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Biocontrol and Growth-promoting Potential of Native *Trichoderma* Isolate from Himalayan Foothills Against Tomato Fungal Pathogens

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ABSTRACT

The study was conducted during August, 2022–March, 2023 in the northwestern region of the Himalayan foothills on isolating and profiling a new *Trichoderma* strain from Himalayan soils for its antagonistic and growth-promoting effects against tomato pathogenic fungi. Soil samples were collected from various locations were subjected to serial dilution to isolate *Trichoderma*. The culture was evaluated morphologically based on colony characteristics, spore shape, and hyphal structure under a microscope. The isolate's DNA was extracted and amplified for genetic identification and profiling. *In vitro* and pot assays were conducted to determine antagonistic activity and plant growth-promoting effects against major tomato fungal pathogens. Morphological features revealed that isolate exhibited rapid colony growth characterized by cottony mycelium and green conidia, showing typical characteristics of genus *Trichoderma*. Molecular identification using ribosomal Deoxy-ribomucleic acid sequencing of the Internal Transcribed Spacer confirmed isolate's taxonomic identity as *Trichoderma* sp. isolate SUB11780421PII. Laboratory tests in Petri plates showed that it inhibited pathogen growth by 33 mm resulted to 52.72% of mycelial inhibition. In pot culture trials, it reduced disease incidence by 20% for *Sclerotium rolfsii*. The reduction was 30% for *Fusarium oxysporum* f. sp. lycopersici, while a more pronounced disease suppression of 35% was observed against *Rhizoctonia solani*. Additionally, this novel isolate from the foothills of the Himalayas promoted root (3.08 cm) and shoot growth (12.39 cm). These outcomes suggested that *Trichoderma* sp. isolate SUB11780421PII showed strong potential as a biological organism for mitigating diseases and supporting resilient agriculture, marking its first report from the region.

KEYWORDS: *Trichoderma*, tomato, growth promotion, biocontrol, characterization, Himalayan foothills

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1. INTRODUCTION

Tomato is a vital crop of great economic value, widely grown across the globe, and plays a crucial role in supporting food systems and nutrition (Naik et al., 2010; Deb and Dutta, 2021). However, its production is impacted by plant pathogens, leading to yield reductions and reduced fruit quality (Collins et al., 2022). These pathogens, encompassing fungi, bacteria, viruses, and nematodes, target tomatoes at various growth stages, causing pre- and post-harvest losses (Khurana and Kumar, 2021; Okey and Okop, 2024). Panth et al. (2020) and Panno et al. (2021) note that these pathogens reduce yield and create economic challenges for farmers and the agricultural sector. Species such as *Fusarium oxysporum*, *Pythium ultimum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Verticillium dahliae*, *Alternaria solani*, and *Phytophthora infestans* cause prevalent diseases (Patil and Sriram, 2020; Bhardwaj et al., 2023b). They cause damping-off, wilting, root rot, blight, and fruit decay, making management difficult due to their resilience (Cacciola and Gullino, 2019; Bhardwaj and Gupta, 2023a). These pathogens thrive in a variety of environmental conditions, including different temperatures and humidity levels. Pathogens spread quickly through soil, water, wind, and human activities, including contaminated tools. Many fungi produce spores and sclerotia, allowing survival in harsh conditions (Ma et al., 2023; Levens et al., 2024). These concerns highlight the need for sustainable, eco-friendly disease management strategies (Khurana and Kumar, 2021; Bhardwaj et al., 2023c). Practices like monoculture, excessive fungicide use, and poor pest management lead to resistant pathogens and reduced ecological diversity (Altieri et al., 2024; Bhardwaj and Chandel, 2024). This results in environmental degradation and increased crop vulnerability. Sharma (2023) advocates for biological control agents as a better alternative. Harnessing natural enemies of pathogens, like *Trichoderma* reduces known for their antifungal properties, reduce chemical use, counter pathogen resistance, and preserve biodiversity (Athira and Anith, 2020; Wongamthing and Sainamole Kurian, 2023).

Trichoderma fungi control pathogenic fungi, by competing for nutrients, and producing bioactive substances (Singh et al., 2023). Seethapathy (2025) highlights chitinases, glucanases, and proteases, produced by *Trichoderma* break down fungal cell walls. Tyskiewicz et al. (2022) report that some species produce peptaibols, trichodermol, viridin, harzianolides that inhibit pathogen growth. They also promote plant growth and soil health by degrading organic matter, mobilizing nutrients, and inducing immunity. These mechanisms are effective for *Pythium ultimum*, *Fusarium oxysporum* and *Rhizoctonia solani*. *Trichoderma* isolates, including those closely related to *T. viride* and *T. harzianum*, manage pathogens and promote growth (Manzar et al., 2022). The Himalayas's vibrant ecosystem supports microbial diversity, with foothills being a hotspot for novel microorganisms with superior biocontrol properties (Rajashekara et al., 2023). Despite growing interest, research on isolating and screening *Trichoderma* against tomato pathogens remains limited. Known species like *T. viride*, *T. atroviride*, *T. hamatum*, *T. asperellum*, and *T.* could benefit from exploring new species (Khan et al., 2018). This could enhance crop resilience, reduce pesticide use, and promote sustainable practices (Alfiky and Weisskopf, 2021; Manzar et al., 2022).

Therefore, the study aims to isolate and characterize a novel *Trichoderma* antagonist from the Himalayan foothills to assess its properties against *Sclerotium rolfsii*, *Fusarium oxysporum f.* sp. *lycopersici*, and *Rhizoctonia solani*. The investigation further explores the impact of the isolate on seedling vigor and development of roots and shoots. Molecular techniques, including ribosomal DNA (rDNA) sequencing of the Internal Transcribed Spacer (ITS) region, will be employed for taxonomic identification. By assessing both the biocontrol efficacy and growth-boosting properties of the isolate, the study intended to present an nature-friendly substitute to synthetic chemical control agents, contributing to self-sustaining farming techniques in managing soil-borne diseases in tomato crop.

2. MATERIALS AND METHODS

2.1. Isolation of trichoderma species from tomato rhizosphere soil for antagonistic assessment

The study was conducted during August, 2022–March, 2023 in the northwestern region of the Himalayan foothills. Soil samples (250 g each) were collected from various locations in the rhizosphere of tomato plants in the foothills of the Himalayas, at a depth of 5–10 cm, using an auger during august month of year 2022. The samples were placed in sterile plastic bags. Serial dilutions (10^{-1} to 10^{-6}) of the soil were plated on Potato Dextrose Agar (PDA) supplemented with streptomycin (50 mg l^{-1}) to inhibit bacterial growth. Petri dishes (9 cm) were incubated at 25°C for 5–7 days. Fungal colonies with characteristics resembling *Trichoderma* were selected. Pure cultures were established by subculturing individual colonies onto fresh PDA plates. The colony growth was evaluated for typical *Trichoderma* features, such as colony color, texture, edges, and growth rate. Microscopic examination was performed to assess hyphal structure, conidiophores, conidia, phialides, and chlamydospores (Games and Bissett, 2002). The pathogens *Pythium ultimum*, *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, and *Sclerotium rolfsii* were isolated from diseased tomato plants, identified by cultural

and morphological traits, and confirmed pathogenic through soil inoculation and mass multiplication methods (Bhardwaj and Chandel, 2024). The antagonistic activity of the *Trichoderma* isolate was tested using a dual culture method. The target pathogen was inoculated on one side of the PDA plate, with *Trichoderma* placed on the opposite side and the plates were incubated at 25°C for 3–7 days. The zone of inhibition around the *Trichoderma* colony was measured to evaluate its antagonistic effect (Bhardwaj et al., 2023a). The isolate's effectiveness was compared with controls to confirm its biocontrol potential.

2.2. Molecular identification and DNA extraction

Around 0.1 g of the sample was placed in a mortar and homogenized with 1 ml of extraction buffer. The homogenate was transferred to a 2 ml microfuge tube, and an equal volume of Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added. The tubes were mixed gently by shaking. The mixture was then centrifuged at room temperature for 15 min at 14,000 rpm. The upper aqueous phase was transferred to a new tube, and an equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed. The tubes were centrifuged again at room temperature for 10 min at 14,000 rpm, and the upper aqueous phase was transferred to a new tube. DNA was precipitated by adding 0.1 volume of 3 M sodium acetate (pH 7.0) and 0.7 volume of isopropanol. After incubation at room temperature for 15 min, the tubes were centrifuged at 4°C for 15 min at 14,000 rpm. The DNA pellet was washed twice with 70% ethanol and then briefly with 100% ethanol, followed by air drying. The DNA was dissolved in TE buffer (Tris-Cl 10 mM, pH 8.0, EDTA 1 mM). To remove RNA, 5 µl of RNase-free RNase A (10 mg ml^{-1}) was added to the DNA (Mistry and Bariya, 2022).

2.3. PCR conditions, cycling and amplification

The PCR amplification of the ITS gene was performed using 127 ng of extracted DNA and 10 µM of each primer. The Taq Master Mix was prepared with high-fidelity DNA polymerase, 0.5 mM dNTPs, 3.2 mM $MgCl_2$, and PCR enzyme buffer to ensure optimal conditions for amplification. The ITS region was amplified for species-level identification. Primers used were, ITS Forward (5'-TCCGTAGGTGAACCTGCGG-3') and ITS Reverse (5'-TCCTCCGCTTATTGATATGC-3'), to target the fungal rRNA gene. PCR conditions were set as follows: 94 °C for 3 min (initial denaturation), followed by 30 cycles of 94 °C for 30 s (denaturation), 50°C for 1 min (annealing), 72 °C for 2 min (extension), with a final extension at 72 °C for 7 min. The reaction mix was prepared with 1 µl of DNA, 2 µl of each primer, 4 µl of dNTPs (2.5 mM), 10 µl of 10X Taq buffer, 1 µl of Taq polymerase (3 U ml^{-1}), and 30 µl of water, making a total volume of 50 µl. After amplification, the PCR product get purified to remove any remaining primers, dNTPs, enzymes, and other contaminants that might interfere with sequencing. The purified PCR product was used as a template for the sequencing reaction. The sequencing reaction is then performed, which uses chain-terminating di-deoxynucleotides (ddNTPs) to produce fragments of different lengths, each terminating at a specific nucleotide. he reaction typically included thermal cycling steps (After the sequencing reaction, the products were purified to remove excess Big Dye terminator chemicals and unincorporated nucleotides, annealing, and extension) to allow DNA synthesis and incorporation of ddNTPs. The obtained ITS sequences were subjected to a BLAST search in the NCBI GenBank database to identify closely related sequences and confirm the species-level identification based on sequence similarity (Cai and Druzhinina, 2021).

2.4. Phylogenetic analysis

Phylogentic Tree Builder uses sequences aligned with System Software aligner. A distance matrix is generated using the Jukes-Cantor corrected distance model. When generating the distance matrix, only alignment model positions were used, alignment inserts were ignored and the minimum comparable position is 200. The tree was created using Weighbor with alphabet size 4 and length size 1000. In phylogenetic tree construction, pseudoalignments were created by randomly selecting positions from the original alignment. This process was repeated 100 times, generating a majority consensus tree showing the frequency of groups on each side of a branch (Asis et al., 2021).

2.5. Biocontrol assays- effect on plant growth

The pot experiments were conducted with four tomato seedlings of Solan Lalima pot^{-1} (5–6 inches), each filled with approximately 1.2 kg of soil, sterilized by the method of Bhardwaj and Chandel (2024). Every pot was inoculated with 10–20 ml of a *Trichoderma* sp. spore suspension (10^8spores ml^{-1}) shortly after seedling emergence. Pathogen inoculation was applied 7 days before *Trichoderma* (10^6 to 10^8 spores ml^{-1} pot^{-1}). A second *Trichoderma* inoculation was applied 2 weeks later to enhance fungal colonization. A control group was maintained where no antagonist was applied and pathogen inoculation was performed. Pots were placed in an uncontrolled glasshouse at 25°C for 4 weeks, and growth parameters (shoot length, root length, seedling weight, seedling vigor I and II) and disease incidence (based on pathogen symptoms) were recorded. Five replications were used for statistical analysis.

2.6. Methods of data analysis

All experiments were conducted in a completely randomized design with five replications, including a control group without treatment. Data were analyzed using ANOVA (Opstat software), followed by Duncan's test for multiple

comparisons. Similar letters indicated no significant difference, while different letters represented significant differences. Statistical significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Identification of trichoderma isolate

Morphological observations showed that the isolate initially produced a cotton-like, pale white mycelium. Over time, the mycelium darkened to a dull green colour as conidia production commenced. This colour change indicates the maturation of reproductive structures within the fungal culture. The texture of the mycelium was initially cottony, with smooth and thin edges. As the isolate matured, the mycelium became denser and more compact in appearance. This change in texture reflects the growth and development of the fungal colony over time. The isolate exhibited rapid growth, fully covering the surface of the petri plates within 48 to 96 hours at a temperature range of 25–28°C on PDA medium. This fast growth rate indicates the isolate's robust ability to colonize and spread in favourable conditions. The hyphal structure of the isolate was smooth-walled, septate, branched, and hyaline. The conidiophores exhibited irregular branching, typically cylindrical or occasionally pyramidal in shape. The conidia were either spherical (2.5–5 µm in diameter) or ovoid, measuring 3–5 µm in width and 4–7 µm in length. Phialides were cylindrical or ampuliform, producing conidia in a single layer at the tip of the conidiophore. The hyphae were septate, thin-walled, and exhibited rapid growth in culture. However, under laboratory conditions, the isolate did not produce chlamydospores.

India, the seventh-largest country, in South Asia, has climates ranging from icy north to humid south and lies in close proximity to the Himalayas (Singh et al., 2016; Sharma and Dube, 2018; Dihidar, 2024). These mountains are the world's highest mountain range, shape India's climate, soil, and ecosystems (Tiwari et al., 2017; Tiwari et al., 2021). The region supports diverse ecosystems, with a rich variety of plants, animals, and microbial life including unique prokaryotes, fungi, and other microorganisms that aid soil health (Azam et al., 2021; Patel et al., 2024). These microorganisms often bear antagonistic properties, contributing to the suppression of soil-borne pathogens of tomato (Dhakar and Pandey, 2020; Suyal et al., 2021). The area's biodiversity creates a range of ecological niches, from which fifteen samples were selected. In a similar study, 20 *Trichoderma* species from rhizospheric soils in northwestern India (Uttarakhand) were collected and evaluated for *Colletotrichum graminicola* in sorghum (Manzar et al., 2021). Similarly, Kannangara et al. (2017) gathered soil samples from Sri Lanka's North Western Province to investigate the efficacy of *Trichoderma* as biocontrol agents (BCAs) against *Ceratocystis paradoxa* in coconut.

Trichoderma species are known for their distinctive morphological characteristics (Pandian et al., 2016; Paul and Kumar, 2021b). These include rapid growth and the formation of cotton-like colonies initially, sparse or dense, which transition to floccose growth, as conidia develop. The species produce green pigments (Figure 3a), mainly due to terpenoid compounds like trichodermin, giving them their distinctive color (Sharma et al., 2014; Dutta et al., 2022). These pigments are metabolites help protect them from environmental stresses like UV radiation and microbial competition, with enhanced defense mechanisms (Sureshrao et al., 2016; Alfiky and Weisskopf, 2021). The green color observed in the isolate suggests it belong to *Trichoderma*, as this is characteristic of *T.* species, which exhibits similar fast colony growth, dense or sparse mycelium, and creamish pigmentation on the reverse side of Potato Dextrose Agar (Moya et al., 2020; Paul et al., 2021a). Also it exhibits smooth, thin edges, floccose growth and less dense colonies, (Figure 3b), typically seen in some of *Trichoderma* isolates (Kumar et al., 2020; Jaklitsch, 2009). While the green pigmentation and antagonistic behavior are strong indicators, confirming the preliminary identity of the isolate belonging to genus *Trichoderma* (Guardiola-Marquez et al., 2023).

Identification of *Trichoderma* species requires careful examination of mycelium and reproductive structures, as the genus is highly diverse (Jambhulkar et al., 2024). *Trichoderma* typically exhibits septate hyphae, divided by cross-walls (septa) that regulate cytoplasm flow, aiding growth and adaptability. This septation, with absence of clamp connections, helps distinguish *Trichoderma* from similar genera (Intana et al., 2024). These also produce conidiophores, typically upright and branched with swollen tips that bear conidia-asexual spores. Conidia are smooth, hyaline (transparent), and often arranged in chains or clusters, that distinguish *Trichoderma* from other fungi, whereas some have spherical or obvoid condia. Additionally, chlamydospores production, provide ability to compete in specific environments and boost their biocontrol capabilities (Alfiky and Weisskopf, 2021; Correa et al., 2021). Some species has branched conidiophores with monophialidic or polyphialidic phialides that produce conidia in chains. However, in this study, the isolate showed unbranched or less branched conidiophores, fewer phialides, conidia arranged in clusters, with some scattered (Figure 3c), suggesting it is more similar to a different species despite initial resemblance to *T. harzianum*. In hilly areas, fluctuating temperatures may cause fungi to produce simpler, less branched conidiophores as they adapt to temperature

stresses. These observations align with Barrera et al. (2021), Qiao et al. (2018), and Zheng et al. (2021), indicating that the isolates are morphologically and microscopically similar to *T. harzianum*, but genetically distinct.

3.2. Antagonistic activity of trichoderma isolate

The antagonistic activity of the *Trichoderma* isolate was evaluated against several tomato pathogens (Table 1). The results of the experiment showed that the isolate exhibited varying levels of antagonistic activity against different pathogenic fungi. When co-cultured with *Trichoderma*, the mycelial growth of *Pythium ultimum* was inhibited by 51.79%, resulting in a reduced growth of 34.40 mm. *Rhizoctonia solani* showed a 56.22% reduction in mycelial growth, resulting in a growth of only 27.80 mm. *Trichoderma* demonstrated the greatest effectiveness against *Sclerotium rolfsii*, with a mycelial inhibition of 60.63%. This significant reduction in growth led to the pathogen's mycelium extending only up to 21.80 mm, highlighting *Trichoderma*'s strong antagonistic capability. On the other hand, *Fusarium oxysporum* f. sp. *lycopersici* exhibited a mycelial inhibition of 52.72%, with its growth reaching 33.00 mm. This indicates that while *Trichoderma* effectively suppressed the pathogen's growth, the level of inhibition was slightly lower compared to other pathogens, suggesting varying degrees of antagonistic efficiency depending on the fungal species. Overall, these results revealed that the isolate has strong biocontrol potential, particularly against *Sclerotium rolfsii* and *Rhizoctonia solani*.

Previous research demonstrated the pathogen-suppression ability of novel *Trichoderma* isolates from soil of different geographical areas against various pathogens. For instance, *Trichoderma ghanense* inhibited *Pythium aphanidermatum* growth by 44.6% (Elshahawy et al., 2019), while *Trichoderma* strains reduced *Fusarium oxysporum* mycelial growth by 50.59% to 92.94% (Xu et al., 2022). *Trichoderma* isolate PBT13 showed 72.97% inhibition of *Fusarium oxysporum* f.sp. *ciceri*, and PBT3 and PBT4 reduced growth by 72.23% and 59.3%, respectively (Kumari et al., 2024). Similarly, *Trichoderma* isolates CEN281 and CEN287 exhibited strong biocontrol against *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (Montalvao et al., 2023). Novel isolates *T. asperellum* AFP, *T. asperellum* MC1, *T. brevicompactum* MF1, and *T. harzianum* CH1 were effective against *Fusarium oxysporum*, *Rhizoctonia solani*, and *Phytophthora capsici* (Singh et al., 2016), while *T. pseudoharzianum* T17 was effective against *Fusarium oxysporum* (Zhou et al., 2020). The present results are consistent with the findings of these researchers (Table 1).

Considerable genetic and metabolic variation in *Trichoderma* species leads to differences in their levels of antagonistic activity (Pandian et al., 2016). Some isolates produce gliotoxin, xylanases, or cellulase at varying levels to inhibit pathogen growth, while temperature, pH, and nutrient availability, also influence their antifungal activity (Sharma et al., 2014). Moreover, the effectiveness of these isolates depends on the target pathogens' susceptibility to the bioactive compounds, with some pathogens being more resistant than others. *Trichoderma* isolate SUB11780421PII demonstrated strong inhibitory effects on *Rhizoctonia solani* (Figure 3d), *Fusarium oxysporum* f.sp. *lycopersici* (Figure 3e), *Pythium ultimum* (Figure 3f), and *Sclerotium rolfsii* (Figure 3g), suggesting a potent combination of antifungal agents. The biocontrol mechanisms of *Trichoderma* include pathogen colonization, pathogen parasitization, production of secondary metabolites, and volatile organic compounds. Not all *Trichoderma* isolates exhibit the same level of efficacy, emphasizing the importance of selective screening (Mishra et al., 2020).

The table presents the mycelial growth and mycelial inhibition of *Pythium ultimum* (Py), *Rhizoctonia solani* (Rz), *Sclerotium rolfsii* (Sc), and *Fusarium oxysporum* f. sp. *lycopersici* (Fs) when treated with *Trichoderma* sp. isolate SUB11780421PII. Significance of data was analyzed using Duncan's test at the 5% level, with different letters indicating significant differences and the same letters

Table 1: Effect of *Trichoderma* isolate on mycelial growth and inhibition of various pathogenic fungi of tomato

Treatments	Mycelial growth (mm)	Mycelial inhibition (%)
Pythium ultimum (Py)+*Trichoderma* sp. isolate SUB11780421PII	34.40^{c}	51.79^{a}
Rhizoctonia solani (Rz)+*Trichoderma* sp. isolate SUB11780421PII	27.80^{b}	56.22^{b}
Sclerotium rolfsii (Sc)+*Trichoderma* sp. isolate SUB11780421PII	21.80^{a}	60.63^{c}
Fusarium oxysporum f. sp *lycopersici* (Fs)+*Trichoderma* sp. isolate SUB11780421 PII	33.00^{c}	52.72^{a}
CD (p=0.05)	4.81	3.45
SEm±	1.59	1.14
SE(d)	2.25	1.61
C.V.	12.17	4.61

indicating no significant difference. Statistical parameters include Critical Difference (0.05), Standard Error of the Mean (SE(m)), Standard Error of the Difference (SE(d)), and Coefficient of Variation (C.V.).

3.3. Molecular identification and phylogenetic analysis

Molecular identification of *Trichoderma* isolate revealed ≥98% similarity with *Trichoderma* species in the NCBI GenBank database. It showed 99.11% similarity to *Trichoderma* sp. SDAS203586 and various *T. harzianum* strains (QT22092, QT22053, QT22003). This high similarity suggested the isolate could be *T. harzianum*, but other *Trichoderma* species shared similar sequences. The match to isolates like SDAS203584 and yi1427_1 confirmed its belonging to the *Trichoderma* genus, though exact species identification remained uncertain. The phylogenetic tree based on these molecular markers, likely showed the isolate

Table 2: Similarity of *Trichoderma* isolate to related *Trichoderma* species based on ITS and rRNA gene sequences

Organism name	Accession number	%
Trichoderma sp. isolate SDAS203586 internal transcribed spacer 1	MK870952.1	99.11%
Trichoderma sp. isolate SDAS203584 internal transcribed spacer 1	MK870941.1	99.11%
Trichoderma sp. isolate yi1427_1 small subunit ribosomal RNA gene	MH284833.1	99.11%
Trichoderma sp. isolate yi1404_1 internal transcribed spacer 1	MH284826.1	99.11%
Trichoderma sp. isolate yi1438_1 internal transcribed spacer 1	MH284795.1	99.11%
Trichoderma harzianum strain QT22092 18S ribosomal RNA gene	KY225649.1	99.11%
Trichoderma harzianum strain QT22053 18S ribosomal RNA gene	KY225616.1	99.11%
Trichoderma harzianum strain QT22003 18S ribosomal RNA gene	KY225600.1	99.11%
Trichoderma sp. strain T1H2 internal transcribed spacer 1	MT250831.1	98.88%
Trichoderma sp. strain Tricho_19 internal transcribed spacer 1	MK680276.1	98.88%

clustering with known *T. harzianum* strains and other *Trichoderma* species (Figure 2). It also shows a 98.88% match with strains T1H2 and Tricho_19 indicating that the isolate might differ from those, but remained closely related. Based on these results, the isolate was tentatively identified as *Trichoderma* sp. (Table 2). However, further genetic analysis is needed for definitive species identification. Morphologically and culturally, it resembled *T. harzianum*. The present study focused on its biocontrol activity, and the isolate is named *Trichoderma* sp. isolate SUB11780421PII (submitted to GenBank). This isolate showed exceptional pathogen control, which was the primary objective of the study. Genetic markers were not pursued, and the isolate's disease control efficacy was evaluated in pot experiments.

Molecular identification of *Trichoderma* species using DNA-based techniques like Polymerase Chain Reaction (PCR), targeting the Internal Transcribed Spacer (ITS) region, helps differentiate species and assess genetic diversity (Figure 1) (Kredics et al., 2018). These tools have resolved taxonomic ambiguities and enabled phylogenetic tree construction to map the genus's evolutionary relationships among *Trichoderma* species. The ITS region, variable between species but conserved within them, is a reliable marker for distinguishing closely related species that are morphologically similar. Similarly, the 18S rRNA gene is highly conserved part, particularly within closely related species, which accounts for the observed genetic similarity

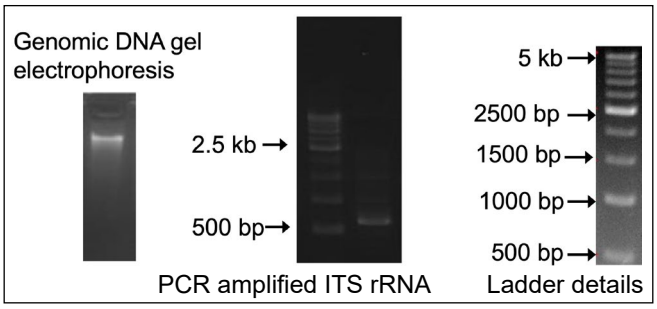

Figure 1: The gel electrophoresis image shows genomic Deoxyribonucleic Acid (DNA) that has been Polymerase Chain Reaction (PCR) amplified, producing two prominent bands at sizes of 2.5 kb (2500 bp) and 500 bp. It also includes a 500 bp DNA ladder, which contains 10 distinct DNA fragments of the following sizes: 500 bp, 1000 bp, 1500 bp, 2000 bp, 2500 bp, 3000 bp, 3500 bp, 4000 bp, 4500 bp, and 5000 bp. The 2.5 kb PCR product aligns with the 2500 bp fragment in the ladder, while the 500 bp band corresponds to the 500 bp marker in the ladder, helping to confirm the sizes of the PCR-amplified products

(Cai and Druzhinina, 2021). The present isolate shows 99.11% similarity with *Trichoderma* sp. isolate SDAS203586 (ITS1) and 99.11% similarity with *Trichoderma* harzianum strain QT22053 (18S ribosomal RNA gene) (Table 2). It is due to the conserved nature of these genetic regions within the *Trichoderma* genus that suggests that the present isolate shares a close genetic relationship with these strains.

However, the slight difference of around 0.89% suggests that it could represent a distinct strain or a slightly divergent species within the *Trichoderma* genus (Kubicek et al., 2019; Hewedy et al., 2020).

Many cryptic species in the *Trichoderma* genus are genetically similar but differ morphologically or adapt to different ecological niches, as seen in the present isolate. Consequently, isolates that show high genetic similarity may still belong to separate species or distinct ecotypes (Jambhulkar et al., 2024). This genetic overlap indicates that the present isolate likely belongs to the same or a very closely related species as *Trichoderma* sp. SDAS203586 and *T. harzianum* QT22053, pointing to similar ecological roles, functional traits, and potential applications in biocontrol or other biotechnological processes (Figure 2). Muthu and Sharma (2011), Kumar et al. (2020), Gajera et al. (2023) also employed ITS and 18S rRNA markers to identify *Trichoderma* isolates and assess their biocontrol potential against pathogens.

Sequence Comparison of *Trichoderma* strains based on Internal Transcribed Spacer (ITS) and Ribosomal RNA gene regions reveals a high similarity to GenBank reference sequences. Accession numbers and corresponding % matches of various *Trichoderma* strains to reference sequences, showing high identity (99.11%) across most isolates, with slight variation (98.88%) are provided.

3.4. Biocontrol assays: effect on pathogen suppression and tomato plant growth in pot culture

The biocontrol efficacy of *Trichoderma* sp. isolate SUB11780421 PII was evaluated in pot culture experiments (Table 3). In the case of *Sclerotium rolfsii*, the disease incidence dropped to 20%. It showed the longest seedling length (12.39 cm), root length (2.88 cm), and seed weight (2.22 g), with significantly high seedling vigor I (1,171.20) and seedling vigor II (220.60). For *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani*, the disease incidences were reduced to 30% and 35%, respectively. The treatment resulted in a seedling length of 11.71 cm for *Fusarium* and 11.42 cm for *Rhizoctonia*, with root lengths of 2.84 cm and 3.03 cm, respectively. Seed weight was 2.03 g for *Fusarium* and 1.72 g for *Rhizoctonia*. SVI was 1,239.40 for *Fusarium*

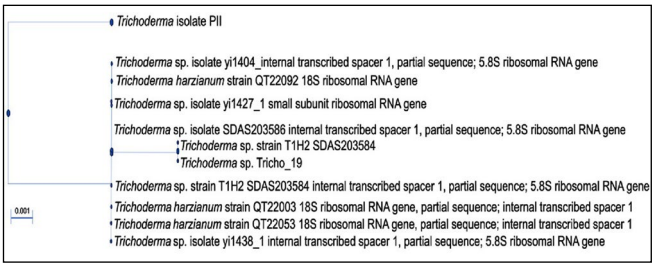

Figure 2: Phylogenetic tree is constructed using Weighbor with a Jukes-Cantor corrected distance matrix based on aligned sequences. Alignment inserts were excluded, and only positions with a minimum length of 200 were considered. The tree was built with an alphabet size of 4 and a sequence length of 1000. Bootstrapping (100 iterations) was applied to assess tree reliability, with a majority consensus tree showing the frequency of groupings. Bootstrap values are shown at the nodes, indicating the support for each branch. The scale used is 0.001. The Jukes-Cantor model corrects for multiple substitutions to prevent distance underestimation

Table 3: Effect of *Trichoderma* sp. isolate SUB11780421PII on plant growth parameters and disease incidence

Treatments	DI (%)	SL (cm)	RL (cm)	SW (g)	SVI	SVII
Pythium ultimum (Py)+*Trichoderma* sp. isolate SUB11780421PII	40.00^{b} (38.98)	10.86^{b}	2.64^{c}	1.56^{a}	1,142.40^{a}	168.60^{b}
Fusarium oxysporum f. sp *lycopersici* (Fs)+*Trichoderma* sp. isolate SUB11780421PII	30.00^{a} (32.98)	11.71^{b}	2.84^{c}	2.03^{b}	1,239.40^{a}	200.20^{c}
Rhizoctonia solani (Rz)+*Trichoderma* sp. isolate SUB11780421PII	35.00^{a} (35.98)	11.42^{b}	3.03^{c}	1.72^{a}	1,071.40^{a}	172.60^{b}
Sclerotium rolfsii (Sc)+*Trichoderma* sp. isolate SUB11780421PII	20.00^{a} (23.99)	12.39^{c}	2.88^{c}	2.22^{b}	1,171.20^{a}	220.60^{c}
Py+Fs+Rz+Sc+*Trichoderma* sp. isolate SUB11780421PII	40.00^{b} (38.98)	10.71^{b}	2.33^{b}	1.66^{a}	1,086.00^{a}	134.80^{a}
Control (No Treatment)	95.00^{c} (83.99)	8.87^{a}	2.13^{a}	1.04^{a}	1,071.40^{a}	64.40^{a}
CD (p=0.05)	13.21	1.55	0.59	0.65	537.95	42.60
SEm±	4.50	0.52	0.20	0.22	167.30	21.32
SE(d)	6.36	0.74	0.28	0.31	56.98	30.15
C.V.	23.68	10.74	17.16	29.09	80.58	29.39

and 1,071.40 for *Rhizoctonia*, while SVII was 200.20 and 172.60, respectively. These results indicate strong growth promotion and pathogen suppression by the isolate for both pathogens. The results demonstrated for *Pythium ultimum* the disease incidence was reduced to 40%, significantly lower than the control group (95%). While seedling length was 10.86 cm and root length was 2.64 cm, both parameters were still lower than other treatments. However, seed weight was relatively high at 1.56 g, and the seed vigor index reached 1,142.40, indicating moderate growth enhancement. The seedling vigor index was 168.60, further suggesting positive effects on plant vigor.

The combination of all four pathogens (Py+Fs+Rz+Sc) with *Trichoderma* sp. isolate SUB11780421PII led to a disease incidence of 40%, with seedling length of 10.71 cm, root length of 2.33 cm, and seed weight of 1.66 g (Table 3). SVI was 1,086.00, and SVII was 134.80, showing that even in the presence of multiple pathogens, the isolate still had a moderate impact on disease suppression and plant growth. The control group (no treatment) exhibited a disease incidence of 95%, with the lowest seedling length (8.87 cm), root length (2.13 cm), and seed weight (1.04 g). Both SVI (1,071.40) and SVII (64.40) were also the lowest, indicating poor growth and pathogen damage. In conclusion, *Trichoderma* sp. isolate SUB11780421PII showed significant potential for biocontrol, effectively suppressing pathogens and promoting tomato plant growth, particularly in treatments with *Fusarium oxysporum* f. sp. l*ycopersici* and *Sclerotium rolfsii*. The isolate's application in pot culture demonstrated its effectiveness as a biocontrol agent for enhancing plant health and reducing the impact of soil-borne pathogens.

The reduced disease incidence from 20 to 40% by application of *Trichoderma* sp. isolate SUB11780421PII due to antifungal metabolites, such as enzymes and secondary metabolites. It also stimulates the plant's immune system through induced systemic resistance (ISR), enhancing its defense responses against infections. *Trichoderma* sp. isolate SUB11780421PII reduces *Sclerotium rolfsii* by up to 20% in pots, which is in agreement to Sumi and Ao (2015 Islam et al. (2017)) who used *T. harzianum* TR05 in sunflower tomato seedlings, leading to a minimum of 5.365.31% disease incidence (Table 3). Also, Azad et al. (2016) Dania and Henry (2021) managed Sclerotium rolfsii (22.2%) with *T.* species application in cowpea. The isolate also reduces wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* by 30% and *Rhizoctonia solani* by 35%, consistent with the findings of Younesi et al. (2021), who reported 66.1% and 69.5% wilt control efficiency with *T. harzianum* KT9 and KT10, respectively, in chickpeas. Similar to the present study, *Trichoderma* Ta41 promoted tomato growth by increasing plant height, root length, and shoot/root weights, with reduced disease incidence by Rhizoctonia solani (Heflish et al., 2021).

However, the shoot and root length of 12.39 cm, 3.03 cm, respectively, indicates that *Trichoderma* sp. isolate SUB11780421PII have promoted plant growth despite the presence of the pathogen. This is due to the growth-promoting properties of *Trichoderma*, like auxins, cytokinins, and gibberellins, which stimulate root growth, increase nutrient uptake, and improve overall seedling health (Figure 3i). Even in the presence of pathogens, *Trichoderma* colonizes the roots, forming a protective biofilm that acts as a barrier against infection, while also enhancing the plant's immune response. This protective and growth-promoting action allows tomato seedlings to survive and thrive despite the presence of soil-borne pathogens (Figure 3h). Whereas, poor performance of the control treatment is likely due to the unprotected root system, allowing unchecked pathogen growth, reduced nutrient uptake, and seedling decline (Figure 3j). The absence of *Trichoderma*'s protective and growth-promoting effects likely led to higher disease incidence and stunted growth (Adedayo and Babalola, 2023; Singh et al., 2023; Santoyo et al., 2024).

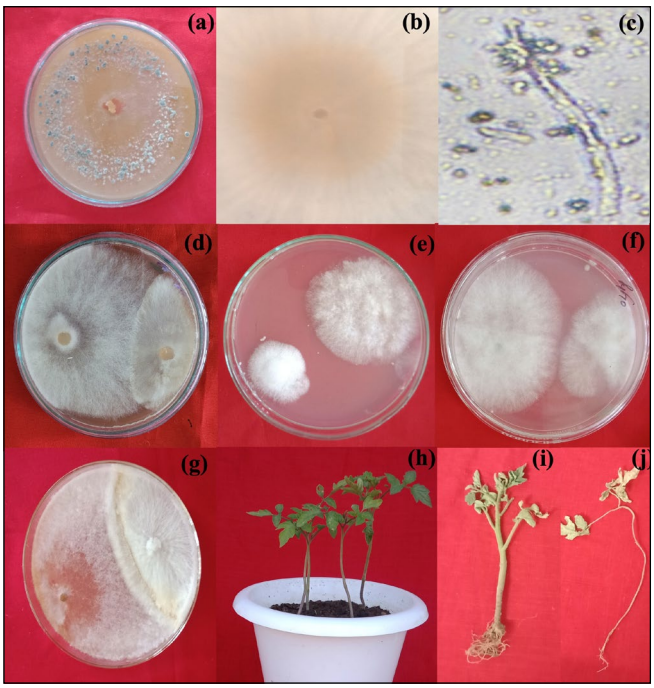

Figure 3: (a) *Trichoderma* sp. isolate SUB11780421PII growth in Petri plate (b) dorsal view (c) less branched conidiophores and conidia in chains (d) *Trichoderma* sp. isolate SUB11780421PII showing antagonistic activity with *Rhizoctonia solani* (e) with *Fusarium oxysporum* f.sp. *lycopersici* (f) with *Pythium ultimum* (g) with *Sclerotium rolfsii* (h) tomato seedlings without disease in pots after application of *Trichoderma* sp. isolate SUB11780421PII (i) seedling exhibits strong root growth and vigorous shoot development (j) diseased seedlings, severely affected by pathogens, exhibit poor root development and stunted shoots

Table represents disease incidence (DI), shoot length (SL), root length (RL), seedling weight (SW), and seedling vigor (SVI, SVII) in tomato seedlings (Solan Lalima) after application of *Trichoderma* sp. isolate SUB11780421PII. These measurements were taken after exposure to individual pathogens (*Pythium ultimum*, *Fusarium oxysporum* f.sp. *lycopersici*, *Rhizoctonia solani*, *Sclerotium rolfsii*), a combined pathogen mixture (Py+Fs+Rz+Sc), and a control group with no treatment. Values represent the mean of each parameter, with DI values are transformed using arcsine square root transformation, presented in parenthesis. Different letters (a, b, c) indicate significant differences at the 0.05 level based on Duncan's test. Values represent treatment effects with corresponding statistical parameters: Critical Difference (at 0.05 significance), Standard Error of the Mean (SE(m)), Standard Error of the Difference (SE(d)), and Coefficient of Variation (C.V.) using OP stat software.

4. CONCLUSION

Trichoderma sp. isolate SUB11780421PII from underexplored areas of the northwestern Himalayan region was proved as a effective biocontrol agent. The isolate demonstrated broad-spectrum antagonistic activity against tomato pathogens in both laboratory and pot culture conditions, reducing disease incidence across all pathogens. Despite up to 40% disease levels, it significantly improved root and shoot growth, seedling weight, and vigor, showcasing its potential for promoting plant growth under moderate disease pressure.

5. ACKNOWLEDGEMENT

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6. REFERENCES

Adedayo, A.A., Babalola, O.O., 2023. Fungi that promote plant growth in the rhizosphere boost crop growth. Journal of Fungi 9(2), 239. https://doi.org/10.3390/jof9020239.

Alfiky, A., Weisskopf, L., 2021. Deciphering *Trichoderma*–plant–pathogen interactions for better development of biocontrol applications. Journal of Fungi 7(1), 61. https://doi.org/10.3390/jof7010061.

Altieri, M.A., Nicholls, C.I., Dinelli, G., Negri, L., 2024. Towards an agroecological approach to crop health: reducing pest incidence through synergies between plant diversity and soil microbial ecology. npj Sustainable Agriculture 2(1), 6. https://doi.org/10.1038/s44264-024-00016-2.

Asis, A., Shahriar, S.A., Naher, L., Saallah, S., Fatihah, H.N.N., Kumar, V., Siddiquee, S., 2021. Identification patterns of *Trichoderma* strains using morphological characteristics, phylogenetic analyses, and lignocellulolytic activities. Molecular Biology Reports 48(4), 3285–3301. https://doi.org/10.1007/s11033-021-06321-0.

Athira, S., Anith, K.N., 2020. Plant growth promotion and suppression of bacterial wilt incidence in tomato by rhizobacteria, bacterial endophytes, and the root endophytic fungus *Piriformospora indica*. Indian Phytopathology 73(4), 629–642. https://doi.org/10.1007/s42360-020-00283-2.

Azad, C.S., Singh, R.P., Kumar, A., Rautela, P., Gupta, S., 2016. Management of pre-emergence and post-emergence damping off disease of chilli through seed treatment. International Journal of Bio-resource and Stress Management 7(4), 748–751. https://doi.org/10.5958/0976-4038.2016.00124.X.

Azam, M.F., Kargel, J.S., Shea, J.M., Nepal, S., Haritashya, U.K., Srivastava, S., Bahuguna, I., 2021. Glaciohydrology of the Himalaya-Karakoram. Science 373(6557), 3668. https://doi.org/ 10.1126/science.abf3668.

Barrera, V.A., Iannone, L., Romero, A.I., Chaverri, P., 2021. Expanding the *Trichoderma harzianum* species complex: Three new species from Argentine natural and cultivated ecosystems. Mycologia 113(6), 1136–1155. https://doi.org/10.1080/00275514.2021.1947641.

Bhardwaj, S., Chandel, S., 2024. Evaluating fungicides compatibility with native strains of *Trichoderma* and *Aspergillus* on tomato crop against damping-off and wilt disease under *in-vitro* and *in vivo* conditions. Indian Phytopathology 77(4), 1077–1089. https://doi.org/10.1007/s42360-024-00810-5.

Bhardwaj, S., Gupta, M., 2023a. Phyto-pathogenic fungi associated with damping-off of tomato in Himachal Pradesh. International Journal of Economic Plants 10(1), 001–005. https://doi.org/10.23910/2/2023.0511.

Bhardwaj, S., Gupta, M., Gupta, S.K., 2023b. Probing efficacy of biological control agents, plant extracts, and resistance-inducing chemicals against damping-off of tomato. Indian Phytopathology 76(3), 813–824. https://doi.org/10.1007/s42360-023-00644-7.

Bhardwaj, S., Gupta, M., Gupta, S.K., Mehta, D.K., 2023c. Soil solarization: an eco-friendly management technique of damping-off of tomato. International Journal of Bio-resource and Stress Management 14(4), 506–511. https://doi.org/10.23910/1.2023.3384.

Cacciola, S.O., Gullino, M.L., 2019. Emerging and re-emerging fungus and oomycete soil-borne plant diseases in Italy. Phytopathologia Mediterranea 58(3),

451–472. https://doi.org/10.14601/Phyto-10756.

Cai, F., Druzhinina, I.S., 2021. In honor of John Bissett: authoritative guidelines on molecular identification of *Trichoderma*. Fungal Diversity 107(1), 1–6. https://doi.org/10.1007/s13225-020-00464-4.

Collins, E.J., Bowyer, C., Tsouza, A., Chopra, M., 2022. Tomatoes: An extensive review of the associated health impacts of tomatoes and factors that can affect their cultivation. Biology 11(2), 239. https://doi.org/10.3390/biology11020239.

Correa, M.A., Pinto, M., Molinatti, M., Valbuena, O., Pavone, D., 2021. Quality control of fungal biocontrol agents with emphasis on *Trichoderma*. In: Biopesticides in Organic Farming, CRC Press, 281–284.

Dhakar, K., Pandey, A., 2020. Microbial ecology from the Himalayan cryosphere perspective. Microorganisms 8(2), 257. https://doi.org/10.3390/microorganisms8020257.

Dihidar, A., 2024. India and her Himalayan Neighbours: analysis of India's bilateral relations with Nepal and Bhutan. In: 75 Years of India's Foreign Policy: Bilateral, Conventional and Emerging Trends. Springer Nature Singapore, 233–260.

Deb, L., Dutta, P., 2021. Antagonistic potential of *Beauveria bassiana* (Balsamo) Vuillemin against *Pythium myriotylum* causing damping off of tomato. Indian Phytopathology 74(3), 728. https://doi.org/10.1007/s42360-021-00372-w.

Dutta, P., Deb, L., Pandey, A.K., 2022. *Trichoderma*—from lab bench to field application: Looking back over 50 years. Frontiers in Agronomy 4, 932839. https://doi.org/10.3389/fagro.2022.932839.

Elshahawy, I.E., El-Mohamedy, R.S., 2019. Biological control of Pythium damping-off and root-rot diseases of tomato using *Trichoderma* isolates employed alone or in combination. Journal of Plant Pathology 101, 597–608. https://doi.org/10.1007/s42161-019-00248-z.

Gajera, H.P., Hirpara, D.G., Savaliya, D.D., Parakhia, M.V., 2023. Biochemical and molecular depictions to develop ech42 gene-specific SCAR markers for recognition of chitinolytic *Trichoderma* inhibiting *Macrophomina phaseolina* (Maubl.) Ashby. Archives of Microbiology 205(6), 242. https://doi.org/10.1007/s00203-023-03582-0.

Guardiola-Marquez, C.E., Santos-Ramirez, M.T., Figueroa-Montes, M.L., Valencia-de Los Cobos, E.O., Stamatis-Felix, I.J., Navarro-Lopez, D.E., Jacobo-Velazquez, D.A., 2023. Identification and characterization of beneficial soil microbial strains for the formulation of biofertilizers based on native plant growth-promoting microorganisms isolated from northern Mexico. Plants 12(18), 3262. https://doi.org/10.3390/plants12183262.

Heflish, A.A., Abdelkhalek, A., Al-Askar, A.A., Behiry, S.I., 2021. Protective and curative effects of *Trichoderma asperelloides* Ta41 on tomato root rot caused by Rhizoctonia solani Rs 33. Agronomy 11(6), 1162. https://doi.org/10.3390/agronomy11061162.

Hewedy, O.A., Abdel Lateif, K.S., Seleiman, M.F., Shami, A., Albarakaty, F.M., El-Meihy, R.M., 2020. Phylogenetic diversity of *Trichoderma* strains and their antagonistic potential against soil-borne pathogens under stress conditions. Biology 9(8), 189. https://doi.org/10.3390/biology9080189.

Intana, W., Promwee, A., Wijara, K., Nguyen, H.H., 2024. Enhancement of damping-off disease control in tomatoes using two strains of *Trichoderma asperellum* combined with a plant immune stimulant. Agronomy 14(8), 1655. https://doi.org/10.3390/agronomy14081655.

Jambhulkar, P.P., Singh, B., Raja, M., Ismaiel, A., Lakshman, D.K., Tomar, M., Sharma, P., 2024. Genetic diversity and antagonistic properties of *Trichoderma* strains from the crop rhizospheres in southern Rajasthan, India. Scientific Reports 14(1), 8610. https://doi.org/10.1038/s41598-024-58302-5.

Kannangara, S., Dharmarathna, R.M.G.C., Jayarathna, D.L., 2017. Isolation, identification and characterization of *Trichoderma* species as a potential biocontrol agent against Ceratocystis paradoxa. Journal of Agricultural Sciences-Sri Lanka 12(1). https://doi.org/10.4038/jas.v12i1.8206.

Khan, M.R., Ahmad, I., Ahamad, F., 2018. Effect of pure culture and culture filtrates of *Trichoderma* species on root-knot nematode, Meloidogyne incognita infesting tomato. Indian Phytopathology 71, 265–274. https://doi.org/10.1007/s42360-018-0031-1.

Kredics, L., Chen, L., Kedves, O., Buschner, R., Hatvani, L., Allaga, H., Vagvolgyi, C., 2018. Molecular tools for monitoring *Trichoderma* in agricultural environments. Frontiers in Microbiology 9, 1599. https://doi.org/10.3389/fmicb.2018.01599.

Kubicek, C.P., Steindorff, A.S., Chenthamara, K., Manganiello, G., Henrissat, B., Zhang, J., Druzhinina, I.S., 2019. Evolution and comparative genomics of the most common *Trichoderma* species. BMC Genomics 20, 1–24. https://doi.org/10.1186/s12864-019-5680-7.

Kumar, G., Singh, A., Pandey, S., Singh, J., Chauhan, S.S., Srivastava, M., 2020. Morphomolecular identification of *Trichoderma* sp. and their mycoparasitic activity against soil borne pathogens. International Journal of Bio-resource and Stress Management 11(6), 613–627. https://doi.org/10.23910/1.2020.2131.

Kumari, R., Kumar, V., Arukha, A.P., Rabbee, M.F., Ameen, F., Koul, B., 2024. Screening of the biocontrol efficacy of potent *Trichoderma* strains against *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii* causing wilt and collar rot in chickpea. Microorganisms 12(7), 1280. https://doi.org/10.3390/microorganisms12071280.

Laevens, G.C., Dolson, W.C., Drapeau, M.M., Telhig, S., Ruffell, S.E., Rose, D.M., Stegelmeier, A.A., 2024. The good, the bad, and the fungus: insights into the relationship between plants, fungi, and oomycetes in hydroponics. Biology 13(12), 1014. https://doi.org/10.3390/biology13121014.

Ma, M., Taylor, P.W., Chen, D., Vaghefi, N., He, J.Z., 2023. Major soilborne pathogens of field processing tomatoes and management strategies. Microorganisms 11(2), 263. https://doi.org/10.3390/microorganisms11020263.

Manzar, N., Kashyap, A.S., Goutam, R.S., Rajawat, M.V.S., Sharma, P.K., Sharma, S.K., Singh, H.V., 2022. *Trichoderma*: advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. Sustainability 14(19), 12786. https://doi.org/10.3390/su141912786.

Manzar, N., Singh, Y., Kashyap, A.S., Sahu, P.K., Rajawat, M.V.S., Bhowmik, A., Saxena, A.K., 2021. Biocontrol potential of native *Trichoderma* spp. against anthracnose of great millet (*Sorghum bicolor* L.) from Tarai and hill regions of India. Biological Control 152, 104474. https://doi.org/10.1016/j.biocontrol.2020.104474.

Mishra, D., Rajput, R.S., Zaidi, N.W., Singh, H.B., 2020. Sheath blight and drought stress management in rice (*Oryza sativa*) through *Trichoderma* spp. Indian Phytopathology 73(1), 71–77. https://doi.org/10.1007/s42360-019-00189-8.

Mistry, H., Bariya, H., 2022. Isolation and identification of *Trichoderma* spp. from different agricultural samples. In: Practical Handbook on Agricultural Microbiology, 131–144. https://doi.org/10.1007/978-1-0716-1724-3_17.

Montalvao, S.C.L., Marques, E., Martins, I., Da Silva, J.P., De Mello, S.C.M., 2023. Suppression of the phytopathogens *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* by *Trichoderma* spp. Biologia 78(10), 2941–2952. https://doi.org/10.1007/s11756-023-01457-9.

Moya, P., Barrera, V., Cipollone, J., Bedoya, C., Kohan, L., Toledo, A., Sisterna, M., 2020. New isolates of *Trichoderma* spp. as biocontrol and plant growth–promoting agents in the pathosystem *Pyrenophora teres*–barley in Argentina. Biological Control 141, 104152. https://doi.org/10.1016/j.biocontrol.2019.104152.

Muthu, K.A., Sharma, P., 2011. Molecular and morphological characters: An appurtenance for antagonism in *Trichoderma* spp. African Journal of Biotechnology 10(22), 4532–4543. https://doi.org/10.5897/AJB10.2089.

Naik, M.K., Prasad, Y., Bhat, K.V., Rani, G.D., 2010. Morphological, physiological, pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. Indian Phytopathology 63(2), 168–173.

Okey, E.N., Okop, I.J., 2024. Control of fungal diseases of tomato (*Lycopersicum esculentum* Mill) fruits using various plant extracts. Dutse Journal of Pure and Applied Sciences 10(3), 202–212. https://doi.org/10.4314/dujopas.v10i3a.19.

Pandian, R.T.P., Raja, M., Kumar, A., Sharma, P., 2016. Morphological and molecular characterization of *Trichoderma asperellum* strain Ta13. Indian Phytopathology 69(3), 297–303.

Panno, S., Davino, S., Caruso, A.G., Bertacca, S., Crnogorac, A., Mandic, A., Matic, S., 2021. A review of the most common and economically important diseases that undermine the cultivation of tomato crop in the mediterranean basin. Agronomy 11(11), 2188. https://doi.org/10.3390/agronomy11112188.

Panth, M., Hassler, S.C., Baysal-Gurel, F., 2020. Methods for management of soilborne diseases in crop production. Agriculture 10(1), 16. https://doi.org/10.3390/agriculture10010016.

Patel, S.K., Sharma, A., Barla, A., Singh, G.S., 2024. Traditional farming in the indian himalayan region: preserving agro-biodiversity and future food security. In: The resilience of traditional knowledge systems for a sustainable future: a focus on agriculture and food practices in the Himalayas. Springer, pp 27–57. https://doi.org/10.1007/978-3-031-56858-92.

Patil, S., Sriram, S., 2020. Biological control of Fusarium wilt in crop plants using non-pathogenic isolates of *Fusarium* species. Indian Phytopathology 73(1), 11–19. https://doi.org/ 10.1007/s42360-020-00202-5.

Paul Khurana, S.M., Kumar, N., 2021. Agricultural sustainability can be ensured by adopting dynamic plant pathology, pedagogy. Indian Phytopathology 74(2), 509–518. https://doi.org/10.1007/s42360-021-00377-5.

Paul, N.C., Park, S., Liu, H., Lee, J.G., Han, G.H., Kim, H., Sang, H., 2021. Fungi associated with postharvest diseases of sweet potato storage roots and in vitro antagonistic assay of *Trichoderma harzianum* against the diseases. Journal of Fungi 7(11), 927. https://doi.org/10.3390/jof7110927.

Qiao, M., Du, X., Zhang, Z., Xu, J., Yu, Z., 2018. Three new species of soil-inhabiting *Trichoderma* from southwest China. MycoKeys 44, 63–80. https://doi.org/10.3897/mycokeys. 44.30295.

Rajashekara, H., Mishra, K.K., Aditya, J.P., Pattanayak, A., Kant, L., 2023. Race distribution pattern of *Magnaporthe oryzae* causing rice blast in mid and high altitudes of North-Western Himalayan region of India. Indian Phytopathology 76(3), 743–751. https://doi.org/10.1007/s42360-023-00665-2.

Santoyo, G., Orozco-Mosqueda, M.D.C., Afridi, M.S., Mitra, D., Valencia-Cantero, E., Macías-Rodríguez, L., 2024. *Trichoderma* and *Bacillus* multifunctional allies for plant growth and health in saline soils: recent advances and future challenges. Frontiers in Microbiology 15, 1423980. https://doi.org/10.3389/fmicb.2024.1423980.

Seethapathy, P., 2025. Potential of *Trichoderma* in Combating Insect Pests. In: The role of entomopathogenic fungi in agriculture, CRC Press, pp 162–182. https://doi.org/10.1201/9781003505228-9.

Sharma, P., 2023. Biocontrol strategies–retrospect and prospects. Indian Phytopathology 76(1), 47–59. https://doi.org/10.1007/s42360-023-00601-4.

Sharma, P., Sharma, M., Raja, M., Shanmugam, V., 2014. Status of *Trichoderma* research in India: A review. Indian Phytopathology 67(1), 1–19.

Sharma, S.K., Dube, L.C., 2018. Climate change and India: impacts on biodiversity. In: Plant Diversity in India. Springer, pp 497–519.

Singh, A.K., Kumar, A., Singh, R., Saini, R., Maanju, S., Leharwan, M., Dixit, P.S., 2023. Revolutionary role of *Trichoderma* in sustainable plant health management: a review. International Journal of Environment and Climate Change 13(11), 4203–4217. https://doi.org/10.9734/IJECC/2023/v13i113600.

Singh, S., Kumar, R., Bhardwaj, A., Sam, L., Shekhar, M., Singh, A., Gupta, A., 2016. Changing climate and glacio-hydrology in Indian Himalayan Region: a review. Wiley Interdisciplinary Reviews: Climate Change 7(3), 393–410. https://doi.org/10.1002/wcc.393.

Singh, A., Srivastava, M., Shahid, M., Pandey, S., Trivedi, S., Kumar, V., Kumar, Y., 2016. *Trichoderma harzianum* (Th Azad): A promising bio-control agent against lentil wilt. International Journal of Bio-resource and Stress Management 7(4), 823–831. https://doi.org/10.23910/IJBSM/2016.7.4.1388c.

Sureshrao, K.S., Pradeeprao, K.T., Dnyanobarao, G.S., Agrawal, T., Kotasthane, A.S., 2016. Efficiency of different *Trichoderma* isolates on plant growth promoting activity in rice (*Oryza sativa* L.). International Journal of Bio-resource and Stress Management 7(3), 489–500. https://doi.org/10.23910/IJBSM/2016.7.3.1544b.

Sumi, K., Ao, N.T., 2015. Integrated management of stem rot of sunflower (*Helianthus annuus* L.) incited by *Sclerotium rolfsii* Sacc. International Journal of Bio-resource and Stress Management 6(6), 673–679, https://doi.org/10.5958/0976-4038.2015.00104.9.

Suyal, D.C., Joshi, D., Kumar, S., Bhatt, P., Narayan, A., Giri, K., 2021. Himalayan microbiomes for agro-environmental sustainability: current perspectives and future challenges. Microbial Ecology 83(3), 1–33. https://doi.org/10.1007/s00248-021-01849-x.

Tiwari, V.P., Verma, R.K., Gadow, V.K., 2017. Climate change effects in the Western Himalayan ecosystems of India: evidence and strategies. Forest Ecosystems 4(1), 1–9. https://doi.org/10.1186/s40663-017-0100-4.

Tiwari, P., Bhattacharya, P., Rawat, G.S., Rai, I.D., Talukdar, G., 2021. Experimental warming increases ecosystem respiration by increasing above-ground respiration in alpine meadows of Western Himalaya. Scientific Reports 11(1), 2640. https://doi.org/10.1038/s41598-021-82065-y.

Tyskiewicz, R., Nowak, A., Ozimek, E., Jaroszuk-Sciseł, J., 2022. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. International Journal of Molecular Sciences 23(4), 2329. https://doi.org/10.3390/ijms23042329.

Wongamthing, R., Sainamole Kurian, P., 2023. The effective antagonistic potential of phylloplane microbes against *Alternaria solani* causing early blight disease in tomato plant. Indian Phytopathology 76(3), 825–834. https://doi.org/10.1007/s42360-023-00645-6.

Xu, H., Yan, L., Zhang, M., Chang, X., Zhu, D., Wei, D., Yang, W., 2022. Changes in the density and composition of rhizosphere pathogenic *Fusarium* and beneficial *Trichoderma* contributing to reduced root rot of intercropped soybean. Pathogens 11(4), 478. https://doi.org/10.3390/pathogens11040478.

Younesi, H., Bazgir, E., Darvishnia, M., Chehri, K., 2021. Selection and control efficiency of *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *ciceris* in Iran. Physiological and Molecular Plant Pathology 116(7-8), 101731. https://doi.org/10.1016/j.pmpp.2021.101731.

Zheng, H., Qiao, M., Lv, Y., Du, X., Zhang, K.Q., Yu, Z., 2021. New species of *Trichoderma* isolated as endophytes and saprobes from Southwest China. Journal of Fungi 7(6), 467. https://doi.org/10.3390/jof7060467.

Zhou, C., Guo, R., Ji, S., Fan, H., Wang, J., Wang, Y., Liu, Z., 2020. Isolation of *Trichoderma* from forestry model base and the antifungal properties of isolate TpsT17 toward Fusarium oxysporum. Microbiological Research 231, 126371. https://doi.org/doi: 10.1016/j.micres.2019.126371.