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Bio-management of Fusarium wilt of tomato (*Fusarium oxysporum* f.sp. *lycopersici*) with multifacial *Trichoderma* species

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Abstract
Fusarium oxysporum f.sp. *lycopersici* (*FOL*), the incitant of the Fusarium wilt of tomato, is a highly damaging and prevalent disease in the majority of tomato growing areas. Keeping into consideration of high disease occurrence and incidence of *FOL* in tomato crop, the present investigation was undertaken to develop an effective bio-management approach to combat this disease. Initially, the studies were conducted to evaluate six multi-facial biocontrol isolates of *Trichoderma* species viz., *Trichoderma harzianum* AMUTH-1, *T. harzianum* AMUTH-2, *T. harzianum* AMUTH-3, *T. asperellum* (= *T. viride*) AMUTV-1, *T. asperellum* AMUTV-3 and *T. virens* (= *Gliocladium virens*) AMUTS-1 against *FOL* in vitro. Among these antagonists, *T. harzianum* AMUTH-1 and *T. asperellum* AMUTV-3 exhibited the maximum inhibitory effect while *T. virens* AMUTS-1 was recorded as the least effective *Trichoderma* isolate against *FOL* in vitro. Interestingly, *T. harzianum* AMUTH-1 and *T. asperellum* AMUTV-3 were found to produce indole acetic acid, siderophore and possess high enzymatic activities (cellulase, chitinase, ligninase and protease) in vitro. Further, pot trials were conducted and the chemical fungicide, carbendazim was used to compare the effectiveness of *Trichoderma* isolates. Pot trials also verified the efficacy of *T. harzianum* AMUTH-1 with 9–28% enhancement in the plant-growth parameters and 15–21% biomass production, and 88% decrease in the soil population of *FOL*. The effect of *T. harzianum* AMUTH-1 was also at par with fungicides, carbendazim.

Keywords Biocontrol · Disease severity · *Fusarium oxysporum* · *Trichoderma* spp.

1 Introduction

Tomato (*Solanum lycopersicum* L. syn. *Lycopesicon esculentum* L.) is a widely cultivated vegetable crop and the poor yield of tomato is attributed to its susceptibility to numerous phytopathogens [1]. Among the diseases caused by fungi, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) is a highly damaging disease under warm soil conditions and causes severe losses to the tomato crop [2, 3]. Infection of *FOL* results in stunting, wilting and finally, death of the plant. Sometimes, the entire fields are wilted or severely damaged before the crop is harvested [1, 4].

Management of Fusarium wilt can be done in many ways. Crop rotation with non-solanaceous crops (non-host) for four to six years is usually recommended to reduce the inoculum level of *FOL* in the field [5]. The crop should be rotated with cereals crops wherever possible [6]. Physical methods are quite effective but important hindrance that limits their

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use is low efficiency and high labour intensive. Synthetic chemicals are commonly used for the management of *FOL* in tomatoes. Treatment with chemical fungicides especially prochloraz, carbendazim, propiconazole, thiabendazole, benomyl, thiophanate, fuberidazole and benzimidazoles considerably reduce the wilt incidence in tomatoes [7, 8]. Khan and Khan [9] reported that the root-dip treatment of carbendazim on tomato seedlings infected with *FOL* led to a 24% increase in yield. However, the use of fungicides is a bit expensive as well as environmentally undesirable due to damaging effects on the agroecosystem, human beings and animals [10]. Under this situation, all hopes are set on the exploration of biological management of plant diseases.

Biological control is one of the best and safest methods of disease management and is devoid of residual toxic effects. This method is based on the idea that pathogen's populations can be decreased by influencing naturally occurring living microbial species, altering the environment, or introducing antagonists. Several antagonists of *FOL* are known and their utilization resulted in a substantial decrease in the disease severity and correspondingly increase in the crop yield. *Trichoderma* spp. are well recognized, potential and frequently used biocontrol agents [11–13]. Till date, 488 species have been identified and most biocontrol agents are from *Hypocrea rufa*, *Trichoderma asperellum* (= *T. viride*), *T. harzianum*, *T. koeningii,* and *T. hamatum* [14]. Application of these *Trichoderma* spp. have successfully controlled *F. oxysporum* f. sp. *lycopersici* in tomato crop [15–18].

Generally, *Trichoderma* isolates/strains are effective antagonists to a particular group of plant pathogens such as true fungi [19], stramenopiles [13] or nematodes [11]. However, their multi-facial potential against different plant pathogens has not been fully explored and it shall be a new and interesting approach to develop effective bio-management strategies to combat many soil-borne pathogens with a single treatment. With these objectives, the present investigations were undertaken to evaluate six multifacial *Trichoderma* isolates (*Trichoderma harzianum* AMUTH-1, *T. harzianum* AMUTH-2, *T. harzianum* AMUTH-3, *T. asperellum* AMUTV-1, *T. asperellum* AMUTV-3 and *T. virens* AMUTS-1) against *Fusarium oxysporum* f. sp. *lycopersici* in vitro and pot conditions. An attempt was also made to comprehend the mechanism of *FOL* suppression by these *Trichoderma* isolates.

2 Materials and methods

2.1 Inoculum and mass production of wilt pathogen, *Fusarium oxysporum* f.sp. *lycopersici*

A pure culture of *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) ITCC-1322 was received from the Indian Type Culture Collection, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. The pure culture was kept in culture tubes on PDA (potato dextrose agar, Himedia™, India) at 5 °C for the study. On sorghum seeds, the pathogen was mass-cultured. For 12 h, the seeds were steeped in a solution of sucrose (5%) and chloramphenicol (0.03%), and transferred in conical flasks of 500 mL capacity. The seeds were autoclaved at 121 °C for 15 kg/cm^2 pressure for 15–20 min. The conical flasks were subsequently inoculated with *FOL* culture and kept in a BOD incubator for ten to fifteen days at 27 °C. To encourage uniform colonisation on seeds, the flasks were manually shaken every day for a short period during incubation. To create the inoculum, double distilled water (DDW) was added with a known weight of *FOL*-colonized seeds [13]. The mixture was then pulverised in an electric mixer. The DDW was added to the ground sterile mixture, and load of the colony-forming units (CFU) load was estimated and standardized to 3.0×10^6 CFU per gram. In the current investigation, a 2 g inoculum was used per pot.

2.2 Mass culture of *Trichoderma* isolates

Six indigenous *Trichoderma* isolates, viz., *Trichoderma harzianum* AMUTH-1 (NCBI GenBank Accessions no. KM435269), *T. harzianum* AMUTH-2 (NAIMCC, ICAR-NBAIM, India Accessions no. NAIMCC-F-04335), *T. harzianum* AMUTH-3 (NCBI accessions no. KY062569), *T. asperellum* (= *T. viride*) AMUTV-1 (NAIMCC accessions no. NAIMCC-F-04337), *T. asperellum* (= *T. viride*) AMUTV-3 (NCBI accessions no. KY062571) and *T. virens* (= *Gliocladium virens*) AMUTS-1 (NAIMCC accessions no. NAIMCC-F-04336) were previously identified and chosen for this study because of their multifacial nature and biocontrol capability [13, 20, 21]. In conical flasks (250 mL), the mass cultures of the aforementioned *Trichoderma* isolates were prepared using potato dextrose broth (PDB, Himedia™, India). To create mycelial suspension, the mycelial-mats were removed from the conical flasks and processed individually in an electric grinder with 1 L DDW. The hyphae were then removed from the suspension by filtering it through a 0.15 mm mesh sieve [22]. Under a microscope, spores were counted using a hemocytometer, and the spore suspension was standardized to $2.0–3.0 \times 10^6$ spores mL^{-1} using DDW.

2.3 Fungicides

To compare the efficacy of *Trichoderma* isolates, carbendazim (Rickstin™, 50 WP, Darrick Insecticides Ltd., India) was added to the soil, already inoculated with *F. oxysporum* f. sp. *lycopersici*, at 1.5 mg a.i./pot one day before the seedlings (four weeks old) were planted. The accepted dose of 8 kg active ingredient /ha was used to compute the fungicide dose [7].

2.4 In vitro antagonism of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici*

The antagonistic potential of six *Trichoderma* isolates against *FOL* was estimated by dual culture technique using a PDA medium [23]. In the Petri plate, a mycelial disc (9 mm diameter) of *FOL* and a *Trichoderma* isolate were positioned 2.5 cm apart on the solidified PDA and incubated for seven days at 27 °C. *Trichoderma* isolates were not inoculated in the control plate. The treatments were replicated three times and the whole experiment was repeated for data accuracy. After seven days of incubation, the pathogen's development towards the *Trichoderma* colony and the inhibitory zone was measured. The pathogen's radial growth was computed, and the percent mycelial inhibition (PI) was estimated as below

$$PI = \{(C-T)/C\} \times 100$$

where C is the growth of test the pathogen (mm) in the control. T is the radial growth of *FOL* in *Trichoderma* spp. treatment

2.5 In vitro estimation of phosphate solubilization and ammonia, hydrogen cyanide, indole acetic acid, siderophore production and oxalic acid detoxification

All six *Trichoderma* isolates were tested in culture broth for their ability to solubilize phosphates [24], produce ammonia [25], siderophore [26], indole acetic acid (IAA) [27], hydrogen cyanide (HCN) [28] and detoxify oxalic acid (OA) [29]. The Lowry et al. [30] method was used to measure the activity of the enzymes (cellulase, chitinase, ligninase and protease) in the supernatant of culture medium.

2.6 Plant treatments and cultures

Nursery of tomato cultivar Pusa Ruby was raised independently in 96 well nursery tray with autoclaved cocoa-peat. Earthen pots (15 cm in diameter) filled with 1 kg autoclaved composted soil (farm yard manure and soil in 3:1) were used for the pot trials. The following nine treatments were maintained separately in an open surface receiving uniform sunlight. T1 = Plant + Un-inoculated soil (Control), T2 = Plant + Inoculated soil (Inoculated control), T3 = Plant + Inoculated soil + *Trichoderma harzianum* AMUTH-1, T4 = Plant + Inoculated soil + *T. harzianum* AMUTH-2, T5 = Plant + Inoculated soil + *T. harzianum* AMUTH-3, T6 = Plant + Inoculated soil + *T. asperellum* AMUTV-1, T7 = Plant + Inoculated soil + *T. asperellum* AMUTV-3, T8 = Plant + Inoculated soil + *T. virens* AMUTS-1 and T9 = Plant + Inoculated soil + carbendazim.

One day before transplanting of tomato seedlings, *FOL* (2 g at 3.0×10^6 CFU g^{-1}) and *Trichoderma* isolates (2 mL at $2.0–3.0 \times 10^6$ spores mL^{-1}) homogenized with 10 mL water separately were mixed to the top-soil of designated pots. On the following day, 3–4 leaf stage (4 weeks old) tomato seedlings cv. Pusa Ruby were planted in the pots (one seedling/pot). Five replicates of each treatment were maintained, and both inoculated and uninoculated controls were kept. The arrangement of the pots was totally randomized. Water (200 mL/pot) was gently given just after planting without overflow. Irrigation was done at one-day breaks and lasted till harvesting. The plants were routinely examined for any obvious disease symptoms. At harvest, plant-length (cm), fresh and dry weight (g), soil population of wilt fungus and *Trichoderma* isolates were calculated. The disease severity (wilt) was calculated at harvest on a 0–5 scale as determined by Lebeda and Buczkowski [31] with slight modification i.e., 0 = symptomless, no wilt visible; 1 = slight wilting with 5% wilted leaves; 2 = limited wilting with 6–10% wilted leaves; 3 = moderate wilting, 11–20% wilted leaves; 4 = severe wilting, 21–50% wilted leaves; 5 = severe witling and plants dead.

2.7 Soil population of *Fusarium oxysporum* f.sp. *lycopersici* and *Trichoderma* isolates

At harvest, the final soil population of *Trichoderma* isolates and *FOL* were estimated. One gram soil sample was taken from the region around the root zone of each infected pot. The sample was processed using serial dilution methods after being diluted to 10^{-5} with DDW. With a sterile pipette, the final dilution of soil suspension (0.5 mL) was put on solidified potato dextrose agar in petri plates. After that, these petri plates were placed in a BOD and maintained at 27 ± 2 °C for 24 h. A colony counter was used to measure the CFU load after 24-h of incubation period.

2.8 Statistical analysis

Both in vitro and pot experiments were repeated and carried out during 2020–21. The data were pooled because the differences in the data gathered throughout the two repeated studies were not significant at $P \leq 0.05$ and were processed to analysis of variance (ANOVA) using MINITAB software for Windows-10. The least significant differences (LSD), degree of freedom, and F values were determined at three levels, $P \leq 0.05$, 0.01, and 0.001. The in vitro antagonism and wilt index were examined through single-factor ANOVA.

3 Results

3.1 In vitro studies

3.1.1 Antagonism of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *lycopersici*

The antagonistic effects of *Trichoderma* spp. were evaluated by dual culture method. Among the six *Trichoderma* isolates tested, *T. harzianum* AMUTH-1 caused maximum inhibition (76.27%) of the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*) over inoculated control (Fig. 1). Next in effectiveness were *T. asperellum* AMUTV-3 (71.34%), *T. harzianum* AMUTH-3 (68.40%) and *T. harzianum* AMUTH-2 (53.70%). The isolates, *T. asperellum* AMUTV-1 (49.45%) and *T. virens* AMUTS-1 (36.32%) showed lowest mycelial inhibition of *FOL* (Fig. 1). Overall the order of inhibition was, *T. harzianum* AMUTH-1 > *T. asperellum* AMUTV-3 > *T. harzianum* AMUTH-3 > *T. harzianum* AMUTH-2 > *T. asperellum* AMUTV-1 > *T. virens* AMUTS-1 (Additional file 1: Figure S1).

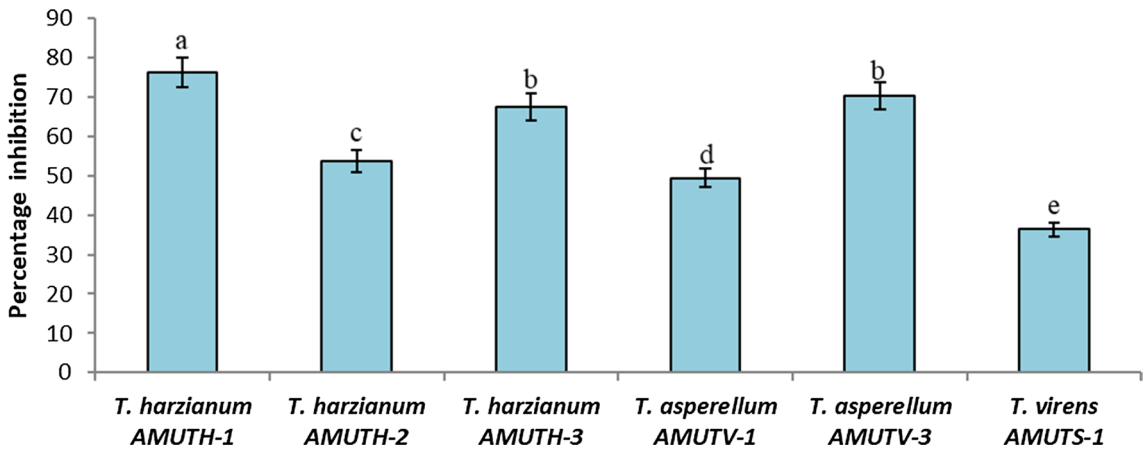

Fig. 1 Effects of *Trichoderma* isolates on the mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* in vitro. Error-bars show standard error. Bars with different alphabets are significantly different at $P \leq 0.05$ according to Tukey's Test

3.1.2 Solubilization of phosphate and production of HCN, IAA, ammonia, siderophore, detoxification of OA and enzyme activities

All *Trichoderma* isolates tested for ammonia, hydrogen cyanide, phosphorus and detoxification of oxalic acid exhibited negative responses and did not produce all these compounds in the medium. However, the isolates positively produced indole acetic acid (IAA) and siderophores in the culture medium with varied responses (Table 1). The greatest IAA production was recorded with *T. harzianum* AMUTH-3 (17.91 µg mL^{-1}) followed by *T. harzianum* AMUTH-1 (17.4 µg mL^{-1}), and *T. asperellum* AMUTV-1 (16.02 µg mL^{-1}; Table 1). The lowest IAA was recorded with *T. harzianum* AMUTH-2 (14.27 µg mL^{-1}) and *T. virens* AMUTS-1 (14.23 µg mL^{-1}). The isolates *T. harzianum* AMUTH-3 and *T. asperellum* AMUTV-3 were recorded as highly positive for siderophore and scored++ (Table 1). The isolate *T. harzianum* AMUTH-1 was also capable of producing siderophores and scored+. While *T. virens* AMUTS-1 and *T. asperellum* AMUTV-1 showed a negative result for siderophores (Table 1).

All isolates of *Trichoderma* exhibited significant enzymatic activities and synthesis cellulase, chitinase, ligninase and protease (Table 1). Highest cellulase (8.99 μg^{-1} protein), chitinase (7.12 μg^{-1} protein), ligninase (6.51 μg^{-1} protein) and protease (6.02 μg^{-1} protein) activity was recorded with *T. harzianum* AMUTH-1. Other *Trichoderma* isolates also exhibited significant enzymatic activity but relatively lower production of the above enzymes (Table 1).

3.2 Pot experiments

3.2.1 Disease severity and symptoms of *Fusarium oxysporum* f.sp. *lycopersici*

Tomato plants inoculated with 2 g *FOL* displayed stunted growth and mild yellowing in inoculated control pots. The chlorosis progressively became noticeable with plant age and at two months after inoculation, the complete plant became wilted with 4.33 wilt severity at 0–5 scale (Fig. 2). However, *Trichoderma* isolates and carbendazim as soil application significantly reduced the wilt severity caused by *FOL* (Fig. 2). The maximum reduction in wilt severity was reported with the treatment of *Trichoderma harzianum* AMUTH-1 (57%) and *T. asperellum* AMUTV-3 (45%) over control (Fig. 2). Next in efficacy, was carbendazim and it suppressed the wilt severity by 42% over inoculated control. Treatment with *T. virens* AMUTS-1 was recorded as least effective in reducing the wilt severity (17%) over inoculated control (Fig. 2).

3.3 Plant growth and biomass

The *F. oxysporum* f. sp. *lycopersici* infection in the tomato plants significantly reduced the plant length (16–20%), fresh weight (9–22%) and dry weight (16–24%) over un-inoculated control (Table 2). In contrast to the untreated inoculation

Table 1 Solubilization of phosphorus and production of indole acetic acid, hydrogen cyanide, ammonia, siderophore, detoxification of oxalic acid, cellulase, chitinase, ligninase and protease enzymes production by *Trichoderma* isolates in the culture broth

Treatments	*T. asperellum* AMUTV-1	*T. asperellum* AMUTV-3	*T. harzianum* AMUTH-1	*T. harzianum* AMUTH-2	*T. harzianum* AMUTH-3	*T. virens* AMUTS-1	LSD (P ≤ 0.05)
Ammonia	–	–	–	–	–	–	–
Detoxification of oxalic acid	–		–	–	–	–	–
Hydrogen cyanide	–	–	–	–	–	–	–
Indole acetic acid (µg mL^{-1})	16.02^{b}	15.61^{b}	17.40^{a}	14.27^{c}	17.91^{a}	14.23^{c}	0.081
Solubilization of phosphorus	–	–	–	–	–	–	–
Siderophore	–	++	+	+	++	–	–
Cellulase production (μg^{-1} protein)	+ (7.21^{c})	+ (9.23^{a})	+ (8.99^{a})	+ (7.09^{c})	+ (9.19^{a})	+ (7.92^{b})	0.71
Chitinase production (μg^{-1} protein)	+ (6.42^{b})	+ (7.45^{a})	+ (7.12^{a})	+ (6.25^{b})	+ (6.87^{s})	+ (6.11^{b})	0.65
Ligninase production (μg^{-1} protein)	+ (6.81^{a})	+ (6.85^{a})	+ (6.51^{a})	+ (6.13^{b})	+ (6.16^{b})	+ (6.04^{b})	0.63
Protease production (μg^{-1} protein)	+ (6.92^{a})	+ (6.67^{a})	+ (6.02^{b})	+ (5.92^{b})	+ (6.32^{b})	+ (5.43^{c})	0.57

+ low, ++ high, – negative

Each value is mean of 10 replicates. According to the Tukey test, values followed by different alphabets in a row are statistically different at P ≤ 0.05

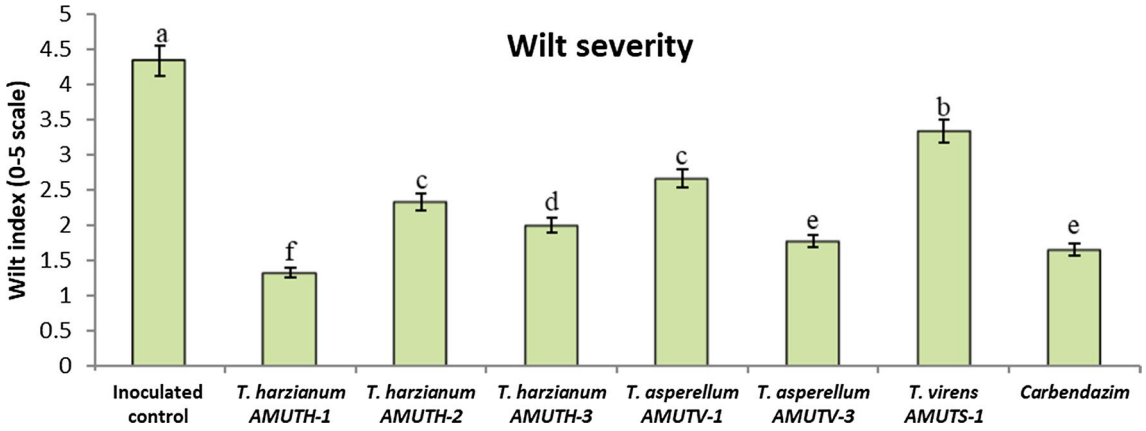

Fig. 2 Effects of *Trichoderma* isolates and fungicides on wilt index on tomato plants inoculated with *Fusarium oxysporum* f.sp. *lycopersici*. Bars show standard error. Bars with different alphabets are significantly different at $P \leq 0.05$ according to Tukey's Test

Table 2 Effects of six *Trichoderma* isolates and carbendazim on plant growth and biomass parameters of tomato plants inoculated with *Fusarium oxysporum* f.sp. *lycopersici* (2 g/kg soil)

Treatment	Plant length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Control (un-inoculated)	56.87^{a}	20.46^{a}	53.57^{a}	16.72^{a}	10.78^{a}	3.19^{a}
Control (inoculated)	47.79^{e}	16.40^{d}	48.90^{d}	13.08^{c}	8.28^{e}	2.69^{d}
T. harzianum AMUTH-1	57.75^{a}	18.92^{b}	53.02^{a}	16.52^{a}	10.56^{b}	3.24^{a}
T. harzianum AMUTH-2	51.7^{c}	17.20^{c}	48.21^{d}	15.32^{b}	9.60^{d}	2.90^{b}
T. harzianum AMUTH-3	52.5^{c}	18.6^{b}	51.30^{b}	15.20^{b}	9.80^{d}	2.90^{b}
T. asperellum AMUTV-1	48.12^{d}	16.28^{d}	49.60^{c}	13.06^{c}	10.25^{b}	2.66^{d}
T. asperellum AMUTV-3	55.30^{b}	18.10^{b}	52.31^{a}	15.00^{b}	10.01^{c}	3.10^{a}
T. virens AMUTS-1	49.80^{d}	16.30^{d}	48.70^{c}	13.10^{c}	10.30^{b}	2.70^{c}
Carbendazim	50.6^{c}	16.4^{c}	50.72^{b}	15.10^{b}	10.10^{b}	2.95^{b}
Df	8	8	8	8	8	8
Sum Sq	109.7	20.67	174.30	4.42	3.178	0.4259
Mean Sq	27.4	5.17	43.57	1.10	0.795	0.1065
F value	19.50	32.302	174.30	12.271	19.863	10.65
Pr(> F)	5.34e−08***	1.77e−10***	< 2e−16***	5e−06***	4.40e−08***	1.72e−05***
LSD $P \leq 0.05$	1.14	0.37	1.48	0.28	0.19	0.09

Each value is mean of 10 replicates (5 each year). According to the Tukey test, values followed by different alphabets in a column are statistically different at $P \leq 0.05$

control, treatment of *Trichoderma* isolates (except *T. asperellum* AMUTV-1 and *T. virens* AMUTS-1) and carbendazim substantially increased all growth parameters viz., plant length (16–21%), fresh weight (8–26%), and shoot and root dry weight (6–28%; Table 2). The fungus-inoculated tomato plants applied with *T. harzianum* AMUTH-1 significantly increased plant growth (15–21%) and biomass (9–28%) compared to the untreated inoculated control (Table 2). The plant growth and biomass metrics also improved by 11–20% and 9–18%, respectively, when *T. asperellum* AMUTV-3 and carbendazim were applied over the inoculated control (Table 2). In contrast to the inoculated control, *T. virens* AMUTS-1 treatment exhibited lowest increase in plant growth ($P \leq 0.05$; Table 2).

3.4 Soil population of *Fusarium oxysporum* f.sp. *lycopersici*

The soil population of *FOL* was amplified over time in untreated pots and it was significantly higher at time of harvesting in comparison to their initial population (Fig. 3). The population was increased up to 380% at harvest in untreated pot over the initial population (Fig. 3). However, application of *Trichoderma* isolates and carbendazim drastically reduced the soil population of wilt fungus in all treated pots (Fig. 3; $P \leq 0.05$). The highest decrease in soil population was recorded with

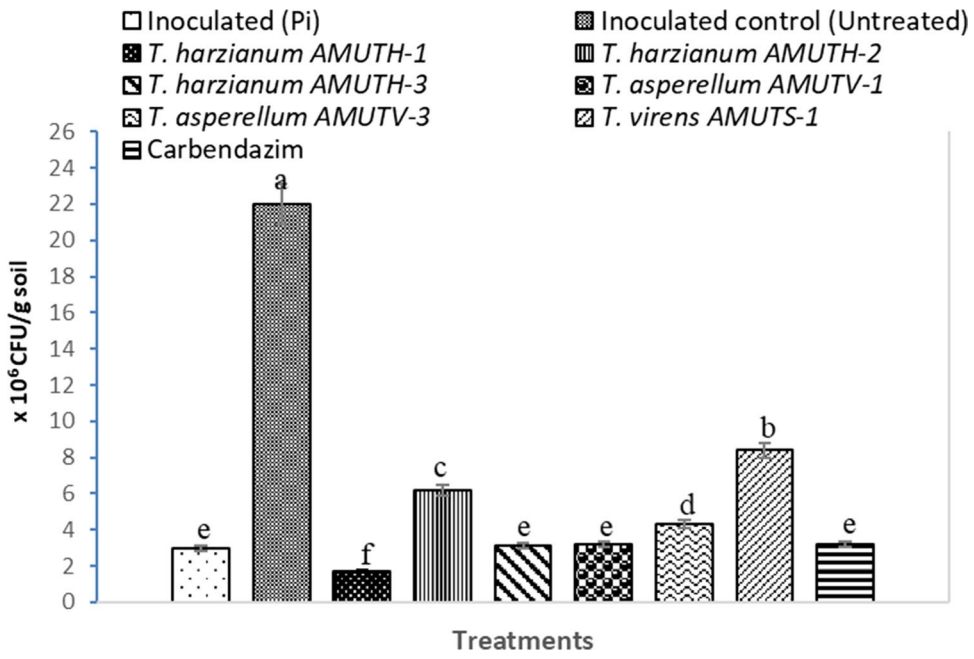

Fig. 3 Effects of *Trichoderma* isolates and carbendazim on the soil population of *Fusarium oxysporum* f. sp. *lycopersici*. Bars show standard error. Bars with different alphabets are significantly different at $P \leq 0.05$ according to Tukey's Test

T. harzianum AMUTH-1 (88%), followed by carbendazim (72%), *T. asperellum* AMUTV-3 (69%) and *T. harzianum* AMUTH-3 (64%). The isolate *T. harzianum* AMUTH-2 also significantly declined the soil population of wilt fungus over inoculated control (Fig. 3).

3.5 Soil population of *Trichoderma* isolates

The soil population of *Trichoderma* isolates were amplified over time, and they were significantly higher in the *Fusarium oxysporum* f.sp. *lycopersici* inoculated pots than their initial population (Fig. 4). Among the six *Trichoderma* isolates, the highest population increase at the time harvest was recorded with *Trichoderma harzianum* AMUTH-1 (560–730%) and *T. asperellum* AMUTV-3 (450–530%) over the initial population at the time of harvesting (Fig. 4). The rhizosphere population

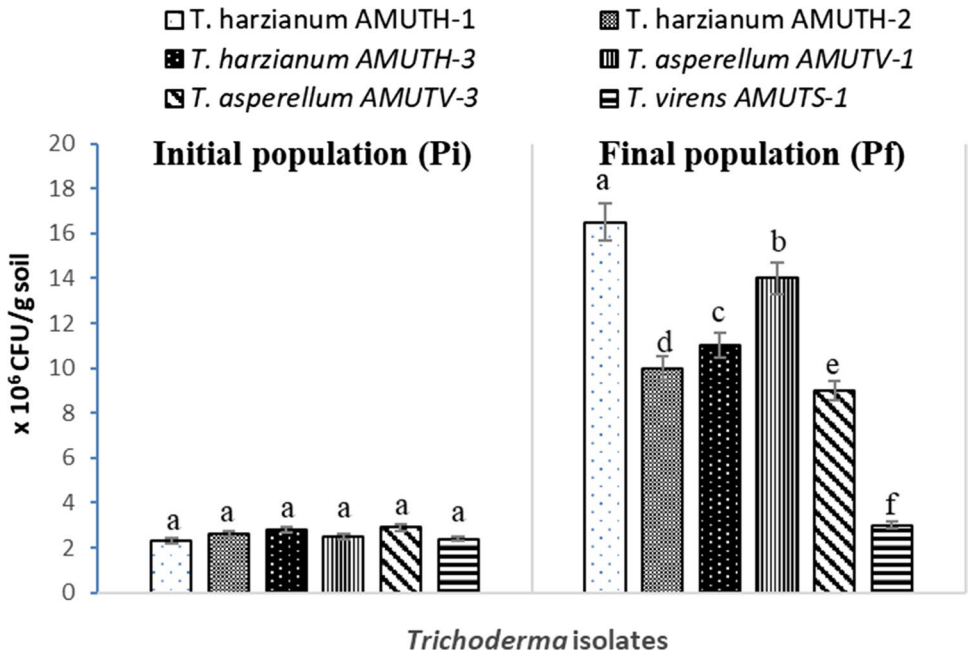

Fig. 4 Initial and final soil population of *Trichoderma* isolates in the pots inoculated with *Fusarium oxysporum* f. sp. *lycopersici*. Bars show standard error. Bars with different alphabets are significantly different at $P \leq 0.05$ according to Tukey's Test

of *T. harzianum* AMUTH-3 and *T. harzianum* AMUTH-2 also increased in the presence of the wilt pathogen in inoculated pots (Fig. 4; $P \leq 0.05$).

4 Discussion

Fusarium-wilt incited by *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*) is highly prevalent and damaging disease of tomatoes [32, 33]. Keeping in view of the high disease occurrence and prevalence of Fusarium wilt, the present study was undertaken to develop an effective biomanagement module, and a series of in vitro and pot trials were conducted. In vitro, six *Trichoderma* isolates were evaluated against *FOL* and the isolates, *T. harzianum* AMUTH-1 and *T. asperellum* AMUTV-3 exhibited the highest inhibitory effect against *FOL* while *T. virens* AMUTS-1 was recorded as the least effective biocontrol. In the current study, the isolate *T. harzianum* AMUTH-1 showed relatively higher inhibition of *FOL* than *T. asperellum* and *T. virens*. *Trichoderma* spp. exhibit varied virulence responses to wilt-fungus, that is characterised by the isolate's antagonism and virulence potential, which may be further expressed through different mechanisms such as rapid colonization/competence [34] and greater toxin production [35]. Greater antagonism by *T. harzianum* than *T. asperellum* and *T. virens* against wilt fungus has also been reported in other researches [15, 18]. Singh et al. [36] also recorded the higher production of antifungal metabolites by *T. harzianum* than *T. asperellum*.

In pot experiments, *FOL*-inoculated tomato plants caused 9–24% decline in biomass production of tomato. However, soil treatments with *Trichoderma* isolates reduced the harmful effects of pathogenic fungus and thus enhanced tomato plant growth and yield. The isolate *T. harzianum* AMUTH-1 was shown to be the most virulent isolate in antagonizing *FOL*. The efficacy of this isolate was also better than *T. asperellum* AMUTV-3 as well as fungicide carbendazim. *Trichoderma* spp. are the best-known biocontrol agent of a wide range of pathogenic fungi and have proven their potential to suppress the diseases in various crops including tomato [15, 16]. This genus contains numerous species that can be recognized as opportunistic endophytes and avirulent symbionts [11, 14]. Other studies also revealed the antagonistic effect of *Trichoderma* spp. especially *T. asperellum, T. harzianum*, *T. virens, T. hamatum,* etc. and successfully controlled *F. oxysporum* f. sp. *lycopersici* on tomato [15, 17, 18].

In this study, an attempt was also made to understand the mechanism of *FOL* suppression by these *Trichoderma* isolates. All *Trichoderma* positively produced IAA and siderophores (except *T. asperellum* AMUTV-1 and *T. virens* AMUTS-1) in the culture medium. The greatest IAA and siderophore production was recorded with *Trichoderma harzianum* AMUTH-3, *T. harzianum* AMUTH-1 and *T. asperellum* AMUTV-3, respectively which also exhibited significant mycelial inhibition and disease suppression. However, the lowest IAA and negative siderophores production was recorded with *T. asperellum* AMUTV-1 and *T. virens* AMUTS-1, which were least effective *Trichoderma* isolates in term of mycelial inhibition and wilt disease suppression, respectively. *Trichoderma* affects phyto-pathogens via various mechanisms, such as enzymatic hydrolysis, direct-parasitism, nutrient competition, antibiosis and induced resistance [11, 37] as recorded in the present study.

In our study, all *Trichoderma* isolates showed significant enzymatic activities and synthesis chitinase, ligninase, protease and cellulase with the overall highest activity recorded with *T. asperellum* AMUTV-3. The hydrolytic enzymes like chitinases, xylanases, cellulases, glucanases, and proteases which break down the fungal cell wall, are produced by *Trichoderma* species in ample amounts [37]. Among these, chitinases, which are released as secondary metabolites, are thought to be of utmost significance against plant pathogens [38]. *T. asperellum* AMUTV-3 and *T. harzianum* AMUTH-1 successfully parasitized *FOL* possibly due to the action of chitinases, glucanases, and proteases. The fungal cell wall is degraded by these chitinolytic enzymes, as their high activity was observed in our study.

The biocontrol applied in the soil to suppress wilt fungus first needs to multiply in the rhizosphere and then colonize the root to become systemic. Most of the *Trichoderma* species generally grow in their natural habitat and colonize root surfaces or become endophytes [39]. In the present study, the *Trichoderma* isolates, particularly *T. harzianum* AMUTH-1, *T. asperellum* AMUTV-3 and *T. harzianum* AMUTH-3 multiplied well in the rhizpsphere as showed by their increased population at harvest.

The study has demonstrated that *Fusarium oxysporum* f.sp. *lycopersici* is a devastating pathogen of tomatoes and inflicted growth and biomass production by 9–24%. Application *Trichoderma harzianum* AMUTH-1 drastically reduced the wilt severity and improved the plant-growth parameters by 9–28% and tomato biomass by 15–21%. The effect of this multifacial isolate was also at par with fungicides, carbendazim. Hence, *T. harzianum* AMUTH-1 may provide an alternative control of tomato diseases in the scenario of multipathogenic attack. This finding could also be used to device suitable

integrated management practices to safeguard tomato from the *FOL*. However, field experiments are required to verify the effectiveness of the above *Trichoderma* isolates before recommending the treatment to the farmers/growers.

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Declarations

Ethics approval and consent to participate Not applicable.

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