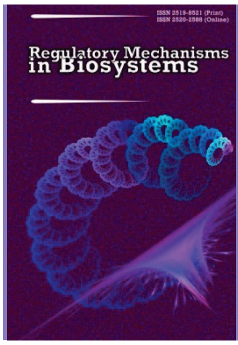

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2022, 13(2), 105–113
doi: 10.15421/022215

Biological activity of soybean seed lectin at the spraying of *Glycine max* plants against the background of seed treatment with pesticide containing fipronil, thiophanate-methyl, pyraclostrobin as active substances and rhizobial bacterization

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Article info

Received 10.03.2022
Received in revised form 04.04.2022
Accepted 05.04.2022

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Kyrychenko, O. V., Kots, S. Y., Khrapova, A. V., & Omelchuk, S. V. (2022). Biological activity of soybean seed lectin at the spraying of Glycine max plants against the background of seed treatment with pesticide containing fipronil, thiophanate-methyl, pyraclostrobin as active substances and rhizobial bacterization. Regulatory Mechanisms in Biosystems, 13(2), 105–113. doi:10.15421/022215

Preparations for protecting plants, particularly those with fungicidal activity, continue to be relevant in agricultural production. They are used to effectively combat phytopathogens and ensure high yield of cultivated plants. However, they are among the anthropogenic factors which impose a heavy chemical load on ecosystems. Data about the effects of pesticides on physiological parameters of plants are essential for understanding the main regulatory mechanisms as preconditions to the phytotoxic state of compounds, as well as greater substantial understanding of the functional condition and implementation of adaptive potential of plants during and post stress. An important and relevant task – both practically and theoretically in the conditions of action of fungicide on seeds - is studying the possibilities of application of phytolectines as biologically active compounds with broad spectrum of action, including fungicidal effect, for spraying legumes in order to stabilize their development, ensure effective functioning of legume-rhizobial symbioses and cause fuller realization of productive potential against the background of decrease in chemical pressure on agrocenoses. Therefore, we aimed to evaluate the biological activity of soybean seed lectin (according to the parameters of productivity and functional activity of soybean-rhizobial symbiosis) at the spraying of *Glycine max* (L.) Merr. plants against the background of seed treatment of pesticide, Standak Top with fungicidal and insecticidal actions on the day of sowing and inoculation with *Bradyrhizobium japonicum* 634b. We used physiological, biochemical, microbiological and statistical methods of studies. We determined that Standak Top, applied on soybeans that were afterwards inoculated with rhizobia, exerted negative tendency on formation of vegetative mass by plants at the beginning of vegetation, though in the following phases of ontogenesis, their development and productivity reached the level of the control. Nitrogenase activity of symbiosis and the condition of photosynthetic pigment complex (content of chlorophyll and carotenoids and their ratio) were at the level or significantly lower than in the control plants. After spraying soybeans with lectin (without use of the fungicide) in the phase of development of two true leaves, there occurred significant increase in functional activity of the symbiotic system (according to total nitrogenase activity of symbiosis, higher by 1.91 and 1.79 times compared with the controls with inoculation and inoculation + fungicide) and the content of photosynthetic pigments (chlorophylls were higher by 1.12–1.45 times, carotenoids by 1.14–1.39 times) and development of strong leaf apparatus (by 1.33–1.42 times). This caused highest level of realization (by 13.9% and 10.1% higher compared with the controls with inoculation and inoculation + fungicide) of productive potential of cultivated plants. After spraying plants with soybean lectin against the background of use of fungicide, notable and reliable increases occurred in the level of absorption of molecular nitrogen (by 1.72 and 1.52 times according to total activity of symbiosis, compared with the controls with inoculation and inoculation + fungicide), content of chlorophyll (1.25–1.64 times) and carotenoids (1.12–1.42 times) in leaves of soybean, and also plants were actively developing during vegetation (1.12–1.40 times), producing yield that exceeded by 12.8% and 9.1% the controls with inoculation and inoculation + fungicide. Therefore, use of soybean seed lectin for spraying plants against the background of seed treatment of pesticide Standak Top on day of sowing can stabilize and even increase the level of realization of symbiotic and productive potential of soybean-rhizobial symbiosis compared both with the control (inoculation with rhizobia) and the variant with treatment of seeds (rhizobia + fungicide). This indicates on the perspectives of further studies of biological activity of phytolectins aiming at decreasing chemical pressure on ecosystems by leveling out or decreasing the negative impact of chemical means of protection on the plants and symbiosis.

Keywords: soybean-rhizobial symbiosis; *Bradyrhizobium japonicum* 634b; fungicide; lectin; nitrogenase activity; photosynthetic pigments; grain productivity.

Introduction

Seeking new ways and means of increasing yield and quality of production of arable farming is one of the main tasks and vectors of development of agrarian production. An important source of increase in the production of competitive products in the system of sustainable arable farming is increase in specific weight of legumes in the structure of cultivated areas through their ability for symbiotic nitrogen fixation – provision of plants with biological nitrogen, resulting in increase in the share of ecologically clean crop production and decrease in chemical pressure on ecosystems (Mazur et al., 2021).

At the current stage of development of the agro-industrial complex of Ukraine, soybean is one of the most profitable crops, cultivation of which is annually increasing (Ilkiv & Stepanus'ko, 2021; Tkalich et al., 2021). It is characterized by high adaptability to climatic conditions and universal use of plant raw material. Soybean is a source of ecologically friendly amino acid-balanced protein. Its grain contain enzymes, vitamins, mineral substances, and are a source of high-quality protein for livestock farming and aquaculture, its oil is used for industries and is a valuable component of the human diet. Because of those properties and high productivity, soybean as a source of plant protein is the leading plant by cultivated area and total grain production from annual legumes and oil crops (Saha &

Mandal, 2019; Chehova, 2021). A determining condition for increase in productivity of soybean fields is development and introduction of new technologies of their cultivation into production, which would completely correspond to genetic specifics of a certain variety and take into account interaction with the plant organism and the overall effect of agro- and hydrothermal conditions, biotic and anthropogenic factors (Vyshnivskyi & Furman, 2020).

One of the important technological methods of cultivation of soybean is treatment seeds by fungicidal preparations, because losses of yield to phytopathogen infections total 40% (Lamichhane et al., 2020). Fungicides are highly selective chemical compounds or biological organisms able to specifically inhibit or cease growth of fungi or fungal spores. Similarly, to medical drugs, they affect the biochemical links that are important for growth and development of pathogens, or stimulate protective mechanisms of plants (Mohamed et al., 2018; Guo et al., 2021; Li et al., 2021).

Most studies of influence of fungicides in agriculture have focused on their efficacy against fungal pathogens or their residuals in yields (Fernandez-Ortuno et al., 2008; Zhang et al., 2020). However, toxicity of fungicides is not always limited to the targeted pest organism. Some studies note that utilization of fungicides has consequences for the physiology of plants, such as decrease in growth, inhibition of development of reproductive organs, change in nitrogen or carbon metabolisms (Saladin, 2003). Other studies indicate that some fungicides can increase protective ability of plants through synthesis of phytoalexin and lignification of the cellular wall, or stimulate activity of enzymes that participate in synthesis of phenol compounds (Dias, 2012). Fungicides can alter content of acids, assimilation of CO_2, effectiveness of photosynthesis, biomass, growth rates, and therefore development of yield (Jakl et al., 2021).

Currently, there is a broad range of chemical substances for protecting plants, which have bactericidal, fungicidal and insecticidal actions, which are broadly used in production of soybean (Lamichhane et al., 2020; Roman et al., 2021). These are protective preparations of multi-oriented spectrum of action (systemic, contact), containing compounds of various chemical classes (triazoles, strobilurins, phenylpyrazoles, benzimidazoles, metalaxyl, phenols and others). The pattern of action of protective substances depends on the chemical nature of the constituents (toxicities of individual components and their combination), as well as concentration, norm, method and period of application of substances (Gupta, 2018; Carvalho et al., 2020; Lamichhane et al., 2020; Shahid et al., 2020). Combined preparations that contain components with various mechanisms and action spectra are increasingly produced based on chemical compounds of systemic action. Therefore, it is important to note Standak Top (BASF, Germany) – an innovative fungicide for control of the main diseases of soybean, which combines fungicidal and insecticidal actions, and also has an effect on physiological processes in plants (Aizemberg & Campo, 2021; Molin et al., 2021).

Treatment seeds of soybean should be correlated with another technological method that is necessarily used in technology of its cultivation – seed inoculation with highly active and effective strains of rhizobia. Seed inoculation with rhizobia results in development of symbiotic apparatus, the fundamental function of which is absorption of molecular nitrogen (Ciampitti & Salvagiotti, 2018), thereby promoting better realization of productive potential of plants (Silva et al., 2018; Hungria et al., 2020; Rodrigues et al., 2020; Kots et al., 2022). Fixation of atmospheric nitrogen by soybean-rhizobial symbiotic systems promotes intensification of arable farming, decrease in energy expenditures, improvement of ecological condition of soils, as well as provision of high level of grain productivity of soybean (Ciampitti & Salvagiotti, 2018). Therefore, study of the possibility of complex application of methods of treatment seeds of fungicides and their inoculation with rhizobia is a relevant issue of development and improvement of technologies of cultivation of soybean (Araujo et al., 2017; Silva et al., 2018; Mostoviak & Kravchenko, 2019).

Currently, we know that against the background of using fungicides, development of plants and formation of their biological and seed productivities significantly changed, and nitrogenase activity of legume-rhizobial symbioses significantly weakened, especially in the first half of the vegetation (Mostoviak & Kravchenko, 2019; Gorshkov et al., 2020; Rodrigues et al., 2020; Kots et al., 2022). Under *in situ* conditions against the background of using fungicides Maksim Star 025 FS and Kinto Duo, symbiotic systems of soybean altered their functional activity, leading to decrease in the share of biological nitrogen in yield. Therefore, treatment of soybean seeds with Maksim Star 025 FS fungicide caused no notable negative effect on symbiotic apparatus of soybean because a negative tendency was only seen toward change in parameters of actual nitrogenase activity of root nodules, whereas Kinto Duo preparation significantly inhibited (1.8 times) their functional ability (Vozniuk et al., 2015).

Fungicidal compounds have been also observed to have a significant impact on plants. Therefore, application of fungicides to seeds can lead to cytotoxic effect and cause disorders in mitotic activity of meristem and changes in genetic apparatus of cells which prevent them being involved in the proliferation, causing growth arrest of soybean seedlings (Melnichuk et al., 2015). Study of transfers of fungicides in plant organism revealed that ^{14}C-pyraclostrobin, ^{14}C-metalaxyl and ^{14}C-carbendazim localize the most in the cotyledons of soybean plants, whereas maximum overall rate of their absorption by root, stems and leaves equaled 15%. Absorption of fungicides also depends on type of soil: higher absorption occurred in soil with lowest amount of organic substances (Sartori et al., 2020).

Biological productivity of plants depends greatly on intensity of photosynthetic activity of their leaf apparatus (Priadkina et al., 2015). Formation of the main elements of the structure of yield (number of beans, number and mass of seeds on plants) directly depends on activity of photosynthesis and influx of assimilates – products of photosynthetic activity – to the flowers and beans in the period of generative development of plants. Fungicides are known to cause significant effect on functioning of photosynthetic apparatus after different ways of application (treatment seeds, spraying plants during vegetation) (Petit et al., 2012). Therefore, depending on active concentration, benzimidazoles and dithiocarbamates inhibited the germination rates of chickpea plants, the length of root and shoot, influenced photosynthesis activity (chlorophyll content), total content of sugar, phenol and activity of antioxidant enzymes (Singh & Sahota, 2018). Comparative analysis of influence of fungicides of stobulurins and triazole classes on physiological parameters of soybean, wheat and barley in various conditions of watering revealed that strobulurins slowed photosynthesis and transpiration, and also decreased concentration of intercellular carbon dioxide (Nason et al., 2007). Toxicity of fungicidal action was confirmed to be dependant on the period of influence on seeds and bacterization (Pavlyshche et al., 2018). Therefore, if 14-days treatment of seeds with Fever and Standak Top fungicides did not inhibit the development of vegetative mass of plants, the treatment of seeds on the day of sowing caused significant decrease in growth of above-ground mass by 18% and 24% (in the phase of flowering) and by 24% and 13% (in the phase of beans formation) respectively. Functional activity of symbiosis decreased against the background of treatment of seeds.

Therefore, the search for methods to stabilize the development of plants and formation of their biological and grain productivities is relevant, as well as the main function of the symbiotic system – absorption of molecular nitrogen, against the background of action of anthropogenic factor of fungicides, especially if seeds had been treated and bacterized on the day they were sown.

Currently, we know that the level of realization of symbiotic and productive potentials of legume-rhizobial symbioses may be regulated by biologically active substances of various nature, including plant lectins (Kyrychenko, 2014; Kandelinskaya et al., 2015; Kyrychenko et al., 2021). Our previous studies, as well as works of other scientists, revealed that phytolectins (or substances based on them) may be used for the pre-sowing treatment of seeds (Kyrychenko, 2014; Erohin, 2019), can be used as an additional biologically active component with a broad range of physiological activity toward rhizobial inoculants with subsequent bacterization of seeds (Kyrychenko, 2014; Kandelinskaya et al., 2015), and also for spraying plants during vegetation (Erohin, 2019; Kyrychenko et al., 2021). Studies also confirmed participation of lectins in regulation of stress-adaptation of plants and the possibility of using them as protective compounds during exogenic action in the conditions of exposure to abiotic and biotic factors (Jiang et al., 2010; Kyrychenko, 2014; Pavlovskaya & Gagarina, 2017; Erohin, 2019).

We hypothesized the use of soybean seed lectin as a biologically active compound of broad range of action to spray plants in order to stabilize their growth and form grain productivity, as well as functioning of soybean-rhizobial symbiosis in the conditions of action of anthropogenic stress factor –fungicide of grains.

Thus, we set ourselves the aim of evaluating biological activity of soybean seed lectin (according to parameters of productivity and functioning of activity of soybean-rhizobial symbiosis) after spraying *Glycine max* (L.) Merr. plants against the background of seeds treatment with Standak Top fungicide with fungicidal-insecticidal action, active substances of which are fipronil, thiophanate-methyl, pyraclostrobin, and inoculation with *Bradyrhizobium japonicum* 634b on the day of sowing.

Materials and methods

The object of the study was soybean-rhizobial symbiosis between soybean plants (*Glycine max* (L.) Merr.) of early-ripening variety Almaz of domestic selection and *B. japonicum* 634b bacteria against the background of treatment seeds with Standak Top fungicide on the day of sowing.

The Almaz variety is cultivated for grains, early-ripening (vegetation period up to 100 days), cold-tolerant, drought-tolerant, tolerant to diseases, has stabilizing vegetative period lasting for 100–105 days. Protein content in seeds is 38–39%, oil – 24–26% (Biliavska & Prysiazhniuk, 2018).

Nodule bacteria *B. japonicum* 634b (collection of strains of symbiotic and associative nitrogen-fixing microorganisms of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine – IPPG of NAS of Ukraine) were cultivated in 28 ºC on agarized mannitol-yeast medium (g/L): K_2HPO_4 – 0.5, $MgSO_4 \times 7H_2O$ – 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 for 10 days, the culture was washed off by sterile water, stirred to homogenous suspension. Then, we determined the number of vital (colony-forming units) bacteria using the classic microbiological method of dilution and inoculation to growth media followed by count of colonies. Titer of bacteria in suspension equaled 10^8 cells/mL.

Standak Top (BASF, Germany) is an innovative fungicide for control of the main soybean diseases, the active substances of which are fipronil (250 g/L, class phenylpyrazoles) + thiophanate-methyl (225 g/L, class benzimidazoles) + pyraclostrobin (25 g/L, class strobilurins). It combines fungicidal and insecticidal effects, and also influences the physiological processes in plants (Yashchuk et al., 2016). Fipronil is a contact broad-spectrum insecticide, which is a highly dangerous pesticide to humans. In soil, it decomposes for 30 days, and products of its breakdown (sulfide, sulfone, sulforide) also have insecticidal action. Protective effects of the substance last for 14 days. Thiophanate-methyl – contact-systemic fungicide of broad action – is efficient against diseases of vegetative organs and seeds, and breaks down in the soil slowly (around 6 months). The protective effect of the fungicide lasts 10–15 days. Pyraclostrobin is a fungicide of contact and deep action, exerts long protective effect by inhibiting germination of conidia, initial growth of mycelium and preventing spore-formation of pathogens. The period of half-life in the soil is up to 37 days. Pesticides that belong to the strobilurins class are identified to biofungicides. This is related to the fact that groups of strobilurins include synthetic substances, structurally similar to fungicidal toxins – strobilurins A and B, isolated from culture of *Strobilurus tenacellus* microorganisms. Plants that had been treated with them were observed to undergo certain changes in physiological processes of the development, particularly slowing of processes of aging, increases in photosynthetic activity and mass of cereals grains (Turenko, 2019).

Seeds were treated with Standak Top (ST) on the day of sowing, using the norm (1.5 L/T of grain) recommended by the manufacturer. One hour later, treated seeds were inoculated for 60 min with suspension of *B. japonicum* 634b and sown in pots (20 pcr/pot). Six plants per each pot were kept until yield, 4 pots in each variant. To spray soybean plants during the phase of two true leaves, we used a solution of commercial soybean seed lectin (SSL, Lektinotest, Lviv, Ukraine) in the concentration of 50 μg/mL, in the calculation of 2 mL/plant (50 mL/m^2).

Peculiarities of plant development, formation of their productivity and functioning of symbiotic systems of soybean after inoculating seeds with rhizobia against the background of treatment using Standak Top and spraying by soybean lectin were studied in the conditions of vegetative experiment, performed at the plot of IPPG of NAS of Ukraine in natural light and temperature in 8 replications in each variant, in 10 kg Wagner's pots in soil (sod-podzolic soil : sand, 3:1) substrate with Hellriegel's nutrient mixture (0.25 of the norm of mineral nitrogen).

The experiment was conducted according to the following scheme:

– inoculation of seeds with rhizobia (control with inoculation);

– inoculation of seeds with rhizobia + spraying with SSL;

– inoculation of seeds with rhizobia + Standak Top (control with fungicide following inoculation);

– inoculation of seeds with rhizobia + Standak Top + spraying with SSL.

We analyzed:

– dynamics of similarity of soybean seeds' germination rate, formation of vegetative mass (above-ground part and root) by plants during vegetation, and generative organs, and also grain productivity of soybean;

– functional (nitrogenase) activity of soybean-rhizobial simbiosis was determined using the acetylene method (Hardy et al., 1968) on Agilent GC System 6850 gas chromotograph (USA). The amount of ethylene which formed from acetylene over 1 h of incubation while exposed to nitrogenase of incubated sample was expressed in molar units of formed ethylene per 1 plant for 1 h: micromole of C_2H_4 / (plant • hour) – actual nitrogenase activity of symbiosis, micromole of C_2H_4 / (g of nodules • hour) – specific nitrogenase activity of symbiosis; nanomole of C_2H_4 / (1 nodule • hour) – actual nitrogenase activity of morpho-structural symbiotic unit (root nodule);

– content of photosynthetic pigments (carotenoids, chlorophyll *a* and *b*) in leaves of soybean was determined by the non-maceration method by extracting pigments from weighed amounts of leaf tissue using dimethyl sulfoxide (Hiscox & Israelstam, 1979) followed by determination of density of the obtained solution on a Smart Spec Plus spectrometer (USA) at the wave lengths of 480, 649 and 665 nm. Calculations were performed using formulas given in the study (Wellburn, 1994).

Plants were selected in the phase of development of one and three true leaves (V1 and V3), start of flowering (R1), formation of beans (R3) and complete ripeness of seeds (R8) (Nleye et al., 2019).

The tables present mean arithmetic values and their standard errors (x ± SE). Significance of differences between selections was evaluated using the method of ANOVA, where differences were considered significant if P-values were less than 0.05 (taking into Bonferroni correction).

Results

Standak Top fungicide that had been applied to seeds of soybean on day of its sowing followed by inoculation with rhizobia did not inhibit the germination of seeds, for no significant differences between seed germination rates in the experimental and control groups were determined throughout the study (Table 1).

Table 1
Dynamics of germination rate (%) of soybean against the background of trearment fungicide and seed inoculation (x ± SE, n = 8)

Variants	Day after sowing			
	12th	13th	16th	18th
Rhizobia (control)	28.5 ± 3.5	33.0 ± 4.0	86.0 ± 3.0	93.5 ± 0.5
Rhizobia + ST	34.5 ± 5.5	32.5 ± 4.5	83.0 ± 2.0	92.0 ± 2.0

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top.

Standak Top that had been used on seeds on the day of sowing somewhat inhibited formation of vegetative mass (above-ground part and root) of soybean plants at the beginning of vegetation (phase V1, Table 2), though there were no significant differences with the control plants. In the phase of development of three true leaves (V3), we observed significant positive change in the parameter of green mass of soybean: differences with the control plants accounted for 15.7% and 20.4% for raw and absolutely dry mass of plants. In the second half of vegetation of soybean (period of generative development, phase of start of flowering – R1, and formation of beans – R3), plants of this variant significantly positively differed from the control by accumulation of green mass: by 18.9% and 19.9% for raw and absolutely dry mass of material respectively in phase R1 and by 11.3% and 6.1% respectively in phase R3, which was insignificant compared with the control plants. The root system of soybean, seeds of which had been treated with the fungicide, was characterized by active development in all the studied phases of ontogenesis. Differences with the control plants were 16.2% and 11.1% (for raw and absolutely dry mass respectively) in phase V3, 18.5% and 57.0% and 36.1% and 40.0% respectively in the period of generative development of soybean (phases R1 and R3 respectively).

Lectin from soybean seeds applied to spraying plants in the phase of two true leaves as a biologically active compound with growth-regulatory action affected accumulation of vegetative mass of plants during all studied phases of vegetation (Table 2). In the phase of development of three true leaves, above-ground mass of soybean (raw and absolutely dry) significantly exceeded the control plants by 24.5% and 31.0%, in the flowering phase – by 31.5% and 44.5%, in the phase of bean formation – by 20.6% and 16.3%; root system respectively by 29.8% and 12.2%, by 37.1% and 73.8%, by 55.8% and 46.8%. Compared with the variant with treatment of seeds with Standak Top fungicide, the difference according to green mass equaled 7.7% and 8.8% in the phase of three true leaves, 10.6% and 20.6% in the phase of flowering and 8.4% and 9.6% in the phase of bean formation, according to root mass – 11.7% (in V3 phase for raw material), 15.7% and 10.7%, 14.4 and 4.9% respectively in R1 and R3 phases.

Spraying plants with specific lectin against the background of treatment their seeds with fungicide activated development of soybean during the second half of vegetation (period of generative development of plants, R). Productivity according to green mass of plants (raw and absolutely dry) increased compared with the control with seed inoculation by 33.9% and 32.2% in phase R1 and by 39.6% and 33.6% in R3 phase respectively (Table 2). At the same time, accumulation of green mass by soybean plants (raw and absolutely dry) was more active by 12.7% and 10.3% than in the variant with using fungicide and by 25.5% and 25.9% than during the phase of flowering and formation of beans; root mass – by 12.0% and 19.6%, 23.3% and 25.1% respectively. In the bean formation phase, we recorded the highest increase in the parameters of biological productivity of soybean plants of this variant compared with all the other variants. In the phase of vegetative growth (V3), soybean plants of this variant almost did not differ from plants of variant rhizobia + fungicide, though they exceeded the control (rhizobia) by 9.5% and 19.0%.

Table 2
Formation of vegetative mass of soybean plants at the seed treatment of rhizobia and Standak Top and lectin-spraying during vegetation (x ± SE, n = 12)

Variant	Weight, g			
	raw material		absolutely dry	
	above-ground part	root	above-ground part	root
Phase of development of one true leaf, V1				
Rhizobia (control)	2.82 ± 0.07[a]	0.422 ± 0.021[a]	0.391 ± 0.011[a]	0.0561 ± 0.000[a]
Rhizobia + ST	2.72 ± 0.07[a]	0.398 ± 0.011[a]	0.377 ± 0.020[a]	0.0558 ± 0.001[a]
Phase of development of three true leaves, V3				
Rhizobia (control)	2.94 ± 0.13[a]	0.382 ± 0.031[a]	0.594 ± 0.044[a]	0.090 ± 0.010[a]
Rhizobia + SSL	3.66 ± 0.16[c]	0.496 ± 0.042[b]	0.778 ± 0.051[b]	0.101 ± 0.010[a]
Rhizobia + ST	3.40 ± 0.26[bc]	0.444 ± 0.022[b]	0.715 ± 0.070[b]	0.100 ± 0.011[a]
Rhizobia + ST + SSL	3.22 ± 0.12[b]	0.487 ± 0.051[b]	0.707 ± 0.072[ab]	0.111 ± 0.021[a]
Phase of flower bud formation – beginning of flowering, R1				
Rhizobia (control)	6.10 ± 0.55[a]	2.011 ± 0.111[a]	1.222 ± 0.102[a]	0.172 ± 0.022[a]
Rhizobia + SSL	8.02 ± 0.39[bc]	2.756 ± 0.101[c]	1.766 ± 0.191[bc]	0.299 ± 0.031[b]
Rhizobia + ST	7.25 ± 0.41[b]	2.383 ± 0.182[b]	1.465 ± 0.133[b]	0.270 ± 0.033[b]
Rhizobia + ST + SSL	8.17 ± 0.42[c]	2.668 ± 0.101[c]	1.616 ± 0.111[b]	0.323 ± 0.031[b]
Phase of bean formation, R3				
Rhizobia (control)	11.47 ± 1.10[a]	3.161 ± 0.211[a]	3.388 ± 0.322[a]	0.453 ± 0.041[a]
Rhizobia + SSL	13.83 ± 0.71[b]	4.924 ± 0.301[bc]	3.939 ± 0.222[b]	0.665 ± 0.080[bc]
Rhizobia + ST	12.76 ± 0.94[ab]	4.303 ± 0.350[b]	3.594 ± 0.251[ab]	0.634 ± 0.061[b]
Rhizobia + ST + SSL	16.01 ± 1.11[c]	5.307 ± 0.501[c]	4.525 ± 0.330[c]	0.793 ± 0.072[c]

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top, SSL – soybean seed lectin; various letters of upper indices [a], [b], [c] in the table indicate values that vary one from another within one column, indicating a certain organ of the plant (above-ground or root) in the corresponding phase of soybean development (V1, V3, R1, R3) as a result of comparison using the Tukey's test ($P < 0.05$) with Bonferroni correction.

We determined a quite significant level of functional abilities of symbiotic systems (Table 3), though it was the lowest when using fungicide. Actual nitrogen-fixing activity of symbiosis against the background of treatment seeds insignificantly exceeded the control during inoculation, specific nitrogenase activity of symbiosis, and also nitrogen-fixing activity of morpho-structural symbiotic units (root nodules) remained at the level of the control or was significantly lower than the control (phase of bean formation).

Spraying plants with lectin from soybean in the phase of two true leaves (without applying fungicide) led to significant and reliable increase in actual nitorgenase activity of soybean-rhizobial symbiosis, compared with the control with inoculation, by 2.86 and 1.89 times respectively in V3 and R3 phases, and also by 2.13 and 1.78 times respectively compared with the variant with inoculation + fungicide. The highest activating effect of lectin on functioning of the nitrogenase complex was determined specifically in the next phase of plants' vegetation (V3): 2.86 and 2.13-fold higher compared with the control (rhizobia) and variant with rhizobia + fungicide (Table 3). Specific nitrogenase activity of symbiosis on vegetating plants during the action of lectin significantly exceeded the control with inoculation and inoculation + fungicide by 2.26 and 1.47 times respectively only in V3 phase. Against the background of utilizing Standak Top fungicide, spraying soybean with specific lectin increased the ability of the symbiotic system to take up molecular nitrogen (Table 3). Actual nitrogenase activity increased compared with both control plants with inoculation (by 1.86 and 1.62 times respectively) and variant inoculation + fungicide (by 1.39 and 1.53 times respectively). Specific nitrogenase activity of this symbiotic system was significantly (1.91 times) higher than the control (rhizobia) only in the phase of three true leaves.

Total actual nitrogenase activity of soybean-rhizobial symbiosis for the studied periods of vegetation of plants (Table 3) in the case of using fungicide for treatment of their seeds remained at the level of control plants, whereas spraying plants with specific lectin led to significant increase in the level of absorption of molecular nitrogen by symbiotic systems both against the background of using fungicide (by 1.63 and 1.52 times compared with the control inoculation + fungicide) and without treatment of seeds (by 1.91 and 1.79 times respectively, Table 3). Maximum increase in actual nitrogenase activity of the soybean-rhizobial symbiotic system was seen in the variant with spraying plants with soybean lectin (specific to soybean plants) against the background of seed inoculation with the specific strain of rhizobia. At the same time, total specific nitrogenase activity of the symbiotic system was at the level of the control (rhizobia), with positive tendency after spraying plants with lectin, and was negative after utilizing fungicide for treatment of seeds. In the variant using lectin against the background of seed treatment, this parameter reached the level of the control (rhizobia) or insignificantly exceeded (13.2%) the value in variant rhizobia + fungicide. Total nitrogenase activity of each root nodule during vegetation after the seeds had been treated with Standak Top was at the level of the control, while spraying plants with lectin led to significant increase in this nitrogen-fixing activity by 1.57 and 1.82 times correspondingly compared with variants inoculation and inoculation + fungicide. Spraying with lectin against the background of using fungicide provided a stable level of activity of each morpho-structural symbiotic unit with positive tendency compared both with the control inoculation (by 7.9%) and inoculation + fungicide (by 25.3%).

Table 3
Nitrogenase activity of soybean-rhizobial symbiosis at the seed inoculation with rhizobia against the background of Standak Top fungicide on day of sowing and spraying plants with soybean lectin (x ± SE, n = 12)

Variant	Phase of three true leaves, V3	Phase of beans formation, R3	Total activity during the period of study
Actual nitrogenase activity, micromole of C_2H_4 / (plant • hour)			
Rhizobia (control)	0.23 ± 0.11^{a}	12.51 ± 1.39^{a}	12.74 ± 1.50^{a}
Rhizobia + SSL	0.66 ± 0.12^{b}	23.65 ± 0.92^{c}	24.31 ± 1.04^{b}
Rhizobia + ST	0.31 ± 0.05^{a}	13.29 ± 1.00^{a}	13.60 ± 1.05^{a}
Rhizobia + ST + SSL	0.43 ± 0.11^{ab}	20.30 ± 1.07^{b}	20.73 ± 1.18^{c}
Specific nitrogenase activity, micromole of C_2H_4/ (1 nodule • hour)			
Rhizobia (control)	9.33 ± 2.03^{a}	33.60 ± 3.83^{b}	42.93 ± 4.86^{ab}
Rhizobia + SSL	21.04 ± 1.50^{d}	26.53 ± 2.39^{a}	47.57 ± 2.90^{b}
Rhizobia + ST	14.32 ± 1.76^{b}	22.88 ± 1.72^{a}	37.20 ± 3.48^{a}
Rhizobia + ST + SSL	17.86 ± 1.50^{c}	24.24 ± 3.84^{a}	42.10 ± 5.35^{ab}
Nitrogenase activity of morpho-structural symbiotic unit, nanomole of C_2H_4 / (1 nodule • hour)			
Rhizobia (control)	27.38 ± 15.11^{a}	936.68 ± 76.62^{b}	964.06 ± 91.73^{ab}
Rhizobia + SSL	195.19 ± 27.04^{c}	1317.99 ± 176.85^{c}	1513.18 ± 203.89^{c}
Rhizobia + ST	104.20 ± 26.79^{b}	726.24 ± 51.31^{a}	830.44 ± 78.10^{a}
Rhizobia + ST + SSL	92.84 ± 14.26^{b}	947.67 ± 67.78^{b}	1040.51 ± 82.04^{b}

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top, SSL – soybean seed lectin; various letters of upper indices ^{a,b,c,d} in the table indicate values that significantly differ one from another within one column, which indicates nitrogen-fixating activity of symbiosis (actual or specific), activity of symbiotic unit (root nodule) in appropriate phase of soybean development (V3, R3) and during the entire period as a result of comparison using the Tukey's test (P < 0.05) taking into account Bonferroni correction.

We determined (Table 4) that treatment of seeds with Standak Top fungicide on the day of sowing hardly influenced the activity of formation of the generative organs (flowers and beans) of soybean plants, because no significant difference was determined according to the number of plants that had formed flowers or beans compared with the control (rhizobia). Spraying plants with lectin (without fungicide) activated processes of flowering and bean formation of soybean plants (Table 4), and also caused increase in the level of realization of grain productivity of plants (Tables 5, 6). We determined a positive tendency compared with the control plants (at the level of 4.0–4.5%) according to the parameters of number of beans and fruiting nodes on plants, as well as number of seeds in bean by 12.8 % (Table 5). At the same time, increase in yield of grain of soybean plants accounted for 13.9% (Table 6). We determined that increase in yield of soybean plants also resulted from significant increase in the number and weight of seeds per plant by 16.6% and 14.0% respectively (Table 6).

In the variant with treatment seeds with Standak Top on the day of sowing, the plants formed yield at the level of control, whereas after spraying soybean plants against the background of using the fungicide, grain yield increased by 12.8% and 9.1% respectively compared with the variants with inoculation and inoculation + fungicide. Lectin caused stabilizing effect (at the level or insignificantly higher, up to 6%) for almost all the parameters of the yield structure compared both with the variant of inoculation and variant with fungicide without lectin spraying. At the same time, significant 15.4% and 12.7% increase – compared with inoculation – was determined for the parameters of number and mass of seeds per plant, and also by 9.0% for mass of grain per plant compared with variant with inoculation + fungicide (Table 6). Mass of 1000 seeds (except the variant with treatment seeds, where significantly lower mass of 1000 seeds was determined, indicating insufficient fullness of grain yield) and index of yield did not change (within experiment error, Table 6).

It is shown (Table 7) that during the soybean vegetation the absolute content of green and yellow photosynthetic pigments (chlorophylls and carotenoids) in the leaves of plants gradually decreased: the maximum values were observed in the development phase of three true leaves (V3), the minimum – in the phase of bean formation (R3). Spraying plants with lectin from soybean seeds promoted significant increase in the level of green and yellow photosynthetic pigments (chlorophylls and carotenoids) in leaves of soybean plants during vegetation. Compared with the control (inoculation), differences according to the contents of chlorophyll *a* equaled 12.4%, 23.9% and 44.8% respectively in the phase of three true leaves, start of flowering and bean formation, chlorophyll *b* – 19.8%, 9.9% and 44.9% respectively, total *a*+*b* respectively – 13.5%, 21.2% and 44.8%. At the same time, in the phase of vegetative growth of the plants (V3), we observed change in ratio of chlorophyll *a* and *b* toward increase in chlorophyll *b* compared with chlorophyll *a*, and also changes in the ratio of total chlorophylls and carotenoids toward increase in the amount of chlorophylls compared with carotenoids. In the period of generative development of soybean, we observed equalizing of ratios of those photosynthetic pigments, while their absolute level was statistically higher compared with the control plants (inoculation and inoculation + fungicide). In soybean plants the seeds of which had been treated, we saw no significant changes in the level and ratio of green and yellow photosynthetic pigments in leaves during vegetation (Table 7).

Table 4
Activity of generative organs formation of plants against the background of Standak Top fungicide, rhizobium inoculation and soybean seed lectin spraying (x ± SE, n = 6)

Variant	% of plants / pot, which formed			
	flowers		beans	
	33rd day	34th day	42nd day	43rd day
Rhizobia (control)	12.5 ± 8.0^{a}	26.0 ± 4.3^{a}	77.8 ± 5.0^{a}	94.4 ± 3.7^{a}
Rhizobia + SSL	25.0 ± 10.4^{ab}	53.1 ± 10.7^{b}	91.7 ± 4.8^{b}	100.0 ± 0.0^{b}
Rhizobia + ST	12.5 ± 4.2^{a}	58.3 ± 10.8^{b}	87.5 ± 8.0^{ab}	95.8 ± 2.2^{a}
Rhizobia + ST + SSL	33.3 ± 11.0^{b}	66.7 ± 11.8^{b}	95.8 ± 4.2^{b}	100.0 ± 0.0^{b}

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top, SSL – soybean seed lectin; different letters of upper indices ^{a,b} in the table present values that significantly differ one from another within one column, which indicates day of formation of flowers or beans as a result of comparison using the Tukey's test (P < 0.05) taking into account Bonferroni correction.

Table 5
Characteristics of soybean beans in the phase of complete ripeness of grain after fungicide coating and inoculation of seeds and spraying plants with specific lectin (x ± SE, n = 24)

Variants	Number			
	beans	fruiting nodes	beans	seeds
	per plant		fruiting node	bean
Rhizobia (control)	10.90 ± 0.21^{ab}	5.33 ± 0.42^{a}	2.01 ± 0.10^{a}	1.72 ± 0.12^{a}
Rhizobia + SSL	11.34 ± 0.40^{b}	5.57 ± 0.22^{a}	2.00 ± 0.11^{a}	1.94 ± 0.01^{b}
Rhizobia + ST	10.51 ± 0.21^{a}	5.80 ± 0.20^{a}	1.84 ± 0.10^{a}	1.90 ± 0.02^{c}
Rhizobia + ST + SSL	11.01 ± 0.64^{ab}	5.61 ± 0.31^{a}	1.96 ± 0.12^{a}	1.88 ± 0.11^{abc}

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top, SSL – soybean seed lectin; different letters of upper indices ^{a,b,c} in the table indicate values that significantly vary one from another within one column for corresponding characteristic of beans as a result of comparison using the Tukey's test (P < 0.05) taking into account Bonferroni correction.

Spraying of soybean plants with lectin against the background of treatment their seeds with fungicide positively influenced the accumulation of green photosynthetic pigments chlorophylls and their ratio in leaves (Table 7). In the period of vegetative growth of soybean (V3 phase), their content was at the level of the control plants, and was significantly higher (chlorophyll *a* – by 25.1% and 61.2%, chlorophyll *b* –23.2% and 79.3%, total chlorophyll – by 24.6% and 64.4%) in the period of generative development (R1 and R3 phases). In these periods of development of soybean (R1 and R3 phases) in the variant where the plants had been sprayed with lectin against the background of treatment seeds with fungicide, the content of chlorophyll in leaves significantly exceeded such in plants in variant with rhizobia + fungicide. Therefore, chlorophyll a level was higher respectively by 27.8% and 42.1%, chlorophyll b – by 12.1% and 54.0%, total chlorophyll – by 26.7% and 44.1%.

It has to be noted that in phase of three true leaves (V3) of soybean, significant change (17.3% and 26.1%) occurred in the ratio of green and yellow photosynthetic pigments, tending toward increase in the level of chlorophyll compared with carotenoids in the variant using lectin for spraying plants (both without and with fungicide respectively) compared with the control plants. Then (period of generative development of soybean, R1 and R3 phases), the pattern in the ratio of green and yellow photosynthetic pigments which we observed remained, though the difference was not that significant (6.2% and 10.9% in R1 phase compared with variants without and with the fungicide, and 4.15 and 15.6% respectively – in R3 phase).

Table 6
Structure of soybean yield in the phase of complete ripeness of grain after treatment of fungicides and inoculation of seeds and spraying of plants with soybean lectin (x ± SE, n = 24)

Variant	Per plant		Yield / vessel, g	Weight of 1000 seeds, g	Index of yield, Hi
	number of seeds	weight of seeds, g			
Rhizobia (control)	15.41 ± 0.51^{a}	3.22 ± 0.07^{a}	19.31 ± 0.45^{a}	220.2 ± 7.1^{b}	0.551 ± 0.032^{a}
Rhizobia + SSL	17.97 ± 0.60^{b}	3.67 ± 0.14^{b}	21.99 ± 0.84^{b}	210.6 ± 7.2^{ab}	0.565 ± 0.021^{a}
Rhizobia + ST	17.22 ± 0.81^{b}	3.33 ± 0.05^{a}	19.98 ± 0.28^{a}	200.2 ± 7.2^{a}	0.553 ± 0.014^{a}
Rhizobia + ST + SSL	17.78 ± 0.90^{b}	3.63 ± 0.11^{b}	21.79 ± 0.65^{b}	210.4 ± 3.3^{ab}	0.558 ± 0.012^{a}

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top, SSL – soybean seed lectin; different letters of upper indices [a, b] in the table indicate values that significantly differ one from another within the column, indicating element of structure of yield of soybean as a result of comparison using the Tukey's test ($P < 0.05$) taking into account Bonferroni correction.

Table 7
Level of photosynthetic pigments in soybean leaves after inoculation and treatment of seeds with fungicide and spraying plants with specific lectine (x ± SE, n = 18)

Variant	Chlorophyll *a*	Chlorophyll *b*	*a/b*	*a + b*	Carotenoids (c)	(*a + b*) / c
	mg / g of raw weight of leaves	mg / g of raw weight of leaves		mg / g of raw weight of leaves	mg / g of raw weight of leaves	
Phase of development of three true leaves, V3						
Rhizobia (control)	2.66 ± 0.07^{a}	0.661 ± 0.022^{a}	4.03	3.33 ± 0.09^{a}	0.622 ± 0.022^{b}	5.37
Rhizobia + SSL	2.99 ± 0.12^{b}	0.792 ± 0.042^{b}	3.78	3.78 ± 0.16^{b}	0.609 ± 0.020^{ab}	6.30
Rhizobia + ST	2.50 ± 0.17^{a}	0.725 ± 0.161^{ab}	3.47	3.21 ± 0.15^{a}	0.578 ± 0.071^{ab}	5.63
Rhizobia + ST + SSL	2.75 ± 0.20^{ab}	0.708 ± 0.066^{ab}	3.93	3.45 ± 0.16^{a}	0.519 ± 0.070^{a}	6.77
Phase of flower bud formation – beginning of flowering, R1						
Rhizobia (control)	2.39 ± 0.05^{a}	0.542 ± 0.046^{a}	4.43	2.93 ± 0.08^{a}	0.572 ± 0.01^{a}	5.14
Rhizobia + SSL	2.96 ± 0.10^{b}	0.596 ± 0.022^{ab}	5.01	3.55 ± 0.11^{b}	0.658 ± 0.021^{b}	5.46
Rhizobia + ST	2.34 ± 0.11^{a}	0.554 ± 0.061^{a}	4.25	2.88 ± 0.10^{a}	0.559 ± 0.020^{a}	5.23
Rhizobia + ST + SSL	2.99 ± 0.19^{b}	0.668 ± 0.052^{b}	4.53	3.65 ± 0.15^{b}	0.642 ± 0.044^{b}	5.70
Phase of formation of beans, R3						
Rhizobia (control)	1.34 ± 0.13^{a}	0.294 ± 0.033^{a}	4.62	1.63 ± 0.17^{a}	0.333 ± 0.031^{a}	4.93
Rhizobia + SSL	1.94 ± 0.15^{b}	0.426 ± 0.131^{ab}	4.61	2.36 ± 0.19^{b}	0.464 ± 0.022^{c}	5.13
Rhizobia + ST	1.52 ± 0.09^{a}	0.342 ± 0.032^{a}	4.47	1.86 ± 0.12^{a}	0.388 ± 0.021^{b}	4.89
Rhizobia + ST + SSL	2.16 ± 0.20^{b}	0.527 ± 0.081^{b}	4.15	2.68 ± 0.18^{b}	0.475 ± 0.062^{c}	5.70

Note: rhizobia – *B. japonicum* 634b, ST – Standak Top, SSL – soybean seed lectin; different letters of upper indices [a, b, c] in the table indicate values that significantly differ one from another within the column for content of different pigments in leaves of plants in certain phases of soybean development (V3, R1, R3), as a result of comparison using the Tukey's test ($P < 0.05$) taking into account Bonferroni correction.

Discussion

The observed activation of processes of growth and development of soybean plants that had been subjected to influence of exogenous lectin from soybean, sprayed during vegetation, depended on a complex of factors both against the background of treatment seeds with Standak Top and without using it. Therefore, treatment seeds may, first, of all result in decrease in the number of pathogenic agents, promoting active development of soybean by decreasing the extent of plants' morbidity, secondly, because of the growth-regulationg effect of certain constituents of Standak Top, particularly pyraclostrobin, which influences physiological processes in plants: namely the process of aging slows, stress tolerance increases, photosynthetic activity and uptake of soil nitrogen by plants improves (Mel'nikova, 2020), and changes, which we mentioned earlier, occur in biological productivity of soybean plants. As is known, light reactions of photosynthesis are quite sensitive to action of pesticide. Thus, a number of systemic and contact fungicides significantly decreased the rates of transport of electrons in chloroplasts, quantum yield of photosystem I, maximum quantum effectiveness of photosystem II, accompanied by decrease in photochemical inhibition of fluorescence (qP) (Kaplaushenko et al., 2016). Comparative analysis of action of fungicides of two different classes – strobilurins and triazoles – on physiological parameters of grain crops such as wheat and barley, as well as cultivated legume, soybean plants, in the conditions of various watering regimes revealed negative impact of strobilurins on functioning of photosynthetic apparatus of plants. In particular, the processes of photosynthesis and transpiration slowed and the concentration of intercellular carbon dioxide decreased (Nason et al., 2007). Deterioration of the physiological condition of plants early on the initial stages of their development causes changes in initial stages of photosynthesis, accompanied by certain quantitative changes in chlorophyll and its optimum properties, as well as changes in carotenoids (Tarnovskyi & Yankovskyi, 2012). There are data that benomyl significantly reduced the concentrations of chlorophyll *a*, chlorophyll *b*, carotenoids and total content of pigments in sunflower plants (Ahmed et al., 1983). Similar results were obtained after treatment of common grape vine by fludioxonil and carbendazim (Garcia et al., 2003; Saladin et al., 2003). According to the results of our studies, application of Standak Top substance, one of the constituents of which is pyraclostrobin (strobilurins), caused no expressed negative effect (though some negative tendencies were present) on the process of seed germination (Table 2), development of soybean plants during vegetation (Table 3), concentration of green and yellow photosynthetic pigments in leaves and their ratio (Table 8), formation of grain productivity of plants (Table 6, 7), but it inhibited functioning of symbiotic apparatus of soybean (Table 4).

Sprayed on plants, lectin from soybean seeds – as a substance with broad range of biological activity (Kyrychenko, 2014; Pavlovskaya & Gagarina, 2017; Lagarda-Diaz et al., 2017) – displays complex action toward soybean-rhizobial symbiosis after fungicide influence. Earlier, we demonstrated that after spraying vegetable plants (tomatoes, cucumbers), soybean seed lectin and wheat germ agglutinin decreased their morbidity and positively influenced development of the plants' productivity (Sergienko et al., 2009; Kyrychenko et al., 2014). Spraying soybean plants, the seeds of which had been inoculated with rhizobia, with solution of lectin from soybean seeds during vegetation led to increase in the nitrogen-fixing activity of the symbiotic system (Kyrychenko et al., 2021), indicating the possibility of realization of biological activity of plant lectin against microsymbiont not only in the conditions of its influence on bacterial cells in the complex inoculant (Sytnikov et al., 2006; Kyrychenko, 2014; Kandelinskaya et al., 2015), but also after spraying plants during vegetation against the background of rhizobium inoculation of seeds.

For soybean plants and other legumes, studies revealed close correlation between the processes of formation of biological productivity and nitrogen-fixing activity of their symbiotic systems, including those the seeds of which had been treated with fungicides (Pavlyshche et al., 2018; Silva et al., 2018; Hungria et al., 2020; Kots et al., 2022). Activation of intensity of nitrogen-fixation of symbiotic system after spraying plants with specific lectin (Table 3), which we determined in the study, may have been caused, first, of all by indirect action of photosynthetic apparatus and metabolism of products of photosynthetic activity of plants, which are a source of energy for functioning of the nitrogenase complex (Kots et al., 2022), because earlier we had determined participation of lectins in coordination of processes of nitrogen-fixation and photosynthesis in phytobacterial systems after exogenous influence on seeds (Sytnikov et al., 2006; Kyrychenko, 2014), and, secondly, by possible direct action of lectin on auxin / cytokinin balance of plants, as indicated after exogenous effect of wheat germ agglutinin on wheat seeds (Kyrychenko, 2014).

Likewise, the activation effect of lectin on the photosynthetic system of soybean (against the background of both using fungicide and using no fungicide) which we saw after spraying plants and which was expressed in significantly higher levels of green and yellow photosynthetic pigments chlorophylls and carotenoids, and also significant changes in their ratios (Table 7) indicates the possibility of formation of a higher level of biological productivity of soybean, and also confirms the direct relationship between two physiologically significant processes – photosynthesis and nitrogen-fixation and participation of lectins (both after exogenous effect on seeds and after spraying plants) in coordination of processes of nitrogen-fixation and photosynthesis in phytobacterial systems, as we determined earlier for legumes and cereals crops (Sytnikov et al., 2006; Kyrychenko, 2014) (Tables 4, 8).

Productivity of plants is greatly determined by their levels of photosynthetic activity, dependent on balanced functioning of photosynthetic pigments chlorophyll *a* and *b* and carotenoids in the leaves (Morgun et al., 2019). The pigment photosynthetic complex manifests a high level of sensitivity to environmental factors, allowing us to classify it to criteria according to which one can determine level of adaptation of plants to natural and anthropogenic stress factors. The same time, for photosynthesis, the ratio was more of a factor than the absolute content of photosynthetic pigments in leaves of plants (chlorophyll *a* to *b*, total chlorophyll to carotenoids), which may indicate disorder in functioning of light-absorbing complexes and reaction centers of photosynthetic systems. Changes in the ratios of chlorophyll *a* to *b* toward increase in chlorophyll *b*, which we determined (Table 7) during all the studied vegetation phases (V3, R1 and R3), as well as changes in green and yellow photosynthetic pigments toward increase in level of chlorophylls – compared with carotenoids in variants using lectin for spraying, significantly distinguished the reaction of photosynthetic apparatus of those plants. This may indicate optimization of the ratio of chlorophyll in soybean leaves and increase in effectiveness of functioning of light-harvesting complexes of photosystems, as well as higher level of adaptive flexibility of the photosynthetic pigment system during influence of the biologically active compound – lectin from soybean seeds, including those plants the seeds of which had been treated with fungicide.

The higher ability of the symbiotic system of soybean to capture molecular nitrogen (Table 3) in variants with spraying plants with lectin of soybean seeds, and thereby higher level of provision of plants with biological nitrogen, and also presence of more powerful photosynthetic apparatus in them (green mass of plants (Table 2) and balanced content of photosynthetic pigments (Table 7)) resulted in a high level of grain productivity of soybean (Tables 5, 6). Increment of grain yield – compared with variants with inoculation of seeds and inoculation + fungicide – equaled 13.9% and 10.1% respectively in plants the seeds of which had not been treated with fungicide and 12.8% and 9.1% respectively against the background of fungicide action.

Studies of biological activity of lectin experimental substance (10^{-4}%), based on lectins from grain-legume crops, including soybean (Pavlovskaya & Gagarina, 2017), after pre-sowing treatment of pea seeds of Faraon and Sofia varieties and additional spraying of vegetative plants with this preparation in the phase of early flowering, indicated (Erohin, 2019) higher level of realization of the productive potential of pea particularly after spraying plants against the background of treatment of their seeds with them. Grain yield of Faraon and Sofia varieties of peas – compared with the variant where no treatment had been carried out – increased by 11% and 14% respectively, the number of beans per plant – by 12% and 11%, number and mass of seeds from plants increased by 13% and 16% and 9% and 14% respectively, mass of 1000 seeds – by 2%. Evaluation of efficiency of method of spraying vegetative plants during pea cultivation compared with treatment of seeds by the preparation prior to sowing indicated that pea yield from Faraon and Sofia varieties increased by 4% and 5% respectively, number of beans per plants – by 4% and 3%, number and mass of seeds from plants – by 2% and 2%, 2% and 3% respectively, mass of 1000 seeds was at the level of the control. However, such increase in the values of almost all the parameters of the structure of pea yield was insignificant. Lectin-based preparation is characterized by anti-stress, growth-activation effects, and also is known to decrease pesticide pressure in agrophytocoenoses, increase field germination rate of seeds and biological productivity of plants, and was therefore proposed as a method of protecting and increasing productivity of pea (Erohin, 2019).

Thus, because of the growth-regulating, bioeffectory, insecticide, bactericide, fungicide activities in plant lectins (Kyrychenko, 2014; Lagarda-Diaz et al., 2017; Pavlovskaya & Gagarina, 2017), they may be considered promising phytoprotective compounds, based on which there may be developed methods of biological control of morbidity of plants with phytopathogens (Sergienko et al., 2009; Erohin, 2019) or regulation of their physiological processes in the conditions of action of biotic, abiotic and anthropogenic factors (Sergienko et al., 2009; Jiang et al., 2010; Kyrychenko, 2014; Pavlovskaya & Gagarina, 2017; Erohin, 2019).

Conclusions

After treatment seeds of soybean on day of sowing with the following rhizobial inoculation, Standak Top fungicide did not inhibit the germination of seeds, but exerted a negative tendency toward formation of vegetative mass by plants (above-ground part and root) at the beginning of vegetation. Later, the plants were actively developing, forming grain yield at the level of the control (rhizobia inoculation). Nitrogenase activity of the symbiotic system, as well as condition of the photosynthetic pigment complex (concentration of chlorophylls and carotenoids in leaves and their ratio) were at the level or significantly lower than in the control plants.

Spraying plants with lectin from soybean seeds in the phase of development of two true leaves (without using Standak Top) promoted increase in functional activity of the symbiotic system during the soybean plants vegetation and development of powerful photosynthetic leaf apparatus with high and balanced content of photosynthetic pigments (chlorophylls and carotenoids), which resulted in maximum level of realization of the productive potential of this plants.

Biological activity of exogenous lectin from soybean seeds after spraying soybean plants against the background of pre-sowing treatment with Standak Top fungicide and inoculation (*B. japonicum* 634b) of seeds manifested in significant and reliable increase in the level of uptake of molecular nitrogen by soybean-rhizobial symbiosis (according to total nitrogenase activity – by 1.63 and 1.52 times compared with the control with inoculation and inoculation + fungicide), sufficiently high content of green (by 1.25–1.61 times) and yellow (by 1.16–1.42 times) photosynthetic pigments in leaves, as well as active development of plants during vegetation (by 1.21–1.40 times) and formation of grain productivity, which by 12.8% and 9.1% exceeded the control with inoculation and inoculation + fungicide.

Thus, spraying soybean plants in the phase of two true leaves with solution of lectin from soybean seeds (50 μg/mL) caused increase in yield of grain of this crop, occurring because of active development of plants, increased uptake of biological nitrogen throughout the vegetation, formation of powerful leaf apparatus with high concentrations of chlorophylls and carotenoids and increase in almost all parameters of the yield structure. Using lectin from soybean seeds for spraying plants against the background of treatment of seeds with Standak Top fungicide on the day of sowing stabilized and even increased the level of realization of symbiotic and productive potentials of soybean-rhizobial symbiosis compared with the control (rhizobia inoculation), as well as the variant with treatment of seeds (inoculation + fungicide). This indicates the high potential of further study of biological activity of substances of natural origin, particularly plant lectins, with the purpose of decreasing chemical load on ecosystems by leveling out or weakening negative impacts of stress factors, particularly such of anthropogenic character – treatment seeds with pesticides prior to sowing – on formation, functioning and effectiveness of legume-rhizobial symbioses.

The study was financed by the National Academy of Sciences of Ukraine (Department of General Biology, program for 2017–2021, "Fundamental basis of predicting and prevention of negative effect of changes in climatic conditions on biotic system of Ukraine", topic "Development of biotechnological means of regulation of adaptibility of soybean-rhizobia symbioses and their introduction into production" State Registration No. 0117U004111).

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