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The influence of microbial preparations on the biological activity of the rhizosphere and the productivity of winter triticale

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Abstract. The article presents the results on the effect of microbial preparations on the biological activity of the rhizosphere and the productivity of winter triticale. The microbiological and biochemical indicators of the biological activity of the winter triticale rhizosphere were studied against the background of the use of microbial preparations agrofil, mizorin, rhizobact of the RZHF brand and the FZhF brand. Data were revealed on the increase in the number of nitrogen-fixing microorganisms and actinomycetes when using agrofil, on the growth of micromycetes, actinomycetes, cellulose-decomposing microorganisms when treated with mizorin, the number of ammonifying microorganisms when treated with rhizobact preparations of the FZhF brand and the RZhF brand. Data on the beneficial effect of microbial preparations on the enzymatic pool of the winter triticale rhizosphere are presented. A high activity of phosphatase and peroxidase was noted when treated with the drug rhizobact brand FZhF, polyphenol oxidase and peroxidase when using the drug rhizobact brand RZHF, invertase and catalase when inoculated with the drug agrofil. Conclusions are formulated on the decrease in urease activity and the increase in indicators of productivity elements for all variants of the experiment.

1 Introduction

Of great importance for obtaining high and stable crop yields is the content in the soil of a sufficient amount of mineral nutrition elements. An alternative to mineral fertilizers, which are widely used in modern agricultural technologies, can be complex microbial preparations based on associative nitrogen-fixing microorganisms, which serve as a powerful factor in increasing soil fertility [1–6].

It is known that the microorganisms that make up such preparations perform a number of functions useful for plants: they increase the fixation of atmospheric nitrogen on the roots of the plant, stimulate the growth and development of plants by producing

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physiologically active substances, suppress the development of phytopathogenic microflora, and increase the resistance of plants to adverse conditions. environment, regulate the accumulation of heavy metals, radionuclides, nitrates and other harmful compounds in plants [1-2;5-6].

Atmospheric nitrogen fixed by microorganisms accounts for more than half of the total amount of this element entering the soil. Unlike nitrogen contained in mineral fertilizers, biological nitrogen is almost completely absorbed by plants and does not pollute the environment. This property necessitates the creation of new agricultural practices that increase the use of the share of biological nitrogen in the crop of agricultural plants.

Biological nitrogen supplied by microorganisms increases soil productivity. The most important role in this belongs to the activity and direction of microbiological and biochemical processes occurring in the soil. There is evidence in the literature that there is a close correlation between the intensity of biological processes, the composition and number of microorganisms, and the content of organic matter and biogenic elements in the soil. It is known that soil microorganisms have an enzymatic apparatus, perform various functions in the cycle of biogenic elements, participate in the formation of soils and maintaining their fertility [7]. Any impact on the soil can change the specifics of the biological processes occurring in it.

When determining the biological activity of soils, due to the greatest influence on its fertility, it is worth considering the number of such ecological and trophic groups of microorganisms as ammonifiers, actinomycetes, micromycetes, cellulites, and from enzymes - oxidoreductases (catalase, peroxidase, polyphenol oxidase) and hydrolases (urease, invertase, phosphatase) .

Winter triticale is an important crop that is widely used in the food and feed industries. Therefore, an important task is to increase the productivity of the grain of this crop [2;8]. To obtain its high and stable yields, the increase in soil fertility is of decisive importance, which largely depends on the activity of biological indicators.

In this regard, the study of the effectiveness of the use of complex microbial preparations on the indicators of the biological activity of the rhizosphere and the elements of the productivity of winter triticale is very important and is of significant scientific and practical interest.

The purpose of the study is to study the features of the influence of microbial preparations on the biological activity of the rhizosphere and the elements of winter triticale productivity.

2 Materials and methods

The studies were carried out on the experimental field of the Leningrad Research Institute of Agriculture "Belogorka" - a branch of the Federal State Budgetary Scientific Institution "Federal Research Center for Potato named after A.G. Lorkha" in a field crop rotation on soddy-podzolic light loamy medium cultivated soil. Spraying of plants with microbial preparations was carried out in the tillering phase with a liquid suspension (titer of preparations - 5 x 109 CFU per 1 ml) at a dose of 100 ml per 1 ha. The area of the experimental plot is 4 m2, repeated three times. The experience was set up as follows:

- Control (without inoculation).
- Agrophile (strain 10 Agrobacterium radiobacter).
- Mizorin (strain 7 Arthrobacter mysorens).
- Rhizobact brand RZHF (PBT strain 7 Corynebacterium sp.).
- Rhizobact brand FZhF (PBT strain 4 Pseudomonas sp.).

Soil samples were taken from the rhizosphere zone of the winter triticale village of Bilinda before harvesting. In the studied variant of the field experiment, the samples were taken with a soil drill in ten places at a depth of 10–15 cm, 100–200 g each. The samples taken were combined, dried, thoroughly mixed, and sieved through a sieve with a mesh size of 2 mm. The number of soil microorganisms was taken into account in the average soil sample. Soil dilutions were carried out by the Koch plate method. The number of ammonifying bacteria was determined on an agar medium consisting of a mixture of MPB and wort. For the cultivation of cellulolytic microorganisms, Hutchinson and Clayton's medium was used. To determine the number of nitrogen fixers (oligonitrophils), Ashby medium was used, micromycetes - Chapek-Dox medium, actinomycetes - KAA (ammonia starch agar) [9-12]. The activity of the urease enzyme was determined by the colorimetric method [11], invertase activity was determined by the method of V.F. Kuprevich [11], phosphatases – by the method of A.Sh. Galstyan and E.A. Harutyunyan. Determination of the activity of peroxidase (PO) and polyphenol oxidase (PPO) was carried out according to the method of K. A. Kozlov [4-5]. Pyrocatechol (0.02 M solution) was used as a substrate for peroxidase and polyphenol oxidase. Titration was carried out with 0.01 N iodine solution. To analyze the structure of the crop, 50 plants were selected from each plot of the experiment. Statistical data processing was carried out in the Microsoft Excel program.

3 Results

Nitrogen is a deficient element for plants, which need significant amounts of it for their development. Its main forms on Earth are bound nitrogen of the lithosphere and molecular nitrogen of the atmosphere. In order for molecular nitrogen to pass into a form accessible to plants, the activity of nitrogen-fixing microorganisms is necessary. The amount of available form of nitrogen in the soil is determined by the activity and quality of microbiological processes occurring in it. When growing crops, much attention is paid to the application of mineral fertilizers. Under natural conditions, the main role in soil enrichment with nitrogen is played by nitrogen fixers and ammonifiers, which can convert into the form of NH4 + or NO3 - organic nitrogen that is not available to plants, contained in plant and animal residues, and humus nitrogen, which account for most of the soil nitrogen. [13].

One of the important indicators of the condition of soils of any type is the presence of nitrogen-fixing bacteria. The activity of nitrogen fixation is one of the integral indicators of biological activity [9;13]. Our data show that the number of nitrogen-fixing microorganisms increases upon inoculation with microbial preparations. The maximum number of nitrogen fixers was noted in the variant with treatment with the microbial preparation agrofil (185 thousand CFU), which is 2.5 times more than in the control variant (table 1).

The process of converting soil organic nitrogen into the ammonium form is called ammonification and is carried out by heterotrophic microorganisms.

Of the ammonifiers in this experiment, the number of spores of bacteria of the genus Bacillus, which play an important role in the mineralization of organic nitrogen compounds, was taken into account. When taking into account the data obtained, there is an increase in their number in the variants with treatment with microbial preparations, the maximum level is in the variant with the use of the drug rhizobact of the RZHF brand, which is 227 CFU per 1 g of soil, which is 29.5% higher than the control variant.

Table 1. Influence of microbial preparations on the number of various ecological and trophic groups of microorganisms in the rhizosphere of winter triticale, thousand CFU per 1 g of soil.

Experience Variant	Nitrogen fixers (oligonitro phils)	Ammonifiers (genus *Bacillus*)	Actinomycetes	Cellular	Micromy cetes
Control	72	160	364	135	198
Mizorin	138	210	389	192	412
agrophile	185	169	760	130	242
Rizobakt brand RZHF	110	227	498	127	151
Ryzobact brand FZhF	103	218	400	123	157
NSR $_{0.5}$	6	14	15	5	12

One of the most important groups of soil microorganisms are actinomycetes. They play an important role in the formation of soil fertility by participating in the decomposition and transformation of complex organic compounds. Our data show that the number of actinomycetes increased in all variants where microbial treatment was carried out. The maximum number of actinomycetes was recorded in the variant with the use of the drug Agrofil (760 thousand CFU), which is 52.1% higher than the control variant (table 1).

Microorganisms that destroy cellulose play an important role in maintaining soil fertility and are called cellulites. They contribute to the enrichment of the soil with available nutrients, and plants - with organic compounds, which has a positive effect on productivity. Table 1 shows that microbial preparations had an ambiguous effect on the change in the number of this group of microorganisms. An increase in the number of cellulites by 29.7% relative to the control (135 thousand CFU) was noted in the variant with the drug mizorin (192 thousand CFU). A slight decrease in the number of this group of bacteria by 3.7% - 8.8% relative to the control was observed in the experimental variants using the preparation Agrofil and rhizobact of the RZHF and FZHF brands.

The largest ecological group that mineralizes the organic remains of plants and animals and the formation of soil humus are micromycetes. The distribution and high activity of micromycetes in the soil are due to their greater resistance to changing environmental conditions compared to other microorganisms. Our studies show that the number of microscopic fungi depended on the mechanism of action of the microbial preparation. The maximum indicator of the number of micromycetes was revealed during treatment with the microbial preparation mizorin (412 thousand CFU). In the variants with microbial treatment with rhizobacts of the FZhF and RZhF brands, a decrease in the number of micromycetes by 20.7–23.7% was observed relative to the control variant (198 CFU) (Table 1).

One of the most important indicators of soil biological activity is enzymatic activity. A special role in the soil belongs to enzymes of the class of hydrolases. With the help of enzymes of this group, macromolecules are decomposed and converted into a more accessible form for microorganisms and plants. The activity of nitrogen metabolism enzymes is an important diagnostic indicator reflecting the intensity of soil nitrogen mobilization processes [11;13].

The enzyme that performs hydrolysis and conversion to the available form of urea nitrogen is urease. Urease activity is one of the most important indicators of soil biological activity. The enzyme is characterized by high specificity, since it acts only on urea, without affecting its other derivatives. Studies on the evaluation of urease activity showed its decrease against the background of the use of microbial preparations (table 2).

Table 2. The effect of microbial preparations on the activity of hydrolytic enzymes in the rhizosphere of winter triticale.

Experience Variant	Urease activity, mg NH_3 per 10 g of soil	Phosphatase activity, mg P_2O_5 per 10 g of soil for 1 hour	Invertase activity, mg of glucose per 1 g of soil in 24 hours
Control	102.0	1.8	1.2
Mizorin	70.0	5.4	1.9
Agrophile	95.3	1.2	3.0
Rizobakt brand RZHF	68.3	2.0	1.6
Ryzobact brand FZhF	63.4	9.6	2.5
NSR $_{0.5}$	1.4	0.2	0.3

Its minimum level (63.4 mg NH3) was noted in the variant with the use of the drug rhizobact brand FZhF, which is 37.9% lower than the control. A decrease in urease activity by 6.5% was noted in the variant with the use of agrophil (95.3 mg NH3). A more significant decrease in urease activity by 31–37.8% relative to the control (102 mg NH3) was noted in the variants with mizorin and rhizobact brand RZHF.

Since, against the background of a high level of urease activity, the process of decomposition of urea in the soil can accelerate, and this, in turn, will lead to a loss of nitrogen (ammonia) and a shift in the acidity of the medium to the alkaline side, then its decrease in variants with the use of microbial preparations can be regarded as a favorable prognostic factor. sign. Therefore, when using these drugs based on associative diazotrophs, which reduce urease activity, possible losses of nitrogen compounds are prevented [14].

The next enzyme from the class of hydrolases, which has the ability to hydrolyze phosphoric acid esters, is phosphatase. Due to the activity of phosphatases (phosphohydrolases), forms of phosphorus inaccessible to plants are absorbed. Phosphatases play an important role in the mobilization of organophosphorus compounds [11].

Data on phosphatase activity in our studies are presented in table 2. The use of rhizobact preparations of the FZhF brand and mizorin stimulated phosphatase activity. Its maximum activity was noted in the variant with the drug rhizobact brand FZhF and amounted to 9.6 mg of phenolphthalein, which is 81.25% higher than the control (1.8 mg of phenolphthalein) (table 2). A sharp decrease in phosphatase activity was noted in the variant with the preparation Agrofil (1.2 mg of phenolphthalein), which is lower than the control variant by 33.3% (0.6 mg of phenolphthalein).

The next representative of the class of hydrolases, a subclass of carbohydrases, is invertase. Its function is to carry out the hydrolysis of sucrose into glucose and fructose. The results of our studies showed that invertase activity increased in all variants relative to the control. Its maximum activity was noted in the variant with the use of the drug Agrofil (3.0 mg glucose), which is 2.5 times higher than the control (1.2 mg glucose). The minimum activity of invertase was noted in the variant with the drug rhizobact brand RZHF (1.6 mg glucose) (table 2).

In the metabolism and energy metabolism in the soil, an important place belongs to redox enzymes (oxidoreductases), which are involved in the synthesis of humus components of the soil [9]. One of the representatives of the class of oxidoreductases is catalase. Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide into oxygen and water. It promotes the development of soil microorganisms, the processes of decomposition of organic substances and their transfer to humus. As a result of our studies, it was found that catalase activity increases in all variants with the use of microbial preparations (table 3).

Table 3. Influence of microbial preparations on the activity of redox enzymes in the rhizosphere of winter triticale.

Experience Variant	Catalase activity, ml O_2 released in 2 min per 1 g of soil	Peroxidase activity, ml I_2/1g absolutely dry soil	Polyphenol oxidase activity, ml I_2/1g absolutely dry soil
Control	1.5	5.0	1.03
Mizorin	2.5	8.0	1.12
Agrophile	3.1	10.0	0.94
Rizobakt brand RZHF	2.0	8.0	1.58
Ryzobact brand FZhF	2.3	11.0	0.56
NSR $_{0.5}$	0.2	0.7	0.05

The maximum activity of catalase was noted in the variant with the Agrofil preparation and amounted to 3.1 ml O2, which is two times higher than in the control variant (1.5 ml O2). The lowest activity of catalase was found in the variant with RZhF brand rhizobact (2.0 ml O2).

The next enzyme belonging to the class of oxidoreductases is peroxidase. Peroxidase oxidizes soil organic matter at the expense of oxygen, hydrogen peroxide, and other organic peroxides formed in the soil as a result of the vital activity of microorganisms [11;15]. The results of our studies have shown that peroxidase activity increases in all variants of the experiment. Its highest activity was noted in the variant with the FZhF rhizobact preparation and amounted to 11 ml of I2, which is 2.2 times higher than the control variant (table 3).

The enzyme involved in the conversion of aromatic organic compounds into humus components is polyphenol oxidase [11]. This enzyme catalyses the oxidation of phenols to quinones in the presence of atmospheric oxygen. Quinones under appropriate conditions, when condensed with amino acids and peptides, form the primary molecules of humic acid. In the course of our study, the maximum activity of polyphenol oxidase was noted in the variant with the preparation of rhizobact brand RZHF (1.58 ml I2), which is 53% higher than the control (1.0 ml I2). In variants with the use of the microbial preparation agrofil and rhizobact of the FZhF brand, the indicators of polyphenol oxidase activity are lower than in the control (0.94 and 0.56 ml of I2) (table 3).

The possibility of the influence of microbial preparations on the productivity of winter triticale deserves special attention. In our studies, all ear productivity indicators increased in all variants of the experiment relative to the control (table 4).

Studies have shown that microbial preparations based on rhizobacteria increase the length of the ear, the number of grains per ear, and the mass of grain per ear. The maximum increase in grain mass and ear length was noted in the variants with the use of the microbial preparation rhizobact of the RZhF and FZhF brands (in terms of grain weight, the increase relative to the control was 59.6 and 59.5%, and in the length of the ear - 19.6 and 5.4% respectively). The largest increase in the number of grains was also noted in the variants with rhizobact preparations of the RZhF brand and the FZhF brand, which amounted to 33.3 and 25.9% respectively.

Table 4. Influence of microbial preparations on the elements of productivity of winter triticale.

Experience options	Spike length, cm	Gain to control, %	Number of grains in an ear, pcs.	Gain to control, %	Weight of grain per ear, g	Gain to control, %
Control	5.6	-	27.0	-	0.8	-
Mizorin	5.8	3.6	29.0	7.4	1.1	39.1
agrophile	5.7	1.8	28.0	7.4	0.8	3.1
Rizobakt brand RZHF	6.7	19.6	36.0	33.3	1.3	59.6
Ryzobact brand FZhF	5.9	5.4	34.0	25.9	1.3	59.5
NSR $_{0.5}$	0.2	-	3.0	-	0.1	-

4 Discussion

The research results show that the treatment of winter triticale plants with microbial preparations has a beneficial effect on the indicators of biological activity (microbial and enzymatic pool) of the rhizosphere, which ultimately leads to an increase in ear productivity elements. The intensity of biochemical processes in the soil and the level of its fertility depend both on the conditions for the existence of living organisms that supply enzymes to the soil, and on factors that contribute to the fixation of enzymes in the soil and regulate their activity (composition and properties of the soil, environmental factors), which indicates , that biological activity is one of the most sensitive indicator manifestations of the soil environment. The results of the conducted studies are confirmed by numerous data from such researchers as Patyka, 1991, Zavalin, Kozhemyakov, 2001, Dorosinsky, 1989, Chistotin, 2001, Mishustin, 2004, Kuznetsov et al., 1999.

5 Conclusion

The microbial preparations studied in our experiment proved to be ambiguous: agrofil and mizorin contributed to an increase in the number of nitrogen fixers, actinomycetes, micromycetes and cellulites, while microbial preparations of the rhizobact brand contributed to an increase in the number of ammonifiers. Enzymatic activity also depended on the type of microbial preparation: agrofil contributed to an increase in the activity of invertase and catalase, mizorin - peroxidase, rhizobact brand RZHF - phosphatase and polyphenol oxidase, rhizobact brand FZhF - phosphatase and peroxidase. The activity of urease decreased in all variants with the use of microbial preparations relative to the control variant, which is a favorable prognostic sign for soil nitrogen exchange. The maximum indicators of ear productivity were noted when treated with a microbial preparation rhizobact brand RZHF, which contributed to an increase in the length of the ear by 19.6%, the number of grains per ear by 33.3%, the mass of grain per ear by 60.8% and grain yield by 63.5 %.

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