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Short Communication

Fusarium Wilt of Tomato Managed by *Trichoderma harzianum* against *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) Synder and Hansen.

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Tomato, (*Solanum lycopersicum*), flowering plant of the nightshade family (Solanaceae), cultivated extensively for its edible fruits. Labelled as a vegetable for nutritional purposes, tomatoes are a good source of vitamin C and the phytochemical lycopene. The world production of tomato is 170.80 million tonnes and China ranks first in tomato production worldwide with the contribution of 31% of global production followed by India. The plants are susceptible to a number of pests and diseases, including bacterial wilt, early blight, mosaic virus, Fusarium wilt, nematodes, and tomato hornworms. The Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) is an economically important and most destructive disease of tomato crop [1]. The fungal antagonist *Trichoderma* sp. effectively manages the Fusarium wilt of tomato. The present studies were undertaken to investigate the effect of fungal antagonistic isolates against tomato Fusarium wilt.

Isolation of native antagonists from tomato rhizosphere soil: Trichoderma harzianum

The *Trichoderma harzianum* was isolated from various soil samples collected during the survey from healthy tomato plants rhizosphere in wilt affected fields of 5 districts of Tamil Nadu. Isolation of *Trichoderma harzianum* was done by serial dilution technique using a Trichoderma selective medium (TSM) [2]. The culture was purified by single hyphal tip method. The *Trichoderma harzianum* culture was maintained on PDA slants for testing the colony colour, colony growth pattern and conidia size on the seventh day of inoculation. The five isolates were designated as Tv1 - Tv5.

Efficacy of antagonists

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Effect on Trichoderma isolates against F. oxysporum f.sp. lycopersici mycelia growth (Dual culture)

The antagonistic effect of *Trichoderma harzianum* isolates (Th1 - Th5) against *F. oxysporum* f.sp. *lycopersici* was tested by dual culture technique [3] 9mm mycelial disc from seven days old culture of *F. oxysporum* f.sp. *Lycopersici* was inoculated at one end of the Petri dish, just away from the edge. Of Petri dish containing 15 ml solidified PDA medium. 9mm of *Trichoderma harzianum* culture disc was placed on the opposite side of plate. Control without antagonist was maintained. The Petri plates were incubated at room temperature (28±2°C) for five days. After the incubation period the radial growth of pathogen and inhibition the zone were recorded and percent inhibition over control was estimated by using the formula [4].

$$\text{Percent inhibition (I)} = C - T / C \times 100$$

Where,
C- Mycelia growth of pathogen in control (mm)
T- Mycelia growth of pathogen in dual plate (mm)

Effect of cultural filtrate of Trichoderma harzianum on the mycelia growth of F. oxysporum f.sp. lycopersici

Six days old culture discs of *Trichoderma harzianum* isolates were inoculated into 50 ml of sterilized potato dextrose broth and kept for 15 days. After 15 days of incubation the culture broth of each isolate was filtered through Whatman No.1 filter paper. The filtered broth was centrifuged at 1500 rpm for 30 mins. in addition, the supernatant solution was collected. The culture filtrate was sterilized and added to the sterilized PDA medium @ 10, 20, 30, 40, and 50 percent. The PDA medium without culture filtrate acted as control. The media were poured in to petridishes separately @ 15 ml and allowed solidify. Seven days old culture of *F. oxysporum* f.sp. *lycopersici* (7mm) was placed in the centre of each plate. carbendazim @ 0.1% was used for comparison. The colony growth of *F. oxysporum* f.sp. *Lycopersici* was recorded the day when any colony fully covered the plates (Solid medium).

Effect of cultural filtrate of Trichoderma harzianum on the mycelia dry weight of F. oxysporum f.sp. lycopersici

The culture filtrate preparation was incorporated into the potato dextrose broth @ 10, 20, 30, 40, 50 percent conc. 7mm mycelial disc of *F. oxysporum* f.sp. *lycopersici* was placed on the broth aseptically and mouth plugged with cotton. PDA broth without culture filtrate acted as control and carbendazim @ 0.1% was used for comparison. Incubate for 15 days at room temperature. The culture was filtered through a pre weighed Whatman No.1 filter paper. They were dried in a hot air oven at 60°C till constant weight and the mycelia dry weight was calculated.

Table 1 Cultural characteristics of *Trichoderma harzianum* native isolates

Isolates	Colony character		Colony growth (mm)	Conidia	
	Third day after inoculation	Seventh day after inoculation		Length (µm)	Breadth (µm)
Th1	White cottony mycelium	Deep green sporulation	85.00[d]	2.35-3.40	2.15-3.10
Th2	Moderate white mycelium	White to dull green sporulation	87.50[c]	2.70-3.50	2.25-3.45
Th3	Profuse white mycelium	Deep green sporulation	90.00[a]	3.07-3.60	2.45-3.67
Th4	Thin white cottony mycelium	Colony fluffy and green sporulation	80.70[e]	2.15-3.20	2.00-3.30
Th5	Moderate white scanty mycelium	White to dull green sporulation	88.40[b]	2.90-3.25	2.30-3.15

*Mean of three replications

*In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Table 2 Antagonistic activity of native *Trichoderma harzianum* isolates against *F.oxysporum* f.sp. *lycopersici* by Dual Culture Techniques

Isolates	Mycelial growth of *F. oxysporum* f.sp.*lycopersici* mm	Inhibition zone Mm	Per cent inhibition over control
Th_1	54.35[d]	6.25	39.61
Th_2	35.40[c]	7.20	60.66
Th_3	19.49[a]	.50	78.35
Th_4	65.17[e]	5.58	27.58
Th_5	21.53[b]	9.76	76.07
Control	90.00[f]	-	-

Cultural characteristics of native isolates of Trichoderma harzianum

The five isolates of *Trichoderma harzianum* was isolated from rhizosphere soil of different tomato growing areas in Krishnagiri district. The morphological character of Th3 showed profuse white mycelium with deep green sporulation Whereas, Th_2 and Th_5 produces moderate white scanty mycelium with white to dull green sporulation (Table 1). The maximum colony growth was observed in isolate Th_3 (90 mm) followed by Th_5 (88.40 mm), Th_2 (87.50 mm), Th_1 (85.00 mm) and Th_4 (80.70 mm). Several workers have reported similar variation in the mycelial growth of *Trichoderma viride* isolates [5]. Isolate of *Trichoderma viride* (GRT1, GRT6, GRT9) produced dark green to bluish green sporulation which was similar to the present research findings [6].

Table 3 Effect of cultural filtrate of *Trichoderma harzianum* on the mycelial growth and mycelial dry weight of *F. oxysporum* f. sp. *lycopersici*

Tr. No	Concentration of culture filtrate (%)	Solid medium		Liquid medium	
		Mycelia growth (mm)	Per cent inhibition over control (%)	Mycelia dry weight(mg)	Per cent inhibition over control (%)
T_1	10	37.50[e]	58.33	150.20[e]	51.09
T_2	20	28.67[d]	68.14	123.58[d]	59.76
T_3	30	17.48[c]	80.57	86.45[c]	71.85
T_4	40	10.95[b]	87.83	30.67[b]	90.01
T_5	50	0.00[a]	100	2.56[a]	99.16
T_6	Carbendazim 50%WP @0.1%	0.00[a]	100	2.47[a]	99.19
T_7	Control	90.00[f]	-	307.15[f]	-

*Mean of three replications

*In a column, means followed by a common letter are not significantly differ at 5% level by Duncan's multiple range test (DMRT)

Antagonistic activity of native Trichoderma harzianum isolates against F. oxysporum f.sp. lycopersici by Dual culture Technique

To test the antagonistic activity of native *Trichoderma harzianum* isolates against Fol through dual culture techniques and the data are recorded in (Table 2). The highest inhibition was recorded by the isolate Th_3, percent inhibition over control (78.35%) followed by Th_5 (76.07%) and Th2 (60.66%). The least inhibition was recorded in isolate Th4 (27.58%). The effective isolate Th_3 is used for further experiments. *Trichoderma harzianum* (ANR-1) effectively reduced the mycelial growth of *Fusarium oxysporum f.sp lycopersici* by

(53%) followed by isolates KGI-3 RTM-5 and KPI-9 recording an inhibition percentage of 38.12%, 31.11% and 27.22% [7]. *Trichoderma asperellum* was effective in inhibiting the Fol pathogen [8]. The highest inhibition percentage ranged between 68-71% from different isolates viz., TS 39, TS 12, TS 42, TS 9, TS 32 and TS 36 respectively. *Trichoderma harzianum* and *Trichoderma viride* effectively reduced the colony growth of Fol, with the inhibition percentage of (29.27% and 34.96%) respectively [9].

Effect of cultural filtrate of Trichoderma harzianum on the mycelial growth and mycelial dry weight of Fusarium oxysporum f. sp. lycopersici

The data presented in Table 3) showed the efficacy of the cultural filtrate of Th_3 isolate at different concentrations against test pathogen Fol by poisoned food technique. Cultural filtrate @ 50% concentration showed complete inhibition of mycelial growth and percent inhibition of mycelia growth over control recorded 100%, which was on par with the chemical check Carbendazim 50% WP @ 0.1%. In liquid broth of the cultural filtrate @ 50% concentration reduced the biomass production to 2.56 mg recording 99.16 per cent of inhibition over control. The culture filtrate of *Trichoderma viride* successfully inhibited the growth of some soil borne pathogens like *Sclerotium rolfsii*, *Macrophomina phaseolina*, and *Colletotrichum capsici* reducing the mycelial growth of all these pathogenic fungi by more than 50% [10]. The culture filtrate of *Trichoderma viride* and *Trichoderma harzianum* effectively reduced the colony growth of *Fusarium solani* by 21.35% and 17.3% respectively [11]. *Trichoderma viride* (TV8) isolates effectively reduce the colony growth of *Fusarium oxysporum f. sp. lycopersici* [12]. The culture filtrates @ concentrations of 10, 20, 30 and 40 percent recorded a colony growth of 36.87, 25.27, 10.85, 0.00mm in solid medium. In liquid medium, at 40% concentration of culture filtrate the mycelia dry weight was restricted to 2.39mg. These reports of earlier workers lend to support present findings.

SUMMARY

The present studies were undertaken to investigate the effect of fungal antagonistic isolates against tomato Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol). The five isolates of *Trichoderma harzianum* was isolated from rhizosphere soil of different tomato growing areas in Krishnagiri district, Tamil Nadu. The *Trichoderma harzianum* isolated showed different morphological characters. The highest inhibition of tomato Fusarium wilt pathogen was recorded by the *Trichoderma harzianum* isolate Th_3, followed by isolate Th_5 and Th_2. The least inhibition was recorded in isolate Th_4. Cultural filtrate Th_3 @ 50% concentration showed complete inhibition of Fol mycelial growth and percent inhibition of mycelia growth over control recorded 100%, which was on par with the comparison fungicide carbendazim 50% WP @ 0.1%.

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