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In vitro Screening of Plant Extracts, *Trichoderma harzianum* and Carbendazim against *Fusarium oxysporium* f. sp. Lycopersici on Tomato

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A B S T R A C T

Fusarium oxysporum f. Sp *lycopersici* is the causal organism that causes wilt disease in tomato all over the world. The antifungal activity of aqueous extract of neem leaf (5%), ginger bulb (5%), *Lantana camara* (5%), *Trichoderma harzianum* (5%), carbendazim (0.01%) and combination of carbendazim+*T. harzianum* (0.01+5%) was investigated against *Fusarium oxysporum* f. sp *lycopersici in vitro*.The least growth of pathogen was recorded in Carbendazim (treated control) (94.00) followed by Neem leaf (35.20) followed by *Lantana camara* (31.11), ginger bulb (26.31) and there was no growth in carbendazim treatment in poison food technique. All treatments were significantly decrease disease incidence.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is economically one of the most important and popular vegetables throughout the world including India (Neela *et al.,* 2014). Tomato is one of economically the most important vegetable crops in India where it is grown both, indoors and outdoors (Ignjatov *et al.,* 2012). Tomato is the second most important vegetable crop next to potato and generally used in soups and stews (Singh *et al.,* 1980). Globally, tomatoes are grown on in area of 45.2 lakh hectares with a production of 12.4 million metric tonnes. Indian contribution to the world's production was 11.97 million tonnes. Tomato crop was grown in area of 0.59 million hectare with a productivity of 19.97 tonnes per hectare. In Uttar Pradesh it occupied an area of 7600 hectare with an annual production of 92500 tones (Anonymus, 2009). Successful cultivation of tomato is hindered by various diseases. *Fusarium* wilt is one of the most serious diseases in tomato throughout the world, especially in upland (Charoenporn *et al.,* 2010). *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is a disease that causes serious economic loss. As compared to other plant parasites, fungi have the greatest impact with regard to diseases and crop production losses. In agriculture,

chemical fungicides are becoming ineffective due to the development of new physiological races of the pathogens (Ocamb *et al.,* 2007). The vegetable growers suffer more than 25.14 – 47.94 % crop losses due to *Fusarium* wilt of tomato in Uttar Pradesh (Enespa and Dwivedi, 2013).

Materials and Methods

Evaluation of leaf extracts and fungicide by Poisoned food technique

Five mm diameter of culture disc of *Fusarium* was kept at the center of each Petriplate of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 27ºC for ten days and colony diameter was recorded. Per cent inhibition of mycelial growth was calculated by using the formula given by (Vincent, 1947). The readings were taken from 24 to168 hrs after inoculation of the pathogen.

Evaluation of bio-agent by dual culture technique

Antagonistic microorganism like, *Trichoderma harzianum* was evaluated for its antagonistic properties against *F. oxysporum* by dual culture technique. Twenty millilitre of PDA was poured into sterile Petriplates. Fungal antagonist was evaluated by inoculating the pathogen at one side of the Petriplate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this actively growing cultures were used. One control was maintained where in only test fungus was grown (Sundaramoorthy and Balabaskar, 2013). The treatments were replicated three times. The plates were incubated for seven days at 27±1ºC. After incubation, the colony diameter of *F. oxysporum* was recorded. Per cent inhibition was calculated by using the formula given by Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100$$

Where,
PI = Per cent inhibition over control
C = Growth of test pathogen with absence of antagonist (mm).
T = Growth of test pathogen with antagonist (mm).

Results and Discussion

The initial day growth least radial growth of *Fusarium oxysporum* f. sp. *lycopersici* was observed in T_2 Neem leaf (5.33 mm) followed by T_4 *Lantana camara* (5.66 mm), and T_3Ginger bulb (6.16 mm) as compared to the treated check T_1 carbendazim (0) and untreated check T_0 (7.33 mm). Among the treatments (T_0), (T_3), (T_4 T_2), (T_1) are statistically non-significant at par with each other at 24 hours of inoculation. The final day radial growth of *Fusarium oxysporum* f. sp. *Lycopersici* was observed in T_2 Neem leaf (29.16 mm) followed by T_4*Lantana camara* (31.00 mm), T_3 Ginger bulb (33.16 mm) as compared to the treated check T_1 carbendazim (0) and untreated check T_0 (45.00 mm). At 144 hours of inoculation the treatments (T0), (T_3), (T_4), (T_2) are statistically non-significant at par with each other.

Maximum per cent inhibition of radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* was observed in T_2 Neem leaf (35.20%) followed by T_4 *Lantana camara* (31.11 %), T_3 Ginger bulb (26.31 %), as compared to the treated check T_1 carbendazim (94.00%) and T_0 untreated check (0%). However, the treatments were significant and statistically at par with each other. Minimum average radial growth of *Trichoderma harzianum* against *Fusarium oxysporum* was observed in T_1 *Trichoderma harzianum* (6.50 mm) followed and compared by T_0 control (7.33mm). Among the treatments (T_1T_0) are

statistically non-significant at par with each other at 24 hours of inoculation. Minimum average radial growth of *Trichoderma harzianum* against *Fusarium oxysporum* was observed in T_1*Trichodermaharzianum* (9.83 mm) followed and compared by T_0 control (13.67mm). Among the treatments (T_1T_0) are statistically non-significant at par with each other at 48 hours of inoculation (Tables 1, 2 and 3).

Table.1 Efficacy of leaf extracts and carbendazim against *Fusarium oxysporum* f. sp. lycopersici by poison food technique

S/N.	Treatments	Radial growth (mm) of *Fusarium oxysporum* f. sp. *Lycopersici*					
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
T_1	Carbendazim (Treated check)	0.00	0.00	0.00	0.00	0.00	0.00
T_2	Neem leaf	5.33	9.00	13.50	17.66	22.83	29.16
T_3	Ginger bulb	6.16	10.33	15.66	21.33	27.00	33.16
T_4	*Lantana camara*	5.66	9.16	15.00	20.16	25.50	31.00
T_0	Control (untreated check)	7.33	11.50	18.16	25.00	33.00	45.00
F-test		**S**	**S**	**S**	**S**	**S**	**S**
S E m=		**0.14**	**0.21**	**0.44**	**0.50**	**0.56**	**0.38**
CD (5%)		**0.47**	**0.66**	**1.38**	**1.57**	**1.78**	**1.19**

Table.2 Per cent inhibition of radial mycelial growth of *Fusarium oxysporum* as affected by treatments

Treatment No.	Treatments	Inhibition Percentage
T_1	Carbendazim (treated control)	94.00
T_2	Neem leaf	35.20
T_3	Ginger bulb	26.31
T_4	*Lantana camara*	31.11
T_0	Control (untreated check)	0.00
F- test		S
	S. Ed. (±)	0.32
	C. D. (P = 0.05)	1.03

Table.3 Mycelial growth (mm) of *Fusarium oxysporum* f. sp. *lycopersici* as affected by *Trichoderma harzianum* (dual culture technique)

S/N.	Treatments	Radial growth (mm) of *Fusarium oxysporum* f. sp. *lycopersici*					
		24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
T_1	*Trichoderma harzianum*	6.50	9.83	15.33	24.00	34.33	34.00
T_0	Control (untreated check)	7.33	13.67	23.50	28.17	36.50	38.00
F-test		**NS**	**NS**	**S**	**NS**	**NS**	**NS**
S E m=		**0.236**	**1.70**	**1.95**	**1.28**	**1.99**	**1.80**
CD (5%)		**0.925**	**6.69**	**7.66**	**5.02**	**7.82**	**7.07**

Plate.1 Growth of *Fusarium oxysporum*in food poison technique at 144 hours

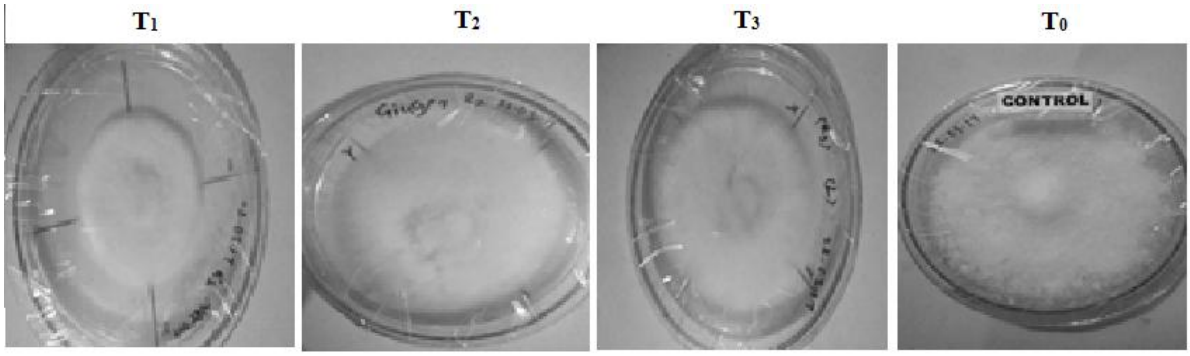
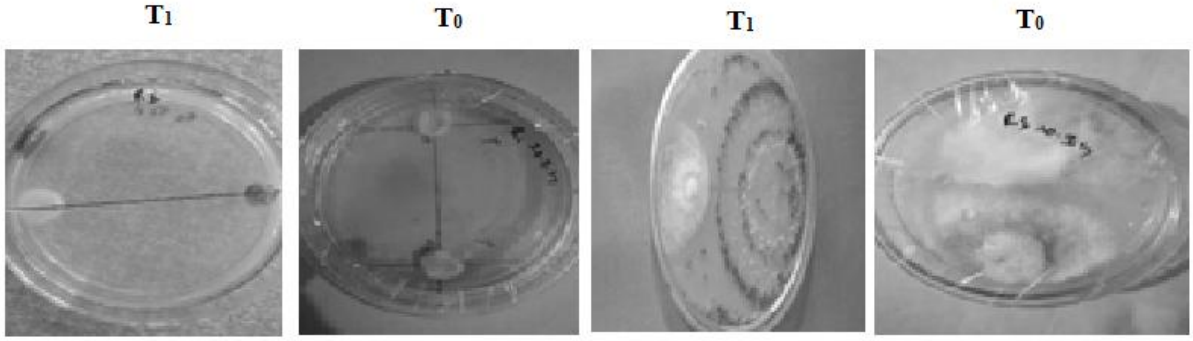


Plate.2 Evaluation of the antagonistic activity of *T. harzianum* on *F. oxysporium* in dual culture technique



Sundaramoorthy and Balabaskar (2013) also reported that *T. harzianum* (ANR-1) isolate exhibited least disease incidence (15.33%) among the fifteen *Trichoderma* isolates under *in vitro.* Rahmah *et al.,* (2013) also reported that *Lantana camera* and *Zizipusspina-christi* were not effective against soil borne fungi of tomato (*Fusarium oxysporium, Pythium aphanidermatum* and *Rhizoctonia solani*) under lab condition. Ali *et al.,* (2013) found that *Aloe-vera* was most effective one in inhibiting the mycelial growth of *F.o.* f.sp.

pisi followed by *Lantana camera* (50.09%). Sultana and Ghaffar (2013) reported that benlate and carbendazim completely inhibited the mycelial growth of *Fusarium oxysporuium* @ 100ppm in poisoned food technique.

Our results are in agreement with Addullah *et al.,* (2013) who reported that carbendazim treatment recorded lowest disease severity (16.9%) followed by *T. harzianum* (15.10%) and Neem leaf extract (20.70%) under pot condition.

Bokkhari *et al.,* (2008) reported that soil application of *T.harzianum* and Topsin-M showed that zero and 0.74% disease intensity respectively. Kouki *et al.,* (2012) revealed that *Trichoderma harzianum* (N-8) isolate showed stimulatory effect on the plant height (70.13cm) and dry weight (265.42g) in comparison to control (54.6cm and 195.5g).

Sundaramoorthy and Balabaskar (2013) who reported that the isolates of *Trichoderma harzianum* significantly enhanced seed germination, reduced disease incidence and promoted plant growth of tomato as compared to control.

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