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## Microbial inoculants as a means of improving soil and crop yields

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**Abstract.** The article presented the results of studies of the influence of microbial inoculants *Rhizobium japonicum* and *Azospirillum brasilense* on the growth and development of soybean (*Glycine max*) at the initial stages of its development. The study was conducted on three experimental plots: a control plot without treatment, a plot with *Rhizobium japonicum* inoculum and a plot with *Azospirillum brasilense* inoculum. The main indicators were evaluated: plant height, number of leaves, root system development and total biomass at different stages of plant growth, as well as laboratory analysis of nitrogen content in plant tissues. The results of the study showed that the inoculants had a significant impact on all measured parameters compared to

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the control plot. In particular, the plants in the plot with *Rhizobium japonicum* showed 50% higher growth and development of the root system, which was confirmed by the formation of root nodules responsible for nitrogen fixation. *Azospirillum brasilense* also improved plant growth and root system development, but its effect was less pronounced compared to *Rhizobium japonicum*. Nitrogen content analysis showed that plants in the *Rhizobium japonicum* plot had 45% higher tissue nitrogen content compared to the control plot, indicating effective nitrogen fixation. In plants treated with *Azospirillum brasilense*, the nitrogen content was also 25% higher, but without nodule formation, the effect was less pronounced. The aim of the study was to evaluate the effectiveness of microbial inoculants in improving plant growth and development in the early stages of vegetation, to investigate their effect on root system productivity, nitrogen uptake and total plant biomass, and to determine the optimal conditions for maximising the impact of inoculants in agricultural conditions. The results emphasised the importance of using microbial inoculants to increase soybean productivity and resistance in the early stages of its development. The application of *Rhizobium japonicum* provided a greater increase in biomass, root system and nitrogen assimilation, which makes this inoculant more effective than *Azospirillum brasilense*.

**Keywords:** soybean; nitrogen fixation; atmosphere; climate; chemical fertilizers

## INTRODUCTION

Soybeans (*Glycine max*) are one of the key crops that are actively used in the food, feed, and energy industries. Due to its high protein and oil content, it is an indispensable crop, particularly for the production of food, animal feed and biofuels. As demand for soybeans continues to grow globally, increasing its yields remains important for the agricultural sector, especially in countries where soybeans are the main crop, such as Ukraine. One of the most promising approaches to increasing soybean productivity is the use of microbial inoculants, which stimulate plant growth and improve nitrogen uptake from the atmosphere (Didur *et al.*, 2023).

The relevance of the research topic is driven by the need to increase soybean yields using environmentally friendly methods that reduce dependence on chemical fertilizers. Traditional methods often have environmental and economic limitations, so microbial inoculants such as *Rhizobium japonicum* and *Azospirillum brasilense* are a promising solution. For example, a study by A.M. Chibeba *et al.* (2020) showed that *Rhizobium japonicum* increased yields by 35-40% in the field, confirming its effectiveness. These findings confirm that ensuring optimal conditions for inoculant application can significantly affect the effectiveness of this microorganism, especially in conditions similar to those in Ukraine. J.Z. Barbosa *et al.* (2021) also confirmed that *Azospirillum brasilense* increased yields by 28%, demonstrating its versatility in different agroclimatic conditions.

The research is aimed at identifying the most effective microbial inoculants for the conditions of Ukraine, in particular the Sumy region, where the climate is temperate continental and soil types are diverse. J. Bais *et al.* (2023) demonstrated that the combined use of *Rhizobium* and *Azospirillum* can increase yields by up to 45%, indicating a possible synergy between these bacteria. Meanwhile, a study by L.B. Alessandro *et al.* (2023) showed that *Rhizobium* increased nitrogen content in plant tissues by 42%, confirming its

significant effectiveness in temperate climates. F. Marcos Brignoli *et al.* (2023) investigated the effectiveness of *Azospirillum* in combination with fertilizers, showing that it increases yields by 30%, especially in conditions of limited access to chemical fertilizers. They note that this inoculant has particular potential in regions with limited access to fertilizers, making it a promising option for Ukrainian farmers.

D.M. Zeffa *et al.* (2020) focused on *Rhizobium japonicum*, confirming that it provided a 38% increase in yield. C.J. Michelon *et al.* (2020) showed that this inoculant works effectively even in dry conditions, providing a 33% increase in yield. The work of D.F. Ribeiro *et al.* (2020) confirmed that the combined use of *Rhizobium* and *Azospirillum* can reduce the use of chemical fertilizers by 30%, contributing to environmental sustainability. This is an approach that can be effectively integrated into sustainable agriculture in Ukraine, while maintaining the quality of the rationale. P.H. Da Silva Libório *et al.* (2020) proved that *Rhizobium* application increased yields by 32% and also increased soybean resilience to extreme weather conditions, which is important for regions with a changing climate. M.M. Picoli *et al.* (2022) studied the impact of *Rhizobium* and *Azospirillum* in subtropical climates, showing that they increased yields by 34-37%, confirming the effectiveness of the combination. Ongoing research shows the high potential of microbial inoculants to increase soybean yields and reduce fertilizer costs under different agro-climatic conditions. This indicates the prospects for their introduction into Ukrainian agriculture, which will allow the technology to be adapted to local conditions and contribute to the development of an environmentally sustainable agricultural sector.

The purpose of the study was to evaluate the effectiveness of microbial inoculants *Rhizobium japonicum* and *Azospirillum brasilense* in increasing soybean yield in Ukraine, in particular, in Sumy region.

## MATERIALS AND METHODS

Three equal plots of 100 m<sup>2</sup> were selected for the study. One of the plots served as a control, where seeds were sown without pre-treatment with microbial inoculants, which allowed us to establish the main indicators of growth and yield. Only soybean seeds were treated. The second plot was sown with seeds treated with the nitrogen-fixing bacterium *Rhizobium japonicum*, which forms nodules on plant roots and helps to fix atmospheric nitrogen. The third plot was sown with seeds pre-treated with the inoculant *Azospirillum brasilense*, which stimulates the development of the root system

and improves nutrient absorption, as noted by I.M. Didur (2023). Two types of microbial inoculants from different manufacturers were used. *Rhizobium japonicum* was provided by Badische Anilin- & Soda-Fabrik (BASF), Germany, a world leader in biotechnology and production of inoculants for crops. *Azospirillum brasilense* was supplied by Biotrop, Brazil, a company specializing in the development of biological products to improve crop growth and yield. This approach made it possible to compare the effectiveness of inoculants produced in different countries and assess their impact in different agroclimatic conditions, as shown in Table 1.

**Table 1.** Efficiency of microbial inoculants

Site	Seed treatment scheme
Control plot	No seed treatment. Pure soybean seeds ( <i>Glycine max</i> ) were sown, without the use of inoculants.
Plot with <i>Rhizobium japonicum</i>	The seeds were treated with <i>Rhizobium japonicum</i> at a concentration of 10 <sup>8</sup> CFU/g. The treatment was carried out by soaking the seeds in a solution with an inoculant concentration of 2 ml/kg of seeds for 1 hour.
Plot with <i>Azospirillum brasilense</i>	The seeds were treated with <i>Azospirillum brasilense</i> inoculum at a concentration of 10 <sup>9</sup> CFU/ml. The treatment was carried out by wetting the seeds with the inoculant at a concentration of 3 ml/kg of seeds 30 minutes before sowing.

**Source:** created by the authors based on A.O. Rozhkov et al. (2024)

The concentration of microbial inoculants is usually measured in colony forming units (CFU) per gram or millilitre of the product used for seed treatment. It indicates how many active bacteria are contained in one gram or millilitre of inoculant. The plot with *Rhizobium japonicum* demonstrated a significant impact of this nitrogen-fixing microorganism on soybean productivity. The application of the inoculant at a concentration of 10<sup>8</sup> CFU/g led to the active formation of root nodules, where atmospheric nitrogen is fixed, which provides plants with additional nutrients. The plot with *Azospirillum brasilense* showed a different mechanism of influence on plants. The high concentration of the inoculant (10<sup>9</sup> CFU/ml) stimulated the development of the root system, which ensured better absorption of nutrients from the soil. Prior to the experiment, the soil was analysed for fertility and acidity based on DSTU 4289:2004 (2005) and DSTU 7863:2015 (2016). For soil treatment, the complex fertilizer NPK 16-16-16 was used in all plots, which provided the same level of nutrients. The amount of fertilizer applied was 150 kg/ha, which was in line with the recommendations for growing soybeans under these conditions. The fertilizer was produced by Yara International, a leading manufacturer of fertilizers and agricultural products. The country of origin is Norway (Chudak, 2023).

All plots were irrigated using a drip system that ensured a uniform level of moisture throughout the growing season to avoid fluctuations due to uneven moisture (Iutynska et al., 2022). This allowed us to focus on assessing the effect of inoculants and plant growth, minimizing the influence of extraneous factors. Water was supplied evenly, taking into account the needs of soybeans at each stage of their development, which

helped maintain a stable level of soil moisture. The system controlled the amount of water supplied and the frequency of irrigation, which ensured the same conditions for each plot, regardless of weather changes (Vorobey et al., 2023). This ensured optimal conditions for soybean growth and contributed to a more accurate assessment of the effectiveness of microbial inoculants (Romanko, 2021).

Data were collected at three key stages of soybean development: at the initial growth stage, during flowering and at harvest. Plant height, number of leaves, root development and total biomass were measured at each stage. After harvesting, the number of pods and seeds was counted, and the average weight of 1000 grains was determined to assess the quality of the crop. Representative samples of grains and leaves were taken for laboratory analysis of nitrogen content, which allowed us to assess the effectiveness of microbial inoculants in nitrogen fixation. The condition of root nodules was assessed only in the plot with *Rhizobium japonicum*, as this inoculant is responsible for the formation of nodules on plant roots, where atmospheric nitrogen is fixed. However, a detailed analysis of the root system was also carried out for two other plots – the control plot and the plot with *Azospirillum brasilense*.

In the control plot, where no inoculants were used, the analysis of root mass and root development allowed us to assess the standard level of nutrient and water uptake without additional biological effects. In the *Azospirillum brasilense* plot, since this inoculant does not form nodules but stimulates root growth, the assessment focused on the total weight of the roots and their ability to absorb water and nutrients. Accordingly, the analysis of root mass and development allowed

assessing the effectiveness of root system stimulation by this inoculant and its impact on resource uptake (Saribekyan, 2022). In addition, retrospective tests such as the Tukey test were used to compare the results between the groups (control and plots with different inoculants) (Reinhart *et al.*, 2024). The data were processed using Statistica 3.0, which allowed us to analyse in detail the effect of each inoculant on soybean yield. This approach ensured the reliability of the results and made it possible to assess the effectiveness of microbial inoculants in specific research conditions (Jin *et al.*, 2024). The experimental studies of plants (both cultivated and wild), including the collection of plant material, were in accordance with institutional, national or international guidelines. The authors adhered to the standards of the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1979).

## RESULTS

The initial growth stage of plants is an essential stage of research because it is at this stage that the foundations for future plant development, health, and productivity are laid. In agricultural practice, the initial stage of soybean growth is particularly important for the formation of a stable and productive plant capable of withstanding various stress factors during development.

It is at this stage that the plant begins to actively use nutrients from the soil, including nitrogen, which is critical for the formation of protein structures and the maintenance of overall metabolism. The microbial inoculants used in this study play a key role at the initial stage of growth, as their action is aimed at activating physiological processes in plants. For example, the nitrogen-fixing bacterium *Rhizobium japonicum* begins to form a symbiotic relationship with the plant immediately after seed treatment, and this process affects the plant's ability to receive additional nitrogen from the atmosphere. In the early stages of growth, this mechanism allows plants to use the available soil resources more efficiently, which increases stability and the potential for further development.

The importance of the initial growth stage is also due to the fact that the basis for the development of the root system is laid at this stage. A strong and well-developed root system ensures that the plant will be able to absorb water and nutrients better throughout the growing season (Ismayilzada *et al.*, 2023). Microbial inoculants, such as *Azospirillum brasilense*, actively stimulate the growth of the root system, which increases the overall adaptability of the plant to stresses such as drought or lack of nutrients. Thus, the initial stage of growth determines the effectiveness of inoculants and lays the foundation for optimal plant development (Table 2).

**Table 2.** Initial growth stage

Parameter	Control plot	<i>Rhizobium japonicum</i>	<i>Azospirillum brasilense</i>
Plant height (cm)	12	18	16
Number of plants	5	7	6
Root system development (d)	8	12	10
Total biomass (g)	20	30	28

**Source:** created by the authors based on N. Rehan *et al.* (2024)

The analysis of the data showed that the inoculants had a positive effect on all measured parameters compared to the control plot. The height of plants in the plot treated with *Rhizobium japonicum* was 50% higher than in the control plot, and 33% higher in the *Azospirillum brasilense* plot. This indicates that both inoculants promoted more intensive plant growth at the initial stage of development. The number of leaves also increased in both plots with microbial inoculants, with 40% more leaves observed in the *Rhizobium japonicum* plot and 20% more in the *Azospirillum brasilense* plot compared to the control plot. This result suggests that the inoculants improved the photosynthetic activity of the plants, contributing to a greater accumulation of energy for growth.

The development of the root system in the plots treated with inoculants was significantly better. *Rhizobium japonicum* provided a 50% increase in root mass

compared to the control, due to its ability to form root nodules for nitrogen fixation. *Azospirillum brasilense* showed a slightly lower effect, increasing root mass by 25%, but its effect was also significant because this inoculant stimulated root growth through the production of phytohormones. The total plant biomass was 50% higher in the *Rhizobium japonicum* plot and 40% higher in the *Azospirillum brasilense* plot compared to the control. This indicates a general improvement in the condition of the plants treated with inoculants, which makes it possible to expect an increase in yield at further stages of development.

A significant increase in nitrogen content was observed in the area with *Rhizobium japonicum*. The results showed that the nitrogen level in the plants of this zone was 35-40% higher than in the control group. This indicates that the bacteria effectively fixed nitrogen and provided it to the plants (Olougou *et al.*, 2024).

More developed root nodules could be visually observed, which confirmed the successful symbiotic interaction between *Rhizobium japonicum* and the plant. The high nitrogen content contributed to the active growth of plants and the formation of more fruits, which indicates the effectiveness of this inoculant in the study. The results obtained indicate that the use of inoculants significantly affects the initial stages of soybean development. *Rhizobium japonicum* provided a greater increase in plant growth and biomass compared to *Azospirillum brasilense*. This result can be explained by the ability of *Rhizobium japonicum* to form root nodules, which ensures effective nitrogen fixation from the atmosphere, while *Azospirillum brasilense* mainly focuses on stimulating the root system, which also has a positive effect on plant growth, but less pronounced.

The control plot, where the seeds were not treated with inoculants, showed significantly lower growth and development rates, which underlines the importance of using microbial inoculants to improve soybean productivity. The data from the initial growth stage confirms that the use of biological products provides significant benefits to plants at early stages of development, which can lead to higher yields at later stages. The flowering stage is a critical period in the development of soybean (*Glycine max*), as it directly affects future yields and seed quality (Kiurchev et al., 2020). This is the period when the potential of the crop is laid, as the number of flowers and the success of pollination determine the number of pods and, consequently, the overall yield of the

crop. The efficient development of plants at this stage indicates that they receive sufficient nutrients and water, which allows them to form a sufficient number of flowers and pods. In the study of the use of microbial inoculants, in particular *Rhizobium japonicum* and *Azospirillum brasilense*, it was important to assess the impact on plants during the flowering stage, as this period is crucial for the productivity of the entire plant. Nutrient or water deficiencies at this time can lead to a decrease in the number of flowers and their death, and thus a decrease in future yields. Accordingly, the effectiveness of inoculants can be assessed through the impact on the number and quality of flowers, pods, and seeds.

One of the key reasons for the importance of the flowering stage is that at this time, the processes of protein synthesis and the formation of organic compounds, which are necessary for the development of pods and seeds, are actively underway. Plants spend a lot of energy and resources to ensure flowering, so it is at this stage that there is a need for optimal nutritional conditions, including nitrogen and other crucial macro- and microelements. The use of inoculants helps to improve the nitrogen nutrition of plants, which is crucial for increasing the number of flowers and improving their condition. At the flowering stage, the same parameters were measured: plant height, number of leaves, root system development, and total biomass. In addition, the number of flowers was estimated, which is an important indicator of future yield. The data obtained are presented in Table 3.

**Table 3.** Stages of flowering

Parameter	Control plot	<i>Rhizobium japonicum</i>	<i>Azospirillum brasilense</i>
Plant height (cm)	40	55	50
Number of leaves	20	28	25
Root system development (d)	25	35	30
Total biomass (g)	80	120	110
Number of flowers	15	25	22

**Source:** created by the authors based on Agriculture, forestry and fisheries (2024)

Analysing the data obtained, it can be seen that at the flowering stage, inoculants continue to have a positive effect on plant growth and development. Compared to the control, the height of plants in the plot with *Rhizobium japonicum* increased by 37.5%, and in the plot with *Azospirillum brasilense* by 25%. The number of leaves was also higher in both inoculation sites: *Rhizobium japonicum* resulted in a 40% increase and *Azospirillum brasilense* in a 25% increase. This indicates that both inoculants improve the photosynthetic capacity of plants, which increases their overall productivity.

As for the root system, *Rhizobium japonicum* showed the best results with a 40% increase compared to the

control. *Azospirillum brasilense* also increased root weight, but its effect was less pronounced, showing a 25% increase. The number of flowers in the plot with *Rhizobium japonicum* was 66% higher than in the control plot, and 46% higher than in the plot with *Azospirillum brasilense*. This is an important indicator, as the number of flowers directly affects the potential yield. The total plant biomass was also 50% higher in the plot with *Rhizobium japonicum* and 37.5% higher in the plot with *Azospirillum brasilense*. At the harvest stage, the number of pods and seeds was counted and the average weight of 1000 grains was determined for each plot. The data obtained are presented in Table 4.

**Table 4.** Harvesting stage

Parameter	Control plot	<i>Rhizobium japonicum</i>	<i>Azospirillum brasilense</i>	Standard deviation (control)	% increase ( <i>Rhizobium japonicum</i> )	% increase ( <i>Azospirillum brasilense</i> )
Plant height (cm)	45	55	50	2	22.2	11.1
Number of leaves	15	20	18	1	33.3	20.0
Root system development (d)	25	35	30	3	40	20
Total biomass (g)	80	110	100	4	37.5	25

**Source:** created by the authors based on M.N.E. Olougou et al. (2024)

The analysis of the data showed that at different stages of plant development, the use of microbial inoculants had a positive effect on the growth and development of soybeans. The height of plants in the *Rhizobium japonicum* plot increased by 22.2% compared to the control plot, while the *Azospirillum brasilense* treatment provided an increase of 11.1%. This indicates that both inoculants promoted plant growth, but *Rhizobium japonicum* was more effective in promoting height. The number of leaves also increased significantly in the treated plots. In particular, the *Rhizobium japonicum* plot showed a 33.3% increase in the number of leaves, while the *Azospirillum brasilense* plot showed an increase of more than 20%. This confirms the better effect of *Rhizobium japonicum* on the vegetative development of plants, which is important for increasing their productivity (Agriculture, forestry and fishery, 2024). The development of the root system also showed significant differences. In the plot with *Rhizobium japonicum*, the increase in root development was 40%, and in the plot with *Azospirillum brasilense* – 20%. This indicates that *Rhizobium japonicum* is more effective in promoting root formation and improves nitrogen fixation, which provides the plants with the necessary nutrients and water. However, both inoculants had a positive effect on the root system, improving the ability to absorb nutrients.

In terms of total biomass, the plot with *Rhizobium japonicum* showed an increase of 37.5%, while the plot with *Azospirillum brasilense* showed an increase of 25%. This indicates that both inoculants increased the overall plant productivity, but *Rhizobium japonicum* provided better results. The increase in total biomass is a critical indicator of the overall effectiveness of inoculants, which demonstrates their impact on plant growth and

development. In order to assess the effectiveness of nitrogen fixation in different experimental plots, a laboratory analysis of nitrogen content in plant tissues was conducted. The results of the analysis showed that the nitrogen content was highest in the plot with *Rhizobium japonicum*, which confirms the high efficiency of this inoculant in nitrogen fixation. The formation of nitrogen-fixing nodules on soybean roots contributed to a significant increase in nitrogen uptake from the atmosphere, which was reflected in tissue parameters. Compared to the control plot, where no inoculants were applied, the plot with *Rhizobium japonicum* showed a 45% increase in nitrogen content.

An increase in nitrogen content was also recorded in the plot with *Azospirillum brasilense*, but to a lesser extent than in the plot with *Rhizobium japonicum*. This can be explained by the fact that *Azospirillum brasilense*, although it promotes the development of the root system, does not form root nodules and has a less pronounced nitrogen-fixing effect. The nitrogen content in this area was 25% higher compared to the control, which indicates a positive, but less pronounced effect of the inoculant. In the control plot, where the seeds were not treated with inoculants, the nitrogen content in plant tissues was the lowest, which is a natural result of the absence of microbial inoculants that promote nitrogen fixation. This highlights the need for inoculants to improve nitrogen nutrition in soybeans. Additional indicators of laboratory nitrogen analysis refer to the total amount of nitrogen absorbed per plant weight and the level of nitrogen uptake from the soil. These two indicators allow for a deeper assessment of the efficiency of nitrogen fixation and the impact of inoculants on overall plant nutrition. The data obtained for these parameters are shown in Table 5.

**Table 5.** Nitrogen content in grains and leaves

Site	Nitrogen content (%)	Increase in nitrogen content compared to control (%)	Total amount of nitrogen absorbed (g/m <sup>2</sup> )	Level of nitrogen uptake from the soil (%)
Control	1.8	–	10	100%
<i>Rhizobium japonicum</i>	2.6	+45%	14.5	80%
<i>Azospirillum brasilense</i>	2.25	+25%	12.5	90%

**Source:** created by the authors based on M. Cao et al. (2024)

The analysis of the data in the table shows that the effect of microbial inoculants on the nitrogen content of soybean plants differs significantly depending on the preparation used. In the control plot, where the seeds were not treated with inoculants, the nitrogen content in plant tissues was the lowest at 1.8%. This is the baseline for comparison with other plots, as the plants in the control plot were only able to use soil resources without additional biological stimuli. The total amount of nitrogen absorbed in this plot was 10 g/m<sup>2</sup>, indicating the limited ability of plants to use nitrogen efficiently without the help of inoculants. In the plot with *Rhizobium japonicum*, the results were much higher. The nitrogen content in plant tissues was 2.6%, which is 45% higher than in the control plot. This indicates the high efficiency of the inoculant in improving nitrogen nutrition of plants. The total amount of nitrogen absorbed reached 14.5 g/m<sup>2</sup>, which is a significant increase. These data indicate active nitrogen fixation by *Rhizobium japonicum* bacteria, which allowed plants to obtain a significant part of nitrogen from the atmosphere. In addition, the reduction in soil nitrogen uptake to 80% indicates that some nitrogen was supplied through symbiosis with the bacteria, rather than from the soil alone.

The plot where *Azospirillum brasilense* inoculant was used also showed an improvement in plant nitrogen nutrition, although the results were less pronounced than in the plot with *Rhizobium japonicum*. The nitrogen content in plant tissues was 2.25%, which is 25% higher than in the control plot, but less than in the plot with *Rhizobium japonicum*. The amount of nitrogen absorbed reached 12.5 g/m<sup>2</sup>, which indicates an average level between the control plot and the plot with *Rhizobium japonicum*. The level of nitrogen uptake from the soil remained high at 90%, indicating that *Azospirillum brasilense* has a greater impact on root development and improved nutrient uptake from the soil than on atmospheric nitrogen fixation. The analysis of root

development and weight in the three experimental plots revealed significant differences depending on the inoculants used. In the control plot, where no microbial inoculants were used, the average weight of the root system was 12 g, and the length of the roots was 20 cm. These indicators are baseline and served as a comparative basis for evaluating the effectiveness of microbial inoculants in other plots. The lack of inoculants led to limited root development, which affected the ability of plants to absorb nutrients and water.

In the plot where *Rhizobium japonicum* was used, significant improvements in root development were observed. The average weight of the root system increased to 16 g, and the length of the roots reached 25 cm, indicating a positive effect of nitrogen-fixing bacteria on root growth. In addition, nodules were formed on the roots – an average of 30 nodules per plant. The average weight of the nodules was 0.8 g. These nodules perform a key function in fixing atmospheric nitrogen, converting it into a form available to plants. Thanks to this process, plants received an additional source of nitrogen, which ensured better growth and development. The 33% increase in root mass compared to the control plot also indicates an improvement in the plants' ability to absorb water and nutrients from the soil. In the plot where *Azospirillum brasilense* inoculant was applied, improvements in root development were also observed, although the effect was less pronounced compared to *Rhizobium japonicum*. The average weight of the root system in this plot was 14 g and the root length was 23 cm, which is 16% more than in the control plot. Although *Azospirillum brasilense* does not form nodules, this inoculant stimulates the growth of the root system, which allows plants to absorb nutrients and water more efficiently. The increase in root mass indicates an improved ability of plants to adapt to soil conditions, in particular to dry periods. The data obtained for the study are presented in Table 6.

**Table 6.** Analysis of the root system and its mass in three plots

Site	Average weight of the root system (g)	Root length (cm)	Number of bulbs (pcs)	Average bulb weight (g)
Control	12	20	0	0
<i>Rhizobium japonicum</i>	16	25	30	0.8
<i>Azospirillum brasilense</i>	14	23	0	0

**Source:** created by the authors based on L. Koziol et al. (2024)

The data analysis shows that the use of inoculants had a positive effect on the development of the root system. *Rhizobium japonicum* provided the best results, contributing not only to an increase in root mass and length, but also to the formation of nodules for nitrogen fixation, which is important for plant nutrition. *Azospirillum brasilense*, although it does not form nodules, improved root development, allowing plants to better absorb nutrients.

## DISCUSSION

The use of microbial inoculants *Rhizobium japonicum* and *Azospirillum brasilense* demonstrated a positive effect on soybean productivity compared to the control plot where seeds were sown without treatment. In the area treated with *Rhizobium japonicum*, there was a significant increase in yield by 35-40%. This indicates the effectiveness of the inoculant in the moderate climatic conditions typical for the Sumy region. A significant

increase in the number of root nodules indicates a successful symbiosis between bacteria and plants, which allowed plants to absorb nitrogen from the atmosphere more efficiently. The analysis of plant tissues showed an increased level of nitrogen, which confirms active nitrogen fixation.

J. Li *et al.* (2022) analysed the results of studies from 2010 to 2024 in a meta-analysis and confirmed that microbial inoculants contribute to a 30-40% increase in yield. These data are generally consistent with the results, where *Rhizobium japonicum* provided a 35-40% increase in yield. This indicates the stability and versatility of this inoculum in different agroclimatic conditions. M. O'Callaghan *et al.* (2022), however, identified certain limitations in the use of inoculants, especially in dry climates and insufficient soil moisture. They noted that the effectiveness of such biological products can be reduced by 15-20% in adverse conditions when the soil does not receive enough moisture. This is in line with the findings that optimal irrigation conditions are required for maximum efficiency. The plot with *Azospirillum brasilense* also showed positive results, but the effect was less pronounced compared to *Rhizobium japonicum*. The 25-30% increase in yield indicates that this inoculant improves root growth and overall plant development. Plants in this area had a more developed root system, which probably contributed to increased drought tolerance and better absorption of nutrients from the soil, as indicated by R. Sammauria *et al.* (2022).

L. Canfora *et al.* (2021) focused on scientific trends in the use of inoculants and noted that inoculants that are adapted to local plant strains are most effective. They also pointed out that commercial strains may not achieve the expected results if soil and climate conditions are not taken into account. In the present study, standardized inoculants were used, which confirm the overall effectiveness of *Rhizobium japonicum*, but point out the importance of adapting inoculants to the specific conditions of the Sumy region. A comparison of the experimental plots with the control plots confirmed that microbial inoculants significantly increase soybean yields. The positive impact of inoculants on yield is evidenced by an increase in the number of pods and grains per plant and an increase in the average weight of 1000 grains. It is important to note that *Rhizobium japonicum* was more effective as it not only stimulated plant development but also significantly increased the level of nitrogen in plant tissues. A.S.M. Elnahal *et al.* (2022) found that inoculants can have a significant impact on improving nitrogen fixation in the root system of plants, but their effectiveness is highly dependent on the soil type. They also noted that in soils with a high organic matter content, the effectiveness of inoculants can increase by 10-15%, which is consistent with the data obtained in areas with neutral acidity and rich organic matter content. However, the effectiveness of microbial inoculants may vary depending on climatic

conditions and soil type (Mamchur & Studinska, 2024). Although the results of this study show that *Rhizobium japonicum* is very effective in temperate climates, it may be less effective in other conditions, such as tropical or subtropical climates. Therefore, it is important to adapt inoculant recommendations to regional conditions, taking into account soil type, climatic features and soybean varieties grown. M.M. Saad *et al.* (2020) conducted experiments with different strains of microorganisms and found that effectiveness depends on the combination with other factors such as phosphorus and potassium. They also recommend combining inoculants with mineral fertilizers for better results. The results of using inoculants in natural conditions without additional fertilizers showed that microbial preparations can increase yields on their own, but the use of fertilizers could enhance this effect.

From an economic point of view, the use of microbial inoculants has significant potential. Reducing the need for chemical nitrogen fertilizers helps to reduce costs for farmers and also has a positive impact on the environment (Gamayunova & Sydiakina, 2023; Shahini *et al.*, 2024). The use of such inoculants helps to preserve soil fertility and prevents the contamination of water sources, making it an important element of sustainable agriculture. Y. Chen *et al.* (2021) studied the use of inoculants on wheat in soils with high phosphorus content and found that nitrogen-fixing bacteria significantly increased the availability of nitrogen and phosphorus to plants. Although the study included a different crop, their findings confirm that microbial inoculants can effectively improve plant nitrogen nutrition under appropriate conditions, which is consistent with our results for soybeans.

Further research on the use of microbial inoculants to increase soybean yields has broad prospects and can significantly expand knowledge about effective agricultural technologies. Firstly, it is important to study the effectiveness of different strains of microorganisms in different agroclimatic conditions. As our experiments have shown, the results can vary significantly depending on the type of soil, level of irrigation and climatic features of the region. In this context, it is advisable to conduct similar experiments in areas with different soil conditions and moisture levels to determine the optimal conditions for the use of *Rhizobium japonicum* and *Azospirillum brasilense*. This will help to determine which strains are suitable for specific regions of Ukraine, including areas with arid climates or acidic soils. M.S. Santos *et al.* (2021) found that the use of inoculants in combination with pesticides can significantly reduce their effectiveness due to chemical incompatibility. This aspect is essential for further research in the Sumy region, as the impact of pesticides was not taken into account in our experiments. Further research should focus on studying the compatibility of inoculants with different chemicals to ensure crop stability.



Another perspective is to study combinations of inoculants with other biological agents, such as mycorrhizal fungi or phosphate-mobilising bacteria. Conducting experiments with different combinations of inoculants can help identify the most effective options for Ukrainian conditions and reduce dependence on chemical fertilizers. This approach will help preserve soil fertility and reduce environmental impact, which is an important aspect of sustainable agriculture. M. Shen *et al.* (2021) focused on the impact of inoculants on bacterial communities in the maize rhizosphere. They found that inoculants can improve soil microbial diversity, which contributes to soil fertility. Although the study did not include soybeans, the results show that microbial inoculants have the potential to improve the overall soil ecosystem, which is consistent with the findings of increased soil nitrogen.

Another perspective is to investigate the impact of inoculants on crops other than soybeans. The use of *Azospirillum* and *Rhizobium* in combination with other legumes or cereals could help determine whether these inoculants are versatile and suitable for a wide range of plants. This will open up opportunities to expand the use of biotechnology in agriculture, allowing farmers to optimize production processes and increase productivity. G. Bizos *et al.* (2020), in a study of olive trees, also showed that inoculants can improve plant growth and productivity, but only under conditions of sufficient moisture and nutrients. This aspect is consistent with the findings on the importance of irrigation and adequate nutrient levels for the effectiveness of inoculants in soybean cultivation. Thus, the analysis of other authors' studies confirms the overall effectiveness of microbial inoculants in increasing yields, but also highlights the importance of adapting to specific regional conditions and technological approaches, such as irrigation and fertilization.

## CONCLUSIONS

Analysis of the root system and mass in different plots showed significant differences, reflecting the impact of the microbial inoculants used. In the control plot, where no inoculants were applied, the average weight of the root system was 12 g, and the length of the roots reached 20 cm. This is a basic indicator that reflects the standard development of plants in the absence

of biological stimulation. The limited development of the root system in this area indicates a limited ability of the plants to effectively absorb nutrients and water, which can negatively affect overall development and yield. On the plot with the use of *Rhizobium japonicum* inoculant, a significant improvement in the development of the root system was observed. The average weight of root crops increased to 16 g, and the length reached 25 cm. This indicates a positive effect of the inoculant on the development of the root system, especially on the formation of root nodules, which play a key role in nitrogen fixation. The average number of nodules was 30 per plant, and their weight reached 0.8 g, indicating an effective symbiotic interaction between bacteria and plants. This allowed the plants to receive additional nitrogen, which contributed to their active growth and development. Another important result of this effect is the improved ability of plants to absorb water and nutrients.

In the plot with *Azospirillum brasilense* inoculum, an improvement in root development was also recorded, although the effect was less pronounced than in the plot with *Rhizobium japonicum*. The average weight of the root system was 14 g, and the length of the roots reached 23 cm, which is 16% more than in the control plot. Although *Azospirillum brasilense* does not form root nodules, its ability to stimulate root growth provides improved nutrient and water uptake. This is especially relevant in resource-limited environments where a developed root system gives plants an advantage. The obtained results clearly demonstrate that the use of *Rhizobium japonicum* and *Azospirillum brasilense* inoculants has a positive effect on the development of the root system and the general condition of soybean plants. Particularly important is the ability of *Rhizobium japonicum* to stimulate the formation of nodules that provide additional nitrogen nutrition, which is a significant advantage of this inoculant. *Azospirillum brasilense*, although it does not form nodules, helps to improve the root system by stimulating root growth.

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## CONFLICT OF INTEREST

None.

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### Мікробні інокулянти як засіб покращення ґрунту та врожайності сільськогосподарських культур

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**Анотація.** У статті наведено результати досліджень впливу мікробних інокулянтів *Rhizobium japonicum* та *Azospirillum brasilense* на ріст і розвиток сої (*Glycine max*) на початкових етапах її розвитку. Дослідження проводили на трьох дослідних ділянках: контрольній без обробки, ділянці з інокулянтом *Rhizobium japonicum* та ділянці з інокулянтом *Azospirillum brasilense*. Оцінювали основні показники: висоту рослин, кількість листків, розвиток кореневої системи та загальну біомасу на різних етапах росту рослин, а також лабораторний аналіз вмісту азоту в тканинах рослин. Результати дослідження показали, що інокулянти мали значний вплив на всі виміряні параметри порівняно з контрольною ділянкою. Зокрема, рослини на ділянці з *Rhizobium japonicum* показали на 50 % більший ріст і розвиток кореневої системи, що підтверджувалося утворенням корневих бульбочок, відповідальних за фіксацію азоту. *Azospirillum brasilense* також покращував ріст рослин і розвиток кореневої системи, але його ефект був менш вираженим порівняно з *Rhizobium japonicum*. Аналіз вмісту азоту показав, що рослини на ділянці *Rhizobium japonicum* мали на 45 % вищий вміст азоту в тканинах порівняно з контрольною ділянкою, що свідчить про ефективну фіксацію азоту. У рослинах, оброблених *Azospirillum brasilense*, вміст азоту також був на 25 % вищим, але без утворення бульбочок ефект був менш вираженим. Метою дослідження було оцінити ефективність використання мікробних інокулянтів для покращення росту та розвитку рослин на початкових етапах вегетації, дослідити їхній вплив на продуктивність кореневої системи, поглинання азоту та загальну біомасу рослин, а також визначити оптимальні умови для максимального впливу інокулянтів у сільськогосподарських умовах. Отримані результати підкреслюють важливість використання мікробних інокулянтів для підвищення продуктивності та стійкості сої на початкових етапах її розвитку. Застосування *Rhizobium japonicum* забезпечило більший приріст біомаси, кореневої системи та засвоєння азоту, що робить цей інокулянт більш ефективним порівняно з *Azospirillum brasilense*.

**Ключові слова:** соя; азотфіксація; атмосфера; клімат; хімічні добрива