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The influence of risogumin on soybean yield components and resistance to abiotic stress

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ABSTRACT

In modern agriculture, the importance of environmentally safe technologies that promote sustainable farming and minimize negative environmental impact is growing. This study investigates the effectiveness of biological preparations in soybean (*Glycine max* L.) cultivation technology, a key crop due to its high protein content and ability to improve soil fertility through nitrogen fixation. Particular attention was paid to the inoculant Rhizogumin, which contains effective microorganisms that enhance the physiological state of plants. Field experiments were conducted from 2022 to 2024 in the field of the North-East Research Institute of Agriculture in the Sumy region of Ukraine. The experiment was two-factorial, involving the application of inoculation and foliar treatments. The obtained results demonstrated that pre-sowing seed treatment with Rhizogumin significantly increased the number and mass of nodules on soybean roots at all developmental stages, indicating successful intensification of symbiotic activity. The best nodulation indicators were observed in variants with pre-sowing inoculation, especially when combined with complex foliar treatments. This approach also positively influenced the formation of soybean yield structure components, particularly increasing the number of pods and seeds per plant, confirming the synergistic effect of the complex treatment. The study emphasizes that while inoculation and foliar treatments significantly improve key physiological indicators and soybean productivity components, the effectiveness of their impact on yield can be negated by abiotic stresses. These findings highlight the need for further research into the interaction of biological preparations with soybean varietal characteristics under changing climatic conditions, as well as the development of integrated strategies aimed at minimizing the impact of stress factors to ensure consistently high yields.

Keywords: soybean, inoculation, rhizogumin, nodulation, yield, abiotic stress, biopreparations, water stress.

INTRODUCTION

In modern agriculture, sustainability-minded farmers are increasingly trying to implement environmentally friendly technologies in their practice [Datsko et al., 2025; Butenko et al., 2025a]. Specifically, they use biofertilizers and plant protection products containing effective microorganisms. In addition, zero or minimum tillage is applied to preserve soil biota, which is necessary for faster decomposition of organic residues in the field. Such steps are essential for increasing crop productivity by preserving or multiplying organic matter in the soil if the above-mentioned practices are applied successfully and correctly [Karpenko et al., 2020; Toleikiene et al., 2021; Kovalenko et al., 2024; Butenko et al., 2025c]. One of the most frequently used methods is inoculation, which involves applying effective microorganisms to the seed surface. These microorganisms improve the physiological state of plants through symbiotic interaction [Lal et al., 2020; Shelest et al., 2023; Butenko et al., 2025b]. This method is based on the natural ability of certain microorganisms, such as nitrogen-fixing bacteria or phosphate-solubilizing microorganisms, to improve nutrient availability for plants or stimulate their growth [Oldfield et al., 2019; Dehodiuk et al., 2024; Kong et al., 2025]. Thanks to inoculation, the need for mineral fertilizers can be significantly reduced, lowering the negative environmental impact and optimizing production costs [Litvinov et al., 2020; Dos Santos Sousa et al., 2022; Karbivska et al., 2023; Kolisnyk et al., 2024]. Inoculation is most commonly used on leguminous crops. Numerous studies confirm its effectiveness, including on soybeans. Specifically, Jabborova et al. [2021] showed that rhizobacteria significantly affect root morphology, increase shoot dry matter, and the number of nodules on the roots. At the same time, Akley et al. [2022] compared the effectiveness of biofertilizers containing Bradyrhizobium bacterial strains. Their research proved a significant impact on soybean plants, particularly on nodule formation and yield increase. Furthermore, the results of Szpunar-Krok et al. [2021] indicate that the Bradyrhizobium strain used to inoculate seeds can also increase the fat content in soybean yield.

Therefore, due to the aforementioned advantages of biological approaches in modern agricultural production, the aim of this study was to evaluate the impact of using the inoculant Rhizogumin on key soybean development indicators. The primary objective was to establish its influence on the intensity of nodule formation on plant roots, which is critically important for nitrogen fixation, as well as on the formation of yield structure and, ultimately, on the final yield of the soybean crop.

MATERIAL AND METHODS

Experimental site

The field experiments took place from 2022 to 2024 at the experimental station of the Institute of Agriculture of the North-East of the National Academy of Agrarian Sciences of Ukraine, located in the Sumy region, Ukraine.

Soil characteristics

The soil on the experimental plots was a typical deep medium-loamy Chernozem. Its key agrochemical characteristics included a humus content of 4.1–4.3% (determined by the Tyurin method) and a pH (salt) between 6.2 and 6.5. The average content of essential nutrients was as follows: nitrogen (Kornfield method) at 128.5 mg kg-1 of soil, phosphorus (Chirikov method) at 211.6 mg kg-1 of soil, and potassium (Chirikov method) at 81.1 mg kg-1 of soil.

Climatic conditions during the growing seasons

Over the three years of the study, the weather conditions varied significantly. In 2022, the total precipitation during the spring durum wheat growing season was 370 mm, which was considerably higher than the long-term average of 237 mm. The monthly distribution was as follows: April – 107 mm, May – 26 mm, June – 155 mm, and July – 82 mm. In 2023, the total precipitation was 222 mm, which was 15 mm below the long-term average. The highest rainfall was in July (80 mm), while May recorded only 17 mm. The 2024 growing season was the driest, with a total of just 150 mm of precipitation, representing a deficit of 87 mm compared to the long-term average. The monthly breakdown included: April – 48 mm, May – 34 mm, June – 51 mm, and July – 17 mm (Figure 1).

Temperature conditions

Throughout the study, average daily temperatures during the spring durum wheat growing

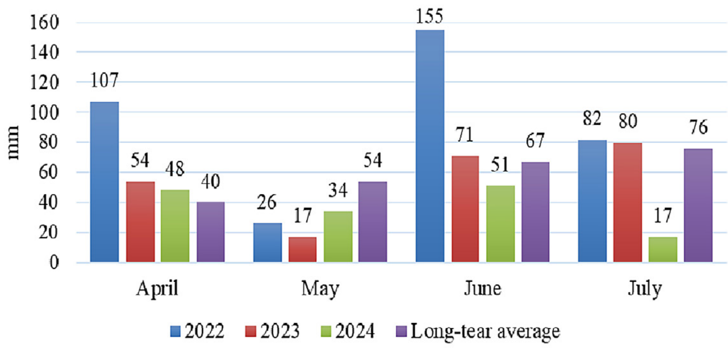

Figure 1. Precipitation during the growing season (2022–2024)

season showed an increasing trend. In 2022, the average temperature was 16.0 °C, slightly above the long-term average of 15.8 °C. The warmest month was July, with an average of 21.3 °C. The following year, 2023, saw a further increase, with the average temperature reaching 16.6 °C, exceeding the long-term average by 0.8 °C. The warmest month was again July, at 21.6 °C. The most significant temperature increase occurred in 2024, when the average daily temperature was a notable 19.2 °C, which was 3.4 °C higher than the long-term average. July of that year was particularly hot, with an average of 25.4 °C (Figure 2).

Meteorological conditions analysis

Overall, 2022 and 2023 were the most favorable years for crop yield development. Conversely, 2024 was marked by predominantly dry conditions, with low precipitation and extreme air temperatures during the growing season. These factors likely had a negative impact on plant development and, consequently, on the final yield.

Experimental design

This study aimed to determine the impact of eco-friendly approaches on improving the symbiotic activity and grain yield of soybeans.

General experimental layout

The study, conducted from 2022 to 2024, consisted of two two-factor experiments using the Siverka soybean variety. The research specifically aimed to evaluate the effectiveness of seed inoculation and foliar treatment on soybean cultivation. Rhizogumin was selected as a standard, commercially available inoculant to serve as a positive control, and the Siverka soybean variety was chosen because it is well-adapted to the local soil and climatic conditions and is widely cultivated in the region. The core of the investigation was to analyze the interaction between two main factors: Factor A, which involved seed treatment, and Factor B, focused on foliar application during the growing season, all following the detailed experimental design outlined in Table 1.

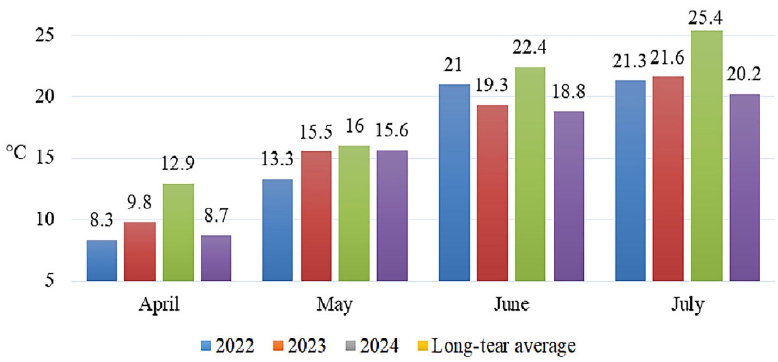

Figure 2. Average daily air temperature during the growing season (2022–2024)

Table 1. Field experiment scheme

Seed treatment (Factor A) plant	Treatment during vegetation (Factor B)
Control (seeds treated with water) – C	No treatment (control) – NT
	Humifield VR-18 (0.4 L ha^{-1}) in the budding phase – BS
	Fulvigrin bor w. s. (0.5 L ha^{-1}) in the flowering phase – FG
	Humifield VR-18 w. s. (0.4 L ha^{-1}) in the budding phase + Fulvigrin bor w. s. (0.5 L ha^{-1}) in the flowering phase BS+FG
Rhizogumin (2 kg t^{-1} seeds) – R	No treatment (control) – NT
	Humifield VR-18 (0.4 L ha^{-1}) in the budding phase – BS
	Fulvigrin bor w. s. (0.5 L ha^{-1}) in the flowering phase – FG
	Humifield VR-18 w. s. (0.4 L ha^{-1}) in the budding phase + Fulvigrin bor w. s. (0.5 L ha^{-1}) in the flowering phase BS+FG

Data collection and measurements

To thoroughly evaluate the field trials, a series of phenological observations were conducted in accordance with the “Methodology of State Variety Testing of Agricultural Crops” and “Methodology for Research in Fodder Production.” Plant growth and development stages were recorded, with the onset of a phase marked when it appeared in 10% of plants and a full phase when it was present in 75%. Plant density was also measured at two key stages: full emergence and before harvest, with three replications for each measurement. Additionally, to assess symbiotic activity, soil monoliths (25 × 25 × 30 cm) were collected to determine the number and mass of rhizobial formations. After the roots were washed, nodules from five plants per replication were separated, counted, and then dried and weighed. Individual plant productivity was assessed by taking a sample sheaf from each variant before harvest. At full maturity, soybean grains were harvested via direct combining at a moisture content of 14–15%, which was verified using a “Wile 55” moisture meter. Plot-wise yield accounting was performed, and samples were collected for further analyses. The 1000-grain weight, a key indicator of grain quality, was determined in accordance with the DSTU – 2949 standard.

Other soil factors were managed by conducting the experiment on a uniform plot of a typical deep medium-loamy Chernozem that was characterized by specific agrochemical properties (e.g., pH 6.2–6.5, humus 4.1–4.3%). The experimental design included a control group (seeds treated with water) to compare the Rhizogumin effect against the natural soil microflora.

Statistical analysis

Mathematical processing of primary data and assessment of reliability were performed using Microsoft Excel. Descriptive statistics were conducted using Statistica 10.0.

RESULTS AND DISCUSSION

The research objectives led to the results described below. Overall, the impact of biofertilizers on the number and mass of nodules on soybean plants, as well as yield structure and, ultimately, crop yield itself, was evaluated.

The first indicator described in Table 2, which reflects changes in the number of nodules on soybean roots by plant development stage, is presented. Specifically, ANOVA results indicate that a significant increase in nodule count is provided by both pre-sowing seed treatment and foliar applications at different stages of crop development. In all variants, a gradual increase in the number of nodules was observed corresponding to the development stage. It’s worth noting that in the variant where seeds were treated with Rhizogumin, the number of nodules was significantly higher compared to the control variant, demonstrating its effectiveness. Thus, the highest number for the investigated parameter was observed with pre-sowing treatment with this preparation and a combination of foliar treatments during the budding and grain-filling stages (BS+FG).

In addition to the number of nodules, their mass on plant roots was also examined during accounting, and the obtained data are presented in Table 3. Across all variants without exception, the highest nodule mass was recorded during the

Table 2. Dynamics of nodule count (pcs./plant) on soybean roots according to development stage

Treatment of seeds	Foliar treatment	BS	F	FG
C	NT	12.0±3.1	16.9±7.8	23.5±5.8
	BS	12.8±3.5	22.4±6.9	26.9±9.5
	FG	12.2±3.8	18.6±7.5	24.0±6.1
	BS+FG	13.1±3.9	22.8±7.6	26.3±9.0
R	NT	25.5±0.9	31.8±4.3	36.0±10.6
	BS	27.9±1.3	37.3±2.3	39.1±12.1
	FG	26.2±2.3	33.4±4.8	35.7±9.6
	BS+FG	27.3±3.0	36.7±2.9	40.5±12.8
Duncans` criterion		F=8.13 p<0.05	F=19.7 p<0.05	F=5.7 p<0.05
		0.064	5.1	10.2

Table 3. Dynamics of nodule mass on soybean roots according to crop development stages

Treatment of seeds	Foliar treatment	BS	F	FG
C	NT	0.066±0.02	0.172±0.12	0.144±0.06
	BS	0.077±0.02	0.208±0.11	0.189±0.09
	FG	0.065±0.02	0.181±0.12	0.158±0.07
	BS+FG	0.084±0.01	0.216±0.10	0.426±0.33
R	NT	0.169±0.05	0.313±0.13	0.248±0.14
	BS	0.202±0.03	0.346±0.12	0.285±0.15
	FG	0.178±0.05	0.323±0.13	0.251±0.12
	BS+FG	0.193±0.04	0.357±0.12	0.293±0.15
Duncans` criterion		F=8.13 p<0.05	F=1.16 p=0.37	F=0.92 p=0.51
		0.064	0.21	0.28

flowering stage of soybean compared to the budding stage. During the grain-filling stage, their mass slightly decreased, but not significantly. A statistically significant difference in nodule mass was observed only during the budding stage, where the Rhizogumin seed treatment variant significantly exceeded the nodule mass indicators of the control variant. No significant effect was observed at later developmental stages.

Many scientists have reported similar findings regarding the increase in nodule count and mass on plant roots in their research. For instance, Moretti et al. [2018] demonstrated that inoculation boosts soybean nodulation and yield, with foliar treatments further enhancing this effect through additional nitrogen fixation by soybean plants. It's also worth noting that the improvement in these indicators can be caused not only by "artificial" bacterial colonization of the seed surface but also by the implementation of soybean monoculture. While this might negatively affect the phytosanitary status of the crop, Halwani et al. [2021] proved that the studied indicators significantly increase in monoculture. It's also important to mention that not only bacterial organisms but also arbuscular mycorrhiza can positively influence this indicator. Ngosong et al. [2022] found that soybean inoculation with effective microorganisms, specifically bacteria and arbuscular mycorrhizal fungi, both individually and in combination, with reduced or no NPK fertilizer application, significantly improves root nodule formation, acid phosphatase activity in the rhizosphere, soybean yield, and nutrient content in the grain.

As seen from the presented studies, an increase in the number of nodule bacteria also leads to higher yields. One of the main yield indicators is the yield structure, which includes the number

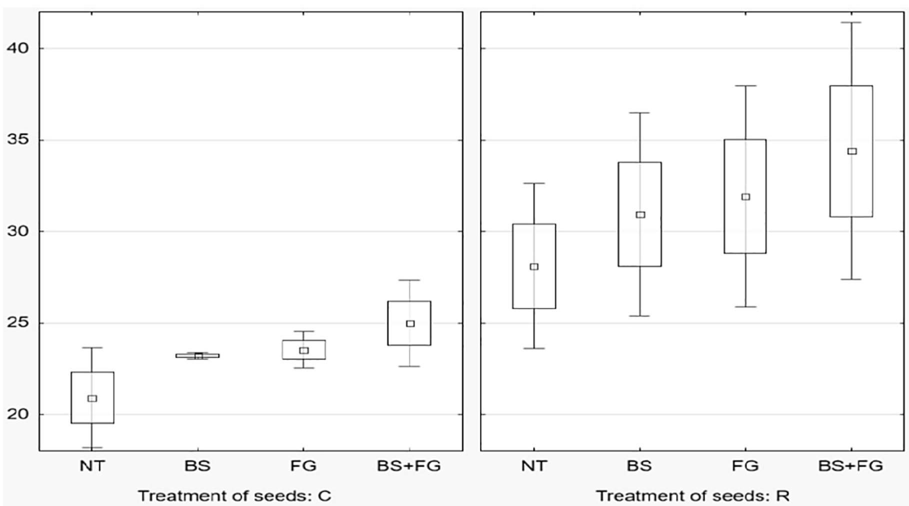

Figure 3. The influence of plant growth regulators on the formation of soybean yield structure components (a – number of pods per plant; b – number of seeds per plant)

of pods in the case of soybeans, 1000-seed weight, and others. Figure 3 shows the dynamics of pod and seed formation on one plant depending on pre-sowing grain treatment and foliar treatments according to growth stages. In the control variant without pre-sowing seed treatment, a gradual increase in the median value of the indicator is observed with the application of foliar treatments, with the maximum value achieved with combined treatment during the budding and grain-filling stages (BS+FG). In contrast, with pre-sowing seed treatment with Rhizogumin, the values of the studied indicator are significantly higher in all foliar treatment variants compared to untreated seeds, with the upward trend maintained in the BS+FG variant. This indicates a synergistic effect of pre-sowing seed treatment and additional foliar treatments on the formation of soybean productivity elements.

According to Table 4, it was established that pre-sowing seed treatment is a critical factor significantly influencing the formation of soybean yield structure components, specifically the number of pods and seeds per plant, providing a substantial increase in these indicators. At the same time, while foliar treatment shows a tendency to improve certain parameters (as seen from the visualization of nodule count and yield components), according to the results of the ANOVA, it does not have a statistically significant independent or synergistic effect with seed treatment on the number of pods and seeds.

The results of pairwise comparisons of mean values for the number of pods and seeds on soybean plants, presented in Table 5 and analyzed using Duncan's criterion, demonstrate significant differences between seed treatment variants. In particular, all variants where seeds were treated with Rhizohumin show a significant increase in the number of seeds and pods compared to the group without seed treatment (control), regardless of foliar feeding. However, within the Ryzohumin-treated variants, there are generally no significant differences in terms of foliar application, nor are there significant differences within the control group. These data confirm the key role of pre-sowing seed treatment in improving yield structure, while the impact of individual foliar treatments is less pronounced or statistically insignificant within a single seed treatment group.

Analysis of the data presented in Table 6 indicates that neither pre-sowing seed treatment nor foliar applications had a statistically significant impact on seed weight per plant or the 1000-seed weight of soybeans. Although a slight increasing trend was observed in groups treated with

Table 4. Analysis of variance of the impact of different seed treatments and foliar applications on the quantitative indicators of pods and seeds on soybean plants

Treatment	Beans				Seeds			
	SS	MS	FF	p	SS	MS	FF	p
Seed	118.8	118.8	31.2	< 0.05	401.6	401.6	27.1	< 0.05
Foliar	12.3	4.1	1.1	0.4	82.2	27.4	1.6	0.2
Seed+Foliar	1.9	0.7	0.2	0.9	4.1	1.4	0.1	0.9

Table 5. Results of pairwise comparisons of mean values for the number of pods and seeds on soybean plants using Duncan's criterion

Seed treatment	Foliar treatment	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
C	NT(1)		0.448	0.430	0.277	0.034	0.006	0.004	0.001
	BS (2)	0.448		0.991	0.694	0.120	0.026	0.018	0.004
	FG (3)	0.430	0.991		0.702	0.128	0.028	0.019	0.004
	BS+FG (4)	0.277	0.694	0.702		0.204	0.048	0.035	0.007
R	NT (5)	0.034	0.120	0.128	0.204		0.375	0.290	0.079
	BS (6)	0.006	0.026	0.028	0.048	0.375		0.811	0.308
	FG (7)	0.004	0.018	0.019	0.035	0.290	0.811		0.398
	BS+FG (8)	0.001	0.004	0.004	0.007	0.079	0.308	0.398	

Table 6. Impact of pre-sowing and foliar treatments on seed weight per plant and 1000-seed weight of soybean

Treatment of seeds	Foliar treatment	Weight of seeds per plant, g	Weight of 1000 seeds, g
C	NT	3.21±0.29	150.0±27.4
	BS	3.41±0.39	153.2±27.7
	FG	3.48±0.36	155.0±28.5
	BS+FG	3.59±0.39	156.1±28.6
R	NT	3.70±0.48	159.2±30.2
	BS	3.81±0.54	159.0±28.9
	FG	3.87±0.53	160.7±30.7
	BS+FG	3.94±0.59	161.9±29.8
Duncans` criterion		F=0.88 p=0.54	F=0.05 p=0.99
		0.80	50.2

Rhizogumin compared to the control, as well as some improvement under the influence of foliar applications, these differences did not reach statistical significance according to the conducted analysis of variance. This suggests that under the experimental conditions, the applied treatment methods did not significantly affect the formation of final productivity indicators characterizing seed size and total seed weight.

Figure 4 illustrates the impact of pre-sowing seed treatment and various foliar application options on soybean yield. It clearly shows that Rhizogumin application increases crop yield compared to the control (C) across all foliar treatment variants. The highest yield is observed with the combination of pre-sowing Rhizogumin treatment and foliar application during the budding and grain-filling stages (BS+FG), which may indicate an additional positive effect of comprehensive treatment. However, the results of the Analysis of variance (Table 7) show no significant difference in yield. Despite many analyzed studies showing a clear correlation between the number of nodule bacteria and crop yield, especially with seed inoculation, the results of this study do not fully support this thesis. Although a significant improvement in soybean yield structure indicators, such as the number of pods per plant or the number of seeds per pod, was recorded, this improvement did not lead to a statistically significant increase in final yield.

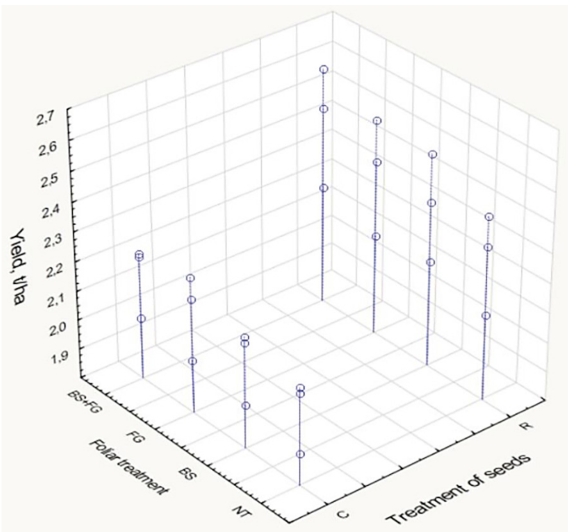

Figure 4. Soybean yield depending on pre-sowing and foliar treatments

This discrepancy can be attributed to several factors. Firstly, the climatic conditions of 2024 were extremely unfavorable, characterized by a significant rainfall deficit and elevated temperatures during the growing season, especially during critical crop development stages. Even with optimal nodulation and an improved yield structure, water stress could have negated the potential yield increase, as moisture availability is a limiting factor for the realization of soybean's genetic potential and photosynthetic efficiency.

The lack of a statistically significant final yield increase to unfavorable weather conditions in 2024, characterized by a significant rainfall

Table 7. Analysis of variance of the impact of different seed treatments and foliar applications on soybean yield

Parameter	SS	MS	FF	p	Dunkan`s criterion
Yield, t ha^{-1}	0.39	0.03	2.15	0.09	0.28

deficit and elevated temperatures. The water stress acted as a limiting factor, potentially negating the productivity gains achieved through improved nodulation and yield structure components.

Secondly, the interaction of microorganisms with soil conditions and other agricultural practices should be considered. It's possible that despite successful nodule formation, the effectiveness of nitrogen fixation or other positive impacts of the Rhizogumin inoculant was limited due to a lack of other nutrients, the specificity of the soil microbiome, or unfavorable physicochemical properties of the soil that could affect the viability of the introduced microorganisms. Furthermore, the type of interaction between the inoculant and the specific soybean variety Siverka may have its own peculiarities that require further investigation.

The results of this study underscore that while improving individual yield structure components is important, it does not always directly and proportionally translate into final yield, especially in the presence of environmental stress factors. This indicates the complex nature of yield formation and the need for an integrated approach, where inoculation is just one element of optimization. Future research should focus on studying the interaction of inoculants with different soybean varieties under changing climate conditions, as well as developing strategies to minimize the negative impact of abiotic stresses for the full realization of the potential of biological preparations.

CONCLUSIONS

This study demonstrated that pre-sowing treatment of soybean seeds with Rhizogumin significantly increases the number and mass of nodules on plant roots at all developmental stages, which is crucial for intensifying symbiotic nitrogen fixation. The best nodulation indicators were observed when Rhizogumin pre-sowing treatment was combined with foliar applications during the budding and grain-filling stages. Furthermore, Rhizogumin application positively impacted the formation of yield structure components, specifically by increasing the number of pods and seeds per plant, showing a synergistic effect between pre-sowing and some foliar treatments.

However, despite the clear improvement in root system biological activity and yield structure, a statistically significant increase in final soybean yield, 1000-seed weight, or seed weight per plant was not achieved. The research highlights the critical importance of pre-sowing seed treatment with Rhizogumin for significantly improving symbiotic activity (nodules) and yield structure components (pods/seeds per plant). This is likely due to unfavorable weather conditions, which acted as a limiting factor and negated the potential productivity increase. The obtained results underscore the importance of a comprehensive approach to soybean cultivation and the need for further research into the interaction of biopreparations with different crop varieties under abiotic stress conditions to fully realize their potential in sustainable agriculture.

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