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THE EFFECTIVENESS OF PHYTOLECTINS AND LECTIN COMPOSITIONS APPLICATION FOR SPRAYING PLANTS DURING VEGETATION

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The effectiveness of the soybean plants solution spraying with the soybean seed lectin solution of during vegetation (against the background of seed inoculation with nodule bacteria and without seed inoculation), as well as the effectiveness of the winter wheat plants spraying with lectin-bacterial composition in green-house and field experiments was investigated respectively. It was found that spraying of soybeans in the phase of two trifoliate leaves development with a specific lectin against the background of pre-sowing seed inoculation with rhizobia caused a significantly positive effect on the functional activity of the symbiotic apparatus. The nitrogen-fixing activity of the rhizosphere microbiota remained unchanged, which may indicate the vector of lectin action when sprayed through the plant. At the same time, the activation of plants vegetative growth was noted, which was maximally manifested by the height of their aboveground part. The activity of exogenous sprayed lectin was less pronounced on the background of seed inoculation with rhizobia compared to non-inoculated plants. Plants spraying with soybean lectin against the background of seed inoculation provided an increase in harvest compared to non-inoculated control by 2.13 g/plant, but by the factor of lectin action this increase was only 0.19 g/plant and was insignificant. Non-inoculated soybean plants when sprayed with lectin formed a harvest that was significantly higher (by 0.64 g/plant) than that of plants in the absence of lectin. At this, the increase by the factor of lectin action was 22%. The spraying of winter wheat plants in the phase of mass spring germinations with the Azolec preparation (without pre-sowing seed inoculation) contributed to a significant increase in harvest by 1.6 c/ha. Therefore, the application of soybean and wheat plants spraying, respectively, with soybean seed lectin and lectin-bacterial Azolec preparation (wheat lectin),without involving pre-sowing seed inoculation, provided a greater degree of plants productive potential realization compared to control (without pre-sowing seed inoculation and plants spraying during vegetation).

Key words: soybean, rhizobia, soybean seed lectin, spraying, inoculation, nitrogen fixation, winter wheat, Azolec preparation, productivity.

Phytolectins are polyfunctional biologically active compounds [1, 2] which have found their application in medicine, pharmacy, veterinary medicine, crop production [3–7]. Active and comprehensive study of scientific, theoretical and applied aspects of these proteins in recent decades [2–4, 7–11] has identified ways of their practical use in plant biotechnology to create of biological preparations [7, 12, 13]. It is shown that the phytolectins, if used for pre-sowing seed treatment, have a growth-regulatory effect on plants and microorganisms, contribute to a fuller realization of the symbiotic and productive potential of plant-microbial systems and have a protective, immunomodulatory and adaptogenic effect on plants [7]. The presence of insecticidal, bactericidal, fungicidal activity in plant lectins [4–7, 11] allows to consider them as perspective phytoprotective compounds, they may be the basis for the methods of biological control of plant diseases development [11, 14, 15]. All foresaid allowed us to offer phytolectins as promising biological agents for the creation of polycomponent inoculants for the purpose of soybean, pea, wheat seed bacterization [7] or vegetable crops spraying [14]. Other researchers have also confirmed the effectiveness of plant lectins in pre-sowing seeds treatment to increase the level of symbiotic and productive potential of legumes realization [10, 13, 15], as well as the use of phytolectins as protective substances [12, 15]. It is shown [11, 12, 15] that lectins isolated from legume seeds– beans, peas, soybeans were characterized by fungicidal properties against the *Fusarim oxysporum* causative agent of peas root rot. Based on these proteins, a preparation was created [12], which has immunomodulatory properties and the use of which not only increases the resistance of peas to the pathogen of root rot, but also reduces the dose of chemical pesticides application as plant protection products [16].

In most cases these results contain the study of the biological activity of phytolectins in the pre-sowing seed treatment, while the assessments of these proteins usage for spraying plants during the vegetation are very few [14, 15].

Therefore, the aim of the study was to investigate the effectiveness of the soybean plants spraying during vegetation with a solution of soybean seed lectin (against the background of seed inoculation with nodule bacteria and without seed inoculation), as well as the spraying of the winter wheat plants with lectin-bacterial composition Azolec.

Materials and Methods

The object of the study was soybean-rhizobial symbiosis formed by soybean plants *Glycine max* (L.) Merr. of early-maturing variety Almaz of domestic selection with bacteria *Bradyrhizobium japonicum* 634b, and of the winter wheat plants *Triticum aestivum* L., variety Podolyanka of domestic selection as well.

The Almaz variety 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 varieties from which it was obtained. The owners of the patent for this variety (No. 07105) are Poltava State Agrarian Academy and Bilyavska L. G. Since 2007 it has been included in the State Register of Plant Varieties of Ukraine [17].

Nodule bacteria *B. japonicum* 634b (symbiotic and associative nitrogen-fixing microorganisms strains collection of the Institute of Plant Physiology and Genetics of the NAS of Ukraine — IPPG of the NAS of Ukraine) were grown at 28 °C on agar mannitol-yeast medium (AMY, 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 liter; pH 6.8–7.0 for 10 days, the culture was washed with sterile water, mixed to a homogeneous suspension and the number of viable (colony-forming units) bacteria was determined by the classical microbiological method of serial dilutions of microorganisms suspension, seeding on AMY and counting grown colonies. The titer of bacteria in suspension was 10^{10} cells/ml. Inoculation of soybean seeds was performed on the day of sowing, keeping the sowing seeds in the inoculant for an hour (1 ml/100 seeds per variant). The absolute control was the variant without seed inoculation (seed treatment with water).

Peculiarities of symbiotic soybean system formation and functioning under conditions of seed inoculation with rhizobia and spraying of vegetative plants in the phase of two trifoliate leaves formation with soybean seed lectin solution (40 µg/ml) calculated as 2 ml/plant (50 ml/m^2) were investigated in the green-house experiment carried out on the plot of IPPG of the NAS of Ukraine under natural lighting and air temperature in 4-fold replicates according to the variants in 10-kg Wagner pots on soil substrate (soil: sand, 3:1) with a Helrigel's nutrient mixture (0.25 norm of mineral nitrogen).

The experiment was laid out according to the following scheme:

Inoculation

1. Without inoculation (seed treatment with water — absolute control).
2. Seed inoculation with rhizobia.

Inoculation + spraying

3. Without inoculation (seed treatment with water).
4. Seed inoculation with rhizobia.

There were tested:

• the formation of symbiosis — by the nodulation ability of soybean rhizobia (the activity of root nodules formation, their number and weight on the plant);

• the functional (nitrogenase) activity of soybean-rhizobial symbioses and rhizosphere soil of plants –by acetylene reductase method according to Hardy et al. [18] on an Agilent GC System 6850 gas chromatograph (USA) with a flame ionization detector. To do this, washed from the plant growth substrate roots with nodules, 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% concentration of acetylene was created. The incubation time of the sample was 1 or 2 hours. After incubation, the gas mixture containing ethylene formed due to acetylene reduction with nitrogenase was analyzed on the device. Gases separation 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 per 1 hour of incubation, when the nitrogenase of the incubated sample is operated, was expressed in molar units of formed ethylene per 1 plant in 1 h. Nitrogenase activity of symbiosis was expressed in micromoles of C_2H_4/(plant · h) — actual activity, in micromoles of C_2H_4/(g of nodules · h) — specific activity. Nitrogenase activity of morpho-structural symbiotic unit — in nanomoles of C_2H_4/(1 nodule · h) and nanomoles of C_2H_4/(1 nodule weight · h). Nitrogenase activity of rhizosphere soil and roots without nodules but with soil was expressed in nanomoles of C_2H_4/(20 g of absolutely dry (a. d.) soil · 2 h) or in nanomoles of C_2H_4/(1 plant with soil · 2 h). Determinations were performed in 4–6 biological replicates;

- the effectiveness of symbiosis by the spraying plants with lectin was evaluated by the soybean vegetative mass formation, activity of the beans formation on the plants and seed productivity of the crop.

Soybean plants were sampled during the development phases of one (V1, 20-day-old plants) and three (V3, 33-day-old plants) trifoliate leaves and full maturity of seeds (105-day-old plants).The rhizosphere soil was carried out in the phase of one (V1, 20-day-old plants), two (V2, 25-day-old plants) and three (V3, 33-day-old plants) trifoliate leaves [19].

To evaluate the effectiveness of the spraying of the winter wheat plants variety Podolyanka with Azolec preparation, we conducted field small-scale experiment (estimated area of one plot was 3.4 m^2, 3-fold replicates in the variant, randomized manual sowing) on the territory of IPPG of the NAS of Ukraine.

Podolyanka variety (originators of variety are IPPG of the NAS of Ukraine and the V.M. Remeslo Myronivka Institute of Wheat of the National Academy of Agrarian Sciences of Ukraine) is high-harvesting intensive type: genetic potential of productivity reaches 113.7 c/ha. It is medium-early, has high winter hardiness, drought resistance, resistance to grain shedding, medium resistance to lodging and damage by powdery mildew, brown leaf rust, root rot. The variety belongs to strong wheat. It is entered in the State Register of Plant Varieties of Ukraine since 2003 for cultivation in Polissya, Forest-Steppe and Steppe of Ukraine [17].

The composition Azolec (IPPG of the NAS of Ukraine) of lectin-bacterial nature [7] contains phytolectin–the wheat germ agglutinin (WGA, Lectinotest, Lviv, Ukraine) and soil microorganisms *Azotobacter chroococcum* T79, which belong to agronomically useful bacteria of the genus *Azotobacter* with a wide range of positive properties regarding of plants and soil, in particular, the ability to fixing of molecular nitrogen, the synthesis of hormones, vitamins, breakdown of sparingly soluble phosphates, soil bioremediation. Azolec composition is registered in Ukraine until 2022 as a bacterial fertilizer Azolec (State Registration Certificate No. A 03582), used for pre-sowing inoculation of spring and winter wheat seeds in order to increase of plant productivity and improve soil microbiological condition due to the development of the useful population of nitrogen-fixing microorganisms in plant rhizosphere.

Spraying of wheat plants was carried out in the phase of mass spring germinations calculated as 3 norms of the preparation/ha (1 norm of the preparation is 100 ml/ha). Previously, the Azolec preparation (bacterial cell titer is 10^8 cells/ml, final WGA, concentration is 5 µg/ml) was diluted in water (*v: v*, 1:360) according to the manufacturer recommendations (1 dose, i.e. 100 ml, of the preparation should be diluted in 2 liters of water).

The experiment was performed according to the following scheme:

1. Control (without spraying).
2. Spraying with Azolec preparation.

Wheat grain harvest was estimated by the weight of grains from the plot of this manual harvesting and subsequent recalculation per quintal per hectare (c/ha).

Statistical processing of experimental data was performed according to generally accepted methods using the software package Microsoft

Excel 2019. The significance of the results between the variants was evaluated using the package ANOVA. Dispersion analysis was used to calculate the Fisher's least significant difference (LSD) test at a probability level of 95%. It was thought that the differences were significant at $P \leq 0.05$.

Results and Discussion

It has been found that a soybean symbiotic apparatus was formed on the plant's roots in the development phase of one trifoliate soybean leaf (V1) by the inoculating seeds with specific rhizobia (Table 1): 68.6% of plants were nodulated (NOD^+) and contained three nodules on the roots an average. In the phase of three trifoliate soybean leaves (V3), all plants, which seeds were inoculated, form root nodules: an average by 14.9 nodules with a total weight of 36.8 mg per plant and the weight of each root nodule of 2.68 mg (Table 1, Inoculation block). Plants of the control variant (without inoculation) did not contain nodules on roots.

Plants spraying in the phase of two trifoliate leaves (V2, Table 1, Inoculation + spraying block) led to a positive trend in the change of values which characterizing of the rhizobia nodulation ability: the number of nodules on the plant increased by 26%, total weight — by 17 % and the weight of one nodule — by 15% (Fig. 1), but this increase was insignificant (Table 1).

Evaluation of the functional activity of the symbiotic apparatus of soybeans in the development phase V3 showed a significant increase in the ability to fixing of molecular nitrogen in the symbiotic system if the plants were treated with a solution of soybean lectin in the phase V2 (Table 2, Fig. 1).

Actual nitrogenase activity (μmol of C_2H_4/(plant · h)) increased 2.7 times, specific activity (μmol of C_2H_4/(g of nodules · h)) increased 2.4 times compared to plants that were not treated with soybean lectin solution. Nevertheless, the functional activity of the morpho-structural symbiotic unit (root nodule) increased 2.3 times (nmol of C_2H_4/nodule per hour) and 2.1 times (nmol of C_2H_4/(nodule weight per hour). The obtained results indicate an increase in the level of actual nitrogenase activity (μmol of C_2H_4/(plant per hour)) not only by increasing the number of symbiotic structures on the roots of soybean plants 1.3 times (Table 1), but also by increasing their functional ability (Table 2).

Thus, spraying soybeans in the development phase of two trifoliate leaves with lectin specific for plants against the background of pre-sowing seed inoculation with nodule bacteria strain 634b had a positive effect on the functional activity of the symbiotic apparatus.

Table 1. **Rhizobia nodulation activity and root nodules characteristics of soybean variety Almaz when the plants were sprayed with soybean lectin against the background of seed inoculation with specific rhizobia**

No.	Variant	NOD^+ plants	Number of nodules per plant	Weight of nodules per plant	Weight of one nodule
		%[1]	pcs.	mg	mg
Inoculation					
Development phase of one trifoliate leaf, V1, 20-day-old plants					
1	Without inoculation	0	0	0	0
2	Rhizobia	68.6	2.7	«–»[2]	«–»
Development phase of three trifoliate leaves, V3, 33-day-old plants					
1	Without inoculation	0	0	0	0
2	Rhizobia	100	13.3	31.50	2.37
Inoculation + spraying (*in the phase of two trifoliate leaves, V2*)					
1	Without inoculation	0	0	0	0
2	Rhizobia	100	16.8	36.83	2.72
[3]$LSD_{0,05}$ 2/2		–	10.76 (–)	8.96 (–)	1.39 (–)

Note. 1.% — % to the total number of plants of one variant; 2. See in the Tables 1 and 4: «–» — was not determined. 3. See in the Tables 1 and 2: $LSD_{0,05}$ 2/2 — reliably ($P \leq 0,05$) to the control when spraying (variant without spraying of plants, with seed inoculation); (+) — the difference is significant, (–) — the difference is insignificant.

Table 2. **Nitrogenase activity of soybean root nodules in the phase of three trifoliate leaves when the plants were sprayed with soybean lectin against the background of seed inoculation with rhizobia**

No.	Variant	Nitrogenase (acetylene-reducing) activity of			
		symbiotic apparatus, µmol of C_2H_4		symbiotic morpho-structural unit, nmol of C_2H_4	
		(plant • h)	(g of nodules • h)	(nodule • h)	(weight of nodule • h)
Inoculation					
2	Rhizobia	0.119	3.795	8.716	50.502
Inoculation + spraying (*in the phase of two trifoliate leaves, V2*)					
2	Rhizobia	0.326	8.919	20.423	109.541
$LSD_{0,05}$ 2/2		0.18 (+)	3.06 (+)	9.58 (+)	52.29 (+)

Evaluation of the nitrogen-fixing ability of soybean rhizosphere soil showed (Table 3) that seed inoculation with rhizobia had a positive effect on the rhizosphere microbiota activity in the early stages of plant ontogenesis (development phase V1 and V2). Nitrogenase activity of roots with soil (in the absence of symbiotic nodules) and rhizosphere soil increased by 56 and 54%, respectively, compared to non-inoculated control. In the development phase V3, the symbiotic system was actively functioning (Table 2), while the difference in the activity of the rhizosphere nitrogen-fixing microbiota between variants with and without seed inoculation alleviated simultaneously with significant growth (almost twice) of absolute values of rhizosphere diazotrophs nitrogenase activity compared to the previous phase of plant development (Table 3, block Inoculation). When spraying soybeans with a solution of specific lectin both against the background of pre-sowing seed inoculation with rhizobia and without seed inoculation, no difference in the ability of rhizosphere diazotrophs to fixing of molecular nitrogen was observed (Table 3, block Inoculation + spraying, Fig. 1).

Thus, in contrast to the functional ability of the soybean symbiotic apparatus, which changed positively under the action of exogenous specific lectin on vegetative plants (Table 2, Fig. 1), the nitrogen-fixing activity of the rhizosphere microbiota remained unchanged, which may indicate the vector of lectin action by the spraying directly through the plant.

Soybean plants in the phase of three trifoliate leaves, by the inoculation seed

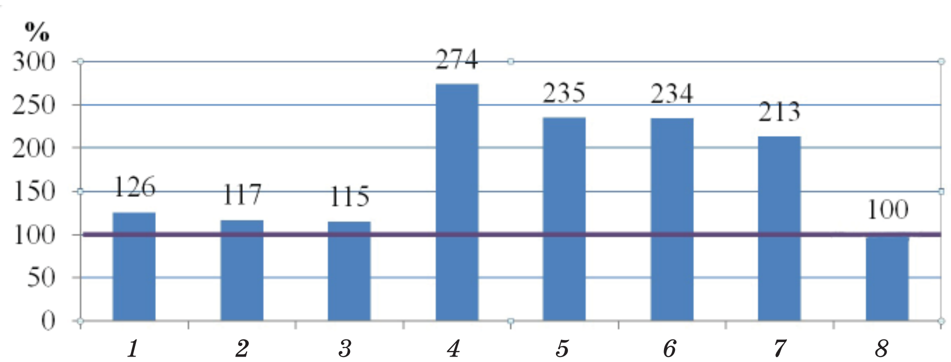

Fig. 1. **Characteristics of the symbiotic potential realization degree of soybean nodule bacteria strain 634b when the plants were sprayed with soybean lectin in the development phase of two trifoliate leaves**
On the Y axis: % to control (without spraying) by the factor of lectin action
On the X axis: *Nodulating ability of rhizobia: 1* — number of nodules per plant; *2* — nodules weight per plant; *3* — weight of one nodule; *Nitrogenase activity of symbiosis*: *4* — actual: C_2H_4/(plant • h); *5* — specific: C_2H_4/(g of nodules • h); *Nitrogenase activity of morpho-structural symbiotic unit: 6* — C_2H_4/(nodule • h); *7* — C_2H_4/(nodule weight • h); *Nitrogenase activity of the rhizosphere microbiota: 8* — C_2H_4/(20 g of a. d. soil • 2 h).

Table 3. **Nitrogenase activity of soybean rhizosphere soil when the plants were sprayed with a specific lectin**

No.	Variant	Nitrogenase activity	
		nmol of C_2H_4/(20 g of a. d. soil · 2 h)	%
Inoculation			
Development phase of one trifoliate leaf, V1, 20-day plants, root without nodules+ soil: nmol of C_2H_4/(plant with soil · 2 h)			
1	Without inoculation	12.650	100
2	Rhizobia	19773	156
$LSD_{0.05}$		7.02 (+)	
Development phase of two trifoliate leaves, V2, 25-day-old plants, soil			
1	Without inoculation	4.108	100
2	Rhizobia	6.352	154
$LSD_{0.05}$		1.03 (+)	
Development phase of three trifoliate leaves, V3, 33-day-old plants, soil			
1	Without inoculation	11.490	100
2	Rhizobia	11.774	103
$LSD_{0.05}$		2.34 (–)	
Inoculation + spraying (*in the phase of two trifoliate leaves, V2*)			
1	Without inoculation	12.384	100
2	Rhizobia	11.795	95
$LSD_{0.05}$		1.52 (–)	
$LSD_{0.05}$ 1/1		1.92 (–)	
$LSD_{0.05}$ 2/2		2.03 (–)	

Note. See in Tables 3–6: $LSD_{0.05}$ 1/1 (variant without seed inoculation); $LSD_{0.05}$2/2 (variant with seed inoculation) — reliably ($P \leq 0{,}05$) to control by spraying (variant without plants spraying); (+) — the difference is significant, (–) — the difference is insignificant.

with rhizobia, had a positive tendency in the formation of green mass: the weight of the aboveground part differed from that of non-inoculated plants by 27 and 37% respectively for raw and absolutely dry matter (Table 4) which may be due to the beginning of the active functioning of the symbiotic system in this period (Table 2). However, the changes in plant vegetative mass we found were insignificant. Similar regularities have been established for the development of soybean root systems: a positive trend of root weigh change in the absence of significant differences. In the phase of one trifoliate leaf, no significant difference was found in the development of the experimental and control plants, which may be due to the same initial level of mineral nitrogen nutrition (0.25 norm of mineral nitrogen in Helrigel's nutrient mixture) in the plant growth substrate. At the same time, it was noted a positive trend in the root system development of soybeans inoculated with rhizobia: raw and dry root mass exceeded the control values by 36 and 14 % in the absence of a significant difference (Table 4).

Soybeans spraying with specific lectin significantly stimulated the growth processes of the plants of control variant (without inoculation), especially the aboveground part: height significantly increased by 55%, weight (raw and absolutely dry) — by 28 and 33%, respectively (Fig. 2). Simultaneously, the root system of sprayed plants developed similarly to plants that were not exposed to soybean lectin. For the plants of the variant with pre-sowing seed inoculation with rhizobia, the difference in the formation of green mass was not so pronounced (no significant difference), while these plants differed significantly from

Table 4. **Formation of soybean plants vegetative and generative organs when the plants were sprayed with specific lectin against the background of seed inoculation with rhizobia**

No	Variant	Vegetative mass of soybeans: aboveground part — AGP, root — R					Formation of soybean beans	
		raw weight of plant		dry weight of plant		Plant height, cm	The total number of beans per plant, pcs.	
		AGP, g	R, g	AGP, g	R, g		*Full seed phase* [19], *72–day–old plants*	*Phase of full seed maturity* [19], *105–day–old plants*
Inoculation								
Development phase of one trifoliate leaf, V1, 20–day–old plants								
1	Without inoculation	1.46	0.14	0.36	0.014	«–»		
2	Rhizobia	1.57	0.19	0.36	0.016	«–»		
$LSD_{0,05}$		0.57 (–)	0.09 (–)	0.24 (–)	0.01 (–)	«–»		
Development phase of three trifoliate leaves, V3, 33–day–old plants								
1	Without inoculation	4.60	2.01	0.76	0.19	24.5	7.9	8.7
2	Rhizobia	5.82	2.36	1.04	0.23	28.2	16.3	16.9
$LSD_{0,05}$		3.32 (–)	0.96 (–)	0.56 (–)	0.11 (–)	7.19 (–)	2.79 (+)	5.10 (+)
Inoculation + spraying (*in the phase of two trifoliate leaves, V2*)								
1	Without inoculation	5.90	1.58	1.01	0.18	37.9	8.6	8.2
2	Rhizobia	6.07	2.17	1.05	0.22	34.1	15.8	15.7
$LSD_{0,05}$		1.84 (–)	0.56 (+)	0.56 (–)	0.11 (–)	6.01 (–)	2.22 (+)	2.58 (+)
$LSD_{0,05}$ 1/1		1.10 (+)	0.75 (–)	0.25 (+)	0.33 (–)	6.65 (+)	0.70 (+)	1.27 (–)
$LSD_{0,05}$ 2/2		2.86 (–)	0.82 (–)	0.80 (–)	0.52 (–)	5.70 (+)	2.74 (–)	4.87 (–)

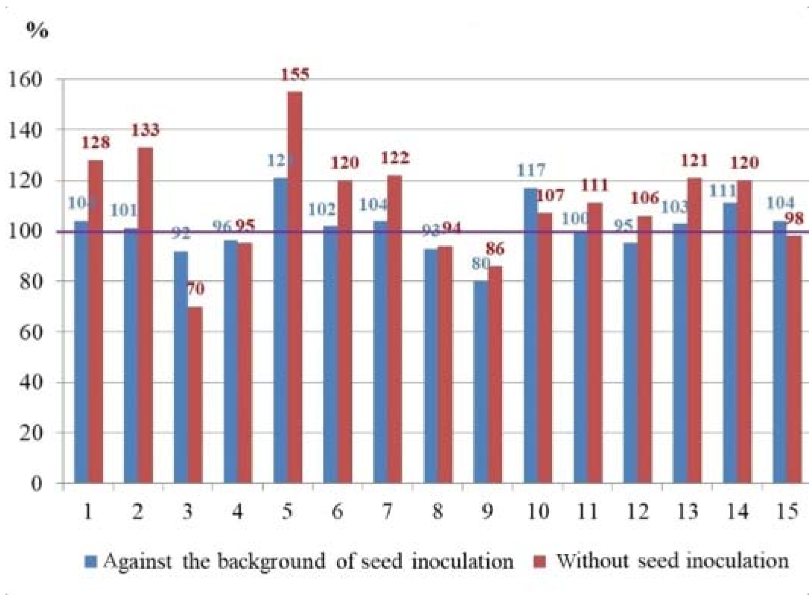

Fig. 2. **Characteristics of the soybeans productive potential realization when the plants were sprayed with specific lectin in the phase of two trifoliate leaves**

On the Y axis: % to control (without spraying) by the factor of lectin action.

On the X axis: *Vegetative mass of the plant: 1* — above ground weight (raw); *2* – above ground weight (dry), *3* — root weight (raw); *4* — root weight (dry); *5* — height of the aboveground part of the plant; *6* — weight of plant (dry);

Crop structure: 7 — crop/pot; 8 –beans number/plant; 9 — internodes number/plant; 10 — beans number/one node; 11 — seeds number/bean; 12 — seeds number/plant; 13 — seeds weight/plant; 14 — 1000 seeds weight; 15 — the harvest index control (without spraying) in height (21%). In the phase of full seed maturity (Table 5, Fig. 2) the soybeans spraying with lectin of non-inoculated plants provided a significant increase in vegetative mass of 20%, while against the background of pre-sowing seed inoculation no significant difference in this indicator change was found.

Our results on the plants vegetative mass formation at the end of the soybean vegetation (the phase of full seed maturity) confirm the regularities of development and different sensitivity of inoculated and non-inoculated plants in response to the sprayed lectin action, which we found in previous phases of soybean ontogenesis (Table 2, Fig. 4).

Activation of soybean growth processes while vegetation under the action of exogenous plant-specific lectin may be due to the regulatory activity of these proteins, activation of photosynthetic processes and the effect on hormonal (auxin/cytokinin) balance of plants which was found in previous studies of phytolectins (soybean seed lectin, wheat germ agglutinin) exogenous action on seed [7, 20].

Therefore, spraying soybeans with a specific lectin solution in the phase of two trifoliate leaves, both against the background of inoculation and without seed inoculation, activates the vegetative growth of plants. Against the background of seed inoculation with rhizobia, the activity of exogenous lectin was less pronounced compared to non-inoculated plants, as the latter were more sensitive to lectin spraying.

Evaluation of soybeans beans formation (Table 4) in the phases of full seed and full seed maturity [19] showed the advantages of inoculated plants in the realization of productive potential compared to non-inoculated ones: the beans number per plant significantly increased 2.1 and 1.9 times, respectively (Table 4, *Inoculation* block). The same regularity was maintained under the action of exogenous lectin against the background of seed inoculation with rhizobia compared to non-inoculated control (Table 4, Inoculation + spraying block, Fig. 2): the number of beans per plant significantly increased 1.8 and 1.9 times, respectively. If we evaluate only the effect of lectin factor, it can be noted that only non-inoculated plants reacted to it, which was manifested in a significant increase

Table 5. **The structure of the soybean variety Almaz harvest when the plants were sprayed with soybean lectin**

No.	Variant	Number of inter-nodes per plant, pcs.	Number of formed beans per plant, pcs.	Number of beans in the one node, pcs.	Number of seeds per plant, pcs.	Weight of seeds per plant, g	Plant weight, g	Weight of 1 000 seeds, g	Index harvest, ih
Inoculation									
1	Without inoculation	6.3	8.0	1.4	14.3	2.96	7.22	200	0.43
2	Rhizobia	9.5	15.0	1.8	29.5	5.54	10.09	190	0.55
$LSD_{0.05}$		2.81 (+)	3.32 (+)	0.43 (−)	9.49 (+)	0.77 (+)	3.25 (−)	40 (−)	0.07 (+)
Inoculation + spraying (*in the phase of two trifoliate leaves, V2*)									
1	Without inoculation	5.4	7.7	1.5	15.1	3.59	8.64	240	0.42
2	Rhizobia	7.6	14.4	2.1	27.9	5.72	10.25	210	0.57
$LSD_{0.05}$		1.00 (+)	1.08 (+)	0.23 (+)	5.51(+)	1.08 (+)	2.28 (−)	10 (+)	0.11 (+)
$LSD_{0.05}$ 1/1		1.62 (−)	3.00 (−)	0.32 (−)	4.59 (−)	0.62 (+)	1.38 (+)	40 (−)	0.12 (−)
$LSD_{0.05}$ 2/2		2.29 (−)	1.80 (−)	0.37 (−)	9.33 (−)	1.41 (−)	3.07 (−)	10 (+)	0.02 (+)

Table 6. **Harvest of soybean Almaz variety when the plants were sprayed with soybean lectin against the background of seed inoculation with rhizobia**

No.	Variant	Harvest/vessel	
		g	± g
Inoculation			
1	Without inoculation	17.70	0
2	Rhizobia	33.21	+ 15.51
$LSD_{0.05}$		10.82 (+)	
Inoculation + spraying (*in the phase of two trifoliate leaves, V2*)			
1	Without inoculation	21.56	0
2	Rhizobia	34.36	+ 12.80
$LSD_{0.05}$		5.38 (+)	
$LSD_{0.05}$ 1/1		3.08 (+)	
$LSD_{0.05}$ 2/2		2.12 (–)	

(by 9%) in the index “beans number per plant” in the phase of full seed (see % by the spraying).

Therefore, spraying of plants with soybean seed lectin against the background of pre-sowing seed inoculation with rhizobia did not affect the activity of beans formation by soybean plants compared to plants that were not affected by lectin.

Rhizobia-inoculated plants fully realized their productive potential, which was manifested in a significant essential increase (by 15.51 g/plant) in the harvest of soybean seeds compared to non-inoculated plants (Table 6, Inoculation block).

Plants spraying with lectin against the background of seed inoculation provided a significant increase in harvest by 12.80 g/plant compared to non-inoculated control (Inoculation + spraying block), which, however, was insignificantly higher (by 1.15 g/vessel) by the factor of exogenous lectin action (Fig. 2). Non-inoculated lectin sprayed plants formed a harvest that was by 3.86 g/plant significantly higher than that of plants without lectin spraying and the increase by the factor of lectin action was 22% (Fig. 2), which also confirms our previously established (Table 4) regularity in the greater sensitivity of non-inoculated plants to their exogenous treatment with specific lectin than inoculated.

Analysis of the soybean harvest structure (Table 5) showed that in non-inoculated plants, lectin spraying led to a positive trend in increasing the number of beans in the node (by 7%), the number of seeds per plant (by 6%) and the weight of 1 000 seeds (by 5%) with an insignificant difference with the control (without spraying). Notably, the seed weight from the plant significantly exceeded (by 21%) the control value. The effect of lectin by the spraying plants against the background of seed inoculation (Fig. 2, Table 5) was manifested in a positive trend to change such indexes of the soybean harvest structure as the beans number in the node (by 17%), seed weight per plant (by 3%) and harvest index (by 4%) with an insignificant difference with control (without spraying). At the same time, the weight of 1 000 seeds significantly increased (by 11%). The harvest index for seed inoculation significantly increased by 28 and 36% compared to the non-inoculated control, respectively, without spraying and with spraying of plants with soybean lectin (Table 5, Fig. 2). In this case, almost all indexes of the soybean harvest structure also increased significantly.

Study of the biological activity of a lectin experimental preparation (10^{-4}%) based on legume lectins [12], which is characterized by anti-stress, growth-activating action, as well as reduces pesticide load in agrophytocenosis, increases seed germination and biological productivity [16] with the pre-sowing treatment Pharaoh and Sofia varieties of peas and additional spraying of vegetative plants in the phase of beginning flowering [19] showed [15] a higher level of productive potential realization by the spraying plants

Table 7. **Harvest of wheat variety Podolyanka when the plants were sprayed with lectin-bacterial preparation Azolec (grain moisture 14%)**

Variant	Grain harvest, c/ha	
	average	increase of harvest
Control (without spraying)	25.8	0
Azolec	27.4	+ 1.6
$LSD_{0.05}$	1.1 (+)	

against the background of seed treatment. The seed harvest of pea varieties Pharaoh and Sophia compared to the variant without any treatment increased by 11 and 14%, respectively, the beans number per plant — by 12 and 11%, the number and weight of seeds per plant — by 13 and 16% and 9 and 14%, respectively, the weight of 1 000 seeds — by 2%. Evaluation of the spraying method of vegetative plants effectiveness as an additional one while growing peas against the background of experimental preparation pre-sowing seed treatment showed that the harvest of peas varieties Pharaoh and Sophia increased by 4 and 5%, respectively, the beans number per plant — by 4 and 3%, number and weight of seeds from the plant — by 2 and 2% and 2 and 3%, respectively, the weight of 1 000 seeds– by 1% and such an increase in the values of almost all indexes of the pea harvest structure was insignificant.

In winter wheat plants Podolyanka without pre-sowing seed inoculation and only with spraying plants in the phase of mass spring germination with the Azolec preparation based on wheat lectin, received a significant increase in grain harvest by 1.6 c/ha (Table 7), which was 6% regarding control (without spraying).

Thus, spraying soybeans of early-maturing variety Almaz in the phase of two trifoliate leaves with a specific lectin against the background of pre-sowing seed inoculation with rhizobia had a significant positive effect on the functional activity of the symbiotic apparatus, while nitrogen-fixing activity of rhizosphere microbiota remained unchanged, which may indicate the vector of lectin action when spraying it just through the plant. At the same time, significant activation of plants vegetative growth was noted. Activity of exogenous sprayed lectin action against the background of seed inoculation with rhizobia was less pronounced compared to non-inoculated plants. Plants spraying with soybean lectin against the background of seed inoculation provided an insignificant increase in harvest (1.15 g/plant) compared to the control without spraying, while non-inoculated soybean plants sprayed with lectin formed a harvest that was significantly larger (by 3.86 g/plant) than in plants in the absence of lectin treatment. In this case the increase in the factor of lectin action was 22%. The spraying of winter wheat plants in the phase of mass spring germinations with the Azolec preparation (without pre-sowing seed inoculation) contributed to a significant increase in harvest by 1.6 c/ha. Therefore, the application of soybean and wheat plants spraying, respectively, with soybean seed lectin and lectin-bacterial Azolec preparation based on wheat lectin without the use of pre-sowing seed inoculation provides a greater degree of plants productive potential realization compared to control (without pre-sowing seed inoculation and plants spraying during vegetation).

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REFERENCES

1. *Sharon N.* Lectins: carbohydrate-specific reagents and biological recognition molecules. *J. Biol. Chem.* 2007, 282 (5), 2753–2764. https://doi.org/10.1074/JBC.X600004200
2. *Van Damme E. J. M., Lannoo N., Peumans W. J.* Plant Lectins. *Adv. Botanical Res.* 2008, V. 48, P. 107–209. https://doi.org/10.1016/S0065-2296(08)00403-5
3. *Hamid R., Masood A., Wani I. H., Rafiq S.* Lectins: Proteins with diverse applications. *J. Appl. Pharm. Sci.* 2013, V. 3, P. 93–103. https://doi.org/10.7324/JAPS.2013.34.S18
4. *Lagarda-Diaz I., Guzman-Partida A., Vazquez-Moreno L.* Legume lectins: proteins with diverse applications. *Int. J. Mol. Sci.* 2017, 18 (6), 1242. https://doi.org/10.3390/ijms18061242
5. *Coelho L. C. B. B., Silva P. M. S., Lima V. L. M., Pontual E. V., Paiva P. M. G., Napoleao Th. H., Correia M. T. S.* Lectins, interconnecting proteins with biotechnological/pharmacological and therapeutic application. *Evid. Based Complement Alter. Med.* 2017, V. 2017. https://doi.org/10.1155/2017/1594074
6. *Mishra A., Behura A.,Mawatwal Sh., Kumar A., Naik L., Mohanty S. S., Manna D., Dokania P., Mishra A., Patra S. K., Dhiman R.* Structure-function and application of plant lectins in disease biology and immunity. *Food Chem. Toxicol.* 2019, V. 134, P. 110827. https://doi.org/10.1016/j.fct.2019.110827
7. *Kyrychenko O. V.* Phytolectins and Diazotrophs are the polyfunctional components of the complex biological compositions. *Biotechnol. acta.* 2014, 7 (1), 40–53. (In Ukrainian). https://doi.org/10.15407/biotech7.01.040
8. *Jiang S., Ma Z., Ramachandran S.* Evolutionary history and stress regulation of the lectin superfamily in higher plants. *BMC Evol. Biol.* 2010, 10 (1), 79–103. https://doi.org/10.1186/1471-2148-10-79
9. *Kovalchuk N. V., Melnykova N. M., Musatenko L. I.* Role of phytolectin in the life cycle of plants. *Biopolymers and cell.* 2012, 28 (3), 171–180. https://doi.org/10.7124/bc.00004A
10. *Kandelinskaya O. L., Grischenko E. R., Ripinskaya K. Ju., Aleschenkova Z. M., Kartizhova L. E., Kuptsov V. N., Kuptsov N. S.* Role of lectins in regulation of legume-rhizobium symbiosis efficiency in lupin. *Botanika (issledovaniya).* Sb. Nauch. Tr.: In-t Experimental noy Botaniki NAN Belarusi. *Minsk: In-t radiobiologii.* 2015, No 44, P. 283–290. (In Russian).
11. *Pavlovskaya N. E., Gagarina I. N.* The physiological properties of plant lectins as a prerequisite for their application in biotechnology. *Khimiya rastitelnogo syriya.* 2017, No 1, P. 21–35. (In Russian).
12. *Pavlovskaya N. E., Gagarina I. N., Rogovin V. V., Borzenkova G. A., Mushtakova V. M., Fomina V. A.* Means for presowing treatment of pea seeds: *Pat. Rus. No 2372763.* Opubl. 20. 11. 2009, Byul. No 33. (In Russian).
13. *Sytnikov D. M.* Economic effect and application of rhizobial preparations modified with homologous lectins. *Microbiol. Biotechnol.* 2012, 1 (17), 75–84. (In Russian).
14. *Sergienko V. G., Kyrychenko O. V., Perkovska G. Yu.* Method of using plant lectins to protect vegetable crops from diseases. *Pat. na korysnu model UA No 41723A01N 63/00, A01C 1/06/.* Zayavnyk i patentovlasnyk Institute of Plant Protection of Ukrainian Academy of Agrarian Sciences. u200812612. Zayavl. 28. 10. 2008, Opubl. 10. 06. 2009, Byul. No 11. (In Ukrainian).
15. *Erohin A. I.* 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. https://doi.org/10.24411/2309-348X-2019-11087
16. *Erohin A. I., Pavlovskaya N. E.* Efficacy of combined application of preparations for pea seeds. *Zemledelie.* 2016, No 4, P. 17–19. (In Russian).
17. State register of plant varieties suitable for dissemination in Ukraine in 2018. *Kyiv: Ministerstvo Ahrarnoi Polityky ta Prodovolstva Ukrainy.* 2018, 447 p. www.sops.gov.ua/uploads/page/5aa63108e441e.pdf (In Ukrainian).
18. *Hardy R. W. F., Burns R. C., Holsten R. D.* Application of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil. Biol. Biochem.* 1973, 5 (1), 41–83. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1086994
19. *Thandiwe Nleye, Peter Sexton, Kyle Gustafson, Janet Moriles Miller.* Soybean Growth Stages. In book: *Grow Soybean: Best management Practices for Soybean Production.* 2019, Ch. 3, P. 1–11. www.researchgate.net
20. *Sytnikov D. M., Kots S. Ya., Malichenko S. M., Kirizii D. A.* Photosynthetic rate and lectin activity of soybean leaves after inoculation with rhizobia together with homologous lectin. *Rus. J. Plant Physiology.* 2006, 53 (2), 169–175. (In Russian).

ЕФЕКТИВНІСТЬ ЗАСТОСУВАННЯ ФІТОЛЕКТИНІВ І ЛЕКТИНОВИХ КОМПОЗИЦІЙ ДЛЯ ОБПРИСКУВАННЯ РОСЛИН У ПЕРІОД ВЕГЕТАЦІЇ

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Досліджено ефективність застосування способу обприскування в період вегетації рослин сої (на фоні інокуляції насіння бульбочковими бактеріями і без інокуляції насіння) розчином лектину насіння сої, а також обприскування рослин озимої пшениці лектинбактеріальною композицією відповідно у вегетаційних і польових умовах. Встановлено, що обприскування сої у фазі розвитку двох трилисників специфічним лектином на фоні передпосівної інокуляції насіння ризобіями спричинило достовірно позитивний вплив на функціональну активність симбіотичного апарату. Азотфіксувальна активність ризосферної мікробіоти лишалась незмінною, що може вказувати на вектор дії лектину за обприскування саме через рослину. При цьому відзначено активацію вегетативного росту рослин, що максимально виявлялося за висотою їхньої надземної частини. На фоні інокуляції насіння ризобіями активність дії екзогенного лектину за обприскування була менш вираженою порівняно з неінокульованими рослинами. Обприскування рослин лектином сої на фоні інокуляції насіння забезпечило приріст урожаю порівняно з неінокульованим контролем на 2,13 г/рослину, однак за фактором дії лектину цей приріст становив лише 0,19 г/рослину і був недостовірним. Неінокульовані рослини сої за обприскування лектином сформували урожай, який на 0,64 г/рослину був достовірно більшим, ніж у рослин за відсутності впливу лектину. При цьому приріст за фактором дії лектину становив 22%. В озимої пшениці за відсутності передпосівної інокуляції насіння обприскування рослин у фазі масових весняних сходів препаратом Азолек на основі лектину пшениці сприяло достовірному збільшенню врожайності на 1,6 ц/га. Отже, застосування способу обприскування вегетуючих рослин сої та пшениці відповідно лектином насіння сої та лектинбактеріальним препаратом Азолек (лектин пшениці) без залучення прийому передпосівної інокуляції насіння забезпечило більший ступінь реалізації продуктивного потенціалу рослин порівняно з контрольними (відсутність передпосівної інокуляції насіння та обприскування під час вегетації).

Ключові слова: соя, ризобії, лектин насіння сої, обприскування, інокуляція, азотфіксація, пшениця озима, препарат Азолек, продуктивність.

ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ ФИТОЛЕКТИНОВ И ЛЕКТИНОВЫХ КОМПОЗИЦИЙ ДЛЯ ОПРЫСКИВАНИЯ РАСТЕНИЙ В ПЕРИОД ВЕГЕТАЦИИ

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Исследована эффективность применения способа опрыскивания в период вегетации растений сои (на фоне инокуляции семян клубеньковыми бактериями и без инокуляции семян) раствором лектина семян сои, а также опрыскивания растений озимой пшеницы лектинбактериальной композицией соответственно в вегетационных и полевых условиях. Установлено, что опрыскивание сои в фазе развития двух трехлистников специфичным лектином на фоне предпосевной инокуляции семян ризобиями оказало достоверно положительное влияние на функциональную активность симбиотического аппарата. Азотфиксирующая активность ризосферной микробиоты оставалась неизменной, что может указывать на вектор действия лектина именно при опрыскивании через растение. При этом отмечена активация вегетативного роста растений, который максимально проявлялся в высоте их надземной части. На фоне инокуляции семян ризобиями активность действия экзогенного лектина при опрыскивании была менее выраженной по сравнению с неинокулированными растениями. Опрыскивание сои лектином на фоне инокуляции семян обеспечило прибавку урожая по сравнению с неинокулированным контролем на 2,13 г/растение, однако по фактору действия лектина эта прибавка составила лишь 0,19 г/растение и была недостоверной. Неинокулированные растения сои при опрыскивании лектином сформировали урожай, который на 0,64 г/растение был достоверно больше, чем у неопрысканных лектином растений. При этом прибавка по фактору действия лектина составила 22%. У пшеницы озимой при отсутствии предпосевной инокуляции семян бактериальными препаратами опрыскивание в фазе массовых весенних всходов препаратом Азолек на основе лектина пшеницы способствовало достоверному увеличению урожая на 1,6 ц/га. Таким образом, применение опрыскивания вегетирующих растений сои и пшеницы соответственно лектином семян сои и лектинбактериальным препаратом Азолек (лектин пшеницы) без использования приема предпосевной инокуляции семян обеспечило более высокую степень реализации продуктивного потенциала растений по сравнению с контрольными (отсутствие предпосевной инокуляции семян и опрыскивания растений во время вегетации).

Ключевые слова: соя, ризобии, лектин семян сои, опрыскивание, инокуляция, азотфиксация, пшеница озимая, препарат Азолек, продуктивность.