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RESEARCH ARTICLE

Possibilities of Stabilizing Soil Biological Activity by Treating Seeds With a Biological Preparation Based on Phosphate Mobilizing Bacteria in the Conditions of the Aral Sea Region of Kazakhstan

Zhanar Zhumadilova[1]*, Laura Tokhetova[1], Shorabayev Yerik[2] , Gulsim Baimbetova[1], Turlybayeva Zere[3]

[1]*Agrotechnology and Soil Science, Kazakh Research Institute of Rice Growing Named After. I. Zhakhaeva, Kazakhstan*
[2]*Industrial Microbiology, Industrial Microbiology LLP, Kazakhstan*
[3]*LLP Research and Production Center of Microbiology and Virology, Almaty, Kazakhstan*

*Corresponding Author: zhanarzhumadilova@list.ru

Abstract: This study examined the effect of Phosphate-Mobilizing Bacteria (PMB) as a pre-sowing treatment for forage crop seeds. The objective was to assess the potential of these bacterial biopreparations to improve both crop productivity and soil fertility. Research methods included agrochemical, microbiological, and conventional field analyses. The findings revealed that pre-sowing seed treatment with PMB increased crop yields by 7.4% to 10.9%. The study concluded that using biological preparations containing local, indigenous PMB strains is particularly effective. These preparations offer a cost-efficient alternative to commercial phosphorus fertilizers, as PMB uniquely convert insoluble soil phosphorus into plant-available forms. This allows farmers to reduce reliance on mineral fertilizers while achieving additional yield gains. These results underscore the importance of bacterial fertilizers for improving plant phosphorus nutrition. Furthermore, they highlight the effectiveness of applying such biopreparations within rice-based cropping systems in the Aral Sea region of Kazakhstan.

Keywords: Phosphate Mobilizing Bacteria, Microorganism, Biopreparation, Soil, Strain, Inoculant, Soil Bacteria

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Introduction

Modern intensive agriculture characterized by deep plowing, the use of heavy machinery, pesticides, mineral fertilizers, and disrupted crop rotations has led to several negative consequences. These include soil compaction in the arable layer, suppression of beneficial microbiota, destruction of soil structure, and environmental pollution from agrochemical residues.

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Global food production is inextricably linked to the use of phosphate fertilizers [1]. Phosphorus is an essential nutrient for plants; it accelerates crop maturation, enhances cold resistance, and improves tolerance to stress factors such as drought and salinity. Low phosphorus availability in soil retards plant growth and development, reduces immunity, and weakens resistance to pests and diseases. To achieve high yields, substantial amounts of phosphate fertilizers are applied annually. However, their excessive and systematic use contributes to water and soil pollution and other negative environmental outcomes [2].

In Kazakhstan, over 70% of arable land is characterized by extremely low levels of plant-available phosphorus compounds [3]. In the southeastern region of the country, the average gross phosphorus content in the arable soil layer is 3.0–5.0 t/ha, yet the amount available to plants does not exceed 0.1–0.2 t/ha. Thus, of the five tons of phosphorus present, crops absorb only 150–200 kg/ha during the growing season [4-5].

Easily assimilated phosphorus compounds from soil and fertilizers hold the greatest nutritional value for plants. A promising approach to improving phosphorus nutrition is biological phosphate mobilization, carried out by soil microorganisms such as bacteria and mycelial fungi. These organisms convert poorly soluble phosphorus compounds into forms accessible to higher plants [6].

In agriculture, the primary consumer of phosphorus compounds, the issue of available phosphorus deficiency is typically addressed through regular mineral fertilizer application. However, only 10–30% of applied phosphorus fertilizers are assimilated by plants [7].

All living organisms require phosphorus to maintain anabolism and homeostasis. Plants and microbes compete for soil phosphorus, making it essential for crops to have access to sufficient amounts to optimize yield and nutritional quality. Phosphorus is critical for plant growth, and its deficiency reduces crop yields. Although phosphorus exists in many forms in soil, plants can only absorb inorganic orthophosphate anions (HPO_4^{2-} and $H_2PO_4^{-}$) through their roots. Unfortunately, this bioavailable form constitutes less than 10% of total soil phosphorus. Despite large reservoirs of total P in most soils, precipitation and fixation with soil elements lead to significant phosphorus deficiency and negatively impact plant productivity [8].

The phosphate-mobilizing ability of bacteria can be harnessed to develop biotechnological methods for mobilizing recalcitrant phosphates and ensuring high crop yields. Seed inoculation with such microorganisms has a beneficial effect on plant growth and development [9].

The application of microbial inoculants with beneficial properties has a multifaceted positive impact on plants. Key factors contributing to increased yields include stimulation of growth processes (hormonal effects), increased availability of mineral nutrients, improved water nutrition, anti-stress action, enhanced plant immunity, and fungistatic effects against pathogenic fungi [10].

Many studies confirm that the combined application of Phosphate Dissolving Bacteria (PDB) and phosphate fertilizers can further reduce phosphorus adsorption in soil and increase its availability, thereby improving the ecological efficiency of fertilizer use. Nevertheless, understanding the dynamic interactions between phosphorus application, soil PDB, and phosphorus utilization remains a relatively unexplored area of research [11].

Research has shown that phosphate-solubilizing bacteria used as soil inoculants represent a suitable strategy for facilitating nutrient uptake by plants and mitigating the effects of environmental stresses [12].

Phosphate-solubilizing rhizobacteria play a vital role in the accumulation and conversion of phosphorus in plant roots. Phosphorus is primarily absorbed during vegetative growth and subsequently translocated to seeds and fruits. Globally, the introduction of phosphate-mobilizing bacterial inoculants has been reported to increase the growth and yield of many crops, including wheat, corn, and sweet potato [13, 14].

Materials and Methods

The experiment was conducted in 2024 on Field 1 of the first rice crop rotation at the "Karaultobe" experimental farm, part of the LLP "I. Zhakaev Kazakh Scientific-Research Institute of Rice Growing." Each plot measured 50 m², with three replications. The distance between adjacent plots was 1 m, and between treatment variations, 2 m. The preceding crop was rice.

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The study focused on forage crops: Mohar (Moharicum), Sudan grass (Sorghum drummondii), and the biological product Fosfobatsirin-S, which is based on the phosphate-mobilizing bacterium Bacillus megaterium 7A (hereinafter referred to as FMB). The product has a minimum titer of 5×10^9 CFU/ml. Fosfobatsirin-S is designed to enhance phosphorus nutrition in agricultural crops, thereby promoting growth and increasing yield. It was developed at the Scientific and Production Center of Microbiology and Virology of the Republic of Kazakhstan.

Pre-sowing seed treatment involves preparing a working solution of Fosfobatsirin-S, which is then sprayed onto the seeds to ensure even coverage. The seeds are left for approximately 30 minutes to allow the biofertilizer to adhere before sowing. Treatment can be performed manually or mechanically using seed treatment equipment such as concrete mixers. A single treatment is applied, with a biofertilizer consumption rate of 2 liters per ton of seeds and 3–5 liters of water used to prepare the working solution.

The physicochemical and microbiological properties of soil and plant samples were analyzed. Soil physicochemical analysis including total humus, total and mobile forms of nitrogen, phosphorus, potassium, mechanical composition, hygroscopic moisture, water extract, CO_2 content, pH, and absorbed calcium and magnesium bases was conducted at the LLP "Kazakh Scientific-Research Institute of Soil Science and Agrochemistry named after U. Uspanov." Soil sampling for chemical analysis involved collecting point samples (five per location, totaling 21 samples) from the arable layer (0–30 cm) of the experimental plot. Each point sample weighed 200–250 g. Analytical methods included [15] for nitrogen, [16] for phosphorus and potassium, [17] for humus, [18] for water extract, and [19] for other parameters.

At the Laboratory of Ecological and Agricultural Microbiology of the LLP "Scientific and Production Center of Microbiology and Virology," the abundance and species composition of microorganisms were assessed. The total microbial count was determined on Meat-Peptone Agar (MPA), fungi were cultured on Sabouraud's medium, nitrogen-fixing microorganisms were quantified and isolated on Mazé's medium, and phosphate-mobilizing microorganisms were quantified and isolated on NBRIP medium. The method was based on counting colonies grown on solid (agarized) nutrient media in Petri dishes.

Method of Inoculation and Counting Microorganisms on Solid Nutrient Media (Koch's Method)

The essence of the method lies in inoculating a specific volume of the investigated suspension onto a solid medium in Petri dishes, followed by counting the grown colonies.

The counting of grown colonies is performed after a specific time following inoculation, which depends on the growth rate of the identified microorganisms on the medium used in the experiment and at the given temperature. It is assumed that one cell forms one colony. The number of colonies grown after inoculation from a specific dilution is counted on three Petri dishes. The results of parallel inoculations are summed, and the average number of colonies grown from this dilution is determined. Colonies are counted without opening the dishes. Each counted colony is marked with a dot on the outer side of the dish bottom using a permanent marker. Dilutions are taken into account, and the number of cells is calculated using the formula.

Determination of the Number of Microorganisms in 1 g (1 ml) of the Original Substrate

The average number of CFU (M) per 1 ml/g is calculated by the formula:

$$M=\frac{a\times10^n}{V},$$

Where:

M : The number of cells in 1 ml/g of the investigated substrate

a : The average number of colonies grown after inoculation from the given dilution

V : The volume of the suspension taken for inoculation, ml

10^(n) : The dilution factor of the investigated substrate

The standard deviation is calculated using the formula:

$$\sigma = \pm\frac{\sqrt{\sum b}}{c}$$

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Where $\sum b$ – the total number of counted colonies when inoculating a given dilution; c the number of replicates.

The nutritional value of the forage crops was assessed at the LLP "Kazakh Scientific-Research Institute of Animal Husbandry and Feed Production." The following parameters were determined: content of protein, fat, fiber, Nitrogen-Free Extract (NFE), starch, sugar, ash, metabolizable energy, carotene, and feed units. Analytical methods included crude protein determination according to [19], metabolizable energy according to [20] and feed units according to [21].

Phenological observations were carried out using the estimation method, identifying both the initial (10%) and full (70%) stages of plant development. Plant density at emergence and the number of surviving plants were recorded using a five-replicate method for annual crops and a three-replicate method for perennial grasses on each plot within the same treatment. Plant height was measured on 20 plants using a measuring ruler, with 10 plants measured in two repetitions. Yield was determined by harvesting ten square meters twice on each plot, following the methodology of [22].

Results

To stimulate growth and increase yield, seeds of the experimental crops were treated with the biological preparation based on Phosphate Mobilizing Bacteria (PMB) before sowing.

To determine the effect of the biopreparation, biometric studies of the forage crops were carried out (tillering, jointing, heading). The results of the analysis are presented in Table 1.

Table 1: Biometric analysis of experimental crops conducted to determine the effect of the biological preparation (average value)

Cultures	Experience options	Biometric indicators					
		Tillering – Stem elongation		Heading			
		Plant height, cm	Number of stems, pcs	Plant height, cm	Number of stems, pcs	Number of spikes, pcs	Spike length, cm
Mogar (Moharicum)	Control (untreated)	16,3	3	55,25	8	4	11,5
	Treated with PMB	21,4	8	89,54	13	13	19,14
Sudan grass (Sorghum drummondii)	Control (untreated)	35,4	2	147,5	5	4	27,25
	Treated with PMB	42,7	5	178,5	8	8	37,63

The biometric analysis revealed that variants treated with Phosphate-Mobilizing Bacteria (PMB) exhibited superior performance compared to the control variants. During the tillering stem elongation stage, the height of Mogar millet in the PMB-treated group reached 21.4 cm, and by the heading stage, it increased to 89.54 cm. This represents an increase of 5.1–34.29 cm over the control group, which measured 16.3 cm and 55.25 cm, respectively.

The PMB treatment also resulted in higher values for both the number and length of spikes. Specifically, the number of stems and spikes per plant was 5–9 units greater than in the control. Additionally, in the PMB-treated variant, three shoots formed above the first node, and the root system developed more intensively.

Similar trends were observed in Sudan grass. During the tillering stem elongation stage, plant height in the PMB variant reached 42.7 cm, which was 7.3 cm taller than the control. At the heading stage, the PMB-treated plants again demonstrated superior results in terms of stem number, spike number, and spike length. In Sudan grass, three shoots also formed above the first node on a single stem, and notable differences were observed in root system development. These findings indicate adequate phosphorus supply to the plants, attributed to the active mobilization and delivery of phosphorus by the phosphate-mobilizing bacteria.

To assess aboveground biomass, yield calculations were performed for the forage crops, and the results are presented in Table 2.

The results of the aboveground yield study for forage crops showed that the control group of Sudan grass produced a yield of 178.3 c/ha, which was 13.2 c/ha lower than the variant treated with Phosphate-Mobilizing Bacteria (PMB). For Mogar millet, the PMB-treated variant also demonstrated a higher yield, reaching 143.2 c/ha.

Table 2: Yield of forage crops (mean values)

Cultures	Experience options	Yield, centners/ha	Dry matter collection, c/ha	Increase			
				Green mass		hay	
				c/ha	%	c/ha	%
Sudan grass (Sorghum drummondii)	Control (untreated)	178,3	41,3	-	-	-	-
	Treated with PMB	191,5	47,8	13,2	7,4	6,5	15,7
Mohar (Moharicum)	Control (untreated)	129,1	51,6	-	-	-	-
	Treated with PMB	143,2	58,9	14,1	10,9	7,3	14,1
NSR 0.05 c/ha		0,37					

To assess plant nutritional parameters, samples were collected and analyzed in the laboratory of the Kazakh Research Institute of Animal Husbandry and Forage Production. The results of this analysis are presented in Table 3.

Table 3: Forage value parameters of the experimental crops

Indicators	Cultures	
	Sudan grass (Sorghum drummondii)	Mohar (Moharicum)
Initial moisture	7,52	8,09
Hygroscopic moisture	3,11	2,81
Total moisture	10,40	10,67
Dry matter	89,60	89,33
Protein, %	9,95	19,53
Fat, %	1,59	3,92
Fiber, %	32,48	33,22
NFE (Nitrogen-free extract), %	38,29	27,55
Sugar, %	1,64	-
Starch, %	-	1,82
Ash, %	7,30	5,12
Ca, %	1,04	1,74
P, %	0,24	0,42
Carotene, mg	28,94	30,96
Feed unit, kg	0,49	0,55
Digestible protein, g	58,71	115,23
Metabolizable energy, MJ	7,29	8,06
FEU (Feed energy unit)	0,73	0,81

The experimental forage crops demonstrated high nutritional value. Metabolizable energy a key criterion for assessing the energy content of feed represents the amount of energy absorbed by the body from nutrients. Among the crops studied, mogar exhibited a higher metabolizable energy value of 8.06 MJ. The feed unit, defined as the nutritional value equivalent to 1 kg of medium-quality oats, was similarly elevated for both crops: mogar recorded 0.49 feed units, and sudangrass also reached 0.49 feed units.

To evaluate soil conditions, analyses were conducted to determine the salinity and agrochemical composition of the soil in the experimental plot. The results of these analyses are presented in Tables 4 and 5.

Based on the dry matter content, the soils are classified as having medium sulfate salinity, with salinity levels ranging from 0.6% to 1.0%. The primary objective of this research project is to accelerate the introduction of forage crops into agricultural production in the Kyzylorda region, particularly in response to increasing water scarcity. Accordingly, the experimental crops were sown without irrigation.

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As shown in the table, when the soil analysis results from the small-plot experiment are grouped according to the salinity classification of N.N. Balyabo, the average salinity level corresponds to sulfate and chloride-sulfate types.

According to the autumn analysis, no significant changes in total humus content were observed. The potassium content in the topsoil of the cultivated crops remains very high, which is attributed to the potassium-rich soils characteristic of the Aral Sea region of Kazakhstan. The available phosphorus content in the plowed soil layer was found to be 20–21 mg/kg higher for Sudan grass and mogar compared to spring levels. Based on Kirsanov's classification, the available phosphorus content in the soil falls within the average range (51–100 mg/kg).

Table 4: Content of easily soluble salts in the soil of the experimental plot

Sample name	Sampling depth, cm	Dry residue %	Mg-eq. 100 g soil/%						
			HCO_3^-	SO_4^{2-}	Cl^-	Na^+	Ca^{2+}	Mg^{2+}	K^+
Sudan grass (*Sorghum drummondii)*	0-20	0,699	0,017	9,36	1,00	2,45	4,91	3,20	0,08
				0,449	0,035	0,056	0,098	0,039	0,003
	0-40	0,552	0,015	7,27	0,96	1,88	3,82	2,74	0,04
				0,349	0,034	0,043	0,076	0,033	0,001
Mohar (*Moharicum)*	0-20	0,892	0,012	11,45	2,18	4,08	4,82	4,84	0,09
				0,550	0,077	0,094	0,096	0,059	0,004
	0-40	0,496	0,015	6,25	1,18	2,16	2,73	2,74	0,04
				0,300	0,042	0,050	0,055	0,033	0,001

Table 5: Indicators of agrochemical composition of soil in the experimental plot

Sample name	Sampling depth, cm	Total humus	Gross, %			mobile, mg/kg		
			Nitrogen%	Phosphorus %	Potassium %	Nitrogen mg/kg	Phosphorus mg/kg	Potassium mg/kg
Before the experiment was started (spring)	0-20	1,30	0,182	0,175	2,000	44,8	32	210
	20-40	1,41	0,098	0,181	1,875	22,4	52	200
Sudan grass (*Sorghum drummondii)*	0-20	1,43	0,098	0,157	2,099	33,6	52	180
	0-40	0,79	0,070	0,136	0,498	22,4	34	160
Mohar (*Moharicum)*	0-20	0,98	0,126	0,153	1,799	36,4	51	210
	0-40	0,36	0,056	0,119	2,199	33,6	18	130

Given that no phosphorus fertilizers were applied to the experimental fields, it can be concluded that the increase in phosphorus content observed in autumn relative to spring is attributable to the activity of phosphate-mobilizing bacteria. Research by scientist D.R. Itkina supports this finding, demonstrating that phosphate-hydrolyzing bacterial strains stimulate plant growth and development. These bacteria play a key role in mobilizing insoluble phosphorus compounds, making them available for plant nutrition. Furthermore, such bacterial strains exhibit antagonistic activity against phytopathogens, reducing the need for chemical fungicides and expensive fertilizers while simultaneously increasing crop yields. This represents a promising and relevant direction in agricultural research [23].

Soil microorganisms are broadly classified into seven categories: bacteria, fungi, viruses, blue-green algae, actinomycetes, protozoa, and nematodes. Each group possesses distinct characteristics and performs specific functions within the soil ecosystem. Collectively, these microorganisms influence soil biological activity. The composition of the soil microbial community is dynamic and responds to changes in environmental conditions.

Microbial abundance decreases with increasing soil depth, as organic matter content declines. The rhizosphere, however, harbors a higher concentration of microorganisms compared to other soil zones. For this study, soil samples were collected from both the rhizosphere and the 0–20 cm soil layer. The results of the microbiological analysis are presented in Table 6.

The data in the table shows that the total number of microorganisms in the mogar is higher than in the crops under Sudan grass, however, the CFU per 1 g of soil indicates that the soil has a low humus content and the processes of organic decomposition in it are weak.

Microorganism colonies isolated from the soil under the Sudan grass crop are shown in Fig. 1-4.

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Aspergillus flavus is a saprophytic fungus found worldwide in soils and is known to cause diseases in various agriculturally important crops. Common hosts include cereals, legumes, and nuts. Infection may occur in the field, before or after harvest, as well as during storage and transportation. Although the pathogen often colonizes host plants while they are still in the field, symptoms and signs of infection are frequently not visible. A. flavus can infect seedlings by sporulating on damaged seeds. In cereal crops, the fungus can penetrate seed embryos, leading to reduced germination and potential contamination of seeds sown in the field. It may also discolor embryos, damage seedlings, and in some cases kill them, thereby reducing grain quality and market value.

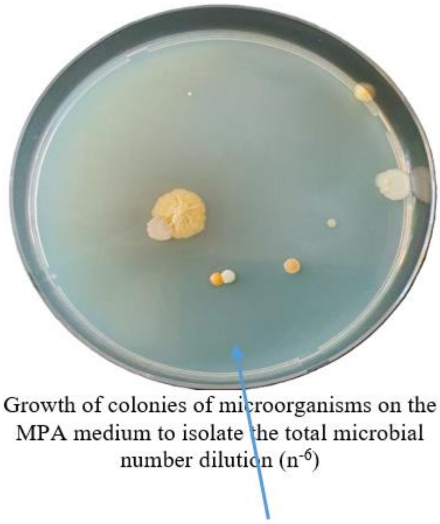


Fig. 1: Koch's method, light zones on MPA medium

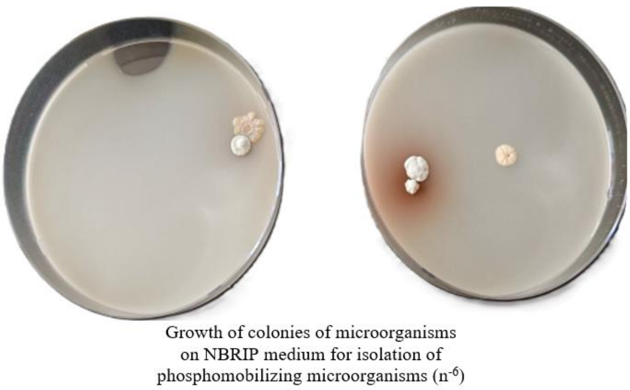


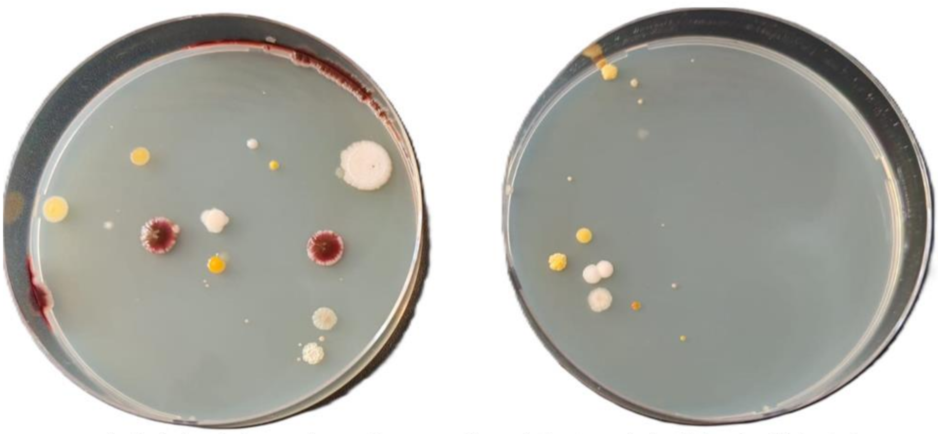
Fig. 2: medium for the isolation of phosphate-mobilizing microorganisms, NBRIP medium

Fig. 3: Colonies for the isolation of nitrogen-fixing microorganisms, Maze medium Agar clearing zones are visible

Growth of colonies of microorganisms on Saburo medium for isolation of yeast and mold fungi (n^{-6})

Fig. 4: Colonies of mold fungi on Sabouraud medium

Colonies of microorganisms isolated from the soil under the Mogar culture are shown in Fig. 5-8.



Growth of colonies of microorganisms on the MPA medium to isolate the total microbial number dilution (n^{-6})

Fig. 5: Koch method, MPA environment

Growth of colonies of microorganisms on NBRIP medium for isolation of phosphomobilizing microorganisms (n^{-6})

Fig. 6: NBRIP medium for the isolation of phosphate-mobilizing microorganism's Visible growth of fungi, bacteria, and actinomycetes

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Fig. 7: Zones of agar clearing on Maze medium for the isolation of nitrogen-fixing microorganisms

Fig. 8: Colonies of bacteria and fungi on Sabouraud medium

A. flavus is unique among fungi due to its thermotolerance, enabling it to survive at temperatures lethal to many other fungal species. It contributes to post-harvest rot, particularly when plant material is stored under high humidity. The fungus thrives in hot and humid climates.

Bacillus subtilis is a Gram-positive, spore forming, facultatively anaerobic bacterium. It acts as an antagonist against pathogenic and opportunistic microorganisms through antibiotic production and environmental acidification. Additionally, B. subtilis functions as a plant growth stimulator.

Determination of microbial abundance using Koch's method revealed that colonies grew on media at the (n-6) dilution level. At higher dilutions, colony counts decreased and were insufficient for reliable quantification according to standard methodology. The (n-6) dilution level is considered indicative of soils poor in humus, with humus content below 1.5–2%. Low soil nitrogen content is suggested by the formation of light zones in Figures 3 and 7 on Maze medium, where colony counts at the (n-6) dilution exceeded those on the general-purpose MPA medium.

On NBRIP medium (Figure 6), no phosphate utilization zones characteristic of phosphate-mobilizing microorganisms were observed. However, active colony growth was noted, exceeding that on MPA medium. Colonies were distinct with clearly visible profiles. Applying Liebig’s Law of the Minimum to microbial growth which states that development is limited by the least

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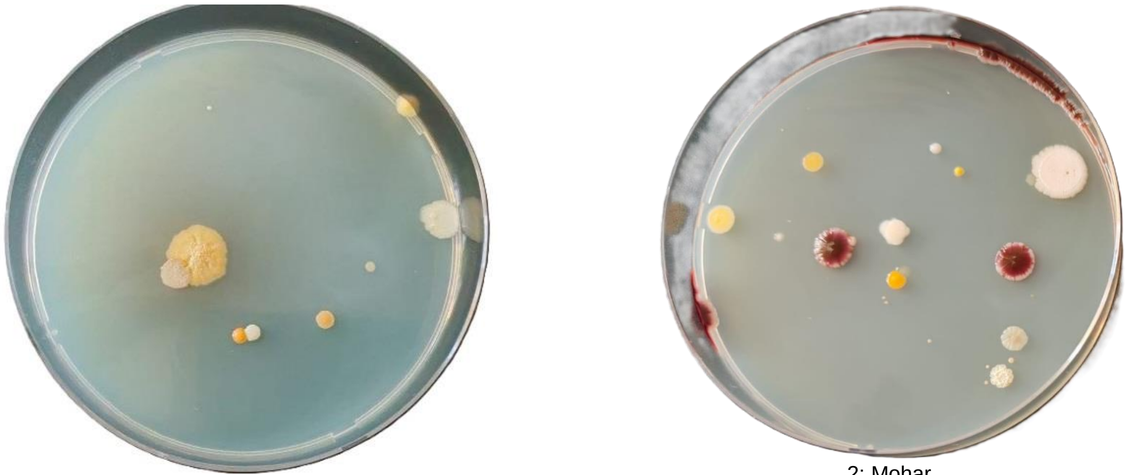
available nutrient it can be concluded that the soil is phosphorus-deficient. Therefore, at early stages of plant development, the application of biofertilizers based on phosphate-mobilizing microorganisms or phosphorus fertilization is recommended.

Figure 9 presents a comparison of microbial colonies associated with Sudan grass and Mogar millet. On plate 2, both the number and species composition of microorganisms were broader, indicating that culture 2 contains a higher nutrient content supporting enhanced microbial growth.



1: Sudan grass 2: Mohar

Growth of colonies of microorganisms on the MPA medium to isolate the total microbial number dilution (n^{-6})

Fig. 9: Colonies of microorganisms under cultures in comparison

In the soil under culture 2, fewer fungal colonies were observed. This suggests that the rhizosphere of Mogar millet may contain compounds capable of suppressing the growth of certain pathogenic fungi, thereby contributing to the maintenance of crop yields. Mogar millet thus appears to function not only as a valuable forage crop but also as a plant that improves soil health by reducing the load of plant-pathogenic fungi.

Figure 9 illustrates active growth of actinomycetes, which appears to have created a barrier limiting the spread of fungal growth. The qualitative composition of actinomycetes in this soil is relatively poor, consisting primarily of ray fungi with white and gray colonies.

Discussion

This research complements the work of other scientists in the field. However, the distinctive feature of this study is that, for the first time, pre-sowing treatment of seeds with phosphate-mobilizing bacteria was carried out on saline soils of the Aral Sea region.

The results obtained demonstrate the importance of utilizing phosphate-mobilizing bacteria in agriculture. Furthermore, the application of biological preparations based on these microorganisms has a significant positive impact not only on agricultural productivity but also on soil protection. In conventional agricultural practice, continuous use of mineral fertilizers leads to soil contamination, as only 30–40% of applied fertilizers are absorbed by plants, while the remainder accumulates in the soil year after year. This repeated cycle results in environmental pollution, particularly of soil resources.

Incorporating these findings into recommendations for crop cultivation and plant nutrition will contribute to sustainable and cost-effective agricultural practices.

Conclusion

In conclusion, to enhance the growth and yield of agricultural crops, it is more effective to use a biological preparation containing local, indigenous Phosphate-Mobilizing Bacteria (PMB). Such preparations offer greater cost-effectiveness compared to commercially available phosphorus fertilizers, as only PMB in nature are capable of converting insoluble phosphorus compounds into forms accessible to plants. This approach enables farmers to reduce expenditures on phosphorus fertilizers while simultaneously achieving additional yield benefits.

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Author’s Contributions

Zhanar Zhumadilova: Performed phenological and botanical observations and analyzed plant morphological traits.
Laura Tokhetova: Conceived and developed the study, designed the methodology, and provided strategic guidance.
Shorabayev Yerik: Conducted field experiments and collected agronomic and yield data.
Gulsim Baimbetova: Conducted field experiments and reviewed and edited the manuscript.
Zere Turlybayeva: Provided guidance and critical analysis of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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