

THE IMPACT OF BIOPREPARATIONS AND PHYTOPATHOGENIC BACTERIA OF THE *PSEUDOMONAS* GENUS ON L-PHENYLALANINE AMMONIA-LYASE ACTIVITY IN SOYBEAN AND LUPINE PLANTS

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It is known that plant protection against diseases is based mainly on the use of pesticides. These chemicals or their degradation products have a detrimental effect on the environment and human health. Due to this, the search for methods of plant protection that are safe for the environment is becoming increasingly popular. Induction of plant resistance to disease is one of the promising non-chemical ways of protection, in which plant enzymes play a key role. It was shown that in response to pathogen invasion, plants enhance protective properties by inducing the activity of a wide range of enzymes that slow the spread of infection, in particular: peroxidases, β -1,3-glucanases, chitinases, polyphenol oxidases and L-phenylalanine ammonia-lyase (PAL). **The aim** of the research was to study the change in PAL activity in soybean and lupine plants under conditions of artificial infection with some phytopathogenic bacteria of the *Pseudomonas* genus and under the action of Ecovital and EPAA-M biological preparations. **Methods.** PAL activity was determined spectrophotometrically. Changes of soybean (*Glycine max*) and lupine (*Lupinus luteus* L.) plants resistance to diseases caused by bacteria of the *Pseudomonas* genus were evaluated using phytopathological methods. Statistical processing of the research results was performed using MS Excel computer program with Student's *t*-test and estimation of least significant difference (LSD). **Results.** It was found that under conditions of soybean and lupine plants infection with phytopathogenic bacteria of the *Pseudomonas* genus on the background of pre-sowing seed treatment with Ekovital and EPAA-M biological preparations, composition based on them and synthetic standard – salicylic acid a significant increase in FAL activity in the aboveground and the degree of infectious roots – reducing of plants with pathogens was observed. It has been established that the growth of PAL activity under the condition of infection with phytopathogenic bacteria of the *Pseudomonas* genus occurs after 2–6 h and lasts up to 7 days from the moment of phytopathogen invasion into the plant. The most increasing of PAL activity was after treatment of seeds with Ecovital and EPAA-M composition – by 38.1–73.3% (*Lupinus luteus* L.) and 60.4–110 % (*Glycine max*) compared to the control. Treatment with the composition of biological products also helped to reduce the degree of plant damage. PAL activity increasing and reducing the area of soybean and lupine leaves affected surface can indicate the induction of protective reactions in plants. **Conclusions.** The use of the composition of EPAA-M with the microbial preparation Ecovital contribute to the increase of PAL activity and the formation of resistance to phytopathogenic bacteria of the genus *Pseudomonas* in *Glycine max* and *Lupinus luteus* L plants.

Keywords: L-phenylalanine ammonia-lyase activity, phytopathogenic bacteria of the *Pseudomonas* genus, degree of damage, plant resistance.

The intensive technology of plant protection against disease is based mainly on the use of pesticides. The harmful effects of these chemicals or products of their breakdown on the environment and human health are undeniable

[1]. Therefore, the search for alternative measures aimed at minimizing their harmful effects on the environment is becoming increasingly popular. Induced plant resistance is one of the promising non-chemical strategies for effective preventing the

spread of disease [2].

The influence of various biotic agents (bacteria, micromycetes, viruses) on plants causes protective biochemical and physiological changes, in particular, the strengthening of the cell wall due to lignification, suberizations and deposition of callose, prevention of pathogen invasion due to the synthesis of phenolic compounds, phytoalexins and pathogenesis-related phytotoxins (pathogenesis-related (PR) proteins). It should also be emphasized that the plants enhance protective reactions in response to pathogen invasion by inducing the activity of a wide range of infection-inhibiting enzymes, including peroxidase, β -1,3-glucanase, chitinase, polyphenol oxidase and L-phenylalanine ammonia-lyase (PAL, EC 4.3.1.24 (formerly EC 4.3.1.5)) [3]. The known forms of induced resistance in plants are systemic acquired resistance (SAR) and induced systemic resistance (ISR) [4]. The ISR can be caused by substances with antagonistic action and is transported through the plant, directly inactivating the pathogen. The induced resistance may not be systemic, but the local resistance may be formed as a result of induced resistance. It is established that ISR is formed with the participation of rhizobacteria that promote plant growth and development. The rhizobacteria synthesize the signaling molecules that induce protection in tissues outside the root system where the microorganism that produce biologically active substance with antagonistic action is. The ISR is formed regardless of the effect of salicylic acid, but stays under the action of jasmonic acid and/or ethylene, that produced by some non-pathogenic rhizobacteria. In addition, plants can become resistant to disease by various biological agents, including pathogenic and non-pathogenic bacteria and fungi, etc. [5].

SAR is a cascade of induced protective reactions. During SAR, a moving signal is generated at the site of microorganism invasion and moves within the plant, resulting in the generalization of induced state in all tissues. This type of resistance induction in plants provides long-term protection against a wide range of microorganisms. SAR development requires salicylic acid, which acts as a signaling molecule. The development of SAR is associated with various reactions in a plant cell: synthesis of PR proteins and phytoalexins, rapid changes in the biochemical composition of the cell wall, increased activity of various protective enzymes, including PAL. SAR is systemically induced after pathogen invasion or the use of certain chemicals, in particular salicylic acid [6]. Some rhizobacteria

can stimulate and increase plant resistance against pathogens [2, 7]. Induction of these pathways at the same time can significantly improve plant protection against biotic agents in general, and phytopathogens in particular [3].

As noted earlier, PAL along with peroxidase, polyphenol oxidase, β -1,3-glucanase and chitinase is among the protective enzymes associated with the induction of resistance in plants [3, 8]. This compound is the key enzyme responsible for binding the primary metabolism of aromatic amino acids with the secondary products of metabolism. PAL catalyzes the non-oxidative deamination of phenylalanine into *trans*-cinnamic acid and ammonia, which is the initial step in phenolic compounds biosynthesis. This enzyme is one of the most widely studied in plants due to investigation of synthesis of various phenolic compounds, as well as anthocyanins, which are responsible for plant resistance to pathogens [9]. Changes in PAL activity can occur during the development of infectious process, wounding and cooling of a plant, as well as the action of ozone. The PAL activity can be induced by the signaling molecules of plant ethylene hormone, as well as salicylic and jasmonic acids. The PAL activity can also be regulated by changing of PAL gene expression [3].

The biological method of plant protection, which is mainly based on the use of biosynthetic potential of microorganisms [10], becomes more popular recently. Ecovital is a complex highly effective multifunctional preparation based on symbiotic nitrogen-fixing (*Bradyrhizobium japonicum* for soybean, *Rhizobium lupini* for lupine) and phosphate-mobilizing bacteria (*Bacillus megaterium*), intended for pre-sowing of legume seeds [11]; the EPAA-M is a biological-derived sticky-carrier [12]. We have previously studied and patented composition of Ecovital and EPAA sticky-carrier for joint inoculation of legume seeds [13]. The long-term use of this composition and its individual components in the field revealed a decrease in the susceptibility of plants to various pathogens, although none of the components has a direct antagonistic effect on the pathogenic microorganisms [10]. So, it is very important to study the effects of seed treatment with Ecovital and EPAA-M biological products on the protective reactions in plants.

The aim of the study was to investigate the change in PAL activity in soybean (*Glycine max*) and lupine (*Lupinus luteus* L.) plants in the formation of induced resistance to diseases caused by *Pseudomonas syringae* pv. *syringae*

and *Pseudomonas savastanoi* pv. *glycinea* phytopathogenic bacteria in cases of Ecovital and EPAA-M biological products action.

Materials and Methods

Modeling system for determining the effect of different types of seed treatment with biological products and based on them compositions. The studies were performed on modeling systems created on young soybean (*Glycine max*) plants of the Gorlitsa cultivar infected with phytopathogenic bacteria *Pseudomonas savastanoi* pv. *glycinea* IMV B-8571 (Collection of plant pathogenic bacteria, Department of Phytopathogenic Bacteria of IMV NASU) strain and lupine (*Lupinus luteus* L.) plants of the Obrij cultivar infected with *Pseudomonas syringae* pv. *syringae* IMV B-8535 strain. The following biological products and their compositions have been used for the treatment of soybean and lupine seeds:

– Ecovital preparation based on nitrogen-fixing nodule bacteria *Bradyrhizobium japonicum* UCM B-6018 for soybean, *Rhizobium lupini* UCM B-6080 (Ukrainian Collection of Microorganisms) for lupine and phosphate-mobilizing bacteria *Bacillus megaterium* UCM B-5724 [11];

– sticky-carrier composition based on EPAA-M obtained from the copolymerization reaction of xampan exopolysaccharide (produced by *Xanthomonas campestris* pv. *campestris*) or enposan (produced by *Paenibacillus polymyxa*) and acrylamide in the presence of 0.5–2.0 % carbonic acid diamide [12];

– composition of Ecovital and EPAA-M in the ratio 1:10 [13].

Treatment of plants with salicylic acid at a concentration of 0.05 mM, which is optimal for stimulating the growth of seedlings, was also used as a known standard [2]. The sterile piped water was used as a control.

Investigation of the effectiveness of treatment with biological products and based on them compositions. Determination of treatment effectiveness of the above biological products and based on them compositions against the causative agents of brown bacterial lupine spot and angular soybean spot was carried out in laboratory conditions. Soybean and lupine seeds were previously sterilized with 10 % hydrogen peroxide before sowing. The treatment was performed with Ekovital preparation (cell titer 5×10^9 CFU (colony-forming units)) at the concentration of 1 liter per 1 ton of seeds and EPAA-M preparation at the concentration of 100 ml per 1 ton of seeds.

Compositions of Ecovital with EPAA-M were used for seeds treatment at the dilution of 1:10. For this purpose, the seeds were treated once (within 30 minutes) with biological products and their compositions before sowing. After sterilization and treatment with biological preparations, the seeds were germinated in sterile sand for 12–14 days in room conditions under natural light; they were twice fed with Knop's solution, diluted twice with water. Plants in the group treated with salicylic acid in the phase of first leaf appearance (12–14 days) were treated by watering in the sand of 0.05 mM salicylic acid solution. In the phase of second leaf appearance plants were infected with a suspension of daily cultures of phytopathogenic bacteria (10^7 cells/ml). After infection with the pathogens (after 2, 4, 6 and 8 hours), roots and shoots of soybean and lupine were fixed in liquid nitrogen and stored at -24° C for further analysis of enzymatic activity.

The studies of treatment effectiveness of the biological products and based on them compositions were also conducted in the green-house conditions (3-year experiment). For this purpose, the seeds were treated prior to sowing as described previously. The plants were grown in vessels with sterile soil until the stage of flowering and start of beans formation, and then they were artificially infected with pathogens. An aqueous suspension of daily phytopathogen culture in a titer of 10^7 cells/ml grown on potato agar was used for artificial infection. Sterile piped water was used as a control. Plants were infected with a threefold tissues injection with bacterial suspension. The accounting of the disease development was performed after the symptoms appearance on the 7th day after infection using the developed 10-point scale. The disease development was considered as low when the symptoms manifestation was estimated at 1–4 points, average – from 5 to 6, high – from 7 to 9 [14]. The images of development of the disease for better resolution were processed with Adobe ©Photoshop® CS5 Extended Version 12.0x32 (Publisher: © 1990–2010. Adobe Systems Incorporated) after symptoms appearance at the leaves on the 7th day after infection. Three leaves of three plants of each variant were cut, fixed and scanned with resolution of 600 dpi.

The area of the affected surface was expressed as a percentage of the total leaf area. On the 7th day after infection the plants were collected and fixed in liquid nitrogen and stored at -24° C for further PAL activity analysis.

PAL activity determination. The sources of the enzymes were buffer extracts from the roots and

shoots of soybean and lupine. The plant material was homogenized in liquid nitrogen in a mortar cooled to +4° C, 10-fold volume of 0.1 M borate buffer (pH 8.8) containing 1.0 mM EDTA and 1.0 mM DTT was added. The extraction was continued for 40–60 min with systematic stirring at +4° C. The homogenate was centrifuged at +4° C for 20 min at 12 000 g, the precipitate was discarded, and the supernatant was used as a source of cytosolic enzymes. Protein content was determined by Bradford method [14]. PAL activity was determined spectrophotometrically at 290 nm by the formation of *trans*-cinnamic acid [15].

Measurements were performed using spectrophotometer Beckman UV 5240 (Beckman Coulter, Inc., USA). The reaction mixture (final volume of 3 ml) contained 0.1 M of borate buffer (pH 8.8), 15 µmol of L-phenylalanine, and 50–300 µl of enzyme extract. The incubation was carried out for 1 h at +37 °C. The reaction was stopped by the addition of 0.5 ml of 1 M trichloroacetic acid into the reaction mixture. The reaction was performed six times.

Statistical analysis. Statistical processing of the research results was performed using MS Excel computer program Microsoft® Excel (Version 14.0.7237.5000 (32-bit)) with Student's t-test. The difference was considered statistically significant at $P < 0.05$.

Results. As a result of a model experiment, it was found that PAL activity in soybean and lupine seedlings changes both under the influence of biological products treatment and when infected with phytopathogenic bacteria. It is established that the growth of PAL activity under the condition of infection with phytopathogenic bacteria of the *Pseudomonas* genus occurs after 2–6 h from the moment of phytopathogen invasion into the plant. It should be noted that the growth of enzymatic activity occurs after all types of plant treatment and is a direct “response” of the plant to the pathogen's penetration.

In addition, the trends of PAL activity increase in the shoots and roots in response to infection with phytopathogens are different (Fig. 1, 2). A sharp decrease in PAL activity compared to the control variant (water treatment) was observed in the roots of experimental plants 2 hours after the phytopathogen invasion, and then a gradual increase in PAL activity was observed 4–6 hours after phytopathogen invasion. Instead, we found a gradual increase in PAL activity in shoots from 2 hours after plant infection. A slight decrease in

PAL activity occurs in the shoots and roots only 8 hours after pathogen injection.

In our opinion, observed patterns of PAL activity change indicate the initiation of protective reactions involving some protective enzymes related to the resistance induction. We believe that the decrease in PAL activity in roots 2 hours after infection with the pathogen is probably due to the redistribution of phenolic compounds synthesis in the plant with increasing of their concentration at the site of phytopathogen invasion in order to form local resistance.

The research also revealed the effect of different types of seed treatment on PAL activity. Thus, the highest levels of enzymatic activity in plants that were infected with phytopathogenic bacteria of *Pseudomonas* genus were observed in the case of seed treatment with the composition of Ecovital and EPAA-M. In particular, the average increase of enzymatic activity for this type of treatment ranged from 38.1 % (roots) to 73.3 % (shoots) in lupine seedlings and from 110 % (roots) to 60.4 % (shoots) in soybean seedlings as compared to controls.

A sufficiently high level of PAL activity was also observed after seeds treatment with salicylic acid which is a standard inducer of plant defense reactions. Thus, an increase in enzymatic activity from 37.8 % (roots) to 52.6 % (shoots) for lupine seedlings and from 70.8 % (roots) to 50.5 % (shoots) for soybean seedlings was observed in this treatment variant. Separate treatment of seeds with EPAA-M or Ecovital less stimulated PAL activity. Thus, PAL activity increased on average from 30.6 % (roots) to 24.2 % (shoots) for lupine seedlings and from 51.8 % (roots) to 24.0 % (shoots) for soybean seedlings after phytopathogen invasion in the variant of Ecovital seeds treatment. EPAA-M treatment increased PAL activity on average from 22.1 % (roots) to 20.2 % (shoots) in lupine seedlings and from 45.5 % (roots) to 9.02 % (shoots) in soybean seedlings.

The results obtained in the model laboratory experiment correlate with the experiment conducted in green-house conditions. In particular, simultaneous treatment with Ecovital and EPAA-M biological preparations had the highest effect on PAL activity on the 7th day from the moment of infection with the *Pseudomonas* genus bacteria (Fig. 3). Thus, treatment of seeds with the composition of biological preparations increases the PAL activity from 83.3 % (roots) to 97.3 % (leaves) in soybean plants and from 61.7 % (roots) to 75.0 % (leaves) in lupine plants in comparison

with control. Plant treatment with salicylic acid also improves this indicator compared to controls (water) from 70.9 % (roots) to 95.8 % (leaves) in soybean plants and from 60.6 % (roots) to 65.7 % (leaves) – in lupine plants. Seed treatment with the above preparations separately also increase PAL activity compared to the control variant. We also noted a slightly lower level of PAL activity in the

roots of plants than in the leaves. In general, the quantitative indicators of PAL activity obtained in the green-house experiment are slightly lower than in the model laboratory experiment.

We also found in our research a decrease in the degree of damage and increase in yield in cases of plant seeds treatment with the biological preparations (Table 1).

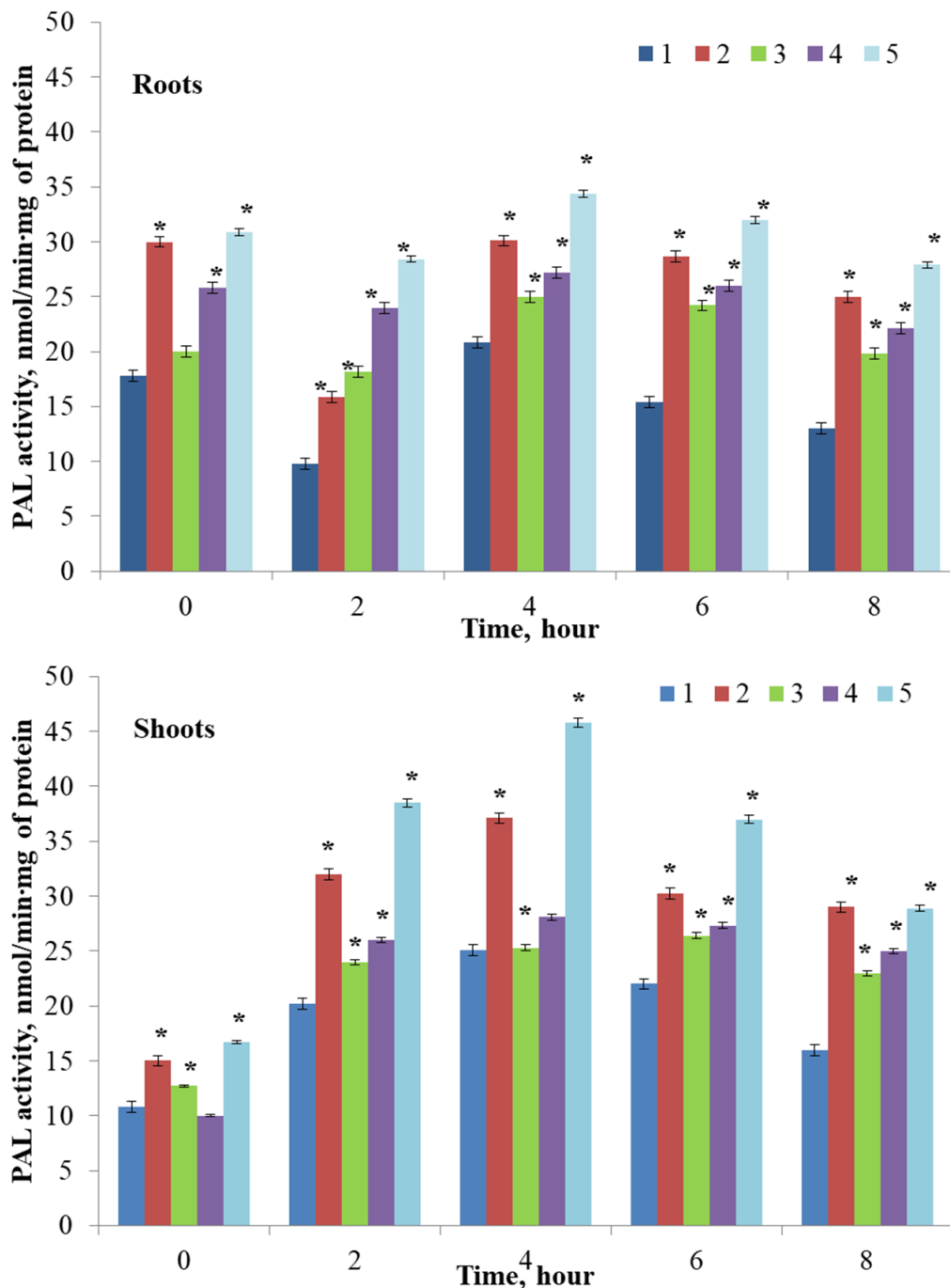


Fig. 1. Changes in PAL activity in seedlings of soybean (*Glycine max*) plants after treatment with biological preparations when infected with *Pseudomonas savastanoi* pv. *glycinea* 8571 strain: 1 – control (water treatment); 2 – composition based on EPAA-M + Ecovital; 3 – EPAA-M; 4 – Ecovital; 5 – salicylic acid. (M ± m, n = 6). *Significantly different from the control (water); P < 0.05 was considered as statistically significant

Thus, the treatment of legumes seeds with Ecovital and EPAA-M composition reduced the affected surface area of lupine and soybean plants by 1.5 times, and with salicylic acid – by 1.1 times compared to the control (water). Treatment of lupine and soybean seeds with Ecovital and EPAA-M biological preparations separately also reduced the degree of plant damage. In particular,

the area of the affected surface decreased by 1.2–1.4 (lupine) and 1.4–1.3 (soybean) times compared to the control.

Pre-sowing treatment of soybean and lupine seeds with Ecovital and EPAA-M biological preparations significantly increased PAL activity in the aboveground part and the roots of plants infected with phytopathogenic bacteria of the

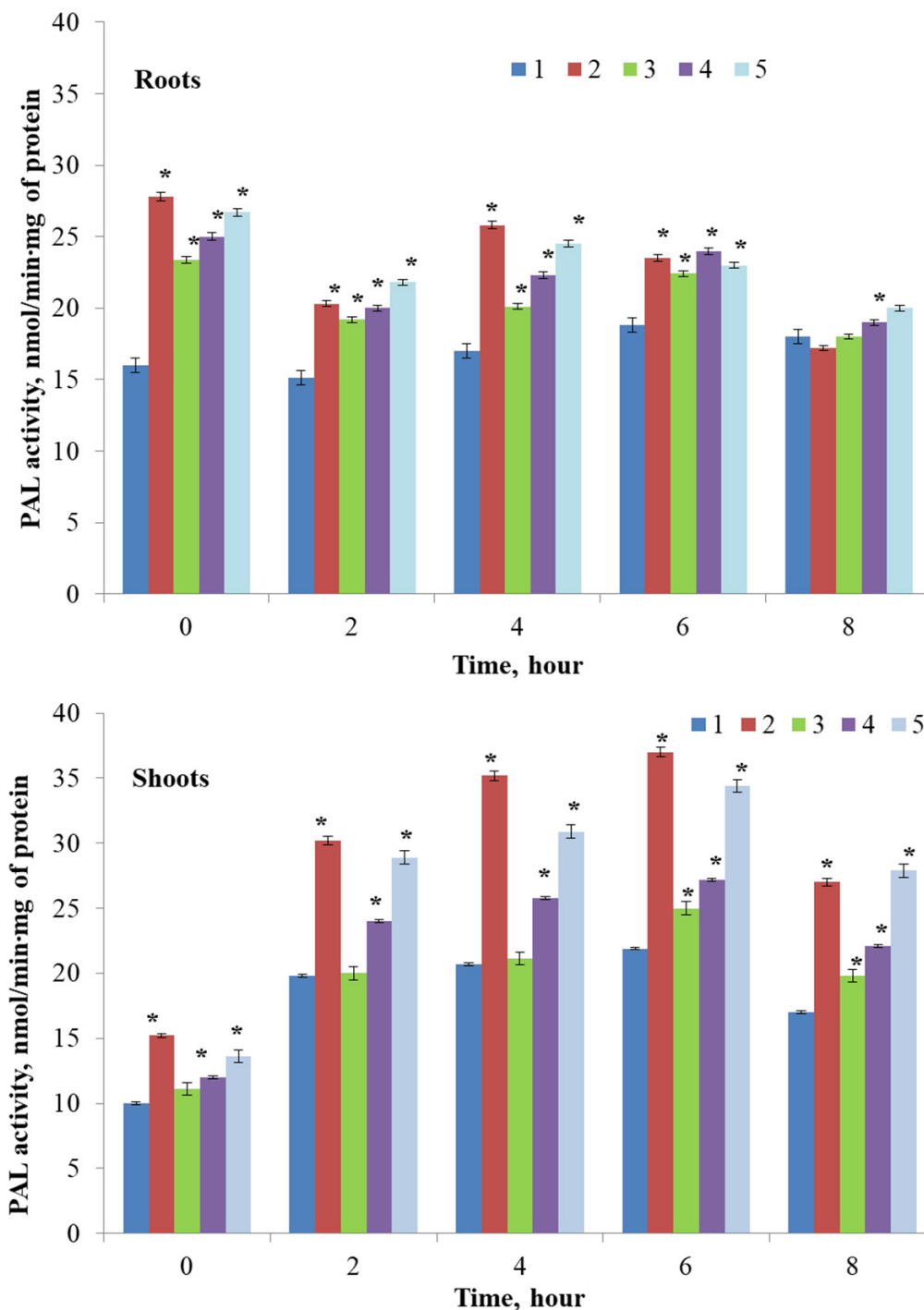


Fig. 2. Changes in PAL activity of lupine (*Lupinus luteus* L.) plants seedlings after treatment with biological preparations when infected with *Pseudomonas syringae* pv. *syringae* 8535 strain: 1 – control (water treatment); 2 – composition based on EPAA-M + Ecovital; 3 – EPAA-M; 4 – Ecovital; 5 – salicylic acid ($M \pm m$, $n = 6$). *Significantly different from the control (water); $P < 0.05$ was considered as statistically significant

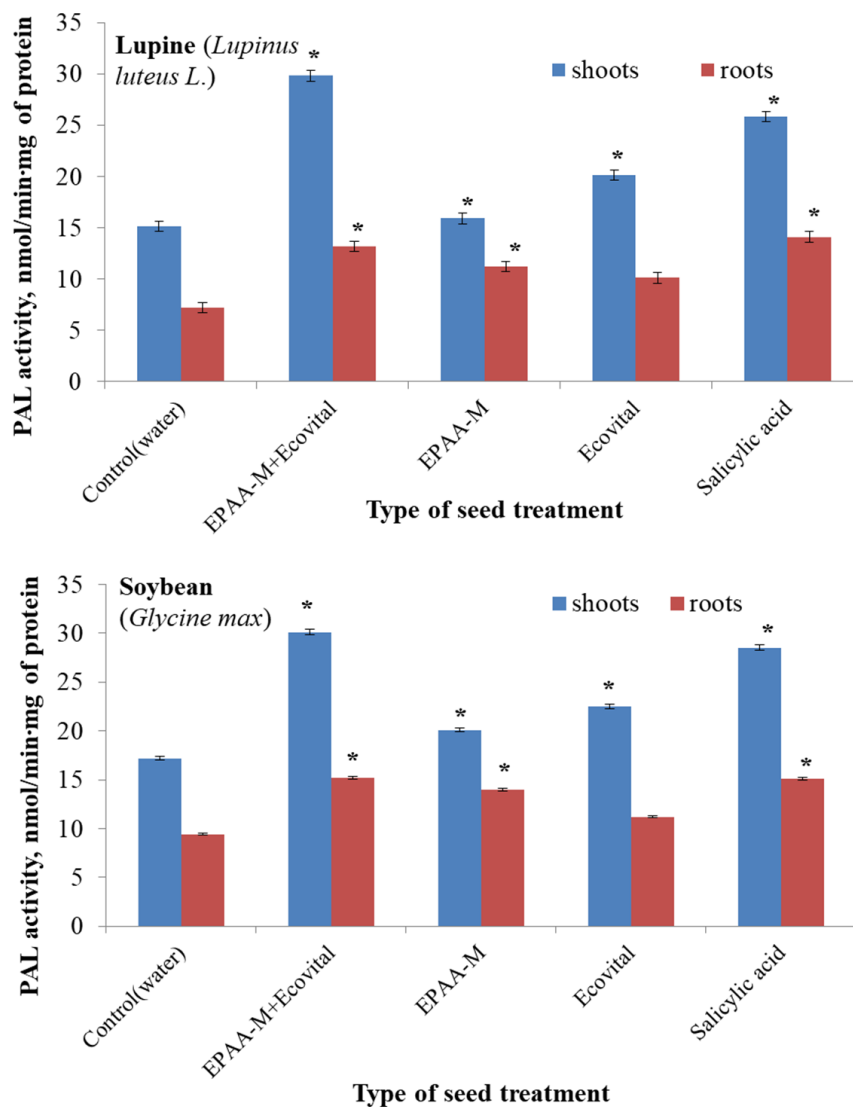


Fig. 3. PAL activity in soybean (*Glycine max*) and lupine (*Lupinus luteus* L.) plants treated with Ecovital and EPAA biological products in cases of artificial infection with *Pseudomonas* bacteria (7 days after infection) ($M \pm m$, $n = 6$). *Significantly different from the control (water); $P < 0.05$ was considered as statistically significant

Table 1
Degree of damage of lupine and soybean plants infected with *P. syringae* pv. *syringae* 8535 and *P. savastanoi* pv. *glycinea* 8571 phytopathogenic bacteria under different types of seeds treatment

Variant of seeds treatment	Degree of damage, points	Area of affected leaf surface,%
Lupine (<i>Lupinus luteus</i> L.)		
Control (water)	8.0	25.5 ± 0.02
Salicylic acid	4.0–6.0	18.0 ± 0.04
EPAA-M	7.0	21.3 ± 0.02
Ecovital	6.0–7.0	20.1 ± 0.04
EPAA-M+ Ecovital	6.0–7.0	17.0 ± 0.01
Soybean (<i>Glycine max</i>)		
Control (water)	9.0	28.0 ± 0.01
Salicylic acid	4–5	20.2 ± 0.03
EPAA-M	7–8	23.4 ± 0.02
Ecovital	7.0	21.3 ± 0.04
EPAA-M+ Ecovital	5–6	19.1 ± 0.01

Pseudomonas genus, and in the subsequent period decreased the degree of infection spread. The change in PAL activity was the most significant after seeds treatment with the Ecovital and EPAA-M composition. Thus, the increase in enzymatic activity for this type of treatment ranged from 38.1 % (roots) to 73.3 % (shoots) in lupine seedlings and from 110 % (roots) to 60.4 % (shoots) in soybean seedlings compared to the control (water).

This seed treatment variant also reduced the area of the affected lupine and soybean leaves surface by 1.5 times and increased the grain weight by almost 1.4–1.5 times and the protein content in the grain by 1.7–2.4 %.

Discussion. The study of plant resistance formation to phytopathogenic bacteria is relevant given the development of bacteriosis in modern agrocenoses [16]. The study of PAL activity as a marker of induced plant resistance makes it possible to assess the impact of various biological means to increase plant resistance to biotic and abiotic stresses. The results of our experiments showed that seeds treatment with the composition based on Ecovital and EPAA-M preparations had maximum positive influence on PAL activity. In our view, PAL activity increase can be explained by several aspects. It is known that symbiotic nitrogen-fixing bacteria are capable to produce a lot of biologically active substances, in particular phytohormones (auxins, cytokinins, gibberellins, ethylene) [16, 17]. Researchers have shown that strawberry plants treatment with abscisic acid increases the activity of anthocyanin and PAL [18]. As noted earlier, one of the forms of induced plant resistance, namely ISR, is significantly affected by ethylene produced by non-pathogenic rhizobacteria, including strains that are part of Ecovital preparation.

It should be noted that EPAA-M has adhesive properties and promotes better fixation of bacteria and microbial metabolites of Ecovital on the seed surface. Due to this, biologically active metabolites promote better development of seedlings with higher resistance to pathogens. EPAA-M copolymer can be destroyed in the soil by saprotrophic microorganisms and decomposed into components, the main of which are exopolysaccharides. EPAA-M contains xampan exopolysaccharide synthesized by *X. campestris* pv. *campestris* phytopathogenic bacterium. It is known from the literature that the role of exopolysaccharides (EPS) of phytopathogenic bacteria has not been fully elucidated, they are considered to be molecules capable to escape or delay activation of plant

protection, or act as signaling molecules in plant-pathogen interaction [19, 20]. Some researchers have found that EPS of individual phytopathogenic bacteria, which do not have the same chemical structure, induce an increase in the synthesis of PAL, a marker enzyme of plant defense responses against stress, while other bacteria EPS do not have this effect. EPS that influence on PAL activity also induce an increase in hydrogen peroxide synthesis and alter the metabolism of ascorbate in plants, which are also involved in individual plant defense reactions [20]. In our opinion, an increase in PAL synthesis, in combination with biological treatment, is to synergize the protective reactions in plants that are induced by the action of each of the preparations separately. High PAL activity was also detected in the case of salicylic acid treatment. The effect of this compound on the induction of SAR as a signaling molecule is known and undeniable [21]. The treatment of legumes seeds with Ecovital and EPAA-M preparations separately also increased PAL activity compared to the control but lower than in the previous variants. Based on the above, we believe that plant seeds treatment with Ecovital and EPAA-M composition has the highest influence on PAL activity, since, probably, this process occurs in several ways.

Conclusions. PAL activity as biochemical marker and the system of lupine or soybean infection with *Pseudomonas syringae* pv. *syringae* and *Pseudomonas savastanoi* pv. *glycinea* may subsequently be used as model systems in the study, in particular, of phytoimmunity inducers and mechanisms of systemic resistance inducing in plants. The increase in PAL activity along with the decrease in the area of affected surface of soybean and lupine leaves indicates the induction of protective reactions in plants under application of promising Ecovital and EPAA-M biopreparations.

ВПЛИВ БІОПРЕПАРАТІВ І ФІТОПАТОГЕННИХ БАКТЕРІЙ РОДУ *PSEUDOMONAS* НА L-ФЕНІЛАЛАНІН-АМОНІЙ-ЛІАЗНУ АКТИВНІСТЬ У РОСЛИНАХ СОЇ І ЛЮПИНУ

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Резюме

Відомо, що захист рослин від хвороб базується, головним чином, на використанні пестицидів. Ці хімічні речовини або продукти їх деградації здійснюють шкідливий вплив на навколишнє середовище та здоров'я людей. Зважаючи на це все, більшої популярності набуває пошук методів захисту рослин, які є безпечними для довкілля. Індукція стійкості рослин до хвороб є одним із перспективних нехімічних шляхів захисту, в яких ключову роль відіграють рослинні ензими. Відомо, що у відповідь на інвазію патогена рослини посилюють захисні реакції, індукуючи активність широкого спектра ензимів, що сповільнюють розповсюдження інфекції, зокрема: пероксидаз, β -1,3-глюканаз, хітиназ, поліфенолоксидаз і L-фенілаланін-амоній-ліази (ФАЛ). Метою наших досліджень було вивчення зміни ФАЛ активності у рослинах сої і люпину за умов штучного інфікування окремими штамми фітопатогенних бактерій роду *Pseudomonas* та за дії біопрепаратів Ековітал і ЕПАА-М. Методи. ФАЛ активність вивчали спектрометричним методом. Зміни стійкості рослин сої і люпину до хвороб, спричинених бактеріями роду *Pseudomonas*, оцінювали фітопатологічними методами. Статистичну обробку результатів досліджень проводили за допомогою MS Excel

із застосуванням критерія Стьюдента. **Результати.** Показано, що за умов інфікування сої і люпину фітопатогенними бактеріями роду *Pseudomonas* на фоні передпосівної обробки насіння біопрепаратами Ековітал і ЕПАА-М, композицією на їх основі та синтетичним стандартом – саліциловою кислотою відбувалось суттєве зростання ФАЛ активності у надземній частині та коренях рослин, а в подальшому – зменшення ступеня інфікування рослин збудниками. Так, зростання рівня ФАЛ активності за умови інфікування фітопатогенними бактеріями роду *Pseudomonas* відбувалося на 2–6-у годину та тривало до 7-ї доби з моменту інвазії фітопатогена у рослину. Найбільше, порівняно з контролем, ФАЛ активність підвищувалася за обробки насіння композицією Ековіталу з ЕПАА-М: на 38,1–73,3 % (люпин) та 60,4–110 % (соя). Обробка композицією біопрепаратів не тільки сприяла підвищенню ФАЛ активності, а й зменшенню площі ураженої поверхні листя та ступеня ураження рослин в цілому, що свідчить про індукцію захисних реакцій у рослин. **Висновки.** Застосування композиції ЕПАА-М з мікробним препаратом Ековітал активує формування резистентності до фітопатогенних бактерій роду *Pseudomonas* у рослин *Glycine max* і *Lupinus luteus* L.

Ключові слова: L-фенілаланін-амоній-ліаза активність, фітопатогенні бактерії роду *Pseudomonas*, ступінь ураження, резистентність рослин.

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