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Biological control of seedling diseases in forest nurseries in Kerala

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ABSTRACT: ***Trichoderma harzianum***, ***T. viride***, **and** ***Pseudomonas fluorescens*** **were screened in forest nursery in two consecutive years to study the efficacy against the seedling damping-off pathogens,** ***Rhizoctonia solani*** **and** ***Cylindrocladium quinqueseptatum*****. Solarization of nursery beds by tarping the moistened soil by thick polythene sheets was also carried out as a part of the experiment. The antagonists were introduced in the nursery beds by soil amendment and seed coating either singly or in different combinations using** ***Eucalyptus tereticornis*** **and** ***E. grandis*** **as the host plants. Solarization increased the temperature in the top layer of soil (2.5-5 cm depth) up to 51°C and reduced the pathogen inoculum considerably. Among various treatments,** ***T. harzianum*** **as soil application was the most effective one against damping-off followed by the combination of soil solarization and** ***T. harzianum*** **soil application.**

KEY WORDS: Biological control, *Cylindrocladium quinqueseptatum*, *Rhizoctonia solani*, seedling damping-off, soil solarization, *Trichoderma harzianum*, *T. viride*

INTRODUCTION

In forest nurseries, damping-off caused by *Rhizoctonia solani* Kuhn and *Cylindrocladium quinqueseptatum* Boedijn & Reitsma poses a major threat to production of planting stock of teak, eucalyptus, acacia, etc. in the Kerala State and thereby affects the planting programme considerably (Mohanan and Sharma, 1985; Sharma and Mohanan, 1991a). *R. solani* is an aggressive pathogen and existence of different Anastomosis Groups (AG), AG1-IA, AG1-IC, AG2-I, AG2-2IV in forest nursery soil has been reported (Mohanan, 2001), and different physiological strains of *C. quinqueseptatum* have also been reported from the state (Sharma and Mohanan, 1991b). Earlier, control of these pathogens in forest nurseries has been achieved through systemic fungicides like carboxin and carbendazim (Mohanan, 2000; Sharma and Mohanan, 1991c, 1992).

Introduction of antagonistic organisms like *Trichoderma* spp. and *Pseudomonas fluorescens* into the nursery soil is a good approach to control the seedling diseases caused by soil-borne pathogens. Several species of *Trichoderma* have been used against diseases caused by different pathogens in the laboratory and field (Papavizas and Lewis, 1981; Papavizas, 1985; Adams, 1990; Whipps, 2001). Damping-off disease requires comparatively a short window of protection during the first month of seedling germination. Protection can be offered not only by application of biological control agents directly onto the seed, but also by exclusion of the inocula of the soil-borne pathogen(s) by the process of solarization of the

nursery beds (Katan *et al.*, 1976; Katan, 1987; Pullman *et al.*, 1981; Sharma and Mohanan, 1991a). The present study was aimed at achieving biological control of damping-off in forest nurseries.

MATERIALS AND METHODS

Nursery trials

The experimental nursery was raised at Chandhanathodu (Wayanad Forest Division) and trials were carried out in two consecutive years, 2000 and 2001. The nursery soil (pH 6.7) was thoroughly worked and 54 seedbeds of 2 x 1 x 0.3 m were prepared at a distance of 60 cm. Twelve nursery beds were selected randomly and watered regularly to make the soil moistened. Each bed was then covered with a polythene sheet and edges sealed with mud on all sides. Soil thermometers were inserted in the beds at different depths (2.5 cm, 5 cm, 10 cm, 15 cm). Soil temperatures were recorded between 08.00 and 17.00 h at 1h interval. The setups were maintained and observations on soil temperature recorded for 18 days. The polythene sheets were removed after 18 days of solarization treatment.

Antagonistic organisms

Isolates of *Trichoderma harzianum*, *T. viride* and *P. fluorescens* found efficient against various strains of *R. solani* and *C. quinqueseptatum* in the laboratory and glasshouse trials were utilized for the nursery trials (Mohanan, 2001). Inoculum of *T. harzianum* (TH3), *T. viride* (TV5) and *P. fluorescens* was prepared on tapioca rinder substrate (Kausalya Gangadharan and Jayarajan, 1988; Mohanan, 2001) and applied in the nursery beds @ 120g / m^2. The inoculum was mixed thoroughly with the top layer of soil (5 cm depth).

Eucalyptus grandis and *E. tereticornis* seeds (20g / m^2) were sown after seven days of antagonistic fungal treatments in the nursery beds. Seed coating with *T. harzianum* and *T. viride* suspension (2 x 10 ^{9}cfu / ml) and *P. fluorescens* suspension (2 x 10^7 cfu / ml) was carried out by soaking 100 g seeds in 250 ml respective fungal/ bacterial suspension for 6 h and air-dried. To regulate the shade over the nursery beds, (75%) shade nets were provided. Water regimes in the seedbeds were regulated at the rate of 30 l per day. Details of various treatments are given in Table 1.

Table 1. Details of various biological control treatments

Treatment code	Treatment	Year	
		2000	2001
PFSC	*Pseudomonas fluorescens* seed coating	√	√
THSC	*Trichoderma harzianum* seed coating	√	√
TVSC	*Trichoderma viride* seed coating	√	√
THSA	*Trichoderma harzianum* soil amendment	√	√
TVSA	*Trichoderma viride* soil amendment	√	√
SS	Soil solarization	√	√
SS+THSC	Combination of 2 treatments (1:1 ratio)	x	√
SS+THSC+TVSC+PFSC	Combination of 4 treatments (1:1:1:1 ratio)	√	x
THSC+TVSC+PFSC	Combination of 3 treatments (1:1:1 ratio)	√	x
C	Control	√	√

x: treatment not done

Disease incidence

Seed germination and disease incidence in each seedbed were recorded daily. Each damping-off patch was marked by aluminium tags or coloured splinters. Observation on number of infection foci, its spread, number of seedlings affected, etc. were recorded from each treatment daily for 30 days.

Evaluation of microbial population

Soil samples from the nursery beds were collected before and 30 days after the treatment. These samples were transported to the laboratory for isolation and analysis of fungal flora, including pathogen inoculum and persistence of the introduced antagonistic organisms by soil dilution plate method was followed. Rose Bengal agar (RBA), Potato dextrose agar (PDA), and Nutrient agar (NA) media were used for isolation. The microbial population was assessed as colony forming units (cfu) per gram of dry soil.

Statistical analysis

All the experiments were arranged in a randomized complete block design and treatments were replicated thrice. Analyses of data were done by ANOVA with separation of means by Duncan's Multiple Range Test. Log transformation was carried out before performing the ANOVA. Data expressed in percentage were subjected to angular transformation before analyses.

RESULTS AND DISCUSSION

The maximum temperature recorded was 51°C, 48°C and 45°C at 2.5, 5 and 10 cm soil depth, respectively, in the nursery beds subjected to solarization during the 2000 trial. During the 2nd year trial (2001), maximum temperature of 45°C was recorded at 5 cm soil depth. Peak temperature was attained between 13.00 and 14.00 h in both the trials. In soil depths of 10 cm and 15 cm, the temperatures attained were much lower than those in top layer.

Evaluation of fungal population before and after the solarization treatment revealed a significant decrease in population in solarized nursery beds at 0-2 cm soil depth in both years. During 2000 trial, fungal population (cfu / g) before soil solarization was 39.13 x 10^3 / g and after solarization, cfu count increased to 25.27 x 10^3 / g; these figures for 2001 trial were 42.85 x 10^3 / g and 29.03 x 10^3 / g, respectively. A total of 14 fungal genera were recorded in the soil before the treatment in 2001 trial and after solarization the number of genera was reduced to 11. Microsclerotia and sclerotia forming damping-off pathogens like *Cylindrocladium* spp., *Fusarium* spp., and *Rhizoctonia solani* were found reduced in treated soil in both the years. However, an increase in population of *Penicillium, Aspergillus* and *Trichoderma* was recorded in solarized soil. An increase of population of *Trichoderma* spp. was recorded from 5.55 to 30.43 % in the first year trial, while in the second year trial, the figures were 7.69 to 18.51%, respectively, before and after solarization. The changes in fungal population in terms of fungal floral composition and number of cfu brought about by the treatment may have contributed towards the protection of seedlings from damping-off pathogens.

Incidence of damping-off in solarized nursery beds was comparatively less than that in the control beds in first and second year trials. In the first year trial, mean damped-off patches or the infection foci were 9.50 in *E. tereticornis* and 11.79 in *E. grandis*, while in the second year trial, disease incidence was found much higher than that in the first year and the mean infection foci observed was 16.18 (Tables 2 and 3). In each infection foci, the number of damped-off seedlings varied from 10 to 15. In many cases, merging of individual patches with the adjoining patches also occurred. In treatments where soil solarization was followed by addition of antagonistic fungal inoculum, disease incidence was found much more reduced than that occurred in solar heating alone. However, in the first year trial, when solar heating was followed by soil amendment with *T. harzianum* (TH3) and *T. viride* (TV5), disease incidence was found much higher than that in treatment with antagonists alone (Table 2).

Inactivation of spores of many soil-borne pathogens like *R. solani, Sclerotium rolfsii* Sacc.,

Table 2. Effect of various treatments on incidence of damping-off in 2000 trial

Treatment	Mean No. of damped-off patches			
	ET*	±SE	EG	±SE
SS	9.5002^{a}	1.1222	11.7984^{a}	1.1701
THSA	10.5298^{ab}	1.2420	15.3722^{ab}	1.2080
TVSA	15.6037^{bc}	1.2082	18.7288^{ab}	1.1121
PFSC	35.3610^{d}	1.1809	37.1574^{c}	1.1042
SS + THSA + TVSA	19.3561^{c}	1.0328	20.0246^{b}	1.3727
THSA + TVSA + PFSC	23.4885^{cd}	1.2162	22.7022^{b}	1.1412
C	93.7761^{e}	1.1116	113.3706^{d}	1.1806

*ET: *Eucalyptus tereticornis*; EG: *E. grandis*; SE: Standard error; values in columns sharing the same superscript(s) do not differ significantly at P = 0.05

Verticillium dahliae Kleb., *Pythium* spp., etc. at temperatures between 40°C and 50°C has been reported by many workers (Pullman *et al.*, 1981; Katan, 1987). Sherwood (1970) reported inactivation and killing of *R. solani* at 45°C. A time–temperature (dosage) relationship for the thermal killing of *R. solani* and *Fusarium* sp. was reported by Elad *et al.* (1981). In the present study, soil temperature of 51°C and 45°C at soil depths of 2.5 and 5 cm, respectively, usually was retained for an average of eight to nine hours per day. This was found close to or greater than the thermal death temperature for *R. solani* reported by Sherwood (1970). It is evident that *R. solani*, a very efficient saprophytic competitor, is not suppressed completely by the treatment and is capable of re-colonizing quickly under conducive soil environment and causes infection. This shows that the success of the treatment largely depends on the microclimatic conditions prevailing in the nursery immediately after the treatment. However, the results support that soil solarization can be used as a promising tool to control the damping-off in forest nurseries.

Antagonistic organisms

The overall performance of various biocontrol treatments in two-year trials was good and soil amendment with the antagonists was found more effective than seed coating. In ANOVA, interaction of various treatments was found highly significant (P = 0.05) and DMRT showed significantly different groups among the treatments (Tables 3 and 4). Soil amendment with *T. harzianum* (TH3) was found better than that with *T. viride* (TV5) in both 2000 and 2001 trials.

Table 3. Effect of various treatments on incidence of damping-off in 2001 trial

Treatment	Mean No. of Damped-off patches*	SE
SS	16.1779^{a}	1.3576
SS + THSA	14.7870^{a}	1.1509
THSA	19.0458^{a}	1.0729
TVSA	36.6037^{bc}	1.1584
THSC	32.5881^{b}	1.0901
TVSC	51.6065^{cd}	1.0894
PFSC	63.8188^{d}	1.0907
C	111.3578^{e}	1.2196

* Values in columns sharing the same superscript(s) do not differ significantly at P = 0.05

Among the various treatments tried in 2000 and 2001, either combination(s) of antagonists or delivery methods was not as effective as those tried singly. For example, solar heating was effective in reducing the damping-off in seedbeds in 2000 trial, while solar heating combined with soil application of antagonists was less effective. However, in 2001 trial, the only treatment combination tried was solar heating and soil application of *T. harzianum* (TH3), which gave a better control of damping-off than solar heating alone. This treatment turned out to be the best among the seven treatments tried in 2001 trial and the mean infection foci recorded was 14.78 against 111.35 mean infection foci in control. However, DMRT showed that the three treatments, solar heating, solar heating + *T. harzianum* (TH3) soil amendment, and *T. harzianum* (TH3) soil amendment are not significantly different from each other (Table 4). In 2000 trial, in the treatment in which *T. harzianum* and *T. viride* were incorporated as soil application, while *P. fluorescens* was introduced as seed coating, results were not very encouraging, however, the disease incidence was less than that in *P. fluorescens* treatment. A strategy that has received recent attention is the use of microbial inoculants, which contain multiple species or strains rather than a single strain of *Trichoderma* or *Pseudomonas*. The rationale behind this strategy is that multiple strain/species inoculants are perceived to represent more closely the natural biological control that occurs in the field.

Even though seed coating with the same antagonists gave promising results in seedling bioassays in glasshouse (Mohanan, 2001), in nursery trials, the results were not very encouraging possibly due to the partial failure of suppression of pathogen inoculum by the antagonists. In nurseries, under natural conditions, many influencing factors may be playing their role in the development of disease. As far as the persistence of the introduced antagonists in the nursery bed is concerned, an increase in population of *T. harzianum*, *T. viride* and *P. fluorescens* was recorded in their respective treatments (Table 4). *T. viride* showed the maximum number of colony forming units (cfu 38.42 x 10^4 / g). Persistence as well as increase in population of *Trichoderma* species recorded was more in treatment with soil amendment than in seed coating.

Analysis of two years' nursery data revealed that damping-off can be controlled or disease incidence can be reduced to a minimum level by application of *T. harzianum* (TH3) isolates as soil application well before the sowing of the seeds. Depending on the host species being raised and prevalent edaphic and environmental conditions, the nursery cultural practices have to be modified to accomplish optimum results from the manipulation of biological control agents.

The biological control trials carried out in forest nursery in two consecutive years suggest that management of damping-off disease can be achieved through soil application of an efficient

Table 4. Persistence of antagonists in various biological control treatments in 2001 trial

Treatment	Microbial population (cfu / g)	
	Before treatment	After treatment
SS +THSA	*Trichoderma* sp. 3.72 x 10^3 / g	*T. harzianum* 24.03 x 10^4 / g
THSA	*Trichoderma* sp. 4.26 x 10^3 / g	*T. harzianum* 32.06 x 10^4 / g
TVSA	*Trichoderma* sp. 3.68 x 10^3 / g	*T. viride* 38.42 x 10^4 / g
THSC	*Trichoderma* sp. 4.18 x 10^3 / g	*T. harzianum* 12.06 x 10^4 / g
TVSC	*Trichoderma* sp. 5.04 x 10^3 / g	*T. viride* 13.08 x 10^4 / g
PF SC	-	*P. fluorescens* 68.04 x 10^7 / g

local strain of *T. harzianum* (TH3). Solarization of nursery beds is also a good treatment for managing the seedling damping-off. Optimal application of biological control is possible only when the operating mechanisms are fully understood. To achieve best results in disease control in forest nursery, an integrated approach involving application of biological control agents, and modification of nursery cultural practices is more desirable.

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