



Agrobiological and Environmental Evaluation of Biomodified Monoammonium Phosphate in the Cultivation of Cotton (*Gossypium hirsutum* L.)



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SUSTAINABLE cotton production in Central Asia is constrained by phosphorus deficiency and strong fixation in calcareous soils. Conventional phosphorus fertilizers, such as monoammonium phosphate (MAP), exhibit low use efficiency due to rapid immobilization by soil carbonates. Sustainable cotton production in Central Asia is constrained by phosphorus. This study evaluates the agrobiological and agroecological performance of biomodified MAP (BM-MAP) enriched with *Bacillus subtilis* BS-26, a phosphate-mobilizing bacterium, under field and laboratory conditions in sierozem soils of the Tashkent region. Structural and elemental characterization was performed using X-ray diffraction (XRD) and energy-dispersive spectroscopy (EDS). Soil incubation experiments were conducted to assess the dynamics of available phosphorus (P_2O_5) and alkaline phosphatase activity over a 30-day period. A randomized complete block design (RCBD) field experiment ($n = 4$, plot size 100 m²) was used to evaluate plant growth and yield. Biomodification reduced fertilizer crystallinity (from 85–90% to 50–60%) and increased the amorphous fraction (up to 25%), facilitating controlled phosphorus release. BM-MAP treatments significantly enhanced soil biological activity, increasing alkaline phosphatase activity by 56–62%, and maintained higher levels of available phosphorus (90–98 mg·kg⁻¹) compared to conventional MAP (34–36 mg·kg⁻¹) after 30 days. Field results demonstrated improved cotton performance, with yield reaching 4.15 t·ha⁻¹ (40.2% higher than MAP), along with increased plant height and reproductive development. The findings indicate that BM-MAP is an effective bio-mineral fertilizer that integrates structural modification and microbial functionality to enhance phosphorus use efficiency and crop productivity in calcareous soils.

Keywords: Biomodified fertilizers; *Bacillus subtilis*; Phosphatase activity; Available phosphorus; yield; Sierozem soils; Central Asia.

1. Introduction

Phosphorus (P) is an essential macronutrient required for plant growth, playing a central role in energy transfer, nucleic acid synthesis, and cellular signaling. Despite its importance, phosphorus availability in agricultural soils is often severely limited due to rapid fixation processes that convert soluble phosphate into insoluble forms shortly after application. Globally, only 8–25% of applied phosphorus fertilizers is utilized by crops, while the remainder is immobilized through precipitation with calcium, iron, and aluminum or adsorption onto mineral

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surfaces (García-Berumen *et al.*, 2024; Wang *et al.*, 2021; Zhang *et al.*, 2020). This inefficiency not only constrains crop productivity but also contributes to long-term soil nutrient imbalance and environmental risks, including eutrophication (Wu *et al.*, 2024; Withers *et al.*, 2018). These challenges are particularly pronounced in arid and semi-arid regions such as Central Asia, where agricultural systems are dominated by calcareous soils with high carbonate content and alkaline pH. In Uzbekistan, irrigated sierozem soils are characterized by low phosphorus bioavailability due to strong fixation by CaCO_3 , which limits the agronomic efficiency of conventional fertilizers such as monoammonium phosphate (MAP) (Karimov *et al.*, 2021; Tuntrachanida *et al.*, 2022; Adnan *et al.*, 2025; Jahan *et al.*, 2025). Cotton (*Gossypium hirsutum* L.), a major strategic crop in the region, has a high demand for phosphorus during critical growth stages, including flowering and boll formation (Rashidov, 2018; Peng *et al.*, 2024). However, under these soil conditions, the agronomic efficiency of conventional phosphorus fertilizers, such as monoammonium phosphate (MAP) and PS-Agro, is severely limited due to the rapid chemical fixation of phosphorus into poorly soluble calcium phosphates, which frequently necessitates repeated applications, raising production costs and ecological risks. In recent years, increasing attention has been directed toward biomodified phosphorus fertilizers that integrate mineral nutrient carriers with phosphate-solubilizing microorganisms (PSMs). These microorganisms, including species of *Bacillus*, *Pseudomonas*, and *Azotobacter*, enhance phosphorus availability through the secretion of organic acids and phosphatases, thereby mobilizing sparingly soluble phosphate compounds in the rhizosphere (Djumaniyazova *et al.*, 2018; Zhao *et al.*, 2022). By generating organic acids and phosphatases, these microorganisms increase the bioavailability of phosphorus by releasing insoluble phosphate compounds in the rhizosphere. These microbiological inoculants can form bioactive coatings that control phosphorus release, increase soil enzymatic activity, and encourage plant growth when added to fertilizer granules (Pang *et al.*, 2024; Jindo *et al.*, 2023). However, despite promising results, the combined physicochemical and biological mechanisms governing such systems remain insufficiently understood, particularly under alkaline conditions typical of irrigated soils in Central Asia.

To enhance phosphorus efficiency in calcareous soils, we present an integrated agrobiological and agroecological evaluation of a biomodified monoammonium phosphate (BM-MAP) fertilizer enriched with the phosphate-mobilizing bacterium *Bacillus subtilis* BS-26. Because carbonate-rich, alkaline sierozem soils quickly immobilize soluble phosphate into sparingly available calcium phosphates, this method is especially crucial for irrigated agriculture in Central Asia. Excessive phosphorus application can have negative environmental effects, raise production costs, and reduce fertilizer use efficiency. Therefore, the objective of this study was to evaluate the effectiveness of biomodified monoammonium phosphate (BM-MAP) enriched with *B. subtilis* BS-26 in improving phosphorus availability, soil biological activity, and cotton productivity in calcareous sierozem soils. Specifically, the study aimed to: (i) assess structural and elemental changes in the fertilizer following biomodification; (ii) investigate the dynamics of available phosphorus in soil over time; (iii) evaluate changes in soil enzymatic activity as an indicator of biological functioning; and (iv) determine the effects of BM-MAP on cotton growth and yield under field conditions. It is hypothesized that biomodification alters both the structural properties of MAP and its interaction with soil–microbial systems, resulting in improved phosphorus use efficiency and enhanced crop performance. By integrating material characterization, soil biochemical analysis, and field-scale evaluation, this study provides a mechanistic framework for the development of next-generation bio-mineral fertilizers suitable for calcareous agroecosystems.

2. Materials and Methods

The study was conducted within a sequential, multi-stage experimental framework including fertilizer preparation, physicochemical characterization, laboratory soil incubation, field evaluation, and statistical integration. A schematic overview of the experimental workflow used in this study is presented in Figure 1. The experimental workflow comprised six sequential stages (Fig. 1). In Stage I, three phosphorus fertilizers were prepared, including the biomodified formulation (BM-MAP) obtained by inoculating conventional MAP granules with *B. subtilis* BS-26. In Stage II, the structural and elemental properties of MAP and BM-MAP were compared using X-ray diffraction (XRD) and energy-dispersive spectroscopy (EDS). Stage III involved laboratory soil incubation experiments to monitor the dynamics of available phosphorus (P_2O_5) and alkaline phosphatase activity over a 30-day period. In Stage IV, a field experiment was established under a randomized complete block design (RCBD) with four fertilizer treatments and four replications on irrigated calcareous sierozem soil in the Tashkent region. Stage V encompassed data collection, including biometric observations at key phenological stages and yield assessment through three consecutive pickings. Finally, in Stage VI, all data were subjected to statistical analysis using ANOVA and Duncan's multiple range test ($p < 0.05$) in R (v4.2.1).

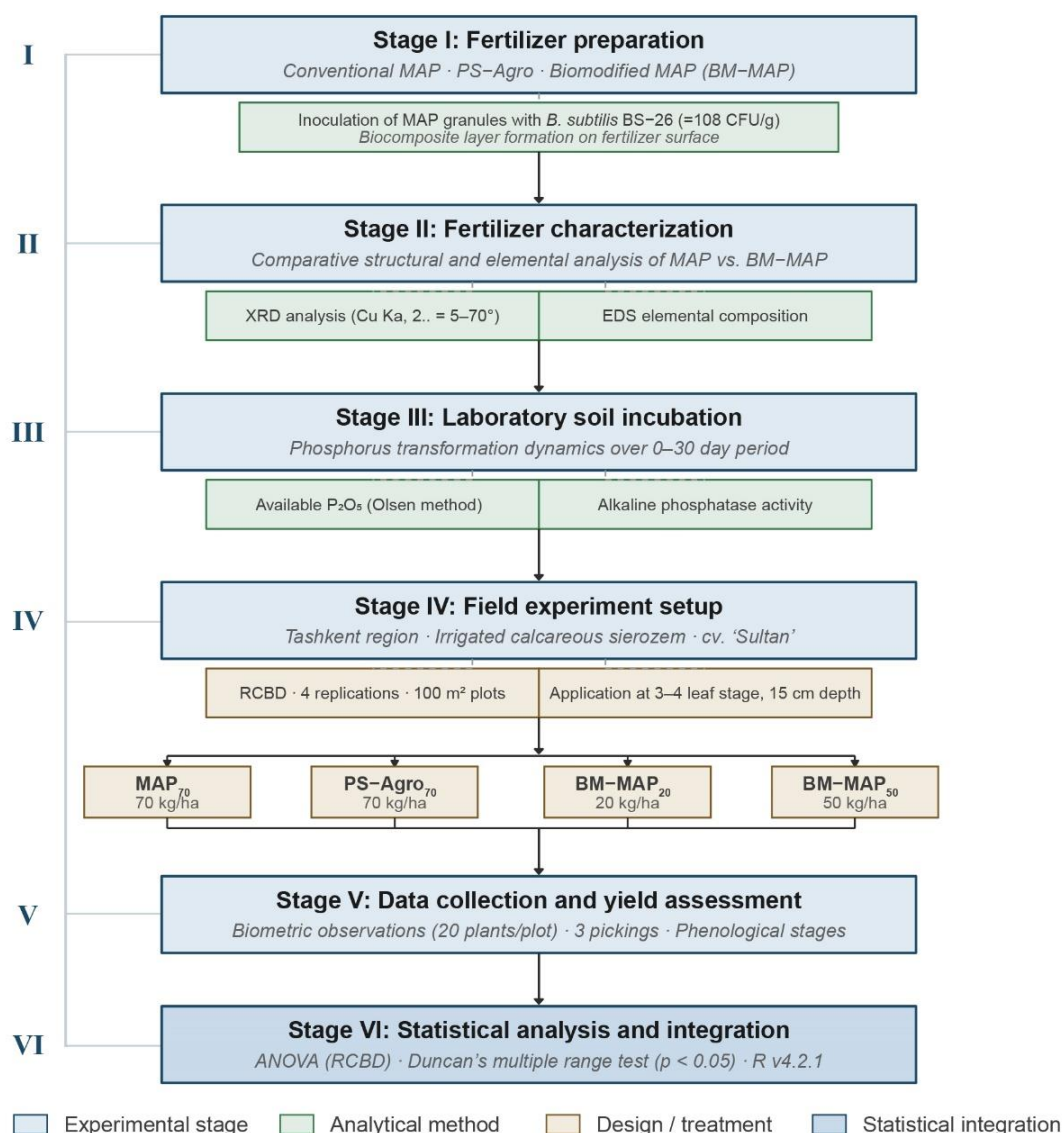


Fig. 1. Schematic representation of the experimental workflow used to evaluate biomodified monoammonium phosphate (BM-MAP) in cotton cultivation. The study comprised six sequential stages: (I) fertilizer preparation, including biomodification of conventional MAP with *B. subtilis* BS-26; (II) fertilizer characterization by XRD and EDS analyses; (III) laboratory soil incubation to assess phosphorus transformation dynamics, available P₂O₅, and alkaline phosphatase activity; (IV) field experiment setup under randomized complete block design (RCBD) with four fertilizer treatments; (V) data collection and yield assessment based on biometric, phenological, and productivity-related parameters; and (VI) statistical analysis and data integration using ANOVA and Duncan’s multiple range test.

2.1. Experimental objects and fertilizer types

The experimental object of the study was field-grown cotton (*G. hirsutum* L., cv. “Sultan”), cultivated under irrigated conditions in the Tashkent region. Three phosphorus fertilizers were evaluated (Table 1): (i) conventional monoammonium phosphate (MAP; NH₄H₂PO₄, 46% P₂O₅ and 11% N); (ii) industrial PS-Agro fertilizer, a mixture of NH₄H₂PO₄ and (NH₄)₂HPO₄ produced by JSC “Ammofos-Maxam” from Kyzylkum phosphorites; and (iii) biomodified monoammonium phosphate (BM-MAP), obtained by inoculating MAP granules with the phosphate-solubilizing bacterial strain *B. subtilis* BS-26.

Table 1. Phosphorus fertilizers used in the study, including their chemical composition and P₂O₅ content. BM-MAP refers to monoammonium phosphate (MAP) biomodified with *B. subtilis* BS-26.

Fertilizer	Chemical formula / composition	Mass fraction of P ₂ O ₅ , %
Ammonium phosphate (MAP)	NH ₄ H ₂ PO ₄	46 ± 1.0
PS-Agro	NH ₄ H ₂ PO ₄ + (NH ₄) ₂ HPO ₄	41 ± 1.0
BM-MAP	MAP (46:11)	46 ± 1.0

The biomodified formulation was designed to enhance phosphorus availability through microbial activity. The concentration of viable *B. subtilis* BS-26 cells in BM-MAP granules was maintained at $\geq 1 \times 10^8$ CFU·g⁻¹ and verified prior to application using standard plate count methods. This bacterial strain is known to produce organic acids and phosphatases, which facilitate the conversion of sparingly soluble phosphorus compounds into plant-available forms in the rhizosphere.

2.2. Characteristics of the fertilizers

BM-MAP fertilizer was prepared by coating MAP granules with a biocomposite layer containing a suspension of *B. subtilis* BS-26 ($\geq 1 \times 10^8$ CFU·g⁻¹). The coating process involved uniform spraying of the bacterial suspension onto the surface of fertilizer granules, followed by air-drying at room temperature (25 ± 2 °C) until a constant mass was achieved. The applied bacterial strain belongs to a functional group of phosphate-mobilizing microorganisms capable of transforming insoluble phosphate compounds into plant-available forms through enzymatic activity and the production of organic acids.

To ensure reproducibility, all fertilizer samples were stored under sterile and dry conditions prior to analysis. Structural characterization of the fertilizers was performed using X-ray diffraction (XRD) on an XRD-6100 diffractometer (Shimadzu, Japan) with Cu K α radiation ($\lambda = 1.5406$ Å). Diffraction data were collected over a 2 θ range of 5–70° with a step size of 0.020°. All diffraction patterns were corrected for background and peak displacement prior to analysis. Phase identification and semi-quantitative analysis were performed using MATCH! software. Quantitative phase analysis was carried out using the Rietveld refinement method implemented in BGMN/Profex software, including refinement of instrumental parameters and evaluation of R-factors to ensure model reliability. To minimize preferred orientation effects and ensure analytical accuracy, all samples were finely ground and homogenized in an agate mortar to obtain a uniform particle size of 1–5 µm. All analyses were performed in triplicate to ensure reproducibility of the results.

2.3. Soil and climatic conditions of the experimental site

Field experiments were conducted in the Tashkent region at an experimental research site under irrigated conditions. The soils of the experimental plots were classified as typical irrigated sierozems (WRB: Calcisols), characterized by a loamy texture, high carbonate content (10–12%), and slightly alkaline pH (7.8–8.3). The humus horizon thickness ranged from 50 to 80 cm, with humus content of 1.2–3.0% and cation exchange capacity of 9–12 cmol(+)/kg (FAO, 2021).

Table 2. Agrochemical and physiochemical properties of the experimental soil.

Parameter	Unit	Value
Soil type	—	Typical irrigated sierozem, loam
pH (H ₂ O, 1:2.5)	—	7.8–8.3
EC (1:5 water extract)	dS/m	0.8–1.5
CaCO ₃ content (mass fraction)	% of dry soil	10–12
Organic carbon (SOC)	%	0.7–1.8
Humus (calculated)	%	1.2–3.0
Cation exchange capacity (CEC)	meq/100 g soil	90–120
Available phosphorus (Olsen P)	mg/kg P	8–18
Exchangeable potassium	mg/kg K	150–220
Mineral nitrogen (NO ₃ ⁻ + NH ₄ ⁺)	mg/kg N	30–60
Soil texture	—	Loam

Baseline soil samples were collected prior to fertilizer application from the 0–20 cm layer using a composite sampling approach (five subsamples per plot). The climate of the region is arid and strongly continental, with a mean annual temperature of 14.5 °C. During the growing season (April–September), average temperatures range from 22 to 28 °C. Annual precipitation is 250–300 mm, with less than 20% occurring during the growing season. Irrigation was applied uniformly across all treatments according to regional agronomic practices to ensure comparable conditions.

2.4. Experimental design

The field experiment included four fertilizer treatments: MAP₇₀, PS-Agro₇₀, BM-MAP₂₀, and BM-MAP₅₀, representing different fertilizer types and phosphorus application rates (kg·ha⁻¹ of active ingredient) (Table 3). A non-fertilized control treatment was not included; therefore, conventional MAP (MAP₇₀) was used as the reference treatment for comparison (Table 3).

Table 3. Description of fertilizer treatments applied in the field experiment, including fertilizer type and application rate based on active phosphorus content.

Treatment	Fertilizer type	Application rate (active ingredient)
MAP ₇₀	Monoammonium phosphate (MAP 46:11)	70 kg/ha
PS-Agro ₇₀	PS-Agro	70 kg/ha
BM-MAP ₂₀	Bio-modified MAP (<i>Bacillus subtilis</i> BS-26)	20 kg/ha
BM-MAP ₅₀	Bio-modified MAP (<i>Bacillus subtilis</i> BS-26)	50 kg/ha

All treatments were applied mechanically during the 3–4 leaf stage, inter-row, at a depth of 15 cm using a KHM-4 cultivator. Each experimental plot measured 100 m². The trial followed a randomized complete block design (RCBD) with four replicates (Table 4).

Table 4. Experimental layout of the field trial arranged in a randomized block design with four replications, showing the distribution of fertilizer treatments across plots.

Replication I	Replication II	Replication III	Replication IV
MAP ₇₀	BM-MAP ₅₀	PS-Agro ₇₀	BM-MAP ₂₀
PS-Agro ₇₀	MAP ₇₀	BM-MAP ₂₀	BM-MAP ₅₀
BM-MAP ₅₀	BM-MAP ₂₀	MAP ₇₀	PS-Agro ₇₀
BM-MAP ₂₀	PS-Agro ₇₀	BM-MAP ₅₀	MAP ₇₀

Plots were separated by 1.5 m buffer zones to minimize cross-contamination between treatments. All agronomic practices (irrigation, weed control, pest management) were applied uniformly across treatments to isolate the effect of fertilizers. The experimental design followed established field experimentation protocols (Dospekhov, 1985).

2.5. Determination of available phosphorus

Available phosphorus (P₂O₅) in soil was determined using the Olsen method (ISO 11263:2021), adapted for calcareous soils. Soil samples were collected from the 0–20 cm layer at the fertilizer placement zone. At each sampling time, composite samples (n = 4 per treatment) were collected, air-dried, and sieved (<2 mm) prior to analysis. The extraction procedure involved 5 g of soil and 100 mL of 0.5 M NaHCO₃ solution (pH 8.5), with the addition of activated carbon. Samples were shaken for 30 min at 25 ± 1 °C and subsequently filtered. An aliquot of the extract was neutralized with 10% HCl using p-nitrophenol as an indicator, followed by color development using molybdate–vanadate reagents. Absorbance was measured at 430 nm using a spectrophotometer (Murphy and Riley, 1962). Sampling was conducted before fertilizer application and at 15 min, 1 h, 3, 10, and 30 days after application. All measurements were performed in triplicate, and analytical precision was verified using standard reference solutions.

2.6. Determination of soil enzymatic activity (Phosphatase activity)

Alkaline phosphatase activity was measured to evaluate the biological activity of the soil. This enzyme is frequently used as a bioindicator of plant phosphorus availability (Khaziev, 2005). Samples of soil were taken from the same depth as the P₂O₅ analysis. After putting one gram of air-dried soil into a 50 mL Erlenmeyer flask, four milliliters of universal buffer (pH 6.5), 0.25 milliliters of toluene, and one milliliter of p-nitrophenyl phosphate sodium solution (0.115 M) were added. After shaking the mixture and incubating it for an hour at 37 °C, 1 mL of 0.5 M NaOH was added, and the mixture was filtered through ashless paper. The release of p-nitrophenol caused the filtrate to turn yellow, as determined by a photocolormeter set to 400–420 nm. According to Khaziev (2005), phosphatase activity was measured in mg of p-nitrophenol per kilogram of soil per hour. In order to compare the chemical and biological transformation of phosphorus, the analyses were conducted at the same intervals as phosphorus extraction (0; 15 min; 1 h; 3; 10; 30 days).

2.7. Assessment of biometric parameters and yield structure

In accordance with the guidelines and methodological recommendations of UzCRI, biometric observations of cotton plants (*G. hirsutum* L., cv. “Sultan”) were performed on 20 randomly chosen plants from each plot at significant phenological stages (Uzbekistan Cotton Research Institute, 2007). To describe growth dynamics and

productivity formation, measurements were taken of the height of the plants, the number of fruiting branches, buds, flowers, and open bolls. Three consecutive pickings were used to manually harvest the seed cotton. The yield ($\text{t}\cdot\text{ha}^{-1}$) was calculated using Dospekhov's procedure (Dospekhov, 1985) and the full collection method from accounting plots.

2.8. Statistical data processing

Analysis of variance (ANOVA) was used to examine the effects of fertilizer treatment on experimental data. At each measurement time, a one-way ANOVA was conducted independently for the time-course variables (available P_2O_5 and alkaline phosphatase activity), and Duncan's multiple range test was used to separate treatment means at $p < 0.05$ ($n = 4$). A randomized complete block design (RCBD) ANOVA was used to assess treatment effects at $p < 0.05$ for agronomic traits and yield. The model $\text{Value} \sim \text{Fertilizer} + \text{Block}$ (four replicates) was used for each trait and sampling date. The results are displayed as mean $\pm$ standard error (SE). An initial agro-economic evaluation was conducted utilizing yield data from the field trial. Supplementary output was assessed in relation to the MAP_{70} treatment, and projected additional gross income was determined using a nominal seed-cotton price of 50–60 USD per centner. Costs associated with fertilizer production, biomodification, and field application were not included. All statistical analyses and graphics were performed in R (v4.2.1).

3. Results

3.1. Structural and Elemental Characterization of Biomodified MAP Fertilizer

X-ray diffraction analysis revealed that biomodification significantly altered the structural properties of monoammonium phosphate (MAP) (Figure 2).

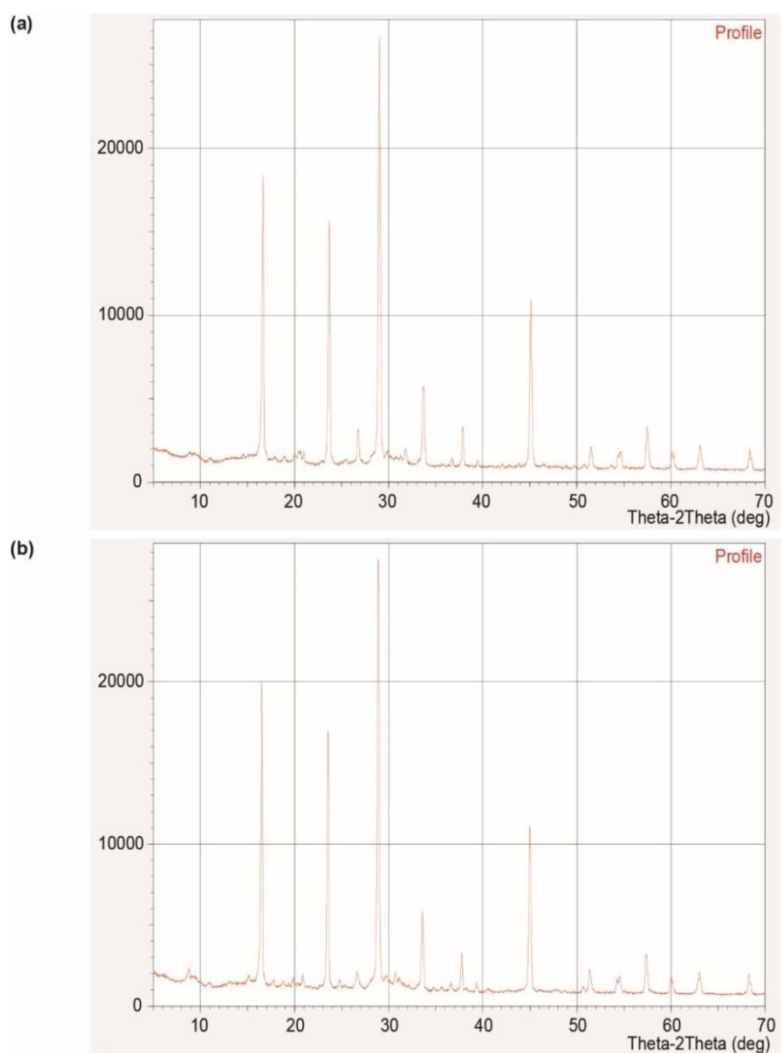


Fig. 2. X-ray diffraction (XRD) peak intensity profiles of conventional monoammonium phosphate (MAP) and biomodified MAP (BM-MAP) samples, illustrating changes in crystallinity and amorphous phase content following biomodification with *B. subtilis* BS-26.

phosphorus availability. Energy-dispersive X-ray spectroscopy (EDS) analysis indicated that both fertilizers were primarily composed of oxygen, phosphorus, and nitrogen (Figure 3). The mass percentages of O, P, and N were 55.1–55.3 wt%, 23.5–24.4 wt%, and 10.6–11.0 wt%, respectively. BM-MAP had a small increase in these important elements and a small decrease in these less important elements (S, Al, Fe, F, Mg, Ca) compared to regular MAP. This shows that the biomodified fertilizer had better chemical homogeneity.

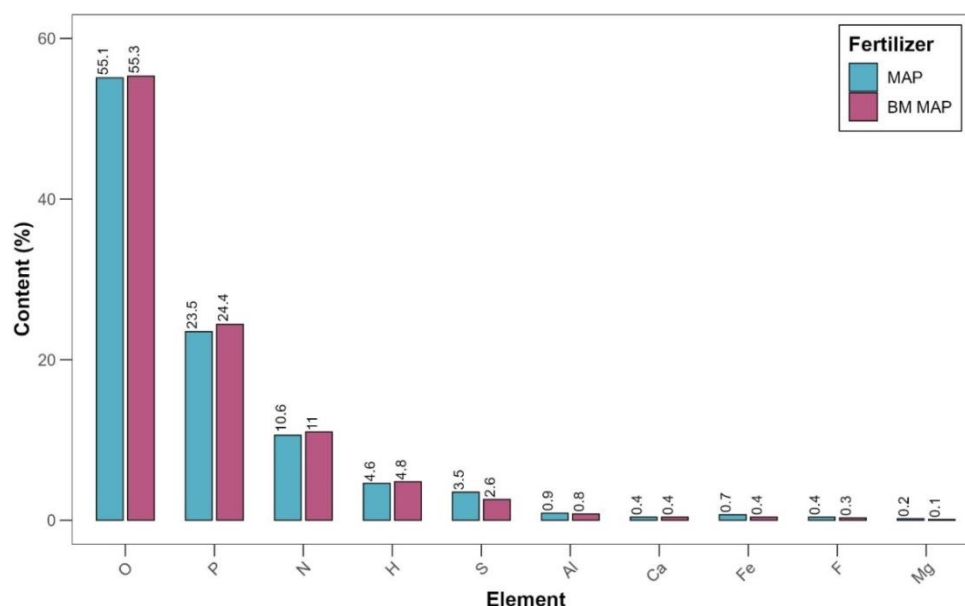


Fig. 3. Elemental composition (wt%) of conventional MAP and biomodified MAP (BM-MAP) as determined by energy-dispersive X-ray spectroscopy (EDS), highlighting the reduction of impurity elements and enrichment with functional organic groups after microbial treatment.

3.2. Dynamics of Available Phosphorus (P_2O_5) in Soil

The application of all phosphorus fertilizers caused the available P_2O_5 to rise quickly, going from 25–25.2 $mg \cdot kg^{-1}$ to 151–153 $mg \cdot kg^{-1}$ in the first 15 minutes (Figure 4). This shows that the water-soluble phosphate in the fertilizers dissolved right away.

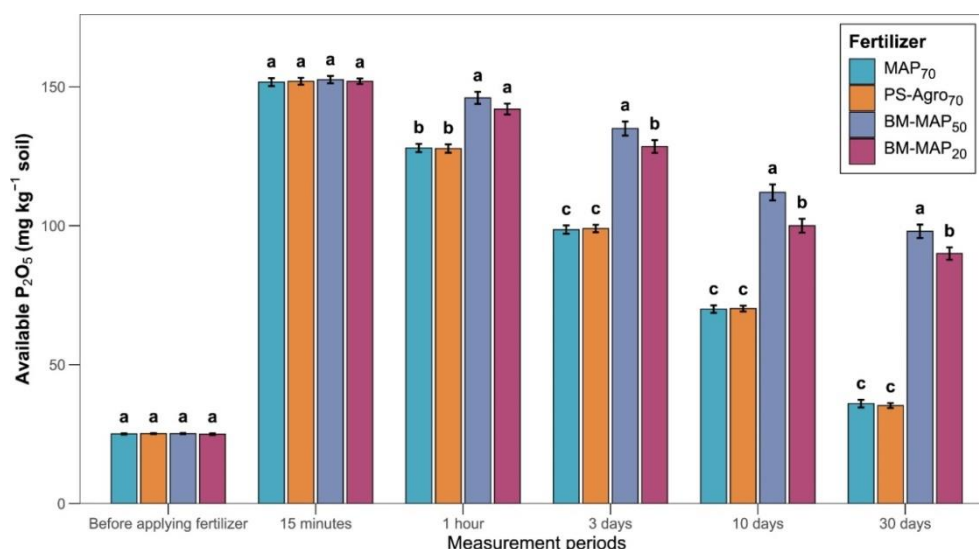


Fig. 4. Changes in the amount of phosphorus (P_2O_5) in the soil over a 30-day incubation period after using regular fertilizers (MAP₇₀, PS-Agro₇₀) and biomodified fertilizers (BM-MAP₅₀, BM-MAP₂₀). The values are the mean $\pm$ SE ($n = 4$). A one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$) shows that the different letters above the bars show that there are significant differences between fertilizer treatments at each sampling time. At later stages of incubation, BM-MAP treatments showed much higher phosphorus retention and sustained release than regular fertilizers.

The phosphorus dynamics that followed varied significantly between treatments. The first three to ten days saw a sharp decline in the amount of P_2O_5 that was available in soils treated with conventional fertilizers (MAP₇₀ and PS-Agro₇₀). P_2O_5 concentrations dropped to 98.6–99 mg·kg⁻¹ after three days, and then to 35–36 mg·kg⁻¹ by day thirty. Conversely, over the course of the 30-day period, the levels of available phosphorus in soils treated with biomodified fertilizers remained noticeably higher. P_2O_5 concentrations in the BM-MAP₅₀ treatment and the BM-MAP₂₀ treatment reached 135 mg·kg⁻¹ and 128.5 mg·kg⁻¹, respectively, after three days. The biomodified treatments continued to have 30–50% more available phosphorus than the conventional treatments after ten days. By day 30, 98 and 90 mg·kg⁻¹ of available P_2O_5 were retained by BM-MAP₅₀ and BM-MAP₂₀, respectively.

3.3. Soil Enzymatic Activity (Alkaline Phosphatase)

Soil biological activity, as measured by alkaline phosphatase activity, was significantly influenced by fertilizer type (Figure 5). Prior to fertilizer application, initial alkaline phosphatase activity was comparable for all treatments and averaged about 243 mg p-nitrophenol·kg⁻¹·h⁻¹ (Figure 5).

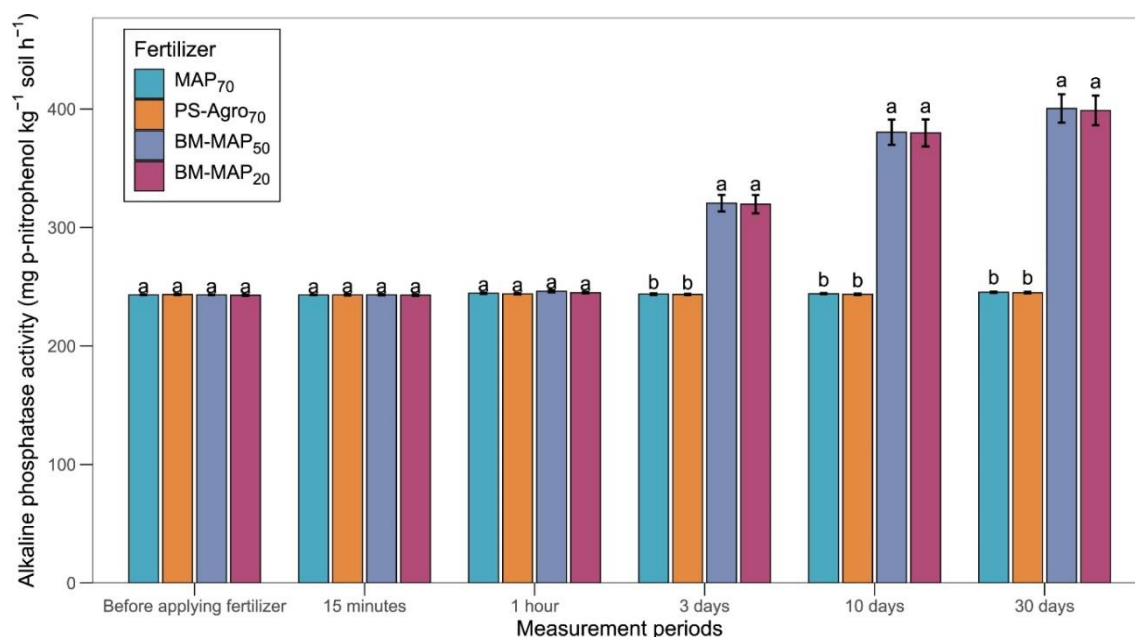


Fig. 5. Changes over time in the activity of alkaline phosphatase in soil (mg p-nitrophenol·kg⁻¹·h⁻¹) after using regular (MAP₇₀, PS-Agro₇₀) and biomodified (BM-MAP₅₀, BM-MAP₂₀) fertilizers. The values show the mean ± SE (n = 4). Different letters show that there are big differences between fertilizer treatments at each sampling time, according to a one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). Biomodified fertilizers significantly increased phosphatase activity starting on day 3.

In all variants, phosphatase activity stayed near baseline values for the first hour following application (15 minutes to 1 hour). Phosphatase activity in soils treated with biomodified fertilizers increased significantly after three days, reaching 320–321 mg p-nitrophenol·kg⁻¹·h⁻¹. Conversely, soils treated with PS-Agro₇₀ and MAP₇₀ only slightly increased, reaching about 244 mg p-nitrophenol·kg⁻¹·h⁻¹. While conventional treatments stayed close to baseline levels, phosphatase activity in BM-MAP treatments increased further by day 10 to about 380 mg p-nitrophenol·kg⁻¹·h⁻¹. While MAP₇₀ and PS-Agro₇₀ stayed at roughly 245 mg p-nitrophenol·kg⁻¹·h⁻¹ on day 30, BM-MAP treatments reached maximum activities of 399–401 mg p-nitrophenol·kg⁻¹·h⁻¹. BM-MAP treatments resulted in a 56–62% increase in alkaline phosphatase activity compared to the MAP treatment. This indicates enhanced microbial activity and suggests improved biological mobilization of phosphorus in the soil system.

3.4. Effects on Cotton Growth and Yield

The application of biomodified fertilizers had a pronounced effect on cotton growth and productivity (Table 5). Plants treated with BM-MAP exhibited increased plant height, improved vegetative growth, and enhanced reproductive development compared to conventional MAP. Plants in the BM-MAP₅₀ treatment grew to a height of 78–79 cm by August 1st, while those in the MAP₇₀ and PS-Agro₇₀ treatments grew to 60–61 cm. Under BM-MAP₅₀, each plant had 12–13 fruiting branches, as opposed to 8–9 under conventional treatments. Additionally, more bolls were produced when biomodified fertilizer was applied. During the final evaluation on September 1,

the BM-MAP₅₀ treatment had the highest plant height (115.8 cm), the most fruiting branches (16.3), and the most open bolls (10.1). The highest yield was recorded in the BM-MAP treatment, reaching 4.15 t·ha⁻¹, which is 40.2% higher than the MAP treatment. This yield improvement is consistent with enhanced phosphorus availability and improved synchronization between nutrient release and plant demand.

Table 5. Effects of conventional and biomodified MAP (BM-MAP) fertilizers on cotton (*G. hirsutum* L., cv. ‘Sultan’) growth, development, and yield across three sampling dates. Values are mean ± standard error (SE) ($n = 4$). Within each row, means followed by different letters are significantly different according to Duncan’s multiple range test at $p < 0.05$ after RCBD ANOVA (Value ~ Fertilizer + Block).

Traits	Values of the studied traits in experimental treatments (Mean ± SE / Duncan Letter)				p-value
	MAP ₇₀	PS-Agro ₇₀	BM-MAP ₂₀	BM-MAP ₅₀	
1 st July					
Plant height, cm	37.3 ± 0.6c	38.1 ± 0.5c	43.8 ± 0.6b	53.9 ± 0.9a	<0.001
Number of fruiting branches, pcs	6.2 ± 0.3c	6.3 ± 0.2c	7.3 ± 0.3b	9.6 ± 0.3a	<0.001
Number of buds, pcs	6.0 ± 0.3c	5.9 ± 0.2c	6.6 ± 0.2b	9.8 ± 0.4a	<0.001
Number of flowers, pcs	1.2 ± 0.1c	2.2 ± 0.1b	2.2 ± 0.2b	3.9 ± 0.1a	<0.001
1 st August					
Plant height, cm	60.1 ± 0.8d	61.1 ± 0.6c	67.9 ± 0.7b	78.8 ± 0.8a	<0.001
Number of fruiting branches, pcs	8.4 ± 0.2c	8.1 ± 0.3c	10.3 ± 0.4b	12.9 ± 0.4a	<0.001
Number of buds, pcs	0.6 ± 0.0c	0.6 ± 0.0c	0.9 ± 0.0b	2.2 ± 0.0a	<0.001
Number of flowers, pcs	0.2 ± 0.0b	0.2 ± 0.0b	0.3 ± 0.0b	1.3 ± 0.0 a	<0.001
Number of bolls, pcs	6.6 ± 0.2b	6.4 ± 0.2b	8.1 ± 0.3a	8.8 ± 0.3a	<0.001
1 st September					
Plant height, cm	78.7 ± 0.7c	78.1 ± 0.7c	89.6 ± 1.1b	115.8 ± 1.5a	<0.001
Number of fruiting branches, pcs	11.2 ± 0.1c	11.1 ± 0.0c	13.5 ± 0.0b	16.3 ± 0.0a	<0.001
Number of unopened bolls, pcs	2.1 ± 0.0b	2.1 ± 0.0b	2.1 ± 0.0b	2.5 ± 0.0a	<0.001
Number of open bolls, pcs	6.4 ± 0.0c	6.4 ± 0.0c	7.3 ± 0.0b	10.1 ± 0.0a	<0.001
Yield, t·ha ⁻¹	2.96 ± 0.6c	2.93 ± 0.5c	3.56 ± 0.8b	4.15 ± 0.9a	<0.001

3.5. Preliminary Agro-economic Assessment

Besides its agronomic benefits, BM-MAP has significant practical promise for resource utilization. This study’s field results indicate that the use of biomodified monoammonium phosphate enhanced cotton production compared to traditional MAP treatment, while concurrently decreasing the fertilizer application rate. In comparison to MAP₇₀ (2.96 t·ha⁻¹), BM-MAP₂₀ and BM-MAP₅₀ yielded 3.56 and 4.15 t·ha⁻¹, reflecting yield enhancements of 20.3% and 40.2%, respectively. The fertilizer application rate was concurrently decreased from 70 kg·ha⁻¹ to 20 and 50 kg·ha⁻¹, representing decreases of 71.4% and 28.6%, respectively (Table 6).

Table 6. Preliminary agro-economic assessment of BM-MAP application in cotton cultivation relative to the conventional MAP treatment.

Parameter	MAP ₇₀ (reference)	BM-MAP ₂₀	BM-MAP ₅₀
Yield, t·ha ⁻¹	2.96	3.56	4.15
Yield, c·ha ⁻¹	29.6	35.6	41.5
Yield increase, c·ha ⁻¹	–	6.0	11.9
Yield increase, %	–	20.3	40.2
Fertilizer application rate, kg·ha ⁻¹	70	20	50
Fertilizer dose reduction, %	–	71.4	28.6
Estimated additional gross income, USD·ha ⁻¹ *	–	300–360	595–714

The supplementary yield was $6.0 \text{ c}\cdot\text{ha}^{-1}$ for BM-MAP₂₀ and $11.9 \text{ c}\cdot\text{ha}^{-1}$ for BM-MAP₅₀ compared to MAP₇₀. With a nominal seed-cotton price of 50–60 USD per centner, this results in an expected increased gross income of 300–360 USD·ha⁻¹ and 595–714 USD·ha⁻¹, respectively. The calculations demonstrate that BM-MAP can enhance yield formation and phosphorus input efficiency in irrigated calcareous sierozem settings. Nonetheless, as production expenses related to fertilizer manufacturing, biomodification, and field application were omitted, these estimates should be considered a preliminary agro-economic assessment rather than a comprehensive cost-benefit analysis.

4. Discussion

The results of this study demonstrate that biomodification fundamentally alters both the physicochemical properties and the biological functionality of monoammonium phosphate (MAP) in calcareous soils. The observed reduction in crystallinity (from 85–90% to 50–60%) and increase in the amorphous fraction indicate partial structural disordering of the $\text{NH}_4\text{H}_2\text{PO}_4$ matrix. This structural transformation enhances the reactive surface area and promotes controlled phosphorus release, which is particularly important in calcareous soils where rapid fixation typically limits phosphorus availability (Zhang, 2020; Wang *et al.*, 2021).

In such soils, soluble phosphate is rapidly immobilized through precipitation with Ca^{2+} (Karimov *et al.*, 2021; Withers *et al.*, 2018; Ge *et al.*, 2019; Hilger *et al.*, 2020; Jindo *et al.*, 2023; Yuldoshev *et al.*, 2023a). In calcareous systems, soluble phosphate ions rapidly interact with Ca^{2+} to form sparingly soluble dicalcium and octacalcium phosphates, eventually transforming into hydroxyapatite-like compounds (Carino *et al.*, 2018; Rashidov, 2018; Johan *et al.*, 2021). However, the sustained phosphorus availability observed in BM-MAP treatments suggests that biomodification modifies this pathway through both physicochemical and biological mechanisms (Figure 6). The sustained P_2O_5 availability observed in BM-MAP treatments suggests that biomodification modifies this pathway at multiple levels. From a physicochemical perspective, the formation of a biocomposite coating containing functional groups ($-\text{OH}$, $-\text{COOH}$, $-\text{NH}_2$) likely acts as a diffusion-regulating layer, reducing immediate phosphorus fixation.

Similar mechanisms have been described for bio-coated and polymer-modified fertilizers, where surface functionalization enhances nutrient retention and mitigates fixation reactions (Bargaz *et al.*, 2018; Zhao *et al.*, 2022). From a biological perspective, the role of *Bacillus subtilis* BS-26 is critical. Phosphate-solubilizing bacteria are known to secrete low-molecular-weight organic acids (gluconic, citric, oxalic, lactic acids), which decrease pH locally and chelate Ca^{2+} , Mg^{2+} , Fe^{3+} , and Al^{3+} ions, thereby dissolving mineral phosphates and preventing re-precipitation (Sharma *et al.*, 2013; Lazo *et al.*, 2017; Saeid *et al.*, 2018; Zhao *et al.*, 2022). This strain is capable of producing low-molecular-weight organic acids (e.g., gluconic, citric, and oxalic acids), which locally reduce pH and chelate Ca^{2+} ions, thereby enhancing phosphate solubilization.

Although direct quantification of organic acid production was not performed in this study, the significant increase in alkaline phosphatase activity (56–62%) and sustained phosphorus availability provide indirect but strong evidence of enhanced microbial-mediated phosphorus mobilization. In alkaline soils, even micro-scale acidification in the rhizosphere or fertilizer microsites can substantially shift equilibrium reactions toward soluble phosphate forms. The observed maintenance of 90–98 $\text{mg}\cdot\text{kg}^{-1}$ available P_2O_5 after 30 days, compared with 35–36 $\text{mg}\cdot\text{kg}^{-1}$ in conventional treatments, indicates that BM-MAP alters the kinetics of phosphorus transformation rather than merely increasing initial solubility.

The increase in alkaline phosphatase activity further indicates that BM-MAP stimulates soil biological functioning, particularly the mineralization of organic phosphorus compounds.

Phosphatases catalyze the hydrolysis of organic phosphorus esters, releasing orthophosphate into soil solution (Khaziev, 2005; Gupta *et al.*, 2015). The 56–62% enhancement in enzymatic activity under BM-MAP reflects not only microbial inoculant activity but also likely stimulation of native soil microbiota. Similar synergistic responses have been reported in calcareous and saline soils where inoculation with phosphate-solubilizing bacteria enhanced both enzymatic activity and Olsen-P levels (Bargaz *et al.*, 2018; Egamberdieva *et al.*, 2020; Shukurov *et al.*, 2020; Alimova *et al.*, 2019; Yuldoshev *et al.*, 2023b). Importantly, the persistence of elevated phosphatase activity up to 30 days suggests that BM-MAP functions as a biologically active carrier supporting microbial viability within the soil matrix, consistent with the concept of integrated bio-mineral fertilizers (Backer *et al.*, 2018).

Beyond chemical mobilization, microbial colonization of fertilizer granules may contribute to biofilm formation and micro-aggregation, which can improve nutrient retention and spatial proximity between phosphate release and root uptake. Recent studies indicate that microbial biofilms on fertilizer surfaces enhance nutrient use efficiency by synchronizing release dynamics with plant demand (Bargaz *et al.*, 2018; Wang *et al.*, 2021). This synchronization is particularly critical in cotton cultivation, where phosphorus demand peaks during flowering and boll development.

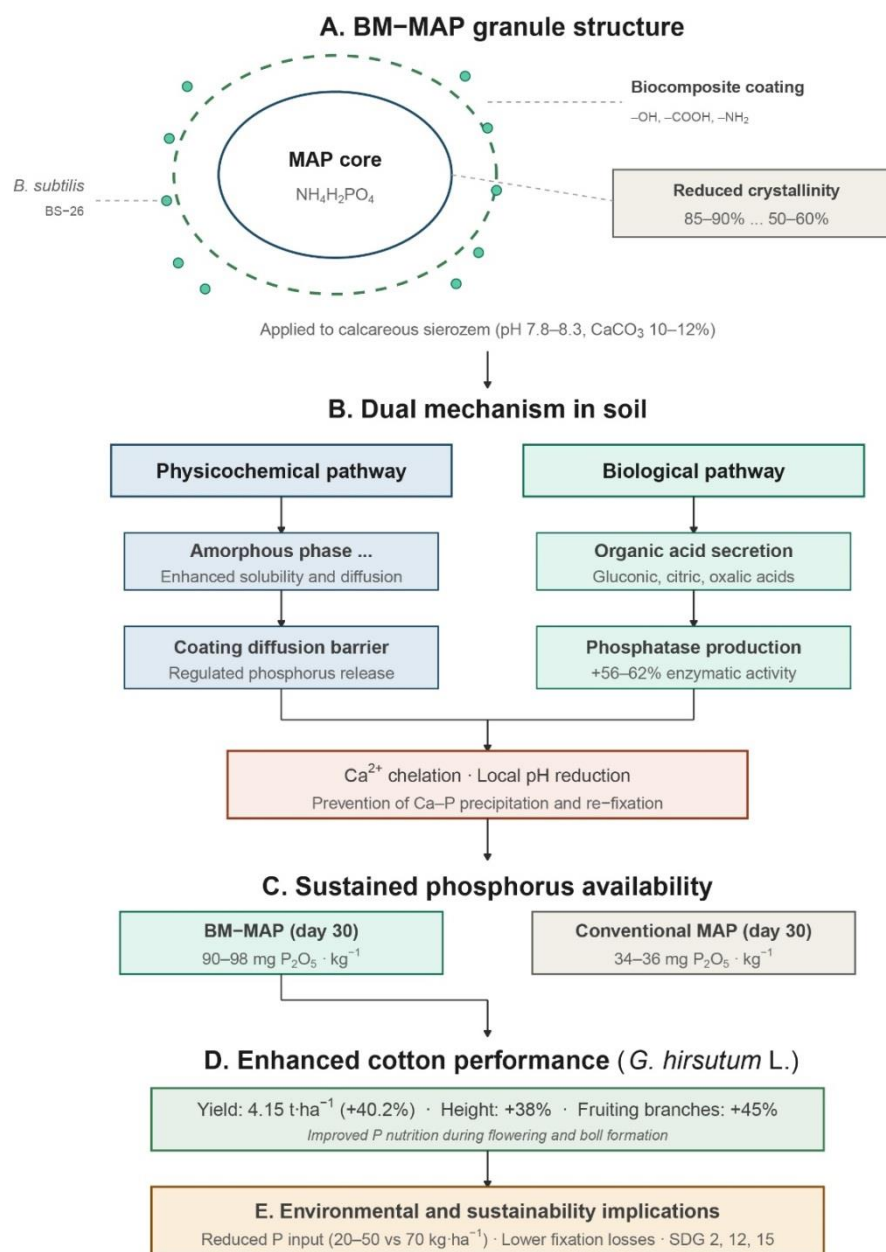


Fig. 6. Proposed mechanism of biomodified monoammonium phosphate (BM-MAP) action in calcareous sierozem soil. (A) Structural modification and microbial colonization of the fertilizer granule following biomodification with *B. subtilis* BS-26, showing reduced crystallinity (85–90% → 50–60%) and formation of a biocomposite coating with functional groups ($-\text{OH}$, $-\text{COOH}$, $-\text{NH}_2$); (B) Dual physicochemical–biological pathways of phosphorus mobilization in soil, including enhanced solubility through amorphous phase increase and regulated release via the diffusion barrier (physicochemical), and organic acid secretion and phosphatase production (+56–62%) by *B. subtilis* BS-26 (biological); (C) Convergence of both pathways through Ca^{2+} chelation and local pH reduction, resulting in sustained available P_2O_5 (90–98 $\text{mg} \cdot \text{kg}^{-1}$ at day 30 vs. 34–36 $\text{mg} \cdot \text{kg}^{-1}$ for conventional MAP); (D) Enhanced cotton (*G. hirsutum* L.) performance, including yield increase of 40.2%, plant height increase of 38%, and fruiting branch increase of 45%; (E) Environmental and sustainability implications, including reduced phosphorus input rates and alignment with SDG 2, 12, and 15.

The agronomic responses observed in this study align with these mechanistic interpretations. Enhanced early vegetative growth, increased number of fruiting branches, and accelerated reproductive differentiation under BM-MAP treatments indicate improved phosphorus nutrition during key ontogenetic stages. Cotton is highly responsive to phosphorus availability during the transition from vegetative to reproductive growth, as

phosphorus directly influences ATP synthesis, nucleic acid formation, and assimilate translocation to developing bolls (Hussain *et al.*, 2019; Karimov *et al.*, 2021). The 40.2% yield increase at 50 kg·ha⁻¹, and comparable productivity at reduced doses, demonstrate substantial improvement in phosphorus use efficiency (PUE).

From an environmental perspective, reducing application rates while maintaining or increasing yield is particularly significant in regions facing both nutrient inefficiency and ecological vulnerability. In calcareous irrigated soils, excessive phosphorus application can lead to nutrient accumulation in surface layers and potential runoff into irrigation networks (Withers *et al.*, 2018; Haque, 2021; Liu *et al.*, 2021; McDowell & Haygarth, 2024). The ability of BM-MAP to sustain plant-available phosphorus with lower input rates suggests reduced risk of secondary environmental impacts and improved long-term nutrient balance.

The findings also contribute to the broader paradigm shift from purely chemical fertilization strategies toward integrated biological–mineral systems. While previous studies have evaluated either controlled-release fertilizers or microbial inoculants separately, this work demonstrates that structural modification of the mineral matrix combined with targeted microbial functionality can produce synergistic effects exceeding those of either approach alone (Bargaz *et al.*, 2018; Zhao *et al.*, 2022).

Compared to other advanced phosphorus fertilizers, such as controlled-release fertilizers and nano-fertilizers, BM-MAP demonstrates a hybrid mode of action, combining structural modification with microbial activation. While controlled-release fertilizers primarily regulate nutrient diffusion and nano-fertilizers enhance surface reactivity, BM-MAP integrates both mechanisms, potentially providing superior performance in calcareous soils. However, direct experimental comparisons with these fertilizer types were not conducted in this study, and further research is required to validate this assumption. The agronomic responses observed, including increased plant growth and yield, are consistent with improved phosphorus nutrition. The yield increase of up to 40.2% suggests improved synchronization between nutrient release and plant demand, particularly during critical growth stages such as flowering and boll formation.

From a sustainability perspective, these findings are aligned with several Sustainable Development Goals (SDGs), including SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land). By enhancing phosphorus use efficiency and reducing potential nutrient losses, BM-MAP represents a more environmentally sustainable alternative to conventional fertilizers.

Despite these promising results, several limitations should be acknowledged. The study did not include a non-fertilized control or treatments with microbial inoculant alone, which limits the ability to fully distinguish between the effects of structural modification and microbial activity. In addition, comparisons with other enhanced-efficiency fertilizers (e.g., nano- or controlled-release formulations) were not experimentally performed. Furthermore, molecular-level characterization of microbial activity (e.g., organic acid profiling or gene expression analysis) was beyond the scope of this study. These limitations should be considered when interpreting the results, and future research should include expanded experimental designs and advanced analytical techniques. Overall, the results support the hypothesis that biomodification transforms MAP into a controlled-release, biologically active nutrient system, improving phosphorus use efficiency and crop productivity under calcareous soil conditions.

The present study evaluated phosphorus dynamics over a 30-day incubation period and one growing season. Longer-term field trials are required to assess cumulative effects on soil phosphorus fractions, microbial community structure, and potential shifts in Ca–P equilibrium forms. Additionally, molecular characterization of microbial survival and colonization dynamics (e.g., qPCR-based quantification of inoculant persistence) would further clarify the longevity and functional stability of the biomodified formulation. Future research should also investigate interactions with nitrogen and potassium regimes, as well as performance under varying irrigation and salinity gradients typical of Central Asian agroecosystems.

Overall, the results support the hypothesis that biomodification transforms MAP into a controlled-release, biologically active nutrient system (Fig. 6), improving phosphorus use efficiency and crop productivity under calcareous soil conditions

5. Conclusions

Integrated laboratory and field experiments have shown that biomodified monoammonium phosphate (BM-MAP), which contains the phosphate-mobilizing strain *B. subtilis* BS-26, works as a good bio-mineral fertilizer that improves the performance of agrochemicals and the ecological sustainability of calcareous sierozem soils. XRD analysis verified that biomodification diminishes the crystallinity of NH₄H₂PO₄ while augmenting its amorphous content, facilitating regulated phosphorus release. The results of EDS showed that a biocomposite coating with functional groups was formed, which got rid of impurities and made nutrients more pure. BM-MAP kept the levels of available P₂O₅ nearly three times higher than those of regular MAP and PS-Agro after 30 days. It also raised alkaline phosphatase activity by 56–62%. Field trials demonstrated significant improvements in

morphogenetic characteristics, such as plant height, fruiting branches, and boll count, resulting in a 40.2% increase in yield at 50 kg·ha⁻¹ and comparable productivity at lower doses. These effects are due to the synergistic interaction of microbial activity, enzymatic phosphorus mobilization, and better nutrient bioavailability. This makes BM-MAP a promising eco-friendly solution for growing cotton sustainably in Central Asia, where phosphorus is limited.

List of abbreviations:

MAP: monoammonium phosphate

BM-MAP: biomodified monoammonium phosphate

XRD: X-ray diffraction

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

Availability of data and material: Not applicable.

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