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LAB TESTS ON EFFICIENCY OF A BIOLOGICAL FERTILIZER BASED ON NITROGEN-FIXING AND PHOSPHATE-MOBILIZING BACTERIA

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Abstract

Soil microbiota has a direct impact on ыoil fertility and composition and, as a consequence, on plant productivity. Currently, agricultural production requirements focus on biological farming the essence of which is to use the potential capabilities of natural ecosystems, in particular soil microorganisms. The most extensive and diverse group of soil microorganisms in properties are free-living and symbiotic nitrogen-fixing bacteria. Another group, phosphate-mobilizing soil microorganisms, participate in the conversion of hard-to-reach inorganic and organic phosphates into water-soluble forms assimilated by plants. Environmentally friendly and safe complex biological fertilizers based on isolates of microorganisms from local natural biogeocenoses are important to increase crop yields and improve soil fertility in the conditions of Kazakhstan. The purpose of the submitted work was a lab assessment of the effectiveness of a combined biological fertilizer based on phosphate-mobilizing and nitrogen-fixing bacteria and its compatibility with some fungicidal and herbicide preparations used in the Republic of Kazakhstan. In pot trials, the biological fertilizer BioAzoPhosfit based on nitrogen-fixing bacteria *Raoultella oxytoca* MS and phosphate-mobilizing bacteria *Serratia plymuthica* MS was tested on cucumber seedlings of the Meva variety and spring wheat seeds of the Akmola 2 variety. Tests confirmed the effectiveness of the combined biological fertilizer on cucumber plants according to the main biometric indicators (e.g., length of stem and internodes, number and size of fruits). It was revealed that the average fruit length in the treatment was 12.4 % ($p < 0.05$) greater than in the control, and the total weight of harvested fruits in the treatment was 20.3 % ($p < 0.05$) greater than in control. Biofertilizer BioAzoPhosfit showed effectiveness on spring wheat after pre-sowing seed treatment. The treated seeds produced more vigorous shoots (the proportion of seedlings that sprouted by the set date was 5.2 % higher), and the plants from the treated seeds were significantly ($p < 0.05$) ahead of the control ones in growth and development throughout the entire observation period (by 12-31 %). In lab tests with the main fungicidal (Flamingo, Dividend Extreme) and herbicide (Assoluta, Tribune, Glyphosate and Smerch) preparations used on wheat in Kazakhstan, the combined biofertilizer BioAzoPhosfit showed a decrease in the viability of living microorganisms under the influence of broad-spectrum herbicides Glyphosate and Smerch. All other fungicides and herbicides tested slightly suppressed microbial cultures. Therefore, we recommend not using biofertilizers based on live microorganisms with broad-spectrum herbicides. The results of the tests allow us to recommend the growth and productivity parameters we used in this work for laboratory express testing the quality of microbiological preparation batches.

Keywords: rhizobacteria, nitrogen-fixing bacteria, phosphate-mobilizing bacteria, biological fertilizer, fertility, fungicide, herbicide

The economic and environmental crisis, the decline in the quality of crop products, and the deterioration of natural soil fertility stimulate attention to biological farming, the essence of which is to use the potential of natural ecosystems, in particular soil microorganisms [1, 2].

Soil microbiota has a direct impact on the soil fertility and composition and, thereby, on plant productivity. Soil microorganisms improve the structure of the soil, accumulate nutrients in it, and mineralize various organic compounds, transforming them into forms easily digestible by plants. To stimulate these processes, various bacterial fertilizers are used based on beneficial microorganisms that enrich the rhizosphere layer of soil with nutrients [3, 4]. Microorganisms used for preparation biologicals improve the supply of plants with mineral nutrition elements, e.g., nitrogen and phosphorus, and physiologically active substances, the phytohormones, vitamins, etc. The positive effect of many biologicals is also due to their phytosanitary function by displacing pathogenic soil microorganisms and inhibiting their reproduction [5, 6].

Free-living and symbiotic nitrogen-fixing bacteria that assimilate the nitrogen of the air are the largest and most diverse group of soil microorganisms. They are associated with the root system or the aboveground vegetative part of plants in cereals, nightshades, oilseeds and other plant families (7, 8).

It has been experimentally revealed that nitrogen fixed by microorganisms is 100% absorbed by plants, while nitrogen from mineral fertilizers is only 50% absorbed. In addition, since bacteria use the energy of organic substances synthesized by plants during photosynthesis to carry out nitrogen fixation, biological nitrogen is practically 'unpayable' [9, 10].

Phosphorus is a biogenic element that participates in the synthesis of nucleic acids, proteins, carbohydrates and energy metabolism in animals and plants. In addition, phosphorus is the main limiting nutritional element for plants in the soil. The lack of phosphorus during early development cannot be compensated by abundant phosphorus nutrition in subsequent growth stages [11-13]. Phosphate-mobilizing soil microorganisms are involved in the conversion of hard-to-reach inorganic and organic phosphates into water-soluble forms assimilable by plants [14, 15].

The various biochemical processes occurring in the soil are caused by the activity of microorganisms. Evolutionally, between soil microbiota and plants both symbiotic and antagonistic complex relationships have been esyablished. The symbiosis between plants and soil bacteria is mainly trophic. The vital activity of microorganisms, in turn, largely determines the regime of root nutrition, plant resistance to diseases and unfavorable environmental conditions, and ultimately, productivity [16-18].

Every year, researchers discovered new strains of soil microorganisms from various families with useful phosphate-mobilizing and nitrogen-fixing properties and, based on these microorganisms, develop monocomponent and complex biological fertilizers. Biological fertilizers have a pronounced effect as growth stimulants and protectants for different plant families, mainly grain and leguminous crops, e,g,, wheat, barley, and soybeans [19-22].

In the conditions of Kazakhstan, to increase crop yields and improve soil fertility, the creation and practical use of environmentally friendly and safe complex biological fertilizers based on isolates from local biogeocenoses is relevant. Therefore, assessment of effectiveness and quality of such biologicals, as well as the developemt of practical regulations for their use are in the focuse.

This work submits the first lab test data on the efficacy of combined biological fertilizer BioAzoPhosfit in cucumbers, variety Meva and spring wheat, variety Akmola 2 and the susceptabilty of the microorganisms making the base of BioAzoPhosfit to a number of fungicides and herbicides. The used biometric parameters we propose as indicators to rapidly assess in lab tests the effectiveness of microbiologicals for grain and vegetable crops, and the quality of factory batches of such preparations.

The purpose of our research was to evaluate the effectiveness of a com-

bined biological fertilizer based on phosphate-mobilizing and nitrogen-fixing bacteria and its compatibility with some fungicidal and herbicidal preparations applicable in the Republic of Kazakhstan.

Materials and methods. Complex biological fertilizer BioAzoPhosfit based on bacterial strains *Raoultella oxytoca* MS (nitrogen-fixing component, NFS) and *Serratia plymuthica* MS (phosphate mobilizing strain, FMS) (23) (series Nos. 2, 3 and 4) were produced by BIOTRON GROUP LLP (Republic of Kazakhstan). Commercial strains *R. oxytoca* and *S. plymuthica* were isolated in 2017 from dark chestnut soils in the Akmola region (Northern Kazakhstan), identified (BIOTRON Progress LLP), and in 2018 deposited under the numbers B-RKM 0833 and B-RKM 0832, respectively in the Republican State Enterprise State Collection of Microorganisms. Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

For biofertilizer BioAzoPhosfit series No. 2, the strains were grown in a mixed culture, the total drug titer was 3.0×10^9 CFU/ml. Strains for series No. 3 and No. 4 were cultured separately and then combined into a two-component preparation of NFS and PMS. For No. 3, the titer of the strains was 1.15×10^{10} and 1.48×10^9 CFU/ml, respectively, for No. 4, 3.65×10^9 and 2.18×10^9 CFU/ml.

Biofertilizer BioAzoPhosfit No. 2 was used in lab tests to increase seed germination of spring wheat (*Triticum aestivum* L.) variety Akmola 2 (TNK Agrofirm LLP, Akmola region, Republic of Kazakhstan) and stimulate the growth of seedlings in cucumber (*Cucumis sativus* L.) variety Meva (Greenhouse Technologies of Kazakhstan LLP, Stepnogorsk, Republic of Kazakhstan) at the first true leaf stage. The research was carried out at BIOTRON Progress LLP (Akmola region, Republic of Kazakhstan). In experiments with wheat, artificial beds were pre-formed, each bed was a 90×50×15 cm pallet. The soil is dark chestnut with humus content from 3 to 4%, pH 7.46-7.49 (slightly alkaline), 0.15-0.21% of total nitrogen in horizon A, 2.6-4.2 mg-eq. freely hydrolyzable nitrogen, and up to 0.10-0.13% of gross phosphoric acid. The amount of total phosphorus in the soil-forming rock reached 0.18%, the content of total potassium along the horizon profile ranged within 1.6-2.2%.

Cucumber were planted from a shipping container into the ground 15 days after germination (2021), 3 plants per each of 6 pots in the control and in the test (n = 18 each). In the test variant, plants were treated only with biofertilizer during the growing season. The instructions for using the biologicals on vegetable crops provide the following regulations: a dosage of 1.0 l/t for pre-sowing seed treatment and 1.0 l/ha for treatment during the growing season; an aqueous solution of the biologicals was prepared before use at the rate of 5-7 l/t of seeds and 200-250 l/ha. In the control, the plants did not receive any fertilizers in the form of chemical monocomponents, mineral (NPK) or biological fertilizers. The regimes of insolation and soil watering (as the soil dries out) were the same in the test and control variants. During the experiment (70 days), growth and development indicators were recorded weekly. Statistical assessment of the results was based on biometric indicators (number of fruits, fruit length, length of internodes, leaf area, and fruit weight) on days 50 and 62 during active fruiting.

On spring wheat variety Akmola 2, pre-sowing seed treatment with biofertilizer was carried out for 4 h, followed by a single treatment during the growing season. The instructions for using the biologicals product on grain crops provide the following regulations: a dosage of 1.5 l/t for pre-sowing seed treatment and 1.5 l/ha for treatment during the growing season; an aqueous solution of the biological product was prepared before use at the rate of 10-15 l/t of seeds and 250-300 l/ha. In the experiment, only biofertilizer was used when growing wheat.

In the control, tap water was used to soak seeds for 4 h and to treat vegetative plants. Seeds were sown on artificial beds on April 1, 2021 with the same seeding rate in the test and control. Control plants did not receive any fertilizing in the form of chemical monocomponents, mineral (NPK) or biological fertilizers. The regimes of insolation and soil watering (as the soil dried out) were the same in the test and control variants. From sowing the seeds to day 10 of growth, visual observation and biometric measurements (uniformity of germination, leaf length) were performed dayly from day 4. Measurements were carried out twice, randomly, in different parts of the bed, according to the envelope design at five points, 4 measurements in each. In statistical processing, for 20 plants in each of two adjacent beds, the measurement of the leaf having the maximum length was used.

The survival and compatibility of phosphate-mobilizing and nitrogen-fixing bacteria of the BioAzoPhosfit preparation was assessed with the fungicides Flamingo, Dividend Extreme (Syngenta LLC, Russia), herbicides Assoluta, Tribune (Agro Expert Group LLC, Russia), Glyphosate (AFD Chemicals, USA) and Smerch (Astana-Nan LLP, Republic of Kazakhstan). The two-component fungicide Flamingo is used at the rate of 0.4 l/t of seeds in 10 l of water (working solution 1:25). Fungicide protectant Dividend Extreme is used at the rate of 0.5 l/t of seeds in 10 l of water (working solution 1:20). Assoluta preparation is used at 0.5 l/ha of crops in 150 l of water (working solution 1:300). Post-emergence herbicide Tribune with the active ingredient tribenuron-methyl is used at 20 g/ha of crops in 150 l of water (working solution 1:7500). Systemic herbicide Glyphosate (glyphosate, isopropylamine salt) is used at 5 l/ha of crops in 150 l of water (working solution 1:30). Systemic herbicide Smerch (glyphosate, isopropylamine salt) was dissolved in 150 l of water providing 5 l/ha of crops (working solution 1:30).

Biofertilizers No. 3 and No. 4 were mixed with the drugs in their working concentration (1:1, exposure in one test up to 4 h). The titers of the *R. oxytoca* MS and *S. plymuthica* MS strains were determined by serial dilutions, from 10^{-6} to 10^{-9}, on Ashby agar and MPA (meat peptone agar) based on counting colonies in 48-h cultures grown at 29 °C. The counting was carried out in triplicate, 2 Petri dishes each, an average of 18 dishes per preparation. The viable cell number per 1 cm^3 suspension was calculated according to OFS.1.7.2.0008.15 "Determination of the concentration of microbial cells" as (n6 + n7)/1.1, where C is the number of viable cells per 1 cm^3 of the drug, $\times 10^6$; *n*6 and *n*7 are the mean numbers of colonies derived from the 10^{-6} and 10^{-7} dilutions, respectively; and 1.1 is a constant coefficient. A decrease in the titer (ΔC) of NFS and PMS by no more than 1.0×10^1 CFU/ml ($\Delta C < 1.0$ lg) was taken as a positive compatibility indicator; a negative result was a decrease in the titer of strains by more than one order of magnitude ($\Delta C > 1.0$ lg).

Statistical processing was carried out in Microsoft Office Excel 2016 using a statistical package for data analysis and in the STATISTICA 8.0 program (StatSoft, Inc., USA). The significance level of all presented values was $p < 0.05$. We used generally accepted statistical processing methods for biotechnological research. Variance, means (*M*), standard deviations (±SD), confidence intervals of means were determined, and a two-sample *t*-test with equal variances was performed [24].

Results. The *R. oxytoca* MS strain belongs to the associative soil nitrogen fixers of the *Enterobacteriaceae* family. Amplification of a gene region with primers nifH-1F and nifH-1R we previously performed showed that a specific ~ 430 bp PCR product is present in *R. oxytoca* MS, confirming thah the strain is a nitrogen fixer (Report on the research work "Isolate and select strains of phos-

phate-mobilizing and nitrogen-fixing bacteria in order to obtain a complex biofertilizer based on them" within the framework of the EurAsEC Interstate Target Program, 2014). The bacteria *S. plymuthica* MS belong to free-living soil microorganisms of the *Enterobacteriaceae* family. Phosphate-mobilizing *S. plymuthica* MS is capable of dissolving inorganic and organic phosphates into digestible water-soluble compounds, which was studied on a selective glucose-aspartic agar medium containing inorganic phosphorus and on PSM medium with calcium phytate. The capability of phosphate mobilizer strains to dissolve inorganic phosphates ranged as 35-52 mg P/l. Based on the gene bank data, *S. plymuthica* has been identified as a phosphate-mobilizing bacterium [23].

In cucumbers on day 50 after germination (Table 1), the average fruit length upon BioAzoPhosphit applicatiton was 17.25% greater than in the control, the average length of internodes was 5.6% greater than in the control. The average leaf area (LA), an indicator of photosynthetic biomass) was 2183 cm^2 upon the treatment vs. 1872 cm^2 in the control, or 16.6% greater. The average number of fruits on day 50 was 16.8±0.65 vs. 14.7±1.36 in the control, exceeding the indicator without the use of biofertilizer by 14.28%.

1. Plant biometric parameters of cucumber (*Cucumis sativus* L.) Meva variety upon reatment with the combined biofertilizer BioAzoPhosphit based on *Raoultella oxytoca* MS and *Serratia plymuthica* MS on days 50 and 62 of growth (n = 18)

Parameter	Treatment	Control	To control, %
	Day 50		
Main stem length, cm:			
M±SD	131.5±6.54	127.3±5.20	+3.30
Sample variance, D[X]	40.2	23.4	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0330 < given significance level p = 0.05			
Fruit number:			
M±SD	16.8±0.65	14.7±1.36	+14.28
Sample variance, D[X]	0.85	1.62	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0029 < given significance level p = 0.05			
Fruit length, cm:			
M±SD	13.3±0.91	12.6±0.82	+5.50
Sample variance, D[X]	1.15	0.94	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0160 < given significance level p = 0.05			
Leaf area, cm^2:			
M±SD	2183.0±34.70	1872.0±29.11	+16.60
Sample variance, D[X]	765.7	573.7	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0311 < given significance level p = 0.05			
	Day 62		
Main stem length, cm:			
M±SD	142.5±7.40	134.4±4.50	+6.00
Sample variance, D[X]	46.7	21.2	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0337 < given significance level p = 0.05			
Fruit number:			
M±SD	18.5±0.55	15.7±1.36	+17.80
Sample variance, D[X]	0.92	1.86	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0025 < given significance level p = 0.05			
Fruit length, cm:			
M±SD	16.3±0.86	14.4±0.78	+13.20
Sample variance, D[X]	0.74	0.62	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.010 < given significance level p = 0.05			
Leaf area, cm^2:			
M±SD	2586.0±42.18	2130.0±31.45	+21.40
Sample variance, D[X]	996.2	747.6	
Two-sample *t*-test with equal variances, p(T ≤ *t*) two-tailed 0.0241 < given significance level p = 0.05			

Note. For each parameter in the table, the data upon treatment are statistically significantly different from the control at p < 0.05. Statistical processing included testing hypotheses of the difference in the mean values of two distributions with equal variances and their two-tailed comparison at a given significance level of 5 %.

On day 62 (see Table 1), the average length of the fruits was 16.3 cm vs. 14.4 cm in the control, that is, 13.2% longer, the number of fruits was on average 17.8% greater than in the control, the average leaf area was 2586 cm^2 vs. 2130 cm^2 in the control. In general, the complex biofertilizer significantly in-

creases the plant biometric parameters (see Table 1), which was confirmed in a two-sample *t*-test by the *t*-critical two-tailed at p(T ≤ *t*) two-tailed < a given significance level p = 0.05. The total weight of the harvested fruits in the experiment was 20.3% (p < 0.05) greater vs. the control.

Cucumber plants treated with complex biofertilizer, even upon visual inspection, were ahead of the control plants in terms of development (plant height, leaf area, length and number of fruits) (Fig. 1). That is, the yield potential per plant turned out to be greater when treated with BioAzoPhosfit biofertilizer.

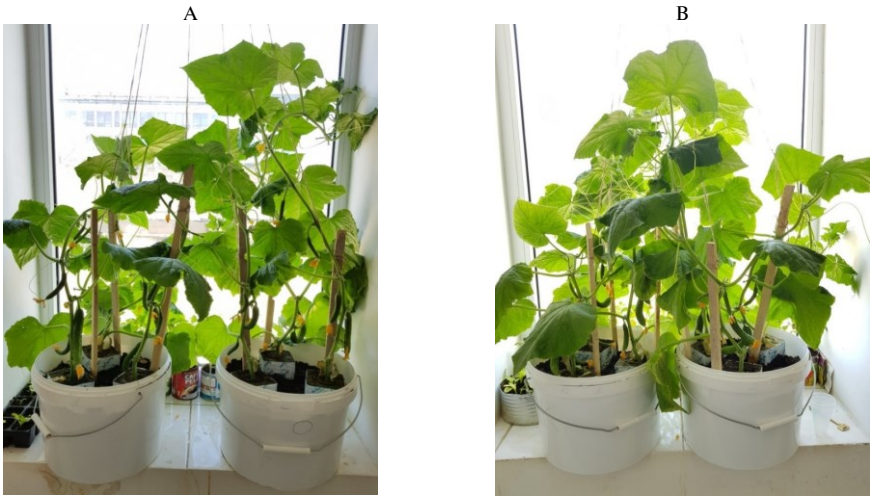

Fig. 1. Cucumber (*Cucumis sativus* L.) Meva variety plants treated (A) **and untreated** (B) **with the combined biological fertilizer BioAzoPhosfit based on the bacterial strains *Raoultella oxytoca* MS and *Serratia plymuthica* MS, 40 days after germination.**

On spring wheat variety Akmola 2, seed germination on days 1-2 was 96.7% vs. 91.5% in the control. In terms of growth intensity, already from 4-5 days after germination, the treated plants were statistically significantly ahead of the control ones (p < 0.05) (Table 2).

2. Leaf length (cm) **in spring wheat (*Triticum aestivum* L.) variety Akmola 2 upon the treatment with the combined biological fertilizer BioAzoPhosfit based on the bacterial strains *Raoultella oxytoca* MS and *Serratia plymuthica* MS** (*n* = 40, *M*±SD)

Variant	Days						
	4	5	6	7	8	9	10
Treatment	4.83±1.011	7.75±2.085	11.14±2.056	14.32±2.562	16.18±1.822	18.65±1.134	21.04±1.215
Control	3.68±0.838	6.29±1.874	9.94±2.206	12.22±3.034	14.14±1.681	16.63±1.573	18.54±1.299
To control, %	+31.25	+23.31	+12.10	+17.23	+14.43	+12.15	+13.49

N o t e. For each parameter in the table, the data upon treatment are statistically significantly different from the control at p < 0.05. Calculation data are given for 20 leaves for 20 plants from each of two adjacent beds (see section Materials and methods).

The average leaf length on day 4 upon the treatment was 4.83 cm, which was 31.25% longer than in the control; on day 7, the indicator was 17.23% higher (see Table 2). The excess of the average leaf length upon the treatment vs. the control remained at approximately the same level and by the end of observation (day 10) amounted to 13.49%. The biofertilizer BioAzoPhosfit statistically significantly increased the green mass growth of wheat sprouts by 12.1-31.25% (*t*-statistics 3.58610 > *t*-critical two-tailed 2.64691, p(T ≤ *t*) two-tailed 0.0241 < given level significance p = 0.05). In addition, spring wheat plants, when pre-sowing seeds were treated with biofertilizer, produced more vigorous shoots, the number of sprouted seeds by the due date was 5.2% higher.

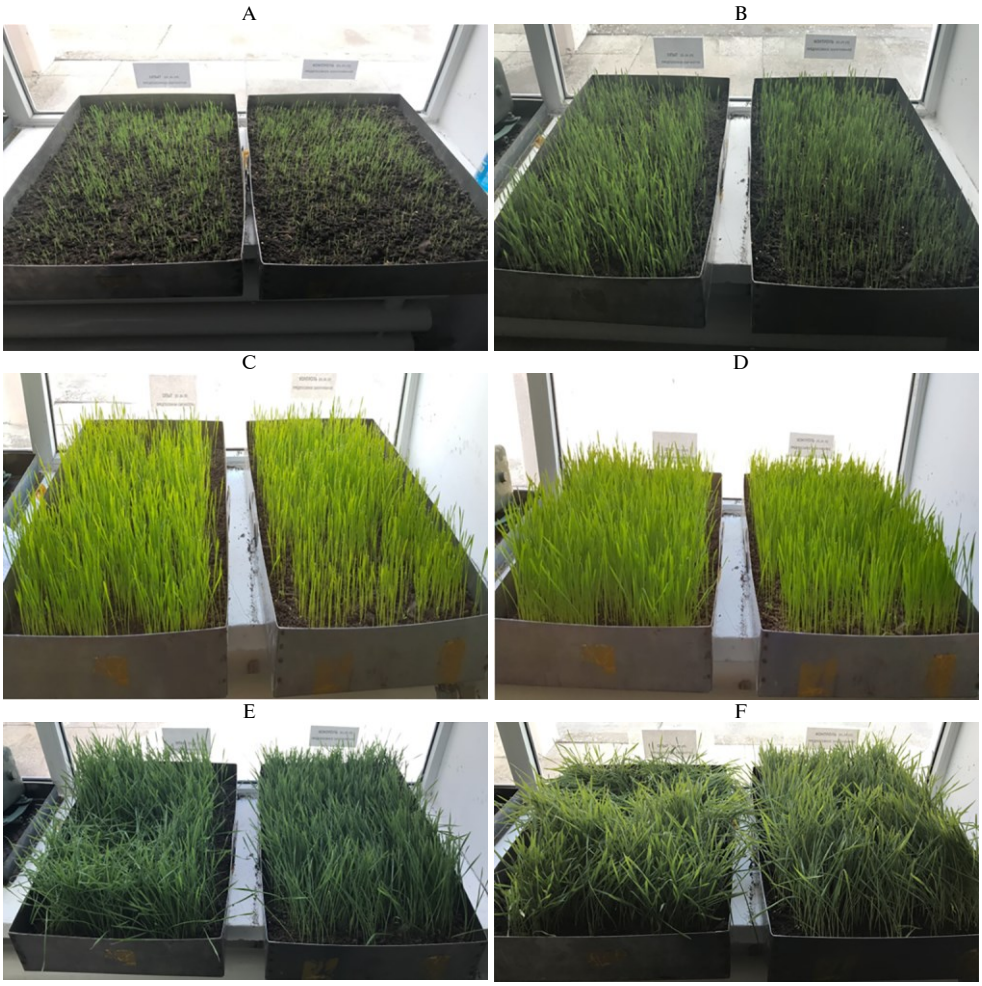

Fig. 2. Spring wheat (*Triticum aestivum* L.) variety Akmola 2 plants treated (left) **and untreated** (right) **with the combined biological fertilizer BioAzoPhosfit based on the bacterial strains Raoultella oxytoca MS and Serratia plymuthica MS:** A — 3-day old, B — 6 day old, C — 8-day old, D — 9-day old, E — 10-day old, F — 11-day old.

It is known that the release of bacterial auxins by nitrogen-fixing strains has a positive effect on the initiation and elongation of roots, the development of lateral roots and root hairs. This accelerates growth, improves nutrient uptake, and increases plant resistance to stress [5, 16, 17]. Visual observations (Fig. 2) confirmed our results of assessing the advanced growth of treated wheat plants. From day 10, massive lodging of wheat seedlings occurred, to a greater extent in the control, probably due to the low depth of seed placement in the soil. Further observation and recording were difficult and therefore stopped.

In general, the results of pot test showed that pre-sowing treatment of seeds and spraying of vegetative wheat plants with complex biofertilizer BioAzo-Phosfit stimulated the leaf growth. The data we obtained on the influence of the nitrogen and phosphate components of the biofertilizer BioAzoPhosfit on the formation of the photosynthetic surface of plants (vegetative biomass) are consistent with the conclusions of other authors [9, 14, 17] for biologicals Bioplant-K (NPO Bioprom LLC, Russia) and Extrasol (Bisolbi Plus LLC, Russia) used on various varieties of spring soft wheat. V.S. Kursakova et al. [17] established the effect of nitrogen biofertilizer and a direct relationship between the development of the photosynthetic surface and increased yield in different wheat varie-

ties. In addition, the assessment of biological fertilizer effectiveness by vegetative mass and average plant height, number and weight of seeds has been reported [3, 25], which is generally consistent with the results of lab tests we performed.

Thus, we believe that the lab test we used on different crops (cucumbers and wheat) to stafy the effectiveness of biofertilizers is quite informative as a rapid assessment of their effect.

In studies on grain crops of two-component biologicals and their various combinations [21], the combination of biofertilizers Azotobacterin and Phosphatobacterin (Innovative Company Bioinvest-Agro, Ukraine) was found the most effective, as well as the addition of organic fertilizer Gumat K (Chemistry and Technology LLP, Kazakhstan) which had qualitative and quantitative positive effects on grain crops. These results confirm the correctness of the strategy we chosed for the development of a complex biological fertilizer BioAzoPhosfit base on nitrogen-fixing and phosphate-mobilizing components.

When developing regulations for the use of microbiological preparations, their compatibility with the fungicides and herbicides is critical. Thus, the two-component fungicide Flamingo of systemic contact action based on tebuconazole and prochloraz is used to protect grain seeds from a complex of diseases transmitted through seeds and soil (hard, dusty, stone smut, helminthosporium and fusarium root rots, seed molding). The fungicide-protectant Dividend Extreme based on difenoconazole and mefenoxam is active against smut and loose smut, fusarium root rot, helminthosporium root rot, seed mold and other pathogens. Assoluta is a systemic herbicide active against annual dicotyledons, including those resistant to 2,4-D (dichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxyacetic acid), and some perennial weeds in wheat, barley and corn crops. Tribune post-emergence herbicide is designed to control a wide range of dicotyledonous weeds in cereal crops. Glyphosate is a continuous-action systemic herbicide for the control of annual and perennial cereal and dicotyledonous weeds, as well as for desiccation; Smerch is a continuous-action systemic herbicide for the destruction of vegetative plants.

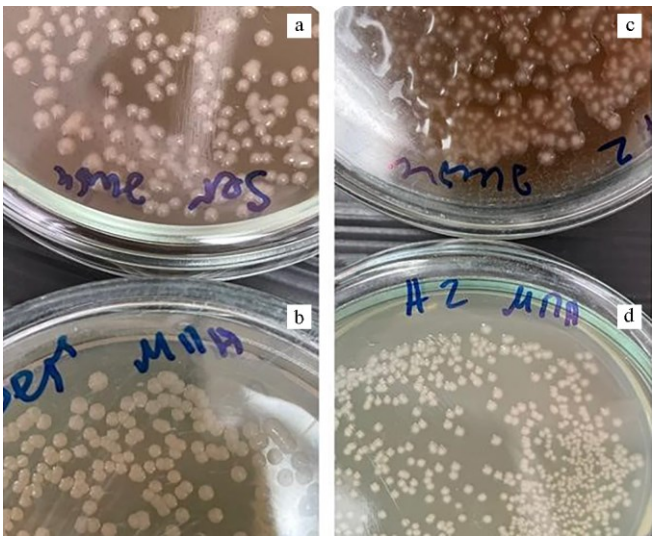

Fig. 3. Growth of *Serratia plymuthica* MS (a, b) **and *Raoultella oxytoca* MS** (c, d) **on Ashby's medium** (a, c) **and meat peptone agar** (b, d)**.**

Testing the compatibility of the BioAzoPhosfit biofertilizer with the fungicides Flamingo and Dividend Extreme revealed that these drugs did not have a significant effect on the strains (Table 3). Native biological products of the same series served as a control (Fig. 3) with the addition of equal volumes of sterile

distilled water. The activity of each component was above the minimum threshold of compliance with the manufacturer's standard (STO) (1.0×10^8 CFU/ml). Accordingly, they can be freely used in the tank mixture during pre-sowing seed treatment.

3. Tests on the compatibility of BioAzoPhosfit biofertilizer with systemic contact fungicides (n = 18 per preparation, $M\pm$SD)

Preparation	Activity, CFU/ml	Preparation	Activity, CFU/ml
Raoultella oxytoca, control (batch No. 4)	$(3.65\pm0.650)\times10^9$	*Serratia plymuthica,* контроль (batch No. 4)	$(2.18\pm0.522)\times10^9$
Raoultella oxytoca (batch No. 4) + Flamingo	$(1.48\pm0.315)\times10^9$	*Serratia plymuthica* (batch No. 4) + Flamingo	$(1.40\pm0.248)\times10^9$
Raoultella oxytoca (batch No. 4) + Dividend Extream	$(1.83\pm0.288)\times10^9$	*Serratia plymuthica* (batch No. 4) + Dividend Extream	$(1.25\pm0.154)\times10^9$
Raoultella oxytoca (batch No. 3) + Flamingo	$(6.35\pm0.731)\times10^9$	*Serratia plymuthica* (batch No. 3) + Flamingo	$(7.42\pm0.436)\times10^8$
Raoultella oxytoca, control (batch No. 3)	$(1.15\pm0.347)\times10^{10}$	*Serratia plymuthica,* контроль (batch No. 3)	$(1.48\pm0.271)\times10^9$

N o t e. For each parameter in the table, the data upon treatment are statistically significantly different from the corresponding control at $p < 0.05$.

4. Tests on the compatibility of BioAzoPhosfit biofertilizer (No. 3) with systemic fungicides and continuous action herbicides (n = 18 per preparation, $M\pm$SD)

Preparation	Activity, CFU/ml	Preparation	Activity, CFU/ml
Raoultella oxytoca, control	$(1.15\pm0.170)\times10^{10}$	*Serratia plymuthica,* control	$(1.48\pm0.125)\times10^9$
Raoultella oxytoca + Tribune	$(5.20\pm0.286)\times10^9$	*Serratia plymuthica* + Tribune	$(8.46\pm0.228)\times10^8$
Raoultella oxytoca + Assoluta	$(3.26\pm0.130)\times10^9$	*Serratia plymuthica* + Assoluta	$(1.21\pm0.147)\times10^9$
Raoultella oxytoca + Glyphosate	$(1.63\pm0.181)\times10^8$	*Serratia plymuthica* + Glyphosate	$(1.00\pm0.092)\times10^7$
Raoultella oxytoca + Smerch	$(2.37\pm0.272)\times10^7$	*Serratia plymuthica* + Smerch	$(1.84\pm0.426)\times10^7$
Raoultella oxytoca + Smerch (duble)	$(6.00\pm0.321)\times10^7$	*Serratia plymuthica* + Smerch (duble)	$(3.72\pm0.361)\times10^6$

N o t e. For each parameter in the table, the data upon treatment are statistically significantly different from the corresponding control at $p < 0.05$.

Systemic herbicides Tribune and Assoluta also did not have a significant effect on the BioAzoPhosfit strains (Table 4). The activity of each component was above the minimum threshold for compliance with the manufacturer's STO (1.0×10^8 CFU/ml), theregore, their use in a tank mixture is possible for treating seedlings by irrigation. Continuous action herbicides Glyphosate and Smerch caused a significant decrease in strain titers, by 2-3 lg, and the residual cell concentration was low for the effective use of biofertilizer. Therefore, we do not recommend biofertilizers based on live microorganisms for use with broad-spectrum herbicides.

Many authors [26-30] confirm the weak toxic effect of herbicides and fungicides on microbiologicals and recommend their combined use. An assessment in planta of the compatibility of biological and chemical means on cucumber showed a slight suppressive effect of the drugs on the culture of microorganisms [26]. The effectiveness and feasibility of combining all used plant protection products of both chemical and biological origin has been shown. It has been established [27, 28] that it is acceptable to combine herbicides of different chemical composition groups and their tank mixtures with biological preparations that are resistant to chemical attack. The use of herbicides with the anti-stress biologicals Fitosporin M [29] affects the biological effectiveness of the preparations themselves, the phytotoxicity of the herbicides does not manifest itself, and as a result, the yield and quality of grain significantly increases. However, some chemicals notably reduce the activity of microbiological fertilizers, which must be accounted when preparing a tank mixture or when spraying seedlings [30, 31].

Thus, in lab tests we reliably ($p < 0.05$) confirmed the effectiveness of the combined biological fertilizer BioAzoPhosfit based on phosphate-mobilizing bacteria *Serratia plymuthica* MS and nitrogen-fixing bacteria *Raoultella oxytoca*

MS on cucumber seedlings of the Meva variety and seeds and sprouts of spring wheat variety Akmola 2. In cucumber, we measured the length of the stem and internodes, the number and size of fruits, and the leaf area as biometric indicators. Our data indicate that the average length of cucmver fruits was 13.2% greater vs. the control, and the total weight of fruits collected from plants was 20.3% greater. Spring wheat seeds, upon a pre-sowing treatment with the biofertilizer BioAzoPhosfit, produced more vigorous shoots, the proportion of germinated seeds within the prescribed period was 5.2% higher, and in terms of growth and development, the treated plants were 12-31% ahead of the control ones over the entire observation period. These biometric indicators can be used in lab express tests to assess the quality of microbiological preparations for grain and vegetable crops, and to control the quality of factory batches of the product. Regarding the compatibility of the biofertilizer BioAzoPhosfit with the main fungicidal and herbicide preparations used on cereal crops, it is not recommended to simultaneously use a biofertilizer based on living microorganisms and broad-spectrum herbicides, for example Glyphosate and Smerch (if necessary, they should be used separately). The remaining fungicides and herbicides that we tested had an insignificant suppressive effect on microbial cultures.

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