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Effectiveness of Soybean Inoculation with Nodule Bacteria and Mycorrhizal Fungi in the Conditions of the Right Bank Forest Steppe of Ukraine

Abstract. The relevance of this study is determined by a complex of factors that shape soybean grain productivity – the effectiveness of symbiotic systems formed with nodule bacteria and mycorrhizal fungi, the activity of growth processes and the formation of vegetative organs by soybean plants, as well as their adaptive plasticity under the influence of various external factors. The purpose of this study was to investigate the morphobiometric characteristics of soybean plants, the productivity of the crop after pre-sowing treatment with biological preparations Rhizoline, Mycofriend, and Rhizosave. This paper presents the results of research on the use of soybean seed treatment with biological preparations to improve the growth and development of soybean plants and increase crop productivity. Field, laboratory, mathematical-statistical, and calculation methods were employed for this study. It was found that seed inoculation positively affects the leaf surface area in the budding phase, it increased from 5,313 cm^2 in the control to 6,426 cm^2 with the Rhizoline + Rhizosave + Mycofriend option, and in the phase of pod formation, respectively, from 8,704 cm^2 to 1,113 cm^2. The joint use of Rhizoline + Mycofriend + Rhizosave preparations most contributed to the increase in the mass of the root with nodules (+0.95 g), the number of nodules on the plant (+32 pcs), and the mass of nodules from the plant (+0.3 g). It was found that a comparable situation occurred during the phase of bean formation. The regularity of yield growth during the years under study showed that the average increase in yield varied from 0.41 t/ha with Rhizoline + Rhizosave inoculation to 0.6 t/ha with the combined use of Rhizoline + Rhizosave and the mycorrhizal preparation Mycofriend. It was substantiated that under appropriate conditions, the intensity of symbiotic nitrogen fixation increases thanks to nodule bacteria, as well as the absorption of water and nutrients by the root system of plants due to the activity of mycorrhizal fungi, the entry of amino acids, phytohormones, and vitamins into the plant, which positively affects the growth and development of soybean plants. The materials of this paper are of practical value for farmers in the development of new plant nutrition strategies involving biological preparations in the cultivation technology

Keywords: soybean seeds, biometric indicators, symbiotic productivity, number of nodules, biological preparation, productivity

INTRODUCTION

Increasing the production of high-quality products and increasing the level of soil fertility is one of the priority tasks of modern agricultural production [1]. Soybean is a unique protein and oil crop that is in great demand on the world and Ukrainian markets. Ukraine has great opportunities to increase the production of soybean seeds and obtain greater profits from its sale. Modern technologies do not fully provide an opportunity to fulfil their productive potential in connection with the parity of prices for fuel, protection products, fertilizers – this increases technological costs and encourages the search for new technologies for growing soybeans [2]. The main measure aimed at the implementation of these tasks is the use of microbial preparations to restore microbiological

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processes in the soil [3-5]. Microorganisms are one of the important components of the soil formation and a link that ensures the ecological balance of the soil ecosystem [6; 7]. The productivity of this culture depends on the performance of many technological operations, namely inoculation. Nodule bacteria fix molecular nitrogen for plants and convert it into a form available for plants. Nodule bacteria synthesize amino acids and vitamins of group B and other biologically active substances. Therefore, optimization of the elements of soybean cultivation technology will help increase the yield of the crop and increase the efficiency of its cultivation.

Pre-sowing treatment of seeds with inoculants is one of the main measures for fulfilling the genetic potential of new soybean varieties. Thanks to the biological fixation of atmospheric molecular nitrogen, plants are supplied with nitrogen. A considerable role is played by nodule bacteria, which contribute to the formation of nitrogen-fixing nodules on the roots of leguminous plants, which also plays a significant role in restoring soil fertility [8; 9]. The use of biological preparations in the fertilization system for growing agricultural crops allows optimising the level of mineral nitrogen from fertilizers to nitrogen biologically fixed by microorganisms from the air [10-12]. Using inoculation with nodule bacteria and treatment with fungicides, seed germination increases by 20%, plant resistance to diseases increases, and the formation of the symbiotic apparatus is stimulated by 28%, the structure of the crop (number of beans, weight of 1000 seeds) improves by up to 10%. [13; 14]. As a result of the activity of soil microorganisms, not only humus and nitrogen accumulate in the soil and the biological activity of the soil improves, but also mobile forms of nutrients, such as phosphorus and potassium [15-17].

A vital role in this process is played by mycorrhizal fungi, together with rhizospheric bacteria, which improve the supply of phosphorus and potassium to plants, improve water consumption, produce antibiotic substances and phytohormones, which positively affect their growth and development [18-20]. Research proved that under the action of biological preparations, the growth and development of soybean plants is activated, the area of its leaf surface increases, which contributes to an increase in the yield of the crop by 0.21 t/ha [21]. The use of biological preparations also positively increases biological activity, reducing the content of nitrates, heavy metal ions and radionuclides, intensifying the development of nitrogen-fixing and phosphate-mobilizing bacteria, therefore the ability to fix molecular nitrogen by plants is considered a valuable genetic resource of soybeans in the technology of its cultivation [22-24].

The use of biological preparations contributes to the supply of biological nitrogen, stimulates the growth and protection of plants from diseases, which allows obtaining high yields of soybean seeds without synthetic fertilizers and negative anthropogenic impact on the environment [25-27]. Some researchers experimentally prove and theoretically substantiate that the maximum fulfilment of the potential of plant-microbial interactions is possible only with the correct selection of complementary pairs of plant varieties – strains of microorganisms [28-30]. However, many aspects of the complex application of biological preparations of different functionality remain understudied. Therefore, research aimed at improving the elements of crop cultivation technologies stays relevant.

The purpose of this study was to find the effect of the joint action of nodule bacteria and mycorrhizal fungi on the growth and development of plants, as well as the symbiotic and grain productivity of soybeans.

MATERIALS AND METHODS

Field work was carried out in 2017-2019 at the Khmelnytskyi State Agricultural Research Station Institute of Fodder and Agriculture of the Podillia National Academy of Sciences (north-western part of the Right Bank Forest Steppe of Ukraine).

In the experiment, soybeans of the Siverka variety were sown at 900,000 pieces/ha, selected by the NSC "Institute of Agriculture of the National Academy of Sciences". This is a precocious variety that ripens in 92-97 days. The predecessor was oats. The area of the accounting plot was 30 m^2, the sowing area was 42 m^2, the repetition was four times. Agricultural machinery generally accepted for the zone: the main cultivation is ploughing, mineral fertilizers are applied at kg/ha in a.s. – $N_{20}P_{40}K_{40}$ (ammonium nitrate, superphosphate, potassium chloride). Protection of crops from weeds was carried out – herbicides Basagran (2 l/ha), Harmony (8 g/ha) + surfactant, Trend 90 (0.2 l/ha), Cannoneer Duo insecticide (0.10 l/ha), Acanto Plus fungicide (0.7 l/ha).

Variants of treatment of soybean seeds with the following biological preparations were investigated:

1. Without inoculation (control).
2. Rhizoline 2 l/t + Rhizosave 2 l/t.
3. Mycofriend 1.5 l/t.
4. Rhizoline 2 l/t + Rhizosave 2 l/t + Mycofriend 1.5 l/t.

Biopreparations manufacturer "BTU-Tsentr".

Seeds were processed on the day of sowing. The proposed preparations differed in composition and properties. The Rhizoline biopreparation is a bioinoculant intended for pre-sowing inoculation of soybean, pea, and other legume seeds. Designed for rapid colonization of the legume root system with nodule bacteria and the formation of nodules on the roots, which enables plants to absorb molecular nitrogen from the atmosphere. The preparation is a concentrate of viable cells of nodule bacteria*: Bradyrhizobium japonicum* – symbiotic to soybeans, *Rhizobium leguminosarum* – symbiotic to peas and other strains of nodule bacteria, symbiotic to certain legumes. The bacterial titre is from 2.0×109 CFU/cm^3. The preparation also includes macro- and microelements, biologically active products of bacterial life (vitamins, heteroauxins, gibberellins, etc.).

The Mycofriend biopreparation is a mycorrhizal preparation intended for seed treatment, seedlings, in-row application, and fertigation. Created for rapid colonization of the root system of plants with mycorrhizal fungi and microorganisms of the rhizosphere of plants. Increases absorption of water and nutrients by the root system of plants, protects roots from diseases, provides plants with vitamins, phytohormones, and amino acids. The composition of the preparation includes microorganisms that support the formation of mycorrhizae and the rhizosphere of plants: *Streptomyces sp.*, *Pseudomonas fluorescens*; as well as phosphate-mobilizing bacteria: *Bacillius megaterium var. phosphaticum*, *Bacillus subtilis*, *Bacillus mucilоginosus*, *Enterobacter sp.*, mycorrhizal fungi: *Glomus VS*, *Trichoderma harzianum*; The total number of viable cells is (1.0-1.5)×108 CFU/cm^3. The preparation includes biologically active substances: amino acids, phytohormones, vitamins.

The soil of the experimental site is medium-loamy chernozem with slightly podzol. Agrochemical characteristics of the soil: humus – 3.05-3.5%, pH – 5.75-6.2; hydrolytic acidity 1.9-2.35 mg/eq. per 100 g; hydrolysed nitrogen 170-136 mg/kg, mobile forms of phosphorus and potassium (according to Chirikov) (DSTU 4115:2002) [31] – 208-243 and 80-136 mg/kg, respectively.

According to the data of the Ukrainian Agrometeorological Centre [32], the weather conditions during the years of research differed from the average multi-year data. During the growing season of soybeans, the air temperature exceeded the average long-term indicators. On average, over the years of research, the increase was 2.29°C in 2017, 4.03°C in 2018, and 4.19°C in 2019 (Fig. 1; 2).

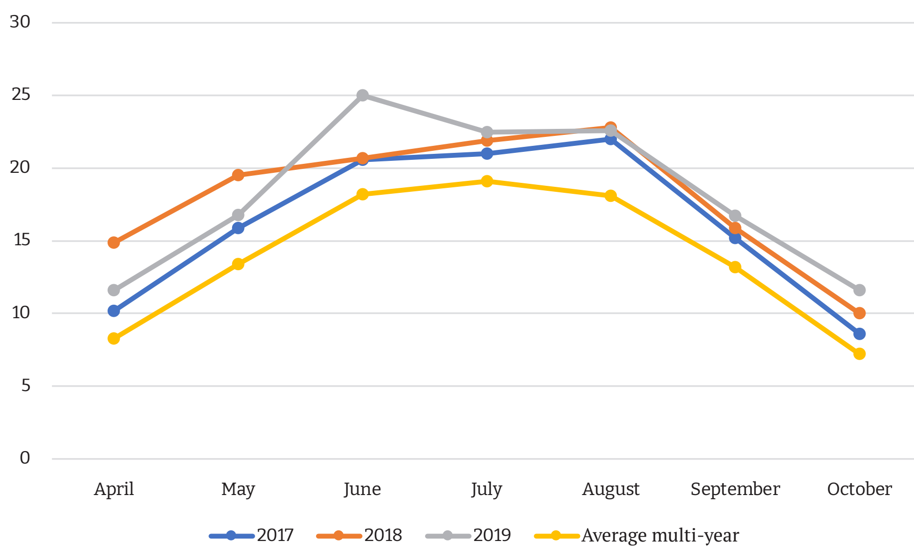

Figure 1. Dynamics of the average daily air temperature during the growing season of soybeans for 2017-2019 compared to the average multi-year data

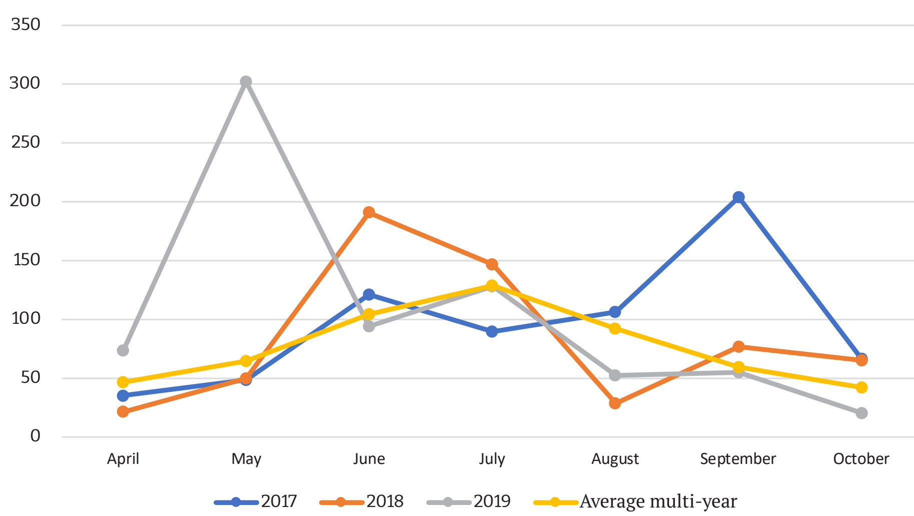

Figure 2. Precipitation dynamics for 2017-2019 compared to average long-term data

During the reporting period, precipitation exceeded the average multi-year data, the excess amounted to 131.8 mm in 2017, 40.9 mm in 2018, and 187.3 mm in 2019, while the unevenness of their precipitation was observed. Thus, a lack of precipitation was observed in April, May 2017-2018, July 2017, and August 2018-2019.

RESULTS AND DISCUSSION

The results of studies on the growth dynamics of soybean plants showed that seed treatment with nodule bacteria and mycorrhizal fungi in the budding phase promotes plant growth, the height increased from 64.95 cm in the control to 70.8 cm in the Rhizoline + Rhizosave + Mycofriend seed treatment. An analogous situation was observed in the phase of bean formation. In the control group, the weight of the plant and the weight of the leaves in the budding phase increased proportionately to the height of the plants and were 20.85 g and 3.9 g, respectively. Upon using the Rhizoline inoculant with the Rhizosave bioprotector, these indicators increased to 24.1 g and 4.15 g, respectively. Some authors note that seed inoculation is a profitable technique in soybean cultivation, which improves the conditions of plant growth and development, the formation of a larger area of leaves, plant height, the number of branches, nodes, beans, seeds, and the growth of productivity up to 2.04 t/ha [33; 34].

Treatment of seeds with the Mycofriend mycorrhizal preparation did not substantially affect the weight of the plant and leaves, and the combined use of nodule bacteria (Rhizoline) and mycorrhizal fungi (Mycofriend) contributed to an increase in these indicators, respectively, to 29.7 g and 5.2 g, respectively, by 8.85 g and 1.3 g more than in the control. The corresponding regularity was also observed during the pod formation phase. Seed inoculation had a positive effect on the leaf surface area in the budding phase, it increased from 5,313 cm^2 in the control to 6,426 cm^2 in the Rhizoline + Rhizosave + Mycofriend variant, and in the phase of pod formation, respectively, from 8,704 cm^2 to 1,113 cm^2, which is 17% and 13%, respectively (Table 1).

Table 1. Biometric indicators of plants after treatment of soybean seeds with nodule bacteria and mycorrhizal fungi

Research variants	Plant height, cm	Plant weight, g	Mass of leaves, g	Leaf surface area, cm^2	Plant height, cm	Plant mass, g	Mass of leaves, g	Leaf surface area, cm^2
	Budding phase				Bean formation phase			
1. Without inoculation (control)	65.0	20.9	3.9	5,313	80.7	48.0	9.0	8,704
2. Rhizoline + Rhizosave (2 l/t+2 l/t)	65.0	24.1	4.2	5,398	83.9	51.2	9.75	9,889
3. Mycofriend (1.5 l/t)	67.1	24.3	4.7	6,402	85.0	53.9	11.1	1,044
4. Rhizoline + Rhizosave (2 l/t+2 l/t)+Mycofriend (1.5 l/t)	70.8	29.7	5.2	6,426	84.0	59.2	12.1	1,113
Average ± S_x	67.2±1.3	24.7±1.8	4.5± 0.3	5,884±306	83.4±0.9	53.0±2.4	10.5±9.8	10,044±514
V, %	3.8	14.8	12.9	10.4	2.2	8.91	13.2	10.3

It was established that modern biological preparations contribute to the formation of a developed root system and an active nitrogen-fixing symbiosis, activate the nitrogenase complex of bacteria and increase the resistance of plants to adverse conditions, under the action of biological preparations, plants formed a larger number of beans, seeds, nodules on the root system, and the area of the leaf surface increased [35]. In the experiment, the use of nodule bacteria and mycorrhizal fungi influenced the growth and development of roots and the formation of nodules. The root length of the control variant in the budding phase was 7.75 cm, the mass of the root with nodules was 2.1 g, the number of nodules on the plant was 45.8 pcs, and the mass of nodules per plant was 0.54 g. The use of Rhizoline + Rhizosave increased the root length by 3.65 cm, root mass with nodules by 0.45 g, number of nodules per plant 12.7 pcs, and mass of nodules per plant 0.205 g. Treatment of seeds with mycorrhizal fungi (Mycofriend) compared to the use of Rhizoline + Rhizosave decreased root mass with nodules and mass of nodules, but a tendency to increase the number of nodules by inoculation of soybeans with aboriginal strains of nodule bacteria by 14% in the budding phase and 6% in the phase of bean formation was noted. The joint use of the preparations Rhizoline + Mycofriend + Rhizosave most contributed to the increase in the mass of the root with nodules (+0.95 g), the number of nodules on the plant (+32 pcs) and the mass of nodules from the plant (+0.3 g). Thus, in the phase of bean formation, the number of nodules on the plant with the simultaneous application of preparations (Rhizoline + Rhizosave + Mycofriend) increased by 1.8 times compared to the control (without inoculation) – 56.1 pcs (Table 2).

Table 2. Symbiotic performance of soybean under seed treatment with nodule bacteria and mycorrhizal fungi

Research variants	Root length, cm	Root mass with nodules, g	Number of nodules on the plant, pcs	Mass of nodules on the plant, g	Root length, cm	Root mass with nodules, g	Number of nodules on the plant, pcs	Mass of nodules on the plant, g
	Budding phase				Bean formation phase			
1. Without inoculation (control)	7.75	2.10	45.8	0.54	10.4	5.00	56.1	1.01
2. Rhizoline + Rhizosave (2 l/t+2 l/t)	11.4	2.55	58.5	0.745	13.1	5.15	84.9	1.76
3. Mycofriend (1.5 l/t)	10.8	2.35	67.8	0.66	12.75	5.9	90	1.58
4. Rhizoline + Rhizosave (2 l/t+2 l/t) + Mycofriend (1.5 l/t)	11.8	3.05	77.8	0.84	13.1	6.45	102.3	2.19
Average ± S_x	10.44±0.92	2.51±0.2	62.48±6.82	0.7±0.07	12.34±0.65	5.63±0.34	83.33±9.78	1.64±0.25
V, %	17.61	16.03	21.81	18.31	10.55	12.02	23.48	29.91

Seed inoculation with highly effective and active strains of nodule bacteria provides a prominent level of nitrogen-fixing capacity of symbiotic systems, which causes the intensification of soybean growth and development and, as a result, increases its yield up to 12% in growing technologies up to 38% [36-38]. According to the research carried out in 2017, soybean productivity increased from 2.18 t/ha in the control and up to 2.63 t/ha when using the Rhizoline biological preparation with the Rhizosave protector. The use of the mycorrhizal Mycofriend preparation increased the yield of the crop to 2.73 t/ha. By combining the Rhizoline biological preparation and the Rhizosave protector with the mycorrhiza-forming Mycofriend preparation, the yield level increased to 2.76 t/ha, respectively, the increase compared to the control (without inoculation) was 0.58 t/ha. The analysis of research results in 2018 showed a comparable trend, the yield level fluctuated, respectively, from 2.13 t/ha in control to 2.72 t/ha with the joint use of biological preparations. The regularity of yield growth due to the introduction of nodule bacteria and mycorrhizal fungi, which was observed in 2017-2018, was confirmed in 2019 as well. Productivity increased from 2.26 t/ha in the control to 2.88 t/ha for the combination of biological preparations (Table 3).

Table 3. Soybean yield depending on seed inoculation with nodule bacteria and mycorrhizal fungi

Variant	Productivity, t/ha			Average for 2017-2018	b_i	V
	2017	2018	2019			
1. Without inoculation (control)	2.18	2.13	2.26	2.19	1.36	3.19
2. Rhizoline + Rhizosave (2 l/t+2 l/t)	2.63	2.58	2.58	2.6	0.12	1.15
3. Mycofriend (1.5 l/t)	2.73	2.64	2.73	2.7	1.07	1.85
4. Rhizoline + Rhizosave (2 l/t+2 l/t)+Mycofriend (1.5 l/t)	2.76	2.72	2.88	2.79	1.62	2.87
LSD_{05}, t/ha	0.04	0.10	0.04			

On average, over three years, the yield increase varied from 0.41 t/ha with Rhizoline + Rhizosave inoculation to 0.6 t/ha with the joint use of Rhizoline + Rhizosave with the Mycofriend mycorrhizal preparation with an excess of up to 22%. The analysis of the regression coefficient (bi), which characterizes the average reaction of the research variant to the change in environmental conditions and allows predicting the change in the characteristic under study (yield), within the conditions available in the experiment, showed how biological preparations affect the reaction rate of the variety. Elevated productivity stability (b_i=0.12) of the crop was observed upon using the Rhizoline inoculant with the Rhizosave bioprotector, i.e., regardless of the growing conditions, the yield was at the level of previous years and consistently exceeded the control. The medium ecological plasticity (b_i=1.07) was characterized by the variant when treating seeds with the Mycofriend mycorrhizal preparation (1.5 l/t), while this variant consistently exceeded the control in terms of productivity. Joint use of Rhizoline inoculant with Mycofriend mycorrhizal preparation and Rhizosave bioprotector increases the plasticity of the variety (b_i=1.62). That is, when the conditions change towards improvement, the intensity of symbiotic nitrogen fixation due to nodule bacteria increases, as well as the absorption of water and nutrients by the root system of plants due to the activity of mycorrhizal fungi and the entry of vitamins, phytohormones, and amino acids produced by microorganisms into the plant. This leads to an increase in crop productivity by 0.6 t/ha compared to the control level of 2.19 t/ha.

CONCLUSIONS

The use of nodule bacteria and mycorrhizal fungi for the processing of soybean seeds contributes to the growth of symbiotic productivity, increasing the absorption of water and nutrients by the root system of plants, which ensures an increase in the leaf surface area and photosynthetic activity, which is reflected in the improvement of economic and valuable indicators and an increase in the yield of the crop. The best option for seed treatment turned out to be the variant of the combined use of Rhizoline (2 l/t) + Rhizosave (2 l/t) + Mycofriend (1.5 l/t), which is characterized by high productivity (2.79 t/ha) and ecological plasticity (b_i=1.62). It was proved that with the joint use of biological preparations, the leaf surface area increased by 13%, and the number of nodules per plant increased by 1.8 times comparing to the control level, the yield increased by 22% (2.19 t/ha in the control).

The obtained results are valuable from both a practical and a theoretical standpoint for improving ways of increasing the grain productivity of soybean plants by increasing the activity of symbiotic nitrogen fixation, the absorption area of the plant root system, and improving the availability of plant nutrients through the use of nitrogen-fixing bacteria and mycorrhizal fungi for the pre-sowing treatment of seeds of this strategic bean crop for Ukraine. The introduction of biological preparations into soybean cultivation technologies opens wide opportunities for economical use of resources and reducing the load of agrochemicals in fertilization systems.

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Ефективність інокуляції сої бульбочковими бактеріями та мікоризними грибами в умовах Правобережного Лісостепу України

Анотація. Актуальність досліджень зумовлена комплексом факторів, що формують зернову продуктивність сої – ефективністю утворюваних із бульбочковими бактеріями і мікоризними грибами симбіотичних систем, активністю ростових процесів та формуванням вегетативних органів рослинами сої, а також їхньою адаптаційною пластичністю за дії різних зовнішніх чинників. Мета наукової роботи – дослідити морфобіометричну характеристику рослин сої, продуктивність культури за передпосівної обробки біопрепаратами Різолайн, Мікофренд і Різосейв. У статті представлені результати досліджень використання обробки насіння сої біопрепаратами для покращення росту і розвитку рослин сої та підвищення продуктивності культури. Методи польові, лабораторні, математично-статистичні і розрахункові. Визначено, що інокуляція насіння позитивно вплинула на площу листкової поверхні у фазу бутонізації, вона зростала від 5313 см^2 на контролі до 6426 см^2, варіант Різолайн + Різосейв + Мікофренд, а у фазу утворення стручків відповідно від 8704 см^2 до 1113 см^2. Сумісне використання препаратів Різолайн + Мікофренд + Різосейв найбільше сприяло підвищенню маси кореня з бульбочками (+0,95 г), кількості бульбочок на рослині (+32 шт.), та маси бульбочок з рослини (+0,3 г). З'ясовано, що аналогічна ситуація склалась і у фазу утворення бобів. Закономірність зростання урожайності протягом досліджуваних років, показала, що середньому приріст урожайності варіював від 0,41 т/га за інокуляції Різолайн + Різосейв до 0,6 т/га за сумісного використання Різолайн + Різосейв з мікоризним препаратом Мікофренд. Обґрунтовано, що за відповідних умов підвищується інтенсивність симбіотичної азотфіксації завдяки бульбочковим бактеріям, а також поглинання кореневою системою рослин води та елементів живлення за рахунок діяльності мікоризних грибів, надходження в рослину амінокислот, фітогормонів, вітамінів, що позитивно впливає на ріст та розвиток рослин сої. Матеріали статті становлять практичну цінність для аграріїв у розробці нових стратегій живлення рослин із залучення у технології вирощування біологічних препаратів

Ключові слова: насіння сої, біометричні показники, симбіотична продуктивність, кількість бульбочок, біопрепарат, урожайність