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RESEARCH **P**APER

Compatibility study of isolates of *Trichoderma* spp. with plant extracts

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The potential *Trichoderma* is an exceptionally good model of bio-control agent as it is ubiquitous, easy to isolate and culture multiply rapidly on many substrates. There are several mechanisms involved in *Trichoderma* antagonism namely antibiosis whereby the antagonistic fungus shows production of volatile metabolites including ethylene and acetone as well as diffusible antibiotics and environmentally safe and economically viable strategy for control of various plant diseases has led to an increased plant based products in agriculture. The results of neem leaves, garlic and onion bulb extracts significantly reduced the growth of isolates of *Trichoderma* species. It was also noted that an increase in the concentration (5 % to 15 %) resulted in subsequent decrease in growth of the isolates. The inhibition per cent at lower concentration *i.e.*, 5 per cent of neem, garlic and onion extracts was ranging from 20.89 to 27.99, 9.51 to 17.11 and 7.95 to 12.5, respectively. Whereas, at higher concentration *i.e.*, 15 per cent, it was 44.53 to 55.09, 34.59 to 39.86 and 31.56 to 39.55, respectively. The average per cent inhibition in mycelial growth of isolates species wise indicated that all the seven species were more or less similar regarding sensitivity towards tested plant extracts.

Key words : *Trichoderma* spp., Plant extracts, Compatibility

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INTRODUCTION

Biological control of plant pathogens was an outgrowth of research on soil borne pathogens and on the ecology of the rich microbial flora and found in the rhizosphere. This work began during the second half of the 20th century and now forms the basis for a unique plant pathological province of biological control, based heavily on microbial and microbe plant interactions (Nelson, 1989). A variety of soil micro organisms have demonstrated activity in the control of various soil borne plant pathogens. The known groups of bio-control fungi, such as *Trichoderma* and *Gliocladium* spp. have been used to control a variety of fungal pathogens *viz.*, *Fusarium*, *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Sclerotium* (Harman, 1991; Taylor *et al.*, 1994 and Lewis *et al.*, 1996).

Trichoderma is an exceptionally good model of bio-control agent as it is ubiquitous, easy to isolate and culture, multiply rapidly on many substrates. There are several mechanisms involved in *Trichoderma antagonism* namely antibiosis whereby the antagonistic fungus shows production of volatile metabolites including ethylene and acetone as well as diffusible antibiotics. Plant extract are environmentally safe and economically viable strategy for control of various plant diseases has led to an increased plant based products in agriculture.

RESEARCH METHODOLOGY

Isolation of isolates of *Trichoderma* spp. :

The isolation of *Trichoderma* species were made by soil dilution plate technique (Johnson and Curl, 1972). From each soil sample, 10g of closely associated rhizosphere/rhizoplane soil was mixed thoroughly with 90 ml sterile distilled water to prepare stock solution and serially diluted up to 10^{-5} (Harris and Sommers, 1968). One ml of suspension from the soil dilutions were plated on solidified *Trichoderma* selective medium (TSM) and gently shaken to spread evenly. These Petriplates were incubated at 28°±1°C temperature for one week with periodic observation for the development of colonies of *Trichoderma* species. The early growing colonies of different morphology were examined critically, picked-up and transferred to Potato dextrose agar slants. Finally the cultures were purified and maintained on PDA slants at low temperature (5°C) in refrigerator in the Department of Plant Pathology, C.P. College of Agriculture, S.D. Agricultural

University, Sardarkrushinagar, for further activities. The isolates were identified with the help of their microscopic structure and compared with taxonomic keys of *Trichoderma* species (Cook and Baker, 1983 and Rifai, 1969).

Compatibility of isolates of *Trichoderma* spp. with plant extracts :

The effect of phytoextracts of three plant species belonging to different families was evaluated on the growth and sporulation of *Trichoderma* spp. *in vitro* by poison food technique. Healthy fresh leaves and bulbs are listed in Table A was collected for evaluation of compatibility of *Trichoderma* spp. isolates at desired concentration of 5.00, 10.00 and 15.00 per cent.

Table A : Plant extracts tested for compatibility with isolates of *Trichoderma* spp. *in vitro*

Sr. No.	Plant	Botanical name	Family	Plant part
1.	Neem	*Azadirachta indica*	Meliaceae	Leaf
2.	Garlic	*Allium sativum* L.	Lilliaceae	Bulb
3.	Onion	*Allium cepa* L.	Lilliaceae	Bulb

Fresh and healthy 100g plant parts of each plant species were crushed in grinder by adding 100 ml distilled water to obtained 1 : 1 extract. The material was homogenized for five minutes and filtered through double layer sterilized muslin cloth. Then the filtrates were centrifuged at 5000 rpm for 15 minutes. The clear supernant was collected and was considered as cent per cent concentration (standard solution).

For evaluation, desired concentration (5.00, 10.00 and 15.00 %) were obtained by adding appropriate amount of standard solution of plant extract to 100 ml PDA medium in 250 ml conical flasks. Then about 20 ml extract mixed PDA medium was poured in sterilized petriplates.

With the help of a sterile cork borer, discs of 5 mm diameter were cut out from actively growing culture of the isolate (3 day old) and transferred to the centre of Petri plates containing the desired media inoculated with the different concentrations of plant extracts. The inoculated plates were incubated at 28 ± 1^{0}C. Control plates without any plant extracts were also inoculated and incubated simultaneously for comparison. Three replicates in PDA medium were maintained for each treatment including control.

The growth of colony in each treatment was measured in two directions at right angles to each other and per cent inhibition was calculated by using formula given by Vincent (1947). Completely randomized and DNMRT design were followed for the analysis of the data obtained.

Table 1 : Effect of plant extracts on growth of isolates of *Trichoderma* spp.

Plant extracts	Sr. No.	Isolates	Concentration					
			5 per cent		10 per cent		15 per cent	
			Growth (mm)*	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)
Neem leaves extract	1.	Th_1	67.67	24.25	60.67	31.58	49.00	44.53
	2.	Th_2	70.67	20.89	60.00	32.33	46.67	47.16
	3.	Th_3	68.00	23.88	59.67	32.71	41.33	53.21
	4.	Th_4	64.33	27.99	58.67	33.83	42.67	51.69
	5.	Th_5	68.67	23.13	56.67	36.09	42.67	51.69
	6.	Th_6	68.67	23.13	53.33	39.86	39.67	55.09
	7.	Th_7	68.00	23.88	60.00	32.33	39.67	55.09
	8.	Control	88.33	-	88.67	-	88.33	-
Garlic extract	1.	Th_1	78.33	10.65	69.67	21.13	55.33	37.60
	2.	Th_2	73.67	15.97	68.67	22.26	53.33	39.86
	3.	Th_3	79.33	9.51	66.33	24.91	54.33	38.73
	4.	Th_4	76.33	12.93	70.00	20.75	58.00	34.59
	5.	Th_5	72.67	17.11	71.00	19.62	54.33	38.73
	6.	Th_6	73.33	16.36	65.00	26.41	55.33	37.60
	7.	Th_7	76.33	12.93	66.00	25.28	53.67	39.47
	8.	Control	87.67	-	88.33	-	86.67	-
Onion bulb extract	1.	Th_1	80.33	9.47	75.57	14.33	59.67	31.94
	2.	Th_2	79.67	9.46	71.67	18.86	59.67	31.94
	3.	Th_3	81.00	7.95	75.00	15.09	60.00	31.56
	4.	Th_4	78.67	10.60	71.00	19.62	59.33	32.33
	5.	Th_5	79.00	10.23	70.00	20.75	57.33	34.61
	6.	Th_6	77.67	11.74	69.67	21.13	53.00	39.55
	7.	Th_7	77.00	12.5	69.00	21.88	53.33	39.17
	8.	Control	88.00	-	88.33	-	87.67	-

* and ** indicates of significance of values at P=0.01 and P=0.05, respectively

RESEARCH FINDINGS AND ANALYSIS

The findings of the present study as well as relevant discussion have been presented under the following heads :

Isolation of *Trichoderma* spp. isolates from rhizosphere :

On the basis of early growing colonies of different morphology, seven isolates of *Trichoderma* spp. were obtained on *Trichoderma* selective medium (TSM) from the rhizosphere of castor plant by soil dilution plate technique (10^{-5} dilution) after incubation period of one week at $28°\pm1^0$C. Out of 20 soil samples collected from castor field, 7 isolates (Th1 to Th7) of *Trichoderma* species (*Trichoderma harzanium*) were obtained. The results are in accordance with the methodology adopted by Sivan and Chet (1989), D'souza *et al.* (2001), Vyas and Mathur (2002), Sangle and Bambawale (2004), Kavitha *et al.* (2004) and Kapil and Kapoor (2005).

Compatibility of isolates of *Trichoderma* spp. with plant extracts :

The search for an environmentally safe and economically viable strategy for control of various plant diseases has led to an increased plant based products in agriculture. Neem based plant products are one of the most popular products using by the farmers. Hence, in the present study, antifungal activity of neem leaves, garlic and onion bulb's extracts at 5, 10 and 15 per cent concentrations against isolates of *Trichoderma* species were studied.

The results presented in Table 1 revealed that neem leaves, garlic and onion bulb extracts significantly reduced the growth of isolates of *Trichoderma* species. It was also noted that an increase in the concentration (5 % to 15 %) resulted in subsequent decrease in growth of the isolates. The inhibition per cent at lower concentration *i.e.*, 5 per cent of neem, garlic and onion extracts ranged from 20.89 to 27.99, 9.51 to 17.11 and 7.95 to 12.5, respectively. Whereas, at higher concentration *i.e.*, 15 per cent, it was 44.53 to 55.09, 34.59 to 39.86 and 31.56 to 39.55, respectively. The average per cent inhibition in mycelial growth of isolates species wise indicated that all the seven species were more or less similar regarding sensitivity towards tested plant extracts.

The results are in agreement with the reports related to screening and reviewing of plant extracts for antimicrobial activities (Osborn, 1943; Misra and Dixit, 1976; Kurucheve *et al.*, 1997; Sharma, 1998; Sengupta *et al.*, 2004 and Bohra *et al.*, 2006).

Conclusion :

The inhibition per cent at lower concentration *i.e.*, 5 per cent of neem, garlic and onion extracts was ranging from 20.89 to 27.99, 9.51 to 17.11 and 7.95 to 12.5, respectively. Whereas, at higher concentration *i.e.*, 15 per cent, it was 44.53 to 55.09, 34.59 to 39.86 and 31.56 to 39.55, respectively. The average per cent inhibition in mycelial growth of isolates species wise indicated that all the seven species were more or less similar regarding sensitivity towards tested plant extracts.

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