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In vitro evaluation of biocontrol agents and botanicals against Wilt incidence of watermelon, tomato and marigold

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Efficacy of saprophytically competitive bioagents and some plants leaf extract against *Fusarium* spp. were studied *in vitro*. Six bioagents viz., *Trichoderma viride, Gliocladium virens, T. harzianum, T. hamatum, Aspergillus niger, Psuedomonas fluorescens* and five plant extracts viz., *Azadirachta indica, Pongamia pinnata, Parthenium hysterophorus, Calotropis gigantia* and *Annona squamosa* were assessed for their efficacy against *Fusarium* spp. by using dual culture technique. The suppression of the growth of the pathogen was significantly higher with *T. viride, T. harzianum, T. hamatum, Aspergillus niger, A. flavus* which were at per among themselves. The minimum inhibition was seen with *Psuedomonas fluorescens*. In case of plants leaf extracts, the growth of pathogen was significantly lowest on *Azadirachta indica* followed by *Pongamia pinnata, Parthenium hysterophoras, Calotropis gigantia* and *Annona squamosa* leaf extracts. It was concluded that all the bioagents reduced the wilt incidence improving seed germination, shoot length and root length over control. Seed treatment with *Trichoderma viride* and *T.harzianum* gave significantly better results in respect to seed germination, shoot length, root length, and wilt incidence. The growth of pathogen was significantly lowest on *Azadirachta indica* followed by *Pongamia pinnata, Parthenium hysterophorus, Calotropis gigantia* and *Annona squamosa* leaf extracts.

Key words: Bioagents, botanicals, pathogen, seed treatment, wilt disease

INTRODUCTION

Fusarium wilts, caused by *formae speciales* of the soil-borne fungus *Fusarium oxysporum*, is a major problem on many crops (Ghini *et al.* 2000). *Fusarium* wilt diseases attack many popular garden and greenhouse flowers, numerous vegetables, fruits, field crops, and trees plus a wide range of other plants (Pataky, 1988).

Several practices including the use of resistant cultivars, crop rotation, and fumigation to reduce the damage of *Fusarium* wilt have been suggested (Yu, 2001). Although the use of resistant cultivars is a viable opportunity, the occurrence and development of new pathogenic races is a continuous problem (Jarvis, 1998). Moreover, traditionally crop rotation has proved to control many soil-borne diseases, but because most of these pathogens (like *F. oxysporum*) can survive for long period of time, the effectiveness of this practice is limited once disease outbreak occurs (King *et al.*, 2008). Hence, application of fungicides is a normal practice, which may not be very effective since the disease appears late in the crop growth and the persistence of fungicides throughout the crop growth is always doubtful (Srivastava *et al.*, 2010). Thus, biocontrol is alternative strategy and it has a potential for the management of *Fusarium* wilt disease (Larkin and Fravel, 1998). Furthermore, it is ideal, safe, cheap, long lasting and eco-friendly as compared with chemicals. In this respect a variety of microorgan-

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isms have been isolated from rhizosphere of cultivated plants, and have demonstrated antagonistic activity against the soil-borne plant pathogens, including *Fusarium* wilt (Srivastava *et al.*, 2010).

Biological control provides an alternative to the use of synthetic pesticides with the advantages of greater public acceptance and reduced environmental impact (Reino *et al.*, 2008). *Trichoderma* spp. have gained wide acceptance as effective biocontrol agents against several phytopathogens (Whipps and Lumsden, 2001). Strains of *T. harzianum* are well known for their efficiency to control wide range of disease such as *Sclerotium rolfsii* (Benhamou and Chet, 1996), *Sclerotium cepivorum* (Kay and Stewart, 1994), *Botrytis cinerea* (Bélager *et al.*, 1995), *Fusarium solani* (Chakraborty and Chatterjee, 2008) and *Fusarium oxysporum* (Hervàs *et al.*, 1998; Dubey *et al.*, 1996; Shanmugum *et al.*, 2008). Biological control of plant pathogens using antagonistic bacteria is a promising strategy for plant protection (Kloepper *et al.*, 1999). Other than biocontrol agents several commercial formulations of botanical extracts and essential oils are being investigated as possible alternatives to soil fumigation for control of *Fusarium* wilt diseases (Bowers and Locke, 2000).

The objective of the present study is to evaluate the efficacy of some biocontrol agents and botanicals against wilt disease of watermelon, tomato and marigold.

MATERIALS AND METHODS

In vitro evaluation of antagonistic fungi against F. oxysporum spp. by dual culture assay

Six bioagents viz., *Trichoderma viride, Gliocladium virens, T harzianum, T. hamatum, Aspergillus niger, Psuedomonas fluorescens* were assessed for their efficacy against *Fusarium* spp. by using dual culture technique. A 5 mm disc of test fungus and the antagonistic fungi, cut from the edge of seven days old culture plate were placed in such a manner that test fungus was placed 72 h before bioagents placements, on PDA in Petri plate. The test fungus and bioagents were placed opposite each other at a distance of 5 mm from periphery of Petri plate. Same disc of test fungus on PDA plates was used as control. Each treatment was replicated four times and incubated at 25±1^{0}C. The data were recorded after 96 h of bioagents placement, when the inhibition zones formed and expressed as per cent inhibition are given in Table 1.

In vitro evaluation of botanicals against F. oxysporum spp. by dual culture assay

The relative efficacy of five plant extracts *viz., Azadirachta indica, Pongamia pinnata, Parthenium hysterophorus, Calotropis gigantia* and *Annona squamosa* were tested against the pathogens in the laboratory by dual culture technique devised by Morton and Strouble (1955). Leaves of plants were separately washed two-three times in tap water then in distilled water and were separately homogenized with distilled water at 1.1 (w/w) in a pestle and mortar, and mixture filtered through muslin cloth. Preparation was 100% plant extract solution. Plant extracts prepared were heated at 62^{0}C for 15 minutes for destruction of other microbial contamination. Medium was then poured in 90 mm sterilized Petri dishes with three replications of each treatment and allowed to solidify. A circular disc of 5 mm diameter was taken from 15 days old culture of the pathogen, cut by sterilized cork borer and placed in the center of each Petri plate containing solidified plant leaves extract medium and control without any plant leaves extract. The plates were incubated at 25±1^{0}C.

For studying the effect of plant leaves extract on sporulation of *Fusarium* sp. The fungus was grown on Potato Dextrose agar for 48 h at 20^{0}C and 1 cm diameter dishes of mycelium were cut and placed in Petri plates. Aqueous plants leaves extract were placed on these dishes and incubated under light for 24 h in controls. The dishes were covered with distilled water. Number of conidia produced per microscopic field was counted. The average for 6 microscopic fields for each replication was counted and compared with that the control. The results obtained are presented in Table 5.

RESULTS AND DISCUSSION

The results presented in Table 1 revealed that all the bioagents suppressed the colony growth of *Fusarium* spp. The suppression of the growth of the pathogen was significantly higher with *T. viride, T. harzianum, T. hamatum, Aspergillus niger, A. flavus* which were at per among themselves. The minimum inhibition was seen with *Psuedomonas fluorescens*.

Table 1 : Per cent inhibition of *Fusarium* spp. over control in presence of bioagents

Treatments	*Fusarium oxysporum* f.sp. *niveum*		*Fusarium oxysporum* f.sp. *lycopersici*	
	Radial growth (mm)	Per cent inhibition	Radial growth (mm)	Per cent inhibition
Trichoderma viride	14.0	78.85(62.7)	15.75	77.15(61.5)
T. harzianum	18.0	72.85(58.6)	18.65	70.38(57.1)
T. hamatum	19.0	71.76(57.9)	20.34	67.44(55.2)
Aspergillus niger	20.0	68.34(55.8)	21.54	60.66(51.2)
Aspergillus flavus	28.0	55.43(48.1)	27.34	53.51(47.0)
Psuedomonas fluorescens	25.0	50.36(45.2)	26.84	54.37(47.5)
Control	65	0.00	65	0.00

Figures in parentheses are angular transformed "Arc" values

Table 2 : Effect of seed treatment with bioagents on seed germination, shoot length, root length and wilt incidence of water melon

Treatment	Doses (g/kg seed)	% Seed germination	Shoot Length (cm)	Root length (cm)	% Wilt Incidence
Trichoderma viride	4	88	5.3	8.8	21
		(69.8)			(27.3)
T.harzianum	4	86	8.4	8.0	27
		(68.1)			(31.3)
T.hamatum	4	82	4.3	7.5	35
		(64.9)			(36.6)
Aspergillus niger	4	72	4.2	7.1	42
		(58.1)			(40.4)
Aspergillus flavus	4	74	3.8	6.8	46
		(59.4)			(42.7)
Psuedomonas fluorescens	4	70	3.5	6.1	50
		(56.8)			(45.0)
Control	--	65	4.0	5.0	60
		(53.8)			(50.8)

Figures in parentheses are angular transformed "Arc" values

The effect of seed treatment with bioagents on seed germination, shoot length, root length and wilt incidence were tested. The seeds were treated with spores and mycelial suspension of the bioagents were obtained from the liquid broth and mixed with 10% carboxymethyl cellulose by coating on the seeds. The seeds were sown in pots and data recorded on seeds germination after 2 weeks and 4 weeks and wilt incidence were observed at 10 weeks after sowing. The data are presented in Tables 2, 3 and 4.

The result showed that all the bioagents reduced the wilt incidence improving seed germination, shoot length and root length over control. Seed treatment with *Trichoderma viride* and *T. harzianum* gave significantly better results in respect to seed germination, shoot length, root length, and wilt incidence.

The radial growth of the *Fusarium oxysporum* f. sp. *niveum, F. oxysporum* f. sp *lycopersici,* was found to vary significantly with respect to plant leaf

Table 3 : Effect of seed treatment with bioagents on seed germination, shoot length, root length and wilt incidence of tomato

Treatment	Doses (g/kg seed)	% Seed germination	Shoot length (cm)	Root length (cm)	% Wilt incidence
Trichoderma viridae	4	88	8.3	8.8	24
		(69.8)			(29.3)
T.harzianum	4	83	7.5	8.3	26
		(65.7)			(30.7)
T.hamatum	4	80	7.3	8.0	28
		(63.8)			(32.0)
Aspergillus niger	4	70	7.0	7.8	38
		(56.8)			(38.1)
Aspergillus flavus	4	78	6.8	7.6	40
		(62.1)			(39.3)
Psuedomonas fluorescens	4	73	6.7	7.2	43
		(58.8)			(41.0)
Control	--	65	4.0	5.0	60
		(53.72)			(50.76)

Figures in parentheses are angular transformed "Arc" values

Table 4 : Effect of seed treatment with bioagents on seed germination, shoot length, root length and wilt incidence of marigold

Treatment	Doses (g/kg seed)	% Seed germination	Shoot length (cm)	Root length (cm)	% Wilt incidence
Trichoderma viride	4	86	7.5	8.0	25
		(68.1)			(30.0)
T.harzianum	4	83	7.35	8.1	26
		(65.7)			(30.7)
T.hamatum	4	80	7.12	7.6	29
		(63.5)			(32.6)
Aspergillus niger	4	78	6.42	7.1	34
		(62.1)			(35.7)
Aspergillus flavus	4	77	6.21	6.6	38
		(61.4)			(38.1)
Psuedomonas fluorescens	4	76	5.95	6.3	41
		(60.7)			(39.8)
Control	--	60	4.0	5.0	60
		(50.8)			(50.8)

Figures in parentheses are angular transformed "Arc" values

extracts (Table 5). The growth of pathogen was significantly lowest on *Azadirachta indica* followed by *Pongamia pinnata, Parthenium Hysterophorus, Calotropis gigantia* and *Annona squamosa* leaf extracts. The lowest sporulation was found on *Azadirachta indica* and *Pongamia pinnata* leaf ex-

Table 5 : Influence of some plant leaf extracts on growth and sporulation of *Fusarium* spp. causing wilt of watermelon, tomato

Plant leaf extracts	*F. oxysporum* f.sp. *niveum*		*F. oxvsporum* f.sp. *lycopersici*	
	Average sporulation	Colony Diameter (mm)	Average sporulation	Colony diameter (mm)
Azadirachta indica	28	2.8	29	3.0
Pongamia pinnata	40	1.8	38	20.0
Parthenium hysterophorus	46	5.0	48	5.0
Calotropis gigantia	58	14	58	10.0
Annona squamosa	63	11.0	63	12.0
Control	85	26	85	26.0

tracts. However, maximum sporulation was found in *Annona squamosa* leaf extract.

The effect of seed treatment with bioagents on seed germination, shoot length, root length and wilt incidence was studied and it was observed that all the bioagents reduced the wilt incidence improving seed germination, shoot length and root length over control. Seed treatment with *Trichoderma viride* and *T.hazianum* significantly gave better results with respect to seed germination, shoot length, root length and wilt incidence. Above finding has close concurrence with finding of antifungal (Henis *et al.,* 1978; Upadhyay and Mukhopadhyay, 1983;Papavizas,1985;Mukhopadhyay,1987, Mukhopadhyay and Kaur 1990; Sawant and Mukhopadhyay,1990;Vyas, 1994; Sharma and Mishra 1995; Dubey *et al.,* 1996, Dubey, 1997 ; and Dubey et al. 2000).

The presence of antifungal compounds in higher plants has long been recognized as an important factor to disease control (Mahadevan, 1982). Such compounds being biodegradable and selective in toxicity are considered valuable for controlling some plant diseases (Singh and Dwivedi, 1987). Mamatha and Ravishankar Rai (2004) have observed that the leaf extracts of *Lantana camara* followed by *Azadirachta indica, Acalypha indica* and *Bacopa monnieir* are found to be equally effective in inhibiting the growth of *Fusarium solani in vitro.* Leaf extracts of *Lantana camara* has been reported to exhibit maximum toxicity against spore germination of *Erysiphe cichoracearum* and inhibit the growth of and germination of spores of *Curvularia tuberculata* (Kumar *et al,.* 1997). The leaf extract of *Azadirachta indica* at 100% completely controls the spore germination of *Fusarium oxysporum* followed by *Lantana camara* (Singh and Dwivedi, 1990; Nair and Arora, 1996; Dwivedi and Shukla 2000; Gupta and Bansal, 2003).

From the above results and references it was concluded that although chemicals are spectacular, impressive, quick and convincing even to an uneducated farmer, but developing countries with low gross national product (GNP) cannot beneficially use capital intensive chemical control without economic strain on national budget. Secondary, the intensified worldwide concern about environmental pollution due to escalated use of hazardous pesticide will force us to further retract the chemicals and use sustainable agriculture attainable through biological control. For this reason, biological antagonistic interactions have been emphasized sufficiently so that economic threshold densities required for predicting diseases development and potential' crop loss can accurately be determined. Second objective is the biological protection of germinating seeds, roots or emerging shoots.

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