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# Antagonistic Activity of *Trichoderma* spp. Against *Pythium aphanidermatum* causing Damping-off in Tomato

***Gokul R.V.[1], Akshaya S.B.[1*], Karpagavalli S.[1] and Melvin Joe M.[2]***
*[1]Department of Plant Pathology, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayanpettai, Chengalpattu Dt. (Tamil Nadu), India.*
*[2]Department of Soil Sciences and Agricultural Chemistry, SRM College of Agricultural Sciences, SRM Institute of Science and Technology, Baburayanpettai, Chengalpattu Dt. (Tamil Nadu), India.*

*(Corresponding author: Akshaya S.B.*)*
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**ABSTRACT: Damping-off caused by *Pythium aphanidermatum* is a serious threat to tomato seedlings and significantly hampers both germination and plant vigour. For effective management of plant disease, *Trichoderma* spp are used as potential biocontrol agents against phytopathogens. The present study aimed to evaluate the antagonistic efficacy of native *Trichoderma* spp. isolated from rhizosphere soils across Tamil Nadu was against *P. aphanidermatum*. A total of 19 *Trichoderma* isolates were morphologically characterized and screened under *in vitro* conditions using quadrium and dual plate assays. Among the isolates tested, the highest mycelial growth inhibition was noticed in isolates T9 (74.81 per cent) and T18 (67.41 per cent), which showed a significant difference between the other treatments tested. The isolate T18 showed hyperparasitic activity against *P. aphanidermatum*, characterized by hyphal coiling and enzymatic degradation. These findings highlight the potential of specific *Trichoderma* strains as eco-friendly biocontrol agents for managing damping-off in tomato nurseries, offering an effective alternative to chemical fungicides.**

**Keywords:** *Trichoderma*, *Pythium aphanidermatum*, damping-off, tomato, biocontrol, dual culture, hyper parasitism.

## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops grown worldwide, valued for its nutritional content and culinary versatility. However, its productivity is severely threatened by soil-borne pathogens, which can significantly impact crop yield and quality (Kumar *et al*., 2022). Among the soil-borne pathogens, one of the most destructive diseases affecting the tomato crop is damping-off by a complex of pathogenic fungi, including such as *Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp., and *Sclerotinia* spp occurs in two forms: pre-emergence (rotting of seeds before germination) and post-emergence (wilting, stem girdling, drooping and collapse). These infections result in patchy stands, reduced seedling vigour, and substantial economic losses in both nursery and field conditions (Thakur *et al*., 2022; Biam, 2019). Conventional approaches to managing damping-off and other soil-borne diseases, such as soil solarisation and application of chemical fungicides, are widely adopted due to their immediate efficacy in reducing pathogen incidence. However, these methods have certain limitations because the soil solarisation will suppress the plant pathogens but disrupts native microbial communities and beneficial soil biota, potentially compromising long-term soil health and ecological balance. Indiscriminate use of fungicides to control plant diseases poses a severe threat to the environment, humans, and plant and animal health (Thakur *et al*., 2022). Alternatively, eco-friendly and sustainable approaches, like biocontrol agents such as *Bacillus* spp., *Pseudomonas* spp., and *Trichoderma* spp., are used to control soil-borne diseases. Among these, *Trichoderma* spp. has emerged as a highly effective antagonist against damping-off pathogens. Their mechanisms of action include competition for nutrients and space, mycoparasitism, secretion of cell-wall degrading enzymes and antifungal metabolites, and induction of systemic resistance in host plants (Marthin Kalay *et al*., 2023; Guzmán-Guzmán *et al*., 2025). Additionally, *Trichoderma* spp. contributes to plant growth promotion through enhanced nutrient uptake and phytohormone production, notably auxins and gibberellins (Rana & Gupta 2021; Joseph, 2025). *Several studies have shown that Trichoderma harzianum*, *T. viride*, and *T. asperellum* effectively suppress *Pythium*-induced damping-off in tomato by actively colonising the rhizosphere and secreting cell wall-degrading enzymes such as chitinases, β-1,3-glucanases, and proteases, which contribute to pathogen inhibition and enhanced seedling protection (El-Katatny *et al*., 2001;

Mukhopadhyay & Kumar 2020). Furthermore, *Trichoderma* spp. is known to produce secondary metabolites such as peptaibols and gliotoxins, which contribute to pathogen inhibition (Sivasithamparam & Ghisalberti 2002). These biocontrol agents also enhance seedling growth by promoting nutrient uptake and producing phytohormones like auxins (Gupta, 2021). Biam (2019) screened 97 *Trichoderma* isolates from rhizosphere and organic sources; isolates TR55, TR66, TR122, and TR136 suppressed *Pythium* and *R. solani* and seed biopriming improved germination and vigour indices in tomatoes. Correspondingly, Rana & Gupta (2021) reported that combining *T. viride* soil treatment seedling with soil drench achieved reduced seed and seedling infection. Intana *et al*. (2024) demonstrated that the combined application of *Trichoderma asperellum* strains with calcium carbonate significantly reduced the incidence of damping-off in tomato seedlings. Therefore, the present investigation aims to test the antagonistic potential and their mechanism of *Trichoderma* spp against damping off in tomato.

## MATERIALS AND METHODS

*A. Collection and isolation of the pathogen*

Infected tomato seedlings (var. shivam) were collected from Dharmapuri district (lat: 12.177653º; long; 78.239665º), one of the major tomatoes growing district of Tamil Nadu. From the collected tomato seedlings, the infected plant parts along with healthy portion are were cut into small bits and surface sterilization with 1% of sodium hypochlorite for 60 seconds followed by three rinses with sterile water and air dried. The disinfected tissue bits were transferred into sterile petri dish containing PDA medium (Potato Dextrose Agar medium). The plates were incubated for 2 to 4 days at 28± 2°C to observe the growth of the pathogen and the hyphal tip of the pathogen were used for pure culture (Thangaraj *et al.*, 2023).

*B. Morphological identification of Pythium sp*

The *Pythium* isolates were identified based on colony morphology and microscopic features. Cultures were grown on PDA at 25 ± 2°C for 2–4 days, and colony characteristics such as colour, texture, and growth rate were recorded. Microscopic examination under 40x magnification was used to observe hyphal structure, sporangium type, oogonia, oospore shape and colour, and antheridia arrangement. The recent studies (Ajrakhia & Hussain 2022; Singh *et al*., 2023), which helped differentiate species such as *P. aphanidermatum*, *P. ultimum*, and *P. debaryanum*. While morphology provides useful preliminary data, molecular tools are recommended for precise identification (Kumar *et al*., 2023; Sharma *et al.*, 2023).

*C. Pathogenicity*

The pathogen *Pythium* sp was multiplied in Sand Maize Medium and incubated at room temperature for 15 days for pathogen multiplication (Koch & Patocka 2017). The pot mixture was prepared by thoroughly mixing red soil, farm yard manure and sand at the ratio of 1:1:1 was sterilized in autoclave at 121 c for 15 psi for 2 hours. The pathogen multiplied in Sand Maize Media mixed with soil (100g/kg) and transferred into earthen pots (Adhikari *et al.*, 2024). The tomato seeds (var. Shivam) were sown in earthen pots and three replications were maintained. Later, the symptoms of pre and post emergence damping-off were observed after seven to fourteen days after sowing and the Percent Disease Incidence (PDI) was determined by using the following formulas given by Wheeler (1969).

PDI (Pre-emergence) = Number of seeds germinated / Total number of seeds sown × 100

PDI (Post-emergence) = Number of seedlings affected / Total number of seeds germinated × 100

*D. Isolation of Trichoderma spp.*

The rhizosphere soil samples were collected from major tomato growing districts across Tamil Nadu such as Dharmapuri, Krishnagiri, Kanyakumari, Salem, Coimbatore, Madurai, Trichy and Dindigul. The samples were isolated in Trichoderma selective medium using $10^{-2}$ to $10^{-4}$ by dilution plate technique. The plates were incubated at room temperature (28±2°C) for 5 days. The morphologically different colonies were selected and purified in the PDA medium. The purified isolates were maintained in 4 c for further study.

*E. Morphological characterization of Trichoderma spp.*

The purified *Trichoderma* isolates were identified based on the morphological characters such as colony colour, appearance, mycelial growth, phialide and conidial characters were observed under 40x compound (Rahman *et al.,* 2011).

*F. Screening of Trichoderma isolates against Pythium sp*

**(i) Preliminary screening.** A total 19 isolates of *Trichoderma* were screened against *Pythium aphanidermatum* by quadrium plate method. A 6mm mycelial disc of *Trichoderma* isolates were placed at the four corners, consistently a 6mm mycelial disc of the pathogen was kept at the center of the Petri dish. The mycelial disc of pathogen alone was maintained as a control and the plates were incubated at room temperature (28±2°C) for 4 days. Based on the mycelial growth inhibition the effective isolates were selected for dual plate assay.

**(ii) Dual plate assay.** A total of 7 effective *Trichoderma* isolates were screened against *P. aphanidermatum* by dual plate assay (Mannai & Boughalleb-M'hamdi 2023). A 9mm mycelial disc of the antagonist was placed at one edge of the petri dish correspondingly the 9mm mycelial disc of pathogen was placed at another edge of the petri dish. The mycelial disc of the pathogen alone maintained as control and each treatment was performed with three replications. The plates were incubated at room temperature (28±2°C) for 5 days. The percent disease reduction over control was analyzed using the formula (Vincent, 1947).

Percent Inhibition (%) = (C − T) / C × 100

Where:

I = Inhibition of mycelial growth over control

C = Mycelial growth in control (mm)

T = Mycelial growth in treatment (mm)

**(iii) Statical analysis.** The statistical analysis of variance (ANOVA) the percentage values of the disease index were transferred into arcsine. All data were undergone through analysis of variance (ANOVA) at the significant levels ($P < 0.05$) and means were compared by the Duncan's Multiple Range Test (DMRT) using Statistical Software Package (SPSS).

## RESULTS AND DISCUSSIONS

**Cultural and morphological characters of *Pythium aphanidermatum.*** The results of current study revealed that the pathogen culture was isolated on PDA medium exhibited dense, white, fluffy aerial mycelial growth. Microscopic observation revealed hyaline, aseptate mycelium measuring 2.4 and 4.1 µm in diameter. Additionally, lobed sporangia, indeterminate paragynous terminal globose oogonia, and sac-shaped antheridia and the production of hyaline globose thick-walled oospores within the oogonia were observed (Fig. 1). Hence the pathogen was identified as *Pythium aphanidermatum.* Similarly, the morphological characters of *P. aphanidermatum* was reported by Ashwathi *et al*. (2017). Notably, variation in colony colour, appearance and the morphological characters was observed in *Pythium* spp by Thangaraj *et al.* (2023). Recent studies by Khan *et al*. (2022); Chakraborty *et al*. (2024) have highlighted the importance of morphological traits such as oospore, features of antheridium and oogonium, and Sporangial characters are essential for distinguishing the *Pythium* spp. they also noted the same colony appearance and spore structures. In addition, more recent studies by Khan *et al.* (2022); Chakraborty *et al.* (2024) confirmed that these are reliable traits for identifying *Pythium* species under the microscope.

**Pathogenicity.** The damping off infected plants showed brown to dark brown lesions on collar region, stem gridling and toppling down of the seedlings was observed on 14$^{th}$ day after sowing (Fig. 2). The percent disease incidence of pre-emergence and post-emergence damping off is 56% and 50% respectively. Likewise, the pathogenicity of *Pythium* spp causing seed rot in solanaceous host under controlled conditions (Adhikari *et al*., 2024). Earlier studies reported that, pathogen exhibit both saprophytic and parasitic lifestyle and the favourable condition occurred for the host, the pathogen can induce pre-emergence and post-emergence damping off in seed and seedlings (Ashwathi *et al*., 2017). However, the infection is mainly initiated through germ tube produced by zoospores or via appressoria that penetrate the host tissues (Muthukumar *et al*., 2010).

**Cultural and Morphological characters of *Trichoderma* spp.** A total of 19 *Trichoderma* isolates were cultured on *Trichoderma* Selective Medium (TSM) and assessed for their colony and microscopic features after 5 to 7 days (Fig. 3). The isolates showed noticeable variation in colony colour, growth pattern, mycelial characteristics, and conidiation were appended in Table 2. The colony colours ranged from white to light green, yellowish green, to dark green, which are typical characteristics of the *Trichoderma* genus. However, KAN 1 (T1) and SLM (T13) isolates exhibited white to light green colonies with concentric rings noticed in the colony margin. While, other isolates such as such as KRI 3 (T7), DIN 2 (T11), and MDU 1(T18) showed light to dark green and yellow to dark green pigmentation. These variations in pigmentation may reflect differences in sporulation intensity, metabolic activity, or secondary metabolite production. The morphological diversity among the isolates aligns with Rahman *et al*. (2011), who state that colony colour and texture in *Trichoderma* species are influenced by genetic variation and external factors like culture medium and incubation conditions. The variation in pigmentation helps with initial identification and indicate physiological traits such as sporulation capacity and metabolite production. These characteristics are closely linked to the ecological adaptability and biocontrol efficiency of *Trichoderma* strains, highlighting the importance of colony morphology in strain selection and characterization.

The colony margins among the *Trichoderma* isolates varied significantly. Isolates like KRI-1 and MDU-1(2) had smooth, well-defined edges, while KAN-1 ST-3, CBE-1(2), and TRI-1(1) showed irregular or wavy margins. These observations align with Mannan and Boughalleb Mhamdi (2023), who noted that *Trichoderma* colonies can have radial, patchy, or uneven margins based on their growth dynamics and sporulation patterns. Notably, the DIN-1(2) isolate showed radial expansion and produced fluorescent pigments, suggesting active secondary metabolite synthesis, as mentioned by Marthin Kalay *et al*. (2023). These metabolites are known for their antagonistic properties against soilborne pathogens like *Pythium* spp. All the *Trichoderma* isolates produced conidia that were globose to subglobose, which is characteristic of the genus. However, the SLM-1(1) isolate displayed a distinct morphology, with conidia ranging from oval to ellipsoidal. This variation in conidial shape may indicate species-level differences or specific ecological adaptations.

***In vitro* screening of *Trichoderma* isolates against *Pythium aphanidermatum.*** The preliminary screening results indicated that out of the nineteen isolates of *Trichoderma* spp. tested against *P. aphanidermatum*, only seven isolates exhibited significant mycelial inhibition toward the pathogen, while the remaining isolates were overgrown by the pathogen. Consequently, the seven most effective isolates were further evaluated for their antagonistic properties against *P. aphanidermatum* by the dual plate technique (Fig. 4). Among them, the highest mycelial growth inhibition was noticed in isolates T9 (74.81 per cent) and T18 (67.41 per cent), which showed a significant difference between the other treatments tested (Table 3). This was further followed by T16 and T10 (62.96 per cent and 59.63 respectively). The least inhibition was recorded by T17 (45.00 per cent). More fascinatingly, isolate T18 demonstrated hyperparasitic activity against *P. aphanidermatum*, wherein the antagonist exhibited directed hyphal growth toward the pathogen, followed by coiling, penetration, and subsequent lysis of the host hyphae (Fig. 5). This interaction was marked by the secretion of hydrolytic

enzymes, including chitinases and β-1,3-glucanases, which facilitated cell wall degradation and structural collapse of the pathogen. This aligns with observations by Jha *et al*. (2022), who reported that *T. harzianum* enveloped and degraded the hyphae of *Pythium*, leading to the shrinkage and collapse of fungal structures. Similarly, Kharte *et al*. (2022) stated that the *T. harzianum* and *T. viride* inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lentis* under *in vitro* assay. In the current study, similar signs of hyphal damage were noted, with twisted, vacuolated, and fragmented mycelia observed in the interaction zone.

**Table 1: Collection of Trichoderma isolates from major tomato growing areas of Tamil Nadu.**

| Sr. No. | Isolates | Village | District | Latitude and Longitude |
|---|---|---|---|---|
| 1. | T1 (KAN 1) | Kallankuzhi | Kanyakumari | 8.303559° N, 77.299790° E |
| 2. | T2 (KAN 2) | Thiruvarambu | Kanyakumari | 8.358563° N, 77.264181° E |
| 3. | T3 (KAN3) | Manalikkarai | Kanyakumari | 8.297841° N, 77.316413° E |
| 4. | T4 (HSR) | Hosur | Krishnagiri | 12.734839° N, 77.812334° E |
| 5. | T5 (KRI 1) | Mathur | Krishnagiri | 12.382259° N, 78.416642° E |
| 6. | T6 (KRI 2) | Pochampalli | Krishnagiri | 13.0938995º N ,80.292356º E |
| 7. | T7 (KRI 3) | Puliyur | Krishnagiri | 12.284249º N, 78.322033º E |
| 8. | T8 (KRI 4) | Samalpatti | Krishnagiri | 12.308464º N, 78.488200º E |
| 9. | T9 (KRI 5) | Uttangarai | Krishnagiri | 12.267422º N, 78.537720º E |
| 10. | T10 (DIN 1) | Batlagundu | Dindigul | 10.162166º N, 77.758435º E |
| 11. | T11 (DIN 2) | Oddanchatram | Dindigul | 10.485993º N, 77.755267º E |
| 12. | T12 (DIN 3) | Odaipatty | Dindigul | 10.565336º N, 77.765009º E |
| 13. | T13 (SLM) | Ammapet | Salem | 11.654705º N, 78.190735º E |
| 14. | T14 (DHM) | Lakkiyampatty | Dharmapuri | 12.109356º N, 78.154322º E |
| 15. | T15 (CBE) | Nagammapudur | Coimbatore | 11.231365º N, 77.117320º E |
| 16. | T16 (TRY 1) | Thalakudi | Trichy | 10.874743º N, 78.716625º E |
| 17. | T17 (TRY 2) | Thuraiyur | Trichy | 11.148497º N, 78.588246º E |
| 18. | T18 (MDU 1) | Karuppayurani | Madurai | 9.933662º N, 78.177498º E |
| 19. | T19 (MDU 2) | Karumathur | Madurai | 9.915092º N, 77.921935º E |

**Table 2: Cultural and Morphological Characters of *Trichoderma* isolates.**

| Sr. No. | Isolates | Colony Colour | Colony Edge | Mycelial Colour | Conidiation |
|---|---|---|---|---|---|
| 1. | **T1 (KAN 1)** | White to light green | Irregular | White | Irregular spreading like pustules |
| 2. | **T2 (KAN 2)** | Yellow to light green | Irregular | White | Mass pustules at the point of inoculation |
| 3. | **T3 (KAN 3)** | White to dark green | Wavy | White | Irregular spreading along the colony margin |
| 4. | **T4 (HSR)** | White to dark green | Irregular | Watery white | Spreading greenish pustules along the colony margin |
| 5. | **T5 (KRI 1)** | Yellow to light green | Smooth | Watery white | Uniformly throughout the plate without forming pustules |
| 6. | **T6 (KRI 2)** | White to dark green | Smooth | White | Spreading along the colony margin |
| 7. | **T7 (KRI 3)** | Light green to dark green | Irregular | White | Formation of green conidia only around the point of inoculum |
| 8. | **T8 (KRI 4)** | White to dark green | Radial | White | Spreading along the edges of the colony |
| 9. | **T9 (KRI 5)** | White to dark green | Smooth | White | Formation of greenish conidial crust with dense conidiation |
| 10. | **T10 (DIN 1)** | Yellowish green to dark green | Patchy and irregular | White | Produced conidia irregularly throughout the plate, forming pustules |
| 11. | **T11 (DIN 2)** | White to dark green | Irregular | White | Yellowish to dark green dense conidiation along the colony margin |
| 12. | **T12 (DIN 3)** | Yellowish white | smooth | White | Tends to grow in a radial shape and produce a pale-yellow pigment of fluorescent pigment |
| 13. | **T13 (SLM)** | White to light green | Radial | White | Formation of concentric rings along the colony margin |
| 14. | **T14 (DHM)** | Yellowish green to dark green | Irregular | White | Showed effuse to dense conidiation over the centre of colonies |
| 15. | **T15 (CBE)** | White to light green | Wavy | White | Appeared to be granular or powdery near the edges of the plate |
| 16. | **T16 (TRY 1)** | White to dark green | Irregular | White | Exhibited slightly cottony colonies along the colony margin |
| 17. | **T17 (TRY 2)** | Yellow to dark green | Patchy | White | Formation of concentric rings with yellow to green conidial production |
| 18. | **T18(MDU 1)** | Light green to yellowish green | Smooth | White | Formation of greenish yellow conidial crust with dense conidiation |
| 19. | **T19 (MDU 2)** | Yellowish green to dark green | Smooth | Watery white | Produced conidia uniformly throughout the plate without forming pustules |

**Table 3: *In vitro* screening effective *Trichoderma* isolates against *P. aphanidermatum.***

| Treatment | Average radial mycelial growth (mm) | PDI (%) |
|---|---|---|
| T1 (KAN 1) | 34.33$^{c}$ (35.86) | 55.67 |
| T9 (KRI 5) | 22.67$^{a}$ (28.43) | 74.81 |
| T10 (DIN 1) | 36.33$^{bc}$ (37.06) | 59.63 |
| T13 (SLM 1) | 42.67$^{c}$ (40.78) | 52.58 |
| T16 (TRI 1) | 33.33$^{b}$ (35.26) | 62.96 |
| T18 (MDU1) | 29.33$^{ab}$ (32.79) | 67.41 |
| T17 (TRI 2) | 45.00$^{d}$ (42.13) | 45.00 |
| Control | 90.00$^{e}$ (71.56) | 0.00 |
| **SED** | **0.51** | - |
| **CD (0.05)** | **1.07** | - |

Culture of *Pythium aphanidermatum*


Hyaline Aseptate Hyphae


Formation of Vesicles


Oogonium with Oospore

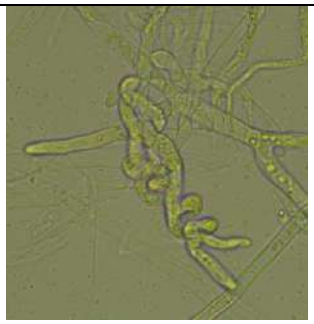
Lobed Sporangia

**Fig. 1.** Cultural and Morphological Characters of *Pythium aphanidermatum.*

**Fig. 2.** Pathogenicity test of *P. aphanidermatum.*

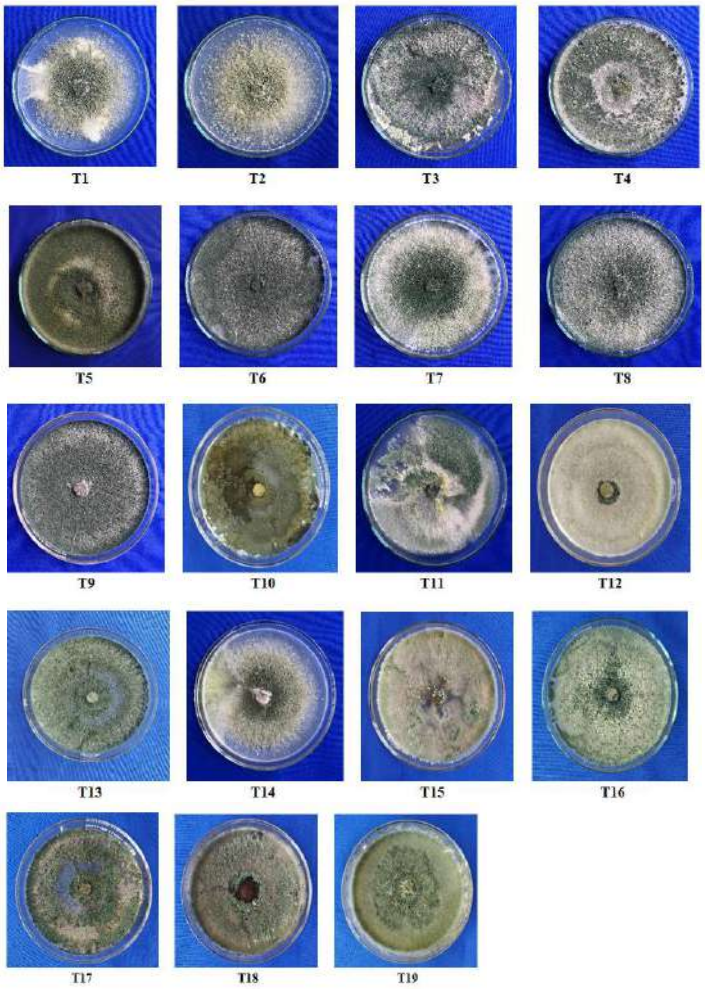


**Fig. 3.** Cultural Characters of *Trichoderma* isolates.

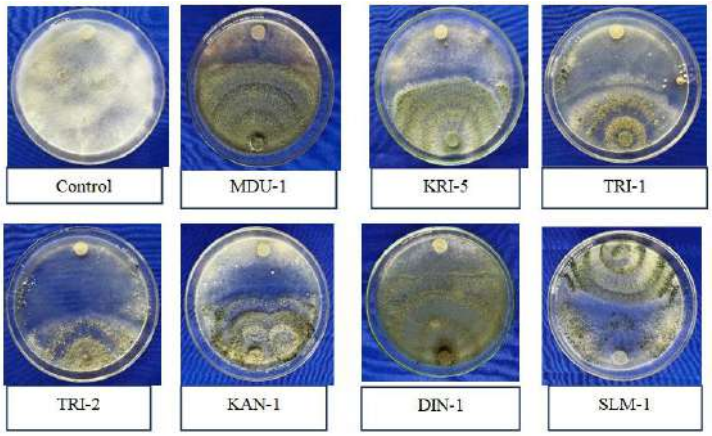


**Fig. 4.** Dual plate assay of *Trichoderma* isolates against *Pythium aphanidermatum.*

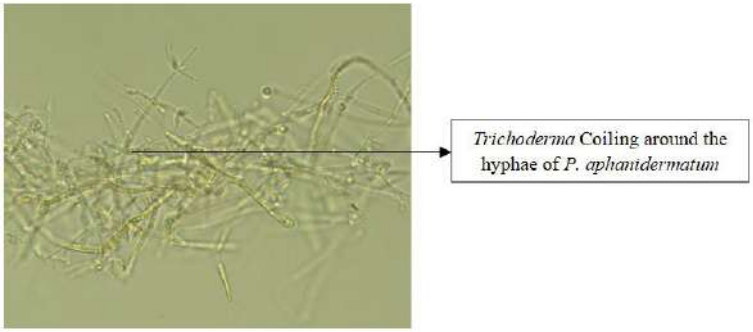


**Fig. 5.** Mycoparasitism of *Trichoderma* sp - T18 (MDU 1) against *P. aphanidermatum.*

## CONCLUSIONS

The present study clearly showed that *Pythium aphanidermatum* is a major cause of damping-off disease in tomato seedlings, leading to severe losses during early growth stages. The pathogen was identified based on its typical cultural and microscopic features. Among the 19 native *Trichoderma* isolates tested, a few showed strong antagonistic activity against the pathogen, especially isolates T9 and T18, which significantly suppressed its growth in dual culture assays. Isolate T18 also showed hyperparasite behaviour, indicating its potential as an effective biocontrol agent. These findings suggest that certain native Trichoderma strains could be developed into eco-friendly alternatives to chemical fungicides for managing damping-off in tomato.

## FUTURE SCOPE

*Trichoderma* offers great potential for controlling *Pythium aphanidermatum* because of its strong biocontrol abilities and eco-friendly traits. Recent improvements in formulation methods, like nano-encapsulation and using multiple strains together, should improve its effectiveness in the field. Additionally, molecular tools and omics approaches will help create genetically improved strains with better resistance to pests and environmental stress. Using *Trichoderma* in sustainable plant protection strategies could significantly reduce the need for chemical fungicides.

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**Conflicts of Interest.** None.

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