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SCREENHOUSE EVALUATION OF *Trichoderma viride* AND *Trichoderma harzianum* FOR THE INTEGRATED MANAGEMENT OF CUCUMBER MOSAIC VIRUS AND FUSARIUM WILT IN TOMATO IN CALABAR, CROSS RIVER STATE, NIGERIA.

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ABSTRACT

The dependence on synthetic fungicides in smallholder vegetable production creates both an economic and environmental concerns in sub-Saharan Africa. This study evaluated the biocontrol potential of two molecularly identified *Trichoderma* isolates, *T. viride* and *T. harzianum*, against *Fusarium oxysporum* and Cucumber mosaic virus (CMV) in tomato under controlled screenhouse conditions. The isolates were characterized using morphological traits and confirmed by ITS and tef1 sequencing. In vitro antagonism against *F. oxysporum* was assessed using serial spore concentrations, while in vivo efficacy was evaluated through pathogen challenge experiments measuring disease incidence, severity, plant growth, chlorophyll content, biomass, and root colonization over 12 weeks. Both isolates significantly suppressed pathogen activity, with *T. viride* showing stronger overall performance. In vitro, *T. viride* achieved 94.3% inhibition of *F. oxysporum* at 10^9 CFU mL^{-1} after 120 hours, compared with 91.7% for *T. harzianum* at same end point. Under screenhouse conditions, *T. viride* reduced Fusarium wilt incidence by 67% and CMV severity by 67%, whereas *T. harzianum* reduced Fusarium wilt incidence by 60% and CMV severity by 59% relative to pathogen-inoculated controls. Plants treated with *T. viride* also exhibited the greatest plant height, chlorophyll content, biomass accumulation, and root colonization, indicating enhanced plant vigour. In contrast, carbendazim effectively suppressed Fusarium wilt but provided no protection against CMV. These findings demonstrate that both Trichoderma species function as multifunctional bioagents capable of simultaneously suppressing fungal and viral diseases while promoting tomato growth, with *T. viride* showing superior overall efficacy and strong potential for sustainable integrated disease management.

Keywords: *Trichoderma viride; Trichoderma harzianum*; integrated disease management; molecular identification; tomato; *Fusarium oxysporum*; Cucumber mosaic virus; controlled environment study

INTRODUCTION

Vegetable production remains central to food security and household livelihoods among smallholder farmers in sub-Saharan Africa (SSA). Tomato (*Solanum lycopersicum* L.) is one of the most economically important solanaceous crops in the region, and Nigeria alone produces about 2.2 million metric tonnes of tomato and 67,000 tonnes of pepper annually (FAO, 2017, 2020). Even so, productivity is still heavily constrained by biotic stresses, particularly soil-borne fungal pathogens and viral diseases, which can cause yield losses of 50–84% in tropical production systems (Sikora and Fernandez, 2005; Pernezny et al., 2003).

Among these constraints, *Fusarium oxysporum* f. sp. lycopersici, the causal agent of Fusarium wilt, is one of the most destructive soil-borne pathogens affecting solanaceous crops worldwide (Agrios, 2005; Dean et al., 2012). Cucumber mosaic virus (CMV) is also of major importance. It is transmitted in a non-persistent manner by more than 80 aphid species (Palukaitis and García-Arenal, 2003) and causes mosaic, leaf distortion, and severe stunting, with yield losses ranging from 10% to 80% depending on the stage of infection and the susceptibility of the cultivar (Jacquemond, 2012; Zitter et al., 1996). In many smallholder systems, disease management still depends largely on synthetic fungicides and pesticides, despite their well-known limitations (Ugwu and Omoloye, 2015; Mengistie et al., 2014). These inputs may account for 30–40% of production costs, placing them beyond the reach of many resource-limited farmers. Their indiscriminate use has also contributed to pathogen resistance, environmental contamination, disruption of beneficial soil microflora, and risks to human health from pesticide residues (Attia et al., 2022; Guzman-Guzman et al., 2023).

Biological control using plant growth-promoting fungi offers a more sustainable alternative. Species of Trichoderma, particularly *T. viride* and *T. harzianum*, are well documented as biocontrol agents against a broad range of phytopathogens through mechanisms such as mycoparasitism, antibiosis, competition, and induced systemic resistance (ISR) (Harman et al., 2004; Vinale et al., 2008; Mukherjee et al., 2013). Although both species are effective, their modes of action are not identical. *T. viride* is often associated with rapid niche colonization and competitive exclusion, whereas *T. harzianum* is noted for producing potent antimicrobial metabolites, including gliotoxin and harzianic acid, as well as cell wall-degrading enzymes early in the interaction process (Benitez et al., 2004; Vinale et al., 2008). While laboratory studies have demonstrated the promise of Trichoderma-based control, fewer studies have compared multiple species side by side under the same controlled conditions relevant to tropical smallholder agriculture.

Furthermore, environmental variability often affects field performance and, thus, strict controlled environment testing with molecularly identified isolates of Trichoderma is a pre-

requisite to the field investment (Harman., 2021). Using tomato as the test crop, this research aimed to investigate the dual role of both *T. viride* and *T. harzianum* not only in the management of Cucumber mosaic virus (CMV) and controlling Fusarium wilt but also in promotion of plant health. Specifically, the study aimed to isolate and molecularly identify both Trichoderma species via ITS and tef1 sequencing; test their in vitro antagonism against *F. oxysporum*; determine their in vivo dose-response effectiveness (10^8 CFU g^{-1}) against both pathogens in tomato; compare their efficacy with carbendazim for practical applicability; and assess their impact on tomato growth, chlorophyll content, and root colonization.

MATERIALS AND METHODS

Experimental site and conditions

The study was conducted from March to August 2025 at the Department of Plant Science and Biotechnology botanic garden screenhouse, University of Cross River State, Calabar, Nigeria (located geographically between latitude 4°28′–6°55′ N and longitude 7°50′–9°28′ E). Microclimatic conditions inside the screenhouse were maintained at temperatures ranging from 25°C to 32°C, with a relative humidity level of 70–85%, and natural lighting. These conditions simulate the typical microenvironment of tropical smallholder vegetable production systems in Cross River State and neighbouring states.

Plant materials, sources, and crop management

Certified and quality seeds of tomato (the Roma VF cultivar) were procured from the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. These specific cultivars were selected based on their high susceptibility to both fungal wilt and viral infections. The soil matrix used for the trials was sourced from the agricultural research fields of the University of Cross River State, Calabar. The sandy loam soil was thoroughly homogenized, sterilized by autoclaving at 121°C for 2 hours, and allowed to cool and stabilize for 48 hours prior to planting. Physical and chemical analyses confirmed a baseline pH of 6.3 and a total organic carbon (TOC) content of 1.42%.

Tomato seeds were surface-sterilized by immersion in a 1% sodium hypochlorite solution for 2 minutes, followed by five successive rinses with sterile distilled water to remove chemical residues. The sterilized seeds were sown directly into a seven litre (7 L) plastic pots filled with 5 kg of the autoclaved sandy loam soil. Upon seedling emergence, the plants were thinned systematically to maintain exactly one vigorous, uniform seedling per pot. The pots were arranged within an insect- proof screenhouse to completely eliminate potential contamination or viral transmission by insect vectors such as aphids or whiteflies. Crop management was performed manually throughout the duration of the study, which involved irrigating each pot every two days with 200 mL of sterile distilled water. In order to simulate the low-input agricultural conditions typical of regional smallholder farming, no chemical or organic fertilizers were applied to the soil throughout the experimental timeline.

Fungal isolation and identification

The antagonistic biocontrol fungi, *T. viride* and *T. harzianum*, were isolated from rhizospheric soils collected from the botanical gardens of the University of Cross River State, Calabar. Genomic DNA was extracted from pure *Fusarium oxysporum* mycelia and purified spores using a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Japan) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were determined spectrophotometrically by measuring absorbance at 260 and 280 nm, and the DNA was stored at −20°C until use. Molecular identification of *F. oxysporum* was performed by PCR amplification of the internal transcribed spacer (ITS) region using the species-specific primer pair Fc-1 (5′-CATACCACTTGTTGCCTC-3′) and Fc-2 (5′-ATTAACGCGAGTCCCACC-3′). PCR was carried out in a 25 µL reaction mixture containing 10 ng genomic DNA under optimized amplification conditions, and the amplicons were separated on 1% agarose gel stained with GelRed and visualized under UV illumination. Representative PCR products were cloned, sequenced.

The Trichoderma isolates were morphologically characterized via light microscopy, observing colony morphology, conidiophore branching patterns, phialide organization, and the size and shape of conidia. Under these diagnostic criteria, *T. viride* exhibited rapidly expanding colonies with dark green conidial sectors, pyramidal conidiophores bearing paired phialides, and ellipsoid conidia measuring 3.5–4.5 × 2.5–3.0 µm. Conversely, *T. harzianum* was characterized by rapid growth with light green conidial zones, conidiophores bearing dense whorls of phialides, and sub-globose conidia measuring 3.0–4.0 × 2.5–3.0 µm.

Taxonomic identities were confirmed using molecular characterization. Genomic DNA was extracted from young mycelia using the Cetyltrimethylammonium Bromide (CTAB) extraction method. The ribosomal Internal Transcribed Spacer (ITS) region was amplified using the universal primers ITS1 and ITS4 (5′-CTTGGTCATTTAGAGGAAGTAA-3′). Furthermore, a portion of the translation elongation factor 1-alpha (*tef1*) gene was amplified using the primers EF1-728F (5′-CATCGAGAAGTTCGAGAAGG-3′) and TEF1LLErev (5′- TACTTGAAGGAACCCTTACC-3′). Polymerase chain reaction (PCR) amplifications were performed under optimized thermal cycling conditions (White et al., 1990; O'Donnell et al., 2004; Dar et al., 2025). The resulting PCR products were purified and sequenced at Inqaba Biotec (Ibadan, Nigeria).

The raw nucleotide sequences were edited, aligned, and analyzed via the Basic Local Alignment Search Tool nucleotide (BLASTn) against the National Center for Biotechnology Information (NCBI) GenBank database to secure high confidence species-level identifications.

CMV source, extraction, identification, and transmission

Cucumber Mosaic Virus (CMV) was sourced from naturally infected cucumber (Cucumis sativus) plants exhibiting characteristic systemic mosaic, leaf narrowing (shoestring symptoms), and severe leaf blistering in Calabar, Cross River State, Nigeria. To ensure viral purity and confirm the identity of the causal agent, symptomatic leaf tissues were subjected to Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) using CMV-specific polyclonal antibodies according to standard manufacturer guidelines. The viral presence was further validated using Reverse Transcription Polymerase Chain Reaction (RT-PCR) with specific primers CMVF4 (5'-GCCGTAAGCTGGA-3') CMVR4 (5'-CCGCTTGTGCGTTTAATGGC-3') targeting the highly conserved coat protein (CP) gene of CMV, producing the expected 519 bp diagnostic amplicon (Ekpiken et al., 2021).

To prepare the viral inoculum, 1 g of confirmed CMV-infected leaf tissue was mechanically macerated in a prechilled pestle and mortar containing 10 mL of 0.01 M phosphate buffer (pH 7.0) supplemented with 0.1% 2-mercaptoethanol to preserve viral infectivity. The homogenate was filtered through a double layer of sterile muslin cloth to remove coarse particulate debris, and the clarified, infectious sap was maintained on ice at 4°C until immediate inoculation.

Mechanical transmission of CMV was executed when the tomato plants reached the 2 to 4 leaf stage.

The adaxial surfaces of the target leaves were dusted lightly with 600-mesh carborundum powder, which served as an abrasive to facilitate entry through microscopic epidermal wounds. The prepared viral sap was gently rubbed onto the dusted leaves using a sterile gloved finger. Control plants were rubbed similarly with phosphate buffer only.

Approximately five minutes following inoculation, the treated leaves were gently rinsed with sterile distilled water to remove excess sap and abrasive material, minimizing non-specific necrotic physical damage. Successful systemic viral infection and multiplication in the test crops were monitored daily for symptom expression and verified 21 days post inoculation using DAS-ELISA on newly emerged, non-inoculated leaves.

Inoculum preparation for fungal agents

Millet grains were utilized as carriers for fungal inoculum preparation. Healthy and intact millet grains (100 g per 500 mL bottle) were soaked in distilled water for 24 hours, drained thoroughly, sterilized by autoclaving at 121°C for 15 minutes, and subsequently inoculated with three 5 mm mycelial discs obtained from the active growing margins of respective Trichoderma species or *F. oxysporum* cultures. The inoculated grain carriers were incubated at 28 ± 2°C for 10–14 days to ensure uniform and complete fungal colonization.

Standardized inoculum concentrations were established prior to the commencement of the screenhouse trials to ensure reproducibility and consistency. The Trichoderma high-dose inoculum was formulated to reach a final concentration of 1×10^8 colony-forming units per gram (CFU g^1). Similarly, the *F. oxysporum* inoculum was standardized to an equivalent concentration of 1×10^8 CFU g^1, enabling direct comparative analyses between antagonist and pathogen applications within the rhizosphere.

In vitro antagonism assay

The antagonistic activity of *T. viride* and *T. harzianum* against *Fusarium oxysporum* was evaluated using the dual culture technique. A 5-mm mycelial disc of *F. oxysporum* was placed approximately 1 cm from the edge of a Potato Dextrose Agar (PDA) plate, while a 5-mm disc of the respective Trichoderma isolate was placed directly opposite at an equal distance from the plate margin. Control plates contained only *F. oxysporum*. Each treatment was replicated five times and incubated at 28 ± 2°C. Radial growth of the pathogen and antagonistic interactions were assessed at 24, 48, 72, 96 and 120 hours after inoculation. Percentage inhibition of radial growth (PIRG) was calculated using:

$$PIRG = \frac{R1 - R2}{R1} \times 100$$

where (R1) is radial growth of the pathogen in the control and (R_2) is radial growth in the dual culture. Antagonistic responses were classified using the inhibition scale shown in Table 1.

Table 1: Antagonism rating scale for *Trichoderma* isolates against *F. oxysporum*

Symbol	Category	Growth Inhibition Range
0	No observable inhibition	0% (no inhibition)
+	Slight restriction	≤ 25%
++	Moderate inhibition	26 – 50%
+++	Strong inhibition	51 – 75%
++++	Complete suppression	> 75%

Screen house experimental design

A Completely Randomized Design (CRD) with nine treatments and five replications (n = 45 pots per crop):

Table 2. Experimental Treatments

Code	Treatment	Dose	Application Timing
C	Control (sterile soil)	---	---
Tv	*T. viride*	10^8 CFU g^{-1}	At planting
Th	*T. harzianum*	10^8 CFU g^{-1}	At planting
F	*F. oxysporum*	10^8 CFU g^{-1}	At planting
Tv+F	*T. viride* + *F. oxysporum*	10^8 + 10^8 CFU g^{-1}	*T. viride* 7 days pre-pathogen
Th+F	*T. harzianum* + *F. oxysporum*	10^8 + 10^8 CFU g^{-1}	*T. harzianum* 7 days pre-pathogen
CMV	Cucumber Mosaic Virus	Crude extract	2–4 leaf stage
Tv+CMV	*T. viride* + CMV	10^8 CFU g^{-1} + virus	*T. viride* at planting, virus at 2–4 leaf
Th+CMV	*T. harzianum* + CMV	10^8 CFU g^{-1} + virus	*T. harzianum* at planting, virus at 2–4 leaf
CBZ	Carbendazim (fungicide control)	0.1% a.i.	Biweekly soil drench

Tomato seeds were surface-sterilized (1% NaOCl, 2 min), rinsed, and sown in 7 L pots with 5 kg sterile sandy loam soil (pH 6.3, TOC 1.42%). Pots were arranged in insect-proof screen house with 2-day irrigation (200 mL). No fertilizer was applied to simulate low-input smallholder conditions.

Data collection and analysis

Growth parameters, including plant height, number of leaves, and number of branches, were recorded at two-week intervals from 2 to 12 weeks after inoculation (WAI). Physiological parameters such as total chlorophyll content, fresh and dry biomass, as well as root length, were determined at 12 WAI following the method described by Lichtenthaler and Buschmann (2001).

Disease assessment was carried out by determining both disease incidence and severity. Disease incidence was calculated as the percentage of symptomatic plants relative to the total number of plants assessed using the formula:

Incidence (%) = (Number of symptomatic plants / Total number of plants) × 100.

Disease severity was evaluated using a 1–5 rating scale, where 1 indicated no visible symptoms, 2 represented less than 25% leaf area affected, 3 indicated approximately 50% leaf area affected, 4 represented complete (100%) leaf area infection, and 5 denoted severe stunting or plant death.

Table 3: Disease Severity Rating Scale Used for Symptom Assessment

Rating	Category	Description
1	No visible symptoms	No disease symptoms observed
2	Mild infection	< 25% leaf area affected
3	Moderate infection	~50% leaf area affected
4	Severe infection	100% (complete) leaf area infection
5	Very severe / lethal infection	Severe stunting or plant death

Root colonization was assessed at 12 WAI. Root segments were first cleared in 10% potassium hydroxide (KOH), acidified with 1% hydrochloric acid (HCl), and stained using 0.05% trypan blue according to the procedure of Phillips and Hayman (1970). The stained root samples were then examined microscopically at magnifications of ×100 and ×400 to determine the percentage of fields colonized by *Trichoderma* hyphae and conidia, as well as structures of *Fusarium oxysporum*.

All experimental data were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics. Treatment means were separated using Duncan's Multiple Range Test at a significance level of $P \leq 0.05$. In addition, Pearson correlation analysis was conducted to evaluate the relationship between colonization density and disease suppression.

RESULTS

Molecular Identification Confirms Species Identity

BLASTn analysis confirmed the identities of the isolates as *Trichoderma viride* (ITS: 99.2% similarity to the ex-type strain; *tef1*: 98.7% similarity to GenBank accession ON184718) and *Trichoderma harzianum* (ITS: 98.9% similarity; *tef1*: 99.1% similarity to GenBank accession KY496191). The *F. oxysporium* isolate was found to be 98.9% similar with GenBank accession DQ452450. The CMV isolate shared a 97% similarity with GenBank accession MT380910.

In Vitro Antagonism: Comparative Efficacy

As shown in Figure 1, the in vitro dual culture assay revealed a time-dependent increase in mycelial growth inhibition for

both *Trichoderma* species, with distinct inhibition patterns across the 120h evaluation period. At 24 h (Figure 1), *T. harzianum* demonstrated greater early inhibition of pathogen growth (38.5 ± 5.2%) than *T viride* (22.3 ± 4.5%). Figure 1 shows that *T. harzianum* recorded the highest percentage inhibition at 48 h, achieving 62.1 ± 6.8% inhibition, while *T. viride* recorded increased inhibition compared with the previous observation (45.6 ± 7.1%). At the 72 h timepoint (Figure 1), both species attained a three-plus (+++) antagonism rating, with *T. viride* surpassing *T. harzianum* (71.2 ± 6.9% vs. 68.4 ± 7.1%), with *T. viride* recording a slightly higher inhibition than *T. harzianum*. Beyond this point, Figure 1 further reveals that *T. viride* exhibited a more aggressive inhibitory trajectory, reaching near-complete suppression at 96 h (88.4 ± 5.2%) and highest biocontrol efficacy at 120 h (94.3 ± 3.7%), marginally outperforming *T. harzianum* (91.7 ± 4.1%) at the same endpoint. These findings indicate that *T. harzianum* produced greater inhibition during the early stages of the assay, whereas *T. viride* achieved higher inhibition at the later stages. The convergence of both isolates at a four-plus (++++) rating at 120 h (Figure 1) underscores the effectiveness of both bioagents for sustained pathogen suppression under in vitro conditions.

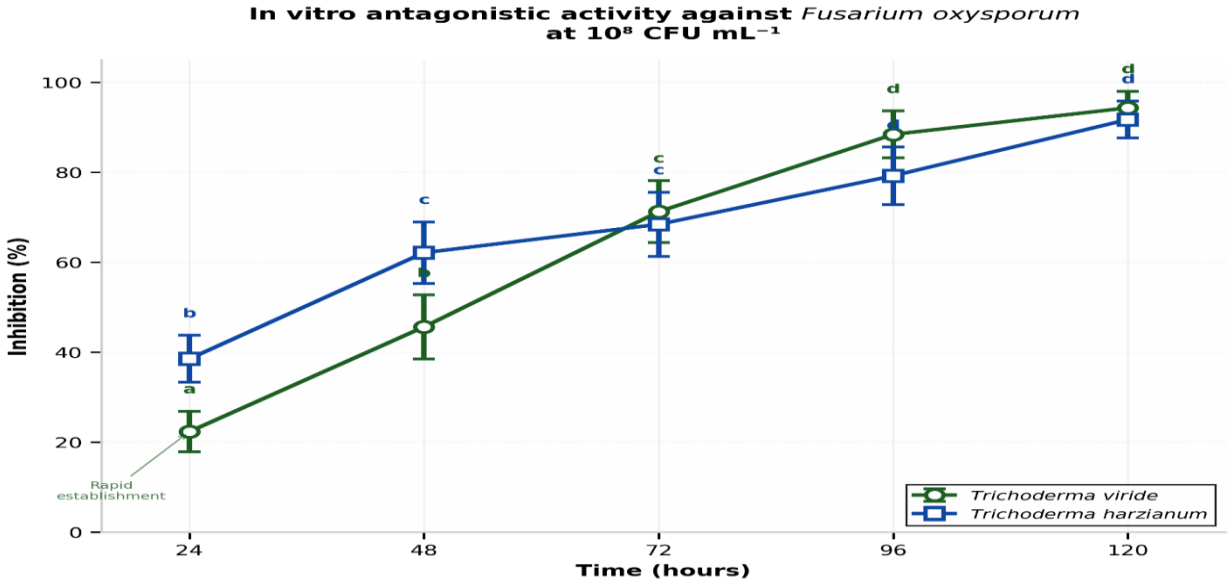

Figure 1: Comparative In vitro Antagonistic Activity at 10^8 CFU mL^{-1}

In Vivo Growth Promotion: Comparative Analysis

Plant height measurements presented in Figure 2, recorded across 12 weeks after inoculation (WAI), revealed marked differences among treatment groups, highlighting the growth-promoting and growth-suppressing effects of the bioagents and pathogen, respectively. As indicated in Figure 2, plants treated with *T. viride* alone consistently recorded the greatest heights throughout the evaluation period, reaching 120.4 ± 43.4 cm at 12 WAI, followed closely by *T. harzianum*-treated plants at 115.8 ± 41.2 cm, both significantly exceeding the uninoculated control (69.0 ± 38.6 cm). Plants treated with *Trichoderma* spp. consistently recorded greater plant height than the uninoculated control throughout the evaluation period. Figure 2 also reveals that the *Fusarium*-inoculated treatment (F) caused severe growth retardation across all sampling periods, yielding the shortest plants at 12 WAI (44.9 ± 26.2 cm), resulting in the shortest plants among all treatments. As further shown in Figure 2, bioagent-combined treatments (Tv+F and Th+F) partially mitigated this growth suppression, with Th+F slightly outperforming Tv+F at 12 WAI (95.4 ± 34.8 cm vs. 89.2 ± 31.6 cm), with *T. harzianum* recording slightly greater plant height than *T. viride* under *Fusarium* challenge. The carbendazim-treated group (CBZ) in Figure 2 performed comparably to the uninoculated control (72.3 ± 39.1 cm), with plant height comparable to the uninoculated control. Overall, the bioagent-only treatments recorded the greatest plant heights by 12 WAI, whereas the *Fusarium*-only treatment recorded the lowest.

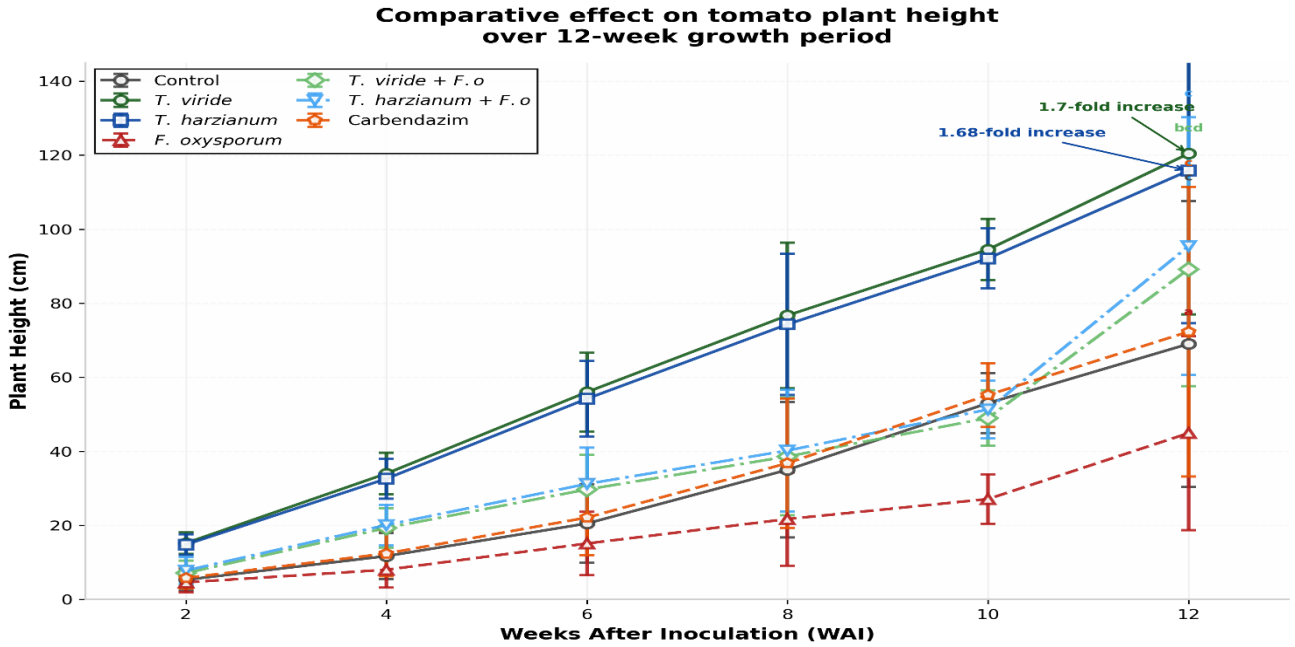

Figure 2: Comparative Effect on Tomato Plant Height (cm)

The analysis of chlorophyll content, dry biomass partitioning, and root colonisation data presented in Figure 3 at 12 WAI provided further evidence of the physiological benefits conferred by *Trichoderma* inoculation. As shown, *T. viride*-treated plants recorded the highest total chlorophyll content (81.2 ± 44.3 mg g^{-1}), total dry weight (6.67 ± 2.87 g), and stem dry weight (4.31 ± 1.94 g), while *T. harzianum*-treated plants yielded comparable values (78.9 ± 42.1 mg g^{-1} and 6.21 ± 2.65 g, respectively), both significantly exceeding all other treatment groups. Figure 3 further shows that the *Fusarium*-inoculated control (F) produced the lowest values across all physiological parameters namely, total chlorophyll (43.4 ± 25.4 mg g^{-1}), leaf dry weight (0.26 ± 0.15 g), and total dry weight (1.20 ± 0.59 g). As shown in Figure 3, plants inoculated solely with *F. oxysporum* recorded a root colonisation value of 88.3 ± 7.1%. This treatment also recorded the lowest chlorophyll content and biomass values. In contrast, plants treated with *T. viride* and *T. harzianum* recorded high levels of beneficial root colonization (94.7 ± 4.2% and 89.3 ± 6.8%, respectively). These treatments also recorded higher chlorophyll content and biomass than the pathogen-only treatment. No root colonisation was observed in the carbendazim-treated plants.

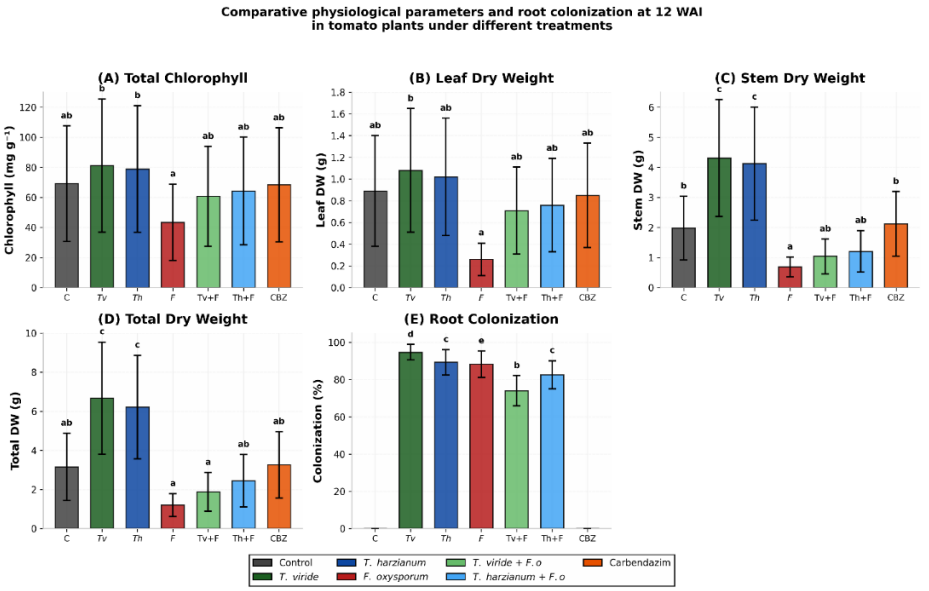

Figure 3: Comparative Chlorophyll Content and Biomass at 12 WAI

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Disease Suppression: Species Comparison

The disease suppression data presented in Figure 4 demonstrated that both *Trichoderma* species effectively reduced *Fusarium* wilt incidence and Cucumber Mosaic Virus (CMV) severity index, though with differential efficacy across pathogen types. As shown in Figure 4, under *Fusarium* challenge, *T. viride* combined treatment (Tv+F) achieved a 67% reduction in disease incidence (22.4 ± 11.9%) relative to the *Fusarium*-only control (67.2 ± 39.6%), while *T. harzianum* (Th+F) yielded a 60% reduction (26.8 ± 14.2%). In Figure 4, he reductions observed in the *Trichoderma*-treated plants were comparable to those recorded for the carbendazim treatment. Against CMV, Figure 4 shows that *T. viride* (Tv+CMV) reduced disease severity index by 67% (24.8 ± 14.9%), slightly outperforming *T. harzianum* (Th+CMV), which achieved a 59% reduction (31.2 ± 18.6%), both relative to the CMV-only control (75.9 ± 45.0%). No reduction in CMV severity was observed in the carbendazim-treated plants. Overall, *T. viride* recorded slightly lower disease incidence and severity than *T. harzianum*.

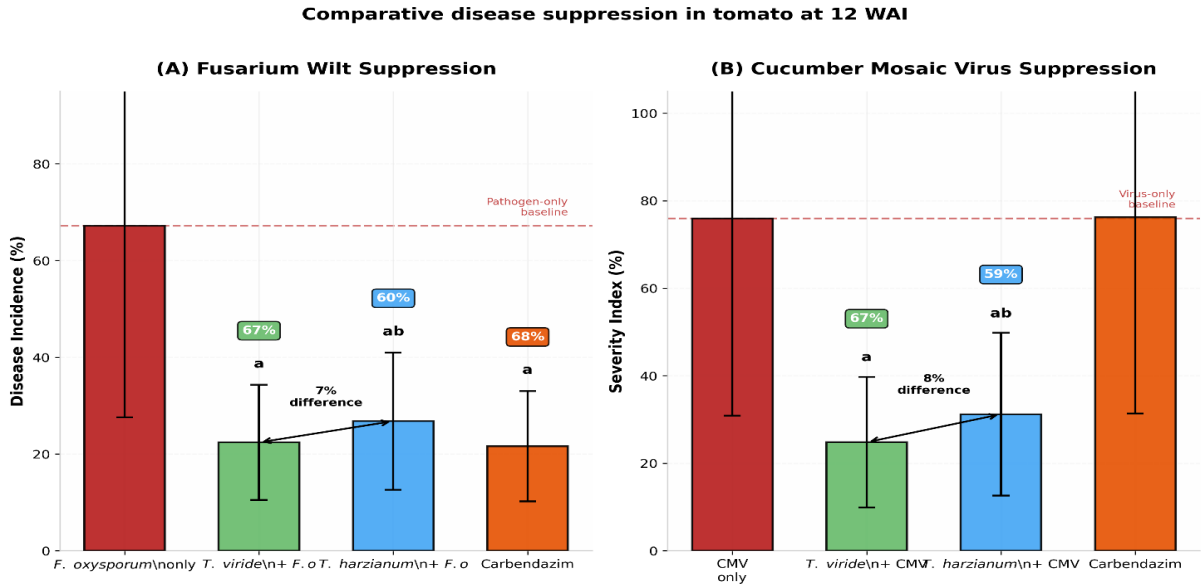

Figure 4: Comparative Disease Suppression in Tomato

DISCUSSION

The present study showed that the molecularly identified *T. viride* and *T. harzianum* have strong potential as dual-purpose biocontrol agents for suppressing *F. oxysporum* and Cucumber mosaic virus (CMV) while also improving tomato growth under screenhouse conditions. Both species enhanced plant performance and reduced disease development, but *T. viride* consistently showed better long-term antagonism, stronger root colonization, greater growth promotion, and higher disease suppression than *T. harzianum*. This supports previous reports that Trichoderma species are not only antagonists of plant pathogens but also plant growth-promoting fungi that improve crop productivity through mycoparasitism, antibiosis, competition for nutrients and space, and induction of plant defense responses (Harman et al., 2004; Vinale et al., 2008; Mukherjee et al., 2013).

Molecular confirmation of the isolates strengthened the reliability of the study by ensuring that the bioagents were correctly identified before evaluation. Identification using the ITS and translation elongation factor-1α (tef1) regions provided confidence in distinguishing *T. viride* and *T. harzianum*, while RT-PCR confirmation of CMV ensured that the symptoms observed were caused by the intended virus. This is important because morphological features alone may not reliably separate closely related Trichoderma species, and correct identification is necessary for reproducible biological control studies.

The dual culture assay revealed different antagonistic patterns between the two species. *T. harzianum* showed stronger inhibition during the first 48 h, whereas *T. viride* increased its activity over time and achieved the highest inhibition after 120 h. The early suppression by *T. harzianum* may be linked to rapid production of antimicrobial metabolites and hydrolytic enzymes, while the sustained inhibition by *T. viride* suggests stronger competitive ability after establishment in the medium. Previous studies have shown that *T. harzianum* produces compounds such as gliotoxin, harzianic acid, peptaibols, chitinases, and glucanases that inhibit fungal growth (Benítez et al., 2004; Vinale et al., 2008). Similar observations were reported by Yassin et al. (2021), who found that *T. viride* ultimately suppressed Fusarium species more effectively than *T. harzianum*. The near-complete inhibition observed in both isolates confirms their strong antifungal potential.

Beyond pathogen suppression, both Trichoderma species improved tomato growth. Plants treated with *T. viride*

recorded the highest plant height, chlorophyll content, and dry biomass, followed by those treated with *T. harzianum*, while plants inoculated with *F. oxysporum* alone showed severe growth reduction. These results agree with earlier reports that Trichoderma spp. enhance nutrient uptake, stimulate root development, and produce phytohormones such as indole-3-acetic acid and gibberellins (Harman et al., 2004; Vinale et al., 2008). The increase in chlorophyll content suggests that the fungi helped maintain photosynthetic capacity, thereby supporting greater biomass accumulation. Similar improvements were reported by Vitti et al. (2015) and Salami et al. (2025) in virus-infected tomato plants treated with Trichoderma.

Root colonization appears to be an important reason for the improved growth and disease suppression observed in this study. *T. viride* colonized tomato roots more extensively than *T. harzianum,* while *F. oxysporum* colonization was strongly reduced in plants receiving either bioagent. Effective rhizosphere colonization allows beneficial fungi to occupy infection sites, compete for nutrients, and maintain close associations with plant roots that support long-term biological activity (Harman et al., 2004). Continuous colonization also helps sustain growth promotion and defense activation. The superior colonization by *T. viride* likely contributed to its stronger overall performance.

Both bioagents significantly reduced Fusarium wilt incidence, with *T. viride* giving slightly better suppression than *T. harzianum*. This is consistent with the known mechanisms of Trichoderma, including mycoparasitism, secretion of cell wall-degrading enzymes, production of antifungal metabolites, and competition for nutrients and ecological niches (Harman et al., 2004; Yassin et al., 2021). Their performance was comparable to carbendazim against Fusarium wilt, showing that biological control can serve as a practical alternative to chemical fungicides. This is especially important given concerns about fungicide resistance and environmental contamination.

A major finding of this study was the strong reduction in CMV severity after inoculation with both Trichoderma species. Since viruses multiply inside living host tissues, they cannot be directly destroyed by mycoparasitism or lysis. The observed reduction in CMV severity is therefore most likely due to activation of the plant's innate defense system rather than direct antiviral action. Earlier studies have shown that Trichoderma root colonization can induce systemic resistance through salicylic acid-, jasmonic acid-, and ethylene-dependent pathways, leading to increased expression of defense-related genes and reduced virus multiplication (Vitti et al., 2015; Rizk et al., 2025; Salami et al., 2025).

The greater reduction in CMV severity recorded for *T. viride* may be linked to its stronger root colonization and longer persistence in the rhizosphere. This would allow more continuous activation of systemic defense responses throughout plant growth. Vitti et al. (2015) reported that *T. harzianum* reduced CMV symptom severity by improving antioxidant enzyme activity, including superoxide dismutase, catalase, and ascorbate peroxidase. Salami et al. (2025) also showed that *T. harzianum* increased the expression of defense-related genes such as PR1, AGO2a, and HSP90, resulting in reduced virus accumulation. Rizk et al. (2025) further demonstrated that Trichoderma culture filtrates reduced Potato virus Y accumulation while increasing PR1-b expression and antioxidant activity. Although such mechanisms were not directly measured in this study, the strong reduction in CMV severity suggests that similar induced resistance processes were involved.

The role of reactive oxygen species (ROS) as secondary messengers in Trichoderma-mediated defense against CMV has been documented in some detail. Vitti et al. (2015) reported that *T. harzianum* T-22 not only modulated CMV symptoms but also suppressed the viral RNA-dependent RNA polymerase gene (RdRp), with ROS serving as a critical relay in the defense response. Histochemical work in that study showed that CMV-infected plants accumulated high levels of superoxide anion and hydrogen peroxide, both of which fell dramatically after T22 application. Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were also implicated in shielding the photosynthetic apparatus and acting as signals that trigger defensive gene expression (Vitti et al., 2016).

The inability of carbendazim to reduce CMV severity highlights the advantage of biological control over conventional fungicides. While carbendazim reduced Fusarium wilt by directly inhibiting fungal growth, it offered no protection against CMV because viruses are not affected by fungicides. In contrast, Trichoderma improved plant health through pathogen suppression, growth promotion, enhanced physiological performance, and activation of host immunity. This multifunctional action makes it especially valuable in low-input farming systems where reducing chemical use while maintaining crop productivity is important. The protection Trichoderma provides against CMV is plant-mediated, not direct antagonism, as shown by the cross-talk among salicylic acid, jasmonic acid, ethylene, and abscisic acid signaling pathways (Martínez-Medina et al., 2010; Vitti et al., 2016).

The bigger picture of Trichoderma-induced resistance starts with recognition of microbe-associated molecular patterns (MAMPs) released by the fungus, which switch on plant immune responses and set systemic resistance in motion. More than twenty elicitors produced by Trichoderma have been characterized (antitoxins, polypeptides, lipopeptides, cellulases, hydrophobic proteins, terpenoids) and these trigger defense enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) (Khan et al., 2023). In the Trichoderma-tomato interaction, jasmonic acid, salicylic acid, and ethylene levels all shift to varying degrees, indirectly strengthening

resistance (Zeilinger and Omann 2007). Volatile secondary metabolites from Trichoderma also serve as elicitors, driving up-regulated expression of defense-related transcription factors such as *MYB72* and activating jasmonic acid-regulated defense responses (Hermosa et al., 2013).

Recent work has continued to broaden the view of Trichoderma as a biocontrol agent against viral diseases. Abdelkhalek et al. (2022) showed that *T. harzanium* strain Th23 promotes tomato growth and induces systemic resistance against tobacco mosaic virus, adding further weight to the versatility of Trichoderma species in managing viral pathogens. The capacity of *T. harzianum* T-22 to control CMV has already been established in model plants, with reports that the fungus enhances resistance in various dicots and monocots against bacterial, fungal, and viral infections including CMV (Contreras-Cornejo et al., 2011; Salas-Marina et al., 2011; Yoshioka et al., 2012; Lorito et al., 2010).

Overall, the findings indicate that both Trichoderma species have strong biocontrol potential, but *T. viride* provided the best overall performance. Its stronger long-term antagonism, better root colonization, greater growth promotion, and higher suppression of both Fusarium wilt and CMV suggest that it is better suited for sustained biological control under the conditions of this study. However, the rapid early antagonism of *T. harzianum* suggests that both species may complement each other. Future studies should evaluate combined formulations of *T. viride* and *T. harzianum*, explore the molecular basis of induced antiviral resistance under field conditions, and improve formulation and delivery methods for wider use in integrated disease management.

CONCLUSION

This study demonstrated that *T. viride* and *T. harzianum* are effective multifunctional bioagents capable of suppressing both *F. oxysporum* and Cucumber mosaic virus while promoting tomato growth under screenhouse conditions. Although both species reduced disease incidence and severity and improved plant growth, *T. viride* consistently showed stronger root colonization, greater long-term antagonistic activity, better physiological performance, and higher suppression of both pathogens. Disease control achieved by the Trichoderma treatments was comparable to carbendazim against Fusarium wilt, while also providing protection against CMV and improving plant growth.

These findings highlight the potential of *T. viride*, and to a lesser extent *T. harzianum*, as environmentally sustainable alternatives to synthetic pesticides for integrated management of fungal and viral diseases in tomato production. Future research should focus on field validation, optimization of application methods, clarification of the molecular mechanisms involved in induced resistance, and evaluation of combined *T. viride* and *T. harzianum* formulations to maximize disease suppression and crop productivity.

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Appendix

Table for Figure 1. Comparative In vitro Antagonistic Activity at 10^8 CFU mL^{-1}

Time (h)	*T. viride* Rating	% Inhibition	Mechanism	*T. harzianum* Rating	% Inhibition	Mechanism
24	+	22.3 ± 4.5[a]	Rapid establishment	++	38.5 ± 5.2[b]	Early antibiosis
48	++	45.6 ± 7.1[b]	Enhanced competition	+++	62.1 ± 6.8[c]	Chitinase peak
72	+++	71.2 ± 6.9[c]	Advanced overgrowth	+++	68.4 ± 7.1[c]	Mycoparasitism
96	+++	88.4 ± 5.2[d]	Near-complete suppression	+++	79.2 ± 6.4[d]	Sustained antibiosis
120	++++	94.3 ± 3.7[d]	Maximum biocontrol	++++	91.7 ± 4.1[d]	Complete suppression

Table for Figure 2. Comparative Effect on Tomato Plant Height (cm)

Treatment	2 WAI	4 WAI	6 WAI	8 WAI	10 WAI	12 WAI
Control	5.3 ± 3.0^{a}	11.7 ± 6.2^{ab}	20.5 ± 10.6^{a}	35.0 ± 18.3^{c}	53.0 ± 8.1^{bc}	69.0 ± 38.6^{c}
T. viride	15.2 ± 2.9^{e}	34.0 ± 5.6^{c}	56.0 ± 10.6^{d}	76.7 ± 19.7^{d}	94.5 ± 8.3^{d}	120.4 ± 43.4^{d}
T. harzianum	14.8 ± 2.8^{e}	32.6 ± 5.4^{c}	54.2 ± 10.2^{d}	74.3 ± 19.1^{d}	92.1 ± 8.1^{d}	115.8 ± 41.2^{cd}
F	4.6 ± 2.7^{a}	8.0 ± 4.8^{a}	15.1 ± 8.6^{a}	21.7 ± 12.6^{a}	27.1 ± 6.7^{a}	44.9 ± 26.2^{a}
Tv+F	7.1 ± 3.4^{ab}	19.3 ± 5.3^{abc}	29.7 ± 9.3^{ab}	38.6 ± 15.9^{c}	49.0 ± 7.5^{bc}	89.2 ± 31.6^{bcd}
Th+F	7.8 ± 3.6^{ab}	20.1 ± 5.5^{bc}	31.2 ± 9.8^{bc}	40.2 ± 16.4^{c}	51.3 ± 7.8^{c}	95.4 ± 34.8^{c}
CBZ	5.8 ± 2.9^{a}	12.4 ± 6.1^{ab}	22.1 ± 10.2^{a}	36.8 ± 17.5^{c}	55.2 ± 8.6^{bc}	72.3 ± 39.1^{c}

Table for Figure 3. Comparative Chlorophyll Content and Biomass at 12 WAI

Treatment	Total Chl (mg g^{-1})	Leaf DW (g)	Stem DW (g)	Total DW (g)	Root Colonization (%)
Control	69.2 ± 38.4^{ab}	0.89 ± 0.51^{ab}	1.98 ± 1.06^{b}	3.16 ± 1.72^{ab}	0.0 ± 0.0^{a}
T. viride	81.2 ± 44.3^{b}	1.08 ± 0.57^{b}	4.31 ± 1.94^{c}	6.67 ± 2.87^{c}	94.7 ± 4.2^{d}
T. harzianum	78.9 ± 42.1^{b}	1.02 ± 0.54^{ab}	4.12 ± 1.88^{c}	6.21 ± 2.65^{c}	89.3 ± 6.8^{c}
F	43.4 ± 25.4^{a}	0.26 ± 0.15^{a}	0.69 ± 0.33^{a}	1.20 ± 0.59^{a}	88.3 ± 7.1^{c}
Tv+F	60.7 ± 33.3^{ab}	0.71 ± 0.40^{ab}	1.04 ± 0.58^{ab}	1.88 ± 0.99^{a}	74.0 ± 8.2^{b}
Th+F	64.3 ± 35.8^{ab}	0.76 ± 0.43^{ab}	1.21 ± 0.69^{ab}	2.45 ± 1.34^{ab}	82.6 ± 7.5^{c}
CBZ	68.4 ± 37.9^{ab}	0.85 ± 0.48^{ab}	2.12 ± 1.08^{b}	3.26 ± 1.70^{ab}	0.0 ± 0.0^{a}

Table for Figure 4. Comparative Disease Suppression in Tomato

Treatment	Fusarium Incidence (%)	Reduction vs. F	CMV Severity Index (%)	Reduction vs. CMV
F only	67.2 ± 39.6^{c}	---	---	---
CMV only	---	---	75.9 ± 45.0^{c}	---
Tv+F	22.4 ± 11.9^{a}	67%	---	---
Th+F	26.8 ± 14.2^{ab}	60%	---	---
Tv+CMV	---	---	24.8 ± 14.9^{a}	67%
Th+CMV	---	---	31.2 ± 18.6^{ab}	59%
CBZ	21.6 ± 11.4^{a}	68%	76.2 ± 44.8^{c}	0%