the biofungicides Mycosan-N and Phytocide-r. Microbiological, physiological, statistical methods, and gas chromatography were employed in the research.

Results. The impact of biological fungicides on the nodulation activity of nodule bacteria has been identified. It was determined that with comprehensive treatment of soybean seeds with *B. japonicum* PC07 and Mycosan-N, the mass of nodules formed on the roots exceeded that of the control plants by 7–22 %, and under the influence of Phytocide-r, the mass was lower by 6–20 % throughout the vegetation period. With the combined application of *B. japonicum* PC09 and Mycosan-N, a decrease in the number of root nodules by 8–25 % and a reduction in their mass by 18–35 % was observed throughout the vegetation period. Under the influence of Phytocide-r, with the inoculation of rhizobia strain PC09, the number of nodules was lower compared to the control plants only in the full flowering stage. At the same time, the reduction in their mass was



6–20 % throughout the entire observation period. A stimulating effect of both biofungicides on the formation of the symbiotic apparatus involving strains *B. japonicum* B78 and B144 has been identified. With the combined application of *B. japonicum* PC07 and Phytocide-r, nitrogenase activity of symbiotic systems decreased by 7 % only in the stage of three true leaves compared to plants whose seeds were treated only with rhizobia. The nitrogenase activity of symbiotic systems formed with the participation of *B. japonicum* B78 and under the influence of Mycosan-N increased by 28 %, 15 %, and 12 % in the stages of three true leaves, budding, and full flowering, respectively. The action of Phytocide-r, with the inoculation by the nodule bacteria B78, resulted in an increase in nitrogenase activity by 14 % only in the budding stage. Under the comprehensive treatment of soybean seeds with involved biofungicides and *B. japonicum* B144, an increase in the intensity of N₂ assimilation was noted by 29–34 % in the stage of three true leaves and by 10–16 % in the budding stage.

Conclusions. The application of a scientifically justified selection of rhizobial strains and biological plant protection agents for pre-sowing treatment of soybean seeds will enable a more complete realization of their nitrogen-fixing potential and phytoprotective effects.

Keywords: *Bradyrhizobium japonicum*, soybeans, inoculation, biological fungicides, number and mass of nodules, nitrogenase activity

INTRODUCTION

Soybeans are one of the main protein-oil crops with a wide range of applications in the food, feed, and industrial sectors. It helps increase soil fertility and is one of the best precursors for other crops (Zhuikov *et al.*, 2020).

Soybean plants in symbiosis with rhizobial bacteria are capable of assimilating atmospheric nitrogen into forms that are accessible to plants. (Raza *et al.*, 2020). Inoculating soybean seeds with active, competitive strains of rhizobia contributes to increased volumes of biologically fixed N₂, grain productivity, and raw protein content. There is a direct relationship and strong positive correlation between the amount of assimilated nitrogen and the productivity of soybean varieties (Kots *et al.*, 2011; Petrychenko, *et al.*, 2018). Therefore, pre-sowing seed treatment with biopreparations made from selected nitrogen-fixing microorganisms is widely used in soybean cultivation technologies (Kots *et al.*, 2016; Andrews, & Andrews, 2017; Kots *et al.*, 2024).

The drive for the intensification of agricultural production through the use of chemical agents leads to a range of environmental problems. It results in soil degradation and the accumulation of residues of pesticides, chemical fertilizers, and other elements in the obtained products in unacceptable quantities. The main environmentally safe method of controlling pathogens and pests in agricultural crops is biological control (Dovgan, 2016). It involves the use of microbial-based products and their by-products to reduce or maintain harmful organism populations at a safe level. The general advantage of microbial preparations over chemical ones is their lack of accumulative properties (Thakur *et al.*, 2020).

Bacillus sp. bacteria (*B. subtilis*, *B. circulans*, *B. thuringiensis*, *B. pumilus*, *B. valislimortis*, *B. amyloliquefaciens*, *B. licheniformis* *et al.*) are used as a basis for biological fungicides. The effectiveness of using biological plant protection agents to limit the development of pathogenic micromycetes *Pythium* sp., *Fusarium* sp., *Phomopsis* sp.,

Rhizoctonia solani, *Aspergillus flavus*, *Botrytis cinerea*, *Alternaria alternata* et al. has been noted. Some of the bio-preparations created based on strains *Bacillus* sp. are capable of restricting the development of phytopathogenic bacteria belonging to the genera *Xanthomonas*, *Pseudomonas*, *Agrobacterium*, *Erwinia*, *Pectobacterium* et al. The implementation of the phytoprotective action of such preparations can occur through mechanisms of competition, direct antibiosis between microorganisms, or by inducing increased plant resistance to disease pathogens (Shafi et al., 2017). The most important biologically active molecules of *Bacillus* genus bacteria include non-ribosomally synthesized peptides and lipopeptides, bacteriocins, and siderophores. Their antagonistic action can be realized against a wide range of phytopathogens (Fira et al., 2018).

Evidence suggests a successful integrated use of biofungicides with preparations based on nitrogen-fixing microorganisms. It has been found that treating soybean seeds with 2.0 liters per hectare of Rhizobofit + 1.0 liter per ton of Phytodoctor (*Bacillus subtilis*, $5 \cdot 10^9$ CFU/mL) and spraying plants at the budding stage with 2.0 liters per hectare of Trichodermin (*Trichoderma viride* (*lignorum*)) enhances plant nitrogen nutrition and provides comprehensive biological protection for crops of this culture against the agents of downy mildew, septoria leaf spot, and ascochyta blight. The use of these bio-preparations ensures a 13 % increase in soybean grain yield (Zadorozhnyi et al., 2019).

The comprehensive inoculation of soybeans with strains of *Bradyrhizobium* and *Bacillus subtilis* is an effective strategy for enhancing plant growth, thanks to its beneficial impact on root architecture. This allows for increased absorption of water and nutrients by the roots (Araujo et al., 2021).

According to Monroy-Guerrero et al. (2022), the co-inoculation of *Bacillus* sp. B02 + *Bradyrhizobium* spp. LMHZ L8 and LMHZ L3 promotes the formation of more nodules on the roots of lupin (*Lupinus mutabilis* Sweet), increases chlorophyll content in leaves, enhances plant growth throughout the vegetation period, reduces the susceptibility of the crop to anthracnose, and contributes to an increase in grain productivity.

Leading countries and organizations are directing their efforts towards the production of environmentally friendly products, actively engaging in innovative activities in the development of agricultural biologization means, and restricting the use of traditional intensive technologies in agriculture (Krutiakova et al., 2018). Therefore, there is a growing trend each year towards expanding the range of microbial preparations.

The expediency of combining seed bacterization processes with nitrogen-fixing microorganisms and seed treatment with environmentally friendly plant protection agents is beyond doubt. Therefore, research on the impact of biopreparations with various properties and mechanisms of action (including fungicidal activity) on the formation and functioning of symbiotic systems such as soybean-*Bradyrhizobium japonicum* is relevant.

MATERIALS AND METHODS

Vegetation experiment was conducted with soybean plants (*Glycine max* (L.) Merr.) of the Khutoryanochka variety, which has been included in the Register of Plant Varieties of Ukraine since 2010. It is early-ripening and recommended for cultivation in the Forest-Steppe, Polissia and Steppe zones (State register of plant varieties suitable for dissemination in Ukraine in 2023).

In the study were used strains of nodular bacteria obtained through analytical selection methods – *Bradyrhizobium japonicum* PC07, PC09 and Tn5 mutants B78,

B144. It was obtained by the method of transposon mutagenesis of *B. japonicum* 646 with *Escherichia coli* S17-1 containing the plasmid pSUP5011::Tn5 *mob*.

These nodule bacteria are stored in the collection of symbiotic and associative nitrogen-fixing microorganisms of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine. The following biofungicides were used in the studies: Mycosan-N (7 L/t) – 3% alkaline extract of the ascomycete fungus *Fomes fomentarius* and Phytocide-r (2 L/t), which contains viable cells *Bacillus subtilis* no less than $1 \cdot 10^9$ colony forming units/cm³.

The scheme of the experiment included the following variants for pre-sowing seed treatment: 1) *B. japonicum* PC07 (control); 2) *B. japonicum* PC07 + Mycosan-N; 3) *B. japonicum* PC07 + Phytocide-r; 4) *B. japonicum* PC09 (control); 5) *B. japonicum* PC09 + Mycosan-N; 6) *B. japonicum* PC09 + Phytocide-r; 7) *B. japonicum* B78 (control); 8) *B. japonicum* B78 + Mycosan-N; 9) *B. japonicum* B78 + Phytocide-r; 10) *B. japonicum* B144 (control); 11) *B. japonicum* B144 + Mycosan-N; 12) *B. japonicum* B144 + Phytocide-r; 13) Without inoculation and biofungicides.

The duration of seed bacterization was 60 minutes. The infectious load was 10^8 cells/mL. In the control groups, the inoculated seeds were not treated with biofungicides.

Soybeans were grown in pots (eight plants per pot) with the capacity of 4 kg, pre-sterilized with a 20 % H₂O₂ solution, on river sand with the introduction of Hellriegel's nutrient mixture enriched with microelements such as molybdenum, boron, and copper, and depleted in nitrogen, under natural light conditions and optimal (60 %) watering. The pots with plants were placed on a specially equipped site of the Institute of Plant Physiology and Genetics NAS of Ukraine. The repeatability in the variants of the experiment was six times.

Nitrogenase activity was determined by acetylene method in terms of acetylene regeneration activity by root nodules of soybean (Hardy *et. al.*, 1968). A gas mixture containing ethylene, formed as a result of acetylene reduction by nitrogenase, was analyzed on Agilent Technologies 6850 Network GC System (USA) gas chromatograph with a flame ionization detector. Separation of gases was performed on a column Supelco Porapak N at thermostat temperature of +55 °C and detector +150 °C. The gas carrier was nitrogen (50 mL per 1 min). Sampling capacity for analysis was 1 cm³. Experiment with nitrogenase activity determination was repeated 4 times.

The mass of the above-ground part of the plants, roots, and nodules was determined with tenfold repetition.

The obtained data were processed by generally accepted methods of variation statistics using Microsoft Excel. The significance of the difference between treatments was evaluated using ANOVA. The results were presented in the form of mean values and standard error ($x \pm SE$). Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The research results indicate that when soybean seeds were inoculated with rhizobia of strain PC07 in combination with biofungicides, the number of nodules in the stage of three true leaves was comparable to the plants of the corresponding control (grown without the use of plant protection agents). Under the influence of Mycosan-N, the investigated indicator in the flowering and full flowering stages in plants of this variant exceeded the controls by 8 and 29 %, respectively. In soybeans treated with Phytocide-r

before sowing, the number of nodules in the budding stage was lower by 15 % compared to the control plants, while in the full flowering stage, it was at their level.

The weight of nodules formed with the participation of strain PC07 while using Mycosan-N was greater compared to control plants by 7-22 % throughout the vegetation period. At the same time, a decrease in this indicator by 6-20 % was noted under the influence of Phytocide-r.

Inoculation of soybean seeds of the Khutoryanochka variety with rhizobia of strain PC09 resulted in a reduction in the number of nodules on the roots under the influence of Mycosan-N by 25 %, 13 %, and 8 % in the stages of the three true leaves, budding, and full flowering, respectively. The action of Phytocide-r led to a decrease in this indicator by 13 % only in the stage of full flowering of soybeans. The mass of root nodules formed with the participation of *B. japonicum* PC09 decreased during the vegetation period by 6-20 % under the influence of both biofungicides compared to the respective controls.

A stimulating effect on the formation of the symbiotic apparatus was observed when the seeds were treated with plant protection biological agents and *B. japonicum* B78. With the combined application of nitrogen-fixing microorganisms of this strain and Mycosan-N, the number of nodules on the plant roots was higher compared to the control plants by 26 % in the stage of three true leaves, 24 % in the budding stage, and 23 % in the full flowering stage. The mass of nodules on the roots of soybeans in this variant throughout the vegetation period exceeded that of the control plants by 14–31 %. Under the influence of Phytocide-r during the inoculation with B78, there was an increase in the number of nodules by 13–36 % and their mass by 14–26 % compared to inoculation without biofungicides.

Under the inoculation of soybean seeds with *B. japonicum* B144, the number of root nodules on plants throughout the vegetation period while using Mycosan-N was higher compared to the control plants (by 26-45 %). The mass of nodules in plants of this variant exceeded the control plants by 38 and 15 % in the stage of three true leaves and budding. However, during the full flowering stage, the investigated indicator was at the level of the control plants. Phytocide-r was found to have a stimulating effect on the number of nodules formed by *B. japonicum* B144. Their mass was greater compared to the control plants by 16 %, 10 %, and 9 % in the stages of the three true leaves, budding, and full flowering, respectively (**Table 1**).

With the comprehensive application of *B. japonicum* PC07 and biofungicides, nitrogenase activity in symbiotic systems decreased by only 7 % in the stage of the three true leaves compared to plants whose seeds were treated with nodule bacteria (without plant protection agents). A decrease in nitrogenase activity was observed in plants inoculated with rhizobia strain PC09 under the influence of Mycosan-N, ranging from 17 % to 24 % during the vegetation period. Under the influence of Phytocide-r in plants inoculated with this strain, nitrogenase activity during the stages of the three true leaves and budding was at the level of control plants and decreased by 22 % in the stage of full flowering.

The stimulating effect of Mycosan-N on the nitrogenase activity level of symbiotic systems formed with the participation of *B. japonicum* B78 has been established. This is evidenced by the increase in the investigated indicator compared to inoculation without biofungicides by 28 %, 15 %, and 12 % in the stages of three true leaves, budding, and full flowering, respectively. The combined treatment with Phytocide-r and nodule bacte-

Table 1. The number and weight of root nodules in soybeans of the Khutoryanochka variety after seed treatment with biofungicides and inoculation with nodule bacteria (per 1 plant)

Treatment	Stage of ontogenesis:					
	3 true leaves		budding		full flowering	
	number of nodules	weight of nodules	number of nodules	weight of nodules	number of nodules	weight of nodules
PC07	11.7 ± 0.9	0.095 ± 0.004	24.0 ± 1.2	0.356 ± 0.018	28.0 ± 1.5	0.581 ± 0.029
PC07 + Mycosan-N	12.3 ± 1.2	0.116* ± 0.007	26.0 ± 2.0	0.382 ± 0.021	36.0* ± 2.9	0.659* ± 0.033
PC07 + Phytocide-r	10.7 ± 0.8	0.076* ± 0.004	20.3* ± 0.9	0.314* ± 0.015	29.5 ± 1.7	0.547 ± 0.030
PC09	8.0 ± 0.4	0.133 ± 0.007	21.3 ± 1.0	0.213 ± 0.010	46.3 ± 3.0	0.320 ± 0.016
PC09 + Mycosan-N	6.0* ± 0.5	0.086* ± 0.003	18.5 ± 1.0	0.156* ± 0.008	42.7 ± 2.3	0.261* ± 0.013
PC09 + Phytocide-r	10.0* ± 1.0	0.101* ± 0.005	28.1* ± 2.5	0.200 ± 0.009	40.1* ± 2.0	0.255* ± 0.012
B78	10.3 ± 0.8	0.085 ± 0.004	22.9 ± 1.3	0.230 ± 0.012	30.7 ± 1.6	0.304 ± 0.017
B78 + Mycosan-N	13.0 ± 1.5	0.097 ± 0.003	28.3* ± 1.4	0.271* ± 0.016	37.7* ± 1.8	0.399* ± 0.021
B78 + Phytocide-r	14.0* ± 1.5	0.107* ± 0.005	26.8* ± 1.5	0.274* ± 0.015	34.7 ± 2.0	0.345 ± 0.019
B144	9.8 ± 0.7	0.064 ± 0.003	21.2 ± 1.3	0.339 ± 0.017	23.7 ± 1.2	0.389 ± 0.021
B144 + Mycosan-N	12.3* ± 1.0	0.088* ± 0.005	30.1* ± 1.5	0.388* ± 0.019	34.3* ± 2.0	0.385 ± 0.018
B144 + Phytocide-r	13.0* ± 1.2	0.074 ± 0.004	29.0* ± 2.0	0.374 ± 0.017	33.3* ± 1.5	0.425* ± 0.021
Without inoculation and biofungicides	0	0	0	0	0	0

Note: $x \pm$ standard error, $n = 10$; * significant difference compared to the inoculation of seeds by the same strain without biofungicides at $P < 0.05$ within one column

ria B78 provided an increase in nitrogenase activity by 14 % in the budding stage and a decrease of this indicator to the level of the control plants in the full flowering stage.

The comprehensive treatment of soybean seeds with biofungicides and *B. japonicum* B144 resulted in an increase in N_2 assimilation intensity by 29–34 % at the three true leaves stage and by 10–16 % at the flowering stage. During the full flowering stage of soybeans under the influence of Mycosan-N, the nitrogenase activity indicator of symbiotic systems formed with the participation of B144 rhizobium strain was higher compared to the control plants by only 5 % (see **Figure**).

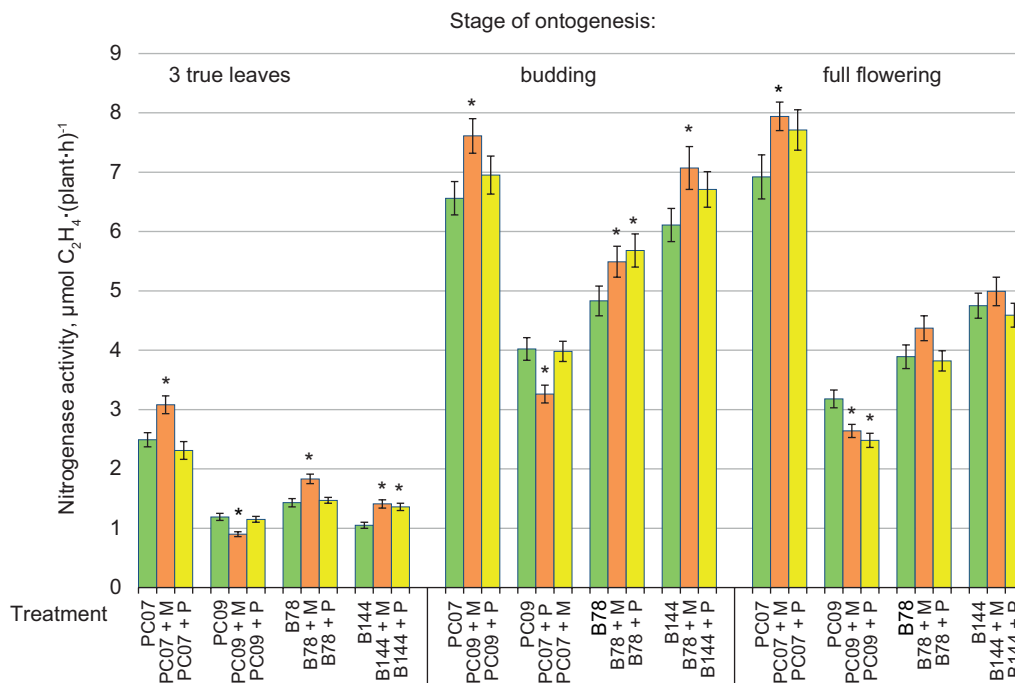


Fig. Nitrogenase activity of soybean plants of the Khutoryanochka variety ($\mu\text{mol C}_2\text{H}_4 \cdot (\text{plant} \cdot \text{h})^{-1}$) after seed treatment with biofungicides and inoculation with nodule bacteria

Note: $x \pm$ standard error, $n = 4$; * significant difference compared to the inoculation of seeds by the same strain without biofungicides at $P < 0.05$ in groups of three columns

The pre-sowing treatment of soybean seeds with Mycosan-N reduces plant infection with bacterial diseases under conditions of natural and artificial contamination, stimulates plant growth and development, increases the content of chlorophyll, nitrogen, phosphorus, and potassium in plants, and ensures an increase in crop yield and grain quality (Teslyuk *et al.*, 2010).

Inoculation of seeds with nodule bacteria and the combined application of rhizobia with biofungicides contribute to an increase in the aboveground and root mass of plants compared to plants without this treatment. In the stage of the three true leaves, regardless of the strain of nitrogen-fixing microorganisms used for inoculation and the biofungicide applied, the root mass of soybeans was at the level of plants in the control variants (without the use of biological plant protection agents).

During the budding stage of soybean plants, inoculation with strain PC07, in combination with Mycosan-N, resulted in a decrease in aboveground mass and root mass by 11 % and 6 %, respectively. During the full flowering stage, there was an increase in these indicators in plants of this variant by 7 % and 12 %. Under the influence of Phytocide-r in conjunction with the treatment with nodule bacteria PC07 during the budding and full flowering stages, a 13–27 % increase in soybean root mass was established compared to plants of the respective control.

The comprehensive treatment of soybean seeds with rhizobia of PC09 strain and Mycosan-N contributed to an increase in the aboveground mass of plants by 15 % and 8 %, and root mass by 7 % and 15 % at the budding and full flowering stages, respectively. At the same time, the action of Phytocide-r and the specified inoculant provided an increase in aboveground mass by only 6 % and 5 % at the budding and full flowering stages, and an increase in root mass by 21 % during the flowering stage (**Table 2**).

There are results of research on the biological properties of the fungus *Fomes fomentarius* and its application in medicine, agriculture, cosmetics production, etc. Various methods of grinding and suspending the fungus are being investigated, which is associated with significant interest in its practical application and the multifaceted nature of its biological activity (Darkal *et al.*, 2021; Kalitukha, & Sari, 2022). The basis of the biofungicide Mycosan is a biologically active composition of fungal glucans. To obtain chitin-glucan complexes from higher basidiomycete fungi, they are extracted with an alkali. The biological effectiveness of the product was studied in various soil and climatic zones of Ukraine on multiple agricultural crops. As a result of the research, it is recommended for seed treatment and plant application during the vegetation period of spring and winter wheat, barley, corn, peas, soybeans, sugar beets, sunflowers, and certain fruit and vegetable crops (Teslyuk, 2011).

In agricultural production, replacing synthetic pesticides with biological ones will reduce soil contamination with chemical residues, halt the development of pest resistance to plant protection agents, restore and enhance soil quality, and increase the productivity of agricultural crops without a negative impact on the environment (Krutiakova *et al.*, 2018).

In leguminous crops, resistance to pathogens is increased, plant biomass is enhanced, and grain productivity is improved through pre-sowing seed treatment with selected strains of nodule bacteria (Volpiano *et al.*, 2019). The functioning of the legume-rhizobial symbiosis brings about specific changes in the metabolism of the host plant. The specific activity of key enzymes involved in the metabolism of phenols in nodules, roots, and leaves of soybeans in symbiotic systems with varying efficiency has been noted (Nyzhnyk, & Kots, 2023).

The influence of nitrogen-fixing microorganisms on plants and pathogenesis is associated with the enzymatic activity of bacterial cells and the production of secondary metabolites with antimicrobial properties, especially concerning the pathogens that cause damage to the root system. In addition to the action of antifungal substances, the restriction of pathogen development can be attributed to the stimulation of plant growth through the active functioning of symbiosis. It has been found that rhizobia enhance systemic resistance to phytopathogens by inducing immune responses in plants. This is a crucial process because, during the vegetation period, the leaf apparatus serves as a favorable substrate for the development and multiplication of pathogenic fungi, bacteria, and viruses, leading to various diseases with different symptoms and con-

Table 2. Aboveground fresh weight and root fresh weight (g/plant) of soybean plants grown under the action of biofungicides and inoculated with nodule bacteria *Bradyrhizobium japonicum*

Treatment	Stage of ontogenesis:					
	3 true leaves		budding		full flowering	
	aboveground weight	root weight	aboveground weight	root weight	aboveground weight	root weight
PC07	3.45 ± 0.14	1.84 ± 0.07	5.55 ± 0.24	3.52 ± 0.16	6.58 ± 0.29	4.46 ± 0.21
PC07 + Mycosan-N	3.29 ± 0.15	1.82 ± 0.07	4.93 ± 0.20	3.31 ± 0.13	7.03 ± 0.31	5.01* ± 0.20
PC07 + Phytocide-r	3.35 ± 0.16	1.79 ± 0.06	5.01 ± 0.24	3.98 ± 0.17	6.79 ± 0.29	5.68* ± 0.28
PC09	3.11 ± 0.12	1.69 ± 0.06	4.06 ± 0.16	3.60 ± 0.15	4.75 ± 0.21	3.53 ± 0.16
PC09 + Mycosan-N	2.86 ± 0.12	1.63 ± 0.06	4.67* ± 0.22	3.84 ± 0.17	5.11 ± 0.24	4.07* ± 0.18
PC09 + Phytocide-r	2.75 ± 0.10	1.68 ± 0.07	4.30 ± 0.19	3.21 ± 0.15	4.99 ± 0.22	4.26* ± 0.19
B78	3.13 ± 0.15	1.72 ± 0.08	4.36 ± 0.20	3.56 ± 0.16	4.97 ± 0.23	4.02 ± 0.20
B78 + Mycosan-N	2.98 ± 0.14	1.77 ± 0.07	4.65 ± 0.21	3.61 ± 0.17	5.32 ± 0.25	3.88 ± 0.16
B78 + Phytocide-r	2.88 ± 0.13	1.69 ± 0.06	4.26 ± 0.18	3.70 ± 0.17	6.08* ± 0.27	4.00 ± 0.18
B144	3.17 ± 0.14	1.61 ± 0.07	5.19 ± 0.25	4.04 ± 0.18	6.41 ± 0.29	4.57 ± 0.21
B144 + Mycosan-N	3.26 ± 0.15	1.64 ± 0.07	4.60* ± 0.22	3.69 ± 0.17	6.05 ± 0.28	3.94* ± 0.17
B144 + Phytocide-r	3.25 ± 0.13	1.61 ± 0.07	4.60* ± 0.22	3.09* ± 0.13	6.59 ± 0.31	4.33 ± 0.20
Without inoculation and biofungicides	2.47 ± 0.11	1.58 ± 0.06	3.31 ± 0.16	2.58 ± 0.12	4.75 ± 0.21	3.40 ± 0.16

Note: $x \pm$ standard error, $n = 10$; * significant difference compared to the inoculation of seeds by the same strain without biofungicides at $P < 0.05$ within one column

sequences for plants (Volpiano *et al.*, 2019). The mechanism of action of biological plant protection agents may be similar to the influence of diazotrophic microorganisms on the plant organism. For example, *Bacillus* strains often demonstrate their ability for biocontrol through inhibitory activity against the growth of phytopathogens. They also induce systemic resistance in plants and compete for ecological niches with pathogenic microorganisms. (Fira *et al.*, 2018).

Tonelli *et al.* (2017) demonstrated that co-inoculation with bacteria *Bacillus* sp. CHEP5, inducing resistance against *Cercospora sojina* in soybeans, and *B. japonicum* E109 reduced the leaf infection by cercosporosis compared to the application of the biocontrol agent alone. This method of pre-sowing treatment did not have a negative impact on the formation and functioning of symbiotic systems and the flavonoid composition of root exudates in plants.

CONCLUSION

The research results indicate that a scientifically substantiated selection of effective strains of nodule bacteria and biological fungicides allows for the implementation of the primary functions of these microbial preparations and contributes to the significant preservation of ecological balance in agroecosystems.

As the world faces the need for rational environmentally-oriented nature management, it is advisable to use soybean-based preparations with biological plant protection products in soybean cultivation technologies. This will reduce the anthropogenic (chemical) load on the soil, help preserve its fertility and biodiversity, and introduce microorganisms with agronomically useful properties into agrobiocenoses. In addition, the integrated use of biological products with different mechanisms of action in agricultural production is a way to biologization of agriculture and production of environmentally-friendly crops.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights. This article does not contain any studies with human subjects performed by any of the authors.

Animal Studies. This article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [K.P.;N.A.]; methodology, [P.P.; K.P.; N.A.]; validation, [K.P.]; formal analysis, [P.P.; K.P.]; investigation, [P.P.; K.P.; N.A.; T.A.]; resources, [P.P.; K.P.]; data curation, [K.P.; N.A.; T.A.]; writing – original draft preparation, [K.P.; N.A.]; writing – review and editing, [P.P.; K.P.; T.A.]; transleting, [T.A.]; visualization, [P.P.; K.P.; N.A.] supervision, [P.P.].

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ВПЛИВ БІОЛОГІЧНИХ ФУНГІЦИДІВ НА ФОРМУВАННЯ ТА ФУНКЦІОНУВАННЯ СИМБІОТИЧНИХ СИСТЕМ СОЯ–*BRADYRHIZOBIUM JAPONICUM*

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Вступ. Із метою зниження негативного впливу на агроєкосистеми мінеральних добрив і пестицидів у технологіях вирощування сої дедалі частіше застосовують екологічно-безпечні системи захисту рослин і забезпечення їх основними елементами живлення.

Матеріали й методи. У вегетаційних дослідах вивчали процеси формування та функціонування симбіотичних систем сої з активними штамми бульбочкових бактерій *Bradyrhizobium japonicum* за впливу передпосівної обробки насіння біофунгіцидами Мікосан-Н і Фітоцид-р. Застосовували мікробіологічні, фізіологічні, статистичні методи та газову хроматографію.

Результати. Виявлено вплив біологічних фунгіцидів на нодуляційну активність бульбочкових бактерій. Встановлено, що за комплексної обробки насіння сої *B. japonicum* PC07 із Мікосаном-Н маса сформованих на коренях бульбочок перевищувала контрольні рослини на 7,3–22,1 %, а за дії Фітоциду-р була меншою порівняно з ними на 5,9–20 % упродовж вегетації.

За сумісного застосування *B. japonicum* PC09 і Мікосану-Н відмічали зниження кількості кореневих бульбочок на 7,8–25,0 % і їхньої маси на 18,4–35,3 % упродовж вегетації. За дії Фітоциду-р на тлі інокуляції ризобіями штаму PC09 кількість бульбочок була меншою порівняно з контрольними рослинами лише у фазу повного цвітіння, водночас зниження їхньої маси становило 6,1–20,3 % упродовж усього періоду спостережень. Виявлено стимулювальний вплив обох біофунгіцидів на формування симбіотичного апарату за участю штамів *B. japonicum* B78 і B144. За комплексного застосування *B. japonicum* PC07 і біофунгіцидів азотфіксувальна активність сформованих симбіотичних систем знизилася на 7,2 % лише у фазу трьох справжніх листків за впливу Фітоциду-р порівняно з рослинами, насіння яких обробляли тільки ризобіями. Встановлено підвищення азотфіксувальної активності симбіотичних систем, сформованих за участю *B. japonicum* B78, та впливу

Мікосану-Н на 28,0, 14,5 і 12,3 % у фази трьох справжніх листків, бутонізації та повного цвітіння відповідно. Дія Фітоциду-р на тлі інокуляції бульбочковими бактеріями В78 забезпечувала підвищення азотфіксувальної активності на 13,6 % лише у фазу бутонізації. За комплексної обробки насіння сої залученими у роботу біофунгіцидами та *V. japonicum* В144 відмічали підвищення інтенсивності асиміляції N₂ на 29,3–34,2 % у фазу трьох справжніх листків і на 9,8–15,7 % у фазу бутонізації.

Висновки. Застосування науково обґрунтованого добору штамів бульбочкових бактерій і біологічних засобів захисту рослин для передпосівної обробки насіння сої дасть змогу забезпечити більшою мірою реалізацію їхнього азотфіксувального потенціалу та фітопротекторної дії.

Ключові слова: *Bradyrhizobium japonicum*, соя, інокуляція, біологічні фунгіциди, кількість і маса бульбочок, азотфіксувальна активність