

SENSITIVITY OF RHIZOBACTERIA OF DIFFERENT SPECIES TO THE INFLUENCE OF SOIL HERBICIDES UNDER *IN VITRO* CONDITIONS

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Objective. To investigate the sensitivity of *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii*, *Azospirillum brasilense*, *Pseudomonas fluorescens*, and *Azotobacter chroococcum* to the effects of soil-applied herbicides under *in vitro* conditions. **Methods.** Microbiological and statistical. The sensitivity of rhizobacteria of different species to herbicidal preparations was studied using the well diffusion method on agarized nutrient medium plates. **Results.** As a result of the laboratory experiments series, it was established that the rhizobial strains *Bradyrhizobium japonicum* 634b, PC07, PC08, B20, T66, T21-2, B78 and *Bradyrhizobium elkanii* SAF18 were not sensitive to average recommended rate herbicide formulations containing 312.5 g/l S-metolachlor + 187 g/l terbuthylazine, 900 g/l acetochlor and 960 g/l S-metolachlor. A low level of sensitivity was observed in certain rhizobial strains to a herbicide formulation containing 500 g/l prometryn. Under the influence of the specified active ingredient, the growth inhibition zones on bacterial lawns of the analytically selected strains *B. elkanii* SAF18 and *B. japonicum* PC07 measured 5.0 mm and 11.0 mm, respectively. Exposure to prometryn at the tested concentration resulted in the formation of growth inhibition zones measuring 8.0 mm and 6.5 mm on bacterial lawns of Tn5 mutants of *B. japonicum* strains B20 and B78, respectively. As a result of examining the growth characteristics of *A. brasilense* 410, *A. chroococcum* T79, and *P. fluorescens* strains 33 and 267 under the influence of soil-applied herbicides, no growth inhibition zones were observed on the bacterial lawns of any of the tested strains. The formation and appearance of bacterial colonies were characteristic of each of the species studied. **Conclusions.** It was found that, under *in vitro* conditions, the chemical plant protection products used in the study did not exhibit bactericidal activity against rhizobacteria of various species under the conditions of applying the manufacturer's recommended average rate. In vegetation and field experiments, it is advisable to determine the effect of herbicidal compounds on the realization of the symbiotic potential of rhizobia. It is also important to assess how these substances influence the plant growth-promoting properties of *Pseudomonas*, *Azotobacter* and *Azospirillum* strains. Such studies will contribute to resolving the issue of compatibility between the application of chemical plant protection products and complex bacterial inoculation.

Key words: *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii*, *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, soil-applied herbicide, sensitivity, growth inhibition zone.

Introduction. In soybean cultivation technologies, it is common practice to introduce microorganisms into agroecosystem that are capable of providing plants with nutrients and

synthesizing various biologically active substances to stimulate the growth and development of the cultivated crop. At the same time, soybeans are very sensitive to weeds, especially in the early stages of vegetation. This necessitates the use of effective chemical plant protection products (PPPs) to reduce the number of weeds in crops. Synthetic chemical compounds may negatively affect the viability and biological traits of microorganisms possessing agronomically beneficial properties. Therefore, in production conditions, it is important to study their sensitivity to herbicides *in vitro*.

Analysis of recent studies and publications. Soybean (*Glycine max* (L.) Merr.) originates from China. Its cultivation history spans over 5,000 years. Under modern agricultural conditions, soybean holds the largest cultivated area among leguminous crops worldwide and serves as an important source of edible oil and high-quality plant protein, as well as a raw material for animal feed production [1].

Given the need to restore and preserve soil fertility, as well as to produce environmentally safe agricultural products, scientific interest in the processes of biological nitrogen fixation is steadily increasing. Particular attention is paid to the study of symbiotic and associative interactions between diazotrophic microorganisms and plants [2; 3].

Soybean plants, in symbiosis with root nodule bacteria, are capable of fixing atmospheric nitrogen. This accounts for approximately 77 % of the total amount of this element biologically assimilated by all leguminous crops [4].

The intensity of biological nitrogen fixation in soybean agroecosystems is influenced by numerous factors, including soil temperature, moisture, aeration, pH, salinity, the amount of available nitrogen, the properties of the rhizobial strain and plant genotype, as well as the application of pesticides and other agrochemicals [5; 6].

In Ukraine, the typical microsymbiont of soybean is *Bradyrhizobium japonicum*. Numerous strains of this species have been isolated from soils in various climatic zones of the country, and they show considerable differences in their phenotypic and genotypic characteristics [7; 8]. Ongoing research focuses on a more detailed study of strains obtained both through analytical selection and by means of genetic engineering. The virulence of new strains, the

intensity of N₂ assimilation by root nodules formed with their participation, and the effects of inoculation on plant growth, development, and grain productivity are evaluated. Based on these assessments, rhizobia with improved properties are selected for use as effective microsymbionts [9].

Among the bacteria commonly used for seed inoculation of both grain and leguminous crops are representatives of the genus *Azospirillum*. The most extensively studied among them are *Azospirillum brasilense* and *Azospirillum lipoferum*. The benefits of introducing azospirilla to plants are mainly attributed to their ability to fix atmospheric nitrogen and to synthesize phytohormones. In recent years, researchers have also highlighted the important role of *Azospirillum* in enhancing plant tolerance to abiotic and biotic stresses [10]. Rhizobacteria are continuously screened for the development of bio-preparations, as they are often characterized by the synthesis of plant-beneficial metabolites and can provide both growth-promoting and phyto-protective functions by acting against pathogens of various etiologies [11]. To preserve the viability and biological activity of microbial agents, various carrier materials, sterilization methods, and storage temperatures for the final products are developed [12].

There are reports in the literature on the effectiveness of combined inoculation of soybean seeds with root nodule bacteria *B. japonicum* together with rhizobacteria, including *A. brasilense* [13], *P. fluorescens* [14], and *A. chroococcum* [15]. These studies confirmed the synergistic effect resulting from the combined functions of the applied microorganisms, which contributed to an increase in soybean grain yield.

One of the factors affecting the viability and realization of the nitrogen-fixing potential of microorganisms introduced into agroecosystems is the impact of chemical plant protection products. In soybean weed control systems, soil-applied herbicides play a key role by providing prolonged control over weed germination, thereby creating more favorable conditions for the growth and development of the cultivated crop [16; 17].

It should be taken into account that herbicides, as highly active chemical compounds, may affect the viability of microorganisms with agronomically beneficial properties even at

production-recommended application rates under *in vitro* conditions. They can also alter the abundance and activity of these microorganisms in soil [18]. Researchers have analyzed a substantial body of scientific literature addressing the effects of herbicidal compounds on rhizobia and have pointed out the potential impact on the host plant as well as on the establishment and development of symbiosis *in vivo* [19].

The investigation of rhizobacterial sensitivity to soil-applied herbicides under laboratory conditions is relevant, particularly for forecasting the potential impact of chemical preparations on the efficiency of plant-microbe interactions when applied simultaneously within agroecosystems.

Materials and methods. The following strains were used in the study: soybean nodule bacteria *B. japonicum* 634b, PC07, PC08, B20, B78, T66, and T21-2; *B. elkanii* SAF18; rhizobacteria *A. brasilense* 410; *P. fluorescens* 10 and 267; and *A. chroococcum* T79. All strains are maintained in the collection of symbiotic and associative nitrogen-fixing microorganisms at the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (IPPG NASU).

The nodule bacteria *B. japonicum* and *B. elkanii* were grown on a mineral medium of yeast mannitol agar (YMA), which contained: K_2HPO_4 — 0.5 g/l; $MgSO_4 \cdot 7H_2O$ — 0.4 g/l; NaCl — 0.1 g/l; mannitol — 10 g/l; yeast extract — 0.5 g/l; agar — 15 g/l, distilled water, pH 6.4. Strains *A. brasilense* and *P. fluorescens* were cultivated on LPG medium, which contained yeast extract — 3 g/l; bacillus peptone — 5 g/l; glucose — 5 g/l; agar — 16 g/l, distilled water, pH 7.0. *A. chroococcum* bacteria were grown on Ashby nutrient medium, which included K_2HPO_4 — 0.2 g/l; sucrose — 20 g/l; $MgSO_4 \cdot 7H_2O$ — 0.2 g/l; NaCl — 0.2 g/l; K_2SO_4 — 0.1 g/l; $CaCO_3$ — 5 g/l; microelements (according to Fedorov) — 1 ml/l; agar — 16 g/l; distilled water, pH 7.2–7.4.

The sensitivity of the microorganisms used in this study to soil-applied herbicides was assessed using a modified well-diffusion method [20]. In Petri dishes containing nutrient medium under aseptic conditions, wells with a diameter of 10 mm were cut using a metal cylinder. According to standard procedures, the surface of the nutrient medium in the dishes was inoculated with a uniform bacterial lawn using

a microbial cell suspension with a titer of 10^6 CFU/mL [21]. Each well was filled with 80 μ L of the herbicide solution and incubated in a thermostat for 5–7 days at a temperature of 28 °C. The control consisted of wells in the center of the agar medium filled with 80 μ L of sterile tap water. All experimental variants were performed in triplicate.

Table 1. List and characteristics of the pesticides used in the study [22]

Trade Name	Active Ingredient	Recommended Application Rate
Gezagard 500 FW SC	Prometryn, 500 g/L	2.0–5.0 L/ha
Primextra TZ Gold 500 SC	S-metolachlor, 312.5 g/L + terbuthylazine, 187.5 g/L	4.0–4.5 L/ha
Harness EC	Acetochlor, 900 g/L	1.5–2.5 L/ha
Dual Gold 900 EC	S-metolachlor, 960 g/L	1.0–1.6 L/ha

Working solutions of the herbicides were prepared using the average concentration recommended by the manufacturer for application in soybean cultivation. The study also took into account and applied the recommended amount of water (250 L/ha) for dissolving each of the herbicides. The sensitivity of *B. japonicum*, *B. elkanii*, *A. brasilense*, *P. fluorescens*, and *A. chroococcum* strains to the herbicides in laboratory experiments was assessed by measuring the size of the growth inhibition zones of the bacterial culture around wells containing the chemical compounds. The intensity of bacterial growth around the wells was evaluated visually: «+ + +» — intensive growth, «+ + -» — slight inhibition, «+ - -» — stronger inhibition, «- - -» — complete absence of bacterial growth. Intensive bacterial growth and the absence of growth inhibition zones around the wells with herbicides indicated the resistance of the tested microorganisms to the applied formulations.

Results and discussion. It was established that all the strains of root nodule bacteria involved in the study — *B. japonicum* 634b, PC07, PC08, B20, T66, T21-2, B78, and *B. elkanii* SAF18 — were not sensitive to the

action the manufacturer's recommended average rate of herbicide formulations containing 312.5 g/L S-metolachlor + 187.5 g/L terbuthylazine, 900 g/L acetochlor, and 960 g/L S-metolachlor.

The soil-applied herbicide based on 500 g/L prometryn caused growth inhibition zones on the bacterial lawn measuring 5.0 mm and 11.0 mm for the analytically selected strains *B. elkanii* SAF18 and *B. japonicum* PC07, respectively (Fig. 1). Under the influence of this active ingredient, growth inhibition zones measuring 8.0 mm and 6.5 mm were observed

on the bacterial lawns of *B. japonicum* B20 and B78, respectively (Table 2).

The formation of growth inhibition zones on the bacterial lawn with diameters of up to 15 mm in various strains indicates their low sensitivity, in pure culture, to the action of the herbicidal substances used in this study.

An examination of the growth characteristics of *A. brasilense* 410, *A. chroococcum* T79, and *P. fluorescens* strains 33 and 267 on agar nutrient media with soil-applied herbicides added to the wells showed that none of the strains exhibited any growth inhibition zones on

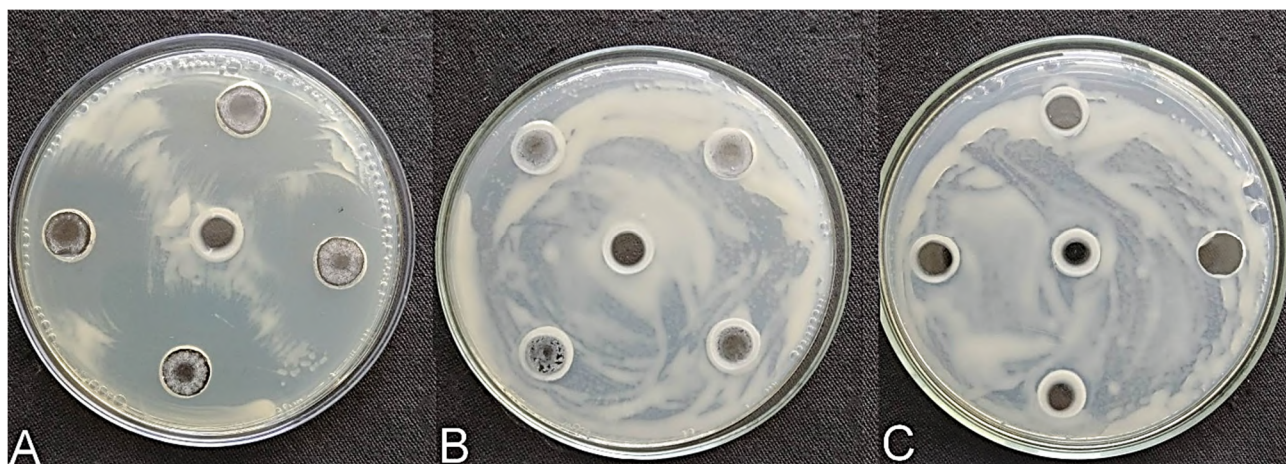


Fig. 1. Growth inhibition zones on bacterial lawns of *Bradyrhizobium japonicum* PC07 under the influence of soil-applied herbicides: A — 500 g/L prometryn; B — 312.5 g/L S-metolachlor + 187.5 g/L terbuthylazine; C — 900 g/L acetochlor.

Table 2. Sensitivity of soybean nodule bacteria to the production-rate application of soil-applied herbicides

Bacterial strain	Method of production	Prometryn, 500 g/L	S-metolachlor, 312.5 g/L + terbuthylazine, 187.5 g/L	Acetochlor, 900 g/L	S-metolachlor, 960 g/L
		Size of growth inhibition zones on the bacterial lawn around wells containing the herbicide, mm / bacterial growth rate			
<i>B. japonicum</i> 634b	Analytical selection	0 / +++	0 / +++	0 / +++	0 / +++
<i>B. japonicum</i> PC07		11.0 / +++	0 / +++	0 / +++	0 / +++
<i>B. japonicum</i> PC08		0 / +++	0 / +++	0 / +++	0 / +++
<i>B. elkanii</i> SAF18		5.0 / ++-	0 / +++	0 / +++	0 / +++
<i>B. japonicum</i> T66	<i>B. japonicum</i> 646 + <i>E. coli</i> S17-1 (pSUP2021::Tn5)	0 / +++	0 / +++	0 / +++	0 / +++
<i>B. japonicum</i> T21-2		0 / +++	0 / +++	0 / +++	0 / +++
<i>B. japonicum</i> B20	<i>B. japonicum</i> 646 + <i>E. coli</i> S17-1 (pSUP5011::Tn5 <i>mob</i>)	8.0 / +++	0 / +++	0 / +++	0 / +++
<i>B. japonicum</i> B78		6.5 / +++	0 / +++	0 / +++	0 / +++

Note. In tables 2 and 3: «+ + +» — intensive growth, «+ + -» — slight inhibition, «+ - -» — stronger inhibition, «- - -» — complete absence of bacterial growth.

the bacterial lawn (Table 3). The formation of bacterial colonies was typical for each of the specified species.

As an example, Fig. 2 shows the growth of the bacterial lawn of *A. chroococcum* T79 under the influence of the soil-applied herbicides used in this study.

There is a great diversity of microorganisms used as the basis for biopreparations, as well as a wide range of chemical compounds applied to control weeds in agricultural crops, including soybean. Therefore, the literature contains numerous studies investigating the effects of various herbicidal substances on different groups of beneficial microbiota. It should also be noted that there are considerable differences in the research methodologies employed, particularly under laboratory conditions.

The response of *Bradyrhizobium* and *Azotobacter* strains to different concentrations of herbicides in liquid nutrient media was studied by Ubogu and Akponah. An inhibitory effect of atrazine at concentrations above 1.5 % on the

viability of *Azotobacter* was observed. When 2.0 % glyphosate was added to the cultivation medium of rhizobia, a decrease in the number of viable cells was recorded after 24 hours of co-cultivation, followed by recovery and stimulation of growth. Under similar conditions, growth activity of pure cultures of *Azotobacter* and *Bradyrhizobium* was suppressed by paraquat. The toxic effect of paraquat was evident at all tested concentrations (0.5 %, 1.0 %, 1.5 %, and 2.0 %). Root nodule bacteria are more sensitive to the effects of herbicides than free-living diazotrophs [23]. In our study, strains of *Pseudomonas*, *Azospirillum*, and *Azotobacter* also demonstrated resistance to the action — to the average recommended rate of soil herbicides based on 500 g/L prometryn, 312.5 g/L S-metolachlor + 187.5 g/L terbuthylazine, 900 g/L acetochlor, and 960 g/L S-metolachlor. Prometryn caused slight growth inhibition zones (ranging from 5 to 11 mm) in rhizobial strains differing in origin and activity. Despite the slight sensitivity to this active ingredient observed in some

Table 3. Sensitivity of various rhizobacterial species to production-rate applications of soil-applied herbicides

Bacterial strain	Method of production	Prometryn, 500 g/L	S-metolachlor, 312.5 g/L + terbuthylazine, 187.5 g/L	Acetochlor, 900 g/L	S-metolachlor, 960 g/L
		Size of growth inhibition zones on the bacterial lawn around wells containing the herbicide, mm / bacterial growth rate			
<i>A. brasilense</i> 410	Analytical selection	0 / +++	0 / +++	0 / +++	0 / +++
<i>A. chroococcum</i> T79		0 / +++	0 / ++-	0 / +++	0 / +++
<i>P. fluorescens</i> 33		0 / +++	0 / +++	0 / +++	0 / +++
<i>P. fluorescens</i> 267		0 / +++	0 / +++	0 / +++	0 / +++

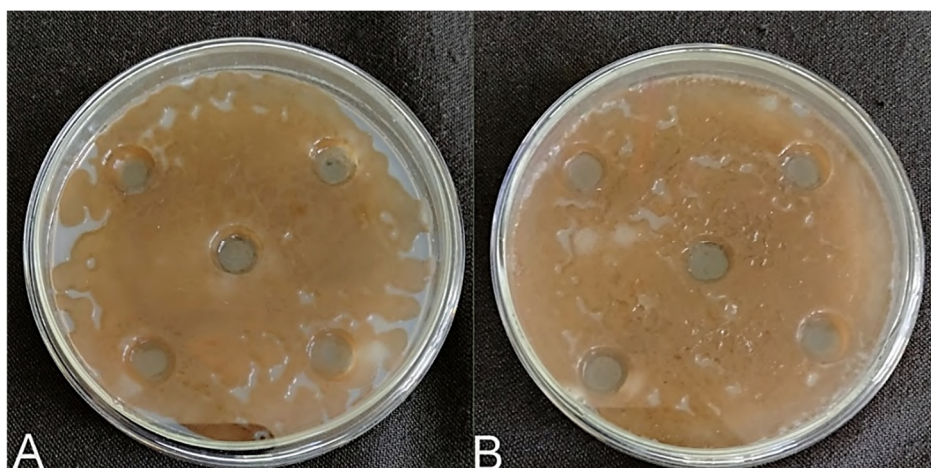


Fig. 2. Growth of the bacterial lawn of *Azotobacter chroococcum* T79 under exposure to: A — 500 g/L prometryn; B — 960 g/L S-metolachlor.

strains such as *B. japonicum* PC07, B20, B78, and *B. elkanii* SAF18, the production of polysaccharide slime by these strains remained intensive in all tested treatments. According to the literature, extracellular polymers such as exopolysaccharides provide microorganisms with an adaptive advantage in the environment. The functions of this heteropolymer include protection against various stresses, biofilm formation, and attachment to the roots of host plants [24]. Rhizobia defective in polysaccharide synthesis are unable to establish an effective symbiosis [25].

We previously evaluated the effect of fungicides on soybean root nodule bacteria in pure culture. Growth inhibition zones measuring 13.0 and 14.0 mm were observed in *B. japonicum* strains B20 and B78, respectively, when exposed to the recommended field dose of a formulation containing 500 g/kg benomyl. Upon doubling this dose, strain *B. japonicum* B20 was classified as slightly sensitive, whereas the growth of *B. japonicum* B78 was completely suppressed, exhibiting an inhibition zone ≥ 25 mm [26].

A difference was noted in the levels of herbicide tolerance between *B. japonicum* and *B. elkanii* strains, which is attributed to differences in the absorption characteristics of these substances by the respective species [27]. It was found that the *P. fluorescens* strain CMA55, isolated from water used for washing chemical pesticide containers, exhibited changes in the activity of antioxidant enzymes and produced biofilms when exposed to certain herbicides. The authors therefore suggested the presence of a specific model of phenotypic plasticity in these microorganisms for adaptation to environments contaminated with xenobiotics. This should be considered in studies on herbicide bioremediation [28]. Some strains have shown resistance to chemical plant protection products of various types. For example, *P. fluorescens* 8 demonstrated high resistance to ten different insecticides and herbicides (quizalofop ethyl 5 % EC, sodium pyriithiobac 10 % EC, oxyfluorfen 3.5 % EC, cyhalofop-butyl 10 % EC, glyphosate + ammonium sulfate 71SG, pendimethalin 30 % EC, imazethapyr 10 % EC, atrazine 50 % WP, glyphosate 41 % SL) when applied at concentrations of 100, 500, and 1000 mg/L in agar nutrient medium. Among fungicidal substances, azoxystrobin and a mixture of car-

boxin with thiram did not affect the viability of this strain, regardless of the concentration used. The authors consider it highly probable that there is an additive effect when using biopreparations based on *P. fluorescens* 8 together with the tested pesticides [29].

Researchers working with the pesticide-resistant strain *Azotobacter vinelandii* AZ6 noted that herbicides at elevated concentrations were more toxic compared to fungicidal and insecticidal formulations. The effects of kitazin, hexaconazole, metalaxyl, glyphosate, quizalofop, atrazine, fipronil, monocrotophos, and imidacloprid on the synthesis by the bacteria of indole-3-acetic, salicylic, and 2,3-dihydroxybenzoic acids were determined. With increasing pesticide concentrations, a decrease in their solubilization capacity was observed, which was attributed to changes in the pH of the nutrient medium. Microorganisms of this strain continued to synthesize biologically active compounds even under the influence of high pesticide concentrations, indicating their potential for use as effective inoculants for agricultural crops [30]. Therefore, it is advisable to determine the effects of herbicides on the biological properties of the studied rhizosphere microorganisms and their production of plant growth-promoting and antibiotic substances.

In some cases, the addition of herbicides to the bacterial cultivation medium results in reduced growth intensity of bacteria with agronomically beneficial properties. This is associated with a decreased efficiency in resource utilization by the bacteria. Under stress conditions, a portion of the energy available to the organism is expended to maintain cellular and biochemical mechanisms of tolerance to the stressor [31].

Singh & Wright investigated the effects of terbutryn with terbuthylazine, trietazine with simazine, prometryn, and bentazon on the growth of *R. leguminosarum*, using herbicide concentrations significantly higher than those recommended by the manufacturers. The reduction in microorganism viability observed under in vitro conditions is considered unlikely to occur under field conditions. They explain this by noting that even in cases of non-compliance with herbicide application regulations or gradual accumulation of residues over the years, the amounts present in soils decrease due to adsorption processes, degradation, leaching, and other factors [32]. In general, a number of

scientific studies emphasize the high tolerance of certain representatives of the rhizosphere microbiota to xenobiotics.

It is scientifically justified to limit the use of herbicides that have demonstrated an inhibitory effect on beneficial microbiota or on the physiological and biochemical processes in plants. The use of compounds that are highly persistent in the environment, capable of remaining in the soil for years and affecting the effectiveness of plant-microbe interactions over a prolonged period, should be avoided [19].

Conclusions. It was found that, under in vitro conditions, the chemical plant protection products under the conditions of the average manufacturer-recommended rate used in this study containing 312.5 g/L S-metolachlor + 187.5 g/L terbuthylazine, 900 g/L acetochlor, 960 g/L S-metolachlor, and 500 g/L prometryn did not exhibit bactericidal activity against *B. japonicum* strains 634b, PC07, PC08, B20, B78, T66, T21-2; *B. elkanii* SAF18; the rhizobacteria *A. brasilense* 410, *P. fluorescens* 10, 267, and *A. chroococcum* T79. The growth inhibition zones ranging from 5 to 11 mm caused by the tested concentration of prometryn in certain soybean root nodule bacterial strains indicate their low sensitivity to this herbicide.

In future research, it should be taken into account that, in soil, changes in the viability or reproduction intensity of microorganisms depend on the processes of pesticide detoxification. Therefore, in vegetation and field experiments, it is advisable to determine the effect of herbicidal compounds on the realization of the symbiotic potential of rhizobia and the growth-promoting properties of *Pseudomonas*, *Azotobacter* and *Azospirillum*. This will help address the issue of compatibility between the application of chemical plant protection products and pre-sowing bacterial inoculation.

REFERENCES

1. Fang, C., & Kong, F. (2022). Soybean. *Current Biology*, 32(17), R902–R904. <https://doi.org/10.1111/10.1016/j.cub.2022.06.054>
2. Patyka, V. P., Hnatyuk, T. T., Buletsa, N. M., & Kyrylenko, L. V. (2015). Biologichnyi azot u systemi zemlerobstva [Biological nitrogen in the agricultural system]. *Zemlerobstvo — Agriculture*, 2, 12–20 [in Ukrainian].
3. Chakraborty, S., Venkataraman, M., Infante, V., Pflieger, B. F., & Ané, J. M. (2024). Scripting a new dialogue between diazotrophs and crops. *Trends in Microbiology*, 32(6), 577–589. <https://doi.org/10.1016/j.tim.2023.08.007>
4. Herridge, D. F., Peoples, M. B., & Boddey, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil*, 311, 1–18. <https://doi.org/10.1007/s11104-008-9668-3>
5. Santachiara, G., Salvagiotti, F., & Rotundo, J. L. (2019). Nutritional and environmental effects on biological nitrogen fixation in soybean: A meta-analysis. *Field Crops Research*, 240, 106–115. <https://doi.org/10.1016/j.fcr.2019.05.006>
6. Jinturkar, B. P. (2019). An analytical approach on pesticides of Rhizobia and the legume — Rhizobium. *Accent Journal of Economics Ecology & Engineering*, 4(5), 1–7.
7. Malichenko, S. M., Omelchuk, S. V., Mamenko, P. M., & Kots, S Ya. (2013). Efektyvnist, konkurentospromozhnist i tekhnolohichnist novykh analitychno selektsionovanykh shtamiv bulbochkovykh bakterii soi [Efficiency, competitiveness and technological effectiveness of new analytically selected strains of soybean nodule bacteria]. *Fiziologiya i biokhimiya kul'turnikh rasteniy — Physiology and biochemistry of cultivated plants*, 45(1), 53–60 [in Ukrainian].
8. Krutylo, D., Leonova, N., & Nadkernychna, O. (2020). Characterization of bradyrhizobia associated with soybean plants grown in Ukraine. *Journal of microbiology, biotechnology and food sciences*, 9(5), 983–987. <https://doi.org/10.15414/jmbfs.2020.9.5.983-987>
9. Vorobey, N. A. (2013). Seleksiia henetychno markovanykh bulbochkovykh bakterii Bradyrhizobium japonicum za symbiotychnymy vlastyvos-tiamy [Selection of genetically marked nodule bacteria Bradyrhizobium japonicum for symbiotic properties]. *Fiziologiya i biokhimiya kul'turnikh rasteniy — Physiology and biochemistry of cultivated plants*, 45(2), 173–182 [in Ukrainian].
10. Fukami, J., Cerezini, P., & Hungria, M. (2018). *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *Amb Express*, 8, 73. <https://doi.org/10.1186/s13568-018-0608-1>
11. David, B. V., Chandrasehar, G., & Selvam, P. N. (2018). *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In *Crop improvement through microbial biotechnology* (pp. 221–243). Elsevier. <https://doi.org/10.1016/B978-0-444-63987-5.00010-4>
12. Abd El-Fattah, D. A., Eweda, W. E., Zayed, M. S., & Hassanein, M. K. (2013). Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant. *Annals of Agricultural Sciences*, 58(2), 111–118. <https://doi.org/10.1016/j.aos.2013.07.001>

13. Hungria, M., Nogueira, M. A., & Araujo, R. S. (2015). Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. *American Journal of Plant Sciences*, 6(6), 811–817. <https://doi.org/10.4236/ajps.2015.66087>
14. Pawar, P. U., Kumbhar, C. T., Patil, V. S., & Khot, G. G. (2018). Effect of co-inoculation of *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* on growth, yield and nutrient uptake in soybean [*Glycine max* (L.) Merrill]. *Crop Research*, 53(1–2), 57–62. <https://doi.org/10.5958/2454-1761.2018.00009.8>
15. Marinkovic, J., Bjelic, D., Tintor, B., Miladinovic, J., Dukic, V., & Dorđevic, V. (2018). Effects of soybean co-inoculation with plant growth promoting rhizobacteria in field trial. *Romanian Biotechnological Letters*, 23(2), 13401–13408.
16. Sorokina, S. I., Rodzevich, Ye. P., & Mordeker, Ye. Yu. (2011). Efektyvnist kontroliuvannia burianiv ta selektyvnist shchodo roslyn soi za kompleksnoho zastosuvannia herbicidiv metrybuzynu, metakloru, tryfluralinu [Effectiveness of weeds control and selectivity for soybean plants at complex application of herbicides metribuzin, metolachlor, trifluralin]. *Fiziologiya i biokhimiya kul'turnikh rasteny — Physiology and biochemistry of cultivated plants*, 43(4), 287–296 [in Ukrainian].
17. Nevmerzhytska, O. M., Plotnytska, N. M., Gurmanchuk, O. V., & Skolub, S. M. (2019). Efektyvnist zastosuvannia gruntovykh herbicidiv u posivakh soi [Efficiency of application of herbicides in soy crops]. *Tavriiskyi naukovyi visnyk — Taurida Scientific Bulletin*, 109(1), 90–94 [in Ukrainian]. <https://doi.org/10.32851/2226-0099.2019.109-1.14>
18. Ruuskanen, S., Fuchs, B., Nissinen, R., Puigbò, P., Rainio, M., Saikkonen, K., & Helander, M. (2023). Ecosystem consequences of herbicides: the role of microbiome. *Trends in Ecology & Evolution*, 38(1), 35–43. <https://doi.org/10.1016/j.tree.2022.09.009>
19. Burul, F., Barić, K., Lakić, J. & Milanović-Litre, A. (2022). Herbicides effects on symbiotic nitrogen-fixing bacteria. *Journal of Central European Agriculture*, 23(1), 89–102. <https://doi.org/10.5513/JCEA01/23.1.3320>
20. Patyka, V. P., Pasichnyk, L. A., Hvozdiak, R. I., Petrychenko, V. F., Korniiichuk, O. V., Kalinichenko, A. V. ... Tomashuk, O. V. (2017). *Fitopatohenni bakterii. Metody doslidzhen* [Phytopathogenic bacteria. Research methods]. Vol. 2. Vinnytsia: Vindruk [in Ukrainian].
21. Antypchuk, A. F., Piliashenko-Novokhatnyi, A. I., & Yevdokymenko, T. M. (2011). *Praktykum z mikrobiologii* [Workshop on Microbiology]. Kyiv: Universytet “Ukraina” [in Ukrainian].
22. Derzhavnyi reiestr pestytsydiv i ahrokhimikativ, dozvolenykh do vykorystannia v Ukraini. Ministerstvo zakhystu dovkillia ta pryrodnykh resursiv [State register of pesticides and agrochemicals authorized for use in Ukraine. Ministry of Environmental Protection and Natural Resources of Ukraine] (March, 2025) [in Ukrainian]. Retrieved from <https://mepr.gov.ua/upravlinnya-vidhodamy/derzhavnyj-reiestr-pestytsydiv-i-agrohikativ-dozvolenykh-do-vykorystannya-v-ukrayini>
23. Ubogu, M., & Akponah, E. (2022). Diazotrophic Bacterial Response to Herbicide Toxicity: In vitro Analysis. *Asian Plant Research Journal*, 10(4), 13–20. <https://doi.org/10.9734/APRJ/2022/v10i4196>
24. Downie, J. A. (2010). The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. *FEMS Microbiol. Rev.*, 34(2), 150–170. <https://doi.org/10.1111/j.1574-6976.2009.00205.x>
25. Skorupska, A., Janczarek, M., Marczak, M., Mazur, A., & Król, J. (2006). Rhizobial exopolysaccharides: Genetic control and symbiotic functions. *Microb. Cell Fact*, 5, 7. <https://doi.org/10.1186/1475-2859-5-7>
26. Kukol, K. P., Vorobey, N. A., & Kots, S. Ya. (2019). Chutlyvist chystykh kultur *Bradyrhizobium japonicum* do vplyvu riznykh norm funhitsydiv [Sensitivity of pure cultures of *Bradyrhizobium japonicum* to the effects of different fungicide rates]. *Silskohospodarska mikrobiologhiia — Agricultural microbiology*, 30, 20–31. <https://doi.org/10.35868/1997-3004.30.20-31>
27. Procópio, S. D. O., dos Santos, J. B., Jacques, R. J., Kasuya, M. C. M., da Silva, A. A., & Werlang, R. C. (2004). Crescimento de estirpes de *Bradyrhizobium* sob influência dos herbicidas glyphosate potássico, fomesafen, imazethapyr e carfentrazone-ethyl. *Revista Ceres*, 51(294), 179–188.
28. Freitas, P. N. N., da Silva, Rovida A. F., Silva, C. R., Pileggi, S. A. V., Olchanheski, L. R., & Pileggi, M. (2021). Specific quorum sensing molecules are possibly associated with responses to herbicide toxicity in a *Pseudomonas* strain. *Environmental Pollution*, 289, 117896. <https://doi.org/10.1016/j.envpol.2021.117896>
29. Hanuman, L. N., & Bindu Madhavi, G. (2018). Compatibility of *Pseudomonas fluorescens* with pesticides in vitro. *International Journal of Current Microbiology and Applied Sciences*, 7(3), 381. <https://doi.org/10.20546/ijcmas.2018.703.381>
30. Shahid, M., Zaidi, A., Ehtram, A., & Khan, M. S. (2019). In vitro investigation to explore the toxicity of different groups of pesticides for an agronomically important rhizosphere isolate *Azotobacter vinelandii*. *Pesticide biochemistry and physiology*, 157, 33–44. <https://doi.org/10.1016/j.pestbp.2019.03.006>

31. Schimel, J., Balsler, T. C., & Wallenstein, M. (2007). Microbial stress response physiology and its implications for ecosystem function. *Ecology*, 88(6), 1386–1394. <https://doi.org/10.1890/06-0219>

32. Singh, G., & Wright, D. (2002). In vitro studies on the effects of herbicides on the growth of rhizobia. *Letters in Applied Microbiology*, 35(1), 12–16. <https://doi.org/10.1046/j.1472-765X.2002.01117.x>

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ЧУТЛИВІСТЬ РИЗОБАКТЕРІЙ РІЗНИХ ВИДІВ ДО ВПЛИВУ ГРУНТОВИХ ГЕРБИЦИДІВ В УМОВАХ *IN VITRO*

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Мета. Дослідити чутливість бактерій *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii*, *Azospirillum brasilense*, *Pseudomonas fluorescens* та *Azotobacter chroococcum* до впливу ґрунтових гербицидів в умовах *in vitro*. **Методи.** Мікробіологічні, статистичні. Зокрема чутливість ризобактерій різних видів до впливу препаратів з гербицидною активністю вивчали методом лунок, висічених у пластинках агаризованого живильного середовища. **Результати.** У серії лабораторних досліджень встановлено, що бульбочкові бактерії *B. japonicum* 634б, PC07, PC08, B20, T66, T21-2, B78 і *B. elkanii* САФ18 є не чутливими до дії середньої рекомендованої норми препаратів на основі 312,5 г/л S-метолахлору + 187 г/л тербутилазину; 900 г/л ацетохлору та 960 г/л S-метолахлору. Водночас виявлено слабку чутливість в окремих штамів ризобій до гербициду, в складі якого міститься 500 г/л прометрину. Зокрема, за впливу вказаної діючої речовини зони затримки росту бактеріального газону в аналітично селекціонованих штамів *B. elkanii* САФ18 та *B. japonicum* PC07 становили 5,0 і 11,0 мм відповідно. Окрім цього, за дії прометрину в досліджуваній концентрації відзначали наявність зон затримки росту бактеріального газону розміром 8,0 та 6,5 мм у Tn5-мутантів *B. japonicum* B20 і B78. Дослідивши особливості росту *A. brasilense* 410, *A. chroococcum* T79 та *P. fluorescens* 33 і 267 за дії ґрунтових гербицидів, відзначили відсутність зон затримки росту бактеріального газону у всіх вивчених штамів. Формування бактеріальних колоній було типовим для кожного із вказаних видів. **Висновки.** Таким чином з'ясовано, що в умовах *in vitro* залучені у роботу хімічні ЗЗР за умов застосування середньої рекомендованої виробником норми не чинять бактерицидної дії на ризобактерії різних видів. Водночас у вегетаційних та польових дослідках доцільно з'ясувати вплив речовин із гербицидною активністю на реалізацію симбіотичного потенціалу ризобій і ростостимулювальні властивості псевдомонад, азотобактера й азоспірил, що сприятиме розв'язанню питання сумісності застосування хімічних ЗЗР із комплексною бактеризацією.

Ключові слова: *Bradyrhizobium japonicum*, *Bradyrhizobium elkanii*, *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Azotobacter chroococcum*, ґрунтовий гербицид, чутливість, зона затримки росту.

ЦИТОВАНА ЛІТЕРАТУРА

1. Fang C., Kong F. Soybean. *Current Biology*. 2022. № 32(17). P. R902–R904. <https://doi.org/10.1016/j.cub.2022.06.054>
2. Патика В. П., Гнатюк Т. Т., Булеца Н. М., Кириленко Л. В. Біологічний азот у системі землеробства. *Землеробство*. 2015. Вип. 2. С. 12–20.
3. Chakraborty S., Venkataraman M., Infante V., Pflieger B. F., Ané J. M. Scripting a new dialogue between diazotrophs and crops. *Trends in Microbiology*. 2024. Vol. 32, № 6. P. 577–589. <https://doi.org/10.1016/j.tim.2023.08.007>
4. Herridge D. F., Peoples M. B., Boddey R. M. Global inputs of biological nitrogen fixation in agricultural systems. *Plant Soil*. 2008. Vol. 311. P. 1–18. <https://doi.org/10.1007/s11104-008-9668-3>
5. Santachiara G., Salvagiotti F., Rotundo J. L. Nutritional and environmental effects on biological nitrogen fixation in soybean: A meta-analysis. *Field Crops Research*. 2019. Vol. 240. P. 106–115. <https://doi.org/10.1016/j.fcr.2019.05.006>
6. Jinturkar B. P. An analytical approach on pesticides of Rhizobia and the legume — Rhizobium. *Accent Journal of Economics Ecology & Engineering*. 2019. Vol. 4, № 5. P. 1–7.
7. Маліченко С. М., Омельчук С. В., Маменко П. М., Коць С. Я. Ефективність, конкурентоспроможність і технологічність нових аналітично селекціонованих штамів бульбочкових бактерій сої. *Физиология и биохимия культурных растений*. 2013. Т. 45, № 1. С. 53–60.
8. Krutylo D., Leonova N., Nadkernychna O. Characterization of bradyrhizobia associated with soybean plants grown in Ukraine. *Journal of microbiology, biotechnology and food sciences*. 2020. Vol. 9, № 5. P. 983–987. <https://doi.org/10.15414/jmbfs.2020.9.5.983-987>
9. Воробей Н. А. Селекція генетично маркованих бульбочкових бактерій *Bradyrhizobium japonicum* за симбіотичними властивостями. *Физиология и биохимия культурных растений*. 2013. Т. 45, № 2. С. 173–182.
10. Fukami J., Cerezini P., Hungria M. Azospirillum: benefits that go far beyond biological nitrogen fixation. *Amb Express*. 2018. Vol. 8. 73. <https://doi.org/10.1186/s13568-018-0608-1>
11. David B. V., Chandrasehar G., Selvam P. N. *Pseudomonas fluorescens*: a plant-growth-promoting rhizobacterium (PGPR) with potential role in biocontrol of pests of crops. In *Crop improvement through microbial biotechnology*. Elsevier, 2018. P. 221–243. <https://doi.org/10.1016/B978-0-444-63987-5.00010-4>
12. Abd El-Fattah D. A., Eweda W. E., Zayed M. S., Hassanein M. K. Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant. *Annals of Agricultural Sciences*. 2013. Vol. 58, № 2. P. 111–118. <https://doi.org/10.1016/j.aos.2013.07.001>
13. Hungria M., Nogueira M. A., Araujo R. S. Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: a new biotechnological tool to improve yield and sustainability. *American Journal of Plant Sciences*. 2015. Vol. 6, № 6. P. 811–817. <https://doi.org/10.4236/ajps.2015.66087>
14. Pawar P. U., Kumbhar C. T., Patil V. S., Khot G. G. Effect of co-inoculation of *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* on growth, yield and nutrient uptake in soybean [*Glycine max* (L.) Merrill]. *Crop Research*. 2018. Vol. 53, № 1–2. P. 57–62. <https://doi.org/10.5958/2454-1761.2018.00009.8>
15. Marinkovic J., Bjelic D., Tintor B., Miladinovic J., Dukic V., Dordevic V. Effects of soybean co-inoculation with plant growth promoting rhizobacteria in field trial. *Romanian Biotechnological Letters*. 2018. Vol. 23, № 2. P. 13401–13408.
16. Сорокіна С. І., Родзевич О. П., Мордерер Є. Ю. Ефективність контролювання бур'янів та селективність щодо рослин сої за комплексного застосування гербіцидів метрибузину, метолахлору, трифлураліну. *Физиология и биохимия культурных растений*. 2011. Т. 43, № 4. С. 287–296.
17. Невмержицька О. М., Плотницька Н. М., Гурманчук О. В., Сколуб С. М. Ефективність застосування ґрунтових гербіцидів у посівах сої. *Таврійський науковий вісник*. 2019. № 109. Ч. 1. С. 90–94. <https://doi.org/10.32851/2226-0099.2019.109-1.14>
18. Ruuskanen S., Fuchs B., Nissinen R., Puigbò P., Rainio M., Saikkonen K., Helander M. Ecosystem consequences of herbicides: the role of microbiome. *Trends in Ecology & Evolution*. 2023. Vol. 38, № 1. P. 35–43. <https://doi.org/10.1016/j.tree.2022.09.009>
19. Burul F., Barić K., Lakić J., Milanović-Litre A. Herbicides effects on symbiotic nitrogen-fixing bacteria. *Journal of Central European Agriculture*. 2022. Vol. 23, № 1. P. 89–102. <https://doi.org/10.5513/JCEA01/23.1.3320>
20. Патика В. П., Пасічник Л. А., Гвоздяк Р. І., Петриченко В. Ф., Корнійчук О. В., Калініченко А. В. ... Томашук О. В. Фітопатогенні бактерії. *Методи досліджень*. Т. 2. Вінниця : Віндрук, 2017. 432 с.
21. Антипчук А. Ф., Піляшенко-Новохатний А. І., Євдокименко Т. М. Практикум з мікробіології. Київ : Університет «Україна», 2011. 156 с.
22. Державний реєстр пестицидів і агрохімікатів, дозволених до використання в Україні. Міністерство захисту довкілля та природних ресурсів. URL: <https://mepr.gov.ua/upravlinnya-vidhodamy/derzhavnyj-reyestr-pestytsydiv-i-agrohimiaktiv-doz>

volenyh-do-vykorystannya-v-ukrayini/ (дата звернення 17.03.2025)

23. Ubogu M., Akponah E. Diazotrophic Bacterial Response to Herbicide Toxicity: In vitro Analysis. *Asian Plant Research Journal*. 2022. Vol. 10, № 4. P. 13–20. <https://doi.org/10.9734/APRJ/2022/v10i4196>

24. Downie J. A. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. *FEMS Microbiol. Rev.* 2010. Vol. 34, № 2. P. 150–170. <https://doi.org/10.1111/j.1574-6976.2009.00205.x>

25. Skorupska A., Janczarek M., Marczak M., Mazur A., Król J. Rhizobial exopolysaccharides: Genetic control and symbiotic functions. *Microb. Cell Fact.* 2006. Vol. 5. 7. <https://doi.org/10.1186/1475-2859-5-7>

26. Кукол К. П., Воробей Н. А., Коць С. Я. Чутливість чистих культур *Bradyrhizobium japonicum* до впливу різних норм фунгіцидів. *Сільськогосподарська мікробіологія*. 2019. Вип. 30. С. 20–31. <https://doi.org/10.35868/1997-3004.30.20-31>

27. Procópio S. D. O., dos Santos J. B., Jacques R. J., Kasuya M. C. M., da Silva A. A., Werlang R. C. Crescimento de estirpes de *Bradyrhizobium* sob influência dos herbicidas glyphosate potássico, fomesafen, imazethapyr e carfentrazone-ethyl. *Revista Ceres*. 2004. Vol. 51, № 294. P. 179–188.

28. Freitas P. N. N., da Silva Rovida A. F., Silva C. R., Pileggi S. A. V., Olchanheski L. R., Pileggi M. Specific quorum sensing molecules are possibly associated with responses to herbicide toxicity in a *Pseudomonas* strain. *Environmental Pollution*. 2021. Vol. 289. 117896. <https://doi.org/10.1016/j.envpol.2021.117896>

29. Hanuman L. N., Bindu Madhavi G. Compatibility of *Pseudomonas fluorescens* with pesticides in vitro. *International Journal of Current Microbiology and Applied Sciences*. 2018. Vol. 7, № 3. <https://doi.org/10.20546/ijcmas.2018.703.xx>

30. Shahid M., Zaidi A., Ehtram A., Khan M. S. In vitro investigation to explore the toxicity of different groups of pesticides for an agronomically important rhizosphere isolate *Azotobacter vinelandii*. *Pesticide biochemistry and physiology*. 2019. Vol. 157. P. 33–44. <https://doi.org/10.1016/j.pestbp.2019.03.006>

31. Schimel J., Balsler T. C., Wallenstein M. Microbial stress response physiology and its implications for ecosystem function. *Ecology*. 2007. Vol. 88, № 6. P. 1386–1394. <https://doi.org/10.1890/06-0219>

32. Singh G., Wright D. In vitro studies on the effects of herbicides on the growth of rhizobia. *Letters in Applied Microbiology*. 2002. Vol. 35, № 1. P. 12–16. <https://doi.org/10.1046/j.1472-765X.2002.01117.x>

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