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IN VITRO EFFICACY OF SOME FUNGAL ANTAGONISTS AGAINST *FUSARIUM SOLANI* AND *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* CAUSING BRINJAL AND TOMATO WILT

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ABSTRACT

Wilt of *Solanum melongena* and *Lycopersicon esculantum* are very serious soil - borne diseases caused by *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici*. A laboratory study was undertaken to study the possibility of controlling the disease using eight biocontrol agents viz., four species of *Aspergillus* (*A. niger, A. flavus, A. sulphureus, A. luchuensis),* two species of *Trichoderma (T. viride, T. koningii)* and two species of *Penicillium (P. citrinum, P. italicum).* The assessment of fungitoxicity was carried out by poisoned food technique at three different concentrations i.e., 25, 50, 75% (v/v) against the test fungi. Assessment was carried out in terms of percent mycelial growth inhibition. All the bioagents showed significant reduction in the growth of the pathogens. Among different bioagents, *Aspergillus luchuensis* against *Fusarium oxysporum* f. sp. *lycopersici* was found significantly superior to the rest in checking the growth of pathogen and showed 100% inhibition at all the concentrations, while *Aspergillus niger* against *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* was most effective and completly inhibited the mycelial growth at 50 and 75% concentration. On the other hand, *Aspergillus luchuensis* against *Fusarium solani*; and *Aspergillus sulphureus* against *Fusarium oxysporum* f. sp. *lycopersici* was most effective and completly inhibited the mycelial growth at 75% concentration followed by *A. flavus, T. koningii, T. viride, P. italicum* and *P. citrinum*.

Key Words: *Fusarium solani, Fusarium oxysporum* f. sp. *lycopersici*, Wilt disease, Fungal antagonists.

INTRODUCTION

The excessive misuse of a wide range of chemical fungicides is being used to suppress the disease but these chemicals have a negative impact on human health and are hazardous to the environment (Özgönen *et al*., 2001). A better alternative of chemicals are the soil microbes such as *Trichoderma, Penicillium* and *Aspergillus spp.* etc. residing in the rhizosphere of crop plants that have the ability to suppress the pathogens (Hyakumachi *et al.*, 1994; Fravel *et al*., 2003) and stimulate plant growth by the production of phytohormones (Hasan, 2002).

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The antagonistic nature of *T. virens* and *Aspergillus* against *Phytophthora capsici* causing foot- root disease of black pepper has been reported (Noveriza *et al*., 2004). Metabolites of *T. harzianum. T. viride* and *T. virens* have been found to inhibit the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* causing wilt disease in chick pea (Dubey *et al*., 2007).

There were many reports on bio-control agents to control *Fusarium* wilt pathogen; some bioactive compounds which were extracted from antagonistic fungi have been found to inhibit *Fusarium* wilt of tomato and brinjal (Kanokmedhakul *et al*., 2003, 2006; Khan *et al*., 2007).

The application of *Trichoderma* species can control a large number of foliar and soil- borne fungi i.e. *Fusarium*

spp., *Rhizoctonia solani, Pythium* spp., *Sclerotium rolfsii* in vegetables, fruit and industrial crops (Ngo *et al.*, 2006). *Trichoderma* is directed to achieve effective mycoparasitic strains as biocontrol agents against plant fungal pathogens under a wide range of adverse environmental conditions (Manczinger *et al.*, 2002).

The *F. oxysporum* f. sp. *lycopersici* and *F. solani* are major pathogens which causes wilt disease and also economic losses in tomato and brinjal crops (Snyder and Hansen, 1940; Bondad-Reantaso *et al*., 2005). Keeping in view the hazardous nature of chemicals that are being presently used to control wilt diseases in these crops, the present study was undertaken to evaluate the antagonistic potentiality of naturally occurring *Trichoderma* spp., *i.e., T. koningii* and *T. viride*; *Penicillium spp., i.e., P. italicum, P. citrinum* and *Aspergillus spp., i.e., Aspergillus flavus, A. niger, A. luchuensis, A. sulphureus* as bio-control agents against *F. oxysporum* f. sp. *lycopersici and Fusarium solani*, the causal organism of wilt disease in tomato and brinjal respectively.

MATERIALS AND METHODS

Fungal isolates and growth conditions

The isolate of *Trichoderma spp., Penicillium spp.,* and *Aspergillus spp.* were used throughout the study. *Fusarium oxysporum* f. sp. *lycopersici* Schelect and *Fusarium solani* f. sp. *melongena* were isolated from diseased tomato and brinjal plants. Fungal isolates were maintained on Czapek's Dox agar medium (CZA) (Thom and Raper, 1945) at 25±2°C. Czapek's Dox broth (CZB) medium was used to harvest the fungal culture filtrates.

Test fungal strains

The pathogenic fungus, *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium solani* were isolated from the wilt affected tomato and brinjal plants and from soil also. The antagonists *Trichoderma* spp., (*T. koningii* and *T. viride*), *Penicillium spp*., (*P. italicum, P. citrinum*) and *Aspergillus spp*., (*Aspergillus flavus, A. niger, A. luchuensis*) were isolated from the tomato and brinjal crop field using standard pathological techniques. The medium Czapek's Dox agar was used throughout the study. The fungitoxicity was studied by poisoned food technique (Grover and Moore, 1962).

Pathogenic fungal growth measurement

Dry weight method

One hundred ml of broth amended with different filtrate of antagonistic fungi individually in 250 ml Erlenmeyer flask were inoculated with 5 mm agar discs. The flasks were inoculated with 5 mm discs. The flasks were incubated at 25±2°C for 7-10 days. The fungal mats were removed by filtration (Whatman no. one and then 42 filter paper) and dried at 60±3°C for 24 hour; dry weight was recorded as g.

Linear growth method

The filtrates of the eight antagonistic fungi were taken under sterilized condition and added to autoclaved CZA medium to give fungal concentration of 25, 50 and 75% (v/v). The plates were inoculated with 5 mm disc of *F. solani* and *F. oxysporum* f. sp. *lycopersici* separately in the centre of each of the plate. Plates were incubated at 25±2°C for 7 days. Three replicates were maintained in each of the experiment. The growth of fungus was measured on 7th day and mean of colony growth dia (mm) was recorded and percentage reduction was calculated as compared to control (Gaspar *et al*., 2004).

Statistical analysis

All values were expressed as mean ± SD, n = 3 and the results on the effect of different filtrates were analysed by analysis of variance (two-way ANOVA with replication), P= 0.001 was considered statistically significant. Statistical evaluation was carried out using SAS system and the mean values were compared using the Least Significant Difference (LSD) at P<0.05.

RESULTS

The antagonistic activity of *Aspergillus* spp., *i.e., A. flavus, A. luchuensis, A. niger, A. sulphureus, Penicillium* spp. *i.e., P.* citrinum, *P. italicum* and *Trichoderma* spp., *i.e., T. Koningii and T. viride* against *Fusarium solani* and *Fusarium oxysporum* f. sp. *lycopersici* showed reduction in the growth of pathogens (P<0.05). The *Aspergillus spp.* showed best ability to inhibit the pathogens compared to *Trichoderma* and *Penicillium* species. Amongst then *Aspergillus luchuensis* against *Fusarium oxysporum* f. sp. *lycopersici* was found significantly superior to the rest in checking the growth of pathogens and showed 100% inhibition at all the concentrations (25, 50 and 75%).

Growth reduction of *F. solani* and *F. oxysporum* f. sp. *lycopersici* by *A. flavus, A. luchuensis, A. niger* and *A. sulphurous*

The filtrates of *A. luchuensis, A. niger, A. flavus* and *A. sulphureus* showed a good potency against the growth of *F. solani* and *F. oxysporum* f. sp. *lycopersici* (Table 1). It was clear that all concentrations had inhibitory effects on the fungal growth and caused appreciable reduction in the colony diameter of the pathogens. The reduction in colony diameter increased with the increase in concentration of fungal filtrates. The highest concentration (75%) of *A. luchuensis, A.niger, A. flavus and A. sulphureus* filtrates revealed a significant (P<0.001) reduction in colony diameter of the *F. solani* (100, 100, 72.91 and 83.98%, respectively). At 50% concentration of the same antagonists against *F. solani*, the reduction of colony diameter was 93.82, 100, 59.37 and 76.67% (P<0.001) respectively. Even at low concentration (25%), the results showed the high efficacy of *A. luchuensis*

(88.04%), *A. niger* (75.72%), *A. flavus* (67.71%) and *A. sulphureus* (53.05%) significant at (P<0.01) to suppress the growth of *F. solani* (Figure 1).

On the other hand, the highest concentration of *A. luchuensis, A.niger, A. flavus* and *A. sulphureus* filtrates (75%) revealed a significant (P<0.001) reduction in colony diameter of the *F. oxysporum* f. sp. *lycopersici* (100, 100, 88.08 and 100%, respectively). At 50% concentration of the same antagonists against *F. oxysporum* f. sp. *lycopersici*, the reduction in colony diameter was 100, 100, 81.66 and 84.36%, respectively. While at low concentration (25%), the results showed that the growth of *F. oxysporum* f. sp. *lycopersici* was suppressed 100% by *A. luchuensis*, 81.52% by *A. niger*, 72.71% by *A. flavus* and 59.38% by *A. sulphurous* (Figure 2).

Growth reduction of *F. solani and F. oxysporum* f. sp. *lycopersici* by *P. citrinum* and *P. italicum*

The percentage reduction in colony growth of *F. solani* and *F. oxysporum* by different culture filtrates of *P. citrinum* and *P. italicum* 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 concentration of both the fungal filtrates. *P. citrinum* and *P. italicum* at 75% concentration inhibited the growth of *F. solani* by 42.09 and 40.91%, and *F. oxysporum* f. sp. *lycopersici* by 67.54 and 66.48%, respectively. While at 50% concentration, *P. citrinum* and *P. italicum* inhibited the growth of *F. solani* by 40.57 and 37.71% and *F. oxysporum* f. sp. *lycopersici* by 59.62 and 56.38%, respectively. On the other hand, at lower concentration (25%), *P. citrinum* and *P. italicum* were least effective and inhibited the growth of *F. solani* by 36.57 and 37.03% and *F. oxysporum* f. sp. *lycopersici* by 52.76 and 48.57%, respectively compared to control after 7 days of inoculation (Figure 3).

Growth reduction of *F. solani and F. oxysporum* f. sp. *lycopersici* by *T. Koningii and T. viride*

The percentage reduction *F. solani* and *F. oxysporum* at different concentration of culture filtrates of *T. Koningii and T. viride* is presented in Table 3. The data revealed a significance increase (P<0.001) in colony growth reduction of *F. solani* and *F. oxysporum* f. sp. *lycopersici* with increasing both the concentration of fungal filtrates. *T. Koningii* and *T. viride* filtrates inhibited the growth of *F. solani* by 64.49 and 64.18% and *F. oxysporum* f. sp. *lycopersici* by 65.26 and 62.64% respectively at 75% concentration. While at 50% concentration, *T. koningii* and *T. viride* inhibited the growth of *F. solani* by 49.71 and 51.86%, and *F. oxysporum* f. sp. *lycopersici* by 51.51 and 50.61% respectively. On the other hand, at lower concentration (25%), *T. Koningii* and *T. viride* were least effective and inhibited the growth of *F. solani* by 39.05 (P<0.01) and 39.00%, and *F. oxysporum* f. sp. *lycopersici* by 39.01 and 36.70%, respectively compared to control after 7 days of inoculation (Figure 4).

Table 1. Effect of *Aspergillus spp.* against *Fusarium solani* (FS) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at different concentration after 7 days of inoculation

Fungal antagonists	Pathogens	Concentration (%) / Mean colony dia.(mm)			Control	Oven dry weight of Fungal antagonists
		25	50	75		
Aspergillus flavus	FS	25.83±1.44	32.50±2.50	21.67±1.44	80.00±0.00	2.009 g
	FOL	21.83±0.76	14.67±0.29	9.53±1.15	80.00±0.00	
Aspergillus luchuensis	FS	10.00±0.00	5.17±0.29	0.00±0.00	83.67±3.40	2.68 g
	FOL	0.00±0.00	0.00±0.00	0.00±0.00	83.83±3.21	
Aspergillus niger	FS	19.67±0.58	0.00±0.00	0.00±0.00	81.00±1.00	1.85 g
	FOL	14.97±0.93	0.00±0.00	0.00±0.00	81.00±1.00	
Aspergillus sulphureus	FS	38.03±4.48	18.90±2.08	12.97±1.50	81.00±1.00	1.24 g
	FOL	32.90±0.85	12.67±0.29	0.00±0.00	81.00±1.00	

Values shown are the mean ± SD of 3 replicates, significant at p≤0.05

Table 2. Effect of *Penicillium spp.* against *Fusarium solani* (FS) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at different concentration after 7 days of inoculation

Fungal antagonists	Pathogens	Concentration (%) / Mean colony dia.(mm)			Control	Oven dry weight of Fungal antagonists
		25	50	75		
Penicillium citrinum	FS	55.50±0.87	52.00±1.00	50.67±0.58	87.50±0.00	2.96 g
	FOL	41.33±0.29	35.33±0.76	28.40±1.85	87.50±0.00	
Penicillium italicum	FS	55.10±1.71	54.50±0.00	51.70±0.00	87.50±0.00	1.71 g
	FOL	45.00±0.50	38.17±0.58	29.33±1.15	87.50±0.00	

Values shown are the mean ± SD of 3 replicates, significant at p≤0.05

Table 3. Effect of *Penicillium spp.* against *Fusarium solani* (FS) and *Fusarium oxysporum* f. sp. *lycopersici* (FOL) at different concentration after 7 days of inoculation

Fungal antagonists	Pathogens	Concentration (%) / Mean colony dia.(mm)			Control	Oven dry weight of Fungal antagonists
		25	50	75		
Trichoderma viride	FS	53.47±2.64	42.20±1.80	31.40±1.39	87.67±0.29	2.75 g
	FOL	55.07±0.51	42.97±1.55	32.50±1.32	87.00±0.00	
Trichoderma koningii	FS	53.33±0.58	44.00±0.50	31.07±0.51	87.50±0.00	1.45 g
	FOL	53.67±0.29	42.67±1.04	30.57±0.12	88.00±0.00	

Values shown are the mean ± SD of 3 replicates, significant at p≤0.05

Figure 1. Percentage inhibition of *F. solani* f. sp. *melongena* at different concentration (%) of *Aspergillus spp*. after 7 days of inoculation

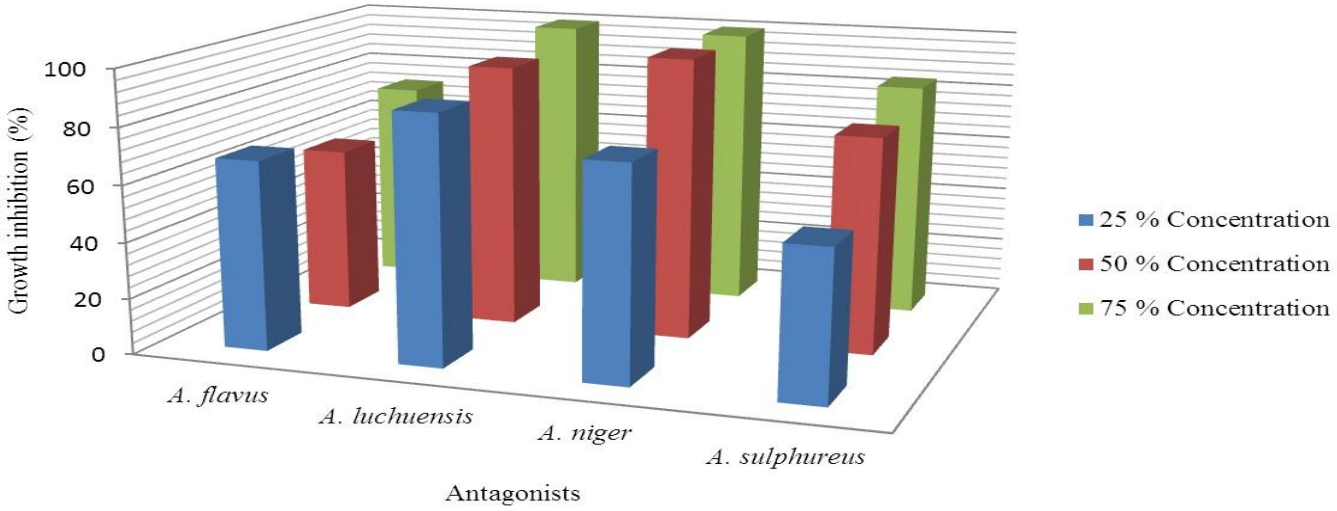


Figure 2. Percentage inhibition of *F. oxysporum* f. sp. *lycopersici* at different concentration (%) of *Aspergillus spp*. after 7 days of inoculation

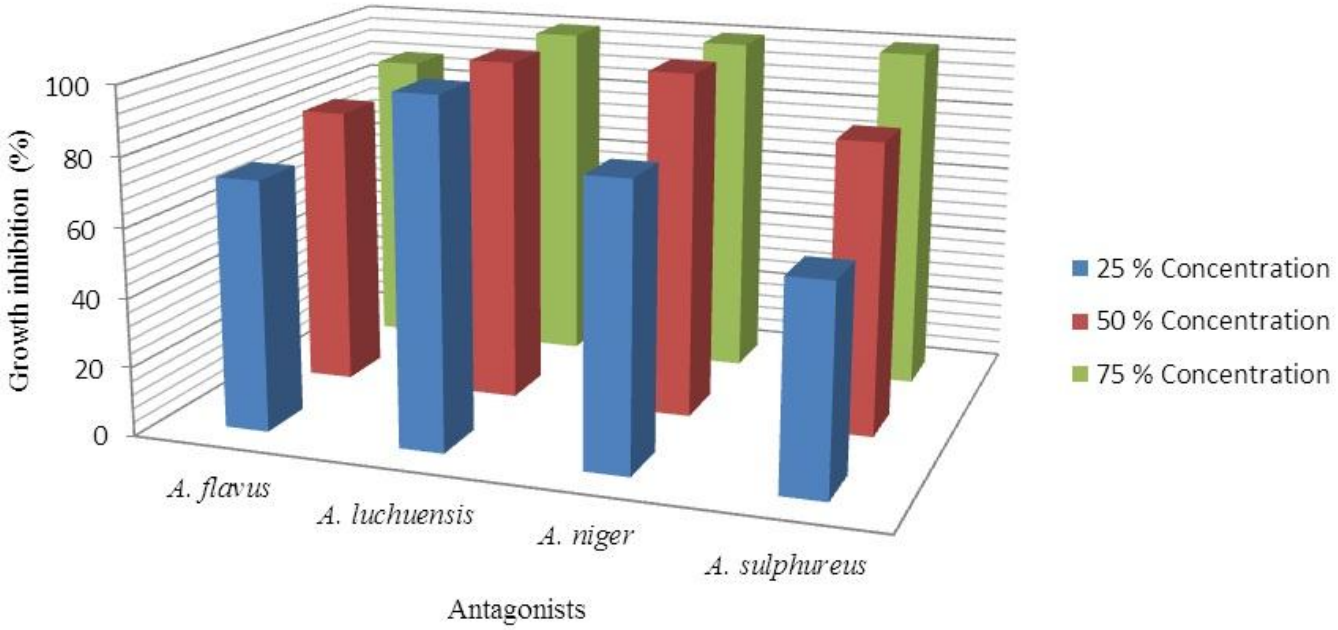


Figure 3. Percentage inhibition of *F. solani* f. sp. *melongena* and *F. oxysporum* f. sp. *lycopersici* at different concentration (%) of *Penicillium spp*. after 7 days of inoculation

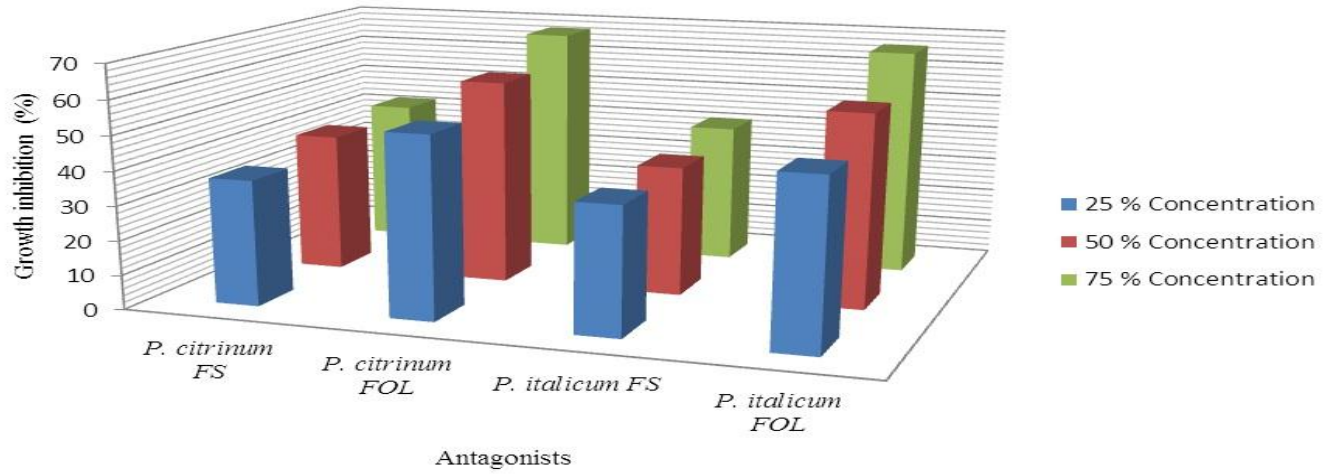
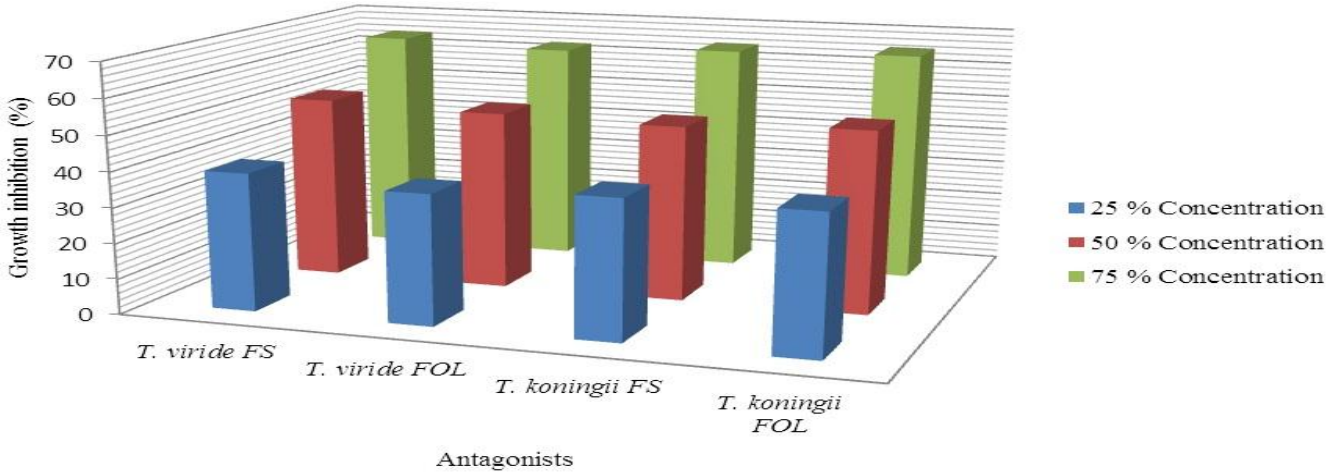


Figure 4. Percentage inhibition of *F. solani* f. sp. *melongena* and *F. oxysporum* f. sp. *lycopersici* at different concentration (%) of *Trichoderma spp*. after 7 days of inoculation



DISCUSSION

In the present study, the significant inhibitory effect of filtrates of *T. koningii* and *T. viride* against both the test pathogens and also the appreciable reduction in the colony diameter (64.49 and 62.26% respectively) particularly at 75% concentration was observed. It might be due to production of antibiotic by *Trichoderma.* Furthermore, they involve various processes such as colonization, plant growth stimulation, bio-control of diverse plant pathogens, decomposition of organic matter, symbiosis, and nutrient exchange (Howell, 2003; Harman, 2006). *Trichoderma* species also exert a property that is known as rhizosphere competence (Saravanan and Jayaraaj, 2004; Anwar *et al*., 2008).

It was evident that the colony diameter of the test pathogenic fungi was significantly decreased even at low concentration of *Aspergillus spp.* It caused a maximum inhibition of colony diameter (100%) of *F. solani* and *F. oxysporum* f. sp. *lycopersici* at 75% concentration. *Aspergillus spp.* have been also reported inhibitory to several plant pathogens (Getha *et al*., 2005; Gachomo and Kotchoni, 2008). In this respect, many workers have reported that *A. japonicas* produce a wide variety of enzymes which may be involved in antifungal activity (Simoes and Tornisielo, 2006).

The colony diameter of both the pathogenic fungi was significantly decreased at higher concentration (75%) of *Penicillium spp*. The percentage inhibition of colony diameter of *F. solani* and *F. oxysporum* f. sp. *lycopersici* was 42.09 and 67.54%, respectively. Bioagents like *P. fluorescens, P. putida*, *T. harzianum* and *B. subtilis* have been widely exploited in the management of soil-borne diseases (Fahri and Murat, 2007; Jayaraj *et al*., 2007).

Among the antagonistic microorganisms, *T. Koningii, T. viride, P. citrinum, P. italicum, A. flavus, A. niger, A. sulphureus* and *Aspergillus luchuensis* have proved their effectiveness.

In fact, it has been reported that biocontrol agents having both antagonistic and plant growth promoting activity, could be more effective in controlling plant diseases (Akkopru and Demir, 2005; Borrero *et al.*, 2006) and suppression of deleterious microorganisms in the rhizosphere (Sabuquillo *et al.*, 2006).

The results of the present study supports earlier findings on biological control in tomato and other field crops (Mujeebur and Shahana, 2002; Moretti *et al*., 2008). Recent studies have also indicated that these fungi can induce systemic resistance in plants, thus increasing the plant defence response to diverse pathogen attack (Harman *et al*., 2004).

CONCLUSION

The antifungal activities of *Aspergillus spp., Penicillium spp. and Trichoderma* species play an important role in controlling soil-borne fungal pathogens (*F. solani* and *F. oxysporum* f. sp. *lycopersici*). The *Aspergillus* species were the best antagonists followed by *Penicillium* spp. and *Trichoderma* spp. for controlling the wilt of tomato and brinjal crops. The use of these bio-agents are not only safe for the farmers and consumers, but also eco-friendly, cost effective, easy to produce and easy to apply the formulations.

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