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Antagonistic Efficacy of Trichoderma Species on Sclerotium Rolfsii in Vitro

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Abstract: *wo Trichoderma isolates viz., T. harzianum Th_4 with fast radial growth, simultaneous lysis and over growth on Sclerotium rolfsii and T. virens Tv_5 with slow radial growth over S. rolfsii and subsequent lysis were evaluated for their antagonistic potential in vitro. Volatile metabolites of Tv_5 isolate were more effective against S. rolfsii growth (54.6% inhibition) compared to Th_4 (16.3% inhibition) while non volatile metabolites of Th_4 were more effective against S. rolfsii with 100% growth inhibition at 60 and 80% concentration compared to 69.3% inhibition at 80% concentration of Tv_5 culture filtrate.*

Key words : *Antagonism, Trichoderma, Sclerotium rolfsii, Volatiles, and Non volatiles.*

I. Introduction

Sclerotium rolfsii is a non-specialized soil borne fungal pathogen of worldwide importance and has a host range spread over 500 species (Punja 1985). Biological control is proved to be a promising disease management technology against soil borne plant pathogens, when applied either alone or in combination with other management practices (Papavizas, 1985). Species of Trichoderma are very effective biological control agents against soil borne plant pathogens such as S. rolfsii. However, variation existed in different isolates of Trichoderma in their biocontrol efficacy (Elad et al. 1980; Patibanda and Prasad, 2004, Jash and Pan 2007 and Devi et al. 2012). The present study is aimed at studying variation in the effect of volatile and nonvolatile metabolites of two isolates of Trichoderma species that differed in their in vitro antagonistic potential in dual culture against S. rolfsii.

II. Material And Methods

In the present investigation two isolates viz., Th_4 of T. harzianum and Tv_5 of T. virens, available in the Department of Plant Pathology, Agricultural College, Bapatla, that differed in their in vitro antagonistic potential against S. rolfsii were studied for their volatile and nonvolatile metabolites on the growth of S. rolfsii in vitro.

Effect of volatile metabolites was tested by inoculating sterile PDA plates with 2mm culture disc of either S. rolfsii or individual Trichoderma spp. at the centre. The bottom plates of Petri dish containing S. rolfsii and either of the Trichoderma isolates were paired together and sealed with a cello tape to trap volatiles within Petri plates. Such paired plates were incubated at 29 ± 1^0C. Appropriate controls were maintained with only test pathogen or test antagonist on one side and the other side with only PDA (uninoculated). Observations were recorded on the radial growth of S. rolfsii and Trichoderma in comparison with control plates.

Effect of diffusible non volatile metabolites were assessed using culture filtrates of Trichoderma isolates, obtained by inoculating individual Trichoderma isolate in to 250 ml Erlenmeyer conical flask containing 100 ml of potato dextrose broth that was incubated for a week and filtered through sterilized Whatman No. 1 filter paper followed by G3 filters. The culture filtrate was used at 10, 20, 40, 60 and 80% concentration by diluting with appropriate quantity of autoclaved potato dextrose agar. Such PDA medium amended with culture filtrate was poured in to Petri plates and allowed to solidify, inoculated with 2mm discs of S. rolfsii culture and incubated at 29 ± 1^0c. Plates inoculated with S. rolfsii on PDA alone (without culture filtrate) served as control and observations were recorded on the radial growth of S. *rolfsii* at first, second, third and fourth day after inoculation.

Per cent inhibition of the pathogen over control was calculated by adopting the following formula (Nene and Thapliyal, 1982).

I (%) = ((C-T)/ C) x 100

I = Percent growth inhibition

C= Growth in control

T= Growth in treatment

III. Results And Discussion

Isolate T. harzianum Th_4 with faster radial growth in monoculture plate (compared to T. virens Tv_5) overgrew on S. rolfsii in dual culture plate with simultaneous lysis of S. rolfsii mycelium. Isolate Tv_5 showed slower radial growth in monoculture (compared to Th_4) caused lysis of S. rolfsii mycelium followed by overgrowth.

In paired plates, with both the isolates of Trichoderma, significant inhibition in the growth of S. rolfsii was observed in comparison to its check plate through out the period of incubation (Table 1). Further the inhibition was higher with Tv_5 compared to Th_4 volatiles. With Th_4, the inhibition in the growth of S. rolfsii was maximum after two days of inoculation (21.1%). In case of Tv_5, the inhibition in S. rolfsii growth was maximum on fifth day of incubation (54.7%). Thus the data revealed that the volatile metabolites of Tv_5 were more antagonistic to S. rolfsii compared to Th_4 volatiles.

Studies on the effect of S. rolfsii volatiles on Trichoderma isolates (reverse antibiosis) has revealed insignificant differences in the growth of Th_4 when comparisons were made between check plate and paired plate up to three days after inoculation. However, four days of incubation resulted in 7.8% inhibition in the growth of Th_4 when compared with its check plate. In case of Tv_5, presence of S. rolfsii in paired plate along with Tv_5 resulted in promotion in the growth of Tv_5 (a maximum of 38.9% one day after inoculation) compared to its check plate up to three days of incubation. By fourth day the growth of Tv_5 in paired plate obtained a maximum of 9.0 cm, i.e., Tv_5 could occupy entire Petri plate. This indicated that Tv_5 and S. rolfsii interaction in paired plate resulted in an early boost in Tv_5 growth and significant reduction in S. rolfsii growth. Further, Tv_5 volatiles were found more effective compared to that of Th_4 volatiles in inhibiting the growth of S. rolfsii. Several reports were published on the involvement of volatiles in Trichoderma antibiosis against S. rolfsii (Dennis and Webster, 1971b; Upadhyay and Mukhopadhyay 1983 and Uma Maheshwari et al., 2002).

When culture filtrates of Trichoderma isolates were assessed against the growth of S. rolfsii using poisoned food technique, 100% inhibition in the growth of S. rolfsii was obtained with Th_4 culture filtrate at and above 60% concentration through out the period of observation (Table 2). Further, higher inhibition was recorded on first day compared to other days at 10, 20 and 40% concentrations of Th_4 culture filtrate. This indicated that at lower concentrations, S. rolfsii got adopted to Th_4 culture filtrate and continued to grow through out the period of observation though at a decreased pace compared to check. In case of Tv_5, even at 80% concentration could not inhibit the growth of S. rolfsii completely (Table 3). Maximum inhibition of 65.4% was recorded with 80% concentration on day 1. At 20 to 60% concentration of Tv_5 culture filtrate could not show significant inhibition in the growth of S. rolfsii compared to its check plate on respective days. Unlike in Th_4, with Tv_5 culture filtrate growth of S. rolfsii was not completely stopped even at the highest concentration of 80%.

The results presented above indicated that non volatile diffusible metabolites of Th_4 were very effective compared to that of Tv_5 and production of toxic diffusible metabolites and their accumulation at toxic concentrations was found much higher in Th_4 than that of Tv_5. Further, lower concentration of either Th_4 or Tv_5 nonvolatile diffusates could slow down the growth of S. rolfsii. Reports on inhibitory effect of Trichoderma culture filtrate and non volatiles on the growth of S. rolfsii were reported earlier (Dennis and Webster (1971a), Upadhyay and Mukhopadhyay (1983) and Rudresh et al., (2005)).

The present investigation indicated variation in the production of toxic volatile and nonvolatile metabolites of two different Trichoderma isolates that differed in their antagonistic potential in dual culture in vitro. Isolate Th_4 through production of strong diffusible toxic nonvolatile metabolites could kill (lysis) and overgrow upon S. rolfsii simultaneously. This character was further facilitated by its faster radial growth. Isolate Tv_5 aided with only stronger volatile metabolites needed to wait till the mycelium of S. rolfsii got lysed due to the combined effect of volatile and nonvolatile metabolites before it could overgrow on S. rolfsii. The present investigation also revealed that faster growth of the antagonistic isolate facilitates the organism in its ability to produce nonvolatile metabolites.

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Test fungi	Interaction with	Days after inoculation							
		Day 1		Day 2		Day 3		Day 4	
		Radial growth (cm)	Growth over control	Radial growth (cm)	Growth over control	Radial growth (cm)	Growth over control	Radial growth (cm)	Growth over control
S. rolfsii	Check	1.5^{c}		3.8^{c}		6.1^{c}		8.6^{ab}	
	Th_4	1.2^{d}	20.0	3.0^{d}	21.1	5.1^{d}	16.4	7.2^{c}	16.3
	Tv_5	1.3^{cd}	13.3	2.5^{e}	34.2	3.6^{e}	41.0	3.9^{d}	54.7
T. harzianum (Th_4)	Check	1.2^{d}		5.0^{ab}		6.9^{bc}		9.0^{a}	
	Sr	1.1^{d}	7.7	5.4^{a}	-8.0	6.5^{c}	5.8	8.3^{b}	7.8
T. virens (Tv_5)	Check	1.8^{b}		4.0^{c}		7.4^{b}		9.0^{a}	
	Sr	2.5^{a}	-38.9	4.6^{b}	-15.0	8.2^{a}	-10.8	9.0^{a}	0.0
SEm ±		0.1		0.1		0.2		0.2	
CD (P=0.01)		0.2		0.4		0.7		0.5	
CV (%)		10.3		6.8		7.2		3.9	

Table 1: Effect of volatiles on the radial growth of test fungi in paired plates.

Figures with similar alphabets do not differ significantly.
Positive values represent inhibition in growth and negative values represent increase in growth in comparison with growth in respective check plate.

Table 2: Effect of Th_4 culture filtrate on the growth of *Sclerotium rolfsii in vitro*.

Concentration (%)	Day 1		Day 2		Day 3		Day 4	
	Radial Growth (cm)	Growth over control (%)	Radial Growth (cm)	Growth over control (%)	Radial Growth (cm)	Growth over control (%)	Radial Growth (cm)	Growth over control (%)
10	1.6	41.0 (39.8)^{d}	3.6	12.0 (20.1)^{d}	5.3	20.5 (26.9)^{d}	7.3	12.2 (20.4)^{d}
20	1.2	56.0 (48.5)^{c}	3.0	27.2 (31.4)^{c}	4.1	39.1 (38.7)^{c}	6.2	28 (31.9)^{c}
40	0.9	64.5 (53.3)^{b}	2.1	50.2 (45.0)^{b}	3.5	48.1 (43.9)^{b}	4.0	53.2 (46.8)^{b}
60	0.0	100.0 (90.0)^{a}	0.0	100.0 (90.0)^{a}	0.0	100 (90.0)^{a}	0.0	100 (90.0)^{a}
80	0.0	100.0 (90.0)^{a}	0.0	100.0 (90.0)^{a}	0.0	100 (90.0)^{a}	0.0	100 (90.0)^{a}
Check	2.4		4.0		6.4		8.7	
SEm ±		1.4		1.0		0.6		0.7
CD (P=0.01)		4.5		3.8		2.0		2.6
CV (%)		4.2		3.1		1.8		2.1

Figures with similar alphabets do not differ significantly
Figures in parenthesis are Arcsine transformed values

Table 3: Effect of Tv_5 culture filtrate on the growth of *Sclerotium rolfsii in vitro*.

Concentration (%)	Day 1		Day 2		Day 3		Day 4	
	Radial Growth (cm)	Growth over control (%)	Radial Growth (cm)	Growth over control (%)	Radial Growth (cm)	Growth over control (%)	Radial Growth (cm)	Growth over control (%)
10	1.8	22.5 (27.8)^{d}	3.7	6.1 (10.2)^{d}	5.6	12.1 (20.1)^{a}	7.5	13.4 (21.1)^{d}
20	1.4	39.5 (38.9)^{bc}	3.1	21.2 (27.1)^{c}	4.6	27.5 (31.6)^{d}	6.2	28.1 (31.9)^{c}
40	1.2	52.1 (46.2)^{b}	2.1	45.9 (42.6)^{b}	3.5	44.3 (41.7)^{c}	4.6	46.8 (43.1)^{b}
60	1.3	44.8 (42.0)^{bc}	1.8	54.6 (47.6)^{ab}	2.7	57.6 (49.4)^{b}	3.9	54.5 (47.6)^{b}
80	0.8	65.4 (54.2)^{a}	1.3	67.1 (55.1)^{a}	2.0	68.6 (55.9)^{a}	2.7	69.3 (56.4)^{a}
Check	2.4		4.0		6.4		8.7	
SEm ±		2.5		3.1		1.2		1.6
CD (P=0.01)		12.0		17.4		5.6		8.2
CV (%)		7.4		9.3		3.5		4.8

Figures with similar alphabets do not differ significantly
Figures in parenthesis are Arcsine transformed values