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The influence of microbiological fertilisers on the productivity and quality of winter wheat

Yuri Koryagin[1], Evgeniya Kulikova[1], Saniya Efremova[2], Nadezhda Sukhova[2]*

[1]Ministry of Agriculture, Faculty of Agronomy, Penza State Agrarian University, Penza, Russia
[2]Ministry of Education and Science, Faculty of Biotechnology, Penza State Technological University, Penza, Russia
**Corresponding author: kuleg@mail.ru*

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Abstract: The study was aimed at assessing the yield and quality of winter wheat grains inoculated with *Beijerinckia fluminensis* (Azotovit) and *Paenibacillus mucilaginosus* (Phosphatovit) in a three-year experiment on leached Chernozem in the forest-steppe zone of the Middle Volga region. The seeds of the plants were treated before sowing with microbiological fertilisers, both individually and together at a dose of 2 L/t. Bacteria *Beijerinckia fluminensis* and *Paenibacillus mucilaginosus* contained in fertilisers increased the resistance of plants to adverse conditions: seedling completeness increased by 5.4%, winter hardiness by 17.4%, and harvestability by 15.0%. The use of fertilisers led to an increase in the productivity of winter wheat grain to 32.4%. The technological parameters characterising the baking properties were improved: the content of crude gluten in the grain of winter wheat has increased to 29.1% at 75 GDI (gluten deformation index) units (I group (good) of gluten quality).

Keywords: *Triticum aestivum* L.; inoculation; nitrogen-fixing and phosphate-mobilising properties; phytopathogen

The provision of high-quality plant products requires the introduction of environmental-friendly technologies for growing grain, industrial and vegetable crops, which provide for the gradual replacement of some agrochemicals with microbiological preparations (Hogenhout et al. 2009, Koryagina et al. 2016, Kulikova and Klimova 2018, Li et al. 2019).

Currently, a new generation of biological products was developed the world that have a wide range of actions such as stimulating growth and inhibiting the development of phytopathogens, increasing resistance to various environmental factors, reducing the intake of heavy metals, pesticides and radionuclides into plants (Nadezhkina and Silnova 2001, Kulikova 2005, Çakmakçı et al. 2007, Stamenov et al. 2012).

One of the most important crops used in bakery is winter wheat. Getting high yield is impossible without increasing the agricultural background with the help of a set of agricultural measures (Baldani and Baldani 2005, Chandra et al. 2019, Zaheer et al. 2019).

The use of mineral fertilisers is partly associated with environmental hazards and their cost. The use of inoculants containing bacteria is an alternative to increase the efficiency of nitrogen and phosphorus fertilisers and it can reduce the volume of their use (Holečková et al. 2018).

Among the main nutrients, phosphorus is the least accessible and mobile in most soil conditions. The content of its organic and inorganic forms in soils is often the main limiting factor for plant growth. In the rhizosphere, the bioavailability of inorganic phosphorus varies significantly depending on the nutritional status of the soil, plant species and environmental conditions. To overcome phosphorus deficiency, phosphate-mobilising microorganisms can play an important role in providing plants with phosphorus in a more environmentally friendly and sustainable way (Khan et al. 2007).

The universality of their use in any technological operation of cultivating crops is an advantage of the microbiological fertilisers and has a positive effect on any plant organism (Koryagin and Koryagina 2018).

Nitrogen-fixing bacteria are generally well known for their ability to fix atmospheric nitrogen and are widely used around the world to inoculate leg-

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umes. However, phosphorus release ability has also been found in some nitrogen-fixing genera such as *Rhizobium*, *Bradyrhizobium* and *Sinorhizobium* (Flajšman et al. 2019).

The effect of new microbiological fertilisers with nitrogen-fixing and phosphate-mobilising properties (Azotovit and Phosphatovit) on the productivity of winter wheat and the quality of the resulting products was studied in this work.

MATERIAL AND METHODS

A study on the influence of the microbiological fertilisers Azotovit and Phosphatovit was carried out on winter wheat of the Bezenchukskaya 380 cultivar in a field experiment. In terms of particle size composition, the soil of the experimental plot is heavy-loamy leached Chernozem. The average content of organic matter in the arable layer is 5.92%, the soil solution in the arable horizon is slightly acidic (pH soil 5.0–5.1), the content of alkaline hydrolyzable nitrogen (according to the Cornfield method: hydrolysis of soil organic compounds with NaOH solution) is very low – from 81 to 98 mg/kg DM (dry matter), the content of mobile phosphorus from 63 to 67 mg/kg DM (average) and the content of potassium from 96 to 104 mg/kg DM (elevated). Determination of mobile compounds of phosphorus and potassium was carried out by the Chirikov method by extracting them with a solution of acetic acid and subsequent quantitative determination of phosphorus on a photoelectrocolorimeter, and potassium on a flame photometer.

The composition of Azotovit includes living bacterial cells (*Beijerinckia fluminensis*) with a concentration of at least 1×10^9 CFU (colony forming unit)/cm^3. The bacteria fix molecular nitrogen and, according to the drug manufacturer, during a series of transformations convert it to ammonium, nitrite and nitrate forms, which are easily absorbed by plants and prevent the loss of mineral nitrogen during its conversion in the soil. The composition of Phosphatovit includes spores and living cells of bacteria *Paenibacillus mucilaginosus* with a concentration of at least 0.12×10^9 CFU/cm^3. Organic acids secreted by the bacteria *Paenibacillus mucilaginosus* mobilise inaccessible phosphorus and potassium from insoluble compounds in the plant rhizosphere (according to the drug manufacturer).

The experiment was laid out at plots with a total area of 15 000 m^2, the size of the experimental plots was 10 000 m^2, the repetition was threefold, the placement of options was randomised according to the following scheme: (1) inoculation of seeds with water (control) (2 L/t); (2) inoculation of seeds with Azotovit (2 L/t); (3) inoculation of seeds with Phosphatovit (2 L/t); (4) inoculation of seeds with Azotovit + Phosphatovit. The treatment of plant seeds before sowing with microbiological fertilisers was carried out at a dose of 2 L/t (2 + 2 L/t).

Agricultural technology: the main tillage was carried out in the third decade of August – disking (with black fallow as a forecrop). In spring harrowing and three cultivations were carried out. Mineral N, P and K were applied as ammonium nitrate (34.5% N), double superphosphate (18.9% P) and potassium chloride (49.8% K) at the doses 120, 26 and 50 kg/ha, respectively (pure nutrient inputs). The nitrogen was applied in three equal dressings (in autumn before sowing, in April for regeneration and in May for grain production). Fertilisers P and K were applied before sowing. Sowing was carried out according to the experimental scheme in the first ten days of September, with an inter-row spacing of 15 cm and a sowing rate of 5.5 million germinating seeds per 1 ha. Packing was done after sowing. Harvesting was carried out by the continuous method. The grain yield was recalculated to standard humidity and the obtained data were processed by the method of dispersion analysis.

The bakery qualities of wheat grains were evaluated by raw gluten and its physical properties. The amount of raw gluten in flour is the ratio of the weight of the washed raw gluten to the weight of the flour, expressed as a percentage. Its quality (deformation value, when the moulded ball (4 g) is compressed) is expressed in standard units of the IDK-3M device (Gluten strain meter, Krasnoobsk, Russia) and is classified into groups.

Over the entire period of the research (2016–2018), meteorological conditions were generally favourable for the growth and development of winter wheat. Humidity and thermal conditions during the experiment are shown in Figure 1.

Statistical analysis. An analysis of variance was performed. The least significant differences (*LSD*) were calculated using the Tukey's test ($\alpha = 0.05$). Principal component analysis (PCA) was applied to show relationships between the analysed parameters and treatments. The data analysis software system Statistica, ver. 10 (TIBCO Software Inc., Palo Alto, USA) was used.

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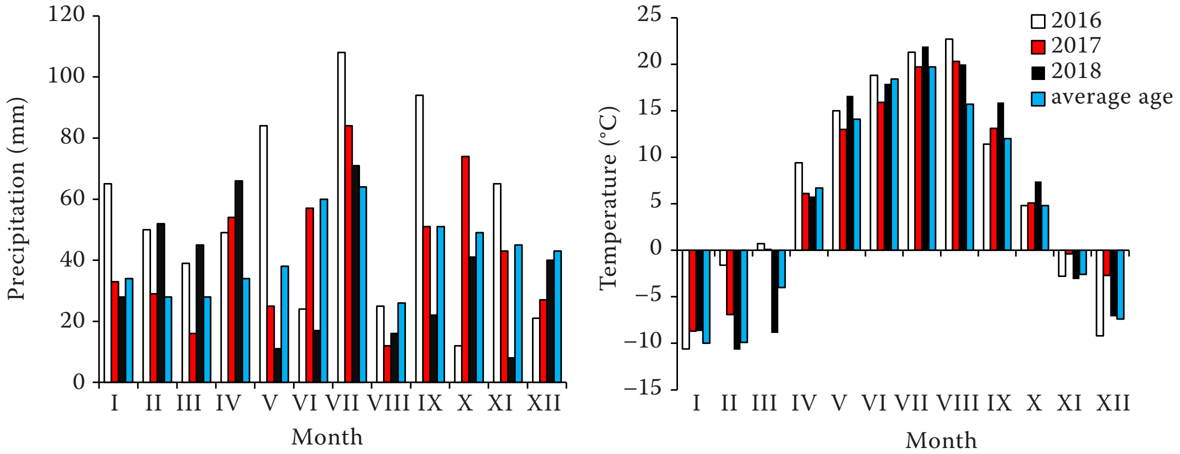

Figure 1. Total precipitation and average air temperature during wheat growth

RESULTS AND DISCUSSIONS

The biological stability of winter wheat to the weather conditions of the growing season is characterised by the most important economically useful signs: seedling completeness, winter hardiness and plant survival.

The seedling completeness on average over the years of research ranged for all options from 450 to 480 pcs/m^2 (out of 550 pcs/m^2 of sown seeds) (Table 1). Its greatest value was recorded in the variant with seeds treated with the Azotovit and Phosphatovit biological preparations before sowing – 87.2% together, which was 5.3% higher compared to the control and 0.7–0.9% compared to separate seed treatment with biological preparations before sowing.

Winter hardiness for all treatments of the experiment was in the range of 328–430 pcs/m^2. The highest percentage of overwintered plants was noted while treating seeds with the biological preparations together – 89.6%. Almost the same ability was characterised by seed treatment with Azotovit (87.2%) and Phosphatovit (87.8%). The lowest rate was observed in winter wheat plants in the control variant – 72.2%.

The largest percentage of plants preserved for harvesting after overwintering, on average over the research period, was noted while treating seeds with the biological preparations together – 93.0%. That was 15.0% more compared to the control and 1.1–1.7% more than their separate use. The highest survival rate of plants was also observed during the joint processing of winter wheat seeds with the biological preparations – 83.3%, and in the control variant – 53.9%.

When analysing winter wheat sheaf material, it was noted that the tallest plants were formed in the variant with the pre-sowing seed treatment with Azotovit – 79.5 cm, which was 7.8 cm more compared to the control, and compared with the use of Phosphatovit and their combination – by 1.7 cm and 3.9 cm, respectively. Productive bushiness in the experiment

Table 1. The effect of microbiological fertilisers on the seedlings completeness, the percentage of overwintered and preserved for harvesting winter wheat plants

Treatment	Seedlings completeness (%)	The amount of overwintered plants per (pcs/m^2)				The percentage of overwintering (%)	The number of plants before harvesting (pcs/m^2)				The percentage of plants preserved for harvesting (%)
		2016	2017	2018	average		2016	2017	2018	average	
1	81.9	312	329	343	328	72.2	244	257	267	256	78.0
2	86.3	415	396	431	414	87.2	390	361	383	378	91.3
3	86.5	399	418	438	418	87.8	367	383	402	384	91.9
4	87.2	430	409	451	430	89.6	400	381	419	400	93.0
Average	–	389	388	416	–	–	350	346	368	–	–
		P = 0.00051; $LSD_{0.05}$ = 34.8912					P = 0.00001; $LSD_{0.05}$ = 30.259				

Inoculation of seeds before sowing with: 1 – water (control) (2 L/t); 2 – Azotovit (2 L/t); 3 – Phosphatovit (2 L/t); 4 – Azotovit + Phosphatovit (2 + 2 L/t); *LSD* – least significant difference

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Table 2. The effect of microbiological fertilisers on the elements of the structure of the grain yield of winter wheat

Treatment	The mass of 1 000 grains (g)				Yield (t/ha)			
	2016	2017	2018	average	2016	2017	2018	average
1	41.8	40.2	40.6	40.9	2.98	2.62	2.83	2.81
2	44.6	40.3	42.0	42.3	3.74	3.29	3.54	3.52
3	45.0	44.5	42.5	43.7	3.79	3.42	3.59	3.60
4	45.3	47.1	43.6	44.5	3.94	3.47	3.75	3.72
Average	44.2	40.2	42.2	–	3.61	3.2	3.40	–
	P = 0.03990; $LSD_{0.05}$ = 3.011				P = 0.00275; $LSD_{0.05}$ = 0.393			

Inoculation of seeds before sowing with: 1 – water (control) (2 L/t); 2 – Azotovit (2 L/t); 3 – Phosphatovit (2 L/t); 4 – Azotovit + Phosphatovit (2 + 2 L/t); *LSD* – least significant difference

ranged from 1.3 to 1.7, with a maximum of 1.7 being observed during seed treatment before sowing with Azotovit together with Phosphatovit. The number of formed grains on one plant with the use of the biological preparations ranged from 35.0 to 36.3 grains per plant, with a spike length of 6.7–7.1 cm, which was 1.7–4.5% and 3.1–10.9% higher than in the control, respectively. The most heavy grain was also obtained in the treatment with joint processing of seeds before sowing – the mass of 1 000 grains was 44.5 g. This was 3.6 g more compared to the control, and by 0.8 g and 2.2 g compared to separate treatment of seeds with Azotovit and Phosphatovit (Table 2).

The grain yield of winter wheat over the years of research ranged from 2.81 t/ha to 3.72 t/ha, which was confirmed by the elements of the yield structure. A low yield (2.81 t/ha) was observed in the control variant, and the highest yield was obtained in the variant with complex seed treatment before sowing with the biological preparations Azotovit and Phosphatovit – 3.72 t/ha.

Many authors carried out studies and experiments where they applied microbiological preparations. Holečková et al. (2018) considered the effect of microbiological preparations on corn yield, but the effect of using a bioeffector on increasing dry matter yield was not confirmed. Kifle and Laing (2016) carried out the study, which included experiments with corn where they applied the *Pseudomonas* bacteria. The results showed that the application had a positive effect only on seed germination, not on increased grain yield, dry matter or plant height.

Grain quality indicators of winter wheat are important for characterising technological parameters and baking properties (Table 3).

The obtained data on the combined effect of the microbiological fertilisers Azotovit and Phosphatovit on the grain nature indicate an increase of this indicator by 14.4 g/L compared with the control, and by 11.3 g/L and 12.9 g/L compared with the separate treatment of winter wheat seeds with Azotovit and Phosphatovit, respectively. The highest protein content (14.5%) was also found with the combined use of the preparations, which was 2.6% higher than in the control and 1.0–1.4% compared to their separate use.

Also, in the experiment with the joint treatment of winter wheat seeds, the largest percentage of hardness was recorded before sowing – 63.8%, which was by 11.5% higher than in the control and by 3.6% and

Table 3. The influence of microbiological fertilisers on technological parameters of winter wheat grain

Treatment	Grain unit (g/L)				Total hardness (%)				Protein content (%)			
	2016	2017	2018	average	2016	2017	2018	average	2016	2017	2018	average
1	822.0	824.0	825.6	824.0	54.9	52.5	49.5	52.3	12.7	11.0	12.1	11.9
2	822.7	825.3	828.4	825.5	62.9	60.3	57.4	60.2	12.8	13.4	13.1	13.1
3	829.2	824.8	827.2	827.1	62.8	60.1	58.6	60.5	12.9	13.5	14.0	13.5
4	843.5	835.1	836.6	838.4	66.4	63.5	61.5	63.8	14.5	13.7	15.3	14.5
Average	831.8	827.9	830.7	–	61.7	59.1	56.7	–	13.2	12.9	13.6	–
	P = 0.00138; $LSD_{0.05}$ = 5.68				P = 0.00308; $LSD_{0.05}$ = 4.779				P = 0.01005; $LSD_{0.05}$ = 1.254			

Inoculation of seeds before sowing with: 1 – water (control) (2 L/t); 2 – Azotovit (2 L/t); 3 – Phosphatovit (2 L/t); 4 – Azotovit + Phosphatovit (2 + 2 L/t); *LSD* – least significant difference

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Table 4. The effect of microbiological fertilisers on baking properties of winter wheat grain

Treatment	Crude gluten content (%)				Indication of the GDI device in standard units				Gluten quality group
	2016	2017	2018	average	2016	2017	2018	average	
1	22.9	21.0	21.8	21.9	84.9	77.9	80.8	81.2	II group (satisfactory weak)
2	24.7	22.5	23.6	23.6	85.5	82.8	83.6	84.0	II group (satisfactory weak)
3	25.0	23.4	23.9	24.1	81.4	76.2	77.8	78.5	II group (satisfactory weak)
4	30.4	28.1	28.8	29.1	78.5	72.4	74.2	75.0	I group (good)
Average	25.7	23.7	24.5	–	82.6	77.3	79.1	–	–
	P = 0.00014; $LSD_{0.05}$ = 1.927				P = 0.02286; $LSD_{0.05}$ = 5.265				

Inoculation of seeds before sowing with: 1 – water (control) (2 L/t); 2 – Azotovit (2 L/t); 3 – Phosphatovit (2 L/t); 4 – Azotovit + Phosphatovit (2 + 2 L/t); *LSD* – least significant difference; GDI – gluten deformation index

3.3% compared with separate seed treatment with Azotovit and Phosphatovit, respectively.

The same tendency was observed in the effect on the crude gluten content in the grain of winter wheat. The highest content of crude gluten (29.1% at 75 units of GDI (gluten deformation index) and group I (good)) was noted in the variant of joint seed treatment before sowing with the biological preparations Azotovit and Phosphatovit, which was 7.2% more compared to control and 5.5% and 5.0% more compared to the treatment of winter wheat seeds with Azotovit and Phosphatovit, respectively (Table 4).

Thus, inoculation of wheat seeds with *Beijerinckia fluminensis* and *Paenibacillus mucilaginosus* and their combination is a promising method for increasing the yield and quality of winter wheat.

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