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Potential of *Trichoderma asperellum* against root-rot caused by *Fusarium equiseti* in tomato plants

Potencial de *Trichoderma asperellum* contra a podridão radicular causada por *Fusarium equiseti* em plantas de tomate

Potencial de *Trichoderma asperellum* contra la pudrición de la raíz causada por *Fusarium equiseti* en plantas de tomate

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Abstract
The aim of this study was to evaluate the effects of *Trichoderma asperellum*, Rhizolex-T (a chemical fungicide), and their combinations with Fusarium equiseti on fruit yield and disease inhibition in plants. *Trichoderma asperellum* and *Fusarium equiseti* were isolated from the soil surrounding robust tomato roots in various parts of Brazil, and molecularly identified by 5.8S-ITS region sequencing. The biocontrol agent *T. asperellum* exhibited strong antagonistic activity, surpassing the efficacy of the chemical fungicide Rhizolex-T. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analysis confirmed that most *T. asperellum* exhibited potent antagonistic activity in terms of mechanistic insights. Fresh fruit weight increased by 14.70%, dry fruit weight increased by 14.81%, fruit size increased by 3.75%, and the number of fruits per plant increased by 12.50% as a consequence of the application of *T. asperellum* (T). Additionally, antioxidant activity and total phenol contents increased in response to *T. asperellum* treatment. These results highlight the potential of *T. asperellum* as a sustainable, eco-friendly alternative to chemical fungicides for managing Fusarium wilt in tomatoes. The study advocates for the integration of biocontrol agents into disease management strategies to reduce chemical inputs and promote sustainable agriculture.
Keywords: *Solanum lycopersicum*; *Fusarium equiseti*; Biological control; Fungal infections; Plant-growth promoting.

Resumo

O objetivo deste estudo foi avaliar os efeitos de *Trichoderma asperellum*, Rhizolex-T (um fungicida químico) e suas combinações com *Fusarium equiseti* na produção de frutos e na inibição de doenças em plantas. *Trichoderma asperellum* e *Fusarium equiseti* foram isolados do solo ao redor de raízes robustas de tomate em diversas regiões do país, e identificados molecularmente por sequenciamento da região 5.8S-ITS. O agente de biocontrole *T. asperellum* exibiu forte atividade antagônica, superando a eficácia do fungicida químico Rhizolex-T. Análises por microscopia eletrônica de transmissão (MET) e microscopia eletrônica de varredura (MEV) confirmaram que a maioria dos isolados de *T. asperellum* apresentou potente atividade antagônica, fornecendo informações sobre os mecanismos envolvidos. O peso dos frutos frescos aumentou 14.70%, o peso dos frutos secos aumentou 14.81%, o tamanho dos frutos aumentou 3.75% e o número de frutos por planta aumentou 12.50% como consequência da aplicação de *T. asperellum* (T). Além disso, a atividade antioxidante e o teor de fenóis totais aumentaram em resposta ao tratamento com *T. asperellum*. Esses resultados destacam o potencial de *T. asperellum* como uma alternativa sustentável e ecologicamente correta aos fungicidas químicos para o manejo de *Fusarium* em tomates.

Palavras-chave: *Solanum lycopersicum; Fusarium equiseti*; Controle biológico; Infecções fúngicas; Promotor de crescimento.

Resumen

El objetivo de este estudio fue evaluar los efectos de *Trichoderma asperellum*, del fungicida químico Rhizolex-T y de sus combinaciones con *Fusarium equiseti* sobre el rendimiento de los frutos y la inhibición de enfermedades en plantas de tomate. *Trichoderma asperellum* y *Fusarium equiseti* fueron aislados del suelo alrededor de raíces vigorosas de tomate en distintas regiones del país y se identificaron molecularmente mediante la secuenciación de la región 5.8S-ITS. El agente de biocontrol *T. asperellum* mostró una marcada actividad antagónica, superando la eficacia del fungicida químico Rhizolex-T. Los análisis mediante microscopía electrónica de transmisión (MET) y microscopía electrónica de barrido (MEB) confirmaron que la mayoría de los aislamientos de *T. asperellum* presentaron una potente actividad antagónica, aportando información relevante sobre los mecanismos involucrados. La aplicación de *T. asperellum* (T) incrementó el peso de los frutos frescos en un 14.70 %, el de los frutos secos en un 14.81 %, el tamaño de los frutos en un 3.75 % y el número de frutos por planta en un 12.50 %. Además, la actividad antioxidante y el contenido total de fenoles aumentaron en respuesta al tratamiento con *T. asperellum*. Estos resultados destacan el potencial de *T. asperellum* como una alternativa sostenible y ambientalmente segura a los fungicidas químicos para el control de *Fusarium* en cultivos de tomate.

Palabras clave: *Solanum lycopersicum*; *Fusarium equiseti*; Control biológico; Infecciones fúngicas; Promotor del crecimiento.

1. Introduction

Plants are vulnerable to a number of biotic stresses generated by various organisms, resulting in a variety of diseases, infections, and crop plant damage, ultimately affecting agricultural output (Awad-Allah et al., 2021; Gowtham et al., 2024). The tomato (*Solanum lycopersicum* Mill.) is one of the world's most significant crops, particularly in Brazil (Chanthini et al., 2018; Olaoluwa et al., 2024; Joseph et al. 2025a). It accounts for approximately 32% of all growing land and 16% of all vegetable production (Elshahawy et al., 2018). According to tomato production, Brazil is America's biggest producer, followed by Bolivia, while Peru is the least prolific (Dube et al., 2020; Ajenifujah-Solebo et al., 2025). However, its yield has been significantly reduced due to a variety of circumstances, including biotic limitations. When sufficient conditions exist, attacks by numerous viral, arthropod pests, bacterial, and fungal diseases are poorly managed, contagious, and can rapidly spread from plant to plant in a tomato crop (Ochilo et al., 2019; Petrov et al., 2024).

Soilborne fungal pathogens such as *Rhizoctonia solani, Fusarium solani, Sclerotium rolfii* and *Phytophora* sp., frequently cause tomatoes root rot, which slows plant development, decreases harvest yield and quality, and eventually kills infected plants (Ajilogba et al., 2013; Hamza et al., 2016; Kashyap et al., 2020; Akber & Fang, 2024). Root rot is a serious threat to agriculture, continually decreasing output and jeopardizing crop life. This illness may infect entire fields, depending on the causal agent, host vulnerability, and environmental variables (Bodah, 2017; Joseph et al., 2022; Mogazy et al., 2024). In addition, Liu et al. (2024) studied the potentiality of *Trichoderma asperellum* CMT10 biocontrol against root rot disease in strawberry. According to Nirmaladevi et al. (2016), root rot caused by *Fusarium* spp. is the most damaging pathogen in tomatoes. Tomato yield can be reduced by as much as 25% in wealthy nations and by more than 50% in impoverished

countries due to root rot, according to (Nicastro & Carillo, 2021). Given the disease's multiple pathogenic causes and inadequate management tools, this presents a significant issue.

The employment of biological control agents, among other strategies, has proven remarkably effective in combating plant diseases (Ons et al., 2020; Sheoran et al., 2025). Many affluent countries have already begun via fungal organisms as biological control agents is an important, ecologically safe, commercially viable, and long-term technique for plant disease management (Figlan et al., 2020; Olowe et al., 2020; Manoharmayum et al., 2025). Microorganisms belonging to the genus *Trichoderma* are frequently used in cutting-edge biotechnology (Sharma & Sharma, 2020) They have demonstrated success in managing plants exposed to biotic and abiotic challenges such as biopesticides, biofertilizers, and bio stimulants. They are crucial in preventing economically damaging plant diseases like tomato root rot (Olowe et al., 2020) and *Trichoderma asperellum* has antifungal and plant-growth stimulating properties against *Fusarium* wilt on tomatoes (Selva Amala et al., 2024; Joseph et al., 2025b). Outstanding capacity for competition in the rhizosphere, severe aggression towards phytopathogenic fungi, and useful suppressive processes make them valuable bioagents (Filizola et al., 2019; Thangaraj et al., 2025; Joseph et al., 2025c). They can thrive in harsh environments, use all soil nutrients efficiently, increase plant development, and have a high resistance to agrochemicals applied to the soil (Köhl et al., 2019; Elshahawy et al., 2024). The aim of this study was to evaluate the effects of *Trichoderma asperellum*, Rhizolex-T (a chemical fungicide), and their combinations with *Fusarium equiseti* on fruit yield and disease inhibition in plants. Specifically, the study sought to quantify the percent inhibition and yield increment associated with each treatment, in order to assess the potential of biological and chemical control strategies in mitigating the pathogenic impact of *Fusarium* and enhancing tomato productivity.

2. Methodology

2.1 Isolation and purification of fungal isolation from soil

Several *Fusarium* isolates were isolated from the soil surrounding robust tomato roots in various parts of Brazil, using the technique described in (Davet &Rouxel, 2000). An experimental study was conducted, partly in the field and partly in the laboratory, of a qualitative and quantitative nature (Pereira et al., 2018) using descriptive statistics with data classes and mean and standard deviation values and using statistical criteria (Bekman & Costa Neto, 2009).

2.2 DNA extraction and amplification

Fungal isolates were cultured on potato dextrose agar, and DNA was extracted from freeze-dried mycelia using a modified protocol (Panabieres et al., 1989). The process involved cell lysis, buffer treatments, and organic solvent extraction, yielding high-purity DNA stored at −80°C.

2.3 ITS Region Amplification

The study involved amplifying the internal transcribed spacer (ITS) regions of rDNA using two primers, ITS-1 and ITS4 (Table 1), based on conserved areas of the eukaryotic rRNA gene according to White et al. (1990). PCR conditions included cycles with specific denaturation, annealing, and elongation steps. Amplified products were separated via agarose gel electrophoresis and sequenced using an ABI 3730XL DNA Analyzer. Sequences were edited and aligned using Finch software. The phylogenetic investigation of the isolates' 18S rRNA gene sequences was conducted using NCBI run BLAST algorithm at the National Centre for Biotechnology Information (NCBI) using the neighbor-joining method (Saitou & Nei, 1987). The data was entered into the NCBI Gene Bank database to generate accession numbers. The phylogenetic trees were generated using the Mega 11 program (Tamura et al., 2021).

Table 1- Details of primer sequences used in PCR experiments. Tm: temperature of melting and GC content %: Guanine (G) to cytosine.

Prime name	Sequence	Tm (°C)	GC content (%)
ITS1	TCC GTA GGT GAA CCT GCG G	53	58
ITS4	TCC TCC GCT TAT TGA TAT GC	50	45

Source: Research data (2025).

2.4 *Trichoderma asperellum* antagonistic behavior in dual cultivation

Trichoderma asperellum antagonistic ability against *Fusarium equiseti* was assessed in vitro using a dual culture method. Before being used as an inoculum, *T. asperellum* and *F. equiseti* were both grown for six days on PDA medium. *T. asperellum* discs of 5 mm in diameter from each isolate were infected in a Petri dish with PDA media on one side and a *F. equiseti* inoculum on the other. Three replicates of each treatment were used, with infected plates that contained only *F. equiseti* acting as the pathogen. The measured pathogen developed linearly after five days of incubation at 28°C (Sallam et al., 2009; Nunes et al., 2023).

2.5 Ultra-structural studies: Microscopic characterization

2.5.1 Scanning Electron Microscopy (SEM)

Specimens were prepared using a modified protocol of Morris (1965), buffered overnight, washed multiple times, post-fixed in osmium tetroxide, dehydrated in ethanol, contrasted with 70% acetone and 0.5% uranyl acetate, and immersed in various concentrations of ethanol. After drying, the specimens were coated with gold-palladium membranes and studied using a microscope (JSM-6510 L.V, JEOL Ltd., Tokyo, Japan) at 30 KV at Federal University of Tocantins Electron Microscopy Unit in Brazil.

2.5.2 Transmission Electron Microscopy (TEM)

Fixing specimens were performed in primary fixative 4F1G (1% glutaraldehyde and 4% formaldehyde in 0.1 M phosphate buffer PB, PH 7.4) for 2 hours, washing them three times in 0.1 M PB, and post-fixing them in 1% osmium tetroxide. The samples were dehydrated using ethanol concentrations and acetone as a substitute. The specimens were embedded in pure Embed 812 for 24 hours, trimmed, and cut into ultrathin pieces using ultramicrotome (RMC-power tome XL / USA). High-quality portions were cut and flattened before being picked up on grids. Sections were stained twice with uranyl acetate and lead citrate, and then cleaned and dried before analysis (Reynolds, 1963). The sections were examined using an electron microscope (JEOL JEM 2100, Tokyo, Japan) at Federal University of Tocantins.

2.6 Pot experimente

Tomato (*Solanum lycopersicum* L.) (Hybrid K 186 cultivar) seeds, were gained from the Agriculture Research Centre (ARC) in Tocantins, Brazil. A pot experiment was conducted in the greenhouse of the Federal University of Tocantins to evaluate the potential of the tested biological control (*T. asperellum*) as well as the chemical control (fungicide Rizolex-T 50% (thiram 30% + Tolclofos-methyl 20%), to control root rot of tomato caused by *F. equiseti*. The soil used was autoclaved prior to inoculation to eliminate native microbial populations and ensure experimental consistency. Clay pots with a 20 cm diameter of each were infected with an inoculum of *F. equiseti* at a weight-to-volume ratio of 1:1:1 (except the negative control) and included sterilized clay, sand, and peat moss. For a week, the containers were kept moist to encourage the spread of the

causative agent and its colonization of the soil. Tomato seeds were put in the diseased ground. As a seed dressing, 3 g of the fungicide Rizolex-T 50% (thiram 30% + Tolclofos-methyl 20%) was used. For application of *T. asperellum*, tomato seedlings were 28 days old when they were transplanted into pots, seven days after the pathogen infestation in the soil. The pathogen (*F. equiseti*) was inoculated into the soil at a concentration of 1×10^6 CFU/g soil. The antagonist was applied at 1% w/w, with a CFU load of approximately 1×10^7 CFU/g soil before the tomato plants were put into the pots. These values were determined using serial dilution and plate count methods. For each treatment, ten replicas (ten pots) were employed.

Six sets of pots were arranged in a complete randomized plot design as follows: Control (negative control; without fungi) (C), *F. equiseti* treatment (Positive control; pathogen) (P), *T. asperellum* treatment (T), Rhizolex-T (Chemical control; fungicide) (F), *T. asperellum* + *F. equiseti* treatment (T+P) and Rhizolex-T + *F. equiseti* treatment (F+P). Tomato plantlets were cultivated on 23 January 2024. The pots were kept in regular day/night circumstances, with temperatures ranging from 25–30°C and relative humidity of 60–70%. They irrigated with equal amounts of water until the experiment ended. On the 15th of May, the sampling of treated and untreated plants took place, which represented the fruiting stage.

2.7 Measurements of growth parameters, inhibition and yield recovery in tomato under *Fusarium* infection

Growth parameters include shoot fresh and dry weight/plant, root fresh and dry weight/plant, shoot and root length/plant, number of leaves/plant and number of branches/plants were measured. In addition to yield attributes (fresh and dry weight of fruit/plant, number of tomato fruits/plant and size of fruit/plant) were also determined. Some inhibition and yield recovery assessments were measured including firstly percent inhibition which measures how much a parameter (e.g., growth and yield) is reduced due to *Fusarium* infection as the following formula:

$$\% \text{ Inhibition} = (\text{Control} - \text{Treated} / \text{Control}) \times 100$$

Where control: measurement from healthy (non-infected) tomato plants and treated: measurement from treated tomato plants. Secondly, tomato yield increment which is used when evaluating treatments or resistance that improves yield despite infection according to the following formula:

$$\text{Yield Increment (\%)} = (\text{Treated Yield} - \text{Control Yield} / \text{Control Yield}) \times 100$$

Where treated yield: yield from plants treated to resist Fusarium or recover from infection and control yield: yield from untreated, uninfected plants.

2.7.1 Total antioxidant activity via DPPH assay

The antioxidant ability of the evaluated substances was examined using the DPPH (2,2- diphenyl-1-picrylhydrazyl) colorimetric technique with ascorbic acid as a reference according to the assay published by (Kitts et al., 2000). Samples were serially diluted with methanol, DPPH solution was added, and samples were left in the dark for 30 minutes at room temperature. Absorbance was measured at 517 nm, and DPPH remaining was calculated using an equation:

$$\% \text{ DPPH remaining} = [\text{DPPH}]T / [\text{DPPH}]T = 0 \times 100$$

The study used an exponential curve to plot the percentage of DPPH remaining against the sample concentration, revealing an inverse association with the sample's antioxidant capability (Parejo et al., 2000).

2.8 Total Phenols

Total phenols in tomato fruit tissue were determined using Ainsworth and Gillespie 2007 protocol. The tissue (0.2 g) was homogenized with 5 mL of 95% methanol, stored, centrifuged at 13000 g for 5 minutes, and mixed with 4 mL of 700 mM sodium carbonate and 1mL of 10% Folin-Ciocalteu reagent. Methanol was utilized as a blank. The absorbance was measured at 765 nm, and gallic acid solutions were used to create a standard curve for total phenols calculation.

2.9 Data analysis

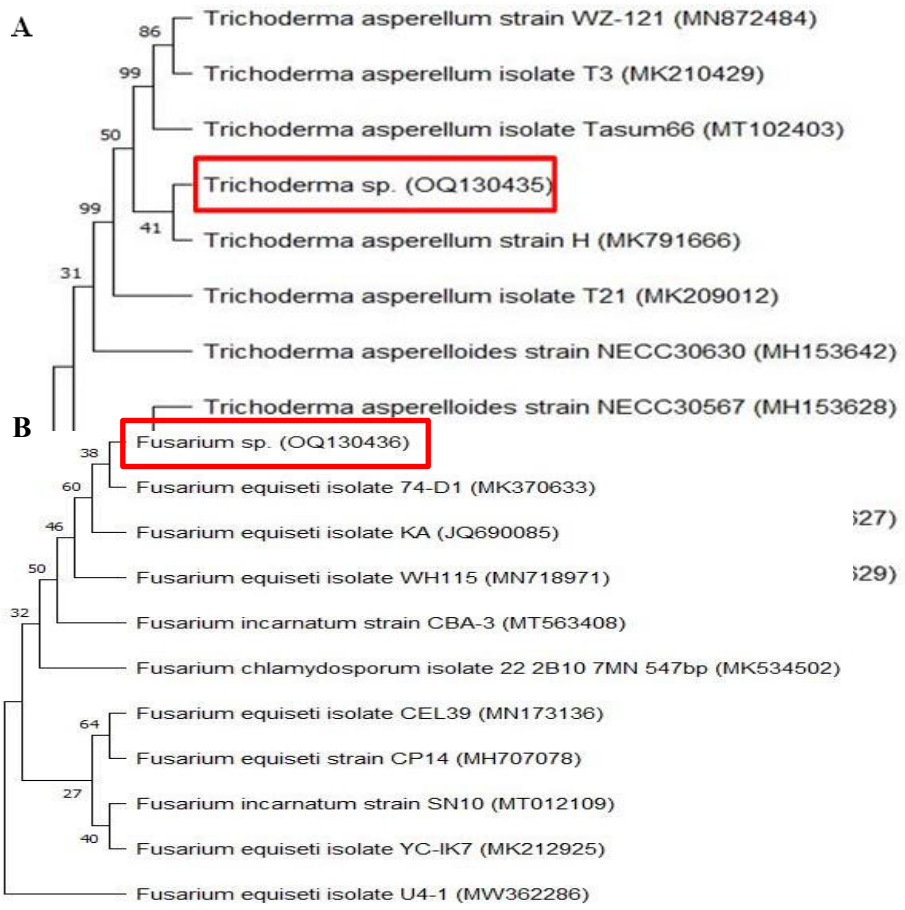
The normality of the dataset was evaluated using the Shapiro-Wilk test. In addition, a Levene's test was also performed to verify the homogeneity of variance. Once the normal distribution had been checked, a one-way analysis of variance (ANOVA) was performed. At a 5% significance level, treatments of the same clone were compared using the Duncan's Multiple Range Test using R version 4.3.3 (R Core Team 2024, Vienna, Austria). Data were visualized using GrapPad Prism version 8.1 software (San Diego, California, USA) (Cardinali & Nason, 2013).

3. Results

3.1 Molecular identification of the fungal strains

Genomic DNA from *Trichoderma* and *Fusarium* isolates was successfully isolated and sequenced using universal primers ITS 1 and ITS 4, and BLAST analysis identified the isolates and submitted to NCBI GenBank as (accession numbers OQ130435- OQ130436), respectively (Figure 1A–B). A phylogenetic tree was created using MEGA X software to identify the genetic relationship between *Trichoderma* sp. and other related species. The tree showed a similar percentage of 100.00% with *Trichoderma asperellum*, indicating H shared one clade (Figure 1A). As a result, strain *Trichoderma* sp. was identified as *Trichoderma asperellum*. Furthermore, the *Fusarium* sp. strain was separated from other related species, with a similarity percentage of 99.82% to *Fusarium equiseti*, and the phylogenetic tree revealed that strain Fusarium sp. isolate 74-D1 and *Fusarium equiseti* shared a clade. Thus, the strain Fusarium sp. was identified as *Fusarium equiseti* (Figure 1B).

Figure 1- Phylogenetic trees analysis based on 18S rDNA sequence alignment. (**A**) *Trichoderma asperellum* and (**B**) *Fusarium equiseti* with some other related species, which possessed the highest similarity. The neighbour-joining of internal transcribed spacer ITS regions was performed using at bootstrap values of 1000 replicates. Abbreviations in brackets indicate accession numbers and the red square represents the studied isolate.

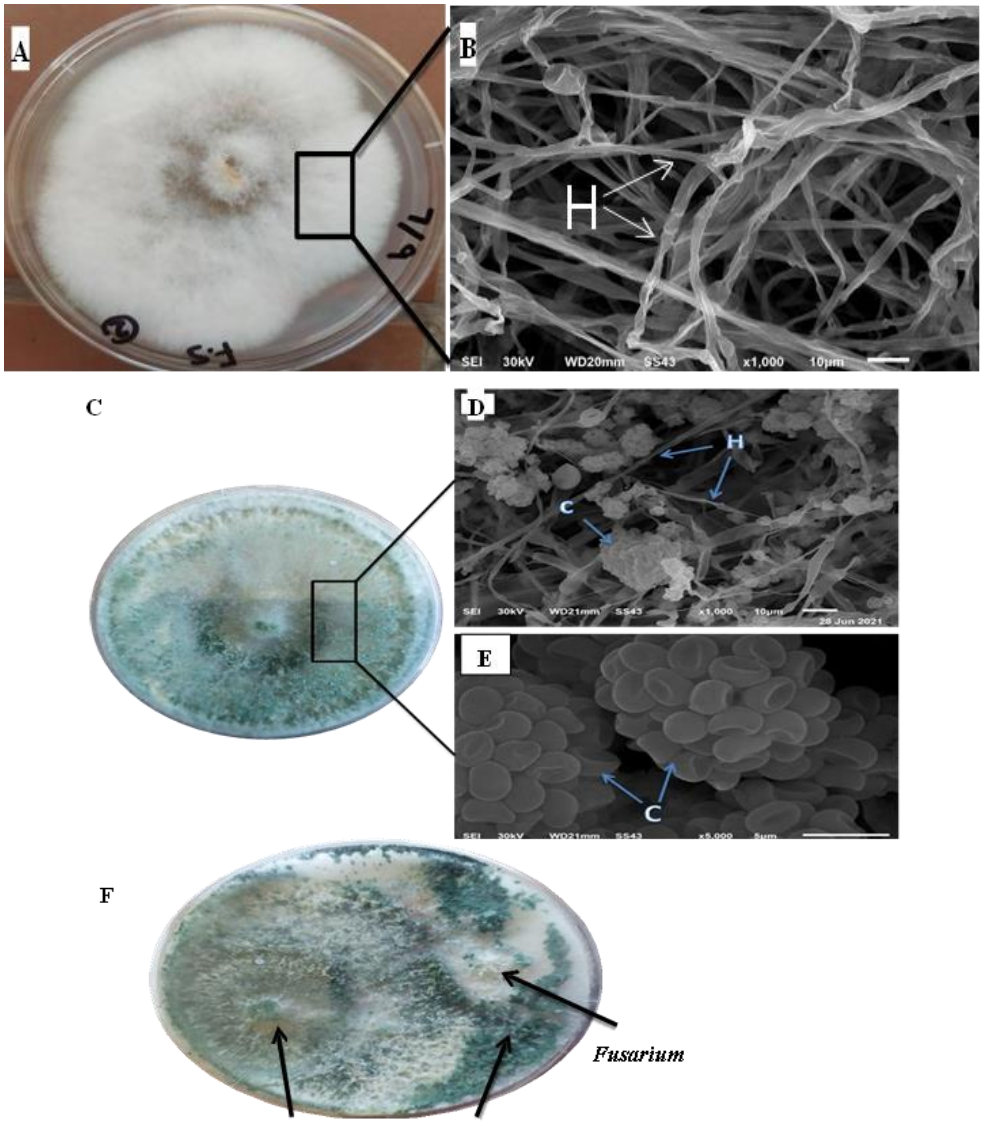


Source: Research data (2025).

3.2 Morphological characterization of *T. asperellum* and *F. equiseti*

In vitro antagonism assay of the mycoparasitic nature of *T. asperellum* and *F. equiseti* was observed in the Figure 2. Figure (2A-B) shows the spread of *F. equiseti* throughout the entire Petri dish with no obvious hyphae damage. *T. asperellum* colonies are uniformly dispersed, not pustulate or in conflict, with a sharply demarcated and dense central disc where most conidia form. They are green after sporulation and have a radius of 42-60 mm after three days of incubation. Hyphae hairy to floccose white, sharply demarcated with a more or less dense central disc within which most conidia form; mycelia formed homogenous mat (Figure 2C–D). On the PDA medium, the potency of local *T. asperellum* isolate was assessed to block *F. equiseti* mycelial development in dual culture, and the inhibitory effect was remarkably begun on the third day of the confronting test. *T. asperellum* completely invaded the mycelia of *F. equiseti* and sporulated on the latter after 7 days of incubation (Figure 2F).

Figure 2- Morphology the mycoparasistic nature of *F. equiseti* (pathogen). (**A**) Colony characteristics of *F. equiseti* on a petri dish; (**B**) SEM micrograph of *F. equiseti* hyphae (H) Bars = 10 µm; (**C**) Colony characteristics of *T. asperellum* on a petri dish; (**D**) SEM micrograph of the mycoparasitic nature of *T. asperellum* showing hyphae (H) and conidiophores; (**E**) Clusters of *T. asperellum* conidia (C). Bars = 10 µm (D) and 5 µm (E). (**F**) The antagonistic action of *T. asperellum* (left and right, dark colour) on the mycelium of *F. equiseti* (right, light colour) showing overgrowth and heavy sporulation.



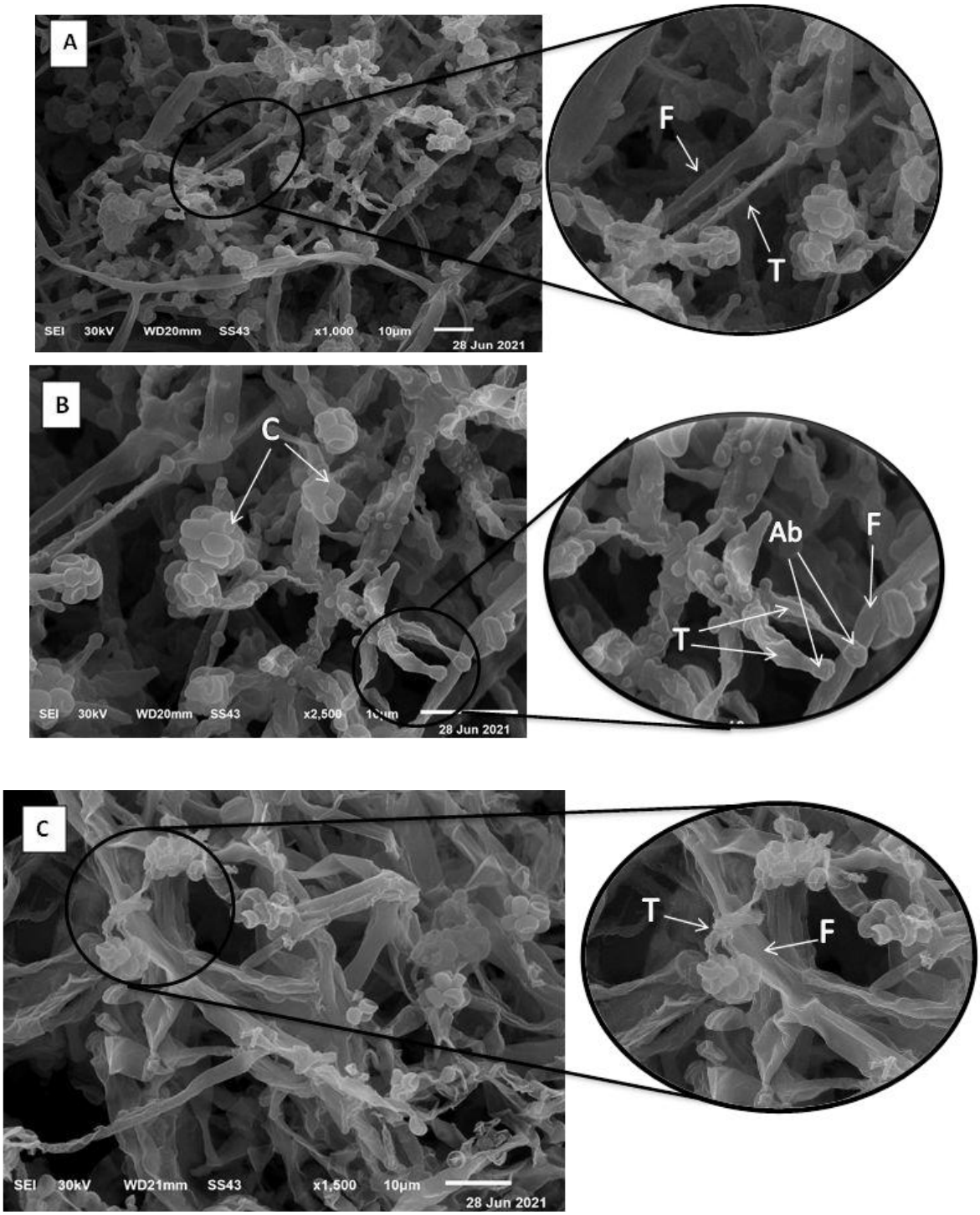
Source: Research data (2025).

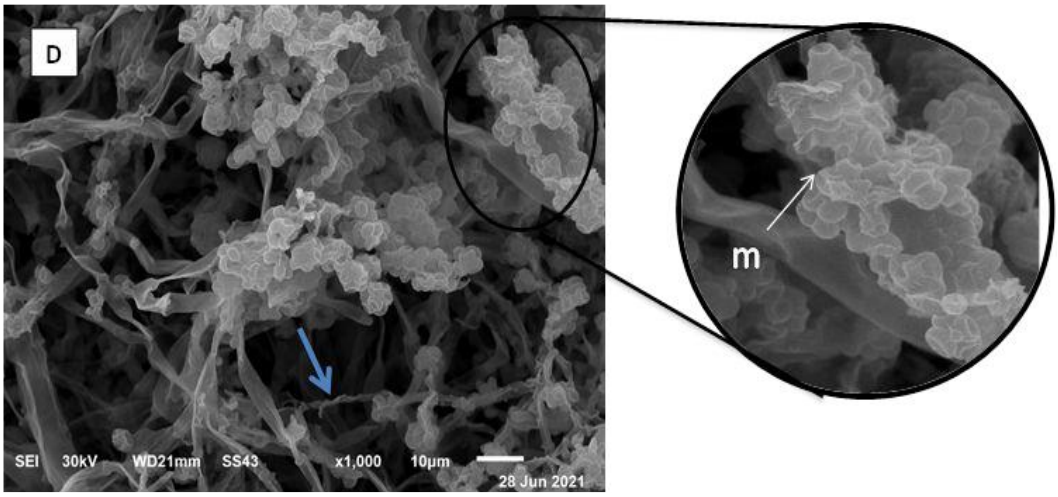
3.3 Scanning electron micrographs (SEM) of *T. asperellum* (T) interacting with cells of *F. equiseti* (F) in dual cultures

SEM micrographs of *F. equiseti* (F) showed that its hyphae appeared homogenous, linear shaped with smooth walls as observed in Figure 3. *T. asperellum* cells displayed as grape-like clusters and round, undamaged cells. Conidiophores branching is unilateral, with paired branching systems and phialide 6.0-9.7 µm long, sinuous or straight, sometimes hooked, whorls of 2-4, often and narrow cylindrical neck. Conidia are sub-globose to ovoidal, and chlamydospores are abundant within 7 days terminal or intercalary. The mycoparasistic characteristics of *T. asperellum* on *F. equiseti* as dual culture was observed by SEM **(**Figure 3**)**. The mechanism steps of antagonism between *T. asperellum and F. equiseti:* (1) Two hyphae parallel to each *F. equiseti* (thick) *T. asperellum* (thin) (Figure 3A). (2) the development of appressor-like structures ″Ab″ on the surface of *Fusarium* hyphae (Figure 3B). (3) helicoidization and coiling of *T. asperellum* around endophytic hyphae *F. equiseti,* which

wrapped around *Fusarium* hyphae, suggested a mechanical inhibition by strangulation (Figure 3C). (4) Formation mass, then, *Fusarium* lysis was eventually seen with blue arrow (Figure 3D).

Figure 3- Scanning electron microscopy (SEM) micrographs showing the direct antagonism of the putative biocontrol agent *T. asperellum* by hyphal penetration into *F. equiseti* hypha. **(A)** *T. asperellum* hyphae in parallel direction to *F. equiseti* hyphae; (**B**) formation of appressoria like structures, "Ab". Bars = 10 µm; (**C**) Coiling of *T. asperellum* around *F. equiseti* (F); (**D**) Sticking of *T. asperellum* hyphae (T) with *F. equiseti* hyphae (F), formed a mass (m) and the *F. equiseti* wall lysed (blue arrow in panel D). Bar = 10 µm.



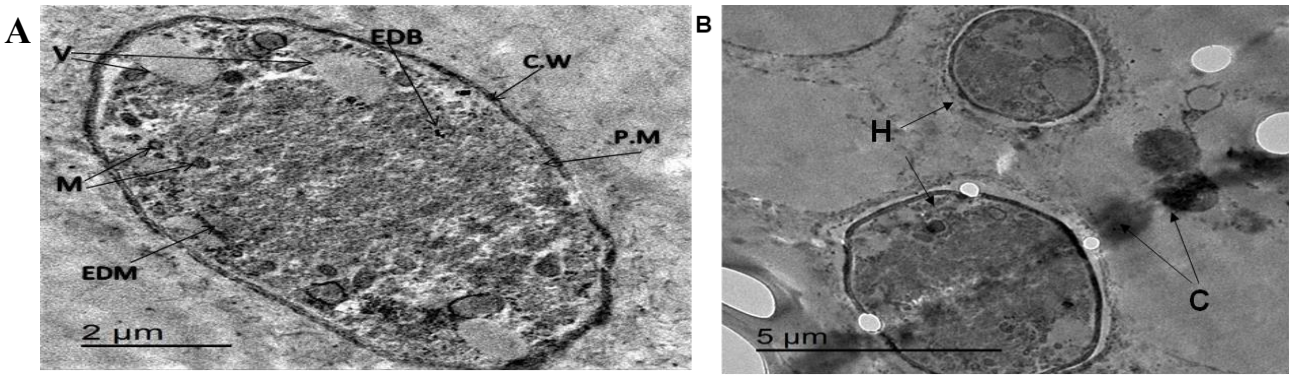


Source: Research data (2025).

3.4 Transmission electron micrographs

Transmission electron micrographs (TEM) of *F. equiseti* cells (control), *T. asperellum* cells (control) and the antagonism between them were observed in Figure (4A–B). The *F. equiseti* cells (control) were spherical, had intact cell walls, and had vacuoles. The DNA- and ribosome-rich areas of these cells are discernible. Cytoplasm dense, abundant in cytoplasmic organelles, and enclosed by a thick wall that is closely linked to the plasma membrane (Figure 4A). *T. asperellum* control cells are spherical, characterized by intact cell walls, a well-defined membrane, DNA-rich and ribosome-rich areas, dense cytoplasm, and a tightly linked plasma membrane (Figure 4B).

Figure 4- Transmission electron (TEM) photomicrographs of *F. equiseti* mycelium (control). Black arrows indicate the cell wall (CW) that is tightly attached with plasma membrane (PM), mitochondria (M), vacuoles (V), electron dense bodies (EDB) and electrons dense materials (EDM). Bar = 2 µm. B) TEM photomicrograph of *T. asperellum* (control): showing the conidia (C) and the hypha (H). Bar = 5 µm.

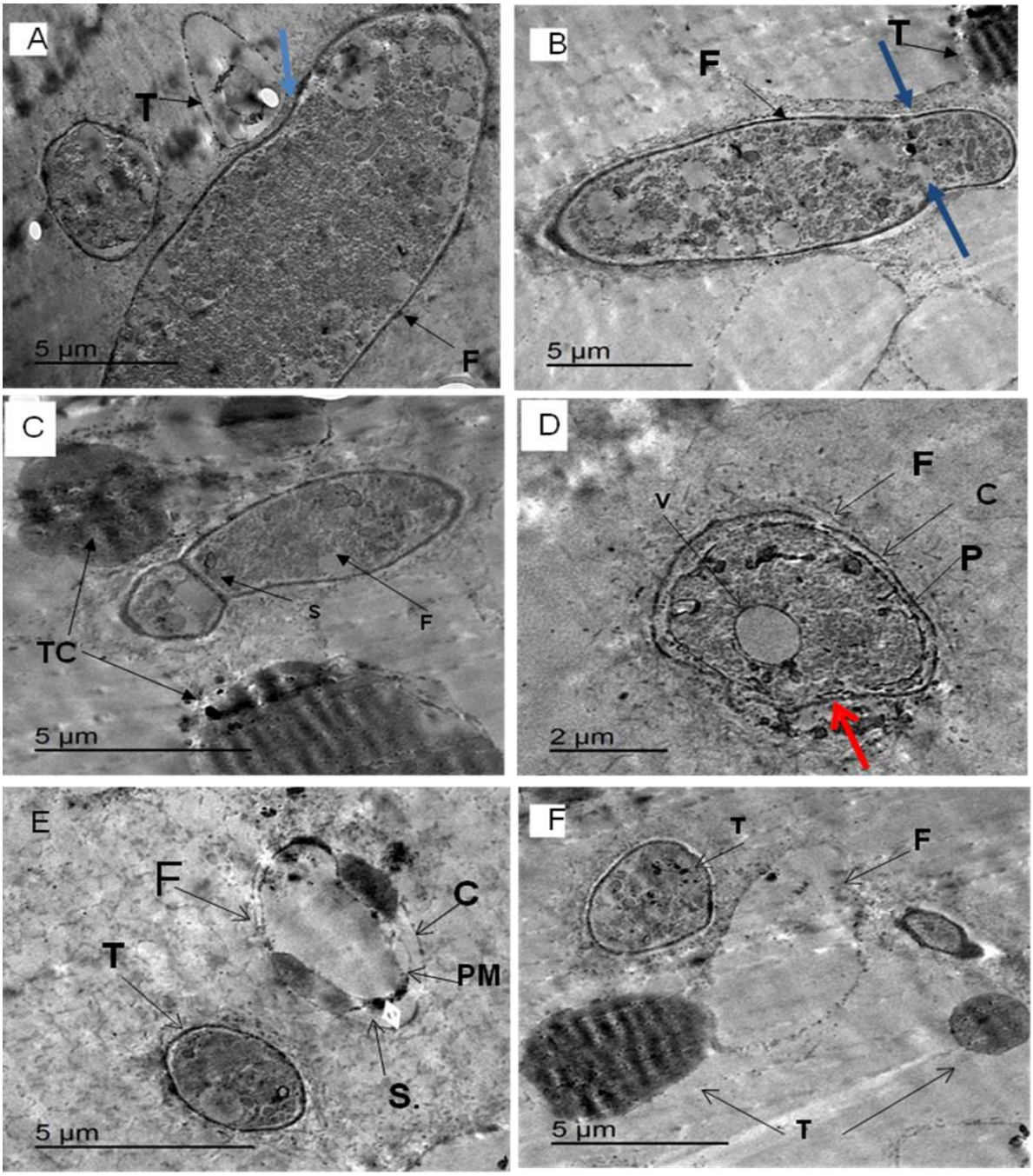


Source: Research data (2025).

Ultrathin sections from 3-day-old dual cultures showed *F. equiseti* cells encircled by *T. asperellum* hyphae undergoing changes, including increased vacuolation and retraction of the plasma membrane at potential antagonist penetration sites (Figure 5A). The antagonist's cells enter the space previously inhabited by the host cytoplasm and apply mechanical pressure to the host cell wall (blue arrow). *Trichoderma* penetration causes loosening and even breakdown of wall-like deposits (Figure 5B). The antagonist's attempts to penetrate this newly produced substance were frequently successful, resulting in a decrease in

labelling intensity along the penetration channel. High-magnification observations indicated that, in most cases, retraction of the plasma membrane was accompanied by the deposition of an amorphous matrix in which tiny electron-dense structures were embedded (Figure 5C). *F. equiseti* -treated cells displayed striking cytoplasm breakdown, cell organelle destruction (notable mitochondrial deformation), and vacuolation increase (Figure 5D). The detachment of the plasma membrane from the cell wall because of the development of large gaps was the most prevalent modification observed (Figure 5E). As a result, the cell membrane and cell wall detached, allowing some cells to discharge their contents. Dramatic cellular alterations were seen in *F. equiseti* hyphal cells treated with *T. asperellum*. Interaction 7 days after inoculation and due to the cell wall being damaged, more treated cells appeared as empty pleiomorphic shells or "ghost cells" due to disruption of the cell wall (Figure 5F).

Figure 5- TEM photomicrographs of *T*. *asperellum* hypha (TH) and conidia (TC) interacting with cells of *F*. *equiseti* (F) in dual cultures. (**A**) Local retraction of the plasma membrane (blue arrow in panel b) was visible. (**B**) Dissolution of the wall-like deposits was seen in areas of *Trichoderma* penetration (**C**) septum (S) was observed. (**D**) Structural changes were characterized by an increase in the number of vacuoles (V) and a local retraction of the plasma membrane (red arrow in panel D) were visible. (**E**) Detachment of the cell membrane from the cell wall due to formation of a wide space (S.P.) and *F*. *equiseti* hyphae appear as empty shells; (**F**) Autolysis of whole *Fusarium* cell. Bars: A, B, C, E and F = 5 µm and D= 2 µm.



Source: Research data (2025).

3.5 Changes in growth parameters of treated tomato plants

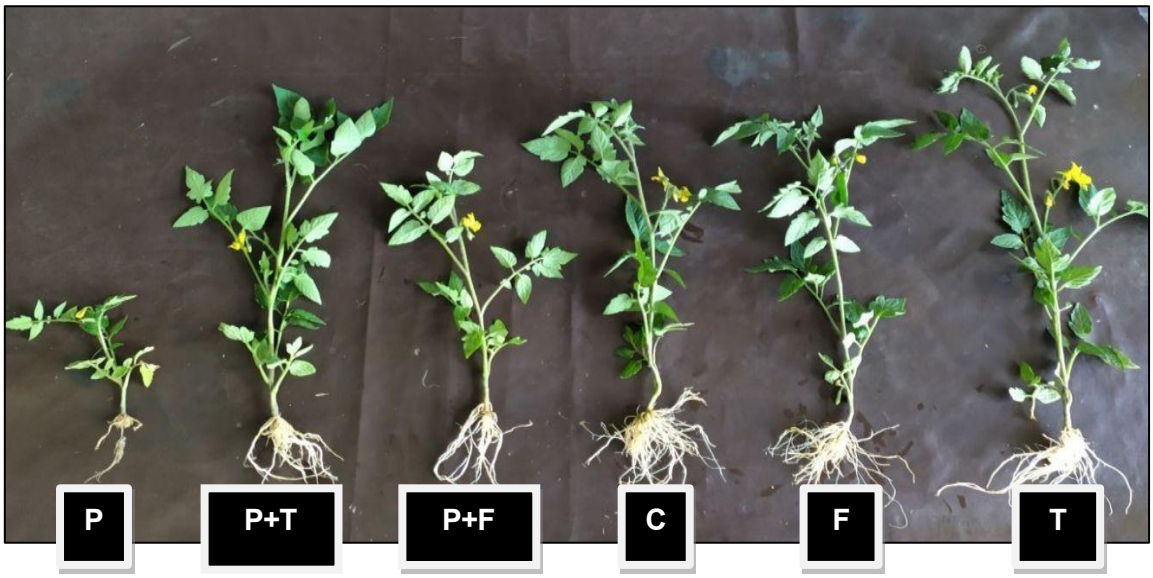
Table 2 and Figure 6 show the impact of fungicide and biocide on the incidence of *Fusarium* infection in tomato plants; this pathogen causes root rot in plants during fruiting stage. Both shoot and root fresh and dry weights of tomato plants are significantly affected by the studied treatments; healthy plants treated with *T. asperellum* recorded the highest values, compared to the other treatments and the lowest values for plants infected with pathogen, but infected plants treated with *T. asperellum* scored an increase compared to pathogen + fungicide. So, from the data it was found that *T. asperellum* was the most superior in plants infected with root rot disease.

Table 2- Fresh and dry weight of tomato shoot and root in soil infested with *Fusarium equiseti* as affected by biocide and chemical fungicide treatment during fruiting stage. Means of (n=10) replicates ± standard deviations and values with the same letter in the column are not statistically different, according to Duncan's multiple range test and Least Significant Difference (LSD). Abbreviations: Control (negative control; without fungi) ©, *F. equiseti* treatment (Positive control; pathogen) (P), *T. asperellum* treatment (T), Rhizolex-T (Chemical control; fungicide) (F), *T. asperellum* + *F. equiseti* treatment (T+P) and Rhizolex-T + *F. equiseti* treatment (F+P).

Treatments	Shoot		Root	
	Fresh weight g/plant	Dry weight g/plant	Fresh weight g/plant	Dry weight g/plant
C	168.0b ± 2.60	20.05b ± 0.72	4.22c ± 0.13	1.90b ± 0.06
T	184.0a ± 1.60	21.85a ± 0.60	5.70a ± 0.14	2.20a ± 013
F	142.1c ± 3.00	17.75c ± 0.64	4.01d ± 0.11	1.88b ± 0.14
P	92.0f ± 4.40	11.00e ± 0.95	3.40e ± 0.13	0.90d ± 0.06
P+T	136.0d ± 0.90	17.85c ± 0.73	4.41b ± 0.17	1.74b ± 0.06
P+F	102.0e ± 0.92	15.10d ± 0.79	3.92d ± 0.12	1.50c ± 0.05
LSD at 5%	4.70	1.25	0.16	0.16

Data is represented by means with standard deviation bars and with different superscript letters indicating significant differences ($P \leq 0.05$) based on Duncan's multiple range test Source: Research data (2025).

Figure 6- Growth parameters of tomato plants in soil infested with *F. equiseti* as affected by *T. asperellum* and chemical fungicide treatment. Abbreviations: Control (negative control; without fungi) (C), *F. equiseti* treatment (Positive control; pathogen) (P), *T. asperellum* treatment (T), Rhizolex-T (Chemical control; fungicide) (F), *T. asperellum* + *F. equiseti* treatment (T+P) and Rhizolex-T + *F. equiseti* treatment (F+P).



Source: Research data (2025).

Table 3 shows the impact of *T. asperellum* as a biocide and Rhizolex-T as a chemical fungicide on tomato plant length for shoot and root, number of leaves, and number of branches under biotic stress from root rot disease during fruiting stage. In

comparison to untreated plants, all treatments investigated considerably improved tomato plant development parameters and reduced the biotic stress caused by root rot. The greatest mean values of the attributes were reported during the stage by healthy plants treated with *T. asperellum*. Every parameter showed considerable improvement over the control. Regarding the plants affected with root rot disease, it was discovered that the vegetative qualities decreased, meanwhile the plants treated with *T. asperellum* and Rhizolex-T showed an improvement in comparison to the infected ones. Therefore, based on the data, it was determined that *T. asperellum* was the best treatment for plants with root rot disease.

Table 3- Plant length of shoot and root, leaves and branches number of tomato plants in soil infested with *F. equiseti* as affected by biocide and chemical fungicide treatment. Means of (n=10) replicates ± standard deviations and values with the same letter in the column are not statistically different, according to Duncan's multiple range test and Least Significant Difference (LSD). Abbreviations: Control (negative control; without fungi), *F. equiseti* treatment (Positive control; pathogen) (P), T. *asperellum* treatment (T), Rhizolex-T (Chemical control; fungicide) (F), *T. asperellum* + *F. equiseti* treatment (T+P) and Rhizolex-T + *F. equiseti* treatment (F+P).

Treatments	Plant length (cm)		Number of leaves/plant	Number of branches/plant
	Shoot	Root		
C	49.00b ± 0.51	16.00b ± 0.26	45.00b ± 2.0	12.00a ± 1.0
T	53.00a ± 0.65	20.00a ± 0.54	51.00a ± 2.0	14.00a ± 1.0
F	47.00c ± 0.74	14.00c ± 0.67	38.00c ± 3.0	9.00b ± 2.0
P	40.00f ± 0.75	10.50e ± 0.66	33.00d ± 3.0	6.00c ± 2.0
P+T	45.00d ± 0.64	13.00cd ± 0.16	41.00c ± 1.0	8.00bc ± 1.0
P+F	42.00e ± 0.46	12.00d ± 0.55	39.00c ± 3.0	7.00bc ± 1.0
LSD at 5%	0.84	1.01	3.64	2.67

Data is represented by means with standard deviation bars and with different superscript letters indicating significant differences ($P \leq 0.05$) based on Duncan's multiple range test. Source: Research data (2025).

3.6 Inhibition and yield recovery in tomato under *Fusarium* infection

The effect of *T. asperellum* as biocide and Rhizolex-T as chemical fungicide alone or in combination treatments on the yield attributes (fresh and dry weight of tomato fruits, size and number of fruits/plant) as affected by the different treatments are presented in Table 4. Fresh and dry weight, fruit size and number of tomato fruits were significantly reduced by the infected plants with pathogen treatment, compared to the control, while the application of *T. asperellum* non-significantly increased this parameter and significantly decreased by the other treatments. Compared to the control, *T. asperellum* promoted the mentioned parameters of tomato than the application of fungicide.

The calculated values for percent inhibition (compared to the control group) and yield increment (compared to the control group) based on the yield attributes (fresh and dry weight of tomato fruits, size and number of fruits/plant) data were showed in Table 5. For treatment T (*T. asperellum*), the yield increment compared to the control was 14.70% for fresh weight, 14.81% for dry weight, 3.75% for fruit size, and 12.50% for number of fruits. This indicates that *T. asperellum* enhanced all yield parameters, suggesting its beneficial role in promoting plant growth and productivity. Conversely, for treatment F (Rhizolex-T), the percent inhibition values of fresh and dry weight of tomato fruits, size and number of fruits/plants were 58.42%, 49.97%, 31.25%, and 45.83%, respectively. Rhizolex-T treatment resulted in substantial inhibition across all parameters, indicating potential phytotoxic effects or limited efficacy when used alone. In addition, treatment P (*F. equiseti*)

caused the most severe inhibition, with yield reductions of −94.90% for fresh fruit weight, −93.54% for dry fruit weight, −68.75% for fruit size, and −83.33% for number of fruits. The percent inhibition values mirrored these results, confirming *Fusarium*'s pathogenic nature and its detrimental impact on plant yield.

In the P+T treatment (combination of *T. asperellum* and *F. equiseti*), the yield increment was −46.79% for fresh fruit weight, −50.86% for dry fruit weight, −35.00% for fruit size, and −29.17% for number of fruits. This combination showed partial mitigation of *F. equiseti* 's effects, suggesting a protective role of *T. asperellum*. For the P+F treatment (Rhizolex-T + *F. equiseti*), the percent inhibition was 69.79% for fresh fruit weight, 68.54% for dry fruit weight, 43.75% for fruit size, and 37.50% for number of fruits. This combination also showed partial recovery, though it was less effective than *T. asperellum* in alleviating *Fusarium*-induced stress.

Table 4- Yield of tomato plants in soil infested with *F. equiseti* as affected by biocide and chemical fungicide treatment. Means of (n=10) replicates ± standard deviations and values with the same letter in the column are not statistically different, according to Duncan's multiple range test and Least Significant Difference (LSD). Abbreviations: Control (negative control; without fungi) (C), F. equiseti treatment (Positive control; pathogen) (P), T. *asperellum* treatment (T), Rhizolex-T (Chemical control; fungicide) (F), *T. asperellum* + *F. equiseti* treatment (T+P) and Rhizolex-T + *F. equiseti* treatment (F+P).

Treatments	Fresh weight of fruits/plant(g)	Dry weight of fruits/plant(g)	Fruit size (cm3)	Number of fruits/plants
C	1565.15a ± 8.85	111.07a ± 1.62	80.00b ± 0.82	24.00a ± 2.0
T	1795.19a ± 12.96	127.52a ± 2.87	83.00a ± 0.46	27.00a ± 4.0
F	650.81bc ± 7.31	55.57b ± 1.93	55.00c ± 0.87	13.00c ± 2.0
P	79.89d ± 6.65	7.17c ± 1.52	25.00f ± 0.31	4.00d ± 1.0
P+T	832.87b ± 12.18	54.58b ± 2.93	52.00d ± 0.26	17.00b ± 3.0
P+F	472.89c ± 7.35	34.94b ± 3.40	45.00e ± 0.35	15.00bc ± 3.0
LSD at 5%	23.80	2.20	1.24	3.88

Data is represented by means with standard deviation bars and with different superscript letters indicating significant differences ($P \leq 0.05$) based on Duncan's multiple range test. Source: Research data (2025).

Table 5- Calculated values for percentage inhibition (vs control) and yield increment (vs control) for the yield parameters: fresh fruit weight, dry fruit weight, fruit size, and number of fruits. Means of (n=10) replicates ± standard deviations and values with the same letter in the column are not statistically different, according to Duncan's multiple range test and Least Significant Difference (LSD). Abbreviations: Control (negative control; without fungi) (C), F. equiseti treatment (Positive control; pathogen) (P), T. *asperellum* treatment (T), Rhizolex-T (Chemical control; fungicide) (F), *T. asperellum* + *F. equiseti* treatment (T+P) and Rhizolex-T + *F. equiseti* treatment (F+P).

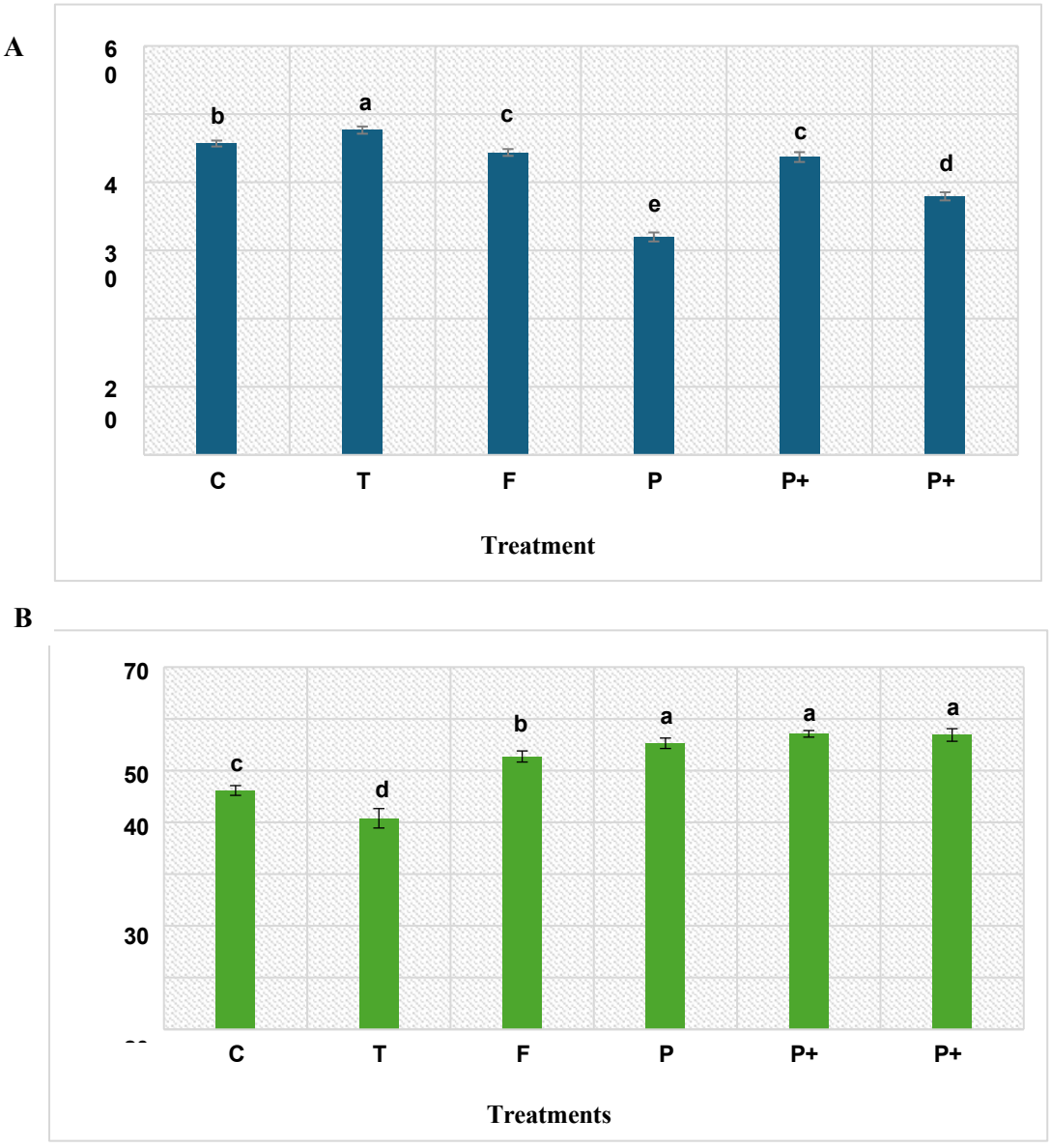
Treatments	Yield increment (%)				Inhibition (%)			
	Fresh Fruit Weight	Dry Fruit Weight	Fruit Size	Number of Fruits	Fresh Weight	Dry Weight	Fruit Size	Number of Fruits
T	14.7	14.81	3.75	12.5	-14.7	-14.81	-3.75	-12.5
F	-58.42	-49.97	-31.25	-45.83	58.42	49.97	31.25	45.83
P	-94.9	-93.55	-68.75	-83.33	94.9	93.55	68.75	83.33
P+T	-46.79	-50.86	-35	-29.17	46.79	50.86	35	29.17
P+F	-69.79	-68.54	-43.75	-37.5	69.79	68.54	43.75	37.5

Source: Research data (2025).

3.7 Assessment of the antioxidant activity (DPPH %) and total phenols of treated tomato plants

Data in Figure 7A indicated the DPPH activity in tomato fruits as affected by *T. asperellum* and fungicide treatment. All treatments significantly affected DPPH content. Application of *T. asperellum* increased DPPH activity in plants, which recorded the highest values, compared the control value then followed by the application of fungicide. The other treatments in addition to fungicide decrease this parameter compared to the control value, 356 with the lowest value detected by pathogen treatment. The effects of *T. asperellum* as biocide and Rizolex-T as chemical fungicide treatment on total phenol content of tomato plants are indicated in Figure 7B thus, comparing to the control, all treatments under investigation significantly affected the total phenol content. It was found an increase in total phenol content in pathogen treatment. The pathogenic plants treated with *T. asperellum* recorded the highest value of total phenol content followed by pathogen + fungicide. While treatment with *T. asperellum* alone decreased this metabolite significantly.

Figure 7- Effect of *T. asperellum* and chemical fungicide treatment. (**A**) antioxidant activity (DPPH %); (**B**) total phenolics (mg g^{-1}) in tomato plant infected by *F. equiseti*.



Source: Research data (2025).

4. Discussion

Root rot infections are sometimes referred to as root rot complexes since they are commonly caused by many pathogen species (Kumari & Katoch, 2020; Khan et al., 2025). *Trichoderma* spp. was discovered to be an efficient biological control agent for protecting a number of agricultural plants from damage caused by *Fusarium* spp. in greenhouse conditions, as revealed in the studies by (Marzano et al., 2013; Redda et al., 2018; Tkalenko et al., 2020; Thangaraj et al., 2025). Because *Trichoderma* spp. have very limited morphological traits and a limited range of difference that could lead to overlap and incorrect identification of the isolates, the information gleaned from the morphological analysis alone was insufficient to properly identify *Trichoderma* spp. (Anees et al., 2010). In addition to these restrictions on morphological features, they are also affected by culturing circumstances like temperature and the caliber of the media that is utilized. As a result, molecular methods had to be used in the identification of these species. One of the most accurate loci for a strain's species-level identification is the ITS region (Hassan et al., 2019; Karimov et al., 2024). Therefore, in the present study, the two fungal isolates were identified based on their ITS sequences (ITS 1 and ITS 4) and a phylogenetic analysis as *Trichoderma asperellum* and *Fusarium equiseti* with accession numbers (OQ130435- OQ130436), respectively. ITS sequences are widely used as molecular markers due to their diversity and ease of PCR amplification (Nilsson et al., 2008; Sushma et al., 2024). The ITS region is a formal fungal barcode widely used for phylogenetic inference and systematics, and is the primary choice for molecular identification of fungi from various sources (Schoch et al., 2012; Seo et al., 2025). ITS genes are typically used to identify fungal strains. With respect to these facts, the recent characterization of the fungal strains was accomplished using the 5.8S gene and the ITS1 and ITS2 portions (Nilsson et al., 2008; Khan et al., 2009; Schoch et al., 2012; Hesham et al., 2017; Elansky et al., 2024).

In this study, SEM micrographs showed the antagonism between *T. asperellum* and *F. equiseti* informed *T. asperellum* as mycoparasitic on *F. equiseti*. It was observed the hyphae of *T. asperellum* attached to the hyphae of *F. equiseti* and formation of appressoria structures winding around the pathogen then destroyed these pathogenic hyphae. It is widely acknowledged that a variety of compounds produced by biocontrol agents, such as enzymes, toxic substances, and volatile metabolites, encourage plant antagonistic activity and the development of defense systems in plants. These enzymes might hydrolyze the cell walls of phytopathogenic fungus. *Trichoderma* has been opposed by manufacturing antibiotics against Fusarium sp. and by mycoparasites (El-Sobky et al., 2019; Elshahawy & Marrez, 2024). In addition, Mazrou et al. (2020); Muhorakeye et al. (2024), showed that partial cell wall disintegration could be seen as the confrontational challenge progressed. It is well recognized that a variety of chemicals produced by biocontrol agents, such as poisonous substances, enzymes, and volatile metabolites, mediate antagonistic interactions and the development of plant defense systems. These enzymes might hydrolyze the cell wall of phytopathogenic fungus. The results of the present study are consistent with those reported by (Nofal et al., 2021; Joseph et al., 2023) investigated the mycoparasitic nature of *T. atroviride* on *F. oxysporum* as a dual culture using SEM. *T. asperellum* hyphae developed alongside *F. equiseti* hyphae, followed by rapid and extensive coiling and the production of appressoria-like structures on the surface of *F. equiseti* hyphae.

On the other hand, TEM observations showed *T. asperellum* treatment altered fungal cell components and morphology, destroying plasma membrane, organelles, and mitochondrial membrane, leading to mitochondrial dysfunction, cytoplasmic leakage, vacuolation increased, and cell death, which agreed with (Latz et al., 2018). Mycoparasitism explains this antifusarial action by disrupting fungal cell synthesis of cell wall-lytic enzymes and altering cytoplasmic components in accordance with (Dai et al., 1993; Hasna et al., 2025). In a study by Al-Surhanee, (2022) demonstrated how *Trichoderma* treatment impacted the ultrastructural properties of *F. oxysporum* mycelium, macroconidia, and microconidia. *Trichoderma*

caused moderate damage to *Fusarium* structure by extending macroconidia and causing an irregular cell wall, altering cell wall and membrane morphology compared to control.

Changes in the growth and yield parameters of tomato plants at the fruiting stage of growth and development in response to different treatments revealed that the application of *T. asperellum* significantly increased all evaluated growth parameters in healthy and infected tomato plants when compared to untreated control plants. Infected plants had the lowest value compared to the other treatments. This result is in agreement with the findings of (Nofal et al., 2021; Hu et al., 2025). Another study emphasized that the biocontrol agent *Trichoderma harzianum* strain T-203's capacity to stimulate a 30% increase in seedling emergence and a substantial increase in shoot length (45%), dry weight (80%), and leaf area (80%) in cucumber plants (Yedidia et al., 2001). Moreover, Nofal et al. (2021) reported that plant growth was also boosted in plants treated with *T. atroviride*, which might be attributed to infection suppression, plant resistance encouragement, high nutrient uptake, and plant development promotion. The current findings were in conformity with (Rinu et al., 2014), by using *T. gamsi* against a variety of plant pathogenic fungi, including *F. oxysporum* and *F. solani*. The current findings are confirmed by previous studies which ensured *Trichoderma* spp.'s potential to colonize plant roots, establish symbiotic relationships with various host plants, and enhance plant growth and development (Shoresh et al., 2010; Harman, 2011; Thangaraj et al. 2025). Similar results were demonstrated (Patel & Saraf, 2017), *T. asperellum* MSST improved tomato S-22 growth and yield indices while also managing *Fusarium* wilt disease both *in vitro* and in vivo.

Understanding the extent of yield inhibition and the potential for recovery through biocontrol or chemical treatments is essential for developing integrated disease management (IDM) strategies. This study explores the physiological and agronomic impacts of *Fusarium* infection on tomatoes and evaluates the effectiveness of various treatments in mitigating these effects. The effects of different treatments on fruit yield and inhibition were evaluated by comparing each to the untreated control. In this study, *F. equiseti* infection led to a 94.9% inhibition in fresh fruit weight and similarly high reductions in dry weight, fruit size, and number of fruits. *F. equiseti* caused the most severe inhibition, confirming its strong pathogenicity. These results are consistent with the well- documented impact of *Fusarium* wilt on tomato productivity, where vascular blockage and toxin production severely impair plant physiology and yield. *T. asperellum* demonstrated exceptional efficacy, reducing inhibition and even enhancing yield beyond the control group (Joseph et al., 2022; Joseph et al., 2023; Hasna et al., 2025).

In this investigation, fresh and dry fruit weight are most responsive to treatments, especially *T. asperellum*, showing strong positive increments. However, fruit size was more sensitive to *Fusarium* stress, with notable reductions in P and P+F. Moreover, the number of fruits was a clear indicator of treatment efficacy in which Trichoderma alone or in combination shows the best recovery. *T. asperellum* significantly improved all yield parameters, confirming its role as a beneficial biocontrol agent. *T. asperellum* treatment (T) resulted in a yield increment of 14.70% in fresh fruit weight, 14.81% in dry fruit weight, 3.75% in fruit size, and 12.50% in the number of fruits per plant. These positive values were accompanied by negative inhibition percentages, indicating a growth-promoting effect. This aligns with previous findings that *Trichoderma* species enhance plant growth through mechanisms such as mycoparasitism, competition, and production of growth-promoting metabolites (Sharma & Sharma, 2020; Ramírez-Cariño et al., 2025). For instance, Awal et al. (2024) highlighted *Trichoderma*'s ability to significantly suppress *Fusarium* growth and improve crop yield in horticultural systems.

Rhizolex-T, a fungicide containing tolclofos-methyl, caused substantial inhibition, suggesting phytotoxic effects or insufficient protection when used alone. In this study, it led to a 58.4% inhibition in fresh fruit weight compared to control and provided some protection but was less effective than *Trichoderma* in reducing *Fusarium*'s impact. These findings support the potential of biological control agents as more sustainable and effective alternatives to chemical fungicides in managing soil-

borne pathogens (Saldaña-Mendoza et al., 2023). Abdel-Monaim et al. (2012) similarly reported that Rhizolex-T reduced disease incidence and improved yield by 40–50% in tomato fields. In this investigation, the combination treatments (P+T and P+F) showed intermediate effects, suggesting synergistic but not fully additive benefits. *T. asperellum* partially mitigated Fusarium's effects, showing a protective role but not full recovery. This supports the concept of integrated disease management, where combining biocontrol agents with chemical fungicides can enhance disease suppression while reducing chemical inputs. A study by El-Khallal (2007) found that *Trichoderma viride* reduced *Fusarium* wilt severity by 75% and increased tomato yield by 60%. Similarly, Singh et al. (2024) demonstrated that *Trichoderma harzianum* combined with organic amendments improved tomato yield by 2–3 times under *Fusarium* infection.

Biochemical analyses revealed elevated phenolic content and antioxidant activity in treated plants, indicating enhanced defense responses. Tomato plants protected with biocontrol agents, such as *T. asperellum*, showed improved total phenolic content, in addition to increase in DPPH activity in plant, which recorded the highest mean values over the control then followed by the application of Rizolex-T (Sharma et al., 2012; Mona et al., 2017; Trotta et al., 2024). Increased phenolic content was the primary factor contributing to reduced wilt intensity in tomato plants treated with Trichoderma. More phenolics contribute to the formation of cell walls and other defense structures, serving as free radical scavengers and protecting the damaged plant (Ahanger et al., 2013; Hashem et al., 2016; Sorahinobar et al., 2025). *T. asperellum* stimulation of total phenol content may play an important role in conferring resistance to *F. equiseti*. These outcomes support that plant cells infected undergo a change that shifts primary metabolism into secondary defense pathways, stimulating numerous genes encoding defense enzymes (Farrag et al., 2017; Tarkowski et al., 2020; Sorahinobar et al., 2025). Others have also reported an enrichment in antioxidant activity as (Alhaithloul et al., 2019; Elkelish et al., 2020; Zaheer et al., 2020). *Trichoderma* species have the ability to develop systemic resistance in host plants by increasing phenolic content (Radjacommare et al., 2010; Zheng et al., 2024). However, the study did not investigate pathogenesis-related (*PR*) gene expression or reactive oxygen species (ROS) bursts, which are critical early indicators of plant immune activation. The SA pathway, triggered mainly by biotrophic pathogens, is involved in systemic acquired resistance (SAR), which induces the expression of the *PR1*, *PR2*, and *PR5* genes and prevents the spread of infection to healthy tissues (Ali et al., 2018; Joseph et al., 2022). *Trichoderma* promotes plant growth and development while inducing a significant immunological response in plants (Hermosa et al., 2013; Dutta et al., 2023). In this situation, the Nox protein is very important. In a study utilizing a *Trichoderma atroviride* mutant expressing the NoxR protein, co-culture with Arabidopsis reduced feeder root growth and phyto-stimulation as compared to the wild-type strain. However, when compared to treatment with wild-type *T. atroviride*, this increased the plant's response to jasmonic acid-mediated systemic resistance (Villalobos-Escobedo et al., 2020).

5. Conclusion

This study successfully identified endemic *Fusarium* species affecting commercial tomato crops through integrated morphological, molecular, and biotechnological approaches. The locally isolated biocontrol agent *Trichoderma asperellum* demonstrated superior antagonistic activity, outperforming the chemical fungicide Rhizolex-T in suppressing disease symptoms and enhancing plant performance. Treatment with *T. asperellum* significantly improved key agronomic traits, including fresh and dry fruit weight, fruit size, and number of fruits, with yield increments exceeding 14% compared to control. These findings underscore the potential of *T. asperellum* as a sustainable and eco-friendly alternative to chemical fungicides in managing *Fusarium* wilt in tomato. The integration of such biocontrol agents into disease management strategies offers a promising path toward reducing chemical inputs, enhancing crop resilience, and supporting sustainable agriculture in pathogen-prone regions. Future research should incorporate gene expression profiling and ROS quantification to better

understand the underlying mechanisms of *T. asperellum*-mediated resistance. Such insights would strengthen the case for integrating biocontrol agents into sustainable tomato disease management strategies.

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