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ORIGINAL ARTICLE

Synergistic effects of *Rhizobium*, *Bacillus* and Arbuscular Mycorrhizal fungi on enhancing cotton growth

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ABSTRACT

Background and Objectives: Telangana district is renowned for its prominence in cotton production, a crop vital to the livelihoods of local farmers. For years, synthetic fertilizers have been relied upon to bolster yields, but escalating costs have shifted focus towards biofertilizers as a cost-effective and sustainable alternative.

Materials and Methods: A microbial consortium comprising *Rhizobium* sp. PKS, *Bacillus* sp. PU-7, and *Funneliformis mosseae* AMF was employed. Microbial identification was performed using 16S rRNA gene sequencing. Biochemical evaluations of consortium-inoculated plants included measurements of protein, sugar, proline, and chlorophyll levels, along with IAA quantification.

Results: A consortium of *Bacillus* sp. PU-7, *Rhizobium* sp. Pks [NCBI OK663003, NCMR-MCC4960], and *Funneliformis mosseae* enhanced Mahyco cultivar growth. Treatment increased plant height, fresh and dry weight, and improved biochemical profiles (reduced proline, elevated IAA, protein, chlorophyll, and sugars). Soil field trials were undertaken in four cotton-producing regions of Mahabubnagar region confirmed for efficacy, with deep black soil promoting phytohormone synthesis (IAA-917.66 ± 2.51) and light black soil (IAA- 802 ± 2) enhancing plant growth.

Conclusion: Given these outcomes, the application of the tested bioinoculants and AMF spores is suggested as an effective strategy to enhance cotton development and yield in the soils of Mahabubnagar, potentially revolutionizing the district's agricultural practices.

Keywords: *Funneliformis mosseae*; *Bacillus*; *Rhizobium*; Microbial consortia; Phytohormones

INTRODUCTION

Cotton, a member of the *Gossypium* genus, stands as a globally important crop, prized for its fibers and oils. In southern India, it is primarily grown during the winter season. Traditional cotton farming practices are heavily dependent on the application of agrochemical practices that have been linked to ecological deterioration and health issues (1). As a response to these concerns, there has been a surge of interest in utilizing mycorrhizae and rhizobiotics to enhance crop yields sustainably (2).

Cotton production remains significant, yet the escalating costs of fertilizers necessitate a shift towards more cost-effective approaches. (3). It is well-known that rhizobiotics and mycorrhizae play crucial roles in enhancing vegetative growth of plant and nutrient uptake (4), with particular emphasis on the *Rhizobium* and *Bacillus* genera (5), alongside AM fungi (6). Lately, there has been a growing fascination with investigating how these advantageous microorganisms influence the growth of cotton plants. Implementing bioinoculants or biofertilizers presents a viable solution for eco-friendly farming (7-9). *Bacillus* and

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Rhizobium microbial inoculants have shown promising results in enhancing yield across various crops, including cotton (10, 11). These inoculants offer protection against root pathogens through the generation of various microbial metabolites such as iron chelators, contributing to the resilience of plants against environmental stressors (12), along with facilitating plant development regulated by heteroauxins (IAA), the process of nitrogen cycling, and the assimilation of phosphorus (P) and other vital minerals. This has been underscored in various studies (13-15).

However, while the individual benefits of these microorganisms are documented, the collective and potentially synergistic effects of such consortia on cotton growth have yet to be fully elucidated (12).

This study fills this knowledge gap by examining the simultaneous inoculation of cotton plants with *Rhizobium*, *Bacillus*, and AM fungi. This research aims to assess the implications of this microbial consortium on the characteristics of a cotton plant, such as the elevation of the shoot and root extent, leaf area, and biomass, alongside nutrient uptake, especially nitrogen and phosphorus (2).

MATERIALS AND METHODS

The experiments took place at the Microbiology Department, Palamuru University, situated in Mahabubnagar, Telangana, India in the year 2022.

Sample procurement. *Rhizobium* assessment began with collecting cotton rhizosphere soil near Eturnagaram forest. The top layer method isolated *Rhizobium*, followed by diluting the soil to 10^{-4} and culturing on YEMA (Yeast Extract Mannitol Agar) medium served as the growth substrate for analytical investigations.

Soil collected from the cotton rhizosphere vicinity of Palamuru University's male student housing was examined for *Bacillus* species. A serial dilution process was carried out, following which a dilution of 10^{-4} was plated on nutrient agar for the purpose of growth. Mycorrhizal spores were isolated by collecting rhizosphere soils from six forest locations: Amrabad-Nagarkurnool, Bhadrachalam-Khammam, Eturunagaram-Warangal, Jannaram-Adilabad, Kataram-Karimnagar, and Narsapur-Medak. The specimens were safely enclosed and conveyed to the lab for analysis.

Preparation of Homogenized Soil Mixture (HMS) for mycorrhizal isolation. To create a standardized soil matrix, soil samples from various locations were aggregated and thoroughly blended to produce a uniform Homogenized Soil Mixture (HMS). This process entailed rigorous mixing, followed by removal of extraneous debris, including roots, pebbles, stones, and gravel. The quartering method was then applied to reduce the bulk soil volume to the desired quantity, ensuring representativeness (16, 17). The resulting HMS served as the foundation for isolating highly efficient mycorrhizae tailored to the Mahyco cultivar of *Gossypium herbaceum*.

Uniform cultures of *Rhizobium* and *Bacillus* variants:

Inoculant preparation. Flasks containing broth culture medium were inoculated with 1 mL of overnight bacterial culture, corresponding to an optical density (OD600) of 0.8-1.0, equivalent to approximately 10^{-8} colony-forming units (CFU)/mL.

Peat was sterilized at a temperature of 121°C and a pressure of 15 psi for a duration of one hour. *Rhizobium* and *Bacillus* sp. broth cultures were incorporated into autoclaved peat at a proportion of 100 ml per kilogram of peat.

The microbial inoculum consisted of *Rhizobium* and *Bacillus* strains, with a bacterial density of 10^{-8} cells/g of peat, as determined by MPN analysis, was utilized for the coating of cotton seeds. These seeds, once coated, were sown and nurtured for a period of 60 days in soil that had been sterilized (18).

Sterilized soil formulation. The sterilization process began with combining sand and red soil in equal proportions, followed by thorough mixing to ensure consistency (18). The resulting blend was then subjected to autoclaving at 121°C and 15 psi for sterilization.

Genomic DNA extraction. Genomic DNA was extracted from *Bacillus* and *Rhizobium* bacterial cells using a modified protocol (19). Bacterial cultures were centrifuged at 12,000 rpm for 2 minutes to collect cell pellets. These pellets were then resuspended in 600 µl of lysis solution containing 10 mM Tris-HCl, 1 mM EDTA, 0.5% SDS, and 100 µg/ml proteinase K (pH 7.5). After adding 5 M NaCl and CTAB/NaCl buffer, the mixture was incubated at 37°C for 1 hour. Subsequent incubations at 65°C for 10 minutes and cooling

to room temperature helped to facilitate cell lysis.

The aqueous phase was separated using chloroform:isoamyl alcohol (24:1) and phenol:chloroform:isoamyl alcohol (25:24:1), followed by centrifugation. DNA precipitation was achieved by adding 0.69 volumes of isopropanol and centrifuging. Finally, the isolated DNA pellets were vacuum-dried and rehydrated in Tris buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) for downstream applications.

PCR study. Amplification of the 16S rRNA gene was performed using universal primers on each sample's culture DNA. The 50 µl PCR reaction mixture consisted of 4 µl of bacterial DNA (~200 ng), 1 µl of Taq DNA polymerase, 5 µl of Taq buffer, 5 µl of 2 mM dNTP mix, 5 µl of forward primer (10 pmol/µl), and 5 µl of reverse primer (10 pmol/µl).

Thermal cycling was conducted in a Bio-Rad thermocycler, comprising 30 cycles of denaturation (94°C, 20 s), annealing (48°C, 20 s), and extension (72°C, 40 s), followed by a final 5-minute extension at 72°C.

The resulting ~1,542 bp DNA fragment was resolved on a 1% agarose gel and subsequently purified using Qiagen spin columns (20, 21).

16S rRNA gene-based phylogeny of *Bacillus* and *Rhizobium* species. For 16S rRNA gene sequencing and phylogenetic analysis of *Bacillus* and *Rhizobium* isolates, DNA was extracted using the Mo Bio microbial DNA isolation kit (22). The 1,502 nucleotide 16S rRNA gene sequences were subjected to BLAST and EzTaxon searches to identify nearest taxa (23, 24). Sequences from the Bacillaceae and Rhizobiaceae families were retrieved from the NCBI database, aligned using CLUSTAL_X (25), and manually corrected. Phylogenetic trees were constructed using maximum likelihood (PhyML) (26), and neighbor-joining (27) (PHYLIP v3.5) methods (28), with tree topologies evaluated by bootstrap analysis (1,000 resamplings) using SEQBOOT and CONSENSE programs. Pairwise evolutionary distances were computed using DNA DIST with the Kimura 2-parameter model (29).

Growth and metabolic characteristics of *Bacillus* sp. PU-7 and *Rhizobium* sp. PKS. Phenotypic characterization of *Bacillus* sp. PU-7 and *Rhizobium* sp. PKS was performed to determine their cellular and physiological properties. Cell morphology and motility were examined using light microscopy, with motility assessed on TSA medium. Growth characteristics, including temperature tolerance, salt sensitivity, and pH range (using buffered NA medium with citric acid-NaOH, phosphate, glycine-NaOH, or Tris buffer), were evaluated. Biochemical properties, carbon assimilation, H2S production, and antibiotic susceptibility were investigated using established methods (30, 31) and commercial kits (Hi25TM Enterobacteriaceae identification and HiCarbohydrateTM kits). The isolates' metabolic traits, temperature tolerance, and antibiotic resistance were also assessed.

Microbial interaction study. A cross-streak method. The cross-streak technique was used to evaluate microbial compatibility, allowing for the identification of potential antagonistic interactions between bacterial strains (32).

Auxin (IAA) synthesis by *Rhizobium* sp. PKS and *Bacillus* sp PU-7. The procedure developed by Brick (33) was employed to evaluate IAA synthesis, with pink coloration serving as a visual indicator of the reaction.

Siderophores synthesis by *Rhizobium* sp. PKS and *Bacillus* sp PU-7. The formation of siderophores was evaluated using a CAS agar plate assay, as described by Schwyn and Neilands (34). The development of an orange-colored zone around the colonies served as a visual indicator of siderophore production.

Phosphate dissolution of *Rhizobium* sp. PKS and *Bacillus* sp PU-7. For this experiment, a pH 7 solution was prepared with specific ingredients like yeast extract, glucose, tricalcium phosphate, actidione and agar. This solution was used to inoculate Petri dishes that contained *Rhizobium* sp. PKS and *Bacillus* sp. PU-7. These dishes were then incubated at a temperature of 28°C for a period of 5 days. The appearance of transparent areas surrounding the colonies suggested the occurrence of phosphate dissolution (35).

Assessment of biochemical properties in cotton plants treated with *Rhizobium* sp. PKS and *Bacillus* sp PU-7: Total protein assay. For the purpose of total protein extraction, 0.5 grams of plant material was mixed with 10 mL volume of 0.2 M perchloric acid solution. The precipitate was subjected to two consecutive extractions with a mixed solvent system consisting of ethanol, ether, and chloroform at a volume ratio of 2:2:1, this was succeeded by a

centrifugation process at 5,000 gravities for a span of 10 minutes at a temperature of 24°C. The remaining residue was subjected to alkaline hydrolysis with 0.2 M NaOH for an extended period (overnight). The supernatant obtained was then used for the estimation of total proteins (36).

Sugar quantification. The process entailed warming 1 gram of plant material with a 0.2% solution of anthrone reagent, followed by the determination of the optical density at 625 nm utilizing a UV-VIS spectrophotometer, specifically the Spectronic D20 model in accordance with the method put forth by Mahadevan and Shridhar (37).

Proline quantification. The method for estimating proline was adapted from previously established protocol (38). A quantity of 0.5 g of fresh leaf tissue was blended with 5 milliliters of 3% solution of sulphosalicylic acid. Post centrifugation, the supernatant was sieved and combined with ninhydrin and acetic acid. The reaction was halted by the addition of toluene after a period of incubation. Colorimetric determination of proline concentration yielded results expressed in milligrams per gram (mg/g).

Chlorophyll assay. Chlorophyll was isolated from a gram of cotton foliage that had been treated with *Rhizobium* sp. PKS and *Bacillus* sp PU-7. The extraction process involved the use of 80% acetone, adhering to the established protocol (39). Filtration was done in the dark. The total chlorophyll content was ascertained by gauging the optical density (OD) at wavelengths of 650 nm and 663 nm, utilizing a UV-VIS spectrophotometer. This measurement was conducted in accordance with Arnon's equation.

***Rhizobium* sp. PKS and *Bacillus* sp PU-7-induced phytohormone synthesis in cotton plants:**

Measurement of IAA levels. A gram of leaf substance was pulverized in conjunction with 1 mL volume of phosphate buffer to create the test preparation. Following centrifugation, the supernatant was treated with two droplets of perchloric acid, which increased the volume to 2 ml, suitable for the Salkowski reagent. After a duration of 25 minutes, the optical density (OD) readings were taken at a wavelength of 530 nm with the aid of a UV-VIS spectrophotometer. The standard graph was constructed by charting the quantity of IAA, expressed in micrograms per milliliter (µg ml^{-1}) against the optical density (OD) measured at 530 nm.

Soil Sample homogenization process. Upon receiving soil samples, the removal of debris was done in the lab. The bulk soil was reduced using the quartering technique, serving as a source for isolating mycorrhizae for the Mahyco cultivar of *Gossypium herbaceum*. Seeds were washed before sowing to remove impurities and bacteria.

Evaluation of mycorrhizal root colonization. Roots were cleaned and fixed in FAA solution, then treated with KOH and autoclaved. After acidification and staining with lactophenol trypan blue, infection percentage was determined by using Giovannetti and Mosse formula (40).

Funnelling trial. Mahyco cotton cultivar was planted by sowing *Funneliformis mosseae* spores in funnels filled with sterilized soil and supplemented with (41).

Extraction and identification of fungal spores. 100 grams of soil were mixed with 1 liter of water, settled, and filtered through sieves of varying sizes. Residues were rinsed onto a filtration sheet, and spores were selected using microscopic observation for identification (42).

Collection of soil samples from diverse cotton farming areas. Soil samples from four distinct cotton fields in Mahabubnagar District were collected. These included shallow black soil from Malleboinpally, red soil from Makthal, deep black soil from Kalwakurthy, and sandy soil from Narayanpet.

Structural and compositional properties of soil in cotton cultivation regions. The available nitrogen in the soil was measured using the alkaline potassium permanganate method (43). The available phosphorus was assessed following the Bray and Kurtz (44) method, while potassium levels were determined using flame photometry according to Jackson (45).

RESULTS

The rhizosphere soil from Eturnagarm forest under-

went testing to isolate efficient *Rhizobium* sp. PKS through the top layer approach which involves the removal and examination of the uppermost layer of soil where *Rhizobium* is most likely to be found (18). Following this, the soil was diluted to a ratio of 10^{-4} and grown on YEMA medium for further study. Additionally, *Bacillus* sp. PU-7 was obtained from the cotton rhizosphere vicinity of Palamuru University's male student housing. Notably, this strain has been associated with improved cotton plant growth in Mahabubnagar District (5).

This investigation focuses on developing a method for isolating host-specific mycorrhizal spores to promote the growth of *Gossypium herbaceum*, leveraging the Homogeneous Mixture of Soil (HMS) technique. The HMS technique enables targeted collection and propagation of mycorrhizal spores, optimizing the symbiotic partnership between the identified *Funneliformis mosseae* spores and Mahyco cotton cultivar.

Research highlights the crucial role of Arbuscular Mycorrhizal Fungi (AMF) in cotton plant development, as demonstrated by studies Sultana and Pindi (46). Moreover, AMF inoculation enhances plant resilience under adverse conditions (47).

In our current study, our primary objective is to create a consortium of bioinoculants and spores specifically tailored for enhancing cotton plant growth in Mahabubnagar District. This region faces challenges such as low rainfall and drought conditions.

We cultured *Rhizobium* sp. PKS and *Bacillus* sp. PU-7 using specific media outlined in the Materials and Methods section. Subsequently, we prepared liquid inoculums of the isolates, serially diluting them to achieve a cell density of 10^{-8} CFU per ml. The isolated strains were tested for their capacity to improve plant growth and development (Table 1).

Utilizing the cross-streak method, we assessed compatibility between two strains *Rhizobium* sp. PKS and *Bacillus* sp. PU-7 which were potential candidates for developing a poly-bioinoculant. Notably, no zone of inhibition was observed in their colonies.

Table 1. Plant growth-enhancing properties of *Rhizobium* sp. PKS and *Bacillus* PU-7

Traits	*Rhizobium* sp PKS	*Bacillus* sp pU-7
Phosphate solubilization assay	+	+
Auxin (IAA) production assay	+	+
Siderophore synthesis assay	+	+

This finding indicates that the isolates did not exhibit antagonistic effects toward each other. In light of this, it appears that all strains are compatible with one another.

Characterization of novel *Bacillus* sp. PU-7 and *Rhizobium* sp. PKS. *Bacillus* sp. PU-7 and *Rhizobium* sp. PKS are two distinct bacterial strains with unique characteristics. *Bacillus* sp. PU-7 is a Gram-positive, motile rod-shaped bacterium (0.6-0.7 μm in width and 1.6-2 μm in length) with a single mono-polar flagellum, growing optimally at 37°C and tolerating up to 9.0% NaCl (w/v) (30) Colonies on nutrient agar are circular, 1-2 mm in diameter, smooth, cream-colored, opaque, and crater-like (Table 2).

Rhizobium sp. PKS, on the other hand, is a Gram-negative, motile bacterium with rod-shaped or irregularly shaped cells, typically occurring singly, in pairs, or short chains (48, 49). It thrives at 25-30°C, with an optimal pH range of 6.0-7.5, and exhibits positive results for catalase, urease, phosphatase, and oxidase assays (Table 3).

Phylogenetic analysis reveals that *Bacillus* sp. PU-7 is closely related to *Bacillus psychrodurans* and *Bacillus psychrotolerans* (96.0-96.2% sequence similarity) (Fig. 1), while *Rhizobium* sp. PKS NCBI-OK663003, NCMR-MCC4960 exhibited 99.13% sequence homology with the *Ensifer adhaerens* HNSQ2 strain.and *Rhizobium* sp. JSM ZJ942 (98.77%) (Fig. 2).

Additionally, we incorporated Mycorrhizal Spores into the consortia. These spores were identified as *Funneliformis mosseae*, following the guidelines outlined in the manual (50).

Table 4 presents data on the biochemical synthesis of plant hormones in eight commonly used cotton cultivars over a 60-day growth period. Notably, among all the cultivars that were inoculated, the Mahyco cultivar exhibited outstanding performance across all measured parameters (Fig. 3).

A subset of four soil samples, chosen for their optimal plant growth conditions, underwent detailed analysis of macro- and micronutrient composition (Table 5).

In the soil samples, either the microbial consortia were deliberately introduced or they were kept uninoculated as a control. Additionally, a positive control was also maintained. Subsequently, the Natural growth patterns of Mahyco cultivar were monitored over 60 days, demonstrating adaptability to four different soil types. This assessment aimed to evaluate the performance of the isolates, which were adminis-

Table 2. *Bacillus* sp. PU-7: A Distinctive Strain within the *Bacillus* Genus

Characteristic	PU-7	*Bacillus psychrodurans* DSM 11713^T	*Bacillus psychrotolerans* DSM 11706^T	*Bacillus insolitus* DSM 5^T
Cell morphology	Rods	Rods	Regular rods	Rods
Cell size (µm)	0.6-0.7 X 1.6-2	0.5-0.6 X 2-5	0.4-1 X 2-7	1.0-1.5 X 1.6-2.7
Nitrate reduction	-	+	-	-
Salinity tolerance (%)	9	5	3	2
Temperature range (°C)	18-40	-2 to 30	-2 to 30	0-25
Anaerobic growth	-	+	-	-
β-Galactosidase	-	W	-	+
Lysine decarboxylase	-	W	W	+
Ornithine decarboxylase	-	+	+	+
Nitrate reduction	-	+	-	+
Hydrolysis of				
DNA	-	+	+	+
Gelatin	-	+	-	+
Starch	-	+	+	-
Tween 60	+	W	+	-
Urea	+	-	-	W

+ positive, - negative, w-weak

Table 3. Growth characteristics and physiological traits of *Rhizobium* sp. PKS

Characteristics	*Rhizobium* sp. PKS
Cell morphology	Rod
Gram's nature	Negative
Cell size (µm)	1.0–1.4 x 3.0–5.4
Temperature range (°C)	23 to 30
pH range	6.0–7.5
Salinity Tolerance	0–2.5
Oxidase	+
Catalase	+
Chitinase	-
Lipase	-
Coagulase	-
Amylase	-
Urease	-

tered in the form of consortia (Fig. 4). Across all soil types, the Mahyco cultivar demonstrated favourable growth concerning plant attributes (Table 6), hormonal output, and biochemical traits (Tables 7, 8 and Figs. 5, 6).

Interestingly red soil exhibited moderate development, while sandy soil displayed the least growth. In contrast, both dark black soil and light black soil demonstrated the most substantial growth.

DISCUSSION

According to research conducted in 2004 (51), it was demonstrated that the inoculation of *Rhizobium* significantly improves several aspects of cotton growth. Noteworthy improvements include augmented nitrogen acquisition, robust plant growth, and improved seedling emergence. The mechanism involves the synthesis of plant hormones, for instance, auxin (IAA) and the efficient solubilization of phosphorus (52). These advantageous impacts enhance the overall robustness and yield of cotton crops. According to the findings of research conducted in 2021 (53), compelling evidence emerged regarding the potential applications of the *Rhizobium* genus in biotechnology. Specifically, *Rhizobium* was identified as a phosphate-solubilizing microorganism exhibiting numerous activities that enhance plant development (54). Notably, it significantly improved both development of plants and phosphate nourishment in non-legume crops, like cotton.

Nitrogen fixation, a critical process for plant growth, relies heavily on *Rhizobium* bacteria, renowned for their symbiotic relationship with leguminous plants (55). As a Plant Growth-Promoting Rhizobacterium (PGPR), *Rhizobium* shares similarities with *Pseudomonas* and *Bacillus* in its beneficial effects on the

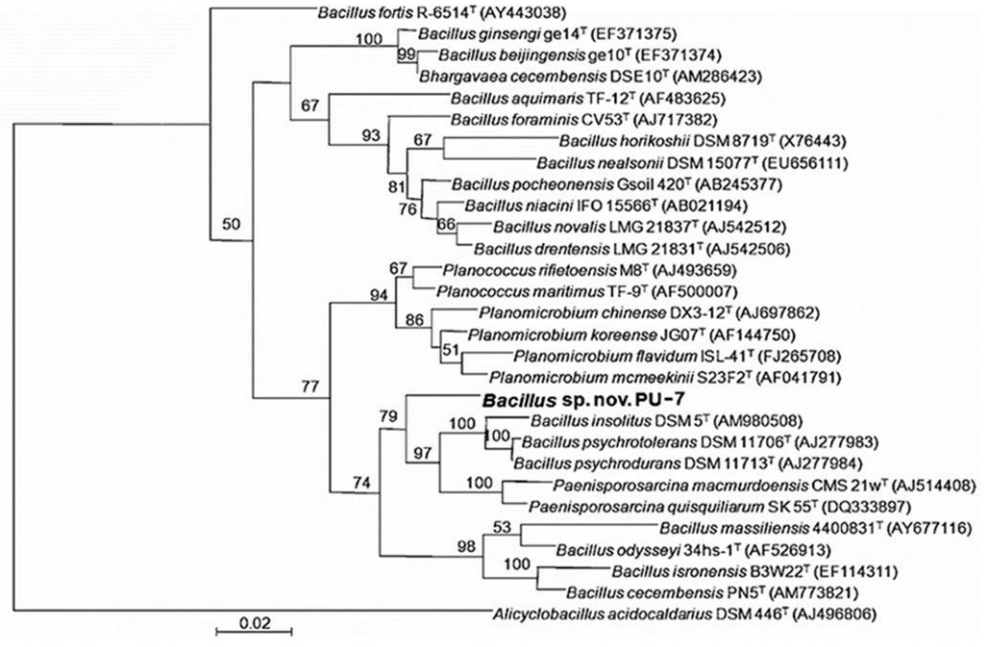

Fig. 1. A maximum likelihood phylogenetic tree constructed from 16S rRNA gene sequences reveals the evolutionary relationships between *Bacillus* sp. PU-7 and other *Bacillus* species, with bootstrap support values indicated at nodes and a scale bar representing 0.02 substitutions per site.

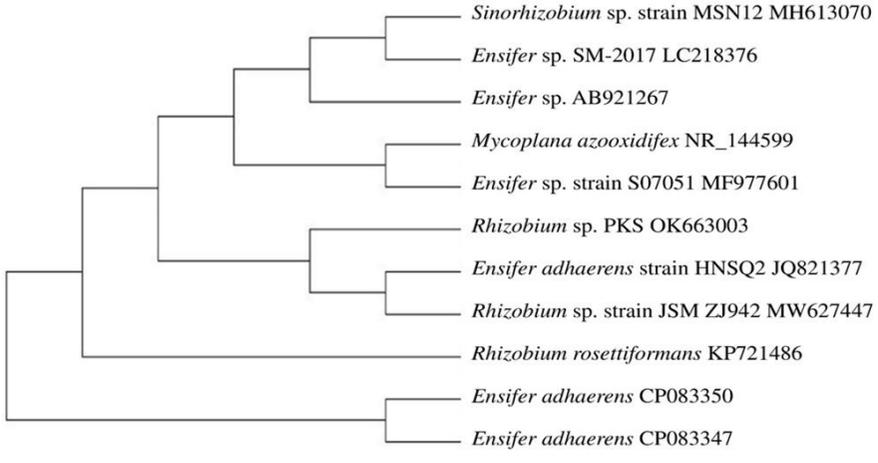

Fig. 2. Evolutionary analysis of *Rhizobium* sp PKS by Maximum Likelihood method

rhizosphere.

Research has consistently shown that specific *Rhizobium* species can significantly enhance crop yields. A wealth of research supports the yield-enhancing potential of specific *Rhizobium* species. The work of multiple researchers (56-59) highlights the significant contributions of *Rhizobium* to improved crop productivity.

According to Díez Méndez and Menéndez (59), non-leguminous plants harbour *Rhizobium* in their rhizosphere, endosphere, and phyllosphere, facilitating beneficial interactions. However, there is limited documentation on *Rhizobium* inoculation in cotton. Research by Romero-Perdomo (53) revealed that *Rhizobium* uniquely promoted cotton development in soils with inadequate phosphorus availability. Rhizobia are adept at colonizing environments with good air exchange and varied metabolic potential,

Table 4. Synthesis of phytohormones and biochemicals in eight varieties of cotton treated with microbial consortia

Variety	Auxin (IAA) (µg/gm)	Proteins (mg/g) 294.26 ± 3.52	Sugars (mg/g)	Chlorophyll (mg/g)	Proline (µg/g)	Mycorrhizal Colonization (%)	N Content (%)	P Content (%)
Mahyco	983.66 ± 1.52	210.13 ± 0.35	198.3 ± 0.45	286.5 ± 1.17	0.03 ± 0	82	1.58 ± 0.07	0.19 ± 0.01
Rashi	820.33 ± 1.52	182.3 ± 0.29	157.1 ± 0.29	240.93 ± 1.02	0.05 ± 0	73	1.27 ± 0.05	0.13 ± 0.01
Marvel	794 ± 2	135.16 ± 0.35	125.1 ± 0.19	186.06 ± 0.25	0.04 ± 0	72	1.08 ± 0.05	0.17 ± 0.01
Nusun	700 ± 2	128.46 ± 0.3	101.99 ± 1.94	157.26 ± 1.29	0.06 ± 0	69	0.74 ± 0.1	0.17 ± 0.01
Kaveri	646 ± 2	105.26 ± 0.3	92.69 ± 0.01	127.4 ± 0.36	0.16 ± 0	72	1.13 ± 0.07	0.15 ± 0.01
Raj seeds	563.66 ± 1.52	95.87 ± 0.06	88.58 ± 0.03	117.6 ± 0.02	0.23 ± 0	60	1.04 ± 0.35	0.11 ± 0.01
Obama	484 ± 2.64	79.4 ± 0.36	79.78 ± 0.03	95.55 ± 0.07	0.24 ± 0	70	0.78 ± 0.06	0.13 ± 0.01
Sunny(NCS-108)	420.66 ± 2.51	(<0.001) **	72.22 ± 0.04	82.57 ± 0.06	0.17 ± 0	59	0.75 ± 0.04	0.09 ± 0.01
p- value	(<0.001) **		(<0.001) **	(< 0.001) **	(< 0.001) **	(< 0.001) **	(< 0.001) **	(< 0.01) *

microbial consortia
** Highly Significant, * Significant

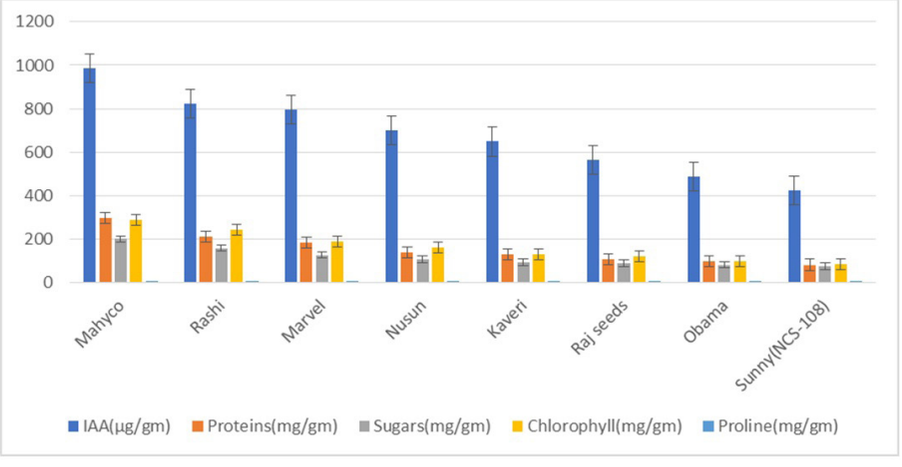

Fig. 3. Graph representing comparative biochemical and phytohormonal synthesis in eight cotton varieties following microbial consortia treatment

Table 5. Evaluation of agricultural soil quality in Mahabubnagar District: Physical-chemical properties, sulfur content, and micronutrient status in four selected soils

Place	Soil variety	Ph	N	P	K	S	Fe	Mn	Zn	Cu
Malleboinpally	Light black soil	8.0	225.14	93.72	115.84	9.1	2.48	26.24	0.30	0.29
Chitteboinpally	Red soil	7.0	192.68	92.15	99.69	12.1	4.98	27.31	1.05	0.27
Kalwakurthy	Dark black	8.0	251.23	105.58	141.12	8.0	2.19	35.21	0.41	0.60
Divtipally	Sandy soil	8.0	189.87	75.92	95.75	3.5	0.18	15.25	0.38	0.05

possessing the unique ability to convert atmospheric nitrogen into ammonia through nitrogen fixation.

The introduction of *Bacillus amyloliquefaciens* to cotton seeds resulted in the broadening of cotyledons, improvement in the growth of primary and lateral roots, and changes in root structure (60). This research also explores the potential of *Bacillus* for promoting cotton growth and mitigating salt stress. When cotton plants were treated with *Bacillus velezensis* (Bv188) and *Bacillus subtilis* (Bs248 and Bs290), they positively affected total nitrogen and phosphorus extraction, total dry matter, and biomass

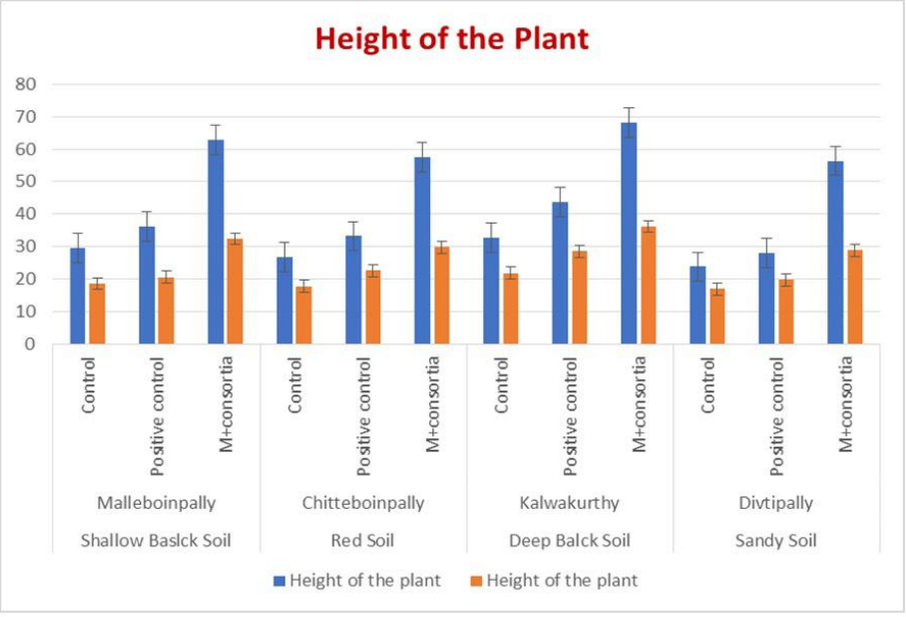

Fig. 4. Graph representing effect of microbial consortia application on mahyco variety plant height across various soil types in Mahbubnagar district. (NC-Negative Control; PC- Positive Control; M+C- Mahyco+Consortia)

Table 6. Plant growth parameters of Mahyco variety in four diverse soil types of Mahbubnagar dist. inoculated with Consortia

Soil variety	Place	Combination	Plant height (cm)		Plant fresh mass (g)		Plant dry mass (g)	
			Shoot	Root	Shoot	Root	Shoot	Root
LightBlack Soil	Malleboinpally	NC	29.4 ± 0.2	18.36 ± 0.25	6.53 ± 0.25	3.15 ± 0.01	1.65 ± 0.02	1.23 ± 0.02
		PC	36.03 ± 0.15	20.33 ± 0.3	8.03 ± 0.25	3.91 ± 0.03	2.83 ± 0.02	1.7 ± 0.2
		M+C	62.43 ± 0.35	31.53 ± 1.33	11.1 ± 0.17	4.8 ± 0.02	3.55 ± 0.2	2.02 ± 0.02
Red Soil	Chitteboinpally	NC	26.53 ± 0.15	17.53 ± 0.25	5.86 ± 0.25	2.6 ± 0.02	1.3 ± 0.02	0.94 ± 0.03
		PC	33 ± 0.2	22.33 ± 0.3	7.56 ± 0.25	3.04 ± 0.12	1.53 ± 0.02	1.13 ± 0.02
		M+C	57.3 ± 0.19	29.53 ± 0.3	8.56 ± 0.35	4.19 ± 0.02	3 ± 0.03	1.72 ± 0.05
Dark Black Soil	Kalwakurthy	NC	32.46 ± 0.3	21.53 ± 0.3	7.13 ± 0.3	3.8 ± 0.02	2.49 ± 0.02	1.81 ± 0.03
		PC	43.36 ± 0.35	28.26 ± 0.3	9.96 ± 0.25	4.96 ± 0.15	3.18 ± 0.03	1.91 ± 0.02
		M+C	64.96 ± 0.25	33.96 ± 0.25	13.36 ± 0.35	5.46 ± 0.35	4.99 ± 0.03	2.42 ± 0.03
Sandy Soil	Divtipally	NC	23.53 ± 0.3	16.6 ± 0.36	5.4 ± 0.36	2.03 ± 0.03	0.94 ± 0.03	0.74 ± 0.04
		PC	27.93 ± 0.2	19.6 ± 0.2	5.96 ± 0.25	2.07 ± 0.08	0.97 ± 0.07	0.94 ± 0.01
		M+C	56.1 ± 0.3	28.46 ± 0.45	7.33 ± 0.3	3.53 ± 0.18	2.43 ± 0.3	1.72 ± 0.05

NC- Negative Control; PC- Positive control; M-Mahyco cultivar; C-Consortia

carbon (61). These *Bacillus* strains show promise for enhancing cotton growth. *Bacillus* sp. PU-7, the novel isolate, holds promise as a bioinoculant for cotton fields, especially in deep black soil (5).

A dual-inoculation study was conducted in 2019 (61) to assess the effects of *Funneliformis mosseae* and *Claroideoglomus etunicatum* on cotton plant growth. The findings indicated that there was a 23–65% increase in root colonization. Under both conditions of soil, whether sterile or non-sterile, there was a noted rise in the ratio of shoot mass to root mass. The cotton plants showed reliance on the species of mycorrhizae and phosphorus nutrition (62), while their dependence on zinc nutrition was less pronounced.

Interactions between plants and microbes are recognized for their positive impact on plant development and nutrient absorption, with particular emphasis on the genera *Rhizobium* and *Bacillus*, alongside AM fungi (6). These organisms are reported to opti-

Table 7. Representing parameters of Mahyco variety in four diverse soil types of Mahbubnagar district inoculated with Consortia

Soil variety	Place	Combination	Mycorrhizal Colonization (%)	N Content (%)	P Content (%)
Light Black Soil	Malleboinpally	NC	-	-	-
		PC	78	1.30 ± 0.02	0.12 ± 0.01
		M+C	82	1.64 ± 0.01	0.2 ± 0.02
Red Soil	Chitteboinpally	NC	-	-	-
		PC	75	1.25 ± 0.02	0.13 ± 0.02
		M+C	80	1.37 ± 0.02	0.2 ± 0.023
Dark Black Soil	Kalwakurthy	NC	-	-	-
		PC	80	1.57 ± 0.02	0.16 ± 0.02
		M+C	85	1.72 ± 0.03	0.23 ± 0.02
Sandy Soil	Divtipally	NC	-	-	-
		PC	72	1.22 ± 0.02	0.09 ± 0.02
		M+C	78	1.35 ± 0.03	0.13 ± 0.01

NC- Negative Control; PC- Positive control; M-Mahyco cultivar; C-Consortia

Table 8. Phytohormone and biochemical production of Mahyco in 4 different soils inoculated with Consortia

Soil variety	Place	Combination	Auxin (IAA) (µg/g)	Proteins (mg/g)	Sugars (mg/g)	Chlorophyll (mg/g)	Proline (µg/g)
Light Black Soil	Malleboinpally	NC	345.66 ± 1.52	41.33 ± 1.15	43.66 ± 2.51	45.3 ± 0.36	0.03 ± 0.01
		PC	598 ± 6.55	69.66 ± 2.3	93.33 ± 3.05	81.93 ± 0.5	0.03 ± 0
		M+C	802 ± 2	184 ± 2	129.33 ± 2.08	170.33 ± 3.51	0.02 ± 0
Red Soil	Chitteboinpally	NC	260 ± 3	40.33 ± 1.52	37.33 ± 0.57	34.33 ± 2.08	0.04 ± 0
		PC	592.66 ± 3.78	73 ± 1.73	85 ± 2.64	75.96 ± 1.96	0.04 ± 0
		M+C	749.66 ± 2.51	140.33 ± 3.51	115 ± 2.64	143 ± 3.6	0.04 ± 0
Dark Black Soil	Kalwakurthy	NC	373.33 ± 4.93	43.66 ± 1.52	48.33 ± 2.51	44.66 ± 2.51	0.03 ± 0
		PC	675 ± 3.6	74.66 ± 0.57	102 ± 5.56	101.66 ± 2.51	0.02 ± 0
		M+C	917.66 ± 2.51	239 ± 3.6	192.66 ± 2.51	223 ± 3.6	0.02 ± 0
Sandy Soil	Divtipally	NC	184.66 ± 1.15	42.66 ± 2.51	30 ± 2	34.66 ± 2.51	0.05 ± 0
		PC	520.33 ± 3.51	100 ± 3	76.33 ± 2.51	64.16 ± 0.2	0.05 ± 0
		M+C	710.33 ± 3.51	109 ± 3.6	94 ± 3	98 ± 3.6	0.05 ± 0

NC- Negative Control; PC- Positive control; M-Mahyco cultivar; C-Consortia

mize the availability of essential nutrients and confer disease resistance, contributing to the resilience of plants against environmental stressors (12). However, while the individual benefits of these microorganisms are documented, the collective and potentially synergistic effects of such consortia on cotton growth have yet to be fully elucidated (12).

A thorough examination of nutrient uptake in cotton plants subsequent to microbial consortium inoculation holds the key to understanding the mechanisms underlying the observed yield enhancements. By dissecting the specific pathways through which these beneficial microorganisms enhance nutrient availability and uptake, we can bolster the evidence supporting their pivotal role in improving cotton yield.

The contribution of advantageous microbes to sustainable farming methods has now been incorporated into the cultivation of cotton, specifically concentrating on boosting the development of plants and the absorption of nutrients. Current studies have emphasized the possibility of utilizing the cooper-

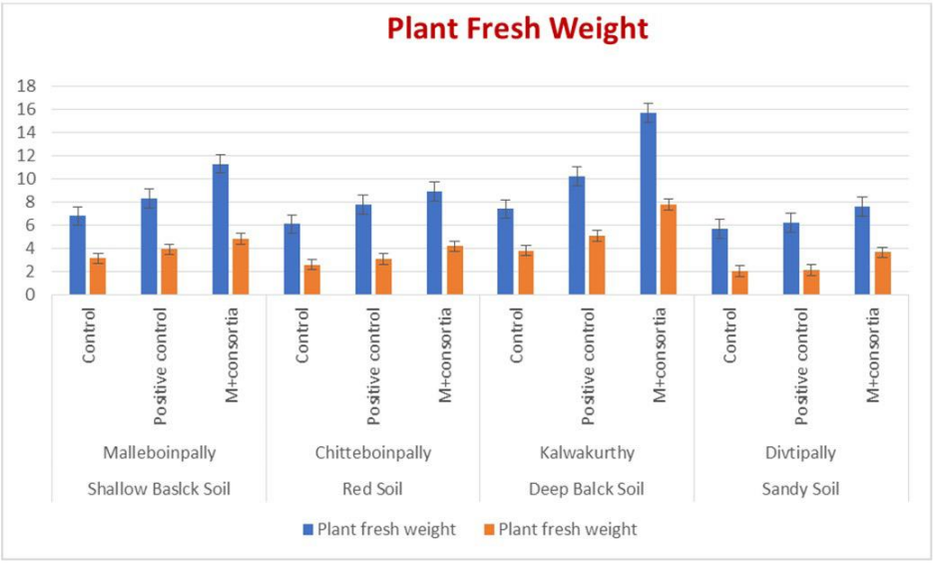

Fig. 5. Graph representing effect of microbial consortia application on Mahyco variety plant fresh mass across various soil types in Mahbubnagar district. (NC-Negative Control; PC- Positive Control; M+C- Mahyco+Consortia)

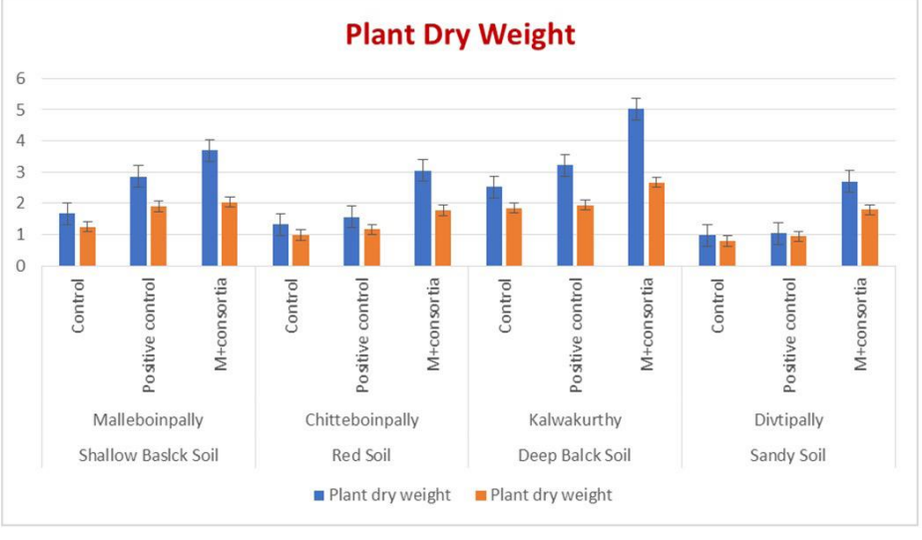

Fig. 6. Graph representing effect of microbial consortia application on Mahyco variety plant dry mass across various soil types in Mahbubnagar district. (NC-Negative Control; PC- Positive Control; M+C- Mahyco+Consortia)

ative interactions among *Rhizobium*, *Bacillus*, and mycorrhizal fungi to enhance the yield and standard of cotton crops.

In summary, the mutual existence of *Rhizobium*, *Bacillus*, and mycorrhizal fungi significantly enhances the growth of cotton plants, nutrient uptake, and total production. These beneficial soil microorganisms assume critical roles in bolstering cotton cultivation, particularly in environmentally demanding regions.

CONCLUSION

The study's outcomes hold significant implications for sustainable agriculture, offering the potential to reduce reliance on synthetic inputs by harnessing the synergies between cotton and soil microbes. These valuable insights shed light on pivotal function of microbial consortia in promoting growth of cotton plants and signal a shift towards ecologically conscious farming practices.

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