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ORIGINAL ARTICLE

Biocontrol potential of *Trichoderma* and *Bacillus* strains against *Macrophomina phaseolina* causing dry root rot in groundnut

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Abstract

Dry root rot caused by *Macrophomina phaseolina* is an emerging and highly destructive disease responsible for significant yield losses in groundnut production, particularly in semi-arid tropics regions of the world. The present study aimed to isolate, characterize and evaluate the antagonistic efficacy of bioagents against *M. phaseolina* via in vitro methods (dual culture, inverted plate and culture filtrate assays) and talc-based formulations of potential *Trichoderma* and *Bacillus* isolates under in vivo conditions. A total of sixteen isolates of each fungal and bacterial bioagents were isolated and screened for their inhibitory effects against *M. phaseolina*. Among these, five *Trichoderma* isolates (PNT01, PNT05, PNT08, PNT11 and PNT16) and five *Bacillus* isolates (PNB01, PNB05, PNB10, PNB13, and PNB16) exhibited the highest level of mycelial growth inhibition under in vitro conditions. Molecular characterization revealed that four *Trichoderma* isolates (PNT01, PNT05, PNT10, and PNT16) were closely related to *Trichoderma harzianum* species complex, and PNT14 was identical to *T. virens*. Similarly, the *Bacillus* isolates PNB05 and PNB10 have similarities with *Bacillus subtilis*, PNB03 (*B. oceanisediminis*), PNB13 (*B. pacificus*), and PNB16 (*B. cereus*). In the culture filtrate assay, PNT05 (*T. harzianum* species complex) and PNB10 (*B. subtilis*) exhibited highest mycelium growth inhibition of *M. phaseolina*. Field results revealed that the seed treatment combined with soil application of *T. harzianum* species complex resulted in the maximum reduction in disease inhibition and significant improvement in plant-growth and yield parameters such as germination percentage, shoot length, root length, number of root nodules per plant, number of pods per plant and pod yield followed by seed treatment combined with soil application of *B. subtilis.* These findings indicate that *T. harzianum* species complex *and B. subtilis* may be used as potential biocontrol agents against *M. phaseolina*, which favours the eco-friendly management of groundnut dry root rot disease.

Keywords *Bacillus subtilis* · Bioagents · Disease inhibition · Dry root rot · *Trichoderma harzianum* species complex

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Introduction

Groundnut (*Arachis hypogaea* L.) is an important oilseed and food crop, widely cultivated in semi-arid tropics (SAT) regions of the world, predominantly by small and marginal farmers under rainfed conditions (Kadiyala et al. 2021). It is a rich source of proteins, essential minerals such as calcium, zinc, magnesium, iron, phosphorus as well as vitamins B and E, along with some biologically active compounds including polyphenols, flavonoids and isoflavones (Desmae et al. 2018). India ranks second after China in both area and production of groundnut, with a cultivated area in India is 5.7 M ha, an annual production of 10.13 MT and a productivity of 1.77 t ha^{-1}. The major groundnut producing states in Indian include Gujarat, Andhra Pradesh, Rajasthan, Karnataka, Madhya Pradesh, Tamil Nadu, Maharashtra and Telangana (Directorate of Economics and Statistics 2023). Groundnut production is frequently challenged by various biotic stresses such as diseases and pests, which can significantly reduce both yield and quality (Kumar et al. 2020). Among diseases, dry root rot caused by the soil-borne pathogen *Macrophomina phaseolina* (Tassi) Goid is one of the most destructive diseases, affecting the crop all the growth stages (Khan et al. 2018) causing yield losses of up to 30% in groundnut (Khan et al. 2017), under favourable conditions like high temperature, low soil moisture, and high humidity (Gaige et al. 2010). Long periods of drought and soil temperature intermingled with rains create favourable conditions for disease development (Babu et al. 2007). The fungus is a facultative saprophyte that survives as sclerotia in infected soil for up to 15 years (Kaur et al. 2012), and is disseminated via plant debris, soil, and infected pods. The wind borne pycniospores also act as potential disseminating agents for the pathogen (Mahendra et al. 2022).

Management of *M. phaseolina* is challenging due to its soilborne nature and its propagules are distributed randomly in the soil, it is difficult to be control via fungicides (Degani et al. 2023). Moreover, fungicides are effective only on the active metabolic stage of the pathogen and not on the resting structures (Karthikeyan et al. 2006). The application of chemical fungicides in soil is expensive, highly employed, haphazardly applied and easy to utilize, which causes environmental deterioration and is deleterious to nontarget microflora, and development of resistant pathogen strains (Rafiq et al. 2024). Sustainable agriculture requires to use of effective, economical, safe, and compatible strategies for disease management. Bioagents are feasible, have great potential to ameliorate environmental hazards, reduce the chances of resistance in pathogen and provide the best integrated disease management strategies to reduce yield losses caused by fungal pathogens (Iqbal and Mukhtar 2020). Among fungal and bacterial bioagents, *Trichoderma* species and *Bacillus* species are the most prominent and effective mycoparasites against different soil borne fungal pathogens have been frequently reported (Ali et al. 2020; Zohaib et al. 2024). *Trichoderma* spp. and *Bacillus* spp. are naturally occurring soil microbes that colonize roots, compete with pathogens for nutrients, inhibit pathogen growth, and stimulate plant growth (Khaledi and Taheri 2016). *Trichoderma* species thrives in diverse soil habitats with high population densities and survives various unfavourable conditions (El-Benawy et al. 2020). It can produce diverse antimicrobial compounds, such as trichomycin, chlorotrichomycin and antimicrobial peptides (Marquez et al. 2021). Beyond suppressing pathogens, *Trichoderma* elicits plant systemic resistance, boosting local and systemic defences (Shafqat et al. 2026). Similarly, *Bacillus* species endure adverse environments by forming endospores (Rangel-Montoya et al. 2022). They synthesize antibiotics, toxins, siderophores, and lytic enzymes, while stimulating systemic resistance and plant growth (Khan et al. 2021).

The efficacy of bioagents against *M. phaseolina*, the causal agent of dry root rot has been documented in various crops by earlier workers, notably in chickpea (Manjunatha et al. 2011), blackgram (Latha et al. 2017), sesame (Adhikary et al. 2019) and soybean (Dhawan et al. 2019). The integration of bioagents with chemicals and organic amendments has been reported to reduce disease incidence while improving crop growth and yield (Shafqat et al. 2026). Similarly, Thilagavathi et al. (2007) reported that the combined use of *B. subtilis* and *T. harzianum* significantly suppressed *M. phaseolina* and improved disease resistance in greengram. Seed treatment with talc-based formulations of potential antagonistic fungi and bacteria reduced the incidence of root rot and improved the groundnut yield (Ramesh and Korikanthimath 2010). However, limited information is available on the combined application of seed treatment and soil application of *Trichoderma* spp. and *Bacillus* spp. for managing dry root rot disease in groundnut. Moreover, the use of native antagonistic strains isolated from groundnut rhizosphere may enhance the disease control efficacy, since native strains perform better in rhizosphere than the exotic strains do (Chenniappan et al. 2019). The present study aimed to explore the potential biocontrol efficacy of *Trichoderma* spp. and *Bacillus* spp. isolated from the groundnut rhizosphere of surveyed locations in southern India. The most effective *Trichoderma* and *Bacillus* isolates identified through dual culture, metabolite production, and culture filtrate assays against the virulent isolate of *M. phaseolina*, were characterized to the species level via universal primers. Furthermore, talc-based formulations of potential *Trichoderma* and *Bacillus* isolates were evaluated for their efficacy as seed treatment and soil application against groundnut dry root rot disease under field conditions.

Materials and methods

The present investigations were carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India, from year 2019–2023.

Isolation of the dry root rot pathogen *M. phaseolina*

The pathogen *M. phaseolina* (Tassi) Goid was isolated from dry root rot infected groundnut plants collected from groundnut growing areas of southern India viz. Andhra Pradesh, Telangana, Karnataka, and Tamil Nadu, were identified and confirmed by morphological, pathological and molecular characterization in previously published work (Pamala et al. 2023). The virulent isolate GRB 52 was utilized in the present work.

Isolation and identification of biocontrol agents from the groundnut rhizosphere

Soil samples were collected from the groundnut rhizosphere during a disease survey (southern India). In each field, five randomly selected healthy groundnut rhizosphere soil samples from the surface (10–20 cm) layer were collected and composited, placed in plastic bags, transported to the laboratory and used for *Trichoderma* and *Bacillus* isolation.

Ten grams of rhizospheric soil was transferred to a 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water and kept on a rotary shaker at a speed of 150 rpm for 30 min; after thorough shaking, the suspension was used to isolate bioagents via the soil dilution plate method. The suspensions were further diluted to 10^{-5} (Aneja 2022). For *Trichoderma* spp. isolation, 1 ml of each aliquot was inoculated via the spread plate method on Petri plates containing *Trichoderma* selective medium (HiMedia), incubated for seven days at 28 ± 2 °C and observed for fungal growth at regular time intervals. Colonies with characteristics of *Trichoderma* spp. were subcultured on potato dextrose agar (PDA) and identified on basis of morphological characteristics like colony and spore morphology. For *Bacillus* spp. isolation, one ml of each aliquot was poured onto petri plates containing *Bacillus* differentiation agar medium (HiMedia). The plates were gently rotated clockwise and anticlockwise for uniform distribution, incubated at 28 ± 2 °C for 48 h and observed for bacterial growth. Rough colonies with waxy growth and irregular spreading edges were subcultured on nutrient agar used for identification and confirmation via Gram staining and microscopic observation (100x).

Antagonistic effects of *Trichoderma* and *Bacillus* strains on the mycelial growth of *M. phaseolina*.

Dual culture antagonistic assay

The in vitro inhibition of the mycelial growth of *M. phaseolina* by the *Trichoderma* isolates was evaluated via the dual culture technique described by Meena et al. (2017). Mycelial discs (6 mm) from colony margins of actively growing 3-days old culture was placed 10 mm away from the periphery of PDA containing Petri dishes (90 mm) and same sized mycelial disc of three-day-old test pathogen was placed on the opposite side of *Trichoderma* isolate. Similarly, *Bacillus* isolates were streaked 10 mm away from the periphery of Petri dish, opposite side to test pathogen (Samaras et al. 2021). In the control plates, only mycelial discs of *M. phaseolina* were inoculated and three replications were maintained. The Petri dishes were incubated at 28 ± 2°C until the radial growth of the test pathogen reached 90 mm in control Petri dishes. The percent inhibition of mycelial growth of the pathogen over the control was calculated via the following formula (Vincent 1947).

Effects of antifungal volatile organic metabolites

An inverted plate assay or paired plate assay was used to evaluate the effects of volatile organic compounds released by both *Trichoderma* and *Bacillus* isolates on the mycelial growth of *M. phaseolina* (Meena et al. 2017). A Petri dish containing PDA inoculated with a 6 mm mycelial disc of the test pathogen was inverted over a plate containing a 6 mm mycelial disc of each *Trichoderma* isolate. A Petri plate containing NA medium was inoculated with a loopful of 2-day-old culture of each *Bacillus* isolate. These plates were sealed with parafilm to prevent the volatile compounds from escaping. The control consisted of a test pathogen plate upturned and positioned over an uninoculated PDA plate (no antagonist). The experiment was conducted in triplicate and paired plates were incubated at 28 ± 2 °C. After the incubation period, the mycelial growth of the test pathogen was inhibited in the paired plates, and the percent inhibition of the test pathogen was calculated.

Antagonistic assay of culture filtrates of *Trichoderma* and *Bacillus* isolates

Trichoderma and *Bacillus* isolates showing considerable antagonistic activity in dual culture and inverted plate assays were selected for the culture filtrate assay. The food poisoning technique was used to evaluate the effects of *Trichoderma* and *Bacillus* culture filtrates on the radial growth of *M. phaseolina* (Dugassa et al. 2021). The selected

Trichoderma isolates were cultured in a 250 ml conical flask containing 100 ml of potato dextrose broth (PDB) medium, incubated for eight days at 28 ± 2 °C on an incubator shaker (150 rpm) and filtered through sterilized filter paper (Whatman No. 1) to remove mycelial mats. The culture filtrate was centrifuged at 10,000 rpm (Sorvall RC-5B, GS-3 rotor, DuPont Instruments, Newton, CT, USA) for 15 min, and the obtained supernatants were filtered under laminar flow using 0.45 µm sterile membrane filters (Merck Millipore, Ireland) in sterilized breakers. Similarly, the *Bacillus* isolates were inoculated in nutrient broth and incubated for 5 days in a shaking incubator at 150 rpm. The culture was filtered with Whatman No. 1 filter paper, centrifuged at 10,000 rpm for 15 min and passed through 0.45 µm sterile membrane filters to obtain the cell free filtrate.

Three different concentrations (10%, 25%, 50%) of culture filtrate were prepared and poured into molten PDA medium containing 250 ml sterilized flasks. Approximately 20 ml of medium mixed with different concentrations was poured into 90 mm Petri dishes and the actively growing 6 mm mycelial plugs of *M. phaseolina* were inoculated at the centre of the Petri dishes. PDA plates inoculated with pathogens without culture filtrates served as controls. Three replications were maintained for each concentration and incubated at 28 ± 2 ℃. The percent inhibition of mycelial growth of *M. phaseolina* was calculated.

Molecular characterization of *Trichoderma* spp. and *Bacillus* spp.

The fungal isolates were grown in test tubes containing PDB (HiMedia) for 3 days at 28 ± 2 °C at the 45° slant position, and mycelia were recovered via sterile glass rods. The genomic DNA was isolated using the CTAB method according to Murray and Thompson (1980). The rDNA-ITS region was amplified using 100 ng of genomic DNA and employing universal fungal primers (Integrated DNA Technologies, Coralville, US) namely, ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCCTCCGCTTATTGATA TGC-3') (White et al. 1990), PCR products were electrophoresed, purified, and sequenced as described previously (Pamala et al. 2023).

The bacterial genomic DNA was extracted according to Sharma and Singh (2005). The five isolates were grown in 100 ml conical flasks containing 50 ml of Luria-Bertani (LB) broth (HiMedia) for 24 h at 28 ± 2 °C in a shaker. Two millilitres of culture broth was transferred to 2 ml Eppendorf tubes and centrifuged at 12,857 *g* for 2 min. The supernatant was discarded, and the pellet was dissolved in 567 µl of TE (1 M Tris–HCl buffer and 0.5 M EDTA; pH 8) buffer by repeated pipetting. Thirty microlitres of 10% SDS and 3 µl of 20 mg/ml proteinase K were added, mixed thoroughly and incubated for 1 h at 37 °C, 100 µl of 5 M NaCl and 80 µl of CTAB/NaCl solution were added, mixed thoroughly, and incubated in a water bath at 65 °C for 45 min with gentle shaking intermediately by hand for 30 s. An equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added, and the mixture was gently mixed to denature the proteins and centrifuged at 12,857 *g* for 10 min. The supernatant was transferred to new 2 ml centrifuge tubes and an equal volume of chloroform: isoamyl alcohol (24:1) was added, gently mixed, and centrifuged at 12,857 *g* for 10 min. The DNA was precipitated with 0.6 volume of chilled isopropanol and 0.1 volume of 7.5 M ammonium acetate and centrifuged at 18,514 *g* for 15 min. The DNA pellets were washed twice with chilled 70% ethanol, dried at room temperature, resuspended in 100 µl of sterile TE buffer and stored at -20 °C. The DNA pellet was dissolved in 100 µl of sterile TE buffer and stored at -20 ℃.

The 16S rRNA region was amplified via the universal bacterial primers 27-F (5'- AGAGTTTGATCCTGGCT CAG −3') and 1492-R (5'- TACGGTTACCTTGTTAC GACTT −3') (Kalam et al. 2020). For this, 2X PCR Taq Master mix (Applied Biological Materials, Richmond, Canada) containing dNTPs, DNA polymerase, buffer and $MgCl_2$, primers (Integrated DNA Technologies, Coralville, US) and nuclease-free water were used. The PCR thermal cycler (Bio-Rad, California, US) reaction contained 50 ng of genomic DNA, 1X PCR Taq Master mix and 0.25 µM of each primer (27-F and 1492-R) in a 30 µl reaction volume. The PCR program was, one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min and one cycle at 72 °C for 5 min and then held at 4 °C. The PCR products were analysed on 1.5% agarose gel electrophoresis for 90 min at 60 V in 1X TAE buffer (40 mM Tris base, 20 mM Acetic acid and 1 mM EDTA), stained with ethidium bromide and photographed by using a Gel Documentation System (Bio-Rad, California, US). PCR amplicons were purified via a NucleoSpin Gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) and sequenced at the University of Hyderabad. The sequences were analyzed using BioEdit Sequence Alignment Editor v7.2.5 and deposited in the GenBank (NCBI) database.

For phylogenetic analysis, a nucleotide Basic Local Alignment Search Tool (BLASTn) was used for fungal isolates (ITS rDNA) and bacterial isolates (16S rRNA) sequences. The references sequences (RefSeq) with the highest similarity with the corresponding sequences were downloaded and aligned with ClustalW, and phylogenetic tree was constructed via Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) (Tamura et al. 2021).

Preparation of talc-based formulations of bioagents

Talc-based formulation of the potential isolate *T. harzianum* species complex PNT05 was prepared as described by Mukherjee et al. (2014). Mycelial discs of 3-day-old actively growing culture of *Trichoderma* isolate PNT05 was inoculated into PDB and incubated for seven days at 28 ± 2°C on a rotary shaker at 150 pm. One kg of talc powder mixture contained ten grams of carboxymethyl cellulose (CMC), 1 kg of talc powder (montmorillonite), and 15 g of calcium carbonate ($CaCo_3$), and the mixture was autoclaved for 30 min at 121°C. After seven days, 200 ml of PDB medium (spore suspension containing 2×10^8 CFU/ml) were mixed with the talc mixture and dried under aseptic conditions. Similarly, talc-based formulation of the potential isolate *B. subtilis* PNB 10 was prepared by following the method described by Nandakumar et al. (2001). A loopful culture of a potential isolate *B. subtilis* PNB 10 was inoculated into sterilized NB medium and incubated in a rotary shaker (150 rpm) for 72 h at 28 ± 2 °C. After 72 h, 400 ml of bacterial broth suspension containing 1×10^9 CFU/ml and 1 kg of talc powder mixture were mixed under aseptic conditions. The dried talc powder was packed and used for field experiments. Talc-based formulation of *T. harzianum* species complex and *B. subtilis* were prepared 15 days before field application to multiply on farmyard manure (FYM) and contains $> 2 \times 10^7$ CFU/gm and $> 1 \times 10^8$ CFU/gm, respectively, with no contaminations at 8% moisture content.

Mass multiplication of *M. phaseolina*

Mass multiplication of *M. phaseolina* was done by using sorghum grains. The grains were soaked overnight and air dried at room temperature to remove excess moisture. Subsequently, 250 g of grains were placed in polythene bags and sterilized twice in an autoclave at 121°C for 15 min at 15 lb psi. After cooling, actively growing mycelial discs of *M. phaseolina* were aseptically inoculated under a laminar airflow chamber and incubated at 28 ± 2 °C for 15 days in a BOD incubator (Pamala et al. 2023). The obtained inoculum was applied to the field (3 kg/plot) at 30 days before sowing and 30 days after sowing (DAS) to increase the inoculum load in the soil.

Table 1 Detail of bio-agents used under field conditions

Treatment	Details of Treatments
T1	Seed treatment (ST) with *T. harzianum* species complex
T2	ST with *B. subtilis*
T3	Soil application (SA) with *T. harzianum* species complex
T4	SA with *B. subtilis*
T5	ST + SA with *T. harzianum* species complex
T6	ST + SA with *B. subtilis*
T7	Seed treatment of tebuconazole (2 DS @ 1.5 g/kg)
T8	Untreated control

Where seed treatment @ 10 g/kg of seed and soil application @ 5 g/kg of FYM

Integrated disease management of groundnut dry root rot under field conditions

A field trial was conducted during two successive growing seasons *Kharif*-2022 and *Rabi* 2022-23 at ICRISAT, Patancheru (India) to evaluate integrated disease management options for groundnut dry root rot disease under sick plot conditions. The experiment was carried out with a plot size of 1.5×4.0 m^2 in an RBD with eight treatments and three replications (Table 1) with a spacing of 30×10 cm. The groundnut cultivar K-6 was used and following production practices recommended by ICRISAT, Patancheru, India.

For the seed treatment, the seeds were treated with talc formulation of biocontrol agents at 10 g/kg of seeds and tebuconazole 2 DS at 1.5 g/kg of seeds prior to sowing. For soil application, the talc formulations were mixed with well-decomposed FYM 15 days before sowing at 5 g/kg FYM and applied to the soil of the respective plots before sowing at 2 kg/plot as basal. Observations like percentage of germination at 15 DAS and the percent disease incidence at harvest were recorded. After harvest, observations of growth and yield parameters viz., shoot length (cm), root length (cm), number of nodules/plants, number of pods/plants, pod yield/plot, and 100 kernel weight were recorded. Symptomatic plants were collected from each plot for re-isolated the pathogen and confirmed based morphological character like hyphal branching at the right angle, formation of constriction near the point of branching, presence of microsclerotia.

Statistical analysis

A completely randomized design (CRD) with three replications was used for laboratory experiments. Field experiments were conducted in a randomized block design (RBD) with eight treatments and three replications. The data were statistically analysed using R software version 4.1.0. Before statistical analysis of variance (ANOVA), the percentage values were arcsine transformed. The data were subjected to ANOVA at a significant level, and treatment means were compared using Tukey's multiple range test at $p \leq 0.01$ for lab studies and $p \leq 0.05$ for field experiments. The critical difference (CD) at 1% and 5% probability level was calculated as part of Tukey's multiple range test for mean separation.

Results

Isolation and identification of *Trichoderma* and *Bacillus* isolates

Sixteen isolates of each *Trichoderma* spp. and *Bacillus* spp. were isolated from groundnut rhizosphere soil collected from different groundnut growing areas of southern India using serial dilution technique. Each isolate represents one district of the surveyed locations.

The *Trichoderma* isolates were visually characterized on the basis of their phenotypic characteristics, like colony colour (dark green and cottony whitish-green colonies) and microscopic characteristics, such as conidiophores are long, hyaline and branched, with phialides emerging from small terminal clusters at 90° from the conidiophore; the conidia are hyaline, single-celled and ovoid. The isolates were designated PNT01 to PNT16 for further studies. Similarly, *Bacillus* isolates were initially characterized based on Gram staining and violent colour (gram positive), rod-shaped, and motile cells were observed. The isolates were designated PNB01 to PNB16 for further studies (Table 2).

Dual culture assay of *Trichoderma* and *Bacillus* strains against *M. phaseolina*

The 16 isolates of each *Trichoderma* spp. and *Bacillus* spp. were evaluated against the virulent isolate of *M. phaseolina* (GRb-52) via a dual culture assay. The results revealed that all the *Trichoderma* isolates significantly reduced the mycelial growth of the pathogen, as shown in Table 3 and Fig. 1. The maximum growth inhibition of 67% was recorded for the PNT05 isolate (Fig. 2) which presented the least mycelial growth of the pathogen (29.80 mm), followed by the PNT08 (62%), PNT11 (61%), PNT16 (60%), and PNT01 (60%) isolates, with growth inhibition over the control. Similarly, *Bacillus* isolates also significantly differed in their ability to inhibit the mycelial growth of the pathogen (Table 4 and Fig. 3). The mycelial growth inhibition ranged from 26 to 49%, where the maximum (49%) reduction in mycelial growth was recorded for isolate PNB10 (Fig. 2), followed by 47%, 46%, 46%, and 46% mycelial growth inhibition of the isolates PNB13, PNB01, PNB05, and PNB16, respectively.

Volatile antifungal compounds of *Trichoderma* and *Bacillus* strains against *M. phaseolina*

An inverted plate assay was used to evaluate the efficacy of sixteen *Trichoderma* isolates and *Bacillus* isolates against *M. phaseolina*. The *Trichoderma* results revealed that the PNT05 isolate inhibited 54% mycelial growth of

Table 2 List of *Trichoderma* and *Bacillus* isolates collected from surveyed areas of southern India

State	Districts	Mandal/Taluk	Isolate code	
			Trichoderma sp.	*Bacillus* sp.
Andhra Pradesh	Ananthapuramu	Kadiri	PNT01	PNB01
		Kudair	PNT02	PNB02
	Chittoor	S.R. Puram	PNT03	PNB03
		Yerpedu	PNT04	PNB04
Karnataka	Chitradurga	Challakare	PNT05	PNB05
		Hiriyur	PNT06	PNB06
	Tumakur	Pavagada	PNT07	PNB07
		Sira	PNT08	PNB08
Tamil Nadu	Tiruvannamalai	Arni	PNT09	PNB09
		Polur	PNT10	PNB10
	Villupuram	Gingee	PNT11	PNB11
		Melmalaiyanur	PNT12	PNB12
Telangana	Nagarkurnool	Balmoor	PNT13	PNB13
		Talkapally	PNT14	PNB14
	Wanaparthy	Kothakota	PNT15	PNB15
		Pebbair	PNT16	PNB16

Table 3 Mycelial growth inhibition of *Trichoderma* isolates in dual culture and inverted plate methods

Isolate	Dual culture		Inverted plate	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Control	$90.0^{a} \pm 0.0$	0^{h}	$90.00^{a} \pm 0.0$	0^{g}
PNT01	$36.30^{hij} \pm 1.5$	60^{bcd}	$54.73^{i} \pm 1.6$	39^{b}
PNT02	$45.20^{b} \pm 0.8$	50^{g}	$80.87^{d} \pm 1.9$	10^{e}
PNT03	$37.33^{h} \pm 0.6$	59^{cde}	$83.28^{c} \pm 1.8$	8^{ef}
PNT04	$36.77^{hi} \pm 1.5$	59^{cde}	$85.00^{b} \pm 1.0$	6^{f}
PNT05	$29.80^{l} \pm 1.9$	67^{a}	$41.67^{l} \pm 2.0$	54^{a}
PNT06	$39.73^{g} \pm 2.5$	56^{def}	$74.70^{e} \pm 0.6$	17^{d}
PNT07	$35.67^{ij} \pm 1.3$	60^{bc}	$80.67^{d} \pm 2.1$	10^{e}
PNT08	$34.54^{k} \pm 1.3$	62^{b}	$52.33^{k} \pm 0.6$	42^{b}
PNT09	$42.88^{cd} \pm 1.3$	52^{fg}	$80.71^{d} \pm 1.5$	10^{e}
PNT10	$43.77^{c} \pm 1.6$	51^{fg}	$70.33^{g} \pm 1.0$	22^{c}
PNT11	$35.33^{j} \pm 0.6$	61^{bc}	$56.00^{h} \pm 1.0$	38^{b}
PNT12	$40.33^{fg} \pm 1.5$	55^{def}	$73.77^{f} \pm 1.6$	18^{cd}
PNT13	$42.28^{de} \pm 2.3$	53^{fg}	$84.33^{b} \pm 0.6$	6^{ef}
PNT14	$41.00^{ef} \pm 1.5$	54^{efg}	$84.73^{b} \pm 1.5$	6^{ef}
PNT15	$45.33^{b} \pm 2.0$	50^{g}	$85.58^{b} \pm 1.4$	5^{f}
PNT16	$36.00^{ij} \pm 1.0$	60^{bcd}	$53.33^{j} \pm 1.0$	41^{b}
Sem	0.03	0.41	0.05	0.38
CD (0.01)	0.15	1.6	0.22	1.47
CV%	1.66	1.26	1.43	3.18

Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.01$, according to Tukey's multiple range test

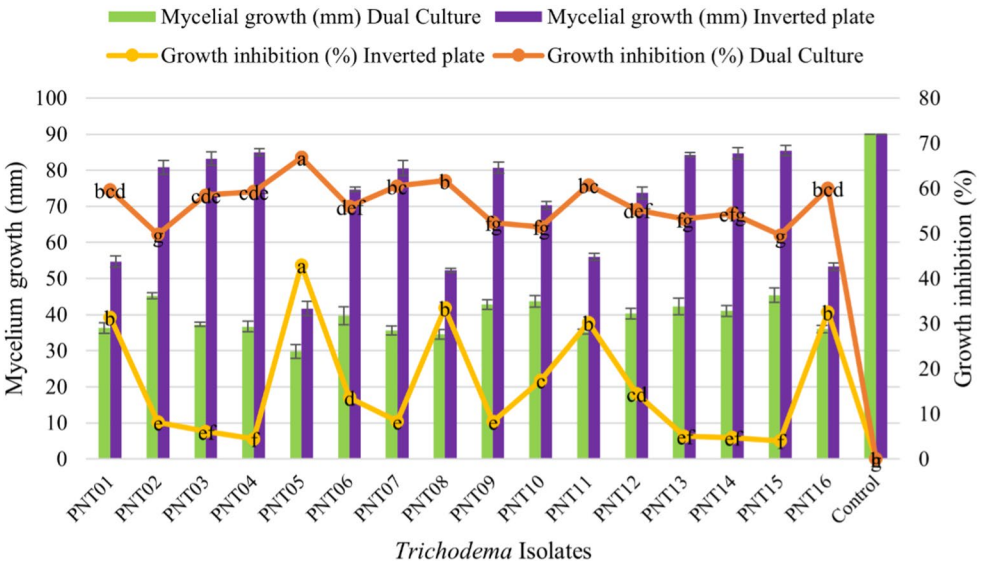


Fig. 1 Effects of *Trichoderma* isolates on mycelial growth of *M. phaseolina* both in dual culture and inverted plate methods. The results are expressed as the mean of three replicate and the vertical bars indicate the ± SD of the mean. Different letters indicate that the values are significantly different from each other ($p \leq 0.01$)

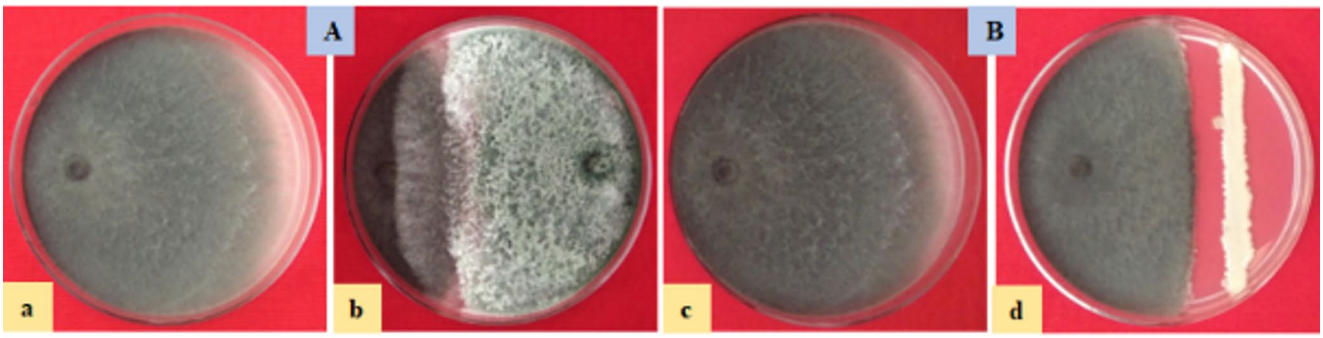


Fig. 2 (**A**) Dual culture of *Trichoderma* against spp. *M. phaseolina* (a) control (*M. phaseolina*) and (b) *Trichoderma* isolate PNT 05 with *M. phaseolina*; (**B**) Dual culture of *Bacillus* spp. against *M. phaseolina* (c) control (*M. phaseolina*) and (d) *Bacillus* isolate PNB 10 with *M. phaseolina*

the pathogen, followed by the PNT08, PNT16, PNT01, and PNT11 isolates, which inhibited mycelial growth by 42%, 41%, 39%, and 38%, respectively (Table 3 and Fig. 1). Among the *Bacillus* isolates, PNB10 isolate was the most efficient at reducing mycelial growth of 44%, followed by the PNB13, PNB16, PNB05, and PNB01 isolates, whose growth inhibition was 40%, 39%, 37%, and 35% respectively, greater than that of the control (Table 4 and Fig. 3). On the basis of the above results, the five *Trichoderma* and five *Bacillus* isolates with highest mycelial growth inhibition ability were selected for the culture filtrate assay.

Non-volatile antifungal metabolites of *Trichoderma* and *Bacillus* strains against *M. phaseolina*

The five tested isolates of *Trichoderma* and *Bacillus* significantly reduced the growth of the pathogen by producing the nonvolatile antifungal secondary metabolites (Table 5 and Fig. 4). Among the *Trichoderma* isolates, the PNT05 isolate showed the highest efficacy, which inhibited the mycelial growth of the pathogen, i.e., 11%, 42%, and 80% at 10%, 25%, and 50% concentrations of culture filtrate, respectively, followed by 4%, 30%, and 56% mycelial growth inhibition was observed in isolate PNT16 at 10%, 25% and 50% concentrations of culture filtrate, respectively. Similarly, five *Bacillus* isolates revealed that the PNB10 isolate was very effective at inhibiting the radial growth of test pathogen to extents of 7%, 34%, and 76% at 10%, 25%, and 50% concentrations of the culture filtrate respectively, followed by the PNB05 isolate at 10%, 25%, and 50% concentrations, which inhibited the mycelial growth of the pathogen by 5%, 26% and 51%, respectively.

The above results, revealed that the *Trichoderma* PNT05 isolate and *Bacillus* PNB10 isolate had maximum antifungal effects on mycelial growth inhibition against test the pathogen. Hence, PNT05 and PNB10 isolates were selected for the preparation of talc-based formulations against *M. phaseolina* under field conditions.

Table 4 Mycelial growth inhibition of *Bacillus* isolates in dual culture and inverted plate methods

Isolate	Dual culture		Inverted plate	
	Mycelial growth (mm)	Growth inhibition (%)	Mycelial growth (mm)	Growth inhibition (%)
Control	$90.00^{a} \pm 0.0$	0^{g}	$90.0^{a} \pm 0.0$	0^{j}
PNB01	$48.33^{jk} \pm 2.0$	46^{a}	$58.67^{i} \pm 1.5$	35^{c}
PNB02	$65.00^{c} \pm 0.0$	28^{ef}	$84.33^{b} \pm 0.8$	6^{i}
PNB03	$64.50^{c} \pm 2.0$	28^{ef}	$82.00^{c} \pm 0.0$	9^{hi}
PNB04	$54.67^{i} \pm 1.5$	39^{b}	$77.00^{e} \pm 1.0$	14^{fg}
PNB05	$49.00^{j} \pm 2.0$	46^{a}	$57.00^{j} \pm 1.5$	37^{bc}
PNB06	$62.00^{ef} \pm 1.5$	31^{cde}	$77.00^{e} \pm 1.3$	14^{fg}
PNB07	$54.33^{i} \pm 0.6$	40^{b}	$72.00^{g} \pm 1.0$	20^{e}
PNB08	$58.00^{h} \pm 1.0$	36^{bc}	$79.67^{d} \pm 0.6$	11^{gh}
PNB09	$67.00^{b} \pm 1.0$	26^{f}	$81.33^{c} \pm 1.5$	10^{hi}
PNB10	$45.67^{l} \pm 1.8$	49^{a}	$50.67^{l} \pm 1.5$	44^{a}
PNB11	$63.00^{de} \pm 1.0$	30^{def}	$64.87^{h} \pm 0.8$	28^{d}
PNB12	$59.33^{g} \pm 1.8$	34^{cd}	$78.83^{d} \pm 1.3$	12^{gh}
PNB13	$47.67^{k} \pm 1.2$	47^{a}	$53.63^{k} \pm 1.2$	40^{ab}
PNB14	$64.00^{cd} \pm 1.0$	29^{ef}	$73.67^{f} \pm 0.6$	18^{ef}
PNB15	$61.67^{f} \pm 1.2$	31^{cde}	$65.63^{h} \pm 1.2$	27^{d}
PNB16	$49.00^{j} \pm 1.0$	46^{a}	$54.83^{k} \pm 1.5$	39^{b}
Sem	0.03	0.38	0.04	0.42
CD (0.01)	0.13	1.47	0.16	1.64
CV%	1.04	1.81	1.08	3.16

Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.01$, according to Tukey's multiple range test

Molecular characterization of *Trichoderma* spp. and *Bacillus* spp.

The molecular characterization of five *Trichoderma* isolates was performed via ITS1 and ITS4 primers, and an approximately 650 bp fragments were amplified (Fig. 5). The size of the PCR products ranged from 632 to 641 bp. The rDNA sequences were deposited in the GenBank database under the accession numbers OK035211 – OK035215. A Blastn search was performed for five amplified sequences in the NCBI database, the results revealed that four (PNT01, PNT05, PNT10, and PNT16) were closely related to *T. harzianum* species complex (THSC), and the PNT14 isolate was 99% identical to *T. virens*. Based on the nucleotide sequence similarity, phylogenetic tree was constructed, revealed that the isolates were distinct from one another in the cluster but identical to each other in terms of homology and divergence with different nodes (Fig. 6).

The DNA sequence of the 16S rRNA gene from five *Bacillus* isolates was analysed via 24 F and 1492 R primers, and fragments of approximately 1500 bp were amplified (Fig. 7). The sequences obtained were deposited in GenBank with the accession numbers OK284526-OK284530 and BLASTn used to determine the closest match and showed 99% similarity with the *Bacillus* sp. sequences. Phylogenetic analysis was performed by comparing the 16S sequences with reference sequences of *Bacillus* sp. available in the NCBI Taxonomy database via MEGA 11 software. The phylogenetic tree revealed that isolates PNB05 and PNB10 have 98% similarity with *Bacillus subtilis*, and isolates PNB03, PNB13, and PNB16 have 99% similarity with *B. oceanisediminis*, *B. pacificus*, and *B. cereus*, respectively (Fig. 8).

Integrated disease management of groundnut dry root rot under field conditions

The field results revealed that, compared to untreated control, all the treatments significantly reduced the disease incidence and increased the percent inhibition of disease in groundnut during both seasons and were pooled data. The minimum dry root rot incidence of 13% with a maximum disease inhibition of 58% was observed in treatment T5, where *T. harzianum* species complex was used as seed treatment and soil application, followed by treatment T6, consisting of the seed treatment and soil application of *B. subtilis* with 15% disease incidence and 51% disease inhibition. These two treatments were highly effective in minimizing the disease incidence and maximum reduction in disease. In contrast, the highest disease and lowest disease control were recorded (22% and 27%, respectively) in T4, which consisting of soil application with *B. subtilis* (Table 6 and Fig. 9).

The growth parameters of the groundnut plants (germination percentage, shoot length, root length, and number of root nodules) were effective and improved during both seasons and pooled data (Table 7). The maximum growth i.e., germination (92%), shoot length (43.1 cm), and root length (17.4 cm) was observed in T5, followed by the T6 treatment (86%, 39.6 cm and 15.6 cm). The maximum nodulation of 125 nodules/plant and highest percentage of root nodules (98%) were recorded in the treatment T5, followed by the T6 treatment (112 root nodules/plant and 76%). The lowest number of root nodules and lowest percent increase in the number of root nodules were observed in the T7 treatment, which consisting of seed treatment with tebuconazole (67 root nodules/plant and 6%) compared to the control (Table 8 and Fig. 10).

The highest pod yield (36.80 q/ha) and highest percent increase in pod yield (97%) were observed in T5 treatment, followed by T6 treatment (31.58 q/ha and 77%, respectively). The lowest pod yield and lowest percent increase in pod yield were recorded in recorded (22.89 q/ha and 28%, respectively) in T4 – consisting of soil application *B. subtilis* (Table 9 and Fig. 11). The number of pods per plant and 100 seed weight revealed that all treatments increased

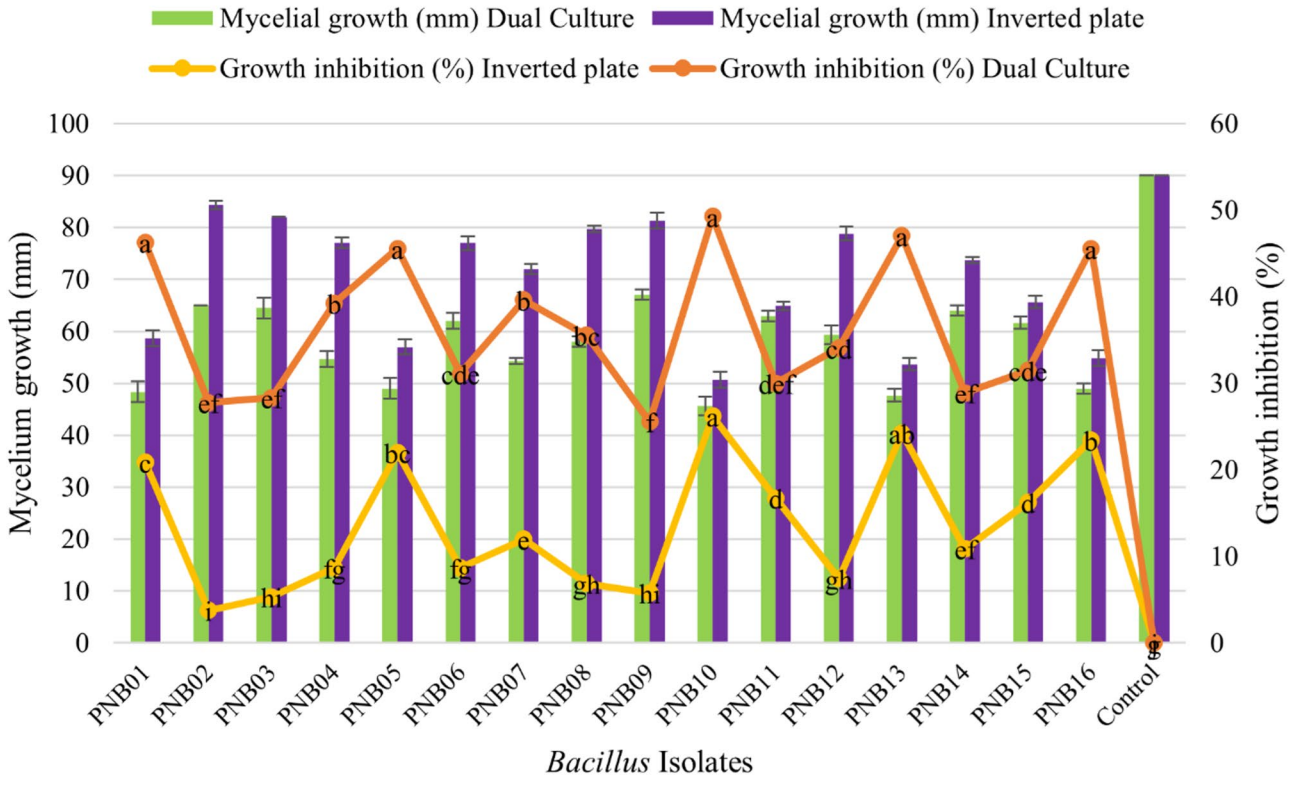


Fig. 3 Effects of *Bacillus* isolates on mycelial growth of *M. phaseolina* both in dual culture and inverted plate methods. The results are expressed as the mean of three replicate and the vertical bars indicate the ± SD of the mean. Different letters indicate that the values are significantly different from each other ($p \leq 0.01$)

Table 5 Effects of the culture filtrate of *Trichoderma* and *Bacillus* strains on mycelial growth of *M. phaseolina*

Bioagents	Culture filtrate concentrations, and mycelial growth (mm), and growth inhibition (%)					
	10% filtrate		25% filtrate		50% filtrate	
	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)	Mycelial growth (mm)	Inhibition (%)
Trichoderma isolates						
Control	$90.00^{a} \pm 0.0$	0^{c}	$90.00^{a} \pm 0.0$	0^{f}	$90.00^{a} \pm 0.0$	0^{f}
PNT01	$90.00^{a} \pm 0.0$	0^{c}	$76.00^{b} \pm 0.6$	15^{e}	$53.00^{b} \pm 0.8$	41^{e}
PNT05	$80.00^{c} \pm 0.6$	11^{a}	$53.00^{f} \pm 0.8$	42^{a}	$18.00^{f} \pm 1.1$	80^{a}
PNT08	$87.00^{b} \pm 1.0$	3^{b}	$70.00^{c} \pm 1.0$	22^{d}	$44.00^{d} \pm 0.6$	51^{c}
PNT11	$90.00^{a} \pm 0.0$	0^{c}	$66.00^{d} \pm 0.3$	26^{c}	$47.00^{c} \pm 1.2$	48^{d}
PNT16	$86.00^{b} \pm 0.6$	4^{b}	$63.00^{e} \pm 0.6$	30^{b}	$39.00^{e} \pm 0.6$	56^{b}
Bacillus isolates						
Control	$90.00^{a} \pm 0.0$	0^{c}	$90.00^{a} \pm 0.0$	0^{e}	$90.00^{a} \pm 0.0$	0^{f}
PNB01	$90.00^{a} \pm 0.0$	0^{c}	$75.00^{b} \pm 1.0$	17^{d}	$55.67^{b} \pm 0.8$	38^{e}
PNB05	$85.67^{b} \pm 0.8$	5^{b}	$66.67^{d} \pm 0.6$	26^{b}	$43.67^{e} \pm 0.6$	51^{b}
PNB10	$83.33^{c} \pm 0.6$	7^{a}	$59.33^{e} \pm 1.2$	34^{a}	$21.33^{f} \pm 1.5$	76^{a}
PNB13	$90.00^{a} \pm 0.0$	0^{c}	$70.00^{c} \pm 0.8$	22^{c}	$48.33^{c} \pm 0.6$	46^{d}
PNB16	$86.67^{b} \pm 0.3$	4^{b}	$68.67^{cd} \pm 0.6$	24^{bc}	$46.00^{d} \pm 1.0$	49^{c}

Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.01$, according to Tukey's multiple range test

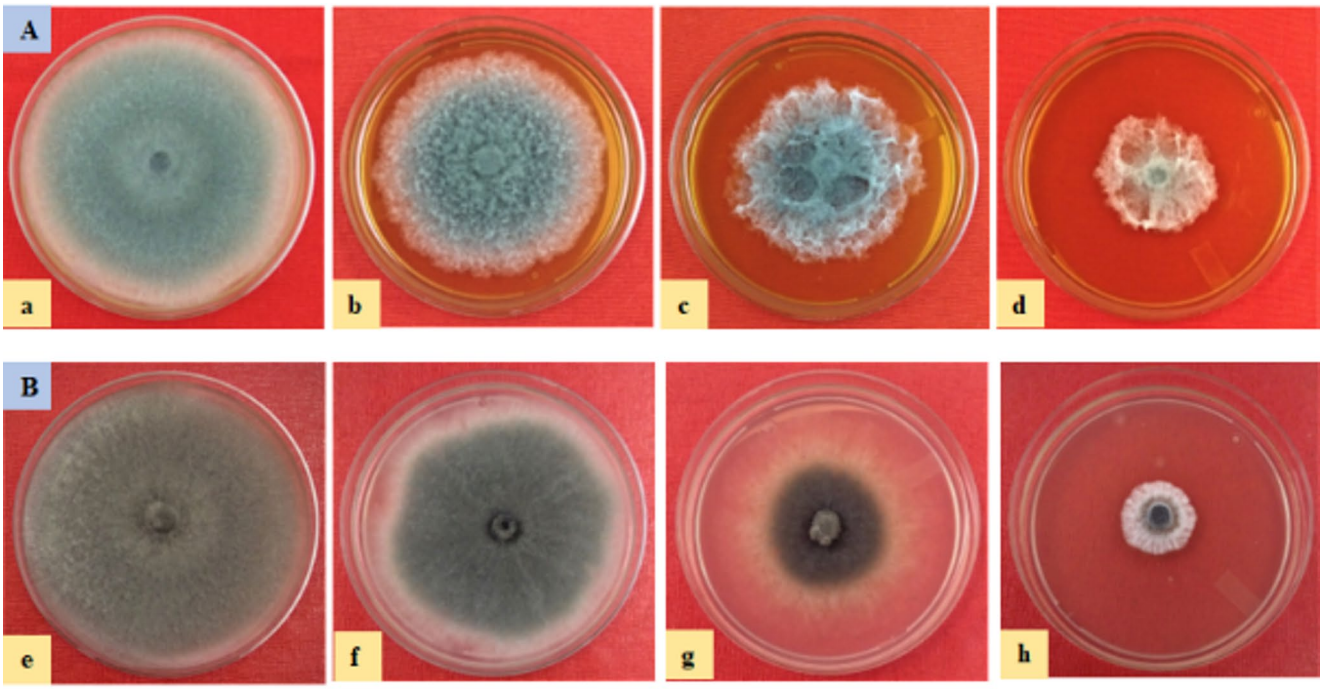


Fig. 4 (**A**) Culture filtrates of *Trichoderma* isolate PNT05 against *M. phaseolina* (a) control *M. phaseolina* b) 10% filtrate with *M. phaseolina* c) 25% filtrate with *M. phaseolina* d) 50% filtrate with *M. phaseolina*; (**B**) Culture filtrates of *Bacillus* isolate PNB10 against *M. phaseolina* (e) control *M. phaseolina* f) 10% filtrate with *M. phaseolina* g) 25% filtrate with *M. phaseolina* h) 50% filtrate with *M. phaseolina*;

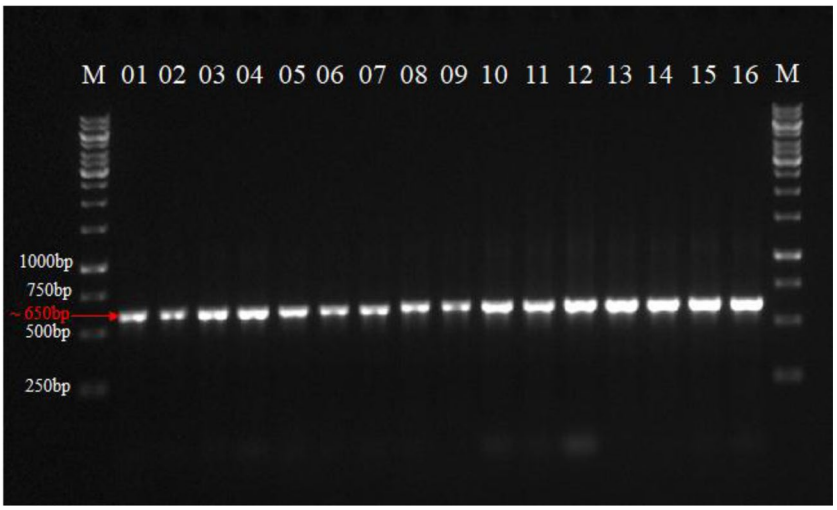


Fig. 5 Identification of *Trichoderma* isolates with universal primers ITS1 and ITS4, Amplified band size of approximately 650 bp. Line 1–16: *Trichoderma* isolates (PNT01-PNT16); M = 1 Kb ladder

significantly in both seasons and pooled data (Table 10). The maximum number of pods per plant (28.44) and seed weight (48.07 g) were recorded in T5 treatment, followed by treatment T6 i.e., 24.60 pods per plant and 44.45 g, which were significantly superior over rest of the treatments.

Discussion

In the present study, fungal and bacterial bioagents were used to manage *M. phaseolina*. *Trichoderma* and *Bacillus* are commonly available antagonistic microorganisms in groundnut rhizospheric soils. Several studies Ramesh and Korikanthimath (2010); Moradia and Khandar (2011); Sreedevi et al. (2011); Rajamohan and Balabaskar (2016); Arya et al. (2017); Mahendra et al. (2022) have demonstrated the antagonistic ability of fungal and bacterial bioagents to suppress groundnut dry root rot disease and observed

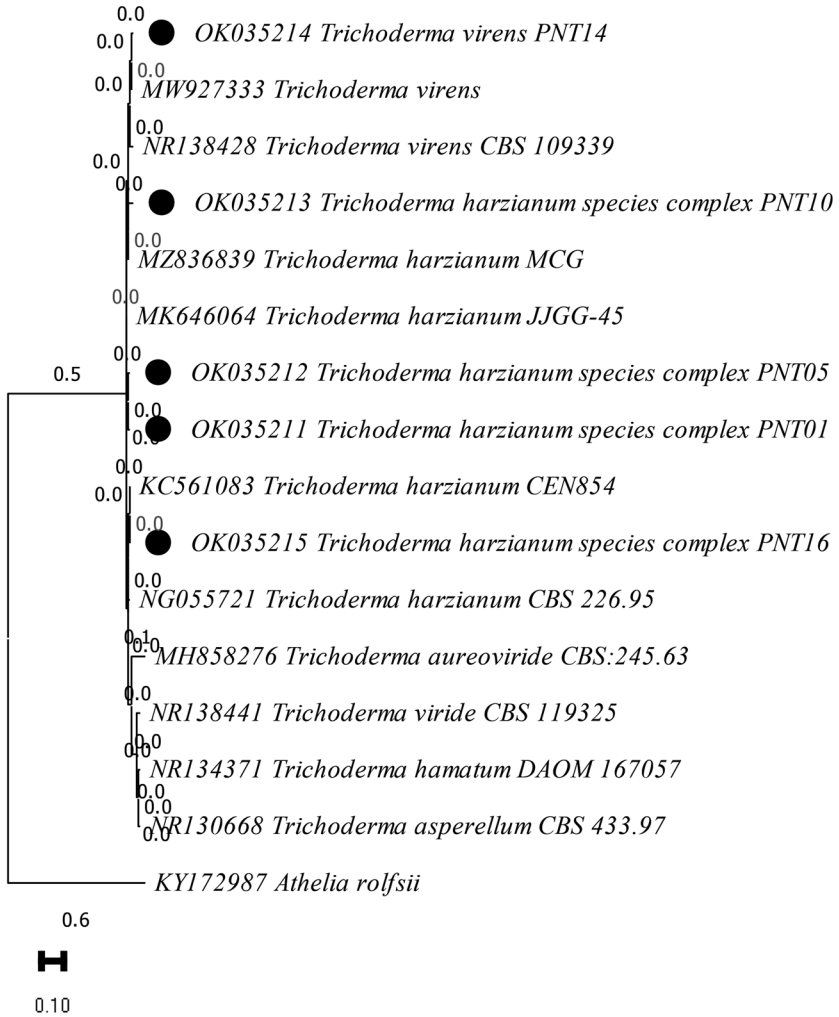


Fig. 6 Phylogenetic tree of the *Trichoderma* spp. inferred by Neighbor Joining method of ITS rDNA gene sequences with 1,000 bootstrapping repetitions using MEGA 11 software (Tamura et al. 2021), and *Athelia rolfsii* was used to assign an outgroup species

significant differences in antagonistic potential among the various groups of bioagents and within the species. Hence, preliminary screening is an important initial step for the selection of potential bioagents for management of plant diseases. We isolated sixteen fungal and bacterial bioagents from the rhizosphere of groundnut plants and screened for their inhibitory effects on *M. phaseolina* in vitro employing dual culture and inverted plate assays and selected five *Trichoderma* isolates (PNT01, PNT05, PNT08, PNT11 and PNT16) and five *Bacillus* isolates (PNB01, PNB05, PNB10, PNB13, and PNB16) with better inhibitory potential on the mycelial growth of *M. phaseolina.* Further, we characterized the five *Trichoderma* isolates based on antagonistic potential (PNT01, PNT05, PNT16) and morphological diversity (PNT10 and PNT14), the results revealed that four *Trichoderma* isolates (PNT01, PNT05, PNT10, and PNT16) were closely related to *Trichoderma harzianum* species complex, and the PNT14 isolate was identical to *T. virens*. Similarly, five *Bacillus* isolates were characterized based on antagonistic potential (PNB05, PNB10, PNB13 and PNB16) and morphological diversity (PNB03), the isolates PNB05 and PNB10 showed similarity to *Bacillus subtilis*, and isolates PNB03, PNB13 and PNB16 showed similarity to *B. oceanisediminis*, *B. pacificus*, and *B. cereus*, respectively. The culture filtrate assay was performed to select the most promising isolate of *Trichoderma* and *Bacillus* and results revealed that *Trichoderma* isolate PNT05 (*T. harzianum* species complex) and *Bacillus* isolate PNB10 (*B. subtilis*) exhibited maximum mycelial growth inhibition of *M. phaseolina* as compared with the other isolates.

The results obtained in this study showed that all *Trichoderma* isolates in all assays (dual culture, inverted plate and culture filtrates) significantly inhibited the mycelial growth of *M. phaseolina* as compared with the control. The mycoparasitism ability or the use of multiple biocontrol mechanisms by *Trichoderma* isolates may account for differences in their antagonistic effects against plant pathogenic fungi (You et al. 2016). Different strains of *Trichoderma* species may be associated with the production of different metabolites with antibiotic potential to inhibit the growth of plant pathogens (Iqbal and Mukhtar 2020). Meena et al. (2017) reported the efficacy of *Trichoderma* spp. in reducing the

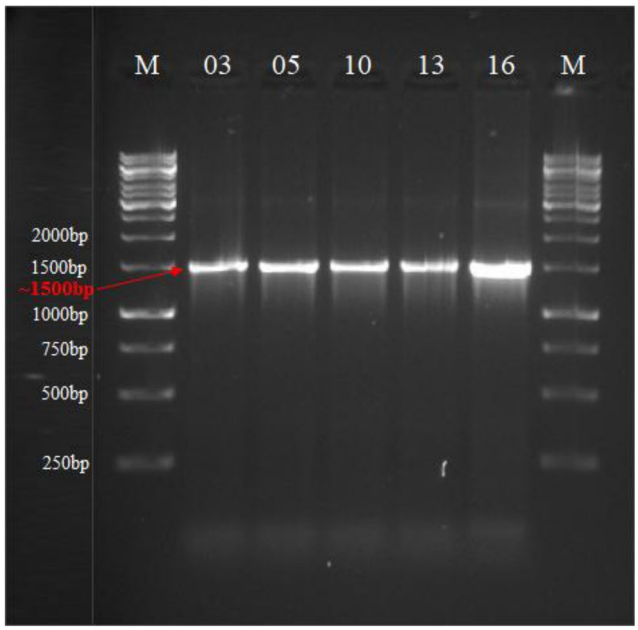


Fig. 7 Identification of *Bacillus* isolates with universal primers 27-F and 1492-R, Amplified band size of approximately 1500 bp. Line 1 (PNB03), Line 2 (PNB05), Line 3 (PNB10), Line 4 (PNB13) and Line 5 (PNB16) *Bacillus* isolates; M = 1 Kb ladder

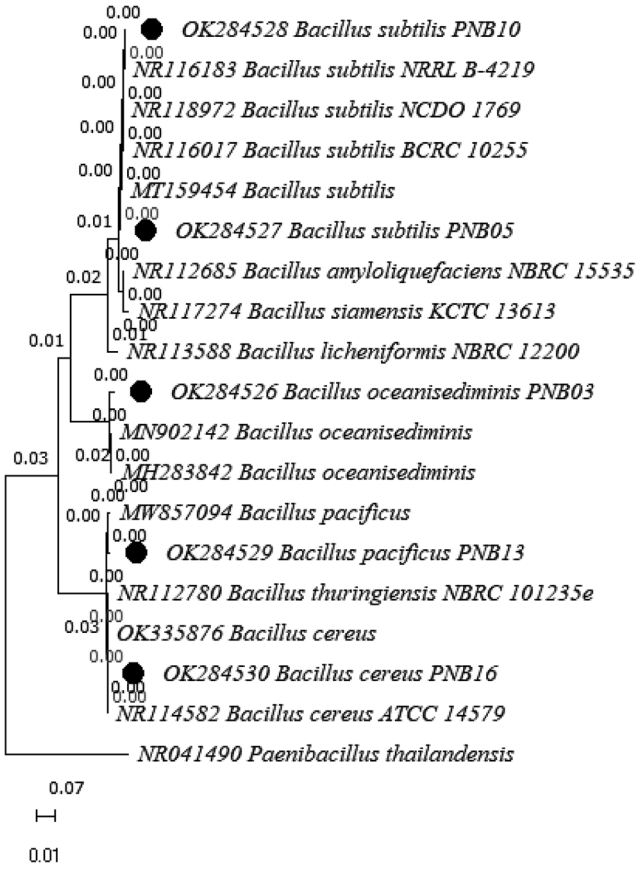


Fig. 8 Phylogenetic tree of the *Bacillus* spp. inferred by Neighbor Joining method of 16S rRNA gene sequences with 1,000 bootstrapping repetitions using MEGA 11 software (Tamura et al. 2021), and *Paenibacillus thailandensis* was used to assign an outgroup species

growth and development of plant pathogens. Sreedevi et al. (2011) reported that in vitro plate assays of *T. harzianum* are more effective at suppressing the growth of groundnut dry root rot with increasing concentrations of culture filtrates of the bioagent, and the radial growth of the test pathogen proportionally decreases because metabolites released by *Trichoderma* spp. are toxic and fungistatic to the pathogen. The increase in the concentration of the culture filtrate resulted in an increase in the inhibition of mycelial growth of the test pathogen. Similarly, Raja Kumar et al. (2021) observed a maximum reduction in the mycelial growth of the pathogen via the dual culture technique and culture filtrates of *T. harzianum* at different concentrations effectively reduced the mycelial growth of the pathogen compared with the control. The volatile compounds produced by *Trichoderma* can alter the structure of hyphae, reduce pathogen growth (Sridharan et al. 2020). *T. harzianum* isolates produce both

Table 6 Disease control efficacy of *T. harzianum* species complex and *B. subtilis* used as seed treatment and soil application against dry root rot disease during two successive seasons under field conditions

Treatments	Disease incidence (%)			Disease inhibition over control (%)
	Kharif 2022	Rabi 2022-23	Pooled	
ST with *T. harzianum* species complex	$18^{cd} \pm 1.5$	$16^{de} \pm 1.2$	$17^{d} \pm 0.9$	42
ST with *B. subtilis*	$20^{bc} \pm 2.5$	$18^{cd} \pm 1.7$	$19^{c} \pm 0.7$	36
SA with *T. harzianum* species complex	$21^{b} \pm 0.8$	$20^{bc} \pm 0.8$	$21^{b} \pm 0.7$	31
SA with *B. subtilis*	$22^{b} \pm 1.4$	$21^{b} \pm 1.3$	$22^{b} \pm 0.6$	27
ST + SA with *T. harzianum* species complex	$13^{f} \pm 1.1$	$12^{f} \pm 0.8$	$13^{f} \pm 0.9$	58
ST + SA with *B. subtilis*	$15^{ef} \pm 1.7$	$15^{e} \pm 0.5$	$15^{e} \pm 1.1$	51
ST with tebuconazole	$17^{de} \pm 1.3$	$18^{d} \pm 1.8$	$16^{d} \pm 1.3$	42
Control	$33^{a} \pm 0.9$	$28^{a} \pm 1.0$	$30^{a} \pm 0.8$	0
SEm	0.71	0.72		
CD (0.05)	2.15	2.18		
CV%	8.61	9.33		

ST—Seed treatment, SA—Soil application; Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.05$, according to Tukey's multiple range test

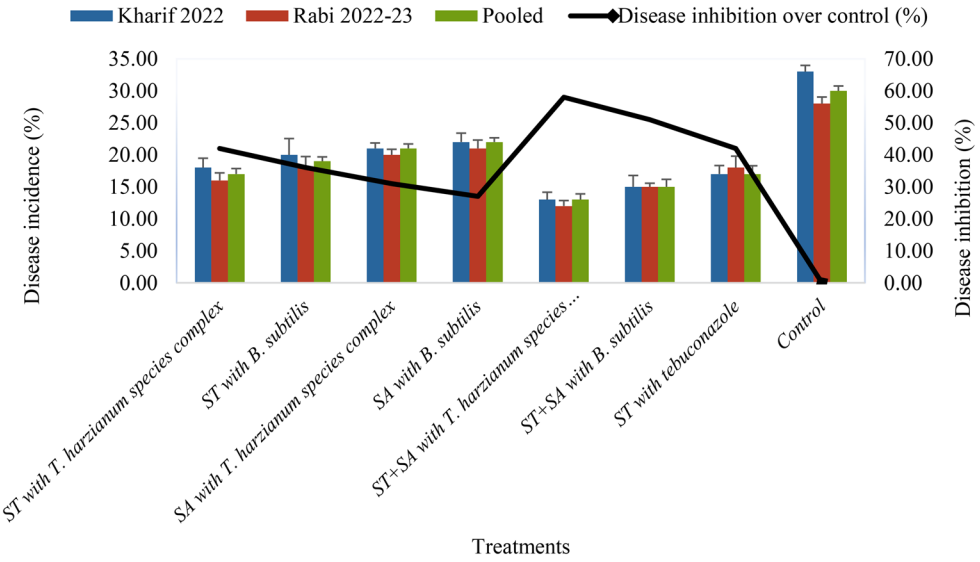


Fig. 9 Disease control efficacy (disease incidence and disease inhibition) of *T. harzianum* species complex and *B. subtilis* against dry root rot disease under field conditions. Values represent mean ($n=3$), and the error bars represent ± standard error of the mean

Table 7 Effects of different treatments on plant growth parameters in groundnut against dry root rot disease under field conditions

Treatments	Germination (%)			Shoot length (cm)			Root length (cm)		
	Kharif 2022	Rabi 2022-23	Pooled	Kharif 2022	Rabi 2022-23	Pooled	Kharif 2022	Rabi 2022-23	Pooled
ST with THSC	$79^{bc}\pm6.1$	$82^{abc}\pm6.4$	$80^{bc}\pm2.6$	$33.8^{bc}\pm3.5$	$38.0^{bc}\pm2.5$	$35.9^{bc}\pm2.3$	$13.0^{bc}\pm1.0$	$15.2^{c}\pm1.1$	$14.1^{c}\pm0.8$
ST with *B. subtilis*	$77^{bcd}\pm7.1$	$80^{abc}\pm6.7$	$79^{bc}\pm6.7$	$32.8^{bc}\pm2.7$	$36.5^{c}\pm3.4$	$34.6^{cd}\pm2.8$	$12.5^{c}\pm1.4$	$14.7^{c}\pm0.7$	$13.6^{cd}\pm1.0$
SA with THSC	$75^{bcd}\pm5.1$	$78^{bc}\pm6.1$	$77^{c}\pm4.1$	$31.8^{c}\pm2.4$	$34.9^{cd}\pm1.3$	$33.3^{cd}\pm1.6$	$11.9^{c}\pm0.7$	$13.6^{cd}\pm0.9$	$12.8^{de}\pm0.7$
SA with *B. subtilis*	$74^{cd}\pm5.6$	$76^{bc}\pm6.7$	$75^{cd}\pm2.5$	$30.7^{cd}\pm2.1$	$33.3^{cd}\pm2.5$	$32.1^{de}\pm1.2$	$11.6^{c}\pm1.1$	$12.9^{de}\pm0.6$	$12.2^{e}\pm0.7$
ST+SA with THSC	$91^{a}\pm5.3$	$92^{a}\pm7.1$	$92^{a}\pm6.1$	$39.6^{a}\pm2.7$	$46.7^{a}\pm4.9$	$43.1^{a}\pm3.1$	$16.1^{a}\pm0.9$	$18.7^{a}\pm1.2$	$17.4^{a}\pm0.9$
ST+SA with *B. subtilis*	$86^{ab}\pm5.7$	$87^{ab}\pm6.6$	$86^{ab}\pm5.9$	$37.0^{ab}\pm2.9$	$42.1^{ab}\pm2.8$	$39.6^{ab}\pm2.6$	$14.2^{b}\pm0.5$	$16.9^{b}\pm0.4$	$15.6^{b}\pm0.4$
ST with tebuconazole	$81^{abc}\pm5.2$	$82^{abc}\pm4.4$	$87^{bc}\pm4.6$	$32.9^{bc}\pm2.3$	$37.1^{c}\pm1.3$	$35.0^{cd}\pm1.7$	$12.6^{c}\pm0.7$	$14.2^{cd}\pm0.5$	$13.4^{de}\pm0.8$
Control	$67^{d}\pm6.5$	$70^{c}\pm8.2$	$68^{d}\pm1.8$	$26.5^{d}\pm1.9$	$30.8^{d}\pm2.2$	$28.7^{e}\pm1.8$	$9.4^{d}\pm0.9$	$11.8^{e}\pm1.3$	$10.6^{f}\pm0.4$
Sem	3.53	3.85		1.56	1.55		0.54	0.72	
CD (0.05)	10.70	11.69		4.72	5.35		1.63	2.18	
CV%	9.09	8.64		8.59	8.61		8.41	9.12	

ST—Seed treatment; SA—Soil application; THSC - *T. harzianum* species complex. Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.05$, according to Tukey's multiple range test

Table 8 Effect of bioagents on root nodules/plant in groundnut against dry root rot disease under field conditions

Treatments	Root nodules/plant			Root nodules increased over control (%)
	Kharif 2022	Rabi 2022-23	Pooled	
ST with *T. harzianum* species complex	$96^{c}\pm6.1$	$107^{bc}\pm11.6$	$101^{c}\pm8.4$	60
ST with *B. subtilis*	$86^{cd}\pm6.0$	$97^{cd}\pm6.1$	$92^{cd}\pm6.1$	45
SA with *T. harzianum* species complex	$79^{de}\pm5.4$	$88^{de}\pm5.1$	$83^{de}\pm4.0$	31
SA with *B. subtilis*	$71^{ef}\pm5.1$	$79^{ef}\pm4.8$	$75^{ef}\pm4.1$	19
ST+SA with *T. harzianum* species complex	$121^{a}\pm8.6$	$130^{a}\pm5.0$	$125^{a}\pm6.8$	98
ST+SA with *B. subtilis*	$107^{b}\pm4.9$	$117^{b}\pm9.8$	$112^{b}\pm7.2$	76
ST with tebuconazole	$64^{f}\pm5.8$	$71^{f}\pm5.5$	$67^{fg}\pm6.3$	6
Control	$60^{f}\pm4.5$	$67^{f}\pm4.1$	$64^{g}\pm5.4$	0
Sem	3.90	4.72		
CD (0.05)	11.82	14.32		
CV%	9.44	9.01		

ST—Seed treatment; SA—Soil application. Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.05$, according to Tukey's multiple range test

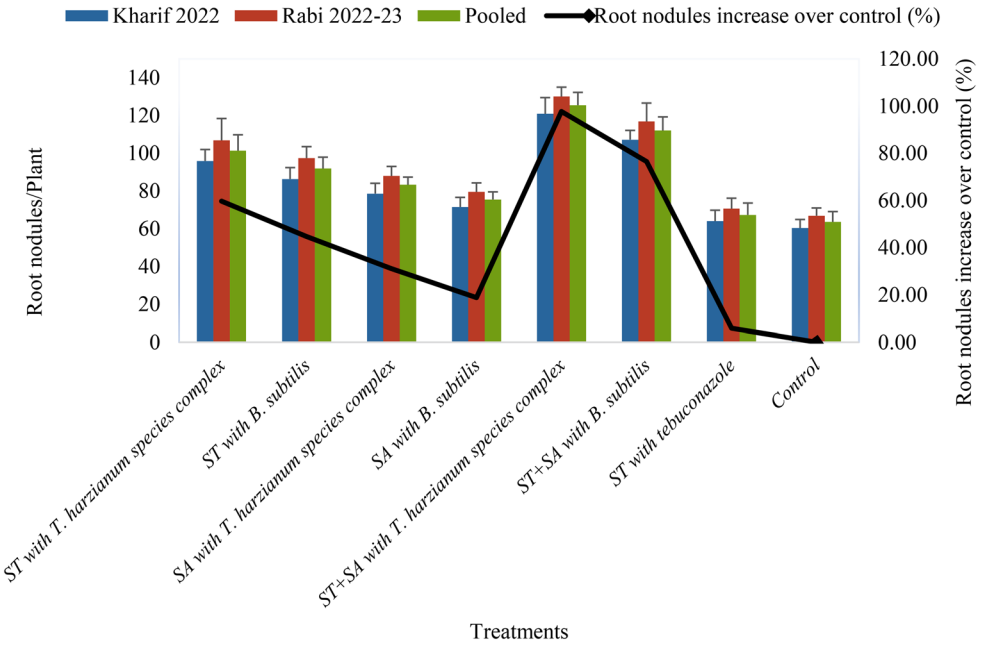


Fig. 10 Effect of bioagents on root nodules production in groundnut plants against dry root rot disease under field conditions. Values represent mean ($n=3$), and the error bars represent ± standard error of the mean

Table 9 Effect of bioagents on pod yield (q/ha) in groundnut against dry root rot disease under field conditions

Treatments	Pod Yield (q/ha)			Pod yield increased over control (%)
	Kharif 2022	Rabi 2022-23	Pooled	
ST with *T. harzianum* species complex	$26.12^{bc} \pm 2.9$	$29.03^{bc} \pm 2.9$	$27.58^{c} \pm 2.9$	54
ST with *B. subtilis*	$24.36^{cd} \pm 1.4$	$28.02^{bc} \pm 3.4$	$26.19^{c} \pm 1.6$	46
SA with *T. harzianum* species complex	$22.4^{cd} \pm 1.1$	$27.5^{bc} \pm 4.7$	$24.95^{cd} \pm 2.7$	40
SA with *B. subtilis*	$20.29^{de} \pm 2.6$	$25.49^{cd} \pm 5.1$	$22.89^{d} \pm 2.5$	28
ST + SA with *T. harzianum* species complex	$33.64^{a} \pm 1.9$	$36.95^{a} \pm 4.9$	$35.29^{a} \pm 2.3$	97
ST + SA with *B. subtilis*	$29.58^{b} \pm 3.8$	$33.58^{ab} \pm 4.4$	$31.58^{b} \pm 2.7$	77
ST with tebuconazole	$25.01^{bcd} \pm 2.7$	$28.38^{bc} \pm 3.3$	$26.7^{c} \pm 1.8$	49
Control	$16.19^{e} \pm 2.3$	$19.56^{d} \pm 4.6$	$17.88^{e} \pm 1.2$	0
Sem	1.47	2.03		
CD (0.05)	4.45	5.68		
CV%	11.84	12.21		

ST—Seed treatment; SA—Soil application. Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.05$, according to Tukey's multiple range test

volatile and non-volatile substances, which may inhibit the growth of *M. phaseolina* (Inayati et al. 2019). Similarly, Lokesh et al. (2021) found that *T. harzianum*, *T. virens* and *B. subtilis* showed the highest average inhibition of *M. phaseolina* mycelial growth.

Bacillus isolates evaluated in this study significantly inhibited the growth of *M. phaseolina* in all of the assays (dual culture, volatile, and non-volatile metabolites). The antagonistic effects on a wide range of plant pathogenic fungi by *Bacillus* isolates may be associated with a diverse range of biocontrol mechanisms viz., the production of hydrolytic enzymes and antibiotics, competition for host nutrients and induction of plant resistance (Ongena et al. 2007). The metabolites and volatile agents produced by *Bacillus* species may be capable of reducing and suppressing the growth of pathogens and promoting the plant defence mechanism (Khan et al. 2021). Hussain and Khan (2020) reported that antifungal compounds produced by *Bacillus* sp. may be useful for managing various fungal pathogens. Similar to our findings, Singh et al. (2008) reported that *B. subtilis* had the highest antagonistic activity against *M. phaseolina*. Vyas and Patel (2015) also reported that *B. subtilis* was effective against dry root rot. In a dual culture assay, *B. subtilis* inhibited the mycelial growth of the groundnut stem rot pathogen (Shifa et al. 2015). *B. subtilis* may produce antibiotics and hydrolytic enzymes that provide a broad spectrum of antifungal activities against the phytopathogenic fungi (Abdelmoteleb et al. 2017). Rangel-Montoya et al. (2022) reported that inoculating cowpea with *B. amyloliquefaciens* reduces *M. phaseolina* incidence and enhances plant growth, likely driven by indole-3-acetic acid and siderophore production.

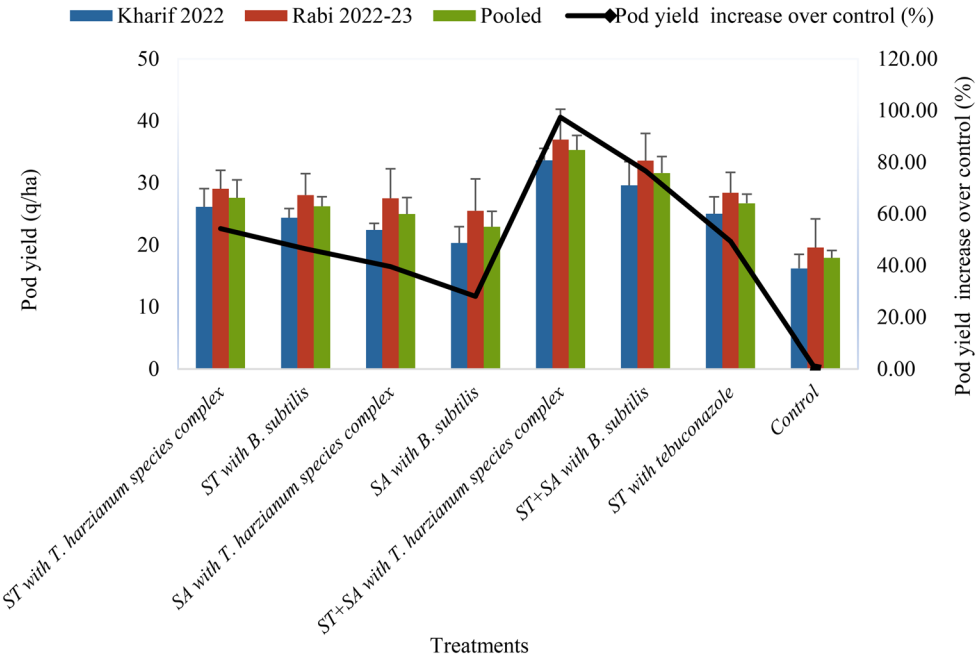


Fig. 11 Effect of bioagents on pod yield in groundnut plants against dry root rot disease under field conditions. Values represent mean ($n=3$), and the error bars represent ± standard error of the mean

Table 10 Effect of different treatments on yield attributing factors in groundnut against dry root rot under field conditions

Treatments	Pods/plant			100 seed weight (g)		
	Kharif 2022	Rabi 2022-23	Pooled	Kharif 2022	Rabi 2022-23	Pooled
ST with *T. harzianum* species complex	$21.6^{bc} \pm 1.9$	$24.4^{bc} \pm 1.9$	$23.04^{bc} \pm 0.8$	$41.27^{b} \pm 3.4$	$43.43^{bc} \pm 2.7$	$42.35^{bc} \pm 2.4$
ST with *B. subtilis*	$20.8^{bc} \pm 1.1$	$23.2^{bc} \pm 2.1$	$22^{cd} \pm 1.6$	$40.6^{b} \pm 1.7$	$42.33^{bc} \pm 3.5$	$41.47^{bc} \pm 1.9$
SA with *T. harzianum* species complex	$20.2^{bc} \pm 1.5$	$22.9y^{bc} \pm 2.4$	$21.6^{cd} \pm 1.9$	$40.13^{b} \pm 2.1$	$40.5^{c} \pm 2.5$	$40.32^{c} \pm 1.0$
SA with *B. subtilis*	$19.6^{cd} \pm 2.1$	$21.8^{c} \pm 1.4$	$20.7^{d} \pm 1.7$	$39.43^{b} \pm 2.1$	$39.47^{cd} \pm 2.4$	$39.45^{c} \pm 2.3$
ST + SA with *T. harzianum* species complex	$28.2^{a} \pm 1.7$	$28.67^{a} \pm 2.0$	$28.44^{a} \pm 1.2$	$47.3^{a} \pm 1.6$	$48.83^{a} \pm 1.5$	$48.07^{a} \pm 1.5$
ST + SA with *B. subtilis*	$23.1^{b} \pm 1.5$	$26.07^{ab} \pm 1.9$	$24.6^{b} \pm 0.9$	$43.2^{ab} \pm 2.7$	$45.7^{ab} \pm 3.2$	$44.45^{b} \pm 1.9$
ST with tebuconazole	$21.2^{bc} \pm 2.2$	$23.73^{bc} \pm 1.7$	$22.5^{bcd} \pm 1.0$	$40.83^{b} \pm 3.2$	$42.63^{bc} \pm 2.6$	$41.73^{bc} \pm 2.6$
Control	$16.5^{d} \pm 1.5$	$17.8^{d} \pm 1.5$	$17.19^{e} \pm 1.5$	$33.53^{c} \pm 1.9$	$35.93^{d} \pm 2.9$	$34.73^{d} \pm 2.2$
Sem	1.02	1.11		1.37	2.04	
CD (0.05)	3.09	3.36		4.17	6.20	
CV%	8.22	9.77		7.70	8.29	

ST—Seed treatment; SA—Soil application. Each value is a mean of three replicates. Means ± standard deviations within a column followed by the different letters indicate significant differences at $p \leq 0.05$, according to Tukey's multiple range test

Field experiments showed that talc based bioagent formulations of *T. harzianum* species complex and *B. subtilis* applied as seed treatment and soil application, significantly reduced disease incidence and increased plant growth. The disease incidence was higher in the *kharif* season than in the *rabi* season, possibly because of the high temperature and low soil moisture conditions during the initial crop growth period. Sharma and Pande (2013) reported that when standing crop is predisposed to high temperature and moisture stress, the incidence of dry root rot disease was increased. Growth and yield parameters were higher in the rabi season than in the kharif season due to lower disease incidence.

Seed treatment and soil application of *T. harzianum* species complex showed maximum disease inhibition, highest plant growth parameters (germination percentage, shoot length, root length, and root nodules/plant), and yield parameters (pods/plant, pod yield, and 100 seed weight) followed by seed treatment and soil application of *B. subtilis*. The seed treatment or soil application of *T. harzianum* species complex showed greater control of dry root rot of groundnut, in comparison to seed treatment or soil application of *B. subtilis*. This reveals the higher efficacy of *T. harzianum* species complex in controlling *M. phaseolina* on groundnut when compared to *B. subtilis*. This difference may be because of the diversity of biocontrol mechanisms produced by the *T. harzianum*, which have an additive effect on the plant pathogen. Our results are similar findings to Kumar et al. (2021) reported that combination of seed treatment and soil application with bioagents were highly effective in minimizing disease incidence, while increasing pod yield, and root nodulation. The application of the talc formulation of bioagent mixtures through seed treatment and

soil application effectively controlled chickpea and pigeonpea wilt (Haveri 2017). Individual or combined applications of *T. harzianum* mitigate disease symptoms and improve plant health (Shafqat et al. 2026). Srivastava et al. (2010) reported when *Trichoderma* spp. interact with plants, it promotes growth, enhances nutrient availability, increases crop yield, and improves disease resistance. Vimala et al. (2000) also reported the lowest degree of root rot in groundnut in response to a combination of seed treatment and soil application of *T. hamatum*. Veena and Reddy (2016) obtained similar results, where seed treatment, and soil application of fungal and bacterial antagonists against chickpea dry root rot resulted in the highest germination percentage, the lowest disease incidence, and the maximum plant height and root length. The seed treatment and soil application of *T. viride* resulted in greater inhibition of root rot incidence then the seed treatment of *T. viride* or *T. harzianum* alone. The combination of seed treatment and soil application with *B. subtilis* increased the root length, shoot length and pod yield, and reduced the incidence of groundnut stem rot (5%) compared with the individual combinations i.e., seed treatment (7.5%) and soil application (30%) (Shifa et al. 2015). Tuber treatment and soil application of talc-based formulations of *B. subtilis* suppressed root rot incidence (Dhanabalan et al. 2024). Similarly, seed treatment with fungicide (tebuconazole) significantly reduced the disease incidence and increased the growth and yield parameters. Jadon et al. (2015) reported that tebuconazole 2 DS at 1.5 g kg^{-1} seed was effective in the management of soil-borne diseases when used separately, with an apparent yield advantage over untreated plots compared with mancozeb 75% WP at 3 g kg^{-1} seed, carbendazim 12% + mancozeb 63% WP at 3 g kg^{-1} seed.

The separate treatments of *T. harzianum* species complex and *B. subtilis* as seed treatments and soil applications also significantly reduced the disease incidence and increased the growth and yield parameters compared to the control. However, in comparison with the soil application of bioagents, seed treatment of bioagents showed better results. Because seed treatment may permit the limited growth of competitive microflora and provides conducive growth for the bioagents and facilitates root colonization, it may help to increase the growth of plants by increasing the uptake of nutrients by roots or by releasing plant hormones increasing yield (Mukhopadhyay and Pan 2012). Soil treatments combining *T. harzianum* and *B. subtilis* effectively suppressed *M. phaseolina* and promoted plant growth (Mannai et al. 2025). Kumar et al. (2021) reported similar findings to our, specifically, that seed treatment was more effective than the soil application of bioagents. The seed treatment with *T. harzianum* and organic amendments resulted in maximum mycelial inhibition and higher germination percentage and morphological parameters like plant height, number of root nodules, number of pods per plant, and 100-seed weight compared to the other treatments against dry root rot disease (Choudhary and Ashraf 2019). Seed bacterization with *Bacillus* reduces the incidence of charcoal rot and enhances the shoot and root length of plants growing from treated seeds (Bojorquez-Armenta et al. 2021).

Conclusion

In the present study, in vitro and in vivo experiments revealed that the native bioagents *T. harzianum* species complex and *B. subtilis* significantly controlled groundnut dry root rot disease. Our results revealed that the percentage disease incidence was lowest when both seed treatment and soil application treatments were combined, compared to sole treatment as seed treatment or soil application. The same pattern was observed for germination percentage, shoot length, root length, number of nodules per plant, number of pods per plant and pod yield. Overall, the results indicate that seed treatment combined with soil application of *T. harzianum* species complex, followed by *B. subtilis* were the most effective in controlling *M. phaseolina* and increasing the growth and pod yield under field conditions. Therefore, *T. harzianum* species complex and *B. subtilis* could be exploited under a biointensive integrated disease management approach for dry root rot disease.

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Author contributions R.S.J and H.K.S. conceived and designed the experiments. P.J.P. performed the experiments and analysed the data and wrote the paper, H.K.S, K.V., R.K.V. and G.M.N. gave valuable suggestions to conduct experiments and edited the manuscript. All authors contributed to the article and approved the submitted version.

Data availability The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

Declarations

Ethical approval Not applicable.

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

References

Abdelmoteleb A, Troncoso-Rojas R, Gonzalez-Soto T, González-Mendoza D (2017) Antifungal activity of autochthonous *Bacillus*

subtilis isolated from *Prosopis juliflora* against phytopathogenic fungi. Mycobiology 45(4):385–391. https://doi.org/10.5941/myco.2017.45.4.385

Adhikary NK, Chowdhury R, Begum T, Mallick R (2019) Integrated management of stem and root rot of sesame (*Sesamum indicum* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. Int J Curr Microbiol Appl Sci 8(4):804–808

Ali A, Javaid A, Shoaib A et al (2020) Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. Egypt J Biol Pest Control 30:102. https://doi.org/10.1186/s41938-020-00305-1

Aneja KR (2022) Experiments in microbiology and plant pathology. New Age International Pvt. Ltd, New Delhi

Arya P, Godara SL, Bimla JA (2017) Efficacy of antagonists against *Macrophomina phaseolina* inciting dry root rot disease of groundnut. J Pharmacogn Phytochem 6(6):1171–1173

Babu BK, Saxena AK, Srivastava AK, Arora DK (2007) Identification and detection of Macrophomina phaseolina by using species-specific oligonucleotide primers and probe. Mycologia 99(6):797–803. https://doi.org/10.1080/15572536.2007.11832511

Bojorquez-Armenta YDJ, Mora-Romero GA, López-Meyer M et al (2021) Evaluation of *Bacillus* spp. isolates as potential Biocontrol agents against charcoal rot caused by *Macrophomina phaseolina* on common bean. J Gen Plant Pathol 87(6):377–386

Chenniappan C, Narayanasamy M, Daniel GM, Ramaraj GB, Ponnusamy P, Sekar J, Ramalingam PV (2019) Biocontrol efficiency of native plant growth promoting rhizobacteria against rhizome rot disease of turmeric. Biol Control 129:55–64. https://doi.org/10.1016/j.biocontrol.2018.07.002

Choudhary A, Ashraf S (2019) Utilizing the combined antifungal potential of *Trichoderma* spp. and organic amendments against dry root rot of mungbean. Egypt J Biol Pest Control 29(1):1–8. https://doi.org/10.1186/s41938-019-0187-8

Degani O, Becher P, Gordani A (2023) Real-time PCR early detection of *Trichoderma* treatments efficiency against cotton charcoal rot disease. J Nat Pestic Res 4:100027. https://doi.org/10.1016/j.napere.2023.100027

Desmae H, Janila P, Okori P et al (2018) Genetics, genomics and breeding of groundnut (*Arachis hypogaea* L). Plant Breed 138:425–444. https://doi.org/10.1111/pbr.12645

Dhanabalan S, Muthusamy K, Iruthayasamy J, Kumaresan PV, Ravikumar C, Kandasamy R et al (2024) Unleashing *Bacillus* species as versatile antagonists: Harnessing the biocontrol potentials of the plant growth-promoting rhizobacteria to combat *Macrophomina phaseolina* infection in *Gloriosa superba*. Microbiol Res 283:127678. https://doi.org/10.1016/j.micres.2024.127678

Dhawan A, Kumar S, Sharma PK, Chugh RK (2019) Effect of different fungicides, organic amendments and bio-control agents on dry root rot of cluster bean (*Cyamopsis tetragonoloba* L. Taub.) caused by *Rhizoctonia bataticola* (Taub.) Butler. Forage Res 44(4):276–281

Directorate of Economics and Statistics (2023) India Agricultural Production, New Delhi, India. https://eands.dacnet.nic.in

Dugassa A, Alemu T, Woldehawariat Y (2021) In-vitro compatibility assay of indigenous *Trichoderma* and *Pseudomonas* species and their antagonistic activities against black root rot disease (*Fusarium solani*) of faba bean (*Vicia faba* L). BMC Microbiol 21:1–11. https://doi.org/10.1186/s12866-021-02181-7

El-Benawy NM, Abdel-Fattah GM, Ghoneem KM, Shabana YM (2020) Antimicrobial activities of *Trichoderma atroviride* against common bean seed-borne *Macrophomina phaseolina* and *Rhizoctonia solani*. Egypt J Basic Appl Sci 7(1):267–280. https://doi.org/10.1080/2314808X

Gaige AR, Ayella A, Shuai B (2010) Methyl jasmonate and ethylene induce partial resistance in *Medicago truncatula* against the charcoal rot pathogen *Macrophomina phaseolina*. Physiol Mol Plant Pathol 74(5–6):412–418. https://doi.org/10.1016/j.pmpp.2010.07.001

Haveri N (2017) Studies on diversity of Sclerotium rolfsii Sacc. and induced systemic resistance in groundnut (Arachis hypogaea L.) against stem rot pathogen. Ph.D. Thesis. Professor Jayashankar Telangana State Agricultural University, Hyderabad, India

Hussain T, Khan AA (2020) Determining the antifungal activity and characterization of *Bacillus siamensis* AMU03 against *Macrophomina phaseolina* (Tassi) Goid. Indian Phytopathol 73:507–516. https://doi.org/10.1007/s42360-020-00239-6

Inayati A, Sulistyowati L, Aini LQ, Yusnawan E (2019) Antifungal activity of volatile organic compounds from *Trichoderma virens*. In: AIP Conf. Proc. AIP Publishing, 2120:080012. https://doi.org/10.1063/1.5115750

Iqbal U, Mukhtar T (2020) Evaluation of Biocontrol potential of seven indigenous *Trichoderma* species against charcoal rot causing fungus, *Macrophomina phaseolina*. Gesunde Pflanzen 72(2):195–202. https://doi.org/10.1007/s10343-020-00501-x

Jadon KS, Thirumalaisamy PP, Kumar V et al (2015) Management of soil borne diseases of groundnut through seed dressing fungicides. Crop Prot 78:198–203. https://doi.org/10.1016/j.cropro.2015.08.021

Kadiyala MDM, Nedumaran S, Padmanabhan J et al (2021) Modeling the potential impacts of climate change and adaptation strategies on groundnut production in India. Sci Total Environ 776:145996. https://doi.org/10.1016/j.scitotenv.2021.145996

Kalam S, Basu A, Podile AR (2020) Functional and molecular characterization of plant growth promoting *Bacillus* isolates from tomato rhizosphere. Heliyon 6:e04734. https://doi.org/10.1016/j.heliyon.2020.e04734

Karthikeyan V, Sankaralingam A, Nakkeeran S (2006) Management of groundnut root rot with biocontrol agents and organic amendments. Arch Phytopathol Plant Prot 39(3):215–223. https://doi.org/10.1080/03235400500094225

Kaur S, Dhillon GS, Brar SK et al (2012) Emerging phytopathogen *Macrophomina phaseolina*: biology, economic importance and current diagnostic trends. Crit Rev Microbiol 38(2):136–151. https://doi.org/10.3109/1040841x.2011.640977

Khaledi N, Taheri P (2016) Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. J Plant Prot Res 56(1):21–31. https://doi.org/10.1515/jppr-2016-0004

Khan AN, Shair F, Malik K et al (2017) Molecular identification and genetic characterization of *Macrophomina phaseolina* strains causing pathogenicity on sunflower and chickpea. Front Microbiol 8:1309. https://doi.org/10.3389/fmicb.2017.01309

Khan N, Martínez-Hidalgo P, Ice TA et al (2018) Antifungal activity of *Bacillus* species against *Fusarium* and analysis of the potential mechanisms used in Biocontrol. Front Microbiol 9:2363. https://doi.org/10.3389/fmicb.2018.02363

Khan M, Salman M, Jan SA (2021) Biological control of fungal phytopathogens: a comprehensive review based on Bacillus species. MOJ Biol Med 6(2):90–92. https://doi.org/10.15406/mojbm.2021.06.00137

Kumar KS, Balabaskar P, Sivakumar T et al (2020) Bio-efficacy of culture filtrate of *Bacillus cereus* against the growth of *Macrophomina phaseolina* causing root rot of groundnut and different organic amendments on the survivability of *Bacillus cereus*. Plant Arch 20:1547–1550

Kumar J, Kumar M, Tomar A et al (2021) Morphological and molecular characterization of *Trichoderma* spp. from rhizosphere soil and their antagonistic activity against *Fusarium* spp. Int J Plant Soil Sci 33(19):100–112

Latha P, Karthikeyan M, Rajeswari E (2017) Development of bioformulations for the management of blackgram dry root rot caused

by *Rhizoctonia bataticola* (Taub.) Butler. Adv Res 9(4):1–12. https://doi.org/10.9734/air/2017/33195
Lokesh R, Rakholiya K, Thesiya M, Madagoudra Y (2021) Evaluation of bioagents against *Macrophomina phaseolina* (Tassi) Goid. causing dry root rot of chickpea (*Cicer arietinum* L). J Mycopathol Res 59(2):185–187
Mahendra M, Reddi Kumar M, Rajan CPD, Sumathi P (2022) Biological management of dry root rot of groundnut using *Trichoderma harzianum* and *Pseudomonas fluorescens* under glasshouse conditions. Biol Forum - Int J 14(3):409–416
Manjunatha SV, Naik MK, Patil MB et al (2011) Prevalence of dry root rot of chickpea in north-eastern Karnataka. Karnataka J Agric Sci 24(3):404–405
Mannai S, Ben Salem I, Boughalleb-M'Hamdi N (2025) Evaluation of *Trichoderma* and *Bacillus* species for the management of watermelon charcoal rot and plant growth promotion. Tunis J Plant Prot 20(2):69–84. https://doi.org/10.4314/tjpp.v20i2.4
Marquez N, Giachero ML, Declerck S, Ducasse DA (2021) *Macrophomina phaseolina*: General characteristics of pathogenicity and methods of control. Front Plant Sci 12:634397. https://doi.org/10.3389/fpls.2021.634397
Meena M, Swapnil P, Zehra A et al (2017) Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. Arch Phytopathol Plant Prot 50(13–14):629–648. https://doi.org/10.1080/03235408.2017.1357360
Moradia AM, Khandar RR (2011) Loss of yield of groundnut (*Arachis hypogaea* L.) due to dry root rot *Macrophomina phaseolina* and their management under in vivo condition. Int J Agric Sci 7(2):282–285
Mukherjee AK, Kumar AS, Kranthi S, Mukherjee PK (2014) Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. 3 Biotech 4(3):275–281. https://doi.org/10.1007/s13205-013-0150-4
Mukhopadhyay R, Pan S (2012) Effect of bipriming of radish (*Raphanus sativus*) seed with some antagonistic isolates of *Trichoderma*. J Plant Prot Sci 4(2):46–50
Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8(19):4321–4326. https://doi.org/10.1093/nar/8.19.4321
Nandakumar R, Babu S, Viswanathan R et al (2001) A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. Biocontrol 46(4):493–510. https://doi.org/10.1023/a:1014131131808
Ongena M, Jourdan E, Adam A et al (2007) Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. Environ Microbiol 9(4):1084–1090. https://doi.org/10.1111/j.1462-2920.2006.01202.x
Pamala PJ, Jayalakshmi RS, Vemana K et al (2023) Prevalence of groundnut dry root rot *Macrophomina phaseolina* (Tassi) Goid. and its pathogenic variability in Southern India. Front Fungal Biol 4:1189043. https://doi.org/10.3389/ffunb.2023.1189043
Rafiq M, Javaid A, Kanwal A, Anwar A, Khan IH, Kanwal Q et al (2024) GC-MS analysis and antifungal potential of flower extract of Acacia nilotica subsp. indica against Macrophomina phaseolina. Microb Pathog 194:106819. https://doi.org/10.1016/j.micpath.2024.106819
Raja Kumar RS, Arulselvi A, Rex Immanuel R et al (2021) Antagonistic activity of fungal and bacterial bio-inoculants against *Sclerotium rolfsii*. Plant Arch 21:1791–1796. https://doi.org/10.51470/plantarchives.2021.v21.no1.245
Rajamohan K, Balabaskar P (2016) In vivo evaluation of AM fungi on dry root rot disease incidence and biometrics of groundnut. Int J Dev Res 6(8):9074–9081
Ramesh R, Korikanthimath VS (2010) Seed treatment with bacterial antagonists - A simple technology to manage groundnut root rot under residual moisture conditions. J Biol Control 24(1):58–64
Rangel-Montoya EA, Delgado-Ramírez CS, Sepulveda E, Hernández-Martínez R (2022) Biocontrol of *Macrophomina phaseolina* using *Bacillus amyloliquefaciens* strains in cowpea (*Vigna unguiculata* L). Agronomy 12:676. https://doi.org/10.3390/agronomy12030676
Samaras A, Roumeliotis E, Ntasiou P, Karaoglanidis G (2021) *Bacillus subtilis* MBI600 promotes growth of tomato plants and induces systemic resistance contributing to the control of soilborne pathogens. Plants 10(6):1113. https://doi.org/10.3390/plants10061113
Shafqat A, Hanif S, Afzal A, Hussain N, Akthar S, Ambreen M, Ikram W (2026) Exploring the potential of *Trichoderma harzianum* and neem (*Azadirachta indica* A. Juss) in controlling charcoal rot in *Zea mays* L. J Plant Pathol 108:985–996. https://doi.org/10.1007/s42161-025-02112-9
Sharma M, Pande S (2013) Unravelling effects of temperature and soil moisture stress response on development of dry root rot Rhizoctonia bataticola (Taub.) Butler in chickpea. Am J Plant Sci 4(3):584–589. https://doi.org/10.4236/ajps.2013.43076
Sharma AD, Singh J (2005) A nonenzymatic method to isolate genomic DNA from bacteria and actinomycete. Anal Biochem 337(2):354–356. https://doi.org/10.1016/j.ab.2004.11.029
Shifa H, Gopalakrishnan C, Velazhahan R (2015) Efficacy of *Bacillus subtilis* G-1 in suppression of stem rot caused by *Sclerotium rolfsii* and growth promotion of groundnut. Int J Agric Environ Biotechnol 8(1):111–118. https://doi.org/10.5958/2230-732x.2015.00015.7
Singh N, Pandey P, Dubey RC, Maheshwari DK (2008) Biological control of root rot fungus Macrophomina phaseolina and growth enhancement of Pinus roxburghii Sarg. by rhizosphere competent Bacillus subtilis BN1. World J Microbiol Biotechnol 24(9):1669–1679. https://doi.org/10.1007/s11274-008-9680-z
Sreedevi B, Charitha Devi M, Saigopal DVR (2011) Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina*. J Agric Technol 7(3):623–635
Sridharan AP, Thankappan S, Karthikeyan G, Uthandi S (2020) Comprehensive profiling of the VOCs of *Trichoderma longibrachiatum* EF5 while interacting with *Sclerotium rolfsii* and *Macrophomina phaseolina*. Microbiol Res 236:1–13. https://doi.org/10.1016/j.micres.2020.126436
Srivastava R, Khalid A, Singh US, Sharma AK (2010) Evaluation of arbuscular mycorrhizal fungus, *fluorescent Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. Biol Control 53(1):24–31. https://doi.org/10.1016/j.biocontrol.2009.11.012
Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38(7):3022–3027. https://doi.org/10.1093/molbev/msab120
Thilagavathi R, Saravanakumar D, Ragupathi N, Samiyappan R (2007) A combination of Biocontrol agents improves the management of dry root rot *Macrophomina phaseolina* in greengram. Phytopathol Mediterr 46(2):157–167
Veena GA, Reddy NE (2016) Integrated disease management of dry root rot of chickpea. Int J Appl Biol Pharm Technol 7(2):45–54
Vimala R, Sheela J, Packiaraj D (2000) Management of root rot disease of groundnut by bioagents. Madras Agric J 87(4/6):352–354
Vincent JM (1947) Distortion of fungal hyphae in the presence of certain inhibitors. Nature 159:850. https://doi.org/10.1038/159850b0
Vyas SC, Patel MC (2015) Integrated biological and chemical control of dry root rot of chickpea. J Mycol Plant Pathol 24(2):132–134
White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications.

Academic, San Diego, pp 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

You J, Zhang J, Wu M et al (2016) Multiple criteria-based screening of *Trichoderma* isolates for biological control of *Botrytis cinerea* on tomato. Biol Control 101:31–38. https://doi.org/10.1016/j.biocontrol.2016.06.006

Zohaib KA, Bashir U, Khan IH, Javaid A, Anwar W (2024) Synergistic effect of plant growth promoting rhizobacteria and *Cirsium arvense* against black scurf disease of potato. Sains Malaysiana 53(9):3071–3083. https://doi.org/10.17576/jsm-2024-5309-13

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