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Biocontrol of *Sclerotium rolfsii* Sacc. Causing Collar Rot of Brinjal*

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ABSTRACT

Out of 54 isolates of fungi and bacteria isolated from soil, three isolates of *Trichoderma harzianum* Rifai (Th-1, Th-2 and Th- 3), two of *T.viride* Pers. fr. (Tv-1 and Tv-2) and one each of *Penicillium* sp., *P.cyclopium* Westling, *Eupenicillium* sp., *Aspergillus* sp., *A. flavus* Link ., *A.fumigatus* Fres., *A.niger* Van Tieghem and an unidentified bacterium were found antagonistic to *Sclerotium rolfsii* Sacc. All the isolates of *T.harzianum, T.viride* and *Gliocladium* sp. were found to be potential antagonists when tested by dual culture technique and culture filtrate methods. Microscopic observations revealed the mycoparasitic activity of *T.harzianum* in dual culture. Inhibitory activity of autoclaved culture filtrate was less effective as compared to filter-sterilized culture filtrate. Culture filtrate of *T.harzianum* (Th-2) and *Gliocladium* sp. were the most effective in inhibiting sclerotial germination. Complete inhibition of sclerotial germination was observed in *T.harzianum* (Th-1, Th-2 and Th-3), *T.viride* (Tv-1 and Tv-2) and *Gliocladium* sp. treated sclerotia after 30 days of incubation in soil. Wheat bran substrate supported maximum growth of *T.harzianum* and *T.viride*. Mixing of wheat bran culture of *T.harzianum* (Th-2), *T.viride* (Tv-2) and *Gliocladium* sp. @ 10 g/kg soil reduced the seedling mortality to 12.71, 14.72 and 17.98 per cent, respectively as compared to 86 per cent in check under green house conditions.

KEY WORDS : *Sclerotium rolfsii*, biocontrol, antagonists, *Aspergillus* spp. *Trichoderma* spp., *Penicillium* spp., *Eupenicillium* spp., *Glioclodium* spp.

Collar rot caused by *Sclerotium rolfsii* Sacc. is one of the important diseases of brinjal (*Solanum melongena* L.). The pathogen attacks the seedlings in nursery beds and grown up plants in transplanted fields. *S.rolfsii* is an unpredictable pathogen because of its soil-borne nature and wide host range. Moreover, the ability of its sclerotia to withstand adverse conditions and aggressiveness of the fungus, under favourable conditions make it a highly successful pathogen. Since its control through host resistance and/or fungicide is difficult to achieve (Datar *et al.*, 1990), biological control is suggested as an alternative method. The present studies were, therefore, undertaken primarily to see the effect of antagonists and their culture filtrates on the mycelial growth and sclerotial germination of *S.rolfsii*, to screen some substrates for mass multiplication and to study the biocontrol potential of the antagonists under green house conditions.

MATERIALS AND METHODS

Soil samples were collected from different brinjal growing areas of Himachal Pradesh and the antagonistic fungi were isolated by serial dilution technique on potato dextrose agar (PDA) plates (Johnson, 1957). The Petri plates were incubated at 25 ± 1^{0}C for 12-36 h. The emerging colonies of fungi were transferred to PDA slants. Cultures were purified by single spore isolations, maintained on PDA slants and identified as per Gilman (1957). The culture of *Gliocladium* sp. was obtained from the Department of Plant pathology at Solan. *S. rolfsii* was isolated from a diseased brinjal plant on PDA.

A dual culture technique (Huang and Hoes, 1976) was used to study the antagonism and

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mode of hyperparasitism. After 96 h of incubation, the per cent inhibition in growth of pathogen was recorded according to Vincent formula (Vincent, 1947). The dual cultures were further incubated for 1-3 weeks and mounted in lactophenol-cotton blue for microscopic observations.

The culture filtrates of potential antagonists were obtained from their liquid culture filtering through whatman No.1 filter paper. The filtrates were then sterilized by autoclaving at 15 lb psi . In another set of experiments, culture filtrates of antagonists were sterilized by filtering through sintered glass filters. Culture filtrate incorporated in double strength PDA was poured in Petri plates, inoculated with 5 mm mycelial disc of *S.rolfsii*, incubated at 25 ± 1^{0}C and colony diameter measured after 96 h.

In order to study the effect of different concentrations of culture filtrate of *T.harzianum* and *T.viride* on pathogen mycelial growth, the filter - sterilized culture filtrates were mixed separately in PDA in different proportions of 0:1, 1:1, 2:1 and 3:1 in such a way that the concentration of ingredients of PDA was not altered.

Sclerotia of *S.rolfsii* were soaked for 12, 24 and 36 h in culture filtrate of antagonists. The sclerotia were removed and washed 5-6 times with sterilized water and plated on PDA Petri plates. Germination counts were taken after 120 h of incubation at 25 ± 1^{0}C. In an other experiment, sclerotia were treated for 30 minutes in spore suspension (1 x 10^8 ml^{-1}) of different antagonists prepared separately in sterilized water from a 9 day - old culture grown on PDA. Sclerotia treated with sterilized water served as check. The treated sclerotia were burried in sterilized soil at 4 cm depth in plastic vials (8.5 x 3.5 cm) and incubated at room temperature (25 - 28 ^{0}C). After 15 and 30 days of incubation, sclerotia were removed from the soil, washed 5-6 times with sterilized water and plated on PDA Petri plates. The Petri plates were incubated at 25 ± 1^{0}C and germination was recorded after 120 h of incubation.

In order to find out the suitable substrate which sustains maximum growth of *Trichoderma* spp., five different easily available and cheap substrates *i.e* shelled cobs, brinjal leaves and stems, saw dust, wheat straw and farm yard manure were taken alongwith wheat bran as a comparison. These were separately crushed to powder in an electric grinder. The screening of substrate was conducted by employing agar plate culture and Ryan Beadle and Tatum tube culture methods.

For biocontrol studies under green house conditions, six treatments of each *T.harzianum, T. viride* and *Gliocladium* sp. namely; seed treatment with dry spore (1:100 w/w), seed dip in spore suspension (10^{8}ml^{-1}), mixing of wheat bran culture in soil (10 g kg^{-1}), soil drenching with 150 ml each of mycelial fragments (10^8 ml^{-1}) and spore suspension (10^8 ml^{-1}) were tested for the control of disease in pots. Mycelial fragments were prepared by homogenizing the mycelial mat in hamogenizer. For mycelial and conidial preparation of antagonists, 2 and 9 days old cultures respectively were used. The concentration of mycelial fragments and conidial suspension were adjusted with the help of a haemocytometer. The sterilized soil was infested with *S.rolfsii* by adding wheat grown inoculum of *S.rolfsii* (10 g kg^{-1} of soil). Fifty seeds of brinjal cv Pusa Purple Long were sown in each pot. The data on seedling mortality were recorded upto 45 days after sowing.

RESULTS AND DISCUSSION

Out of fiftyfour isolates of fungi and bacteria isolated from naturally infested soil of *S. rolfsii*, three isolates of *T.harzianum* Rifai, two isolates of *T.viride* Press. ex. S.F.Gray and one isolate each of *Penicillium cyclopium* Westling, *Eupenicillium* sp., *Aspergillus flavus* Link, *A.fumigatus* Fres., *A.niger* Van Tiegham and *Aspergillus* sp. were identified and found antagonistic to *S. rolfsii*. The data (Table -1) revealed that in dual culture, maximum inhibi-

Table 1. Mycelial inhibition of *S. rolfsii* by different antagonists and their culture filtrates (Dual culture and culture filtrate methods)

Antagonist	Isolate	Per cent inhibition*		
		Dual culture	Culture filtrate	
			Filter-sterilized	Heat-sterilized
Aspergillus flavus		7.03 (15.34)	5.41 (13.56)	5.32
A. fumigatus		25.75 (30.46)	6.79 (15.12)	5.33
A. niger		33.14 (35.12)	12.71 (20.88)	3.55
Aspergillus sp.		7.58 (16.00)	6.09 (14.13)	1.55
Bacterium (unidentified)		2.06 (8.13)	--	--
Eupenicillium sp.		21.85 (27.83)	14.36 (22.46)	4.22
Gliocladium sp.		65.81 (54.97)	54.01 (47.29)	5.11
Penicillium cyclopium		38.14 (38.06)	19.26 (25.99)	1.33
Penicillium sp.		37.22 (37.58)	20.92 (27.13)	5.11
T. harzianum	Th-1	67.22 (55.06)	39.61 (39.00)	4.88
	Th-2	73.40 (58.95)	57.56 (49.31)	7.33
	Th-3	57.23 (49.14)	48.83 (44.03)	3.55
T. viride	Tv-1	53.53 (47.01)	19.20 (25.99)	5.33
	Tv-2	59.62 (50.53)	15.27 (23.03)	1.33
Control		0.00 (0.00)	0.00 (0.00)	0.00
S.E. (d)		(1.03)	(1.23)	1.65
L.S.D (P=0.05)		(2.30)	(2.48)	3.34

* Average of four replications
Figures in parenthesis are angular transformations
-- = Not tested

Table 2. Inhibition of mycelial growth (%) of *S. rolfsii* by different culture filtrates of potential antagonists mixed with PDA in different proportions*.

Filtrate : PDA	*T. harzianum*	*T. viride*
	(Th-2)	(Tv-1)
0 : 1	0.00	0.00
1 : 1	59.65	18.06
2 : 1	82.60	23.03
3 : 1	100.00	35.86
S.E. (d)	8.73	1.93
L.S.D. (P=0.05)	18.06	3.99

* Average of five replications

tion of mycelial growth of *S.rolfsii* was obtained with Th-2 isolate of *T.harzianum* (73.4%) and least with an unidentified bacterium (2.06%). In general, isolates of *T. harzianum, T. viride* and *Gliocladium* sp. showed high antagonistic activity as compared to other species and thus, have considerable biocontrol potential. Antagonism of *T. harzianum, T. viride* and *G. virens* have also been reported by Elad *et al.* (1980) and Lewis *et al.* (1989). Microscopic observations in the present study revealed that *T.harzianum* penetrated the host hyphae. Attempts to reisolate *T.harzianum* from infected hyphae of *S.rolfsii* resulted in recovery of the antagonist. Unlike filter- sterilized culture filtrate, heat-sterilized culture filtrate did not cause much growth inhibition of *S.rolfsii.* However, the trend of inhibition of mycelial growth of *S.rolfsii,* by culture filtrate was same as in the case of direct antagonism in dual culture. Maximum inhibition was observed with Th-2 isolate of *T.harzianum* which was significantly more than the inhibition by the culture filtrate of *Gliocladium* sp. Mycelial inhibition of *S.rolfsii* was increased significantly with the increase in the concentration of culture filtrate of antagonists (Table-2) and the effect was more with *T.harzianum* (Th-2) as compared to *T.viride* (Tv-1).*T.harzianum* excreted β - 1, 3 glucanase and chitinase which affects the growth of *S.rolfsii* (Davet, 1987).

Table 3. Germination (%) of *S. rolfsii* sclerotia treated with conidia of antagonist and burried in sterilized soil for different duration*.

Antagonist	Isolate	Incubation period (days)	
		15	30
Aspergillus flavus		18.75 (25.64)	2.50 (9.20)
A. fumigatus		15.00 (22.83)	1.25 (6.28)
A. niger		49.00 (44.46)	5.00 (12.94)
Aspergillus sp.		68.75 (55.98)	13.75 (21.74)
Eupenicillium sp.		53.00 (46.76)	13.75 (21.70)
Gliocladium sp.		6.25 (14.46)	0.00 (0.003)
Penicillium cyclopium		15.00 (22.80)	2.50 (9.18)
Penicillium sp.		19.25 (25.95)	6.25 (14.50)
T. harzianum	Th-1	3.75 (11.24)	0.00 (0.003)
	Th-2	4.00 (11.60)	0.00 (0.003)
	Th-3	6.25 (14.40)	0.00 (0.003)
T. viride	Tv-1	2.50 (9.15)	0.00 (0.003)
	Tv-2	2.50 (9.20)	0.00 (0.003)
Control		87.50 (69.20)	85.00 (67.23)
S.E. (d)		2.52	1.07
L.S.D (P=0.05)		5.08	2.16

* Average of four replications
Sclerotia (80 per treatment) were suspended in conidial suspension (10^8 ml^{-1}) for 30 minutes before putting in soil for incubation.
Figures in parenthesis are Arc Sin transformed values.

The rate of sclerotial germination decreased with the increase in co-incubation of antagonist and pathogen in soil. *T.harzianum* and *T.viride* - treated sclerotia did not germinate even after 30 days of incubation in soil. The failure of sclerotial germination may be attributed to penetration and utilization of sclerotia contents by the antagonists. Such infected sclerotia yielded mycelial growth of antagonists on PDA. *A. fumigatus* and *Aspergillus* spp. - treated sclerotia gave 1.25 to 13.75 per cent germination of sclerotia as compared to 85 per cent in check after 30 days of incubation in soil (Table-3) .These findings are in conformity with those of earlier workers (Lozano and Pineda, 1977; Modi and Henis, 1989).

Table 4. Sclerotial germination (%) of *S. rolfsii* in the culture filtrate of potential antagonists at different intervals*.

Antagonist	Isolate	8 h	16 h	24 h
Gliocladium sp.		27.75 (31.36)	20.00 (26.57)	7.33 (15.68)
T. harzianum	Th-1	30.77 (33.65)	21.00 (27.27)	12.00 (20.27)
	Th-2	23.00 (28.66)	12.50 (20.70)	7.00 (15.34)
	Th-3	29.00 (32.67)	24.00 (29.33)	16.00 (23.58)
T. viride	Tv-1	48.00 (43.28)	40.00 (39.23)	24.00 (29.33)
	Tv-2	42.66 (40.74)	34.00 (35.67)	23.00 (28.66)
Control		87.00 (68.87)	89.00 (70.63)	85.00 (67.83)
S.E. (d)		(2.12)	(1.89)	(2.39)
L.S.D (P=0.05)		(4.41)	(3.93)	(4.97)

* Average of five replications
Figures in parenthesis are angular transformed values.

Table 5. Colony diameter (mm) of *T. harzianum* and *T. viride* on powdered substrate mixed with agar*.

Substrate	Incubation period (h)					
	T. harzianum			*T. viride*		
	24	48	72	24	48	72
Brinjal leaves and stem	17.2	40.6	58.6	23.6	46.8	67.4
Farm yard manure	13.4	38.0	57.6	33.4	57.6	80.0
Saw dust	16.0	35.0	56.2	17.0	36.2	55.8
Shelled cobs	27.2	67.2	89.0	34.8	73.8	89.6
Wheat bran	28.6	71.2	89.4	36.6	76.8	90.0
Wheat straw	28.0	68.2	88.2	44.0	71.6	90.0
S.E (d)	2.13	2.08	1.52	1.03	2.03	0.75
L.S.D. (P=0.05)	4.39	4.28	3.14	2.12	4.18	1.54

* Average of five replications

T.harzianum (Th-2) and *Gliocladium* sp. reduced the sclerotial germination to 7 per cent as compared to 85 per cent in check after 24 h of dip in culture filtrate (Table-4). The effect of culture filtrate increased with increase in duration of treatment . Elad *et al.* (1983) reported that β - 1, 3 glucanase and chitinase in culture filtrate affected germination of sclerotia.

Wheat bran substrate supported maximum growth of *T.harzianum* and *T.viride* (Table-5 & 6). However, brinjal leaves and stem and saw dust supported minimum growth of *Trichoderma* spp. which might be due to the presence of growth inhibitory substances like phenols and tannins.

All treatments of *T.harzianum* (Th-2), *T.viride* (Tv-2) and *Gliocladium* sp. gave significant control of disease (Table 7). Mixing of wheat bran culture of test antagonists @ 10 g/kg soil was the most effective in reducing the seedling mortality under green house condition. *T.harzianum* gave minimum seedling mortality (12.71%) followed by *T.viride* (14.7%) and *Gliocladium* sp.. (17.98%) as compared to control (86 %). Seed dip in mycelial fragments suspension of *T.harzianum* and *T.viride* gave 19.05 and 20.22 per cent seedling mortality, respectively and was at par with soil drenching. However, seed dip treatment of *Gliocladium* sp. differed significantly from soil drenching. Seed dip in spore suspension of all the test antagonists was inferior to spore and fragments drenching and seed dip in mycelial fragments suspension. Seed mixing in dry spore was least effective. Information regarding the biocontrol of collar rot of brinjal is

Table 6. Linear growth of mycelium (mm) of *T. harzianum* and *T. viride* on different substrates (Ryan Beadle and Tatum tube)*.

Substrate	Incubation period (days)							
	T. harzianum				*T. viride*			
	1	4	8	12	1	4	8	12
Brinjal leaves and stem	6.50	22.75	37.75	49.25	7.00	20.25	36.75	57.75
Farm yard manure	8.75	28.00	62.15	99.25	18.75	34.25	93.00	105.50
Saw dust	5.50	14.50	20.50	41.15	5.50	10.50	29.00	45.25
Shelled cobs	16.50	32.25	66.00	103.75	10.75	28.75	77.25	104.50
Wheat bran	9.75	47.00	95.50	116.00	10.25	44.75	91.50	117.00
Wheat straw	11.50	49.50	98.75	109.75	15.75	34.25	77.75	107.50
S.E (d)	3.06	1.89	2.83	2.75	2.78	3.87	4.02	3.13
L.S.D. (P=0.05)	6.30	3.89	5.83	5.77	5.72	7.97	8.28	6.56

* Average of five replications

Table 7. Effect of different methods of application of potential antagonists on the seedling mortality (%) of brinjal*.

Treatment	*T. harzianum* (Th-2)	*T. viride* (Tv-2)	*Gliocladium* sp.
Seed + dry spore (1:100 W/W)	67.51 (55.24)	68.14 (55.67)	71.27 (57.54)
Seed dip in spore suspension (10^8 ml^{-1})	57.34 (49.24)	61.61 (51.65)	64.87 (53.62)
Seed dip in (Mycelial fragments suspension (10^8 ml^{-1})	19.05 (25.84)	20.22 (26.78)	60.87 (51.24)
Seeds sown in soil drenched with spore suspension (10^8 ml^{-1})	29.61 (32.92)	36.07 (36.93)	52.17 (46.26)
Seeds sown in soil drenched with mycelial fragments (10^8 ml^{-1})	24.12 (29.52)	21.01 (27.26)	36.32 (37.10)
Seeds sown in soil mixed with antagonist grown on wheat bran (10 g/kg soil)	12.71 (20.86)	14.24 (22.15)	17.98 (25.05)
Check-I (*S.rolfsii* - infested sterilized soil)	86.18 (68.20)	86.20 (68.26)	86.29 (68.16)
Check-II (Sterilized soil)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.(d)	(2.16)	(2.08)	(3.01)
L.S.D. (P=0.05)	(4.49)	(4.32)	(6.25)

* Average of four replications
Figures in parenthesis are Arc sin transformed values.

lacking. However, diseases caused by *S.rolfsii* in other crops are reported to be effectively controlled by the soil application of wheat bran culture of *T.harzianum* and *T.viride*. Mathur and Sarbhoy (1978) have reported the effectiveness of wheat bran preparation of *T.harzianum* and *T.viride* in controlling root rot of sugarbeet caused by *S.rolfsii*. Papavizas and Lewis (1989) have reported that *T.harzianum* and *G.virens* reduced the damping -off of Snap bean caused by *S.rolfsii* under green house conditions. This is the first report concerning collar rot of brinjal from India.

KEY WORDS : *Sclerotium rolfsii* antagonists, biocontrol, brinjal.

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