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Variation in some biological characters of wild and mutant isolates of *Gliocladium virens*

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A number of mutant isolates were raised through induced mutation of a potential isolate of *Gliocladium virens* (15 GV1) using γ ray at different doses (50 KR, 75 KR and 100 KR.). According to the hyperparasitic activity of mutant isolates over *Rhizoctonia solani, Sclerotium rolfsii, Fusarium udum* and *Macrophomina phaseolina,* three most efficient mutant isolates (50 KRI, 75 KRV and 100 KRIV) were picked up for this study along with their wild biotype (15 GV_1). The germinability of phialo- and chlamydospores was studied using different sources of carbon (glucose, sucrose, starch) and nitrogen (peptone, ammounium nitrate, potassium nitrate and ammonium sulphate). Among them, peptone was found superior for gemination of both spore types followed by ammonium nitrate for chlamydospores irrespective of isolates. In general, the different spores form of mutant isolates had less germination than the wild isolate. Except in sterilized soil leachates for 50 KRI, both phialo- and chiamydospores of wild isolate exhibited better germination than all other mutant isolates in sterilized as well as nonsteriized soil leachates.

In a different study on the potentiality of different isolates to colonize sclerotia of *R. solani* and *S. rolfsii,* better colonization of sclerotia by all isolates was observed in sterilized soil over natural soil at 30°C. At pH 5.5 mutant isolates performed more efficiently to colonize the sclerotia of both pathogens than the wild isolate. With the increase of pH to alkaline range their efficacy decreased steeply. However, it appeared from the results that there was no correlation between spore germination and sclerotia colonization. Highest colonization of sclerotia by 100 KRIV isolate was observed in spite of low germinability of its both spore forms compared to other isolates studied at pH 5.5.

Key words : *Gliocladium vireus,* wild and mutant isolates, biological characters

INTRODUCTION

The genetic modification of *Trichoderma* and *Gliocladium* through mutation was attempted earlier mainly to enhance their biocontrol potential against soilborne fungal pathogens, fungicide tolerance and antibiotic producing ability with varied success (Papavizas and Lewis, 1981; Howel and Stipanovic, 1983; Ahmed and Baker, 1988 ; Mukherjee and Mukhopadhyay, 1993). As a random phenomenon mutation not only modify the targetted character but some others also that may or may not be desirable for its biology (Kumar and Gupta, 1999). Any abolishment of important biological property of the wild isolate make the mutants less fit to its parental ecosystem. This also may be due to extra metabolic load in mutants toward single/few desirable character (Cook, 1993). Perhaps, due to these reasons several mutants effective *in vitro* were not much successful as biocontrol agent *in vivo.* So, it is necessary to assess some biological properties of the mutants that may increase their ecological fitness in soil. In this study, the changes of some biological characters like germinability of chlamydospore and phialospore under different conditions and the ability for competitive

colonization on dormant fungal propagules by a few efficient mutants of *G. virens* have been studied.

MATERIALS AND METHODS

Production of mutants

A number of isolates of *G. virens* was isolated from different ecological niches and screened for their antagonistic potential against four soilborne plant pathogens viz. *Rhizotonia solani, Sclerotium rolfsii, Fusarium udum* and *Macrophomina phaseolina.* The most efficient isolate 15 GV_1 was selected for mutation using γ-ray. Three doses of γ-ray *i.e.,* 50 KR, 75 KR and 100 KR were used to expose the 4 day-old culture grown in test tube. Twelve mutants were selected based on their phenotypic characters like colony colour, colony structure, growth rate etc. following Pan *et al.* (in press). These mutants were screened against the said four plant pathogens and studied for ten consecutive generations to examine their stability on repeated subculturing. Among these twelve isolates the most efficient three mutants *i.e.* 50 KR I, 75 KR V and 100 KR IV were selected for this study along with the wild isolate for comparison.

Production of phialospore and chlamydospore

The phialospores of the wild and mutant isolates were collected from 9 day-old culture in potato dextrose both (PDB) at 28 ± 1°C by camel hair brush and the suspension was prepared in sterilized distilled water (SDW). For mass production of chlamy-dospores, selected mutants and the wild isolate were grown in glucose-tartarate medium (Brain and Hemming, 1950) for 21 days at 28 ± 1°C The mycelial mat was harvested using filter paper and crushed in a blender using sterile distilled water. The suspension was repeatedly (3 - 4 times) centrifuged at 4000 × g for 10 minutes. The supernatent containing mycelial fragments were carefully decanted. The sediment containing chlamydospores were used in different experiments by resuspending in SDW.

In vitro germination behaviour of phialospores and chlamydospores

The germination behaviour of phialospores and chlamydospores of the wild and mutants were studied in SDW at different pH *i.e.,* 5.5, 7.0 and 8.5, SDW containing different carbon and nitrogen sources and soil leachets. Three pH of the SDW were adjusted either with 10 % lactic acid of 10 % NaOH as required. Carbon source *vig.,* glucose, sucrose, peptone and starch were used separately at 100 ppm and 1000 ppm (W/V). The inorganic compounds such as, ammonium nitrate, potassium nitrate and ammonium sulphate were used as nitrogen sources using the same concentrations.

To prepare soil leachets three types of soils having pH around 5.5, 7.0 and 8.5 were collected discarding the surface layer (upper 2.5 cm) to avoid undecomposed organic matter. The soil was allowed to dry in shade, powdered and sieved with 2 mm mesh. 100 ppm and 500 g of this soil was throughly stirred with 500 ml distilled water and allowed to settle overnight. The supernatant was gently decanted, filtered through filter paper and used for the study. In another preparation, the conical flask containing water and soil of same quantity was free steamed for one hour in autoclave and allowed to settle overnight. The supernatant was collected as described and used for the study. One part of these leachets were sterilized in autoclave and then used.

Competitive colonization ability on pathogens' dormant propagules by wild and mutant isolates

The mycelial mat of the selected mutants and wild isolate were air dried and ground by a sterilized mortar and pastle. The powdery masses avoiding all lumps were collected using spatula. These inocula were separately incorporated into natural and sterilized soils @ 5 mg/100 g of soil, 10 mg/100 g soil and 15 mg/100 g soils in replicated treatments. In each level of inoculum soil mixture, the colony forming unit (cfu) of antagonist per g of soil was determined in modified *Trichoderma* specific medium (TSM, Elad *et al.,* 1981). Fifty sclerotia of *R. solani and S. rolfsii* produced in potato dextrose agar (PDA) plate were buried in separate treatments and incubated at 15°C, 25°C and 30°C as separate batch for 7 days. The experiment was conducted in 200 ml plastic cup keeping soil moisture at 50 % of water holding capacity. The sclerotia were recovered by washing and sieving. The recovered sclero-

tia were surface sterilized by 0.1 % sodium hypochlorite for 2 minute and placed on modified TSM. The competitive colonizing ability of the wild and mutants were determined by studying sclerotia production (%) in present of the colony of the antagonist on TSM.

RESULTS AND DISCUSSION

Germination of phialo- and chlamydospores in distilled water and soil leachets of different pH

The result of spore germination in sterile distilled water supported the fact that wild and all mutants germinated better in acidic pH 5.5 and the spore germination gradually decreased as the pH increased (Table 1.) A similar trend was observed both for phialo- and chlamydospore germination, but at pH 8.5 chlamydospore germination was negligible (Table 2) for wild and all mutants.

Table 1. Germinability of phialospores of wild and mutant isolates of *G. viresns* in different soil leachets and pH at fixed time.

Isolates	Germination of phialospores (%)								
	Soil leachets								
	NB + NS			IB + NS			IB + SS		
	pH 5.5	pH 7.0	pH 8.5	pH8.5	7.0	8.5	5.5	7.0	8.5
$15GV_1$	5.53	0.5	0.5	13.65	10.18	5.12	33.62	11.02	11.97
	(13.56)	(4.05)	(4.05)	(21.72)	(18.63)	(13.05)	(35.43)	(19.37)	(20.27)
50 KRI	0.5	0.5	0.5	11.22	3.3	0.5	33.41	25.63	19.13
	(4.05)	(4.05)	(4.05)	(19.55)	(10.47)	(4.05)	(35.30)	(30.40)	(25.91)
75 KRV	0.5	0.5	0.5	11.49	5.10	0.5	29.77	17.71	0.5
	(4.05)	(4.05)	(4.05)	(19.82)	(13.05)	(4.05)	(33.09)	(24.88)	(4.05)
100 KRIV	5.91	0.5	0.5	6.42	4.09	0.5	21.76	29.37	0.5
	(14.06)	(4.05)	(4.05)	(14.65)	(11.54)	(4.05)	(27.83)	(32.83)	(4.05)
CD (P = 0.05)	For Isolate 0.06			Isolate x pH 0.11					
	pH 0.04			Isolate x soil leachet 0.11					
	Soil leachet 0.04			pH x soil leachet 0.08					
				Isolate x pH x soil leachet 0.2					

Each insertions is an average of 5 observations recorded after 30 h at 28 ± 1° C, NB = nonboiled, NS = nonsterilized, 1B = 1 hr boiled, SS = sterilized

Table 2. Germinability of chlamydospores of wild and mutant isolates of *G. virens* in different soil leachets and pH at fixed time.

Isolates	Germination of chlamydospores (%)								
	Soil leachets								
	NB + NS			IB + NS			IB + SS		
	pH 5.5	pH 7.0	pH 8.5	pH5.5	7.0	8.5	5.5	7.0	8.5
$15GV_1$	0.5	0.5	0.5	4.30	0.5	0.5	12.56	5.34	4.32
	(4.05)	(4.05)	(4.05)	(11.97)	(4.05)	(4.05)	(20.70)	(13.31)	(11.97)
50 KRI	0.5	0.5	0.5	1.20	0.5	0.5	10.10	3.60	1.20
	(4.05)	(4.05)	(4.05)	(6.29)	(4.05)	(4.05)	(18.53)	(10.94)	(6.29)
75 KRV	0.5	0.5	0.5	1.20	0.5	0.5	11.50	1.20	0.5
	(4.05)	(4.05)	(4.05)	(19.82)	(4.05)	(4.05)	(19.82)	(6.29)	(4.05)
100 KRIV	0.5	0.5	0.5	0.5	0.5	0.5	9.59	1.25	0.5
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(18.05)	(6.29)	(4.05)
CD (P = 0.05)	For Isolate 0.06			Issolate x pH 0.11					
	pH 0.04			Isolate x soil leachet 0.11					
	Soil leachet 0.04			pH x soil leachet 0.08					
				Isolate x pH x soil leachet 0.20					

Each insertions is an average of 5 observations recorded after 30 h at 28 ± 1° C, NB = nonboiled, NS = nonsterilized, 1B = 1 hr boiled, SS = sterilized

Table 3. Germinability of phialosporess of wild and mutant isolates of *G. virens* in different sources of energy at fixed time and temperature.

Energy Source	Germination of phialospores (%)							
	Isolate 15 GV$_1$		Isolate KRI		Isolate 75 KRV		Ioşlate 100 KRIV	
	Concentration (ppm)							
	100	1000	100	1000	100	1000	100	1000
Glucose	54.07 (47.29)	66.07 (54.39)	42.88 (40.92)	65.39 (53.97)	41.07 (39.82)	61.98 (51.94)	23.98 (29.33)	42.39 (40.57)
Sucrose	42.39 (40.63)	59.07 (50.24)	37.77 (37.94)	49.72 (44.83)	34.01 (35.67)	41.63 (40.16)	35.04 (36.27)	40.04 (39.23)
Starch	10.88 (19.37)	31.61 (34.20)	10.40 (18.81)	29.55 (32.90)	6.92 (15.23)	26.70 (31.11)	5.29 (13.18)	11.33 (19.64)
Peptone	74.23 (59.47)	100.00 (90.00)	72.87 (58.63)	100.00 (90.00)	72.01 (58.05)	98.42 (82.73)	66.43 (54.57)	87.31 (69.12)
Ammonium nitrate	50.61 (45.34)	82.50 (65.27)	52.60 (46.49)	85.70 (67.78)	47.96 (43.85)	74.03 (43.85)	46.63 (43.05)	74.50 (59.67)
Potassium nitrate	45.99 (45.00)	69.69 (56.60)	42.52 (40.49)	80.31 (63.65)	23.40 (28.93)	62.50 (52.24)	20.59 (26.99)	51.20 (45.69)
Ammcnium sulphate	43.55 (41.32)	51.07 (45.57)	41.01 (39.82)	54.21 (47.41)	43.51 (41.32)	54.33 (47.47)	11.17 (19.55)	33.98 (35.67)
Control	42.89 (40.91)		40.56 (39.52)		37.37 (37.64)		32.65 (34.82)	

CD (P = 0.05)
For Isolate 0.10
Concentration 0.05
Energy source 0.10
Isolate x conc. 0.14
Isolate x energy source 0.20
Energy source x conc. 0.14
Isolate x energy source × conc. 0.29

Table 4. Germinability of chlamydospores of wild and mutant isolate of *G. virens* in different source of energy at fixed time and temperature.

Energy Source	Germination of phialospores (%)							
	Isolate 15 GV$_1$		50 KRI		75 KRV		100 KRIV	
	Concentration (ppm)							
	100	1000	100	1000	100	1000	100	1000
Glusoe	45.33 (42.30)	68.92 (56.10)	36.72 (37.29)	55.37 (48.10)	34.93 (36.21)	49.99 (45.00)	19.97 (26.57)	36.63 (37.23)
Sucrose	31.33 (34.02)	61.11 (51.41)	34.44 (35.91)	54.22 (47.41)	33.99 (35.67)	49.01 (44.43)	19.90 (26.49)	30.05 (33.21)
Starch	5.49 (13.56)	19.05 (25.84)	4.92 (12.79)	19.02 (25.84)	4.86 (12.79)	18.66 (25.62)	1.02 (5.74)	5.99 (14.18)
Peptone	46.42 (42.94)	61.11 (51.41)	56.03 (48.45)	60.92 (51.30)	55.45 (48.16)	56.39 (48.68)	36.63 (37.23)	44.32 (41.73)
Ammonium nitrate	23.66 (29.16)	42.50 (40.69)	17.16 (24.43)	29.30 (32.77)	19.99 (25.91)	27.06 (31.37)	10.09 (18.53)	15.59 (23.26)
Potassium nitrate	34.31 (35.85)	41.03 (39.82)	5.66 (13.81)	18.91 (25.77)	10.67 (19.09)	18.59 (25.55)	10.01 (18.43)	6.70 (15.00)
Ammonium Sulphate	16.76 (24.20)	29.06 (32.65)	5.17 (13.18)	8.75 (17.05)	8.40 (16.85)	10.99 (19.37)	4.32 (11.97)	5.62 (13.69)
Control	34.16 (35.79)		15.35 (23.11)		14.07 (22.06)		10.65 (19.09)	

CD (P = 0.05)
For Isolate 0.15
Concentration 0.09
Energy source 0.13
Isolate x conc. 0.20
Isolate x energy source 0.25
Energy source x conc. 0.17
Isolate x energy source x conc. 0.36

Table 5. Competitive colonization on the sclerotia of *R. solani* by wild and mutant isolates of *G. virens* at different temperature and soil pH 5.5.

Isolates	Soil type	Colonization of sclerotia (%)								
		Temperature (°C)								
		15			25			30		
		Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)		
		5	10	15	5	10	15	5	10	15
15 GV_1	NS	6.03	16.50	19.53	9.02	25.18	28.35	11.40	25.88	27.71
		(14.18)	(23.97)	(26.21)	(17.46)	(30.07)	(32.14)	(19.73)	(30.53)	(31.76)
	SS	43.97	70.07	78.77	63.16	86.65	94.38	65.19	96.86	96.79
		(41.50)	(56.85)	(62.51)	(52.59)	(68.53)	(76.19)	(53.79)	(79.70)	(79.53)
50KRI	NS	4.67	11.60	14.10	7.85	23.20	27.87	8.64	27.04	27.07
		(12.38)	(19.91)	(22.06)	(16.22)	(28.79)	(31.82)	(17.05)	(31.31)	(31.31)
	SS	55.47	81.90	84.83	66.39	86.16	94.16	69.62	97.11	98.01
		(48.10)	(64.82)	(67.05)	(54.51)	(68.11)	(75.94)	(56.54)	(80.20)	(81.87)
75 KRV	NS	4.40	11.93	14.07	7.92	22.96	28.00	8.27	23.92	27.45
		(12.11)	(20.18)	(21.97)	(16.32)	(28.59)	(31.95)	(16.94)	(29.27)	(31.56)
	SS	55.43	81.57	86.03	63.37	85.23	94.88	69.94	96.77	96.97
		(48.10)	(64.53)	(68.03)	(52.71)	(67.37)	(76.82)	(56.37)	(79.53)	(79.86)
100 KRIV	NS	4.13	12.80	14.53	6.83	21.90	27.22	7.22	22.71	26.32
		(11.68)	(20.96)	(22.98)	(15.12)	(27.90)	(31.44)	(15.56)	(28.45)	(30.85)
	SS	55.43	88.80	94.67	66.71	85.63	95.25	72.17	97.52	98.31
		(48.10)	(70.45)	(76.56)	(54.76)	(67.70)	(77.34)	(58.12)	(80.90)	(82.51)
CD (P = 0.05)		For Isolate = 0.32 Soil = 0.23 Inoculum = 0.23 Isolate × soil = 0.46 Isolate × inoculum = 0.61 Soil × inoculum = 0.39 Isolate × soil × inoculum = 0.86			For Isolate = 0.56 Soil = 0.39 Inoculum =0.49 Isolate × soil = 0.79 Isolate × inoculum = 0.98 Isolate × inoculum = 0.68 Isolate × soil × inoculum = 1.38			For Isolate = 0.38 Soil = 0.26 Inoculum = 0.33 Isolate × soil = 0.52 Isolate × inoculum = 0.63 Isolate × inoculum = 0.68 Isolate × soil × inoculum = 0.92		

Table 6. Competitive colonization on the sclerotia of *R. solani* by wild and mutant isolates of *G. virens* at different temperature and soil pH 7.0.

Isolates	Soil type	Colonization of sclerotia (%)								
		Temperature (°C)								
		15			25			30		
		Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)		
		5	10	15	5	10	15	5	10	15
15 GV_1	NS	5.48	12.12	15.91	7.13	21.14	22.11	13.87	22.53	22.98
		(13.44)	(20.36)	(23.50)	(15.45)	(27.35)	(28.04)	(21.81)	(28.32)	(28.59)
	SS	43.26	50.29	61.77	59.63	82.12	83.48	67.56	85.15	85.82
		(41.09)	(45.11)	(51.77)	(50.53)	(64.97)	(65.96)	(55.24)	(67.29)	(67.86)
50 KRI	NS	3.77	7.11	10.17	7.38	9.85	14.79	7.30	11.86	15.13
		(10.47)	(15.45)	(18.53)	(15.68)	(18.24)	(22.54)	(15.68)	(20.09)	(22.87)
	SS	26.23	32.74	39.35	35.17	43.67	58.13	40.49	47.77	59.76
		(30.79)	(34.88)	(38.82)	(36.33)	(41.32)	(49.66)	(39.47)	(43.68)	(50.59)
75 KRV	NS	2.94	7.92	9.78	6.90	9.85	14.15	7.87	11.23	14.92
		(9.80)	(16.32)	(18.15)	(15.23)	(18.24)	(22.06)	(16.22)	(19.55)	(22.71)
	SS	26.99	30.68	38.85	36.95	40.74	56.77	37.95	47.46	57.74
		(31.24)	(33.58)	(38.53)	(37.41)	(39.64)	(48.85)	(38.00)	(43.49)	(49.43)
100 KRIV	NS	2.78	6.13	7.20	6.58	8.95	10.85	5.67	11.11	11.36
		(9.46)	(14.30)	(15.56)	(14.77)	(17.36)	(19.19)	(13.69)	(19.64)	(19.64)
	SS	12.67	24.80	33.72	26.67	32.51	50.72	24.89	32.83	51.87
		(20.79)	(29.87)	(35.49)	(31.05)	(34.76)	(45.40)	(29.97)	(34.94)	(46.03)
CD (P = 0.05)		For Isolate = 0.42 Soil = 0.31 Inoculum = 0.35 Isolate × soil = 0.58 Isolate × inoculum = 0.72 Soil × inoculum = 0.51 Isolate × soil × inoculum = 1.03			For Isolate = 0.52 Soil = 0.38 Inoculum =0.45 Isolate × soil = 0.73 Isolate × inoculum = 0.89 Soil × inoculum = 0.63 Isolate × soil × inoculum = 1.27			For Isolate = 0.45 Soil = 0.31 Inoculum = 0.38 Isolate × soil = 0.61 Isolate × inoculum = 0.75 Soil × inoculum = 0.54 Isolate × soil × inoculum = 1.08		

Table 7. Competitive colonization on the sclerotia of *R. solani* by wild and mutant isolates of *G. virens* at different temperature and soil pH 8.5.

Isolates	Soil type	Colonization of sclerotia (%)								
		Temperature (°C)								
		15			25			30		
		Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)		
		5	10	15	5	10	15	5	10	15
15 GV_1	NS	5.48	11.21	13.55	6.98	19.59	21.36	7.86	22.19	23.64
		(13.44)	(19.55)	(21.56)	(15.23)	(26.21)	(27.49)	(16.22)	(27.9)	(29.06)
	SS	18.54	25.41	28.24	24.95	40.88	42.74	25.81	40.76	45.78
		(25.47)	(30.26)	(32.08)	(29.93)	(39.70)	(40.80)	(30.53)	(39.64)	(42.53)
50 KRI	NS	1.12	3.56	6.24	1.97	5.20	7.46	2.26	5.31	7.89
		(6.02)	(10.78)	(14.42)	(7.92)	(13.18)	(15.79)	(8.53)	(13.31)	(16.22)
	SS	4.84	10.64	12.38	8.03	12.82	14.78	8.92	15.16	19.78
		(12.56)	(10.94)	(20.53)	(16.43)	(20.96)	(22.54)	(17.36)	(22.87)	(26.35)
75 KRV	NS	0.85	3.21	5.61	1.75	5.11	7.34	1.93	5.72	7.53
		(12.92)	(10.30)	(13.69)	(7.49)	(13.05)	(15.68)	(7.92)	(13.81)	(15.89)
	SS	5.02	9.98	11.82	5.93	9.26	12.56	5.81	8.87	12.53
		(4.05)	(18.34)	(20.09)	(14.06)	(17.66)	(20.70)	(13.94)	(17.26)	(20.70)
100 KRIV	NS	0.56	1.66	3.35	1.13	1.61	2.42	0.73	1.74	3.50
		(4.05)	(7.27)	(10.47)	(6.02)	(7.27)	(8.91)	(4.80)	(7.49)	(10.75)
	SS	4.31	9.13	9.59	2.02	5.66	7.16	1.93	5.53	7.76
		(11.97)	(17.56)	(17.95)	(8.13)	(13.69)	(15.45)	(7.92)	(13.56)	(16.22)
CD (P = 0.05)		For Isolate = 0.39			For Isolate = 0.49			For Isolate = 0.42		
		Soil = 0.28			Soil = 0.35			Soil = 0.31		
		Inoculum = 0.35			Inoculum =0.42			Inoculum = 0.35		
		Isolate × soil = 0.70			Isolate × soil = 0.84			Isolate × soil = 0.58		
		Isolate × inoculum = 0.56			Isolate × inoculum = 0.70			Isolate × inoculum = 0.72		
		Soil × inoculum = 0.49			Soil × inoculum = 0.61			Soil × inoculum = 0.51		
		Isolate × soil × inoculum = 0.98			Isolate × soil × inoculum = 1.22			Isolate × soil × inoculum = 1.03		

Table 8. Competitive colonization on the sclerotia of *R. solani* by wild and mutant isolates of *G. virens* at different temperature and soil pH 5.5.

Isolates	Soil type	Colonization of sclerotia (%)								
		Temperature (°C)								
		15			25			30		
		Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)			Inoculum (mg/100 g soil)		
		5	10	15	5	10	15	5	10	15
15 GV_1	NS	5.97	17.10	18.97	5.25	16.07	15.71	5.88	15.95	15.89
		(14.06)	(24.43)	(25.77)	(13.18)	(23.58)	(23.34)	(13.94)	(23.50)	(23.42)
	SS	42.67	71.63	81.30	43.71	61.26	63.45	42.28	60.97	63.21
		(40.74)	(57.80)	(64.38)	(41.38)	(51.47)	(52.77)	(40.51)	(51.31)	(52.65)
50 KRI	NS	3.93	10.10	13.80	3.52	9.02	10.44	4.53	9.17	10.89
		(11.39)	(18.53)	(21.81)	(10.78)	(17.46)	(18.81)	(12.25)	(17.56)	(19.19)
	SS	51.37	79.27	85.93	45.91	61.81	64.13	46.59	61.51	65.01
		(45.74)	(62.87)	(67.94)	(42.65)	(51.83)	(53.19)	(42.99)	(51.65)	(53.73)
75 KRV	NS	4.03	9.03	13.97	3.41	8.77	10.32	4.17	8.87	10.60
		(11.54)	(17.46)	(21.89)	(10.63)	(17.15)	(18.72)	(11.68)	(17.26)	(19.00)
	SS	51.93	80.40	85.73	49.90	61.49	63.81	49.04	61.55	65.49
		(46.09)	(63.72)	(67.78)	(44.94)	(51.59)	(53.01)	(44.43)	(51.65)	(53.97)
100 KRIV	NS	4.07	9.83	14.80	3.88	9.15	13.37	4.73	9.17	13.92
		(11.54)	(18.24)	(22.63)	(11.24)	(17.56)	(21.39)	(12.52)	(17.56)	(21.89)
	SS	52.07	79.37	86.53	48.78	66.22	70.30	48.23	65.48	70.78
		(46.15)	(62.94)	(68.44)	(44.26)	(54.45)	(56.98)	(43.97)	(53.97)	(57.23)
CD (P = 0.05)		For Isolate = 0.37			For Isolate = 0.28			For Isolate = 0.33		
		Soil = 0.28			Soil = 0.18			Soil = 0.23		
		Inoculum = 0.32			Inoculum =0.23			Inoculum = 0.28		
		Isolate × soil = 0.56			Isolate × soil = 0.39			Isolate × soil = 0.47		
		Isolate × inoculum = 0.57			Isolate × inoculum = 0.46			Isolate × inoculum = 0.59		
		Soil × inoculum = 0.46			Soil × inoculum = 0.32			Soil × inoculum = 0.40		
		Isolate × soil × inoculum = 0.95			Isolate × soil × inoculum = 0.68			Isolate × soil × inoculum = 0.82		

Table 9. Competitive colonization on the sclerotia of *R. solani* by wild and mutant isolates of *G. virens* at different temperature and soil pH 7.0.

Isolates	Soil type	Mean percent of colonization of sclerotia								
		Temperature (°C)								
		15			25			30		
		Level of inoculum/100 gm soil			Level of inoculum/100 gm soil			Level of inoculum/100 gm soil		
		5 mg	10 mg	15 mg	5 mg	10 mg	15 mg	5 mg	10 mg	15 mg
15 GV_1	NS	5.91	12.60	18.84	7.95	15.21	24.49	11.04	16.39	26.22
		(14.06)	(20.79)	(25.70)	(16.33)	(22.95)	(29.60)	(19.37)	(23.81)	(30.79)
	SS	41.45	54.67	62.97	50.45	63.78	67.22	54.96	65.49	74.09
		(40.05)	(47.64)	(52.48)	(45.23)	(52.95)	(55.06)	(47.81)	(53.97)	(59.34)
50 KRI	NS	3.18	6.67	12.00	5.79	9.93	13.58	9.45	11.43	14.80
		(10.14)	(14.89)	(20.27)	(13.81)	(18.34)	(21.56)	(17.85)	(19.73)	(22.63)
	SS	25.45	43.77	68.59	32.12	53.67	70.77	36.79	56.54	71.85
		(30.26)	(41.38)	(55.86)	(34.51)	(47.06)	(57.23)	(37.29)	(48.73)	(57.92)
75 KRV	NS	3.60	6.14	11.31	5.94	9.88	15.08	7.97	11.57	14.61
		(10.94)	(14.30)	(19.64)	(14.06)	(18.24)	(22.79)	(16.32)	(19.82)	(22.46)
	SS	24.68	41.57	65.46	33.74	54.86	70.14	37.15	53.78	70.90
		(29.93)	(40.11)	(53.97)	(35.49)	(47.75)	(56.85)	(37.52)	(47.12)	(57.37)
100 KRIV	NS	3.25	6.12	7.93	5.06	8.68	14.63	5.82	9.87	14.34
		(10.30)	(14.30)	(16.32)	(12.92)	(17.06)	(22.46)	(13.94)	(18.24)	(22.22)
	SS	21.65	39.08	59.57	22.24	41.06	60.97	24.45	41.85	60.23
		(27.69)	(38.65)	(50.48)	(28.11)	(39.82)	(51.30)	(29.60)	(40.28)	(50.89)
CD (P = 0.05)		For Isolate = 0.49 Soil = 0.35 Inoculum = 0.42 Isolate × soil = 0.70 Isolate × inoculum = 0.87 Soil × inoculum = 0.61 Isolate × soil × inoculum = 1.22			For Isolate = 0.61 Soil = 0.45 Inoculum =0.54 Isolate × soil = 0.87 Isolate × inoculum = 1.08 Soil × inoculum = 0.75 Isolate × soil × inoculum = 0.1.53			For Isolate = 0.40 Soil = 0.28 Inoculum = 0.35 Isolate × soil = 0.56 Isolate × inoculum = 0.68 Soil × inoculum = 0.49 Isolate × soil × inoculum = 0.99		

Table 10. Competitive colonization on the sclerotia of *S. rolfsii* by wild and mutant isolates of *G. virens* at different temperature and soil pH 8.5

Isolates	Soil type	Mean percent of colonization of sclerotia								
		Temperature (°C)								
		15			25			30		
		Level of inoculum/100 gm soil			Level of inoculum/100 gm soil			Level of inoculum/100 gm soil		
		5 mg	10 mg	15 mg	5 mg	10 mg	15 mg	5 mg	10 mg	15 mg
15 GV_1	NS	3.52	7.79	8.44	3.39	7.81	8.35	4.24	8.32	8.87
		(10.78)	(16.11)	(16.85)	(10.47)	(16.22)	(16.74)	(11.83)	(16.74)	(17.26)
	SS	11.82	21.56	23.89	15.77	21.63	23.27	16.59	21.93	23.90
		(20.00)	(27.62)	(29.20)	(23.34)	(27.69)	(28.79)	(23.97)	(27.90)	(29.27)
50 KRI	NS	0.91	2.19	3.48	1.52	5.29	6.53	2.04	6.71	9.79
		(5.44)	(8.33)	(10.63)	(7.03)	(13.18)	(14.77)	(8.13)	(15.00)	(18.15)
	SS	2.90	5.81	8.53	7.51	11.61	14.45	8.50	13.44	19.12
		(9.80)	(13.94)	(7.92)	(15.89)	(19.91)	(22.30)	(16.95)	(21.47)	(25.91)
75 KRV	NS	0.74	1.28	1.97	1.00	5.62	6.70	1.80	6.02	7.62
		(4.80)	(6.29)	(15.68)	(5.74)	(13.69)	(15.00)	(7.71)	(14.18)	(16.00)
	SS	2.87	4.95	7.34	4.48	8.55	10.59	6.18	11.01	12.73
		(9.63)	(12.79)	(8.13)	(12.11)	(16.95)	(18.91)	(14.30)	(19.37)	(20.88)
100 KRIV	NS	0.11	1.88	2.02	0.00	7.10	7.36	0.17	1.67	2.33
		(1.81)	(7.71)	(14.65)	(0.00)	(5.74)	(15.37)	(1.81)	(7.27)	(8.72)
	SS	1.95	3.54	6.49	3.47	6.35	7.58	4.17	7.21	8.91
		(7.92)	(10.78)	(16.95)	(10.63)	(14.54)	(15.89)	(11.68)	(15.56)	(17.36)
CD (P = 0.05)		For Isolate = 0.42 Soil = 0.31 Inoculum = 0.35 Isolate × soil = 0.58 Isolate × inoculum = 0.72 Soil × inoculum = 0.51 Isolate × soil × inoculum = 1.03			For Isolate = 0.82 Soil = 0.59 Inoculum =0.70 Isolate × soil = 1.17 Isolate × inoculum = 1.43 Soil × inoculum = 1.01 Isolate × soil × inoculum = 2.04			For Isolate = 0.54 Soil = 0.38 Inoculum = 0.27 Isolate × soil = 0.75 Isolate × inoculum = 0.94 Soil × inoculum = 0.66 Isolate × soil × inoculum = 1.34		

In soil leachets, the germination of both spore forms decreased significantly under all experimental parameters. However, among the different types of soil leachets boiled and sterilized leachets was best for germination. In unboiled and unsterilized leachet, spore germination was negligible in all pH except the wild (15 Gv_1) and 100 KR IV at pH 5.5 for phialospores. All unsterilized leachets were unsuitable for chlamydospore germination irrespective of acidity or alkalinity. Though all of the three mutants were less efficient than the wild isolate for all cases, at higher pH the phialo- and chlamydospores of 50 KR I germinated in a comparable proportion to the wild in sterilized condition. Among the isolates studied poorest germination ability was exhibited by 100 KR IV.

It can be concluded that germination of phialospores dominated over chlamydospores. However, it may or may not be significant in certain cases. The information about the factors and processes that govern germination of both spore forms of *Gliocladium* spp. is inadequate. The reason might be due to their mechanisms of germination of differing in many substrates (Papavizas, 1985). This experimental results revealed the pH dependence of the spore germination and favoured by acidic pH over alkaline. There were contradictory reports on the effects of soil pH upon germinability of spores of this biocontrol agent, *Gliocladium* sp. Highly acidic nature (pH 2.8 – 4.9) of the germination fluid did not inhibit germination of spores (Jackson, 1958). However, subsequent investigations made by Lingappa and Lockwood (1963) did not upheld Jackson's findings. The biological factors responsible for imposition of static effect on spore germination might be several : (i) operation of natural soil static effect, (ii) high soil moisture which might be another physical factor influencing biotic component of the responsible soil for its inhibitory action, and (iii) the inhibitory/toxic metabolites produced by the microorganisms in soil causing inhibition of germination. Thus, the causes for reduction in spore germination can be attributed to their greater dependency on pH and audophilic nature. Moreover, lysis of different spore forms may convincingly justify reduced germination percentage of both the spore forms and in the present experiment also appeared equally true for both wild and mutant isolates.

Germinability in different sources of carbon and nitrogen

Spore germination being nutrient indepedent in nature, it was presumed from the poor germination ability of both phialo- and chlamydospores could be overcome by addition of some exogenous energy sources. Among all sources, peptone performed best to increase germination percentage over control, may be due to its dual role as a source of both carbon and nitrogen (Tables 3 & 4). Other two carbon sources glucose and sucrose increased the germination percentage of all isolates. But most remarkable effect was observed in chlamydospores of the mutant isolates where more than three fold increase in spore germination over control was observed. Starch showed the opposite effect and decreased the germination percentage over control in all cases except chlamydospores of 50 KR I and 75 KR V. As a nitrogen source, ammonium nitrate proved to be the most favourable for both spore forms compared to potassium nitrate and ammonium sulphate. Though all the three nitrogen sources increased germination in most cases (except ammonium sulphate in chlamydospore of all isolates).

The use of exogenous sources of carbon and nitrogen induced better germination in most of the cases. Starch being a complex carbohydrate was not satisfactorily metabolized by the isolates and germination of spores mainly phialospores were very low. Inspite of supplying nutrient starch affected the osmotic gradient between spore and fluid that might have reduced the inhibition rate of water more adversely in thin walled phialospores.

Competitive colonization ability of antagonists

The colonization by wild and mutant isolates over sclerotia of *R. solani* was clearly affected by temperature and soil pH (Tables 5-7). At 30°C and pH 5.5 the per cent (Tables 5-7) colonization of sclerotia at highest inoculum level (15 mg/100 g soil) was maximum followed by the inoculum 10 mg/100 g soil. In unsterilized condition, the colonization by mutant isolates were lower than the wild biotypes at all temperature and pH. But in acidic condition,

mutant showed higher colonizing ability than wild in the sterilized soil. The mean per cent colonization decreased steeply with increasing pH of soil and at 8.5 all isolates of the antagonist exhibited a poor result, however, wild isolate performed much better.

Tests using the sclerotia of *S. rolfsii* followed identical pattern of sclerotial colonization (Tables 8-10). Not probing into the minute details it could be inferred that although temperature fluctuation from 15°C to 30°C definitely increased colonization; the ultimate dependency on soil pH played the key role in determining satistically significant level of sclerotial colonization.

Thus it can be concluded that the colonizing ability of the antagonists were always higher when sterilized soil was used and the static effect of the soil was either partly or fully eliminated by hot steam that ultimately determined biocontrol ability of the mutants and the wild biotype. The use of live baits and their penetration/colonization by antagonist reflected their much higher parasitic ability than saprophytic attributes. The use of sclerotia was intentional as they are protected by melanoid rind that provides mechanical as well as biochemical barrier as a by product of phenyl propanoid. As to how the antagonist *Gliocladium* availed access inside these structures had been amply demonstrated (Hennis *et al.*, 1983 ; Papavizas and Collins, 1990). Besides, under natural conditions the fungal spores were known to succumb to the static effect of soil to different degrees (Lockwood, 1977). Their possible annulment strategies were also known (Linderman and Gilbert, 1973 ; Hennis and Papavizas, 1983 ; Maiti and Sen, 1985). It had been demonstrated that germinating sclerotia of *S. rolfsii* secreted amino acids and sugars (Smith, 1972b). Increased secretion of these substances by damaged sclerotia could easily invite attack by antagonist (Smith, 1972a). Gilbert and Linderman (1971) had also reported increased acitivity of soil microorganisms near dried sclerotia of *S. rolfsii,* that created nutrient rich microsites around them.

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