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EFFECT OF *GLIOCLADIUM VIRENS, TRICHODERMA* SP. AND ORGANIC AMENDMENTS ON THE INOCULUM POTENTIAL OF *THIELAVIOPSIS PARADOXA* IN SOIL.

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ABSTRACT

The efficacy of four antagonistic fungi and two organic amendments in inhibiting the pathogen of coconut stem bleeding disease (*T.paradoxa*) in soil was studied using pot experiments. Among the nine treatments tested, *Gliocladium virens* inoculum @ 10.0g/pot + neem cake (50g/pot) + FYM (500g/pot) showed maximum reduction in *T. paradoxa* population (50.6%). *Trichoderma harzianum, T. viride* and *T. hamatum,* showed comparatively lesser reductions in *T. paradoxa* population. (31.3, 25.3 and 21.9% respectively). Neem cake + FYM alone (@ 50g & 500g/pot respectively) showed the least reduction in *T. paradoxa* population (6.1%). Among the four antagonistic fungi tested, maximum post-treatment populations were observed with *T. harzianum* (334.67 and 406.67x104 cfu/g for the doses of 5 and 10g/pot respectively), followed by the populations of *G.virens* (66.06 and 82.6 x 104cfu/g for the doses of 5 and 10g/pot respectively). Soils amended with neem cake + FYM showed increased levels of native populations of *G. virens* (0.0262 x 104 cfu/g) and *T. harzianum* (3.8187 x 104 cfu/g) compared to their populations in the un-amended soils (0.0029 and 0.0921 x 104 cfu/g respectively).

INTRODUCTION

Stem bleeding disease of coconut caused by the soil borne fungus *Thielaviopsis paradoxa* (de Seynes) von Hohnel is a debilitating disease prevalent in almost all coconut growing areas of the country causing considerable economic loss. The fungus infects coconut stem through growth cracks or wounds that occur on the stem near the ground level and cause extensive bleeding patches on the stem. The affected palm shows gradual decline in yield. Control of the disease through phytosanitation using systemic fungicides like tridemorph (Calixin) and carbendazim (Bavistin) have been reported with limited success(Ramanujam *et al.*, 1993). Since the pathogen is soil borne, it is essential to adopt an integrated approach involving antagonistic organisms along with other control measures for effective disease management. *Gliocladium virens* (Uduma isolate), *Trichoderma harzianum* (Kallangai isolate), *T.viride* (Calicut isolate) *and T.hamatum* (IARI, New Delhi isolate) were identified as potential antagonists of *Thielaviopsis paradoxa* based on *in vitro* and *in vivo* screening of several fungi (Ramanujam, 1997). The present investigations were taken up to test the efficacy of these four antagonistic fungi in inhibiting the *T.paradoxa* in the soil and also to study the proliferation and survival of the introduced antagonists in *T.paradoxa* infected soil.

MATERIALS AND METHODS

Gliocladium virens (Uduma isolate), *Trichoderma harzianum* (Kallangai isolate), *T.viride* (Calicut isolate) *and T.hamatum* (IARI, New Delhi isolate) were used in inhibiting *T. paradoxa* in the infected soil and also for studying the proliferation and survival of the introduced antagonistic fungi in the infected soil. For this purpose, the infected laterite soil (pH 5.6) collected from a stem bleeding affected garden in Kudlu, Kasaragod district (Kerala) was used. Earthen pots of 12 kg capacity (30 cm diameter) were used in the pot experiment. In each pot 10.6 kg of infected soil (equivalent to 1/100th of the soil present in a palm basin around 1.8 meter radius and up to 30.0 cm depth was used). The experiment was started in the month of October was and continued for one year.

Preparation of antagonistic fungus for soil application: The antagonistic fungus was grown on rice bran (RB) + neem cake (NC) (1:1W/W) for 15 days at 25° ± 2°C. Two doses of antagonistic fungal inoculum grown on RB + NC *viz.,* @ 5 g/pot (equivalent to 0.5 kg/palm) 10.0 g/pot (equivalent to 1.0 kg/palm) were tested. Neem cake @ 50 g/pot (equivalent to 5 kg/palm) and Farm Yard manure (FYM) @ 500 g/plot (equivalent to 50 kg/palm) were used as carrier cum food base for the antagonistic fungus in soil. The respective dose of antagonistic fungus (5 or 10 g/pot) was thoroughly mixed with 50 g of NC and 500 g of FYM and the mixture was moistened (50% W/V) and kept for five days for rapid enrichment before application to the soil.

Preparation of pots: 10.6 kg of infected soil was thoroughly mixed with the respective dose of antagonist-enriched neem cake and farm yard manure and filled into each pot. The three treatments used in the pot experiment are 5g, 10g, Nil antagonistic fungus inoculum + neem cake @ 50 g and farm yard manure @ 500 g/pot. Control was without antagonistic fungus, neem cake and farm yard manure

Three replications were maintained for each treatment/sampling period. The filled pots were buried in the trenches taken in a coconut garden in such a manner that the brims of the pots were above the soil level.

Sampling for population studies: Sampling was done at the start of the experiment (October) and subsequently at quarterly intervals (January, April, July and September). *T. paradoxa* population was assessed in terms of percentage of baits (coconut rachis bits) infected by *T. paradoxa* in the soil in each treatment. For this, 50 g soil in three replications were taken in sterile Petri plates (90 mm diameter) and was wetted with 5 ml of sterile distilled water. Ten sterilized pieces of coconut petiole tissues (5 x 5 x 10 mm) were kept in each plate as baits and incubated at 30°C for 48 hr. After this, the baits were recovered and washed in sterile water. A thin layer of tissues from all surfaces of each bait piece was excised with a sterile scalpel and the baits were plated on sugarcane juice agar medium (Sugarcane juice 200 ml, Agar 20 g, distilled water 800 ml, pH 5.5) and incubated at 30°C for 48 hr. The number of baits yielding *T. paradoxa* colonies were counted. The number of baits infected by *T. paradoxa* in the different treatments were compared with the number of baits infected by *T. paradoxa* in the control soil (no treatment) at quarterly intervals. Based on this, the percentage reduction in the *T. paradoxa* bait infection in the antagonist + NC+ FYM/ Neemcake+ FYM treated soils was estimated.

% Reduction in bait infection

= No. baits infected in control - No. baits infected in treatment X 100

No. baits infected in control

The post- treatment populations of introduced antagonistic fungus in the treated soils and the native populations of *G. virens* and *T. harzianum* in the NC and FYM amended soils and in the untreated soils were estimated at quarterly intervals for a period of one year using soil dilution plate method on *Trichoderma* selective medium (TSM).

RESULTS AND DISCUSSION

Effect on *T.paradoxa* Population: The number of baits infected by *T.paradoxa* and the percentage reduction in the bait infection in the nine treatments in comparison with control (un-amended soils) are presented in Table1. Reductions in the populations of *T. paradoxa* were seen in all the nine treatments, as evidenced by reduced bait infection. The percentages of reduction in the *T. paradoxa* bait infection during post-treatment was maximum in the case of *G.virens* at the dose of 10.0g/pot (50.59%) followed by *G.virens* at the dose of 5.0g/pot (46.75%). The percentages of reduction observed after three, six, nine and 12 months of treatment did not vary significantly from each other in the soil treated either at the dose of 5g/pot (43.75, 45.54, 48.90 and 48.90% respectively) or at the dose of 10g/pot (47.90, 50.01, 51.12 and 53.34% respectively). However, maximum reductions in the bait infection by *T.paradoxa* were observed after 12 months of treatment in both doses tested

(53.34% and 48.90% reductions for the doses of 10.0g and 5.0g/pot respectively) and the minimum reductions were noticed during three months of treatment in the two doses tested (47.90% and 43.75% reductions for the doses of 10.0g and 5.0g/pot respectively). A gradual increase in the percentage reduction in *T.paradoxa* bait infection was observed after three months of treatment and reached peak level during 12 months of treatment.

The next efficient antagonist was *T. harzianum* with 31.33 and 26.42% reductions at the doses of 10 and 5g/pot respectively followed by *T. viride* (25.29 and 20.33% for the doses of 10 and 5g/pot) and *T. hamatum* (21.93 and 17.58% for the doses of 10 and 5g/pot respectively). This indicated the superiority of *G. virens* over the other antagonists in the suppression of *T. paradoxa* in the soil. Since, *G. virens* was found to produce effective diffusible inhibitory metabolites against *T. paradoxa* in the *in vitro* studies, the same might have contributed to the higher suppression of *T. paradoxa* in the soil. *G. virens* was reported to produce antifungal metabolites in the organic matter against *R. solani* and *P. aphanidermatum* (Lumsden *et al.*, 1992). In the present study, two organics *viz.*, neem cake and farm yard manure were used along with *G. virens* in the pot experiment, which not only would have served as food base for *G. virens* but also might have led to the production of antifungal metabolites by *G. virens*. Although, *T. harzianum* (Kallangai), *T. viride* (Calicut) and *T. hamatum* (Delhi) were found to be highly inhibitory to *T. paradoxa* in the *in vitro* antibiosis test (64.2-78.8% inhibition) and *in vivo* tests (57.7-66.9% inhibition), (Ramanujam, 1997), their efficacy in the suppression of *T. paradoxa* in soil was found to be low (21.9-31.3%) even at the highest dose tested. Based on these studies, *G. virens* (Uduma) was found to be superior to other antagonistic fungi tested and hence this was selected as the potential antagonist of *T. paradoxa* for the use in the field trials.

Soils amended with neem cake @ 50g + FYM @ 500g /pot showed 6.06 % inhibition of *T.paradoxa* during the post-treatment. The percentages of reduction observed after three, six, nine and 12 months of treatment (6.24, 6.81, 6.74 and 4.46% respectively) did not vary significantly from each other in the soil treated with NC+ FYM. However, maximum reduction in the bait infection by *T.paradoxa* was observed after six months of treatment. In the earlier studies, 10% aqueous extract of neem cake was found to show 55% inhibition of *T.paradoxa in vitro* studies (Ramanujam,1997). But in the present study neem cake could inhibit only 6.06% of *T.paradoxa* in soil.

Proliferation and survival of introduced antagonistic fungi in the soil: The populations of *G. virens* (Uduma), *T. harzianum* (Kallangai), *T.viride* (Calicut) and *T. hamatum* (Delhi) in the soils during post-treatment (after augmentation) are presented in Table-2. Among the four antagonistic fungi tested, maximum mean post-treatment population of the introduced antagonist was observed with *T. harzianum* (406.67 and 334.67 X 104 cfu/g for the doses of 10 and 5g/pot respectively) followed by that of *G. virens, T. viride,* and *T. hamatum*. The post-treatment population of *T.harzianum* in the soils treated with *T.harzianum* (Kallangai) was found to be slightly higher during initial stage, three and six months of treatment (766.67, 833.33 and 303.33 X 104 cfu/g) and thereafte the the populations decreased after nine and 12 months after treatment (93.33and 36.67 X 104 cfu/g). Similar trend was observed with the dose of 5.0 g/pot. Although, *T. harzianum* showed higher post-treatment soil population, it was found to be less effective in suppressing *T. paradoxa* population in the soil, compared to the inhibition observed with *G. virens* (Table 2).

Post treatment populations of *G. virens* in the amended soils at the doses of 5 and 10g/pot increased sharply to the levels of 123.33 and 163.33 X 104 cfu/g respectively from the pre-treatment level of 0.003 X 104 cfu/g immediately after mixing with the respective dose of *G. virens* and reached the peak levels (143.33 and 170.00 X 104 cfu/g respectively) three months after augmentation. This indicated the ability of *G. virens* (Uduma) to proliferate in the amended soils at the doses of 5 and 10g/pot. The populations of *G. virens* showed a declining trend after three

Table 1. Interaction of four antagonistic fungi with T.paradoxa in infected soil-Effect on T.paradoxa population levels (Pot experiment)

Sl.No.	Treatment	% of baits infected by T-Paradoxa						%reductioninthe T-paradoxa bait infection				
		initial (Oct)	after 3M	after 6M	after9 M	after12 M	Mean	3 M	6 M	9 M	12 M	Mean
1.	G.virens(Uduma) 5g+NC 50g and FYM 500g	53.33	30.00	26.67	25.56	25.56	32.22	43.75	45.44	48.90	48.90	46.75
2.	G Virens (Uduma)10 g+Nc50g and FYM 500g/pot	52.22	27.78	24.44	24.44	23.33	30.44	47.90	50.01	51.12	53.34	50.59
3.	T.harzianum (kallangai) 5g + NC 50g and FYM 500g/pot	53.33	41.11	35.56	34.45	37.79	40.45	22.92	27.28	31.08	24.42	26.42
4.	T.Harzianum (Kallangai) 10g + NC 50g and FYM 500g/pot	52.22	38.89	32.22	32.22	35.56	38.22	27.07	34.09	35.32	28.86	31.33
5.	T. viride (Calcut) 5g + NC 50g and FYM 500 g/pot	51.11	42.22	37.78	40.00	41.11	42.44	20.83	22.72	19.98	17.78	20.32
6.	T. viride (Calicut) 10 g + NC 50g and FYM 500g/pot	53.33	40.00	34.45	37.79	38.89	40.89	25.00	28.55	24.42	22.20	25.29
7.	T. hamatum (Delhi) 5g + NC 50g and FYM 500g/pot	52.22	43.33	38.89	41.11	43.33	43.78	18.75	20.45	17.78	13.34	17.58
8.	T.hamatum (Delhi) 10g + NC 50g and FYM 500g/pot	51.11	40.00	36.67	38.89	42.22	41.79	25.00	24.99	22.20	15.56	21.93
9.	Neem cake 50g and FYM 500g/pot	53..	50.00	45.56	46.67	47.78	48.67	6.24	6.81	6.74	4.46	6.06
10.	Control (No. treatment)	52.22	53.33	48.89	50.00	50.00	50.89	-	-	-	-	-
	Mean	52.44	40.67	36.11	37.11	38.56	-	26.38	29.04	28.61	25.42	-

C.D (P=0.05)	
Treatments	3.09
Sampling periods	2.19
Treatments X sampling periods	NS

M - Months

* Before amendment with the antagnistic fungus + NC + FYM/NC+FYM. Expt. started in October.

** % reduction in T paradoxa bait infection in the treatment was calculated compared to the bait infection in the control

months of treatment and decreased gradually, reaching the minimal levels during 12 months of treatment (2.33 and 3.67X 104 cfu/g for the doses of 5 and 10g/pot respectively). Although the populations of *G. virens* decreased after three months of treatment, its ability to suppress *T. paradoxa* continued till 12 months of treatment in both the doses tested. This indicated that the post-treatment populations of *G. virens* were found to be sufficiently high enough through out the year to show the suppressive effect on *T. paradoxa*. *G. virens* (Uduma) was found to

Table 2. Interaction of four antagonistic fungi with *T.paradoxa* in infected soil- Post- treatment population levels of introduced antagonistic fungi in the soil (Pot experiment)

Sl. No.	Treatment	Post- treatment population of intrduced antagonistic fungi in the soil (x 10^4cfu/g)					
		Initial* (Oct.)	After 3 months	After 6 months	After 9 months	After 12 months	Mean
1	*G.virens* (Uduma) 5g + NC 50g and FYM 500g/pot	123.33	143.33	53.33	8.0	2.33	66.06
2	*G.virens* (Uduma) 10 g + NC 50g and FYM 500g/pot	163.33	170.00	66.67	9.33	3.67	82.60
3	*T.harzianum* (Kallangai) 5g + NC 50g and FYM 500g/pot	633.33	700.00	223.33	86.67	30.00	334.67
4	*T.harzianum* (kallangai) 10g + NC 50g and FYM 500g/pot	766.67	833.33	303.33	93.33	36.67	406.67
5	*T.viride* (Calicut) 5g + NC 50g and FYM 500g/pot	93.33	86.67	17.55	2.67	0.57	40.16
6	*T.viride* (Calicut) 10g + NC 50g and FYM 500g/pot	131.33	117.67	21.77	4.56	0.70	55.17
7	*.hamatum* (Delhi) 5g +NC 50g and FYM 500g/pot	74.44	63.33	12.67	2.11	0.43	30.60
8	*T.hamatum* (Delhi) 10g + NC 50g and FYM 500g/pot	107.77	90.00	17.11	1.67	0.37	43.38
	Mean	**245.27**	**275.54**	**89.47**	**26.04**	**9.34**	

C.D.(P=0.05)

Treatments	49.54
Sampling periods	39.16
Treaments X Sampling periods	110.77

* Population count taken immediately after augmentation with the respective antagonistic fungus. The intial native population

* levels of *G.virens* and *T.harzianum* in the pre-treatment soil were 0.0033 and 0.0867 x 10^4 cfu/g soil respectively (Table 3).

produce chlamydospores, which enabled the fungus to survive and persist for longer periods.

The post-treatment populations of exotic antagonistic fungi like *T. viride* (Calicut) and *T. hamatum* (Delhi) were found to be low as they might be less adapted to local soils, compared to the local isolates of *G. virens* (Uduma) and *T. harzianum* (Kallangai). which have withstood the local conditions for ages and have evolved here due to the selection pressure from pathogen.

In the pot experiment, it was observed that application of neem cake (50g/pot) and FYM (500g/pot) to the soil increased the native populations of *G. virens* and *T. harzianum* to the levels of 0.7333 and 9.667 X 104 respectively after three months of treatment from the initial levels of 0.0033 and 0.0867 X 10^4 cfu/g respectively (Table 3). Thereafter, the native population levels of *G. virens* and *T. harzianum* in the amended soils showed a declining trend and reached the minimal levels during 12 months of treatment (0.027 and 1.333 X104 cfu/g respectively). However, the population levels of these organisms remained significantly higher throughout the post-treatment period, compared to the levels of populations observed in the un-amended soils. This may be due to the growth promoting effect of neem cake and FYM on these organisms. Neem cake was found to encourage the native populations of *Trichoderma* in the amended soils (Bhaskaran *et al.*, 1993). In the present study, neem cake was found to encourage not only native population of *T.*

Table 3. Population dynamics of native populations of G.virens and T.harzianum in the neem cake and FYM amended and control soils (Pot experiment)

Sl. No.	Treatment	Native population of G. virens (x 10⁴ cfu/g)						Native population of T.harzianum (x 10⁴ cfu/g)					
		Intial (Oct)	3M	6M	9M	12M	Mean	Intial (Oct)	3M	6M	9M	12M	Mean
1.	Neem cake 50g and FYM 500g	0.004	0.7333	0.5000	0.0430	0.0270	0.0262	0.0933	9.667	5.3333	2.6667	1.3333	3.8187
2.	Control	0.003	0.0037	0.0013	0.0032	0.0030	0.0029	0.0867	0.1170	0.0800	0.0933	0.0833	0.0921
	C.D. (P=0.05)												
	Treaments	0.006	0.0495										
	sampling periods	0.004	0.0392										
	Treat x samp.period	0.011	1.1080										

harzianum and *G. virens* but also was found to be inhibitory to *T. paradoxa* in the *in vitro* studies and pot experiment. Hence, neem cake along with FYM was selected as carrier cum food base for the delivery of the antagonistic fungus to the soil in the field trials.

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