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RESEARCH PAPER

Evaluation of *Trichoderma* Isolates for their Biocontrol Potential and Plant Growth Promoting Activity

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ABSTRACT

Trichoderma species are well-known for their biocontrol potential and plant growth-promoting abilities. The research conducted used ITS sequencing analysis to pinpoint eight different *Trichoderma* isolates. A phylogenetic tree confirmed their genetic relationships with known species of *Trichoderma*, including *T. yunnanense*, *T. atrobrunneum*, *T. longibrachiatum*, and *T. harzianum*. During Dual culture tests, *T. harzianum* (SPT6) showed the highest level of inhibition (79.91%) against *Sclerotinia sclerotiorum*. The ability to solubilize phosphate, produce IAA, and exhibit siderophore activity differed among the various isolates, with SPT6 demonstrating the greatest amounts of soluble phosphorus and IAA (45.95 µg/ml). The volatile substances released by *Trichoderma* isolates significantly hindered the growth of *S. sclerotiorum* mycelium (38 to 62.43%). In a greenhouse study, tomato seeds treated with *T. harzianum* in treatment T1 achieved the highest germination rates (93.66%) and seedling vigor (2088.77). T1 reduced the damping off of seedlings, resulting in the lowest mortality rate (1.66%) among seeds treated with *Trichoderma*. There were improvements in growth factors such as shoot and root length, as well as fresh and dry weight, due to *Trichoderma* treatment, with SPT6 showing the most significant impact compared to other treatments. These findings highlight the potential of *Trichoderma* isolates as effective biocontrol agents and their ability to enhance plant growth, suggesting they could be useful in sustainable farming practices.

HIGHLIGHTS

- *Trichoderma harzianum* (SPT6) showed the maximum inhibition of *Sclerotinia sclerotiorum* and the highest phosphate solubilization, IAA and siderophore production. Seed treatment with SPT6 improved tomato germination, vigour and significantly reduced damping off, highlighting its strong biocontrol and growth promoting potential.

Keywords: Biocontrol agent, *Sclerotinia sclerotiorum*, phosphate solubilization, IAA, *Trichoderma*

Plant diseases pose an enormous threat to world agriculture, resulting in large crop losses. Chemical pesticides have long been employed to alleviate this problem. However, their indiscriminate use has had a number of negative consequences, including environmental contamination, health hazards due to residual toxicity, pathogen resistance, secondary pathogen outbreaks, and the elimination of beneficial microbes (Mathre *et al.* 1999 and Tyagi *et al.* 2024). Biological control agents (BCAs) are emerging as an environmentally friendly, cost-effective, and reliable tool for plant disease management (Leadbeater *et*

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al. 2016; Palmieri *et al.* 2022 and El-Saadony *et al.* 2022). Among these, antagonistic microorganisms represent a promising non-chemical strategy to disease prevention.

Trichoderma spp. (teleomorph: Hypocrea) is a filamentous fungus categorized as Division Ascomycota, Sub-division Pezizomycotina, Order Hypocreales, Class Sordariomycetes, and Family Hypocreaceae (Chaverri *et al.* 2003 and Druzhinina *et al.* 2011). *Trichoderma* species are fast-growing, free-living, facultatively aerobic, and opportunistic fungus that live in a variety of environments. Their mycelium, which is commonly green, yellow, or white, has a distinct fuzzy or cottony texture (Gams *et al.* 2002). They reproduce asexually by producing conidia at the tips of either branched or unbranched conidiophores. These conidia are usually single-celled, hyaline, and have a smooth or rough surface. *Trichoderma* has biocontrol potential through both direct and indirect mechanisms. It directly causes mycoparasitism, produces cell wall-lytic enzymes, and secretes antibacterial chemicals. Indirectly, it competes for resources and space, modifies environmental conditions, promotes plant development, and triggers host plant defensive responses (Benítez *et al.* 2004; Alfiky *et al.* 2021 and Ferreira *et al.* 2021).

Tomato (*Solanum lycopersicum* L.), a member of the Solanaceae family, is one of the most commonly grown and economically important vegetable crops in the world. About 182.3 million tons of tomato fruits are produced annually on 4.85 million hectares, making the tomato the second most important fruit or vegetable crop after potatoes (FAOSTAT, 2019). It is high in lycopene, an antioxidant, as well as vital vitamins like vitamin C and ascorbic acid. Despite its nutritional and economic value, tomato production is severely hampered by a variety of biotic and abiotic stressors, including *Sclerotinia sclerotiorum*, a necrotrophic ascomycetous pathogen with a host range of over 600 plant species, poses a significant danger to tomato production (Willetts *et al.* 1980 and Liang *et al.* 2015). It causes Sclerotinia stem rot (SSR), also called cottony rot, crown rot, blossom blight, watery soft rot, and white mold (Bolton *et al.* 2006 and Mazumdar *et al.* 2021). SSR was originally detected in tomato in California in 1952 (Purdy and Bardin, 1953). Rodríguez *et al.* (2004) reported that the pathogen infects flowers and causes water-soaked sores at stem joints near the soil surface, fungal infections. As the infection spreads, white cottony mycelium covers the stem and fruit, causing tissue hardness, discolouration, wilting, and eventual plant death (Bolton *et al.* 2006).

The pathogen lives in soil for long periods of time via pigmented, multi-hyphal sclerotia that can germinate myceliogenically or carpogenically (Duncan *et al.* 2006). These resilient structures allow the pathogen to survive for up to ten years in agricultural areas (Harper *et al.* 2002). SSR can reduce tomato yield by more than 50%. Although fungicides are available to manage *Sclerotinia sclerotiorum*, their negative environmental and health consequences need alternate approaches. The use of *Trichoderma* as a biocontrol agent has gained popularity as a sustainable solution. Several investigations have shown that *Trichoderma* is effective against *S. sclerotiorum* (Abdullah *et al.* 2008; Jone *et al.* 2014; Lopes *et al.* 2012; Menendez and Godeas, 1998 and Troian *et al.* 2014). Furthermore, *Trichoderma* has been shown to have dual effects in tomatoes, reducing infections and promoting plant growth (Fontenelle *et al.* 2011 and Zaw *et al.* 2020). *T. harzianum*, for example, has been demonstrated to increase rhizospheric bacteria, hence increasing nutrient availability and uptake in tomato crops (Cai *et al.* 2015).

The purpose of this study is to assess the biocontrol capability and plant growth-promoting activity of several *Trichoderma* isolates against *S. sclerotiorum* in tomatoes, hence offering insight into their efficacy as environmentally friendly alternatives to conventional fungicides.

MATERIALS AND METHODS

Isolation of *Trichoderma* isolates

Trichoderma strains were isolated from rhizospheric soil. About 500 g soil samples were collected and fungal strains were isolated by the serial dilution method using the *Trichoderma* Selective Medium (TSM) (Elad *et al.* 1981). Potential cultures were purified by subculturing on potato dextrose agar (PDA).

Determination of *Trichoderma* species

For each isolate, 100 mg mycelial mat was harvested from potato dextrose broth (PDB). The fungal DNA was extracted according to Raeder and Broda (1985).

The purified DNA is used as the template for PCR to amplify a segment of about 600bp of the ITS gene sequence. A single PCR sample containing 1µl of DNA was amplified in a 20µl reaction mixture containing 10X Buffer, each deoxynucleotide triphosphate at 0.2 mM, each primer at 0.5 µM, and 2 µl of Taq polymerase (R001C TaKaRa Taq™) per ml. PCRs were subjected to Initial denaturation for 3 mins at 95°C, 32 cycles (denaturation, 30 s at 95°C; annealing, 30 s at 50°C; extension, 1 min at 72°C) and 1 final extension cycle at 72°C for 10 min. Twenty microliters of the reaction mixtures were analysed on 1.5% agarose in the presence of 0.5 µg of ethidium bromide per ml and photographed under UV illumination. The Positive amplicons were purified by using Favor Prep™ GEL/PCR Purification Kit (Cat No. FAGCK 001). The Purified Product was sequenced with Sanger's method of DNA sequencing for both directions using universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al.* 1990). All sequences were deposited in GenBank under the accession number listed on Table 1.

Phylogenetic analyses

ITS sequences were analysed using NCBI nucleotide BLAST to find related species sequences. Using MEGA version 11.0, phylogenetic trees were created using maximum likelihood method and Tamura-Nei model. The bootstrap value was calculated using 1,000 bootstrap resampling of the initial alignments of nucleotide sequences.

***In vitro* dual confrontation assays**

All *Trichoderma* isolates were screened for their antagonistic activity against *Sclerotinia sclerotiorum* by using dual culture method (Morton and Stroube, 1955) in the laboratory to screen out most potent isolate. Fresh cultures of *Trichoderma* isolates and pathogen were inoculated on PDA Petri dish 50mm distance from each other in three replications for each isolate. These dishes were incubated at 27±1° and observed regularly. The inhibition percentage over control was calculated according to Dennis and Webster *et al.* (1971).

Phosphorus solubilizing screening

The quantitative assay of phosphate solubilization by *Trichoderma* isolates was carried out by determining the solubility of tricalcium phosphate in liquid medium using the method proposed by King (1932). The *Trichoderma* isolates were inoculated in 25 ml Pikovskaya broth (Pikovskaya, 1948) containing 2.5g insoluble tricalcium phosphate, 0.5g (NH_4) SO_4, 13g glucose, 0.2g NaCl, 0.2g KCl, 0.5g Yeast Extract, 0.1g $MgSO_4.7H_2O$, $MnSO_4$ trace, $FeSO_4.7H_2O$ trace, pH was adjusted to 7.2 and dissolved in 1000 mL distilled water (DW) and incubated at 27±1°C for 72 hours. Then the broth cultures were centrifuged for 10 min at 10000 rpm. 1 ml of supernatant was added to 10 ml of chloromolibidic acid and thereafter 0.25 ml Chlorostannous acid was added and final volume was made up to 50 ml by DW. The developing blue colour's absorbance was measured at 600 nm.

Indole 3-acetic acid production

The quantification of Indole acetic acid (IAA) production ability of *Trichoderma* isolates was carried out by using the method described by Brick *et al.* (1991). *Trichoderma* isolates were inoculated in a medium containing 500µg Tryptophan per ml as precursor. To estimate the quantity of IAA, broth cultures were centrifuged for five minutes at 10,000 rpm. 1ml of supernatant was collected in a test tube, two drop of orthophosphoric acid was added, and then 2ml of Salkowski's reagent (1 ml of 0.5M $FeCl_3$ in 50 ml of 35% perchloric acid) was also added. The test tubes were incubated for 20 minutes and appearance of pink colour indicated the production of IAA, and a UV-bis spectrophotometer was used to detect the absorbance at 530 nm.

Determination of Siderophore production by *Trichoderma* isolates

Siderophore production will be measured by using the universal C.A.S. assay method published by Schwyn and Neilands (1987), which has been modified by Milagres (1999) to determine the ability of *Trichoderma* isolates to produce iron chelating compounds i.e. siderophore in solid medium.

Preparation of the C.A.S. (Chrome Azurol S) Blue Agar

For preparing 1 L of CAS (Chrome Azurol S) blue agar, 60 mg CAS was dissolved in 50 ml of deionized distilled water and mixed with 10 ml

iron (III) solution (1 mM $FeCl_3.6H_2O$ was added in 10ml of 10 mM HCl). This solution was slowly added to conical flask containing 72.9 mg Hexa decyl tri methyl-ammonium bromide (H.D.T.M.A.) dissolved in 40 ml distilled water. The resultant blue dye solution was autoclaved at 121° for 15 minutes. 900 ml water agar was also prepared, autoclaved consisting of 15 g agar and 30.24g Pipes. The final pH was adjusted to 6.8 using 50% (w/v) NaOH solution. The dye solution was added slowly in molten water agar medium along the glass wall before pouring, stir with care to prevent foaming. Then, 30 ml PDA was poured into 90 mm Petri dish and after solidification, the half of the medium was cut and replaced with 15 ml CAS blue agar. Trichoderma were inoculated on PDA halves and incubated at 27±1° for seven days in the dark. The C.A.S. reaction rate was calculated by measuring the advance of the colour-change front in the C.A.S. blue agar, beginning at the boundary between the two mediums. The colour changed from blue to dark purplish red or purple. Results were indicated by positive and negative on the basis of reaction rate of CAS.

Determination of effect of volatile metabolites

The isolates of *Trichoderma* were evaluated for effect of their volatile metabolites by the technique illustrated by (Denis and Webster, 1971) with slight modification. *Trichoderma* and the test pathogen were separately inoculated in two identically sized Petri dishes containing PDA. The bottom of these two dishes were attached mouth-by-mouth and sealed with Parafilm. The control sets just have a PDA in the place of antagonists. The dishes were incubated at 27±1°. The growth was recorded regularly and the inhibition percentage was computed using the formula—

$$\text{Inhibition \%} = \{(\text{mean growth of the control} - \text{mean growth of the pathogen}) / \text{mean growth of the control}\} \times 100$$

Evaluation of germination percentage and seedling vigour in Tomato plants

Surface sterilized tomato seeds were treated with formulation of best performing *Trichoderma* isolates according to the treatments and tested for their germination ability using standard roll towel method (ISTA, 1993). The germination percentage of seeds were recorded. Vigour index was calculated as per Baki and Anderson, (1973), using the formula:

$$\text{Vigour index} = \text{Percent germination} \times \text{seedling length (shoot length + root length).}$$

$$\text{Germination percentage} = (\text{Number of seeds germinated} / \text{Total number of seeds}) \times 100$$

Assessment of disease and plant growth Response of *Trichoderma*

To evaluate the growth promoting and disease suppression potential of the best performing *Trichoderma* isolate a greenhouse experiment was conducted. The following treatment were designed i.e. T1- Tomato seeds were treated with *Trichoderma*, T2- Challenged with pathogen (*Sclerotinia sclerotiorum*), T3-Seeds were treated with *Trichoderma* and challenged with pathogen and T4-Control (without pathogen and *Trichoderma*).

The growth parameters like emergence of seedling, increase plant height, fresh and dry weight were tested. For this, pot trials were carried out under the glasshouse conditions. The pathogen inoculum was combined with 1.5 kg of sterilized soil in pots. The inoculum-containing pots were regularly watered and stirred to encourage the growth of fungi in the soil kept at room temperature for seven days. In accordance with the treatments, treated dry tomatoes seeds were sown in pots of 15 cm diameter. Each pot had one hundred seeds, which were assessed for germination and seedling damping off. At regular intervals, the number of seedlings that emerged was counted. Thirty days after seeding, the seedlings germination and cumulative damping off were noted.

Fresh and dry weight of shoot and root for each replicate were recorded. In order to remove soil from the roots of uprooted plants were washed under running tap water. Following the recording of weights of shoot and root, the plants were subsequently dried in a hot air oven at 60 °C. Plant dry weights were calculated after 72 hours.

Statistical analysis

Tomato plant growth promotion and pathogenicity experiments were carried out using fully randomized block designs with three replicates for dual confrontation assays. One-way analysis of variance (ANOVA) was used to statistically assess the experimental data. Analysis of variance and Tukey's multiple range test at $p < 0.05$ were used to compute and statistically analyse means and standard deviations using IBM SPSS Statistics 26.

RESULTS

Identification of Trichoderma from soil samples

Eight *Trichoderma* isolates were isolated from rhizospheric soil and based on internal transcribed spacer (ITS) sequence analysis they were identified up to the species level. Primers ITS4 and ITS1 were used to amplify the fungal 18s rDNA's ITS region. Table 1 displays the results of analyses done using the Basic Local Alignment Search Tool (BLAST) (NCBI GenBank) along with their accession number.

Table 1: *Trichoderma* isolates and their GenBank accession number

Sl. No.	Isolate Code	Isolate name	Gene bank Accession No.
1	SPT-1	*Trichoderma longibrachiatum*	PV202391
2	SPT-2	*Trichoderma yunnanense*	PV202392
3	SPT-3	*Trichoderma atrobrunneum*	PV202388
4	SPT-4	*Trichoderma yunnanense*	PV202395
5	SPT-5	*Trichoderma yunnanense*	PV202396
6	SPT-6	*Trichoderma harzianum*	PV202398
7	SPT-7	*Trichoderma yunnanense*	PV202400
8	SPT-8	*Trichoderma longibrachiatum*	PV235255

Phylogenetic analysis

To investigate the evolutionary relationships of the isolated *Trichoderma* strains (SPT isolates) with reference species, a Maximum Likelihood (ML) phylogenetic analysis was done using MEGA 11 and 1000 bootstrap replicates. Nineteen sequences, comprising those of eight isolated Trichoderma species, eleven Trichoderma species from GenBank, and an outgroup sequence from *Fusarium solani* (KM52873), were analysed to create a phylogenetic tree. The phylogenetic tree clearly reveals that the SPT isolates are spread across various *Trichoderma* species, confirming their genetic relationship.

With substantial bootstrap support (≥98%), SPT-2, SPT-4, SPT-5, and SPT-7 cluster within the *T. yunnanense* clade, showing a close evolutionary relationship with known strains like *T. yunnanense* Ft125 and *T. yunnanense* KByT1. Similarly, strong bootstrap values support the classification of SPT-1 and SPT-8 as belonging to the *T. longibrachiatum* cluster, while SPT-3 and SPT-6 are associated with *T. atrobrunneum* and *T. harzianum*, respectively (Fig. 1).

The presence of well-supported clades (bootstrap values ≥87%) validates the robustness of the tree, confirming that these isolates belong to distinct *Trichoderma* species. The outgroup, *Fusarium solani*, is appropriately positioned, supporting the overall tree structure. The isolates' taxonomic identity is supported by molecular evidence from the phylogenetic study, which also supports the isolates possible use in biological control and plant growth promotion.

Dual culture assay between *Trichoderma* and *Sclerotinia sclerotiorum*

In the dual culture assay between *Trichoderma* and *Sclerotinia*, *Trichoderma harzianum* exhibited the highest level of inhibition (79.91%), followed by *Trichoderma yunnanense* (SPT-2) and *Trichoderma yunnanense* SPT-4. The lowest inhibition was demonstrated by *Trichoderma longibrachiatum* isolate SPT-1 and SPT-8 (Table 2).

Quantification of soluble phosphorus

The soluble phosphorus was recorded maximum in case of isolate SPT-6 followed by SPT-8, SPT-1 and SPT-3. The Least phosphate solubilizing activity has shown by SPT-7 and SPT-5 (Table 2).

IAA production by Trichoderma isolates

The maximum IAA synthesis was exhibited by *Trichoderma harzianum* SPT-6 (45.95 µg/ml) followed by SPT-8, SPT-1 and SPT-4 whereas the isolate SPT-7 and SPT-5 has shown the minimum IAA production ability respectively (Table 2).

Determination of Siderophore production by Trichoderma isolates

Trichoderma siderophore production ability was

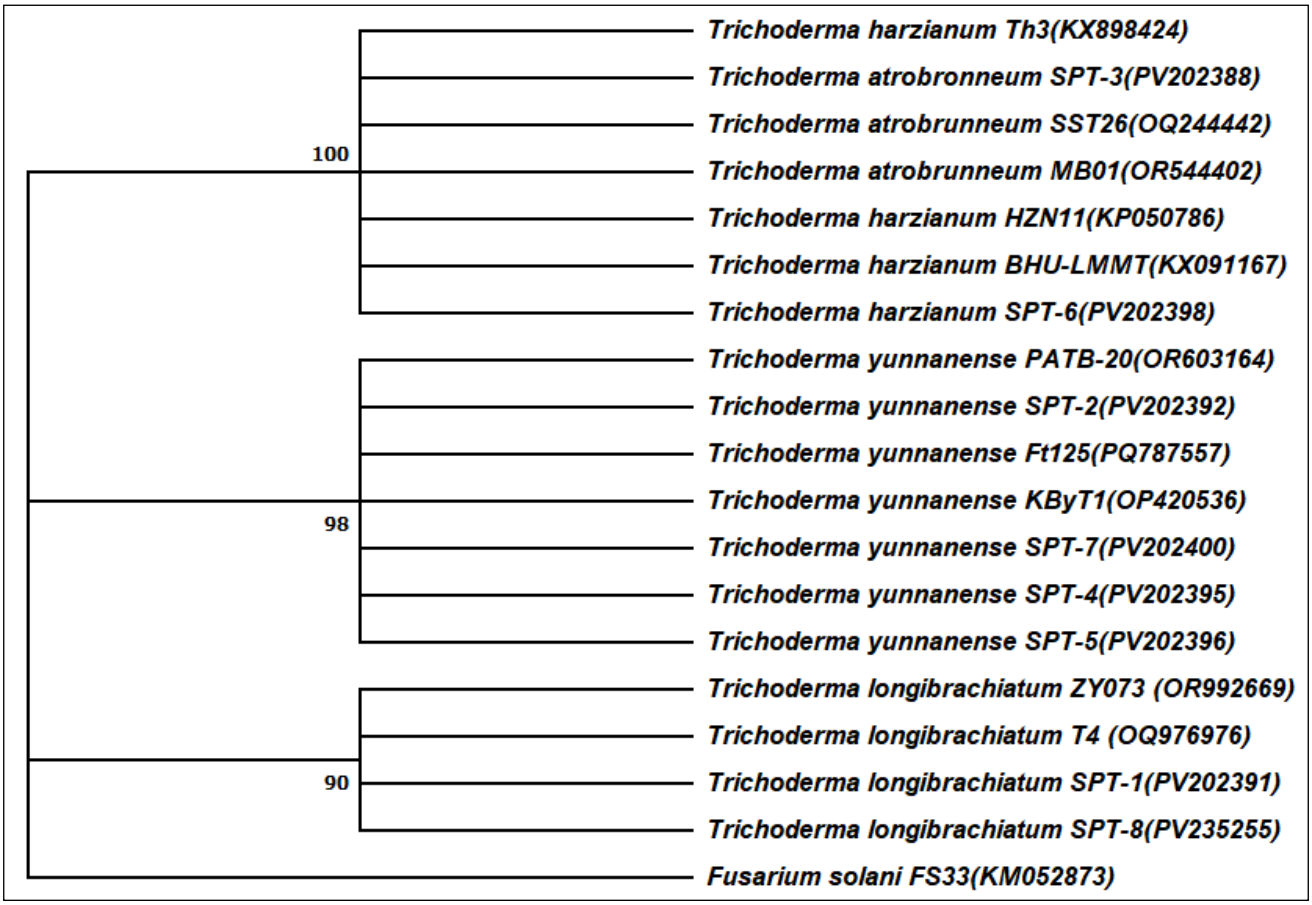

Fig.1: Phylogenetic tree based on the ITS sequences of *Trichoderma* isolates (SPT1-SPT8) prepared using maximum likelihood method in MEGA 11

investigated using CAS blue agar assay. All the Trichoderma isolates showed positive result by changing colour of CAS from blue to purple or purple pink confirming the positive results (Table 2).

Volatile compound

Trichoderma volatile metabolites reduced the mycelial growth of *S. sclerotiorum* by 38 to 62.43%. The SPT-6 (62.43%), SPT-1 (59.13%), and SPT-8 (58.10%) showed the maximum reduction in mycelial growth of *S. sclerotiorum* by *Trichoderma* volatile metabolites (Table 2).

Effects of *Trichoderma* on plant growth and pathogenicity

1. Germination percentage

In vivo investigation of the effect of plant growth-promoting *Trichoderma* on tomato plant growth revealed the effect of seed treatment with *Trichoderma* on seed germination. Tomato seeds treated with *Trichoderma* isolates were monitored for one week for seed germination. Among all the treatments seed priming with SPT-6 i.e. *Trichoderma harzianum* has shown the maximum seed germination (93.66%) followed by control (91.66%). Treatment T3 has exhibited higher germination percentage having both *Trichoderma* and pathogen (90.66%) compared to treatment T2 which was only challenged with pathogen (84.66%) (Table 3).

2. Damping off of seedling / Mortality percentage

Damping off of tomato seedling was also recorded during the greenhouse experiment (Table 3). The observation revealed that least mortality was observed in treatment T1 *Trichoderma* primed seed (1.66%) followed by T4 (4.33%) and T3 (15%) whereas maximum mortality was exhibited by T2 (29.33%).

Table 2: Exhibiting the growth promoting ability and antagonistic effect of different isolates of *Trichoderma* against *Sclerotinia sclerotiorum*

Isolate Code	Isolate name	Phosphate solubilization (µg/ml)	IAA production (µg/ml)	Dual culture % inhibition	Volatile % inhibition	Siderophore production
SPT-1	*Trichoderma longibrachiatum*	291.20 ± 9.509^{ab}	41.73 ± 3.526^{ab}	57.44 ± 5.410^{c}	59.13 ± 3.042^{ab}	+
SPT-2	*Trichoderma yunnanense*	279.19 ± 8.800^{b}	33.10 ± 2.571^{b}	78.65 ± 1.770^{ab}	51.20 ± 1.835^{b}	+
SPT-3	*Trichoderma atrobronneum*	284.08 ± 7.232^{b}	37.12 ± 4.106^{b}	63.20 ± 3.984^{c}	57.00 ± 4.684^{ab}	+
SPT-4	*Trichoderma yunnanense*	269.43 ± 8.069^{bc}	32.78 ± 2.034^{b}	77.70 ± 2.157^{ab}	40.03 ± 5.292^{c}	+
SPT-5	*Trichoderma yunnanense*	265.24 ± 14.498^{bc}	32.32 ± 2.712b	71.10 ± 4.393^{b}	45.21 ± 3.966^{bc}	+
SPT-6	*Trichoderma harzianum*	306.69 ± 12.923^{a}	45.95 ± 4.351^{a}	79.91 ± 3.774^{ab}	62.43 ± 6.119^{a}	+
SPT-7	*Trichoderma yunnanense*	258.49 ± 11.560^{c}	31.52 ± 1.561^{b}	73.67 ± 2.362^{ab}	38.48 ± 5.474^{c}	+
SPT-8	*Trichoderma longibrachiatum*	292.17 ± 7.950^{ab}	43.46 ± 2.302^{a}	56.27 ± 1.879^{c}	58.10 ± 8.000^{ab}	+
	CD at 5 %	20.05	5.89	6.69	9.9	–

The data represented in the table are the mean value of three replicates adjusted to two decimal values; means with the same letter(s) for each parameter did not differ significantly according to complete randomized design.

Table 3: Exhibiting the result of growth parameters of different treatments

Treatments	Germination %	Mortality %	Vigour index	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
T1	93.66 ± 1.366^{a}	1.66 ± 0.516^{c}	2088.77	15.71 ± 0.342^{a}	6.59 ± 0.360^{a}	821.03 ± 13.800^{a}	41.69 ± 0.865^{a}	44.89 ± 1.769^{a}	6.16 ± 0.342^{a}
T2	84.66 ± 2.251^{b}	29.33 ± 3.614^{a}	1506.88	13.28 ± 1.006^{b}	4.52 ± 0.501^{c}	458.23 ± 5.045^{c}	28.71 ± 1.624^{c}	36.60 ± 2.052^{b}	3.94 ± 0.178^{c}
T3	90.66 ± 1.033^{a}	15.00 ± 1.788^{b}	1786.39	14.70 ± 0.854^{ab}	5 .00 ± 0.200^{bc}	526.37 ± 54.967^{b}	33.87 ± 1.604^{b}	40.38 ± 1.797^{a}	4.95 ± 0.348^{b}
T4	91.66 ± 1.366 ^{a}	4.33 ± 1.366^{c}	1807.87	14.45 ± 0.236^{b}	5.27 ± 0.202^{b}	567.37 ± 5.991^{b}	36.19 ± 1.386^{b}	42.76 ± 1.772^{a}	5.26 ± 0.212^{b}
CD at 5%	3.04	4.15	—	1.302	0.639	53.868	2.642	3.486	0.527

T1- Tomato seeds were treated with Trichoderma, T2- Challenged with pathogen (Sclerotinia sclerotiorum) T3- Seeds were treated with Trichoderma and challenged with pathogen, T4- Control (without pathogen and Trichoderma), The data represented in the table are the mean value of three replicates adjusted to two decimal values; means with the same letter(s) for each parameter did not differ significantly according to complete randomized design.

3. Vigour Index

Vigour index result has shown maximum vigour in Treatment T1 (2088.77) followed by Control, T4 (1807.87) whereas minimum vigour index was observed in T2 (1506.88) and T3 (1786.39) respectively. The pathogen challenged seedling along with *Trichoderma* treatment had shown better result than only pathogen challenged treatment.

4. Assessment of plant growth Response of *Trichoderma*

Trichoderma treated seeds, treatment T1 has increased overall growth of the tomato plant compared to control and has shown highest value of all-growth parameters among all the treatments i.e. maximum shoot length (15.71 cm), root length (6.59 cm), shoot fresh weight (821.03 mg), shoot

dry weight (41.69 mg), root fresh weight (44.89 mg) and root dry weight (6.16 mg). The pathogen challenged treatment T2 has shown the minimum increase in growth parameters compared to control and treatment T1 i.e. minimum shoot length (13.28 cm), root length (4.52), shoot fresh weight (458.23 mg), shoot dry weight (28.71mg), root fresh weight (36.60 mg) and root dry weight (3.94 mg). Treatment T3, *Trichoderma* treated plants with pathogen inoculation had exhibited higher value compared to only pathogen challenged, T2 such as higher shoot length (14.70 cm), root length (5 cm), shoot fresh weight (526.37 mg), shoot dry weight (33.87 mg), root fresh weight (40.38 mg) and root dry weight (4.95 mg). Treatment T1 has shown a significant increase in fresh weight of shoot and root and dry weight of shoot and root over control (Table 3).

DISCUSSION

The most effective alternative approach for managing plant diseases is biological control. *Trichoderma* is one of the most important fungal biocontrol agents because of its microparasitic potential against a number of fungal infections, including *B. cinerea*, *Pythium* spp., *Fusarium* spp., *Sclerotium rolfsii*, *S. sclerotiorum*, and *R. solani* (Sarma *et al*. 2015). According to research, *Trichoderma* spp. is among the most widely utilized microbial biological control agents in agriculture and are also extensively investigated fungi (Woo *et al.* 2014). Still, there is a lot of interest in identifying novel isolates of *Trichoderma* with more potent antifungal activity in order to create more effective biocontrol agents (Herath *et al.* 2015 and Alwadai *et al.* 2024).

To create a new strain for widespread application of *Trichoderma* and enhance the commercialization of *Trichoderma* for biological control in the future, we investigated the growth promoting ability and antagonistic effect of eight *Trichoderma* isolates against pathogen. On the basis of their performance, we selected the best performing *T. harzianum* strain SPT-6. One of the main strategies that *T. harzianum* uses to counteract plant-pathogenic fungus has been suggested to be mycoparasitism (Almeida *et al.* 2007). Dual culture tests are commonly used to assess Trichoderma's mycoparasitism of phytopathogenic fungi (Vieira *et al.* 2018; Steindorff *et al.* 2014). We used a dual culture test to explore *T. harzianum* antagonistic activity against *S. sclerotiorum*. We discovered that *T. harzianum* suppressed *S. sclerotiorum* mycelial growth.

Trichoderma species are widely known to boost the growth response of a variety of crops (Mwangi *et al.* 2011 and Cai *et al.* 2015; De Palma *et al.* 2016 and Degani *et al.* 2021). *Trichoderma* isolates have shown IAA, siderophore and volatile compounds production along with phosphate solubilization ability (Nieto-Jacobo *et al.* 2017). *Trichoderma harzianum* shown the maximum potential for all the test attributes viz. IAA production, siderophore, phosphate solubilization and VOCs. Li *et al.* 2015 reported that inoculation of *Trichoderma harzianum* strain SQR-T037 significantly improved the nutrient uptake of P, Fe, Cu, Mn and Zn, and biomass of tomato seedling in nutrient deficient soil. Similar results have been found by Bader *et al.* 2020 in their study with *T. harzianum* which produce indole-3 acetic acid and phosphorus solubilizing, promote growth and control wilt disease on tomato.

In present study we found that seed treatment with *T. harzianum* resulted in the highest seed germination (93.66%) of tomato seeds. Similar results were also reported with *T. harzianum* T969 which showed the highest seed germination rate (95.8%) and increased length, fresh and dry weight of shoot and root of wheat plant (Hajieghrari *et al.* 2016). Many Trichoderma species has been reported to reduce damping off of seedlings as well as disease suppression against *Sclerotinia sclerotiorum* (Sharma *et al.* 2004; Intana *et al.* 2024).

Among all the treatments, T1 i.e. *T. harzianum* treated seeds had shown the least damping off of tomato seedling. Similarly, Sivan *et al.* (1984) found that application of *T. harzianum* in rooting mixture effectively reduced the damping off of tomatoes, peas, cucumbers and peppers and disease was reduced by up to 85% in tomato plants (Sivan *et al.* 1984). This study showed that the microbial agent *Trichoderma harzianum* could clearly promote tomato plant growth, which was directly linked to the microorganisms' successful colonization and survival in the tomato rhizosphere.

CONCLUSION

In present study out of eight isolated Trichoderma were identified as *T. longibrachiatum*, *T. atrobrunneum* and *T. harzianum* and *T. yunnanense*. We found that the *T. harzianum* isolate SPT-6, a beneficial

fungus that increased the growth by phosphate solubilization, production of IAA and siderophore and reduced the disease incidence of *Sclerotinia sclerotiorum*. Because of their strong biological control potential, these strains can be used in production and their field control effects can be evaluated in the future.

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REFERENCES

Abdul-Baki, A.A. and Anderson, J.D. 1973. Vigor determination in soybean seed by multiple criteria 1. *Crop Science*, **13**(6): 630-633.

Abdullah, M.T., Ali, N.Y. and Suleman, P. 2008. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. *Crop Protection*, **27**(10): 1354-1359.

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

Almeida, F.B.D.R., Cerqueira, F.M., Silva, R.D.N., Ulhoa, C.J. and Lima, A.L. 2007. Mycoparasitism studies of *Trichoderma harzianum* strains against *Rhizoctonia solani*: evaluation of coiling and hydrolytic enzyme production. *Biotechnology Letters*, **29**: 1189-1193.

Alwadai, A.S., Al Wahibi, M.S., Alsayed, M.F., Alshaikh, N.A., Perveen, K. and Elsayim, R. 2024. Molecular characterization of plant growth-promoting *Trichoderma* from Saudi Arabia. *Scientific Reports*, **14**(1): 23236.

Bader, A.N., Salerno, G.L., Covacevich, F. and Consolo, V.F. 2020. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). *Journal of King Saud University-Science*, **32**(1): 867-873.

Benítez, T., Rincón, A.M., Limón, M.C. and Codon, A.C. 2004. Biocontrol mechanisms of Trichoderma strains. *International Microbiology*, **7**(4): 249-260.

Bhatt, B. and Sharma, G. 2024. Studying the aggressiveness of *Sclerotinia Sclerotiorum* (Lib.) de Bary isolates from multiple hosts on tomato cultivars. *Indian Phytopathology*, **77**(2): 355-361.

Bolton, M.D., Thomma, B.P.H.J. and Nelson, B.D. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Mol. Plant Pathol.*, **7**: 1–16.

Bonanomi, G., Lorito, M., Vinale, F. and Woo, S.L. 2018. Organic amendments, beneficial microbes, and soil microbiota: toward a unified framework for disease suppression. *Annual Review of Phytopathology*, **56**(1): 1-20.

Brick, J.M., Bostock, R.M. and Silverstone, S.E. 1991. Rapid in-situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Appl. Environ. Microbiol.*, **57**: 535–538.

Burton-Freeman, B. and Reimers, K. 2011. Tomato consumption and health: emerging benefits. *Am. J. Lifestyle Med.*, **5**: 182–191.

Cai, F., Chen, W., Wei, Z., Pang, G., Li, R., Ran, W. and Shen, Q. 2015. Colonization of *Trichoderma harzianum* strain SQR-T037 on tomato roots and its relationship to plant growth, nutrient availability and soil microflora. *Plant and Soil*, **388**: 337-350.

Chaverri, P., Castlebury, L.A., Overton, B.E. and Samuels, G.J. 2003. Hypocrea/Trichoderma: species with conidiophore elongations and green conidia. *Mycologia* **95**: 1100–1140.

De Palma, M., D'Agostino, N., Proietti, S., Bertini, L., Lorito, M., Ruocco, M. ... and Tucci, M. 2016. Suppression subtractive hybridization analysis provides new insights into the tomato (*Solanum lycopersicum* L.) response to the plant probiotic microorganism *Trichoderma longibrachiatum* MK1. *Journal of Plant Physiology*, **190**: 79-94.

Degani, O., Rabinovitz, O., Becher, P., Gordani, A. and Chen, A. 2021. *Trichoderma longibrachiatum* and *Trichoderma asperellum* confer growth promotion and protection against late wilt disease in the field. *Journal of Fungi*, **7**(6): 444.

Dennis, D.C. and Webster, J. 1971 Antagonistic properties of species-groups of *Trichoderma*. III. Hyphal interactions. *Trans Brit. Mycol. Soc.*, **57**: 363–369.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, **57**(1): 25-IN3.

Di Matteo, A., Sacco, A., Anacleria, M., Pezzotti, M., Delledonne, M., Ferrarini, A., Frusciante, L. and Barone, A. 2010. The ascorbic acid con tent of tomato fruits is associated with the expression of genes involved in pectin degradation. *BMC Plant Biol.*, **10**: 163.

Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E. ... and Kubicek, C.P. 2011. *Trichoderma*: the genomics of opportunistic success. *Nature Reviews Microbiology*, **9**(10): 749-759.

Duncan, R.W., Fernando, W.D. and Rashid, K.Y. 2006. Time and burial depth influencing the viability and bacterial colonization of sclerotia of *Sclerotinia sclerotiorum*. *Soil Biol. Biochem.*, **38**: 275–284.

Elad, Y., Chet, I. and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. *Phytoparasitica*, **9**: 59-67.

El-Saadony, M.T., Saad, A.M., Soliman, S.M., Salem, H.M., Ahmed, A.I., Mahmood, M. ... and AbuQamar, S.F. 2022.

Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. *Frontiers in Plant Science*, **13**: 923880.

FAOSTAT. 2019. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 12 September 2021).

Ferreira, F.V. and Musumeci, M.A. 2021. Trichoderma as biological control agent: Scope and prospects to improve efficacy. *World Journal of Microbiology and Biotechnology*, **37**(5): 90.

Fontenelle, A.D.B., Guzzo, S.D., Lucon, C.M.M. and Harakava, R. 2011. Growth promotion and induction of resistance in tomato plant against *Xanthomonas euvesicatoria* and *Alternaria solani* by *Trichoderma* spp. *Crop Prot.*, **30**: 1492–1500.

Gams, W. and Bissett, J. 2002. Morphology and identification of Trichoderma. *Trichoderma and Gliocladium*, **1**: 3-34.

Gomaa, N.A., Mahdy, A.M.M., Fawzy, R.N. and Ahmed, G.A. 2016. Integrated management of tomato white mold disease caused by *Sclerotinia sclerotiorum* using the combined treatments of compost, chemical inducers and fungicides. *Middle East J. Agric.*, **5**: 479–486.

Haddad, P.E., Leite, L.G., Lucon, C.M.M. and Harakava, R. 2017. Selection of *Trichoderma* spp. strains for the control of *Sclerotinia sclerotiorum* in soybean. *Pesquisa Agropecuária Brasileira*, **52**(12): 1140-1148.

Hajieghrari, B. and Mohammadi, M. 2016. Growth-promoting activity of indigenous Trichoderma isolates on wheat seed germination, seedling growth and yield. *Australian Journal of Crop Science*, **10**(9): 1339-1347.

Hampton, J.G. 1993. The ISTA perspective of seed vigor testing. *Journal of Seed Technol.*, **17**(2): 105-109.

Harman, G.E. and Uphoff, N. 2019. Symbiotic root-endophytic soil microbes improve crop productivity and provide environmental benefits. *Scientifica,* (1): 9106395.

Harper, G.E., Frampton, C.M. and Stewart, A. 2002. Factors influencing sur vival of sclerotia of *Sclerotium cepivorum* in New Zealand soils. *N Z J. Crop Hortic. Sci.*, **30**: 29–35.

Herath, H.H.M.A.U., Wijesundera, R.L.C., Chandrasekharan, N.V., Wijesundera, W.S.S. and Kathriarachchi, H.S. 2015. Isolation and characterization of *Trichoderma erinaceum* for antagonistic activity against plant pathogenic fungi. *Current Research in Environmental and Applied Mycology*, **5**(2): 120-127.

Intana, W., Promwee, A., Wijara, K. and 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.

Jone, E.E., Rabeendran, N. and Stewart, A. 2014. Biocontrol of *Sclerotinia sclerotiorum* infection of cabbage by *Coniothyrium minitans* and *Trichoderma spp*. *Biocontrol Sci. Technol*., **24**: 1363e1382.

King, E.J. 1932. The colorimetric determination of phosphorus. *Biochem. J.,* **26**(2): 292.

Leadbeater, A. 2016. Recent Developments and Challenges in Chemical Disease Control—A Review. *Plant Protect. Sci.,* **51**: 163–169.

Li, R.X., Cai, F., Pang, G., Shen, Q.R., Li, R. and Chen, W. 2015. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *PloS One*, **10**(6): e0130081.

Liang, X., Moomaw, E.W. and Rollins, J.A. (2015b) Fungal oxalate decarboxylase activity contributes to *Sclerotinia sclerotiorum* early infection by affecting both compound appressoria development and function. *Mol. Plant Pathol.,* **16**: 825–836.

Liu, B., Ji, S., Zhang, H., Wang, Y. and Liu, Z. 2020. Isolation of Trichoderma in the rhizosphere soil of *Syringa oblata* from Harbin and their biocontrol and growth promotion function. *Microbiological Res.*, **235**: 126445.

Lopes, F.A., Steindorff, A.S., Geraldin, A.M., Brandao, R.S., Monteiro, V.N., Lobo, M.J., Coelho, A.S., Ulhoa, C.J. and Silva, R.N. 2012. Biochemical and metabolic profiles of Trichoderma strains isolated from common bean crops in the Brazilian Cerrado, and potential antagonism against *Sclerotinia sclerotiorum*. *Fungal Biol*., **116**: 815e824.

Mahde, B.Y., Fayyadh, M.A. and Al-Luaibi, S.S. 2019. Evaluation of biofungicide formulation of *Trichoderma longibrachiatum* in controlling of tomato seedling damping-off caused by *Rhizoctonia solani*. *Basrah J. of Agricultural Sci.*, **32**(2): 135-149.

Mazumdar, P. 2021. Sclerotinia stem rot in tomato: A review on biology, pathogenicity, disease management and future research priorities. *J. of Plant Diseases and Protection*, **128**(6): 1403-1431.

Mathre, D.E., Cook, R.J. and Callan, N.W. 1999. From discovery to use: traversing the world of commercializing biocontrol agents for plant disease control. *Plant Disease*, **83**(11): 972-983.

Menendez, A.B. and Godeas, A. 1998. Biological control of *Sclerotinia sclerotiorum* attacking soybean plants. Degradation of the cell walls of this pathogen by *Trichoderma harzianum* (BAFC 742). *Mycopathologic,* **142**: 153e160.

Milagres, A.M., Machuca, A. and Napoleao, D. 1999. Detection of siderophore production from several fungi and bacteria by a modification of chrome azurol S (CAS) agar plate assay. *Journal of Microbiological Methods*, **37**(1): 1-6.

Morton, D.J. and Stroube, W.H. 1955. Antagonistic and stimulating effects of soil microorganisms upon *Sclerotium rolfsii*.

Mwangi, M.W., Monda, E.O., Okoth, S.A. and Jefwa, J.M. 2011. Inoculation of tomato seedlings with *Trichoderma harzianum* and *Arbuscular mycorrhizal* fungi and their effect on growth and control of wilt in tomato seedlings. *Brazilian Journal of Microb.*, **42**: 508-513.

Natsiopoulos, D., Tziolias, A., Lagogiannis, I., Mantzoukas, S. and Eliopoulos, P.A. 2022. Growth-promoting and protective effect of *Trichoderma atrobrunneum* and *T.*

simmonsii on tomato against soil-borne fungal pathogens. *Crops,* **2**(3): 202-217.

Nieto-Jacobo, M.F., Steyaert, J.M., Salazar-Badillo, F.B., Nguyen, D.V., Rostás, M., Braithwaite, M. ... and Mendoza-Mendoza, A. 2017. Environmental growth conditions of *Trichoderma spp*. affect indole acetic acid derivatives, volatile organic compounds, and plant growth promotion. *Frontiers in Plant Sci.*, **8**: 102.

Palmieri, D., Ianiri, G., Del Grosso, C., Barone, G., De Curtis, F., Castoria, R. and Lima, G. 2022. Advances and perspectives in the use of biocontrol agents against fungal plant diseases. *Horticulturae*, **8**(7): 577.

Pikovskaya, R. 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiology,* **17**: 362–370.

Purdy, L.H. and Bardin, R. 1953. Mode of infection of tomato plants by the ascospores of *Sclerotinia sclerotiorum*. *Plant Dis. Rep.*, **37**: 6.

Raeder, U. and Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. *Letters in Appl. Microb.*, **1**(1): 17-20.

Rodríguez, M.A., Venedikian, N., Bazzalo, M.E. and Godeas, A. 2004. Histopathology of *Sclerotinia sclerotiorum* attack on flower parts of *Helianthus annuus* heads in tolerant and susceptible varieties. *Mycopathologia,* **157**: 291–302.

Sarma, B.K., Yadav, S.K., Singh, S. and Singh, H.B. 2015. Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. *Soil Biology and Biochem.*, **87**: 25-33.

Schwyn, B. and Neilands, J. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochem.*, **160**(1): 47-56.

Sharma, P. and Sain, S.K. 2004. Induction of systemic resistance in tomato and cauliflower by *Trichoderma* spp. against stalk rot pathogen, *Sclerotinia sclerotiorum* (Lib) de Bary. *Journal of Biological Control*, pp. 21-28.

Sivan, A., Elad, Y. and Chet, I. 1984. Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. *Phytopathology*, **74**(4): 498-501.

Steindorff, A.S., Ramada, M.H.S., Coelho, A.S.G., Miller, R.N.G., Pappas, G.J., Ulhoa, C.J. and Noronha, E.F. 2014. Identification of mycoparasitism-related genes against the phytopathogen *Sclerotinia sclerotiorum* through transcriptome and expression profile analysis in *Trichoderma harzianum*. *BMC Genomics*, **15**: 1-14.

Troian, R.F., Steindorff, A.S., Ramada, M.H.S., Arruda, W. and Ulhoa, C.J. 2014. Myco parasitism studies of *Trichoderma harianum* against *Sclerotinia sclerotiorum*: evaluation of antagonism and expression of cell wall-degrading enzymes genes. *Biotechnol. Lett.*, **36**: 2095e2101.

Tsahouridou, P.C. and Thanassoulopoulos, C.C. 2002. Proliferation of *Trichoderma koningii* in the tomato rhizosphere and the suppression of damping-off by *Sclerotium rolfsii*. *Soil Biology and Biochem.*, **34**(6): 767-776.

Tyagi, A., Lama Tamang, T., Kashtoh, H., Mir, R.A., Mir, Z.A., Manzoor, S. ... and Ali, S. 2024. A Review on biocontrol agents as sustainable approach for crop disease management: Applications, production, and future perspectives. *Horticulturae*, **10**(8): 805.

Vieira, P.M., Zeilinger, S., Brandão, R.S., Vianna, G.R., Georg, R.C., Gruber, S. ... and Ulhoa, C.J. 2018. Overexpression of an aquaglyceroporin gene in *Trichoderma harzianum* affects stress tolerance, pathogen antagonism and *Phaseolus vulgaris* development. *Biological Control*, **126**: 185-191.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics *In:* Innis MA, Gelfand DH, Sninsky JJ, White (Eds.) PCR protocols: a guide to methods and applications. 18: 315-322.

Willetts, H.J. and Wong, J.A.L. 1980. The biology of *Sclerotinia sclerotiorum, S. trifoliorum*, and *S. minor* with emphasis on specific nomenclature. *The Botanical Rev.*, **46**: 101-165.

Woo, S.L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N. ... and Lorito, M. 2014. Trichoderma-based products and their widespread use in agriculture. *Open Mycol. J.*, **8**(1): 71-126.

Zaw, M. and Matsumotom, M. 2020. Plant growth promotion of *Trichoderma virens*, Tv911 on some vegetables and its antagonistic effect on Fusarium wilt of tomato. *Environ. Control Biol.*, **58**.