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REALIZATION OF NODULATION AND NITROGEN-FIXING ACTIVITIES OF *BRADYRHIZOBIUM JAPONICUM* AND RHIZOSPHERE MICROBIOTA THROUGH SEED TREATMENT WITH PESTICIDE STANDAK TOP AND SPRAYING PLANTS WITH SOYBEAN SEED LECTIN

The idea of the study *was to use soybean lectin as a biologically active compound with a broad spectrum of action to spray soybean plants for stabilization of the formation and functioning of the soybean-rhizobium symbiosis as well as the nitrogen-fixing activity of rhizosphere microbiota against the background of seed treatment with chemical plant protection product Standak Top — an innovative pesticide with fungicidal and insecticidal activity for the control of major diseases and pests of soybean plants.* ***Aim.*** *To study the peculiarities of formation and functioning of soybean-rhizobium symbiosis as well as the nitrogen-fixing activity of rhizosphere microbiota under spraying plants with specific soybean seed lectin on the background of seed treatment with Standak Top and inoculation with nodule bacteria Bradyrhizobium japonicum 634b on the sowing day in the conditions of pot experiments with soil as a substrate.* ***Methods****. Physiological, microbiological, gas chromatography, and statistical methods were used.* ***Results****. It was shown that after seed treatment with Standak Top (1.5 L/ton of seeds) on the sowing day, there was observed suppression of the process of nodule formation on the roots in the period of soybean vegetative growth. The nitrogen-fixing activity of the symbiotic system was at the control level, while the functional activity of soil diazotrophs was suppressed (by 1.2—2.2 times). Spraying plants in the phase of two trifoliate leaves (V2) with soybean seed lectin (without pesticide) led to an increase in the total mass of nodules on the plant (by 1.5 and 1.9 times as well as by 2.3 and 2.0 times compared to the control of inoculation in the phase of three trifoliate leaves (V3) and beginning of pod formation (R3), respectively). The increase in the total mass of the symbiotic apparatus on soybean roots in the phases V3 and R3 respectively was by 1.4 and 1.5 times in comparison with seed treatment with Standak Top, and the mass of one nodule was higher by 1.3 and 1.6 times, respectively. Soybean seed lectin led to a significant increase in the actual nitrogenase activity of the soybean-rhizobium symbiosis. It was 2.9 and 1.9 times higher compared to control of*

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inoculation and 2.1 and 1.8 times compared to the variant of inoculation + pesticide in the V3 and R3 phases, respectively. The functional activity of soil nitrogen-fixing microorganisms did not change significantly. The use of soybean seed lectin against the background of the seed treatment with Standak Top and inoculation contributed to the stabilization and increase in the rhizobia nodulation ability, the suppression of which was due to the influence of such an anthropogenic factor as pesticides. There was observed an increase in the number (by 1.6 and 1.2 times) and mass of root nodules (by 2.2 and 1.5 times and 1.4 and 1.2 times, respectively, compared to the controls of inoculation and inoculation + pesticide). Soybean seed lectin significantly increased the nitrogenase activity of the symbiosis against the background of Standak Top (by 1.9 and 1.6 times and 1.4 and 1.5 times, respectively, in the V3 and R3 phases of soybean ontogenesis) compared to the control of inoculation and inoculation + Standak Top. ***Conclusions****. The obtained results suggest the possibility of using the method of spraying plants with specific lectin as a means of leveling (or mitigating) the negative effect of pesticides used for the seed treatment on the formation and functioning of the symbiosis and rhizosphere diazotrophic microbiota. This indicates the prospects of studying the biological activity of phytolectins in spraying plants in order to regulate the formation and functioning of phytobacterial systems, as well as their responses to various environmental or anthropogenic stress factors, in particular, to the effect of chemical plant-protecting products used for the seed treatment.*

Keywords: *soybean-rhizobium symbiosis, Standak Top, soybean seed lectin, nodulation, nitrogenase activity, rhizosphere diazotrophs.*

Bacterization and treatment with pesticides of legume seeds, including soybeans, are two interrelated technological methods used in the technology for their cultivation [1—3]. The presowing treatment of seeds with fungicides minimizes plant diseases caused by the presence of phytopathogens in them at the early stages of ontogenesis, and also ensures the complete development of plants in the subsequent growing phases of ontogenesis [1, 2]. Inoculation of soybean seeds with microbial preparations based on nodule bacteria causes the formation of symbiotic systems, the functional significance of which is determined by the level of molecular nitrogen fixation, which contributes to a better realization of the productive potential of this legume [4]. Symbiotic characteristics of nodule bacteria, such as the nodule-forming (nodulating) ability, nitrogen-fixing activity, and specificity to the host plant are the main features that determine the effectiveness of legume-rhizobium symbiosis under inoculation of seeds [4—6]. It has been found that the nodulation ability of soybean nodule bacteria and nitrogenase activity of symbiotic soybean-rhizobium systems against the background of using fungicides are significantly weakened, especially in the first half of the growth [3, 7, 8]. Therefore, it is important to find the ways to stabilize the symbiotic potential of rhizobia against the background of such anthropogenic factors as chemical plant-protecting products used for seed treatment, especially if such a treatment is carried out on the sowing day.

As known, the level of realization of the symbiotic properties of nodule bacteria in the formation of legume-rhizobium systems can be regulated by biologically active substances of different nature, including plant lectins [9—11].

Lectins are a group of proteins that combine the ability to bind carbohydrates and carbohydrate determinants of glycosylated biopolymers without causing their chemical transformations [12, 13]. Phytolectins, including legumes lectins [14, 15], are multifunctional biologically active compounds, which today have found their application in various areas of medicine, pharmacy, veterinary medicine, and crop production [9, 15—19]. The insecticidal, bactericidal, and fungicidal activities of plant lectins [9, 15—17, 19] allow us to consider them as promising phytoprotective compounds, on the basis of which efficient methods can be developed for biological control of plant diseases [20—22] or regulation of plant physiological processes in the conditions of biotic and abiotic stresses [9, 11, 20—23]. The study of the possibility of using soybean seed lectin (100 μg/mL) in the pre-sowing seed treatment as a compound with possible antidote activity or its introduction into the rhizobial inoculum has shown the ambiguity of the action of this biologically active compound, which depends on the development of soybean plant's phase and the nature of fungicide applied [8]. Thus, the use of Standak Top and inoculum based on rhizobia and soybean seed lectin or the seed treatment with lectin followed by rhizobia inoculation resulted in a significant reduction in the nodule formation on plant roots and nitrogen-fixing activity of the symbiotic system. At the same time, against the background of fungicides Maxim XL and Fever, a weaker toxic effect is established. Basing on the results, the authors conclude that it is impossible to use soybean seed lectin (100 μg/mL) for the action on seeds or bacterial culture as an antidote to fungicides.

The aim of the work was to study the peculiarities of the formation and functioning of symbiotic systems of soybean plants with nodule bacteria *Bradyrhizobium japonicum* 634b as well as the nitrogen-fixing activity of rhizosphere microbiota under the seed treatment with the pesticide Standak Top on the sowing day and spraying vegetative plants with soybean seed lectin.

Materials and methods. The objects of the study were the soybean-rhizobium symbiosis formed by soybean plants (*Glycine max* (L.) Merr.) of Ukraine's breeding (variety Almaz) [24] and nodule bacteria *B. japonicum* 634b against the background of seed treatment with Standak Top on the sowing day and spraying vegetative plants with soybean seed lectin during the developmental phase of two trifoliate leaves.

Variety Almaz, obtained by hybridization of the varieties Beal'tsi 3/86-x and Fiskebv-840-5-3, significantly exceeded (by 6—8 c/ha) the harvest of the parent varieties. The owners of the patent for this variety (N 07105) are the Poltava State Agrarian Academy and L.G. Bilyavska. In 2007, it was included in the State Register of Plant Varieties of Ukraine [24]. Variety Almaz is a grain, early ripening, drought-resistant, and resistant to diseases, which provides a stable growing season lasting 100—105 days. The content of protein in the seeds was 38—39% and of oil — 24—26% [24].

Nitrogen-fixing nodule bacteria *B. japonicum* 634b (symbiotic and associative nitrogen-fixing microorganisms strains from the collection of the Institute of Plant Physiology and Genetics of the NAS of Ukraine (IPPG) were grown for 10 days at 28 °C on an agar mannitol-yeast medium (g/L): K_2HPO_4 — 0.5; $MgSO_4 \cdot 7H_2O$ — 0.4; NaCl — 0.1; mannitol — 10.0; yeast extract — 0.5; agar-agar — 16.0; distilled water — 1 L; pH — 6.8—7.0. The culture was washed with sterile water and admixed into a homogeneous suspension. The number of viable bacteria (colony-forming units) was determined by classical microbiological methods of serial dilutions of bacteria suspension, seeding on an agar mannitol-yeast medium, and counting of grown colonies. The titer of rhizobia in the suspension was 10^8 cells/mL.

Standak Top (BASF, Germany) is an innovative pesticide with fungicidal and insecticidal activities for the control of major diseases and pests of soybean plants with active substances (Fipronil — 250 g/L; Thiophanate-methyl — 225 g/L; Pyraclostrobin — 25 g/L) belonging to chemical classes of phenylpyrazoles, benzimidazoles, and strobilurins, respectively [25]. The chemical plant-protecting product Standak Top, which is recommended to reduce the damage to seeds and plants by phytopathogens under seed treatment, was used as one of the technological means in soybean cultivation. Seed treatment with Standak Top was performed on the day of sowing, using the recommended dose of 1.5 L / ton of seeds. Inoculation of soybean seeds (1 mL / 120 seeds in the variant for 1 h) was carried out 1 h later the seed treatment with fungicide and followed by sowing in pots (20 pcs/pot). A solution of commercial soybean seed lectin (SSL, Lectinotest, Lviv, Ukraine) at a concentration of 50 μg/mL and a dose of 2 mL/plant (50 mL/m^2) was used to spray soybean plants in their development phase of two trifoliate leaves (V2, 33-day-old plants).

The features of formation and functioning of soybean-rhizobium symbiosis to the seed treatment with fungicide on the day of sowing and spraying vegetative plants with soybean seed lectin were studied in a pot experiment with the soil culture. The pot experiments were carried out at the IPPG under natural light and air temperature in 10 kg Wagner's pots on a soil substrate (soil: sand — 3:1) using Gelrigel's nutrient mixture with 0.25 norms of mineral nitrogen according to the following variants of seed inoculation:

- with bacteria (control for inoculation)
- with bacteria + spraying SSL
- with bacteria + Standak Top (control for fungicide and inoculation)
- with bacteria + Standak Top + spraying SSL.

There were tested:

- formation of symbiosis owing to the nodulation ability of rhizobia: the activity of root nodule formation, their number and mass per plant, the mass of 1 nodule, the nodulation scale;
- functional (nitrogenase) activity of soybean-rhizobium symbiosis and soybean rhizosphere soil — by an acetylene reductase method according to Hardy et al. (1973) [26] using an Agilent GC System 6850 gas chromatograph (USA) with a flame ionization detector. To do this, roots with nodules, washed from the plant growth substrate, or roots without nodules with soil or clean soil (20 g) were placed in hermetically sealed glass vials with a volume of 75 mL, in which 10% acetylene was created. The incubation time of the sample was 1 or 2 h. After incubation, the gas mixture containing ethylene formed due to acetylene reduction with nitrogenase was analyzed on the device. Separation of gases was performed on a Supelco Porapak N column at a furnace temperature of 55 °C and a detector temperature of 150 °C. The carrier gas was helium (50 mL per 1 min). The analyzed sample volume of the gas mixture was 1 mL. Pure ethylene (Sigma-Aldrich, N536164, USA) was used as a standard. The amount of ethylene formed from acetylene for 1 h of incubation, when the nitrogenase of the incubated sample is operated, was expressed in molar units of formed ethylene per 1 plant for 1 h. Nitrogenase activity of symbiosis was expressed in micromoles of C_2H_4 / (plant·h) — actual activity. Nitrogenase activity of rhizosphere soil was expressed in nanomoles of C_2H_4 / (20 g of absolutely dry (a. d.) soil·h). Nitrogenase activity of symbiotic systems and soil microbiota in the different phases of soybean ontogenesis was determined through 4—6 biological replicates.

Soybean plants were sampled at the growth stages of one (V1, 30-day-old plants) and three (V3, 36-day-old plants) trifoliate leaves, and the beginning of pod formation (R3, 56-day-old plants). The rhizosphere soil sampling was carried out at the stage of one trifoliate leaf (V1, 30-day-old plants), beginning flowering (R1, 44-day-old plants), and beginning pod formation (R3, 56-day-old plants) [27].

Statistical processing of experimental data was performed according to generally accepted methods using the software package Microsoft Excel 2019. The significance of the results among the variants was evaluated using the package ANOVA. Then, the differences between the means were compared by Fisher's Least Significant Difference (LSD) test at a probability level of 95%. Significance levels were expressed as $P = 0.05$ and data were significant when $P \leq 0.05$.

Results. It was found (Fig. 1, Table 1) that the use of pesticide Standak Top for seed treatment on the sowing day did not inhibit the process of infection of soybean roots with nodule bacteria at the beginning of the formation of symbiotic structures (phase of development of one trifoliate leaf, V1): the number of plants nodulated with rhizobia was 79.2% vs. 74.8% in the control, and the number of plants that formed more than 5 nodules — 9.8% compared to 3.7% in the control. However, in the phase of three trifoliate leaves (V3), despite the fact that the number of plants nodulated with rhizobia was at the control level (Fig. 1), the number of nodules on the plant was significantly less (by 24%, Table 2). This may indicate the suppression of the process of nodule formation on soybeans by Sandak Top

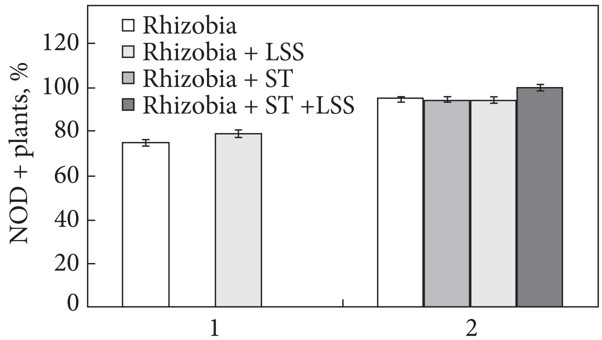

Fig. 1. Nodulation activity of *B. japonicum* after the seed treatment with Standak Top (ST) and spraying with lectin of soybean seeds (LSS). *X axis*: Phases of plant development: 1 — one trifoliate leaf (V1), 2 — three trifoliate leaves (V3). *Y axis:* Part NOD$^+$ plants, %. NOD$^+$ plants — the plants with root nodules

directly in the first half of the period of plant growth (the period of vegetative growth) because in the phase of the beginning of pod formation (R3, the period of reproductive growth), which is far away in time from the application of the pesticide, the number of nodules on plant roots exceeded the control value by 1.4 times (Table 2).

Spraying soybean plants in the phase of two trifoliate leaves (V2) with a solution of soybean seed lectin on the background of Sandak Top contributed to a great extent to the activation of both the process of nodulating plants with rhizobia (Fig. 1) and the process of root nodules formation (Table 2). The number of rhizobia-nodulated plants (NOD $^+$) increased by 6% (Fig. 1) in phase V3, the plants that formed more than 5 nodules were 45% against 19.1 and 15.8% in the control variants with inoculation and inoculation + Sandak Top, respectively (Table 1). A similar pattern was also observed in phase R3: the number of plants that formed more than 20 nodules was 83.3% compared to 16.7 and 33.4% in the control variants (rhizobium and rhizobium + ST) respectively (Table 1). The formation of a larger number of symbi-

Table 1. **Dynamics of nodule formation on soybean roots due to inoculation with rhizobia under the action of Standak Top on seeds and spraying plants with lectin**

Treatment	Nodulation scale (number of nodules per plant, pcs.)							
	0	1—5	6—10	11—15	16—20	21—25	26—30	31—35
	Part of plants with the appropriate number of nodules,%							
Phase of one trifoliate leaf's development (V1)								
Rhizobium (control)	25.2	70.0	3.7	0	0	0	0	0
Rhizobium + ST	20.8	68.1	9.8	0	0	0	0	0
Phase of three trifoliate leaves' development (V3)								
Rhizobium (control)	4.8	76.2	19.1	0	0	0	0	0
Rhizobium + SSL	5.3	84.2	10.5		0	0	0	0
Rhizobium + ST	5.3	78.9	15.8	0	0	0	0	0
Rhizobium + ST + SSL	0	55.0	45.0	0	0	0	0	0
Phase of the beginning of pod formation (R3)								
Rhizobium (control)	0	0	16.7	66.7	0	16.7	0	0
Rhizobium + SSL	0	0	16.7	16.7	33.3	16.7	16.7	0
Rhizobium + ST	0	0	16.7	16.7	33.3	16.7	16.7	0
Rhizobium + ST + SSL	0	0	0	16.7	0	83.3	0	0

Note: ST — Standak Top, SSL — soybean seed lectin.

otic structures on soybean roots with a larger total mass on the plant and the mass of one nodule were recorded as well (Table 2). The number and mass of nodules increased by 1.2 and 1.6 times and by 12 and 7%, respectively in phase V3 of soybean development, compared with the controls of inoculation and inoculation +ST. During phase R3 of soybean plants' ontogenesis, the numbers of root nodules were by 57 and 15% higher compared to the control of inoculation and inoculation + ST. At the same time, their total mass and the mass of one nodule increased by 2.3 and 1.5 times and by 1.5 and 1.2 times, respectively.

Spraying plants with soybean seed lectin (without fungicides) led to an increase in the total mass of nodules on the plant and the mass of one root nodule (Table 2). In the phase of three trifoliate leaves (V3), these indexes increased significantly by 1.5 and 1.9 times compared to the control of inoculation, and in the phase of the beginning of pod formation (R3) — by 2.3 and 2.0 times. The increase in the total mass of the symbiotic appa-

Table 2. **Characteristics of soybean root nodules under inoculation of seeds with nodule bacteria on the background of Standak Top's action and spraying plants with lectin**

Treatment	Units	% to control	Units	% to control
Soybean ontogenesis phase [27]	Phase of three trifoliate leaves (V3)		Phase of beginning of pod formation (R3)	
Number of nodules per plant, pcs.				
Rhizobium (control)	4.1	100	13.7 ± 1.1	100
Rhizobium + SSL	3.3^{b}	81/107	18.5 ± 2.5^{a}	135/99
Rhizobium + ST	3.1^{b}	76/100st	18.7 ± 2.4^{a}	137/100st
Rhizobium + ST + SSL	4.9^{ac}	120/158	21.5 ± 1.4^{a}	157/115
$LSD^{ab}_{0.05}$	0.3		1.1	
$LSD^{c}_{0.05}$	0.3		1.4	
Mass of nodules per plant, mg				
Rhizobium (control)	21.04	100	387.17	100
Rhizobium + SSL	31.24^{ac}	149/143	892.67^{ac}	233/153
Rhizobium + ST	21.89	104/100st	585.50^{a}	153/100st
Rhizobium + ST + SSL	23.49	112/107	872.00^{ac}	228/149
$LSD^{ab}_{0.05}$	4.73		82.04	
$LSD^{c}_{0.05}$	4.41		64.41	
Mass of one nodule, mg				
Rhizobium (control)	4.90	100	26.95	100
Rhizobium + SSL	9.30^{ac}	190/127	53.43^{ac}	198/160
Rhizobium + ST	7.34^{a}	150/100st	33.47	124/100st
Rhizobium + ST + SSL	5.14	105/70	40.14^{a}	149/120
$LSD^{ab}_{0.05}$	0.38		5.50	
$LSD^{c}_{0.05}$	1.64		5.24	

Note: in Tables 2—4:% before the line — relative to the control (Rhizobium, 100%), after the line — relative to the variant Rhizobium + Standak Top (ST, 100%). [a] — significantly (P ≤ 0.05) more than the control of Rhizobium; [b] — significantly (P ≤ 0.05) less than the control of Rhizobium; [c] — significantly (P ≤ 0.05) more than the variant of Rhizobium + ST.

ratus on soybean roots was by 1.4 and 1.5 times in phases V3 and R3 respectively in comparison with the seed treatment with Standak Top, and the mass of one nodule was higher by 1.3 and 1.6 times, respectively. The number of root nodules in the period of vegetative growth of soybean was less than that for the control with inoculation, while in the phase of the beginning of pod formation —by 1.4 times greater than the control value.

A sufficiently high level of nitrogenase activity of symbiotic apparatus formed by soybean plants and nodule bacteria was established during all studied phases of soybean vegetation (Table 3). However, it was the lowest for Standak Top: the actual nitrogen-fixing activity of such a symbiosis was unreliably (by 1.4 and 1.1 times) higher than the inoculation control for phases V3 and R3, respectively.

Spraying plants with soybean seed lectin in the phase of two trifoliate leaves (without fungicide) led to a significant increase in the actual nitrogenase activity of the soybean-rhizobium symbiosis. It was 2.9 and 1.9 times higher in the development phases V3 and R3 compared to the control of inoculation and by 2.1 and 1.8 times respectively compared to the variant of inoculation + Standak Top. The maximum activating effect of lectin on the functioning of the nitrogenase complex was established immediately in the following soybean ontogenesis period after treatment (by 2.9 and 2.1 times more compared to the control of inoculation and inoculation + fungicide, Table 3). Spraying soybean plants with specific lectin against the background of Standak Top led to a higher level of the symbiotic system ability to fixing molecular nitrogen during all phases of plant vegetation (Table 3) compared to the inoculation control (by 1.9 and 1.6 times, respectively) and to the variant of inoculation + ST (by 1.4 and 1.5 times, respectively).

Table 3. **Nitrogenase activity of soybean-rhizobium symbiosis after seed treatment with Standak Top and inoculation and spraying vegetative plants with soybean seed lectin**

Treatment	Actual nitrogenase activity of symbiosis	
	Micromoles of C_2H_4 / (plant·h)	% to control
Phase of three trifoliate leaves (V3)		
Rhizobium (control)	0.23	100
Rhizobium + SSL	0.66^{ac}	287/213
Rhizobium + ST	0.31	$135/100^{st}$
Rhizobium + ST + SSL	0.43^{ac}	187/139
$LSD^{ab}_{0.05}$	0.11	
$LSD^{c}_{0.05}$	0.05	
Phase of the beginning of pod formation (R3)		
Rhizobium (control)	12.51	100
Rhizobium + SSL	23.65^{ac}	189/178
Rhizobium + ST	13.29	$106/100^{st}$
Rhizobium + ST + SSL	20.30^{ac}	162/153
$LSD^{ab}_{0.05}$	1.39	
$LSD^{c}_{0.05}$	1.07	

Evaluation of the nitrogen-fixing activity of soil microbiota of diazotrophs during soybean vegetation showed that seed treatment with Standak Top resulted in the inhibition of nitrogen-fixing microorganisms' activity both at the beginning of plant vegetation and in the subsequent phases of soybean ontogenesis (Table 4).

Spraying plants with soybean seed lectin against the background of pesticide action stabilized the nitrogenase activity of diazotrophic microorganisms compared to the variant of inoculation + ST in the phase of the beginning of flowering (R1) and did not affect the activity of diazotrophs in the phase of the beginning of pod formation (R3). A significant decrease in the nitrogen-fixing activity of soil microorganisms in the variants with the use of Standak Top compared to the inoculation control was recorded in the period of reproductive growth of soybeans (R3).

Soybean seed lectin in spraying plants in the phase of two trifoliate leaves (without seed treatment with pesticide) did not significantly affect the nitrogenase activity of soil diazotrophic microorganisms. However, the nitrogen-fixing activity of soil diazotrophs in this variant was slightly higher (by 1.1 and 1.4 times) in phases R1 and R3 compared to the variant of seed treatment with Standak Top. These results indicate a negative effect of the chemical pesticide in the seed treatment on the capacity of the diazotrophic microbiota to the molecular nitrogen conversion, while the use of soybean seed lectin for spraying plants did not show a significant difference in the ability of microorganisms to molecular nitrogen fixing compared to the inoculation control.

Discussion. It is known that a decrease in the degree of damage to seeds and plants by phytopathogens and the best rooting of plants in the soil due to the accelerated growth and development of the root system as well as an increase in the leaf's assimilation surface and the maximum disclosure of the biological potential of the plants are observed due to the treatment of seeds with Standak Top [25]. Our results suggest that the application of the pesticide Standak Top for to soybean seed treatment on the sowing day does not inhibit the process of soybean roots nodulation with *B. japonicum* at the beginning of the formation of symbiotic structures (the phase of development of one trifoliate leaf), however, the number of nodules on theplant was less in the phase of the development of three trifoliate leaves. This may indicate the suppressive action of Sandak Top directly on the process of nodule formation on soybean roots in the period of soybean vegetative growth. Other researchers have also shown that treatment of seeds with pesticides of different action spectra leads to inhibition of the nodulation ability of soybean nodule bacteria and nitrogenase activity of symbiotic soybean-rhizobium systems, which is most pronounced in the first half of the growing stages of plants [1, 3, 7, 8]. Spraying soybean plants in the phase of two trifoliate leaves with a solution of soybean seed lectin on the background of pesticide contributed greatly to the activation of both the process of nodulating plants with rhizobia and the process of forming root nodules. The formation of a larger number of symbiotic structures on soybean roots with a larger total mass on the plant and the mass of one root nodule was recorded as well.

Thus, the application of a solution of soybean seed lectin at a concentration of 50 μg/mL on the background of seed treatment with Standak Top and inoculation has contributed to a higher level of realization of the rhizobia nodulation ability, the decrease in which was due to an anthropogenic factor — pesticide. At the same time, the nodules with the bigger mass on plants were formed.

Spraying plants with soybean seed lectin (without the use of Standak Top) led to an increase in the realization of the rhizobia nodule-forming

Table 4. **Nitrogenase activity of soil diazotrophic soybean microbiota after inoculation of seeds with rhizobia against the background of Standak Top action and spraying vegetative plants with specific lectin**

Treatment	Nitrogenase activity of soil	
	nanomoles of C_2H_4 / (20 g of a. d. soil · h)	% to control
Phase of one trifoliate leaf (V1)		
Rhizobium (control)	1.91	100
Rhizobium + ST	1.48^b	$78/100^{st}$
$LSD^{ab}_{0.05}$	0.21	
Phase of the beginning of flowering (R1)		
Rhizobium (control)	5.56	100
Rhizobium + SSL	5.07	91/111
Rhizobium + ST	4.55^b	$82/100^{st}$
Rhizobium + ST + SSL	4.83	87/106
$LSD^{ab}_{0.05}$	0.29	
$LSD^{c}_{0.05}$	0.54	
Phase of the beginning of pod formation (R3)		
Rhizobium (control)	3.99	100
Rhizobium + SSL	2.44^c	61/140
Rhizobium + ST	1.74^b	$44/100^{st}$
Rhizobium + ST + SSL	1.14^b	29/66
$LSD^{ab}_{0.05}$	0.99	
$LSD^{c}_{0.05}$	0.34	

ability, which was manifested by an increase in the total mass of nodules on the plant and in the mass of one root nodule. Thus, spraying soybean plants with a specific lectin affected the mass of root nodules to a greater extent than the process of their direct formation. This may be explained by the ability of phytolectins to regulate division of cells and increase their mass [28].

A high enough level of nitrogenase capacity of symbiotic systems formed by soybean plants and specific for plants nodule bacteria was established in all studied phases of the development of soybean plants. However, it was the lowest in the case of using Standak Top for seed treatment on the sowing day. Spraying plants with soybean seed lectin in the phase of two trifoliate leaves (without pesticide) led to a significant increase in the actual nitrogenase activity of the soybean-rhizobium symbiosis compared to the control of inoculation and inoculation + Standak Top during soybean ontogenesis. The maximum activating effect of lectin on the functioning of the nitrogenase complex was established immediately in the following soybean ontogenesis period after spraying. Earlier we showed that spraying soybean plants in the phase of two trifoliate leaves with soybean seed lectin led to an increase in the nodule-forming ability of rhizobia and nitrogen-fixing activity of the symbiotic system soybean-*B. japonicum* 634b [29]. The results of this work confirm the possibility of realization of plant lectin biological activity against microsymbionts not only under the conditions of its influence on bacterial cells in a complex inoculant [9] but also under spraying plants against the background of the rhizobium inoculation of seeds.

Spraying soybean plants with specific lectin against the background of the application of Standak Top led to a higher level of the symbiotic system's ability to molecular nitrogen fixing during all phases of plant vegetation compared to the controls of inoculation and inoculation + pesticide. The activity of lectin in the case of spraying plants on the background of seed treatment with Standak Top and inoculation with rhizobia was lower compared with that in the case of seed lectin inoculation. The actual nitrogenase activity of soybean-rhizobium symbiosis during the studied periods of plant vegetation in the case of seed treatment with Standak Top on the sowing day remained at the control level. Spraying plants with specific lectin led to significant growth of the nitrogen-fixing level of symbiotic systems both against the background of pesticide application and without seed treatment with Standak Top. The maximum increase in nitrogenase activity of the soybean-rhizobium symbiotic system was recorded in the variant with spraying plants with soybean seed lectin (specific to soybean plants) against the background of seed inoculation with a specific strain of nodule bacteria.

Activation of the nitrogenase capacity of the soybean-rhizobium symbiotic system by spraying plants with specific lectin may be due to its indirect effect on the functioning of the plant's photosynthetic apparatus and metabolism of photosynthetic products, which are a source of energy for the functioning of the nitrogenase complex. We have previously established the participation of phytolectins with exogenous effects on seeds in the coordination of two significant physiological processes in plants, namely nitrogen fixation and photosynthesis in phytobacterial systems [9, 30], as well as the possible direct effect of plant lectin on the auxin/cytokinin balance in plants, which has been shown in previous studies on the exogenous action of wheat germ agglutinin on the wheat seeds [9].

The functioning of nitrogen-fixing soil microorganisms was suppressed both at the beginning of plant vegetation and during the subsequent phases of soybean ontogenesis under the treatment of seeds with Standak Top followed by inoculation with rhizobia. Spraying of plants with soybean seed lectin against the background of pesticide action stabilized the functional activity of nitrogen-fixing microorganisms compared to the variant of inoculation + Standak Top in the phase of the beginning of flowering and did not affect the activity of diazotrophs in the phase of the beginning of pod formation of soybeans. Spraying plants with soybean seed lectin in the phase of two trifoliate leaves (without seed treatment with Standak Top) did not significantly influence the nitrogen-fixing activity of soil diazotrophic microorganisms. This may indicate the absence of direct action of this biologically active substance onto the soil microbiota in this method of lectin application. However, the nitrogen-fixing activity of soil diazotrophs was slightly higher in the phases of beginning flowering and beginning pod formation compared to the variant with seed treatment with fungicide. This may be caused by the influence of exogenous lectin on the functioning of the photosynthetic apparatus of plants [9, 30] and the possible indirect regulation of the quantitative and qualitative compositions of their root metabolites — products of photosynthetic activity, which largely determine the development and functional activity of soil microorganisms [31].

Thus, the process of nodule formation on the soybean roots was suppressed in the first half period of the soybean growing, while the process of nodulating plants with rhizobia remained stable during the seed treatment with Standak Top on the sowing day. The nitrogen-fixing activity of the symbiotic system was at the control level, while the nitrogenase activity of soil diazotrophs was suppressed. Spraying plants in the phase of two trifoliate leaves with soybean seed lectin (without using pesticide) led to an increase in the total mass of nodules on the plant and the mass of one root nodule, as well as to a significant increase in the nitrogenase activity of the soybean-rhizobium symbiosis with the maximum (almost threefold) activating action directly in the phase of soybean vegetation following the processing. Spraying plants with soybean seed lectin in the phase of two trifoliate leaves (without seed treatment with Standak Top) did not significantly affect the nitrogen-fixing activity of soil diazotrophic microorganisms. The application of a solution of soybean seed lectin at a concentration of 50 μg/mL on the background of seed treatment with Standak Top (1.5 L/ton of seeds) and inoculation with *B. japonicum* 634b (10^8 cells/mL) contributed to the stabilization and higher level of realization of the rhizobia nodulation ability, the decrease in which was due to such an anthropogenic factor as pesticides. At the same time, the root nodules with bigger masses on plants were formed. The maximum activating effect of lectin on the functioning of the soybean-rhizobium nitrogenase complex was established immediately in the the following soybean ontogenesis period after spraying. Soybean seed lectin significantly increased the nitrogenase activity of the symbiosis under the action of Standak Top compared to the inoculation + pesticide control.

Thus, the obtained results suggest the possibility of using the method of spraying plants with specific lectin as a means for leveling (or mitigating) the negative effect of seed treatment with pesticides (e.g. Standak Top) on the formation and functioning of the symbiosis and nitrogen-fixing activity of rhizosphere diazotrophic microbiota. This indicates the prospects of studying the biological activity of phytolectins through spraying plants in order to regulate processes of the formation and functioning of phytobacterial systems, as well as their response to various environmental or anthropogenic stress factors, in particular, the effect of chemical plant- protecting products applied to the seed treatment.

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REFERENCES

1. Kintschev MR, Goulart ACP, Mercante FM. Compatibility between rhizobium inoculation and fungicide application in seeds of common beans. Summa Phytopathologica. 2014; 40(4):338—346.
2. Saenko GM, Bushneva NA. [Compatibility of fungicide dresser and inoculators on soybean. Oil Crops]. Scientific and Technical Bulletin of the All-Russian Scientific Research Institute of Oil Crops by Pustovoit VS. 2018; 3(175):124—127. Russian.
3. Mostoviak II, Kravchenko OV. Symbiotic apparatus of soya under the application of different types of fungicides and microbial preparation. Tavriys'kyy naukovyy visnyk. 2019; 108:72—77.
4. Morgun VV, Kots SYa. [The role of biological nitrogen in nitrogen nutrition of plants]. Visnyk of the National Academy of Sciences of Ukraine. 2018; 1:62—74. Ukrainian.
5. Wang D, Yang Sh, Tang F, Zhu H. Symbiosis specificity in the legume — rhizobial mutualism. Cellular Microbiology. 2012; 14(3):334—342.
6. Suzaki T, Kawaguchi M. Root nodulation: a developmental program involving cell fate conversion triggered by symbiotic bacteria. Current Opinion in Plant Biology. 2014; 21(10):16—22.
7. Vozniuk SV, Tytova LV, Lyaska SI, Iutynska GO. [Influence of fungicides complex inoculum Ekovital on rhizosphere microbiocenosis, diseases resistance and soybean productivity]. Mikrobiol Z. 2015; 77(4):8—14. Ukrainian.
8. Pavlyshche AV, Zhemojda AV, Kiriziy DA, Rybachenko LI. [The functioning of the symbiotic and photosynthetic apparatus of soya under the influence of chemical agents with fungicidal action and exogenic lectin]. Plant physiology and genetics. 2019; 51(6):517—528. Ukrainian.
9. Kyrychenko OV. [Phytolectins and diazotrophs are the polyfunctional components of the complex biological compositions]. Biotechnologia Acta. 2014; 7(1):40—53. Ukrainian.
10. Kandelinskaya OL, Grischenko ER, Ripinskaya KJu, Aleschenkova ZM, Kartizhova LE, Kuptsov VN, Kuptsov NS. [Role of lectins in regulation of legume-rhizobium symbiosis efficiency in lupin]. Botanika (issledovaniya). Sb. Nauch. Tr.: In-t Experimentalnoy Botaniki NAN Belarusi. Minsk: In-t radiobiologii. 2015; 44:283—290. Russian.
11. Kots SYa, Mykhalkiv LM, Mamenko PM, Veselovska LI. The use of soybean seed lectin to regulate the formation and functioning of legume-rhizobial symbiosis of alfalfa and soybeans under drought: pat. n*а ко*rysnu model UA No 102077 A01N25 / 00, A01N65 / 20. Publ. 26.10.15. Bull. N 20. Ukrainian.
12. Sharon N. Lectins: carbohydrate-specific reagents and biological recognition molecules. Journal of Biological Chemistry. 2007; 282:2753—2764.
13. Gabius HJ. The sugar code: why glycans are so important. Bio Systems. 2018; 164:102—111.
14. Van Damme EJM, Lannoo N, Peumans WJ. Plant Lectins. Advances in Botanical Research. 2008; 48:107—209.
15. Lagarda-Diaz I, Guzman-Partida A, Vazquez-Moreno L. Legume lectins: proteins with diverse applications. International Journal of Molecular Sciences. 2017; 18(6):1242.
16. Coelho LCBB, Silva PMS, Lima VLM, Pontual EV, Paiva PMG, Napoleao ThH, Correia MTS. Lectins, interconnecting proteins with biotechnological / pharmacological and therapeutic application. Evidence-Based Complementary and Alternative Medicine. 2017; ID 1594074.
17. Pavlovskaya NE, Gagarina IN. [The physiological properties of plant lectins as a prerequisite for their application in biotechnology]. Khimiya rastitelnogo syriya. 2017; 1:21—35. Russian.
18. Hendrickson OD, Zherdev AV. Analytical application of lectins. Critical Reviews in Analytical Chemistry. 2018; 48(4):279—292.
19. Mishra A, Behura A, Mawatwal Sh, Kumar A, Naik L, Mohanty SS, e.a. Structure-function and application of plant lectins in disease biology and immunity. Food and Chemical Toxicology. 2019; 134:110827.
20. Pavlovskaya NE, Gagarina IN, Rogovin VV, Borzenkova GA, Mushtakova VM, Fomina VA. Means for presowing treatment of pea seeds: Pat. Rus. No 2372763. Publ. 20.11.2009. Byul. No 33. Russian.
21. Sergienko VG, Kyrychenko OV, Perkovska GYu. Method of using plant lectins to protect vegetable crops from diseases: pat. UA No 41723 A01N 63/00, A01C 1/06. Publ. 10.06.2009. Byul. No 11. Ukrainian.
22. Erohin AI. [The effectiveness of the preparation on the basis of lectins on leguminous crops in presowing treatment of seeds and vegetating pea plants]. Zernobobovyye i krupyanyye kul'tury. 2019; 2(30):48—53. Russian.
23. Jiang S, Ma Z, Ramachandran S. Evolutionary history and stress regulation of the lectin superfamily in higher plants. BMC Evolutionary Biology. 2010; 10(1):79.
24. [State Register of Plant Varieties Suitable for Dissemination in Ukraine in 2018]. Ministry of Agrarian Policy and Food of Ukraine. 2018. 447. Ukrainian.
25. Plant protection products — Bayer, Basf, Syngenta, Dupon, Avgust. Product catalog. URL: http://www.demetra-agra.com.ua and http://www.cropscience.bayer.ua

26. Hardy RWF, Burns RC, Holsten RD. Application of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry. 1973; 5(1):41—83.
27. Thandiwe N, Sexton P, Gustafson K, Miller JM. Soybean Growth Stages. In book: iGrow Soybean: Best management Practices for Soybean Production. 2019; 3:1—11. www.researchgate.net
28. Shakirova PhM, Bezrukova MV. [Current understanding of the putative function of plant lectins]. Journal of General Biology. 2007; 68(2):109—125. Russian.
29. Kyrychenko OV, Kots SY, Pukhtaevich PP. The effectiveness of phytolectins and lectin compositions for spraying plants during the growing. Biotechnologia Acta. 2021; 14(1):57—68.
30. Sytnikov DM, Kots SYa, Malichenko SM, Kirizii DA. [Photosynthetic rate and lectin activity of soybean leaves after inoculation with rhizobia together with homologous lectin]. Russian Journal of Plant Physiology. 2006; 53(2):169—175. Russian.
31. McNear JrDH. The Rhizosphere. Roots, soil and everything in between. Nature Education Knowledge. 2013; 4(3):1—20.

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РЕАЛІЗАЦІЯ НОДУЛЯЦІЙНОЇ ТА АЗОТФІКСУВАЛЬНОЇ АКТИВНОСТЕЙ *BRADYRHIZOBIUM JAPONICUM* І РИЗОСФЕРНОЇ МІКРОБІОТИ ЗА ОБРОБКИ НАСІННЯ ПЕСТИЦИДОМ СТАНДАК ТОПОМ ТА ОБПРИСКУВАННЯ РОСЛИН ЛЕКТИНОМ НАСІННЯ СОЇ

Ідея дослідження полягала у використанні соєвого лектину, як біологічно активної сполуки широкого спектру дії, для обприскування рослин сої з метою стабілізації формування та функціонування соєво-ризобіального симбіозу, а також азотфіксуючої активності ризосферної мікробіоти на тлі обробки насіння хімічним засобом захисту рослин Стандак Топ — інноваційним протруйником із фунгіцидною та інсектицидною дією для боротьби з основними хворобами та шкідниками рослин сої. **Мета.** У вегетаційних умовах із ґрунтовою культурою дослідити особливості формування та функціонування соєво-ризобіального симбіозу, а також азотфіксуючу активність ризосферної мікробіоти за обприскування рослин специфічним лектином насіння сої на тлі обробки насіння Стандак Топом та інокуляції бульбочковими бактеріями *Bradyrhizobium japonicum* 634б у день посіву. **Методи.** Фізіологічні, мікробіологічні, газова хроматографія та статистичні. **Результати.** Показано, що при обробці насіння Стандак Топом (1,5 л/т насіння) у день посіву відбувалося пригнічення процесу бульбочкоутворення на коренях у період вегетативного росту сої. Азотфіксуюча активність симбіотичної системи була на рівні контролю, а нітрогеназна активність діазотрофів ґрунту пригнічена (в 1,2—2,2 рази). Обприскування рослин лектином насіння сої (варіант без пестициду) у фазу двох трійчастих листків (V2) привело до збільшення загальної маси бульбочок на рослині (в 1,5 і 1,9 рази та в 2,3 і 2,0 рази порівняно з контролем інокуляції у фази розвитку трьох трійчастих листків (V3) та початку формування бобів (R3) відповідно). Збільшення загальної маси симбіотичного апарату на коренях сої при цьому становило відповідно в 1,4 і 1,5 рази в порівнянні з обробкою насіння Стандак Топом, а маси однієї бульбочки — в 1,3 і 1,6 рази. Лектин насіння сої сприяв значному зростанню фактичної нітрогеназної активності соєво-ризобіального симбіозу, яка в 2,9 і 1,9 рази первищувала контроль (інокуляція) та у 2,1 і 1,8 рази — рослини варіанту інокуляція + пестицид у фазах онтогенезу сої V3 та R3 відповідно. Функціональна активність ґрунтових азотфіксуючих мікроорганізмів істотно не змінювалась. Використання лектину насіння сої на тлі обробки насіння Стандак Топом та інокуляції сприяло стабілізації та підвищенню бульбочкоутворюючої здатності ризобій, пригнічення якої було зумовлено впливом антропогенного фактора — пестициду. Відзначається збільшення кількості (в 1,6 і 1,2 рази) та маси кореневих бульбочок (у 2,2 і 1,5 рази та в 1,4 і 1,2 рази відповідно у порівнянні з контролем інокуляції та інокуляції + пестицид). Лектин насіння сої сприяв значному підвищенню нітрогеназної активності симбіозу на фоні Стандак Топу (в 1,9 і 1,6 рази та в 1,4 і 1,5 рази відповідно у фазах V3 і R3) порівняно з контролем інокуляції та інокуляції + пестицид. **Висновки.** Отримані результати дозволяють припустити можливість використання способу обприскування рослин специфічним лектином як засобу, що нівелює (або пом'якшує) негативну дію пестициду за обробки насіння на формування та функціонування симбіозу, а також функціонування ризосферної діазотрофної мікробіоти. Це вказує на перспективність вивчення біологічної активності фітолектинів за обприскування рослин із метою регуляції процесів формування та функціонування фітобактеріальних систем, а також їхньої реакції на різноманітні екологічні або антропогенні стрес-фактори, зокрема на дію хімічних засобів захисту рослин при обробці насіння.

Ключові слова: *соєво-ризобіальний симбіоз, Стандак Топ, лектин насіння сої, нодуляція, нітрогеназна активність, ризосферні діазотрофи.*