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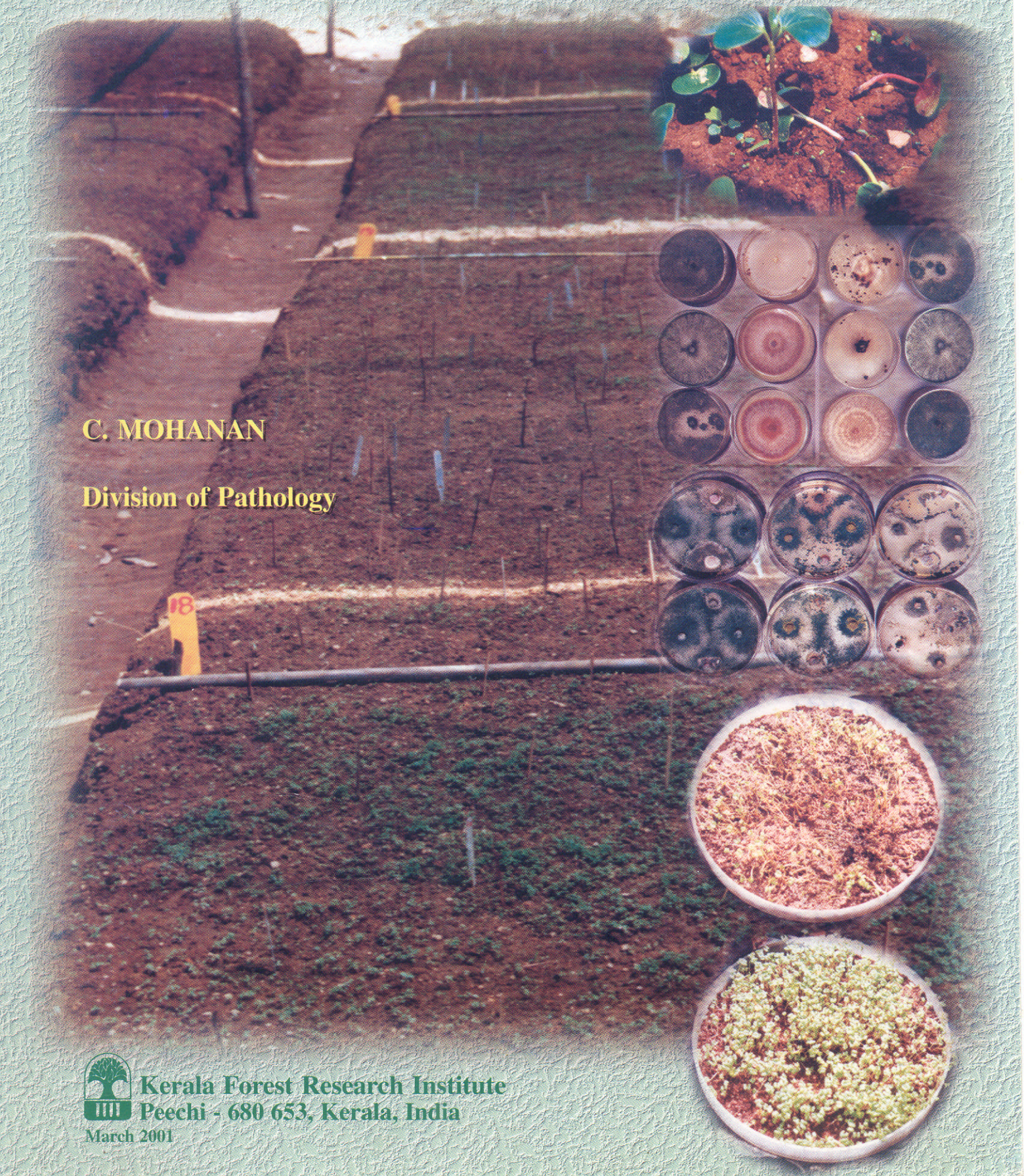

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BIOLOGICAL CONTROL OF DAMPING-OFF IN FOREST NURSERIES IN KERALA

(Final Report of the Research Project KFRI 275/98)
ICFRE No. 37-2/96-ICFRE (R)

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ABSTRACT OF PROJECT PROPOSAL

1. Project No. : KFRI 275/98
2. Title : Biological control of damping-off in forest nurseries in Kerala

3. Objectives :

   i. To isolate, identify and characterize the important damping-off pathogens in forest nurseries

   ii. To isolate, identify and evaluate the potential microbial candidates against damping-off pathogens

   iii. To develop biocontrol method(s) to manage the disease in forest nurseries.

4. Date of commencement : June 1997
5. Scheduled date of completion : December 2000
6. Project Team

   Principal Investigator : Dr. C. Mohanan (Division of Pathology)

   Research Fellow : Smt. S. Ampili (May 1998 to November 1999)

7. Funding Agency : ICFRE (Under ICFRE –World Bank FREE Project)

CONTENTS

ABSTRACT

Disease monitoring in forest nurseries in Kerala State revealed that damping-off disease poses major threat to seedling production and thereby affects the planting programme considerably. *Rhizoctonia solani, Cylindrocladium quinqueseptatum, Fusarium oxysporum,* and *Pythium myriotylum* are the important pathogens associated with the damping-off disease and most forestry species raised in seedbed and container nurseries are affected by them. In rootrainer nurseries surveyed, very low incidence of damping-off was recorded and the new technology seems to have a major impact on production and protection of planting stock. Among the damping-off pathogens, *R. solani* and *C. quinqueseptatum* are the most serious ones, which occur in different strains of varying virulence. Characterization of 31 isolates of *R. solani* and 23 isolates of *C. qinqueseptatum* obtained from different nurseries, revealed existence of different Anastomosis Groups (AG) in *R. solani*, viz., AG1-IA, AG1-IC, AG2-I, and AG2-2IV, and physiological strains among *C. quinqueseptatum.* Isolates of antagonistic organisms viz. *Trichoderma harzianum, T. vride, T. koningii, Gliocladium virens,* and *Pseudomonas fluorescens* retrieved from nursery soil and also obtained form other Biocontrol Laboratories were screened against different isolates of *R. solani, C. quinqueseptatum, F. oxysporum, Pythium* sp. and *P. myriotylum* employing slide culture technique, Petri dish and seedling bioassays. In all these tests, isolates of *T. harzianum* (TH3) and *T. viride* (TV5) were found most effective in controlling the damping-off pathogens. Mass culturing of the promising antagonistic fungi on tapioca rinder substrate and maize-meal-perlite media was standardized and studies on shelf-life of the fungal inoculum revealed that the inoculum raised in both the media in polypropylene bags can be stored at 25 ± 2^{0} C for 3 to 4 months without adversely affecting its viability and virulence. The selected promising candidates of biocontrol agents were further tested in experimental nursery raised at Chandhanathodu, Wayanad in two consecutive years, i.e. 1998 and 1999. The antagonists were introduced in the nursery beds by soil amendment and seed coating. *Eucalyptus tereticornis* and *E. grandis* were used as the major host plants in biocontrol treatments, while thirteen other forestry species were also tried as test plants. Solarization of nursery beds by tarping the moistened soil by thick polythene sheets for 18 days was also

carried out as a part of the biocontrol experiment. Solarization increased the soil temperature in top layer of soil (2.5 cm to 5 cm depth) up to 51^{0}C and reduced the pathogen inoculum considerably. Biocontrol agents viz., *Trichoderma harzianum*, *T. viride* and *P. fluorescens* were applied in nursery beds either singly or in different combinations. Two years' nursery data showed that among various treatments tried, *T. harzianum* (TH3) as soil application was the most effective one followed by a treatment combination of soil solarization and *T. harzianum* soil application. Seed coating with *T. viride* and *P. fluorescens* was not effective, however, the treatments were better than control. The studies on persistence of introduced antagonists in the nursery beds revealed that population of antagonists increased to a considerable level and both the carrier materials, viz., tapioca rinder substrate and maize-meal-perlite were equally effective. The study confirms that biological control of damping-off in forest nursery can be succeeded through application of efficient local strains of antagonistic fungus like *Trichoderma harzianum* (TH3) as nursery soil amendment.

1. INTRODUCTION

Planting programme starts with raising sufficient planting stock in nurseries. Due to the warm-humid climate prevailing in Kerala State, forest nurseries are often confronted with a series of disease problems. In nurseries, diseases appear right from the seedling emergence phase till planting out. Damping-off, the disease which occurs first in the nursery, causes pre-emergence or post-emergence damage and affects seed or young seedlings. Until 1980s, damping-off was a very common and major disease in forest nurseries in the State. Its prevalence has decreased with the introduction of direct seeding of many species into the polythene containers as well as in soil-less or soil-free medium in rootrainers, very recently. However, damping-off disease persists still in seedbed nurseries and poses major threat, especially those raised in high rainfall areas. The loss due to damping-off cannot be predicted as the disease may destroy the entire emerging seedlings in the seedbeds within 10 to 20 days. Mortality of seedlings may occur at two distinct phases of their growth due to pre-emergence and post-emergence damping-off. In pre-emergence damping-off, the developing seedlings are attacked and killed before they emerge above the ground and hence the emergent crop becomes sparse. In post-emergence damping-off, the seedlings are attacked on the hypocotyle or on roots or both while still in cotyledon stage, which causes seedlings either wilt completely or fall over on the ground due to rotting of tissues at the base.

Damping-off disease affects most forestry species raised in nurseries. Many fungal pathogens are involved with the disease. Species of *Rhizoctonia, Cylindrocladium, Fusarium, Pythium, Phytophthora*, etc. are associated with the disease either singly or in-groups. Depending upon the edaphic and micro-climatic conditions in the nursery and the forestry species raised, association of the pathogen as well as severity of the disease may vary. Among various fungi associated with the disease, *Rhizoctonia solani* Kuhn state of *Thanatephorus cucumeris* (Frank) Donk has emerged as the most important and potential pathogen in forest nurseries in the State. Even though, damping-off pathogens do not show any host specificity, *Cylindrocladium* spp. are usually found associated with the eucalypts, acacias and pines.

Disease management in forest nurseries is often achieved through application of chemical pesticides and also by modification of the nursery cultural practices. Regulating the shade over the seedbeds, water regime, and reducing seedling density by selecting appropriate sowing rate, etc. are some of the important nursery practices which are likely to have distinct impact upon the incidence and severity of damping-off. Protection of seedlings in forest nurseries against diseases has been much relied on chemical pesticides, because of the assured results. However, widespread and indiscriminate applications of chemical pesticides in forest nurseries have resulted in the emergence of problems related to pesticide resistance in pathogens, toxicity to non-target organisms, environmental contamination, etc. which have greatly reduced the desirability of chemical pesticides.

Biological control of plant pathogens has gained considerable attention over the past 30 years and much of this interest stems from the desire to decrease the use of chemical pesticides. Disease management through biological means can result from the reduction of pathogen inoculum, protection of the infection court, reduction of infection of the host, or reduction of disease progression or severity (Cook, 1993; Cook and Baker, 1983). A major mechanism, which plays a role in many biological control situations, is the competition for nutrient as well as space, which can result in niche exclusion of pathogen. An example of a specialized form of nutrient competition, which has offered disease protection in some plant/pathogen systems, involves the production of siderophores (Liang *et al.,* 1996; Loper, 1988). Siderophores are iron-chelating compounds produced by microorganisms like *Pseudomonas fluorescens* and can cause iron deprivation of the pathogen. Additional mechanisms which may be involved in biological control include antibiosis (Fravel *et al.*, 1998; Thomashow and Weller, 1998), parasitism or predation (Papavizas and Lumsden, 1980), induced host resistance or cross protection and hypovirulence (Kuc, 1987). Several mechanisms may operate jointly to reduce disease through biological control and it is difficult to determine the mechanisms, which are most influencial. Furthermore, microbial communities are complex and several members of the community are likely to rescue in decreasing disease development or progression. The biological control of plant diseases can include several approaches such as the protection of the infection court or the eradication of a virulent pathogen. Introduction of antagonistic organisms into the nursery soil is a good approach in

biocontrol of seedling diseases caused by soil-borne microorganisms. The genera of fungi most commonly evaluated for their potential, as biocontrol organisms are *Trichoderma* and *Gliocladium*. Several species of *Trichoderma* including *T. harzianum* Rifai, *T. hamatum* (Bonord) Bain., and to a lesser extent *T. koningii* Oudem, *T. polysporum* (Link ex Pers.) Rifai and *T. viride* Pers. ex Gray have been used against diseases caused by several pathogens in the laboratory and field (Papavizas and Lewis, 1981; Papavizas, 1985). Knowledge on the disease and pathogen(s) associated with it is very important prior to embark upon developing a biological control strategy. Damping-off disease requires comparatively a short window of protection during the first month of seedling emergence. Protection can be offered by application of biocontrol agents directly onto the seed, or infection court. Protection can also be achieved by exclusion of the inoculum 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, 1991). The present project is aimed at achieving biocontrol of damping-off in forest nurseries with the following specific objectives:

- To isolate, identify and characterize the important damping-off pathogens in forest nurseries
- To isolate, identify and evaluate the potential microbial candidates against damping-off pathogens
- To develop biological control method (s) to manage the disease in forest nurseries

For collecting information on occurrence, spread, and severity of disease, pathogens associated with the disease, etc. a disease monitoring in forest nurseries raised in the State was carried out during 1997 to 1999. Antagonistic organisms isolated from native nursery soils and procured from different Biocontrol Laboratories were utilized for laboratory screening and field application. Solarization of the seedbeds was also tried. Bioassays were carried out in laboratory and greenhouse and the results were further tested in experimental forest nurseries raised during 1998 and 1999.

2. METERIALS AND METHODS

2.1. Disease Monitoring in Forest Nurseries

Seedbed nurseries raised at various localities in Kerala State and rootrainer nurseries raised at Kulathupuzha (Central Nursery, Thenmala Forest Division), Valluvassery (Central Nursery, Nilambur North Forest Division), and Cheruvanchery (Central Nursery, Kannur Forest Division), were monitored during 1997-'99. Disease incidence, severity, and spread were recorded using a disease scoring scale (Table 1). Disease specimens and sick soil samples collected from the nurseries were brought to the laboratory.

2.2. Isolation and Identification of Causal Agents of Damping-off

Isolation of causal agents from the damped-off specimens was carried out by standard procedures. Employing seedling bait method isolation of damping-off pathogens from sick soil was made. Sick soil (1-2 kg) collected from different nurseries was kept in Aluminium trays (30 x 30-x 2 cm) and *Eucalyptus tereticornis and E. grandis* seeds (5g per tray) were sown. Trays were watered and seedling emergence observed. Damped-off seedlings and seedlings showing damping-off symptoms were collected, and causal agent(s) isolated. Identification of causal agents was made up to species level. All the fungal isolates were pure cultured and maintained on Potato dextrose agar (PDA) medium. Bacterial cultures were maintained in Nutrient agar medium.

Table 1. Disease scoring scale

Disease severity	Disease severity scale	
	Percent seedling affected	No. of disease patch/m^2seedbed
Nil	0	0
Low-L	1-10	1-10
Medium-M	11-30 (11-20% seedlings dead)	11-30
Severe - S	31-75 or more >30% seedlings dead	>30

2.3. Characterization of Damping-off Fungi

2.3.1. Rhizoctonia solani Kuhn

A total of thirty one isolates of *Rhizoctonia solani* Kuhn recovered from diseased seedlings / infested soil (sick soil), collected from various nurseries situated in different locations in the State (Table 2) were tested for determining their Anastomosis Group as well as nuclear conditions. Authentic tester isolates of *R. solani* belonging to various Anastomosis Groups (AG) (Table 3) obtained from Dr. Akira Ogoshi, Hokkaido University, Japan were employed. Anastomosis was tested by opposing isolates of *R. solani* on sterilized glass slides (76 x 26 mm) sprayed with 1.5% water agar (Yokoyama and Ogoshi, 1986). Aniline blue (0.2%) in 50% glycerol, slightly acidified with HCl (Tu and Kimbrough, 1973) was used to stain the fungal hyphae and also to observe the nuclear conditions.

Further, these *R. solani* isolates were designated into eight groups based on their similarity in colony colour, aerial mycelium, and production of sclerotia on Potato dextrose agar (PDA) medium. From each of these group one isolate was selected and thus eight isolates were used for detailed investigation on linear growth, cultural characters, relative virulence, etc. Linear growth, cultural and morphological characters of the selected eight isolates of *R. solani,* viz., RS1, RS2, RS3, RS4, RS5, RS6, RS7 and RS8 were studied on PDA. Flat bottomed assay Petri dishes (90-mm dia) containing 15 ml of medium were used for the study. Mycelial disk (5-mm dia) cut from the margin of actively growing 5-day-old cultures was used for inoculation. Linear growth of the fungus was recorded at 2h interval up to 54 h of incubation. The cultural characters such as colony colour, type of aerial mycelium, sclerotial production, etc. were recorded after 12 days of growth. The development of sclerotia was rated according to the relative abundance and density of sclerotia on the agar medium as 0, (absent), 1 poor (widely scattered), 2 numerous (moderate), 3 good (abundant), 4 excellent (closely aggregated).

Table 2. List of forest nurseries in Kerala surveyed during 1997 to 1999

Locality	Forest Range	Species raised
Kulathupuzha – Central nursery	Kulathupuzha	*Tectona grandis, Eucalyptus grandis, E. tereticornis, Acacia mangium, A. auriculiformis*
Valluvassery – Central nursery	Nilambur	*T. grandis, E. grandis, E. tereticornis, Acacia mangium, A. auriculiformis*
Cheruvanchery- Central nursery	Kannavam	*T. grandis, E. tereticornis*
Nilambur	KFRISub-Centre, Nilambur	*Dalbergia latifolia, Bixa orientalis, Pterocarpus marsupium, Swietenia macrophylla, A. mangium*
Thalapuzha	Mananthavady	*Gmelina arborea, Swietenia macrophylla, Artocarpus hirsutus, Lagerstroemia speciosa*
Periya	Mananthavady	*Melia dubia, Acrocarpus fraxinifolius*
Varayal	Manathavady	*Bambusa bambos*
Pulpally	Sulthan Battery	*Cassia fistula, Dalbergia latifolia*
Choodal	Thenmala	*T. grandis, Bombax ceiba*
Rajampara	Ranni	*P. marsupium, T. grandis, D. latifolia*
Kanamana	Ranni	*G.arborea, Hardwickia binata, Acrocarpus fraxinifolius, Pongamia pinnata, Azadirachta indica, Ailanthes triphysa*
Punalur	Punalur	*S. macrophylla, E. tereticornis*
Karulai	Nilambur	*Tectona grandis, E. tereticornis*
Arippa	Kulathupuzha	*E. tereticornis, G. arborea, Paraserianthes falcataria*
Peechi	KFRI Campus	*E. tereticornis, E. grandis, Casuarina equisetifolia*
Chandanathodu	Mananthavady	*E. tereticornis, E. grandis*
Thetroad	Begur	*E. grandis*
Nellikutha	Nilambur	*E. tereticornis*
Pattikkad	Trichur	*T. grandis, S. macrophylla*

Table 3. Details on tester isolates of *Rhizoctonia solani* used in AG determination

AG & ISG*	Isolate	Year of isolation
AG 1- IA	CS-Ka Hokkaido Univ.	1961
AG1-IB	B-19 Hokkaido Univ.	1954
AG 1-IC	BV7 Natl. Inst. Agri. Sci.	1961
AG 2-1	PS-4 Hokkaido Univ.	1973
AG 2-2 III B	C-96 Oniki	1972
AG 2-2 IV	RI-64 Ibraki Agr. Exp. Statn.	1960
AG- 3	ST-11-6 Hokkaido Univ.	1981
AG- 4 HG I	AHI- Hokkaido Univ.	1968
AG- 5	GM-10 Hokkaido Univ.	1970
AG- 6 HG I	OHT1-1 Kuninaga	1970
AG -7	HO- 1556 Homma	1979
AG- BI	TS-2-4 Kuninaga	1977

*AG: Anastomosis Group; ISG: Intraspecific Group

2.3.2. Cylindrocladium quinqueseptatum Boedijn & Reitsma

Of the twenty-three isolates of *Cylindrocladium* species retrieved from the damped-off seedlings of *Eucalyptus tereticornis* and *E. grandis* collected from various nurseries, eight isolates of *Cylindrocladium quinqueseptatum*, viz., CY1, CY5, CY6, CY7, CY8, CY10, CY12, and CY15 were selected for detailed study. For each isolate, three replicates of flat bottom assay Petri dishes containing 15 ml of medium were kept. In the center of each dish, a mycelial disk (5 mm dia) cut from the margin of 7-day-old culture of *C. quinqueseptatum* was inoculated and incubated at 25 ± 2^0 C. From each replicate dish, linear growth of the fungus was recorded on 4^{th}, 8^{th}, and 12^{th} day of incubation. Cultural characters such as colony colour, aerial mycelium, sporulation and mycrosclerotial production, etc. were recorded after 12 days of incubation. Five random observations on average number of conidial heads per microscopic field (10 x) were taken under a Stereobinocular microscope for scoring sporulation, and numerical rating was done as: 0, conidial heads absent, 1, poor (1-20 heads), 2, moderate (21-50); abundant (51-90), profuse (>90). The development of microsclerotia was rated as in the case of *R. solani.*

2.3.3. **Pythium myriotylum** Drechsler

Pythium isolates obtained from the damped-off seedlings of different forestry species and also retrieved from the sick soil collected from nurseries were plated on Oat meal agar (OMA) medium and their cultural and morphological characteristics were studied. Three isolates of *Pythium myriotylum* (PM1, PM2, and PM3) and an unidentified isolate of *Pythium* sp. (PS) were selected for detailed study.

2.3.4. **Fusarium oxysporum** Schlecht.

Fusarium isolates obtained from the damped-off seedlings of different forestry species and also retrieved from the sick soil collected from nurseries were plated on Potato sucrose agar (PSA) medium and their cultural and morphological characteristics were studied. Four isolates of *Fusarium oxysporum,* viz., FO1, FO2, FO3, FO4 were selected for further investigation.

2.4. Isolation of Antagonistic Fungi from Forest Nursery Soil

Soil samples collected from different nurseries were air-dried, compacted and used for isolation of antagonistic fungi. Glucose ammonium nitrate agar (GANA) and Rose bengal agar (RBA) media were used and soil plate technique (Warcup, 1950), and soil dilution plate techniques were employed. The isolates of *Trichoderma* spp. and *Gliocladium* spp. were purified, cultural and morphological characters studied and identified up to species level. All isolates of *Trichoderma* spp. and *Gliocladium* spp. were sub-cultured and maintained on PDA medium.

2.5. Procurement and Collection of Antagonistic Organisms from Other Sources

Antagonistic fungi, viz., *Trichoderma harzianum* Rifai, *T. viride* Pers. ex Grey, *Gliocladium virens* Miller *et.al.*, and a fluorescent pseudomonad, *Pseudomonas fluorescens* were procured from Biocontrol Laboratory, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Isolates of *T. harzianum, T. viride, T. koningii*, *Gliocladium virens,* were also collected from Biological Control Laboratory, Indian Institute of Spices Research, Calicut, Kerala. All the procured isolates of antagonistic fungi were maintained on PDA and the fluorescent pseudomonad on Nutrient agar medium.

2.6. Screening of the Efficacy of Antagonistic Fungi

The antagonistic fungal isolates cultured from native forest soil as well as procured and collected from other sources were screened for their antagonistic efficacy against selected isolates of *Rhizoctonia solani, Cylindrocladium quinqueseptatum*, *Fusarium oxysporum*, *Pythium myriotylum* and *Pythium* sp. Petri dish dual culture method, Slide culture technique and Seedling bioassay were employed for the efficacy screening.

2.6.1. Slide Culture Technique

Mycelial disks (5mm dia) cut from the margin of actively growing 4-day-old cultures of *Trichoderma harzianum, T. viride, T. koningii, Gliocladium virens* and those of *R. solani*, *F. oxysporum*, *C. quinqueseptatum*, *Pythium myriotylum* and *Pythium* sp. were placed four centimeter apart on the sterilized microscopic slide coated with 1.5% water agar. The inoculated slides were placed over a U shaped glass tube kept in the Petri dish (90 mm dia) having moistened sterile blotter disks. The set ups were incubated at 24 ± 2° C for 2 to 4 days. The process of antagonism, parasitism, lysis, coiling, cell degradation, etc. were observed under Leitz Dialux 20 microscope. Per cent hyphal interaction (PHI) was calculated by following formula:

$$PHI = \frac{A \times 100}{B}$$

Where, A is the sum of parasitized pathogen hyphae in 15 microscopic field (40 x 10x) and B is the sum of pathogen (*R. solani, Fusarium oxysporum, C. quinqueseptatum* and *Pythium myriotylum, Pythium* sp.), hyphae in 15 microscopic field. The most efficient antagonists (mycoparasites) among *T. harzianum, T. viride, T. koningii* and *Gliocladium virens* were selected on the basis of their respective PHI. Thirteen isolates of *T. harzianum,* five isolates

of *T.viride*, one isolate each of *T. koningii* and *G. virens* (Table 4) were screened by this method. Eight isolates of *R. solani* belonging to different anastomosis groups and showing different cultural and morphological characteristics, eight isolates of *C. quinqueseptatum* and four isolates each of *Fusarium* and *Pythium* species were used as test pathogens.

Table 4. Isolates of *Trichoderma* and *Gliocladium* species employed for screening

Sl. No.	Isolate	Isolate No.	Source
1.	*Trichoderma harzianum*	TH1	Nursery soil, Periya, Wayanad
2.	*T. harzianum*	TH2	Nursery soil, Pattikkad, Trichur
3.	*T. harzianum*	TH3	Nursery soil, Varayal, Wayanad
4.	*T. harzianum*	TH4	Nursery soil, Arippa, Trivandrum
5.	*T. harzianum*	TH5	Nursery soil, Trichur
6.	*T. harzianum*	TH6	T N A U, Coimbatore
7.	*T. harzianum*	TH7	Nursery soil, KFRI Campus
8.	*T. harzianum*	TH8	"
9.	*T. harzianum*	TH11	"
10.	*T. harzianum*	TH12	Nursery soil, Ranni
11.	*T. harzianum*	TH13	Nursery soil, Nilambur
12.	*T. harzianum*	TH17	"
13.	*T. harzianum*	TH18	Nursery soil, Begur, Wayanad
14.	*T. viride*	TV1	Nursery soil, Choodal, Thenmala
15.	*T. viride*	TV2	T N A U, Coimbatore
16.	*T. viride*	TV3	Nursery soil,Nilambur
17.	*T. viride*	TV4	"
18.	*T. viride*	TV5	Nursery soil, Valluvassery, Nilambur
19.	*T. koningii*	TK	IISR, Calicut
20.	*Gliocladium virens*	GV	IISR, Calicut

2.6.2. Petri Dish Bioassay

Antagonistic ability of all the isolates of *Trichoderma harzianum, T. viride, T. koningii, Gliocladium virens* was tested in Petri dish dual culture method. Selected isolates of *R. solani* (RS1 to RS8), *C. quinqueseptatum* (CY1, CY5, CY6, CY7,CY8,CY10, CY12, CY15), *F. oxysporum* (FO1 to FO4), *Pythium myriotylum* (PM1 to PM3) and *Pythium* sp. (PS) were used as test isolates. Disks (5 mm dia) taken from actively growing 5-day-old cultures

of antagonist was placed at one side of the sterile PDA medium in 90 mm Petri dish and at the opposite side with the test isolates of pathogens. In one Petri dish 2 to 4 disks of antagonist and pathogen were kept, incubated for 7-10 days at 24 ± 2°C and observations recorded on antagonism, predation, hyperparasitism, lysis of hyphae, etc. Isolates of *R. solani* and *C. quinqueseptatum* were placed on agar plates 24 h and 48 h respectively before seeding *Trichoderma* and *Gliocladium* mycelial disks. *Fusarium oxysporum* and *Pythium* isolates were placed simultaneously with *Trichoderma* and *Gliocladium* mycelial disks.

2.6.3. Mass Culturing of Antagonists and Test Isolates of Pathogens and Inoculum Production

Isolates of *T. harzianum* (TH3, TH5, TH6) and *T. viride* (TV4, TV5), selected on the basis of their antagonistic efficiency in Petri dish and Slide bioassays against isolates of *R. solani, C. quinqueseptatum, F. oxysporum, P. myriotylum* and *Pythium* sp. were mass cultured on tapioca rinder substrate (Kausalya Gangadharan and Jayarajan, 1988), maize meal-perlite medium and maize meal-sand medium. Powdered tapioca rinder (220g) was mixed with 750 ml water, filled in polypropylene bags at the rate of 200 g per bag (30 x 20 cm) and sealed by flaming. Perlite (1 kg), steam sterilized for 2 h was added to powdered maize (1 kg), sprinkled with water (500 ml) and steamed in open pan for 30 min. The steamed mixture was thoroughly mixed and filled in polypropylene bags at the rate of 200 g/ polypropylene bag (30 x 20 cm), moisture content was adjusted to 50 % and sealed. The medium was sterilized in autoclave (1.4 kg/cm^2) for 1 h on two consecutive days. Maize sand medium was also prepared in similar way. Ten millimeter disks cut from 4-day-old actively growing cultures of *Trichoderma harzianum* and *T. viride* were transferred in the medium separately, bags were resealed and incubated for 20 days.

Inocula of *R. solani, F. oxysporum, C. quinqueseptatum and P. myriotylum* were prepared separately in maize meal-sand medium (powdered maize 1000 g; washed sand, 1000 g, water 750 ml) in polypropylene bags (30 x 20 cm). The media inoculated with disks cut from actively growing cultures of *R. solani, C. quinqueseptatum, F. oxysporum* and *P. myriotylum* separately were incubated for 18 to 20 days, air dried and powdered using Remi Automixer.

2.6.4. Seedling Bioassay

Soil Amendment with *Trichoderma* species: Inocula of *R. solani, C. quinqueseptatum, F. oxysporum* and *P. myriotylum* prepared in maize meal-sand medium were added to sterile forest soil (200 g) at the rate 1:100 (w/w) in plastic dishes (180 mm dia), separately and mixed thoroughly, watered, covered with lid and incubated for 15 days. In the infested soil, isolates of *Trichoderma harzianum* (TH3, TH5, TH6) and *T. viride* (TV4, TV5), raised on maize-sand medium were added separately at the rate of 10 g per dish. The treated soil was mixed thoroughly and incubated for 8 days. *Eucalyptus tereticornis* seeds having 95% germinability were sown at the rate of 1 g per dish. A thin layer of sterile soil was put to cover the seeds. All the treatments were replicated thrice. Controls were maintained without adding the antagonists in the soil infested with the test pathogen. The whole set ups were kept in incubation chamber fitted with fluorescent lights. Observations on per cent seedling emergence, incidence of disease and spread in various treatments were recorded for 20 days.

Seed Treatment with *Trichoderma* spp. and *Pseudomonas fluorescens*: *Trichoderma harzianum* (TH3) and *T. viride* (TV5) cultures were raised on PDA medium and incubated for 12 days. Sterile distilled water (10 ml) was added to each Petri dish and conidia dislodged with a sterile brush and fungal conidial suspension prepared, passed through cheese cloth, centrifuged at 2500 g for 10 min. and re-suspended in sterile water (Ahmad and Baker, 1987). The density of the conidia was adjusted to 10 x 10^6 / ml with the aid of Haemocytometer. To 15 ml of conidial suspension 15 ml of 2% carboxy methyl cellulose (CMC) was added to get final spore load of 5 x10^6/ml. This conidial suspension was used for soaking 250 g of *E. tereticornis* seeds, mixed thoroughly by shaking vigorously and then air-dried (30 min).

Pseudomonas fluorescens culture grown in nutrient agar broth for 10 days was used for preparing the spore suspension of 2 x 10^7 cfu /ml. Seeds of *E. tereticornis* were soaked in bacterial suspension for 5 min. and air dried. The treated seeds were sown at the rate of 1 g per plastic dish (180 mm dia) in soil infested with *R. solani, C. quinqueseptatum, F. oxysporum and P. myriotylum* (1:100) separately. All the treatments were replicated thrice.

Control sets without antagonistic organisms were also maintained. Observations on per cent seedling emergence, disease incidence, spread, etc. were recorded for 20 days.

2.7. Selection of Antagonistic Organisms for Nursery Trials and Mass Production of Inoculum

The best performed isolates of *T. harzianum* (TH3) and *T. viride* (TV5) in *in vitro* and seedling bioassays were selected and mass cultured on maize meal-perlite medium / tapioca rinder substrate medium and maintained for nursery trials.

2.8. Shelf-life of Antagonistic Fungal Preparations

Efficient isolates of *T. harzianum* (TH3) and *T. viride* (TV5), were mass cultured on tapioca rinder substrate and maize-meal-perlite media and maintained for 4 months in laboratory under different temperature regimes (5 ^{0}C, 10 ^{0} C, 25 ± 2^{0}C). Samples were taken out periodically and tested the efficacy of the antagonists employing seedling bioassay (*E. tereticornis)*.

2.9. Ectomycorrhizal application in rootrainers

Fructifications of ectomycorrhizal (EM) fungus, *Pisolithus tinctorius* were collected from eucalypt plantations in Trichur Forest Division and processed and inoculum prepared for studying their role in seedling disease control. Fungal spore suspension (slurry) and sodium alginate pellets of mycelial bits were prepared and inoculated in rootrainers and trays. *E. tereticornis* seeds were sown in EM fungus treated rootrainers and trays and observations recorded on disease incidence.

2.10. Nursery Trial 1998

An experimental nursery was raised at Chandhanathodu, Periya (Wayanad Forest Division). The area is 800 m a.m.s.l. and receives an annual rainfall of 5000 mm or more. The area

records a very high relative humidity through out the year with mean minimum and maximum daily temperatures of 13^0 C and 31^0C, respectively. The nursery soil is slightly acidic (pH 6.7). The soil of the selected area was thoroughly worked and 60 experimental seedbeds of 2 x 1 x 0.3 m were prepared at a spacement of 60 cm.

2.10.1. Soil Solarization

Soil solarization was carried out by covering the raised seedbeds with thin polythene sheets. Twelve nursery beds were selected randomly and watered profusely to make the soil moistened. Then each bed was 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), and soil temperatures were recorded during 08.00 to 17.00 h at 2 h interval. The set ups were maintained and observations on soil temperature recorded for 15 days. The polythene sheets were removed after 15 days of solarization treatment.

2.10.2. Application of Biocontrol Agents

Seed Treatment: *Pseudomonas fluorescens* cultures raised in Nutrient agar broth (250 ml) for 15 days was used for seed coating by adjusting the bacterial suspension to 2 x 10^7 cfu /ml. For seed coating, 120 g each of *Eucalyptus grandis* and *E. tereticornis* seeds were used.

Soil Amendment: Isolates of *Trichoderma harzianum* (TH3) and *T. viride* (TV5), raised on tapioca rinder substrate medium was applied in the nursery beds separately at the rate of 120 g/m^2. The inoculum was mixed thoroughly with the top layer of soil (5 cm depth). The treated beds were watered regularly (30 l/day) and seeds of *Eucalyptus grandis* and *E. tereticornis* were sown at the rate of 20 g/m^2 after seven days of antagonistic fungal treatment.

Table 5: Details of various treatments during 1998 trials

Sl. No.	Treatment code	Treatment	Species EG	ET*
			Replication	
1.	PF	*Pseudomonas fluorescens* seed coating	3	3
2.	TH	*Trichoderma harzianum* soil amendment	3	3
3.	TV	*Trichoderma viride* soil amendment	3	3
4.	SS	Soil solarization	3	3
5.	SHTHTVPF	Combination of 4 treatments	3	3
6.	THTVPF	Combination of 3 treatments	3	3
7.	C	Control	3	3

*EG: *Eucalyptus grandis*, ET: *Eucalyptus tereticornis*

2.10.3. Evaluation of Microbial Population and Persistence of Introduced Antagonists in the Nursery Beds

Soil samples from the nursery beds were collected before and after various treatments, viz., soil solarization, soil amendment and seed coating with antagonists, etc. and transported to the laboratory for isolation and analysis of fungal flora, including pathogen inoculum and also persistence of the introduced antagonistic organisms. Soil dilution plate technique was followed and 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.

2.10.4. Other Forestry Species Raised

Important forestry species, viz., *Tectona grandis, Gmelina arborea, Bombax ceiba, Acacia nilotica, Albizia lebbeck, Terminalia arjuna, Pongamia pinnata, Hardwickia binata,* and *Leucaena leucocephala* were also raised in the nursery beds to study the incidence and spread of disease. Seeds of the above species were sown after appropriate seed pre-treatments (dormancy breaking). Three beds for *Tectona grandis* and *Gmelina arborea* and two beds each for the other species were maintained.

2.11. Nursery Trial 1999

Experimental nursery was raised at Chandhanathodu, (Wayanad Forest Division) during 1999 at the same site where the nursery trial was conducted in 1998. The soil was thoroughly worked and 54 seedbeds of 2 x 1 x 0.3 m were prepared at a spacement of 60 cm. To regulate the shade over the nursery beds shade nets (75%) was provided in the nursery. Six beds were selected at random and soil solarization was carried out as done in the previous year. *Eucalyptus tereticornis* seeds were sown in all the biocontrol seedbeds. Ten other forestry species were also raised in the experimental nursery for studying the disease incidence, pathogen(s) associated, and severity (Table 6).

Table 6. Forestry species other than eucalypts raised in the nursery during 1999

Sl.No.	Species	No. of beds
1.	*Ailanthes excelsa*	3
2.	*Acacia nilotica*	3
3.	*Paraserianthes falcataria*	3
4.	*Dalbergia sissoides*	3
5.	*Eucalyptus grandis*	3
6.	*Leucaena leucocephala*	3
7.	*Pongamia pinnata*	3
8.	*Swietenia macrophylla*	3
9.	*Terminalia arjuna*	3
10.	*Tectona grandis*	3

2.11.1. Biocontrol Treatments in Nursery

The treatments of biocontrol agents include: seed coating with *Pseudomonas fluorescens* (PF), *Trichoderma harzianum* (TH3), *T. viride* (TV5), soil amendment with *Trichoderma harzianum* (TH3) and *T. viride* (TV5), soil solarization (SS), and a combination of soil solarization and seed coating with *Trichoderma harzianum* (TH3). Control beds without any treatment were kept. *Eucalyptus tereticornis* (ET) seeds (20 g /m^2) were sown. For each treatment three replications were kept (Table 7).

Table 7: Details on various biocontrol treatments during 1999 trials

Sl.No.	Treatment code	Treatment	Replication
1.	PFSC	*Pseudomonas fluorescens* seed coating	3
2.	THSC	*Trichoderma harzianum* seed coating	3
3.	TVSC	*Trichoderma viride* seed coating	3
4.	THSA	*Trichoderma harzianum* soil amendment	3
5.	TVSA	*Trichoderma viride* soil amendment	3
6.	SS	Soil solarization	3
7.	SS+THSC	Combination of 2 treatments	3
8.	C	Control	3

2.12. Recording Observations on Disease incidence and Severity

Water regimes in the seedbeds were regulated at the rate of 30 litre per day. Shade was regulated by providing shadenets (75%) over the nursery beds. Seed germination, disease incidence in each seedbed, etc. were recorded daily. Each damping-off patch was marked by aluminium tags or coloured splinters. Number of infection foci its spread, number of seedlings affected, etc. were recorded from each treatment daily. Disease incidence in other forestry species was recorded and disease specimens collected and isolation of causal organisms made.

2.13. Meteorological Data

Data on rainfall and ambient temperature for the year 1998 and 1999 were gathered from a weather station (Navarathna Estate) near the experimental nursery. Wet and dry bulb temperatures in the nursery were recorded daily at 08.00 h, 12.00 h, and 16.00 h and respective relative humidity (r.h.) was deduced. Daily maximum and minimum temperatures in the nursery were also recorded.

2.14. Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1. Disease Monitoring in Forest Nurseries

Disease monitoring in conventional and rootrainer nurseries at various localities in the State revealed a large number of diseases affecting the seedlings of most of the forestry species raised in both conventional and rootrainer nurseries. The important seedling diseases encountered in conventional nurseries are damping-off, collar rot, wilt, stem infection, web blight, seedling blight, and foliage infection. Incidence and severity of each disease depends on the host species, microclimatic conditions in the nursery as well as nursery management practices followed. Damping-off caused mortality of seedlings during the first two to three weeks after germination. Pre-emergence damping-off was difficult to diagnose because the affected seeds were not visible. The classic symptoms of post-emergence damping-off is decay of hypocotyl at the ground level, but the point where infection starts is non-specific. In many cases there was no clear separation between post-emergence damping-off and root rot on young seedlings. Indeed, the same fungal species invading succulent seedling tissue can cause both damping-off and root rot. Many fungi were found associated with damping-off disease and most of them are not host-specific, except *Cylindrocladium* spp. which show host specificity to eucalypts and acacias. *Rhizoctonia solani* Kuhn, *Pythium myriotylum* Drechsler, *Fusarium oxysporum* Schlecht., *Cylindrocladium quinqueseptatum* Boedijn & Reitsma were the most prevalent damping-off fungi in the seedbed nurseries raised throughout the State. Of these *R. solani* was found associated with the damping-off of most forestry species. The survey recorded a severe incidence of damping-off in *E. tereticornis* nursery at Nellikkutha (Nilambur Forest Division) and in *E. grandis* nursery at Thetroad, Begur Range (Wayanad Forest Division) during 1998 and 1999, respectively, which resulted in complete failure of the nurseries and necessitated re-sowing the beds.

Web blight was the other major disease recorded in bareroot nurseries. The disease caused by *Rhizoctonia solani* was severe in *Hardwickia binata* Roxb., *Paraserianthes falcataria* (L.) Nielson, *Eucalyptus tereticornis* Sm., *E. grandis* Hills ex Maiden, *Bambusa bambos* (L.) Voss, *Acacia auriculiformis* A. Cunn., *A. mangium* Willd., *A. nilotica* (L.) Willd. Ex Del.,

Luecaena leucocephala (Lam.) De Wit., *Casuarina equisetifolia* Forst., *Albizia procera* Benth., and *Albizia lebbeck* Benth. Seedling blight caused by *Cylindrocladium quinqueseptatum, C. ilicicola* (Hawley) Boedijn & Reitsma, and *C. clavatum* Hodges & May was also encountered in all the eucalypts nurseries raised in the State. *Sclerotium rolfsii* causing seedling wilt was observed in *Swietenia macrophylla* King, *Pterocarpus santalinus* L.f., *Dalbergia latifolia* Roxb., *Eucalyptus tereticornis* and *E. grandis* nurseries. Collar rot caused by *R. solani* was also prevalent in most of the conventional nurseries and was severe in *Ailanthes triphysa* (Dennst.) Alston, *Tectona grandis* L., *Bombax ceiba* L., and *Terminalia arjuna* (Roxb. Ex DC.) Wt. & Arn. nurseries. Foliage infection caused by *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Walker) Boed., *Alternaria alternata* (Fr.) Kiessler, *Exserohilum rostratum* (Drech.) Leonard & Suggs, *Phomopsis* spp., *Bipolaris* spp., and *Guignardia* sp. recorded in most of the forestry species and disease severity ranged from low to severe (Table 8). Leaf rust was severe in *Tectona grandis* (caused by *Olivea tectonae* (T.S. & K. Ramakr.) Mulder, *Dalbergia latifolia* (caused by *Uredo sissoo* Syd. & Butler), *Bambusa bambos* (caused by *Dasturella divina* (Syd.) Mundk. & Khes.) and *Bombax ceiba* (caused by *Uredo bombacis* Petch.). Leaf mosaic caused possibly a virus and leaf littling caused by phytoplasma were also observed in eucalypts, casuarina and acacia bareroot seedlings. Bacterial wilt incited by *Ralstonia solanacearum* Yabuuchi *et al.* (= *Burkholderia solanacearum* (Smith) Yabuuchi) (Yabuuchi *et al.*, 1998) was severe in teak nurseries raised in Trivandrum, Konni, Ranni, Punalur, Trichur, Nilambur and Wayanad Forest Divisions of the State. Disease was also observed in *Eucalyptus tereticornis* nurseries in Punalur Forest Division. In general, incidence and severity of diseases in conventional nurseries raised throughout the State was severe during 1998 than 1999. In all the rotrainer nurseries surveyed, comparatively a low disease incidence was recorded. Damping-off was observed in *Tectona grandis, Eucalyptus grandis, D. latifolia, C. equisetifolia*, and *E. tereticornis* and the incidence and severity of disease was very low (Table 8). Other seedling diseases were seldom observed in rootrainer seedlings, except mild foliage infection in eucalypts and teak seedlings.

Conventional nurseries raised in the State are subjected to disease hazards due to various factors. Build-up of pathogen inoculum potential in nurseries due to raising of nurseries year

after year in the same sites is one of the factors leading to the severe incidence and spread of the disease. Build-up of pesticide resistance and emergence of new strains of pathogens is another factor for the incidence and often outbreak of diseases to an epidemic proportion. High soil water regimes and high seedling density together with poor nursery management practices are the other possible factors for the disease havoc. The strategies followed to combat the diseases in conventional nurseries at different localities are different, however, chemical measures are always adopted for protecting the seedlings from diseases and for maintaining the seedling health. The introduction of rootrainer technology in forest nurseries has had a tremendous impact on production and protection of forestry planting stock. In rootrainer nurseries no major disease, except very low incidence of damping-off caused by *Rhizoctonia solani* and *Pythium* sp. and bacterial wilt caused by *Ralstonia solanacearum,* possibly contaminated through seeds or potting medium was observed which shows that the new technology has major impact on seedling disease management. The technology offers tremendous scope for improving the forestry seedling production system as well as minimizing the disease pressure over the forest nurseries (Mohanan, 2000). On the other hand, the conventional seedbed and container nurseries which supply seedlings for large-scale planting programmes in the State, still suffer from threat of damping-off disease. Association of different pathogens with damping-off disease, viz., *R. solani, Cylindrocladium* spp., *Fusarium* spp., *Pythium* spp., and existence of virulent strains of pathogens also pose practical problems in nursery disease management.

Species of *Pythium, Rhizoctonia, Fusarium* and *Phytophthora* cause damping-off throughout the world (Vaartaja and Cram, 1956; Vaartaja, 1967; Bakshi *et al.*, 1972; Perrin and Sampagni, 1986; Huang and Kuhlman, 1990; Sutherland and Davis, 1991; Sharma and Mohanan, 1991; Arja *et al.*, 1999; Mohanan, 2000b). Damping-off fungi can survive in soil or seeds as mycelia, sclerotia, or spores. They can be transmitted through air, water and soil and the inoculum becomes active and pathogenic when the environmental conditions such as temperature, relative humidity, soil moisture content, soil pH become favourable (Vaartaja, 1952). Disease management can be achieved through cultural, chemical or biological measures.

Table 8. Incidence and severity of diseases in conventional and rootrainer nurseries

Tree species	Disease	Incidence & severity	
		Rootrainer	Conventional
Tectona grandis	Damping-off	0-L	L
	Cotyledon rot	L	0
	Collar rot	0-L	L-M
	Bacterial rot	L-M	L
	Foliage infection	0-L	M-S
Eucalyptus spp.	Damping-off	0-L	L-S
	Web blight	0	M-S
	Seedling blight	0	M-S
	Wilt	0	L-S
	Collar rot & stem infection	0-L	M-S
	Foliage infection	0-L	L-S
Paraserianthes falcataria	Damping-off	0	L-M
	Web blight	0	M-S
	Foliage infection	0-L	L
Gmelina arborea	Damping-off	0	L
	Wilt	0	L
	Foliage infection	0-L	L-M
Swietenia macrophylla	Damping-off	0	L
	Seedling wilt	0	L-M
	Foliage infection	0-L	L
Ailanthus triphysa	Damping-off	0	M-S
	Collar rot	0	M-S
	Foliage infection	0-L	L
Acacia mangium	Damping-off	0	L
	Collar rot	0-L	L-M
	Foliage infection	L	L-M
Acacia auriculiformis	Damping-off	0	L
	Web blight	0	L-M
	Foliage infection	0-L	L-M
Terminalia arjuna	Damping-off	0	L
	Collar rot	0	L-M
Pterocarpus santalinus	Damping-off	0	L
	Seedling wilt	0	L-M
	Foliage infection	0-L	L-M
Dalbergia latifolia	Damping-off	L	L
	Collar rot	0	L
	Foliage infection	0	L-M
Casuarina equisetifolia	Damping-off	L	L
	Collar rot- stem infection	0	L-M
	Web blight	0	L-M

3.2. Damping-off Organisms - Isolation and Identification

Isolation of damping-off pathogens from damped-off specimens and sick nursery soil collected from different parts of the State yielded a large number of fungi belonging to different genera. These include: 31 isolates of *Rhizoctonia solani,* 19 isolates of *Cylindrocladium quinqueseptatum,* 2 isolates of *C. parvum* Anderson, 1 isolate each of *C. camelliae* Venkataramani & Venkataram and *C. ilicicola,* 3 isolates of *Pythium myriotylum*, 1 isolate of *Pythium* sp. (unidentified), 9 isolates of *Fusarium oxysporum*, and 1 isolate of *F. solani* (Mart.) Sacc. All the pathogens isolated were earlier recorded as associated with damping-off disease of various forestry species in Kerala (Sharma *et al.*, 1985; Sharma and Mohanan, 1991; Mohanan, 2000a,b). Of these, *Cylindrocladium* species were found associated with the damping-off of *Eucalyptus tereticornis, E. grandis* and *Acacia mangium*, while all the other pathogens do not show any host specificity and recorded on most forestry species raised in the nurseries in the State. Among the damping-off pathogens isolated, *R. solani* was found associated with all the forestry species raised both in conventional as well as rootrainer nurseries.

3.3. Characterization of Damping-off Pathogens

3.3.1. *Rhizoctonia solani* isolates

Thirty one isolates of *Rhizoctonia solani* obtained from damped-off seedlings of different forestry species showed difference in cultural and morphological characteristics. These isolates were screened for Anastomosis Grouping (AG). Anastomosis occurred between hyphal tips, hyphal tip and side branches and also between side branches. Anastomosis appeared to follow random cell contact but sometimes this was the result of attraction between hyphae which eventually anastomosed. Usually, three types of hyphal fusion were observed; perfect fusion, imperfect fusion, and contact fusion. Anastomosis Group was assigned to an isolate, when the paired isolate showed imperfect fusion with the authentic AG tester isolates. The imperfect fusion between the hyphae resulted in granulation of the cytoplasm of the fused cell, vacuolation and collapse of the cytoplasm, resulting in killing of

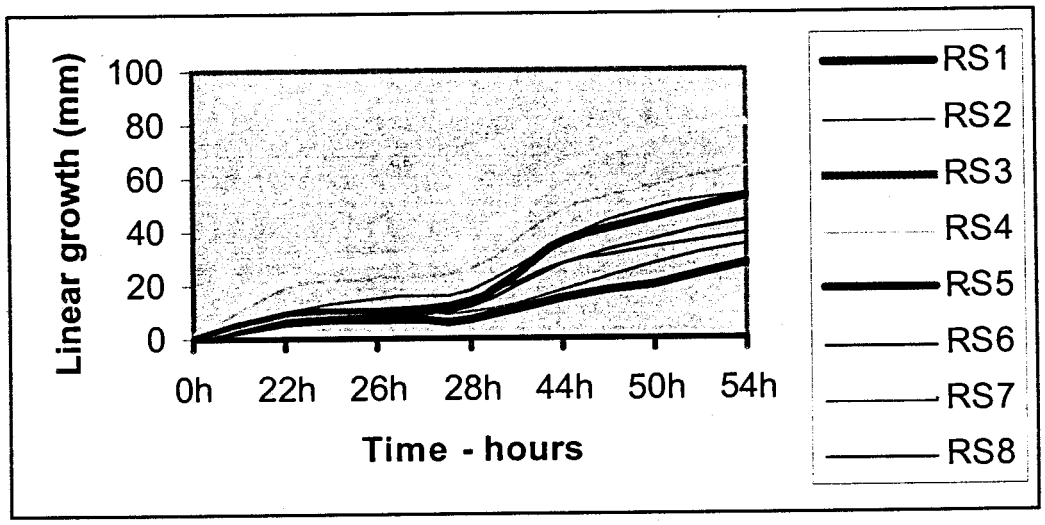
the fused cells and adjacent cells of the paired isolates. Among the 31 isolates of *R. solani* tested, 14 belonged to AG1-IA, 9 isolates AG1-IC, 3 isolates AG2-1 and 2 isolates AG2-2IV. Three isolates did not anastomose with any of the available tester isolates. Anastomosis Groups, AG1-IA and AG1-IC were the predominant groups in the forest nurseries and found distributed widely in the State. The isolates from the same nursery belonged to different Anastomosis Groups or strains. All the *R. solani* isolates were multinucleated and Aniline blue staining showed deep blue coloured nuclei in contrast to the light blue cytoplasm.

Rhizoctonia solani, mycelial state of *Thanatephorus cucumeris* (Frank) Donk is considered to be made up of divergent group of populations (Parmeter and Whitney, 1970; Adams, 1990; Liu and Sinclair, 1991). The lack of understanding of the relationship among populations has often hampered the studies on the fungus as well as disease management in nurseries. In nature *R. solani* occurs as an aggregate of strains that differ in cultural characteristics, Anastomosis Grouping, physiology and virulence (Sherwood, 1970; Parmeter *et. al.*, 1969). Even though, Anastomosis Grouping is considered to be the most meaningful and currently accepted form of grouping of *R. solani* (Anderson, 1982; Sneh *et al.*, 1991), in the present study, *R. solani* isolates belonging to the same Anastomosis Group also showed considerable variation in cultural and morphological characteristics. Earlier, possibility of occurrence of two biotypes in *R. solani* was reported by Mehrotra (1990). Occurrence of *R. solani* belonging to AG1-IA, AG1-IC, and AG2-2IV in forest nurseries in Kerala was earlier reported by Mohanan (1995). As 31 isolates of *R. solani* belonged to four different Anastomosis Groups and also isolates within the same AG differ considerably in cultural and morphological characteristics, a total of eight isolates belonging to the same or different Anastomosis Groups, were selected for further studies.

All the eight isolates of *R. solani* showed different growth characteristics on PDA medium. Isolate RS3 (AG1-IA) was the fastest among the test isolates which attained 81 mm growth within 54 h of incubation. RS1 (AG2-2IV) was the slow growing strain among the isolates tested (Fig.1). *R. solani* isolates showed considerable variation in one or other cultural characteristics, such as mycelial growth, colony colour, sclerotial production, size, etc. Colony growth varied from submerged to cottony growth and colony colour ranged from

pale yellow to dark brown. Sclerotial production and their relative abundance also varied among the isolates. Isolate RS3 produced abundant, large (1-5 mm dia) dark brown sclerotia, while isolates RS1 (AG1-IC), RS2 (AG1-IC), RS4 (AG1-IC) produced scanty small sclerotia (0.30-0.7 mm dia). Isolate RS6 (AG1-IA) produced dark brown large sclerotia mostly at the periphery of the colony. Isolate RS8 (AG1-IA) produced dark brown to black, large sclerotia (1-4 mm dia), usually aggregated at the center of the colony.

Figure 1. Linear growth of different *R. solani* isolates on PDA medium



3.3.2. *Cylindrocladium quinqueseptatum* isolates

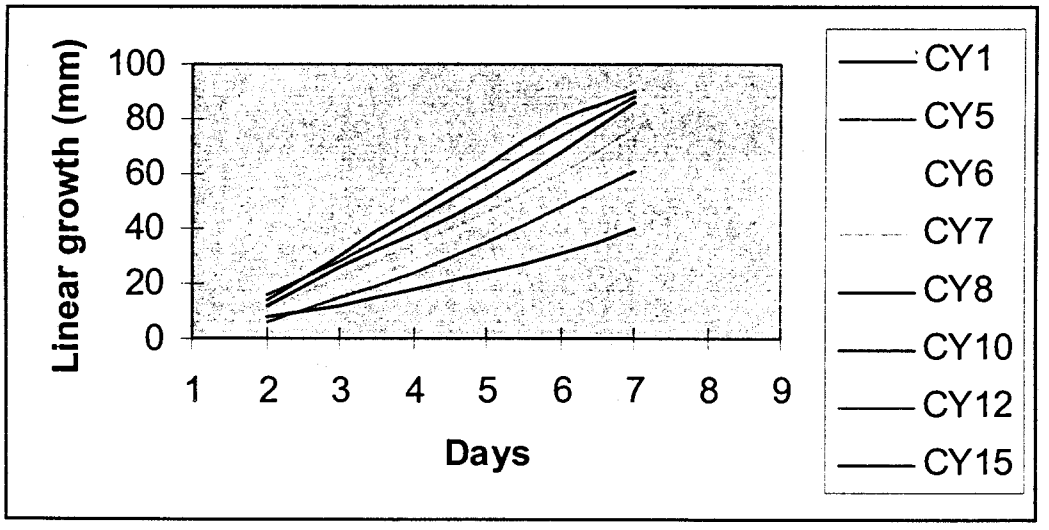
Among 19 isolates of *Cylindrocladium quinqueseptatum* obtained from the damped-off specimens, eight isolates showing distinct cultural and morphological characteristics were selected and further characterized on the basis of their growth on PDA, sporulation and microsclerotial production. Isolate CY7 showed maximum linear growth of 90 mm in 12 days of incubation and isolates CY12 and CY15 were comparatively slow growing (Table 9) (Fig.2). The slow growing isolates showed sparse sporulation, while microsclerotial production was abundant and profuse.

Table 9. Colony characteristics of *C. quinqueseptatum* isolates

Isolate No.	Linear growth(mm) Mean value for 12 days	Sporulation rating	Microsclerotia production
CY1	61.00	2	2*
CY5	75.50	3	3
CY6	77.60	2	1
CY7	90.00	4	3
CY8	86.4	3	2
CY10	88.00	2	2
CY12	39.80	1	3
CY15	36.80	1	4

* 0: absent;1: poor; 2: moderate; 3: abundant; 4: profuse

Figure 2. Linear growth of *C. quinqueseptatum* isolates on PDA medium



Cylindrocladium quinqueseptatum is the most important pathogen in *Eucalyptus* nurseries in the State, which causes various diseases including damping-off. Other species of little significance, as far as damping-off is concerned are: *Cylindrocladium ilicicola* (Hawley) Boedijn & Reitsma, *C. floridanum* Sobers & Seym., *C. parvum* Anderson, *C. camelliae* Venkataramani & Venkataram, *C.theae* (Petch) Alf. & Sob., and *C. scoparium* Morg. (Sharma and Mohanan, 1982; Mohanan and Sharma, 1985). Earlier studies confirmed the occurrence of wide range of pathogenic variability among the natural population of *C.*

quinqueseptatum and the existence of different physiological strains (Sharma and Mohanan, 1992). The present study also revealed considerable variation in cultural and morphological characteristics among the *C. quinqueseptatum* isolates. Hence, a large number of isolates of *C. quinqueseptatum* representing the natural population were selected for screening against the antagonistic organisms.

Four isolates of *Fusarium oxysporum*, three isolates of *Pythium myriotylum* and an unidentified *Pythium* isolate were purified and studied their cultural characteristic in Potato sucrose agar (PSA) and Oat meal agar media respectively. As *Fusarium* and *Pythium* isolates showed marked difference in their growth characteristics in the respective culture medium, all the isolates were selected for screening against antagonists.

3.4. Antagonistic Organisms – Isolation and Identification

A total of 49 isolates of *Trichoderma* species were recovered from nursery soil collected from different localities in the State. Of these, 32 isolates were identified as *T. harzianum* and 17 isolates as *T. viride*. Propagules of *Trichoderma* generally formed 3 to 5% of the total fungal propagules in most of the forest nursery soils. For screening against damping-off pathogens, 13 isolates of *T. harzianum* and 5 isolates of *T. viride* were selected on the basis of their cultural and morphological variations. *T. koningii, Gliocladium virens*, and *P. fluorescens* obtained from different biocontrol Laboratories were also employed for bioassays.

3.5. Antagonistic Organisms - Evaluation of Biocontrol Efficiency

3.5.1. Slide Bioassay

The antagonists, *Trichoderma harzianum, T. viride, T. koningii* and *Gliocladium virens* hyperparasitized all the isolates of *Rhizoctonia solani, Cylindrocladium quinqueseptatum, Fusarium oxysporum, Pythium myriotylum*, and *Pythium* sp. within 52 to 76 h after pairing on the glass slide. However, the level and nature of parasitism varied greatly among the antagonists depending on the pathogen isolates. Antagonistic activity of the different fungi

against the pathogen isolates was exhibited by hyphal coiling, penetration of the pathogen hyphae, vacuolation, granulation, lysis and disintegration of the affected hyphae. The slender hyphae of *Trichoderma* and *Gliocladium* species grew alongside the broad hyphae of *Rhizoctonia solani* and *C. quinqueseptatum* isolates, often penetrated them at certain points and further extended through the inner cavity of hyphae (Fig. 3). Breakage of affected hyphae at septal plates as in the case of *R. solani* isolates and leakage of cytoplasmic contents were common.

In the case of *R. solani* isolates, *T. harzianum* isolate TH3 (nursery soil, Varayal, Wayanad) showed highest per cent hyphal interaction against all the eight RS isolates. Highest interaction (96.8%) was recorded for RS6 x TH3. *T. viride* (TV5) isolate (nursery soil, Valluvassery, Nilambur), also showed highest per cent interaction against all the eight RS isolates (Table 10). Highest interaction recorded was 94% for RS1 x TV5. Even though, other *Trichoderma* isolates also showed hyphal interaction ranging from 10 to 90% against various RS isolates, the isolates TH3 and TV5 were the only ones effective against all the eight *R. solani* isolates tested. *T. koningii* and *G. virens* were found equally ineffective against most of the *R. solani* isolates tested.

In *Cylindrocladium quinqueseptatum – Trichoderma* species interaction also a similar picture emerged. Maximum hyphal interaction for all the eight isolates of *C. quinqueseptatum* was recorded for *T. harzianum* (TH3) and *T. viride* (TV5). In the case of *T. harzianum* (TH5), highest per cent hyphal interaction of 96.60 was recorded for CY10 (Table 11). *Gliocladium virens* was found to be ineffective against all the CY isolates, except CY10.

Fusarium oxysporum and *Pythium* isolates also showed almost similar trend with the antagonists. However, per cent interaction was comparatively low (66 to 78) in the case of *Fusarium* isolates against *T. harzianum*. *T. viride* isolates also gave similar interaction rate, but with a higher figure (92%) for FO4 and PM2 isolates (Table 12). Hyphal interactions of selected antagonists against RS, CY, FO, and PM isolates are given in Figures 4,5 and 6.

Table 10. Antagonistic behaviour of *Trichoderma harzianum, T. viride and Gliocladium virens* isolates against *Rhizoctonia solani* isolates

Trichoderma isolates	Percent hyphal interaction							
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8
T.harzianum (TH1)	32.02	43.00	56.02	22.08	29.00	**80.71**	64.00	49.00
T. harzianum (H2)	45.06	34.78	33.05	67.00	46.00	32.00	23.08	24.00
T. harzianum (TH3)	**78.06**	**94.95**	**86.00**	**78.06**	**92.04**	**96.00**	**88.40**	**96.80**
T. harzianum (TH4)	64.08	29.00	56.00	**78.00**	46.06	**82.00**	42.80	34.00
T. Harzianum (TH5)	46.80	61.90	45.00	**76.39**	53.00	52.00	34.00	31.00
T. harzianum (TH6)	48.00	46.80	42.00	54.00	56.00	28.08	32.10	32.00
T. harzianum (TH7)	64.00	54.08	**78.00**	34.00	43.00	29.00	60.78	54.00
T. harzianum (TH8)	54.00	56.08	32.00	44.03	43.00	**82.00**	32.00	43.00
T. harzianum (TH11)	34.00	32.00	**71.00**	**77.53**	54.60	58.00	32.10	28.70
T.harzianum (TH12)	67.80	58.00	**70.00**	54.00	32.00	36.00	28.00	**90.71**
T. harzianum (TH13)	32.00	24.60	39.00	**76.00**	18.00	34.00	65.00	62.00
T. harzianum (TH17)	12.00	34.00	46.00	11.50	22.00	23.78	22.00	12.46
T. harzianum (TH18)	23.60	26.00	49.00	10.00	16.80	22.00	29.80	11.50
T. koningii (TK)	46.80	34.60	**76.00**	34.00	29.08	22.06	42.10	36.00
T. viride (TV1)	48.00	65.00	68.00	54.00	36.00	48.80	76.00	83.01
T. viride (TV2)	68.00	56.78	46.00	51.00	46.00	32.00	57.80	61.29
T. viride (TV3)	62.00	43.20	42.00	**75.00**	64.00	54.00	32.00	29.00
T. viride (TV4)	**88.00**	64.00	**86.00**	43.00	48.00	**78.00**	42.00	46.08
T. viride (TV5)	**94.00**	**88.00**	**92.60**	**86.00**	**90.64**	**92.00**	**86.78**	**90.00**
G. virens (GV)	46.00	62.48	68.00	64.00	42.00	**86.00**	47.80	42.00

*RS: *Rhizoctonia solani* isolates

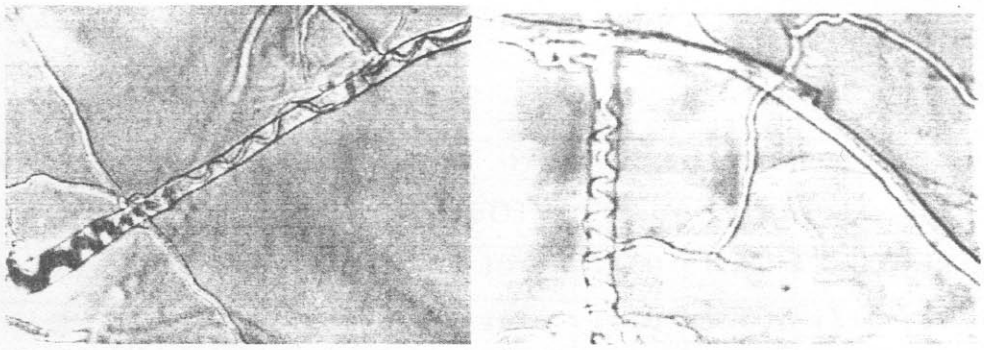


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Figure 3. a. Hyphal coiling and parasitism by *T. harzianum* in *R. solani* ; b. *T. viride* in *R. solani* hyphae

Table 11. Antagonistic behaviour of *Trichoderma harzianum, T. viride and Gliocladium virens* isolates against *Cylindrocladium quinqueseptatum* isolates

Trichoderma isolates	Percent hyphal interaction							
	CY1*	CY5	CY6	CY7	CY8	CY10	CY12	CY 15
T.harzianum (TH1)	62.42	**93.00**	56.00	28.00	32.80	21.00	23.00	12.00
T. harzianum (TH2)	45.06	12.80	**12.00**	12.80	34.00	27.00	45.80	26.00
T. harzianum (TH3)	**89.00**	**93.73**	**76.00**	**88.00**	**82.00**	**96.60**	**82.40**	**86.00**
T. harzianum (TH4)	28.00	34.80	29.00	35.00	74.80	12.00	16.00	14.60
T. Harzianum (TH5)	**88.72**	24.00	12.00	28.00	68.00	**86.00**	**90.00**	14.20
T. harzianum (TH7)	26.00	28.00	24.00	10.20	18.00	62.00	08.00	12.80
T. harzianum (TH8)	**86.60**	26.00	**76.80**	65.00	**78.00**	43.00	21.00	33.70
T. viride (TV4)	67.00	23.00	72.80	**81.00**	12.00	10.00	16.00	18.00
T. viride (TV5)	**76.80**	**81.00**	**80.00**	**78.60**	**92.00**	**78.00**	74.00	**89.00**
G. virens (GV)	16.00	34.60	24.00	22.00	10.20	**79.00**	32.00	36.00

- CY: *Cylindrocladium quinqueseptatum* isolates

Table 12. Antagonistic behaviour of *Trichoderma harzianum, T. viride and Gliocladium virens* isolates against *Fusarium oxysporum*, *Pythium* isolates

Trichoderma isolates	Percent hyphal interaction							
	FO1	FO2	FO3	FO4	PM1	PM2	PM3	PS*
T.harzianum (TH1)	22.00	12.60	10.00	12.00	11.80	16.00	18.60	22.00
T. harzianum (TH2)	11.20	15.80	**16.00**	14.00	36.00	12.00	16.00	18.80
T. harzianum (TH3)	**66.80**	**72.00**	**76.00**	**78.00**	**94.00**	**88.00**	**84.80**	**78.00**
T. harzianum (TH4)	24.60	22.00	12.00	18.00	16.00	30.00	40.80	62.00
T. harzianum (TH5)	28.00	34.00	12.00	10.00	13.00	35.00	48.80	64.00
T. harzianum (TH7)	24.00	21.00	12.80	16.00	18.00	60.00	54.00	56.00
T. harzianum (TH8)	22.00	12.00	17.00	18.60	12.50	44.00	62.00	45.80
T. viride (TV4)	12.00	16.70	19.00	46.00	68.00	76.00	62.00	38.00
T. viride (TV5)	**72.00**	69.00	**78.00**	**92.00**	**76.00**	**92.00**	**87.00**	**88.80**
G. virens (GV)	18.00	22.00	42.00	61.00	18.00	26.00	29.80	34.00

- FO: *Fusarium oxysporum*; PM: *Pythium myriotylum*; PS: *Pythium* sp.

Figure 4. Hyphal interactions of selected isolates of *Trichoderma* and *R. solani* isolates

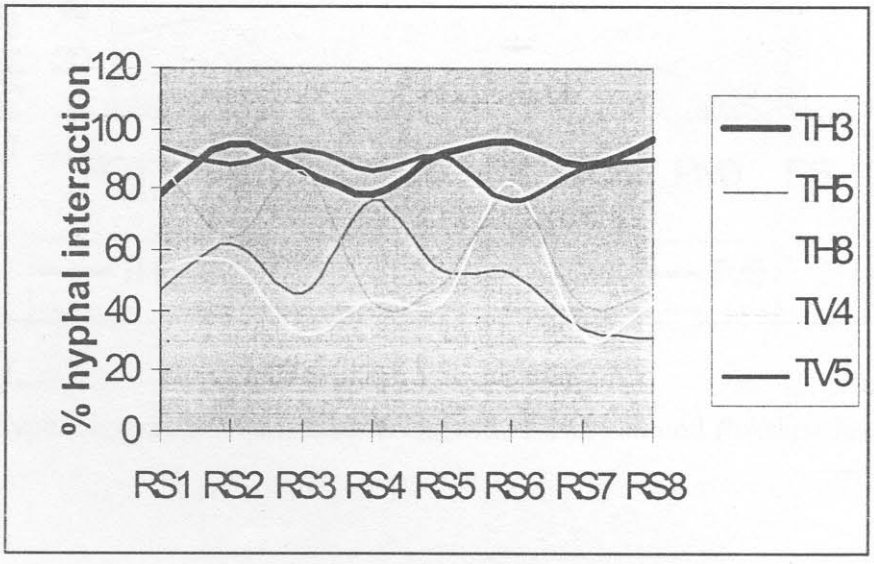
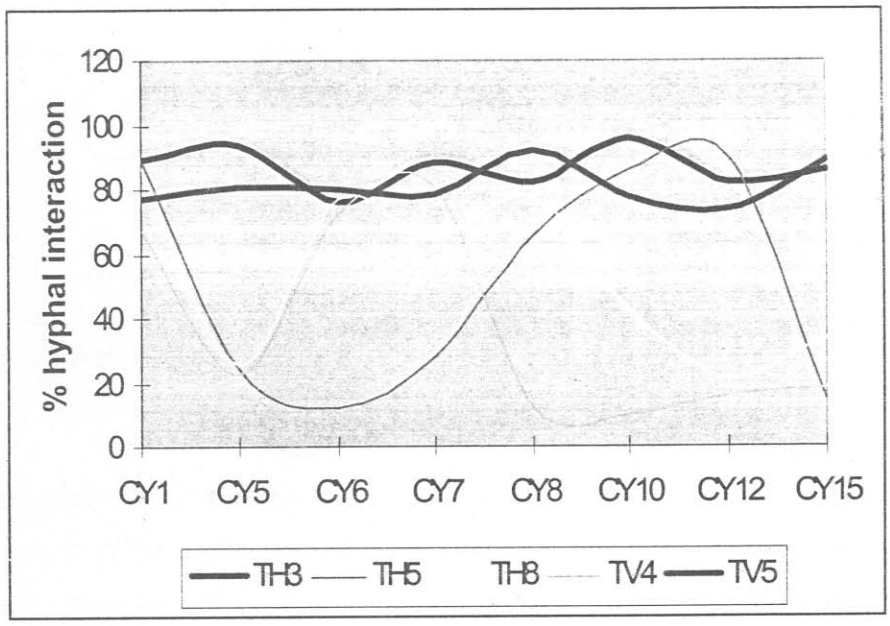


Figure 5. Hyphal interactions of *Trichoderma* and *C. quinqueseptatum* isolates



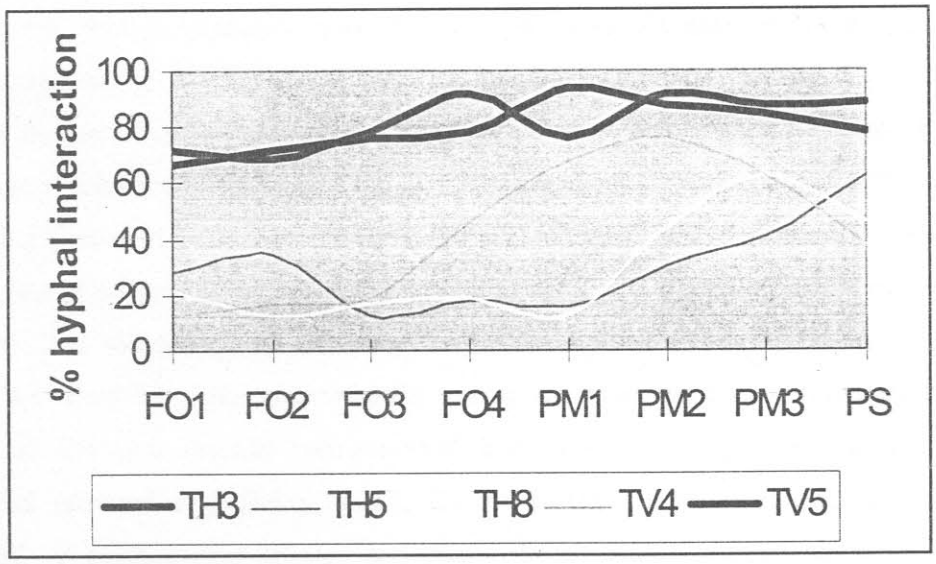
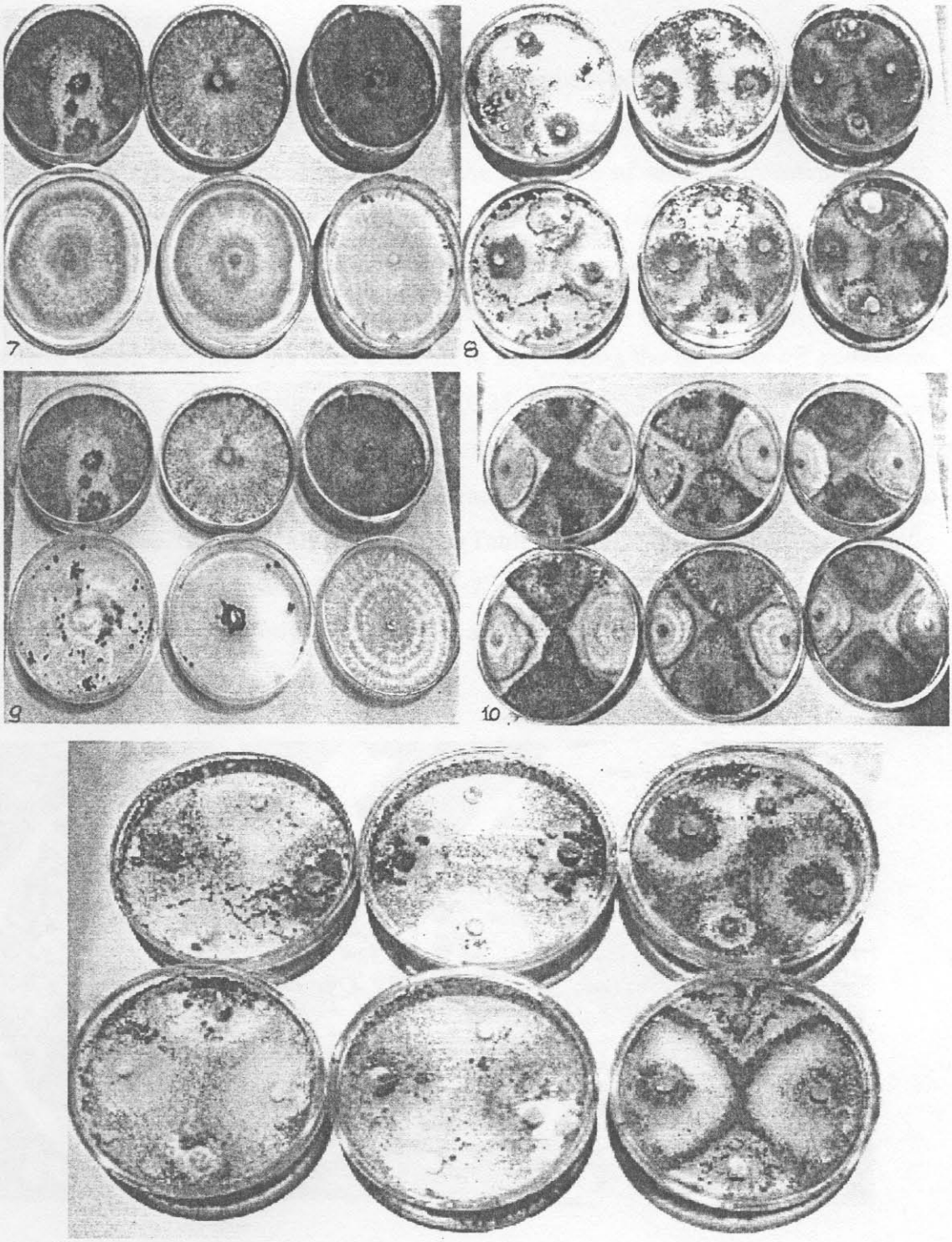


Figure 6. Hyphal interaction of *Trichoderma* with *Fusarium* and *Pythium* isolates

On the basis of efficacy of hyphal interactions with the representative damping-off pathogen isolates, promising isolates of *Trichoderma harzianum* (TH3) and *T. viride* (TV5) were selected for further screening. Though, all the antagonistic fungi screened exhibited their hyperparasitic behaviour, the level of interaction showed considerable variation and thereby the selection of promising candidates of antagonists becomes very easy. This technique offers a quick and easy method for screening large number of antagonists within a short period of time.

3.5.2. Petri Dish Bioassay

Observations on Petri dish bioassay revealed that *Trichoderma harzianum* and *T. viride* interacted with all the pathogen isolates as evidenced by clear growth inhibition zones as well as over growth, parasitism and profuse sporulation over the predated colonies. Growth inhibition zones of 3-9 mm wide was observed for *T. harzianum* seeded Petri dishes. In the case of *T. viride* seeded Petri dishes, the inhibition zones ranged from 3-7 mm wide. Restriction of linear growth of pathogen colonies was observed in the dual culture of TH3 and TV5 isolates and *R. solani* and *C. quinqueseptatum* isolates. In most cases, the antagonists overgrew the pathogen colonies and profusely sporulated over the predated colonies. In the case of *Fusarium* and *Pythium* isolates, hyperparasitism and suppression of colony growth were observed (Figures 7-11). Colony inhibition rate among the *Trichoderma* species varied and *Trichoderma harzianum* (TH3) and *T. viride* (TV5) were the most efficient isolates against all the pathogen isolates tested. The hyphae of all the pathogenic fungi nearest to the *Trichoderma* colony showed morphological changes. It is suggested that some antifungal metabolites are produced by these *Trichoderma* species which inhibited the growth of the pathogens. Production of fungal metabolites by many species of *Trichoderma* has been reported and their role in lysis of pathogen cell has been elucidated (Ahmad and Baker, 1987; Baker, 1991). Hydrolytic and chitinilytic enzymes produced by *Trichoderma* species and their role in mycoparasitism has been investigated by many workers (Horavath *et al.*, 1995; Lora *et al.*, 1995). In the present study, of the many potential antagonistic fungi screened, *T. harzianum* (TH3) and *T. viride* (TV5) isolates consistently and effectively checked the growth of all the representative isolates of damping off pathogens.



Figures 7-11. Petridish Bioassay. 7: Cultures of *Trichoderma* species, *C. quinqueseptatum* and *R. solani*. 8: *P. myriotylum* and *Trichoderma* spp.; 9: Cultures of *Trichoderma* spp. and *R. solani* 10: *C. quinqueseptatum* and *Trichoderma* spp.; 11: *R.solani* and *Trichoderma* spp.

3.5.3. Seedling Bioassay

Soil amendment with *Trichoderma* species: Soil amendment with *Trichoderma harzianum* and *T. viride* isolates in damping-off pathogens (*R. solani, C. quinqueseptatum, F. oxysporum, P. myriotylum*) infested soil reduced the incidence of seedling infection than the respective controls (Figure 12). Among the three isolates *of T. harzianum,* viz., TH3, TH5, TH6 tested, TH3 was the most efficient one which showed only a very low per cent seedling infection (0.37 to 0.71%), in all the pathogen infested treatments (Table 13). Of the two *T. viride* isolates tested, TV5 was the most efficient in suppressing the damping-off pathogens, as only very low per cent seedling infection (0.25 to 0.57%) was recorded in all the treatments. Disease incidence was very high in controls, especially in *R. solani* (52.76%) and *C. quinqueseptatum* (62.27%) infested soils. Interaction of *Trichoderma* isolates with all the pathogen isolates was found highly significant (Table 14). DMRT showed significantly different groups among the treatments (P=0.05). *T. harzianum* (TH3) and *T. viride* (TV5) treatments were found significantly different from all the other treatments (P=0.05).

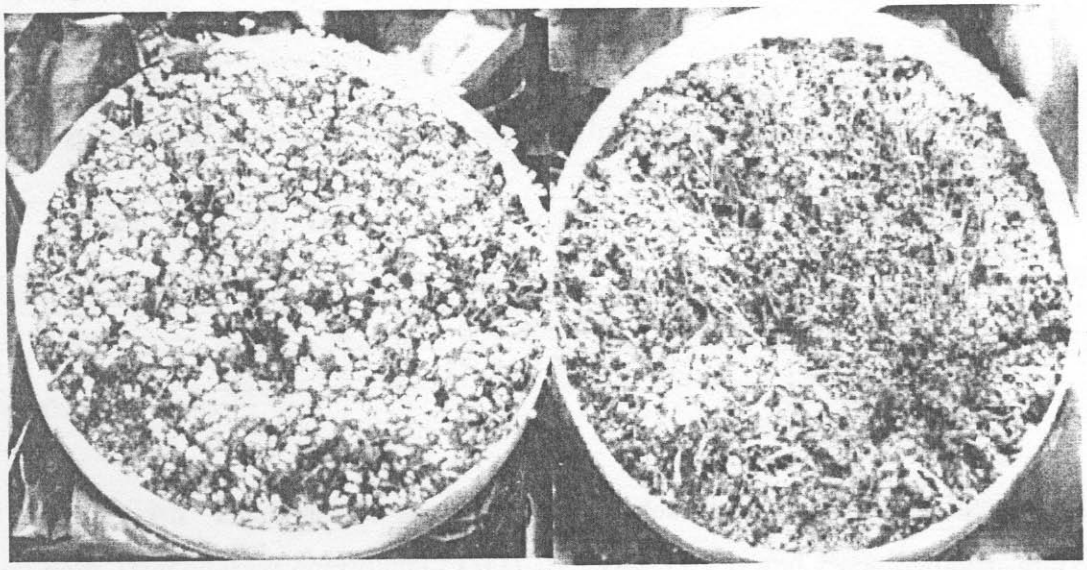


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Figure 12. Seedling bioassay- a: *Eucalyptus tereticornis* seedlings in *Trichoderma* (TH3) treatment, b: control

Table 13. Seedling bioassay : Soil amendment with antagonists

Treatment Antagonists	Per cent seedlings affected Inoculated with pathogens RS		CY		FO		PM	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TH3	0.71[a]	0.0011	0.59[a]	0.0012	0.46[a]	0.0025	0.37[a]	0.0115
TH5	4.43[b]	0.0116	5.22[b]	0.0031	5.64[b]	0.0092	6.14[b]	0.0157
TH6	6.32[b]	0.0063	6.58[b]	0.0082	7.21[b]	0.0189	8.03[b]	0.0071
TV4	7.55[b]	0.0028	8.68[b]	0.0056	6.27[b]	0.0069	6.45[b]	0.0034
TV5	0.57[a]	0.0074	0.25[a]	0.0038	0.46[a]	0.0031	0.50[a]	0.0021
Control	52.76[cd]	0.7503	62.27[d]	1.2386	45.61[c]	0.6747	43.81[c]	0.5700

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

TH3, TH5, TH6: *Trichoderma harzianum* isolates. TV4, TV5: *Trichoderma viride* isolates; RS: *R. solani*,CY: *C. quinqueseptatum*, FO:*F. oxyxsporum*, PM: *P. myriotylum*

Table 14. Analysis of Variance – data on seedling bioassay (soil amendment)

Source	D.F.	Sum of Squares	Mean Squares	F Ratio
Between Groups	23	14358.3901	624.2778	45.0736*
Within Groups	48	664.8094	13.8502	
Total	71	15021.1995		

- Significant at P= 0.05

Seed coating with *Trichoderma* species and *Pseudomonas fluorescens*: Results on seed coating with *Trichoderma harzianum* (TH3), *T. viride* (TV5) and *Pseudomonas fluorescens* revealed effective in reducing disease incidence in the pathogen infested soil. In controls a very high per cent seedling infection of 73.47% in *R. solani* and *Pythium myriotylum* infested treatments was noticed. However in *Fusarium* and *Cylindrocladium* infested soil, comparatively low per cent seedling infection and mortality was observed (Table 15). The results were encouraging that the introduction of antagonists in the infection court through

seed coating offers protection against the damping-off pathogens to a certain level. In *Pseudomonas fluorescens* treated soil, per cent infection ranged from 5.30 to 6.18. In the ANOVA, interaction of antagonists treatment and pathogen isolates was found highly significant and DMRT showed significantly different groups among the treatments (Table 16). Of the potential biocontrol agents screened in greenhouse, *T. harzianum* (TH3) and *T. viride* (TV5) most consistently and effectively controlled all the four damping-off pathogens. *Pseudomonas fluorescens* treatment was also found effective in reducing the disease incidence. The wide range of responses of the antagonists to the four isolates of different damping-off pathogens is striking. This range of activity suggests a complicated mechanism involving more than one metabolites or mode of antagonistic action operating in the system. Since the greenhouse environment is relatively controlled and growth medium is relatively uniform, the ecological interaction between pathogen-host-antagonist and resident microbes should be least complicated. The situation will be more realistic and suspected to be more complicated in the forest nurseries.

Table 15. Seedling bioassay: Seed coating with antagonists

Treatment with Antagonists	Per cent seedlings affected Inoculated with pathogens RS		CY		FO		PM	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TH3	1.44^{a}	0.0080	2.04^{ab}	0.0288	2.97^{abc}	0.0006	1.00^{a}	0.0155
TV5	2.74^{abc}	0.0015	3.16^{abc}	0.0000	3.69^{abc}	0.0406	1.44^{a}	0.0048
PF	5.30^{bc}	0.0033	5.33^{bc}	0.0235	6.18^{c}	0.0175	5.37^{bc}	0.0411
Control	73.47^{e}	0.3148	43.56^{d}	0.0078	39.31^{d}	0.7250	70.11^{e}	0.4078

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

TH3: *Trichoderma harzianum* isolate; TV5: *Trichoderma viride* isolate; RS: *R. solani*, CY:*C. quinqueseptatum*, FO:*F. oxysporum*, PM: *P. myriotylum*

Table 16. Analysis of Variance – data on seedling bioassay (seed coating)

Source	D.F.	Sum of Squares	Mean Squares	F Ratio
Between Groups	15	14774.3534	984.9569	97.3729*
Within Groups	32	323.6900	10.1153	
Total	47	15098.0434		

* Significant at P= 0.05

3.6. Mass Production of Inoculum of Antagonists and Evaluation of their Shelf-life

Tapioca rinder substrate and Maize-meal-perlite were found effective as carrier material and for large-scale production of inoculum of *T. harzianum* and *T. viride*. Experiment on the shelf-life of the mass cultured inoculum of antagonists showed that the cultures can be maintained in polypropylene bags for a period of 4 months in all the temperatures , i.e. 5 ^{0}C, 10^{0}C and 25± ^{0}C tried without affecting their physical and physiological qualities. However, storage of mass cultured inoculum of antagonists at 25 ± 2 ^{0}C is found more desirable than storage at lower temperatures, as far as direct application of inoculum in forest nursery is concerned.

3.7. Disease Control in Rootrainers through Ectomycorrhizal Manipulation

With a view to manage the damping-off in rootrainer seedlings of *Eucalyptus* and *Acacia*, and to boost the growth of seedlings, *Pisolithus tinctorius* (Pers.) Coker & Couch [Syn. = *P. arhizus* (Scop.& Pers.) Rauschert], an ectomycorrhizal (ECM) fungus was applied. Fungal mycelial bits prepared in sodium alginate pellets and spore slurry application in rootrainers reduced the incidence of disease. *Ralstonia solanacearum (= Burkholderia solanacearum)* is the major pathogen in rootrainers which causes damping-off, root rot and foliage infection. Since soil-less or soil-free media are used in rootrainers, chances of occurrence of damping-off caused by soil-borne fungi like *R. solani* or *Pythium* spp. are less. *P. tinctorius* is a common ectomycorrhizal associate of eucalypts and acacias and application of this

symbiotic organism in rootrainers reduced the incidence of bacterial disease of emerging seedlings. Recently ECM fungi have been exploited as biocontrol agents to suppress the seedling diseases caused *by R. solani* (Niemi *et al.*, 2000). ECM fungi may form a protective mantle over roots, which mechanically prevents the invading pathogen (Marx, 1970), produce antifungal compounds against pathogens (Duchesne *et al.*, 1988; Chakravarty and Hwang, 1991), induce plant resistant reactions (Buscot *et al.*, 1992), alter conditions in the rhizosphere (Rasanayagan and Jeffries, 1992), or support rhizosphere microbial populations detrimental to pathogens (Schelkle and Peterson, 1996). The ECM fungus constantly compete with other microorganisms for living space and also antagonize pathogens. A clear knowledge on competitive interactions between ECM fungus and the soil-borne damping-off pathogen will allow us to select appropriate ECM fungal species or isolate for their ability to dominate root system upon inoculation and continue to provide selected benefits to the inoculated seedlings when outplanted.

3.8. Nursery Trials

Success of forest nursery in raising sufficient number of healthy planting stock depends largely on the proper nursery management measures practiced. Adopting appropriate cultural measures suitable to the forestry species raised, edaphic and microclimatic factors, occurrence of diseases in nursery and their severity can be reduced. However, protection against possible incidence of potential disease(s) can be offered through chemical or biological means.

Nursery disease management through biological agents has been recognized as a plausible approach and many attempts on these lines have been made in forest nurseries in temperate situations. However, examples of success of disease management through biological measures in forest nurseries in tropical conditions are meagre. Introduction of antagonistic organisms into the nursery soil by different delivery methods and reduction of pathogen inoculum potential by increasing the soil temperature sub-lethal to lethal to soil-borne

pathogen propagules by solar heating are the methods adopted to attain disease management through biological means.

Antagonistic efficiency proven isolates of *Trichoderma harzianum* (TH3) and *T. viride* (TV5) through different laboratory and greenhouse screening were exploited in nursery trials for further confirmation of their effectiveness and possibility of utilizing in large-scale nursery operations. *Pseudomonas fluorescens*, though its biocontrol potential screened in greenhouse trial was not very encouraging, was also tried in nursery. Soil solarization by tarping the moistened nursery beds was also included. Nursery trials were conducted during 1998 and 1999.

3.8.1. Soil Solarization

Soil solarization of the nursery beds recorded a maximum temperature of 51°C at 2.5 cm soil depth, followed by 48 °C at 5 cm and 45 °C at 10 cm soil depth respectively during the 1998 trial. However, average maximum soil temperature attained in the beds in these three soil depths were 44.61^0 C, 42.38^0 C, and 40.3 ^{0} C respectively (Table 17).

Table 17: Mean soil temperature at different soil depths in solar heated beds (1998 trial)

Soil depth (cm)	Mean temperature (^{0}C)* Hours				
	8.00	10.00	12.00	14.00	16.00
2.5	28.84	30.46	38.92	44.61	43
5.0	26.69	31.61	36.69	42.38	42.16
10	27.23	29.30	34.76	40.30	38.53

- Mean values of 18 consecutive days

Soil solarization of nursery beds conducted during 1999 recorded a maximum temperature of 45 ^{0} C at 5 cm soil depth. However, average maximum temperature for 15 days recorded

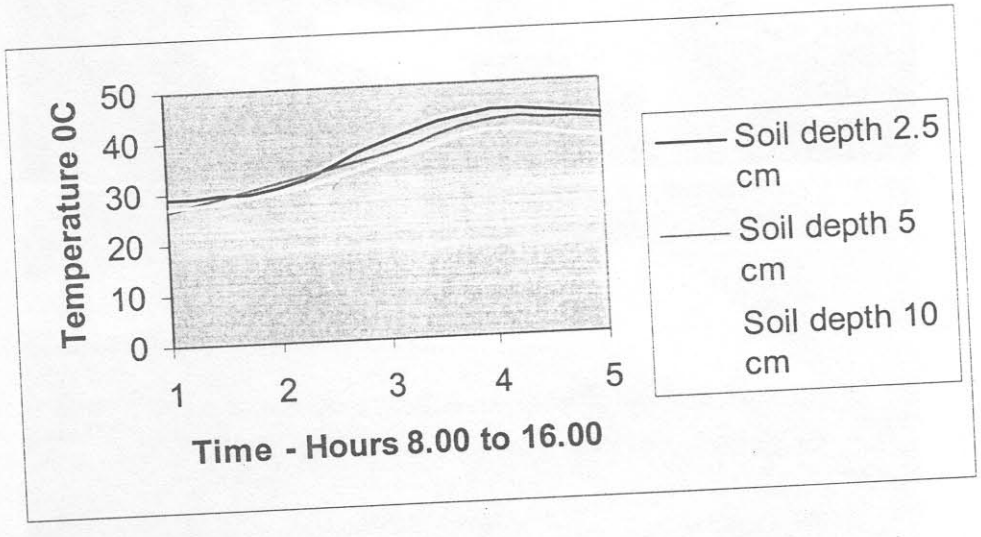
was only 40.27 ^{0} C at 5cm depth (Table 18). Peak temperature was attained between 13.00 and 14.00 h in both the trials. In soil depths of 10 cm and 15 cm, the temperatures attained were much lower than those in top layer (Figures 13,14).

Table 18: Mean soil temperature at different soil depths in solar heated beds (1999 trial)

Soil depth (cm)	Mean temperature (^{0}C)* Hours									
	8.00	9.00	10.00	11.00	12.00	13.00	14.00	15.00	16.00	17.00
5	19.90	20.18	24.81	29.45	33.68	36.31	40.27	38.27	35.59	33.81
10	21.40	22.18	24.59	28.27	32.27	34.54	36.81	35.77	33.80	32.81
15	26.36	27.54	28.72	29.81	30.31	32.31	32.95	33.18	32.90	31.13

- Mean value for 18 consecutive days

Figure 13. Mean soil temperature at different soil depths during 1998 trial



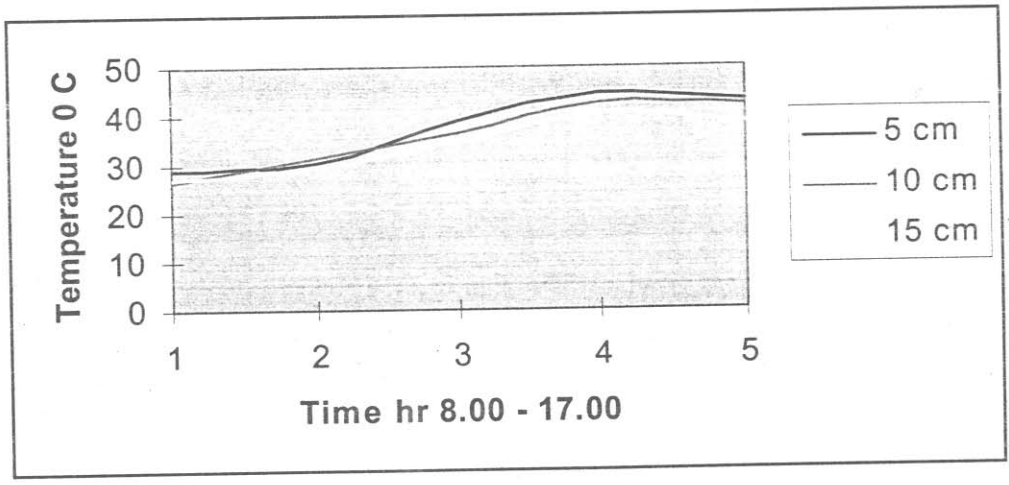


Figure 14. Mean soil temperature at different soil depths during 1999 trial

b

Figure 15. Soil solarization of nursery beds. a: Tarping the moistened beds with polythene sheets.b: Seedbeds prepared for solarization

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 1998 and 1999 trials (Table 19). A drastic reduction in number of fungal genera was also observed (Tables 20-21). Damping-off pathogens like *Pythium* and *Fusarium* were eliminated from the top layer (0-2 cm depth) of the nursery beds in both the trials, while population of *R. solani* and *C. quinqueseptatum* could be considerably reduced by the solarization treatment. However, an increase in population of *Penicillium, Aspergillus* and *Trichoderma* was recorded in solarized soil. An increase of population of *Trichoderma* spp. from 5.55% to 30.43 % was recorded in 1998 (Table 20), while in 1999 trial the figures were 7.69% and 18.51% respectively for before and after solarization treatment (Table 21).

A total of 14 fungal genera were recorded in the soil before the treatment in 1999 trial and after solarization the number of genera was found 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 (Table 20,21). 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.

Table 19: Fungal population (cfu/g) in experimental nursery beds before and after soil solarization

Nursery trial	Fungal population (cfu /g)	
	Before solarization	After solarization
1998	39.13×10^3 /g	25.27×10^3 / g
1999	42.85×10^3 /g	29.03×10^3 / g

Table 20: Fungal flora in the nursery soil before and after soil solarization (1998 trial)

Sl. No.	Fungus	Mean % to the total population	
		Before solarization	After solarization
1.	*Absida* sp.	2.77	-
2.	*Acremonium* sp.	5.55	-
3.	*Aspergillus* spp.	13.88	26.08
4.	*Cladosporium* spp.	5.55	8.69
5.	*Cunninghamella* sp.	2.77	-
6.	*Cylindrocladium quinqueseptatum*	13.88	4.34
7.	*Fusarium* spp.	11.11	-
8.	*Mucor* sp.	2.77	-
9.	*Penicillium* spp.	11.11	26.08
10.	*Pythium* sp.	8.33	-
11.	*Rhizoctonia solani*	16.66	4.34
12.	*Trichoderma* spp.	5.55	30.43

Table 21: Fungal flora in the nursery soil before and after soil solarization (1999 trial)

Sl.No.	Fungus	Mean % to the total population	
		Before solarization	After solarization
1.	*Acremonium* sp.	5.12	3.70
2.	*Aspergillus niger*	5.12	14.81
3.	*Aspergillus* spp.	7.69	18.51
4.	*Cladosporium* spp.	5.12	3.70
5.	*Cunninghamella* sp.	2.56	-
6.	*Curvularia* sp.	2.56	-
7.	*Cylindrocladium quinqueseptatum*	17.94	3.70
8.	*Fusarium* spp.	12.82	-
9.	*Geotrichum* sp.	-	3.70
10.	*Gongronella* sp.	-	3.70
11.	*Mucor* sp.	2.56	3.70
12.	*Paecilimyces* sp.	2.56	-
13.	*Penicillium* spp.	5.12	22.22
14.	*Pythium* sp.	2.56	-
15.	*Rhizoctonia solani*	20.51	3.70
16.	*Trichoderma* spp.	7.69	18.51

Disease incidence in solarized nursery beds: Incidence of damping-off in solarized nursery beds was comparatively less than that in the control beds in both 1998 and 1999 trials. In 1998 trial, mean damped-off patches or the infection foci was 9.50 in *E. tereticornis* and 11.79 in *E. grandis*. While in 1999 trial, disease incidence was found much higher than that occurred in 1998 and the mean infection foci observed was 16.1779 (Tables 22,25). In each infection foci, the number of damped-off seedlings varies from 10 to 15. In many cases merging of individual patches with the adjoining patches was also occurred. In treatments where soil solarization was followed by addition of antagonistic fungal inoculum, disease incidence was found much reduced than that occurred in solar heating alone. However, in 1998 trial, in treatment where solar heating was followed by soil amendment with both the antagonistic fungi (TH3 and TV5), disease incidence was found much higher than that occurred in treatment with antagonists alone (Table 22).

Soil temperatures in the solarized nursery beds reached up to 51^0C in 1998 trial and 45^0 C in 1999 trial which are sufficient for inactivating the spores and propagules of damping-off pathogens. Inactivation of spores of many soil-borne pathogens like *R. solani, Sclerotium rolfsii* Sacc., *Verticillium dahliae* Kleb., *Pythium* spp., etc. at temperature between 40^0C and 50^0C has been reported by many workers (Pullman *et al.*, 1981; Katan, 1987). Sherwood (1970) reported inactivation and killing of *R. solani* at 45 ^{0} 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 experiments, soil temperature of 51^0C and 45^0C at soil depths of 2.5 cm and 5 cm, usually retained for several hours with 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 recolonizing quickly under conducive soil environment and cause 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.

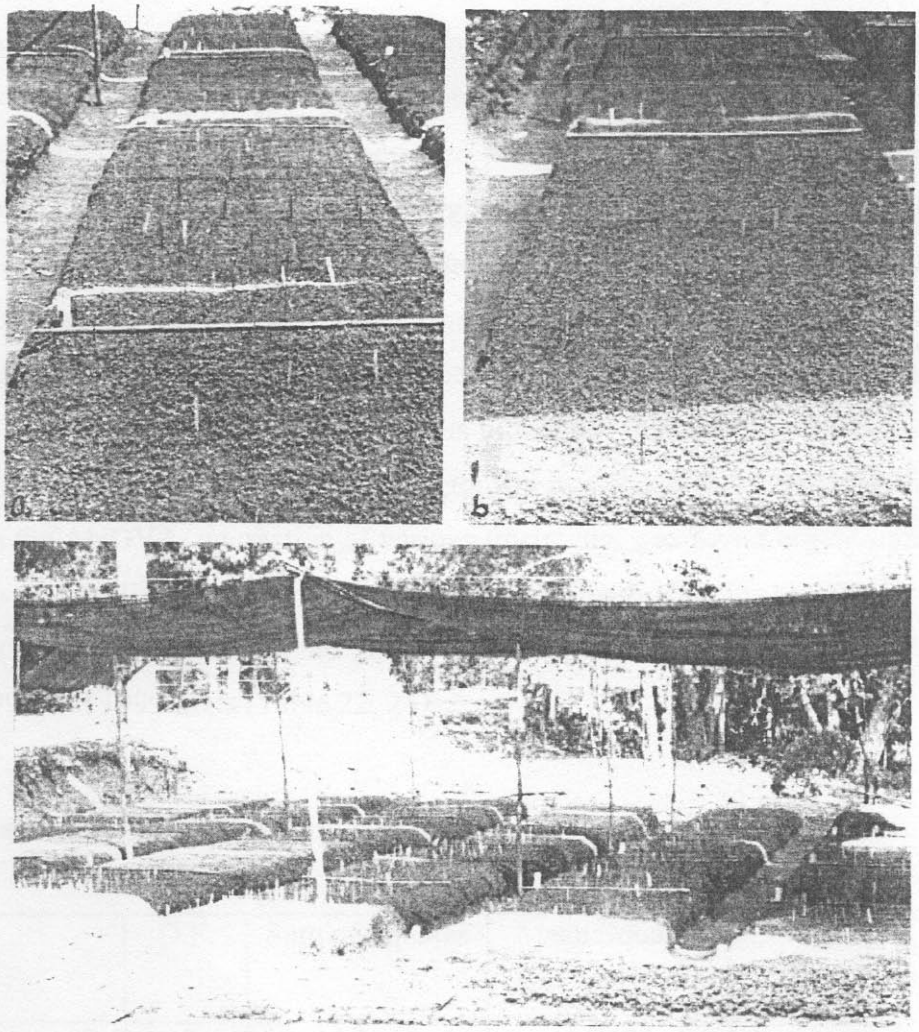


Figure 16. a: Experimental nursery raised at Chandhanathodu, Wayanad. b,c: Seedbeds under different treatments during 1998 and 1999.

3.8.2. Biological Control Through Manipulation of Antagonistic Organisms

Soil amendment and seed coating is the two delivery methods employed for introduction of the antagonistic organisms, viz., *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* into the nursery beds. Besides application of the promising antagonists singly, different combinations such as solar heating + soil amendment with *Trichoderma harzianum* + soil amendment with *T. viride*, soil amendment with both the *Trichoderma* species + seed coating with *P. fluorescens*, etc. were tried. Introduction of antagonists into the nursery beds

through soil amendment much before the sowing of seeds allows their multiplication, competition with the other native soil microflora for the nutrition and space, predation over the soil saprophytes and pathogens and establishment of dominance among the soil microbes. In an ideal nursery conditions, the above mentioned processes are expected to operate fully and thus offer protection of rhizosphere of emerging seedlings from invading pathogens. However, in the case of seed coating with the antagonists, development and establishment of introduced microorganisms in the system take place along with the seedling emergence and thus offers protection of individual emerging seedling against the threat from potential pathogens. The overall performance of the various biocontrol treatments in 1998 and 1999 trials was good and soil amendment with the antagonists was found more effective than the seed coating. In the ANOVA, interaction of various treatments was found highly significant and DMRT showed significant different groups among the treatments (Tables 22-26). Soil amendment with *Trichoderma harzianum* (TH3) was found better than that with *T. viride* (TV5) in both 1998 and 1999 trials (Table 22, 24).

Table 22. Analysis of Variance of data on nursery trial 1998-*E.tereticornis* seedbeds

Source	D.F.	Sum of Squares	Mean Squares	F Ratio
Between Groups	6	11.1615	1.8602	31.9798*
Within Groups	14	0.8144	0.0582	
Total	20	11.9759		

- Significant at P = 0.05

Table 23. Analysis of Variance of data on nursery trial 1998 - *E. grandis* seedbeds

Source	D.F.	Sum of Squares	Mean Squares	F Ratio
Between Groups	6	10.1533	1.6922	23.5245*
Within Groups	14	1.0071	0.0719	
Total	20	11.1604		

- Significant at P = 0.05

Table 24. Effect of various biocontrol treatments on disease incidence in 1998 Nursery trial

Sl. No.	Treatment	Mean No. of damped-off patches			
		ET*	SE	EG	SE
1.	SH- solar heating	9.5002[a]	1.1222	11.7984[a]	1.1701
2.	TH3 –soil amendment	10.5298[ab]	1.2420	15.3722[ab]	1.2080
3.	TV5 – soil amendment	15.6037[bc]	1.2082	18.7288[ab]	1.1121
4.	PF seed coating	35.3610[d]	1.1809	37.1574[c]	1.1042
5.	SH + TH3 + TV5 soil amendment	19.3561[c]	1.0328	20.0246[b]	1.3727
6.	TH3 + TV5 soil amendment + PF seed coating	23.4885[cd]	1.2162	22.7022[b]	1.1412
7.	C- control	93.7761[e]	1.1116	113.3706[d]	1.1806

*ET: *Eucalyptus tereticornis*; EG: *E. grandis*; SH: solar heating; TH3; *Trichoderma harzianum*; TV5: *T. viride*; PF: *Pseudomonas fluorescens;* SE: Standard error

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

Table 25. Biocontrol treatments and their effect on incidence of damping-off in 1999 nursery trial

Sl. No.	Treatment	Mean No. of Damped-off patches	SE
1.	Solar heating (SH)	16.1779[a]	1.3576
2.	SH+ *T. harzianum* (TH3) soil amendment	14.7870[a]	1.1509
3.	*T. harzianum* (TH3) soil amendment	19.0458[a]	1.0729
4.	*T. viride* (TV5) soil amendment	36.6037[bc]	1.1584
5.	*T. harzianum* (TH3) seed coating	32.5881[b]	1.0901
6.	*T. viride* (TV5) seed coating	51.6065[cd]	1.0894
7.	*P. fluorescens* (PF) seed coating	63.8188[d]	1.0907
8.	Control	111.3578[e]	1.2196

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

Table 26. Analysis of Variance of data on nursery trial 1999

Source	D.F.	Sum of Squares	Mean Squares	F Ratio
Between Groups	7	10.6934	1.5276	26.2128*
Within Groups	16	0.9324	0.0583	
Total	23	11.6258		

- Significant at P = 0.05

Among the various treatments tried in both 1998 and 1999 trials, 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 1998 trial, while solar heating combined with application of antagonists (Treatment No.5) was less effective. However, in 1999 trial, the only treatment combination tried was solar heating and application of *T. harzianum* (TH3) as soil amendment, which gave a better control of damping-off than solar heating alone (Table 23). This treatment turned out to be the best among the seven treatment tried in 1999 trial and the mean infection foci recorded was 14.78 against 111.35 mean infection foci in control. However, DMRT performed shows that the three treatments, viz. solar heating, solar heating + TH3 soil amendment, and TH3 soil amendment are not significantly different from each other (Table 25). In 1998 trial, a treatment in which *T. harzianum, 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 found less than that occurred in *Pseudomonas 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. Multiple strains/species release allows for the deployment of several different biocontrol mechanisms simultaneously. And biocontrol could be enhanced if synergistic mode of action is incorporated into the strains/species mixture.

Even though, the seed coating with the antagonists gave promising results in seedling bioassays in greenhouse, in nursery trials, the results were not very encouraging and may possibly be 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, the studies carried out during 1999 trial showed an increase in population of *T. harzianum*, *T. viride* and *P. fluorescens* in their respective treatments (Table 26). *Trichoderma viride* showed a maximum number of colony forming units (cfu 38. 42 x 10^3/g) in treatment with TV5 as soil amendment (Table26). Persistence as well as increase in population of *Trichoderma* species recorded was more in treatment with soil amendment than in seed coating.

Table 27. Persistence of antagonists in various biocontrol treatments: Nursery trial 1999

Treatment	Microbial population (cfu/g)	
	Before treatment	After treatment
SH +TH3 soil amendment	*Trichoderma* sp. 3.72 x 10^3/g	*T. harzianum* 24.03 x 10^4/g
TH3 soil amendment	*Trichoderma* sp. 4.26 x 10^3/g	*T. harzianum* 32.06 x 10^4 /g
TV5 soil amendment	*Trichoderma* sp. 3.68 x 10^3 /g	*T. viride* 38.42 x 10^4/g
TH3 seed coating	*Trichoderma* sp. 4.18 x 10^3/g	*T. harzianum* 12.06 x 10^4/g
TV5 seed coating	*Trichoderma* sp. 5..04 x 10^3/g	*T. viride* 13.08 x 10^4/g
PF seed coating	-	*P. fluorescens* 68.04 x 10^7/g

Of the host species tried, *Eucalyptus tereticornis* was found more susceptible to the disease than *E. grandis*. Among other forestry species raised in the nursery during 1998 and 1999 trials, all the species including *Tectona grandis* were found susceptible to damping-off. Severe damping-off of *T. grandis* caused by *Rhizoctonia solani* and *Pythium myriotylum* was observed and this is the first record of damping-off in teak seedlings. *Bombax ceiba,*

Terminalia arjuna, Leucaena leucocephala, Acacia nilotica, Dalbergia sissoides are the other species which succumbed to severe damage.

Analysis of two years nursery data on biocontrol of damping-off revealed that damping-off can be controlled or disease incidence can be reduced to a minimum level by application of *Trichoderma 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 biocontrol agents.

In forest nurseries, the 'biological balance 'is likely to be drastically altered by either raising monoculture crops extensively year after year and / or by introducing broad spectrum pesticides that disrupt the balance of microorganism populations. Biological control of plant diseases caused by soil-borne pathogen, by introduced microorganisms has been the focus of study for over 80 years (Baker, 1991). Forest ecosystems possess vast microbial communities that are responsible for the natural suppression of plant pathogens. Isolation and selection of potential antagonists and their introduction into the nursery system to suppress the pathogen inoculm potential is a plausible approach in biocontrol of nursery diseases. The genera of fungi most commonly exploited are *Trichoderma* and *Gliocladium.* Among bacteria, *Pseudomonas fluorescens* is the most commonly evaluated. Several species of *Trichoderma* including *T. harzianum, T. hamatum, T. koningii, T. polysporum, T. viride,* etc. have been used in the laboratory and field trials (Papavizas and Lewis, 1981; Papavizas, 1985). In the present study, though many isolates of *T. harzianum, T. viride, T. koningii, G. virens* used for screening their effectiveness, only one isolate each of *T. harzianum and T. viride* turned out to be effective against the representative isolates of damping-off pathogens. In the nursery trials carried out during 1998 and 1999, though the application of antagonists reduced the disease incidence, only soil amendment with *T. harzianum* gave promising results. The same antagonist introduced in the nursery as seed coating responded quite differently. One major problem confronted particularly with seed coating may be the induction of rhizosphere competence by biocontrol agent, defined as the

ability of the agent to grow and proliferate in host seedling rhizosphere (Liu and Baker, 1980; Ahmad and Baker, 1987). This is the zone where protection against pathogens is most critical.

Specific strains of *Pseudomonas fluorescens* introduced as seed inoculants have been shown to control a number of soil-borne fungal diseases including damping-off (Howell and Stipanovic, 1979). Fluorescent pseudomonads employ a number of different mechanisms in the suppression of plant pathogens which include competition, induced resistance, and production of antibacterial substances such as antibiotics, siderophores and hydrogen cyanide. Competition for substrate or site is probably responsible for disease suppression by most fluorescent pseudomonads, but the importance of this mechanism can vary among biocontrol strains and target pathogens (O'Sullivan and Gara, 1992).

Most of the antagonists are not rhizosphere competent as reported by Beagle-Ristanino and Papavizas (1985). However, recent work by Baker (1991) shows that *Trichoderma* spp. exhibit the ability to multiply in the rhizosphere. The results on persistence of introduced antagonists in the nursery beds also suggest that multiplication of the antagonists in the changed environment occurred to a certain extent and the carrier material like tapioca rinder substrate used in 1998 trial and maize-meal-perlite tried in 1999 were equally effective. However, the possible reason for the ineffectiveness of the treatments with the promising candidates of antagonists may be due to the failure of their multiplication beyond a certain limits due to unfavourable edaphic conditions. Under forest nursery system, the potential biocontrol agents which provided consistent control of the target pathogens in greenhouse and laboratory trials, have been inconsistent in suppressing the pathogen population. The variable performance of these antagonists in the nursery system has been attributed to several factors such as, variable colonization of the plant host and rhizosphere soil by the introduced strains/ species, instability or degradation of the active metabolite, failure to produce the metabolite to required threshold concentration at appropriate time and space, and variation in sensitivity of the target organisms to active metabolites produced by the biocontrol agents (Weller and Cook, 1986; O'Sullivan and Gara, 1992). Survival and multiplication of

Trichoderma species in soil is not an inherent character but it is influenced by several edaphic factors, viz., soil pH, soil temperature, soil moisture, texture, and nutrient status. Different reports on biocontrol experiments also indicate variation in the effectiveness of biological control strains under different environmental conditions and among different trials (Cook and Baker, 1983; Bolland, 1990; Baker, 1991). The biocontrol 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 local nursery soil strain of *T. harzianum* (TH3). Optimal application of biocontrol 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 biocontrol agents, and modification of nursery cultural practices is more desirable.

4. CONCLUSION

Damping-off disease poses major problem in conventional seedbed and container nurseries in the State. Among the damping-off pathogens, viz., *Rhizoctonia solani, Cylindrocladium quinqueseptatum, Fusarium oxysporum*, and *Pythium myriotylum* affecting most forestry species raised in nurseries, *R. solani* has emerged as the most potential pathogen. In natural population, *R. solani* exists in different Anastomosis Groups, and among the isolates from forest nurseries in Kerala four Anastomosis Groups, viz., AG1-IA, AG1-IC, AG2-I, and AG2-2IV have been recognized. Of the various isolates of antagonistic organisms screened for their antagonistic efficacy against the representative isolates of different damping-off pathogens employing various techniques, *T. harzianum* (TH3), *T. viride* (TV5) and *P. fluorescens* were found to be the most efficient ones. Inoculum of antagonists can be mass cultured on tapioca rinder substrate or maize-meal-perlite media and maintained at 25 ± 2^0C for 3 to 4 months without adversely affecting its quality. Among various potential biocontrol agents screened in nursery, *T. harzianum* (TH3) introduced by soil amendment most consistently and effectively controlled the damping-off disease. Solarization of nursery beds

is also an efficient tool to reduce the disease incidence. Disease management can be optimized through integrating appropriate cultural measures along with biocontrol measures.

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6. REFERENCES

Adams, P.B. 1990. The potential of mycoparasites for biological control of plant diseases. Annu. Rev. Phytopathol. 28: 59-72.

Ahmad, J.S. and Baker, R. 1987. The potential of mycoparasites for biological control of plant diseases. Annu. Rev. Phytopathol. 28:59-72.

Anderson, N.A. 1982. The genetics and pathology of *Rhizoctonia solani.* Annu. Rev. Phytopathol. 20: 329-347.

Arja, L., Sakari, L., Timo, K. and Risto, R. 1999. Nursery practices and management of fungal diseases in forest nurseries in Finland. A Review. Silva Fennica 31(1): 79-100.

Baker, K.F. 1991. Diversity in Biological Control, Crop Protection, 10:85-94.

Baker, K.F. and Cook, R.J. 1974. Biological Control of Plant Pathogens. W.H. Freeman, San Francisco, 433 pp.

Bakshi, B.K., Reddy, M.A.R., Puri, Y.N. and Sujan Singh 1972. Forest Disease Survey (Final Technical Report). Forest Pathology Branch, FRI, Dehra Dun, 117 pp.

Beagle-Ristano, J.E. and Papavizas, G.C. 1985. Biological control of Rhizoctonia stem canker and black scurf of potato. Phytopathology 75: 560-564.

Bolland, G.J. 1990. Biological control of plant diseases with fungal antagonists: Challenge and opportunities. Canadian Journal of Plant Pathology, 12: 295-299.

Burdon, J.J. and Chilvers, G.A. 1982. Host density as a factor in plant disease ecology. Annual Review of Phytopathology, 20: 143-166.

Buscot, F., Weber, G. and Oberwinkler, F. 1992. Interaction between *Cylindrocarpon destructans* and ectomycorrhizas of *Picea abies* with *Laccaria laccata* and *Paxillius involutus*. Trees 6: 83-90.

Campbell, R. 1986. The search for biological control agents against plant pathogens; A pragmatic approach, Biological Agriculture and Horticulture, 3:317-327.

Chakravarty, P. and Hwang, S.F. 1991. Effect of ectomycorrhizal fungus, *Laccaria laccata*, on *Fusarium* damping-off in *Pinus banksiana* seedlings. Eur. J. For. Pathol., 21: 97-106.

Cook, R.J. 1993. Making greater use of introduced organisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 31: 53-80.

Cook, R.J. and Baker, K.F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. St. Pauls, M.N. American Phytopathological Society, 539 pp.

Duchesne, L.C.. Peterson, L. and Ellis, B.A. 1988. Pine root exudate stimulates the synthesis of antifungal compounds by the ectomycorrhizal fungus *Paxillus involutus*. New Phytologist 108: 471-476.

Elad, Y., Chet, I. and Henis, Y. 1981. Biological control of Rhizoctonia solani in strawberry field by Trichoderma harzianum. Plant and Soil, 60: 245-254.

Fravel, D.R., Connick, W.J.and Lewis, J.A. 1998. Formulation of microorganisms to control plant diseases. In: H.D. Burges (ed.), Formulation of Microbial Biopesticides, Beneficial Microorganisms and Nematodes. Chapman and Hall, London (in Press).

Horavath, E.M., Burgel, J.L., and Messner, K. 1995. The production of antifungal metabolites by the biocontrol fungus *Trichoderma harzianum* in connection with the formation of conidiophores. Material und Organismen 29: 1-14.

Howell, C.R. and Stipanovic, R.D. 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69: 480-482.

Huang, J.W. and Kuhlman, E.G. 1990. Fungi associated with damping-off of Slash pine seedlings in Georgia. Plant Disease 74: 27-30.

Katan, J. 1987. Soil Solarization. In: Innovative Approaches to Plant Disease Control. I. Chet (Ed.) John Wiley & Sons, New York. 77-105.

Katan, J. and Green Berger, A., Alon, H. and Grinstein, A. 1976. Solar heating by polythene mulching for the control of disease caused by soil borne pathogen. Phytopathology 66: 683-688.

Kausalya Gangadharan and Jeyarajan, R. 1988. techniques for mass multiplication of *Trichoderma viride* Pers. F. and *T. harzianum*. In: Proceedings of the National Seminar on Management of Crop Diseases. Rifai (Ed.) Madurai, pp. 32-33.

Kuc, J. 1987. Plant immunization and its applicability for disease control. Pages, 255-274, In: I.Chet (ed.), Innovative Approaches to Plant Disease Control. John Wiley, New York.

Liang, X.Y., Huang, H.C., Yanke, L.J., and Kozub, G.C. 1996. Control of damping-off of safflower by bacterial seed treatment. Can. J. Pl. Pathol. 18: 43-49.

Liu, S.D. and Baker, R. 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani*. Phytopathology 70(5): 404-412.

Liu, Z.L. and Sinclair, J.B. 1991. Isolates of *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. Plant Disease 75: 682-687.

Loper, J.E. 1988. Role of fluorescent siderophores production in biological control of *Pythium ultimum* by a *Pseudomonas flurescens* strain. Phytopathology 78: 166-172.

Lora, J.M., Cruz, J.D., Llobell, A., Benitez, T. and Pintor-toro, J.A. 1995. Molecular characterization and heterologous expression of an endo-B-1,6-glucanase gene from the mycoparasitic fungus *Trichoderma harzianum*. Mol. Gen.Genet. 247: 639-645.

Marx, D.H. 1970. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infection. V. Resistance of mycorrhizae to infection by vegetative mycelium of *Phytophthora cinnamomi*. Phytopathology 60: 1472-1473.

Mohanan, C. 1995. Methods to control important diseases of bamboos. Bamboo (India) Phase II 3-P-90-0198. Final Technical Report, IDRC, Canada, Kerala Forest Research Institute, Peechi.

Mehrotra, M.D. 1990. *Rhizoctonia solani* a potentially dangerous pathogen of khasi pine and hardwoods in forest nurseries in India. Eur. J. For. Path. 20:329-338.

Mohanan, C. 2000. Introduction of rootrainer technology in forestry- impact on nursery disease management. In: Lilja, Sutherland (eds.). Proc. 4th Meeting of IUFRO WP 7.03.04 - Diseases and Insects in Forest Nurseries, Finnish Forest Research Institute Research Papers 781, Suonenjoki, Finland, pp. 39-48.

Mohanan, C. 2000a. Status of forest nursery diseases in India and emerging trends in seedling disease management. In: Lilja, Sutherland (eds.). Proc. 4th Meeting of IUFRO WP 7.03.04 - Diseases and Insects in Forest Nurseries, Finnish Forest Research Institute Research Papers 781, Suonenjoki, Finland, pp. 231-242.

Mohanan, C. 2000b. Epidemiology and integrated management of web blight in bamboo nurseries in India. In: Lilja, Sutherland (eds.). Proc. 4th Meeting of IUFRO WP 7.03.04 - Diseases and Insects in Forest Nurseries, Finnish Forest Research Institute Research Papers 781, Suonenjoki, Finland, pp. 107-121.

Mohanan, C. and Sharma, J.K. 1985. *Cylindrocladium* causing seedling diseases of Eucalyptus in Kerala, India. Trans. Br. Mycol. Soc. 84(3): 538-539.

Niemi, K., Lilja, A., Kasurinen, A. and Heinonen-Tanski, H. 2000. Ectomycorrhizal fungi as biological control agents against Rhizoctonia rot infection of container-grown Scots pine seedlings. In; Lilja, Sutherland (eds.). Proc. 4th Meeting of IUFRO WP 7.03.04-Diseases and Insects in Forest Nurseries, Finnish Forest Research Institute Research Papers 781, Suonenjoki, Finland, pp. 151-162.

O'Sullivan, D.J. and Gara, F. 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol. Rev. 56: 662-676.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Annual Review of Phytopathology, 23: 23-54.

Papavizas, G.C. and Lumsden, R.D. 1980. Biological control of soilborne fungal propagule. Annu. Rev. Phytopathol. 18: 389-413.

Papavizas, G.C., and Lewis, J.A. 1981. Introduction and augmentation of microbial antagonists for the control of soil borne plant pathogens. In: Biological Control in Crop Production, G.C. Papavizas (Ed.). Allanheld, Osman and Co., Totowa, N.J. Pages 305-322.

Parmeter, J.R.Jr. and Whitney, H.S. 1970. Taxonomy of the imperfect state. Pages 7-9. In: *Rhizoctonia solani*: Biology and Pathology. J.R. Parmeter, Jr. (ed.), Univ. Cal. Press, Berkeley.

Parmeter, J.R.Jr, Sherwood, R.T. and Platt, W.D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59: 1270-1278.

Perrin, R. and Sampagni, R. 1986. La fonte des semis en pepiniere forestiere. European Journal of Forest Pathology 16: 309-321.

Pullman, G.S., De Vay, J.E. and Garber, R.H. 1981. Soil solarization and thermal death: A logarithmic relationship between time and temperature for four soil borne plant pathogens. Phytopathology, 71: 959-694.

Rasanayagan, S. and Jeffries, P. 1992. Production of acid is responsible for antibiosis by some ectomycorrhizal fungi. Mycological Research 96: 971-976.

Schelkle, M. and Peterson, R.L. 1996. Suppression of common root pathogens by helper bacteria and ectomycorrhizal fungi *in vitro*. Mycorrhiza 6: 481-485.

Sharma, J.K. and Mohanan, C. 1982. Cylindrocladium spp. associated with various diseases of Eucalyptus in Kerala. Eur. J. Forest Path. 12(3): 129-136.

Sharma, J.K. and Mohanan, C. 1991. Epidemiology and control of diseases of *Eucalyptus* caused by *Cylindrocladium* spp. in Kerala, KFRI Research Report 70, Kerala Forest Research Institute, Peechi Kerala, India, 155 pp.

Sharma, J.K. and Mohanan, C. 1992. Effect of some nursery practices on incidence and severity of disease, and growth of *Eucalyptus grandis* seedlings. European Journal of Forest Pathology 22: 125-135.

Sharma, J.K., Mohanan, C. and Florence, E.J.M. 1984. Nursery diseases for *Eucalyptus* in Kerala. European Journal of Forest Pathology, 14:77-89.

Sharma, J.K., Mohanan, C. and Florence, E.J.M. 1985. Disease survey in nurseries and plantations of forest tree species in Kerala. KFRI Research Report No. 36. Kerala Forest Research Institute, Peechi , Kerala, India, 288 pp

Sherwood, R.T. 1970. Physiology of Rhizoctonia solani, In: Rhizoctonia solani: Biology and Pathology . J.R. Parmeter Jr. (Ed.) University of California Press, Berkeley, pp. 69-92.

Sneh, B., Burpee, L. and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. The American Phytopathological Society, St. Paul, MN. 133 pp.

Sutherland, J.R. and Davis, C. 1991. Diseases and insects in forest nurseries in Canada. In: Sutherland, J.R. & Glover, S.G. (eds.). Proc. 1st Meeting of IUFRO WP S2.07.09 Diseases and Insects in Forest Nurseries. Victoria, British Columbia, August 23-30, 1990. Forestry Canada, Pacific and Yukon region, Pacific Forestry Centre, BC-X-331. P.25-32.

Thomashow, L.S. and Weller, D.M. 1988. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumanomyces graminis* var. *tritici.* J. Bacteriol. 170: 3499-3508.

Tu, C.C. and Kimbrough, J.W. 1973. A rapid staining technique for *Rhizoctonia solani* and related fungi. Mycologia 65: 91-944.

Vaartaja, O. 1952. Forest humus quality and light conditions as factors influencing damping-off. Phytopathology, 46: 391-397.

Vaartaja, O. 1967. Damping-off pathogens in South Australian nurseries. Phytopathology 57: 765-768.

Vaartaja, O. and Cram, W.H. 1956. Damping-off of conifers and of Caragana in Saskatchewan. Phytopathology 46: 505-507.

Warcup, J.H. 1950. The soil-plate method for isolation of fungi from soil. Nature 159: 856.

Weller, D.M. and Cook, R.J. 1986. Increased growth of wheat by seed treatments with fluorescent pseudomonads and implications of *Pythium* control. Can.J. Plant Pathol. 8: 328-334.

Yabuuchi, E., Kosako, Y., Yano,I., Hotta, H. and Nishituchi, Y. 1998. Transfer of two *Burkholderia* and *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1993) comb.nov. *Ralstonia solanacearum* (Smith 1996) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. Nov. Microbiol. Immunol. 39: 897-904.

Yokoyama, K. and Ogoshi, A. 1986. Studies on hyphal anastomosis of *Rhizoctonia solani* IV. Observation on imperfect fusion by light and electron microscopy. Trans. Mycol. Soc. Japan 27: 399-413.