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Harnessing microbial and organic inputs for enhancing sustainable cotton production and soil health

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Abstract

Cotton is a cash crop of arid to semiarid regions, and the present study investigates the integration of biofertilizers with organic amendments and balanced NPK for improving cotton growth, yields, nutritional quality, antioxidant status and soil health. Two local cotton varieties (SS32 and CK9) were evaluated in a completely randomized design (CRD) with three replications in pots. Treatments include control, recommended NPK, compost, poultry manure, biofertilizer +NPK, biofertilizer +compost +NPK and biofertilizer +poultry manure +NPK. The results revealed a significant improvement in cotton growth, nutritional and yield attributes viz., shoot length by 26 and 29%, root length by 28 and 29%, seed cotton yield by 28 and 34%, lint yield by 32 and 38%, shoot nitrogen by 26 and 32%, phosphorus by 31 and 33%, K by 26 and 29%, Zn by 33 and 34% and Fe by 27 and 34% under CK9 and SS32, respectively, by biofertilizer +compost +NPK treatment compared to control. Same treatment also improved microbial biomass carbon (MBC) by 45% and 43%, and CFU by 36% and 28%, under the CK9 and SS32 varieties, respectively. It can be concluded that integrating different nutrient sources offers sustainable cotton production while improving soil quality.

Keywords: Biofertilizers, compost, poultry manure, cotton, crop yield

Introduction

In arid and semi-arid regions of Pakistan, cotton is cultivated as the primary fiber crop. Cotton production significantly supports the agricultural and industrial sectors of cotton-producing countries, which generate vital materials for the textile industry in addition to substantial job opportunities (Rana *et al.*, 2020). Arid and semi-arid regions face significant challenges with nutrient deficiencies, particularly those with low diffusion coefficients (de Araújo *et al.*, 2022). The agricultural output of these regions primarily depends on chemical utilization, as it is considered a crucial aspect of the regional agricultural systems.

The use of synthetic fertilizers is slowly turning the productive agricultural lands to barren, non-productive soils by deteriorating soil health and plant development, which subsequently causes ecosystem destruction (Wei *et al.*, 2024). Moreover, continuous and non-judicious use of chemical fertilizers results in soil degradation and loss of soil fertility *viz.,* erosion, leaching, reduced aggregation, lower water retention, and nutrient depletion (Jaffar *et al.*, 2024). Globally, the excessive reliance of agriculture on agrochemicals is becoming an emerging threat to food production, environmental sustainability, soil degradation, soil quality deterioration and biodiversity loss (Lin *et al.*, 2019; Odewale *et al.,* 2023).

Sustainable soil health improvement and crop productivity lies in the integrated nutrient management by simultaneous application of organic amendments *viz.* biofertilizers, farmyard waste, poultry manure, directly or through processing (biochar and composting), with chemical fertilizers (Jaffar *et al.*, 2024; Pagliarini *et al.*, 2024). Organic amendments enhance the soil quality by enhancing soil carbon, which is not only responsible for flourishing soil flora and fauna but also improves the physiochemical properties of soil, *viz.* stabilization of aggregates, enhancing porosity, water holding capacity, optimization of soil pH, bulk density and improving soil water retention (Bamdad *et al.*, 2022; Mthiyane *et al.*, 2024). Moreover, biofertilizer application with organic amendments is highly promoted under organic agriculture/regenerative agriculture due to the reduction in nutrient losses through leaching, enhancing their use efficiencies and reducing reliance on chemical fertilizers (Niazi *et al.*, 2019). Another advantage of using biofertilizers is the accelerated decomposition of organic

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amendments to enhance not only carbon sequestration but also play significant role in N cycling in terms of nitrogen fixation and mineralization (Wei *et al.*, 2024).

Composting is a process of aerobic decomposition of organic amendments with microbial augmentation to form a partially decomposed and stable organic amendment (Manea *et al.*, 2024), which can reduce the rate of chemical fertilizers by readily providing nutrients to soil along with sequestering carbon in soil and enhancing soil quality (Lhaj *et al.*, 2024). Poultry manure application in soil is responsible for lowering soil compaction and deterioration, along with improving soil structure, porosity, nutritional status and soil quality to enhance crop growth and yields (Pagliarini *et al.*, 2024). Moreover, Adeli *et al.* (2019) also demonstrated that similar improvements are responsible for cotton crop stands, cotton yields and boll weight by poultry manure applications. However, the role of farmyard manure in the growth improvement of crops is due to enhancing mineral contents of crops e.g. Cu, Fe, N, K, P and their use efficiencies through reduced leaching losses and fixation under integrated systems. Moreover, the organic amendments also help in improving soil quality by stabilizing aggregates, reducing bulk density, and improving soil organic carbon and biodiversity (Obia *et al.*, 2016; Liu *et al.*, 2024).

The novelty of this study can be demonstrated under the umbrella of integrated nutrient management (INM), utilizing potential of biofertilizers, organic amendments and chemical fertilizers alone and in integration for sustainable cotton production in arid to semi-arid regions of Pakistan. Moreover, copious literature is available on their individual effects, but limited studies are available to date about the comparison of the individual and integrated effects of biofertilizers, compost and chemical fertilizers for cotton production. It was therefore hypothesized that the integration of bio and chemical fertilizers with organic amendments may synergistically enhance soil health, soil fertility, cotton growth and yield. The objective of the current investigation was to check the efficacy of integrating different biological (biofertilizers, compost, poultry manure) and synthetic nutrient sources in a pot trial to validate an INM approach for cotton production.

Materials and Methods

This study evaluates how biofertilizers (*Bacillus* sp. ZE32 and *Bacillus* sp. IA16), in combination with compost, poultry manure, and recommended NPK fertilizers, influence cotton growth, yield, nutrient uptake, antioxidant enzyme activity, and soil health.

Experimentation

The experiment was conducted in the wire house under natural conditions. Seven treatments of compost, poultry manure, and biofertilizer, along with a recommended dose of NPK and control, were used, and each was replicated three times following a completely randomized design.

The biofertilizer was prepared using *Bacillus sp.* ZE32 (MN003401) and *Bacillus sp.* IA16 (NM005924) has nutrient solubilization ability along with other plant growth-promoting traits, as reported by Iqbal *et al.* (2020) and Ahmad *et al.* (2021a). As per analysis through standard protocols (Estefan *et al.*, 2013), the soil used in the pots was

Table 1: Analysis of compost and poultry manure used for the experiment, n = 3

Characteristic	Unit	Value
	Compost	
EC	dS m^{-1}	2.01 ± 0.03
pH	-	7.40 ± 0.07
Organic matter	%	45.56 ± 1.02
Total carbon	%	26.43 ± 0.71
Total nitrogen	%	1.41 ± 0.02
C/N ratio	-	18.74 ± 0.56
Total phosphorus	%	0.31 ± 0.01
Total potassium	%	0.34 ± 0.01
Zinc	mg kg^{-1}	49.21 ± 1.23
	Poultry Manure	
EC	dS m^{-1}	3.21 ± 0.03
pH	-	6.90 ± 0.05
Moisture contents	%	22.1 ± 0.61
Organic matter	%	50.34 ± 1.34
Organic carbon	%	29.20 ± 0.68
Organic nitrogen	%	2.71 ± 0.04
C/N ratio	-	10.77 ± 0.23
Total nitrogen	%	3.27 ± 0.06
Total phosphorus	%	1.31 ± 0.03
Total Potassium	%	1.53 ± 0.05
Zinc	mg kg^{-1}	273.2 ± 4.71

deficient in nutrients, having 0.26% organic matter, 0.026% total nitrogen, 5.5 mg kg^{-1} phosphorus, and 78 mg kg^{-1} potassium. It had EC_e = 1.62 dS m^{-1}, pH = 7.7 and a sandy loam texture (Table-2).

The air-dried and sieved soil (10 kg) was filled in pots having dimensions of 18″×12″ height and diameter. Seeds of two cotton varieties (CK9 and SS32) were collected from the Regional Agricultural Research Institute, Bahawalpur. Surface sterilization of cotton seed was performed by Abd-Alla *et al.* (2012), by dipping seeds in 70% ethanol for 30 s and soaking for 3 min in 5% NaClO and rinsing six times with distilled water. The biofertilizer was prepared by inoculating the LB-Broth (1L) for each strain and incubated for 48 h at 30 ± 2 °C in a shaking incubator. After incubation, the cultures were centrifuged at 9000 *g,* and pellets were resuspended in the saline buffer. Afterwards, the optical density of the culture was maintained to 0.55 (10^6– 10^8 CFU per mL), and biofertilizer was prepared by taking equal volumes from both strains in 1:1 ratio (200 mL), glycerol (2%) and sugar solution (10%) and rest volume was filled with water up to 2000 mL. The seeds were inoculated by soaking cotton seed for half an hour in biofertilizer at the rate of 2 liters per hectare. Five cotton seeds were sown in each pot. Organic amendments (compost and poultry manure) were analyzed for physio-chemical

Table-2: Pre-sowing analysis of soil used in the experiment

Soil Characteristic	Unit	Value
Soil Texture	-	Sandy loam
Organic matter	%	0.26
EC_e	dS m^{-1}	1.62
pH	-	7.7
Total Nitrogen	%	0.26
Extractable Potassium	mg kg^{-1}	78
Available Phosphorus	mg kg^{-1}	5.5

Table 3: Effect of combined application of biofertilizers and organic amendments on growth attributes of cotton under nutrient deficit soil conditions in a pot trial

Treatment	Shoot length (cm)		Root length (cm)	
	CK9	SS32	CK9	SS32
Control	53 ± 0.45 j	53 ± 0.76 j	32 ± 0.70 gh	31 ± 0.50 h
Recommended NPK	57 ± 1.27 g-i	56 ± 1.04 ij	35 ± 0.76 e	33 ± 1.32 fg
Compost	61 ± 0.81 f-h	63 ± 0.61 e-g	36 ± 0.50 cd	36 ± 0.78 de
Poultry manure (PM)	59 ± 0.27 h-j	62 ± 0.16 f-i	36 ± 0.17 de	34 ± 0.66 f
Biofertilizer + NPK	61 ± 0.94 d-f	64 ± 1.0 de	37 ± 0.56 c	37 ± 0.57 c
Biofertilizer + compost + NPK	67 ± 0.90 ab	69 ± 0.5 a	41 ± 1.25 a	39 ± 0.36 b
Biofertilizer + PM + NPK	64 ± 0.22 cd	64 ± 0.92 bc	39 ± 0.76 b	38 ± 0.5 c
HSD ($p \leq 0.05$)	1.2842		1.2811	
	Shoot dry weight (g)		**Root dry weight (g)**	
	CK9	**SS32**	**CK9**	**SS32**
Control	83 ± 1.25 h	83 ± 2.08 h	11 ± 0.34 j	11 ± 0.48 j
Recommended NPK	89 ± 1.52 fg	87 ± 1.52 g	12 ± 0.34 g-i	12 ± 0.09 i
Compost	92 ± 1 ef	89 ± 1.52 fg	12 ± 0.29 f-h	13 ± 0.96 e-g
Poultry manure (PM)	92 ± 1.52 ef	93 ± 1.52 e	12 ± 0.22 h-j	12 ± 0.27 f-i
Biofertilizer + NPK	98 ± 2 d	99 ± 1.52 d	13 ± 0.15 d-f	13 ± 0.36 de
Biofertilizer + compost + NPK	111 ± 2.08 a	107 ± 2.08 b	14 ± 0.32 ab	15 ± 0.35 a
Biofertilizer + PM + NPK	103 ± 1.52 c	102 ± 2 c	13 ± 0.43 cd	14 ± 0.57 bc
HSD ($p \leq 0.05$)	2.9302		0.7096	

characteristics (Table 1) following the standard protocols as described by Ryan *et al.* (2001) and were applied according to the treatment plan as control (without any fertilizer), recommended NPK, compost, poultry manure, biofertilizer +NPK, biofertilizer +compost +NPK and biofertilizer +poultry manure +NPK. Synthetic fertilizers were applied according to the recommended NPK dose for cotton (310-170-110 kg ha^{-1}). All phosphatic and potassic fertilizers were applied as a basal dose, while nitrogen was applied in 3 equal splits including basal, early flowering, and early boll formation stages. Each pot received 500 mL of water every 3-4 days, adjusted based on pot moisture conditions and crop growth stage. The trial was regularly irrigated with good-quality water, and all agronomic practices were carried out as per the requirements of the cotton crop. At the flowering stage, leaf samples were collected and analyzed for antioxidant enzymes. The picking was carried out at the complete bolls opening stage, and the crop was harvested and analyzed for various growth, yield, and ionic attributes.

Plant analysis

The wet digestion of plant samples was carried out following Wolf's protocol (Wolf, 1982) by taking 0.5 g of the plant sample in a conical flask and soaked in 10 mL of concentrated H_2SO_4 overnight. After 24 h, 21 mL of 30% H_2O_2 was added to the flasks and placed on a hot plate at 350 °C for 30 minutes. Afterwards, the flasks were checked for a clear white solution after removing them from the hot plate. If the contents of the flask were not clear, another 2 mL of 30% H_2O_2 was added and placed on a hot plate for 30 minutes. The procedure was repeated until a colourless appearance of the contents. For nitrogen determination, 5 mL of the digested sample was transferred into a Kjeldahl flask and connected to a Kjeldahl distillation unit. Subsequently, 10 mL of 40% NaOH solution was added. A conical flask containing 5 mL of 4% boric acid was positioned at the receiving end. The conical flask was removed after collecting approximately 30–40 mL of distillate, and 5–10 drops of the mixed indicator were added. The content was titrated against 0.01N standard sulfuric acid, and nitrogen concentration was determined based on the pink endpoint. Phosphorus concentration in the digested plant samples was analyzed by wet digestion and measuring following the method as described by Estefan *et al.* (2013) using a UV-visible spectrophotometer (Model G6860A, Agilent Technologies, USA). Potassium concentration in the digested samples was determined using a flame photometer (Model BWB-XP, BWP Technologies, UK). Standard solutions of known K concentrations were prepared using potassium chloride, a calibration curve was prepared, and the K content in the samples was calculated. The concentrations of iron (Fe) and zinc (Zn) were quantified using an atomic absorption spectrophotometer (Model 240FS AA, Agilent Technologies, USA).

Post-harvest soil analysis

After crop harvest, rhizosphere soil samples were collected from pots and analyzed for various physico-chemical properties. Composite soil samples were air-dried and passed through a 2 mm mesh sieve. The processed samples were stored at 4 °C and analyzed within seven days. Soil organic matter content was determined following the method described by Nelson and Sommers (1982). Available phosphorus and extractable K were determined by following the standard protocols (Estefan *et al.*, 2013). The serial dilution method was used to enumerate the bacterial colony-forming units (CFU) count (Alexander, 1965). Microbial biomass carbon was assessed using the chloroform fumigation-extraction method as described by Jenkinson and Ladd (1981) and Bremner and Kessel (1990). Soil organic carbon was calculated by organic matter using the following formula given by Nelson and Sommers (1982):

$$SOC = \frac{58 * organic\ matter}{100}$$

Statistical analysis

A multivariant analysis of experimental data was performed by constructing ANOVA using a completely randomized design (Steel *et al.*, 1997) in Statistix 8.1 software, and the means were compared using Tukey's HSD at $p \leq 0.05$. The treatment means, and standard errors were calculated in Excel (MS Office 2010 and 365), while the graphs were prepared in MS Office 2010 and version 2024b of Origin Pro (Origin Lab Corporation, Northampton, USA).

Results

Biofertilizer combined with compost and NPK significantly improved cotton growth, yield, and nutrient content (macro- and micronutrients) compared to the control. It also enhanced soil physicochemical and biological properties, notably increasing microbial biomass carbon and total organic carbon.

Effect of biofertilizer in combination with organic amendments on growth and yield attributes of cotton

The biofertilizer, combined with recommended NPK and organic amendments, significantly improved the growth and yield attributes of both cotton varieties under natural

Table 4: Effect of combined application of biofertilizers and organic amendments on yield attributes of cotton under nutrient deficit soil conditions in a pot trial

Treatment	No. of bolls plant^{-1}		Seed cotton yield (g pot^{-1})	
	CK9	SS32	CK9	SS32
Control	13 ± 1.0 de	10 ± 16 g	53 ± 0.25 i	50 ± 0.80 j
Recommended NPK	14 ± 1.15 d	11 ± 0.57 fg	56 ± 1.25 fg	55 ± 1.00 gh
Compost	14 ± 0.57 cd	11 ± 1.15 fg	60 ± 1.05 d	54 ± 1.00 hi
Poultry manure (PM)	14 ± 1.00 cd	11 ± 1.0 fg	58 ± 0.64 e	57 ± 0.57 ef
Biofertilizer + NPK	15 ± 1.52 bc	12 ± 0.57 ef	63 ± 1.31 c	58 ± 1.52 ef
Biofertilizer + compost + NPK	17 ± 0.57 a	13 ± 1.0 de	67 ± 0.57 a	67 ± 1.00 a
Biofertilizer + PM + NPK	16 ± 1.00 ab	12 ± 1.0 ef	65 ± 1.00 b	62 ± 1.52 c
HSD ($p \leq 0.05$)	1.6567		1.6346	
	Lint yield (g pot^{-1})			
	CK9		SS32	
Control	16 ± 0.50 h		16 ± 0.40 gh	
Recommended NPK	16 ± 0.35 gh		17 ± 0.50 gh	
Compost	17 ± 0.72 fg		18 ± 0.35 de	
Poultry manure (PM)	16 ± 0.35 gh		17 ± 0.17 fg	
Biofertilizer + NPK	18 ± 0.50 ef		19 ± 0.76 cd	
Biofertilizer + compost + NPK	21 ± 1.00 a		21 ± 0.76 a	
Biofertilizer + PM + NPK	20 ± 0.50 bc		20 ± 1.00 b	
HSD ($p \leq 0.05$)	1.0206			

Data presented in the table are the mean values of three replications ± SE (standard error). Values with similar letterings represent non-significant results with each other at $p \leq 0.05$

conditions. Results showed that the treatment with combined application of biofertilizer, compost, and NPK significantly improved the shoot length and root length of cotton by 26% and 29% in shoot length, 29% and 28% increase in root length of CK9 and SS32 varieties, respectively, as compared to the control (Table 3). The combined application of biofertilizer, organic amendments, and NPK significantly improved root and shoot dry biomass (Table 3). Similar treatment showed a 34% and 30% increase in shoot dry biomass, while 29% and 35% in root dry biomass of CK9 and SS32, respectively, as compared to the control.

The results in Table 4 demonstrated that the combined use of biofertilizers, organic amendments, and synthetic fertilizers significantly enhanced cotton yield contributing parameters, including the number of bolls per plant, seed cotton yield, and lint yield, as compared to their sole applications. Significantly highest bolls plant^{-1} were recorded in the CK9 variety (17 bolls) and SS32 variety (13 bolls) under biofertilizer +compost +NPK treatment, followed by biofertilizer +poultry manure +NPK treatment, while the sole application of compost was significantly more effective than other individual treatments as compared to control. Similarly, seed cotton yield showed the maximum significant increase under biofertilizer +compost +NPK treatment, which was 28% and 34% increase and in lint yield 38% and 32% increase in CK9 and SS32, respectively, over the control.

Effect of biofertilizer in combination with organic amendments on mineral contents in cotton

The combined application of biofertilizers, organic amendments, and synthetic fertilizers significantly enhanced the nitrogen, phosphorus, and potassium contents in the shoots of both cotton varieties (CK9 and SS32) compared to their sole applications (Figure 1). The highest significant increase in nitrogen concentration was observed under biofertilizer +compost +NPK treatment, which was 32% and 26% in CK9 and SS32, respectively, compared to control (Figure 1-A). Similarly, the significant highest nitrogen contents in roots were observed under biofertilizer +compost +NPK treatment, resulting in a 28% and 29% increase in CK9 and SS32, respectively, compared to control (Figure 1-B).

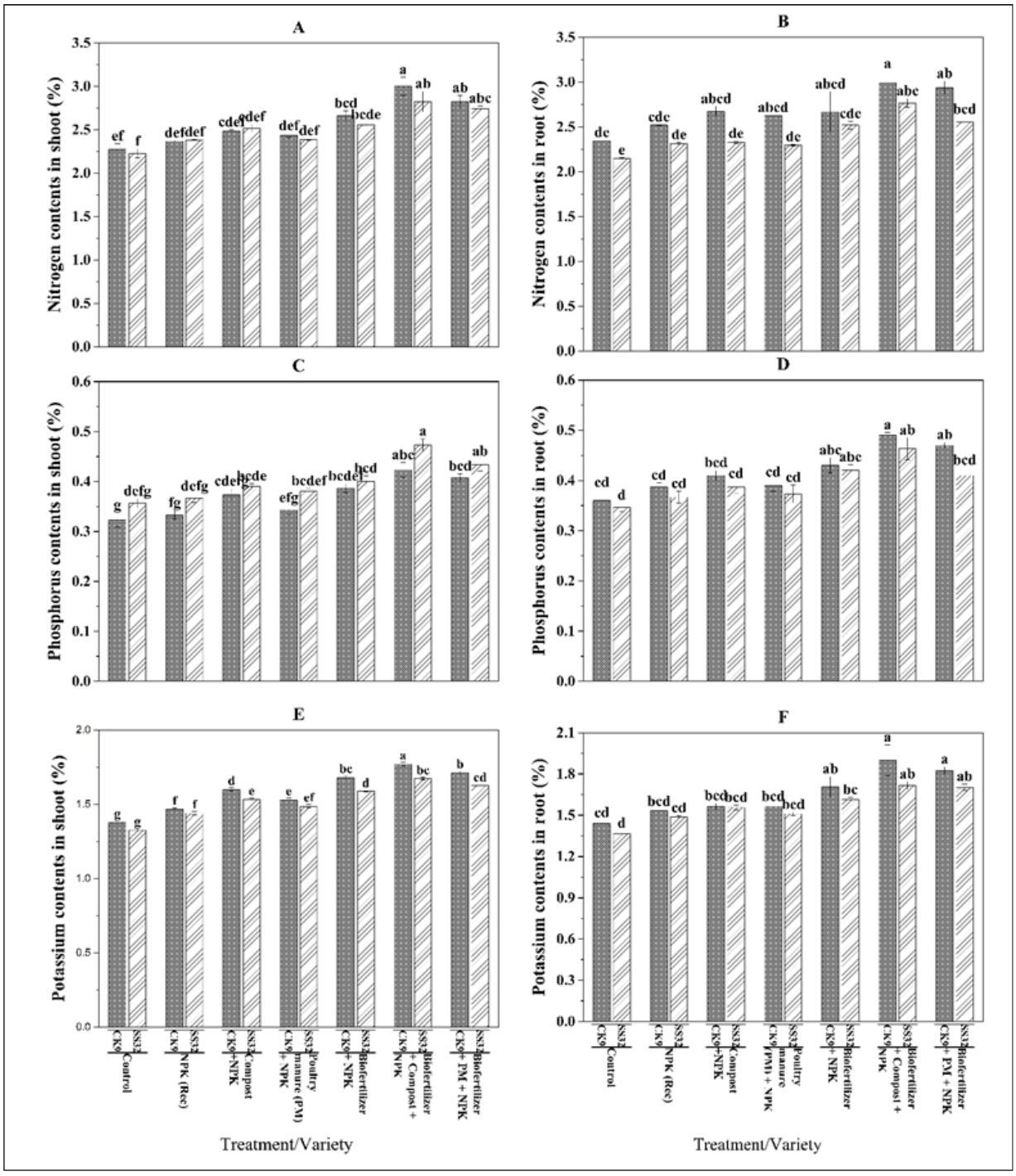


Figure 1: Effect of combined application of biofertilizers and organic amendments on (A) nitrogen contents in the shoot, (B) nitrogen contents in the root, (C) phosphorus contents in the shoot, (D) phosphorus contents in the root, (E) potassium contents in the shoot, (F) potassium contents in root of cotton under nutrient deficit soil conditions in a pot trial. Bars with the same letters show non-significant results at $p \leq 0.05$

The results regarding phosphorus concentration in the shoot showed a significant increase in biofertilizer +compost +NPK treatment with 31% increase in CK9 and 33% in SS32 cotton varieties (Figure 1-C). The maximum phosphorus concentration in the cotton root was also measured in the same treatment (Figure 1-D), showing a significant increase of 36% in CK9 and 34% in SS32 cotton varieties. Similarly, the shoot's potassium contents were also enhanced by biofertilizer +compost +NPK treatment causing a significant increase of 29% and 26% in CK9 and SS32, respectively (Figure 1-E). The results (Figure 1-F) showed that potassium concentration in roots followed a similar trend to that of the other two macronutrients, with biofertilizer +compost +NPK treatment exhibiting the highest significant increase (32% in CK9 and 26% in SS32).

The Fe contents in cotton shoots and roots were significantly enhanced under biofertilizer +compost +NPK treatment as compared to control plants. The combination gave a 27% increase in shoot Fe contents in CK9 and a 34% increase in the SS32 shoot compared to control plants (Figure 2-A). Similarly, the integrated treatment (biofertilizer +compost +NPK) also caused a significant increase of 30% and 33% increase in root Fe contents in CK9 and SS32, respectively, over the control (Figure 2-B).

The combined application of biofertilizers, organic amendments, and synthetic fertilizers significantly enhanced the zinc concentration in the shoot and roots of both cotton varieties (CK9 and SS32) compared to their sole applications. The integrated treatment (biofertilizer

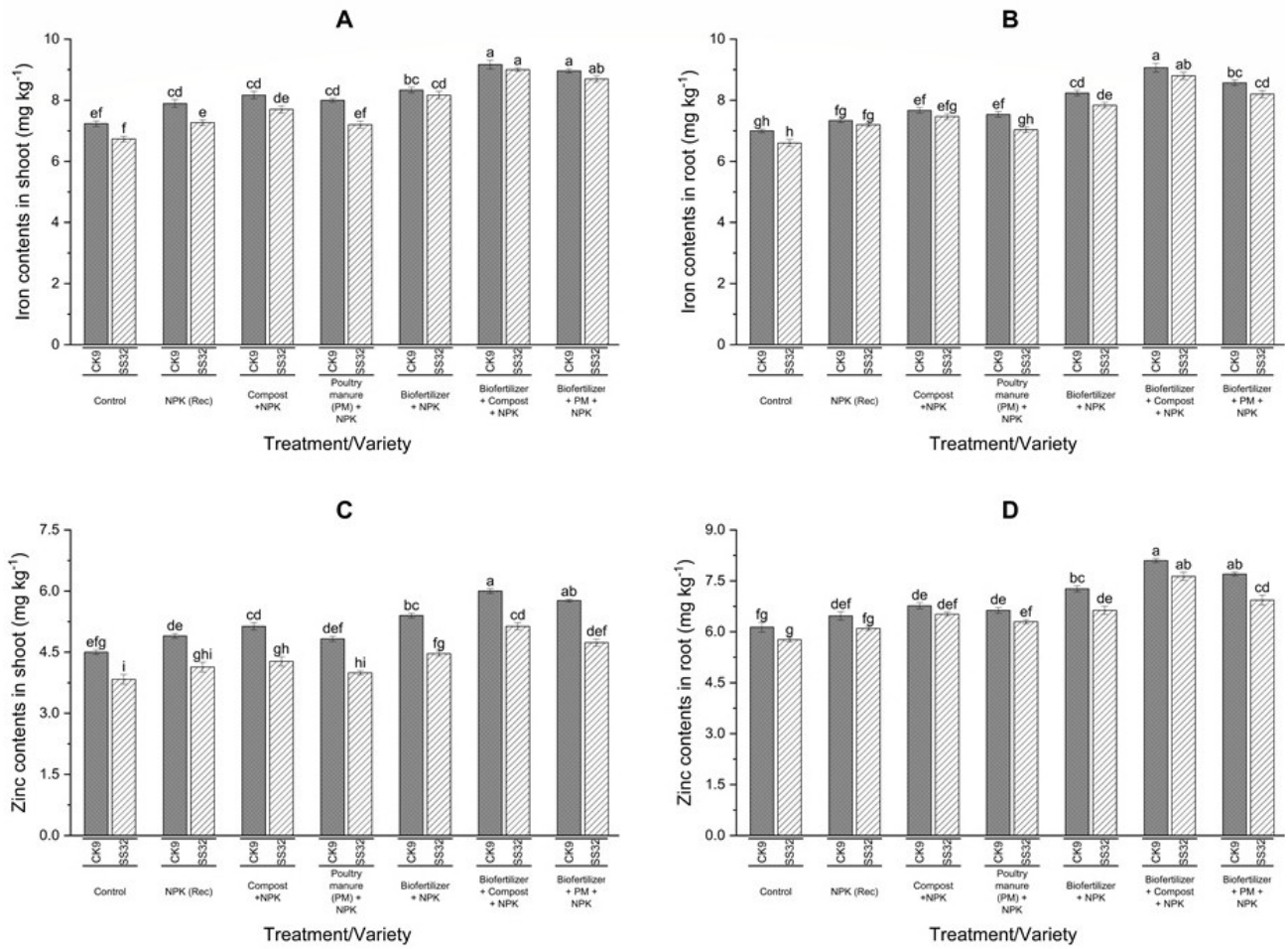


Figure 2: Effect of combined application of biofertilizers and organic amendments on (A) iron contents in the shoot, (B) iron contents in the root, (C) zinc contents in the shoot, (D) zinc contents in the root of cotton under nutrient deficit soil conditions in a pot trial. Bars with the same letters show non-significant results at $p \leq 0.05$.

+compost +NPK) gave 33% and 34% significant increase in Zn concentration in shoots of CK9 and SS32, respectively, over the control treatment (Figure 2-C). Similarly, Zn concentration in roots of CK9 and SS32 was significantly enhanced due to integrated treatment (biofertilizer +compost +NPK) which was 32% and 32%, respectively, over the control (Figure 2-D). The application of compost and poultry manure in combination with NPK fertilizers demonstrated better efficacy than NPK alone, highlighting their potential as effective nutrient sources.

Effect of biofertilizer in combination with organic amendments on soil health

The combined application of biofertilizers, synthetic fertilizers (NPK), and organic amendments demonstrated better efficacy in enhancing various soil physicochemical and biological properties under both CK9 and SS32 cotton varieties. Total soil nitrogen contents were significantly enhanced by the combined application of biofertilizer +compost +NPK, with a 30% increase in CK9 and a 30% increase in SS32, as compared to the control treatment (Figure 3-A). The maximum significant increase in soil available phosphorus and extractable potassium under CK9 was 29% and 27%, while under SS32 it was 29% and 28%, respectively, with biofertilizer +compost +NPK treatment as compared to control (Figure 3-B and 3-C). The data regarding soil organic matter (Figure 3-D) also showed a significant increase in soil organic matter by 27% in both varieties CK9 and SS32 under biofertilizer +compost +NPK treatment as compared to the control.

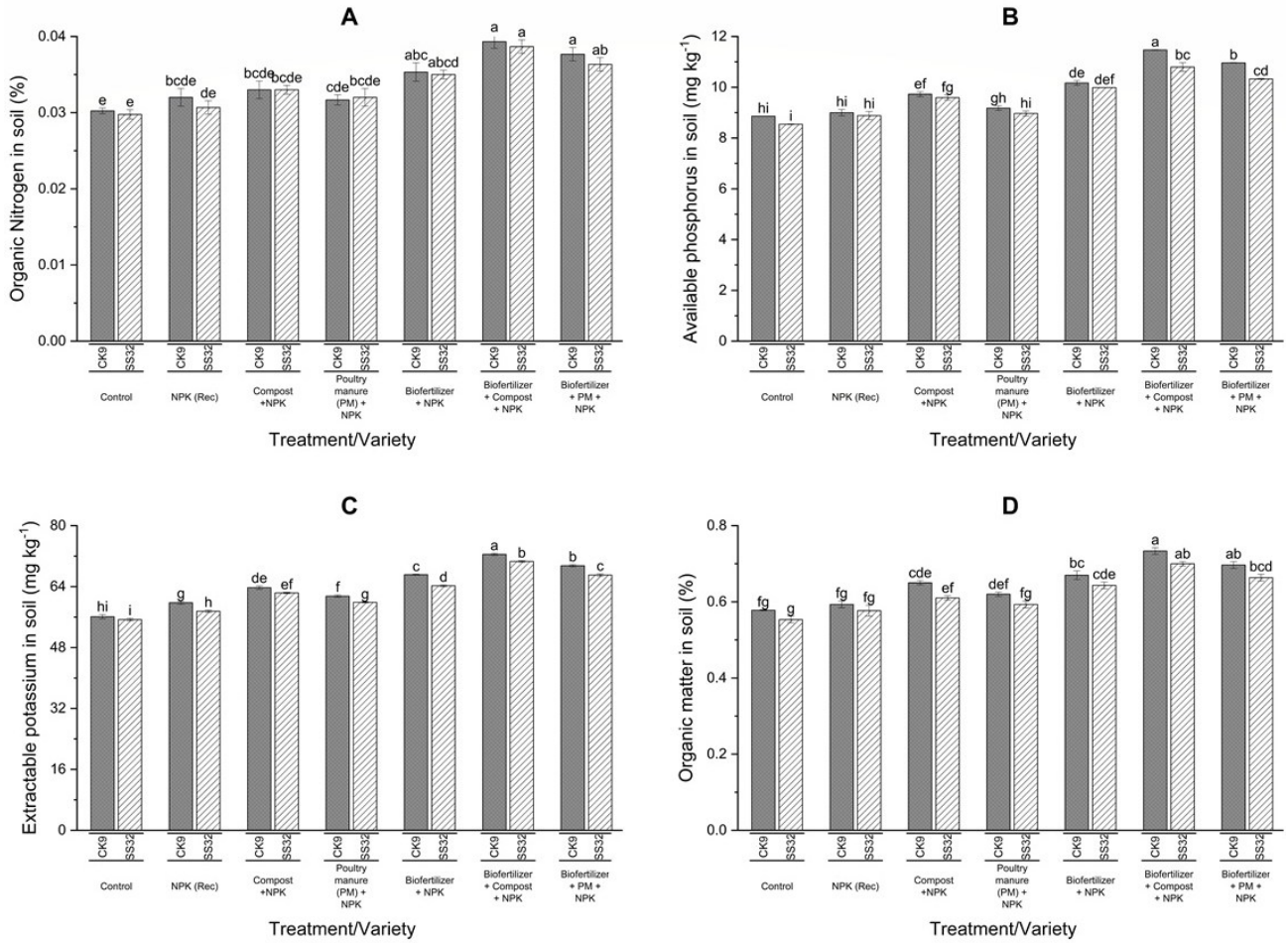


Figure 3: Effect of combined application of biofertilizers and organic amendments on (A) organic nitrogen, (B) available phosphorus, (C) extractable potassium, and (D) organic matter in the soil under cotton in nutrient deficit soil conditions in a pot trial. Bars with the same letters show non-significant results at $p \leq 0.05$

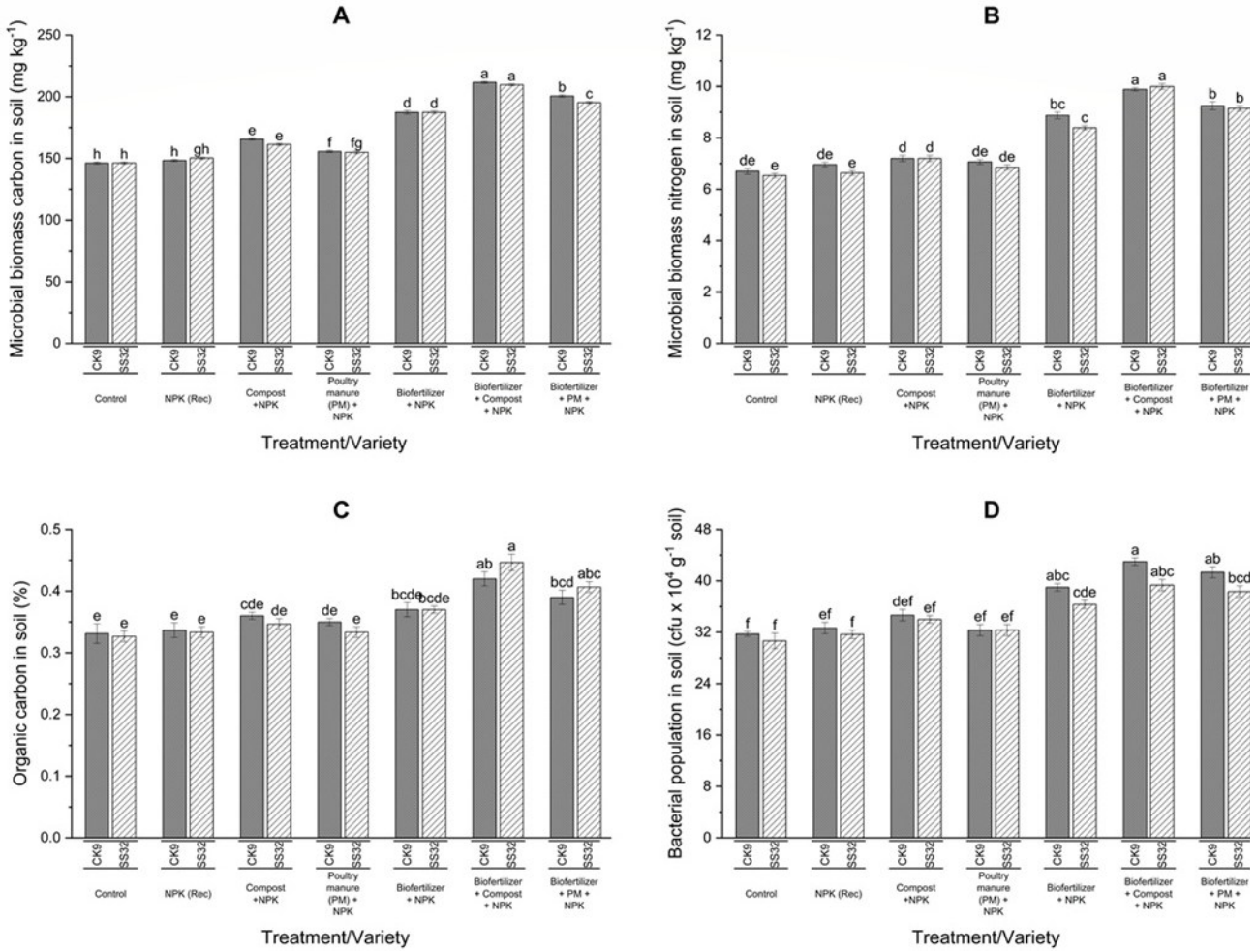


Figure 4: Effect of combined application of biofertilizers and organic amendments on (A) microbial biomass carbon (MBC), (B) microbial biomass nitrogen (MBN), (C) organic carbon, and (D) bacterial population in the soil under cotton in nutrient deficit soil conditions in a pot trial. Bars with the same letters show non-significant results at $p \leq 0.05$

Microbial biomass carbon contents also increased significantly by 45% and 43% in soil under CK9 and SS32, respectively, as compared to the control, where biofertilizer +compost +NPK treatment was applied (Figure 4-A). Similarly, microbial biomass nitrogen contents were significantly improved with the same treatment having biofertilizer +compost +NPK (Figures 4-B and 4-C). Furthermore, bacterial population dynamics in soil indicated a significant increase of 36% in soil under CK9 and 28% in soil under SS32 over the control by integrated application of biofertilizer +compost +NPK (Figure 4-D). These findings collectively highlight that the synergistic use of biofertilizers, organic amendments, and synthetic fertilizers is more effective in improving soil fertility and microbial activity than the individual application of fertilizers.

Discussion

Effect of biofertilizers in combination with organic amendments on growth and yield attributes of cotton crop

The present study reports that biofertilizers (*Bacillus sp.* ZE32 and *Bacillus sp.* IA16) in combination with organic amendments (compost and poultry manure) and NPK fertilizers significantly enhanced growth and yield in two cotton varieties (CK9 and SS32) over control under arid

conditions. The results of this investigation are in line with the findings of Mthiyane *et al.* (2024), who demonstrated that this growth enhancement is attributed to the balanced nutrients uptake and chlorophyll improvement in plants under biofertilizer and organic fertilizer application. The higher nutrient uptake and fertilizer use efficiencies might be due to the nutrient solubilization mechanism of biofertilizers (Schütz *et al.,* 2018). Zhao *et al.* (2025) also demonstrated that the addition of compost improves soil physical (aggregation, porosity, structure) and chemical (pH, EC, soil solution chemistry) conditions for better root proliferation, nutrient uptake, cotton growth and ultimately improve yield . The enhancement in plant height and number of bolls per plant observed under the biofertilizer + compost + NPK treatment can be attributed to the presence of beneficial microorganisms in biofertilizers, including nitrogen-fixing bacteria such as *Rhizobium* and *Azospirillum*, as well as phosphorus-solubilizing bacteria like *Pseudomonas* and *Bacillus* (Shahwar *et al.,* 2023). These microorganisms enhance nutrient acquisition by facilitating the solubilization and availability of essential soil nutrients (Liu *et al.*, 2024), thereby improving plant growth and development (Iniesta-Pallarés *et al.*, 2021).

Morash *et al.* (2024) also demonstrated that yield enhancement through enhancing nutrient use efficiencies might be due to the application of organic fertilizers along with mineral fertilizers which enhances the accessibility of macronutrients (N, P, K), and soil organic carbon (SOC). Several research studies have shown organic amendments used with synthetic fertilizers lead to increased yields of seed cotton and lint according to Adeli *et al.* (2022), Wang *et al.* (2024) and Eliş *et al.* (2026). A study by Shi *et al.* (2023) showed that the combined application of organic and chemical fertilizers increased seed cotton yield in cotton by up to 21%. Another study found that the number of tillers, length of the spike, grain production, and grain weight in rice were significantly improved by biofertilizer integration with chemical fertilizer (Chen *et al.*, 2023).

Effect of biofertilizers in combination with organic amendments on mineral contents of cotton crop

The combination of biofertilizers and organic amendments with NPK fertilizers led to elevated levels of macronutrients (N, P, K) and micronutrients (Zn, Fe) in plant shoots and roots. Organic amendments undergo decomposition and support native and applied microbial communities, providing a consistent supply of nutrients to cotton, leading towards growth, nutritional status, and yield improvement, which might be attributed to minimizing nutrient loss and improving their availability (Ahmad *et al.*, 2022). Ho *et al.* (2022) also demonstrated a similar impact of composted manure application for improving plant's potassium and phosphorus contents. Similarly, Adeli *et al.* (2019) demonstrated that the decomposition of organic amendments might be a reason for boosting essential plant nutrients N, P, K, Zn, Fe, Mn, and Cu in the cotton crop. The findings regarding phosphorus content enhancement under integrated treatment in cotton root and shoot are aligned with the findings of Hussain *et al.* (2020), Bamdad *et al.* (2022) and Peng *et al.* (2025), who demonstrated that the improved nutrient cycling in soil and plant uptake through synergistic microbial interactions with organic amendments. Biofertilizers exhibit several traits for improving plants' growth, including N-fixation, nutrient-solubilization, exopolysaccharides and siderophores production, abiotic stress alleviation, weed inhibition and pathogen protection (Ahmad *et al.*, 2021b; Paul *et al.*, 2024). Hoover *et al.* (2019) clearly demonstrated that the long-term application of poultry manure results in better plant growth development, which might be attributed to the immediate provision of both macro- and micronutrients upon decomposition by native biodiversity.

Overall, our findings suggest that the combined use of organic amendments and bio-fertilizers is an effective approach for improving soil nutrient availability and boosting cotton productivity. Singh *et al.* (2024) also demonstrated that the replacement of inorganic fertilizers with organic fertilizers improves soil structure, which in turn enhances nutrient availability, soil enzymatic activity, and soil biodiversity. Ghazanfar *et al.* (2024) also witnessed that bacterial biofertilizers resulted in increased nutrient uptake, especially Fe in maize crops. The results were also in agreement with the findings of Ozdemir *et al.* (2008), who also reported that increased leaf Fe, Zn, Cu, and Mn concentrations are directly linked with the application of organic nutrient sources in grapevine. The increased nutrient concentration in the shoot may be due to the enhancement of the microbial diversity under organic amendments applied to pots, which is responsible for producing organic acids, *viz.,* citric acid, gluconic acid, and oxalic acid in soils and solubilizing P and K by cation exchange or by dissolving their Al and Fe complexes (Hasan *et al.,* 2021).

Effect of biofertilizers in combination with organic amendments on soil health

Application of organic amendments and biofertilizers along with synthetic fertilizers significantly enhanced available soil total nitrogen, phosphorus, potassium, MBC, MBN, and SOM. The results of the current investigation are in line with the findings of Padbhushan *et al.* (2015), who observed similar variations in soil MBC under different

organic amendment applications. The slower decomposition rate of organic amendments improves soil physicochemical attributes *viz.,* soil aggregation, porosity, along with reduced bulk density are responsible for better growth and nutritional status of soil (Rehman and Thengane, 2025). Haider *et al.* (2015) presented that poultry manure and biochar application significantly increase available soil N and maize yield by improving plant water relations. Increased available soil phosphorus might be attributed to improved soil properties through the addition of P upon decomposition of poultry manure and compost (Zhang et al., 2023). Using organic amendments is key for improving soil health and fertility by building up organic carbon, flourishing biodiversity and solubilizing entrapped minerals. Moreover, the carbon of organic amendments is translated into microbial biomass carbon, as described by Tejada and Benitez (2011), Kumari *et al.* (2011) and Wang *et al.* (2022). Combining organic and inorganic fertilizers balances the uptake and buildup of soil fertility through improving soil total nitrogen, available phosphorus, extractable potassium and soil organic carbon (Iqbal *et al.*, 2019; Wang *et al.*, 2024)

Conclusion

The results of the current investigation demonstrated that the integrated nutrient management (INM) approach in biofertilizer + compost + NPK treatment performed best, significantly enhancing cotton growth, yield, nutrient uptake, and soil biological properties compared to other treatments. These findings will lay a strong foundation for testing the said INM formulation under field conditions for ensuring its practicality for commercialization as a robust strategy for achieving high cotton yields while maintaining long-term soil fertility in arid and semi-arid agroecosystems under the current climate change scenario.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data availability statement

The data set presented in this manuscript is also available at a reasonable request from the corresponding author.

The data regarding molecular identification of strains is available online on the NCBI website as:

1. Bacillus megaterium strain ZE32 under accession No. (MN003401):
https://www.ncbi.nlm.nih.gov/nuccore/MN003401.1/.

2. Bacillus sp. strain IA16 under accession No. (MN005924):
https://www.ncbi.nlm.nih.gov/nuccore/MN005924.1/.

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