J. Biol. Control, 22(1): 43-49, 2008

Characterization of antagonistic potential of *Trichoderma* spp. against some soil borne plant pathogens

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ABSTRACT: **Ten isolates of *Trichoderma* spp. isolated from rhizosphere of different crops were evaluated for their antagonistic potential against five soil borne plant pathogens, viz., *Rhizoctonia solani, Fusarium oxysporum f* sp *radicis-lycopersici, Macrophomina phaseolina* and *Sclerotium rolfsii* using dual culture technique and production of volatile and non-volatile antibiotics. Sclerotial antagonism by the biocontrol strains was tested with *R. solani* and *S. rolfsii*. The isolate T_2, T_4 and T_7 against *Pythium* sp., T_1, T_7 and T_{10} against *F. oxysporum f. sp radicis-lycopersici*, T_3 and T_9 against *R. solani*, T_7 against *S. rolfsii* and T_6 against *M. phaseolina* were the most efficient. The highest percentage inhibition of respective pathogen through the production of certain metabolites of *Trichoderma* isolates was recorded with T_7 against *Pythium* sp. and *Fusarium oxysporum* sp. *radicis-lycopersici*, T_1 against *R. solani*, T_5 and T_6 against *S. rolfsii* and T_9 isolate against *M. phaseolina*. *Trichoderma* isolate T_9 and T_2 were most effective in inhibition of sclerotia formation, production and germination of sclerotia of *R. solani* and *S. rolfsii*, respectively.**

KEY WORDS: Antagonistic potential, sclerotial antagonism, *Trichoderma* spp., volatile and non-volatile metabolites

INTRODUCTION

The focus on the management of plant diseases has beeen shifted from chemical pesticides to more ecofriendly biopesticides to reduce environmental hazards and to minimize the risk of development of pesticide resistant strains of plant pathogens. Many fungi have the potential to reduce the crop loss through biocontrol mechanism. The mechanisms of strains of *Trichoderma* include mycoparasitism, antibiosis, competition for nutrient or space, tolerance to stress through enhanced root and plant development, induced resistance, solublization and sequestration of inorganic nutrients and inactivation of pathogen's enzyme etc. (Harman, 2000). Several workers have reported the inhibitory effect of volatile and non-volatile compounds produced by *Trichoderma* spp. on several soil borne plant pathogens (Bose *et al.*, 2005; Pan and Bhagat, 2007). The indigenous strains of *Trichoderma* spp are seems to function as better antagonist and disease control as they are well adapted to local conditions. Therefore, present study was carried out to isolate the

Trichoderma spp. from diverse crop rhizosphere of West Bengal and to establish their antagonistic potential against five soil borne plant pathogens, isolated from different crops.

MATERIALS AND METHODS

Ten isolates of *Trichoderma* were isolated from the rhizosphere soil of various crops, viz., tomato, chilli, mustard, brinjal, gram, tuberose, cauliflower, cabbage, potato and green gram, by soil dilution plate technique (Harris and Sommers, 1968) on modified *Trichoderma* specific medium (Saha and Pan, 1997) and identified as different species based on Rifai (1969) and Bissett (1991a-c). Pure cultures of *Trichoderma* isolates were maintained on potato dextrose agar (PDA) slants at 4°C for subsequent use. Five soil borne plant pathogens, viz., *Rhizoctonia solani* Kuhn (from sheath blight of rice), *Fusarium oxysporum f* sp *radicis-lycopersici* Jarvis and Shoemaker (fusarial wilted tomato), *Macrophomina phaseolina* Tassi (Goid) (stem rot of jute) and *Sclerotium rolfsii* Sacc. (root and foot rot of tuberose) and *Pythium* sp. (pointed gourd fruit) were isolated by tissue segment method (Rangaswami, 1958), purified and preserved at 4°C for subsequent use.

Dual culture technique

In vitro antagonistic potential of biocontrol agents was evaluated by dual culture technique (Morton and Stroube, 1955) against all five-test pathogens. The growth of both pathogen and antagonist were recorded periodically and rating was done after contact between these two according to modified Bell's scale (Bell *et al.*, 1982).

Effect of volatile and non-volatile substances

The effect of volatile and non-volatile substances produced by antagonistic fungi on respective pathogen was studied by the method followed by Dennis and Webster (1971a-b). The experiment was laid out in completely randomized block design and all treatments were replicated four times. The radial mycelial growth of the pathogen was recorded and inhibition of mycelial growth was calculated according to Vincent (1947).

Sclerotial antagonism

The viability of mature sclerotia from different treatment was tested by slide germination method and plated on solidified potato dextrose agar media. Sixty sclerotia from each treatment was tested for its germination by placing two sclerotia in cavity slide and a drop of serilized distilled water was added. They were incubated in a moist chamber for 24h. The number of germinated sclerotia and hyphae put forth by each sclerotium were counted. In another set of experiment, sixty matured sclerotia from each treatment was placed onto PDA medium with equal space, incubated for 24h and examined under microscope.

RESULTS AND DISCUSSION

The results presented in Table 1 revealed that the *Trichoderma* isolates T_2, T_4 and T_7 were most efficient antagonist than others and they completely overgrew *Pythium* sp. within four days of incubation. Similarly, the isolates T_1, T_7 and T_{10} against *F. oxysporum* sp. *radicis-lycopersici*, T_3 and T_9 against *R. solani*, T_7 against *S. rolfsii* and T_6 against *M. phaseolina* were most efficient in parasitizing mycelial growth of respective pathogen.

T_7 isolate of *T. virens* was most effective in suppression of radial mycelial growth of *Pythium* sp.(57.8%; 68.1 and 82.6%), *F. oxysporum* sp. *radicis-lycopersici* (62.6%; 53.3 and 68.3 %) production of certain metabolites, whereas T_1 isolate of *T. viride* caused highest growth inhibition of *R. solani* (36.3%; 73.0% and 82.6%) and *S. rolfsii* (62.6%; 53.3 and 68.3 %) by producing volatile and non-volatile inhibitors at variable concentrations (Table 2). The isolate T_9 (*R. solani* and *S. rolfsii*), T_3 (*F. oxysporum* sp. *radicis-lycopersici*), T_5 (*R. solani*) and T_8 (*M. phaseolina*) were least effective in growth inhibition of respective pathogen, whereas other isolates were having intermediate effect.

The results of sclerotial antagonism of *Trichoderma* isolates is presented in Table 3, revealed that T_9 isolate showed highest percentage inhibition of sclerotia formation, producing only 21 followed by T_3, T_{10} and T_1 which were statistically

insignificant and T_2 (44.0) isolate was best in case of *S. rolfsii*. The isolate T_3 (40.0%) and T_9 (43.2%) isolates significantly reduced the per cent germination of sclerotia produced by *R. solani* in dual culture as compared to control in cavity slide method, whereas T_3 and T_6 (63.3%) isolates were most effective in case of *S. rolfsii*. Similar result was also recorded in PDA with T_3 (70.0%) and T_6 (75.0%) against *R. solani* and S. *rolfsii*, respectively. The production hypha on germination of sclerotia of both pathogen differed with the test isolates of *Trichoderma*.

The degree of antagonistic potential of microorganisms *in vitro* is not always same under field level, yet such studies are important in screening the candidate strains of antagonists. Present findings revealed that particular species or strain of *Trichoderma* is not always effective against all test pathogens has strong selectivity in their antagonistic potential towards a particular pathogen. The variability in antagonistic potential of different aggregates of *Trichoderma* against different pathogens has been reported (Bose *et al.*, 2005; Pan and Bhagat, 2007). Similarly, Bell *et al.*(1982) reported significant differences in antagonistic potential of 77 isolates of *T. harzianum* against six fungal pathogens.

Table 1. Bell's rating of antagonistic potential of *Trichoderma* isolates against soil borne pathogens

Isolates	*Pythium* sp.	*F. oxysporum sp. radicis-lycopersici*	*R. solani*	*S. rolfsii*	*M. phaseolina*
T_1	6	5	4	7	6
T_2	4	7	5	6	7
T_3	6	8	3	7	5
T_4	4	7	4	6	5
T_5	5	7	4	7	5
T_6	5	6	5	6	4
T_7	4	5	6	5	5
T_8	5	6	4	8	6
T_9	7	6	3	6	5
T_{10}	7	5	4	6	6

The volatile compounds released by *Trichoderma* isolates did not have significant effect on sclerotia production and germination of *R. solani* and *S. rolfsii* (Table 4). Maximum inhibition of sclerotia formation by *R. solani* (47.1%) and *S. rolfsii* (58.1%) was recorded with T_9 isolate followed by T_1 and T_5 (36.6%) and T6 (47.3%), respectively in cavity slide. Similar result was also recorded with the sclerotia germination of *R. solani* and *S. rolfsii*, where highest percentage inhibition of sclerotia germination was noted with T_9 (50.0%) isolate of *G. roseum* followed by T_2 (51.7%) with *R. solani* and T_6 (55.0%) followed by T_2 (63.3%) in case of *S. rolfsii*.

Selective activity of both volatile and non-volatile substances released by *Trichoderma* isolates against the pathogen is well known (Dennis and Webster, 1971a, 1971b; Upadhyay and Mukhopadhyay, 1983). It is clear from the results that there might be production of different types of volatile and non-volatile substances and its amount, which was highly selective in their action. Upadhyay and Mukhopadhyay (1983) has claimed that culture filtrate of *Trichoderma* spp. produces

Table 2. ***In vitro*** **antagonistic potential of** ***Trichoderma*** **isolates against five pathogens through production of volatile and non-volatile metabolites**

Isolate	*Pythium*			F. *oxysporum* f sp *radicis lycopersici*			*R. solani*			*S. rolfsii*			*M. phaseolina*		
	Volatile	Non-volatile		Volatile	Non-volatile		Volatile	Non-volatile		Volatile	Non-volatile		Volatile	Non-volatile	
	Radial mycelial growth	Radial mycelial growth		Radial mycelial growth	Radial mycelial growth		Radial mycelial growth	Radial mycelial growth		Radial mycelial growth	Radial mycelial growth		Radial mycelial growth	Radial mycelial growth	
		5%	10%		5%	10%		5%	10%		5%	10%		5%	10%
T_1	48.3	31.3	36.3	26.3	21.7	34.3	57.3	15.7	24.3	59.3	25.0	31.7	54.3	15.3	20.0
T_2	63.3	20.7	26.3	41.0	22.0	28.7	61.3	24.0	35.7	51.3	25.0	31.0	72.0	18.0	39.3
T_3	55.0	32.7	38.0	43.3	35.0	43.3	72.7	28.7	38.0	49.0	29.3	44.7	70.6	19.0	48.3
T_4	54.0	21.0	25.3	34.7	22.0	32.0	68.0	31.0	44.0	42.2	28.0	48.0	65.0	16.3	22.7
T_5	44.7	36.3	41.7	27.0	24.0	33.7	81.3	36.3	49.0	38.3	37.3	54.7	68.0	23.0	33.3
T_6	72.7	22.3	40.0	35.0	21.7	39.3	69.7	27.0	37.0	69.3	30.0	42.0	61.7	12.0	15.3
T_7	38.0	15.7	28.7	24.3	22.0	37.0	69.3	28.3	39.3	39.3	16.0	29.7	50.0	16.3	25.0
T_8	44.7	22.3	31.7	30.0	23.3	36.3	81.0	27.3	31.7	55.3	13.0	55.0	57.3	38.3	53.3
T_9	72.0	43.7	60.0	29.0	26.0	44.3	60.7	21.7	42.7	66.3	43.3	62.7	47.3	12.7	19.3
T_{10}	63.0	29.3	52.3	31.3	21.3	30.0	60.7	17.0	29.0	48.7	32.7	54.7	72.7	12.0	16.7
Control	90.0	90.0	90.0	65.0	60.0	60.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
Sed	2.20	2.01	1.8	2.72	2.54	1.99	1.74	2.34	2.36	2.39	3.52	2.26	2.70	3.52	1.91
LSD (P=0.05)	7.07	6.8	6.5	9.42	6.18	5.43	7.81	7.26	5.92	7.03	7.30	4.68	5.61	7.30	3.97

The data are means of four replications

Table 3. Effect of antagonists on production and germination of sclerotia of *R. solani* and *S. rolfsii* and hyphal production in dual culture

Isolates	Number of sclerotia		Sclerotial germination (%)				Hyphal production			
	R. solani	*S. rolfsii*	Cavity slide (%)		On PDA		Cavity slide (%)		On PDA	
			R. solani	*S. rolfsii*	*R. solani*	*S. rolfsii*	*R. solani*	*S. rolfsii*	*R. solani*	*S. rolfsii*
T_1	27.7	74.3	73.3 (59.0)	80.0 (63.5)	78.3 (62.3)	90.0 (75.0)	++	+++	++	+++
T_2	34.3	44.0	76.7 (61.2)	73.4 (59.0)	85.0 (67.4)	100.0 (90.0)	+++	+++	+++	+++
T_3	24.3	60.0	40.0 (39.2)	63.3 (52.7)	50.0 (45.0)	76.7 (61.1)	+	++	+	+++
T_4	34.0	64.3	70.0 (56.8)	70.0 (56.8)	76.7 (61.3)	78.3 962.3)	++	++	++	+++
T_5	30.3	66.0	76.6 (61.1)	73.1 (58.9)	93.3 (75.2)	83.4 (65.9)	+++	++	+++	+++
T_6	34.0	51.0	66.7 (54.8)	63.3 (52.7)	83.1 (65.9)	75.0 (60.3)	+++	+	+++	++
T_7	43.7	47.3	65.0 (53.8)	83.4 (65.9)	73.4 (58.9)	100.0 (90.0)	++	+++	++	+++
T_8	33.0	76.0	66.7 (54.8)	78.2 (62.3)	81.7 (64.7)	100.0 (90.0)	+++	+++	+++	+++
T_9	21.0	66.7	43.2 (41.2)	76.7 (61.1)	80.0 (63.5)	88.1 (70.2)	+++	++	+++	++
T_{10}	27.2	59.3	48.3 (44.0)	70.0 (56.8)	70.0 (56.8)	86.7 (68.7)	++	+++	++	+++
Control	53.0	96.0	90.0 (75.0)	91.7 (73.4)	96.7 (81.38)	100.0 (90.0)	+++	+++	+++	+++
Sed3.54	4.46	2.96	2.54	3.10	4.06	-	-	-	-	
LSD (P=0.05)	7.34	9.27	6.14	5.26	6.43	8.43	-	-	-	-

Figures in parentheses are angular transformed values, '+' indicates number of hyphae per germinating sclerotia

Table 4. Effect of volatile compound(s) released by *Trichoderma* isolates on sclerotia production and germination of *R. solani* and *S. rolfsii*

Isolates	Number of sclerotia/plate		% inhibition of sclerotia production		% Sclerotia germination in cavity slide		% inhibition of germination in cavity slide	
	R. solani	*S. rolfsii*	*R. solani*	*S. rolfsii*	*R. solani*	*S. rolfsii*	*R. solani*	*S. rolfsii*
T_1	32.3	55.0	36.6	40.0	58.3 (48.8)	71.7 (57.9)	31.4	21.8
T_2	37.3	55.3	26.8	40.1	51.7 (45.90	63.3 (52.7)	39.2	30.9
T_3	46.3	63.3	9.1	31.4	66.7 (54.8)	73.3 (58.9)	21.6	20.0
T_4	49.7	70.3	2.6	23.8	78.3 (62.30	76.7 (61.2)	7.8	16.4
T_5	32.3	74.3	36.6	19.5	53.3 (46.90	75.0 (60.0)	37.2	18.2
T_6	42.0	48.7	17.6	47.3	76.7 (61.1)	55.0 (47.9)	9.8	40.0
T_7	34.3	77.0	32.7	16.6	60.0 (50.8)	68.3 (55.8)	29.4	25.5
T_8	36.0	53.7	29.4	41.9	75.0 (60.0)	80.0 (63.7)	11.8	12.7
T_9	27.0	38.7	47.1	58.1	50.0 (45.0)	60.0 (50.8)	41.2	34.5
T_{10}	45.0	49.7	11.8	46.2	71.7 (57.9)	71.7 (57.9)	15.7	21.8
Control	51.0	92.3	-	-	85.0 (67.4)	91.7 (73.4)	-	-
SEM±	3.25	4.51	-	-	2.13	2.39	-	-
LSD (0.05)	6.75	9.37	-	-	4.42	4.95	-	-

volatile and non-volatile antibiotics effective against *S. rolfsii* and *R. solani*. Dubey (2000) reported that *T. virens* inhibited 59.8% mycelial growth 70.0% sclerotial production followed by *T. viride* and *T. harzianum*.

However, Bunker and Mathur (2001) reported that *T. harzianum* was most effective causing significant suppression of both growth and sclerotial production of *R. solani in vitro* and they also reported that diffusible non-volatile antibiotic activity of *T. harzianum* was more potential than volatile antibiotics. Muthamilan and Jeyarajan (1992) reported that 67.4 % reduction of sclerotial production in *S. rolfsii* in presence of *T. viride*. The variability in sclerotia production, inhibition of germination and susceptibility to degrade *in vitro* and infectivity of sclerotia of *R. solani* and *S. rolfsii* suggests that there is considerable specificity in biocontrol.

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(Received: 19.04.2007; Revised: 21.09.2007; Accepted: 15.10.2007)