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Antagonistic activity of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *lycopersici* causing Fusarium Wilt of Tomato

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ABSTRACT: Tomato wilt is most devastating disease of tomato, caused by *Fusarium oxysporum* f.sp. *lycopersici* that limiting tomato production severely all over the world. Biological control with *Trichoderma* spp. has emerged as one of the most promising alternatives to the chemical control. The objective of present investigation was to examine the antagonistic activity of five different *Trichoderma* spp. viz. *Trichoderma viride*-5 (IIHR-T.V.-5), *Trichoderma viride*-PCI (T.V. P.C.I.), *Trichoderma aureoviride* (DG. A. 91-5), *Trichoderma harzianum* (J.H.H.89-2) and *Trichoderma harzianum* (ITCC- 7922/10) in laboratory conditions by applying dual culture technique, against *Fusarium oxysporum* f.sp. *lycopersici* causing tomato wilt disease. *In-vitro* test showed that all the *Trichoderma* spp. were antagonistic to the *Fusarium oxysporum* f.sp. *lycopersici*. Among all, *Trichoderma viride*-5 (IIHR-T.V.-5) was found most effective in inhibiting the mycelial growth of the pathogen and showed highest antagonistic activity, followed by *Trichoderma viride*-PCI (T.V. PCI). Application of *Trichoderma* spp. in the field not only serve as disease controlling methods but also advantageous for the vegetative growth of the plant.

Keywords: Trichoderma, tomato, wilt, Fusarium oxysporum f.sp. lycopersici.

INTRODUCTION

Tomato (Lycopersicon esculentum Mill.) is also known as "poor man's apple" and "protective food" (Mehta, 2017). It belongs to the family Solanaceae and viewed as one of the world's most mainstream vegetables (Pritesh and Subramanian 2011). It is an important dietary component and contains high-value in local market among horticultural crops. The fruit contains good amount of nutrients such as, vitamins A (42 μ g), B₁ (0.037 μg), B₃ (0.594 μg), B₆ (0.08 μg), C (14mg), E (0.54 mg), K (7.9 mg), beta-carotene (449 µg) and fat (0.2 g), protein (0.9 g), dietary fibres (1.2g), lycopene (2573µg), sugars (2.6 g) water (94.5g) and carbohydrates (3.9 g), (nutritional value per 100 g) (Anon. 2020) and also contain some minerals such as calcium, phosphorus, potassium and magnesium (Kaushik et al., 2011). A number of biotic and abiotic factors affect the quality and productivity of tomato in which diseases caused by fungi, bacteria, viruses and nematode, are most important. Among which Fusarium wilt caused by Fusarium oxysporum f.sp. lycopersici affect all tomato growing areas all over the world and causes serious economic losses (Singh et al., 2015).

Fusarium wilt or vascular wilt disease of tomato, caused by Fusarium oxysporum f.sp. lycopersici (Sacc.) W.C. Snyder and H.N. Hans is one of the major constraints in tomato production. The disease was first reported by G.E. Massee in 1895 from England. First of all Link (1809) described the genus Fusarium and later classified in fungi imperfecti in the class Hyphomycetes. In India the disease was first reported by Butler, 1918 from Pusa, Bihar as Fusarium oxysporum f.sp. lycopersici according to Shiva et al. (2013). Snyder and Hans (2003) reported that Fusarium oxysporum f.sp. lycopersici (Sacc.) is a soil borne fungus and is specific for tomato causing wilt disease in it. Kapoor (1988) characterized disease by vellowing of leaves with wilted plants with minimum or absent of crop yield also stated that tomato wilt causes 30-40% yield loss and under favorable conditions losses increased to 80%. Biological control with Trichoderma spp. has emerged as one of the most promising alternatives to the chemical control. Application of *Trichoderma* spp. in the field not only serve as disease controlling methods but also advantageous for the vegetative growth of the plants.

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MATERIALS AND METHOD

Isolation, identification and pathogenecity of *Fusarium oxysporum* f.sp. *lycopersici*

Isolation of the pathogen was done by tissue isolation method from the diseased part of the tomato plant. The fungus isolated from diseased tissues was purified by hyphal tip method and identified on the basis of cultural and morphological characters and later confirmed from Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012. Pathogenicity of isolated culture was examined by growing tomato plants containing pathogen infested soil as per Koch's postulates.

Antagonistic activity of *Trichoderma* sps. against *Fusarium oxysporum* f.sp. *lycopersici*

The antagonistic activity of five *Trichoderma* sps. *viz.*, *Trichoderma viride*-5 (IIHR-T.V-5) procured from Indian Institute of Horticulture Research, Bengaluru, *Trichoderma viride*-PCI, procured from PCI: Pest Control India Pvt. Ltd., Bengaluru and three resident bioagent viz; *Trichoderma aureoviride* (DG.A. 91-5), Dungarpur, *Trichoderma harzianum* (JH.H.89-2) and *Trichoderma harzianum* (ITCC- 7922/10) procured from Udaipur were tested against wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici* by using dual culture technique.

Twenty ml of PDA was transferred in each petri plate aseptically and allowed to solidify. Mycelial disc of five mm of each antagonist and test fungus were placed separately at some distance on the periphery of petri plates containing solid PDA medium. A fungus growing pteri plate without antagonist was also kept as control, by placing one bit of the pathogen in the centre of the plate. Five replications of each treatment were maintained. Both inoculated and un-inoculated plates were incubated at $28 \pm 1^{\circ}C$ and observed after seven days to record the growth of antagonist and test pathogen. Antagonistic activity of each bio-control agent was calculated by measuring the linear growth of the test pathogen both in dual culture and control plates. Per cent growth inhibition of pathogen and Index of antagonism were determined in each treatment by using following standard formula (Bell et al., 1982):

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

Where, I= percent growth inhibition zone of pathogen C= Growth of test fungus in control (mm) T= Growth of test fungus in dual culture (mm).

RESULT AND DISCUSSION

Efficacy of five *Trichoderma* spp. viz. *Trichoderma* viride -5(IIHR-T.V-5) procured from Indian Institute of Horticulture Research, Bengaluru, *Trichoderma viride*-PCI, procured from PCI: Pest Control India Pvt. Ltd., Bengaluru and three resident bioagent viz; *Trichoderma aureoviride* (DG.A. 91-5), Dungarpur, *Trichoderma harzianum* (JH.H.89-2) and *Trichoderma harzianum* (ITCC- 7922/10) procured from Udaipur were tested against *Fusarium oxysporum* f.sp. *lycopersici* (UDPA Fo-1) by using dual culture technique.

All the five bio-control agents were significantly reduced the mycelial growth of the pathogen. Results depicted that minimum mycelial growth of pathogen, 19.00mm was found in *Trichoderma viride* -5 (IIHR-T.V-5) with maximum per cent growth inhibition 78.88% followed by *T. viride*-PCI (T.V.-PCI) with 22.60mm mycelial growth and 74.82% growth inhibition. Further, bio-agent *T. harzianum* (JH.H.89-2) showed 26.30mm mycelial growth with 70.77% growth inhibition of pathogen. Maximum mycelial growth of pathogen (30.00mm) was found in *T. aureoviride* (DG.A. 91-5) with minimum per cent growth inhibition (66.66%) followed by *T. harzianum* (ITCC- 7922/10) which showed 29.00mm mycelial growth and 67.77% growth inhibition of pathogen (Table 1, Plate1, Fig. 1).

The present findings are in conformity with Bhujbal et al. (2021), they tested antagonistic activity of six different bio-agents viz. Trichoderma viride, T. harzianum, T. hamatum, T. koningii, Bacillus subtilis and Pseudomonas fluorescens in in vitro conditions against Fusarium oxysprum f.sp. lycopersici. Among them, T. viride (84.84% growth inhibition) was found superior followed by T. harzianum (72.54% growth inhibition) and T. hamatum (69.93% growth inhibition) to inhibit fungal growth of pathogen. Similarly, Kumar et al. (2021) tested antagonistic activity of five bio control agents viz. T. harzianum, T. viride, Chaetomium globosum, Bacillus subtilis and Pseudomonas fluorescence by employing dual culture technique against Fusarium oxysporum f.sp. lentis causing vascular wilt of lentil. Among them, T. viride (76.25%) growth inhibition) found significantly superior followed by Chaetomium globosum (75% growth inhibition) after 144 hours of incubation. Reports of Sara et al. (2018); Devi et al. (2013) also support the present findings. Bio-control through Trichoderma spp. has great potential to reduce the disease in lab as well as in field conditions. Akrami and Yousefi (2015) stated that T. harzianum, T. aspergillum and T. virens has more protective effect against Fusarium solani and Fusarium oxysporum.

 Table 1: Effect of different bio-control agents on mycelial growth of Fusarium oxysporum f.sp. lycopersici in vitro.

Sr. No.					
	Bio-control agents		Mycelial growth (mm*)		Per cent Growth inhibition*
		Isolate code	Control	Dual culture	
1.	T. viride	IIHR-T.V5	90.0	19.00	78.88 (62.65)
2.	T. viride	T.V. P.C.I.	90.0	22.60	74.82 (59.88)
3.	T. aureoviride	D.G A. 91-5	90.0	30.00	66.66 (54.73)
4.	T. harzianum	J.H.H. 89-2	90.0	26.30	70.77 (57.27)
5.	T. harzianum	ITCC-7922/10	90.0	29.00	67.77 (55.39)
SEm±			1.981	0.542	0.548
CD at 5%			6.103	1.671	1.689
C.V. (%)			3.81	3.70	1.64

*Mean of four replications

Figure in parentheses are arcsine \sqrt{per} cent angular transformed values

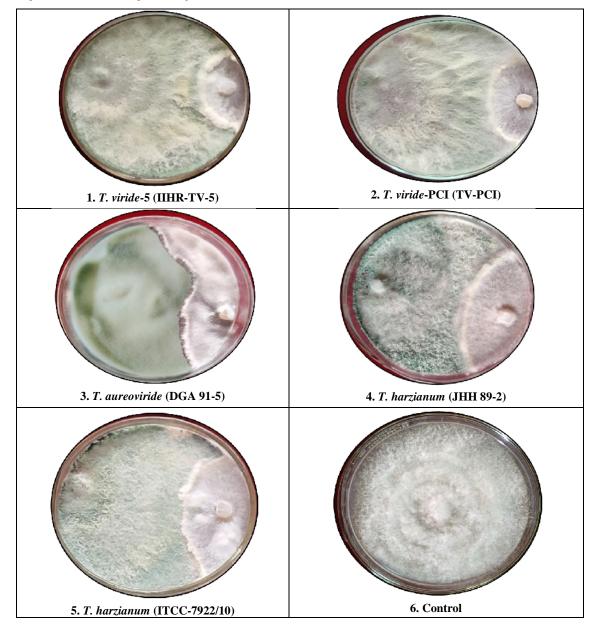


Plate 1: Antagonistic activity of *Trichoderma* sps. against *Fusarium oxysporum* f.sp. *lycopersici* by Dual culture method.

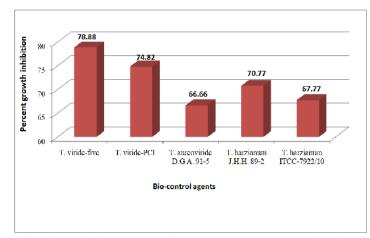


Fig. 1. Antagonistic activity of *Trichoderma* sps. against *Fusarium oxysporum* f.sp. *lycopersici* by Dual culture method.

CONCLUSION

Hence, from the present study and results, it is concluded that in *in vitro* efficacy of biological control agents *Trichoderma viride-5* was found to be most effective for the suppression of mycelia growth of tomato wilt pathogen *Fusarium oxysporum* f.sp. *lycopersici* followed by *Trichoderma viride*-PCI. The use of bio-control agent has proved economic and ecological alternative method to manage different plant disease in effective manner.

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