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ORIGINAL ARTICLE **OPEN ACCESS**

Efficacy of bio-inoculant *Trichoderma asperellum* against Fusarium wilt of tomato incited by *Fusarium solani*

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ABSTRACT
Tomato (Solanum lycopersicum) is one of the most cultivated and popular vegetable crop across the world. Fusarium solani is predominant disease of tomato causing Fusarium wilt which occurs at any stage of the crop particularly flowering and fruiting stage and it causes yield losses up to 10-90%.Trichoderma is potential bio-agent and widely used in the management of soil borne diseases. The objective of this paper is to evacuate the efficacy of T. asperellum against Fusarium solani under invitro condition. The isolates of T. asperellum are designed as Ta_1 - Ta_{10}. The effective isolate Ta_9 recorded the maximum mycelial inhibition (Percent reduction over control) (80.81%) which is followed by the isolates Ta_5which recorded 76.31 per cent inhibition on the mycelial growth and the isolate Ta_7 recorded the minimum inhibition (52.62%). The poison food technique at 30% concentration recorded maximum percent inhibition then 20% and 10% concentration respectively.
Keywords*: Fusarium solani, Trichoderma asperellum*

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INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is a member of the Solanaceae family and one of the most significant vegetable crops grown worldwide due to their nutritional and commercial value (24). Central and South America are believed to be center of origin and diversity of the crop (27). It is a tropical annual herb which can be cultivated under wide range of soil and well adapted to all climatic zones around the globe. It is also called as wolf apple, love apple or poor man's orange (29). The red pigment carotenoid and lycopene, which are significant anti-oxidants, are very valuable constituents of tomato (26). Lycopene is a natural antioxidant produced only by the tomato that works effectively against the growth of the cancerous cells and responsible for their red color (31). India had a total of 0.9 million ha under cultivation for the tomato crop, producing 19.19 million tones with an average yield of 21.20 tons per ha (28). In Tamil Nadu, it is cultivated over 0.5M ha with an annual production of 1.5M tons (21) and they are cultivated in all seasons but Thaippattum (January- February) is most preferable season for its cultivation (33). Tomato is affected by numerous diseases caused by many different agents including fungi, fungus-like organisms, bacteria, viruses and phytoplasma, as well as physiological disorders (10). Among all the diseases of tomato, *Fusarium* wilt is a serious soil borne disease (19) and is caused by *Fusarium solani* and *Fusarium oxysporum* f.sp. l*ycopersici* (30). A genus *Fusarium* is a large group of hyaline filamentous fungi. The genus *Fusarium* was coined by Link in 1809 (20). The pathogen produces three types of asexual spores *viz.,* microconidia, macroconidia and chlamydospores (1). Disease emerges at temperatures of 28^0C in the soil (8). *Fusarium* wilt and nematode colonization are frequently related with nematodes acting as a fungus entry point (7).
Among the different available options for the management, chemicals are neither economically viable, nor safe for the environment. Biological management of plant disease will be given more emphasis using the microflora since chemical pesticides are causing hazards to environment as well as human health. Several biocontrol agents such as *Trichoderma viride, T. harzianum, Bacillus subtilis* were separately studied for the management of *Fusarium* wilt disease on several crop plants (18). Compared to other fungal biocontrol agents, *Trichoderma* is considered the genus most used to control plants diseases caused by fungi (41). *Trichoderma* spp. can produce a wide range of secondary metabolites that protect against phytopathogenic fungi through various mechanisms, including antibiosis and mycoparasitism (40). *Trichoderma* spp. exert their antagonistic effects by secreting antibiotics, such as herzianolide, trichodermin and trichodermol (4).

MATERIAL AND METHODS

Survey and disease assessment

An intensive survey was conducted during 2021 -2022 to assess the wilt incidence in different tomato growing areas *viz.*Krishnagiri, Dharmapuri, Namakkal, Trichy and Pudukkottai districts. In each village, four fields were selected and four plots in each field having an average area of one square meter were marked at random. Percentage of disease incidence was calculated using the following formula suggested by Mayee and Datar in 1986 (23).

$$\text{Disease Severity} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Isolation of *Fusarium solani*

The pathogen was isolated from the diseased plant parts of tomato plant by tissue segment method (34) collected during survey. The plant specimens were washed with tap water, the infected root parts cut into small pieces (5 mm), sterilized with 0.1% Sodium hypochlorite for two minutes and rinsed in sterilize water for three times. The sterilized root pieces were transferred on three replicated Petri dishes containing sterilized PDA (potato dextrose agar) @ 15 ml/plate and incubated at room temperature 28±2°C for 7 days. The fungal isolates were purified following hyphal tip technique (42).

Isolation of native fungi antagonist

For isolation of the *Trichoderma* strains, soil samples of the rhizosphere area were collected and dried at room temperature for eight days. A five- fold serial dilution was followed as described by Singh and Singh (39) to isolate fungal antagonist. From each sample 0.5 ml was poured on the surface of *Trichoderma* selective medium (9). The pure green-colored cultures were maintained on respective agar slants at 4°C. Identification of the antagonistic strains was achieved by morphological characterization of the colonies and conidia dimensions (36).

Dual culture technique (Dennis and Webster, 1971)

To test the efficacy of antagonistic fungus, 15 ml of sterilized melted PDA was plated in Petri plates (90 mm) and allowed to solidify. Mycelial discs measuring nine mm diameter from seven-day old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile Petri plate containing PDA medium. The Petri plate with pathogen inoculated at one end alone, served as control. The Petri plates were then incubated at 28±2°C. Three replications were maintained in each treatment. Growth of antagonists and pathogen were measured after recording full growth of the pathogen in control plate.

Bioassay of culture filtrates of *T. asperellum* on the mycelia growth of *Fusarium solani*

The effective *T. asperellum* isolates were grown for 7 days at room temperature (28 ± 2°C) in Erlenmeyer flasks containing 50 ml of sterilized potato dextrose broth. Then the cultures were filtered through Bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

Effect of culture filtrates on the mycelial growth of *Fusarium solani* (Poisoned food technique) (Groover and Moore, 1962)

The culture filtrates of the fungal antagonists were separately incorporated into sterile PDA medium at 10, 20 and 30 per cent by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @ 15 ml and allowed to solidify. Each plate was inoculated at the centre with a seven-day old (9 mm) PDA culture disc of *F. solani.* Three replications were maintained for each treatment. The diameter of the mycelial growth (in mm) of *F. solani* was measured after 7 days of incubation.

RESULT AND DISCUSSION

Morphological and cultural characteristics of *Fusarium solani* from major tomato growing areas of Tamil Nadu

The colony features of all ten isolates of *Fusarium solani* produced on potato dextrose agar medium varied, with cotton white fluffy growth on the front side and a creamy to dark brown color on the back side (Table 1). Fs_6, Fs_7, and Fs_9 had a cream color, whereas Fs_1, Fs_3, and Fs_5 had a brown color, Fs_2 and Fs_8 had a light brown color, and Fs_4 and Fs_{10} had a dark brown color. Among the ten isolates collected, oval and round-shaped microconidia, cylindrical and sickle-shaped macroconidia are identified. Gopi et al. 2016 (12) while studying variability of *F. solani* isolates found isolates with white sparse growth, three as white cottony growths, two as white fluffy and three as white dense growths. The size of macro conidia and micro conidia ranged from 26.91 to 57.64 × 2.01 to 2.59 µm and 5.62 to 8.44 × 1.86 to 2.71 µm, respectively. The shape of macroconidia is sickle shaped and elongated with blunt ends and microconidia is round to oval shaped. The chlamydospores were oval, intercalary and terminal among the isolates (3). Reddy et al. 2022 (35) observed that the size of the microconidia varied from 5.37 µm ×2.44 µm to 13.58 µm ×3.33 µm, and the size of the macroconidia varied from 16.62 µm ×3.35 µm to 39.05 µm ×4.61 µm.

Table 1: Morphological and cultural characteristics of *Fusarium solani* from major tomato growing areas of Tamil Nadu

S.No	Isolates	Locality	Mycelial growth rate (90cm) (7 DAI)	Conidial stage (10DAI)		Colony character of the plate	
				Micro conidia	Macro conidia	Front side	Rear side
1	Fs_1	Puliyampatti	81.64^{c}	Oval shaped	Sickle shaped	Cottony white	Brown
2	Fs_2	Palacode	85.39^{b}	Oval shaped	Sickle shaped	Cottony white	Light Brown
3	Fs_3	Vaiyampatti	76.96^{de}	Oval shaped	Sickle shaped	Cottony white	Brown
4	Fs_4	T. kovilpatti	82.24^{c}	Oval shaped	Sickle shaped	Cottony white	Dark Brown
5	Fs_5	Shoolagiri	74.59^{ef}	Oval shaped	Sickle shaped	Cottony white	Brown
6	Fs_6	Nedungal	90.00^{a}	Oval shaped	Cylindrical	Cottony white	Creamy
7	Fs_7	Vadavathur	70.24^{g}	Oval shaped	Sickle shaped	Cottony white	Creamy
8	Fs_8	Jambumadai	69.15^{g}	Oval shaped	Cylindrical	Cottony white	Light Brown
9	Fs_9	Vadakadu	72.18^{fg}	Oval shaped	Sickle shaped	Cottony white	Creamy
10	Fs_{10}	Kodikarambai	79.42^{cd}	Oval shaped	Sickle shaped	Cottony white	Dark Brown

Morphological characters of native *Trichoderma asperellum* isolates

Ten *Trichoderma* strains were isolated from different locations (Table 2). Among them Ta_9 collected from Vadakadu recorded maximum sporulation with conidia length of 2.72-3.28 µ and breadth of 2.43-3.44 µ followed by Ta_5 collected from Shoolagiri showed green to dark green sporulation with conidia length of 2.16-3.52µ and breadth of 1.68-3.07 µ.

Trichoderma had many white aerial mycelia, which gradually turned green with yellow pigmentation in *T. koningii* and dark green color in *T. hamntum* (28). Chaverri et al. (5) reported that the genus ***Trichoderma*** is a fast growth in culture medium and development of conidia with green-yellow color. Garcia-Nunez et al. (11) described several ***Trichoderma*** isolates that developed profuse fluffy mycelium and two to three fine defined concentric mycelium (white) and conidia (green) rings. Matas-baca et al. 2022 (22) observed that the microscopic view of ***Trichoderma*** sp. isolate showed dense conidia, branched conidiophores, ampuliform phialides and slightly globose conidia with yellow-green pigmentation.

Table 2: Morphological characters of native *Trichoderma asperellum* isolates

S.NO	Isolates	Locality	Colony morphology (7DAI)	Conidial size	
				Length (µm)	Breath (µm)
1	Ta_1	Puliyampatti	Green to Dark green sporulation	2.28-3.56	2.36-3.18
2	Ta_2	Palacode	Whitish Green to light green sporulation	2.37-3.62	2.18-3.47
3	Ta_3	Vaiyampatti	Dark green sporulation	2.18-3.47	1.89-3.26
4	Ta_4	T. kovilpatti	Green to bright green sporulation	2.81-3.78	2.58-3.20
5	Ta_5	Shoolagiri	Green to Dark green sporulation	2.16-3.52	1.68-3.07
6	Ta_6	Nedungal	Dark green sporulation	2.48-3.67	2.25-3.74
7	Ta_7	Vadavathur	Whitish Green to light green sporulation	2.16-3.91	1.79-3.64
8	Ta_8	Jambumadai	Green to Dark green sporulation	2.32-3.82	2.11-3.26
9	Ta_9	Vadakadu	Dark green sporulation	2.72-3.28	2.43-3.44
10	Ta_{10}	Kodikarambai	Dark green sporulation	2.91-3.62	2.55-3.81

Efficacy of *Trichoderma asperellum* isolates against *Fusarium solani* (Dual culture method)

The antagonistic activity of *Trichoderma asperellum* collected from different regions of tomato growing areas of Tamil Nadu were tested for their antagonistic activity against *Fusarium solani* by dual culture technique (Table

3). Among the tested isolates the *T. asperellum* **(**Ta_9) recorded the maximum inhibition zone (62.11%) followed by *T. asperellum* (Ta_5) which recorded 60.36per cent inhibition on the mycelial growth and the isolate *T. asperellum* (Ta_7) recorded the minimum inhibition (32.63%). *T. asperellum* produces primary metabolites that are precursors of antimicrobial compounds; it also produces a variety of antimicrobial secondary metabolites, including polyketides and alkanes (43). *Trichoderma* spp. had different percentages of inhibition against the tested pathogenic isolate with a range extended from 51.83 to 66.80% (37). The dual culture experiment indicated that the three *T. harzianum* species were able to repress the mycelium growth of *F. culmorum* with a great variability in the level of the antagonist potential among them (16). *Trichoderma asperellum* had the ability to inhibit the mycelial growth of *Fusarium solani* at 56.30% (32).

Table 3: Efficacy of *Trichoderma asperellum* isolates against *Fusarium solani* (Dual culture method)

S.No	Isolates	Mycelial growth (mm)		Percent reduction over control (%)
		Trichoderma asperellum	*Fusarium solani*	
1.	Ta_1	43.21^{e}	46.79	48.01
2.	Ta_2	52.78^{b}	37.22	58.64
3.	Ta_3	49.19^{c}	40.81	54.66
4.	Ta_4	41.78^{e}	48.22	46.42
5.	Ta_5	54.32^{ab}	35.68	60.36
6.	Ta_6	46.82^{d}	43.18	52.02
7.	Ta_7	39.37^{f}	60.63	32.63
8.	Ta_8	53.17^{b}	36.83	59.08
9.	Ta_9	56.26^{a}	33.74	62.11
10.	Ta_{10}	48.69^{cd}	41.31	54.10
11.	Control	-	90.00	-

Effect of nonvolatile compounds produced by *Trichoderma asperellum* isolate on the mycelial growth of *Fusarium solani* (Poisoned food technique)

The mycelial growth of *Fusarium solani* was found reduced with an increase in the concentration of culture filtrates of all the isolates of *Trichoderma asperellum* tested and the reduction was significantly the maximum in the isolate Ta_9 with 34.27, 28.38, 17.27 mm at 10, 20, 30 per cent concentration of the culture filtrate respectively as against the maximum growth of 90 mm mycelia growth of *Fusarium solani* in the control (Table 4). These results are in conformity with the earlier findings of Barari 2016 (2) who reported *Trichoderma harzianum* as most effective against *F.oxysporum* f. sp. *lycopersici* causing wilt disease in tomato crop. *Trichoderma* spp. induces pathogen inhibition by secreting secondary metabolites. Different *Trichoderma* spp. secretes different substances, including isonitrile, diketopiperazines, sesquiterpenes, stemids, polyketides, alkylpyrones, and peptaibols (14). Similarly, Hegde et al. (15) and Mishra et al. (25) reported the efficacy of *T. harzianum* against *F. oxysporum*, causing wilt disease in safflower, tomato, fir, tomato, onion and chilli crops. Kumar et al. 2019 (17) confirmed that the ability of *Trichoderma* species to produce the non-volatile substances and it was found most efficient in reducing the maximum mycelial growth of tested *Fusarium* isolates. It may be due to the presence of trichodermin, gliotoxins, viridin, cell wall-degrading enzymes

CONCLUSION

Ten strains of *Trichoderma* were collected from various *Fusarium* wilt infected tomato fields. Among the ten isolates, Ta_9 from vadakadu recorded the maximum inhibition zone against *Fusarium solani* in *invivo* with maximum sporulation. It may be due to the presence of trichodermin, gliotoxins, viridin, cell wall-degrading enzymes etc. Therefore, to minimize the severity of tomato wilt, it is always preferable to increase the *Trichoderma* population in *Fusarium* infected soil. Moreover, it can promote the plant growth and supports the properties of suppressive soil.

Table 4: Evaluation of non-volatile compounds produced by *Trichoderma asperellum* isolate on the mycelial growth of *Fusarium solani* (Poison food technique)

S.NO	Isolates	Mycelial growth (mm) (7DAI)					
		10%	Percent inhibition over control	20%	Percent inhibition over control	30%	Percent inhibition over control
1	Ta_1	54.92 g	38.98	52.29 g	41.90	39.14 f	56.51
2	Ta_2	42.34 d	52.96	40.23 d	55.30	29.28 d	67.47
3	Ta_3	47.98 e	46.69	46.89 e	47.90	32.72 e	63.64
4	Ta_4	57.36 h	36.27	54.26 h	39.7	40.28 fg	55.24
5	Ta_5	35.93 b	60.08	33.27 b	63.03	21.32 b	76.31
6	Ta_6	51.32 f	42.98	50.23 f	44.19	37.52 f	58.31
7	Ta_7	58.93 h	34.52	55.39 h	38.46	42.64 g	52.62
8	Ta_8	39.21 c	56.43	35.27 c	60.18	25.57 c	71.59
9	Ta_9	34.27 a	61.92	28.38 a	68.47	17.27 a	80.81
10	Ta_{10}	49.25 e	45.28	47.53 e	47.19	34.41 e	61.77
11	Control	90	-	90	-	90	-

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