BIOCONTROL OF RHIZOME ROT OF GINGER

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ABSTRACT

Rhizome rot disease caused by *Pythium aphanidermatum* is a serious problem in ginger. As a part of disease management, field evaluation of biocontrol agents was carried out in *Pythium*-sick soil. Further, the efficacy of these biocontrol agents in combination with soil solarisation was evaluated as a measure of integration. *Trichoderma viride, T. harzianum* I & II, *T. hamatum and Gliocladium virens* were the biocontrol agents used and compared with fungicide mancozeb.

Two years of field trials showed that the isolate *T. harzianum* I was efficient in controlling the disease both in solarised and non-solarised plots. The disease incidence was less and the yield was high in both the years. *T. hamatum* was the second best in both the years. In general, the yield was higher in solarised plots in both the years but significant increase in yield was obtained in the second year only. The weed growth was also suppressed in the solarised plot to an extent of 40%.

INTRODUCTION

Ginger is an important spice and vegetable and is grown in India over an area of 53,020 ha producing 1,52,890 tonnes and Kerala contributes 25.14% of area and 30.34% of production. Rhizome rot (soft rot) caused by *Pythium aphanidermatum* Edson Fitz. is one of the major production constraints in ginger. In general, disease management is mainly confined to fungicide treatments. Concerted efforts have not been made to evolve biological control schedules for the control of rhizome rot of ginger.

The potential of *Trichoderma/ Gliocladium* as biological control agents to check soil-borne root infecting fungal pathogens has been well established. Effective suppression of *Pythium* spp., *Fusarium* spp. and *Rhizoctonia* spp. by *Trichoderma* and *Gliocladium* have been reported (Papavizas, 1985; Mukhopadhyay, 1987; Chet 1987). Katan (1976) reported the technique of soil solarisation that reduced the population of soil-borne pathogens and weeds. Application of neem cake at the time of planting reduced the disease incidence of rhizome rot of ginger to a certain extent and increased the yield (Sadanandan & Iyer, 1986).

In the present study, field trials were conducted in *Pythium* sick soil which were subjected to soil solarisation and later integrated with biocontrol agents. Five biocontrol agents *viz.*, *T. Viride*, *T. harzianum*-I, *T. harzianum*-II, *T. hamatum* and *Gliocladium virens* which were isolated from ginger rhizosphere soils in Kerala were found effective in dual culture technique. These were applied both as seed treatment and soil application and were

compared with mancozeb treatment, a recommended fungicide for rhizome rot control.

MATERIALS AND METHODS

The experiment was laid out at NRCS Farm, Peruvannamuzhi adopting RBD design with 7 treatments and each was replicated six times. The plot size was of two beds of 3 x 1m size. Beds of 3 x 1 m with a height of 15 cm were prepared with an interspace of 50 cm in between beds and 1 m between rows. The beds were prepared after the early monsoon shower to ensure sufficient soil moisture. The beds were covered with polythene sheets (200 gauge) and the edges were sealed with mud. The polythene tarped beds were allowed for solarisation for 30 days. Soil temperature was recorded with the help of soil thermometer at three different depths *viz.*, 5, 15 & 30 cm. during solarisation period. The beds were left uncovered in the non-solarised experimental plots.

Application of the biocontrol agents *viz.*,. *T. viride, T. harzianum* I & II, *T. hamatum* and *G. virens* were the treatments adopted. For comparison a standard fungicide mancozeb was used in the trial. Both seed treatment and soil application of the respective bioagents and mancozeb were done before sowing.

Mass multiplication of biocontrol agents (BCA's)

1) **Seed treatment:** Biocontrol agents were multiplied on sterilised sorghum grains in polypropylene bags. After 15 days of incubation, they were ready for seed treatment. Conidial suspension with 0.2% CMC (Carboxy Methyl Cellulose) was prepared and the seed rhizomes were kept 30 minutes in this suspension. The spore load of each organism was adjusted to get equal strength (x 107) in the conidial suspension. The rhizomes were air dried for one hr. after seed treatment.

2) **Soil application:** Well powdered neem cake was used for multiplication of *Trichoderma/ Gliocladium*. Five hundred grams of neem cake was filled in polypropylene bags and sterilised twice for about 1 hr. (15 PSI/ 121°C). Two culture discs of 1 cm (5 day-old) of the organism were inoculated into each bag and were incubated for 20 days at room temperature (28°-30°C) The inoculum load of the organism was x 10^6 (CFU) in each case. The inoculum was applied in both solarised and nonsolarised beds into each of the planting pits @ 12.5g/pit, @ 40 pits per 3 x 1m bed.

The seed rhizomes of Maran weighing 20-25 g each having one or two buds were selected for planting. Forty seed rhizomes/ bed were planted in four rows at a spacing of 20 cm along the rows and 20-25 cm between the rows. The seed rhizomes were put in shallow planting pits prepared with a hand hoe and covered with farm yard manure and a thin layer of soil and levelled. Fertilizer as per the package of practices was applied in two splits.

3) **Monitoring population of *Pythium* and BCA's:** The population of *Pythium* and the introduced antagonists *Trichoderma/ Gliocladium* were monitored during 30, 60, 90 and 150 days after germination of the seeds. Soil dilution plate method was employed for counting the colony or colony forming units (CFUs). Selective media for both *Pythium* (Tsao and Ocana, 1969) as well as *Trichoderma/ Gliocladium* (Elad & Chet, 1983) were used. This was done both in solarised and non-solarized conditions.

4) **Monitoring Weed biomass:** Weeds were collected from both solarised and non-solarised plots during the crop season at 30 and 90 day intervals. The biomass of the weeds was calculated by taking wet weight. Weeds collected from ten beds were weighed and percentage of reduction calculated. Three replications were maintained for each treatment.

RESULTS

In solarised plot the temperature range was 35.0 to 46.5°C whereas in non-solarised plot, the temperature range was 29.5 to 40.5°C (Table I). *Pythium* population was less in solarised plots compared to non-solarised plots (Tables II to V). With time, the population level increased in both the conditions. However, the *Pythium* population decreased in all the plots where biocontrol agents were applied compared to untreated control (Tables II to V).

In the case of *Trichoderma/ Gliocladium*, the adaptation/survival ability was very high in solarised compared to non-solarised plots. A gradual decrease in population was noticed with time in both the treatments (Tables II to V). Among the treatments in solarised plots *T. harzianum* II showed greater adaptability compared to all other organisms. In the first year even after 150 days, the population was 17.00 x 10^3 cfu/g and in the second year the population was 18.0 x 10^3 cfu/g in solarised plots. The same organism showed 6.50 and 6.00 x 10^3 cfu/g in the same period in non-solarised plots. *T. hamatum* in non-solarised plot showed greater adaptability in both the years compared to all the other biocontrol agents i.e. 9.50 x 10^3 cfu/g in the first year and 7.50 x 10^3 cfu/g in the second year. In the untreated plots the population of *Trichoderma* was negligible under both the conditions and in both the years (Tables II to V).

The results of two years of field trial experiments conducted in solarised and non-solarised plots are presented in the Table VI. Germination percentage in solarised plots was 77.92% whereas in non-solarised plots it was 74.90%. In general, the germination percentage was comparatively less in all the BCA treated plots. Among the BCA's, *T. harzianum* I applied plots recorded maximum germination both in solarised (79.61%) and non-solarised plots (79.43%). In the case of mancozeb, the germination percentage was 79.23% in solarised and 73.89 in non-solarised plots (Tables VI). In the untreated control the germination per cent was 81.33 in solarised and 78.86 in non-solarised plots.

In general, disease incidence ranged from 13.7 to 19.38% in solarised plots treated with BCA's. The disease incidiences were 36.22 and 57.58 percentage in mancozeb and control plots respectively in the solarised plots (Table VI). In non-solarised plots, the disease incidence was higher and ranged from 32.07 to 43.81 in BCA's applied plots whereas it was 46.7 and 53.81% in the mancozeb treated and untreated control plots. In general, the disease incidence was significantly higher in non-solarised plot (41.08%) compared to solarised plot (25.30%). Among BCA's applied in solarised plots, *T. viride* showed minimum disease incidence *i.e.*, 13.70% and *T. harzianum* I applied plots recorded 15.80% of disease incidence. In the case of *G. virens* the disease incidence was 18.27% in solarised plots.

Compared to control plots, all the biocontrol applied plots showed significantly

Table I. Temperature build up in solarised vs. non solarised soil at different depths (°C)

Depth (cm)	Solarised		Non-solarised		Difference
	Range	Mean	Range	Mean	
5	35.0-46.5	40.52	31.0-40.5	34.91	5.61
15	33.0-42.0	37.12	29.5-35.5	31.72	5.40
30	34.0-38.0	34.70	29.5-33.5	31.22	3.48

Average of 24 values recorded at 2 PM.

Table II. Population level of *Pythium* and *Trichoderma/ Gliocladium* sp. in solarised plot (1992-93)

Organism	Period of sampling after germination							
	30 days		60 days		90 days		150 days	
	P*	T**	P	T	P	T	P	T
T. viride	1.00	13.50	1.25	13.5	0.50	11.25	0.75	1.25
T. harzianum I	0.50	18.50	0.75	20.50	0.75	08.25	0.50	8.25
T. harzianum II	0.75	37.50	1.00	31.75	1.25	25.25	1.25	17.00
T. hamatum	1.00	50.25	1.25	56.25	1.25	12.75	1.50	9.50
G. virens	0.25	36.50	0.25	36.50	1.00	14.50	1.00	7.75
Mancozeb	1.50	4.25	1.50	3.00	1.50	1.00	2.00	0.50
Control	1.25	4.25	1.25	6.25	1.75	2.75	2.00	—

Basal population: **Pythium* x 10^2/g, ** *Trichoderma* x 10^3/g
Multiplication factor for CFU of *Pythium* - 1.1 x 10^2/g & *Trichoderma* - 3.2 x 10^2/g

Table III. Population level of *Pythium* and *Trichoderma/Gliocladium virens* in non-solarised plot (1992-93)

Organism	Period of sampling after germination							
	30 days		60 days		90 days		150 days	
	P*	T**	P	T	P	T	P	T
T. viride	1.25	5.25	1.00	4.50	0.25	4.50	1.50	2.25
T. harzianum I	1.50	4.50	1.00	2.75	1.25	6.50	1.50	4.00
T. harzianum II	1.25	9.75	0.25	7.25	0.50	11.75	1.00	6.50
T. hamatum	1.75	8.75	1.00	5.25	1.00	1.50	1.50	9.50
G. virens	1.00	9.50	0.75	5.50	0.50	3.75	0.75	7.00
Mancozeb	1.75	3.75	0.50	2.00	1.00	1.75	1.50	1.00
Control	2.00	1.75	2.00	1.50	1.75	2.50	2.00	1.50

Basal population: * *Pythium* x 10^2/g, *Trichoderma* x 10^3/g
** *Pythium* - 1.9 x 10^2/g, *Trichoderma* - 3.2 x 10^2/g

Table IV. Population level of *Pythium* and *Trichoderma/Gliocladium virens* in solarised plots (1993-94)

Organism	Period of sampling after germination							
	30 days		60 days		90 days		150 days	
	P*	T**	P	T	P	T	P	T
T. viride	1.25	10.50	1.25	9.00	1.00	6.00	1.00	2.00
T. harzianum I	0.25	25.00	0.25	30.00	0.25	20.50	0.25	15.00
T. harzianum II	0.75	30.25	1.00	20.00	0.75	26.00	1.00	18.00
T. hamatum	1.25	45.00	1.00	50.50	0.75	36.00	1.25	14.50
G. virens	1.00	34.00	0.75	30.50	1.00	22.00	1.25	11.50
Mancozeb	2.00	-	1.75	0.25	1.75	1.00	2.00	1.25
Control	1.75	2.00	2.25	3.25	2.25	1.00	2.50	0.00

Basal population: *Pythium* - 1.0 x 10^2/g
Trichoderma - 1.2 x 10^2/g
* *Pythium* x 10^2/g, ** *Trichoderma* x 10^3/g

Table V. Population level of *Pythium* and *Trichoderma/Gliocladium* sp. in non-solarised plots (1993-94)

Organism	Period of sampling after germination							
	30 days		60 days		90 days		150 days	
	P*	T**	P	T	P	T	P	T
T. viride	2.00	6.00	2.50	3.00	2.50	3.00	2.50	1.00
T. harzianum I	1.50	10.00	1.25	11.00	1.00	15.00	1.25	3.00
T. harzianum II	1.75	14.00	0.75	13.00	0.75	19.00	1.25	6.00
T. hamatum	1.75	8.00	1.00	10.00	1.25	9.00	1.50	7.50
G. virens	2.00	12.00	1.75	5.50	1.00	6.50	1.00	4.50
Mancozeb	2.00	1.00	2.00	1.50	1.50	2.00	1.50	1.00
Control	2.00	-	2.00	2.00	1.50	3.00	1.50	1.00

Basal population
Pythium - 2.0 x 10^2/g
Trichoderma - 3.00 x 10^2/g
* *Pythium* x 10^2/g
** *Trichoderma* x 10^3/g

Table VI. Effect of biocontrol agents on germination, disease incidence and yield (pooled data)

Organism	Solarised			Non-solarised		
	G%	D1%	Y%	G%	D1%	Y%
T. viride	77.69	13.70	2.846	71.52	35.25	1.225
T. harzianum I	79.61	15.80	3.552	79.43	32.07	2.673
T. harzianum II	77.87	19.38	2.910	72.91	43.61	1.439
T. hamatum	75.00	16.16	2.744	73.23	39.26	1.602
G. virens	74.48	18.25	2.641	74.43	36.63	1.705
Mancozeb	79.23	36.22	2.260	73.89	46.70	0.818
Control	81.53	57.58	1.692	78.86	53.8	0.992
Mean	77.92	25.30	2.278	74.90	41.08	1.485
CD at 5%	NS	6.26	0.430	NS	6.26	0.430

G = Germination D1 = Disease incidence Y = Yield

lesser disease incidence both under solarised and non-solarised conditions. The disease incidence in non-solarised plots was 32.07 where *T. harzianum* I was applied, whereas in the case of *T. harzianum* II 43.81% disease incidence was recorded. When *G. virens* was applied, the disease incidence was 36.63% (Table VI).

In general, the yield was significantly higher in solarised plots. It showed 2.298 kg/ $3m^2$ in the solarised plot whereas it was only 1.485 kg/$3m^2$ in non-solarised plot. All the biocontrol applied plots showed higher yields both in solarised and non-solarised plots. The yield levels in general are low since the experiments were conducted in *Pythium* sick soils. However, yields as high as 12-18 kg/3x1 m bed have been obtained in non-sick soil compared to low yields in *Pythium* sick soil.

Among the treatments, *T. harzianum* I treatment recorded the highest yield under both the conditions (3.552 kg in solarised plot and 2.673 kg/$3m^2$ in non-solarised plot.) *T. hamatum* applied plots yielded 3.075 kg and 1.659 *kg/$3m^2$* in solarised and non-solarised plots respectively. In the case of *G. virens*, the yield was 2.699 *kg/ $3m^2$* in solarised plots and 1.705 *kg/$3m^2$* in non solarised fields respectively. In the untreated control plots the yield was only 1.692 kg in solarised and 0.932 kg in non solarised beds (Table VI).

Reduction of weeds was also noticed in the solarised plots. There was 100 per cent reduction of weeds at one month and after 90 days the reduction was 40.57%.

DISCUSSION

The efficacy of soil solarisation in reducing the soil-borne plant pathogens is well documented (Katan, 1976). The pathogens like *Fusarium oxysporum*; *Phytophthora cinnamomi* and *Pythium ultimum* could be controlled by soil solarisation method (Katan *et. al.* 1983; Pinkas *et. al.* 1984 and Pullman *et. al.* 1981 a).

Trichoderma sp. and *Gliocladium* sp. are two important BCA's which were used against a number of soil-borne pathogens (Chet, 1987; Mukhopadhyay, 1987 and Papvizjas, 1985). Large scale multiplication methods of the BCA's with suitable substrates have been reported, both as seed treatment and soil application. (Kousalya and Jayarajan, 1990).

The efficacy of BCA's on disease suppression was conspicuous both in solarised and non-solarised plots (Table VI). The suppression of disease incidence was clearly reflected in the yield level also. The yield in solarised plots were significantly high (2.298 kg) compared to non-solarised plot (1.485 kg). However, the germination percentage in solarised plot was on par with non-solarised plot (Table VI). Among the treatments, *T. harzianum* I applied plots gave the maximum yield in both the conditions.

The population of *Trichoderma/ Gliocladium* and *Pythium* were monitored. It indicated that the survival/ adaptation of the BCA's was higher in solarised soil (Tables II to V). Among the spp. tested, *T. harzianum* II survived longer in solarised fields and in non-solarised fields it was *T. hamatum*. However a gradual decrease in population was noticed in all the treatments with time. In the untreated plots, the population of *Trichoderma/ Gliocladium* was noticed, but population level was much lower compared to BCA's augmented plots. Effect of soil solarisation on suppression of *Pythium* propagules was noticed in both the conditions. However its effect was more in solarised plots and was reflected in disease suppression. Its population fluctuated with the BCA's but there was a gradual increase with time. This is in conformation with the earlier reports.

The study clearly brought out the biocontrol efficacy of *Trichoderma* spp. and *G. virens* both under solarised and non-solarised condition. The effects were synergistic in the former, indicating the potential utility of combining both, as an effective disease management strategy, which needs popularisation among farming community.

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