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# FUNCTIONING OF SOYBEAN-RHIZOBIA SYMBIOSIS AFTER VARIOUS METHODS APPLYING TRACE ELEMENTS COMPLEX

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***Objective.*** *Investigate the effect of inoculation of soybean seeds with an active strain of nodule bacteria Bradyrhizobium japonicum T21-2 and treatment of seed or vegetative plants with a complex of trace elements Avatar-2 on the functioning of the symbiotic apparatus and grain productivity.* ***Methods.*** *Physiological, microbiological, gas chromatographic, vegetation experiment, statistical.* ***Results****. As a result of joint treatment of seeds with nodule bacteria and Avatar-2, inhibition of nodulation activity of nitrogen-fixing microorganisms in the stage of three true leaves was revealed in comparison with symbiotic systems formed as a result of standard inoculation of seed with rhizobia of Bradyrhizobium japonicum T21-2 strain. In the stage of budding / beginning of flowering and bean formation on the soybean roots of this variant, the number of nodules exceeded the corresponding figure in plants, bacterized without the use of trace elements by 16.6 % and 24.1 %, respectively. The combination of inoculation of seeds and feeding of plants during the vegetation with Avatar-2 did not have a significant effect on the activity of symbiotic systems in the stage of three true leaves and budding / beginning of flowering. However, in the stage of bean formation, a 38 % increase in nitrogen-fixing activity was detected compared to plants grown from seeds of rhizobia inoculated with Bradyrhizobium japonicum T21-2 strain (without the use of trace elements). Activation of soybean growth processes at all stages of plant development when exposed of seed bacterization and use of Avatar-2 by different methods was noted. Inoculation of seeds and using microfertilizers, both as separate elements of soybean cultivation technology and complex treatments in the conditions of vegetation experiment, resulted in a decrease in grain content of copper, zinc, iron and nickel. It was found that pre-sowing treatment of soybean seeds with a complex of chelated nutrients without bacterization caused an increase in grain weight per plant by 17.8 %, and when feeding non-inoculated growing plants with this fertilizer — by 27.5 % compared to the control. The combination of both factors — inoculation of seeds with a bacterial preparation and different using of trace element complex also had a positive effect on individual productivity of soybean plants, as evidenced by an increase of 14.4 % and 30.2 % compared to plants whose seeds were treated with rhizobia only.* ***Conclusion****. The combination of intensification factors in soybean cultivation technology through the use of different methods of complex microfertilizer and seed treatment with highly active strain of nodule bacteria has a significant impact on plant growth, content of individual trace elements in grain and creates a significant reserve of grain productivity of this crop.*

Key words: *Bradyrhizobium japonicum, inoculation, number and weight of nodules, nitrogen-fixing activity, trace elements, feeding, soybean, grain yield.*

 

**Introduction.** Soybean is among the most valuable agricultural crops in world agriculture. It combines a high content of protein, fat, carbohydrates and enzymes, vitamins and minerals [1]. Soybean yield cropped by domestic farmers in recent years have allowed Ukraine to enter the top ten world producers and exporters. For our country, this is a strategic crop and the process of its production affects both the economic stabilization of agriculture and the accumulation of nitrogen reserves in the soil [2]. The acreage under soybeans is growing intensively, and the products are used in various branches of the national economy (food industry, animal husbandry, pharmacy, medicine, etc.) [3].

The stability of soybean grain production, at the current stage and in the future, can be successfully realized only under the condition of increasing the productivity of this crop through further improvement and introduction of competitive adaptive cultivation technologies with a high level of payback. These growing technologies should be aimed at the maximum use of natural factors, the entire complex of soil and climatic conditions, varietal selection, fertilizers and microelements for the maximum disclosure of the potential of intensive varieties [4].

**Analysis of the novel studies and publications.** The nitrogen-fixing potential of the symbiosis between leguminous crops and native rhizobia is often limited by the low activity of microorganisms or their insufficient number in the rhizosphere, therefore, farms treat seed material with biological preparations made on the basis of selected, competitive strains of nodule bacteria [5]. The use of high-quality inoculants provides stimulation of natural growth processes associated with nitrogen fixation and enables soybean plants to realize their genetic potential to a greater extent [6; 7].

Leguminous plants are quite finicky about the nutrient regime of the soil. At the same time, trace elements play an important role in the growth and development of plants, as well as in the processes of formation and functioning of the symbiosis between soybean and nodule bacteria, resulting in the intensification of the process of binding molecular air nitrogen. Unlike macronutrients, which largely function as structural elements, trace elements in the enzymatic system act as catalysts for many chemical reactions. The main vital processes and metabolism are impossible without them, despite the fact that their necessary amount for plants is minimal (about one or two atoms in the composition of a protein or enzyme molecule). As a key link of enzymes, metal trace elements directly influence the immunity of plants, their viability, resistance to pests and pathogens of various origins [8; 9]. In addition, trace elements are able to increase the resistance of plants to adverse environmental conditions (moisture deficit in the soil, temperature increase or decrease, etc.) [10].

Among the key technologies in the 21$^{st}$ century, which allow to improve traditional agricultural practices and are able to offer sustainable development through changes in management tactics and conservation of resources, are nanotechnologies. The creation and introduction of new environmentally safe nanopreparations with unique physicochemical properties of their components is one of the ways to increase the yield of cultivated crops and improve the quality of the obtained products [11−13].

The researchers have noted the high efficiency of using complex microfertilizers with a balanced amount of nanocarboxylates of microelements for pre-sowing treatment of seeds and feeding crops, which are able to influence biochemical processes in plant cells due to their properties that ensure high digestibility. In case of foliar feeding of winter wheat with a complex preparation containing zinc, magnesium, manganese, iron, copper, cobalt and molybdenum, chelated with citric acid, it was established that the preservation of the activity of the photosynthetic apparatus of crops at the late stages of ontogenesis contributed to the growth of grain productivity. The obtained results were confirmed by a high correlation coefficient between yield and chlorophyll index in milk-wax ripeness stage ($r = 0.96 \pm 0.08$). For example, the average yield increase for the four winter wheat varieties studied, which were grown during the years with different weather conditions, was 6.5 % relative to the control [14]. The literature reviews and summarises the factors that can affect the absorption, transport and penetration of trace elements in the form of nanoparticles into plant cells. It is during the treatment of seeds and introduction of nanopreparations into the soil that their components can interact with some microorganisms that have symbiotic interactions with plants, such as fungi (e. g., mycorrhiza) and bacteria (e. g., rhizobacteria), as well as with various compounds (e. g., humic acids), which can affect their bioavailability in the rhizosphere [15].

The use of Avatar-1 microfertilizer in soybean cultivation technology in combination with pre-sowing seed bacterization with *B. japonicum* 532C-based bioperparation activated the formation of the leaf surface, the nodulation ability of rhizobia and contributed to the increase of individual plant productivity [11].

Considering the above, the study of the feasibility of complex application of biopreparations based on selected nitrogen-fixing microorganisms and multi-component microfertilizers for the formation and functioning of an effective legume-rhizobial symbiosis, providing plants with the necessary nutrients, and therefore increasing the yield of soybeans, is of particular relevance.

**Objective.** Investigate the effect of inoculation of soybean seeds with an active strain of nodule bacteria *Bradyrhizobium japonicum* T21-2 and treatment of seed or vegetative plants with a complex of trace elements Avatar-2 on the functioning of the symbiotic apparatus and grain productivity.

**Materials and methods.** The vegetation experiment was carried out with soybean plants (*Glycine max* (L.) Merr) of the Almaz variety, which has been included in the Register of Plant Varieties of Ukraine since 2007; this variety is early-ripening and recommended for cultivation in the forest-steppe zone [16].

Before sowing, soybean seeds were inoculated for 1 hour with the strain of nodule bacteria *B. japonicum* T21-2 ($10^8$ cells/mL) and treated with a working solution of multicomponent microfertilizer Avatar-2 (200 mL/1 t). According to the developed scheme of the experiment, soybeans were fertilized with Avatar-2 twice during the growing season, calculating the rate of consumption of the working solution based on the manufacturer's recommendation (200 mL/ha).

The scheme of the experiment included the following variants:

1) control (without seed treatment and feeding of vegetative plants);

2) seed inoculation with *B. japonicum* T21-2;

3) seed treatment with Avatar-2;

4) inoculation with *B. japonicum* T21-2 + seed treatment with Avatar-2;

5) feeding of vegetative plants with Avatar-2;

6) inoculation with *B. japonicum* T21-2 + feeding of vegetative plants with Avatar-2.

*B. japonicum* T21-2 strain involved in the work was obtained at the Department of Symbiotic Nitrogen Fixation of the Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine (IPPG, National Academy of Sciences of Ukraine) as a result of transposon mutagenesis between *B. japonicum* 646 and *Escherichia coli* S17-1 containing pSUP2021::Tn5 plasmid. The strain has been patented [17] and is a part of IPPG's collection of nitrogen-fixing microorganisms, and is used for the production of bacterial fertilizers for soybeans in various presentations.

The composition of the complex preparation Avatar-2 (manufactured by AVATAR Scientific and Production Company) includes biogenic trace and ultra-trace elements (Mg, Cu, Zn, Fe, Mn, Co, Mo, La, Ce, Ni, V, Ti , Se, Ge, Ag, Si, K, S, I, B), chelated with natural di- and tricarboxylic organic acids — citric, succinic, tartaric, and malic [18].

Twelve soybean plants were grown in each 15-kilogram vessels, pre-sterilized with a 20 % $H_2O_2$ solution, on river sand with the introduction of Hellrigel nutrient mixture [19], enriched with trace elements, such as molybdenum, boron and copper and depleted of nitrogen — 0.25 of normal rate (one normal rate corresponds to 708 mg of $Ca(NO_3)\cdot 4H_2O$ per 1 kg of sand), under natural lighting and optimal (60 % RH) water supply, on a specially equipped plot at IPPG, National Academy of Sciences of Ukraine. The repeatability in the variants of the experiment is six times.

Nitrogen-fixing activity (NFA) of root nodules was determined by the acetylene method [20]. Roots with nodules were placed in 75 $cm^3$ tightly closed glass vials, where a 10 % concentration of acetylene was achieved. Duration of incubation was 1 hour. After incubation, the gas mixture was analysed using Agilent Technologies 6850 (USA) Network GC System gas chromatograph with a flame ionization detector. Gases were separated on a column (Supelco Porapak N) at a thermostat temperature of +55 °C and a detector temperature of +150 °C. The carrier gas was nitrogen (50 mL per min). The volume of the analysed gas mixture sample was 1 $cm^3$. Pure ethylene obtained from ethyl alcohol and concentrated sulfuric acid when heated to 160 °C was used as a standard. The amount of ethylene formed from acetylene under the action of nitrogenase of the incubated sample was expressed in molar units (μmol). The nodulation activity of rhizobia was determined by calculating the number of nodules on plant roots and their weight.

Biometric parameters — the weight of the raw material of the above-ground part of plants and roots — were determined ten times.

The content of Cu, Zn, Mn, Fe, Ni in the seeds was determined in the Department of Agroecology and Analytical Research of the NSC "Institute of Agriculture of the National Academy of Sciences" by the atomic absorption method according to GOST 30178-96 [21] on an atomic absorption spectrophotometer AAS-3 after acid hydrolysis followed by thermal destruction.

Experimental data were statistically processes using Microsoft Excel 2019 software package. The tables show arithmetic mean data and their standard errors.

**Results and discussion.** Important parameters of the successful symbiosis between leguminous plants and nitrogen-fixing bacteria are the number and weight of nodules on the roots, especially during the period of the greatest hotosynthetic activity of the macrosymbiont (Fig. 1). First of all, it should be noted that the absence of symbiotic structures on the roots was observed in plants of variants without inoculation of soybean seeds with nodule bacteria. Bacterization of seed material with *B. japonicum* T21-2 ensured the formation of a powerful symbiotic apparatus.

As a result of the conducted research, the number and weight of root nodules in the three true leaves stage decreased by 38.9 % and 29.3 %, respectively, under the combined pre-sowing treatment of seeds with *B. japonicum* T21-2 and Avatar-2 microelement complex, compared to pure bacterization. Such a decrease in the studied parameters may result from imbalance of trace elements associated with the rate of their absorption. Also, the increased concentration of trace elements around the seeds can directly affect the rhizobia.

The inhibitory effect of the multicomponent microfertilizer involved in the work with symbiotic systems, detected in three true leaves stage, gradually changed to an activating effect. In particular, in budding / beginning of flowering stage, under the combined pre-sowing treatment of soybeans with nodule bacteria and Avatar-2, an increase in the number of root nodules by 16.6 % was reported compared to plants grown from seeds inoculated with *B. japonicum* T21-2 (without additional application of trace elements). In the bean formation stage in plants of this variant, an increase in both the number and weight of root nodules by 24.1 % and 21.9 %, respectively, was registered.

The multicomponent product Avatar-2 can directly influence rhizobial cells, activating the main enzymatic systems of microorganisms, since it includes a number of elements vital to both symbiotic and free-living forms of rhizobia. In particular, magnesium, iron, cobalt, boron, etc. It is known that nanoparticles of biogenic metals in the composition of microfer-


*Fig. 1. Nodules on the roots of soybean plants of the Almaz variety: A — after seed inoculation with B. japonicum T21-2; B — after complex treatment of seeds with inoculant and Avatar-2; C — after seed inoculation with B. japonicum T21-2 and fertilizing plants during vegetation with Avatar-2 (budding / beginning of flowering stage).*

tilizers influence growth and development of macrosymbionts, the availability and use of mineral nutrition elements by them, interaction with useful and pathogenic microbiota and can be caused by an indirect effect, through the plant, by increasing its stress resistance, forming a more powerful and deeper penetrating root system [13, 22].

The combination of seed inoculation and treatment of soybean plants during the growing season with the Avatar-2 led to a decrease in the weight of root nodules in budding / beginning of flowering stage and bean formation by 28.1 % and 5.9 %, respectively. Under such conditions, their number was at the level of symbiotic systems formed as a result of seed bacterization with *B. japonicum* T21-2 without additional application of trace elements (Table 1). Therefore, this method of treatment led to the formation of smaller root nodules on soybean roots.

Investigating the nitrogen-fixing activity of the formed symbiotic systems, we have found a stimulating effect of the complex treatment of seed material with Avatar-2 and nodule bacteria on the studied parameter. In particular, in budding / beginning of flowering and bean formation stage, an increase in the nitrogen-fixing activity of symbiotic systems in plants of the specified variant by 32.6% and 49.6% was registered, respectively, compared to the standard inoculation of seeds (Table 2). We believe that such activation of the nitrogen-fixing symbiosis

*Table 1.* ***The number and weight of nodules formed on the roots of soybean plants with B. japonicum T21-2 against the background of different methods of application of the trace element complex Avatar-2*** *(per 1 plant)*

| Variants of experiment | Plant development stage | | | | | |
|---|---|---|---|---|---|---|
| | three true leaves | | budding / beginning of flowering | | bean formation | |
| | amount, pcs | weight, g | amount, pcs | weight, g | amount, pcs | weight, g |
| Control (without inoculation) | – | – | – | – | – | – |
| *B. japonicum* T21-2 | 36.0 ± 1.1 | 0.242 ± 0.016 | 25.3 ± 2.0 | 0.392 ± 0.023 | 29.0 ± 1.7 | 0.808 ± 0.045 |
| Avatar-2 (seed treatment) | – | – | – | – | – | – |
| *B. japonicum* T21-2 + Avatar-2 (seed treatment) | 22.0 ± 2.0 | 0.171 ± 0.011 | 29.5 ± 1.4 | 0.398 ± 0.026 | 36.0 ± 1.8 | 0.985 ± 0.068 |
| *B. japonicum* T21-2 + Avatar-2 (feeding) | 38.5 ± 2.4 | 0.238 ± 0.018 | 27.5 ± 1.9 | 0.282 ± 0.026 | 30.8 ± 2.6 | 0.760 ± 0.032 |
| Avatar-2 (feeding) | – | – | – | – | – | – |

*Table 2.* ***Nitrogen-fixing activity*** *(μmol $C_2H_4$ / (plant·h))* ***of soybean symbiotic systems after seed inoculation with B. japonicum T21-2 and different methods of using Avatar-2 microfertilizer***

| Variants of experiment | Plant development stage | | |
|---|---|---|---|
| | three true leaves | budding / beginning of flowering | bean formation |
| Control (without inoculation) | – | – | – |
| *B. japonicum* T21-2 | 4.27 ± 0.23 | 12.35 ± 1.20 | 11.71 ± 1.05 |
| Avatar-2 (seed treatment) | – | – | – |
| *B. japonicum* T21-2 + Avatar-2 (seed treatment) | 4.16 ± 0.29 | 16.37 ± 1.55 | 17.52 ± 1.21 |
| *B. japonicum* T21-2 + Avatar-2 (feeding) | 4.18 ± 0.33 | 11.32 ± 1.10 | 16.23 ± 1.34 |
| Avatar-2 (feeding) | – | – | – |

is achieved due to the synergistic effect of the inoculant strain and trace elements that have gradually been included in metabolic processes.

The combination of seed inoculation and feeding of plants during vegetation with Avatar-2 did not have a significant effect on the activity of symbiotic systems in three true leaves and budding / beginning of flowering stage, however, in bean formation stage, it contributed to an increase in NFA by 38 % compared to plants grown from seeds, inoculated with B. *japonicum* T21-2 (without trace elements).

The intensity of growth may indicate the ability of the plant organism self-regulation under the influence of various environmental factors. It is from the development of the herbage that the supply of plastic substances, necessary for the creation of reproductive organs and the formation of the crop, depends. Therefore, one of the stages of our research was the evaluation of soybean plants vegetative matter accumulation over time.

The research has found that during the bacterization of soybean seeds with the active strain *B. japonicum* T21-2 and the combined use of inoculation and pre-sowing treatment with Avatar-2 throughout the growing season, the raw above-ground mass of plants was 10.6–24.9 % higher compared to the control. After seed pretreatment with a complex of chelated biogenic metals only, the parameter of the above-ground mass of soybeans in three true leaves stage was at the level of control plants, but in budding / beginning of flowering and bean formation stages, it exceeded the latter by 6.4 % and 11.2 %, respectively.

Bacterization with feeding during vegetation and separate feeding of plants also demonstrated the stimulating effect on the growth of *Glycine max* (L) Merr., which is evidenced by higher parameters of the formed vegetative matter in budding / beginning of flowering and bean formation stages compared to control plants. For example, after inoculation and feeding with Avatar-2, the studied parameter was higher compared to control plants by 26.8 % and 29.5 %, feeding of vegetative plants with Avatar-2 ensured an increase in the above-ground mass of soybeans in budding / beginning of flowering and bean formation stage by 9 %, 4 % and 12.9 %, respectively (Table 3).

In our work, the growth of the vegetative matter of soybean plants was registered in most variants of the experiment, where microfertilizer with metal nanoparticles in its composition was applied in various ways. Therefore, it can be assumed that colloidal metal particles, under the conditions of contact with the surface of a biological object, are absorbed by it due to their small size (from 10 to 100 nm) [12; 23]. In this case, further translocation and biological transformation of these particles in plant tissues will be determined by physical properties and the ability to participate in metabolic processes. Thus, a nanosized object can be considered as a carrier of necessary cations. The general

*Table 3.* ***Vegetative matter and root weight (g/plant) of soybean plants grown under the action of the complex trace element preparation Avatar-2 and inoculation with B. japonicum T21-2***

| Variants of experiment | Plant development stage | | | | | |
|---|---|---|---|---|---|---|
| | three true leaves | | budding / beginning of flowering | | bean formation | |
| | above-ground weight | root weight | above-ground weight | root weight | above-ground weight | root weight |
| Control (without inoculation) | 4.91 ± 0.19 | 3.06 ± 0.13 | 5.75 ± 0.22 | 3.22 ± 0.10 | 8.43 ± 0.36 | 3.82 ± 0.14 |
| *B. japonicum* T21-2 | 5.47 ± 0.24 | 3.25 ± 0.11 | 6.36 ± 0.32 | 3.58 ± 0.13 | 10.25 ± 0.47 | 4.29 ± 0.16 |
| Avatar-2 (seed treatment) | 4.80 ± 0.26 | 2.75 ± 0.12 | 6.12 ± 0.29 | 3.27 ± 0.15 | 9.37 ± 0.45 | 4.07 ± 0.16 |
| *B. japonicum* T21-2 + Avatar-2 (seed treatment) | 5.63 ± 0.21 | 2.90 ± 0.09 | 7.01 ± 0.30 | 3.83 ± 0.15 | 10.53 ± 0.51 | 4.72 ± 0.21 |
| *B. japonicum* T21-2 + Avatar-2 (feeding) | 5.50 ± 0.22 | 3.20 ± 0.15 | 6.29 ± 0.34 | 3.36 ± 0.11 | 9.52 ± 0.42 | 3.99 ± 0.15 |
| Avatar-2 (feeding) | 5.10 ± 0.11 | 3.10 ± 0.13 | 7.29 ± 0.37 | 3.60 ± 0.13 | 10.92 ± 0.50 | 4.15 ± 0.20 |

efficiency of the effect of colloidal nanoparticles with different properties lies precisely in their inclusion in biochemical processes.

The raw weight of roots in soybeans bacterized with *B. japonicum* T21-2 (without the introduction of trace elements) exceeded plants of the control variant by 6.2–12.3 % throughout the growing season. After complex pre-sowing inoculation and treatment of seeds with Avatar-2 in three true leaves stage, a decrease in the weight of roots compared to control plants by 5.2 % was noted, however, in budding / beginning of flowering and bean formation stages, the studied parameter exceeded the control values by 18.8 % and 23.6 %, respectively.

A 10.1 % decrease in the weight of roots in three true leaves stage was also found in plants whose seeds were treated with only chelated forms of trace and ultra-trace elements before sowing. After complex bacterization and plant feeding, and in the variant with only soybean feeding during the growing season, the stimulating effect of these treatments on root growth was registered. At the same time, the increase of the studied parameter was 11.8 % and 8.6 % for inoculation and feeding and 4.3 % and 4.5 % when foliar application of a complex of trace elements was applied.

The researchers point out that trace elements chelated by organic acids are very similar to organometallic compounds synthesized in living plant cells. Therefore, when they enter a living cell, they are not perceived by it as foreign elements, which ensures their high biocompatibility and high digestibility [14]. Since Avatar-1 and Avatar-2 are characterized by the presence of nanoparticles of microelements of the same origin in their composition, we can assume the presence of similar mechanisms of their assimilation and influence on agricultural crops due to the effect on the main source of biological energy — photosynthesis.

Soybean holds the top spot among other leguminous crops in terms of the diversity and richness of the chemical composition of the seeds, which contain a wide range of trace elements. Their average content is as follows (mg/kg of dry matter): copper — 12, manganese — 30, boron — 13, zinc — 28, aluminium — 20, barium — 9, chromium — 1.5, cobalt — 0.1, strontium — 0.2, etc. Soybean grain trace elements are part of enzymes or are their activators and influence the synthesis of proteins, fats and carbohydrates. At the same time, the fact that a significant proportion of the mineral elements contained in soybean grains play a positive role in the use of soybean products for food and fodder purposes cannot be ignored [3]. Therefore, we have analysed the grain collected in the experiment to determine the content of a number of trace elements, in particular copper, zinc, manganese, iron and nickel (Table 4).

As a result of the research, it was established that the highest content of trace elements, such as Cu, Zn and Ni, was in the control variant of soybeans. In the grain of bacterized plants

*Table 4.* ***Trace element composition of soybeans grown under the influence of inoculation with rhizobia and different methods of application of the complex preparation Avatar-2***

| Variants of experiment | Copper, Cu | Zinc, Zn | Manganese, Mn | Iron, Fe | Nickel, Ni |
|---|---|---|---|---|---|
| | mg/kg on air-dried basis | | | | |
| Control (without inoculation) | 8.7 | 29.0 | 7.1 | 45.4 | 14.3 |
| *B. japonicum* T21-2 | 4.6 | 20.1 | 7.2 | 42.8 | 8.6 |
| Avatar-2 (seed treatment) | 8.2 | 28.0 | 6.8 | 40.1 | 8.1 |
| *B. japonicum* T21-2 + Avatar-2 (seed treatment) | 5.0 | 21.7 | 7.7 | 38.0 | 4.5 |
| Avatar-2 (feeding) | 6.3 | 27.6 | 6.6 | 40.4 | 6.0 |
| *B. japonicum* T21-2 + Avatar-2 (feeding) | 4.7 | 19.0 | 7.6 | 46.6 | 5.2 |
| *MAC* | 10 | 50 | – | 50 | 0.5 |
| $\overline{X} \pm S\overline{x}$ | *6.2 ± 0.7* | *24.2 ± 1.8* | *7.2 ± 0.2* | *42.2 ± 1.4* | *7.8 ± 1.5* |
| *V, %* | *29.0* | *18.4* | *6.0* | *7.9* | *45.9* |

and after the combination of inoculation of seed material and feeding of the culture during the growing season with a complex of nanocarboxylates, a significant decrease in the content of Cu by 42.5–46 % with a significant variation of V = 29.0 % and Zn by 25.2–34.4 % with an average level of variation V = 18.4 % was registered. It should be noted that according to DSTU 4964:2008 [24], the maximum acceptable content of Cu in soybeans, if used for food and technical needs, should be no more than 10 mg/kg and no more than 30 mg/kg for export; Zn content, regardless of the direction of seed use, should not exceed 50 mg/kg. The scientists have analysed 2,543 batches of soybean seeds for the content of trace elements and found that Zn ranged from 3 to 485 $mg/kg^{-1}$ and Mn — from 5 to 455 $mg/kg^{-1}$ [25].

According to Prasad [26], the content of Mn in soybean grain can be 8 times higher compared to corn. The results of our research showed that the concentration of Mn in the grain of *Glycine max* (L.) Merr had a slight increase by 7.0–8.5 % when it was grown from sterilized seeds against the background of treatment of seed material or vegetative plants with a trace element complex compared to the control.

It is known from the literature that microorganisms of various species have developed a number of adaptation mechanisms in the course of evolution to mitigate the consequences of Fe deficiency, which allow them to optimize the absorption of metals from available sources. Therefore, an important role in the process of Fe absorption by plants can be related to the vital activity of microorganisms capable of interacting with the root system of cultivated crops. For example, Sharma et al. [27] have shown that an increase in Fe content in rice plants is associated with an increase in the activity of rhizobacteria, which promote plant growth. The authors concluded that the use of strains of agronomically useful microorganisms stimulating the growth and development of plants is one of the strategies to combat the problem of Fe deficiency in rice crops.

According to our research, it was established that the range of Fe fluctuations in soybeans collected from the plants of most variants of the experiment was from 38.0 to 42.8 mg/kg (with a low variation of V = 7.9 %), which is 5.7–16.3 % less compared to the control. Only in the grain of vegetation plants bacterized and twice fed with microfertilizer, the amount of this element was close to the control variant.

Serbian scientists found that soybean and sunflower seeds have a tenfold greater Ni accumulation potential compared to corn and wheat [28]. At the same time, a group of researchers found that pre-sowing soybean bacterization with highly active strains of *Bradyrhizobium*, which ensures the effective functioning of legume-rhizobial symbiosis, thanks to which grain yield increases, allows to reduce nickel concentrations in it, in systems of organic cultivation of this crop [29]. Via biological assimilation of the nitrogen from the atmosphere, soybean has an increased need for Ni due to its exclusive role in the composition of urease [30]. Therefore, the decrease in the content of the specified element in the grain of plants bacterized before sowing is probably a consequence of its inclusion in metabolic transformations during the active functioning of the soybean-rhizobial symbiosis.

It was found that seed inoculation with nitrogen-fixing bacteria and nanoparticles of trace- and ultra-trace elements in the conditions of a vegetation experiment, both as individual elements of soybean cultivation technology and as complex treatments, provided a significant effect on the concentration of Ni in the grain. At the same time, its maximum decrease was recorded in the variant, where seed was treated with rhizobia and microfertilizer (3.2 times) and for the combination of bacterization with foliar application of microfertilizer (2.8 times) compared to the control.

The main efficiency parameter of technological techniques used in the cultivation of agricultural crops is the yield of grain. In our work, this parameter reflects the efficiency of functioning of the formed symbiotic systems between soybean and *B. japonicum* and the use of a complex of trace elements in various ways. It is known that trace elements are absorbed by soybean in smaller quantities compared to nitrogen, phosphorus, potassium and sometimes calcium, magnesium and sulphur. Despite this, their value is equally important, and deficiency leads to a significant slowdown in growth rates and a decrease in yield [8; 9].

Our research has found that the pre-sowing treatment of soybean seeds with a complex of chelated biogenic trace elements Avatar-2 without inoculation with nodule bacteria led to an increase in the weight of grains from one plant by 17.8 %, and feeding of vegetative non-inoculated plants with the indicated microfertilizer — by 27.5 % compared with control (Fig. 2). The high efficiency of foliar feeding is due to the fact that readily available nutrients, which are insufficiently supplied by the root system, getting to the vegetative organs of plants, quickly penetrate through the protective wax barrier — cuticle, epidermis and enter the cells.

The researchers have established that improving the conditions of plant nutrition through fertilization and foliar feeding with complex microfertilizers is an effective means of influencing the biosynthesis of chlorophyll in soybean plants, which also positively influences the yield of soybean varieties belonging to different rippening groups [31; 32]. At the same time, a distinctive feature of metal nanoparticles is their lower toxicity compared to metal salts and the ability to activate physiological and biochemical processes in fairly small doses.

In our work, the combination of both factors, inoculation of seeds with *B. japonicum* T21-2 and the use of the multicomponent preparation Avatar-2 with trace and ultra-trace elements in its composition in various ways, also had a positive effect on the individual productivity of soybean plants, as evidenced by the increase in this parameter by 14.4 % and 30.2 % compared to plants whose seeds were treated only with nitrogen-fixing bacteria.

The lowest level of soybean grain productivity in the experiment was observed in control plants, which were grown without pre-sowing treatment of seeds or spraying of plants during vegetation with trace elements. Our findings can be explained by the fact that in our studies, soybeans were grown in the conditions of a model vegetation experiment on river sand and, obviously, the nutrients introduced with Hellrigel nutrient mixture are not enough to realize the potential of its grain productivity, especially in plants of the control variant under nitrogen deficiency.

The fact that soybean unevenly uses nutrients in the process of growth and development (by vegetation stages) is also important. For example, in the initial stages (I–II stages of organogenesis) it needs a small amount of nitrogen, and later the plant's need for this element is provided due to the functioning of leguminous-rhizobial symbiosis [33]. Therefore, increasing the productivity of soybean grain in bacterized plants was ensured thanks to the use of nitrogen fixed by nodules.

**Conclusion.** After dimultaneous treatment of seeds with nodule bacteria *B. japonicum* T21-2 strain and complex preparation Avatar-2, inhibition of the nodulation activity of nitrogen-fixing microorganisms in three true leaves stage was revealed compared to symbiotic systems formed only by inoculation of seed material with rhizobia. However, in the process of plant growth and development, the inhibitory effect of this method of pre-sowing seed treatment decreased, and a gradual increase in both the nodulation activity of rhizobia and the nitrogen-fixing activity of the symbiotic systems formed by them was observed. The combination of seed inoculation and treatment of plants during vegetation with trace element complex Avatar-2 led to the formation of smaller root nodules on the

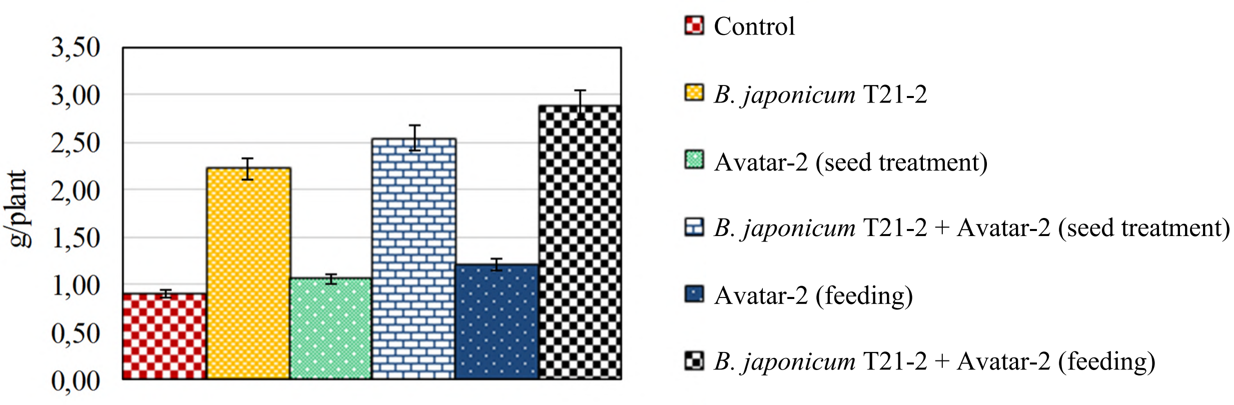


*Fig. 2. Weight of grain from 1 soybean plant (g), grown under the action of the complex trace element preparation Avatar-2 and inoculation with B. japonicum T21-2.*

roots of soybean plants, however, it did not significantly affect the nitrogen-fixing activity of symbiotic systems in three true leaves and budding / beginning of flowering stages.

It was established that the use of nodule bacteria and complex of trace elements for seed treatment or feeding plants during the growing season provided the maximum parameters of plant grain productivity, as well as a decrease in the content of copper, zinc, iron and nickel in soybean grains among all variants.

Thus, the obtained research results are valuable from both a practical and a theoretical point of view, since a scientifically based approach to providing plants with the necessary nutrients due to various methods of applying complex microfertilizer and seed treatment with a highly active strain of nodule bacteria creates a significant reserve in increasing symbiotic activity and grain productivity of this leguminous crop strategic for Ukraine.

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# ФУНКЦІОНУВАННЯ СИМБІОЗУ СОЇ З РИЗОБІЯМИ ЗА РІЗНИХ СПОСОБІВ ЗАСТОСУВАННЯ КОМПЛЕКСУ МІКРОЕЛЕМЕНТІВ

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***Мета***. *Дослідити вплив інокуляції насіння сої активним штамом бульбочкових бактерій Bradyrhizobium japonicum T21-2 та обробки посівного матеріалу або вегетуючих рослин комплексом мікроелементів Аватар-2 на функціонування симбіотичного апарату та зернову продуктивність.* ***Методи***. *Фізіологічні, мікробіологічні, газохроматографічні, вегетаційного досліду, статистичні.* ***Результати***. *За сумісної обробки насіння бульбочковими бактеріями та Аватаром-2 виявлено пригнічення нодуляційної активності азотфіксувальних мікроорганізмів у фазу трьох справжніх листків, як порівняти із симбіотичними системами, сформованими внаслідок стандартної інокуляції посівного матеріалу штамом B. japonicum T21-2. У фази бутонізації-початку цвітіння та формування бобів на коренях сої цього варіанту кількість бульбочок на 16,6 % та 24,1 % перевищувала відповідний показник у рослин, бактеризованих без застосування мікроелементів. Поєднання інокуляції насіння та підживлення рослин по вегетації Аватаром-2 не чинило суттєвого впливу на активність симбіотичних систем у фази трьох справжніх листків та бутонізації-початку цвітіння, проте у фазу формування бобів виявлено підвищення азотфіксувальної активності на 38 % проти рослин, вирощених з насіння, інокульованого штамом B. japonicum T21-2 (без застосування мікроелементів). Відзначено активізацію ростових процесів сої на всіх етапах розвитку рослин за впливу бактеризації насіння та внесення Аватару-2 різними способами. За застосування інокуляції насіння і мікродобрива як окремих елементів технології вирощування сої та як комплексних обробок в умовах вегетаційного досліду виявлено зниження в зерні вмісту міді, цинку, заліза й нікелю. Встановлено, що передпосівна обробка насіння сої комплексом хелатованих біогенних мікроелементів без бактеризації зумовила приріст маси зерен з однієї рослини на 17,8 %, а за підживлення вегетуючих неінокульованих рослин вказаним мікродобривом — на 27,5 % проти контролю. Поєднання обох чинників — інокуляції насіння бактеріальним препаратом і застосування різними способами комплексу мікроелементів також мало позитивний вплив на індивідуальну продуктивність рослин сої, про що свідчить підвищення цього показника на 14,4 % та 30,2 % проти рослин, насіння яких обробляли лише ризобіями.* ***Висновки***. *Поєднання факторів інтенсифікації в технології вирощування сої за рахунок застосування різних способів внесення комплексного мікродобрива та обробки насіння високоактивним штамом бульбочкових бактерій чинить істотний вплив на ріст рослин, уміст окремих мікроелементів у зерні та створює суттєвий резерв у підвищенні симбіотичної активності та зернової продуктивності цієї культури.*

Ключові слова: *Bradyrhizobium japonicum, інокуляція, кількість і маса бульбочок, азотфіксувальна активність, мікроелементи, підживлення, соя, урожай зерна.*

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