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In vitro antagonistic activity of *Trichoderma* species against important soil borne pathogens

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ABSTRACT

The rhizospheric soil samples were collected from different cultivated agricultural fields from Bharuch and Narmada districts and the mycoflora were isolated by serial dilution plate technique. Total eight isolates of *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma longibrachiatum* were isolated on Potato Dextrose Agar medium. The green coloured colonies were identified by comparing with taxonomic key. They were purified by single spore isolation method and maintained on PDA slants at 4^0C in the refrigerator at Department of Pl.Pathology, NMCA, NAU, Navsari. Soil borne Pathogenic fungi *viz., Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium oxysporum* were isolated from the respective diseased plants during field survey in Navsari Agricultural University farm, Navsari. The antagonistic efficacy against test pathogen was evaluated by dual culture plate technique. Among all 8 Trichoderma isolates, The *Trichoderma harzianum* NCJD8 isolate has showed 24.17 mm mycelial growth with 73.15 per cent inhibition of *Sclerotium rolfsii, where in case of Macrophomina phaseolina*, Minimum mycelial growth (32.67 mm) of *test pathogen* was recorded in *T. longibracheatum* NCJD2 isolate with 63.70 per cent inhibition which was statistically at par with *T. viride* NCJD6 (34.50 mm) with 61.67 per cent inhibition and when it comes to *Fusarium oxysporum, T. harzianum* NCJD5 showed minimum mycelial growth and highest per cent growth inhibition (75.56%) with 22.00 mm colony diameter of the pathogen after seven days of incubation which was statistically at par with isolate *T. harzianum* NCJD1 (72.96%) with 24.33 mm colony diameter.

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INTRODUCTION

Injudicious use of pesticide in agriculture leads to environmental pollution, causing hazardous effects to both environment and food quality. Many pathogenic micro-organisms have developed resistance against chemical fungicides (Gaigole *et al.*, 2011). Fungicides pose serious hazards to health and environment. This emphasized an alternative method to control fungal diseases. Bio-control

of plant pathogen is an ecofriendly, safe approach that utilizes antagonistic micro-organisms as a potential means of disease control. Trichoderma is a non-pathogenic bio-control agent having antagonistic properties against many plant pathogens in various degrees (Dennis and Webster, 1971a). To determine the antagonistic property of *Trichoderma* spp. against *Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium oxysporum*, isolates were compared on a medium and at temperature where antagonist and pathogen both can grow well in the laboratory. The present study was undertaken, to find out the bio-control efficacy of *Trichoderma* spp. against above mentioned pathogens.

MATERIAL AND METHODS

Isolation of antagonist:

The rhizospheric soil samples were collected from different cultivated agricultural fields from Bharuch and Narmada districts and the mycoflora were isolated by serial dilution plate technique (Johnson and Curl, 1972). Total eight isolates of *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma longibrachiatum* were isolated on Potato Dextrose Agar medium. The green coloured colonies were identified by comparing with taxonomic key. They were purified by single spore isolation method and maintained on PDA slants at 4^0 C in the refrigerator.

Isolation of pathogenic fungi:

Pathogenic fungi *viz*., Mp was isolated from the diseased plant of sesame, Sr from infected plant of Indian bean and Fo from wilt infected pigeonpea root during field survey in Navsari Agricultural University farm, Navsari of Gujarat State, India (Aneja, 2003). Parts of plants with symptoms of infection were surface sterilised by immersion in 0.1% sodium hypochlorite for 30 seconds and then rinsed thoroughly with sterile distilled water three times. They were transferred to potato dextrose agar (PDA) medium in petri plates and incubated at 26 ± 2^0 C for seven days. They were purified by single spore isolation method and maintained on PDA slants at 4^0 C in the refrigerator.

Dual culture plate technique:

Trichoderma spp. were evaluated against *Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium oxysporum* by the dual culture plate technique (Dennis and Webster, 1971b). The antagonistic efficacy against test pathogen was evaluated on PDA medium. Both pathogen and antagonists were grown on PDA plates separately for 5 days. Mycelial discs of 5 mm in diameter of antagonist was excised from the edge of an actively growing culture plate and inoculated opposite to the pathogenic fungi in the same plate 2 cm away from the edge similarly. For each treatment three replicates were maintained and incubated at 27 ± 2^0 C. Control plates were maintained for test pathogen in triplicate. Both, antagonist and test pathogen were placed equidistant from the periphery so that they would get equal opportunity for their growth. After the incubation period, the radial growth of Sr, Mp and Fo in control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula (Edgington *et al.*, 1971).

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

where I = Percentage inhibition of radial growth of pathogen (%),

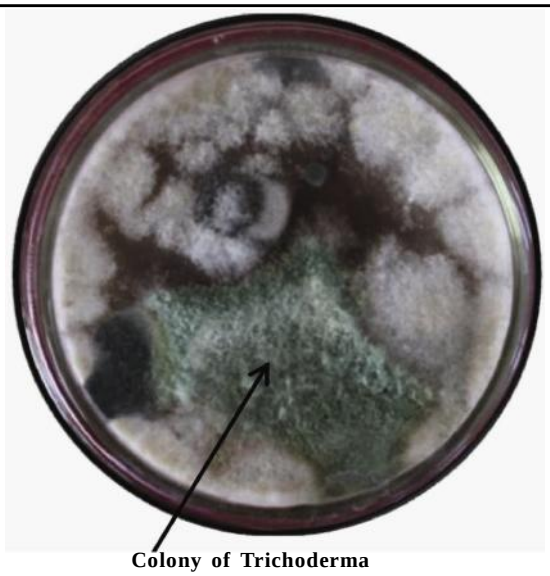
C = Radial growth of the pathogen (mm) in control,

T = Radial growth of the pathogen (mm) in treatment

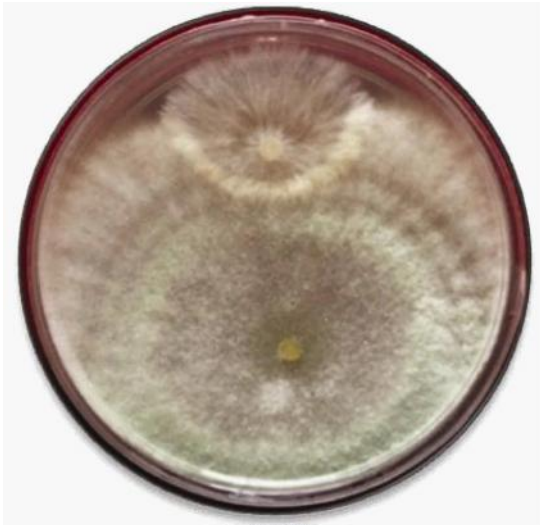
The mycelial mats from zone of interaction in dual culture plate between pathogen and antagonist were placed on glass slide. The glass slides were stained with lacto phenol cotton blue (HiMedia) to improve the visibility of the hyphae and then observed under a light microscope (CH20i Olympus, India). The hyphal interaction between the mycelia of opposite colonies was studied.

RESULTS AND DISCUSSION

Isolates of *Trichoderma* spp. were evaluated for their antifungal activity against *Sclerotium rolfsii, Macrophomina phaseolina* and *Fusarium oxysporum*. Among all 8 Trichoderma isolates, The *Trichoderma harzianum* NCJD8 isolate has showed 24.17 mm mycelial growth with 73.15 per cent inhibition of *Sclerotium rolfsii,* where in case of *Macrophomina phaseolina,* Minimum mycelial growth (32.67 mm) of *test pathogen* was recorded in *T. longibracheatum* NCJD2 isolate with 63.70 per cent inhibition which was statistically at par with *T. viride* NCJD6 (34.50 mm) with 61.67 per cent inhibition and when it comes to *Fusarium oxysporum, T. harzianum* NCJD5 showed minimum mycelial growth and highest per cent growth

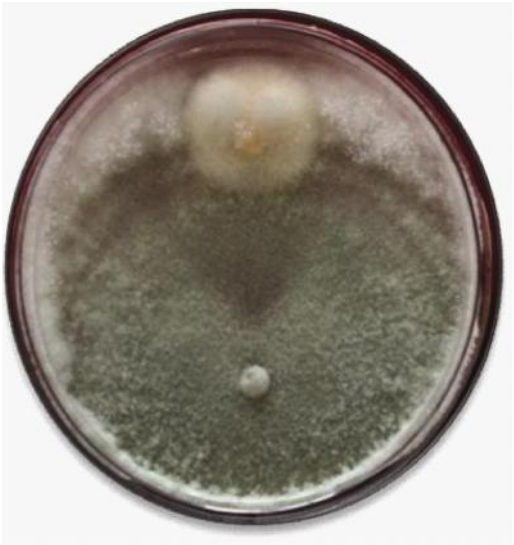


Colony of Trichoderma



Trichoderma harzianum NCJD8 + Sclerotium rolfsii

T. longibracheatum* NCJD2 + *Macrophomina phaseolina



T. harzianum* NCJD5 + *Fusarium oxysporum

Table 1: Antagonistic effect of *Trichoderma* isolates against *S. rolfsii* (Indian bean stem rot)

Sr. No.	Isolates	ACD (mm)	PIMG
1.	*T. harzianum* strain NCJD8	24.17	73.15
2.	*T. harzianum* strain NCJD1	46.67	48.15
3.	*T. longibracheatum* strain NCJD4	29.33	67.41
4.	*T. longibracheatum* strain NCJD2	45.00	50.00
5.	*T. harzianum* strain NCJD5	40.67	54.81
6.	*T. viride* strain NCJD6	31.17	65.37
7.	*T. longibracheatum* strain NCJD7	38.17	57.59
8.	*T. viride* strain NCJD3	43.17	52.04
9.	Control	90	-
	S.E.±	0.13	1.09
	C.D. (P=0.05)	0.39	3.28
	C.V.%	3.71	3.79

Table 2: Antagonistic effect of *Trichoderma* isolates against *M. phaseolina (Chickpea root rot)*

Sr. No.	Isolates	ACD (mm)	PIMG
1.	*T. harzianum* strain NCJD8	42.17	53.15
2.	*T. harzianum* strain NCJD1	43.17	52.04
3.	*T. longibracheatum* strain NCJD4	41.50	53.89
4.	*T. longibracheatum* strain NCJD2	32.67	63.70
5.	*T. harzianum* strain NCJD5	40.33	55.19
6.	*T. viride* strain NCJD6	34.50	61.67
7.	*T. longibracheatum* strain NCJD7	38.17	57.59
8.	*T. viride* strain NCJD3	41.67	53.70
9.	Control	90	-
	S.E.±	0.11	0.88
	C.D. (P=0.05)	0.33	2.64
	C.V.%	3.03	3.13

Table 3: Antagonistic effect of *Trichoderma* isolates against *F. oxysporum f.sp. udum (Pigeonpea wilt)*

Sr. No.	Isolates	ACD (mm)	PIMG
1.	*T. harzianum* strain NCJD8	26.84	70.18
2.	*T. harzianum* strain NCJD1	24.33	72.96
3.	*T. longibracheatum* strain NCJD4	25.70	71.44
4.	*T. longibracheatum* strain NCJD2	28.17	68.70
5.	*T. harzianum* strain NCJD5	22.00	75.56
6.	*T. viride* strain NCJD6	26.50	70.56
7.	*T. longibracheatum* strain NCJD7	25.67	71.48
8.	*T. viride* strain NCJD3	37.17	58.70
9.	Control	90	-
	S.E.±	0.11	0.76
	C.D. (P=0.05)	0.33	2.27
	C.V.%	3.03	2.31

inhibition (75.56%) with 22.00 mm colony diameter of the pathogen after seven days of incubation which was statistically at par with isolate *T. harzianum* NCJD1 (72.96%) with 24.33 mm colony diameter.

Overall experimental results clearly indicated that all the isolates proved effective against the all the pathogens tested. Maximum average inhibitory effect was found with isolate TVMs (*T. viride*) against all three pathogens *viz*., *F. oxysporum* f.sp. *udum, S. rolfsii* and *M. phaseolina* which was followed by THSh2, TLS, TLMa, THMo, TLD, THSh1 and TVH. In general, the results of isolate wise efficacy showed that isolate THMo (*T. harzianum*) was found comparatively superior with an average mycelial growth inhibition of 75.56 per cent as compared to the rest. The variation of different isolates in their efficacy against the fungal pathogens might be due to different levels of secondary metabolites produced by different isolates. The results of the present study also indicated that the effect of bio-control agents may be specific and hence more study on this aspect is required. The present results are in agreement with the earlier results obtained by Gurha (2001); Pan and Bhagat (2007); Vishwanath *et al.* (2008) and Madhusudan *et al.* (2010).

Conclusion:

Plant diseases caused by pathogenic fungi constrain the yields. In agriculture, farmers still depend on the use of chemical fungicides to control plant diseases. However, misuse of these synthetic chemicals cause hazardous to both environment and health. The alternative method for replacement of chemical fungicides has led to the use of biological control agents. Biocontrol of soil borne pathogens is met by the introduction of micro-organisms. Micro-organisms that grow in the rhizosphere are ideal for use as biocontrol agents. Our studies proved that *Trichoderma* spp. have the potential to control *F. oxysporum* f.sp. *udum, S.*

rolfsii and *M. phaseolina in vitro* to the extent of 75.56 per cent, 73.15 and 63.70 per cent, respectively. The potential use of these biocontrol agents can be improved by isolation, formulation and application methods, particularly in the field.

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