PLANT SCIENCE TODAY
Vol 13(sp1): 01–10
https://doi.org/10.14719/pst.11673

eISSN 2348-1900

RESEARCH ARTICLE

Native bioagents of the North-eastern Himalayas for the management of *Sclerotinia sclerotiorum* (Lib.) de Bary

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Received: 08 September 2025; Accepted: 24 December 2025; Available online: Version 1.0: 24 February 2026

Cite this article: Meryhun M, Tasvina RB, Mahesh P, Meetei NT, Ibanrishisha K, Aditee B, Mahasweta C, Pankaj B. Native bioagents of the North-eastern Himalayas for the management of *Sclerotinia sclerotiorum* (Lib.) de Bary. Plant Science Today. 2026;13(sp1):01–10. https://doi.org/10.14719/pst.11673

Abstract

Meghalaya, a biodiversity-rich state in North-eastern India, is increasingly facing challenges from soil-borne pathogens, particularly *Sclerotinia sclerotiorum* (Lib.) de Bary, which has emerged as a major threat to vegetable cultivation in the hill farming systems. This fungus causes significant yield losses and its management through chemical means poses risks to soil health, the environment and long-term sustainability. The present study explored 11 native *Trichoderma* spp. and 5 plant growth-promoting rhizobacteria (PGPR) isolates for the management of *S. sclerotiorum* affecting various horticultural crops in the state. The *Trichoderma* and PGPR isolates obtained from the rhizosphere of different crops grown under the Umiam circle of Ri Bhoi district, Meghalaya, were characterised based on morpho-cultural, biochemical, internal transcribed spacer (ITS) and 16s region amplification, respectively. The potent *Trichoderma* isolates UM-5, UM-10 and the PGPR isolate (PGPR-4) were established as *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, *Trichoderma harzianum Rifai* and *Bacillus subtilis* (Ehrenb.) Cohn. *In vitro* evaluation of the isolates showed that UM-5 and UM-10 exhibited 78.4 %, 87.3 % inhibition, while PGPR-4 showed 67.0 % inhibition of the target pathogen, along with functional and plant growth-promoting attributes in the range higher than 50 % for most of the parameters. The potential biocontrol agents (UM-5, UM-10 and PGPR-4) can be employed alone and in combination with other bio inputs for holistic and eco-friendly management of the soil-borne pathogen.

Keywords: *Bacillus subtilis*; bioagents; eco-friendly management; *Sclerotinia sclerotiorum*; *Trichoderma* spp.

Introduction

A holistic approach to agriculture, organic farming promotes the use of resources that are specific to a given region and are derived from farms, while also emphasising ecological balance, biodiversity and a minimal reliance on synthetic inputs (1). Even though green revolution technologies have greatly improved food production (2, 3), their extended use has resulted in health risks, environmental pollution and soil degradation, which have caused a shift towards organic and eco-friendly practices. The agrarian Northeastern Himalayan region of the country is considered organic by default, with the use of bare minimum synthetic fertilisers and chemicals. The hilly states of the region, rich in biodiversity hotspots, have witnessed the evolution of many beneficial and harmful microbiotas. Evolving from the primitive form of slash-and-burn agriculture to a more settled land use system of terrace cultivation has led to emerging threats of soil-borne pathogens.

Meghalaya, one of the hill states of the region, depends on agriculture and horticulture as a source of earning mostly for the rural economy, with a net sown area of 2.85 lakh ha and vegetable production of 548.146 ton recorded in 2024 is from an area of 18498 ha (4). However, the potential productivity is not achieved, which can be attributed to many factors, like changing weather parameters over the years and biotic stresses. Plant pathogens contribute to yield loss by attacking the crops at almost all stages from sowing to harvest and post-harvest handling. In recent times soil-borne plant pathogens are seen (5) as an emerging threat to vegetable and horticultural crops in the terrace cultivation system. *Sclerotinia sclerotiorum* (Lib.) de Bary is a homothallic, necrotrophic fungus that propagates via airborne ascospores or soil-borne sclerotia. These sclerotia can sprout mycelia or apothecia under suitable conditions and infect over 500 plant species (6–8) with a particular affinity for species in the Leguminosae, Compositae, Solanaceae and other families (9). Although initially found in cool,

moist environments, the pathogen now thrives in warmer, drier climates, owing to its adaptability and genetic variability (10–13).

The excessive application of chemical pesticides to manage soil-borne pathogens has sparked worries about ecological sustainability and food safety, which has boosted interest in biological control methods (14). Through mechanisms including competition, mycoparasitism, enzyme production and induction of plant resistance, bioagents like *Trichoderma* spp. and plant growth-promoting rhizobacteria (PGPR) provide environmentally friendly and efficient disease management (15, 16). Integrated microbial consortia have demonstrated improved reliability in field settings (17). In organic agriculture, plant protection measures rely primarily on the use of bioagents. Introduced biocontrol agents may not perform to their potential owing to the challenges of ecological, edaphic adaptation and competition with the native strains. In the pursuit of becoming an organic state, Meghalaya is well equipped with the cultural and mechanical strategies of plant disease management. However, the native strains of bioagents have been very little explored and exploited for their potential, to be used as biocontrol agents for managing emerging pathogens like *S. sclerotium*. Therefore, the present study focuses on isolating and evaluating the potential of native microbial bioagents for the effective, eco-friendly management of *S. sclerotiorum* under the hill agro-ecosystem of Meghalaya.

Materials and Methods

Isolation and purification of native *Trichoderma* spp. and PGPR

Soil samples were collected randomly from 5 spots per subplots (3 fields of a village) at a depth of 10–15 cm and composite samples were prepared *in situ* from the rhizosphere of various crops such as French bean, cauliflower, ginger, turmeric, cabbage, sweet potato, maize, broccoli and from forest areas across seven villages, viz. Umdohbyrthih, Umeit, Mawpun, Pyllun, Nongsder, Lum Sohpetbneng and Umiam under the Umiam circle of Ri Bhoi district, Meghalaya, during 2023–25. In the laboratory, samples were air-dried and processed for microbial isolation by the serial dilution method. *Trichoderma* spp. was isolated with dilution up to 10^{-4}on Potato Dextrose Agar (PDA) media and incubated at 27 ± 2 °C for 3–5 days. Colonies resembling *Trichoderma* were purified using the hyphal tip technique and maintained on PDA slants, then stored at 4 °C for further use, with regular sub-culturing. PGPR were isolated by serial dilution (10^{-6}), plated on Nutrient Agar (NA) and incubated at 28 ± 2 °C for 24–48 hr. Bacterial colonies with distinct morphology were purified through repeated streaking, maintained on NA slants and stored at 4 °C for future use, with periodic sub-culturing to maintain viability and purity.

Identification and characterisation of *Trichoderma* isolates and PGPR isolates

Pure culture of the *Trichoderma* isolates obtained on PDA through the hyphal tip technique was used for morpho-cultural characterisation of the isolates in triplicate. After 7 days, observations were recorded on colony growth rate, colour (front and reverse), mycelial pattern (dispersed/dense/concentric), pigmentation and pustule formation. PGPR isolates were cultured on NA and incubated at 28 ± 2°C for 24–48 hr. The colonies were examined for morphological traits, including colour, shape, margin, elevation, surface texture and opaqueness.

Microscopic examination of *Trichoderma* isolates was conducted at 10× and 40× magnifications, with observations including hyphal structure, conidiophore branching, phialides and conidia. PGPR isolates were examined through Gram staining and observed at 100× magnification using oil immersion. All observations were recorded using a Leica DM750 microscope.

Molecular identification of *Trichoderma* and PGPR isolates

Extraction of genomic DNA of *Trichoderma* and PGPR isolates was done using the DNeasy® Plant Mini Kit (QIAGEN) following the manufacturers' quick-start protocol. *Trichoderma* isolates were cultured in 100 mL of PDB at 27±2 °C for 3 days on a rotary shaker. Similarly, PGPR isolates were cultured in 100 mL of NB at 27±2 °C on a rotary shaker for 24–48 hr. The required number of samples as per manufacturer specifications was further processed to obtain the DNA of both the bioagent isolates and the quality of the genomic DNA was analysed using agarose gel electrophoresis.

The extracted genomic DNA was amplified using a thermal cycler (Eppendorf AG 22331) through the Polymerase Chain Reaction (PCR) technique. For the amplification of the internal transcribed spacer (ITS) region of fungal DNA, with ITS1 as the forward and ITS4 reverse primer (18) were used. The amplification of the 16S rRNA gene of the PGPR isolates was done using universal primers 27F and 1492R (19). The expected amplicons between 500–700 bp were visualised under UV light using a gel documentation system with a 100 bp DNA ladder used as a molecular size marker to estimate the fragment lengths.

Screening of *Trichoderma* and PGPR isolates for functional and plant growth-promoting attributes

Ammonia production was assessed using peptone water medium (10 g peptone, 5 g NaCl, 1 L distilled water). A 5 mm mycelial disc (*Trichoderma*) or a loopful of PGPR culture was inoculated into 10 mL of peptone water in separate test tubes and incubated at 27±2 °C for *Trichoderma* and 28±2 °C for PGPR for 7 days and 48 hr, respectively. After incubation, 0.5 mL of Nesslers' reagent was added to each tube. The appearance of a brown to yellow colour indicated positive ammonia production.

HCN production was tested on PDA (*Trichoderma*) and NA (PGPR) media supplemented with 4.4 g/L glycine. A 5 mm mycelial disc (*Trichoderma*) or a loopful of PGPR culture was inoculated onto the centre of Petri plates. A Whatman No.1 filter paper strip, soaked in picric acid solution (0.5 % picric acid, 2 % sodium carbonate, 100 mL distilled water), was affixed to the lid. Plates were sealed with parafilm and incubated at 27±2 °C (*Trichoderma*, 10 days) and 28±2 °C (PGPR, 2–4 days). A colour change in the paper from yellow to brown/reddish-brown indicated positive HCN production. *Trichoderma* (mycelial disc) and PGPR (loopful culture) were inoculated in 20 mL broth (PDB for *Trichoderma*, NB for PGPR) supplemented with 1 g/L L-tryptophan and incubated at 27±2 °C (150 rpm, dark). After centrifugation (11000 rpm, 15 min), 1 mL supernatant was mixed with 2 mL Salkowski reagent and kept at room temperature for 30 min. Pink colouration indicated Indole-3-acetic acid (IAA) production.

Siderophore production by *Trichoderma* spp. and PGPR was assessed using a modified Chrome Azurol Sulfonate (CAS) agar

assay. The CAS reagent was prepared by mixing 60.5 mg CAS in 10 mL of 1 mM $FeCl_3 \cdot 6H_2O$ (in 10 mM HCl), then adding 72.9 mg HDTMA with continuous stirring. This solution was autoclaved with 900 mL of Luria Bertani agar. After cooling, 100 mL of the CAS dye was added to the medium. A colour change from blue to orange, purplish-red, or magenta indicated siderophore production. Observations were recorded 5 days post-inoculation. Iron chelation efficiency (%) was calculated by the following formula (20).

$$E\ (\%) = (H-C))/H \times 100 \qquad \text{(Eqn. 1)}$$

where, E (%) =Iron chelation efficiency (%), H= Diameter of colony + Halo zone (mm) and C= Diameter of colony (mm).

Phosphate solubilization by isolates was tested on Pikovskayas' agar with tricalcium phosphate. The inoculated plates were incubated at 27±2°C. A clear halo around the colony indicated solubilization (21).

Efficiency (%) =

(Colony + Halo diameter) / Colony diameter × 100 (Eqn. 2)

Zinc solubilization by isolates was tested on Pikovskayas' agar supplemented with 5g/L ZnO (22). The inoculated plates were incubated at 27±2 °C in the dark. A clear halo around the colony observed on the 3rd day indicated zinc solubilization. Amylase production by *Trichoderma* spp. and PGPR was assessed using starch agar plates. A 5mm disc or bacterial spot from actively growing cultures was inoculated at the centre of sterile plates and incubated at 27±2 °C for 3–5 days. After incubation, plates were flooded with iodine solution; a clear halo around the colony indicated starch hydrolysis and positive amylase activity.

Evaluation of antagonistic potential of native *Trichoderma* and PGPR isolates against *S. sclerotiorum*

The antagonistic activity of *Trichoderma* spp. and PGPR against *S. sclerotiorum* was assessed using the dual culture technique on PDA and NA plates, respectively. A 5 mm mycelial disc of *Trichoderma* and the pathogen were placed 30mm apart, while PGPR was streaked opposite the pathogen. Plates were incubated at 27±2 °C in the dark with three replications and control plates included only the pathogen were maintained. After incubation, radial growth of the pathogen was measured and per cent inhibition (PI) was calculated using the formula as per standard (23).

$$PI\ (\%) = [(R1-R2)/R1] \times 100 \qquad \text{(Eqn. 3)}$$

where R1 = pathogen growth in control, R2 = pathogen growth in treatment.

Compatibility of the effective and potential *Trichoderma* and PGPR isolates was carried out through the dual culture technique. After five days of incubation at 27 ± 2 °C, the growth of bioagent isolates in the dual inoculation plates was assessed by observing the presence or absence of an inhibition zone, indicating incompatibility and compatibility, respectively, between the bioagents. The statistical tool used in the present study was one-way ANOVA, with the experimental design being completely randomised design (CRD). The significant difference, if any, among the treatment means was compared by using the critical difference (CD) at $p = 0.05$ % using SPSS statistical software (24).

Results

A total of 27 rhizospheric soil samples were collected from various crops across seven villages in the Umiam Circle of Ri-Bhoi district, Meghalaya. Isolation of microbial bioagents from the rhizospheric soil samples yielded eleven *Trichoderma* isolates and five PGPR from the different locations (Table 1).

The morphological characterisation of both *Trichoderma* and PGPR isolates revealed significant diversity, indicating their potential for use in biocontrol and plant growth promotion. The *Trichoderma* isolates exhibited considerable variability in colony morphology with growth patterns ranging from densely granular (UM-1, UM-7), dense cottony (UM-3, UM-5, UM-9, UM-11), dispersed dusty/granular with concentric rings (UM-2, UM-4, UM-8, UM-10) to dispersed cottony (UM-6). Colony colour on maturity varied from whitish to yellowish green, lime green, dark green, olivaceous green and pale green. Pigmentation was observed in three isolates (UM-4, UM-6, UM-10), with UM-10 showing olivaceous green colonies along with pigmentation. Colony texture was either rough surfaced (UM-2, UM-4, UM-8, UM-10) or smooth surfaced (UM-1, UM-3, UM-5, UM-6, UM-7, UM-9, UM-11). Pustule formation was recorded in all isolates. PGPR isolates (PGPR-1, PGPR-2 and PGPR-4) were Gram-positive, catalase-positive and exhibited opaque colonies with entire or undulate margins. PGPR-3 showed gram-positive cocci and PGPR-5 as a Gram-negative, creamy yellow colony with an entire margin. The observed variability in cell shape, colony morphology, pigmentation and biochemical traits among both groups (Fig. 1, 2 and Table 2).

Microscopic characterisation of the eleven *Trichoderma* isolates showed distinct variations in conidiophore branching, phialide morphology and conidial characteristics (Table 3). Conidiophores ranged from regularly, irregularly and sparingly branched to compact verticillate and pyramidal types. Phialides

Table 1. *Trichoderma* and PGPR isolates obtained from different crop rhizospheres

Isolate code(s)	GPS coordinates	Associated crops	Place of collection
UM-1, PGPR-1	25°42'36''N 91°57'10''E	French bean	Umeit, Ri-Bhoi, Meghalaya
UM-2, UM-4, UM-10, PGPR-2	25°40'52" N 91°55'9" E	Sweet potato	Nongsder, Ri-Bhoi, Meghalaya
UM-3, PGPR-4	25°42'58''N 91°57'50''E	Ginger	Umdohbyrthih, Ri-Bhoi, Meghalaya
UM-5, UM-11, PGPR-3, PGPR-5	25°41'33''N 91°55'35''E	Cabbage	Pyllun, Ri-Bhoi, Meghalaya
UM-6	25°41'8" N 91°54'39" E	Forest land	Lum Sohpetbneng, Ri-Bhoi, Meghalaya
UM-7	25°42'1''N 91°56'8''E	French bean	Mawpun, Ri-Bhoi, Meghalaya
UM-8	25°40'2" N 91°54'5" E	Chayote	Umiam, Ri-Bhoi, Meghalaya

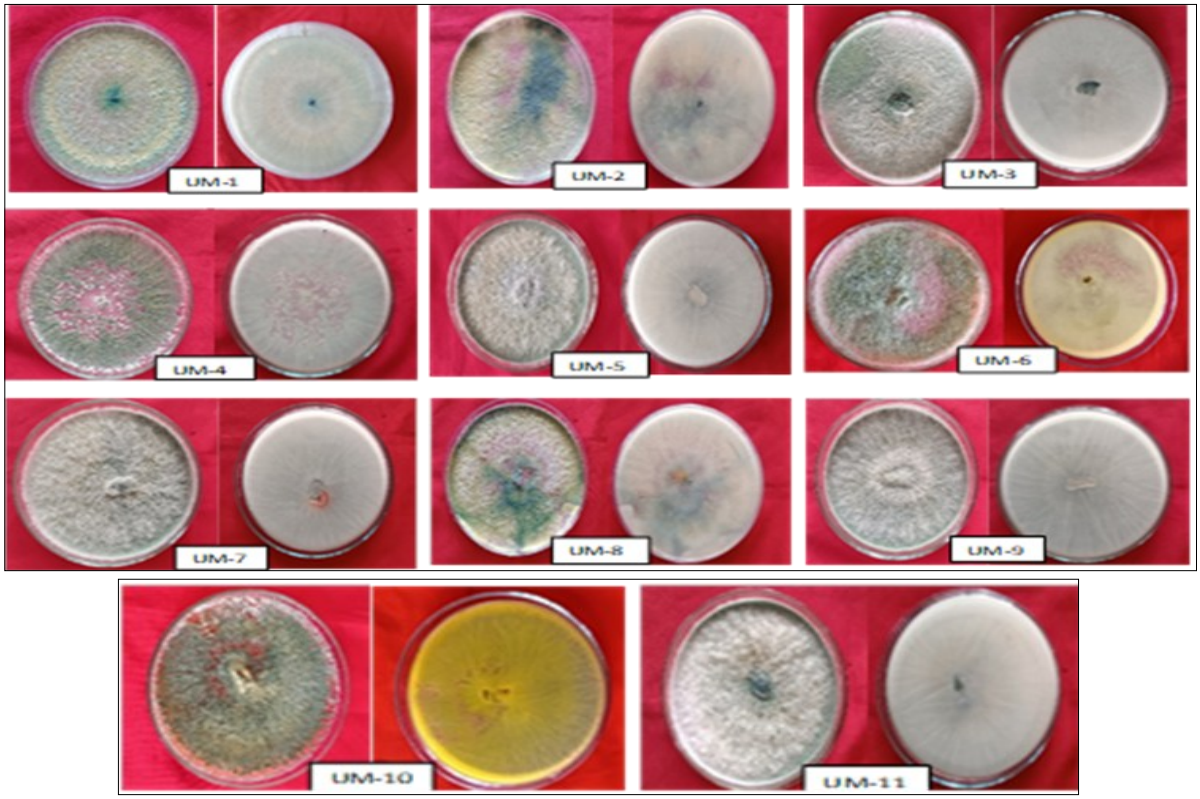

Fig. 1. Growth of *Trichoderma* isolates on potato dextrose agar media.

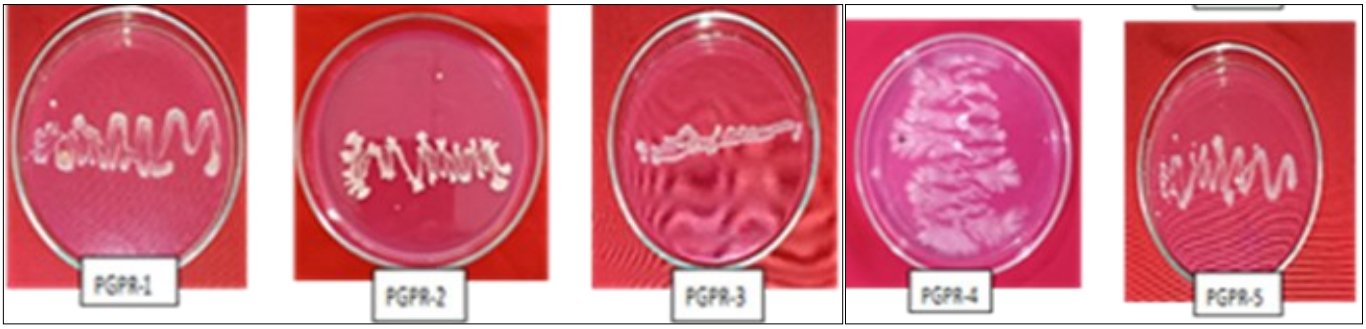

Fig. 2. Growth of plant growth-promoting rhizobacteria on nutrient agar media.

Table 2. Morpho-cultural characteristics of the bioagents

***Trichoderma* isolates**

Sl. No.	Isolate	Colony colour		Mycelial growth pattern	Pigmentation	Pustules
		Front	Reverse			
1.	UM-1	Whitish yellowish green to olivaceous green	Dull white	Granular with dense concentric growth	Non pigmented	Present
2.	UM-2	greenish yellow to light green	Dull white	Granular with concentric growth	Non pigmented	Present
3.	UM-3	Light green to whitish green	Dull White	Dense cottony growth with no concentric rings	Non pigmented	Present
4.	UM-4	Light yellowish to green	Yellowish green	Dusty granular with concentric growth	Pigmented	Present
5.	UM-5	White colour to lime green	Dull white	Dense cottony growth with no concentric growth rings	Non pigmented	Present
6.	UM-6	Light green	Yellowish white	Slightly granular, concentric rings	Pigmented	Present
7.	UM-7	Dark green to olive green	Dull white	Densely granular with no concentric growth	Non pigmented	Present
8.	UM-8	Green to yellowish white	Pale green	Dusty granular with concentric ring	Non pigmented	Present
9.	UM-9	Light green to dark green	White	Dense cottony growth with concentric rings	Non pigmented	Present
10.	UM-10	Olivaceous green	Yellow	Dusty granular dense growth with concentric rings	Pigmented	Present
11.	UM-11	Whitish green to dark green	Dull white	Dense cottony growth with no concentric rings	Non pigmented	Present

PGPR isolates

Sl. No.	Isolate	Gram staining	Catalase test	Cell shape	Colony colour	Colony margin
1.	PGPR-1	+	+	Rod shaped	Off white	Entire
2.	PGPR-2	+	+	Rod shaped	White	Undulate
3.	PGPR-3	+	-	Cocci	Off white	Entire
4.	PGPR-4	+	+	Rod shaped	Creamy white	Entire
5.	PGPR-5	-	-	Rod shaped	Creamy yellow	Entire

Table 3. Microscopic characterisation of *Trichoderma* isolates

Sl. No.	*Trichoderma* isolates	Conidiophore	Phialides	Conidia colour	Size of conidia (µm)	Conidia shape	Species identified
1	UM-1	Regularly branched	Flask-shaped	Green	3.26 × 2.75	Globose to ovoid	*T. asperelloides*
2	UM-2	Irregularly branched	Bottle-shaped	Dark green	3.73 × 2.33	Ellipsoidal to globose	*T. reesei*
3	UM-3	Pyramidal	Flask-shaped	Light green	2.99 × 2.4	Globose to ovoid	*T. asperellum*
4	UM-4	Sparingly branched	Narrow flask-shaped	Yellowish green	3.74 × 1.80	Ellipsoidal to obovoid	*T. asperellum*
5	UM-5	Compact verticillate	Flask-shaped	Yellowish green	3.29 × 2.56	Globose to ovoid	*T. asperellum*
6	UM-6	Sparingly branched	Slender bottle-shaped	Light green	3.3 × 1.73	Ellipsoidal to obovoid	*T. ghanense*
7	UM-7	Verticillate	Flask-shaped	Dark green	3.3 × 2.5	Globose to ovoid	*T. virens*
8	UM-8	Irregularly branched	Bottle- to rod-shaped	Light green	3.45 × 2.37	Ellipsoidal to globose	*T. viride*
9	UM-9	Sparingly branched	Narrow flask-shaped	Yellowish green	3.13 × 1.61	Ellipsoidal to obovoid	*T. asperellum*
10	UM-10	Compact verticillate	Bottle- to rod-shaped	Green	3.70 × 2.4	Ellipsoidal to globose	*T. harzianum*
11	UM-11	Regularly branched	Bottle-shaped	Green	3.35 × 2.4	Ellipsoidal to globose	*T. asperellum*

Fig. 3. PCR amplicons of *Trichoderma* and plant growth-promoting rhizobacteria isolates.

were mostly flask- or bottle-shaped, with some variations like slender and cylindrical forms. Conidia were predominantly green to yellowish green in colour, with shapes varying from globose to ovoid and ellipsoidal to obovoid. Conidial sizes ranged from 2.99 × 2.4 µm (UM-3) to 3.745 × 1.80 µm (UM-4). Gram staining of PGPR isolates showed that PGPR-1, PGPR-2, PGPR-3 and PGPR-4 were Gram-positive (purple colour), whereas PGPR-5 was Gram-negative (pinkish colour).

Molecular characterisation of *Trichoderma* and PGPR isolates with PCR amplification and visualisation of the extracted amplicon (Fig. 3) revealed that the amplicon size of ~ 600 bp confirms the genus of *Trichoderma* for the eleven isolates. Supported by morpho-cultural and microscopic characterisation of the isolates, establish the identity as *Trichoderma asperelloides* (UM-1), *T. reesei* (UM-2), *T. asperellum* (UM-3, UM-4, UM-5, UM-9, UM-11), *T. ghanense* (UM-6), *T. virens* (UM-7), *T. viride* (UM-8) and *T. harzianum* (UM-10). The amplicon size of ~ 1400 for PGPR isolates, along with the morpho-cultural and biochemical characterisation, identifies three PGPRs as *Bacillus* spp. (PGPR-1, PGPR-2 and PGPR-4), while PGPR-3 was identified as *Enterococcus faecium* and PGPR-5 resembled *Azotobacter chroococcum.*

The functional characterisation of both *Trichoderma* and PGPR isolates revealed considerable variability in their plant growth -promoting and biochemical traits. Most *Trichoderma* isolates were positive for ammonia, HCN, IAA, iron chelation and amylase production, with several isolates also exhibiting efficient phosphate and zinc solubilization. Among them, UM-10 stood out with the highest iron chelation (60.66 %) and zinc solubilization (70.1 %), followed by UM-1 and UM-4, which also demonstrated strong multifaceted activity (Table 4). Similarly, all PGPR isolates, except PGPR-5, produced HCN, while all were positive for IAA and ammonia production. PGPR-4 exhibited the most efficient profile, showing high zinc (65.0 %) and phosphate solubilization (62.0 %), significant iron chelation (67.5 %) and positive amylase activity. PGPR-2 recorded the highest iron chelation (75.0 %) but lacked phosphate and zinc solubilization ability (Table 5). Overall, UM-10

Table 4. Functional and plant growth-promoting attributes of *Trichoderma* isolates

Isolate	Ammonia production	HCN production	IAA production	Iron chelation efficiency (%)	Phosphate solubilization efficiency (%)	Zinc solubilization efficiency (%)	Amylase production
UM-1	+	+	+	51.67 ± 0.47^{cd}	40.8 ± 0.37^{a}	56.7 ± 0.59^{c}	+
UM-2	+	+	+	53.46 ± 2.23^{c}	38.6 ± 0.22^{b}	53.7 ± 0.37^{d}	+
UM-3	+	-	+	52.66 ± 0.47^{cd}	37.0 ± 0.25^{c}	53.8 ± 0.36^{d}	+
UM-4	-	-	+	56.00 ± 0.81^{b}	0.0 ± 0.00^{f}	64.5 ± 0.36^{b}	-
UM-5	+	+	+	50.66 ± 0.47^{d}	39.0 ± 0.37^{b}	52.0 ± 0.35^{e}	+
UM-6	-	-	+	51.66 ± 0.94^{cd}	0.0 ± 0.00^{f}	0.0 ± 0.00^{f}	-
UM-7	+	+	+	51.00 ± 0.81^{d}	35.0 ± 0.22^{d}	54.2 ± 0.22^{d}	+
UM-8	-	-	+	41.00 ± 0.81^{e}	33.0 ± 0.16^{e}	54.1 ± 0.24^{d}	-
UM-9	+	+	+	56.00 ± 0.81^{b}	0.0 ± 0.00^{f}	0.0 ± 0.00^{f}	+
UM-10	+	+	+	60.66 ± 0.47^{a}	0.0 ± 0.00^{f}	70.1 ± 0.29^{a}	+
UM-11	+	+	+	43.50 ± 0.81^{e}	40.6 ± 0.35^{a}	53.6 ± 0.20^{d}	+
C.D. (p=0.05)				1.985	0.46	0.097	
S.E.m (±)				0.677	0.158	0.033	

Different letters indicate statistically significant differences ($p < 0.05$) between means or treatments.

Table 5. Functional and plant growth-promoting attributes of PGPR isolates

PGPR isolate	Ammonia production	HCN production	Indole-3-acetic acid production	Iron chelation efficiency (%)	Phosphate solubilization efficiency (%)	Zinc (Zn) solubilization efficiency (%)	Amylase test
PGPR- 1	+	+	+	0.0 ± 0.00^{d}	56.0 ± 0.73^{c}	55.0 ± 0.93^{c}	-
PGPR- 2	+	+	+	75.0 ± 0.29^{a}	0.0 ± 0.00^{e}	0.0 ± 0.00^{d}	+
PGPR- 3	+	+	+	0.0 ± 0.00^{d}	60.0 ± 0.16^{b}	0.0 ± 0.00^{d}	+
PGPR- 4	+	+	+	67.5 ± 0.22^{b}	62.0 ± 0.33^{a}	65.0 ± 0.22^{a}	+
PGPR- 5	+	-	+	66.7 ± 0.22^{c}	55.0 ± 0.57^{d}	63.0 ± 0.36^{b}	-
C.D ($p = 0.05$)				0.42	1.00	1.01	
S.E.(m) ±				0.134	0.316	0.321	

Different letters indicate statistically significant differences ($p < 0.05$) between means or treatments.

among *Trichoderma* and PGPR-4 among PGPR isolates emerged as the most promising candidates due to their consistent and strong plant growth-promoting potential, suggesting their suitability for development as bioinoculants in sustainable agriculture.

The antagonistic activity of *Trichoderma* and PGPR isolates showed significant variation in percent inhibition of the pathogen. Among *Trichoderma* isolates, UM-10 exhibited the highest inhibition (87.30 %), followed by UM-4 (81.00 %) and UM-5 (78.38 %), indicating strong mycoparasitic potential. Moderate inhibition was observed in UM-1, UM-2 and UM-11 (70–76 %), while UM-8 and UM-9 recorded the lowest inhibition (< 60 %). In the case of PGPR isolates, PGPR-3 (67.0 %) and PGPR-2 (66.6 %) showed the highest inhibition, followed by PGPR-4 (63.6 %) and PGPR-1 (62.6 %), whereas PGPR-5 showed the least inhibition (55.7 %) (Table 6 and Fig. 4, 5). In this study, the two potential *Trichoderma* isolates (UM-5 *T. asperellum* and UM-10 *T. harzianum*) and one PGPR isolate (PGPR-4 *Bacillus subtilis*) were tested for compatibility. The results showed no formation of a halo or inhibition zone, indicating that these bioagents (UM5 and PGPR4; UM10 and PGPR4) were compatible with each other. The antagonistic potential and functional attributes of the native *T. asperellum* (UM-5), *T. harzianum* (UM-10) and *Bacillus subtilis* (PGPR-4) isolates make

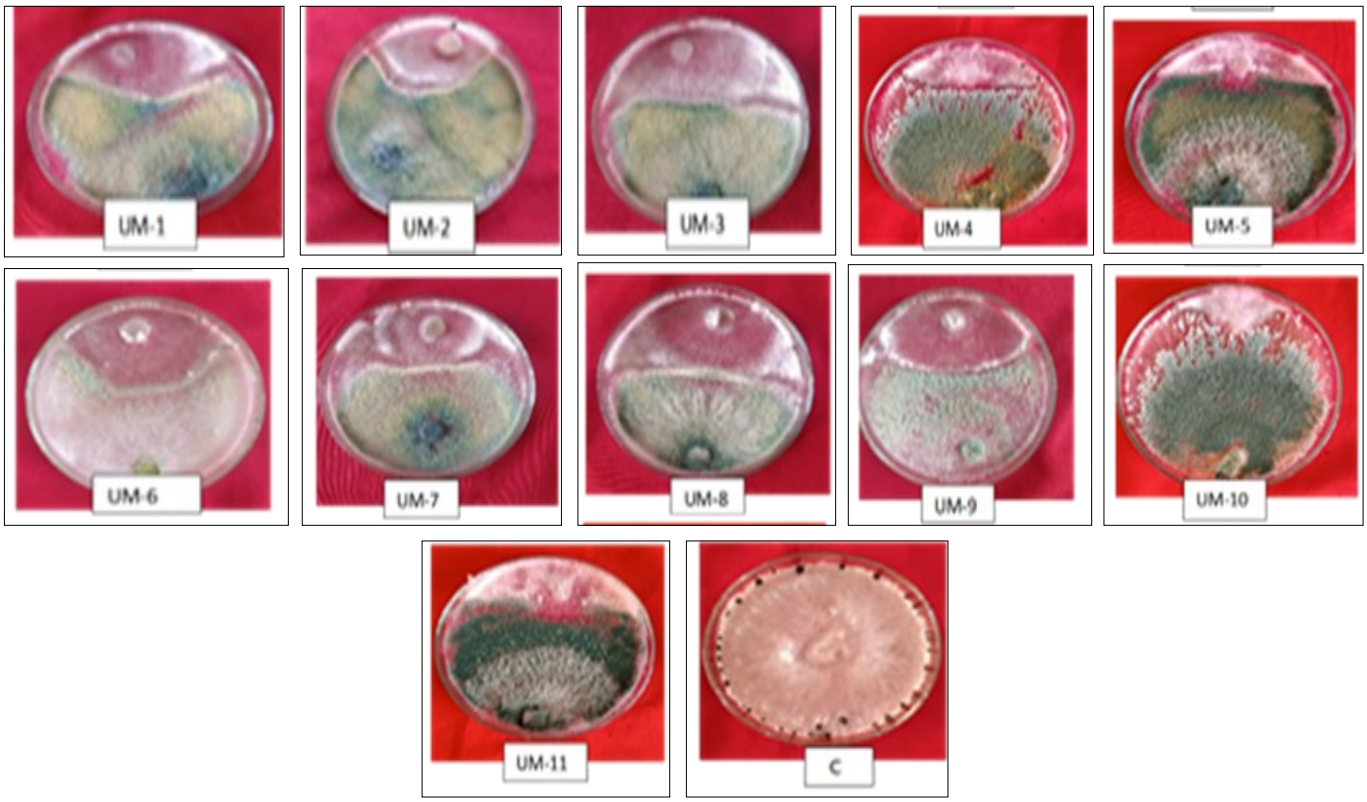

Fig. 4. Dual culture assay with *Trichoderma* spp.

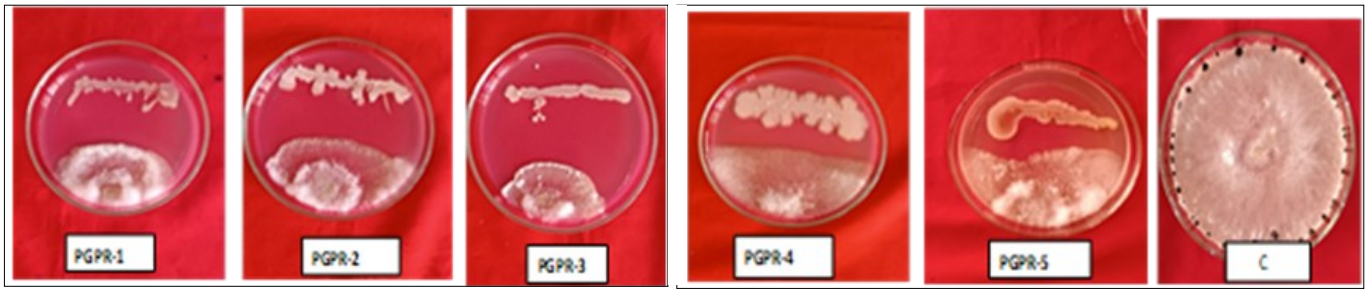

Fig. 5. Dual culture assay with PGPR.

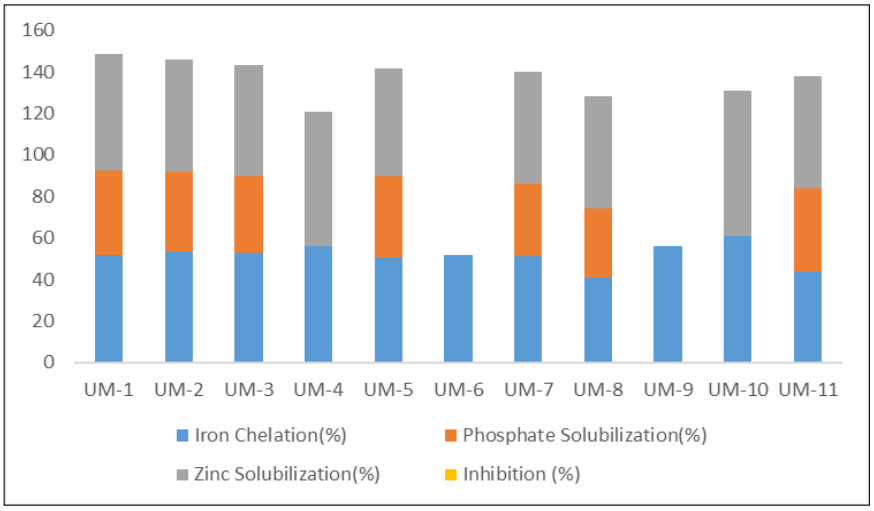

Fig. 6. Antagonistic and functional attributes of *Trichoderma* isolates.

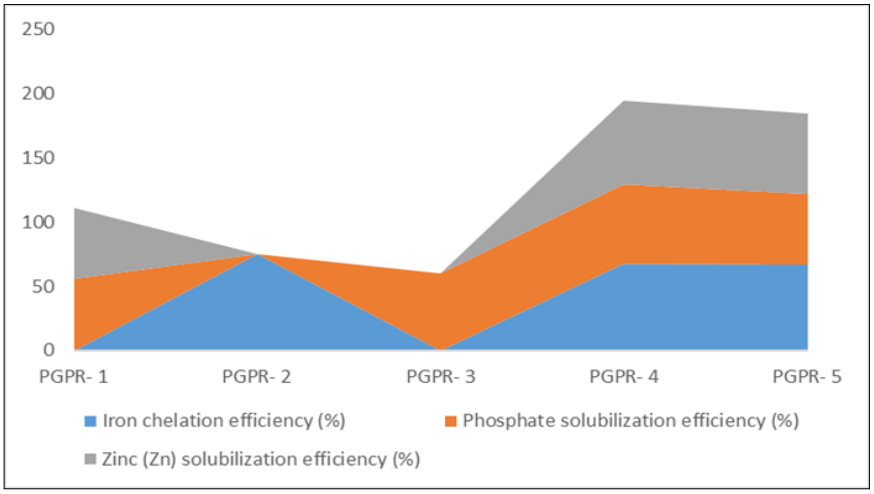

Fig. 7. Antagonistic and functional attributes of PGPR isolates.

Table 6. Antagonistic potential of bioagents against *S. sclerotiorum*

Isolate	Percent inhibition (%)
Trichoderma isolates	
UM-1	70.890 ± 0.057^{f}
UM-2	71.890 ± 0.024^{e}
UM-3	60.267 ± 0.170^{l}
UM-4	81.000 ± 0.816^{b}
UM-5	78.383 ± 0.278^{c}
UM-6	68.400 ± 0.455^{g}
UM-7	66.000 ± 0.057^{h}
UM-8	56.000 ± 0.016^{k}
UM-9	58.420 ± 0.348^{j}
UM-10	87.297 ± 0.421^{a}
UM-11	75.967 ± 0.080^{d}
C.D ($p = 0.05$)	0.710
S.E.(m) ±	0.242
Isolate	**Percent inhibition (%)**
PGPRs	
PGPR-1	62.6 ± 0.36^{c}
PGPR-2	66.6 ± 0.29^{a}
PGPR-3	67.0 ± 0.37^{a}
PGPR-4	63.6 ± 0.29^{b}
PGPR-5	55.7 ± 0.28^{d}
C.D ($p = 0.05$)	0.72
S.E.(m) ±	0.228

Different letters indicate statistically significant differences ($p < 0.05$) between means or treatments.

them efficient bioagents for use in plant disease management strategies of sustainable agriculture (Fig. 6, 7).

Discussion

The present study explored components of eco-friendly strategies for managing *S. sclerotiorum* through the use of native *Trichoderma* spp. and PGPR isolates. 11 *Trichoderma* isolates collected from diverse rhizospheric soils exhibited notable morphological and microscopic diversity in colony colour, texture, ring formation, pigmentation, conidiophore structures and phialide and conidial characteristics traits widely recognised for species-level identification, as reported by previous workers (25–29). All isolates tested positive for ammonia and IAA production, which aligns with studies that show most *Trichoderma* spp. showed a positive reaction for the test (30, 31), while traits like HCN, siderophore, phosphate and zinc solubilization showed variation among strains. Research has indicated antagonism level as a defence regulator and inhibiting pathogens or by chelating iron and depriving the pathogens (32–35). Most isolates also demonstrated amylase activity, indicating their contribution to nutrient cycling and promoting plant growth aligns with the earlier findings (21, 35). *In vitro* antagonism confirmed that all *Trichoderma* isolates effectively suppressed *S. sclerotiorum*, with UM-10 (*T. harzianum*) exhibiting the highest inhibition (87.3 %), surpassing earlier reports of 59.08 % and 69.8 % inhibition (36, 37). Research indicates that some isolates showed 85.3 % inhibition (33, 38). The PGPR isolates also exhibited morphological and functional diversity (39, 40), with most showing positive responses for catalase, IAA, HCN, siderophore, phosphate,

zinc solubilization and amylase production (41–43). *In vitro* antagonistic assays confirmed an inhibition of 55–67 % against *S. sclerotiorum* by PGPR isolates, which is consistent with previous studies ranging from 23 % to 45.19 %, as well as 86.6 % inhibition by *Bacillus* spp. (44–46). Compatibility testing between the two *Trichoderma* isolates (UM-5, *T. asperellum* and UM-10, *T. harzianum*) and a promising PGPR isolate (PGPR-4, *Bacillus subtilis*) using the dual culture technique showed no inhibition zone, indicating mutual compatibility. This suggests their potential for combined application in disease management. Research also observed no antagonistic interaction between *Bacillus* spp. and *Trichoderma* isolates (47). Similarly, several *Bacillus* and *Trichoderma* isolates remained mutually compatible and effective when combined, supporting the idea that appropriate pairing enhances biocontrol efficacy (48).

Conclusion

The present study demonstrates that native *Trichoderma* spp. and PGPR isolates possess significant potential in managing *S. sclerotiorum*. These isolates exhibited key plant growth-promoting traits such as ammonia, IAA, HCN, siderophore production and nutrient solubilization, along with strong antagonistic activity, achieving more than 50 % inhibition of the pathogen. Such attributes highlight their promise as effective bioagents for eco-friendly disease management while simultaneously enhancing crop growth. Future studies focusing on field evaluations, formulation development and integration of these bioagents into sustainable crop production systems could further strengthen their applicability in agricultural disease management.

Acknowledgements

The authors duly acknowledge the support received from ICAR- Research Complex for NEH Region, Umiam, Meghalaya and College of Post Graduate Studies-Agricultural Sciences, Central Agricultural University (CAU-Imphal), Umiam, Meghalaya for conducting and carrying out the research work.

Authors' contributions

MM executed the research work and prepared the manuscript as part of her master's degree. TRB contributed to the formulation of the study and was involved in facilitation, drafting, writing and revising the manuscript. MP and NTM supported the execution of the research. IK and AB assisted in conducting the experiments. MC and PB provided support in the execution of the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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