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Research article

Effect of Phosphorus-Solubilizing Bacteria, Organic Fertilizer, and mineral phosphate fertilization on NPK Concentrations in Cabbage (Brassica oleracea var. capitata) under Calcareous Soil Conditions

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ABSTRACT

A field experiment was conducted in autumn 2025 at the fields of College of Agriculture, University of Wasit to evaluate the effect of inoculation with phosphorus solubilizing bacteria, liquid organic fertilizer Bio-60 and different levels of mineral phosphate fertilizer triple superphosphate (TSP) on nitrogen, phosphorus and potassium concentrations in cabbage plants cv. Club Star. The experiment was conducted in a randomized complete block design (RCBD) in a 2 × 2 × 3 factorial arrangements consisting of three replications. The first factor was the inoculation with phosphorus solubilizing bacteria at two levels; no inoculation (A_0) and 12 mL plant^{-1} (A_1). The second factor consisted of two levels of organic liquid fertilizer (Bio-60) (no application, O_0; 5 mL plant^{-1} in the O_1). Third factor was mineral phosphate fertilizer TSP at three levels 0, 50 and 100 kg ha^{-1} (M_0, M_1 and M_2). The effects of fertilization on all studied traits were significant at $P \leq 0.05$. The control treatment ($A_0O_0M_0$) recorded the lowest values of N, P and K with 0.51, 0.236 and 0.755%, respectively. By contrast, the interaction between all three factors $A_1O_1M_2$ was significantly better and gave the maximum values of 1.89, 0.409, and 2.567% for N, P, and K, respectively. This higher efficiency can be explained by the synergistic effect of biofertilization, organic fertilization, and mineral phosphate fertilization on nutrient availability and on nutrient uptake efficiency. Phosphorus-solubilizing bacteria increase phosphorus availability and stimulate rhizosphere activity, and organic fertilizer enhances soil physical properties and nutrient holding capacity. On the other hand, mineral phosphate fertilizer provides a direct source of available phosphorus. These findings indicate that integrated fertilization is a promising approach to enhance the nutritional status of cabbage plants and the content of macronutrients under calcareous soil conditions.

1. Introduction

Cabbage (Brassica oleracea var. capitata) is considered one of the important vegetable crops from both nutritional and economic perspectives due to its high nutritional value and good content of vitamins, minerals, and dietary fiber, in addition to its role in enhancing food security. Achieving good growth and high productivity of this crop depends on the efficiency of plant nutrition with

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macronutrients, particularly nitrogen, phosphorus, and potassium, because of their essential roles in plant physiological and biochemical processes, such as photosynthesis, protein formation, enzyme activation, regulation of water balance, and improvement of vegetative and root growth efficiency (Hänsch & Mendel, 2009; Marschner, 2012).

Phosphorus is a limiting nutrient for plant growth. Phosphorus has a role in the mineralization of nucleic acids as well as in a root development, energy metabolism, and plant cell energy transfer; it is part of energy-rich compounds such as ATP; and it is essential in regulating the metabolic activities of a plant. Even though it is essential, its availability is frequently limited in calcareous and alkaline soils as a result of fixation and precipitation with calcium into poorly soluble phosphate oligomers. This decreases its efficiency for uptake by plants and also the ability of plants to use the added phosphates from fertilizer (Hinsinger, 2001; Shen et al., 2011). Phosphorus Use Efficiency (PUE) is low in calcareous soils that represent a staple in agriculture. The solubility and mobility of phosphorus is decreased in the soil solution due to their high calcium carbonate content and high pH. On the other hand, mineral phosphate fertilizers such as triple superphosphate (TSP) are extensively employed as sources of phosphorus that are abundant and readily available, but their effectiveness may be undermined in such soils by the rapid fixation of phosphorus following the application of fertilizers. Hence, there is a need to develop more effective and sustainable fertilization practices that enhance nutrients availability while minimizing nutrient loss and fixation (Halajnia et al., 2009; Zhang et al., 2024) . Within this frame, phosphorus-solubilizing bacteria (PSB) have also emerged as efficient biofertilizer candidates for enhancing phosphorus availability in soil. These bacteria solubilize inaccessible phosphates by the production of organic acids and enzymes, thus raising the level of available phosphorus in the soil solution and facilitating its uptake by plants. These microbes are responsible for enhancing the rhizosphere, promoting root system development, and improving the acquisition of different nutrients, and this is well reflected in improved nutrirional status and growth of plants (Richardson & Simpson, 2011; Alori et al., 2017; Abbasi, 2023; Pan et al., 2023) . Organic fertilizer also plays an important role in improving the physical, chemical, and biological properties of soil. It contributes to improving soil structure and aeration, increasing cation exchange capacity, and stimulating microbial activity, in addition to enhancing soil nutrient retention and reducing nutrient losses. Organic materials may also form organic complexes that reduce the fixation of some nutrients in calcareous soils, thereby improving their availability to plants. Therefore, integrating organic fertilizer with biofertilizer and mineral fertilizer sources may represent an effective approach to improving nutrient-use efficiency in sustainable agricultural systems (Marschner, 2012; Khan et al., 2022; Paramesh et al., 2023). Several studies have confirmed the importance of integrating biofertilization, organic fertilization, and mineral phosphate fertilization in improving nutrient availability and increasing plant nutrient uptake efficiency. This integration provides a direct nutrient source through mineral fertilizer, improves root environment and soil properties through organic fertilizer, and stimulates biological processes responsible for phosphorus solubilization and increased availability through phosphorus-solubilizing bacteria. Yasir et al. (2025) reported that inoculation with beneficial microorganisms combined with the addition of organic materials led to a significant increase in the concentrations of N, P, and K in plants. Habeeb et al. (2023) also showed that the type and level of phosphate fertilizer significantly affected phosphorus release in soil, which is reflected in plant nutrition efficiency. Although many studies have investigated the effects of biofertilization, organic fertilization, or mineral phosphate fertilization individually, field studies evaluating the integrated effect of phosphorus-solubilizing bacteria, liquid organic fertilizer Bio-60, and mineral phosphate fertilizer TSP on the concentrations of the macronutrients N, P, and K in cabbage plants under calcareous soil conditions in Iraq remain limited. Therefore, this study was conducted to test the hypothesis that integrating inoculation with phosphorus-solubilizing bacteria, organic fertilizer, and mineral phosphate fertilization can improve nutrient availability and uptake, thereby positively affecting the nutritional status of cabbage plants. Accordingly, this study aimed to evaluate the effects of inoculation with phosphorus-solubilizing bacteria, liquid organic fertilizer Bio-60, different levels of mineral phosphate fertilizer TSP, and their interactions on nitrogen, phosphorus, and potassium concentrations in cabbage plants grown under calcareous soil conditions. The objectives of this work were also to evaluate the effectiveness of combined fertilization in increasing the plant nutritional status and in reducing reliance on mineral phosphate fertilization alone.

2. Materials and Methods

2.1 Experimental Site

The field trial was carried out in the autumn season of 2025 in the fields of the College of Agriculture, University of Wasit, Wasit Governorate, Iraq (32.497105° N, 45.842733° E) with the use of the cultivar Club Star seedlings. Sowing was done on 12th September 2025 and harvesting on 20th December 2025. All the cultivation practices were done as per the scientific recommendations for growing this crop. Weeds were controlled manually when needed, and surface irrigation was applied.

2.2 Soil Sample and Analysis

Before planting, a composite soil sample was taken from the depth of 0–30 cm at the experimental site. The sample was air-dried, pulverized and passed through 2-mm mesh sieve. The sample was analyzed to evaluate a number of physical and chemical properties of the soil such as soil texture; particle density; bulk density; soil reaction (pH); electrical conductivity (EC); cation exchange capacity (CEC); basic cations (Ca, Mg, Na); anions (Cl^-, SO_4^{2-}, CO_3^{2-}, HCO_3^-); available nutrients (NPK); micronutrients (Fe, Cu, Zn); and organic matter content (Table 1) following the procedures of Black (1965).

2.3 Experimental Design and Treatments

The experiment was conducted using a randomized complete block design (RCBD) with three replications in a 2 × 2 × 3 factorial arrangement, comprising 12 treatments. The studied factors were as follows:

A: Inoculation with phosphorus-solubilizing bacteria (PSB) at two levels: 0 or 12-mL plant^{-1}.

O: Liquid organic fertilizer Bio-60 at two levels: 0 or 5-mL plant^{-1}.

M: Mineral phosphate fertilizer, triple superphosphate (TSP), at three levels: 0, 50, or 100 kg ha^{-1}.

The area of each experimental plot was 3 × 3 m. Each plot contained 20 plants, with a spacing of 75 cm between rows and 50 cm between plants. Thus, the total number of plants in the experiment was 720 plants.

2.4 Fertilization and Bacterial Inoculum

A commercial liquid biofertilizer, BactoFeed, produced by Green Tech America Inc. (Miami, Florida, USA), was used. The product contains phosphorus-solubilizing bacterial strains belonging to the genus Bacillus spp., including Bacillus subtilis, Bacillus megaterium, and Bacillus amyloliquefaciens, with a total viable count of 1 × 10^8 CFU mL^{-1}.

The bacterial inoculum was applied once at planting by direct injection into the soil near the root zone at a rate of 12 mL plant^{-1}. The liquid organic fertilizer Bio-60, containing 50% organic matter, was also applied once at planting to the soil near the roots at a rate of 5 mL plant^{-1}. Triple superphosphate (TSP) fertilizer was applied in a single dose at planting according to the specified level for each treatment. Nitrogen fertilizer was applied in the form of urea (46% N) in two equal doses for all treatments. The first dose was applied at planting, while the second dose was applied 30 days after the first application.

Table 1. Chemical and physical properties of the field soil before planting.

Property	Value	Measurement Unit
Soil pH	7.8	—
Electrical Conductivity (EC)	2.62	dS m^{-1}
Cation Exchange Capacity (CEC)	12.7	cmol kg^{-1} soil
Ca^{2+}	6.43	
Mg^{2+}	4.02	
K^{+}	1.04	
Na^{+}	4.70	mmol L^{-1}
Cl^{-}	7.91	
SO_4^{2-}	8.55	
CO_3^{2-}	Nil	
HCO_3^{-}	0.91	
Soil Organic Matter (S.O.M.)	8.03	g kg^{-1} soil
Bulk Density	1.19	Mg m^{-3}
Particle Density	2.56	
Available Nitrogen	36.33	mg kg^{-1} soil
Available Phosphorus	13.8	
Available Potassium	76.7	
Iron (Fe)	0.68	mg kg^{-1} soil
Copper (Cu)	0.17	
Zinc (Zn)	0.25	
Soil Texture	Sandy loam	

2.5 Agricultural Practices

All necessary agricultural practices were carried out throughout the experimental period according to standard agronomic practices. Weed control was performed manually when needed, and surface irrigation was used. Plants were irrigated when approximately 50% of the available soil water had been depleted, with soil moisture restored to field capacity to ensure suitable conditions for plant growth.

2.6 Studied Measurements

Chemical traits were determined at the full maturity stage of cabbage heads (Brassica oleracea var. capitata) based on five representative plants from each experimental unit. Plant samples were collected from leaves and heads, washed with distilled water, and air-dried. They were then dried in an electric oven at 65–70°C until a constant weight was reached. After drying, the samples were ground and stored until chemical analysis. The studied measurements included the following:

1.Nitrogen concentration (N): Nitrogen concentration in plant material was determined using the Kjeldahl method after digestion, and the results were expressed as a percentage.(%)

2.Phosphorus concentration (P): Phosphorus concentration was determined using a spectrophotometer after wet digestion of the plant sample, and the results were expressed as a percentage.(%)

3.Potassium concentration (K): Potassium concentration was determined using a flame photometer, and the results were expressed as a percentage (%).

2.7 Statistical Analysis

The data were statistically analyzed according to the randomized complete block design (RCBD) with a three-factor factorial arrangement of 2 × 2 × 3 to study the effects of the investigated factors and their interactions. Analysis of variance (ANOVA) was performed to determine the significance of differences among treatments. Treatment means were compared using the least significant difference (LSD) test at the probability level of $P \leq 0.05$, using SAS statistical software, version 2018.

3. Results and Discussion

3.1 Nitrogen Concentration

The results showed significant differences in nitrogen concentration (%) among fertilization treatments at the probability level of $P \leq 0.05$. The control treatment $A_0O_0M_0$ recorded the lowest value, reaching 0.51% Table 2. This may be attributed to the low availability of nutrients in calcareous soils in the absence of fertilizer additions, as indicated by Hinsinger (2001) and FAO.(2018)
In contrast, the three-way interaction treatment $A_1O_1M_2$ achieved the highest nitrogen concentration, reaching 1.89%, with a significant difference compared with the other treatments (LSD = 0.038). This result was due to the integrated effect of biofertilization, organic fertilization, and mineral mineral phosphate fertilization. The superiority of this treatment may be attributed to the role of phosphorus-solubilizing bacteria (PSB) in improving phosphorus availability and stimulating root growth, which enhances nitrogen uptake. This finding agrees with Alori et al. (2017) and Calvo et al.(2014) .
The organic fertilizer also helped improve soil properties, increase nutrient retention, and reduce nitrogen losses (Khan et al., 2022), while the mineral fertilizer TSP provided a readily available source of phosphorus, which improved nitrogen uptake efficiency due to the physiological relationship between these two nutrients (Othman & Panhwar, 2014).
Therefore, the superiority of the $A_1O_1M_2$ treatment reflects a synergistic effect among the three fertilization sources, which improved the nutritional status of the plant and increased nitrogen concentration, in agreement with the concept of integrated and sustainable nutrient management (Pan et al., 2023; Dat et al., 2025; Sun et al., 2026).

Table 2. Effect of the interaction between phosphorus-solubilizing bacteria, organic fertilizer, and mineral fertilizer on nitrogen concentration (%) in cabbage plants

Biofertilizer (A)	Organic fertilizer (O)	M0	M1	M2	A × O Mean
A0	O0	0.51	0.69	0.85	0.68
	O1	1.08	1.13	1.25	1.16
A1	O0	1.30	1.30	1.35	1.32

	O1	1.43	1.60	1.89	1.64
LSD (A × O × M)			0.038		LSD (A × O) = 0.022
		A * O			
Biofertilizer × Mineral fertilizer (A × M)	M0	M1	M2		Biofertilizer Mean
A0	0.80	0.91	1.05		0.92
A1	1.37	1.45	1.62		1.48
LSD (A × M)		N.S			LSD (A) = 0.015
		O * M			
Organic fertilizer × Mineral fertilizer (O × M)		M0	M1	M2	Organic Mean
O0		0.91	1.00	1.10	1.00
O1		1.26	1.36	1.57	1.40
LSD (O × M)		0.027			LSD (O) = 0.015
Main effect of mineral fertilizer (M)		M0	M1	M2	
Mean of mineral fertilizer		1.08	1.18	1.34	
LSD (M)			0.019		

3.2 Phosphorus Concentration in Cabbage Plants

The results presented in Table 3 showed significant differences in phosphorus concentration (%) among fertilization treatments at the probability level of $P \leq 0.05$. The control treatment $A_0O_0M_0$ recorded the lowest value, reaching 0.236%. This may be attributed to the low availability of phosphorus in calcareous soils as a result of chemical fixation and the absence of fertilizer additions, which agrees with FAO (2018). In contrast, the three-way interaction treatment $A_1O_1M_2$ recorded the highest phosphorus concentration, reaching 0.409%, with a significant difference compared with the other treatments (LSD = 0.005). This reflects the integrated effect of biofertilization, organic fertilization, and mineral phosphate fertilization. The superiority of this treatment may be attributed to the role of phosphorus-solubilizing bacteria (PSB) in releasing unavailable phosphate through the secretion of organic acids, thereby increasing phosphorus availability and uptake by the plant. This finding is consistent with Alori et al. (2017) and Calvo et al. (2014). The quality of soil was also improved and the content of organic matter was enhanced and phosphorus fixation was suppressed by the organic fertilizer. At the same time, mineral fertilizer acted as a contact source of phosphorus coagulating its concentration in the plant. This is consistent with recent reports highlighting the effect of integration of biological, organic and mineral based sources in enhancing phosphorus uptake efficiency and nutritional status of plants (Othman & Panhwar, 2014; Silva et al., 2023; Pan et al., 2023; Dat et al., 2025; Sun et al., 2026).

Table 3. Effect of the interaction between phosphorus-solubilizing bacteria, organic fertilizer, and mineral fertilizer on phosphorus concentration (%) in cabbage plants.

Biofertilizer (A)	Organic fertilizer (O)	M0	M1	M2	A × O Mean
A0	O0	0.236	0.244	0.283	0.254
	O1	0.273	0.291	0.304	0.289
A1	O0	0.276	0.293	0.308	0.293
	O1	0.325	0.382	0.409	0.372
LSD (A × O × M)			0.005		LSD (A × O) = 0.003
	A * O				
Biofertilizer × Mineral fertilizer (A × M)		M0	M1	M2	Biofertilizer Mean
A0		0.254	0.267	0.293	0.272
A1		0.301	0.337	0.358	0.332
LSD (A × M)		0.004			LSD (A) = 0.002
	O * M				
Organic fertilizer × Mineral fertilizer (O × M)		M0	M1	M2	Organic Mean
O0		0.256	0.269	0.295	0.273
O1		0.299	0.336	0.356	0.330
LSD (O × M)			0.004		LSD (O) = 0.002
	M				
Main effect of mineral fertilizer (M)		M0	M1	M2	
Mean of mineral fertilizer		0.277	0.302	0.326	
LSD (M)			0.003		

3.3 Potassium Concentration in Cabbage Plants

The results presented in Table 4 showed significant differences in potassium concentration (%) in cabbage plants among fertilization treatments at $P \leq 0.05$. The lowest value of 0.755% was observed for the control treatment $A_0O_0M_0$. It shows that the nutrients have low availability when no fertilizer was added, mainly on calcareous soils, which is in line with Hinsinger (2001) and FAO (2018). The highest K concentration was obtained with the three-way interaction treatment $A_1O_1M_2$ (2.567%), which was significantly higher than the other treatments (LSD = 0.092). This finding was attributed to the combined effect of biofertilization and organic and mineral phosphate fertilization. The superiority of this treatment may be attributed to the ability of phosphorus-solubilizing bacteria to enhance rhizosphere activity and root development, thereby improving the uptake efficiency of nutrients, including potassium. This interpretation is consistent with Alori et al. (2017) and Calvo et al. (2014). Organic fertilizer application also improved soil quality and cation exchange capacity, resulting in enhanced soil K retention and less soil K loss. However, the effect of triple superphosphate (TSP) on potassium concentration should be interpreted as indirect, through improved phosphorus availability, root growth, and overall nutrient uptake efficiency, rather than as a direct potassium source. The mean potassium concentration increased from 1.129% under M_0 to 1.586% under M_2, suggesting that integrated fertilization improved the nutritional status of cabbage plants. This is in line with Sai and Paswan (2024) who found higher NPK fertilization levels enhanced the growth and yield of the plants by improving its nutritional status. Thus, the excellent performance of treatment $A_1O_1M_2$ implied a synergistic action among the three fertilization sources, leading to the best improvement on plant nutritional status and potassium uptake efficiency, which is consistent with the idea of integrated and sustainable nutrient management (Pan et al., 2023; Cao et al., 2025; Sun et al., 2026).

Table 4. Effect of the interaction between phosphorus-solubilizing bacteria, organic fertilizer, and mineral fertilizer on potassium concentration (%) in cabbage plants.

Biofertilizer (A)	Organic fertilizer (O)	M0	M1	M2	A × O Mean
A0	O0	0.755	0.836	1.134	0.908
	O1	1.202	1.287	1.319	1.269
A1	O0	1.216	1.233	1.324	1.257
	O1	1.344	2.144	2.567	2.018
LSD (A × O × M)			0.092		LSD (A × O) = 0.053
	A * O				
Biofertilizer × Mineral fertilizer (A × M)		M0	M1	M2	Biofertilizer Mean
A0		0.979	1.061	1.226	1.089
A1		1.280	1.688	1.945	1.638
LSD (A × M)			0.065		LSD (A) = 0.038
	O * M				
Organic fertilizer × Mineral fertilizer (O × M)		M0	M1	M2	Organic Mean
O0		0.986	1.034	1.229	1.083
O1		1.273	1.715	1.943	1.644
LSD (O × M)			0.065		LSD (O) = 0.038
	M				
Main effect of mineral fertilizer (M)		M0	M1	M2	
Mean of mineral fertilizer		1.129	1.375	1.586	
LSD (M)			0.046		

Conclusions

The inoculation of PSB significantly enhanced nitrogen (N), phosphorus (P), and potassium (K) contents in calcareous soil-cultivated cabbage plants. The nutrition status of the plant was best improved by combined application of organic fertilizer (Bio-60) and mineral phosphate fertilizer (TSP) under bio-inoculation among the treatments. The concentrations of macronutrient in plant tissues were markedly synergistically increased by the combined application of bio-organic- mineral phosphate fertilization. The three-way interaction treatment $A_1O_1M_2$ yielded the highest N, P and K concentrations in the cabbage plants followed by $A_1O_1M_1$. It was considered as the best treatment among the treatments under investigation. These findings demonstrate that the enhancement of nutrient availability in the root zone is associated with the enhancement of plant nutrient-use efficiency for macronutrients. An integrated fertilization management, which improves nutritional conditions of the cabbages and reduce reliance on mineral phosphate fertilizationalone on the calcareous soil, could be a practical and sustainable option.

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