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Effectiveness of some Antagonistic Fungi and Botanicals against *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* Infecting Brinjal and Tomato Plants

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ABSTRACT

In the present study, the pathogenic fusaria *viz.*, *Fusarium solani* f. sp. *melongena* and *F. oxysporum* f. sp. *lycopersici* causing brinjal and tomato wilt were isolated from soil as well as from the infected plant parts. *In vitro* efficacy of three medicinal plants *viz.*, *Azadirachta indica* (leaf extract), *Psidium guajava* (leaf extract), *Eucalyptus camaldulensis* (bark extract) and three fungal antagonists *viz.*, *Trichoderma harzianum, T. atroviride* and *T. longibrachiatum* were tested at 25, 50 and 75% (v/v) by poisoned food technique against both the pathogens. The assessment of fungitoxicity was carried out in terms of percent mycelial growth inhibition against the test fungi. Among different medicinal plant extracts, *Azadirachta indica* (leaf) was found significantly superior to the rest in suppressing the growth of *F. oxysporum* f. sp. *lycopersici* as 100% inhibition was recorded at 50 and 75% concentration followed by *Psidium guajava* and *Eucalyptus camaldulensis* on 7th day of inoculation. On the other hand, among different microbial antagonists, *T. longibrachiatum* against both the test fungi was highly effective and there was 100% inhibition of mycelial growth at 50 and 75% concentration, while *T. harzianum* was effective against *F. oxysporum* f. sp. *lycopersici* followed by *T. atroviride* as it completely inhibited the mycelial growth at 75% concentration.

Key words: Wilt disease, *F. solani*, *F. oxysporum* f. sp. *lycopersici*, medicinal plants, fungal antagonists

INTRODUCTION

The Fusarium wilt of tomato (*Lycopersicion esculantum* Mill) caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen (FOL) and Brinjal wilt (*Solanum melongena* L.) caused by *Fusarium solani* f. sp. *melongena* is recognized as a devastating disease in tomato and brinjal growing regions worldwide (Beckman, 1987; Bondad-Reantaso *et al*., 2005) besides in different regions of India in severe to moderate (50-60%) form (Sherf and Macnab, 1986; Jiskani *et al*., 2007; Chakraborty and Chatterjee, 2009). In Kanpur (U. P.), the vegetable growers suffer more than 25.14-47.94% crop losses due to *Fusarium* wilt of tomato and 18.61-33.37% due to *Fusarium* wilt of brinjal in heavily infested fields. Kapoor (2008) has reported that most of the common varieties of tomato and brinjal are susceptible and fungicides are frequently used to control the disease.

However, the soil-borne disease is very difficult and uneconomical to control with chemicals alone. In this context, biological control is an alternative and eco-friendly strategy for disease management (Bowers and Locke, 2000; Momin and Nair, 2001; Eziashi *et al*., 2007).

The extract of medicinal plants (Daferera *et al*., 2000; Manczinger *et al*., 2002; Sridhar *et al*., 2003; Dwivedi and Enespa, 2012) as well as antimicrobial agents (Haggag and Mohamed, 2007; Dwivedi and Enespa, 2013) have been found effective as antifungal agent. The leaf extract of *Azadirachta indica* showed *in vitro* antifungal effect against *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* (Jarvis and Morgan, 2000; Dai *et al.,* 2001; Verma and Kharwar, 2006; Agbenin and Marley, 2006). The antifungal effect (bark extract) of *Eucalyptus camaldulensis* (Pattanaik *et al*., 2002), leaf extract of *Psidium guajava* (Khayungarnnawee *et al*., 2004) against *F. solani* and *F. oxysporum* f. sp. *lycopersici* have also been studied.

On the other hand, the antagonistic fungi especially *Trichoderma* spp. *viz*., *T. harzianum*, *T. atroviride* and *T. longibrachiatum* have been widely used against *F. solani* and *F. oxysporum* f. sp. *lycopersici* (Ahmed, 2011; Hend and Perveen, 2012). Christopher *et al*. (2010) have reported successful reduction of fusarial wilt in many crops with application of different species of *Trichoderma*. Rini and Sulochana (2007) and Kapoor (2008) have found *Trichoderma* spp. to be effective biocontrol agents against *F. solani* and *F. oxysporum*. However, it is also reported that all the isolates of *Trichoderma* spp. are not equally effective in the control of the pathogens *in vitro* and *in vivo* (Moussa *et al*., 2006; Morsy *et al*., 2009). Therefore, a specific effective native *Trichoderma* isolate has to be identified for successful control of a particular pathogen. The purpose of this study was to evaluate the efficacy of medicinal plants as well as fungal antagonists (*Trichoderma* spp.) against *F. solani* and *F. oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Test fungal strains: The pathogenic fusaria *viz*., *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* and fungal antagonists *viz*., *Trichoderma harzianum*, *T. atroviride* and *T. longibrachiatum* were isolated from the wilt affected (soil+plant parts) and healthy tomato and brinjal crop fields of Kanpur, Uttar Pradesh, India, using serial dilution technique as well as direct inoculation of plant parts. The cultures of the fungi were purified by single spore isolation technique on Czapek's Dox Agar medium (Chauhan *et al*., 2002). The isolated pathogenic and biocontrol mycoflora were identified on the basis of cultural and morphological characters (Gilman, 1957; Barnett and Hunter, 1972; Booth, 1985). The fungitoxicity was studied by poisoned food technique (Grover and Moore, 1962).

Preparation of crude plant extracts: Fresh plant materials of *Azadirachta indica* (leaves), *Psidium guajava* (leaves), *Eucalyptus camaldulensis* (bark) were washed thoroughly under running tap water followed by sterilized distilled water. The leaves were air dried and then grinded with the help of pestle and mortar. One mL of extract was added in 1ml distilled water (1:1 v/v) separately for each plant extract; filtered through muslin cloth and 100% plant extract solution was prepared. The extracts were poured in the flasks and further diluted to different concentrations (25, 50 and 75%) by adding sterile distilled water for further studies.

Determination of mycelial growth inhibition

Dry weight method: One hundred ml of broth amended with different filtrate of antagonistic fungi individually were inoculated with 6.0 mm agar discs in 250 mL Erlenmeyer flask. The flasks were then incubated at 25±2°C for 10 days. The fungal mats were removed by filtration (Whatman Filter Study 1 and then 42 Nos.), dried at 60±3°C for 24 h and dry weight (g) was recorded.

Linear growth method: The filtrates of the antagonistic *Trichoderma* spp. *viz.*, *T. harzianum*, *T. atroviride* and *T. longibrachiatum* and extracts of medicinal plant *viz.*, *Azadirachta indica* (leaves), *Psidium guajava* (leaves), *Eucalyptus camaldulensis* (bark) were added to autoclaved CZA medium to give fungal concentration of 25, 50 and 75% (v/v). The plates were inoculated with 6.0 mm disc of *F. solani* and *F. oxysporum* f. sp. *lycopersici* individually in the centre of each of the Petri plates and incubated at 25±2°C for 7 days. Three replicates were maintained for each treatment. The growth of fungus was measured on 7th day and mean of colony growth dia. (mm) was recorded and percentage of mycelial growth inhibition was calculated as compared to control (Gaspar *et al.*, 2004).

Statistical analysis: The Data recorded during the course of investigation was subjected to two-way ANOVA without replication. The conclusion was drawn on the basis of analysis of variance. The calculated value of F was compared with Table value of F at 5% levels of significance for an appropriate degree of freedom.

RESULTS AND DISCUSSION

Evaluation of medicinal plants against fungal pathogens: The mean fungal radial growth (mm) for each treatment is presented in (Table 1) indicate the inhibition zone of different extracts. All the medicinal plant extracts showed significant inhibition in the mycelial growth of the test pathogens. The *A. indica* leaf extract against *F. oxysporum* f. sp. *lycopersici* was most effective as it completely inhibited the mycelial growth at the concentration of 50 and 75%. *A. indica* leaf extract at 75% concentration was also most effective against *F. solani* on 7th day of inoculation. The growth of *Fusarium solani* was inhibited at 25% concentration of *Azadirachta indica* (leaves),

Table 1: Efficacy of medicinal plant extracts against *Fusarium solani* f. sp. *melongena* and *Fusarium oxysporum* f. sp. *lycopersici* at different concentration on 7th day of inoculation

		Pathogens/mean colony dia. (mm)	
Medicinal plants	Concentration (%)	FS	FOL
Azadirachta indica (leaf extract)	25	26.75±1.26	11.50±0.58 aII
	50	18.75±1.50	0.00±0.00 aI
	75	7.25±0.96 aI	0.00±0.00 aI
Psidium guajava (leaf extract)	25	53.75±1.50	58.50±2.38
	50	47.00±2.94	47.75±1.50
	75	44.25±0.00	45.50±0.57
Eucalyptus camaldulensis (bark extract)	25	70.00±1.41	71.75±1.50
	50	56.50±1.73	65.75±0.96
	75	15.50±1.00 aII	62.75±0.50
Control		73.25±0.95	73.00±1.41

Values shown are the Mean±SE of 3 replicates, significant at $p \leq 0.05$. Application of *A. indica* on *F. oxysporum* f. sp. *lycopersici* at 50 and 75% concentration had highest inhibiting effect followed by 75% conc. in *A. indica* on *F. solani*. These biopesticides checked fungal growth by 100, 100 and 90.10%, respectively

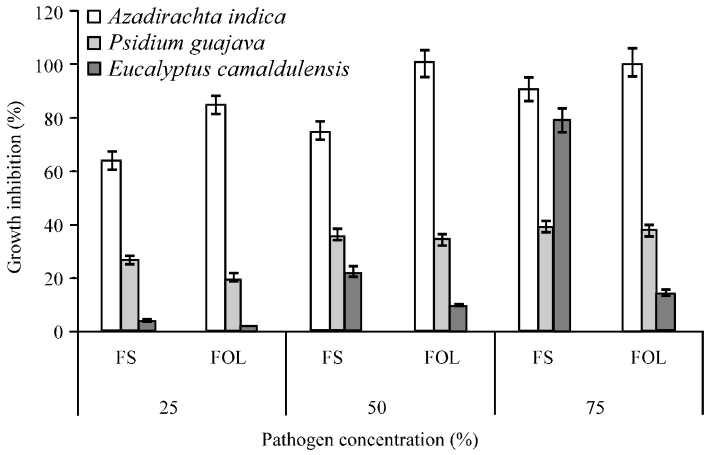

Fig. 1: Effect of medicinal plant extracts on radial growth of *F. solani* and *F. oxysporum* f. sp. *lycopersici* on 7th day of incubation

Psidium guajava (leaves) and *Eucalyptus camaldulensis* (bark) by 63.48, 26.62 and 4.44%, respectively. On the other hand, at 50% concentration of the foresaid medicinal plants inhibited the mycelial growth of *Fusarium solani* by 74.40, 35.84 and 22.87%, respectively. However, maximum inhibition i.e., 90.10, 39.59 and 78.84% was recorded at 75% concentration. The foresaid medicinal plants at 25% concentration inhibited the mycelia growth of *Fusarium oxysporum* f. sp. *lycopersici* by 84.25, 19.86 and 1.72%, respectively. On the other hand, at 50% concentration of these plants inhibited mycelial growth by 100.00, 34.59 and 9.93%, respectively. Maximum percentage inhibition i.e., 100.00, 37.67 and 14.04% was recorded at 75% concentration of *Azadirachta indica* (leaves) against *F. oxysporum* f. sp. *lycopersici* as indicated in Fig. 1.

Our findings are in conformity with Joseph and Priya (2010) with respect to efficacy of guava leaf extract against *Aspergillus niger* and *Aspergillus aculeatus*. Similar results were reported (Mossini *et al*., 2004; Niaz and Kazmi, 2005) with the increase in concentration of the medicinal plants, the greater inhibition of mycelial growth of *Fusarium* spp. and *Aspergillus alternata* at 0.15 and 0.1% concentration. Antifungal activities and chemical properties of *Azadirachta indica* oil and leaf extracts on the growth of *Aspergillus*, *Rhizopus, Alternaria solani* and *Alternaria brassicae* have been also reported (Mondali *et al*., 2009; Suleiman, 2010; Sasode *et al*., 2012). Bajwa and Ifftikhar (2005) evaluated *Eucalyptus camaldulensis* and D*atura tetramera* against fungal pathogens causing wilt in vegetable crops.

Evaluation of *Trichoderma* spp. against fungal pathogens: The percentage reduction of *F. solani* and *F. oxysporum* at different concentration of culture filtrates of *T. harzianum, T. atroviride* and *T. longibrachiatum* is presented in Table 2. The data revealed a significance increase ($p<0.001$) in colony growth reduction of *F. solani* and *F. oxysporum* f. sp. *lycopersici* with increasing the concentration of both the fungal filtrates. *T. harzianum, T. atroviride* and *T. longibrachiatum* filtrates inhibited the growth of *F. solani* by 84.01, 48.91 and 100% and *F. oxysporum* f. sp. *lycopersici* by 100.00, 73.67 and 100.00%, respectively at 75% concentration. At 50% concentration, the foresaid antagonists inhibited the growth of *F. solani* by 74.88, 44.06 and 86.87% and *F. oxysporum* f. sp. *lycopersici* by 92.35, 65.74 and 100.00%, respectively. At lower concentration (25%), *T. harzianum*, *T. atroviride* and *T. longibrachiatum* were least effective and

Table 2: Effect of *Trichoderma* spp. against *Fusarium solani* (FS) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at different concentration on 7th day of inoculation

Fungal antagonists	Concentration (%)	Pathogens/mean colony diameter (mm) FS	Pathogens/mean colony diameter (mm) FOL	Mycelial dry weight (g) of fungal antagonists
T. richoderma harzianum	25	46.25±0.96	19.75±2.50	2.70
	50	22.00±1.82	6.75±0.95 aII	
	75	14.00±1.82 aII	0.00±0.00 aI	
T. richoderma atroviride	25	50.75±0.96	37.75±1.50	1.48
	50	49.00±0.81	30.25±0.50	
	75	44.75±0.50	23.25±1.26	
T. richoderma longibrachiatum	25	21.75±1.26	18.75±0.96	2.16
	50	11.50±0.58 aII	0.00±0.00 aI	
	75	0.00±0.00 aI	0.00±0.00 aI	
Control		87.60±0.14	88.30±0.41	

Values shown are the Mean±SD of 3 replicates, significant at $p \leq 0.05$. Application of *T. longibrachiatum* at 50 and 75% concentration had highest inhibiting effect on both the pathogens followed by *T. harzianum* at 75% concentration on *Fusarium oxysporum*. These fungal antagonists checked fungal growth by 100, 100 and 100%, respectively

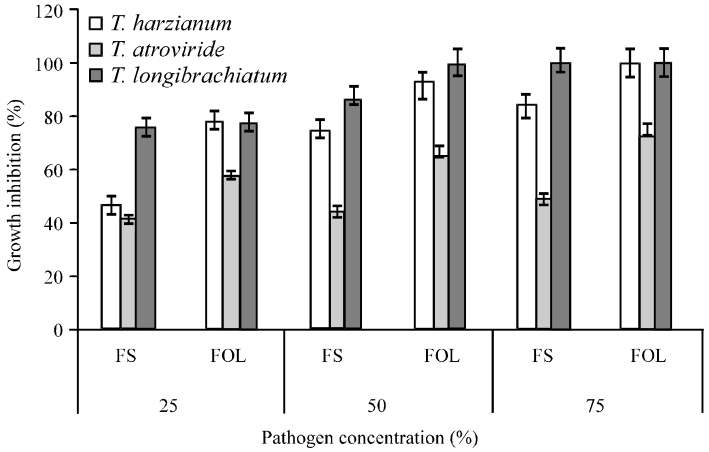

Fig. 2: Percentage inhibition of *F. solani* f. sp. *melongena* and *F. oxysporum* f. sp. *lycopersici* at different concentration of *Trichoderma* spp. on 7th day of inoculation

inhibited the growth of *F. solani* by 47.20, 42.06 and 75.17% and *F. oxysporum* f. sp. *lycopersici* by 77.63, 57.25 and 78.76%, respectively compared to control on 7th day of inoculation (Fig. 2)

Our findings are in conformity with Devi and Singh (2012) with respect to *in vitro* efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici* causing tomato wilt. Antagonistic activity of three isolates of *Trichoderma* spp. against *F. solani* and *F. oxysporum* f. sp. *lycopersici* due to parasitism, competition and antibiosis was reported by Elad (2000); Freeman *et al.* (2004); Dubey *et al.* (2007); Anwar *et al.* (2008); coiling and penetration of antagonistic hyphae of *T. virens* and *T. harzianum* around the hyphae of *F. solani* and their lysis (Kumar and Dubey, 2001; Zaldivar *et al.*, 2001) and production of organic metabolites (Haggag and Mohamed, 2007). It was observed that with the increase in concentration of the culture filtrates of *Trichoderma* spp. there was greater inhibition of the mycelial growth of *Rhizoctonia solani*, *Sclerotinia*, *Fusarium*, *Pythium* (Ashrafizadeh *et al.*, 2005; Yigit and Dikilitas, 2007; Khan and Sinha, 2007).

CONCLUSION

In the present study, the medicinal plant (*Azadirachta indica*) leaf extract and fungal antagonists *viz.*, *T. longibrachiatum* and *T. harzianum* were most effective against both the pathogens. Therefore, these bio-pesticides (botanicals and bio-agents) can be used as an alternative to pesticides to minimize the wilt disease of these crops besides improving the yield as they are environmental safe.

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