Annals of Plant and Soil Research 27(3): 454-463 (2025)
https://doi.org/10.47815/apsr.2025.10490

Microbial solutions to *Phytophthora*-induced *gummosis* in Khasi mandarin (*Citrus reticulata* Blanco)

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Received: October, 2025; Revised accepted: November, 2025

ABSTRACT

Phytophthora gummosis, foot rot, and root rot are major global challenges to the current citrus industry. Despite several studies demonstrating the modulation in rhizosphere microbial communities of host plant challenged by the pathogen, little is known about Phytophthora–microbiome interactions. This study investigated the abundance of plant beneficial microbes in the healthy Khasi mandarin rhizosphere and their role as antagonist to Phytophthora nicotianae. Preliminary screening of culturable microbes isolated from healthy rhizosphere showed 8 fungal and 5 bacterial isolates antagonistic to P. nicotianae, with more than 50% mycelial inhibition. Morphological, cultural and molecular characterization of these promising isolates revealed their identity to two genera, Trichoderma (Trichoderma asperellum DamT22, T. asperellum CST1, T. asperellum CST3, T. asperellum CST5, T. harzianum NAG, T. asperellum KOT22, T. asperellum BH3, T. harzianum BH4) and Bacillus (Bacillus amyloliquefaciens BAC105, B. subtilis JB 5, B. velezensis MH4, B. amyloliquefaciens BAC 1, B. velezensis BAC 3) as citrus rhizosphere-specific antagonists. The antagonists were further screened in vitro, displayed a much greater efficacy of bacterial antagonists with 76.33 to 72.09% inhibition of Phytophthora spp. than fungal isolates showing 70.07-54.16% inhibition. Amongst the bacterial antagonists, Bacillus velezensis MH4-22 (76.78%) was observed as most effective followed by B. subtilis JB5 (75.44%). While, amongst all the Trichoderma strains, T. asperellum CST3 was observed most effective inhibiting 70.07% Phytophthora mycelia followed by T. asperellum BH3 with 69.12% mycelial inhibition. Our study further suggested that the presence of Phytophthora spp. in the rhizosphere altered the microbial community structure, having implications for plant health and productivity. These findings paved the way for microbial antagonists-mediated management of Phytophthora-induced gummosis disease in Khasi mandarin.

Keywords: Biocontrol, *Phytophthora* gummosis, rhizopheric microb

INTRODUCTION

The rhizosphere, defined as the narrow region of soil directly influenced by root secretions and associated soil microorganisms, represents a crucial interface between plant roots and soil microorganisms, playing a significant role in plant growth and health management. This dynamic and complex environment is shaped by various factors, such as plant genotype, soil type and environmental conditions (Srivastava and Bora, 2023). Diverse microbial communities within the rhizosphere, known as the rhizosphere microbiome, have profound implications for plant health, disease resistance and ecosystem processes (Bulgarelli *et al.,* 2013; Mendes *et al.,* 2013; Srivastava *et al.,* 2022). These microorganisms interact with plants through chemical signals as a part of molecular mechanisms, altering soil biological properties and eventually impacting the plant productivity (Handique *et al.,* 2024). Recent research highlighted the role of the rhizosphere in shaping plant-associated microbial communities and their interactions with pathogens. For instance, Yuan *et al.* (2018) demonstrated that the rhizosphere microbiome of maize plants can influence the colonization and virulence of the fungal pathogen *Fusarium verticillioides* by modulating the expression of plant genes involved in jasmonic acid (JA) and salicylic acid (SA) signalling. Similarly, Berg *et al.* (2017) reported that the rhizosphere microbiome of *Arabidopsis thaliana* promoted the growth and biocontrol activity of the fungal endophyte *Serendipita indica,* which enhanced plant resistance to the foliar pathogen *Pseudomonas syringae*. Therefore, any changes in the microbial composition of the rhizosphere can have far-reaching consequences on its ecological roles, plant physiology, and ultimately the plant productivity.

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Phytophthora spp. are soil borne pathogens causing devastating magnitude of disease manifested in form of foot and root rot and gummosis in citrus plants (Malhotra and Srivastava, 2023) . These infections can lead to gum pockets on the bark and wood of trunks and branches, resulting in untimely tree decline. The pathogen can infect roots, trunks and branches, often through wounds or injuries caused by mechanical damage or adverse environmental conditions (Cevik *et al.,* 2019). Recently, researchers have investigated the impact of *Phytophthora* infection on the composition of rhizospheric microbial communities, recognizing the crucial role of *Phytophthora* infection in plant health and productivity. Thangavel *et al.* (2020) examined the effects of *Phytophthora* infection on the rhizospheric microbial community composition of Khasi mandarin plants. Like other citrus cultivars, *Phytophthora* diseases are a major threat to Khasi mandarin, an economically and culturally important citrus cultivar of northeastern region of India, particularly in Meghalaya, Assam, and Arunachal Pradesh (Malhotra and Srivastava, 2023). Despite its significance, the production of Khasi mandarin is threatened seriously by *Phytophthora* spp. Several studies have focused on the importance of Khasi mandarin in India and the risks posed by diseases such as *Phytophthora* root rot. Bhattacharya *et al.* (2020) highlighted Khasi mandarin as the most preferred citrus fruit in Meghalaya due to its unique flavour and aroma, but it is also vulnerable to various diseases, impacting both yield and quality (Srivastava *et al.,* 2025; Nasrin *et al.,* 2020). Similarly, Barman *et al.* (2018) reported susceptibility to pests and diseases, further affecting the productivity and quality of the crop. Our previous study has already reported *P. nicotianae* associated with gummosis and fruit rot of khasi mandarin in Assam identified through morpho-cultural and molecular characterization (Handique *et al.,* 2024). Understanding microbial diversity and its functional contribution to the health and productivity of citrus plants in the rhizosphere is highly important to study plant-microbial community interactions, identifying plant beneficial microbes and developing microbial-based strategies for managing citrus diseases, including *Phytophthora* disease complexes. With the advances in new chemical molecules for *Phytophthora*-induced diseases caused by oomycetes fungi, management of *Phytophthora* diseases has not yet received desired success. Such chemical-based failures for management of *Phytophthora* diseases shifted the concentration of researchers on exploration and usage of microbial antagonists. However, such efforts also produced no satisfactory soluton to virulence of *Phythophthora* diseases in citrus largely due to excessive aggressivity of *Phytophthora*, swift life cycle of the pathogen and perennial nature of the host plant (Srivastava *et al.,* 2011). In this background, our efforts aimed at isolating citrus rhizosphere-based microbial communities and their evaluation for antagonistic ability against major species of *Phytophthora* using Khasi mandarin as host plant.

MATERIALS AND METHODS

Source of *Phytopthora* spp.

Phytophthora nicotinae previously isolated from infected Khasi mandarin plants and well characterized through morpho-cultural and molecular tools with NCBI Accession no. OP753715 (Handique *et al.,* 2024) was collected from the authors laboratory in the Department of Plant Pathology, AAU, Jorhat. The pathogen was sub-cultured in PDA and maintained at 4^0C for further studies in our experiment.

Rhizospheric soil sampling

Soil samples were collected from the apparently disease free, Khasi mandarin rhizosphere of two citrus orchards viz., Motapung and Gelapukhuri of Tinsukia district of Assam for isolation of plant beneficial microbes for onward screening against *P. nicotianae.* The sampling was done following standard procedure. The process involved the collection of 100 grams of soil from four different sides of the tree basin using a 25 mm soil auger. To avoid any potential contamination, we washed the shovel sterilized with 70% ethanol in each sampling. Composite sample of rhizospheric soil was made by mixing the soil adhered to the root system from each sample of a plant. Samples were collected in zip lock polyethene bags and placed in an ice box (4ºC). These samples were then transported to the Biocontrol laboratory, Department of Plant Pathology, Assam Agricultural University, Jorhat for further experiment process.

Isolation of culturable rhizospheric microbes

Culture dependent microbes were isolated from the soil samples following serial dilution method. A 0.5mL of the one-fold to nine-fold dilution soil water sample was transferred to the Petri Plates containing sterilized nutrient agar (NA) medium (HiMedia) through spread plate technique for isolation of bacterial strains. Plates after solidication of NA were then incubated upside-down position at 30±1ºC in the incubator for 48 hours. Three replicates were maintained for each sample. For isolation of fungal colonies, sterilized PDA (HiMedia) medium supplemented with streptomycin was used to prevent bacterial growth. Three replicates were maintained for each sample. The inoculated plates were then incubated upside down at 25±1°C for 5 days in a BOD incubator. The colony form unit (CFU) of fungi and bacteria were estimated by counting the number of fungal and bacterial colonies following the protocol of Chowdhry and Varshney, (2000). The individual colonies with distinct colony color and morphology were further purified in NA and PDA for bacterial and fungal isolates, respectively.

Preliminary *in vitro* screening of rhizospheric isolates

The distinct purified isolates were subjected to preliminary screening for antagonistic potential against *P. nicotianae* using in *vitro* dual culture technique. A mycelia disc of 5mm diameter of the pathogen from a 5- days - old actively growing pathogen culture was taken out with a help of sterilized cork borer and was placed at the centre of PDA media and incubated at 28 ± 2°C for 2 days. Petri dish pre-inoculated with fungal pathogen was divided into four quadrants and each quadrant was inoculated with the bacterial antagonist leaving 1cm from the periphery of the petri dish. The bacterial isolates were inoculated on four sides of the pathogen as a streak line for preliminary screening (Baruah *et al.,* 2024**;** Bora *et al.,* 2020). Similarly, preliminary screening was done for fungal rhizospheric microbes using a 4-point assay following the standard protocols (Saikia *et al.,* 2022). A 5mm mycelial discs from the *P. Nicotiane* was placed at one side of plate, leaving 1 cm away from the periphery ; while the test fungal isolate was placed at the opposite side of the plate. The plates with only pathogen served as control plates. The inoculated plates were then incubated at 28 ± 2°C and observations were made 24, 48 and 72 hours after inoculation. Each treatment was replicated 5 times. Percent inhibition (PI) was calculated by the following formula (Vincent, 1927):

$$\mathbf{PI} = (\mathbf{C} - \mathbf{T})/\mathrm{C} \times \mathbf{100}$$

where, C represents the radial growth of the pathogenic fungus in the control plate and T denotes the radial growth of the pathogenic fungus in the presence of the microbial strain. The isolates were categorized into two categories: Class 1: + (>50% inhibition) and Class 2: ++ (40-50% inhibition).

Characterization of promising rhizospheric isolates

In our investigation, we focused on rhizospheric isolates that exhibited significant inhibition (over 50%) of *Phytophthora nicotianae*. These selected fungal isolates were studied for their morpho-cultural characterization after 7 days of inoculation in pure culture. Morphological and cultural features of each fungal isolate viz., colony color, texture, conidia shape, phialides and chlamydospores were studied by using the slide culture method following the standard protocol of Bawage *et al.,* 2013).Bacterial rhizospheric isolates were studied for their morphological, cultural and biochemical characteristics after 48 hours of inoculation. Morphological characters like the shape of bacteria and Gram reactions were analysed by using the Gram staining method following the protocol of Gephardt *et al.* (1981). Cultural characteristics of bacterial isolates such as colony shape, colour, surface, edges, elevation and opacity were studied in the solid phase of nutrient agar media (Saikia *et al.,* 2020).

Molecular identification of Potential rhizospheric microbes

We adopted a rigorous approach to select promising rhizospheric bacteria with a high level of inhibition (>50%) again.st *Phytophthora nicotianae*. Isolation of bacteria's genomic DNA was done following the protocol of Cardinal *et al.* (1997). To perform molecular characterization of these bacteria, PCR amplification was done using universal primers specifically targeting the 16S rRNA gene for bacteria. We utilized the 16S rRNA gene (5`TACGGYTACCTTGTTACGACTT3`, 5`AGAGTTTGATCMTGGCTCAG3`) (Goswami

et al., 2017) in our study and initial denaturation was done for 3 minutes at 94oC. Fungal DNA was extracted following the CTAB method (Baruah *et al.,* 2025) and further amplified using ITS-1 and ITS-4 primers (Das *et al.,* 2017, Srivastava *et al.,* 2025). DNA amplification was done with an initial denaturation at 94 °C (4 min) followed by 30 cycles of denaturation, annealing, and extension. The amplified DNA was visualized using gel electrophoresis. The sequence data were analysed using the Bio-Edit sequence alignment editor and searched against the NCBI database using nucleotide BLAST. A phylogenetic tree was constructed using MEGA version 11 with the maximum likelihood method and 500 bootstrap replicates (Rahman *et al.,* 2023). This approach enabled us to identify the species that were phylogenetically closest to the obtained sequences.

In vitro bioassay of potential bacterial and fungal isolates against *P. nicotianae*

The promising rhizospheric bacterial and fungal isolates exhibiting mycelial inhibition more than 50% were further carried forward to study their comparative efficacy through dual culture assay method as per protocols stated above. The experiment was laid out in completely randomized design (CRD) with 5 replications against each treatment. Radial growth of the fungal pathogens was measured and Percent inhibition (PI) was calculated against the control plate. All the treatments were replicated five times with a completely randomized design (CRD). The per cent inhibition (PI) was calculated using the formula

$$\mathbf{PI} = (\mathbf{C} - \mathbf{T})/\mathrm{C} \times \mathbf{100}$$

Statistical analysis

Completely Randomised Design (CRD) was used for statistical analysis of data generated out of laboratory studies. Analysis of variance was performed using all the data generated through various experiments using the statistical package MSTAT (Freed and Glover, 1986). Multiple comparisons amongst treatment means were undertaken using DMRT. The analysis of variance (ANOVA) was used to compare the significant difference with the t-test at 5% level of significance.

RESULTS AND DISCUSSION

Identification of rhizospheric microbes from Khasi mandarin rhizosphere

Following serial dilution, pure culture was undertaken for colonies with diverse morphologies and dominant growth patterns from the microbial-rich rhizosphere of Khasi mandarin. In total, we isolated 16 different fungal colonies with distinct colony color, sporulation pattern and 28 bacterial isolates with distinct morphologies from the rhizosphere soil samples for further screening. The isolates were named with some codes till their final identifications.

Preliminary screening of rhizospheric microbes against *Phytophthora nicotianae*

Among the tested bacterial isolates, five out of 28 exhibited more than 50% mycelial inhibition against *Phytophthora nicotianae*, indicating significant antagonistic activity. Similarly, among the fungal isolates, eight (8) demonstrated more than 50% mycelial inhibition, suggesting their potential as biocontrol agents.

Identification of the promising Isolates

Morphological and cultural characterization of the bacterial isolates revealed distinct colony morphologies and gram-positive reactions for all the isolates (Table 1). Molecular characterization via 16S rRNA gene sequencing identified JB4 and BAC3 as *Bacillus velezensis*, (BAC-105) and BAC 1 as *Bacillus amyloliquefaciens*, and JB5 (MH-JB5-BAC) as *Bacillus subtilis.* The abundance of *Bacillus* in soil is largely due to its ability to form resistant endospores, enabling survival under harsh conditions such as desiccation, nutrient scarcity, and temperature or pH extremes an advantage over non-spore-forming bacteria like *Pseudomonas* and *Azotobacter* (Bora *et al.,* 2023). Their metabolic versatility allows utilization of diverse carbon and nitrogen sources, supporting colonization across varied soils and plant hosts. Additionally, *Bacillus* produces antimicrobial metabolites (e.g., surfactin, iturin, fengycin) that suppress pathogens and competitors, enhancing their dominance. Their strong rhizosphere competence through biofilm formation, root

adhesion, and interactions with root exudates further boosts persistence Moreover, *Bacillus* promotes plant growth via phytohormone production, phosphate solubilization, and nutrient enhancement, creating a mutualistic, feedback that sustains their abundance in the rhizosphere (Maslennikova *et al.,* 2023; Lv *et al.,* 2025).

Table 1: Morpho-cultural characteristics of Promising bacterial isolates screened through preliminary assay

Bacterial Isolate	Morpho-Cultural Characteristics							*GR
	Shape	Colony Shape	Surface	Colour	Edge	Elevation	Opacity	
JB4	Rod	Uneven	Rough	Creamy white	Uneven	Flat	Opaque	+
JB5	Rod	Uneven	Rough	Creamy white	Uneven	Flat	Opaque	+
BAC105	Rod	Uneven	Rough	White	Uneven	Flat	Opaque	+
BAC 3	Rod	Uneven	Rough	Creamy white	Uneven	Flat	Opaque	+
BAC 1	Rod	Uneven	Rough	White	Uneven	Flat	Opaque	+

**GR: Gram-reaction*

The fungal isolates exhibited characteristic morphological and cultural characteristics similar to *Trichoderma* spp. Morphological characterization of the NAG and BH4 isolates revealed that the conidia size ranged from 1.7-2.5 × 2.5-3 µm, and the colonies exhibited flat pustules and effuse conidiation, with colours ranging from whitish green to pale green. These observations were consistent with previous studies by Rifai (1969) and Bisset (1991). On the other hand, the KOT, DAMT, CST1, CST3, CST5, and BH3 isolates exhibited conidia sizes ranging from 3-6 × 5-3 µm and were characterized as light green and globose to subglobose in shape, with colony colours mainly being greenish to whitish green, occasionally showing a floral growth pttern, which aligned with findings by Samuels *et al.* (2006) (Table 2).

Table 2: Morpho-cultural Characteristics of promising antagonistic fungal isolates

Isolate	Colony	Conidiophore	Phialide	Conidia
NAG	Whitish green to pale green, floccose, Flat pustules, effuse conidiation	Flexible, right-angled branches uneven, and shorter	Whorls of 2–6 Short, skittle-shaped, subulate, Ampulliform to laginiform in shape	Light green, globose to sub-globose Size: 2.5 × 3µm
KOT	Green to whitish green Growth is flat.	Paired branches regularly branched	Straight, laginiform to ampulliform	Light green, globose to sub-globose Size: 5.0 × 3.0µm
DamT	Dark green spores with profuse white mycelial growth	Paired branches that are regularly branched	Straight, laginiform to ampulliform	Light green, globose to sub-globose Size: 5.4 × 4µm
CST1	White to pale-green to green in colour	Paired branches, regularly branched	Straight, laginiform to ampulliform	Light green, globose to sub-globose Size: 6.0 × 3.5µm
CST3	White to pale-green to green in colour	Paired branches, regularly branched	Straight, laginiform to ampulliform	Light green, globose to sub-globose Size: 6.0 × 3.5µm
CST5	White to pale green coloured mycelial growth	Paired branches, regularly branched	Straight, laginiform to ampulliform	Light green, globose to sub-globose Size: 4.0 × 5.0µm
BH3	Floral growth pattern, Cottony and compact, and green to dark green.	Paired branches, regularly branched	Straight, laginiform to ampulliform	Globose to sub-globose, sparsely ornamented Size: 3.0 × 5.0µm
BH4	Pale green to the dull white-coloured colony with profuse growth	Flexible, Right-angled, Uneven, and shorter, less heavily branched	Whorls of 2–6 that are short, skittle-shaped, Subulate, and ampulliform to laginiform in shape, Narrower at the base.	Light green, globose to sub-globose to shorter oval in shape.Size: 1.7 × 2.5µm Numerous chlamydospores

Molecular characterization using the ITS1 and ITS4 primers indicated that all the isolates belonged to the *Trichoderma* genus. NAG and BH4 were observed closely related to *Trichoderma harzianum*; while KOT, DAMT, CST1, CST3, CST5, and BH3 were closely related to *Trichoderma asperellum.* Phylogenetic analysis indicated that all eight isolates belong to the Trichoderma genus. Notably, the NAG and BH4 isolates exhibited the highest similarity to *Trichoderma harzianum*, while the other six isolates, namely, KOT, DAMT, CST1, CST3, CST5, and BH3, were classified as *Trichoderma asperellum* (Table 3).

Table 3: Molecular identification of efficient isolates with NCBI Gen Bank Accession number

BH3	*Trichoderma asperellum*	ON364135.1
BH4	*Trichoderma harzianum*	ON364137.1
MH4	*Bacillus velezensis*	ON351281.1
JB5	*Bacillus subtilis*	ON398954.1
BAC-105	*Bacillus amyloliquefaciens*	ON351065.1
BAC 3	*Bacillus velezensis*	ON392437.1
BAC 1	*Bacillus amyloliquefaciens*	ON392425.1
BH3	*Trichoderma asperellum*	ON364135.1
BH4	*Trichoderma harzianum*	ON364137.1
MH4	*Bacillus velezensis*	ON351281.1

Trichoderma is a predominant ubiquitous fungal microbe having wide adaptability and multiple growth promoting and biocontrol traits (Rahman *et al.,* 2023). *Trichoderma* outcompetes phytopathogens and other microbes due to high rhizosphere competence, colonizing the rhizosphere and root surfaces, rapidly establish high populations, and thereby out-compete phytopathogens for space and nutrients. Stummer *et al.* (2024) reported that a strain of *Trichoderma gamsii* (A5MH) maintained ~log 5.6 GC/g in soil at crop emergence and persisted above ~log 4.5 GC/g through to harvest while reducing root-pathogen loads. Sucha high rhizosphere competence, combined with efficient root adhesion, biofilm formation and utilization of root exudates, render *Trichoderma* a competitive edge against soil-borne pathogens (Bora *et al.,* 2022) . Additionally, modern reviews highlighted their inclusion in multicomponent inoculants that exploit these strong colonization traits (Bora and Bora, 2020; Bora *et al.,* 2020).

In-vitro bio-effiicacy of microbial isolates against *P. nicotianae*

In the realm of potential bacterial bioagents, *Bacillus* spp. exhibited impressive antagonistic potential against *Phytophthora nicotianae*. Notably, *Bacillus velezensis* MH4 was the most effective, with the highest inhibition rate of 76.78%, followed closely by *Bacillus subtilis* JB5 at 75.44%. Both of these isolates were classified under Belle's antagonistic rating of Class I. These observations align with the results reported by Lee *et al.* (2007), who observed varying levels of antagonism among *Bacillus* spp., with maximum growth inhibition rates of 86.8% and 71%, respectively (Fig. 1).

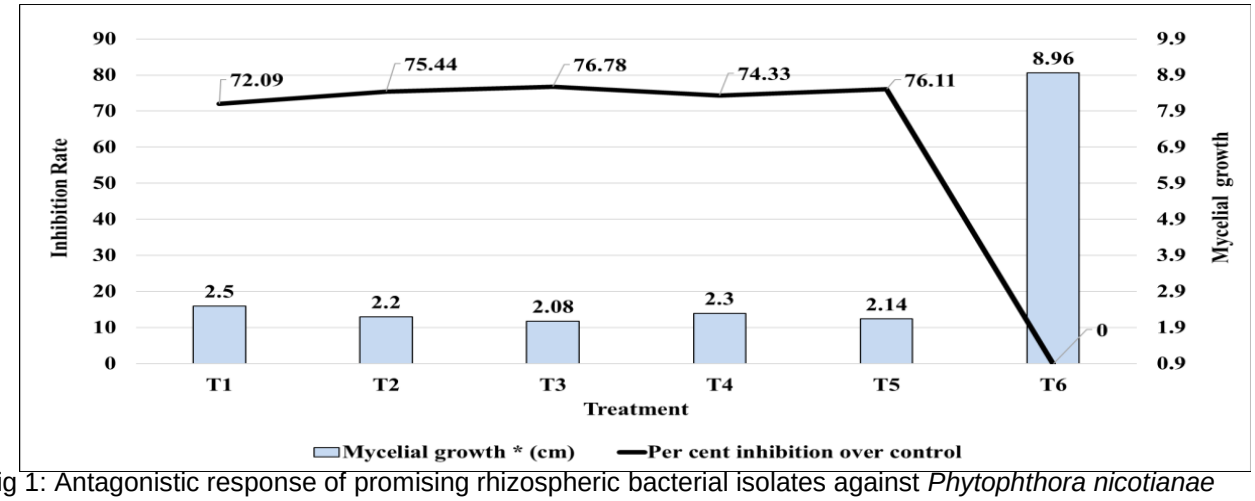

Fig 1: Antagonistic response of promising rhizospheric bacterial isolates against *Phytophthora nicotianae*

Bacillus spp have been widely recognized as plant growth promoter owing to its nutrient solubilising and growth hormone producing ability. The antimicrobial and pesticidal efficacies have also been reported against different pathogens in a variety of crops.

The antagonistic effects of *Bacillus* spp. are attributed to an array of secondary metabolites, including phenol, 2,4-bis (1,1-dimethyl ethyl), 3-hexadecanol, pyrrolo (1,2-a) pyrazine-1,4-dione, hexahydro-3-(2-methyl-propyl)-,5,10-diethoxy-2,3,7,8tetrahydro-1H, 6H-pyrrole (1,2-a:1′,2′d) pyrazine and hexadecenoic acid (Bora *et al.,* 2023, Saha *et al.,* 2025). These compounds effectively inhibited the mycelial growth, spore formation and any possible germination as demonstrated by Prakash and Arora. (2021). Further, it can induce activity of defense enzymes such as PAL, PPO, Peroxidase in host plants (Bora *et al.,* 2024).
T1: *Bacillus amyloliquefaciens* BAC105; T2: *B. subtilis* JB 5; T3: *B. velezensis* MH4; T4: *B. amyloliquefaciens* BAC 1; T5: *B.* velezensis BAC 3; T6: Control (pathogen only)

Turning our attention to *Trichoderma* spp. identified as potential bioagent, all Trichoderma isolates displayed significant levels of antagonism in terms of inhibiting mycelial growth. Notably, *Trichoderma asperellum* CST3 exhibited the highest invitro efficacy against *Phytophthora* nicotianae, with an impressive mycelial growth inhibition of 70.07%, followed closely by *Trichoderma asperellum* BH3 at 69.12%. These findings corroborate the results of a study by Tchameni *et al.* (2017), where *T. asperellum* isolates demonstrated strong antagonism toward *P. megakarya*, resulting in mycelial inhibition rates ranging from 65% to 80%.In a similar vein, the study conducted by Fatima *et al.* (2015) consistently revealed that *T. harzianum* effectively reduced the radial growth of *Phytophthora infestans,* with inhibition rates ranging from 57% to 85% (Fig. 2).

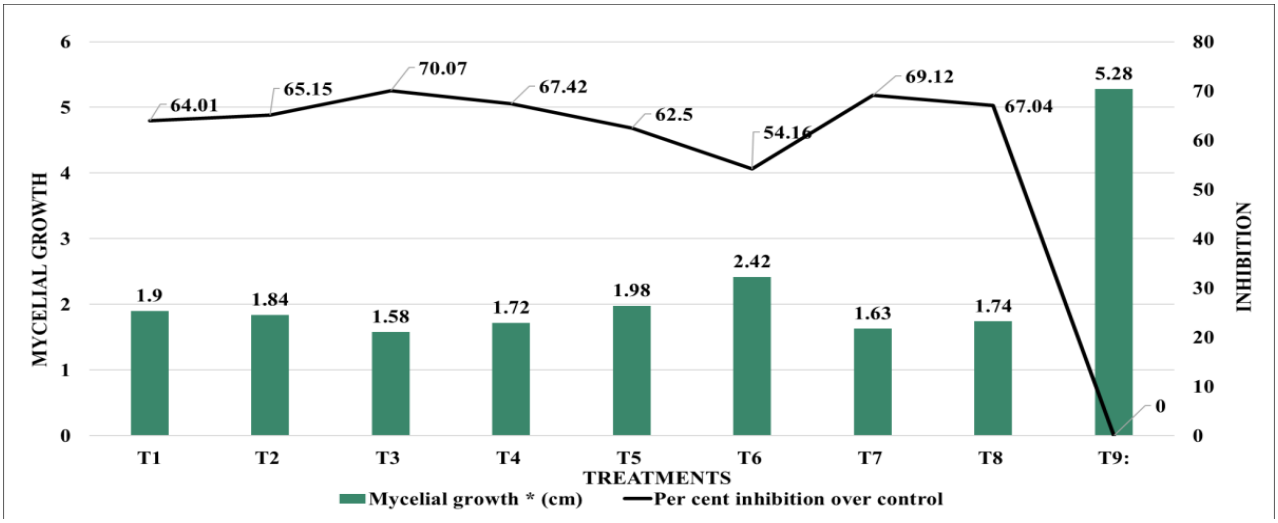

Fig 2: Antagonistic response of promising rhizopheric fungal isolates against *Phytophthora nicotianae*

T1**:** *Trichoderma asperellum* DAM; T2: *T. asperellum* CST1; T3: *T. asperellum* CST3; T4: *T. asperellum* CST5; T5: *T. harzianum* NAG; T6: *T. asperellum* KOT; T7: *T. asperellum* BH3; T8: *T. harzianum* BH4; T9: Control (Pathogen)

The genus Trichoderma is an widely explored antagonist against many fungal and bacterial pathogens and also used as plant growth booster (Bora and Rahman, 2022). Trichoderma can survive in soil, water, phyllosphere, and have high rhizopshere competence, which might be the reason for higher abaundance of the genus in khasi manadarin rhizopshere in our study. Further, Trichoderma spp. can sense and parasitize over the pathogens and can produce many volatile and non volatile antifungal componenets (Saikia *et al.,* 2021), besides producing siderophore and depriving pathogens of vital element Fe in the soil (Sharma and Bora, 2025). In addition to direct antagonism, the bioagent through nutrient competition and through induced host resistance also act indirectly against phytopathogens.

CONCLUSION

In conclusion, our study significantly added a strong conviction about the role of microbial communities in the natural suppression of Phytophthora diseases in Khasi mandarin orchards. The abundance of beneficial rhizospheric microbes antagonistic to the pathogen and the identification of *Bacillus* and

Trichoderma displaying the maximum antagonistic ability, further presenting the promising avenues for developing effective strategies to manage diseases in citrus orchards and other ecological systems. However, developing a suitable carrier based formulations of the citrus specific microbes and field performance study are required for further validation. The insights gained from this study provide a solid foundation for future investigations and advancements in our understanding of plant–microbe interactions in a healthy versus disease infested rhizosphere soils through multi omic tools.

ACKNOWLEDGEMENTS

The authors thank to Department of Plant Pathology and the ICAR-Central Citrus Research Institute in Nagpur, Maharashtra, India for providing the essential assistance and facilities for the study.

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