J.Biol.Control, 5 (2), 105-109, 1991

Antagonism of *Trichoderma harzianum* and *Gliocladium virens* Isolates to *Sclerotium rolfsii* and Biological Control of Stem Rot of Groundnut and Betelvine

D. MAITI, B. DASGUPTA and CHITRESHWAR SEN
Department of Plant Pathology
Bidhan Chandra Krishi Viswavidyalaya
Kalyani - 741 235, Nadia, West Bengal

ABSTRACT

Of the 15 isolates in each of *Trichoderma harzianum* and *Gliocladium virens* tested, one biotype of the former and three of the latter proved to be highly antagonistic to *Sclerotium rolfsii*. These isolates did not secrete any biostatic compound(s) into the medium but produced volatiles inhibitory to the pathogen. Histopathological studies revealed that the hyperparasite, *G. virens* attacked the pathogen hyphae by appressoria, hooks and intercellular growth. Production of the selectively high potential parasite in different solid surface media, their survival potential in such media and their use against *S. rolfsii* causing stem rot of groundnut and betelvine, both in greenhouse and field, are reported.

KEY WORDS : *Trichoderma harzianum, Gliocladium virens, Sclerotium rolfsii,* stem rot, groundnut, betelvine

In spite of striking success in the laboratory, field performance of biocontrol agents against soil borne plant pathogens presented problems that are yet to be resolved. Integrated approaches with cultural practices were more successful (Maiti and Sen, 1985; Sen and Maiti, 1983, 1988), but these were not free of problems because of sensitivity to fungicides (Abd-el-Moity *et al.*, 1982). Other reasons for the erratic field performance were, lack of proper identification of biotypes with antagonistic properties, as all isolates were not equally antagonistic, lack of realisation that a population above threshold level of virulent biotype is to be maintained for sclerotial and hyphal destruction of the pathogen and failure to develop suitable delivery system that will work for sufficient time to maintain the population above the threshold level. This paper reports selection of virulent biotypes, their possible mode of action and their effectiveness in controlling stem rot of groundnut and betelvine both in lab and field.

MATERIALS AND METHODS

Fifteen isolates of *Gliocladium virens* and *Trichoderma harzianum* isolated from soils of W. Bengal were evaluated using Davet's medium (Davet, 1979) and a modified TSM medium (Maiti, 1986). The antagonistic potential of the isolates was tested by the method developed by Bell *et al.* (1982). Observations were recorded after seven days of incubation. The treatments were replicated three times. Their antibiotic production potential was tested using culture filtrates in Richard's medium filtered through UF grade sintered glass filter. The sclerotia were directly soaked or placed in impregnated filter paper and then germination was counted. Test of antagonism through volatile production was tested by the method of Webster and Lomas (1964). *In vivo* mycoparasitisation of sclerotia in soil was tested by isolating sclerotia from inoculated soil and testing for their germinability (Agnihotri *et al.*, 1975).

Different delivery systems were developed using wheat bran-sand (1:2),

Table 1. Efficacy of mycoparasites in dual culture

Code No.of Isolate	Mean score	Code No.of Isolate	Mean score
S_1^1	2.3	S_8^3	4.0
S_1^2	5.0	S_9^1	5.0
S_2^2	4.0	S_9^2	4.0
S_2^3	4.0	S_{10}^1	2.3 (=2.0)
S_3^2	5.0	S_{10}^2	4.0
S_3^8	1.3 (=1.0)	S_{11}^4	1.3 (=1.0)
S_4^4	1.0	S_{11}^6	3.0
S_4^6	4.0	S_{12}^1	5.0
S_5^3	3.6 (=4.0)	S_{12}^2	3.0
S_5^4	5.0	S_{13}^2	4.0
S_6^1	4.3	S_{13}^3	4.0
S_6^2	1.3 (=1.0)	S_{14}^3	5.0
S_7^1	4.6 (=5.0)	S_{14}^4	4.0
S_7^2	4.0	S_{15}^1	4.0
S_8^2	1.3 (=1.0)	S_{15}^2	5.0

Figures in parentheses are the scores assigned by Bell's test

wheat bran-saw dust-water (3:1:6) and chopped straw-acid mineral solution (One kg chopped straw moistened with 2L mineral solution of following composition: $Ca(NO_3)2.4H_2O$- lg; $CaC1_2.2H_2O$- 1g, KNO_3 -0.25g; $MgSO_4.7H_20$- 0.25g; KH_2PO_4- 0.125g; K_2HPO-0.125g; tapwater-1L. The inocula were mixed at the rate of 5.0 g/kg soil and population of *Gliocladium* and *Trichoderma* assessed at 0, 10 and 40 days. In the field trial on groundnut, normal dose of N(80 kg/ha) as Ammonium sulphate, N + 100g *G. virens* S_{44} inoculum on wheat bran saw dust medium/m^2 and *G. virens* alone were tested. The treatments were replicated five times.

For control of betelvine stem rot, four levels of the fungicide Rizolex, two isolates each of *T. harzianum* and *G. virens* were tested. In each pot, ten plants were retained. The treatments were replicated four times. The experiment was repeated over two years. Actively growing mycelia of *S. rolfsii* grown on sand-maize meal medium were mixed with sand in the proportion of 1:5 and placed around one month old seedlings (approximately 20 g culture per kg soil). Ten grams of a culture preparation in wheat bran-sand-water medium of *T. harzianum* and *G. virens* per kg soil were applied around the seedlings immediately after the inoculation with *S. rolfsii*. Drenching and dusting of Rizolex were done 2 days after inoculation with *S. rolfsii*. For soil drench, 250ml of 0.2% and 0.4% suspensions of fungicide were used per pot containing 3 kg soil.

RESULTS AND DISCUSSION

The results of screening of the isolates (Table 1) showed that *G. virens* isolates *viz.*, S_{44}, S_{38}, S_{82}, S_{114} and *T. harzianum* isolate S_{62} had maximum antagonistic potential against *S. rolfsii*. These isolates are deposited with the CMI (IMI nos. 282995, 282993, 282996, 282997 and 282994 respectively). Culture filtrate, hot and cold-sterilised, when tested by soaking sclerotia or placing them in impregnated filter paper did not inhibit the germination. When mixed with growth media, no significant growth inhibition was observed. These isolates produced volatiles that partially inhibited the growth of *S. rolfsii* in

Table 2. Effect of volatiles on mycelial growth of *S. rolfsii* (% decrease in mycelial growth over control)

Isolate	Growth of antagonist before pairing		
	Oh	24h	48h
S_6^2(*T.h*)	20.6 (4.6)	40.0 (6.3)	35.8 (6.0)
S_3^8(*G.v*)	14.0 (3.8)	39.4 (6.3)	37.9 (6.2)
S_4^4(*G.v*)	34.2 (5.9)	44.6 (6.7)	60.2 (7.8)
S_8^2(*G.v*)	0.0 (0.7)	22.0 (4.8)	39.0 (6.2)
S_{11}^4(*G.v*)	19.0 (4.4)	19.6 (4.4)	36.5 (6.1)
CD for treatment x incubation		$t_{0.01, 30}$	1.5

(*T.h* = *T.harzianum* ; *G.v* = *G. virens*)
Data in parentheses are transformed (square root) values

Table 3. Population of *G.virens* in soil (X 10^5/g) after adding inoculum

Treatment	Days after inoculation		
	5	10	40
Wheat bran + tap water (1:2; w/v)	52.9	91.8	100.0
Wheat bran + sawdust + tap water (3:1:6; w/w/v)	159.9	242.5	406.3
Paddy straw + mineral solution	4.0	8.7	13.5
CD for media ^{t}	0.01,19	33.3	
CD for days ^{t}	0.01,19	33.3	
CD for media x days^t	0.01,19	57.5	

culture, the most effective being S_44 of *G. virens* (Table 2). However, the degree of inhibition was not sufficient to cause major effects in disease control. Dual cultures showed overgrowth of antagonist on pathogen. Coiling, penetration and formation of hooks were commonly observed. Hyphal lysis was the end result.

G. virens S_44 was grown on different solid surface media and added to soil. Wheat bran-saw dust tap water mixture was most effective and the population proliferated upto 40 days to the extent of about two and a half times

Table 4. Effect of mycoparasites on the sclerotial population of *Sclerotium rolfsii* in soil (number/ 100g soil)

Isolate No.	Mean sclerotial population			
	Days			
	0	15	30	45
S_6^2 (*T.h*)	100.0	64.0	38.0	28.7
S_4^4 (*G.v*)	98.7	44.7	21.3	4.7
S_3^8 (*G.v*)	99.3	68.7	57.3	41.7
S_8^2 (*G.v*)	99.3	65.3	54.7	49.3
S_{11}^4 (*G.v*)	100.0	70.7	44.7	30.7
Control		100.0	95.3	83.3
CD treatment; ^{t} 0.01, 48				4.2
CD days; ^{t} 0.01, 48				3.2
CD treatment X days; ^{t} 0.01, 48				8.4

(*T.h = T.harzianum; G.v. = G.virens*)

(Table 3). Sclerotia of pathogen and wheat bran-saw dust inoculum of antagonists were mixed with soil and incubated for different periods. The sclerotia were harvested after 0, 15, 30 and 45 days and their viability tested. *G. virens,* S_44 was the most effective followed by *T. harzianum* S_62 (Table 4).

In the field control of groundnut stem rot, *G. virens* alone was very effective and the disease incidence was further reduced by applying the antagonist along with normal dose of nitrogen. It doubled the yield over control (Table 5).

Table 5. Effect of *G.virens* on groundnut stem rot and nut yield

Treatment	% mortality		Nut yield (kg/ha)
Control I (80 kg N/ha)	22.7 (28.5)		514.2
Control II (no N)	37.6 (37.8)		295.8
G.virens + N	7.0 (15.3)		607.5
G.virens	10.9 (19.3)		574.2
C.D. for fertilizer; ^{t} 0.01	2.9	^{t} 0.05	21.7
C.D. for antagonist; ^{t} 0.01	2.7	^{t} 0.05	16.9

In a pot culture experiment, isolates of *T. harzianum* and *G. virens* were tested for two years along with the fungicide Rizolex for the control of betelvine stem rot. Patna isolate of *T. harzianum* at 10g/kg and GBPUAT isolate of *T. harzianum* at 20g/kg recorded no incidence of the disease compared to 16.6 and 93.3 per cent disease incidence in 0.4% Rizolex drenching and untreated control respectively (Table 6).

The present studies proved four isolates of *G. virens* and one of *T. harzianum* to be of promise in parasitising *S. rolfsii*. Since culture filtrates of the antagonists did not inhibit growth of the pathogen or affected germination of sclerotia, toxic principles may not be

Table 6. Effect of antagonists and Rizolex on betelvine stem rot incidence (%)

Treatment	1988	1989
Rizolex 50% WP 0.2% drench	76.7 (62.7)	–
Rizolex 50% WP 0.4% drench	16.7 (15.0)	–
Rizolex 10% dust 5 g/3 kg	58.8 (50.0)	–
Rizolex 10% dust 10 g/3 kg	46.7 (38.9)	
T.harzianum (Patna) 10 g/kg	00.0 (00.0)	–
T.harzianum (GBPUAT) 20 g/kg		0.0 (00.0)
T.harzianum (GBPUAT) 10 g/kg	27.8 (26.7)	40.0 (35.2)
G.virens (Isolate I) 40 g/kg	–	43.7 (41.0)
G.virens (Isolate II) 40 g/kg	–	25.0 (22.5)
Control	93.3 (75.2)	87.5 (77.9)
CD at 5%	(39.3)	(40.2)

Figures in parentheses are the angular transformed values

involved. These antagonists have been reported to produce antibiotics like viridin, gliotoxin, gliovirin, trichovirin A_{40} etc. (Howell and Stipanovic, 1983). Production of inhibitory volatile substances have been noted by Tu (1980) and Howell (1982). They may act as secondary weapons predisposing the pathogen to enzymic degradation, as there is overwhelming evidence that the hyperparasites cause lysis through production of chitinase, β-(1,3)-glucanase and penetrate the hyphae through coiling, appressoria formation and hooks (Chet *et al.*, 1978; Chet and Elad, 1982).

Our observations showed that most soils have threshold population of *Gliocladium, Trichoderma* or both. However, successful control occurs only when the cfu is raised to the level of ca 10^6/g soil. The antagonist is not able to reach this threshold level in the well bufferised agricultural soils. Hence, a good delivery system is necessary. Several delivery systems have been tested by different workers giving variable results (Wells *et al.*, 1972; Backman and Rodriguez-Kabana, 1975; Hadar *et al.*, 1979; Lewis and Papavizas, 1984) but very few studies were conducted to study the cfu as a function of time. We found that wheat-bran: saw dust: inoculum when added to soil, maintained the population above the threshold level for the entire six week test period.

It is known that nitrogenous fertilizers reduce *S. rolfsii* incidence (Huber, 1981; Leach and Davey, 1942; Thakur and Mukhopadhyay, 1972). The addition of the antagonist along with N fertilizer further reduced the disease and brought about integrated control. The excellent control of stem rot of betelvine by *T. harzianum* (Patna) at a low dosage provides a cheap method of managing this disease since it was superior to the fungicide Rizolex. These results clearly showed that biological control of *S. rolfsii* with *G. virens* and *T. harzianum* appear to be very promising provided proper attention is given to selection of virulent isolates and proper delivery system.

ACKNOWLEDGEMENT

The authors are thankful to Dr. S. Maiti, Project Coordinator, AICRP Betelvine, ICAR, Bangalore, and Prof. A.N. Mukhopadhyay of G.B.P.U.A.T., Pantnagar for providing two isolates of *T. harzianum* and to M/s Rallis India for providing samples of Rizolex.

REFERENCES

ABD-EL MOITY, T.H., PAPAVIZAS, G.C. and SHATLA, M.N. 1982. Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. *Phytopathol.*, **72**, 396-400.

AGNIHOTRI, V.P., SEN, C. and SRIVASTAVA, S.N. 1975. Role of fungitoxicants in the control of *Sclerotium* root rot of sugarbeet, *Beta vulgaris* L. *Indian J. Exp. Biol.*, **13**, 89-91.

BACKMAN, P.A. and RODRIGUEZ-KABANA, R. 1975. A system for the growth and delivery of biological control agents to the soil. *Phytopathol.*, **65**, 819-821.

BELL, D.K., WELLS, H.D. and MARKHAM, C.R. 1982. *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. *Phytopathol., 72,* 379- 382.

CHET, I. and ELAD, Y. 1982. Prevention of plant infection by biological means. In "*La Selection des plantes pour la Resistance aux Maladies.*" pp. 195-204. Institute National de la Recherche Agronomique, Paris.

CHET, I., HADAR, Y., ELAD, Y. and HENIS, Y. 1978. Biological control of soil-borne plant pathogens by *Trichoderma harzianum. Proc. 3rd Intern. Congr. Pl. Path.*, 185 pp.

DAVET, P. 1979. Technique pour 1' analyse des populations de *Trichoderma* et de *Gliocladium virens* dans le soil. *Ann. Phytopathol.*, **11**, 529-533.

HADAR, Y., CHET, I. and HENIS, Y. 1979. Biological control of *Rhizoctonia solani* damping- off with wheat-bran culture of *Trichoderma harzianum. Phytopathol.*, **69**, 64-68.

HOWELL, C.R. 1982. Effect of *Gliocladium virens* on *Pythium ultimum, Rhizoctonia solani* and damping-off of cotton seedlings. *Phytopathol.*, **72**, 496-498.

HOWELL, C.R. and STIPANOVIC, R.D. 1983. Gliovirin, a new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum. Can. J. Microbiol.*, **29**, 321-324.

HUBER, D.M. 1981. The use of fertilizers and organic amendments in the control of plant disease. In"*Handbook of Pest Management in Agriculture*", (D. Pimental Ed.) pp. 357-394. CRC Press, Florida.

LEACH, L.D. and DAVEY, A.E. 1942. Reducing southern *Sclerotium* rot of sugarbeets with nitrogenous fertilizers. *J. Agric. Res.*, **64**, 1-18.

LEWIS, J.A. and PAPAVIZAS, G.C. 1984. A new approach to stimulate population proliferation of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. *Phytopathol.*, **74**, 1240-1244.

MAITI, D. 1986. *Studies on the Integration of Cultural Practices in the Biocontrol of Sclerotium rolfsii* Sacc. *in Soil.* Ph.D. Thesis, B.C.K.V., Kalyani, Nadia, W.B. India, pp. 163.

MAITI, D. and SEN, C. 1985. Integrated biocontrol of *Sclerotium rolfsii* with nitrogenous fertilizers and *Trichoderma harzianum. Indian J. Agric. Sci.*, **55**, 464-468.

SEN, C. and MAITI, D. 1983. Integration of cultural practices in the biocontrol of *Sclerotium rolfsii. Proc. 3rd Intern. Symp. Microbiol. Ecol.*, pp. 67 (Abst.). East Lansing, U.S.A.

SEN, C. and MAITI, D. 1988. Biocontrol of *Sclerotium rolfsii* as a component of integrated control. *Proc. Int. Conf. Res. Plant Sci. & its Relevance to Future,* pp. 175 (Abst.), New Delhi.

THAKUR, R.P. and MUKHOPADHYAY, A.N. 1972. Nitrogen fertilization of sugarbeet in relation to sclerotium root rot caused by *Sclerotium rolfsii* Sacc. *Indian J. Agric. Sci.*, **42**, 614-617.

TU, J.C. 1980. *Gliocladium virens,* a destructive mycoparasite of *Sclerotinia sclerotiorum. Phytopathol.*, **70**, 670-674.

WEBSTER, J. and LOMAS, 1964. Does *Trichoderma viride* produce gliotoxin and viridin. *Trans. Brit. Mycol. Soc.*, **47**, 535-540.

WELLS, H.D., BELL, D.K. and JAWORSKI, C.A. 1972. Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii. Phytopathol.*, **62**, 442-447.