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THE EFFECT OF RHIZOGUMIN ON NODULATION, PRODUCTIVITY AND STRESS RESISTANCE OF SOYBEAN (*Glycine max*)

SUMMARY

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. 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. Pre-sowing seed treatment with Rhizogumin significantly increased crop productivity. Without additional treatments, the yield was 3.43 t ha⁻¹, which is 0.19 t ha⁻¹ (5.9%) more than in the control without drugs. Treatment in the budding phase –3.49 t ha⁻¹, an increase of +0.25 t ha⁻¹ (7.7%), in the grain filling phase –3.43 t ha⁻¹, at the level of a single effect of the drug (pre-sowing seed treatment with Rhizogumin). The

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highest yield was provided by the combined application (budding + grain filling) -3.56 t ha^{-1} , which exceeded the control by 0.32 t ha^{-1} or 9.9%. 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: inoculation, rhizogumin, nodulation, yield, foliar feeding, biopreparations

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; Tolekiene *et al.* 2021; Voitovyk *et al.* 2023).

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; 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 were conducted from 2022 to 2025 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 (50°53'22.3"N, 34°42'34.1"E).

Soil Characteristics. The soil at the experimental plots was classified as a typical deep medium-loamy Chernozem. Key agrochemical characteristics were determined as follows: humus content ranged from 4.1% to 4.3% (determined by the Tyurin method), and pH (salt) was between 6.2 and 6.5. The average content of essential nutrients included: 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.* 2022 Growing Season: Total precipitation during the growing season of spring durum wheat amounted to 370 mm, which was 133 mm higher than the long-term average of 237 mm. The monthly precipitation distribution was: April –107 mm, May –26 mm, June –155 mm, and July –82 mm. 2023 Growing Season: The total precipitation recorded during the growing season was 222 mm, falling 15 mm below the long-term average of 237 mm. Monthly distribution was as follows: April –54 mm, May –17 mm, June –71 mm, and July –80 mm. 2024 Growing Season: A total of 150 mm of precipitation was observed during the growing season, representing a deficit of 87 mm compared to the long-term average (237 mm). The monthly breakdown included: April –48 mm, May –34 mm, June –51 mm, and July –17 mm.

Temperature Conditions. 2022 Growing Season: The average daily temperature during the growing season of spring durum wheat was 16.0 °C, which was 0.2 °C higher than the long-term average of 15.8 °C. Monthly temperature distribution was as follows: April –8.3 °C, May –13.3 °C, June –21.0 °C, and July –21.3 °C. 2023 Growing Season: The average daily temperature reached 16.6 °C, exceeding the long-term average by 0.8 °C. Monthly distribution was: April –9.8 °C, May –15.5 °C, June –19.3 °C, and July –21.6 °C. 2024 Growing Season: A significant increase in temperature was observed in 2024, with the average daily temperature reaching 19.2 °C. This was 3.4 °C higher than the long-term average. The monthly temperature breakdown included: April –12.9 °C, May –16.0 °C, June –22.4 °C, and July –25.4 °C.

Meteorological Conditions Analysis. Overall, the years 2022 and 2023 were most favorable for crop yield formation. In contrast, 2024 was characterized by prevailing dry conditions, marked by low precipitation and extreme deviations in air temperature throughout the vegetation period, likely impacting plant development negatively.

The growing season of soybeans in 2025 was generally favorable, but with certain contrasts in temperature and moisture. The average daily air temperature during this period was 19.6°C, which exceeded the average long-term norm by 1.5-2.0°C, and the total number of active temperatures (SAT>+10°C) was 2623°C, which indicates sufficient thermal supply of the crop.

Precipitation during the growing season (May-August) was 226 mm, which is less than the average long-term indicator (234 mm), but their distribution was uneven. In early June, optimal moisture conditions were observed (GTC 1.0-1.3), while in July dry and hot weather prevailed (GTC 0.38-0.68), which could inhibit the growth and formation of beans. Moderate rains fell at the end of August, which partially improved the conditions for grain filling. Overall, the soybean growing season was characterized as warm, moderately dry with short-term periods of moisture deficiency.

The primary objective of this research was to investigate the influence of environmentally friendly approaches on enhancing the symbiotic activity and grain productivity of soybeans. The research program included a two-factor experiment aimed at determining the effectiveness of inoculation and foliar treatment in the cultivation of soybeans.

The studies were conducted using the Siverka soybean variety. The research focused on examining the interaction of two factors: Factor A – Seed Treatment and Factor B – Foliar Application during vegetation, according to the experimental schemes detailed in Table 1.

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) at the budding stage – BS
	Fulvirin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvirin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG
Rhizogumin (2 kg t ⁻¹ seeds) – R	no treatment (control) – NT
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS
	Fulvirin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvirin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG

To significantly evaluate the field trials, phenological observations were conducted according to the "Methodology of State Variety Testing of Agricultural Crops" and "Methodology for Research in Fodder Production." Growth and development phases of plants were recorded. The onset of a phase was established when it occurred in 10% of plants, while a full phase was noted when it occurred in 75% of plants. Additionally, plant density was determined at the full emergence stage and before harvest, with three replications.

To determine the number and mass of rhizobial formations, soil monoliths (25×25×30 cm) were sampled. After washing the roots, 5 plants from each replication were retained, nodules were separated from the roots, their average number per plant was counted, and they were then dried and weighed.

Before harvesting, a sample sheaf was taken from each variant to determine the individual productivity of soybean plants. Soybean grains were harvested directly combining at full maturity, with a moisture content of 14–15%. Moisture content was determined using a "Wile 55" moisture meter, with plot-wise yield accounting and sample collection for further analyses. The 1000-grain weight was determined according to DSTU – 2949.

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 control variant (seed treatment with water) formed the smallest number of nodules, from 7.2 to 16.7 units/plant, with maximum values at two-time treatment of crops (budding + grain filling) (Table 2).

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

Treatment of seeds	Foliar treatment	Budding stage	Flowering stage	At the flowering stage
Control (seeds treated with water) – C	no treatment (control) – NT	7.2	14.6	16.7
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	11.5	18.6	20.6
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	8.1	13.7	17.1
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	10.3	17.4	19.4
Rhizogumin (2 kg t ⁻¹ seeds) – R	no treatment (control) – NT	18.4	29.1	36.8
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	23.2	34.3	38.2
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	19.2	30.3	34.3
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	24.4	36.7	37.7
LSD ₀₅ factor A		4.0	2.9	4.9
LSD ₀₅ factor B		2.1	4.5	0.7

Foliar treatments in the budding phase increased the indicators by +23.3–60% (up to 11.5–20.6 units/plant), in the grain filling phase –by +2.4–12.5% (up to 8.1–17.1 units/plant), and at two-time treatment –by +16.2–43% (up to 10.3–19.4 units/plant) compared to the control.

The use of the drug Rhizohumin significantly increased the number of nodules in all phases: – without fertilizing –18.4–36.8 units/plant, which is 2.0–2.6 times more than the control; in the budding phase to 23.2–38.2 units/plant, where this indicator increased by 2.3–3.2 times; in the grain filling phase to 19.2–34.3 units/plant, (by 2.0–2.7 times); with double treatment –24.4–33.7 units/plant, by 2.3–3.4 times compared to the control.

The mass of nodules on the soybean root system significantly depended on pre-sowing seed treatment and the use of foliar fertilization in different phases of vegetation. In the control variant (seed treatment with water), the mass of nodules varied within 0.038–0.105 g/plant, with minimum values in the budding phase and maximum values in the bean filling phase, which reflected the natural level of symbiotic activity without stimulation (Table 3).

Table 3. Dynamics of nodule mass on soybean roots according to crop development stages, g/plant

Treatment of seeds	Foliar treatment	Budding stage	Flowering stage	At the flowering stage
Control (seeds treated with water) – C	no treatment (control) – NT	0.038	0.085	0.105
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	0.077	0.130	0.141
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	0.047	0.082	0.111
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	0.059	0.108	0.136
Rhizogumin (2 kg t ⁻¹ seeds) – R	no treatment (control) – NT	0.144	0.236	0.261
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	0.195	0.288	0.302
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	0.156	0.261	0.294
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	0.215	0.323	0.345
LSD ₀₅ factor A		0.02	0.04	0.04
LSD ₀₅ factor B		0.01	0.02	0.03

Pre-sowing inoculation of seeds with Rhizohumin provided a significant increase in the mass of nodules compared to the control – without fertilization – 0.144–0.261 g/plant, i.e. 3.8–2.5 times more than in the control.

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 of pods in the case of soybeans, 1000-seed weight, and others. Figure 1 illustrates the dynamics of the number of pods and seeds per plant, depending on pre-sowing seed 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.

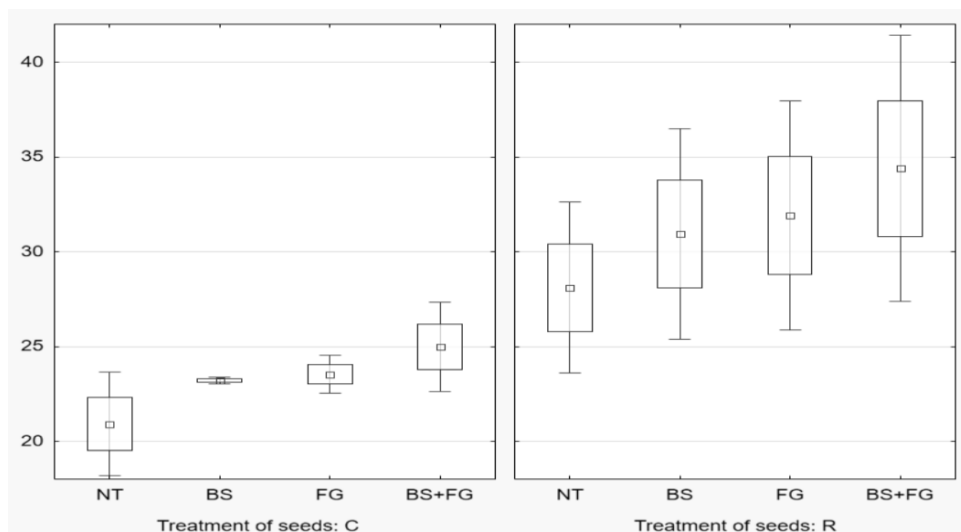


Figure 1. Soybean yield structure components depending on the effect of plant growth regulator (a – number of pods per plant; b – number of seeds per plant)

Analysis of the data presented in Table 4 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 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.

Table 4. 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
Control (seeds treated with water) – C	no treatment (control) – NT	4.52	217.6
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	4.61	220.3
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	4.60	218.3
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	4.64	224.7
Rhizogumin (2 kg t ⁻¹ seeds) – R	no treatment (control) – NT	4.78	228.7
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	4.82	233.6
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	4.73	230.5
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	4.85	236.9
LSD ₀₅ factor A		0.13	3.11
LSD ₀₅ factor B		0.03	3.21

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 (Table 5).

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.

Table 5. Analysis of the impact of different seed treatments and foliar applications on soybean yield

Treatment of seeds (factor A)	Foliar treatment (factor B)	Yield t ha ⁻¹		
		2022-2025	± to factor A, t ha ⁻¹	± to factor B, t ha ⁻¹
Control (seeds treated with water) – C	no treatment (control) – NT	3.24	K	1.00
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	3.30	0.06	1.85
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	3.27	0.03	0.93
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	3.32	0.08	2.47
Rhizogumin (2 kg t ⁻¹ seeds) – R	no treatment (control) – NT	3.43	0.19	5.86
	Humifield VR-18 (0.4 l ha ⁻¹) at the budding stage – BS	3.49	0.25	7.72
	Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage – FG	3.43	0.19	5.86
	Humifield VR-18 w.s. (0.4 l ha ⁻¹) at the budding stage + Fulvigrin Bor w.s. (0.5 l ha ⁻¹) at the flowering stage BS+FG	3.56	0.32	9.88
LSD ₀₅ factor A		0.067		
LSD ₀₅ factor B		0.066		

The yield in the control (without the use of drugs) was 3.24 t ha⁻¹, foliar treatments provided a slight increase in yield: in the budding phase –3.30 t ha⁻¹ (+0.06 t ha⁻¹ or +1.9%), in the grain filling phase –3.27 t ha⁻¹ (+0.03 t ha⁻¹ or +0.9%), with two-time treatment (budding + grain filling) –3.32 t ha⁻¹ (+0.08 t ha⁻¹ or +2.5%)

Pre-sowing seed treatment with Rhizogumin significantly increased crop productivity. Without additional treatments, the yield was 3.43 t ha⁻¹, which is 0.19 t ha⁻¹ (5.9%) more than in the control without drugs.

Treatment in the budding phase –3.49 t ha⁻¹, an increase of +0.25 t ha⁻¹ (7.7%), in the grain filling phase –3.43 t ha⁻¹, at the level of a single effect of the drug (pre-sowing seed treatment with Rhizogumin). The highest yield was provided by the combined application (budding + grain filling) –3.56 t ha⁻¹, which exceeded the control by 0.32 t ha⁻¹ or 9.9%.

Thus, the most effective option was “Rhizohumin + two-time treatment during vegetation” (3.56 t ha⁻¹), which indicates the synergistic effect of factors A and B.

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.

It was determined that the treatment of soybean seeds with the drug Rhizohumin provides a stable increase in soybean productivity. Foliar treatments during the growing season have an additional stimulating effect, especially in the phases of budding and grain filling, ensuring full seed filling. The highest indicators were obtained with the combined action of both factors (Rhizohumin + two-time treatment), which provided an increase in the mass of grain per plant to 4.85 g (+7.3%).

Rhizohumin provided a 6% increase in yield compared to the control. The highest results were recorded for the combination of Rhizohumin + two-time treatment during the vegetation period (budding and grain filling phases) – 3.56 t ha⁻¹, which is 0.32 t ha⁻¹ (10%) more than in the control variant.

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