Biotechnology of biological bacterial preparations used in resource-saving farming

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Abstract. The article presents materials on the use of selenium compounds in the production of biological fertilizers, the main active component of which being artificially cultivated nodule bacteria. The objects of research in this work were various types of bacterial organisms used to prepare bacterial fertilizers: symbiotic nitrogen-fixing bacteria (rhizoagrin, rhizotorfin for galega and pea) and free-living agrobacteria (agrophil). Sodium selenate (Na$_2$SeO$_4$) was used as a source of selenium. The most optimal concentration of selenium ions in the liquid culture of microorganisms was $10^{-4}$ g/kg. A positive effect of selenium on the titer of finished preparations was observed: on average for the study period Rhizobiumpisum (rhizotorfin for peas) and Agrobacter 10 (agrophil) on the variant with selenium exceeded that on the control variant by 35.2 and 26.6%, respectively. The absolute titer values of the finished preparations Rhizobiumpisum (pea rhizotorfin) and Agrobacter 10 (agrophil) on non-sterile peat turned out to be higher than on sterile peat, although the trend remained. The inoculation of pea seeds with bacterial preparations increased the number of nodules in the flowering phase on the roots of pea plants by 1.8–2.1 times compared with the control. The treatment of pea seeds with rhizotorfin activated by selenium compounds during its manufacture increased the grain yield by 0.51 t/ha (17.1%) compared with the control, and by 0.30 t/ha (10.0%) when inoculated with rhizotorfin.

1. Introduction

The weaknesses in the production of bacterial preparations at biofactories of Russia, as well as the practical use of these preparations in field conditions are a relatively low reproduction rate of nodule bacteria and their low viability during storage and use. Like other non-spore microorganisms, nodule bacteria are viable and productive only in an active state [1–4]. This culture is very sensitive to water loss, temperature, it requires good gas exchange and sufficient (mainly carbohydrate) nutrition. The latter served as a decisive factor for the choice of their main carrier – peat. It is the peat form of preparations that is dominant throughout the world today. It is possible that this choice has somewhat aggravated the main disadvantage of biofertilizers – the relatively low viability and productivity of microorganisms. A large amount of phenolic substances contained in peat formed during the decomposition of lignin has a strong inhibitory effect on nodule bacteria.

To solve the problem of increasing the ability of soils to bind atmospheric nitrogen, it is more appropriate to recognize the use of selenium compounds in the production of biofertilizers, the main active component of which are artificially cultivated nodule bacteria [5–8]. The vital activity of the latter can be partially suppressed by the low content of selenium in peat, as well as by the acidic nature of peat, which prevents the absorption of selenium and other microelements by bacteria. Peat enrichment with selenium-containing compounds or the direct effect of selenium on nodule bacteria at the stage of their reproduction can resist all these negative factors [9].
The viability of using selenium compounds on plants belonging to different families was identified by a number of scientists [10–14]. The purpose of this study was to establish the effectiveness of the use of sodium selenate in the production of bacterial preparations and the effect of their inoculation of seeds of field pea in the forest-steppe of the Middle Volga region.

2. Materials and methods
The experiments were conducted in the laboratory of bacterial fertilizers on the basis of OOO “Biofabrika” (LLC) in the town of Kuznetsk in the Penza region.

The objects of research in this work were various types of bacterial organisms used to prepare bacterial fertilizers: symbiotic nitrogen-fixing bacteria (rhizoagrin, rhizotorfin for galega and pea) and free-living agrobacteria (agrophil). During the experiments the strains of microorganisms from the collection of OOO "Biofabrika" were used.

Sodium selenate (Na$_2$SeO$_4$) was used as a source of selenium, which was introduced into various phases of the production process in the form of a solution. Sodium selenate solutions were autoclaved for 1.5 hours (sterilization of Na$_2$SeO$_4$ was confirmed by earlier studies).

At different phases of the process, samples of control and experimental variants were taken to view the results of exposure of selenium ions on bacterial cells through the microscope.

Field studies were conducted on the experimental field of the educational-experimental farm of the Penza State Agrarian University. The soil was leached heavy loamy black (chernozem). The humus content was 6.2%, the hydrolytic acidity was 4.8 (pH – 4.7). The nitrogen content was 12.5 mg, the content of phosphorus was 13.0 and of potassium – 15.0 mg per 100 g of the soil. The estimated soil quality score was 69.

Before sowing the seeds of field peas were treated with the preparations manually, using an adhesive. The plots were placed by a randomized method in six repetitions.

Meteorological conditions for the entire period of research (2016–2018) were generally favorable for the growth and development of peas. The average air temperature was in the range of long-term average values. The largest amount of precipitation during the growing season (239 mm) was noted in 2016. Compared with the average-summer values, the deviation of the indicator was + 24 mm. In 2017, the amount of precipitation during the growing season was 175 mm, which is 40 mm lower than the average annual value. In 2018, the amount of precipitation during the growing season was 137.3 mm, which is 18.7 mm lower than the annual average.

3. Results and discussion
At the initial stage of the experiment, it was necessary to establish the positive effect of selenium compounds on bacterial organisms and to determine the concentration of selenium ions. The most optimal concentration of selenium ions in the liquid culture of microorganisms was 10–4 g/kg (table 1). The number of bacterial cells there exceeded the control variant by 17.8–33.5%. A concentration of 10–2 g/kg was fatal for these organisms. This particular concentration of sodium selenate was used for further research.

Table 1. The effect of various concentrations of selenium ions on microorganisms (Rhizobium) in liquid culture (average for 3 years).

| The strain of microorganisms (biological preparation) | Titer, billion cells in 1 ml | without adding Na$_2$SeO$_4$ solution to the liquid nutrient medium (control) | with the addition of Na$_2$SeO$_4$ solution in a liquid nutrient medium in different concentrations | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
|-----------------------------------------------------|-----------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------|--------|--------|--------|--------|--------|
| Rhizobiumpisum (risotorfin for peas)                 |                             |                                                                                |                                                                                  | 4.50   | –      | 3.70   | 5.30   | –      |
In the case of Rhizobiumpisum (rhizotorfin for peas), 2 experimental variants were studied - with sterile and non-sterile peat. In the first case, selenium was introduced into 3-liter bottles immediately before autoclaving, and the culture liquid was introduced after it, above the burner flame.

In both cases, a positive effect of selenium on the titer of finished preparations was observed. On average for the study period, the titer of the preparations Rhizobiumpisum (rhizotorfin for peas) and Agrobacter 10 (agrophil) in the variant with selenium exceeded that in the control variant by 35.2 and 26.6%, respectively (table 2).

The absolute titer values of the prepared preparations Rhizobiumpisum (rhizotorfin for peas) and Agrobacter 10 (agrophil) on non-sterile peat turned out to be higher than on sterile peat, although the trend remained. Most likely, this was due to the fact that when autoclaving peat, its moisture content decreased significantly, or nutrients under the influence of high temperature turned into a hard-to-reach form.

Table 2. The titer of preparations (peat medium), billion cells in 1 ml (g).

| The strain of microorganisms (biological preparation) | Variant without adding Na$_2$SeO$_4$ solution (control) | with the addition of Na$_2$SeO$_4$ solution in the concentration of 10$^{-4}$ g/l | Deviation from the control billion cells in 1 ml (g) | Deviation from control % | The regulatory values of the titers of the culture liquid |
|----------------------------------------------------------|----------------------------------------------------|---------------------------------|---------------------|-----------------|---------------------------------|
| Sterile peat                                              |                                                    |                                 |                     |                 |                                 |
| Rhizobiumpisum (risotorfin for peas)                      | 2.231                                              | 3.143                           | 0.912               | 40.9           | 2.5                             |
| Agrobacter 10 (agrophil)                                 | 8.407                                              | 11.904                          | 3.497               | 41.6           | 10                              |
| Non-sterile peat                                          |                                                    |                                 |                     |                 |                                 |
| Rhizobiumpisum (risotorfin for peas)                      | 6.625                                              | 9.791                           | 3.166               | 47.8           | 2.5                             |
| Agrobacter 10 (agrophil)                                 | 10.983                                             | 16.387                          | 5.404               | 49.2           | 10                              |

The manufactured microbial fertilization preparations with the addition of selenium compounds after laboratory tests were subjected to a production test in the experimental field of the Penza State Agrarian University.

The conducted scientific studies showed that the inoculation of pea seeds with bacterial preparations increased the number of nodules in the flowering phase on the roots of pea plants by 1.8–2.1 times compared to the control where inoculation was not performed (table 3).

Table 3. The effect of inoculation of pea seeds with bacterial preparations on the quantitative composition of nodules.

| Variants                 | The amount of nodules, pcs./1 plant: | Duration of symbiosis, days |
|--------------------------|-------------------------------------|-----------------------------|
|                          | flowering phase | deviation from control | bean formation phase | deviation from control |
| Control (water treatment)| 25          | – | 39 | – | 73 |
The use of Rhizobia pisi um (Rizotorfin B for peas), activated by selenium compounds in its manufacture (rhizotorfin + selenium) increased the formation of nodules on the pea root system by 2.1 times compared to the control (seed treatment before sowing with water) and by 1.8 times compared with the variant where the pre-sowing inoculation of seeds with Rhizobia pisi um (Rizotorfin for peas) was carried out.

The maximum development of the symbiotic apparatus occurred in the phase of beans formation of pea plants. The treatment of seeds with bacterial preparations contributed to an increase in the number of nodules on the roots of pea plants by 1.5–1.8 times in comparison with the control, where water was not inoculated. The use of Rhizobia pisi um (Rizotorfin B for peas), activated by selenium compounds in its manufacture, increased the formation of nodules on the pea root system by 82% compared to the control and by 16.4% compared to the variant where the pre-sowing inoculation of seeds with non-selenized Rhizobia pisi um (rhizotorfinum for peas) was performed.

The treatment of pea seeds with rhizotorfin, activated by selenium compounds during its manufacture, increased the grain yield by 0.51 t/ha (17.1%) compared with the control, and by 0.30 t/ha (10.0%) when inoculated with rhizotorfin (table 4).

Table 4. The effect of bacterial preparations on pea productivity.

| Variants                                      | Yield, t/ha | Increase |
|-----------------------------------------------|-------------|----------|
| Control (water treatment)                     | 2.98        | –        |
| Rhizobia pisi um (Rizotorfin for peas)        | 3.28        | 0.30     |
| Rhizobia pisi um (Rizotorfin B for peas) + selenium | 3.49    | 0.51     |
| least significant difference (LSD)            | 0.13        |          |

4. Conclusion

The studies established the effectiveness of the use of selenium compounds to stimulate bacterial cells in the production of bacterial fertilizers Rhizobium and Agrobacter. The addition of selenium directly to peat lead to the fact that the titer of the preparations amounted to 50.5% of the control. The inoculation of seeds of agricultural crops with microbial fertilizers with the addition of selenium compounds twice increased the number of nodules on the roots of legumes compared with the control (seed treatment with water) and from 16.4 to 21.7% compared with the variant treated with non-selenized rhizotorfin. The use of bacterial fertilizers significantly increased the productivity of peas. The greatest increase occurred when seeds were inoculated with selenized rhizotorfin – by 17% compared with the control.

Reference

[1] Bekele G, Dechassa N, Tana T and Sharma J 2019 Effects of nitrogen, phosphorus and vermicompost fertilizers on productivity of groundnut (Arachis hypogaea L.) in Babile, Eastern Ethiopia Agronomy Research 17 (4) 1532–46
[2] Dubova L, Senberga A, Alsina I, Strauta L and Cinkmanis I 2019 Development of symbiotic interactions in the faba bean (Vicia faba L.) roots Agronomy Research 17 (4) 1577–90
[3] Nadezhkina E V and Silnova E G 2001 Influence of rhizosphere bacteria on the formation of millet grain yield Agrochemistry 6 40–43
[4] Koryagina N V, Koryagin Yu V, Efremova S Yu and Koryagina E J 2017 Evaluation of the use of environmentally-safe biological bacterial preparations in resource-saving agriculture XXI century: the results of the past and the problems of the present plus: periodical scientific publication 05 (39)/06 (40) 49–56

[5] Blinohvatov A F and Denisova G V 2001 Selenium in the biosphere (Penza: RIO PSAA) p 324

[6] Vikhreva V A and Nadezhkina E V 2012 The accumulation of selenium by plants, depending on its content in leached chernozems of the Penza region Agricultural chemistry 10 46–50

[7] Ilyin D Yu, Ilyina G V and Sashenkov S A 2013 The use of selenium compounds in the cultivation of xylotrophic basidiomycetes of different trophic strategies Niva Povolzhya 3 (28) 9–15

[8] Duran P, Acuna J and Gianfreda L 2015 Endophytic selenobacteria as new inocula for selenium biofortification Applied soil ecology 96 319–326

[9] Renwei F, Chaoyang W and Shuxin T 2013 The roles of selenium in protecting plants against abiotic stresses Environmental and experimental botany 87 58–68

[10] Vogrincic M, Cuderman P and Kreft I 2009 Selenium and Its Species Distribution in Above-ground Plant Parts of Selenium Enriched Buckwheat (Fagopyrum esculentum Moench) Analytical sciences 25 (11) 1357–63

[11] Piekarska A, Kolodziejski D and Pilipczuk T 2014 The influence of selenium addition during germination of Brassica seeds on health-promoting potential of sprouts International journal of food sciences and nutrition 65 (6) 692–702

[12] Golob A, Kavcic J and Stibilj V 2017 The effect of selenium and UV radiation on leaf traits and biomass production in Triticum aestivum L Ecotoxicology and environmental safety 136 142–149

[13] Schiavon M, Leonardo W and Jiang Y 2017 Effects of Selenium on Plant Metabolism and Implications for Crops and Consumers. Selenium in plants: molecular, physiological, ecological and evolutionary aspects Plant Ecophysiology 11 257–275

[14] Hanqin Y, Zhenyu Q and Mengqi L 2019 Selenium forms and methods of application differentially modulate plant growth, photosynthesis, stress tolerance, selenium content and speciation in Oryza sativa L Ecotoxicology and environmental safety 169 911–917