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Biomodified Phosphorus Fertilizers (Bmpfs) are New and Promising Forms of Phosphorus Fertilizers

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Abstract: Traditional and biomodified phosphorus mineral fertilizers were studied in laboratory and field conditions. It has been established that the kinetics of the transition of mobile forms of P2O5 phosphorus fertilizers into an indigestible form depends on the time of interaction of fertilizers with the soil. It was shown that the highest level of mobile forms of P2O5 fertilizers, a month after application, was found in soils applied with biomodified fertilizers BMPF-Ammophos, BMPF-Suprefos NS and BMPF -PS-Agro (48-50 and 34 mg/kg of soil, respectively). While the content of mobile P2O5 in soils with traditional fertilizers on day 30 was measured to be only 24-25 mg/kg of soil.

Changes in the phosphatase activity of the experimental soils ranged from 242.8 to 622.3 mg p-nitrophenol kg-1 soil h-1, while the value of the phosphatase enzyme activity of the control plots changed from an initial value of 243.2 to values varying between 244.6 and 247.2, which indicates the effectiveness of BMPF in increasing the phosphatase activity of soil and, accordingly, a high level of solubilization of indigestible organic phosphates.

The results of field experiments showed that by the end of the growing season in the variants with BMPF, the plant height exceeded the control variant by 31.2-38.1 cm, in the number of sympodial branches - by 3.7-5.0 pcs., in the number of open bolls - by 2.8-4.0 pcs., in terms of yield - by 5.0-9.7 c/ha.

Key words: Mobile forms of P2O5, phosphate-mobilizing microorganisms (PMM), phosphatase, digestibility, phosphorus fertilizers.

Introduction.

Phosphorus is the limiting element of mineral nutrients necessary for the growth, development and reproduction of plants [1]. As a major component of fundamental macromolecules such as nucleic acids and phospholipids, phosphorus plays an important role in energy transfer and regulation of enzymatic reactions. Participates in the control and regulation of metabolic pathways both at the cellular and organismal levels [2,3]. Almost 95% of the extracted phosphorus is used to feed agricultural plants in the form of mineral fertilizers [4]. Critical to agricultural production, as well as experiencing significant price volatility, phosphates and their future availability have received increasing attention from both scientists and the public in recent years [5-9].

Given the strategic importance of this element and society's significant dependence on phosphate rock reserves, researchers around the world have declared an urgent need to apply new approaches to its use. Since extractable phosphate rock is a non-renewable resource, and at the current rate of consumption, humanity may exhaust the known fossil reserves of the resource by the end of this century [7, 8, 9].

The results of chemical and agrochemical studies carried out in recent years have made it possible to find out that, depending on the type of soil and its physical and chemical status, no more than 8-20% of phosphorus added to the soil with fertilizers is absorbed by agricultural crops [10]. When water-soluble forms of phosphorus fertilizers are introduced into the soil, about 30-40% of P_2O_5 becomes a water-insoluble state within 15 minutes [6,11]. Almost complete conversion of phosphorus pentaoxide (95.5%) into insoluble form ($AlPO_4$, $Al(OH)_3PO_4$, $FePO_4$, $Fe_2(OH)_3PO_4$, $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$ and $Ca_5(PO_4)_3OH$) occurs within a month after applying phosphorus fertilizers to the soil.

In this connection, to prevent crop shortages, the application of phosphorus fertilizers to agricultural soils increases annually by 3.2% or more, depending on the condition of the soil [7,11]. Consequently, the demand for phosphorus fertilizers to maintain plant productivity has increased over the years. As a result, depending on the type, condition and biological activity of soils, up to 0.2-0.4% (by soil weight) of indigestible fixed phosphates can accumulate in them, which, in terms of one hectare of arable horizon, will amount to from 3 to 6 or more tons P_20_5 [4,7,8]. At the same time, a huge part of the applied phosphorus fertilizers containing various heavy metals are fixed in the soil, having a detrimental effect on the fertility of agricultural land, the health of animals and people [4,6]. In this connection, there is a need to find alternative approaches to eliminate shortcomings and reduce the application rates of phosphorus mineral fertilizers, without reducing crop yields.

One of the alternative approaches is the biomodification of traditional phosphorus mineral fertilizers based on phosphate-mobilizing microorganisms (PMMs). FMMs are bioinoculants; the main mechanisms ensuring the mobilization of hard-to-reach phosphorus compounds are their production of various organic acids and enzymes [1,12]. The set of such acids is extremely diverse; microorganisms produce gluconic, pyruvic, acetic, oxalic, acetic, malic, lactic, and citric acids.

In addition, the production of organic acids is often associated with the production of phosphatases, most often alkaline, as well as other biologically active metabolites, such as IAA, siderophores, and antifungal metabolites [1,12,13,14]. The accumulated data allow us to draw a conclusion about the fundamental contribution of phosphate-mobilizing microorganisms in the phosphorus nutrition of plants and their influence on growth performance. The efficiency of dissolution of mineral phosphorus compounds can be quite high; data on the amount of available phosphorus can completely satisfy the needs of the plant organism [1,11-14].

Purpose of the work: Study of biomodified phosphorus fertilizers (BMFP).

Objects of research: The objects of research were: BMFU based on FMM (BMFU-Ammophos, BMFU-Suprefos NS, BMFU-PS-Agro), developed by researchers at the Institute of Chemistry of Plant Substances (IHRV) of the Academy of Sciences of the Republic of Uzbekistan. Traditional phosphate mineral fertilizers (Ammophos, PS-Agro and Suprefos-NS) of domestic production. Cotton plants variety S-5707, created by scientists from the Research Institute of Breeding, Seed Growing and Agrotechnology of Cotton Growing. The soil is typical for the soil-climatic zone of the Tashkent region, taken from the depths of the arable layer of cotton fields.

Research methods

Studies of the kinetics of changes in mobile forms of P_2O_5 in soil. To study the kinetics of changes in the mobile forms of P_2O_5 of traditional phosphorus fertilizers and BMFC in the soil, we used a typical sierozem taken from a depth of 15-25 cm in the arable layer. 100 g of soil samples were subjected to heat treatment to sterilize the native microflora and denature their enzymes in a drying oven at 180°C in for 3 hours and stored under sterile conditions until the experiments. To conduct the experiment, soil samples weighing 100 grams were fertilized at the rate of 100 mg/kg of soil, thoroughly mixed and transferred to flasks, and evenly moistened with distilled water to 60% of the total moisture capacity. As a control - soil without fertilizer application. The samples were stored at this humidity until mobile P_2O_5 was determined.

The content of mobile forms of phosphorus (P_2O_5) in the soil was determined using the Olsen method [15]. For this purpose, crushed and passed through a sieve with a hole diameter of 1 - 2 mm, 5 g of soil, previously dried to an air-dry state, is placed in flasks with a capacity of 200 - 250 cm^3. Add 100 cm^3 0.5 N. $NaHCO_3$ solution, add 1-3 g of activated carbon and shake on a rotator for 30 minutes. Then it is filtered and 5-40 cm^3 of colorless transparent filtrate is transferred into 100 cm^3 volumetric flasks, carefully neutralized with 6-dinitrophenol with 10% HC1, stirred to remove the released CO_2 bubbles, and reagents are added to color the solution for photometry.

Soil extracts for the determination of mobile phosphates were prepared at different times: after 15 min; in one hour; in 3 days; 10 days and 30 days after application of fertilizers.

The experiment compared the kinetics of changes in mobile P_2O_5 forms of traditional (Ammophos, Suprefos NS, PS-Agro) and biomodified (BMFU-Ammophos, BMFU-Suprefos NS, BMFU-PS-Agro) fertilizers in the soil.

Research on the effect of BMFU on the phosphatase activity of soil. Phosphatase activity of experimental and control soils was determined according to “Methods of soil enzymology” edited by F.Kh. Khaziev [16]. At the same time, BMFU-Ammophos was applied to the soil under cotton crops between rows at a depth of 15 cm, normally at the rate of 50 kg/ha. Two weeks later, samples were taken from the same depth where BMFU-Ammophos was added to analyze the phosphatase activity of the soil. To do this, place 1 g of air-dry soil in a 50 ml Erlenmeyer flask with a ground stopper, add 4 ml of universal buffer (pH 6.5), 0.25 ml of toluene and 1 ml of 0.115 M solution of sodium p-nitrophenyl phosphate (para-nitrophenyl phosphate- PNFF) and the mixture was stirred for 5 minutes. The flask is closed and placed in a thermostat for 1 hour at a temperature of 37°C. After incubation, add 1 ml of 0.5 M sodium hydroxide and stir for 5 minutes. and the suspension is filtered through an ashless filter into a 50 cm^3 volumetric flask. The filtrate is colored yellow from the liberated p-nitrophenol.

The density of the yellow color of the filtrate is measured on a photocolorimeter with a blue filter (400-420 nm). The amount of p-nitrophenol released is calculated from a calibration curve compiled from standard solutions of p-nitrophenol. Phosphatase activity is expressed in mg p-nitrophenol kg^{-1} soil h^{-1} [17].

Field studies of the BMFU were carried out in the soil and climatic conditions of the Tashkent region, on cotton crops of the S-5707 variety, according to the methods of [18]. The soil of the experimental fields is monotonous, typical irrigated gray soil. The relief of the experimental site is flat. The mechanical composition is medium loamy with a predominance of coarse dust, amounting to 45-50% or more. The soils are quite high-carbonate and have a high absorption capacity - 9-12 mEq. per 100 g of soil, which decreases with depth. In the arable horizon, the humus content is 1.0 - 3.1%. The thickness of the humus layer is 50 – 80 cm.

Scheme of the experiment studying different doses of phosphorus fertilizers:

1. Ammaphos, 70 kg/ha a.i. (control);
2. BMFU-Ammophos - 50 kg/ha a.i.;
3. BMFU-Ammophos - 70 kg/ha a.i.

The placement of options in the field experiment is randomized. Planning and conducting field experiments, as well as statistical processing of the data obtained, were carried out according to the methods of B.A. Dospehov [18].

Research results and discussion.

The results of the study of the kinetics of changes in the digestible forms of P_2O_5 of traditional (Ammofos, Suprefos NS, PS-Agro) and biomodified (BMFU-Ammofos, BMFU-Suprefos NS, BMFU-PS-Agro) fertilizers in the soil showed that the content of mobile forms of P_2O_5 BMFU in the soil in within 30 days decreased from 58-73 at the time of application to 34-50 mg/kg of soil, while the P_2O_5 content of traditional phosphorus fertilizers in the soil decreased from 57-72 to 24-25 mg/kg of soil. In the soils of the control variant (without applying fertilizers), the P_2O_5 content remained practically unchanged during the month (22-23 mg/kg of soil) Fig. 1.

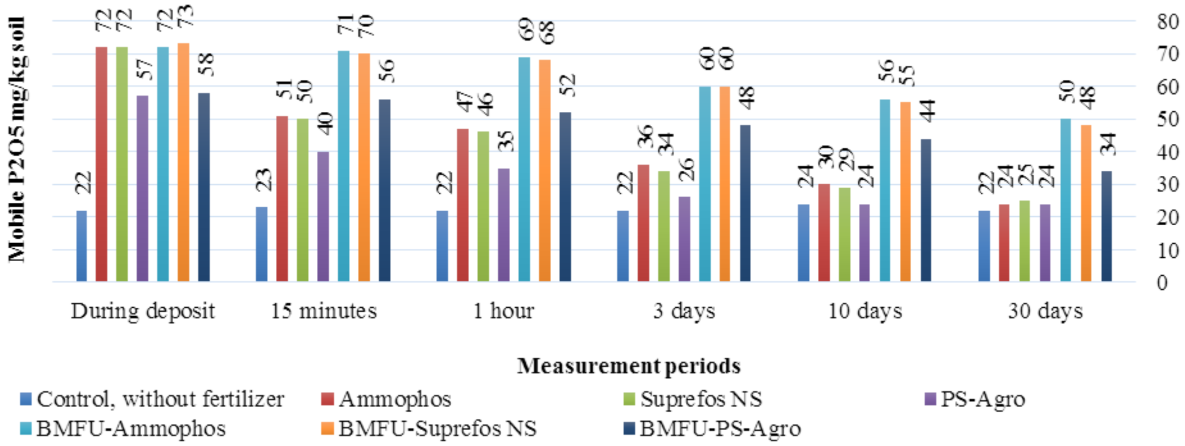

Figure 1. Kinetics of changes in mobile forms (P_2O_5) of traditional and biomodified fertilizers in the soil

According to the results of the experiment, a significant decrease in the mobile forms of P_2O_5 of traditional phosphorus fertilizers begins within 15 minutes after applying fertilizers to the soil. The P_2O_5 content at the time of application was 57-72 mg/kg, after 15 minutes it became 40-51 mg/kg of soil, which is consistent with the results of researchers [10,11], who found that when water-soluble forms of phosphorus fertilizers are introduced into the soil, about 30+ 40% of digestible forms of P_2O_5 are fixed by soil minerals within 15 minutes. At the same time, the degree of fixation of mobile forms of phosphorus strongly depends on the type of soil and soil solutions in the area of application. Thus, in acidic soils, P_2O_5 is associated predominantly with iron and aluminum in the form of $FePO_4$ and A_1PO_4, and in case of an excess of sesquioxides, it is mainly in the form of basic salts - $Fe_2(OH_3)PO_4$ and $A_{12}(OH_3)PO_4$. In alkaline and slightly alkaline soils, the formation of such slightly soluble phosphorus salts as $CaHPO_4$ occurs; $Ca_3(PO_4)_2$; $MgHPO_4$; $Mg_3(PO_4)_2$ and sparingly soluble - hydroxyapatite $Ca_5(PO_4)_3OH$ [2, 20, 30].

The fixation of mobile forms of P_2O_5 BMFC in the soil occurred significantly slowly compared to traditional fertilizers. At the same time, the highest level of mobile forms of P_2O_5 fertilizer, a month after application, was found in the soil applied with BMFU-Ammophos and BMFU-Suprephos NS (48-50 mg/kg of soil, respectively). The content of mobile forms of P_2O_5, BMFU-PS-Agro on the 30th day was measured at 34 mg/kg of soil. The results obtained are consistent with the opinions of researchers [1,10,19], who argue that the main mechanism ensuring the mobilization of hard-to-reach phosphorus compounds is the production of various organic acids by microorganisms, such as gluconic, pyruvic, acetic, oxalic, acetic, malic, lactic , lemon acid. As a rule, the production of organic acids is often associated with the production of phosphatases, most often alkaline, as well as other biologically active metabolites, such as IAA, siderophores, and antifungal metabolites [1,19].

The results of measuring the phosphatase activity of the soil, two weeks after the application of BMFU-Ammophos, showed that the fertilizer significantly increases the phosphatase activity of the soil.

The kinetics of increasing phosphatase activity of soils is observed within 21 days. The level of phosphatase activity in soils increased with increasing period of application of BMFU-Ammophos to the soil (Fig. 2). The highest values of soil phosphatase activity were observed 21 days after application of fertilizers. At the same time, the highest levels of phosphatase activity in test soil samples were observed on the 28th day of measurement.

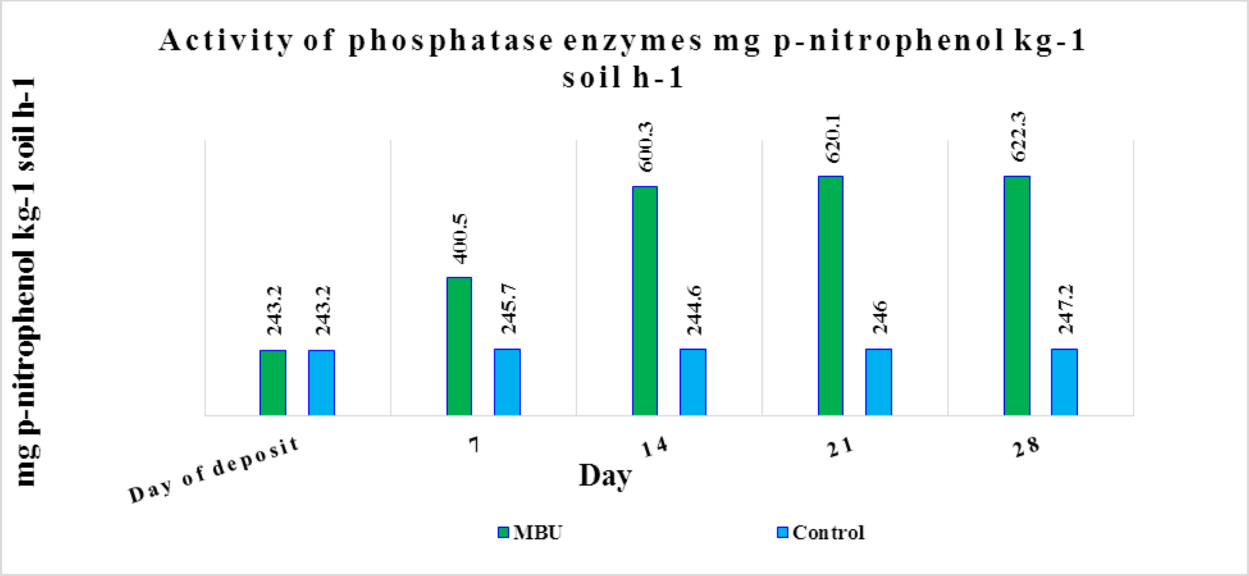

Рис. 2. Активность ферментов фосфатаз, мг п-нитрофенола кг$^{-1}$ почвы час$^{-1}$

Changes in the phosphatase activity of the experimental soils varied from 242.8 on the day of application to 622.3 mg of p-nitrophenol kg-1 soil h-1 on the 28th day of the experiment, while the value of the phosphatase enzyme activity of the control plots changed from the initial value of 243.2 to values varying between 244.6 and 247.2. The phosphatase activity of the experimental plots with the added BMFU-Ammophos was 622.3 mg of p-nitrophenol kg-1 soil h-1 on the 28th day after application, while the initial value of soil activity was equal to 243.2, which indicates the effectiveness of BMFU-Ammophos in an increase in the phosphatase activity of the soil and, accordingly, a high level of solubilization of indigestible organic phosphates.

Phosphatases are known as specialized exoenzymes produced by microbes. They play an important role in the mineralization of organic phosphorus. These enzymes hydrolyze various ester (phosphomonoesters) and phosphoanhydride compounds of phosphorus, mobilizing phosphorus fixed in organic matter that is not digestible by plants [20, 21]. In soils, phosphatases not only mineralize organic phosphorus, but also promote the entry of mineral phosphorus into plant roots. Organophosphorus compounds make up the predominant part of soil phosphorus (from 20 to 80%) and are represented by nucleic compounds, derivatives of adenosine phosphoric acid, phosphohumic complexes and slightly mobile sugar phosphates and glycerophosphates [22]. Organophosphorus compounds in the soil become accessible to plants during enzymatic hydrolysis with the elimination of phosphoric acid residues [23-25].

In field studies, phenological observations and biometric measurements of vegetating plants were carried out. Based on the results of the analysis of the data obtained, it was found that the indicators of plant height, number of sympodial branches, buds, flowers, bolls and yield were higher in all experimental variants compared to the control, Table 1.

Table 1 The influence of BMFU on the growth, development and productivity of cotton plants.

The date of the	Observation Parameters	Options and rate of fertilizer application t/ha		
		Ammaphos (control), 70.0 kg/ha	BMFU-Ammophos, 50.0 kg/ha	BMFU-Ammophos, 70.0 kg/ha
July 1	Plant height, cm	44,4	56,7	63,6
	Number of sympodial branches, pcs.	7,3	9,9	11,5
	Number of buds, pcs.	6,6	7,0	8,8
	Number of flowers, pcs.	1,7	1,0	2,2
	Number of boxes, pcs.	0,9	1,4	2,0

August 1	Plant height, cm	67,9	84,9	94,4
	Number of sympodial branches, pcs.	10,3	12,1	15,6
	Number of buds, pcs.	0,8	2,5	3,0
	Number of flowers, pcs.	0,2	0,6	0,7
	Number of boxes, pcs.	8,6	7,3	10,5
September 1	Plant height, cm	89,5	114,4	128,6
	Number of sympodial branches, pcs.	13,8	16,2	18,8
	Number of buds, pcs.	2,4	3,8	4,4
	Number of flowers, pcs.	7,1	8,1	11,1
Yield ts/ha		38,1	40,8	47,8
Difference relative to control +;-		-	+2,7	+9,7

By the end of the growing season, in the experimental variants with BMFU-Ammophos (50.0-70.0 kg/ha), the plant height exceeded the control variant by 24.9-39.1 cm, and in the number of sympodial branches - by 2.4-5 ,0 pcs., by the number of opened boxes - by 1.4-2.0 pcs., by yield - by 2.7-9.7 c/ha, respectively. Even in the experimental variants, where biomodified fertilizers were applied 20 kg/ha less (50.0 kg/ha) than traditional fertilizers applied to the control fields (70.0 kg/ha, adopted for this crop), the development of fruit organs and plant productivity was higher compared to the control, which indicates the possibility of a significant reduction in application rates. The data obtained indicate the significant effectiveness of biomodified mineral fertilizers for growth, development and productivity, which is consistent with the data of researchers who studied the effects of FMM on the growth and development of tobacco and corn plants [26,27].

Conclusion

A study of traditional and BMFU has shown that the combined use of natural soil bioresources (NSB) and mineral fertilizers can serve as a promising alternative to the currently standard application of inorganic fertilizers. The results of our experiments and recent scientific results are consistent with the idea that FMMs can significantly influence the uptake of phosphorus by plants and play a fundamental role in the life cycle of phosphorus in the soil [1,3,22-27].

Organic acids and phytase enzymes produced by FMM have a positive effect on the phosphorus nutrition of plants, releasing phosphorus fixed by soil cations and chelated by phytate in soil organic matter. Moreover, other positive effects of FMM on plant growth and development have been studied: suppression of diseases caused by plant pathogens (possibly due to competition with pathogenic microorganisms for colonization of roots), microbial synthesis of plant growth regulators and reduction of ethylene levels in root cells [1, 3.22-27].

Modification of traditional phosphorus mineral fertilizers based on effective FMM strains producing organic acids and phytase enzymes can be considered as a possible effective and environmentally friendly approach to increasing the bioavailability of soil phosphorus and reducing the rate of use of inorganic phosphorus fertilizers.

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