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Effect of Antagonistic Fungi on *Sclerotium rolfsii* Causing Root Rot of Groundnut

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ABSTRACT

***In vitro* studies showed that 67.4 per cent reduction of sclerotial production in *Sclerotium rolfsii* was observed in the presence of *Trichoderma viride* Pers. (isolate 2). Mature sclerotia from each dual culture plate measured 647 , 777, and 763 μm with *T.viride* (isolate 3), *T.harzianum* Rifai (isolate2) and *Laetisaria arvalis* Burdsall respectively. In slide germination test, 68.3 and 56.7 per cent reduction of sclerotial germination was observed with *T. viride* (isolate 3) and *T. harzianum* (isolate 2), respectively; whereas when plated in solid media, it was 47.5 per cent with *T.viride* (isolate 3). The number of hyphae put forth by each sclerotium was found to be minimum in sclerotia produced in dual culture with *T. viride* (isolates 2 and 3).**

KEYWORDS : *Trichoderma viride, T.harzianum, Laetisaria arvalis,* dual culture, incubation, germination

The efficacy of *Trichoderma viride* Pers. - based mycofungicide in controlling root rot of lentil caused by *Sclerotium rolfsii* Sacc. was reported by Rodriguez - Kabana *et al.* (1978). Since this is a serious disease in India, work was taken up to select an effective antagonist for the biological control of this disease. We tested eight isolates of antagonistic fungi *in vitro* to find out their inhibitory effect on mycelial growth, number, size and germination of sclerotia of *S. rolfsii*.

MATERIALS AND METHODS

Cultures of *T. viride, T.harzianum* Rifai and *Laetisaria arvalis* Burdsall., were obtained from the Department of Plant Pathology. The different isolates were :

i) *T. viride* Isolate 2 - No.143, I.T.C.C.F., New Delhi

ii) *T. viride* Isolate 3 - Coimbatore

iii) *T. viride* Isolate 4 - Commonwealth Mycological Institute, London

iv) *T. viride* Isolate 5 - Coimbatore

v) *T. viride* Isolate 6 - Netherlands

vi) *T. harzianum* Isolate 1 - Uttar Pradesh

vii) *T. harzianum* Isolate 2 - No.2895, I.T.C.C.F., New Delhi

viii) *Laetisaria arvalis* - Commonwealth Mycological Institute, London

The antagonistic effect of the five isolates of *T. viride* (No. 2,3,4,5 and 6), two isolates of *T. harzianum* (No.1 and 2) and one isolate of *L. arvalis* against *S.rolfsii* was tested by dual culture method on PDA medium (Dennis and Webster, 1971). A 9 mm diameter disc of fungal antagonist was placed at one end of the Petri dish over the PDA medium. After 24 h, just opposite to the antagonists, a 9mm diameter mycelial disc of *S. rolfsii* was placed. The growth of *S. rolfsii* was measured at 24, 48, 72, 96 and 120 h of inoculation. For each antagonist, four replications were maintained. Ten days after inoculation, the mature sclerotia of the pathogen were harvested from each Petri dish with a sterile forceps and counted. The sclerotial size was measured for each treatment. The percentage germination

Table 1. Mycelial growth of *Sclerotium rolfsii* in the dual culture plates at different intervals (mm)

Antagonists	24 h	48 h	72 h	96 h	120 h	Mean
Trichoderma harzianum - 1	11.5	27.0	39.5	52.0	67.3	39.5
T.harzianum - 2	9.0	19.5	25.3	26.5	27.5	21.5
Trichoderma viride - 2	2.0	8.3	14.0	15.8	17.3	11.5
T.viride - 3	6.0	12.8	18.8	19.3	19.3	15.2
T.viride - 4	8.0	14.8	17.8	22.3	23.5	17.3
T.viride - 5	9.0	11.8	20.8	21.3	21.3	16.8
T.viride - 6	8.0	19.8	38.5	47.5	60.3	34.8
Laetisaria arvalis	11.0	20.3	40.8	51.5	66.8	38.1
Control	19.0	31.5	46.0	64.8	80.0	48.3
Mean	9.3	18.4	29.1	35.6	42.6	

CD (P = 0.05)

Treatments - 0.4 Intervals - 0.3 Interaction - 0.9

of sclerotia from different treatments was tested by slide germination method and plating on solid media.

In slide germination method, sixty mature sclerotia from each treatment were tested by placing two sclerotia in cavity slide and a drop of sterilised 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 (Montgomery and Moore, 1938). In another test, sixty mature sclerotia from each treatment were placed over Potato Dextrose Agar (PDA) medium with equal space, incubated for 24h and examined under a microscope. The number of sclerotia germinated and number of hyphae put forth by the sclerotia were counted.

Conidial suspensions (2 ml) of *T. viride* (isolates 2 and 3) and *T.harzianum* (isolate 2) containing 5 x 10^9/ml with 1 per cent carboxy methyl cellulose were used to treat 6.5g groundnut seeds. The treated seeds were shade - dried for 1 h and sown immediately. The seed dresssing fungicide captan 75 WP was used at the rate of 4g/kg seed, 24h before sowing. Artificial infestation of unsterilized soil was accomplished by adding sclerotia of the pathogen at the rate of 0.3g (dry weight)/pot containing 3kg soil (Elad *et al.*, 1980). Five seeds were sown in each pot. Four replications were maintained for each treatment. Seed germination was recorded 15 DAS and plant survival was recorded 45 DAS. The antagonist population in the rhizosphere region was estimated by the method of Papavizas and Davey (1961) by using *Trichoderma* special medium (TSM) developed by Elad and Chet (1983).

RESULTS AND DISCUSSION

T. viride isolate-2 was significantly superior to all the other antagonists in arresting the mycelial growth of *S.rolfsii* (Table 1). This isolate recorded a mean radial growth of only 17.3 mm of pathogen as against 80.0 mm in control, 120 h after inoculation. It was followed by *T.viride* isolates 3, 5 and 4 in efficacy. *L.arvalis* did not appreciably reduce the growth of the pathogen. In view of the fact that the sclerotial population and size play an important role in the number of infected plants, the influence of the antagonists on sclerotial production, size, germination and number of hyphae produced by the sclerotium were investigated. The number of sclerotia produced was reduced to the maximum extent of 68.7 and 67.4 per cent in the presence of *T.viride* isolates 2 and 3 respectively which were on par. It is followed by *T.harzianum* isolate 2 (Table 2). *L.arvalis* did not appreciably reduce the sclerotial number. The sclerotial size was also reduced to 649 μm

Table 2. Effect of antagonists on sclerotial production, size, germination and hyphal production

Antagonists	Number of sclerotia per plate	Sclerotial size (μ)	Sclerotial germination %		Hyphal production	
			Cavity slide	In PDA	Cavity slide	In PDA
Trichoderma harzianum - 1	72.3	783	88.3 (73.4)	100.0 (89.4)	+++	+++
T.harzianum - 2	51.3	777	56.7 (48.9)	73.8 (59.3)	++	++
T.viride - 2	35.8	649	75.0 (60.1)	57.6 (49.3)	+	+
T.viride - 3	37.3	647	68.3 (55.9)	47.5 (43.6)	+	+
T.viride - 4	77.8	770	85.0 (67.7)	100.0 (89.4)	+++	+++
T.viride - 5	56.5	741	83.3 (66.6)	92.5 (76.3)	++	++
T.viride - 6	82.5	775	93.3 (75.2)	100.0 (89.4)	+++	+++
Laetisaria arvalis	91.0	763	81.7 (65.2)	100.0 (89.4)	+++	+++
Control	114.3	779	96.7 (83.5)	100.0 (89.4)	+++	+++
CD (P = 0.05)	6.5	49	14.7	5.3		

Note : Figures in parenthesis indicate mean transformed values
'+' indicates hyphal number produced by each germinating sclerotium

in the presence of *T.viride* isolate 2 from 779 μm in control. It was on par with *T.viride* isolate 3.

It was interesting to note that the percentage germination of sclerotia produced in dual culture with *T.viride* isolates 2 and 3 and *T.harzianum* isolate 2 was significantly reduced compared to control when tested by the cavity slide method. In these treatments, sclerotial germination was 75.0, 68.3 and 56.7 per cent respectively, as against 96.7 per cent in control. When the germination test was conducted in PDA, the germination was only 47.5 per cent in sclerotia produced in the presence of *T.viride* isolate 3 as against 100 per cent in control. It was followed by *T.viride* isolate 2 and *T.harzianum* isolate 2 in efficacy.

When the number of hyphae produced by the germinating sclerotia was examined, it was found to be minimum in sclerotia produced in dual culture with *T.viride* isolates 2 and 3 by the cavity slide method. A similar trend was observed in PDA also.

In the seed pelleting experiment, all the treatments except pathogen alone recorded significant increase in seed germination when compared with infested soil. They were on par (Table 3) and gave increased seed germination of 90-95 per cent as compared to 55 per cent in pathogen alone-inoculated soil. In pathogen alone- inoculated pots, all plants died before 45 DAS. All other treatments including captan were on par. The treated seeds recorded 85 to 95 per cent surviving

Table 3. Effect of seed pelleting with antagonists on seed germination, survival and antagonists population in rhizophere

Treatments	Seed germination (%)	Surviving plants (%) 45 DAS	Antagonists population in rhizophere per gm soil (cfu x 10^3/g) 75 DAS
Trichoderma viride - 2	90 (76.1)*	85 (69.8)	18.3
T.viride - 3	95 (82.4)	90 (79.2)	20.1
T.harzianum - 2	95 (82.4)	95 (82.4)	21.4
Captan	90 (76.1)	90 (76.1)	10.0
Pathogen alone	55 (48.2)	0 (1.3)	1.9
CD (P = 0.05)	20.3	20.5	4.1

* Figures in parenthesis indicate transformed values

plants as against zero in pathogen alone- inoculated soil 45 DAS. The antagonist population in rhizosphere of treated seeds was 7 to 10 times more than in those treated by pathogen alone. Captan- treated seeds recorded five-fold increase compared to pathogen alone. Seeds receiving spore load of 5×10^9 conidia/ml of *T.viride* isolates 2 and 3 and *T.harzianum* isolate 2 were on par with each other (Table 3). Among all treatments, the seed pelleting with *T.harzianum* at 5×10^9 conidia/ml was found to be the best.

Mathur and Sarbhoy (1978) reported that *T.viride* and *T.harzianum* inhibited the growth of *S.rolfsii* by 88 and 86 per cent respectively. Under scanning electron microscope Elad *et al.* (1983) observed that *T.harzianum* attached to the hyphae of *S.rolfsii* either by coiling, hooks or appressoria. The high inhibition of pathogen by *T. viride* isolate 2 indicates the great potential of this isolate in biocontrol of the pathogen. Though *T.viride* and *T.harzianum* isolates showed high level of antagonism towards *S.rolfsii in vitro,* they differed in their mechanism of action against the pathogen. *T.viride* isolates 2, 3, 4 and 5 and *T.harzianum* isolate 2 overgrew *S.rolfsii.* In addition, the latter two secreted an yellowish metabolite and formed an inhibition zone around the pathogen. Some isolates caused drastic reduction in sclerotial production by the pathogen. This may reduce the inoculum potential and subsequently the disease incidence. Since the nutrients for the development and maturity of the sclerotium are to be supplied by the hyphae, the decreased mycelial growth of the pathogen in the presence of antagonist will naturally lead to production of sclerotia of smaller size. The small size of sclerotium indicates less amount of reserve food material stored in them. Sclerotia may not attain maturity when produced in the presence of antagonists. These two factors might have contributed to reduction in sclerotial germination and also the number of hyphae produced by the sclerotium during germination. The granulated medullar cells served as external nutrient reservoir for the germ tubes. The hyphae of *T.harzianum* developed in the medulla of *S.rolfsii* sclerotia (Mutto *et al.,* 1986) and rapidly degenerated the cytoplasm of penetrated host cells.

Seed treatments with *T.viride* and *T.harzianum* were found to be as effective as captan in increasing seed germination (Table 3). In cotton, Alagarsamy *et al.* (1987) reported that seed pelleting with *Trichoderma* spp. increased the germination rate and reduced the post-emergence mortality. Ruppel *et al.* (1983) reported that seed treatment of sugarbeet with *T.harzianum* was as effective as maneb in controlling *Rhizoctonia* root-rot. Seed treatments with *T.viride* and *T.harzianum* were superior to thiram and captan in the control of tomato damping-off disease caused by *Pythium indicum* Balakrishnan (Krishnamoorthy, 1987).

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