

Original Research

# Comparative Efficacy of Coated Diammonium Phosphate Formulations for Improving Crop Productivity and Nutrient Uptake in Maize

Sadia Murad<sup>1</sup>, Maqshoof Ahmad<sup>1</sup>, Azhar Hussain<sup>1\*</sup>, Abubakar Dar<sup>1</sup>, Muhammad Latif<sup>2</sup>, Rashid Iqbal<sup>2,3\*\*</sup>, Mohamed Ragab Abdel Gawwad<sup>4</sup>, M. Ajmal Ali<sup>5</sup>

<sup>1</sup>Department of Soil Science, The Islamia University of Bahawalpur, 63100, Bahawalpur, Pakistan

<sup>2</sup>Department of Agronomy, The Islamia University of Bahawalpur 63100, Pakistan

<sup>3</sup>Department of Life Sciences, Western Caspian University, Baku, Azerbaijan

<sup>4</sup>Department of Genetics and Bioengineering, Faculty of Engineering and Natural Sciences, International University of Sarajevo, 71210 Sarajevo, Bosnia and Herzegovina

<sup>5</sup>Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Received: 29 February 2024

Accepted: 26 April 2024

## Abstract

Fertilizer use efficiency is the major problem behind low crop productivity on calcareous sandy loam soils, mostly for exhaustive crops like maize. Improving maize productivity to meet food requirements is among the priority research areas for scientists. The study explores the efficacy of coated diammonium phosphate (DAP) with phosphate solubilizing bacteria (PSB) and their extracted metabolites to improve maize growth and soil health. For this purpose, the efficacy of conventional DAP is compared with that of coated DAP (C-DAP) to improve soil nutrient dynamics, maize seedling growth, the antioxidant system, and nutrient uptake in grains. Results showed that C-DAP significantly improved maize growth as compared to uncoated DAP. Bacterial and metabolites coated DAP formulations exhibit increased soil available phosphorus by 44% and 41% and extractable potassium by 41% and 43%, respectively, at 100% of the required P dose. Coated DAP treatments also enhanced microbial biomass carbon in rhizospheric soil, indicating a positive influence on soil microbial communities. Furthermore, bacterial and metabolites coated DAP formulations enhanced relative water contents (44% and 40%), membrane stability index (45% and 47%), and chlorophyll contents (37% and 39%), respectively. The present findings conclude that the use of microbial and metabolites coated DAP is an effective strategy to improve maize growth, physiology, and nutrient use efficiency in a sustainable manner to address food security.

**Key Words:** Coated Fertilizers, Maize, Growth, Physiology, Soil Health

\* e-mail: azharhaseen@gmail.com

\*\* e-mail: rashid.iqbal@iub.edu.pk

## Introduction

Maize holds a significant role as a crucial food crop and is a vital component in forage and industrial raw materials [1]. It contributes roughly one-third of global grain production [2], Pakistani soils primarily consist of calcareous sandy compositions, characterized by poor quality due to elevated calcium carbonate levels and limited nutrient accessibility owing to high pH and unfavorable soil traits [3]. Additionally, the prevalence of sand particles in these soils results in a low to medium capacity for retaining water in the upper soil layers [4]. To optimize economic returns from the depleted calcareous sandy soils, it's crucial to promptly discover and implement efficient strategies for fertilization management. Phosphorus (P), an indispensable plant nutrient in farming, is a nonrenewable resource that should be introduced into the soil in both inorganic and/or organic forms to support sustainable cropping systems [5, 6]. Adequate phosphorus in the root zone is crucial for the optimal growth and development of maize, serving as an essential nutrient. It's a requisite factor for achieving a high-quality maize yield [7]. It accelerates the maturation of crops and encourages initial growth and root establishment [8]. Nonetheless, within agricultural crop management, it stands as the second most restrictive nutrient, following nitrogen [9], owing to its strong chemical bonding with soil colloidal surfaces [10]. The transformations of phosphorus via mineralization and immobilization processes modify its presence in topsoil, influencing its accessibility to plants. Different crop scientists report that less than 45% of phosphorus from phosphate fertilizer is typically recovered in the initial year of application [11]. A recent finding uncovered a deficiency of phosphorus in 5.7 billion hectares of agricultural soil globally [12]. The availability of phosphorus in calcareous soils remains low because of chemical surface precipitation and adsorption, although distinguishing between these mechanisms poses a challenge. Furthermore, the presence of P in a negatively charged state renders it inaccessible to plants due to its strong complexation with Fe/Al oxides [13]. Moreover, excessive use of chemical fertilizers has adverse effects on soil structure and communities of microbes and may lead to other issues. Consequently, approximately 80–90% of soil P becomes inaccessible, depending on soil composition and pH [14]. Therefore, finding ways to minimize P loss and enhance PUE (Phosphorus Use Efficiency) is crucial for ensuring optimal maize yields.

A controlled-release phosphate fertilizer with a polymer coating steadily delivers nutrients according to crop needs, reducing phosphorus inefficiency due to soil fixation. This method extends the fertilizer's effectiveness by synchronizing nutrient release with demand. Employing this fertilizer in a single application could sufficiently nourish crops throughout their growth cycle, providing a fresh approach to the challenges posed by traditional fertilization methods. Lu et al. [15] demonstrated the creation of coated diammonium phosphate (CDAP) by

modifying the surface with polyolefin wax and coating it with polyurethane derived from castor oil. This innovative approach showcased improved controlled-release capabilities in the wax-modified CDAP, presenting an ideal "S" shaped release profile when compared to conventional diammonium phosphate (DAP). Sarkar et al. [16] described that the controlled-release P fertilizers led to notable enhancements in wheat yield (ranging from 14.3% to 34.6%) and P uptake (rising by 0.7% to 18.7%) compared to commercial DAP. Likewise, Yaseen et al. [17] have shown similar findings through polymer coating on DAP under a maize-wheat cropping system.

Microbes in soil, crucial for agricultural systems, actively maintain soil health and sustainability [18]. The properties of the soil and the amounts of nutrients, particularly phosphorus (P) and nitrogen (N), have an impact on these microscopic organisms [19]. Zhang, [20], found that the impact of fertilization methods on soil microbes outweighs that of crop rotation. Employing phosphate solubilizing bacteria (PSB) presents a promising biological approach to enhance the effectiveness of applied P fertilizer [21]. Most significantly, the use of PSB inoculants accelerates the dissolution of inorganic P through the release of acidic metabolites, reducing P adsorption and precipitation, and encouraging the mineralization of organic P [22]. Fitriatin et al. [23] examined how utilizing biofertilizers containing a mix of phosphate-solubilizing microbes and nitrogen-fixing bacteria enhances the accessibility of nitrogen and phosphorus in the soil. The presence of phosphorus can be augmented by enveloping it with phosphorus-solubilizing bacteria (PSB) and bacteria that promote plant growth (PGPR) [24, 25]. Research has indicated that soil treated with controlled-release fertilizer demonstrates significantly higher nutrient levels than soil treated with traditional fertilizer [26].

Moreover, alterations in soil microbial community traits, including microbial biomass and the variety and prevalence of bacteria/fungi, are influenced by the introduction of controlled-release fertilizer [27]. The application of bacterial culture and metabolites to coat P fertilizer enhances its effectiveness as a slow-release agent in soil [28]. Recent research has centered on controlled-release diammonium phosphate (C-DAP), which effectively manages the release of both N and P [15, 29], resulting in significant enhancement of plant root growth and aboveground biomass [30]. Nevertheless, the specific impact of C-DAP on plant and microorganism development in continuous cropping scenarios remains inadequately explored.

The present study aims to evaluate the efficacy of coated DAP formulations with PSB and metabolites in enhancing maize growth and soil health on calcareous sandy loam soils. The objectives of the study were to compare the effect of conventional DAP with coated DAP on soil nutrient dynamics, maize seedling growth, the antioxidant system, and nutrient uptake in grains of maize for sustainable maize production. It was hypothesized that coated bacterial and metabolites

coated DAP may improve the growth and nutritional status of the maize crop compared to commercially available DAP.

## Experimental Methods

### Physico-Chemical Soil Analysis

Before sowing, the soil's physico-chemical traits for the pot trial were assessed following standard protocols, with the findings shown in Table 1. The pH, the electrical conductivity (EC), and organic matter were determined through standard protocols [31]. The method of Sarfraz et al. [32] used to calculate saturation percentage by oven drying the saturated paste at 105 °C until it reached a constant weight. Watanabe and Olsen's [33] method was used for available P determination. The soil textural class was determined following the protocol given by Gee and Bauder [34]. The Kjeldhal method by Jackson [35] was used to analyze the total nitrogen %.

### Coating of DAP with Bacterial and Metabolites

Diammonium phosphate fertilizer was coated with phosphorus solubilizing bacterial strains (*Bacillus megaterium* ZR19 and ZE32 and *Bacillus subtilis* ZR3 and ZE15) by Iqbal et al. [36] and metabolites extracted from these strains by following the procedure of Politz et al. [37]. Diammonium phosphate fertilizer was provided by the Soil Microbiology and Biotechnology Laboratory of the Soil Science Department. Homogenous granules were selected with a diameter ranging from 2-4mm and coated with bacterial culture and their extracted metabolites by spraying through a spray bottle on the

Table 1. Pre-sowing physico-chemical analysis of soil (means  $\pm$  SD of three replications)

Characteristics	Value
EC <sub>c</sub> (dSm <sup>-1</sup> )	1.4 $\pm$ 0.02
pH	7.8 $\pm$ 0.03
Organic matter (%)	0.36 $\pm$ 0.02
Saturation percentage (%)	31 $\pm$ 0.71
Available P (mg kg <sup>-1</sup> )	3.5 $\pm$ 0.01
Textural class	Sandy loam
Total N (%)	0.026 $\pm$ 0.002

surface of DAP granules. This procedure was repeated five times on coated DAP (the first coating). Subsequently, the finalized coated DAP fertilizer was stored in polythene bags at ambient temperature.

### Growth Conditions and Experimental Design

A pot trial was designed in the wirehouse to check the effect of coated DAP on maize productivity and the physicochemical and biological properties of rhizospheric soil. The experiment was carried out during February-March in a completely randomized design (CRD) under factorial settings. Un-coated and coated DAP (@120 kg P ha<sup>-1</sup>) was applied in various treatments, as described in Table 2. Sandy loam soil (10 kg per pot) with poor water-holding capacity was used to fill the pots. The recommended dose of N (160 kg ha<sup>-1</sup>) was applied in split form. Half of the recommended nitrogen (N) was administered as a basal dose, while the remaining half was distributed in two splits using urea. The fully recommended K fertilizer (60 kg ha<sup>-1</sup>) was applied in the form of potassium sulfate before sowing as a basal dose.

All agricultural practices, from planting to harvesting, were implemented. Parameters for growth and yield were documented during the crop's harvest. To analyze antioxidant enzymes as well as other physiological and ionic parameters, plant samples were collected. Rhizospheric soil samples were also collected from each pot to determine different physico-chemical and biological parameters.

### Antioxidant Enzyme Activity

The fresh leaf sample (0.5g) was homogenized at 4 °C in 8 mL of extracting buffer (50 mM L<sup>-1</sup> phosphate, pH 7.8). The prepared homogenate was centrifuged at 15,000g for 20 minutes, and the resultant supernatant was analyzed for antioxidant activities. Ascorbate peroxidase (APX) was determined using 2.5 mL phosphate buffer solution at 290nm wavelength on a spectrophotometer by following the method of Prochazkova et al. [38]. Catalase (CAT) activity was assayed by following the protocol of Chance and Maehly [39] at 240nm wavelength. The process explained by Giannopolitis and Ries [40] was employed for assessing the superoxide dismutase (SOD) activity, measured specifically at 560 nm. The activity of peroxidase (POD) was determined using Guaiacol + 0.1 mL H<sub>2</sub>O<sub>2</sub> at 470 nm wavelength.

Table 2. Self-explanation of treatments (% of DAP fertilizer applied with actual amount)

Treatments	Factors		
	Uncoated	Bacterial coated	Metabolites coated
Control	No DAP	No DAP	No DAP
25% DAP	0.75 g DAP pot <sup>-1</sup>	0.75 g DAP pot <sup>-1</sup>	0.75 g DAP pot <sup>-1</sup>
50% DAP	1.49 g DAP pot <sup>-1</sup>	1.49 g DAP pot <sup>-1</sup>	1.49 g DAP pot <sup>-1</sup>
75% DAP	2.24 g DAP pot <sup>-1</sup>	2.24 g DAP pot <sup>-1</sup>	2.24 g DAP pot <sup>-1</sup>
100% DAP	2.97 g DAP pot <sup>-1</sup>	2.97 g DAP pot <sup>-1</sup>	2.97 g DAP pot <sup>-1</sup>

## Physiological Parameters

Relative water contents (RWC) were estimated using the protocol described by Lazcano-Ferrat and Lovatt [41]. According to the method, the fresh weight (FW) of the youngest plant leaf was taken by using an electrical weight balance. The leaves were soaked in distilled water overnight at ambient temperature for turgid weight (TW). Subsequently, the leaves were subjected to 65°C in a hot air oven for drying to record dry weight (DW). RWC was calculated using Equation 1:

$$RWC (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad (1)$$

The method described by Sairam and Saxena [42] was used to analyze the membrane stability index (MSI). The crushed 0.1 g leaf disc was taken in distilled water and heated in the water bath for 30 minutes at 40 °C. An EC meter was used to determine electrical conductivity ( $C_1$ ). After this, the sample was heated again in the water bath at 100 °C for 10 min to record electrical conductivity ( $C_2$ ). Equation 2 was used to calculate MSI:

$$MSI = \left[ 1 - \left( \frac{C_1}{C_2} \right) \right] \times 100 \quad (2)$$

The determination of chlorophyll contents was done by following the process of Arnon [43]. In short, fresh leaf samples weighing 0.1 g were sliced and placed in glass test tubes. Then, 10 mL of 80% acetone was added to each tube, and they were kept in a dark location until the leaf samples completely lost color. Following centrifugation, the absorbance of the resulting supernatant was assessed at 645 and 663 nm using a UV-visible spectrophotometer to determine chlorophyll 'a' and 'b'. Total chlorophyll contents were calculated by using Equation 3:

$$Chl (total) = Chl a + Chl b \quad (3)$$

## Nutritional Analysis of Grains

The protocol described by Wolf [44] was used for grain digestion. The standard protocol by Ryan [45] was used for the determination of phosphorus concentration in grains on a UV-visible spectrophotometer (Agilent Carry 60, USA) and potassium concentration on a flame photometer (Model; Model BWB-XP, BWB Technologies, Newbury, UK). For nitrogen determination, the Kjeldhal apparatus (VELP Sci., Italy) was utilized as described by Jackson [35].

## Post-Harvest Soil Sample Analysis

After crop harvesting, soil rhizospheric samples were collected from pots and analyzed for different physico-chemical properties. Combined soil samples were dried in the air and sifted through a 2mm mesh sieve. They were then kept in a refrigerator at 4 °C and analyzed within a week. Soil pH was determined using the Kent Eil 7015 pH meter, while soil organic matter was assessed following the procedure outlined by Nelson and Sommers [31]. The method of Watanabe and Olsen [33] was used to analyze the available phosphorus. A flame photometer (Model; Model BWB-XP, BWB Technologies, Newbury, UK) was utilized to describe extractable potassium. Chloroform fumigation and extraction methods [46, 47] were used to determine microbial biomass carbon.

## Results

## Effect of Coated DAP on Growth Parameters of Maize Crops

Changes in plant growth parameters measured at the time of maize harvesting are presented in Table 3. Shoot fresh biomass of maize plants under uncoated control treatment was the minimum, while the maximum shoot

Table 3. Effect of coated DAP on growth parameters of maize crop in pot trail

Treatments	Shoot fresh weight (g)			Root fresh weight (g)		
	Uncoated	Bacterial coated	Metabolites coated	Uncoated	Bacterial coated	Metabolites coated
Control	283±0.51 <sup>i</sup>	304±0.68 <sup>h</sup>	303±0.68 <sup>h</sup>	42±0.39 <sup>h</sup>	45±0.39 <sup>gh</sup>	43±0.51 <sup>gh</sup>
25%	318±0.93 <sup>g</sup>	360±1.04 <sup>c</sup>	357±1.29 <sup>c</sup>	47±0.29 <sup>fh</sup>	52±0.64 <sup>d-f</sup>	53±0.51 <sup>c-c</sup>
50%	338±0.78 <sup>f</sup>	384±1.04 <sup>d</sup>	398±0.68 <sup>c</sup>	49±0.51 <sup>e-g</sup>	58±0.64 <sup>b-d</sup>	58±0.25 <sup>bc</sup>
75%	355±0.64 <sup>e</sup>	426±0.90 <sup>b</sup>	423±0.53 <sup>b</sup>	54±0.39 <sup>e-c</sup>	62±0.90 <sup>ab</sup>	62±0.39 <sup>ab</sup>
100%	364±0.39 <sup>c</sup>	431±0.51 <sup>ab</sup>	438±.81 <sup>a</sup>	54±0.53 <sup>e-c</sup>	65±0.53 <sup>a</sup>	62±0.68 <sup>ab</sup>
<b>HSD(p≤0.05)</b>	<b>2.680</b>			<b>1.696</b>		
Treatments	Shoot length (cm)			Root length (cm)		
	Uncoated	Bacterial coated	Metabolites coated	Uncoated	Bacterial coated	Metabolites coated
Control	109±0.51 <sup>i</sup>	118±1.27 <sup>hi</sup>	119±0.68 <sup>h</sup>	36±0.64 <sup>f</sup>	38±0.39 <sup>ef</sup>	37±0.93 <sup>ef</sup>
25%	119±0.64 <sup>h</sup>	135±0.90 <sup>fg</sup>	141±0.78 <sup>ef</sup>	41±0.90 <sup>d-f</sup>	43±0.78 <sup>c-f</sup>	44±0.74 <sup>c-c</sup>
50%	127±1.07 <sup>gh</sup>	146±1.18 <sup>de</sup>	151±0.44 <sup>cd</sup>	43±0.39 <sup>e-f</sup>	48±0.68 <sup>a-d</sup>	49±0.25 <sup>a-c</sup>
75%	134±0.90 <sup>fg</sup>	158±0.51 <sup>bc</sup>	158±0.78 <sup>bc</sup>	45±0.39 <sup>b-d</sup>	52±0.78 <sup>ab</sup>	53±0.53 <sup>ab</sup>
100%	139±0.51 <sup>ef</sup>	164±0.53 <sup>ab</sup>	171±0.78 <sup>a</sup>	47±0.51 <sup>a-d</sup>	54±0.53 <sup>a</sup>	53±0.53 <sup>a</sup>
<b>HSD(p≤0.05)</b>	<b>2.546</b>			<b>2.060</b>		



fresh biomass (438 g) was observed from 100% of metabolites coated DAP application from T5 treatment, followed by the same dose of bacterial coated DAP. Application of coated DAP showed better results even at lower applied concentrations, i.e., 50% and 75% of the required DAP dose. Root fresh biomass was improved by 46% as compared to control when 100% of bacterial coated DAP was applied. Fresh biomass, shoot length, and root length were also significantly enhanced by the application of coated DAP. In the case of uncoated DAP application, the maximum improvement in shoot length was 28% which is 12% and 15% lower than the maximum improvement obtained from bacterial and metabolites coated DAP. The root length of the maize crop was also significantly enhanced by the application of coated DAP, with a 44% increase from T5 and T4 treatment of bacterial and metabolites coated DAP application, respectively, as compared to their respective controls.

#### Effect of Coated DAP on the Antioxidant Enzyme Activity of Maize Crops

Results (Fig. 1) showed that when 100% of coated DAP was applied, POD activity was increased by 40% and 41% with bacterial and metabolites coated DAP, respectively, as compared to their respective controls, while with uncoated DAP, only 30% was increased when the fully required dose was applied (Fig. 1A). When 75% of the required dose was applied, the maximum

increase was 39% due to metabolites coated DAP, while with bacterial and uncoated application, a 37% and 23% increase was observed. Similarly, a 43% and 41% increase were observed in APX with 100% bacterial and metabolites coated DAP, respectively, and a 28% increase was observed when uncoated DAP was applied in the same treatment (Fig. 1B). The SOD activity was increased by 40% and 43% with the full dose of both types of coated DAP, respectively, as compared to their respective controls. When DAP was applied at a 75% rate of fertilizer application, 29%, 38%, and 39% increases in SOD activity were observed in uncoated, bacterial coated, and metabolites coated DAP, respectively, (Fig. 1C). In the case of CAT activity, the maximum increase (43% and 41%) was observed in T5 treatment, where 100% of the required DAP was applied as bacterial coated DAP. Results showed that when 75% of DAP was applied, 22%, 37%, and 40% were observed under uncoated, bacterial coated, and metabolite coated DAP formulations, respectively, (Fig. 1D).

#### Effect of Coated DAP on Leaf Water Contents and Membrane Stability Index of Maize Crops

Results represented in Fig. 2 (2A, 2B) showed that coating DAP significantly enhanced plant physiological parameters in comparison with uncoated DAP. The maximum (44%) increase in relative water contents was observed when bacterial coated DAP was applied as a

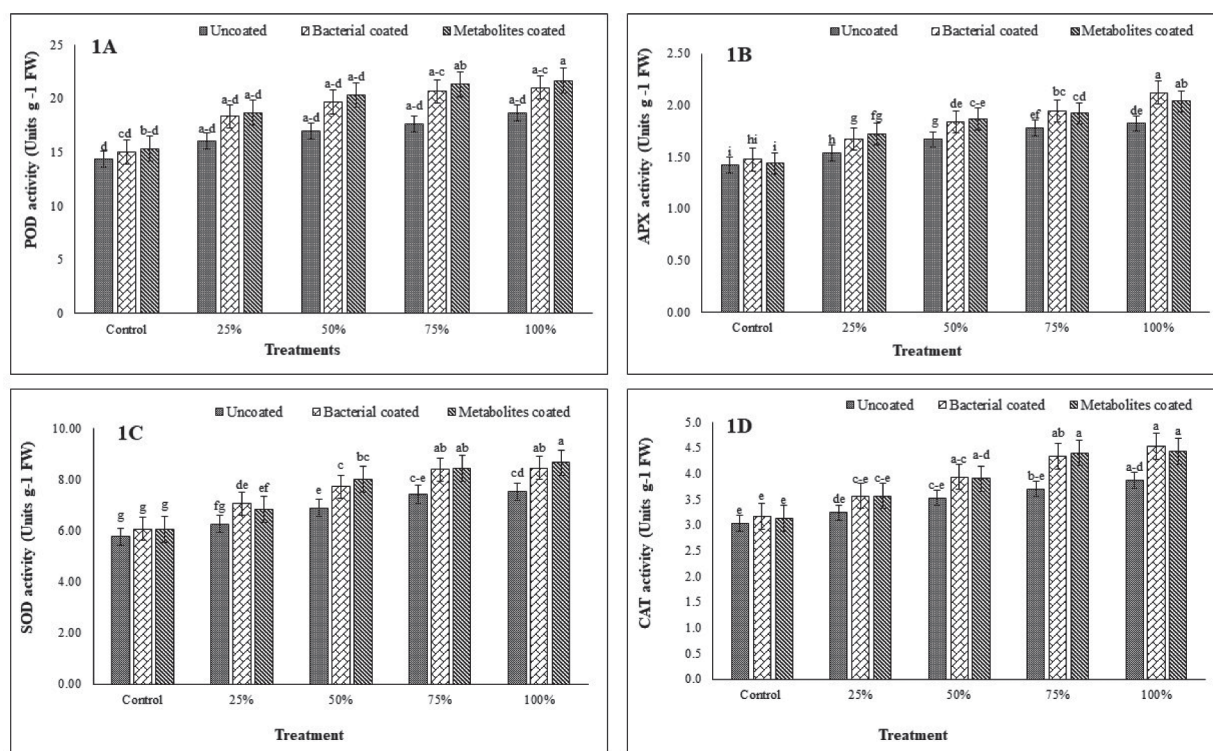


Fig. 1. Effect of Bacterial and metabolites coated DAP on antioxidant enzymes activity of maize crop in pot trail. POD (peroxide dismutase) (1A), APX (ascorbate peroxide) (1B), SOD (superoxide dismutase) (1C), CAT (catalase) (1D). Bars with the same alphabets showed non-significant results with each other at  $p \leq 0.05$

full dose, i.e., followed by a 40% increase from the same treatment of metabolites coated and 75% of the required bacterial coated DAP formulations as compared to their respective controls. Uncoated DAP, when applied as a full dose, increased leaf water contents by 32%, which is equal to the increase obtained from 50% of the required bacterial coated DAP formulation. The coating also showed an expressive effect on membrane stability index (MSI) when 100% of the required DAP was applied as bacterial and metabolites coated DAP. The maximum increase was observed in the case of metabolites coating, i.e., 47% at a 100% DAP application rate. The next highest value in MSI was observed when 75% of the required metabolites coated DAP, with a 41% increase as compared to its control.

### Effect of Coated DAP on the Total Chlorophyll Contents of Maize Crops

Total chlorophyll contents were improved with the application of bacterial and metabolites coated DAP as compared to uncoated DAP (Fig. 2C). Metabolite coated DAP showed better results as compared to bacterial coated DAP and uncoated DAP. The maximum improvement (39%) was observed in T5, i.e., 100% of the required DAP dose as metabolites coated DAP as compared to its control. The same treatment of bacterial coating showed a 37% increase in chlorophyll contents, and uncoated DAP with a full dose showed a 27% improvement. The next best treatment was T4, with 75% of the required DAP application, which resulted in a 30% and 33%

enhancement in chlorophyll contents, which was 3% and 6% more than the increase obtained from the full dose of uncoated DAP. The lowest values were obtained from T1 (25% of the required DAP) treatment from all types of DAP applications, with 9% and 13% increases from uncoated and coated DAP, respectively.

### Effect of Coated DAP on Nutrient Contents in Grains of Maize Crops

The application of coated DAP significantly improves total NPK concentrations in grains of the maize crop, as compared to uncoated DAP as shown in Fig. 3. When 100% of the required DAP was applied as bacterial coated and metabolites coated DAP, the total nitrogen concentration in grains was improved by 40% and 37%, respectively, as compared to their respective controls (Fig. 3A). While uncoated DAP at a similar rate of application showed a 26% increase in grain nitrogen concentration as compared to its control, coated DAP at 75% and 50% of the required DAP dose also showed better results with a 37% and 36% increase from bacterial coated and metabolites coated DAP, respectively. Similarly, phosphorus and potassium concentrations were also significantly improved by the coated DAP application, with a 41% and 40% increase in phosphorus concentrations, as well as a 37% and 36% increase in potassium concentration, respectively, when a full dose of the required DAP was applied in comparison with the control (Fig. 3B, 3C). Conventional DAP showed a 24% and 28% increase in P and K concentrations in grains of maize, respectively.

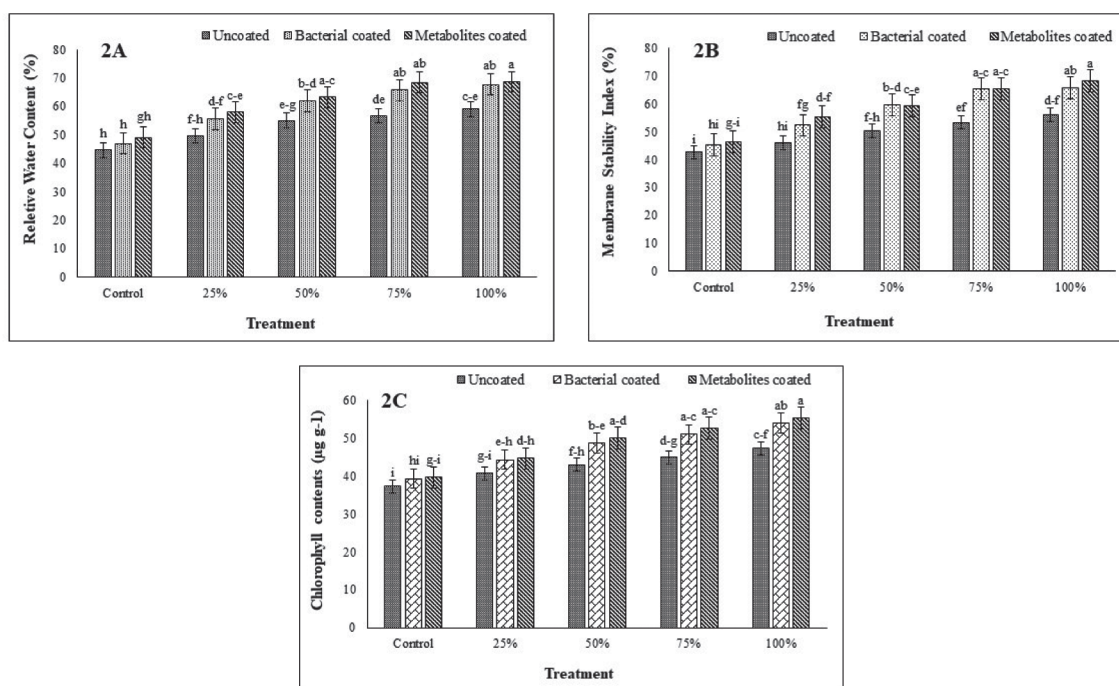


Fig. 2. Effect of Bacterial and metabolites coated DAP physiological parameters of maize crop in pot trail. Relative water contents (2A), membrane stability index (2B), chlorophyll contents (2C). Bars with the same alphabets showed non-significant results with each other at  $p \leq 0.05$ .



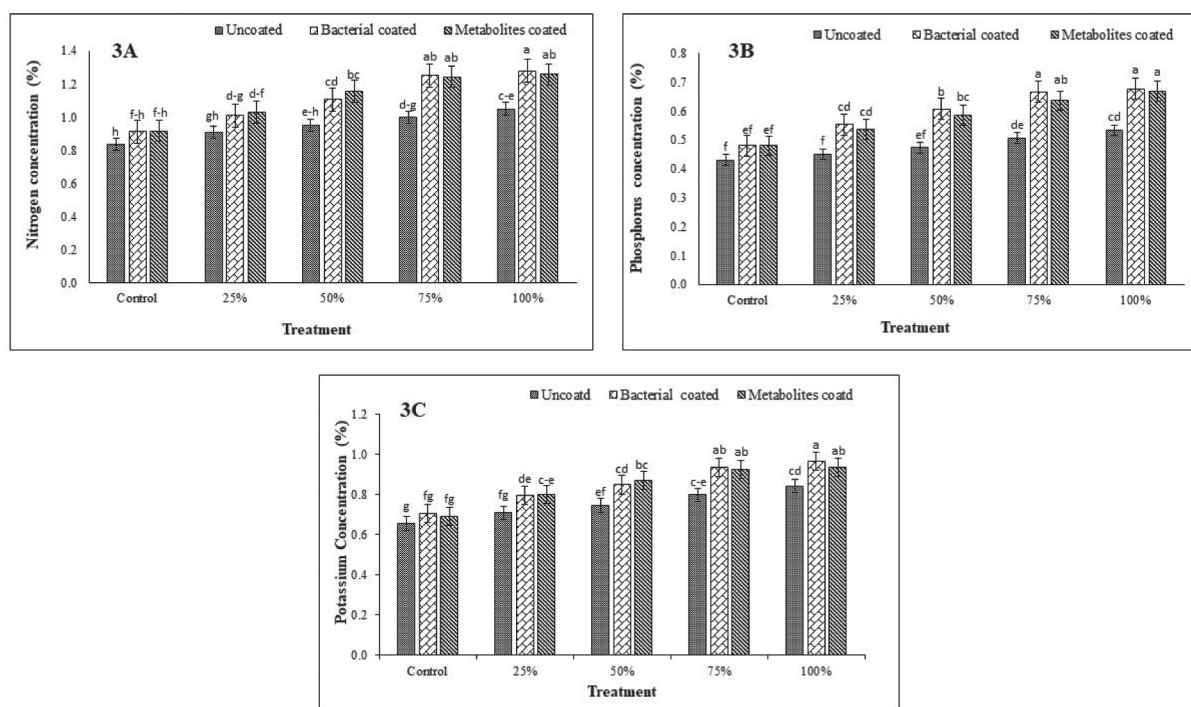


Fig. 3. Effect of Bacterial and metabolites coated DAP on grain nutritional values of maize crop in pot trail. Nitrogen concentration (3A), Phosphorus concentration (3B), Potassium concentration (3C). Bars with the same alphabets showed non-significant results with each other at  $p \leq 0.05$ .

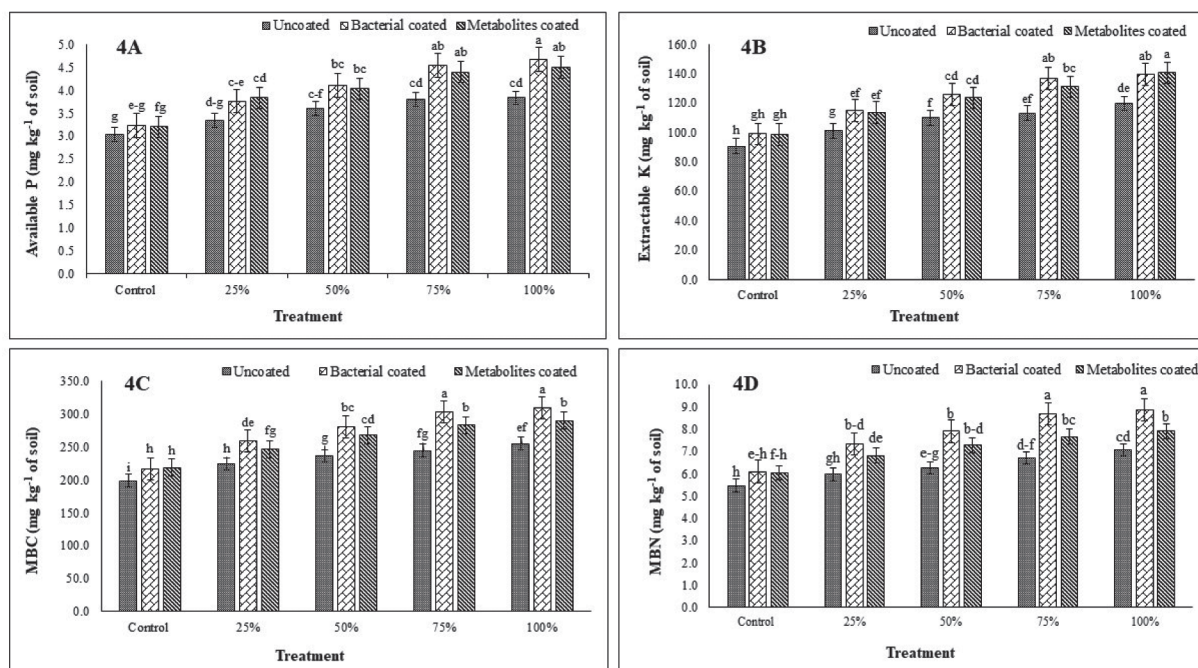


Fig. 4. Effect of Bacterial and metabolites coated DAP on post-harvest rhizospheric soil characteristics of maize crop in pot trail. Available P (4A), Extractable K (4B), MBC (microbial biomass carbon) (4C), MBN (microbial biomass nitrogen) (4D). Bars with the same alphabets showed non-significant results with each other at  $p \leq 0.05$ .

#### Effect of Coated DAP on Post-Harvest Rhizospheric Soil Characteristics

Bacterial coated and metabolites coated DAP significantly improved rhizospheric soil properties in a pot trial (Fig. 4), while uncoated DAP showed non-significant

results. The maximum increase in available P was 44% and 41%, which were obtained from bacterial coated DAP and metabolites coated DAP, respectively, as compared to their respective controls, while a 26% increase was observed in the case of uncoated DAP (Fig. 4A). At a lower application rate, i.e., 75% of the required DAP, the bacterial coated DAP

and metabolites coated DAP showed statistically similar results. Similarly, extractable K was also the maximum in the rhizospheric soil of pots that were treated with coated DAP, with a 41% and 43% increase, respectively, from bacterial coated and metabolite coated DAP formulations. When uncoated DAP at the rate of 100% of the required P dose was applied, a 32% improvement in extractable K was observed, which is statistically at par with metabolites coated DAP formulation at 75% of the recommended P rate (Fig. 4B). Bacterial coated DAP resulted in a 43% increase in microbial biomass carbon (MBC) at 100% of the required P rate, while metabolites coated DAP showed a 33% increase in MBC as compared to their respective controls (Fig. 4C). Similarly, MBN was also significantly improved by the application of bacterial coated DAP with a 45% increase as compared to its control. Meanwhile metabolites coated and uncoated DAP applications at 100% of the required P dose showed statistically similar results but significantly better than the respective controls (Fig. 4D).

## Discussion

Diammonium phosphate stands out as a crucial and costly component in agricultural production. [48]. Meeting the escalating demand for food in the next five decades poses a significant challenge. This challenge is exacerbated by a growing population, diminishing arable land, swift global climate change, escalating water scarcity, and a surge in the cost of agricultural inputs. In this context, the significance of fertilizers has grown, aiming to attain elevated agricultural production per unit area from soils experiencing declining fertility. Consequently, enhancing the efficiency of applied fertilization is crucial to achieving optimal crop yields. In the past three decades, the intersection of agronomy and genetically efficient crop varieties has been instrumental in enhancing Phosphorus Use Efficiency (PUE) and overall crop productivity. More recently, advanced technologies such as polymer-coated P fertilizers, as well as the utilization of phosphorus-solubilizing bacteria and fungi, have been explored [49, 50]. However, inconclusive outcomes have been observed, attributed to challenges such as exorbitant production costs, inadequacies in microbial carriers, and suboptimal survival rates at the designated target sites. The present study was conducted to check the efficiency of bacteria and their metabolites coated DAP to improve the growth and yield of maize crops. The results showed that coated DAP performed better regarding maize growth and yield. Obtained results highlighted enhanced growth parameters such as biomass and root shoot length of maize in pot trail up to 44%, 42%, 40%, and 43% by using bacteria and their extracted metabolites coated DAP, respectively. The increase in shoot length and shoot dry weight may be attributed to the solubilization of insoluble phosphorus (P) in the soil, facilitated by Phosphorus-Solubilizing Bacteria (PSB) through the production of organic acids and enzymes [51]. This process enhances the accessibility

of available phosphorus to the plant [52]. The synthesis of indole-3-acetic acid (IAA), a plant growth-promoting phytohormone, by Phosphorus-Solubilizing Bacteria (PSB) additionally contributes to the enhancement of plant growth. This is achieved through the improvement of root growth and development [53]. The coating of DAP serves a dual purpose. Firstly, it acts as a barrier, preventing direct contact between DAP and the soil, thereby mitigating the risk of phosphorus (P) fixation through processes such as sorption, complexation, and precipitation as outlined by Fertahi et al. [54]. Furthermore, this encapsulation impedes the expeditious dissolution of DAP, thereby mitigating its subsequent loss via surface runoff and subsurface flow [55]. These findings align with the observations of Li et al. [56], who documented that Phosphorus-Solubilizing Bacteria (PSB) in conjunction with chemical fertilizers can enhance root length. P-solubilizing bacteria release hormones that stimulate elongated root growth, consequently improving nutrient uptake [57]. Inoculation with *Bacillus* and *Lysinibacillus* strains across diverse crop types markedly stimulated plant growth, manifested by increases in plant height and biomass, as evidenced in the present investigation. The application of phosphorus-solubilizing bacterial coated Diammonium Phosphate notably augmented plant growth in terms of both root and shoot length [58]. The results of the present study showed that the application of bacterial and their metabolites coated DAP increased plant physiological attributes more prominently as compared to uncoated DAP. This increase in physiological attributes of the maize crop may be due to the increase in the retention of assimilates that are essential for cob production in the stem of the plant [59]. Chlorophyll content is an important indicator of crop health, growth, and nutrient status. In our study, maximum chlorophyll contents were observed by the application of 75% and 100% bacterial and metabolites coated DAP. The optimal uptake of phosphorus (P) is crucial for the biosynthesis of chlorophyll, as it serves as the structural component of phospholipids essential for the formation and stabilization of the thylakoid membrane. This membrane plays a pivotal role in the positioning of chlorophyll molecules, influencing the *f<sub>v</sub>/f<sub>m</sub>* (maximum quantum yield of photosystem II) parameter [60]. This investigation revealed a positive correlation between phosphorus (P) uptake and key growth parameters, including shoot dry weight, shoot height, leaf area, and the photosynthetic efficiency parameter (chlorophyll content). This correlation underscores the significance of accessible phosphorus facilitated by Phosphorus-Solubilizing Bacteria (PSB) in enhancing plant growth, photosynthetic rates, dry weight accumulation, and overall plant development. Our results are consistent with the findings of [61]. Phosphorus uptake was significantly higher in PSB coated DAP applications supplemented with 75% and 100% recommended P fertilizer doses as compared to their respective controls. The augmented P uptake resulting from the synergistic interplay of PSB and an optimal phosphorus fertilizer dosage can be attributed to the enhanced solubilization of insoluble phosphorus compounds. This process improves the availability of



phosphorus to plants, arising from both the chemical fertilizer and indigenous phosphorus in the soil, facilitated by the activities of PSB [62]. These activities include the production of organic acids, participation in cation exchange reactions, and the generation of phosphatase enzymes [63]. The increase in plant biomass production subsequently promoted elevated P uptake by plants. Specific exudates released by rhizospheric bacteria and plant roots within the rhizosphere induce the activation of phosphatase activity, thereby amplifying the efficiency of phosphorus uptake [64]. Besides that, *Bacillus* sp. helped the plant uptake K [65]. Application of PSB and metabolites coated DAP improved CAT, SOD, POD, and APX activities that can protect membrane integrity and mitigate the detrimental impacts of abiotic stresses [66]. Antioxidant enzymes were significantly affected by the application of coated DAP. Our results are in line with the findings of Israr et al. [61] and Ishizawa et al. [67]. Ishizawa et al. [67] demonstrated that the bacterial strains H3 (*Aquitalea magnusonii*) and M12 (*Pseudomonas otitidis*) have caused an elevation of O<sub>2</sub>- contents in plants. One of the immune responses involved in O<sub>2</sub>- elevation was directly triggered by microbially assisted molecular patterns (MAMPs) such as flagellins and chitin [68]. Moreover, it has been proven that the O<sub>2</sub>- generation in response to oxidase activity using the reducing potential of NADPH serves as a signaling molecule to establish symbiosis in plants and bacteria [69]. A rise in soil-available phosphorus, extractable potassium, and soil microbial biomass carbon and nitrogen was documented in pots treated with DAP coated with bacterial strains and their metabolites. Furthermore, the elevated P content observed in pots treated with DAP coated with PSB may be attributed to the increased abundance of PSB and heightened enzymatic activities in the soil. This, in turn, led to the mineralization of soil phosphorus, as evidenced by the noteworthy correlations observed between the microbial population, available phosphorus, and soil enzyme activities in our investigation [70]. Overall, the findings of this research advocate for the application of coated DAP fertilizer, characterized by its efficiency, at the recommended rate, as well as at reduced rates of 75% and 50% of the recommended rate. This approach proves advantageous in maintaining maize grain yield without compromising agricultural productivity when compared to the use of uncoated DAP fertilizer.

### Conclusions

The coated DAP showed better results as compared to uncoated DAP, leading to enhanced growth of maize plants. The coated DAP enhanced the phosphorus use efficiency by increasing the phosphorus availability in the soil. Moreover, bacterial coated DAP showed better results than metabolites coated DAP, however, the difference was non-significant, which means both formulations were equally effective. Coated DAP outperformed at a 100% application rate, but also performed well at lower rates of application, i.e., 50% and 75%, as compared to the full dose of uncoated

DAP. Overall, the study advocates the use of coated DAP fertilizer as an effective tool to improve phosphorus use efficiency. Thus, the investigations support the idea of using coated DAP as a promising strategy to improve fertilizer use efficiency, increase crop productivity, and address the challenges posed by factors such as population growth, decreasing arable land, climate change, water scarcity, and rising agricultural input prices in the coming decades.

### Acknowledgments

The paper is one of the components of Ms. Sadia Murad's PhD thesis and the authors highly acknowledge the Soil Microbiology and Biotechnology Laboratory (SMBL) the Department of Soil Science for providing research facilities. The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R306), King Saud University, Riyadh, Saudi Arabia for provision of funds for analytical work and publication.

### Conflict of Interest

"The authors declare no conflict of interest".

### References

1. GUO J., FAN, J., XIANG Y., ZHANG F., YAN S., ZHANG X., ZHENG J., LI Y., TANG Z., LI Z. Coupling effects of irrigation amount and nitrogen fertilizer type on grain yield, water productivity and nitrogen use efficiency of drip-irrigated maize. *Agricultural Water Management*, **261**, 107389, **2022**.
2. LI G., CHENG G., LI L., LU D., LU, W. Effects of slow-released fertilizer on maize yield, biomass production, and source-sink ratio at different densities. *Journal of Plant Nutrition*, **43** (5), 725, **2020**.
3. GIRALDO P., BENAVENTE E., MANZANO-AGUGLIARO F., GIMENEZ E. Worldwide research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy*, **9** (7), 352, **2019**.
4. BADR M.A., EL-TOHAMY W.A., ZAGHLOULA M. Yield and water use efficiency of potato grown under different irrigation and nitrogen levels in an arid region. *Agricultural Water Management*, **110**, 9, **2012**.
5. GITARI H.I., SHADRACK N., KAMAU S., KARANJA N.N., GACHENE C.K., SCHULTE-GELDERMANN E. Agronomic assessment of phosphorus efficacy for potato (*Solanum tuberosum* L) under legume intercrops. *Journal of Plant Nutrition*, **43** (6), 864, **2020**.
6. LEMMING C., OBERSON A., MAGID J., BRUUN S., SCHEUTZ C., FROSSARD E., JENSEN, L.S. Residual phosphorus availability after long-term soil application of organic waste. *Agriculture, Ecosystems & Environment*, **270**, 65, **2019**.
7. NAOMI M.R., NURMALASARI I.A. Role of phosphate fertilizer on growth and yield of hybrid maize (*Zea mays* L.). In IOP Conference Series: Earth and Environmental Science, **637**, 012070, **2021**.
8. ALAM A., TARIQ M., HAQ I., ALI J., ADNAN M., FAHAD S., MANZOOR AHMAD D., ROMMAN M., HAMZAH SALEEM M., AHMAD S. Co-application of Phosphorus and

- Sulfur Improve Yield, Quality, and Nutrients Uptake in *Nicotiana tabacum* L. Philippine Agricultural Science, **105**, 61, **2022**.
9. SALEEM M.H., ALI S., REHMAN M., RANA M.S., RIZWAN M., KAMRAN M., IMRAN M., RIAZ M., SOLIMAN M.H., ELKELISH A., LIU L. Influence of phosphorus on copper phytoextraction via modulating cellular organelles in two jute (*Corchorus capsularis* L.) varieties grown in a copper mining soil of Hubei Province, China. Chemosphere, **248**, 126032, **2020**.
  10. RASHMI I., BISWAS A.K. Phosphorus Sorption Characteristics of Soils for Managing the Fertilizer Phosphorus in Three Dominant Soil Types of India. Chemical Science Review and Letters, **7** (27), 725, **2018**.
  11. ULLAH I., MUHAMMAD D., MUSSARAT M., KHAN S., ADNAN M., FAHAD S., ISMAIL M., MIAN I.A., ALI A., SALEEM M.H., SAEED M., GUL F., IBRAHIM M., RAZA M.A.S., HAMMAD H.M., NASIM W., SAUD S., KHATTAK J.Z.K., AHMAD M., ALIN., AKBAR R., KHAN S.M., BANOUT J. Comparative effects of biochar and NPK on wheat crops under different management systems. Crop and Pasture Science, **74** (2), **2022**.
  12. MA Q., WEN Y., MA J., MACDONALD A., HILL P.W., CHADWICK D.R., WU L., JONES D.L. Long-term farmyard manure application affects soil organic phosphorus cycling: A combined metagenomic and <sup>33</sup>P/<sup>14</sup>C labelling study. Soil Biology and Biochemistry, **149**, 107959, **2020**.
  13. CHEN M., ALIMN., ZHANG Y., XUN., CAO X. Contrasting effects of biochar nanoparticles on the retention and transport of phosphorus in acidic and alkaline soils. Environmental pollution, **239**, 562, **2018**.
  14. TRENKEL M.E. Slow and controlled-release and stabilized fertilizers: An option for enhancing nutrient use efficiency in agriculture. Paris, France: International Fertilizer Industry Association (IFA), **2010**.
  15. LU H., TIAN H., LIU Z., ZHANG M., ZHAO C., GUO Y., GUAN R., CHEN Q., YU X., WANG H., ZHENG L. Polyolefin wax modification improved characteristics of nutrient release from biopolymer-coated phosphorus fertilizers. ACS omega, **4** (23), 20402, **2019**.
  16. SARKAR A., BISWAS D.R., DATTA S.C., ROY T., MOHARANA P.C., BISWAS S.S., GHOSH A. Polymer coated novel controlled release rock phosphate formulations for improving phosphorus use efficiency by wheat in an Inceptisol. Soil and Tillage Research, **180**, 48, **2018**.
  17. YASEEN M., AZIZ M.Z., MANZOOR A., NAVEED M., HAMID Y., NOOR S., KHALID M.A. Promoting growth, yield, and phosphorus-use efficiency of crops in maize-wheat cropping system by using polymer-coated diammonium phosphate. Communications in soil science and plant analysis, **48** (6), 646, **2017**.
  18. TAN G., LIU Y., PENG S., YIN H., MENG D., TAO J., GU Y., LI J., YANG S., XIAO N., LIU D. Soil potentials to resist continuous cropping obstacle: Three field cases. Environmental research, **200**, 111319, **2021**.
  19. GEISSELER D., SCOW K.M. Long-term effects of mineral fertilizers on soil microorganisms—A review. Soil Biology and Biochemistry, **75**, 54, **2014**.
  20. ZHANG J. The long-term effects of crop rotation and fertilizer applications on soil health and crop productivity in Alberta, **2022**.
  21. ROSA P.A.L., MORTINHO E.S., JALAL A., GALINDO F.S., BUZETTI S., FERNANDES G.C., BARCO NETO M., PAVINATO P.S., TEIXEIRA FILHO M.C.M. Inoculation with growth-promoting bacteria associated with the reduction of phosphate fertilization in sugarcane. Frontiers in Environmental Science, **8**, 32, **2020**.
  22. SOUMARE A., BOUBEKRI K., LYAMLOULI K., HAFIDI M., OUHDOUCH Y., KOUISNI L. Efficacy of phosphate solubilizing Actinobacteria to improve rock phosphate agronomic effectiveness and plant growth promotion. Rhizosphere, **17**, 100284, **2021**.
  23. FITRIATIN B.N., SILPANUS R., SOFYANE T., YUNIARTI A., TURMUKTINI T. Effect of microbial fertilizers and dosage of NPK on growth and yield of upland rice (*Oryza sativa* L.). International Journal of Environment, Agriculture and Biotechnology, **4** (4), **2019**.
  24. WAHID F., FAHAD S., DANISH S., ADNAN M., YUE Z., SAUD S., SIDDIQUI M.H., BRTNICKY M., HAMMERSCHMIEDT T., DATTA R. Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. Agriculture, **10** (8), 334, **2020**.
  25. ZAFAR-UL-HYE M., ZAHRA M.B., DANISH S., ABBAS M., REHIM A., AKBAR M.N., IFTIKHAR A., GUL M., NAZIR I., ABID M., TAHZEEB-UL-HASSAN M. Multi-strain inoculation with pppr producing acc deaminase is more effective than single-strain inoculation to improve wheat (*Triticum aestivum*) growth and yield. Phytion, **89** (2), 405, **2020**.
  26. ZHOU J., TANG S., PAN W., XU M., LIU X., NI L., MAO X., SUN T., FU H., HAN K., MA Q. Long-term application of controlled-release fertilizer enhances rice production and soil quality under non-flooded plastic film mulching cultivation conditions. Agriculture, Ecosystems & Environment, **358**, 108720, **2023**.
  27. LI R., GAO Y., CHEN Q., LI Z., GAO F., MENG Q., LI T., LIU A., WANG Q., WU L., WANG Y. Blended controlled-release nitrogen fertilizer with straw returning improved soil nitrogen availability, soil microbial community, and root morphology of wheat. Soil and Tillage Research, **212**, 105045, **2021**.
  28. DUAN Q., JIANG S., CHEN F., LI Z., MA L., SONG Y., YU X., CHEN Y., LIU H., YU L. Fabrication, evaluation methodologies and models of slow-release fertilizers: a review. Industrial Crops and Products, **192**, 116075, **2023**.
  29. WANG X., YAO Y., WANG G., LU H., MA J., ZHANG M., CHEN X., YIN C., MAO Z. Controlled-Release Diammonium Phosphate Alleviates Apple Replant Disease: An Integrated Analysis of Soil Properties, Plant Growth, and the Soil Microbiome. Journal of Agricultural and Food Chemistry, **70** (29), 8942, **2022**.
  30. CHEN Q., LI Z., QU Z., ZHOU H., QI Y., LIU Z., ZHANG M. Maize yield and root morphological characteristics affected by controlled-release diammonium phosphate and *Paecilomyces variotii* extracts. Field Crops Research, **255**, 107862, **2020**.
  31. NELSON D.W., SOMMERS L.E. Total carbon, organic carbon and organic matter. In Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties, **9**, 539, **1983**.
  32. SARFRAZ M., ASHRAF Y., ASHRAF S. A review: prevalence and antimicrobial susceptibility profile of listeria species in milk products. Matrix Science Medica, **1** (1), 3, **2017**.
  33. WATANABE F.S., OLSEN S.R. Test of an ascorbic acid method for determining phosphorus in water and NaHCO<sub>3</sub> extracts from soil. Soil Science Society of America Journal, **29** (6), 677, **1965**.
  34. GEE G.W., BAUDER J.W. Particle size analysis. In A. Klute (Ed.), Methods of soil analysis. Part 2<sup>nd</sup> (Ed.) Agronomy Monographs, Madison: ASA and SSSA, **5**, 383, **1986**.
  35. JACKSON M.L. Soil Chemical Analysis; Prentice Hall Inc.: New York, NY, USA, **1962**.

36. IQBAL Z., AHMAD M., JAMIL M., AKHTAR MF. Appraising the potential of integrated use of *Bacillus* strains for improving wheat growth. *International Journal of Agriculture and Biology*, **24**, 1439, **2020**.
37. POLITZ M., LENNEN R., PFLEGER B. Quantification of bacterial fatty acids by extraction and methylation. *Bio-protocol*, **3** (21), e950, **2013**.
38. PROCHAZKOVA D., SAIRAM R.K., SRIVASTAVA G.C., SINGH D.V. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Science*, **161** (4), 765, **2001**.
39. CHANCE B., MAELY A.C. Assay of catalase and peroxidase methods. *Enzymology*, **2**, 755, **1955**.
40. GIANNOPOLITIS C.N., RIES S.K. Superoxide dismutases: I. Occurrence in higher plants. *Plant physiology*, **59** (2), 309, **1977**.
41. LAZCANO-FERRAT I., LOVATT C.J. Relationship between relative water content, nitrogen pools, and growth of *Phaseolus vulgaris* L. and *P. acutifolius* A. Gray during water deficit. *Crop Science*, **39** (2), 467, **1999**.
42. SAIRAM R.K., SAXENA D.C. Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *Journal of Agronomy and Crop Science*, **184** (1), 55, **2000**.
43. ARNON D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*, **24** (1), 1, **1949**.
44. WOLF B. A comprehensive system of leaf analyses and its use for diagnosing crop nutrient status. *Communications in Soil Science and Plant Analysis*, **13** (12), 1035, **1982**.
45. RYAN J. *Methods of Soil, Plant, and Water Analysis: A Manual for the West Asia and North Africa Region*; International Center for Agricultural Research in the Dry Areas (ICARDA): Beirut, Lebanon, **2017**.
46. JENKINSON D.S., LADD J.N. Microbial biomass in soil: measurement and turnover. *Soil biochemistry*, **5** (1), 415, **1981**.
47. BREMNER E., KESSEL V. Extractability of microbial 14C and 15N following addition of variable rates of labeled glucose and ammonium sulphate to soil. *Soil Biology and Biochemistry*, **22**, 707, **1990**.
48. AZIZ M.Z., NAVEED M., ABBAS T., SIDDIQUE S., YASEEN M. Alternative fertilizers and sustainable agriculture. *Innovations in Sustainable Agriculture*, 213, **2019**.
49. NAVEED M., AZIZ M.Z., YASEEN M. Microbes for legume improvement. In *Perspectives of Endophytic Microbes for Legume Improvement*; Zaidi, A., Khan, M.S., Musarrat, J., Eds.; Springer: Cham, Switzerland, 277, **2017**.
50. PATEL C., SINGH J., KARUNAKARANA., RAMAKRISHNA W. Evolution of nano-biofertilizer as a green technology for agriculture. *Agriculture*, **13** (10), p.1865, **2023**.
51. SILVA L.I.D., PEREIRA M.C., CARVALHO A.M.X.D., BUTTRÓS V.H., PASQUAL M., DÓRIA J. Phosphorus-Solubilizing Microorganisms: A Key to Sustainable Agriculture. *Agriculture*, **13** (2), 462, **2023**.
52. SULEMAN M., YASMIN S., RASUL M., YAHYA M., ATTA B.M., MIRZA M.S. Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. *PLoS one*, **13** (9), e0204408, **2018**.
53. WANG Z., ZHANG H., LIU L., LI S., XIE J., XUE X., JIANG Y. Screening of phosphate-solubilizing bacteria and their abilities of phosphorus solubilization and wheat growth promotion. *BMC microbiology*, **22** (1), 296, **2022**.
54. FERTAHI S., ILSOUK M., ZEROUAL Y., OUKARROUM A. BARAKAT A. Recent trends in organic coating based on biopolymers and biomass for controlled and slow-release fertilizers. *Journal of controlled release*, **330**, 341, **2021**.
55. CHEN Q., QU Z., LI Z., ZHANG Z., MA G., LIU Z., WANG Y., WU L., FANG F., WEI Z. ZHANG M. Coated diammonium phosphate combined with humic acid improves soil phosphorus availability and photosynthesis and the yield of maize. *Frontiers in Plant Science*, **12**, 759929, **2021**.
56. LI Z., WANG Y., LIU Z., HAN F., CHEN S., ZHOU W. Integrated application of phosphorus-accumulating bacteria and phosphorus-solubilizing bacteria to achieve sustainable phosphorus management in saline soils. *Science of The Total Environment*, **885**, 163971, **2023**.
57. PHRINGPAEN W., AIEDHET W., THITITHANAKUL S., KANJANASOPA D. Ability of Phosphate-Solubilizing Bacteria to Enhance the Growth of Rice in Phosphorus-Deficient Soils. *Trends in Sciences*, **20** (12), 7032, **2023**.
58. KHAN K.S., NAVEED M., QADIR M.F., YASEEN M., SIDDIQUI M.H. Bio-organically acidified product-mediated improvements in phosphorus fertilizer utilization, uptake and yielding of zeas in calcareous soil. *Plants*, **12** (17), 3072, **2023**.
59. ASGHAR M.J., MEHDI S.S. Selection indices for yield and quality traits in sweet corn. *Pakistan Journal of Botany*, **42**, 775, **2010**.
60. RADY M.M., EL-SHEWY A.A., SEIF EL-YAZAL M.A., ABD EL-GAWWAD I.F. Integrative application of soil P-solubilizing bacteria and foliar nano P improves *Phaseolus vulgaris* plant performance and antioxidative defense system components under calcareous soil conditions. *Journal of Soil Science and Plant Nutrition*, **20**, 820, **2020**.
61. ISRAR D., MUSTAFA G., KHAN K.S., SHAHZAD M., AHMAD N., MASOOD S. Interactive effects of phosphorus and *Pseudomonas putida* on chickpea (*Cicer arietinum* L.) growth, nutrient uptake, antioxidant enzymes and organic acids exudation. *Plant physiology and biochemistry*, **108**, 304, **2016**.
62. TIMOFEEVA A., GALYAMOVA M., SEDYKH S. Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants*, **11** (16), 2119, **2022**.
63. KALEEM ABBASI M., MANZOOR M. Biosolubilization of phosphorus from rock phosphate and other P fertilizers in response to phosphate solubilizing bacteria and poultry manure in a silt loam calcareous soil. *Journal of Plant Nutrition and Soil Science*, **181** (3), 345, **2018**.
64. CHEN S., YANG D., WEI Y., HE L., LI Z., YANG S. Changes in Soil Phosphorus Availability and Microbial Community Structures in Rhizospheres of Oilseed Rapes Induced by Intercropping with White Lupins. *Microorganisms*, **11** (2), 326, **2023**.
65. ALI A.M., AWAD, M.Y., HEGAB, S.A., GAWAD, A.M.A.E. EISSA, M.A. Effect of potassium solubilizing bacteria (*Bacillus cereus*) on growth and yield of potato. *Journal of Plant Nutrition*, **44** (3), 411, **2021**.
66. CHEN Y.P., REKHA P.D., ARUN A.B., SHEN F.T., LAI W.A., YOUNG C.C. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied soil ecology*, **34** (1), 33, **2006**.
67. ISHIZAWA H., KURODA M., MORIKAWA M., IKE M. Differential oxidative and antioxidative response of duckweed *Lemna minor* toward plant growth promoting/inhibiting bacteria. *Plant Physiology and Biochemistry*, **118**, 667, **2017**.
68. SMITH D.L., SUBRAMANIAN S., LAMONT J.R., BYWATER-EKEGÄRDM. Signaling in the phytomicrobiome: breadth and potential. *Frontiers in Plant Science*, **6**, 709, **2015**.
69. NANDA A.K., ANDRIO E., MARINO D., PAULY N., DUNAND C. Reactive oxygen species during plant-microorganism early interactions. *Journal of integrative plant biology*, **52** (2), 195, **2010**.
70. SHI X.K., MA J.J., LIU L.J. Effects of phosphate-solubilizing bacteria application on soil phosphorus availability in coal mining subsidence area in Shanxi. *Journal of Plant Interactions*, **12** (1), 137, **2017**.