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The influence of a microbial consortium of lactic acid bacteria and yeast on the grain yield and quality of several important agricultural crops

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Abstract. The use of chemicals in agriculture has a negative impact on the environment, soil, and the yield and quality of harvests. The **purpose** of this research was to study the effect of a microbial consortium based on lactic acid bacteria and yeast on the productivity of several important agricultural crops. The microbial consortium was obtained by co-cultivation of *Lactobacillus casei*, *L. plantarum*, *L. lacti*s, and *Saccharomyces cerevisiae.* **Methods.** The microbial consortium (1 L/ha) was used to treat crops of wheat, corn, soybeans, and rapeseed 1, 2, or 4 times during the growing season compared to treatment of the same crops with water and a widely used microbiological preparation ("Baykal EM-1"). **Results.** A single application of the microbial consortium increased the yield of the treated crops by 25–38 %, while treating the crops in 2 or 4 times affected the quality of the crop rather than its quantity. An increase in protein content of 1.2–3.1 % and oil content of 2.2–4.3 % was observed. The increasing for mineral elements was such as: for copper by 20 %, for zinc by 18.2% and for manganese by 34.4 %. Treatment with the microbial consortium increased the protein yield for wheat, corn, and soybean by 30.2–72.1 % and increased the oil yield for soybean by 30.2–31.7 % and for rapeseed by 40.2–47.7 % depending on the number of treatments. **The scientific novelty.** The microbial consortium with Lactobacillus casei IMB B-7343, Lactobacillus plantarum IMB B-7344, Lactococcus lactis IMB B-7352 and Saccharomyces cerevisiae IMB Y-5046 has been demonstrated the effectiveness for the first time in production conditions, associated not only with an increase in yield, but also with its quality.

Keywords: rapeseed, corn, grain quality, lactic acid bacteria, microbial consortium, soybean, wheat, yield

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Влияние микробного консорциума молочнокислых бактерий и дрожжей на урожай и качество зерна некоторых важных сельскохозяйственных культур

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Аннотация. Применение химических препаратов в сельском хозяйстве оказывает негативное влияние на окружающую среду, почвы, урожай и его качество. **Целью** исследования явилось изучение влияния микробного консорциума на основе молочнокислых бактерий и дрожжей на продуктивность некоторых сельскохозяйственных культур. **Методы.** Микробный консорциум получали путем совместного культивирования *Lactobacillus casei*, *L. plantarum*, *L. lacti*s и *Saccharomyces cerevisiae*. Микробным консорциумом в количестве 1 л/га обрабатывали посевы пшеницы, кукурузы, сои и рапса 1, 2 и 4 раза за вегетационный период и сравнивали с обработкой посевов водой и широко используемым микробиологическим препаратом «Байкал ЭМ-1». **Результаты.** Одноразовое внесение микробного консорциума увеличило урожай исследованных сельхозкультур на 25–38 %, а 2- и 4-разовая обработка посевов оказывает влияние не столько на количество урожая, сколько на его качество: отмечено увеличение содержания белка на 1,2–3,1 %, масла на 2,2–4,3, а минеральных элементов: меди – на 20 %, цинка – на 18, 2%, марганца – на 34,4 %. Микробный консорциум повысил выход белка из зерна пшеницы, кукурузы и сои на 30,2–72,1 %, выход масла из зерна сои увеличился на 30,2–31,7 %, а рапса – на 40,2–47,7 % в зависимости от числа обработок. **Научная новизна.** Впервые в производственных условиях показана эффективность микробного консорциума *Lactobacillus casei* IMB B-7343, *Lactobacillus plantarum* IMB B-7344, *Lactococcus lactis* IMB B-7352 и *Saccharomyces cerevisiae* IMB Y-5046, связанная не только с увеличением урожая, но и с его качеством.

Ключевые слова: микробный консорциум, молочнокислые бактерии, урожай, качество зерна, пшеница, рапс, кукуруза, соя

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Introduction

Using of chemical pesticides, fertilizers, growth regulators, and plant protection agents in agriculture causes irreparable ecological damage to ecosystems worldwide and reduces soil fertility [1]. Biological agents comprise only a small percentage of the total volume of such preparations [2]. Using the biological components based on microorganisms is a promising solution to the damage caused by chemical substances. Their use would increase plant productivity and crop quality while preserving the natural fertility of soils without deteriorating the ecological state of the environment [3].

Application of microbiological preparations is known to have many benefits. These include allowing regulation of the number and activity of beneficial microflora in the rhizosphere, accelerating the initial development of plants, helping to neutralize the partial toxic effects of chemical agents, increasing the overall immunity and adaptive capacity of the culture as a result of adverse impacts on environmental factors, and improving the availability of plant use of micro- and macronutrients from the soil [4–8].

Usage of microbiological preparations in combination with modern agricultural engineering allows the soil and climatic potential of the agrolandscape to be realized by 60–80 % (instead of the current 20–30 %), as well as the biological potential of agricultural plants, which is also not being used effectively [9–11]. Many experiments have shown an increase in the quality of crop production and profitability of agricultural enterprises by 30–50 % when biological products are used [12–15].

The effectiveness of microbiological preparations based on lactic acid bacteria and yeast is due to their ability to suppress pathogenic microorganisms, improve soil fertility, and stimulate plant growth [16–18].

Metagenomic analysis of plant microbiomes, including rhizobacteria, has revealed lactic acid bacteria in the plant-soil ecosystem, although they are difficult to detect due to their low numbers [19; 20].

Lactic acid bacteria suppress pathogens by means of synthesized high molecular-weight antimicrobial substances (bacteriocins and fungicins) as well as low molecular-weight metabolites (hydrogen peroxide, carbon dioxide, diacetyl, reuterin, alcohols, organic acids, etc.) [21]. Intestinal bacteria can penetrate plants, migrate, and infect all their organs (including fruits); human consumption of these contaminated agricultural products may cause intestinal infections [22; 23]. Bacteriocins produced by lactic acid bacteria [24] inhibit the growth of intestinal pathogenic and opportunistic bacteria, phytopathogenic bacteria, and mycelial fungi [25–27].

Stimulation of plant growth by lactic acid bacteria is due to their ability to produce phytohormones [11; 14; 17], to convert insoluble phosphorus compounds into forms accessible to plants [28; 29], and to accelerate mineralization processes in the soil [30].

In recent decades, lactic acid bacteria, due to the synthesis of phytohormones and/or their precursors and metabolites suppressing phytopathogens, have come to be considered plant growth-promoting bacteria capable of stimulating plant growth (PGPB) [31].

The aim of our research was to study the effect of a microbial consortium, based on lactic acid bacteria and yeast, on the productivity of several important crops.

Methods

Creation of a microbial consortium based on lactic acid bacteria and yeast was the research object. The microbial consortium was obtained by co-cultivation of lactic acid bacteria and yeast on MRS nutrient medium. Microorganisms were cultivated in a 4-L Minifors 2 bioreactor (Switzerland) at 32 °C and 50 rev/min during over 3 days.

Bacterial cultures grown for 24 h were used to produce the inoculum: *Lactobacillus casei* (IMB B-7343; 2.4 × 10^8 CFU/mL); *Lactobacillus plantarum* (IMB B-7344; 5.1 × 10^8 CFU/mL); *Lactococcus lacti*s (IMB B-7352; 1.7 × 10^8 CFU/mL), and a 2-day culture of *Saccharomyces cerevisiae* (IMB Y-5046; 3.1 × 10^5 CFU/mL) in a ratio of 1:1:1:0.5. *Lactobacillus spp.* were cultivated on MRS nutrient medium [32] in a MaxQ 4500 shaker-incubator (ThermoFisher Scientific, United States) at 100 rev/min at 36 °C; *Lactococcus* at 30 °C. The *S. cerevisiae* was cultivated on Sabouraud's medium [33] in an incubator shaker at 200 rev/min at 28 °C.

To control the quality of the consortium produced, the quantity of microorganisms in the consortium was counted after 3 days of joint cultivation using the dilution limit method [34]: *L. casei* (IMB B-7343) 6.2 × 10^8 CFU/mL; *L. plantarum* (IMB B-7344) 7.4 × 10^8 CFU/mL; *L. lacti*s (IMB B-7352) 1.2 × 10^9 CFU/mL; *S. cerevisiae* (IMB Y-5046) 2.7 × 10^6 CFU/mL.

The molecular genetic identification of culture strains as a component of the microbial consortium was carried out by analyzing the genes encoding 16S rRNA. For this purpose, conservative primers were used to process genes encoding 16S rRNA – 8f – aga gtt tga tcc tgg ctc ag and 926r – ccg tca att cct ttr agt tt. Sequencing was performed on an automatic sequencer AE 3000. The culture of strains of the microbial consortium was seeded to a separate colony and biomass was obtained for the analysis of genes encoding 16S rRNA. Primary screening for the 16S rRNA gene of the microbial consortium strain No. 6 according to the GenBank and RDP-II database showed that the studied strain belongs to the following systematic groups: Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus. Its homology with the bacterium *L. casei* (IMB B-7343), *L. plantarum* (IMB B-7344), *L. lacti*s (IMB B-7352) and *S. cerevisiae* (IMB Y-5046) is shown to be 98 %.

The effectiveness of the microbial consortium that we developed was compared with control variant 1 (water) and control 2, a widely used commercial biopreparation of similar composition, "Baykal EM-1". This microbiological preparation, which is produced by Research and Production Association EM-Center, LLC (registration number 226(227, 228)-19-156-1), comprises *Lactobacillus casei* (IMB B-11360), *Lactococcus lactis* (IMB B11341), and *Saccharomyces cerevisiae* (IMB Y-3964) [35].

The effectiveness of the microbial consortium was tested on the following crop plants: Nador winter wheat (*Triticum aestivum* L.; Poaceae), a 1st generation hybrid of Goverla corn (*Zea mays* L.; Poaceae), Horlitsa soybean (*Glycine max* L. *Merr.*; Fabaceae*),* and Magnate rapeseed (*Brassica napus* L.; Brassicaceae).

The research was conducted of the Republic of Crimea. The size of the experimental plot for each experimental variant was 20–25 m^2, and each experiment was repeated four times. Sowing was carried out using the optimal technology recommended for crops in this area. The ontogenesis of the plants was marked by the onset of the main phenological phases and the differences between the specimens in the experimental plot and those in plots for controls 1 and 2.

According to agrochemical analysis, the physical and chemical parameters of the arable layer of soil (0–25 cm) of the experimental plot was as follows: humus content, 1.48 %; content of labile forms of nitrogen, 103 mg/kg; labile phosphorus and exchangeable potassium, 214 mg/kg and 160 mg/kg of soil, respectively; pH of the salt extract, 5.0; and the hydrolytic acidity, 1.34 mg-eq/100 g of soil [36].

We applied 60 kg/ha of phosphorus fertilizer (granulated superphosphate) and 90 kg/ha of potassium fertilizer (potassium chloride) under the main tillage during pre-sowing cultivation before sowing all crops under study. In the spring, 90 kg/ha of ammonium nitrate was applied during pre-sowing cultivation.

The average annual temperature for the 2019 and 2020 growing seasons was 9.2 °C and 10.6 °C, and the average annual precipitation was 53.0 mm and 77.9 mm, respectively (Table 1).

Experiment options:

Control 1 (water).

Control 2 (“Baykal EM-1”).

Microbial consortium: one treatment.

Microbial consortium: two treatments.

Microbial consortium: four treatments.

Sowing experiments are presented in Table 2.

The microbial consortium was applied to all crops under moist soil conditions. During each treatment, 1 L of microbial consortium dissolved in water was applied per hectare of crops. The microbiological preparation “Baykal EM-1” was applied once in the amount of 1 L/ha. After each application, the upper layer of the soil (2–3 cm) was loosened.

Soil samples for research were taken before the experiments. The content of mobile forms of nitrogen, phosphorus, and potassium, pH, and humus were determined in average soil samples. On the day of harvest, samples of plant material were taken to determine the content of major mineral elements (N, P, K) and trace elements (Zn, Mn, Cu).

In plant samples of wheat and corn grain, we determined the content of protein and mineral elements; in soybean grain, protein and fat; and in in rapeseed, fat [35].

Analysis of soil biological activity and quantitative characteristics of the soil microorganisms was performed using conventional techniques [37–41].

Statistical analysis of the data was performed by calculating the least significant difference (LSD) using the Excel program [42]. Data were processed, standard deviation and correlation analyses were performed using the STATISTICA version 8 software package (Statsoft Inc.). ANOVA was used to analyze statistically significant differences within and between test groups. The degree of statistical significance of the results was calculated using the GraphPad Prism 9 software (intergroup statistical significance was fixed at $p \leq 0.05$; Tukey and Šídák criteria were used).

Table 1

Agrometeorological indicators of the 2019–2020 growing season

Month	Growing season 2019				Growing season 2020			
	Mean temperature, °C	Minimum temperature, °C	Maximum temperature, °C	Total rainfall, mm	Mean temperature, °C	Minimum temperature, °C	Maximum temperature, °C	Total rainfall, mm
January	0.8	–4.7	7	20.2	–4.5	–14.0	3.5	70.4
February	2.5	–8.2	10.2	52.4	0.6	–7.6	10.5	57.4
March	6.5	–4.2	18.5	13.8	5.1	–5.7	19.1	44.0
April	9.9	–1.8	23.3	42.1	10.6	1	22.4	63.8
May	12.4	2.1	26.7	141.7	17.0	5.7	22.6	126.8
June	21.7	5.7	32.7	53.5	23.6	13	33.7	90.9
July	21.9	11.8	32.3	42.9	19.8	11.7	32.5	89.5
August	21.4	11.1	32.8	45.4	20.7	12.1	34.0	61.9
September	18.4	7.1	34.3	36.0	15.9	3.1	30.7	26.1
October	12.5	2.9	21.2	94.3	11.1	–2.0	24.0	25.5
November	3.8	–3.1	11.1	21.0	4.6	–9.7	18.7	43.7
December	–0.5	–9.5	8.1	73.1	2.7	–4.9	14.6	54.1

Table 2

Treatment options for experimental agricultural crops

Subsequence activities	Plant cultures and event dates			
	Nador wheat	Magnat rapeseed	Goverla corn hybrid	Gorlitsa soybean
Plant culture sowing	06.10.19	06.10.19	08.05.20	08.05.20
First introduction of the tested microbial consortium	10.10.19	10.10.19	25.06.20	25.06.20
First introduction of the tested microbial consortium	17.10.19	17.10.19	07.07.20	07.07.20
First introduction of the tested microbial consortium	25.10.19	25.10.19	19.07.20	19.07.20
First introduction of the tested microbial consortium	30.10.19	30.10.19	24.07.20	24.07.20

Table 3
The effect of microbial consortium on yield, grain protein content, and protein yield of Nador winter wheat

Experiment options	*Grain yield, t/ha*	*Gain to control 1, %*	*Gain to control 2, %*	*Grain protein content, %*	*Gain to control 1, ±*	*Gain to control 2, ±*	*Protein yield, t/ha*	*Gain to control 1, %*	*Gain to control 2, %*
Control (water)	2.16	-	9.2	9.90	–	0.98	0.214	–	17.4
Control 2 ("Baykal EM-1")	2.38	10.2	–	10.88	0.98	–	0.259	21.0	–
Microbial consortium: 1 treatment	3.00	38.9	26.0	11.13	1.23	0.25	0.334	56.1	46.3
Microbial consortium: 2 treatments	3.04	40.7	27.7	11.47	1.57	0.69	0.349	63.1	52.1
Microbial consortium: 4 treatments	3.06	41.7	28.6	11.78	1.88	0.90	0.360	68.2	56.4
LSD_{05}	0.13								

Table 4
The effect of microbial consortium on yield, content, and oil yield of Magnat rapeseed grain

Experiment options	*Grain yield, t/ha*	*Gain to control 1, %*	*Gain to control 2, %*	*Oil content in grain, %*	*Gain to control 1, ±*	*Gain to control 2, ±*	*Oil yield, t/ha*	*Gain to control 1, %*	*Gain to control 2, %*
Control (water)	1.05	–	13.9	41.67	–	0.33	0.438	–	14.1
Control 2 ("Baykal EM-1")	1.22	16.2	–	41.81	0.30	–	0.510	16.4	–
Microbial consortium: 1 treatment	1.42	35.2	16.4	43.23	3.74	3.39	0.614	40.2	20.4
Microbial consortium: 2 treatments	1.46	39.0	19.7	43.48	4.34	3.99	0.635	45.0	24.5
Microbial consortium: 4 treatments	1.52	44.8	24.6	42.58	2.18	1.84	0.647	47.7	26.3
LSD_{05}	0.07								

Results

A single application of microbial consortium in an amount of 1 L/ha increased the grain yield of winter Nador wheat by 38.9 % (0.84 t/ha), the protein content by 1.23 %, and the protein yield by 56.1 % (0.12 t/ha) compared to control 1 (Table 3). Application of the microbiological preparation "Baykal EM-1" (control 2) resulted in an increase in the grain yield of 10.2 % (0.22 t/ha), the protein content of 0.98 %, and the protein yield of 21.0 % (0.259 t/ha) compared to control 1. A single application of the microbial consortium exceeded the performance of the commercially available microbial preparation "Baykal EM-1" by 26.0 % for grain yield, by 0.25 % for grain protein content, and by 46.3 % for the protein yield from grain.

Increasing the number of microbial consortium treatments of the wheat crops had a stimulating effect on protein content and grain yield. Following treatment of crops two and four times with the microbial consortium, the yield of wheat increased in comparison with that of single treatment by 1.8–2.8 %; its grain protein content increased by 0.34–0.65 % and its grain yield increased by 7.0–12.1 %.

The results of our research showed that the optimal amount of microbial consortium applied to the black meadow soil under the conditions of the 2019–2020 growing season was 2–4 L/ha. During this time, the grain yield of winter wheat increased by 40.7–41.7 % (0.88–0.90 t/ha), the protein content by 1.57–1.88 %, and the protein yield by 63.1–68.2 % (0.135–0.146 t/ha) compared to control 1.

A single treatment with the microbial consortium (1 L/ha) increased the Magnat rapeseed yield by 35.2 % (0.37 t/ha), the oil content by 1.56 %, and the crude oil yield by 40.2 % (0.176 t/ha) compared to control 1 (Table 4). The microbiological preparation "Baykal EM-1" increased these indicators by 16.2 %, 0.30 %, and 16.4 %, respectively. Comparison of the effectiveness of our microbial consortium with that of "Baykal EM-1" showed that the consortium we developed increased the rapeseed yield compared to the registered preparation by 19 %, the crude oil content by 1.42 %, and its yield by 23.8 %.

Two- and four-time treatment of the crops with the microbial consortium increased the rapeseed yield compared to a single application by 3.8–9.6 %. It was noted that the four-time treatment decreased the oil content compared to the double treatment; nevertheless, it remained higher than that of control 1 by 2.18 % and control 2 by 1.84 %. Increasing the number of treatments resulted in a 4.8–7.5 % increase in the yield of crude oil. Multiple treatments of rapeseed with the microbial consortium increased both grain yield and the content and yield of crude oil.

Thus, the optimal amount of microbial consortium application for treatment of rapeseed is 2 L/ha. Application of this amount of the microbial consortium increased the rapeseed grain yield by 39.0 % (0.41 t/ha) and the oil yield by 45.0 % (0.197 t/ha) in comparison to control 1.

It was shown that treatment of Goverla corn hybrid crops with the microbial consortium (1 L/ha) increased the yield of green mass by 5.1 % (1.6 t/ha), protein content by 0.10 %, and protein yield by 3.9 % (0.10 t/ha) compared to control 1. These figures were lower than those of control 2 (Table 5).

Two- and four-time treatment of corn crops with the microbial consortium increased the yield of green mass by 19.7–27.9 % compared to control 1 and by 10.9–18.5 % compared to control 2. The protein content in the corn grain increased by 0.63-0.91% with two- and four-time treatment compared to control 1 and by 0.47–0.70 % compared to control 2. Increasing the number of treatments of the corn with the microbial consortium also increased the protein yield by 29.8–42.2 % compared to control 1 and by 19.0–31.4 % compared to control 2. The highest yield of corn green matter (40.3 t/ha) was obtained with four-time treatment with the microbial consortium, accompanied by a protein content that reached 9.11 %.

When the corn was harvested for grain, the control yielded 6.22 t/ha with a protein content of 6.23 % (Table 6). When treated with the microbial consortium, compared to the control, the corn yield increased by 25.1–46.9 % (1.56–2.92 t/ha), the protein content by 0.25–1.06 %, and the protein yield by 30.2–72.1 % (0.117–0.273 t/ha) depending on the number of treatments.

Our research showed that under the conditions of the 2020 growing season, four-time treatment of corn crops with the microbial consortium (1 L/ha) resulted in the yield of green mass exceeding that of control 1 by 27.9 % and the grain yield by 46.9 %.

Table 5

The effect of microbial consortium on green matter yield, protein content, and its yield of Goverla corn hybrid

Experiment options	Green matter yield, t/ha	Gain to control 1, %	Gain to control 2, %	Protein content in grain, %	Gain to control 1, ±	Gain to control 2, ±	Protein yield, t/ha	Gain to control 1, %	Gain to control 2, %
Control (water)	31.5	–	7.3	8.20	–	0.21	2.58	–	9.8
Control 2 (“Baykal EM-1”)	34.0	7.9	–	8.41	0.21	–	2.86	10.8	–
Microbial consortium: 1 treatment	33.1	5.1	2.6	8.30	0.10	0.11	2.68	3.9	6.3
Microbial consortium: 2 treatments	37.7	19.7	10.9	8.88	0.63	0.47	3.35	29.8	19.0
Microbial consortium: 4 treatments	40.3	27.9	18.5	9.11	0.91	0.70	3.67	42.2	31.4
LSD_{05}	0.14								

Table 6

The effect of microbial consortium on grain yield, protein content, and grain yield of Goverla corn hybrid

Experiment options	Grain yield, t/ha	Gain to control 1, %	Gain to control 2, %	Protein content in grain, %	Gain to control 1, ±	Gain to control 2, ±	Protein yield, t/ha	Gain to control 1, %	Gain to control 2, %
Control (water)	6.22	–	21.8	6.23	–	0.50	0.387	–	27.8
Control 2 (“Baykal EM-1”)	7.96	28.0	–	6.73	0.50	–	0.536	38.5	–
Microbial consortium: 1 treatment	7.78	25.1	0	6.48	0.25	0.25	0.504	30.2	2.0
Microbial consortium: 2 treatments	8.39	34.9	5.4	7.11	0.88	0.38	0.596	54.0	11.2
Microbial consortium: 4 treatments	9.14	46.9	14.8	7.29	1.06	0.56	0.666	72.1	24.2
LSD_{05}	0.26								

Table 7

The effect of microbial consortium on grain yield, protein content, and grain yield of Gorlitsa soybean

Experiment options	Grain yield, t/ha	Gain to control 1, %	Gain to control 2, %	Protein content in grain, %	Gain to control 1, ±	Gain to control 2, ±	Protein yield, t/ha	Gain to control 1, %	Gain to control 2, %
Control (water)	1.95	–	7.1	20.14	–	1.13	0.392	–	12.3
Control 2 (“Baykal EM-1”)	2.10	7.7	–	21.27	1.13	–	0.447	14.0	–
Microbial consortium: 1 treatment	2.55	30.8	21.4	22.83	2.69	1.56	0.582	48.5	30.2
Microbial consortium: 2 treatments	2.60	33.3	23.8	23.24	3.10	1.97	0.604	54.1	35.1
Microbial consortium: 4 treatments	2.69	37.9	28.1	23.23	3.09	7.96	0.625	54.9	39.8
LSD_{05}	0.15								

Table 8

The effect of microbial consortium on oil content and oil yield from Gorlitsa soybean

Experiment options	Oil content in the grain, %	Gain to control 1, ±	Gain to control 2, ±	Oil outlet, t/ha	Gain to control 1, %	Gain to control 2, %
Control (water)	21.00	–	0.75	0.410	–	10.5
Control 2 (“Baykal EM-1”)	21.82	0.75	–	0.458	11.7	–
Microbial consortium: 1 treatment	20.93*	0.14	0.89	0.534*	30.2	16.6
Microbial consortium: 2 treatments	20.63*	0.44	1.19	0.536*	30.5	17.1
Microbial consortium: 4 treatments	20.09*	0.98	1.73	0.540*	31.7	17.9

*Note. * Statistically significant $p < 0.05$.*

The grain yield of Gorlitsa soybean increased by 30.8 % (0.6 t/ha) compared to control 1 and by 21.1 % (0.45 t/ha) compared to control 2 (Table 7). The grain protein content increased by 2.69 % compared to control 1 and by 1.56 % compared to control 2, and the protein yield increased by 48.5 % compared to control 1 and by 30.2 % compared to control 2. The oil content of the soybean grain treated with the microbial consortium was lower than that of both control 1 and control 2 (Table 8). The oil yield of soybean treated once with the microbial consortium increased by 30.2 % compared to control 1 and by 16.6 % compared to control 2.

Two- and four-time treatment with the microbial consortium increased the soybean yield by 33.3–37.9 % (0.65–0.74 t/ha), the protein content by 3.10–3.09 %, and the yield by 54.1–54.9 %. Increasing the number of treatments did not lead to significant differences in the content or yield of oil.

Thus, the microbial consortium has the maximum effect following four-time treatment for soybean, which resulted in the greatest increase in crop yield and protein yield from the seed.

Analyses showed that treatment with the microbial consortium had almost no effect on the content of micronutrients in wheat grain; the amounts of copper, zinc, and manganese were equal to or slightly higher than those of control 1 and control 2.

Treatment with the microbial consortium had a significant effect on the content of microelements in corn grain (Fig. 1). In the control variant 1, the content of copper in corn grain was 2.5 mg/kg; treatment with “Baykal EM-1” microbial preparation increased the content of this microelement to 2.7 mg/kg (8 %), and treatment with the microbial consortium to 3.0 mg/kg (20.0 %). When corn crops were treated with “Baykal EM-1”, the zinc content was 20.0 mg/kg, and with the microbial consortium 16.2 mg/kg; the content in the control was 13.7 mg/kg. The content of manganese in the control sample of corn was 6.1 mg/kg; treatment with “Baykal EM-1” resulted in an increase of this microelement to 6.9 mg/kg (13.1 %), while treatment with the microbial consortium increased it to 8.2 mg/kg, which was 34.4 % higher than in control 1.

Increasing the number of treatments of the wheat and corn crops led to an accumulation of macronutrients in the cereal grains. The wheat grain initially contained 1.87 % nitrogen; after treatment with “Baykal EM-1”, its content increased to 2.06 %, and after treatment with the microbial consortium, depending on the number of treatments, this figure increased to 2.10–2.23 % (Fig. 2, *a*). A similar trend was observed in corn seeds; when treated with the microbial consortium, the nitrogen content was 1.29–1.37 % compared with 1–1.16 % in the control.

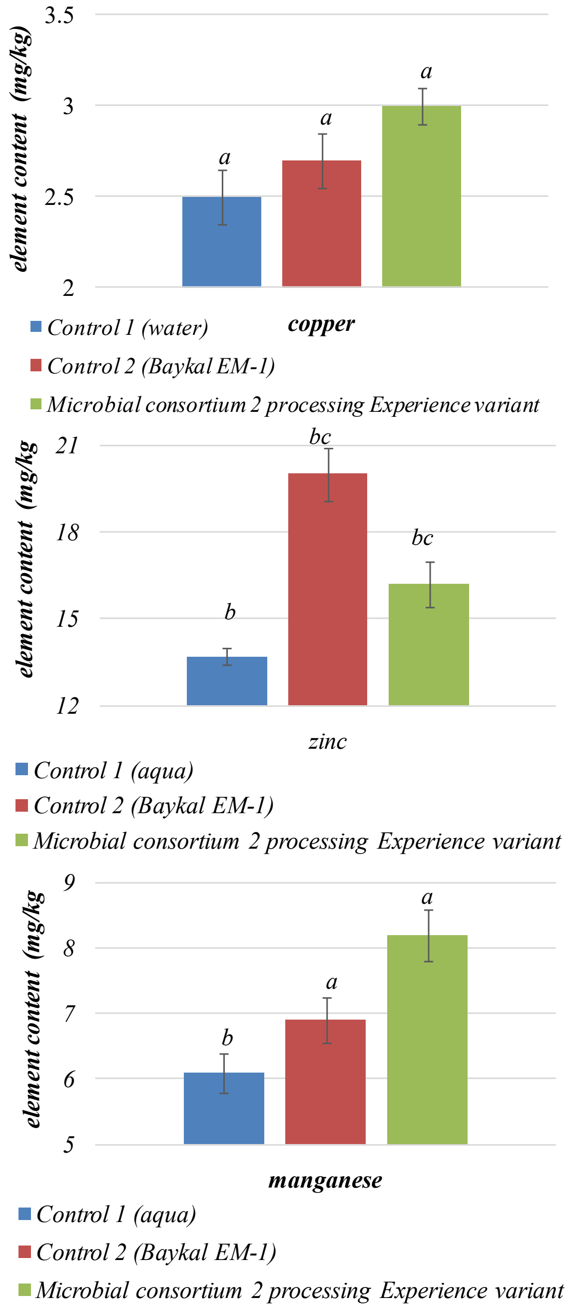

Fig. 1. The effect of treatment with the microbial consortium on the content of microelements in corn grain (mg/kg)

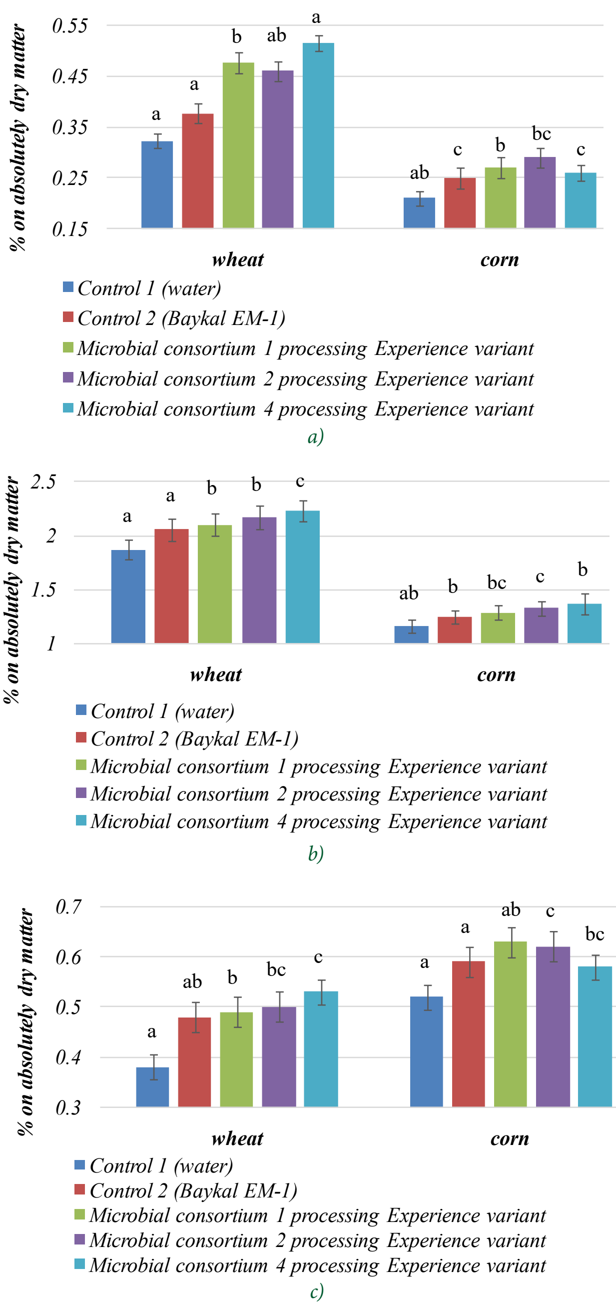

Fig 2. The effect of microbial consortium on nitrogen (a), phosphorus (b), and potassium (c) content in wheat and corn grains (% on absolute dry matter)

The phosphorus content in the control variant 1 wheat grain was 0.323 %, while following treatment with "Baykal EM-1", it increased to 0.377 %, and as a result of 1–4 treatments with the microbial consortium, it increased to 0.460–0.516 %. In corn grain, the phosphorus content resulting from 1–4 treatments with the microbial consortium was 0.26–0.29 % compared to 0.21 % in control 1 (Fig. 2, *b*).

The potassium content for wheat grain was 0.38 % in control 1, 0.48 % in control 2, and 0.49–0.53 % after one to four treatments with the microbial consortium. In corn grain, several treatments with microbial consortium resulted in a potassium content of 0.50–0.63 % compared to 0.52 % in control 1 (Fig. 2, *c*).

Thus, while treatment with the microbial consortium had no effect on the accumulation of microelements in wheat grain, it increased it in corn grain: copper by 20.0 %, zinc by 18.3 %, and manganese by 34.4 % compared to control 1. Treatment of wheat and corn crops led to accumulation of mineral elements in the grain of both cereals.

The microbial consortium had a positive effect on microbiological processes in the soil of the root zone because breathing, destruction of organic matter, the total content of the microbial mass, and the increased development of nitrogen-fixing microorganisms (Tables 9 and 10).

Table 9

Biological activity of the soil rhizosphere of Goverla corn hybrid and Gorlitsa soybean

Parameters	Experience options		
	Control 1 (water)	Control 2 ("Baykal EM-1")	Microbial consortium 2 processing experience variant
Corn rhizosphere			
Soil respiration rate (mg CO_2/g soil)	29.75 ± 1.91	30.20 ± 3.08	33.31 ± 2.21
Total microbial count of soil (μg C/g soil)	99.32 ± 3.99	102.89 ± 6.36	120.47 ± 8.17
Cellulose-destroying activity of soil (%)	19.99 ± 0.17	20.16 ± 0.65	21.25 ± 0.58
Phytotoxicity of soil (% of inhibition of seed germination)	5.21 ± 0.83	8.33 ± 0.64	1.09 ± 0.07
Soybean rhizosphere			
Soil respiration rate (mg CO_2/g soil)	31.98 ± 1.66	35.31 ± 2.21	36.43 ± 1.12
Total microbial count of soil (μg C/g soil)	114.40 ± 3.33	117.50 ± 4.26	123.67 ± 3.74
Cellulose-destroying activity of soil (%)	39.91 ± 0.94	40.47 ± 1.54	42.77 ± 0.63
Phytotoxicity of soil (% of inhibition of seed germination)	7.29 ± 0.25	5.21 ± 0.19	1.04 ± 0.09

Table 10

Quantitative characterization of soil microorganisms

Parameters	Experience options		
	Control 1 (water)	Control 2 ("Baykal EM-1")	Microbial consortium 2 processing experience variant
Corn rhizosphere			
Ammonificators (million CFU/g soil)	1.98 ± 0.16	2.07 ± 0.22	3.25 ± 0.41
Nitrogen fixers and oligonitrophils (million CFU/g soil)	1.53 ± 0.41	1.84 ± 0.11	1.94 ± 0.47
Micromycetes (thousand CFU/g of soil)	6.16 ± 0.32	66.0 ± 0.21	49.9 ± 0.22
Soybean rhizosphere			
Ammonificators (million CFU/g soil)	1.53 ± 0.41	3.92 ± 0.92	4.74 ± 1.01
Nitrogen fixers and oligonitrophils (million CFU/g soil)	0.71 ± 0.07	0.63 ± 0.19	1.05 ± 0.12
Micromycetes (thousand CFU/g of soil)	36.4 ± 5.7	28.2 ± 6.1	41.8 ± 9.8

The microbial consortium reduced soil phytotoxicity, which was assessed by its effect on the germination of test-culture seeds. Under the action of the microbial consortium, the root zone of the soybean stimulated germination of the test crop.

The microbiological preparation "Baykal EM-1" had no effect on the biological activity of the soil in which corn was grown or the development of nitrogen transforming microorganisms. Soybean was found to be more sensitive to treatment with the microbiological preparation "Baykal EM-1"; the activity of organic residue degradation due to cellulose-destroying microorganisms and ammonificators increased, while the number of micromycetes and nitrogen fixers was lower than in control 1.

Discussion and Conclusion

Many studies in the literature show the effectiveness of using the microbiological preparations on various crops. The authors discuss below several promising uses of these preparations to improve the quantity and quality of crop production.

Preparations based on nodule bacteria, such as Nitragin, Rizotrophin, Rizobofit, Rizoaktiv, and Rizogumin, are currently used to treat legume crops. Their application in the cultivation of legume crops has resulted in an increase in protein harvest and increased yields of 11–40 %. Application of the microbiological preparation Azoriz in combination with $N_{15}P_{30}K_{30}$ increased soybean yield by 29 % and increased the protein content in the beans by 2.4–3.1 % [25]. The experimental use of the microbiological preparations Nitrogen Bacterial Fertilizer + Bacterial Phosphorus Fertilizer, Phosphatovit + Nitovit, and Extrasol on spring wheat Moscow 35 resulted in increased yields of 40 %, 23.3 %, and 36.7 % respectively, and increases in gluten content of 1.0 %, 3.0 %, and 0.5 %, respectively [6]. In addition, treatment with cultures of *Klebsiella variicola* and *Rhizophagus intraradices* increased the content of inulin in topinambour [21], treatment of wheat with a biofertilizer containing *Rhizophagus irregularis* together with *Bacillus megaterium* and *Frateuria aurantia* increased the content of wheat gluten proteins [22], and treatment with PGPB (*Mesorhizobium spp.*, *Burkholderia spp.,* and *Pseudomonas spp.*) and AMF (*Rhizophagus irregularis*, *Funneliformis geosporum*, and *Claroideoglomus claroideum*) increased the protein content in chickpeas in a field experiment [23].

Studies of repeated treatment of soil and plant crops with microbiological preparations have been carried out by a number of researchers. The growth stimulation of chickpea inoculated with PGPB (*Mesorhizobium spp.*, *Burkholderia spp.*, and *Pseudomonas spp.*) and AMF (*Rhizophagus irregularis*, *Funneliformis geosporum*, and *Claroideoglomus claroideum*) increased the yield of chickpeas by 6 % compared to a single inoculation and by 24 % compared to the control variant [23]. Mrkovački and others [29] performed inoculation of the soil with three strains of *Azotobacter chrooccum* before and after sowing sugar beet. Single inoculation influenced the increase in root crop yield by 20 %, and repeated inoculation increased it by 23 %. Higher yields of polarized crystallized sugar obtained after inoculation ranged between 20 and 21 %; after repeated inoculation, this increased to 22–23 % [29].

The use of the microbial consortium that we developed had a significant effect after even a single treatment, increasing the yields by 25–38 %; the two- and four-treatment variations improved the quality of the products, increasing the protein content by 1.23–3.10 % and the oil content by 2.18–4.34 %. Depending on the number of treatments, the use of the microbial consortium increased the protein yield from wheat, corn, and soybean by 56.1–68.2 %, 30.2–72.1 %, and 48.5–54.9 %, respectively, and the oil yield from soybean by 30.2–31.7 % and from rapeseed by 40.2–47.7 %.

In our previous studies, it was shown that a consortium of lactic acid bacteria and saccharomycetes converts forms of trisubstituted phosphates inaccessible to plants into soluble compounds [43], thereby increasing the amount of available phosphorus. Also lactic acid bacteria activate the work of the proton pump, increasing the supply of nutrients and increasing the biological productivity of plants.

Active microbial colonization of plant roots by lactic acid bacteria and saccharomycetes is associated with the consumption by microorganisms of the studied consortium of root exometabolites as sources of energy and carbon. In turn, the microorganisms of the consortium synthesize metabolites that stimulate plant growth [44]. Colonizing the surface of the rhizosphere, lactic acid bacteria compete with phytopathogens for adhesion sites and nutrients, and also secrete metabolites with bactericidal and fungicidal properties: squalene, dimethyl fumarate, capric acid, lactic acid, acetic acid, caprylic acid, fumaric acid, butyric acid, decanol, butanol, pentanol, β-phenylethanol [45].

The use of a microbial consortium stimulates the development of significant agronomic groups of microorganisms in the soil, and also increases the enzymatic activity of the soil [46].

A particular focus of interest in our studies is a comparative characterization of the effect of the microbial consortium we developed and that of the microbial preparation “Baykal EM-1”, which is included in the state catalog of pesticides and agrochemicals approved for use in the Russian Federation (2020). Treatment with our investigational microbial consortium, in comparison with the preparation “Baykal EM-1”, increased the corn yield by 5.4 % and 14.8 %, respectively; the wheat yield by 26.0 %, the soybean yield by 21.4 %, and the rapeseed yield by 16.4 %.

A single treatment with the microbial consortium increased the protein content in wheat grain by 0.25 %, in soybean by 1.56 %, and increased the protein yields by 46.3 % and 30.2 %, respectively. Additionally, we observed an increase in crude oil content and yield in rapeseed grain of 3.39 % and 20.4 %, and an increase in soybean of 16.6 %.

Our study showed that treatment with the microbial consortium stimulated indicators of soil biological activity such as respiration intensity, cellulose-destroying activity, and total microbial number, as well as increasing the number of nitrogen-modifying soil microorganisms. The microbial consortium lowered soil phytotoxicity, i. e., the percentage inhibition of seed germination.

In the course of the experiment, the microbial consortium we developed was not only as effective as the registered microbiological preparation, but was in fact more effective when adminstered as two- and four-time treatments. When taking stock of the advantages of the microbial consortium, we feel confident that it shows great promise for use in agricultural practice as an alternative to the microbiological preparations currently in use and the damaging chemicals still, also, in use.

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