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Research Note

Effect of *Trichoderma* spp. on the growth of *Sclerotium rolfsii* Sacc.

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ABSTRACT: **Six antagonists were screened *in vitro* for their effect in suppressing the growth of *Sclerotium rolfsii* Sacc. *Trichoderma harzianum* Rifai was found more effective in suppressing the growth of *S. rolfsii* in dual culture followed by *Trichoderma viride* Pers. Fr. Studies on production of volatile and non-volatile antibiotics revealed that *T. harzianum* and *T. viride* were highly effective in reducing the radial growth of *S. rolfsii*.**

KEY WORDS: Antagonists, antibiotics, *Sclerotium rolfsii*, *Trichoderma harzianum*

Sclerotium rolfsii Sacc. is a soil borne pathogen having broad host range and persists for longer period in soil by resistant resting structures (sclerotia). For the control of *S. rolfsii* by using fungicides provide certain degree of control but the same time pollutes the environment and affecting the beneficial soil microorganisms. Considering the above facts biocontrol agents are being used for disease management in the present day crop husbandry in an increasing scale. Volatile and non-volatile antibiotics produced by *Trichoderma viride* Pers.Fr. in agar culture completely inhibit the growth of *Macrophomina phaseolina* (Tassi.) Goid (Mathur and Bhatnagar, 1994). Antagonism is mediated by specific or non-specific metabolites of microbial origin, *viz.*, lytic enzymes, volatile compounds or other toxic substances. Upadhyay and Mukhopadhyay (1983) reported that *Trichoderma harzianum* Rifai produced the diffusible antibiotics at varying amounts in the medium, which was detrimental to the growth *S. rolfsii*. The literature on mode of action of antagonists on *S. rolfsii* is meager. Therefore, in the present study, effect of *Trichoderma* spp. on the growth of *S. rolfsii* was studied.

The antagonists, *viz.*, *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* Migula were collected from Department of Plant Pathology, Dharwad while *Trichoderma koningii* Oudem and *Trichoderma virens* Miller were from Agricultural Research Station, Hebballi, Dharwad and *Aspergillus niger* Van Tiegh and *Penicillium* sp. were isolated from the soil.

Interaction of antagonists with *S. rolfsii* in dual culture

Antagonists were screened for their antagonistic activity in dual culture on Potato Dextrose Agar (PDA) in Petri-plates. Discs of five mm size were cut from the margin of young vigorously growing cultures, placed in opposite points in the plates containing Potato Dextrose Agar at the same time. Each treatment was replicated

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thrice and all the plates were incubated at 27±1°C for five days and also appropriate control was maintained. Observations on colony diameter of *S. rolfsii* in all plates were recorded and the per cent inhibition of mycelial growth over the control was calculated (Vincent, 1927).

In dual culture technique, maximum inhibition of mycelial growth of *S. rolfsii* was recorded in *T. harzianum* (60.7%) and was superior to other antagonists tested. While minimum inhibition of 18.5 per cent mycelial growth was noticed in *Penicillium* sp. (Table 1). All the antagonists, namely, *T. harzianum, T. viride, T. koningii* and *T. virens* inhibited the mycelial growth of *S. rolfsii*. Similar observations were made by earlier workers while working with on collar and root rot diseases of crop plants caused by *S. rolfsii* (Iqbal *et al.*, 1995; Prasad *et al.*, 1999).

Effect of non-volatile substances against S. rolfsii

The production of non-volatile compounds of antagonists was tested by an "agar layer technique" (Dennis and Webster, 1971a). Twenty ml of sterilized PDA was aseptically poured into sterilized Petri-plates. A single sterile cellophane paper was placed aseptically on the solidified PDA in Petri-plates and left over night. Disc of five mm size was taken from the margin of three days old culture of each antagonist and placed at the centre. These plates were incubated at 27±1°C for two days. Then cellophane paper along with adhering fungus was removed carefully. Five mm disc of the test pathogen was placed immediately on the medium at the centre and appropriate control was maintained. Each treatment was replicated thrice. After three days of incubation colony diameter of the test fungus was recorded and data were analysed statistically.

Data presented in Table 1 showed significant effect of non-volatile compounds produced by antagonists on the test pathogen. *T. harzianum* (40.5%) showed maximum reduction in mycelial growth of test pathogen. *T. harzianum* and *T. viride* (37.8%) did not differed significantly with each other but differed significantly from rest of the antagonists tested. The next best treatment was *T. koningii* (33.7%). The least reduction of mycelial growth was noticed in *Penicillium* sp. (13.5%).

Effect of volatile substances against S. rolfsii

Antagonists were also tested for production of volatile compounds by "inverted plate technique" adopted by Dennis and Webster (1971b). Agar disc of five mm size was cut from the actively growing margin of the antagonists and laid on the centre of Petri-plates containing 20 ml of PDA. Mycelial disc of five mm size was removed from the actively growing culture of *S. rolfsii* and inoculated in similar manner to Petri-plates having 20 ml of PDA then inverted over the plates inoculated with antagonists. Two plates were sealed together (mouth to mouth) with adhesive tape. Control plates consist of *S. rolfsii* inverted over uninoculated PDA plates. The plates were incubated at 27±1°C and three replications were maintained for each treatment. After three and seven days of incubation, the colony diameter of the test pathogen was measured and compared with the control.

Data on radial growth of the test pathogen affected by the inhibitory substances produced by the antagonists are presented in Table 1. On 3rd day of incubation, the highest per cent reduction of mycelial growth over control was observed in *T. harzianum* (36.0%) and *T. viride* (33.3%) and were significantly superior to other antagonists tested. However, there was no significant difference between the two. Some morphological effects like more branching and abundant aerial growth of mycelium towards the antagonists were observed.

On 7th day also *T. harzianum* was consistently found most effective with 25.9 per cent reduction in mycelial growth over control and significantly superior to all other antagonists. The next best treatments were *T. viride* (21.71) and *T. koningii* (20.9%), which were on par with each other. The least reduction of mycelial growth of 10.2 per cent and 4.0 per cent on 3rd and 7th day, respectively, was recorded in *Penicillium* sp.

Table 1. Effect of antagonistic micro-organisms on the growth of *S. rolfsii*

Antagonist	Growth reduction over control (%)			
	Dual culture	Non-volatile compound	Non-volatile compound	
		72 h	3rd day	7th day
Trichoderma koningii	52.9 (46.6)**	33.7 (35.5)	29.2 (32.7)	20.2 (27.1)
Trichoderma harzianum	60.7 (51.1)	40.5 (39.5)	36.0 (36.9)	25.9 (30.6)
Trichoderma viride	58.5 (49.9)	37.8 (37.9)	33.3 (35.2)	21.7 (27.7)
Trichoderma virens	58.8 (50.1)	27.7 (31.7)	19.0 (25.8)	12.4 (20.6)
Penicillium sp.	18.5 (25.4)	13.5 (21.5)	10.2 (18.6)	4.0 (11.4)
Aspergillus niger	30.0 (33.1)	17.5 (24.7)	14.2 (22.2)	5.0 (12.5)
Control	0.0	0.0	0.0	0.0
SEM±	0.56	0.83	0.71	0.93
CD (P=0.01)	2.3	3.5	3.0	3.9

** Figure in parentheses are arcsine transformed values.

The present study revealed that toxic substances produced by antagonists were also effective for inhibiting the pathogen and physical contact between antagonists and pathogen may not be necessary for effective antibiosis. Several workers reported the inhibitory effects of both volatile and non-volatile substances produced by *Trichoderma* spp. on several soil borne plant pathogens (Dennis and Webster, 1971a, 1971b; Hutchinson and Cowan, 1972; Upadhyay and Mukhopadhyay, 1983; Mathur and Bhatnagar, 1994). Production of volatile antibiotics by *A. niger* was reported by Deb (1990).

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