

## Synergistic effects of Arbuscular Mycorrhizal Fungi and PGPR Consortia on growth, soil biological activity, and nutrient dynamics in *Citrus reticulata* under Himalayan conditions

KIRAN SUNAR<sup>1\*</sup>, ARUN KUMAR RAI<sup>2</sup>, KESHAB DAS<sup>1</sup> AND SAURAV ANAND GURUNG<sup>2</sup>

<sup>1</sup>Department of Botany, Balurghat Mahila Mahavidyalaya, Balurghat, D.Dinajpur- 733101, West Bengal

<sup>2</sup>Department of Botany, School of Life Sciences, Sikkim University, Gangtok- 737102, Sikkim

Received : 02.05.2026

Accepted : 02.06.2026

Published : 29.06.2026

Mandarin (*Citrus reticulata* Blanco) cultivation in the Eastern Himalayan region, particularly in Sikkim, is constrained by declining soil fertility, nutrient imbalances, and environmental stresses. The present study evaluated the synergistic effects of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) consortia on plant growth, soil biological properties, enzymatic activities, and nutrient dynamics. A pot experiment was conducted with six treatments: control, AMF alone, and AMF combined with four compatible PGPR consortia (BC-X01, BC-X02, BC-X03, and BC-X04). The results demonstrated that combined inoculation significantly enhanced AMF colonization, glomalin content, plant biomass, and soil enzyme activities compared to control and AMF alone. Maximum AMF colonization (62.33%) and glomalin content (10.44 mg g<sup>-1</sup> soil) were observed in the AMF + BC-X01 treatment. Root and shoot biomass also increased substantially under AMF-PGPR combinations, indicating improved plant growth. Soil enzymatic activities, particularly acid and alkaline phosphatase, were significantly elevated, with the highest alkaline phosphatase activity (135.25 µg p-nitrophenol g<sup>-1</sup> soil h<sup>-1</sup>) recorded in AMF + BC-X01. Macro- and micronutrient availability was markedly improved under microbial treatments. Nitrogen (36.88), phosphorus (43.44), and potassium (47.36) contents were highest in AMF + BC-X01 and AMF + BC-X02 treatments, while micronutrients such as Cu<sup>2+</sup> (56.22) and Zn<sup>2+</sup> (42.44) were maximized under AMF + BC-X01. These improvements reflect enhanced nutrient mobilization through microbial activity, including phosphate solubilization and siderophore production. Correlation analysis revealed strong positive relationships among AMF colonization, glomalin, enzymatic activities, nutrient availability, and plant biomass, indicating functional interdependence. Overall, AMF-PGPR consortia significantly improved soil health, nutrient dynamics, and growth of *Citrus reticulata*, highlighting their potential as a sustainable strategy for mandarin cultivation in the Sikkim Himalaya.

**Keywords** : AMF, *Citrus reticulata*, PGPR, Sikkim Himalaya, soil health sustainable agriculture

### INTRODUCTION

*Citrus reticulata* Blanco (Mandarin orange) is one of the most economically important fruit crops cultivated in the Eastern Himalayan region of India, particularly in Sikkim. The crop plays a vital role in the livelihood of farmers and contributes significantly to regional horticultural production.

However, mandarin cultivation in Sikkim faces multiple constraints, including declining soil

fertility, nutrient imbalances, erratic rainfall patterns, and increasing biotic and abiotic stresses, which have led to reduced productivity and orchard decline. Sikkim being an organic state, sustainable agricultural approaches focusing on biological inputs have gained attention as alternatives to chemical fertilizers. Beneficial soil microorganisms such as Arbuscular Mycorrhizal Fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) play a crucial role in enhancing plant growth and soil health (Sunar *et al.* 2017). AMF form symbiotic associations with plant roots, improving nutrient uptake—particularly phosphorus—and enhancing water relations and soil structure through the

\* Correspondence : kiran.sunar@gmail.com

production of glomalin (Brundrett and Tedersoo, 2018; Huang *et al.* 2020). Studies in *Citrus* have demonstrated that AMF significantly improve nutrient acquisition, stress tolerance, and overall plant performance (Wu *et al.* 2017; Wu *et al.* 2019; Chakraborty *et al.* 2016). PGPR promote plant growth through multiple mechanisms, including phosphate solubilization, nitrogen fixation, siderophore production, phytohormone synthesis, and induction of systemic resistance (Hakim *et al.* 2021; Merakly and Memon, 2020). They also influence root architecture and enhance nutrient availability in the rhizosphere, thereby supporting plant development under both normal and stress conditions (Sun *et al.* 2021; Pandey *et al.* 2017). The integration of PGPR into crop management systems has been shown to improve plant productivity and resilience, particularly under adverse environmental conditions (Chakraborty *et al.* 2013; Vidal *et al.* 2022). The combined application of AMF and PGPR often results in synergistic interactions that enhance plant growth beyond the effects of individual inoculants. PGPR can stimulate AMF colonization by modifying root exudates and improving rhizosphere conditions, while AMF enhance nutrient uptake efficiency, creating a complementary system (Pan *et al.* 2020; Raturi *et al.* 2023). Such interactions have been shown to improve nutrient cycling, soil enzyme activity, and plant biomass production. Additionally, bioinoculant-based approaches can improve soil microbial diversity and ecological stability, which are critical for sustainable agriculture (Nicotra *et al.* 2024; Singh *et al.* 2011; Sunar *et al.* 2020, 2013). In perennial fruit crops like mandarin, the rhizosphere represents a dynamic interface where microbial interactions strongly influence plant health and productivity. In the fragile ecosystems of Sikkim, characterized by steep slopes, high rainfall, and soil erosion, maintaining soil structure and fertility is particularly important. Indigenous AMF communities have been reported to vary across altitudinal gradients in Sikkim mandarin orchards, indicating their ecological significance in nutrient cycling and plant adaptation (Sunar *et al.* 2025). Despite growing global evidence on AMF–PGPR interactions, limited information is available on their combined effects in mandarin cultivation under Himalayan agro-climatic conditions. Therefore, the present study aims to evaluate the effectiveness of AMF

and PGPR consortia in improving plant growth, soil biological properties, and nutrient dynamics in *Citrus reticulata* under the high altitude Himalayan conditions of the organic state of Sikkim.

## MATERIALS AND METHODS

### *Isolation and identification of bacterial strains*

Soil samples were collected from different Mandarin Orange Orchards of North Sikkim which included both the rhizosphere soils, roots and soils closely adhering to the roots. Bacterial Isolates were isolated on Nutrient Agar Medium using soil plate and direct soil plating methods (Warcup, 1950). Pure cultures of bacterial isolates were streaked on NA plates for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation. The Gram reactions of all the isolated bacteria were recorded according to Buchanan and Gibbson (1974) and Gram positive/ negative reactions, shape of cells observed were recorded.

### *In vitro assays for plant growth promoting traits*

Plant growth promoting activities of the bacterial isolates were analyzed following standard procedures and techniques. Phosphate solubilization by the bacterial isolates was tested on Pikovskaya's (PVK) agar supplemented with 5% tricalcium phosphate (Pikovskaya, 1948). Production of siderophore was detected using blue indicator chromeazurol S (CAS) as described by Schwyn and Neiland (1987). Production of indole acetic acid (IAA) in the culture supernatant by the bacterium was quantified spectrophotometrically as described by Pilet and Chollet (1970). Hydrocyanic acid (HCN) production was tested on 35-mm petri dish containing Nutrient agar medium amended with 4.4 g glycine/l with filter paper dipped in picric acid in the upper lid and sealed with parafilm as described by Reddy *et al.* (2008). ACC deaminase activity was assayed with respect to the amount of  $\mu\text{mol}$  of  $\alpha$ -ketobutyrate produced upon the hydrolysis of ACC as described by Honma and Shimomura (1978).

### **Identification of PGPR using ribosomal DNA sequences**

Potential PGPR isolates were identified on the basis of 16S rDNA sequences. Genomic DNA extraction and 16S rDNA-PCR amplification were carried out following the method of Stafford *et al.* (2005). The universal bacterial 16S rDNA originally designated as F243 primer set with forward primer (5' to 3') GGATGAGCC-CGCGGCCTA and 16S Reverse (5' to 3') CGGTGTGTACAAGGCCCGG. were used (Heuer *et al.* 1997) PCR and gene sequencing were conducted by Biokart India Pvt. Ltd, Bangalore, India and rDNA sequences were deposited to NCBI GenBank through Bank .It sequence submission procedure and submitted to NCBI GenBank database.

### **Isolation of AMF and Monosporal cultures**

Required amount of soil from soil samples collected from various location of study sites (stored in the proper condition) were taken for isolation of AM spores. Spores were isolated by wet-sieving and decanting method (Gerdemann and Nicolson, 1963) as suggested by Pacioni *et al.* (1992). Trap cultures and pure cultures of AMF ( *in vitro* cultures developed from single spores) were developed and characterized as outlined by Ghorui *et al.* 2024 and Sunar *et al.* 2025.

### **Colonization and infectivity assessment**

The root clearing and staining technique proposed by Phillip and Hayman (1970) were employed to investigate mycorrhizal root colonization. Roots were cleared by treating 10% KOH at room temperature for 24 hrs. Following this, the KOH solution was decanted, and the root pieces were washed with water until the brown color disappeared. Subsequently, the roots were acidified using a 1% HCl solution for 3-5 minutes, and the acid was then decanted. The root segments were stained with a 0.5% Trypan blue solution for 24 hours. After staining, the Trypan blue solution was removed. The colonization was assessed by frequency distribution method (Biermann and Lindermann, 1981).

### **In vitro test for compatibility among PGPR isolates**

Selected PGPR isolates were tested for their compatibility between them by a cross-streak assay method as outlined by Al- Hussini *et al.* 2019 on Nutrient Agar medium. One isolate of PGPR was streaked in the centre of Petri dish containing NA medium in parallel lines and other test bacterial isolates were streaked at right angle to the first bacterial isolate. The plates were incubated at room temperature  $27\pm 1^\circ\text{C}$  for 48 hrs and the growth of the bacteria at the intersection was observed for possible merger of bacteria. The zone of inhibition was analysed and recorded as incompatible (-) for the presence of inhibition zone, whereas compatible (+/++) for the absence of the inhibition zone.

### **Bio-formulation of compatible AMF and PGPR**

AMF inoculum was prepared by mass multiplication of AMF species in *Sorghum* as a host plant. Pure AMF spores obtained from monosporal cultures were inoculated to pots filled sterile planting material (sand:soil; 1:1) and seeded with surface sterilized *Sorghum* seeds. After a growth cycle of 3 months, spores and colonizes roots were harvested, washed with sterile water and air dried for 2-3 days. With moisture content of the root less than 20%, the roots were finely chopped to about 1 cm in length and used as AMF inoculums (1 cm root colonized more than 50% was counted as 1 propagule and 1 AMF spore as 1 propagule). Compatible PGPR isolates belonging to different categories (BC-X01, BC-X02, BC-X03, and BC-X 04) were mass multiplied in a liquid medium (Nutrient Broth-13g/L; Tryptophan 0.2%; Glycerol 5mM; Trehalose 10mM; Polyvinylpyrrolidone (PVP) 1%) with a culture conditions of incubation with rotation at 120 rpm, temperature of  $25^\circ$  to  $28^\circ\text{C}$  and a growth period of 12-72 h. Bacterial cells harvested after the optimum growth period was diluted to maintain a minimum concentration of  $10^8$  c.f.u. /ml (Manikandan 2010). These bacterial bioformulations were then co-inoculated with AMF to the 2 years old nursery grown seedlings of *Citrus reticulata* in small scale green house based pot experiment with complete randomized block

design (CRD) with ten replicates. Arbuscular mycorrhizal fungi (AMF) inoculum was applied at the time of transplantation to the pots by placing it below the root zone to facilitate root colonization. The compatible PGPR consortia (BC-X01 to BC-X04) were applied as soil inoculation at a standardized concentration of  $10 \times 10^8$  CFU mL<sup>-1</sup>. Combined treatments received both AMF and respective PGPR inocula simultaneously. Plants were grown in pots containing a fixed quantity of soil, and recommended agronomic practices were followed throughout the experiment. The experiment included six treatments viz- Untreated control, T-1: Only AMF (*Glomus* sp, *Claroideoglomus etunicatum*, *Rhizophagus irregularis* and *Funneliformis mosseae*); T-2: AMF + PGPR group BC-X01; T-3: AMF + PGPR group BX-02; T-4: AMF + PGPR group BX-03; T-5: AMF+PGPR group BX-04.

#### **Evaluation of effect of microbial inoculation**

Effect of PGPR-AMF inoculation to the rhizosphere of *Citrus reticulata* was evaluated after 90 days of inoculation (3 months) and growth increment was measured in terms of seedling height, root shoot biomass and percent root colonization. Uprooted seedlings were separated into root and shoot portions. Samples were oven-dried at 65–70°C until constant weight and weighed to determine root biomass and shoot biomass. Seedling height was recorded at the time of uprooting. Root samples were collected at harvest, washed thoroughly, and cleared with KOH followed by staining using trypan blue. The percentage of root colonization was determined using the gridline intersect method as described by Phillips and Hayman (1970) and Giovannetti and Mosse (1980). Soil glomalin was extracted using citrate buffer (pH 8.0) under autoclaving conditions and quantified spectrophotometrically following the method described by Wright and Upadhyaya (1998). Results were expressed as mg g<sup>-1</sup> soil. Soil phosphatase activities were determined using p-nitrophenyl phosphate as substrate following the method of Tabatabai and Bremner (1969). Acid phosphatase activity was measured at pH 6.5, while alkaline phosphatase activity was measured at pH 11. Results were expressed as µg p-nitrophenol released g<sup>-1</sup> soil

h<sup>-1</sup> (Tominaga and Takeshi 1974). Soil phosphate content was measured as outlined by (Knudsen and Beegle (1988). For macro and micro nutrient analysis, the soil samples were collected from the surface layer (0–15 cm depth). The soil was air-dried, homogenized, and passed through a 2 mm sieve prior to use. Initial physicochemical properties of the soil, including pH, electrical conductivity (EC), organic carbon, and available macro- and micronutrients, were determined using standard procedures as outlined by (Tandon, 1993; Nannipieri *et al.* 2012). [Available Nitrogen (N): Determined by the Kjeldahl method; Available Phosphorus (P): Olsen's method using spectrophotometry; Available Potassium (K): Flame photometry; Micronutrients (Fe, Zn, Cu, Mn): Extracted using DTPA and measured by Atomic Absorption Spectrophotometry (AAS)].

#### **Statistical Analysis**

Data were subjected to analysis of variance (ANOVA) using standard statistical software. Treatment means were compared using the least significant difference (LSD) test at p < 0.05. Pearson correlation analysis was performed to evaluate relationships among variables. Hierarchical clustering and heatmap analysis were conducted using Ward's method based on correlation distance matrices to identify functional groupings among soil and plant parameters.

## **RESULT AND DISCUSSION**

#### **Plant Growth Promoting activities of bacterial strains and identification**

The present investigation was carried out to isolate potential PGPR and AMF from the rhizosphere of *Citrus reticulata* and to utilize them collectively for growth promotion and enhance nutrient mobilization in the nursery grown seedlings of mandarin orange of Sikkim Himalayas, out of more than 200 bacterial isolates, ten most efficient strains were analyzed in this study. *In vitro* tests for plant growth promoting activity presented in Table 1, shows that all the tested isolates demonstrated phosphate solubilization, siderophore production, ACC deaminase activity and phytohormone production. Indole-3-acetic acid

**Table 1:** Screening for Plant Growth Promoting Traits *in vitro* of isolates with multiple PGP Traits and identified using 16S rDNA sequences.

Isolate number*	Phosphate solubilization	HCN production	IAA ( $\mu\text{g/ml}$ )	Siderophores	ACC deaminase	Identified as	NCBI GenBank Accession number
C/RH/RA/B-02	+	-	236.277	+	+	<i>Bacillus aryabhatai</i>	PP410310
C/RH/RA/B-07	+	+	236.833	+	+	<i>Enterobacter cloacae</i>	PP423091
C/RH/DZ(01)/B-27	+	+	134.055	+	+	<i>Staphylococcus haemolyticus</i>	PQ270276
C/RH/DZ(01)/B-28	+	-	192.388	+	+	<i>Bacillus thuringiensis</i>	PQ270285
C/RP/DZ(02)/B-49	+	-	110.722	+	+	<i>Staphylococcus sciuri</i>	PQ474403
C/Rh/DZ(03)/B-68	+	+	162.944	+	+	<i>Staphylococcus warneri</i>	PQ474424
C/Rh/DZ(04)/B-83	+	+	64.055	+	+	<i>Bacillus altitudinis</i>	PQ474446
C/Rh/DZ(04)/B-84	+	+	29.055	+	+	<i>Bacillus pumilus</i>	PQ474550
C/Rh/DZ(05)/B-89	+	-	61.277	+	+	<i>Bacillus subtilis</i>	PQ474634
C/Rh/DZ(05)/B-101	+	-	113.23	+	+	<i>Bacillus oceanisediminis</i>	PQ474642

\* Bacterial isolates obtained from the Rhizoplane soils collected from East and North Sikkim

**Table 2 :** The bacterial compatibility matrix showing interaction among the PGPR isolates. indicates antagonism.

Bacterial isolate	C/RH/RA/B-02	C/RH/RA/B-07	C/RH/01/B-27	C/RH/01/B-28	C/RP/DZ(02)/B-49	C/RP/DZ(04)/B-68	C/RH/DZ(04)/B-83	C/RH/DZ(04)/B-84	C/RH/DZ(05)/B-89	C/RH/DZ(04)/B-101
C/RH/RA/B-02	0	-	+	+	-	-	+	+	+	+
C/RH/RA/B-07	-	0	-	+	+	-	+	+	+	+
C/RH/01/B-27	-	+	0	-	-	-	+	+	-	+
C/RH/01/B-28	+	-	-	0	-	-	-	-	-	+
C/RP/DZ(02)/B-49	-	+	-	-	0	-	+	-	-	-
C/RP/DZ(04)/B-68	-	-	-	-	-	0	-	+	+	+
C/RH/DZ(04)/B-83	+	+	+	-	+	+	0	-	-	-
C/RH/DZ(04)/B-84	+	-	+	-	-	+	-	0	+	-
C/RH/DZ(05)/B-89	+	+	-	-	-	+	-	-	0	-
C/RH/DZ(04)/B-101	+	+	+	+	-	+	-	-	-	0
Consortia	BC-X01	BC-X02	BC-X03			BC-X04				
BC-X01	<i>Bacillus aryabhatai</i> (PP410310), <i>Bacillus thuringiensis</i> (PQ270285), <i>Bacillus altitudinis</i> (PQ474446), <i>Bacillus pumilus</i> (PQ474550), <i>Bacillus subtilis</i> (PQ474634), <i>Bacillus oceanisediminis</i> (PQ474642)									
BC-X02	<i>Enterobacter cloacae</i> (PP423091), <i>Staphylococcus haemolyticus</i> (PQ270276), <i>Staphylococcus sciuri</i> (PQ474403), <i>Bacillus altitudinis</i> (PQ474446), <i>Bacillus subtilis</i> (PQ474634), <i>Bacillus oceanisediminis</i> (PQ474642)									
BC-X03	<i>Staphylococcus haemolyticus</i> (PQ270276), <i>Bacillus altitudinis</i> (PQ474446), <i>Bacillus pumilus</i> (PQ474550), <i>Bacillus subtilis</i> (PQ474634)									
BC-X04	<i>Bacillus altitudinis</i> (PQ474446), <i>Bacillus pumilus</i> (PQ474550), <i>Bacillus subtilis</i> (PQ474634), <i>Bacillus oceanisediminis</i> (PQ474642)									

(IAA) production varied significantly among the isolates, ranging from 29.055  $\mu\text{g/ml}$  (*Bacillus pumilus* C/Rh/DZ(04)/B-84) to a maximum of 236.833  $\mu\text{g/ml}$  (*Enterobacter cloacae* C/RH/RA/B-07). Hydrogen cyanide (HCN) production, a key biocontrol trait, was observed in 50% of the isolates, specifically in *E. cloacae*, *S. haemolyticus*, *S. warneri*, *B. altitudinis*, and *B. pumilus*. 16S rDNA sequence analysis identified the isolates as belonging to three major genera:

*Bacillus* (60%), *Staphylococcus* (30%), and *Enterobacter* (10%). The NCBI GenBank accession numbers were successfully assigned. The rhizoplane environment of the Sikkim Himalaya serves as a reservoir for diverse Plant Growth-Promoting Rhizobacteria (PGPR). The current study identified *Bacillus* as the dominant genus, consistent with previous findings that *Bacillus* species are highly resilient and common in high-altitude soil ecosystems

**Table 3:** Macro and Micro nutrient profiling of the rhizospheric soil of *C. reticulata* post treatment (mg/Kg)

Treatments	N	P	K+	Cu <sup>2+</sup>	Zn <sup>2+</sup>	Fe <sup>2+</sup>
Untreated Control	28.43±3.22	20.44±2.36	23.44±1.11	14.46±3.44	22.43±2.22	11.77±1.68
T-1:only AMF	31.33±4.33	28.44±3.33	37.86±2.44	38.47±3.22	38.36±3.38	14.33±2.43
T-2:AMF + BC-X01	36.88±2.12	43.44±3.42	46.44±3.44	56.22±4.24	42.44±4.47	18.22±2.86
T-3:AMF + BC-X02	33.44±2.33	32.73±4.88	47.36±3.56	47.48±3.48	26.33±4.16	13.42±2.47
T-4:AMF + BC-X03	32.43±2.24	31.44±4.54	38.19±3.22	45.23±3.26	21.68±3.18	12.33±2.18
T-5:AMF + BC-X04	28.12±2.18	22.36±3.66	26.43±2.16	32.33±2.18	24.28±3.33	17.86±3.36

Average of 10 replicate plants; ±= SE

**Table 4 :** Comparison of nutrient profiles of one of the most significant treatments AMF-BC-X01 with AMF only treatment and Control

Nutrient	Control Baseline	AMF-BC-X01 Value	% Increase (vs Control)	% Increase (vs AMF Only)
Nitrogen (N)	28.43±3.22	336.88±2.12	+29.7%	+17.7%
Phosphorus (P)	20.44±2.36	43.44±3.42	+112.5%	+52.7%
Potassium (K+)	23.44±1.11	46.44±3.44	+98.1%	+22.6%
Copper (Cu <sup>2+</sup> )	14.46±3.44	56.22±4.24	+288.7%	+46.1%
Zinc (Zn <sup>2+</sup> )	22.43±2.22	42.44±4.47	+89.2%	+10.6%
Iron (Fe <sup>2+</sup> )	11.77±1.68	18.22±2.86	+54.8%	+27.1%

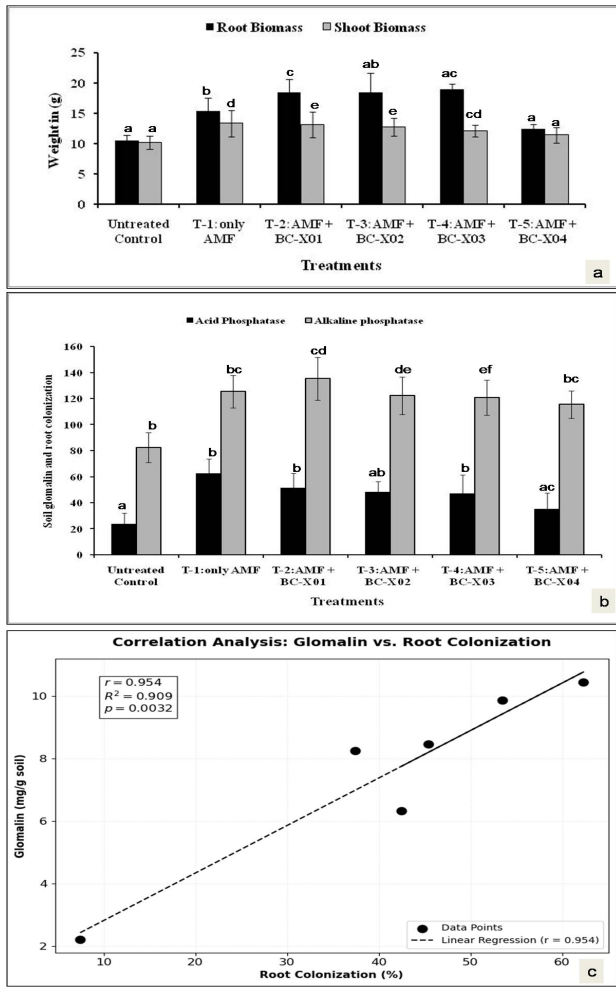
Average of 10 replicate plants; ±= SE

(Chakraborty *et al.* 2010; Sunar *et al.* 2013; Chhetri *et al.* 2024). The universal presence of phosphate solubilization and siderophore production across all isolates suggests their vital role in nutrient cycling. Siderophores not only facilitate iron uptake for the plant but also act as a biocontrol mechanism by sequestering iron away from soil-borne pathogens (Ahmad *et al.* 2008). Furthermore, the 100% prevalence of ACC deaminase activity indicates that these strains are well-adapted to mitigate environmental stress. ACC deaminase lowers ethylene levels in plants, thereby preventing growth inhibition under stressful conditions, a trait frequently observed in high-altitude microbes (Glick, 2014). The high levels of IAA production observed in *Enterobacter cloacae* (C/RH/RA/B-07) and *Bacillus aryabhatai* (C/RH/RA/B-02) are significant. IAA is a primary auxin that triggers root hair elongation and increases the root surface area for nutrient absorption. The concentration of IAA produced by *E. cloacae* in this study is notably higher than many previously reported rhizospheric strains, marking it as a potent candidate for bio-inoculant

formulation (Spaepen & Vanderleyden, 2011). The production of HCN by five isolates suggests a synergistic effect where the bacteria not only promote growth but also protect the host plant. HCN is a volatile secondary metabolite that inhibits the cytochrome oxidase system of fungal pathogens, providing a natural defense mechanism.

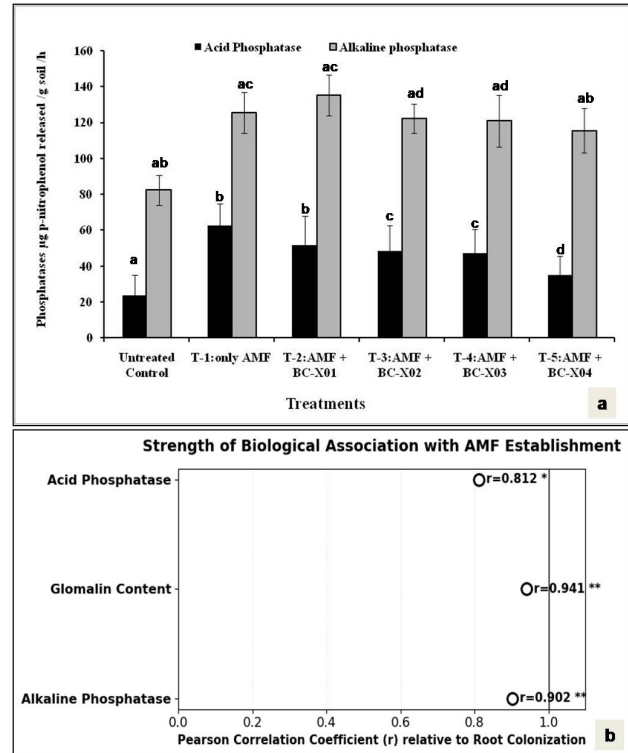
#### **Isolation of Arbuscular Mycorrhizal Fungi (AMF) and identification**

Rhizospheric soils were used to isolate AMF using trap and monospore cultures. Individual spores were carefully segregated and grouped on the basis of their shape, size and ornamentation. On the basis of dominance and spore abundance few AMF isolates were used for this current study. Morphological characters like reaction of wall layers towards Melzer's and PVLG, sporocarp, spore surface ornamentations, wall structures, nature of subtending hyphae and attachments were taken into considerations as outlined by Wu *et al.* 2019. Identification keys were prepared and



**Fig 1:** Synergistic interaction between indigenous AMF and bacterial consortia: Comparative analysis of glomalin production and root colonization efficiency (b), Average of ten replicate plants, bars represent SE. Bars with different letters indicate significant differences between control and treated at P =0.01. Similar letters indicate insignificant (a-b). Linear regression showing the correlation between intraradical colonization and extraradical glomalin secretion. All data points were analyzed at the level of "p d" 0.05 significance. Similar letters indicate insignificant (c).

were compared with reference to the species descriptions and manuals by INVAM (The International Culture Collection of Vesicular/ Arbuscular Mycorrhizal Fungi, The University of Kansas, invam.ku.edu), AMF spore morphotypes were identified. Viability of spores extracted from monospore culture pots were tested by MTT assay (0.25% MTT) On the basis of morphological identification few successful AMF were identified as C/RH/DZ/AMF-M-22-*Funneliformis mosseae* (Ref-INVAM-UK-115); C/ RH/DZ (02)/AMF-M-56- *Rhizophagus irregularis* (Ref-INVAM-PL-112); C/RH/DZ (02) /AMF-M-28-*Claroideoglomus etunicatum* (Ref-INVAM-NE-

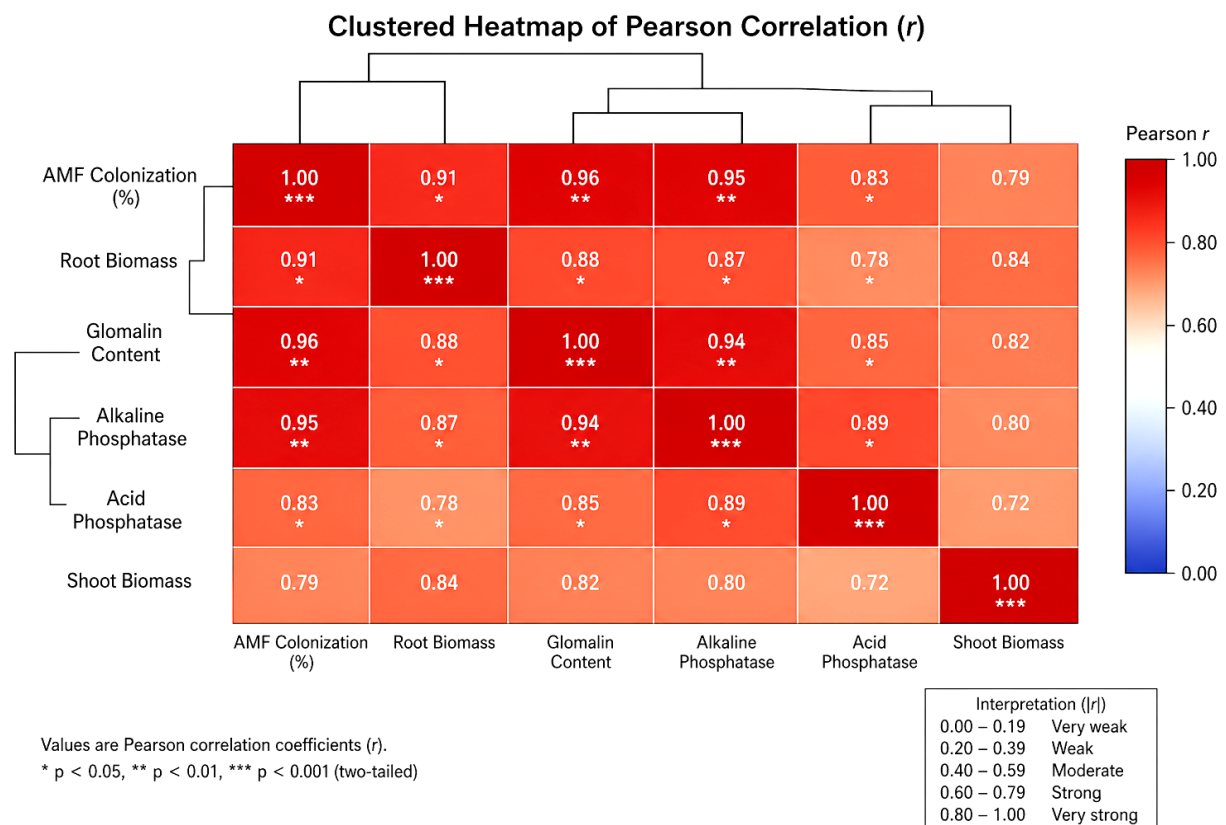


**Fig 2 :** Acid and Alkaline phosphatase activities of the rhizospheric soils of *C. reticulata* after inoculation, Average of ten replicate plants, bars represent SE. Bars with different letters indicate significant differences between control and treated at P =0.01. Similar letters indicate insignificant (a); Coefficient plot illustrating the strength of association between AMF root colonization and soil biochemical parameters. The data points represent the Pearson correlation coefficient (r), where values closer to 1.0 indicate a near-perfect linear relationship. Asterisks represent levels of significance \*\* of "p d" 0.01, \* of "p d" 0.05 (b)

108A); C/RH/DZ (02)/AMF-M-35-*Glomus* spp (Ref-INVAM-W-5581).

**Bacterial Compatibility tests and consortia**

The bacterial compatibility matrix presented in Table 2 shows how each PGPR isolate interacts with every other PGPR isolate. Positive (+) sign indicates compatibility. These are the candidates for a consortium. Negative (-) sign indicates antagonism. One strain is likely producing inhibitory metabolites (like bacteriocins or antibiotics) that prevent the growth of the other. It was observed that there is a high degree of antagonism between C/RH/RA/B-07 (*Enterobacter cloacae*) and C/RH/RA/B-02 (*Bacillus aryabhatai*). Based on the compatibility results, the PGPR strains were grouped into four distinct Bacterial Consortia (BC). Each consortium is designed to be "antagonism-free." BC-X01 (The "All-Bacillus" Group): This consortium focuses



**Fig 3:** Hierarchically clustered heatmap illustrating Pearson correlation coefficients (r) among soil biological and plant growth parameters across treatments. Color gradients represent the strength and direction of correlations, while dendrograms depict similarity among variables using Ward's linkage method. Two major clusters are evident: (i) AMF colonization and root biomass, representing belowground biological interactions, and (ii) glomalin, phosphatase activities, and shoot biomass, reflecting soil biochemical functioning and plant productivity.

exclusively on the genus *Bacillus*. These are hardy, spore-forming bacteria that are excellent for long-term storage and high-altitude survival in Sikkim. BC-X02 (The Diverse Group): This mixes *Enterobacter*, *Staphylococcus*, and *Bacillus*. This group likely offers a wider range of PGP traits (like the high IAA from *Enterobacter* combined with the stress tolerance of *Bacillus*). BC-X03 (The Compact Group): A smaller, four-strain mix focusing on *Staphylococcus* and *Bacillus*. BC-X04 (The Core *Bacillus* Group): A refined version of BC-X01, excluding *B. aryabhatai* and *B. thuringiensis*, potentially focusing on the most mutually compatible strains from the DZ (04/05) collection sites. The development of an effective microbial inoculant requires the selection of compatible strains to avoid intra-consortium antagonism (Ahmad *et al.* 2008; Rizzo *et al.* 2023). In this study, a compatibility matrix revealed specific inhibitory interactions, particularly between *E. cloacae* and certain *Bacillus* spp. Based on these results, four consortia (BC-X01

to BC-X04) were formulated. BC-X01 and BC-X04 leverage the resilience of the *Bacillus* genus, while BC-X02 provides a taxonomically diverse assembly of PGPR. Such multi-strain consortia are shown to provide more consistent plant growth promotion than single-strain inoculants by occupying diverse ecological niches in the rhizosphere (Seneviratne and Jayasinghearachchi, 2003; Vassilev *et al.* 2015).

### **Effect of AMF–PGPR Consortia on growth and biological parameters**

All the morphological tests and biochemical assays were carried out 90 days post inoculation. Effect of AMF-PGPR inoculation to the rhizosphere of *C. reticulata* seedlings on the root and shoot biomass alongside soil glomalin content presented in Fig. 1 illustrates the synergistic effect of the bacterial consortia on Arbuscular Mycorrhizal Fungal (AMF) performance. There is a significant increase in

root colonization when AMF is paired with bacterial consortia compared to the “AMF Only” treatment. Treatment T-2: AMF + BC-X01 achieved the highest colonization rate at 62.33%, this confirms that the *Bacillus*-only consortium (BC-X01) acts as a potent Mycorrhiza-Helper Bacteria (MHB) group, likely facilitating faster hyphal penetration into the root cortex. Glomalin levels increased from a baseline of 2.22 mg/g in the control to a peak of 10.44 mg/g in the BC-X01 group. There is a clear positive correlation between colonization percentage and glomalin content. Higher colonization leads to higher metabolic activity of the fungi, which results in more glomalin being secreted into the soil. Co-inoculation of indigenous AMF with bacterial consortia significantly enhanced both root colonization and glomalin-related soil protein (GRSP) production. The consortium BC-X01 demonstrated the highest synergy, increasing root colonization by 66.4% compared to the AMF-only treatment. This synergistic response suggests that the *Bacillus* strains within BC-X01 likely produce volatile organic compounds or metabolites that stimulate fungal spore germination and hyphal branching, a hallmark of Mycorrhiza-Helper Bacteria (Frey-Klett *et al.* 2007). Glomalin, a glycoprotein produced by AMF hyphae, is known to enhance soil structure and carbon stability (Brundrett and Tedersoo, 2018). Pearson correlation analysis revealed a highly significant positive relationship between AMF root colonization and glomalin production which suggests the tight physiological link between intraradical fungal development and subsequent soil protein secretion (Liang, 2021; Yang *et al.* 2025).

#### **Effect of AMF-PGPR on soil phosphatases**

Inoculation with indigenous AMF and bacterial consortia significantly enhanced soil enzymatic activity within the rhizosphere of *C. reticulata* within a time frame of 90 days post inoculation. Data presented in Fig, 2 (a) shows Acid phosphatase (ACP) activity to be the highly significant in the T-1 (only AMF) treatment, suggesting a strong fungal-mediated P-mineralization pathway. Conversely, alkaline phosphatase (ALP) peaked in the T-2: AMF + BC-X01 consortium. This dual-enzyme enhancement

indicates that AMF facilitated the acid-driven mineralization and the *Bacillus*-based consortium drives the alkaline-driven pathways, thereby maximizing phosphorus availability across a broader rhizosphere niche. Alkaline phosphatase is predominantly of microbial (bacterial) origin. The fact that this reaches its highest point with BC-X01 confirms that this consortium (mostly *Bacillus* species) is highly active and synergistic. It effectively complements the fungal ACP production with its own ALP production (Kalam *et al.* 2017; Srivastava *et al.* 2017). Correlation analysis revealed a highly significant positive relationship between AMF root colonization and glomalin production ( $r = 0.941$ ;  $p < 0.01$ ), as well as alkaline phosphatase activity ( $r = 0.902$ ;  $p < 0.01$ ) (Fig.2-b). These strong coefficients indicate the physiological synergy of the tripartite association (Plant-AMF-PGPB).

#### **Effect of AMF-PGPR on soil nutrient mobilization**

Nutrient profiling of the rhizospheric soil of *C. reticulata* post treatment revealed a significant enhancement in both macro- and micronutrient availability as shown in (Table. 3). The tripartite symbiosis of AMF + BC-X01 demonstrated the highest efficiency, nearly doubling phosphorus (P) availability and significantly increasing copper ( $\text{Cu}^{2+}$ ) and zinc ( $\text{Zn}^{2+}$ ) concentrations compared to the untreated control. The superior mobilization of micronutrients in the BC-X01 and BC-X02 treatments reflects a synergistic interplay between bacterial siderophore production and expanded fungal hyphal reach, suggesting a high potential for these consortia in biofortification and soil fertility management in nutrient-limited Himalayan soils. The significant enhancement of macro- (N, P, K) and micronutrient (Cu, Zn, Fe) availability in the rhizospheric soil following inoculation confirms the efficacy of “eco-adapted” microbial consortia. The consortium BC-X01 (comprised primarily of *Bacillus* species) alongside indigenous AMF emerged as the most potent combination, doubling phosphorus (P) availability (43.44 mg/kg) compared to the control (Table 4). The synergy between AMF hyphae, which expand the soil volume and the P-solubilizing *Bacillus* strains in BC-X01, facilitates the mineralization of organic P that is otherwise unavailable in the acidic soils

of the Himalayan regions for citrus cultivation (Chakraborty *et al.* 2011; Srivastava *et al.* 2017). Furthermore, the increase in Nitrogen (N) suggests that these PGPR may promote a “priming effect,” enhancing the mineralisation of soil organic matter or facilitating better root architecture for nitrate capture (Rana *et al.* 2011). One of the most significant findings is the massive spike in Cu<sup>2+</sup> (56.22) and Zn<sup>2+</sup> (42.44) in the BC-X01 treatment. This is likely driven by the production of siderophores and organic acids by the bacterial isolates (specifically *B. aryabhatai* and *B. thuringiensis*), which chelate poorly soluble micronutrients. The high correlation between glomalin production and these nutrient levels suggests that the fungal “pipeline” is essential for the delivery of these bacterial-mobilized ions (Wu *et al.* 2019).

### Consortium Specificity and Compatibility

The poor performance of BC-X04 relative to BC-X01- despite both being *Bacillus* based-highlights the importance of strain compatibility. BC-X01 contains a balanced assembly of microbes that do not inhibit one another, allowing for a sustained “biochemical push” in the rhizosphere. This supports the hypothesis that a well-characterized, multi-strain consortium is more resilient and effective than random microbial assemblies (Vassilev *et al.* 2015). Results showed strong positive correlations among AMF colonization, glomalin content, biomass production, and phosphatase activities. Clustered heatmap and dendrogram analyses presented in Fig. 3 revealed distinct functional groupings, indicating synergistic interactions between AMF and PGPR. The findings suggest that AMF-PGPR combinations enhance soil functionality and plant productivity through coordinated biological and biochemical mechanisms.

### CONCLUSION

The present study reports and highlights the potential of using microbial partnerships to revitalize nutrient-limited soils in the Sikkim Himalaya. By integrating indigenous Arbuscular Mycorrhizal Fungi (AMF) with specifically characterized Plant Growth-Promoting Rhizobacteria (PGPR), we have identified a high-

performance bio-inoculant system tailored for high-altitude organic farming. Among the four formulated consortia, AMF-BC-X01 (a *Bacillus*-dominant assembly) emerged as a superior Mycorrhiza-Helper group. The tripartite association (Plant + AMF + BC-X01) effectively optimized phosphorus mineralization through a dual-enzyme pathway. While AMF drove Acid Phosphatase activity, the addition of BC-X01 significantly enhanced Alkaline Phosphatase ensuring phosphorus availability across a wider pH range. The resilience of the *Bacillus* strains and adoptability of AMF used in AMF-BC-X01 makes this consortium particularly suitable for the challenging climatic conditions of the Eastern Himalayas.

### ACKNOWLEDGEMENT

Financial assistance under the research grant number- BT/PR40047/NER/95/1662/2020 received from the Department of Biotechnology, Ministry of Science and Technology, Government of India is gratefully acknowledged.

### DECLARATION

Conflict of Interest. All the authors of collaborating institutions declare no conflict of interest.

### REFERENCES

- Ahmad, F., Ahmad, I., Khan, M.S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.* **163**: 173–181.
- Al-Hussini, H.S., Al-Rawahi, A.Y., Al-Marhoon, A.A., Al-Abri, S.A., Al-Mahmooli, I.H., Al-Sadi, A.M., Velazhahan, R. 2019. Biological control of damping-off of tomato caused by *Pythium aphanidermatum* using native antagonistic rhizobacteria. *J. Plant Pathol.* **101**: 315–322. Doi.10.1007/s42161-018-0184-x.
- Biermann, B., Linderman, R.G. 1981. Quantifying Vesicular–Arbuscular Mycorrhizae: A proposed method towards standardization. *New Phytol.* **87**: 63–67. Doi.10.1111/j.1469-8137.1981.tb01690.x
- Brundrett, M.C., Tedersoo, L. 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* **220**: 1108–1115. Doi.10.1111/nph.14976.
- Buchanan, R.E., Gibbons, N.E. 1974. Bergey’s manual of determinative bacteriology (8th ed.). Williams & Wilkins, Baltimore, USA.
- Chakraborty, B.N., Allay, S., Chakraborty, A.P., Chakraborty, U. 2016. PGPR in managing root rot disease and enhancing growth in Mandarin (*Citrus reticulata* Blanco.) seedlings. *J. Hortic. Sci.* **11**: 104–115. Doi.10.24154/jhs.v11i2.80
- Chakraborty, B.N., Chakraborty, U., Saha, A., Dey, P.L., Sunar, K. 2010. Evaluation of phosphate solubilizer from soil of North Bengal and their diversity analysis. *World J. Agric. Sci.* **6**: 195-200.

- Chakraborty, U., Chakraborty, B.N., Chakraborty, A.P., Sunar, K., Dey, P. 2013. Plant Growth Promoting Rhizobacteria mediated improvement of health status of Tea plants. *Indian J. Biotechnol.* **12**: 20-31.
- Chakraborty, U., Chakraborty, B., Allay, S., de, U., Chakraborty, A.P. 2011. Dual application of *Bacillus pumilus* and *Glomus mosseae* for improvement of health status of Mandarin plants. *Acta. Hort.* **892**: 215-230.
- Chhetri, S., Sherpa, M.T., Sharma, L. 2024. Characterization of Plant Growth Promoting Bacteria isolated from rhizosphere of Tomato cultivated in Sikkim Himalaya and their potential use as biofertilizer. *Sci. Rep.* **15**: 98953.
- Frey-Klett, P., Garbaye, J., Tarkka, M. 2007. The Mycorrhiza Helper Bacteria: a new dimension to the Mycorrhizal symbiosis. *New Phytol.* **175**: 2-14.
- Gerdemann, J.W., Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**: 235-244.
- Ghorui, M., Chowdhury, S., Prakash, B., Suman, S., Das, K., Sunar, K. 2024. Morpho-biochemical and functional comparison of Arbuscular Mycorrhizal Fungal spores cultured under two conditions. *J. Mycopathol. Res.* **62**: 55-65.
- Giovannetti, M., Mosse, B. 1980. An evaluation of techniques for measuring Vesicular-Arbuscular Mycorrhizal infection in roots. *New Phytol.* **84**: 489-500. Doi.10.1111/j.1469-8137.1980.tb04556.x
- Glick, B.R. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol. Res.* **169**: 30-39.
- Hakim, S., Naqqash, T., Nawaz, M.S., Laraib, I., Siddique, M.J. 2021. Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and sustainability. *Front. Sustain. Food Syst.* **5**: 617157. Doi.10.3389/fsufs.2021.617157
- Heuer, H., Krsek, M., Baker, P., Smalla, K., Wellington, E.M. 1997. Analysis of actinomycete communities by specific amplification of 16S ribosomal DNA and denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* **63**: 3233-3241.
- Honma, M., Shimomura, T. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem.* **42**: 1825-1831.
- Huang, D., Ma, M., Wang, Q., Zhang, M., Ma, F. 2020. Arbuscular Mycorrhizal Fungi enhance drought resistance in apple by regulating MAPK pathway genes. *Plant Physiol. Biochem.* **149**: 245-255. Doi.10.1016/j.plaphy.2020.02.020
- Kalam, S., Basu, A., Subramanian, S. 2017. Phosphatase activity and plant growth promotion of *Bacillus* and *Pseudomonas* species. *J. Appl. Microbiol.* **123**: 1230-1241.
- Knudsen, D., Beegle, D. 1988. Recommended phosphorus tests. In: Dahnke, W.C. (Ed.), Recommended chemical soil test procedures for the North Central Region. North Dakota Agricultural Experiment Station, North Dakota, USA, pp. 12-15.
- Liang, S.M. 2021. Correlation coefficient between Mycorrhizal growth and soil properties. *J. Soil Sci.* **11**: 353185196.
- Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T., Samiyappan, R. 2010. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 against *Fusarium* wilt of tomato. *Biol. Control.* **54**: 78-82.
- Merakly, N., Memon, A.R. 2020. Role of plant growth-promoting bacteria in plant growth and soil-plant relationships. *Turk. J. Agric. Food Sci. Technol.* **8**: 2590-2602.
- Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G. 2012. Microbial diversity and soil functions. *Soil Biol. Biochem.* **48**: 1-13.
- Nicotra, D., Ghadamgahi, F., Ghosh, S., Anzalone, A., Dimaria, G., Mosca, A. 2024. Genomic insights and biocontrol potential of bacterial strains from tomato microbiome. *Front. Plant Sci.* **15**: 1437947. Doi.10.3389/fpls.2024.1437947
- Pacioni, G. 1992. Wet-sieving and decanting techniques for the extraction of spores of Vesicular Arbuscular Fungi. *Meth. Microbiol.* **24**: 317-322.
- Pan, J., Huang, C., Peng, F., Zhang, W., Luo, J., Ma, S., Xue, X. 2020. Effect of AMF and PGPR inoculations on plant growth under saline soil conditions. *Appl. Sci.* **10**: 945.
- Pandey, P., Irulappan, V., Bagavathiannan, M.V., Senthil-Kumar, M. 2017. Impact of combined abiotic and biotic stresses on plant growth. *Front. Plant Sci.* **8**: 537. Doi.10.3389/fpls.2017.00537
- Phillips, J.M., Hayman, D.S. 1970. Improved procedures for clearing and staining parasitic and Vesicular-Arbuscular Mycorrhizal fungi in roots. *Trans. Br. Mycol. Soc.* **55**: 158-161. Doi.10.1016/S0007-1536(70)80110-3
- Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil by microbial activity. *Microbiologia* **17**: 362-370.
- Pilet, P.E., Chollet, R. 1970. Sur le dosage colorimétrique de l'acide indolylacétique. *C.R. Acad. Sci. Ser.* **271**: 1675-1678.
- Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K., Nain, L. 2011. Identification of multi-trait PGPR from wheat rhizosphere and their characterization as h-producers. *Microbiol. Res.* **166**: 400-407.
- Raturi, P., Rai, R., Sharma, A.K., Singh, A.K., Dimri, D.C. 2023. Effects of PGPR and AMF on morpho-physiological parameters of crops under stress conditions. *Int. J. Environ. Clim. Change* **13**: 2707-2713. Doi.10.9734/ijeccl/2023/v13i92502
- Reddy, B.P., Reddy, K.R.N., Subba Rao, M., Rao, K.S. 2008. Antimicrobial metabolites of *Pseudomonas fluorescens* against rice pathogens. *Curr. Trends Biotechnol. Pharm.* **2**: 178-182.
- Rizzo, G.F., Al Achkar, N., Treccarichi, S., Malgioglio, G., Infurna, M.G., Nigro, S. 2023. Bioinoculants influence yield of snap bean under deficit irrigation. *Agriculture* **13**: 865. Doi.10.3390/agriculture13040865
- Schwyn, B., Neilands, J.B. 1987. Universal assay for detection of siderophores. *Anal. Biochem.* **160**: 47-56. Doi.10.1016/0003-2697(87)90612-9
- Seneviratne, G., Jayasinghearachchi, H.S. 2003. Mycorrhizal colonization and seedling growth of *Pueraria phaseoloides* as affected by a rhizobial-fungal consortium. *Biol. Fertil. Soils.* **39**: 119-123.
- Singh, J.S., Pandey, V.C., Singh, D.P. 2011. Efficient soil microorganisms for sustainable agriculture. *Agric. Ecosyst. Environ.* **140**: 339-353. Doi.10.1016/j.agee.2011.01.017
- Spaepen, S., Vanderleyden, J. 2011. Auxin and plant-microbe interactions. *Perspect. Biol.* **3**: a004572. Cold Spring Harb
- Srivastava, A.K., Wu, Q.S., Ngullie, L. 2017. Synergistic response of PGPR and AMF in citrus: A review. *Indian J. Agric. Sci.* **87**: 1435-1444.
- Stafford, W.H.L., Baker, G.C., Brown, S.A. 2005. Bacterial diversity in rhizosphere of Proteaceae. *Environ. Microbiol.* **7**: 1755-1768. Doi.10.1111/j.1462-2920.2005.00847.x
- Sun, H., Jiang, S., Jiang, C., Wu, C., Gao, M., Wang, Q. 2021. Root exudates and rhizosphere microbiome interactions in crop production. *Environ. Sci. Pollut. Res.* **28**: 54497-54510. Doi.10.1007/s11356-021-14878-3
- Sunar, K., Chakraborty, U., Chakraborty, B.N. 2020. *Bacillus* spp. induce resistance in *Brassica juncea* against seedling blight. *J. Mycopathol. Res.* **58**: 167-179.
- Sunar, K., Chakraborty, U., Chakraborty, B.N. 2017. Influence of Indigenous Bacilli Isolated from Darjeeling Hills on Phosphate Mobilization and Induction of resistance against Sclerotial blight disease of Tea cultivars. *Int. J. Basic and Appl. Biol.* **4**: 128-134.

- Sunar, K., Dey, P.L., Chakraborty, U., Chakraborty, B.N. 2013. Biocontrol efficacy and plant growth promoting activity of *Bacillus altitudinis* isolated from Darjeeling hills, India. *J. Basic Microbiol.* **55**: 91-104.
- Sunar, K., Rai, A.K., Das, K., Ghorui, M., Gurung, S.A. 2025. Altitudinal variation and colonization patterns of arbuscular mycorrhizal fungi associated with organically grown mandarin (*Citrus reticulata*) in Sikkim Himalayas. *J. Mycopathol. Res.* **63**: 317–328.
- Tabatabai, M.A., Bremner, J.M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1**: 301–307. Doi.10.1016/0038-0717(69)90012-1
- Tandon, H.L.S. 1993. *Methods of analysis of soils, plants, waters and fertilizers*. FDCO, New Delhi.
- Tominaga, N., Takeshi, M. 1974. A sulfite-dependent acid phosphatase of *Thiobacillus thiooxidans*. *J. Biochem.* **76**: 419–428. Doi.10.1093/oxfordjournals.jbchem.a130581
- Vassilev, N., Vassileva, M., Lopez, A., Martos, V., Reyes, A., Pascual, J.A. 2015. Unexploited potential of some non-conventional microbial consortia for enhancing plant growth. *Bioresour. Technol.* **184**: 402–410.
- Vidal, C., González, F., Santander, C., Pérez, R., Gallardo, V. 2022. Rhizosphere microbiota management under drought stress: A review. *Plants* **11**: 2437. Doi.10.3390/plants11182437
- Warcup, J.H. 1950. Isolation of fungi from soil hyphae. *Nature* **175**: 953. Doi.10.1038/175953a0
- Wright, S.F., Upadhyaya, A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil.* **198**: 97–107. Doi.10.1023/A:1004347701584
- Wu, Q.S., He, J.D., Srivastava, A.K., Zhang, F., Zou, Y.N. 2019. Development of propagation technique of indigenous AMF and their inoculation response in *Citrus*. *Indian J. Agric. Sci.* **89**: 1190–1194. Doi.10.56093/ijas.v89i7.91696
- Wu, Q.S., Srivastava, A.K., Zou, Y.N. 2017. Mycorrhizas in *Citrus*: Beyond soil fertility and plant nutrition. *Indian J. Agric. Sci.* **87**: 1190–1194.
- Yang, S., Yuan, T., Duan, T., Zhu, H., Zhang, X., Zhang, H. 2025. Integrated omics analysis of PGPR and AMF effects on soil microbiota and root metabolites. *Front. Microbiomes* **4**: 1709335. Doi.10.3389/frmbi.2025.1709335