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In vitro antagonism of three *Trichoderma* spp. against *Sclerotium rolfsii* Sacc., a collar-rot pathogen in elephant foot yam

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ABSTRACT: **The present investigation focuses on the screening of potential biocontrol agents against the collar-rot pathogen, *Sclerotium rolfsii* Sacc. on elephant foot yam, an intercrop of coconut in Andhra Pradesh. Among the three *antagonists screened, Trichoderma viride* Pers. Fr., *T. harzianum* Rifai and *T. hamatum* (Bon.) Bain. isolated and screened, *T. hamatum* inhibited the radial growth of *S. rolfsii* to an extent of 52.22 per cent followed by *T. viride* (44.11%) and *T. harzianum* (38.87%). All the three *Trichoderma* spp. were very effective in producing volatile and non-volatile metabolites that were suppressive to *S. rolfsii*. Viability tests on sclerotia of *S. rolfsii* parasitized by *Trichoderma* spp. revealed complete colonization and replacement of sclerotial contents by 7th day of parasitisation in case of *T. hamatum* and by 10th day in case the of *T. viride* and *T. harzianum*.**

KEY WORDS: Antagonism, elephant foot yam, *Sclerotium rolfsii, Trichoderma*

Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst) Nicolson) is an important tuber crop grown as intercrop in coconut ecosystem. The crop is often affected by collar-rot or foot-rot disease caused by the soil-borne fungus, *Sclerotium rolfsii* Sacc. The symptoms include water soaked lesions on the stem near the collar region. In the advanced stages, the stem collapses due to rotting and a thick, white mycelial mat is seen. Biological control is an ecofriendly and easily adoptable technology to manage soil-borne diseases. *Trichoderma* spp. have been found antagonistic to *S. rolfsii* and *Rhizoctonia solani* Kuhn (Chet *et al.*, 1979; Elad *et al.*, 1983) and are successfully used for the control of these pathogens in several crops under greenhouse and field conditions (Wells *et al.*,1972; Mathur and Sarbhoy, 1978; Chet *et al.*, 1979; Hadar *et al.*, 1979 and Elad *et al.*, 1980). Upadhyay and Mukhopadhyay (1983) reported that *T. harzianum* produced diffusible antibiotics at varying amounts under *in vitro* conditions, which was detrimental

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to the growth of *S. rolfsii*. Hence the present investigation was taken up to screen different native antagonistic fungi against the collar-rot pathogen *S. rolfsii* and the methods of antagonism by these antagonists in controlling the pathogen.

Isolation of *Trichoderma* spp.

Rhizosphere soil saples were collected from healthy plants surrounding the diseased plants of elephant-foot yam. The soil samples were then dried and later sieved to fine powder and serially diluted in sterilized distilled water (SDW) to 10^{-3} and 10^{-4} concentrations. Then, 500 µl of each dilution was spread on Petri-dishes containing *Trichoderma* specific medium.

Two plates were maintained for each dilution. The plates were incubated for 4 days at 28°c and typical *Trichoderma* spp. colonies were identified according to the identification key based on the branching of conidiophores, shape of the phialides, emergence of phialophores and phialospores (Rifai, 1969).

Dual culture studies

Dual cultures of the antagonists and the test pathogen *S. rolfsii* were prepared by inoculating PDA discs from the growing margins of fresh fungal cultures on to Petri-dishes containing PDA (Gams *et al.*, 1980) and incubating at 29°C. The dual cultures were observed for antibiosis and agar blocks from the regions where the colonies merged were observed for hyphal interaction under the light microscope.

Viability of sclerotial bodies

The sclerotial bodies of the test pathogen from dual-culture plates that were parasitised by the *Trichoderma* spp. are then subjected to viability studies (Morton & Stroube, 1955; Abd-El Moity and Shatla, 1981). The parasitized sclerotia were surface sterilized with mercuric chloride (0.1%), transferred onto sterilized wet towel paper and incubated at 30°C temperature for 72 hours and finally plated on PDA for isolation of the pathogen. Sclerotial bodies exposed to different time intervals were subjected to viability studies.

Inhibition by volatile metabolites

Production and inhibitory effect of volatile metabolites by the antagonists were tested against the test pathogen by using procedure given by Dennis and Webster (1971). The pathogen growth was measured after 4 days of incubation at 29°C.

Inhibition by non-volatile metabolites

The antagonists that had shown inhibition in dual-culture studies were grown on potato dextrose broth to test the effect of the culture filtrates (non-volatile antibiotics) on the test pathogen by food poisoning technique (Khara and Hadwan, 1990). The culture filtrates were purified by autoclaving and the sterilized filtrate was incorporated in the medium for observing fungal growth and inhibition at different concentrations (50% and 100%). The PDA mixed filtrate in all the cases were poured (20ml each) into sterilized Petri-dishes and the same were inoculated with fresh disc of the test pathogen, *S.rolfsii*. Control plates were maintained simultaneously and per cent inhibition was calculated.

Mycelial interactions between *Trichoderma* spp. and *S. rolfsii*

Growth of *S. rolfsii* in Petri-dish dual-culture was suppressed by all the three *Trichoderma* spp. (*T. viride*, *T. harzianum* and *T. hamatum*). Highest inhibition was recorded in case of *T. hamatum* (52.22%) followed by *T. viride* (44.11%) and *T. harzianum* (38.87%) (Table 1). Inhibition zone was observed in the case of *T. viride* and *T. hamatum* followed by mycoparasitism whereas only mycoparasitism was noticed in case *T. harzianum*. In the case of *T. hamatum*, a yellow halo prevailed up to 10 days of interaction followed by mycoparasitism. In all the three cases, the *S. rolfsii* mycelium did not grow when transferred on to fresh media indicating its death. Microscopic observations made from the mycelial interaction zone revealed frequent adpressing zones of *Trichoderma* spp. mycelia on *S. rolfsii* mycelia.

Viability of Sclerotial bodies

Viability studies carried out on sclerotial

Table 1. Dual-culture studies between *Trichoderma* spp. and *S. rolfsii*

Biocontrol agent	Mycelial growth of *S. rolfsii* (mm)	Per cent inhibition of *S. rolfsii* mycelial growth	Mode of action
T. viride	50.3	44.11[b]	Antibiosis followed by mycoparasitism
T. harzianum	55.0	38.87[a]	Mycoparasitism
T. hamatum	43.0	52.22[c]	Antibiosis (Yellow halo formation) followed by mycoparasitism
Control	90	-	-

* Numbers in each column followed by the different letters are significantly different (P=0.05).

bodies of *S. rolfsii* revealed that sclerotial body germination was affected when parasitised by *Trichoderma* spp. in dual culture studies. Sclerotial bodies gave rise to respective *Trichoderma* spp. mycelia when subjected to germination tests after 10 days of parasitisation and above. However, *T. hamatum* was found to be superior among the other *Trichoderma* spp., since parasitised sclerotial bodies from *T. hamatum* vs *S. rolfsii* dual culture plates gave rise to *T. hamatum* mycelia when subjected to germination tests after 7 days.

Inhibition by volatile metabolites of 0, 15 and 25 day old cultures of *Trichoderma* spp.

Among the *Trichoderma* spp., *T. harzianum* proved very effective in producing volatile antibiotics specific against *S. rolfsii* at all the three stages of exposure. This was followed by *T. hamatum* and *T. viride*, which were also effective in producing volatile substances against *S. rolfsii*. An increase in reduction was evident with an increase in the age of *Trichoderma* spp. cultures (Table 2). The results indicated the production of effective volatile antibiotics by all the antagonistic species of *Trichoderma*. Hyphae from the exposed cultures of *S. rolfsii* when transferred to fresh medium did not grow. Hence, the volatile metabolites produced by the *Trichoderma* spp. i.e., *T. viride*, *T. harzianum* and *T. hamatum* were both fungicidal and fungistatic (Claydon *et al.*, 1987). Sawant and Mukhopadhyay (1990) while working on damping off of sugarbeet reported that older cultures of *T. harzianum* had a greater inhibitory effect on the mycelial growth of *Pythium aphanidermatum* as compared to that of younger cultures.

Inhibition by non-volatile metabolites

Culture or cell free filtrates of all the three *Trichoderma* spp., *viz. T. viride*, *T. harzianum* and *T. hamatum* were suppressive to the radial growth of *S. rolfsii* (Table 2). With an increase in the concentration of the culture filtrate of the *Trichoderma* spp., a corresponding increase in per cent inhibition of the mycelial growth of *S. rolfsii* was noticed. Similar results were noted with an increase in concentration of the culture filtrate of *T. harzianum* and inhibition of *Pythium aphanidermatum* on tobacco by Devaki *et al.* (1992). However, cell-free filtrates of *T. hamatum* were found to be highly effective at both the 50 per cent and 100 per cent concentration under study thus inhibiting the *S. rolfsii* mycelium completely. Narasimha Rao and Kulkarni (2003) reported that *T.* viride and *T. harzianum* are very effective in reducing the radial growth of *S. rolfsii* and also in the production of volatile and non-volatile antibiotics against the pathogen.

From the present investigations, it is evident that all the three *Trichoderma* spp., *viz. T. viride*, *T. harzianum* and *T. hamatum* are very effective against *S. rolfsii* under *in-vitro* conditions. Further, all the *Trichoderma* spp. are found to produce substantial quantities of volatile and non-volatile

Table 2. Effect of volatile and non-volatile metabolites of *Trichoderma* spp. on *S. rolfsii* under *in vitro* conditions

Antagonist	Inhibition of *S. rolfsii* (%)				
	Volatile metabolites Age of antagonist (days)			Non-volatile metabolites Concentration of culture filtrate (%)	
	0	15	25	50	100
T. viride	4.44[a]	20.00[a]	46.67[a]	45.00[a]	65.00[a]
T. harzianum	28.89[a]	55.56[b]	73.33[c]	61.67[b]	71.66[b]
T. hamatum	5.56[a]	33.33[c]	51.11[b]	100.00[c]	100.00[c]

• Numbers in each column followed by the same letter are not significantly different.

compounds, which are detrimental to the growth of *S. rolfsii*. Moreover, all the *Trichoderma* spp. replaced the sclerotial contents of the test pathogen within 7 to 10 days by parasitization. Going by the present results, the collar-rot pathogen in elephant foot yam can be effectively checked by application of these antagonists to the soil either singly or in combination.

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