ETIOLOGY AND ECOFRIENDLY MANAGEMENT OF FUNGAL DISEASES OF THIPPALI (*Piper longum* L.)

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DECLARATION

I hereby declare that this thesis entitled **"Etiology and ecofriendly management of fungal diseases of Thippali (*Piper longum* L.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

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CERTIFICATE

Certified that this thesis entitled **"Etiology and ecofriendly management of fungal diseases of Thippali (*Piper longum* L.)"** is a record of research work done independently by Miss. Poornima. R (2004-11-50) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to

The Farming

Community

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LIST OF ABBREVIATIONS

%	Percent
@	At the rate of
^{0}C	Degree Celsius
CD	Critical difference
cfu	Colony forming units
cm	Centimetre
DAT	Days After Treatment application
dia.	Diametre
DRE	Defence Related Enzymes
et al.	And others
f.sp.	Forme species
Fig.	Figure
g	Gram
hrs.	Hours
i.e.	That is
lbs	Pounds
mg	Milligram
min.	Minute
ml	Millilitre
mM	Millimolar
PDI	Percentage Disease Index
ppm	Parts per million
pv.	Pathovar
spp.	Species
v/v	Volume per volume
viz.	Namely
w/v	Weight per volume
µg	Microgram
µl	Microlitre
µm	Micrometre

Introduction

1. INTRODUCTION

The Indian long pepper (*Piper longum* L.), commonly known as thippali in Malayalam belongs to the family Piperaceae. It is a slender aromatic climber (plate 1). The spikes of this plant contain the alkaloids mainly piperine (4-5%), piplatin, piperolactum A, piperolactum B and piporadione. The roots contain the alkaloids piperlonguminine (0.2-0.25%) and piperlongumine (0.02%) besides piperine. There are other alkaloids and lignins which add to its medicinal values. It also yields 0.7% essential oil with a spicy odour resembling that of pepper oil and ginger oil. Its roots and fruiting spikes are used in treating diarrhoea, indigestion, jaundice, asthma, hiccough, malaria etc. (Farooqi and Sreeramu, 2001).

Incidence of diseases and pests is a major hurdle in the successful cultivation of any crop plant. Incidence of disease may adversely affect the pharmacological value of thippali and reduce the market value. A preliminary study conducted in the medicinal garden of College of Agriculture, Vellayani, Thiruvananthapuram revealed that the thippali plants grown in the garden are affected by a severe leaf spot / blight disease (Plate 2a & 2b). Adoption of chemical methods of disease management have an adverse effect on the pharmacological components of the medicinal plant. Hence emphasis has to be given to organic or ecofriendly management to ensure quality of the produce. With this view, the present investigation has been carried out with the following objectives.

1. Survey on the fungal diseases of thippali in Thiruvananthapuram district.
2. Detailed study on the symptomatology and development of disease.
3. Isolation of the pathogens associated, its characterisation and maintenance.
4. Identification of the pathogens based on colony and conidial morphology at the species level.
5. Study on the symptomatology, nature of damage and survival of the most commonly observed pathogen.
6. Proving Koch's postulate with the isolated pathogens.

Plate1 **Thippali plant**

Plate 2a **Field symptom of leaf spot affected thippali plants**

Plate 2b **Advanced stage of the disease showing defoliation**

Plate 1

Plate 2a

Plate 2b

7. Isolation of the phyllosphere / rhizosphere microflora of healthy thippali plants.
8. *In vitro* analysis of promising antagonistic fungi or bacteria, selected plant based chemicals and selected resistance inducers against the pathogen and mass multiplication of the promising biocontrol agents.
9. *In vivo* evaluation of the promising biocontrol agents, plant based chemicals, resistance inducers and neem cake on disease suppression.
10. Biochemical analysis of defense related enzymes *viz*., polyphenol oxidase, peroxidase and phenyl alanine ammonia lyase.

Review of Literature

2. REVIEW OF LITERATURE

Literature on the fungal diseases of thippali are meagre. Hence the present attempt was made to study the etiology of the major fungal diseases of thippali and their ecofriendly management.

Farooqi and Sreeramu (2001) reported a leaf and vine rotting in thippali during monsoon caused by *Colletotrichum* sp. They also reported a necrotic leaf spot and blight caused by *Colletotrichum* sp. along with *Cercospora* sp. during warmer months. *Colletotrichum* sp. are found to cause anthracnose in other crops of Piperaceae family viz., black pepper and betel vine (Chattopadhyay and Satyabrata, 1990). The disease in black pepper was first identified in North Malabar of Kerala as 'berry spot' and 'berry split' (Ayyar, 1921). Later, the causal orgsanism was identified as *C.gloeosporioides* and the disease was named as 'fungal pollu' (Rao, 1926). Loss due to fungal pollu in black pepper in some years was as high as 50 per cent of the marketable produce (Cattopadhyay and Satyabrata, 1990). In betel vine *Colletotrichum piperis* Petch., *C dasturi* Roy. and *C capsici* caused anthracnose (Dastur, 1935; Chattopadhyay and Satyabrata, 1990). Singh and Joshi (1973) reported extensive damage on betel vine due to *C capsici* in Madhya Pradesh. Maiti and Sen (1982) reported that *C.capsici* (Syd.) Butler & Bisby is of major importance in West Bengal and may cause damage up to 25 to 90 per cent in betel vine. No reports are available on the incidence of leaf spot or blight on thippali due to *C.gloeosporioides*.

2.1 THE PATHOGEN

During the survey, the major disease observed was leaf spot / blight. On isolation, the pathogen was found to be *C.gloeosporioides* (Penz.) Penz and Sacc. This fungus has been described by Sutton (1992) as follows.

Teleomorph: *Glomerella cingulata* (Stonem.) Spauld and van Schrenk. Colonies variable, greyish white to dark grey, aerial mycelium even and felted or in tufts associated with conidiomata, reverse unevenly white to grey or darker especially with age. Setae present or absent but immature ascomata may be mistaken

for sclerotia. Appressoria clavate, ovate, obovate, sometimes lobed, setae brown, 60-20 × 4-12 µm. Conidia formed in pale salmon masses, straight, cylindrical, apex obtuse, base truncate 12-17 × 3.5-6 µm.

2.2 CROSS INOCULATION STUDIES

Quimio and Quimio (1975) reported that isolates of *C.gloeosporioides* from guava could cross infect grape, egg plant and black pepper.

2.3 SURVIVAL OF THE PATHOGEN

Colletotrichum can survive in the soil as well as in plant debris for varying periods depending on the environmental conditions. *C capsici* (Syd.) Butler and Bisby causing anthracnose of betel vine survived through competitive saprophytic ability (Dasgupta, 1989). *C.gloeosporioides* has been isolated from anthracnose affected dried arecanut leaves on the ground for more than eight months (Hegde *et al.*, 1989). Viability of *C curcuma* (Syd.) retained upto nine months when infested turmeric leaves were buried in the soil (Palarpawar and Ghurde, 1989). The survival of *C.acutatum* Simmonds ex. Simmonds in plant debris and in soil varied depending on soil moisture and temperature (Eastburn and Gublerg, 1992). Bean anthracnose fungus *C.lindemuthianum* (Sacc. and Magnus) Briosi and Cav. overwintered for four months in bean debris placed 0-20 cm. deep in soil in New York State (Dillard and Cobb, 1993). When infected leaves were stored in nylon bags buried in soil, sorghum anthracnose pathogen *C.graminicola* survived for five months (Misra and Sinha, 1996). Yoshida and Shirata (1999) studied the survival of *C.dematium* (Pers.:Fr.) Grove., the pathogen of mulberry anthracnose. They observed that the pathogen can overwinter in the infected mulberry leaves and served as primary source of inoculum for the following year. *C.coccodes* (Walker.) S. Hughes. survived in infected roots and fruits of tomato plants, which served as the primary source of inoculum (Dillard and Cobb,1998). Anoop and Kumari (2002) reported that *C.gloeosporioides* can survive in the infested plant parts in soil for upto 90 days and upto 150 days in the infested materials inside paper cover under laboratory conditions.

2.4 ANTAGONISTIC MICROORGANISMS OF *Colletotrichum*

Biological control of plant pathogens is gaining momentum since chemicals raise environmental issues. Chemical control of thippali disease is problematic. The fungicides cause adverse effect on the pharmacological compounds of this medicinal plant. So emphasis was mainly on biological methods of disease control using micro organisms. The most widely studied and used biocontrol agent is *Trichoderma* spp. The antagonistic property of *Trichoderma* spp. was first demonstrated by Weindling (1932) on *Rhizoctonia solani* Kühn. Elad *et al.* (1983) reported that *T.harzianum* has antagonistic property against a wide variety of plant pathogens. Gokulapalan and Nair (1984) reported that *Aspergillus niger* Van Tieghem, *A.flavus* Link. and *T.viride* Pers.ex.S.F. Gray have inhibitory action on *R.solani* Kühn infecting rice. *Aspergillus niger* isolated from the rhizosphere of coffee seedlings was antagonistic to the collar rot pathogen *R.solani in vitro* and hyperparasitised the pathogen completely in dual culture (Venkatasubbiah and Safeeulla,1984). Cristinzio (1987) reported that *Trichoderma* spp. and *Chaetomium* spp. had antagonistic property against *Phytophthora capsici* Leonian. Antagonistic effect of *A. niger* and *T. harzianum* against *R. solani* under *in vitro* condition was studied by Padmakumari (1989). Gabje and Lanjewar (1991) showed that under dual culture experiments, *A. niger* showed antagonism to a variety of plant pathogens. Antagonistic effect of *A. flavus* and *A. niger* on sugar cane smut whip after 10-15 days of the onset of monsoon was studied by Vaishnav *et al.*, (1992). Mukherjee and Sen (1992) reported that *Aspergillus terreus* Thom., *A. fumigatus* Fres. and *Penicillium citrinum* Thom. were antagonistic under *in vitro* conditions to *Macrophomina phaseolina*, a soil borne plant pathogen. *Trichoderma* spp. was effective in controlling many plant disease (Chet and Inbar, 1994; Sen, 2000). Aujla and Kaur (1993) studied the effect of culture filtrate of *T.viride* and *Aspergillus* spp. on germination of teliospores of *Neovossia indica* (Karnal bunt pathogen). They reported that the culture filtrate of all the three antagonists were effective in reducing the teliospore germination significantly under laboratory conditions. The sheath blight pathogen of rice, *R. solani* was controlled by the application of spore suspension of *A. terreus* Thom (Gogoi and Roy, 1993). Jeyarajan *et al.* (1994) reported that the talc based

formulation of *T.viride* and *Bacillus subtilis* were effective for controlling the root rot disease of groundnut. A report by Sarma *et al* (1996) revealed integrated disease management (IDM) with *T. harzianum* as a component for the management of foot rot of black pepper. The disease incidence was reduced from 25 percent to 15 per cent in the field. Jubina and Girija (1997) reported that a combination of fungal antagonists *Aspergillus* spp. and *Penicilliuum* spp. was found to be effective in delaying foliar infection in black pepper by *Phytophthora capsici.* Suriachandraselvan (1997) reported that soil application of *T.viride* in talc based formulation reduced the infection of *Macrophomina phaseolina* on sunflower to 33.33 per cent compared to 66.67 percent in control. Reghuchander *et al.* (1998) reported that seed treatment of *Trichoderma* spp. is effective for controlling dry rot of soyabean caused by *Macrophomina phaseolina. T.viride* and *T. harzianum* caused the maximum inhibition of mycelial growth of seed borne *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and cav. In dual culture studies (Ravi *et al.* ,1999). Shanmugam and Varma (1999) reported that *A. niger, A. flavus* and *T. viride* were found to be potential antagonists of ginger rhizome rot pathogen *viz., Pythium aphanidermatum* (Edison) Fitzpatrik by dual culture and cell free culture filtrate studies. Red rot of sugar cane caused by *Colletotrichum fulcatum* Went. was controlled by *Trichoderma* isolated from sugarcane rhizosphere (Nallathambi *et al.*, 2000). Maximum inhibition of growth of *Alternaria solani* (Ell and Mart) Jones and Grout was observed with metabolites of *Aspergillus flavus* and *A. terreus* of the phyllosphere under laboratory conditions (Singh and Singh, 2000). Mathivanan *et al.* (2000) reported that *T. viride* was equally effective as that of fungicides in controlling early and late leaf spots and rust in ground nut. Under field conditions, application of *A. terreus* into pressmud amended neutral soil showed highest efficiency in reducing sheath blight of rice caused by *R. solani* (Das and Roy, 2000). Anith and Manomohandas (2001) reported that combination of *T.harzianum* and *Alcaligens* sp. strain AMB 8 reduced the nursery rot disease of black pepper caused by *Phytophthora capsici* to 14.81 per cent as compared to 74.01 per cent disease in control. Alvira D'souza *et al.* (2001) reported that the three isolates of *T.harzianum* T_1, T_2, and T_3 had highest potential against the major betel vine pathogens. Dubey

and Patel (2001) reported that *T. harzianum* and *T. viride* was effective in inhibiting mycelial growth and sclerotia of *Thanatephorus cucumeris* in dual culture. Pant and Mukopadhyay (2001) reported that biocontrol agents like *T. harzianum* antagonize pathogens by antibiosis, competition mycoparasitism or other forms of direct exploitation. Anoop (2002) reported that talc based formulations of *T. harzianum* isolates T_1 and T_2 and *A. niger* isolates A_1 and A_2 were effective in controlling anthracnose of black pepper caused by *C.gloeosporioides.* Rajan *et al.* (2002a) reported that on screening a number of *Trichoderma* isolates *in vitro* and *in vivo* against foot rot of black pepper, *T.virens*-12 and *T. harzianum*-26 were found to be more effective and the isolate *T. harzianum*-26 was most adaptive to rhizosphere of black pepper. Rajan *et al.* (2002b) reported that *T.harzianum* isolated from Sikkim was found effective in controlling ginger rhizome rot disease substantially and controlled rhizome rot, root rot and mortality caused by both fungal and bacterial pathogens. *T. viride* Pers.ex.S.F. Gray.is effective in controlling *S.rolfsii* associated with jasmine wilt. Antagonists inhibit the sclerotial germination of *S.rolfsii in vitro* (Umamaheswari *et al.*, 2002). *T.viride* overgrew the colony of *Alternaria tenuisima* within 72 hrs. of incubation in dual culture, and caused lysis and protoplasmic disintegration of the mycelia of this pathogen (Lal and Upadhyay, 2002). Mohanbabu *et al.* (2002) reported that biological control efficiency of *T.viride* was increased by addition of neem cake. In cauliflower and tomato *T. harzianum* and *T. viride* at the site spatially separated from *S. sclerotiorum* inoculation resulted in a 30-70 percent suppression of stalk rot symptom caused by delay or suppression of spreading , blighting or blackening formation (Sharma and Sain, 2004). Sivaprasad *et al.* (2005) reported that *Trichoderma* spp. were among the most promising biocontrol agents for large scale application. Species of this genus were found highly successful for biocontrol of wide range of pathogens including *Armillaria mellea, Pythium* spp., *Phytophthora* spp., *R.solani, Sclerotium rolfsii, Sclerotinia* spp., *Fusarium* spp., *Heterobasidium* spp., *Aphanomyses* spp. etc. Patibanda and Sen (2005) reported that *Aspergillus niger* isolate AN 27 is effective against muskmelon wilt pathogen *Fusarium oxysporum f.sp. melonis.* Sivaprasad *et al.* (2005) reported that *T.viride* was effective against rhizome rot of ginger caused by *Pythium*

aphanidermatum. Ayana *et al.* (2005) reported that *T. viride, T. harzianum* and *A. niger* were effective in controlling damping off of Brinjal caused by *Pythium aphanidermatum.*

2.5 RESISTANCE INDUCERS

Plants can activate their resistance mechanisms upon contact with invaders. This is termed as induced or acquired resistance. Chemical activators of systemic acquired resistance (SAR) comprises inorganic compounds, natural compounds and synthetic compounds (Sticher *et al.,* 1997). The induction of disease resistance in plants in response to biotic and abiotic inducers is usually systemic in nature and is called as SAR. Criteria for a chemical to be considered as an activator is given by Rohilla *et al.* (2001). Kuc (2001) observed that SAR is the phenomenon by which defense mechanisms in plants are activated by pathogen or their metabolites or by a diverse group of structurally unrelated organic and inorganic compounds. There are several forms of induced resistance: the hypersensitive reaction (HR), SAR and induced systemic resistance (ISR) (Huang and Hsu, 2003). They have also studied different mechanisms involved in the development of systemic resistance.

2.5.1 Acibenzolar-S- methyl (ASM)

Fredrich *et al.* (1996) observed that ASM has demonstrated efficiency in reducing diseases of plants caused by a variety of bacterial, fungal and viral pathogens. ASM gave protection against wheat powdery mildew (Gorlach *et al.,* 1996). Ishii *et al.*, (1999) reported that ASM increased the resistance of Japanese pear to rust (*Gymnosporangium asiaticum*) and scab (*Venturia nashicola*). Foliar application of ASM protected the rock melons and hami melons from post harvest fungal diseases (Huang *et al.*, 2000). Bell pepper sprayed with ASM (Actigard 50 WG) showed resistance to subsequent infection with bacterial spot pathogen (*Xanthomonas axonopodis* pv. *vesicatoria*) (Romero *et al.,* 2001). Resistance activator ASM (Bion) activated resistance in many crops against broad spectrum of diseases caused by fungi, bacteria and viruses, at low concentrations (Oostendrop *et al.*,2001). Defense response is induced in cowpea against *Colletotrichum destructum*

after seed treatment with ASM (Akinwunmi *et al.,* 2001). Nair (2005) reported that ASM 37.5 ppm concentration showed maximum inhibition of mycelial growth of *R. solani* causing foliar blight of Amaranthus and minimum disease severity in combined treatment with *Bacillus subtilis* strain GBO3 and ASM. Nair (2005) conducted studies on the treatment of bacterial blight of Anthurium using chemical activators ASM, Salicylic acid etc. and reported that ASM 25 ppm and 50 ppm were effective in controlling the disease.

2.5.2 Salicylic acid (SA)

White (1976) reported that exogenous application of SA to tobacco plants resulted in disease resistance which has correlated with PR gene expression. SA induced a range of defense genes most notably those encoding the PR proteins, several of which have been shown to possess antifungal and antimicrobial properties (Bowels, 1990). Gaffney *et al.*, (1993) found out that the development of SAR is associated with the accumulation of SA derived from enhanced phenyl propanoid biosynthesis. Delaney *et al.* (1995) reported that SA is a natural phenolic compound present in many plants and is an important compound in the signal transduction pathway, involved in local and systemic resistance to pathogens. Vernooji *et al.*, (1995) found out that the exogenous application of SA was shown to mimic certain aspects of a pathogen infection resulting in both SAR gene expression and induction of SAR. SA plays an essential role in the establishment of SAR induced by a variety of pathogens or pathogen elicitors (Ryals *et al.*, 1996). Kalix *et al.*(1996) reported that SA could induce resistance in cucumber against *Cladosporium cucumerinum.* Studies conducted by Dempsey *et al.* (1999) revealed that the mechanism through which SA mediated SAR include alterations in the activity or synthesis of certain enzymes that increased the defense response and the generation of free radicals. Studies on diseases of tomato by Speltzer *et al.* (1999) showed that SA induced resistance against *Alternaria parasitica* and *A. solani.* Meena *et al.* (2001) observed that SA is involved in signal transduction pathway and is required for establishment of SAR. They also observed that foliar application of SA at the concentration of one µM significantly reduced the leaf spot disease intensity and increased the pod yield

under green house condition. Amarersh *et al.* (2001) reported that root rot caused by *R. solani* in cowpea was reduced by foliar treatment with SA 0.02 percent solution. Rohilla *et al.* (2001) reported that SA played a key role in the induction and maintenance of plant resistance against a wide range of pathogens.

2.5.3 Hydrogen peroxide (H_2O_2)

Chen *et al.* (1993) reported that H_2O_2 induced expression of defense related genes associated with SAR. Thus the action of SA in SAR is likely mediated by elevated amounts of H_2O_2. H_2O_2 induced the accumulation of free benzoic acid and SA in tobacco (Leon *et al.*, 1995). Studies conducted by Bestwick *et al.* (1997) revealed that inoculation with wild type cells of *Pseudomonas syringe* pv *phaseolicola* caused rapid hypersensitive reaction (HR) during which highly localised accumulation of H_2O_2 was found in the plant cells adjacent to cells attacked by the bacteria. Steven *et al.* (2003) reported that H_2O_2 played a central role in launching the defense response during stress in plants. Amirsadegi *et al.* (2006) observed that SA specifically inhibited the catalase activity *in vitro* and induced an increase in H_2O_2 concentration *in vivo*.

2.5.4 Calcium chloride ($CaCl_2$)

Foyer *et al.* (1997) reported that many of the stress induced pathways induced SA and calcium ions. Calcium ions have been implicated in signaling in response to a variety of stress (Sanders *et al.* 1999; Knight, 2000; Jane and Marc 2002).

2.6 PLANT BASED CHEMICALS

The major plants used for extracting their products for plant disease management are *Azadiracta indica* (neem), *Ocimum* spp. (tulsi), *Lantana camera* (Lantana) etc. Singh and Bhatt (2002) reported that neem based formulation PJM-C was very effective in managing rust of French bean along with fungicides. Pramod and Singh (2005) reported that marketed products of neem like Neemgold and Neemachook were found to be highly effective in controlling leaf blight of wheat.

Surendran (2005) reported that newer botanicals like Tricure, Achook, Wanis and Neemgold were highly effective against rice sheath blight. Bharathi *et al.* (2005) reported that application of Wanis combined with *P. fluorescens* on tomato induced PO, PPO and PAL and enhanced accumulation of phenol when challenged inoculated with leaf blight pathogen. Sunder *et al.* (2005) studied the effect of various formulations like Achook, Neemazal, Neemgold, Spictaf etc. and reported that Spictaf reduced the bacterial blight of rice considerably. Sahayarani *et al.* (2005) reported that the extract of Lantana reduced the spore germination of *Oidium phyllanthii.* Shan *et al.* (2005) also studied the effect of Lantana on spore germination of *Macrophomina phaseolina.*

2.7 NEEM CAKE

Neem based products, neem extracts and neem cake were reported to be very effective in containing plant diseases. They also helped to enhance the activity of antagonistic micro organisms. Kannayan and Prasad (1983) reported that seedling infection of *R. solani* is reduced by using neem cake. Meena and Mariappan (1993) reported that neem extract inhibited the mycelial growth and spore germination of *Fusarium* spp. infecting pea seeds. Diyora and Khander (1995); Lodha (1995) reported that neem cake is effective in controlling cumin wilt caused by *F. oxysporum f.sp. cumini.* Neem cake and FYM application were found to enhance antagonistic fungi in soil and helped in bringing the suppression of wilt of cowpea (Ushamalini *et al.*, 1997). Haque *et al.* (1998) showed that neem cake was very effective in controlling *R. solani* infecting sunflower. Padmodaya and Reddy (1999) reported that organic amendments like neem cake, FYM etc. significantly reduced the seedling disease of tomato caused by *F. oxysporum f. sp. lycopersici.* Meena and Muthusamy (1999) reported that neem cake gave very good suppression of *R.solani* affecting rice under glass house conditions. Sachin *et al.* (2002) studied the effectiveness of neem cake in reducing the root rot incidence of cotton by amending it in the soil both in glass house and in the field. Neelamegam (2005) reported that wheat bran based inoculum of *T. viride* and neem leaf powder and their combined application is effective in reducing damping off of tomato to 85.71%. Rajani *et al.*

(2005) observed that minimum root rot incidence (21.06 %) and maximum seed yield (2128 kg/ha) were recorded in soil application of neem cake (500 kg/ha) with *T. harzianum* formulation (5 kg containing 10^6cfug^{-1}). Vidyasagar and Akbar (2005) reported that application of 10% neem cake effectively controlled anthracnose of oil palm caused by *C.gloeosporioides.*

2.8 MECHANISM OF PARASITISM

Brian and Mc Grawn (1945) reported that *T. viride* produced viridin which is a second highly fungistatic antibiotic. Dennis and Webster (1976) found that *T. viride* and *T. polysporum* produced Trichodermin (chloroform soluble antibiotic). *T. hamatum* produced peptided antibiotics. Papavizas (1985) reported that *Trichoderma* and *Glyocladium* were not only good sources of various toxic metabolites and antibiotics, but also of various enzymes such as exo and endo glucanases cellobiases and chitinases. *T. harzianum* produced cell wall lytic enzymes such as cellulose, chitinase and β-1,3- glucanase and caused lysis of mycelia of *R. solani* (Elad *et al.*, 1983; Jones *et al.*, 1974; Tronsmo *et al.* 1993). Zeppa *et al.*, (1990) reported that the volatile metabolites produced by *T. viride* are lactones, alcohols and terpene derivatives. A new tetracyclic diterpene $C_2OH_28O_2$ was isolated from the culture filtrate of a strain of *T. viride* that exhibited antifungal activity against *S. rolfsii* (Mannia *et al.*, 1997). *A. niger* AN 27 produced different hydroxamate and catecholate group of compounds effective against different soil borne pathogens (Mondal and Sen, 1999). Studies conducted by Umamaheshwari *et al.* (2002) reported that the volatile metabolites produced by *T. viride* was effective against *S. rolfsii* causing jasmine wilt. The percentage disease inhibition was 61.11%. *T. harzianum* and *A.niger* causes excessive granulation, vacoulation and disintegration of hyphae of *C.gloeosporioides. T. harzianum* isolates also found to coil around and penetrate the hyphae of *C.gloeosporioides* (Anoop, 2002). Roy (2003) reported that the potential of fungal isolates to produce mycotoxins also vary widely with the variation of substrates as well as with the changes of the strains. *A. flavus* strains mainly produced Afl B_1 and Afl B_2 *A. ochraceous* produced Ochr A.

2.9 MASS MULTIPLICATION OF FUNGAL ANTAGONISTS

Papavizas *et al.* (1984) reported that for biological control of plant pathogens, it is necessary to mass produce the promising antagonists rapidly in the form of spores, mycelia or mixture. Further studies by Papavizas (1985) revealed that inorder to improve the biocontrol efficiency, several formulations of biocontrol agents have been developed. Several isolates of *Trichoderma* spp. produced large amounts of biomass containing conidia and chlamydospores in both liquid and solid media, containing inexpensive ingredients like molasses and brewers yeast (Lewis and Papavizas, 1985). The standardization of storage condition for increasing shelf life of *Trichoderma* formulations was done by Nakkeeran *et al.* (1997). The superiority of PDA over molasses, brewer's yeast etc. as a medium for biomass production was demonstrated by Prasad *et al.* (1997). Prasad and Rangeshwaran (2000) observed that kaoline and talc were better carriers of *T. harzianum*. Cultures multiplied by liquid fermentation are mixed with carrier materials such as talc, gypsum and peat and packed for marketing. Umamaheshwari *et al.* (2002) described the method for preparation of talc based formulation of *T. viride*. Anoop (2002) reported that talc based formulation of *T. harzianum* isolates T_1 and T_2 and *A.niger* isolates A_1 and A_2 were effective in controlling anthracnose of black pepper.

2.10 METHOD OF APPLICATION OF BIOCONTROL AGENTS

Formulated products of biocontrol agents were applied to the plants by different methods. Aqueous suspension of conidia of *T. viride* and *T. polysporum* (Link ex Pers.) Rifai were used for spraying the plants in the field at early flowering to control storage rot in strawberry (Tronsmo and Dennis, 1977). Coating of mung bean seeds with a culture of bacterium, *Bacillus megaterium* could control damping off disease (Tschen and Kuo,1981). De Oliveira *et al.* (1984) reported that the applicatiomn of *T. harzianum* to the soil as conidial suspension @10^6 conidia per ml during the transplanting period significantly reduced the severity of white rot of garlic caused by *Sclerotium sepivorum* Berk. Foliar application of *T. harzianum* @10^8 spores per ml reduced the leaf spot of wheat caused by *Cochliobolus sativus* (Ito & Kuribayashi) Drechs. Ex. Dast. (Biles and Hill,1988). Zhou and Reeleder

(1989) reported that the foliar application of spore suspension of *Epicoccum purpurascens* Link significantly reduced white mold incidence on snap bean. Gogoi and Roy (1993) reported that the application of spore suspension of *A.terreus* Thom. reduced the infection by sheath blight pathogen, *R.solani* in rice. Foliar application of *Aspergillus* spp. @10^6 spores per ml reduced the incidence of bacterial leaf blight of rice (Saikia and Chowdhary, 1993). Tosi and Zazzerini (1994) reported that there was an increased antagonistic effect when fungal isolates were added to soil as air dried inoculum rather than as seed treatment in rust infected safflower seeds. Spore suspension of *Penicillium chrysogenum* Thom. isolates significantly reduced the development of chocolate lesions of *Botrytis fabe* Delacr. on faba beans (*Jackson et al.*, 1994). Angappan *et al.* (1996) observed that muskmelon seedlings raised from soils treated with Kalisena SL, a formulation of *A.niger* AN 27, showed resistance to *F. xoysporum melonis*. Foliar spray of *T. harzianum* strain T-22 controlled powdery mildews on Cathranthus and pumpkins and *Botrytis cinerea* Pers. on strawberry and grapes (Harman, 2000). Fusarium wilt of musk melon was controlled by treating the seeds with Kalisena SD @ 8g per kg and soil with Kalisena SL @30g per pit (Sen, 2000). Susheelabhai *et al.* (2000) reported that soil application of *T. harzianum* in carrier medium @ 1kg per plant at 23 × 10^8 $cfug^{-1}$ twice a year was effective in reducing the soil population of *Phytophthora spp.* and reducing the capsule rot of cardamom. Anoop (2002) reported that foliar as well as soil application of T.harzianum and *A. niger* were effective in reducing anthracnose of black pepper.

2.11 DEFENSE RELATED ENZYMES (DRE)

2.11.1 Peroxidase (PO)

Bonner (1950) reported that PO is one of the key enzymes involved in disease resistance. It has got an important role in the biosynthesis of lignin, oxidation of many mono and diphenolic compound and aromatic amines to highly toxic quinines in the presence of H_2O_2. Macko *et al.* (1968); Urs and Dunkavy (1974) found that the enzyme PO itself is toxic to many pathogens. PO activity was frequently increased in plants infected by pathogens and its activity is closely related with disease resistance (Kosuge, 1969). Friend and Thornton (1974) reported that

PO activity increased in both resistant and susceptible varieties of potato tubers inoculated with *P. infestans.* Quantitative and qualitative changes in isoperoxidase activity in susceptible and resistant cultivars of *Capsicum annuum* L. to *P. capsici* was demonstrated by Alcazar *et al.* (1995). Pereira *et al.* (2000); Guleria *et al.* (2001) reported the increase in PO activity in cassava inoculated with *Xanthomonas* spp. and pea inoculated with *Erysiphe pisi* respectively. Howell *et al.* (2000) studied the biochemistry of biocontrol of damping off of cotton by *T. virens* and reported an increase in host resistance due to enhanced PO activity and terpenoid synthesis. Hammerschmidt *et al.* (1982) reported that inoculation of the pathogen *C.lagenarium* enhances PO activity in cucumber. Yamamoto *et al.* (1978) observed that activity of PO increased in resistant oat plant inoculated with crown rust pathogen. Rahayuningsih (1990) studied PO activity in relation to *Phytophthora palmivora* in *Piper hirsutum* and *P. nigrum.* He observed that *P. hirsutum* was highly resistant to *P. palmivora* and had high PO activity. *P. nigrum* was highly susceptible to *P.palmivora* and PO activity was very low in this. Lizzy and Coulomb (1991) reported that *P. capsici* infection enhanced PO and catalase activity in *C. annuum.*

2.11.2 Poly phenol oxidase (PPO)

Kosuge (1969) reported that PPO is a copper containing enzyme that oxidized phenolics to highly toxic quinones and involved in the terminal oxidation of the diseased plant tissue. This was attributed for its role in disease resistance. PO activity is closely related with disease resistance. Avdiushko *et al.* (1993) reported enhanced PO activity in cucumber leaves due to infection by *Colletotrichum lagenarium* or tobacco necrosis virus.

2.11.3 Phenyl alanine ammonia lyase (PAL)

Dickerson *et al.* (1984) described the procedure for determining PAL activity in plants. Studies conducted by Crammer *et al.*, (1985) revealed that PAL and other enzymes were induced in plants when challenged by pathogens. Meena *et al.*, (2001) reported that PAL is the first enzyme in phenyl propenoid metabolism in higher

plants and it has been suggested to play a significant role in regulating the accumulation of phenolics, phytoalexins and lignins. These are the key factors responsible for disease resistance. Jebakumar *et al.* (2001) observed the PAL activity in both leaf and root tissues of three black pepper varieties (tolerant P24 and susceptible Panniyoor and Subhakara) in healthy and *P. capsici* infected tissues. They concluded that the infection *of P. capsici* enhanced PAL activity and the variety P24 expressed maximum activity Chakraborty *et al.* (2002) reported that healthy leaves of grey mildew resistant plants exhibited less PAL activity than the healthy leaves of susceptible variety. Jha and Jalali (2005) reported that exposure of seedlings of pea to root rot pathogen *F.solani* increased PAL activity. Increased expression of PAL activity by *T.viride* may play a vital role in protecting pea plants against the invasion of *F.solani f.sp. pisi.*

Materials and Methods

3. MATERIALS AND METHODS

3.1 SURVEY ON THE INCIDENCE OF DISEASES OF THIPPALI

Fortnightly survey was conducted in the medicinal garden, of College of Agriculture, Vellayani, Thiruvananthapuram and Ayurveda Research Institute, Poojappura, Thiruvananthapuram during January to December 2005 to study the fungal diseases affecting thippali. Diseased plant samples were collected from both the above locations and the symptomatology were studied.

3.2 ISOLATION OF THE PATHOGEN

The pathogen causing leaf spot of thippali was isolated from the naturally infected leaves from the medicinal garden of College of Agriculture, Vellayani and Ayurveda Research Institute, Poojappura. Isolation of the pathogen was done following standard tissue isolation technique on Potato Dextrose Agar (PDA) medium. Fungal growth from the plated tissue were transferred to PDA slants. The culture of pathogen was maintained by subculturing at periodical intervals and stored under refrigeration for further studies.

3.3 PATHOGENICITY TEST

The pathogenicity of the isolate was proved following Koch's postulates. Healthy thippali plants were inoculated on the upper surface of the leaves with seven day old sporulated culture of the pathogen with little wounds. Humidity was provided by placing a thin layer of moist cotton over it. The fungus was then reisolated from the necrotic portion after development of the symptom.

3.4 MORPHOLOGY OF *C.gloeosporioides* of THIPPALI

3.4.1 Colony morphology

A 5 mm disc of *C.gloeosporioides* from an actively growing seven day old sporulated culture was inoculated at the centre of a 90 mm Petri plate with sterile PDA and incubated at room temperature (28±2°C). Colony colour and sporulation

density were observed after 10 days. For determining mycelial dry weight, 50 ml potato dextrose broth was poured in 250 ml conical flask and autoclaved at 1.04 kg cm^{-2} for 20 min. Each isolate was inoculated separately in this broth and flasks were incubated at room temperature (28±2^{0}C) for 10 days. Mycelium was filtered on Watman No.1 filter paper and dried in the oven at 60°C. It was weighed from the next day till constant weight was observed.

3.4.2 Conidial morphology

Conidial size was measured using a standardized microscope with micrometer. The average size of 100 conidia were taken.

3.5 ISOLATION OF *Colletotrichum spp.* FROM BLACK PEPPER AND BETEL VINE

Since black pepper and betel vine belong to Piperaceae and were reported to be the hosts of *Colletotrichum* spp. a study was made to compare the two isolates of black pepper and betel vine with that of thippali. The diseased samples were collected from the black pepper and betel vine plantations of the College of Agriculture, Vellayani, Thiruvananthapuram. Isolation of the pathogen was done as in 3.2.

3.5.1 Colony and conidial morphology of Black pepper and Betel vine isolates

Colony and conidial morphology of black pepper and betel vine isolates were studied as described in 3.4.

3.6 CROSS INOCULATION STUDIES

The three *C.gloeosporioides* isolates were cross inoculated *i.e.*, thippali isolate was inoculated on black pepper and betel vine plants. Black pepper isolate was inoculated on thippali and betel vine plants and betel vine isolate was inoculated on thippali and black pepper plants. The inoculations were made using one week old sporulated cultures of the pathogens on PDA. Humidity was also provided.

Observations were taken on the lesion development in all the leaves inoculated on fourth, seventh and tenth day after inoculation.

3.7 SURVIVAL OF *C.gloeosporioides*

The method described by Yoshida and Shirata (1999) was followed for this study with slight modification.

3.7.1 Survival of *C.gloeosporioides* on infected leaves in the soil

Diseased leaves were collected from the medicinal gardens of Instructional farm, College of Agriculture, Vellayani. The infected leaves were washed thoroughly in running water and were cut into small bits. Three earthen pots were filled with nonsterilized soil from the thippali field. Approximately 2 kg. soil was taken in each pots. Two hundred and fifty g. infected leaf bits were placed in each pot at a depth of 30 cm from the surface and covered with soil. The pots were then placed at out doors and watered on alternate days.

Leaf bits were collected from the pots at an interval of 15 days and plated on PDA, after surface sterilization. This was followed up to 60 days. Later serial dilution of the soil from the pots were made and 10^{-4} dilution was plated on PDA, since leaf bits could not be traced out. Plates were incubated at room temperature. Survival of the pathogen was determined based on the development of viable fungal colonies. The experiment was continued for 165 days.

3.7.2 Survival of *C.gloeosporioides* on thippali leaves under laboratory conditions

Naturally infected thippali leaves were collected from medicinal garden of Instructional farm College of Agriculture, Vellayani, Thiruvananthapuram. After thoroughly washing in running water these were air dried for 24 hours The air dried leaves were placed in brown paper cover of size 32x26 cm sealed with staples and kept under laboratory conditions. Samples were taken from these covers at 15 days

interval for a period of 165 days. The survival of the pathogen was determined as described under 3.7.1.

3.8 ISOLATION OF MICROORGANISMS FROM RHIZOZSPHERE AND PHYLLOSPHERE OF THIPPALI

3.8.1 Isolation of mycoflora from thippali phyllosphere

Dilution plate technique was used for the isolation of mycoflora from the thippali leaf surface (Timonin,1940). Disease free leaf samples collected from plants growing in the infected fields of College of Agriculture,Vellayani and Ayurveda Research Institute, Poojappura were used for the isolation. Leaf bits were cut using sterilized blades. One gram of the leaf bits were transferred to 100ml of sterile distilled water in a 250ml conical flask and shaken for 20 min. in a rotary shaker. Serial dilutions were made and the final dilution of 10^{-4} was used for the study. One ml of this dilution was plated on Martin's Rose Bengal Agar medium for isolating fungi present in the phyllosphere. Replications were also maintained. The plates were then incubated at room temperature for 48-72 hrs. The fungal colonies developed were examined and transferred to PDA plates by the hyphal tip culture method. The purified cultures were then stored in PDA slants under refrigerated condition for identification and subsequent antagonism studies. The same experiment was repeated for confirmation.

3.8.2 Isolation of mycoflora from thippali rhizosphere

Soil samples were collected from the rhizosphere of healthy thippali plants from both the above locations. The rhizosphere mycoflora were obtained by the dilution plate technique as described earlier using one gram rhizosphere soil in 100 ml distilled water. Pure culture of the fungi were maintained for identification and antagonistic studies. The same experiment was repeated for confirmation.

3.8.3 Isolation of bacteria from thippali phyllosphere

The procedure described earlier was followed. A 10^{-6} dilution was used for the study. This dilution was plated on soil extract agar medium. The plates were then incubated at room temperature for 48-72 hrs. The bacterial colonies developed were examined and transferred to nutrient agar plates by streaking and subsequently to nutrient agar slants. The purified cultures were then stored under refrigerated condition for identification and subsequent antagonism studies. The same experiment was repeated for confirmation.

3.8.4 Isolation of bacteria from thippali rhizosphere

The procedure described in 3.8.2 was followed. A dilution of 10^{-6} was used for plating. The bacteria were maintained for identification and antagonism studies. The same experiment was repeated once more for confirmation.

3.9 *IN VITRO* STUDIES

3.9.1 *In vitro* screening of fungal isolates against *C.gloeosporioides*

The fungal isolates were tested for their antagonistic effect on *C.gloeosporioides* by the dual culture technique (Dickson and Skidmore 1976). Agar disc of 5 mm dia. were cut from the edge of a vigorously growing seven day old sporulated culture of *C.gloeosporioides* and this was placed 2 mm away from the periphery of Petri plates. On the opposite side, 2 mm away from the periphery, 5 mm culture disc of antagonistic fungi cut from a vigorously growing one week old culture was placed. Three replications were also maintained. The plates were incubated at room temperature (28 ±2°C) for five days. Control was kept with 5 mm disc of *C.gloeosporioides* at the centre of PDA plate. Antagonists that exhibited highest percentage inhibition within a short period was selected. Percentage inhibition of mycelial growth was calculated using the formula

$$I=100\frac{(C-T)}{C}$$

Where I - Percentage inhibition of mycelial growth

C - Growth of the pathogen in the control plates (cm).

T - Growth of the pathogen in dual culture (cm).

3.9.2 *In vitro* screening of bacterial isolates.

Bacterial isolates were tested for their antagonistic effect on *C.gloeosporioides* by dual culture technique. Procedure described earlier was used. The bacterial isolate was streaked 2 mm away from the periphery opposite to pathogen disc. Replications were also maintained. Pathogen inhibition was calculated as earlier.

3.9.3 *In vitro* screening of resistance inducers

Five chemicals such as Salicylic acid (SA), Azibenzolar-S methyl (ASM), Hydrogen peroxide (H_2O_2), Calcium chloride ($CaCl_2$) and Borax which are reported to have systemic resistance inducing capacity were tested against *C.gloeosporioides* Each chemical was tested at three concentrations as follows.

Chemicals	Concentration			Reference
	C_1	C_2	C_3	
SA	1 g/l.	2 g/l.	3 g/l.	Sunder *et al.*(2005) Meena *et al.* (2001)
ASM	10 ppm.	25 ppm.	50 ppm.	Huang and Hsu (2003)
H_2O_2	1 ml/l	2 ml/l	3 ml/l	Sticher *et al.* (1997)
$CaCl_2$	1 g/l	2 g/l	3 g/l	Sunder *et al.* (2005)
Borax	1 g/l	2 g/l	3 g/l	Sunder *et al.* (2005)

All these methodologies were adopted with slight modification to suit this experiment. PDA was prepared and sterilized. These chemicals were then added independently to the media to get the required concentrations. After plating the

sterile needle. One such block was placed at the centre of a sterile microscopic slide. All the four sides of the agar blocks were inoculated with one antagonistic fungal spore. In the same method another fungal block was inoculated with the second antagonist. Both the blocks were then covered with sterile cover slilps. The slides were incubated separately in moist chamber for 48 hrs. at room temperature (28±2°C). The cover slip was then gently lifted and a drop of 15 percent alcohol was placed at the centre and before drying the cover slips were mounted on lactophenol cotton blue. The slides were then observed under microscope. Based on their spore morphology, they were identified at generic level. To continue the experiment a correct identification of the antagonists were made at MACS, Pune. These were identified as *T. viride* Per. : S. F. Gray and *A. terreus* Gr.

3.10.1.2 Mycoparasitism of the selected fungal antagonists

The mechanism of mycoparasitism of the selected fungal antagonist was studied adopting dual culture technique used by Anoop (2002)

3.10.1.3 Antibiosis

Effect of volatile and nonvolatile compounds produced by antagonists on inhibition of the pathogen was studied adopting the method developed by Pant and Mukopadhyay (2001) with slight modifications.

3.10.1.3.1 Effect of volatiles

The selected two antagonists were centrally inoculated by placing 5 mm culture disc from seven day old culture cut with sterilized cork borer on separate PDA plates. The top of each petri plates was replaced with bottom of petri plate inoculated with 5 mm disc of *C.gloeosporioides*. The paired plates were sealed with cellophane adhesive tape and incubated at room temperature. Control was kept by keeping pathogen inoculated plate over uninoculated plate. Replications were also maintained. Percentage suppression was calculated as earlier.

3.10.1.3.2 Effect of non volatiles

5 mm culture disc of both the selected antagonists were separately inoculated into 100 ml sterilized Potato Dextrose Broth (PDB) in 250 ml conical flask. The flasks were then plugged tightly using cotton and incubated at room temperature for 10 days. The culture filtrate of both the antagonists were obtained by passing the broth successively through Whatman No. 1 filter paper. This filtrate was then sterilized for complete removal the spores. These were then separately added to sterilized, melted PDA so as to get the final concentration 10 percentage v/v. The medium was poured into Petri plates (20 ml / plate) and plates were inoculated with 5 mm culture disc of *C. gloeosporioides*. PDA plates inoculated with *C. gloeosporioides* but not amended with culture filtrate of antagonists served as check. The plates were then incubated at room temperature. Observations were made and percentage suppression was calculated as in 3.9.1

3.11 INOCULUM PREPARATION AND APPLICATION

3.11.1 Preparation of pathogen inoculum for soil application.

The pathogen was mass multiplied on rice bran. The composition was as follows.

Rice bran	-	250g.
Sucrose	-	25g.
Multivtamine tablets	-	3 Nos.
Distilled water	-	100ml.

This was filled in poly propylene bags and autoclaved at 1.04 kg cm^{-2} for 20 min. After autoclaving, the propylene bags were inoculated with agar discs of 5 mm dia. (15 Nos.) cut from the edge of a vigorously growing culture of *C.gloeosporioides*. The propylene bags were then incubated at room temperature for 15 days. When the propylene bags showed full growth of mycelium, this was taken for field application. Pathogen inoculum was given at the rate of 10 g per pot. A second application was given after 10 days of first application of inoculum.

3.11.1.1 Preparation of pathogen inoculum for foliar application

The procedure described by Laha and Venkataramanan (2001) was adopted with slight modification. 5 mm culture disc of pathogen was inoculated in 250 ml conical flask containing 100 ml sterile water enriched with 0.5 g peptone. When the mycelial mat completely covered the liquid surface, it was thoroughly agitated and the liquid was filtered through a muslin cloth. Foliar spray was done twice using this conidial suspension of *C. gloeosporioides* containing 10^6 conidia per ml on the same day of soil application of pathogen

3.11.2 Preparation of the talc based formulation of the selected fungal antagonists

The fermented biomass of selected antagonists were prepared by a slightly modified liquid fermentation process of Papavizas *et al.* (1984). Five hundred ml. potato dextrose broth each was taken in 1L conical flasks and autoclaved. After that it was inoculated with 5 mm culture disc of antagonists separately. This was incubated at room temperature for 15 days to obtain sufficient propagules in the mycelial mat. The mat was then carefully taken and pressed in filter paper to remove moisture content and then air dried. It was sun dried for an hour and then powdered in a mixer grinder. The resulting powder was mixed with sterilized talc at the rate of 25 g per 100 g of talc. One percent of Carboxy Methyl Cellulose (CMC) was added to this and stored at room temperature.

3.11.3 Layout of pot culture experiment for the ecofriendly management of leaf spot or blight of thippali

A pot culture experiment was laid out at the Department of Plant Pathology College of Agriculture, Vellayani. 12" pots were filled with 2kg potting mixture. The pots were planted with three uniform cuttings of thippali showing approximately uniform leaf spot symptom. Experiment was repeated twice. Details of the experiment are as follows.

Treatment Details

Design : CRD

Treatments : 17 (15 + 2)

Replication : 3

Variety : Viswam

T_1 - *Trichoderma viride* talc based formulation (soil application @10 g/kg potting mixture + foliar applicatoion @ 1g/l)

T_2 - *Aspergillus terreus* talc based formulation (soil application @10 g/kg potting mixture + foliar applicatoion @ 1g/l)

T_3 - Ovis @ 1 g/l (foliar application)

T_4 - SA @ 1 g/l (foliar application)

T_5 - Neem cake 10g/kg potting mixture (soil application)

T_6 - T_1T_2 - *T.viride* +*A.terreus*

T_7 - T_1T_3 - *T.viride* + Ovis

T_8 - T_1T_4 - *T.viride* + SA

T_9 - T_1T_5 - *T.viride* + Neem cake

T_{10} - T_2T_3 - *A.terreus* + Ovis

T_{11} - T_2T_4 - *A. terreus* + SA

T_{12} - T_2T_5 - *A. terreus* + Neem cake

T_{13} - T_3T_4 - Ovis + SA

T_{14} - T_3T_5 - Ovis + Neem cake

T_{15} - T_4T_5 - SA + Neem cake

C - Control (Pathogen inoculated)

AC - Control (Uninoculated)

3.11.4 Application of the pathogen inoculum

3.11.4.1 Application of pathogen inoculum to soil

When the rice bran in the polypropylene cover was completely covered with mycelium (after 20 days of incubation), this was taken for field application.

Pathogen inoculum was given at the rate of 10 g per pot. A second application was given 10 days after first application.

3.11.4.2 Foliar application of the pathogen inoculum

Foliar spray was done twice using the conidial suspension, on the same day of soil application. This contained 10^6 conidia per ml.

3.11.5 Determination of percentage disease index

Disease index was calculated using a 0 - 9 score, just prior to application of treatments. The score chart was developed based on the score developed by Anoop (2002) for scoring the anthracnose of black pepper.

Description

0 - No infection

1 - 1-3 lesions

3 - 3-5 lesions

5 - 5-7 lesions

7 - 7-9 lesions

9 - More than 9 lesions with blighting

Percentage disease index (PDI) was calculated using the formula of Mayee Dater (1986)

$$PDI = \frac{\text{Sum of grades of each leaf}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

3.12 *FIELD APPLICATION* OF ECOFRINDLY MATERIALS AND THEIR COMBINATIONS

All the treatments were applied twice at 15 days interval after 10 days of application of pathogen inoculum.

3.12.1 Soil application of the antagonists

Talc based formulation of the fungal antagonists applied to the soil at the base of thippali cuttings twice at 15 days interval starting from 10 days after second application of the pathogen inoculum. The antagonists inoculum was applied at the rate of 10 g per kg of soil after raking the soil without disturbing the roots of thippali plants. This was then properly mixed with soil.

3.12.1.1 Foliar application of the fungal antagonists

For this one percent aqueous suspension of the formulated product was prepared and sprayed using a hand sprayer. The disease index was recorded at periodic intervals.

3.12.2 Foliar application of resistance inducers

The resistance inducers showing least inhibition under *in vitro* condition was selected for field application. Concentrations was also fixed on the criteria of least pathogen inhibition. Disease index was recorded at periodic intervals as earlier.

3.12.3 Foliar application of plant based chemicals

Plant based chemical showing maximum inhibition under *in vitro* condition was selected for field application. Concentration was also fixed on the basis of maximum inhibition. Disease index was recorded at periodic intervals as earlier.

3.12.4 Soil application of neem cake

Neem cake was applied to the pots at the rate of 10g/kg potting mixture as fixed in the technical programme. Disease index was recorded at periodic intervals.

3.12.5 Combination treatments

Combination treatments were given as determined in 3.11.3.

3.13 ESTIMATION OF DEFENSE RELATED ENZYMES

3.13.1 Estimation of Peroxidase (PO)

Peroxidase activity was determined according to the procedure described by Srivastava (1987).

3.13.2 Estimation of Poly Phenol Oxidase (PPO)

PPO activity was determined as per the procedure given by Mayer *et al,*. (1965).

3.13.3 Estimation of Phenyl alanine ammonia lyase (PAL)

PAL activity was analysed based on the procedure described by Dickerson *et al.* (1984).

All the above enzymes were estimated 45 DAT to the plants. Details of buffers used were presented in appendix 1.

Results

4. RESULTS

4.1 SURVEY ON THE INCIDENCE OF DISEASES OF THIPPALI.

Survey conducted for a period of one year revealed that in both the locations leafspot and blight were the major disease on thippali. The major fungal disease observed at both these locations was leaf spot which ultimately leads to shot hole and blighting. Leaves were the major site of infection. Occasionally spike infection was also observed. The locations surveyed and disease observed was presented in Table 1.

4.1.1 Symptomatology of the disease.

The disease appeared as small brown spots of 1-2 mm dia. which enlarged upto 7-8mm dia. with an yellow hallow around it (Plate-3). Small pinhead like acervuli of the fungus can also be seen on the spots. The spots coalesced to give blighted appearance. In the later phase of disease development, shot hole like symptoms and defoliation occurred. The pathogen also affected the spikes. But the incidence of spike infection was very less during the study. Blighting reduced the photosynthetic capacity of the leaves.

4.2. ISOLATION OF THE PATHOGEN

The pathogen was isolated from naturally infected thippali leaves following standard tissue isolation procedure on Potato Dextrose Agar (PDA) medium. The pathogen was identified as *Colletotrichum gloeosporioides* by studying the cultural and conidial morphology.

4.3. PATHOGENICITY TEST.

Pathogenicity was proved following Koch's postulate on thippali plants, variety Viswam. Typical symptom of leaf spot (necrotic lesion of 0.8 cm dia. with a distinct yellow hallow) was reproduced within six days.

Plate3 **Thippali leaf showing leaf spot with yellow hallow**

Plate 4 **Thippali isolate of *C. gloeosporioides* on PDA**

Plate 5 **Conidia of thippali isolate of *C. gloeosporioides*** ***(100x)***

Plate 3

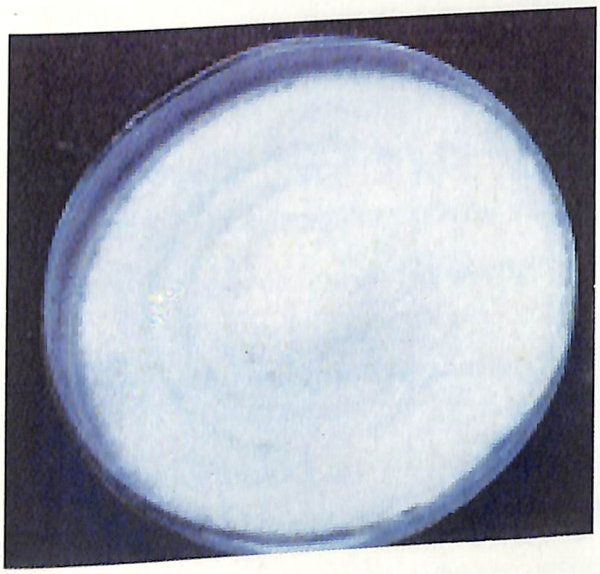


Plate 4

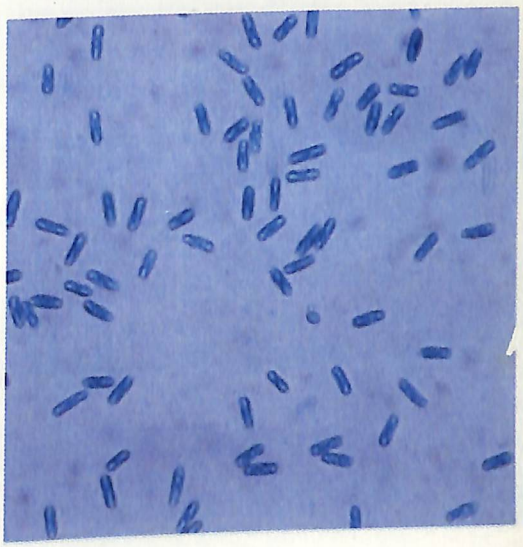


Plate 5

4.4 MORPHOLOGY OF *C.gleosperioides* OF THIPPALI

4.4.1 Colony morphology.

Colony morphology of the pathogen was studied in PDA (Plate 4). The details are given in Table 2. The fungal colony was evenly felty with evenly white in colour, with shy sporulation Average colony diameter was 8.9 cm and mycelial dry weight was 280.1mg.

4.4.2. Conidial morphology

Conidial morphology of the pathogen was studied. The data are presented in Table 2 (Plate-5). The conidia were cylindrical, hyaline with oil globule at the centre. The average size of conidia was 12.69 x 4.7µm with a range of 14.1-18.8x 4.7µm.

4.5. ISOLATION OF *C.gloeosporioides* FROM BLACK PEPPER AND BETEL VINE.

Since black pepper and betel vine are reported to be hosts of *Colletotrichum,* pathogen associated with fungal pollu of black pepper and anthracnose of betel vine were isolated. Based on the cultural characteristics and conidial morphology, both the isolates were identified as *C.gloeosporioides.*

4.5.1 Colony and conidial morphology of black pepper and betel vine isolates.

Colony morphology of both the isolates are described in Table 3 (Plate 6a,b&7a,b). Black pepper isolate was slightly fluffy unevenly white with grey margins. Spore mass is light salmon coloured and it is a moderately sporulating fungus. Betel vine isolate is felty, evenly white with dark salmon coloured spore mass. It was a highly sporulating fungus. Average conidial size of black pepper isolate was 14.63x4.7µm with a range of 14.1-23.5 x 4.7µm. The average conidial size of betel vine isolate was 15.52 x 4.7µm with a range of 14.1-23.5x4.7µm.

Table 1. Survey of diseases on thippali

Location	Disease observed
Medicinal garden, College of Agriculture, Vellayani, Thiruvananthapuram	Leaf spot / blight
Medicinal garden, Ayurveda Research Institute, Poojappura, Thiruvananthapuram.	Leaf spot / blight

Table 2. Colony and conidial morphology of *C.gloeosporioides* isolate of thippali

Sl.no	Characters	Description
1	Type of growth	Evenly felty
2	Colony colour	Evenly white
3	Spore mass colour	Salmon
4	Sporulation	Shy sporulation
5	Colony diameter (cm)*	8.9
6	Mycelial dry weight (mg)*	280.1
7	Length of conidia (µm)**	12.69
8	Breadth of conidia (µm)**	4.7

* Average of 3 replications
** Average of 100 spores

Table 3. Colony and conidial morphology of *C.gloeosporioides* isolates of black pepper and betel vine

Sl.no	Characters	Description	
		C2	C3
1	Type of growth	Slightly fluffy	Felty
2	Colony colour	Unevenly white with grey margins	Evenly white
3	Spore mass colour	Light salmon	Dark salmon
4	Sporulation	Moderately sporulating	Highly sporulating
5	Colony diameter (cm)*	9.00	8.7
6	Mycelial dry weight (mg)*	301.4	288.3
7	Length of conidia (µm)**	14.63	15.52
8	Breadth of conidia (µm)**	4.7	4.7

C2-black pepper isolate
C3-betel vine isolate
*Average of 3 replications
**Average of 100 spores

Plate6a **Black pepper isolate of *C. gloeosporioides* on PDA**

Plate 6b **Conidia of black pepper isolate of *C. gloeosporioides*** **(100x)**

Plate 7a **Betel vine isolate of *C. gloeosporioides* on PDA**

Plate 7b **Conidia of betel vine isolate of *C. gloeosporioides*** **(100x)**

Plate 6a

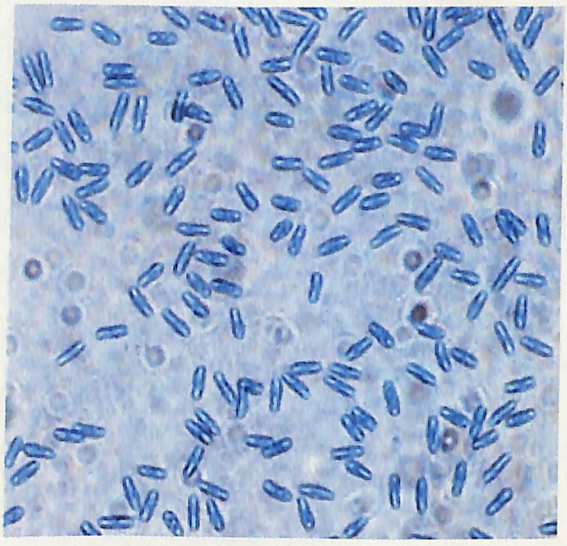


Plate 6b

Plate 7a

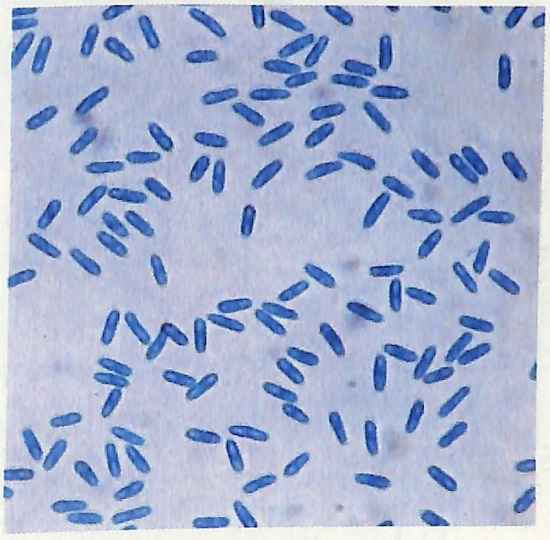


Plate 7b

4.6 CROSS INOCULATION STUDIES.

Cross inoculation of the three (thippali, black pepper and betel vine) isolates of *C.gloeosporioides* showed difference in symptom expression. The thippali isolate failed to produce any symptom on both black pepper and betel vine. The black pepper and betel vine isolates were found to develop the characteristic leaf spot symptom with yellow hallow on cross inoculation. Black pepper isolate on betel vine leaf produced the largest lesion with 4 cm dia. with an yellow hallow around it. Betel vine isolate on black pepper produced a necrotic lesion with 3.60cm dia. with a slight yellow hallow. Both black pepper and betel vine isolates produced similar symptoms (necrotic lesion with 0.8 cm dia and distinct yellow hallow) on thippali leaves. Details are given in Table 4 (Plates 8-13).

4.7. SURVIVAL OF *C.gloeosporioides*

Studies on the survival of *C.gloeosporioides* of thippali showed that the fungus survived for 105 days in infected thippali leaves under laboratory conditions in paper bags. In the soil the organism survived for 150 days. Data are given in Table 5 (Fig.1)

4.8. ISOLATION OF MICRO ORGANISMS FROM RHIZOSPHERE AND PHYLLOSPHERE OF THIPPALI

From the rhizosphere eight fungi and two bacteria were isolated. They were designated from R1 to R8 (fungi) and B1 and B2 (bacteria) respectively. It was observed that the fungus *Aspergillus* sp was found to be the most predominant one in the rhizosphere. Both the bacteria were Gram negative. From thippali phyllosphere, three fungi and two bacteria were isolated. They were designed from P1 to P3 (fungi) and B3 and B4 (bacteria). Among fungi *Penicillium* spp was most predominant. Both the bacteria were Gram positive. Data are presented in Table 6a and 6b.

Table 4. Cross inoculation studies

Isolate	Inoculated host	Nature of symptom		Lesion dia. (cm)*
		7days after inoculation	10 days after inoculation	
C1	Black pepper	No symptom	No symptom	-
	Betel vine	No symptom	No symptom	-
C2	Thippali	Small pale necrotic lesion with yellow hallow	Lesion enlarged	0.80
	Betel vine	Large dark necrotic lesion with pale yellow hallow	Lesion enlarged	4.00
C3	Thippali	Small pale necrotic lesion with light yellow hallow	Lesion enlarged	0.80
	Black pepper	Large dark necrotic lesion with pale yellow hallow	Lesion enlarged	3.60

*Average of 3 replications
C1-Thippali isolate
C2-Black pepper isolate
C3-Betel vine isolate

Table 5. Survival of *C.gloeosporioides*

Days after incubation	Percentage recovery	
	In soil	In the infected leaves
0	100	100
15	90	85
30	88	73
45	70	58
60	66	43
75	55	31
90	47	20
105	31	**8**
120	20	0
135	15	0
150	**7**	0
165	0	0

Table 6a. Fungal isolates from thippali rhizosphere and phyllosphere

Designation given to isolates	Source	Suspected genera
R1	Rhizosphere of thippali	*Syncephellastrum* sp.
R2	"	*Rhizopus* sp.
R3	"	*Penicillium* sp.
R4	"	***Aspergillus* sp.**
R5	"	*Choanephora* sp.
R6	"	***Aspergillus* sp.**
R7	"	*Fusarium* sp.
R8	"	*Mucor* sp.
P1	Phyllosphere of thippali	*Trichoderma* sp.
P2	"	***Penicillium* sp.**
P3	"	***Penicillium* sp.**

Table 6b. Bacterial isolates from thippali rhizosphere and phyllosphere

Designation given to isolates	Source	Gram reaction
B1	Rhizosphere of thippali	–
B2	"	–
B3	Phyllosphere of thippali	+
B4	"	+

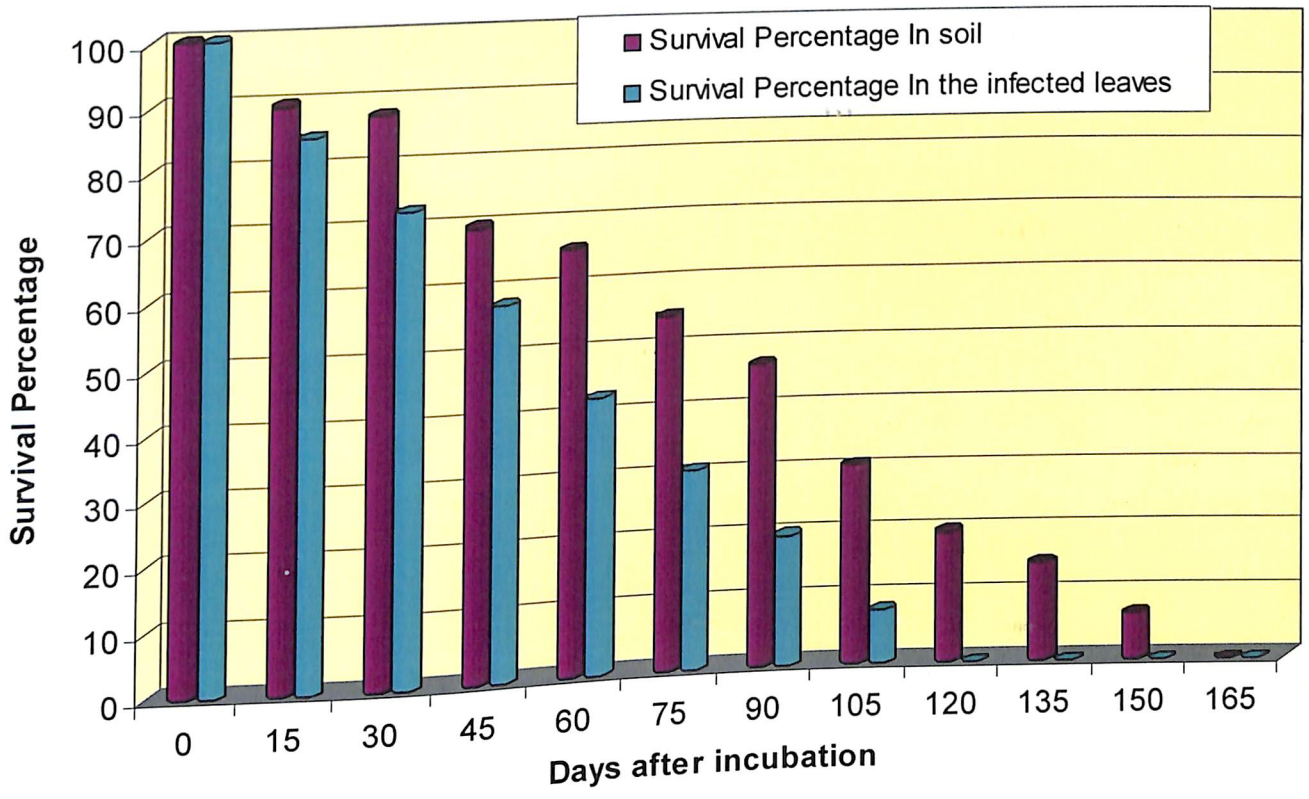


Fig. 1. Survival of *C.gloeosporioides*

Plate8 **Thippali isolate inoculated on black pepper**

Plate 9 **Thippali isolate inoculated on betel vine**

Plate 10 **Black pepper isolate inoculated on thippali**

Plate 11 **Black pepper isolate inoculated on betel vine**

Plate 12 **Betel vine isolate inoculated on thippali**

Plate 13 **Betel vine isolate inoculated on black pepper**

Plate 8

Plate 9

Plate 10

Plate 11

Plate 12

Plate 13

4.9 *IN VITRO* STUDIES

4.9. 1 *In vitro* screening of fungal isolates against *C. gloeosporioides*

All the eleven fungal isolates were effective against *C. gloeosporioides* in dual culture experiment. P1 was the most effective (84.3%) in suppressing the pathogen. Inhibition of R4 (80%) was also on par with P1. All the antagonists showed significant difference with the control. Effectiveness of R3, R6, R8 and P2 were on par. The antagonists R5 was least effective (56.7%) in inhibiting the pathogen. Data are given in Table 7 (Plate 14-17, Fig. 2).

4.9.2 *In vitro* screening of bacterial isolates

All the four bacterial isolates failed to inhibit the fungal growth *in vitro* (Plate 18).

4.9.3. *In vitro* screening of resistance inducers

Five different resistance inducers were tried at three levels. The different treatments showed considerable difference in their effect on the pathogen. H_2O_2 at all the levels gave complete (100 percent) suppression of the pathogen. SA showed least suppression on the growth of the pathogen. Among the different levels of SA tested, 1 g/l gave zero percent suppression (Plate 19). Hence this level of SA was selected for field application as it had no direct effect on the pathogen. Details of the *in vitro* analysis of resistance inducers are given in Table 8 (Fig.3)

4.9.4. *In vitro* screening of plant based chemicals

The data on plant based chemicals tested are presented on Table 9 (Fig. 4). Statistical analysis of the data showed significant difference between the two treatments. Ovis exhibited highest per cent suppression of the pathogen at 2 g/l and 5 g/l and was significantly superior when compared to Amruth neem (Plate 20a & 20b). There was no significant difference between the three levels of ovis tested. Hence lower dose (1g/l) was selected for field application (Fig.4).

Table 7. *In vitro* inhibition of C.*gloeosporioides* by fungal antagonists

Isolate	Percentage inhibition*
R1	79 (8.94)
R2	74.32 (8.68)
R3	67.62 (8.28)
R4	**80 (9.00)**
R5	56.65 (7.59)
R6	73.31 (8.62)
R7	76.99 (8.83)
R8	68.99 (8.37)
P1	**84.31 (9.24)**
P2	68.98 (8.37)
P3	76.65 (8.81)
CD	0.25

*Average of 3 replications

Figures in parentheses are $\sqrt{x+1}$ transformed values

Table 8. *In vitro* inhibition of *C. gloeosporioides* by resistance inducers

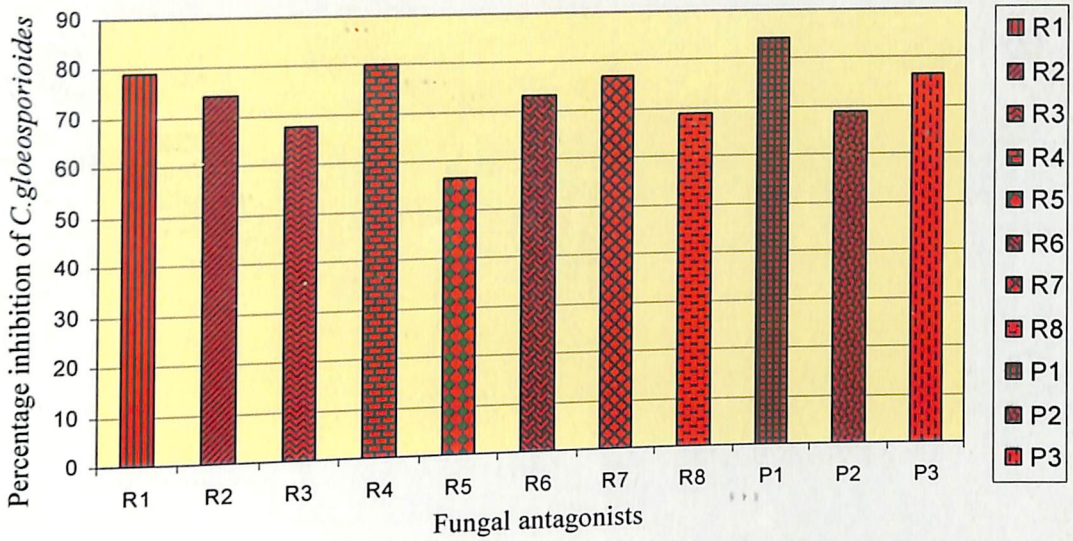
Resistance inducer	Concentration	Percentage inhibition*
$CaCl_2$	1 g/l	31.66
	2 g/l	32.62
	3 g/l	47.67
H_2O_2	1 ml/l	100.00
	2 ml/l	100.00
	3 ml/l	100.00
Borax	1 g/l	36.98
	2 g/l	38.33
	3 g/l	42.97
SA	1 g/l	0.00
	2 g/l	4.89
	3 g/l	15.62
ASM	10 ppm	27.22
	25 ppm	41.32
	50 ppm	46.66
CD		4.55

*Average of 3 replications

Table 9. *In vitro* inhibition of *C. gloeosporioides* by Plant Based Chemicals

Chemical	Concentration	Percentage inhibition *
Amruth neem	(ml/l)	
	C1 1	8.33
	C2 3	21.00
	C3 5	30.00
Ovis	(g/l)	
	C1 1	94.33
	C2 3	100
	C3 5	100
CD	6.93	

*Average of 3 replications



R1 - *Syncephellastrum* sp.
R2 - *Rhizopus* sp.
R3 - *Penicillium* sp.
R4 - *Aspergillus* sp.
R5 - *Choanephora* sp.
R6 - *Aspergillus* sp.
R7 - *Fusarium* sp.
R8 - *Mucor* sp.
P1 - *Trichoderma* sp.
P2 - *Penicillium* sp.
P3 - *Penicillium* sp.

Fig. 2 *In vitro* inhibition of *C.gloeosporioides* by different fungal antagonists

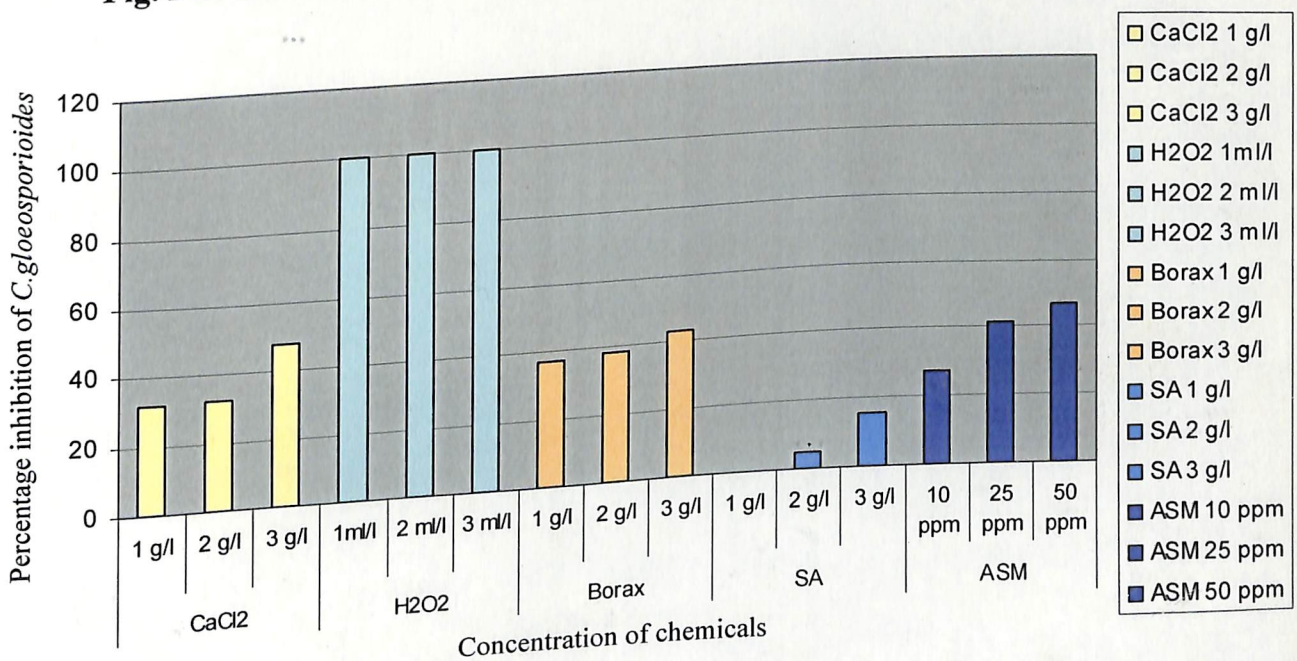


Fig. 3 *In vitro* inhibition of *C. gloeosporioides* by different resistance inducers

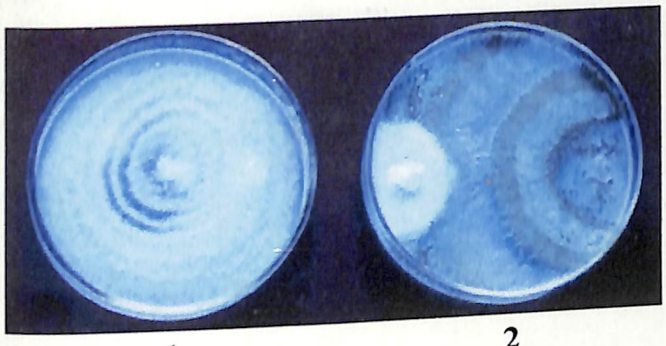
Plate 14 **Inhibition of *C. gloeosporioides* by *T. viride***
1: Control
2: *C. gloeosporioides* X *T. viride*

Plate 15 **Inhibition of *C. gloeosporioides* by *A. terreus***
1: Control
2: *C. gloeosporioides* X *A. terreus*

Plate 16 **Inhibition of *C. gloeosporioides* by *Fusarium* sp.**
1: Control
2: *C. gloeosporioides* X *Fusarium* sp.

Plate 17 **Inhibition of *C. gloeosporioides* by *Penicillium* sp.**
1: Control
2: *C. gloeosporioides* X *Penicillium* sp.

Plate 18 **Inhibition of *C. gloeosporioides* by bacterial isolate (B_1)**
1: Control
2. *C. gloeosporioides* X B_1 (Bacteria)



1 2

Plate 14

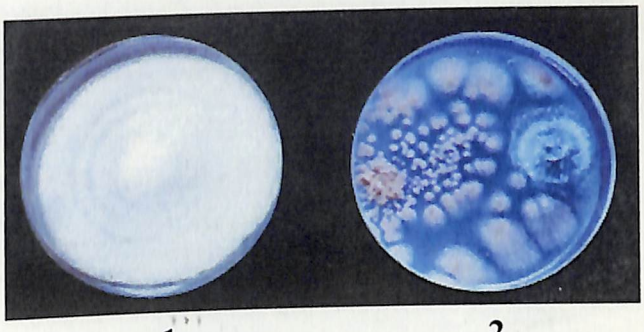


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Plate 15

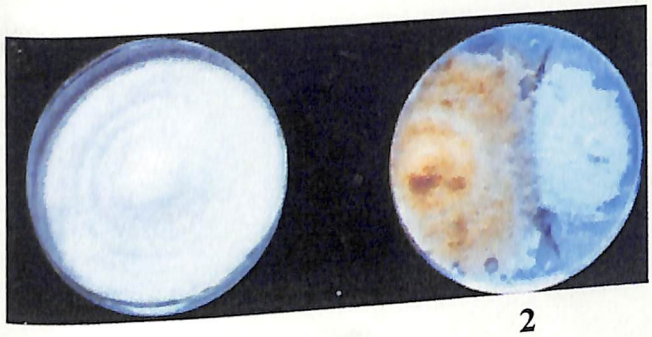

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Plate 16

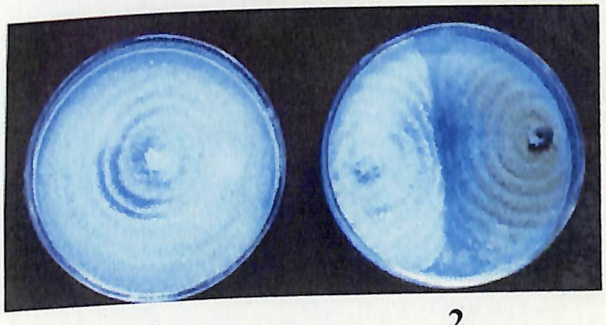

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Plate 17

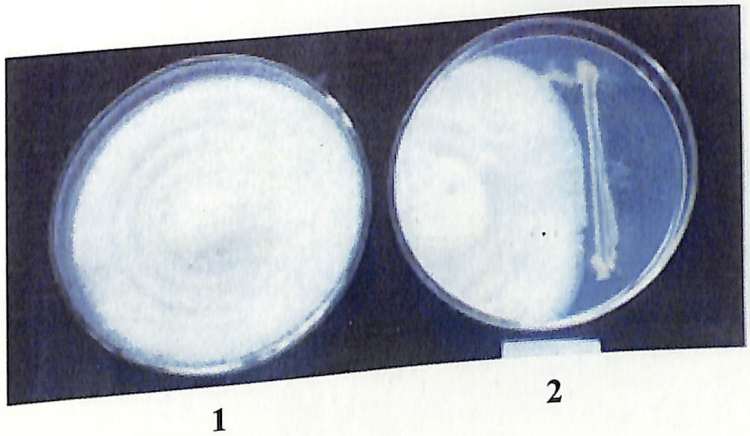

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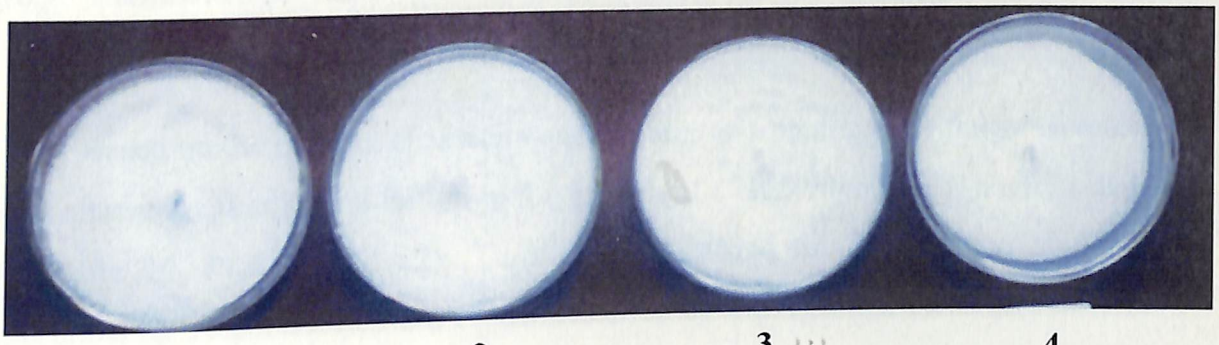
Plate 18

Plate 19 **Inhibition of *C. gloeosporioides* with SA**
1: Control
2: SA 1 g/l
3: SA 2 g/l
4: SA 3 g/l

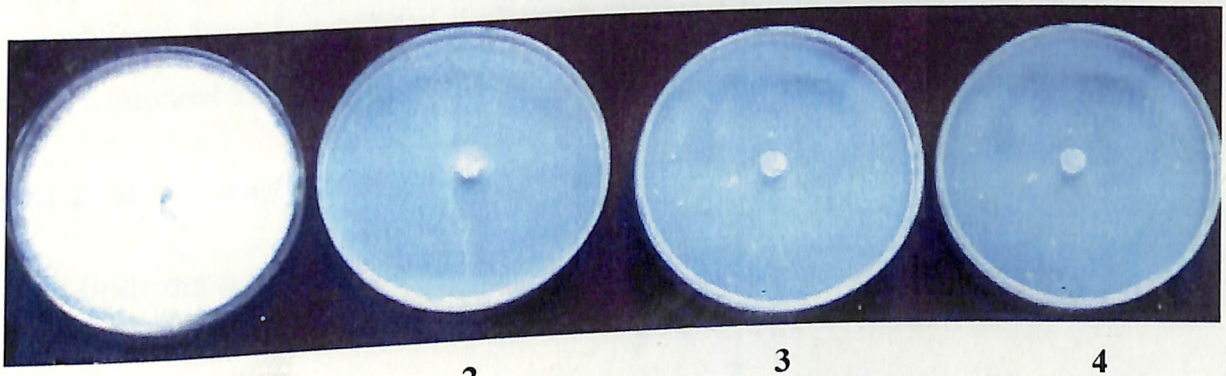
Plate 20a **Inhibition of *C. gloeosporioides* with Ovis**
1: Control
2: Ovis 1 g/l
3: Ovis 3 g/l
4: Ovis 5 g/l

Plate 20b **Inhibition of *C. gloeosporioides* with Amruth neem**
1: Control
2: Amruth neem 1 ml/l
3: Amruth neem 3 ml/l
4: Amruth neem 5 ml/l



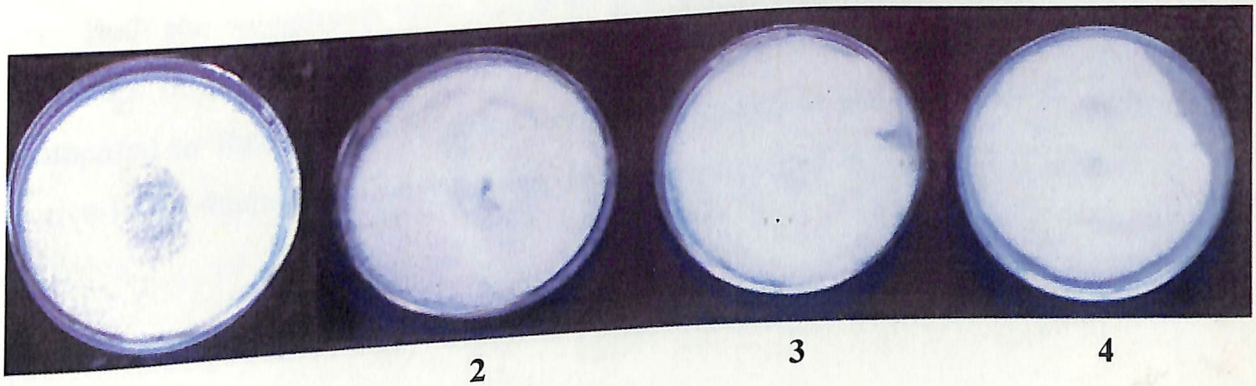
1 2 3 4

Plate 19



1 2 3 4

Plate 20a



1 2 3 4

Plate 20b

4.10. STUDIES ON THE SELECTED FUNGAL ANTAGONIST

4.10.1 Identification and characterization of the antagonists exhibiting maximum inhibition of *C. gloeosporioides*

Based on the cultural characters and conidial morphology, the fungal isolates which showed maximum inhibition of the growth of *C. gloeosporioides* were P1 and R4 (Table 10, Plate 21 & 22). They were identified as *Trichoderma* sp. and *Aspergillus* sp. respectively. Further identification by the Agharkar research institute MACS, Pune, Maharashtra confirmed that the isolates were *Trichoderma viride* Per.: S.F. Gray and *Aspergillus terreus Gr.* respectively. *A.terreus* exhibited yellow spore mass whereas *T.viride* exhibited dull green spores. *T.viride* is found to be faster in growth compared to *A.terreus*

4.10.1.2 Mycoparasitism of selected fungal antagonists with *C. gloeosporioides*

Both the antagonists were found to coil around the pathogen hyphae and caused breakdown of it (Plate 23 & 24).

4.10.1.3 Antibiosis of the selected fungal antagonists

Both the antagonists were found to produce both volatile and non volatile compounds. Inhibition of growth of pathogen by the volatiles was found to be less as compared to that by non volatile compounds. *A.terreus* was found to be more effective in production of non volatiles. Data are presented in Table 11 (Plates 25 and 26).

4.11 INOCULUM PREPARATION AND APPLICATION

4.11.1 Preparation and application of pathogen inoculum

C. gloeosporioides mass multiplied on rice bran (Plate 27) was applied to pots of thippali plants. Foliar application of *C. gloeosporioides* with 0.5 percent peptone water was also given. After 15 days of initial pathogen application, all the

Table 10. Cultural and morphological characters of selected fungal antagonists

Antagonist	Colony colour	Colony dia. (cm)*	Nature of sporulation
Aspergillus terreus	Yellowish brown	8.50	Highly sporulating
Trichoderma viride	Dull green	9.00	Highly sporulating

*Average of 3 replications 7days after inoculation

Table-11 Effect of volatiles and nonvolatiles of selected antagonists on *C.gloeosporioides*

Antagonists	Effect of volatiles* (% suppression of colony)	Effect of non volatiles* (% suppression of colony)
Aspergillus terreus	15	28
Trichoderma viride	13	20

*Average of 3 replications

Plate 21 **Fungal antagonist *Trichoderma viride***

Plate 22 **Fungal antagonist *Aspergillus terreus***

Plate 23 **Hyphal interaction of *C. gloeosporioides* with *Trichoderma viride***

Plate 24 **Hyphal interaction of *C. gloeosporioides* with *Aspergillus terreus***

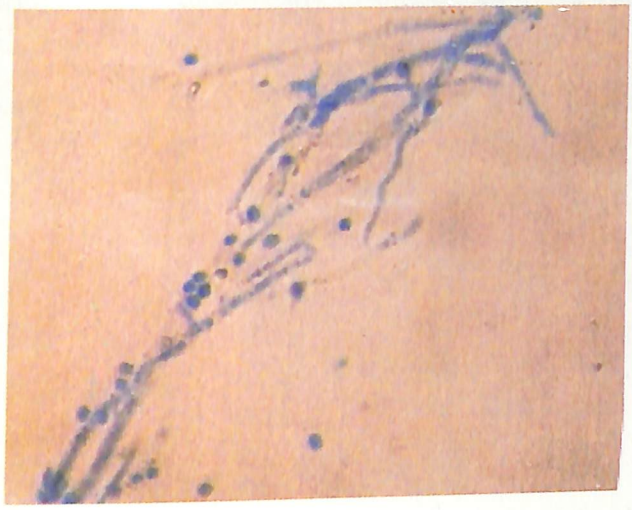


Plate 21

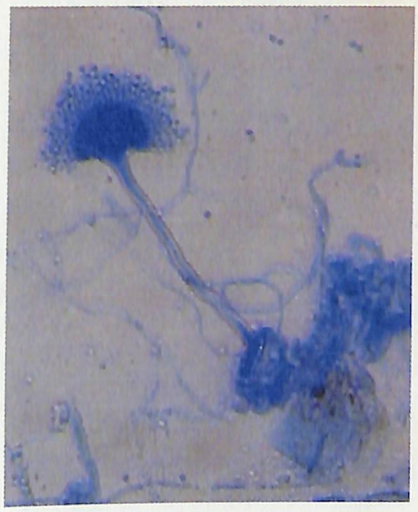


Plate 22

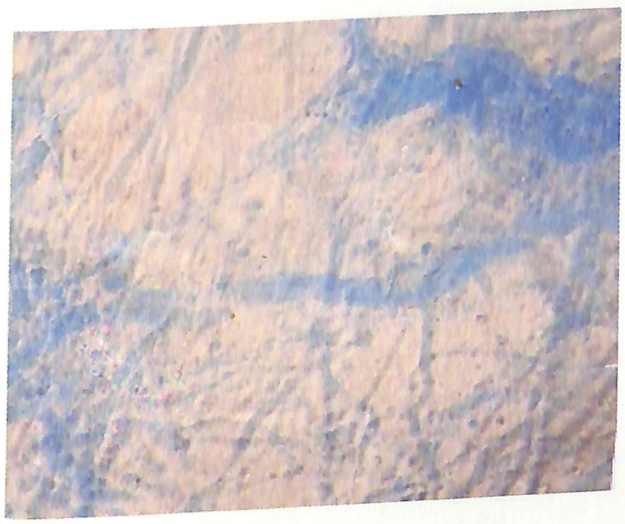


Plate 23

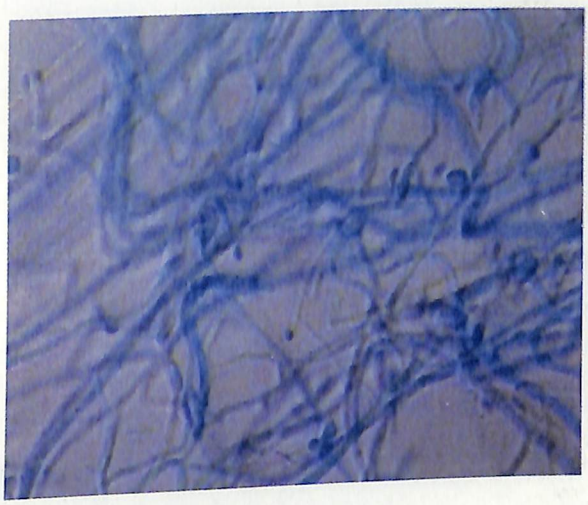


Plate 24

Plate 25 **Effect of nonvolatiles of *T. viride* on *C. gloeosporioides***
1: Control
2: Inhibition of C. *gloeosporioides* by non volatiles of *T. viride*

Plate 26 **Effect of nonvolatiles of *A. terreus* on *C. gloeosporioides***
1: Control
2: Inhibition of C. *gloeosporioides* by non volatiles of *A. terreus*

Plate 27 ***C. gloeosporioides* multiplied on rice bran for field application**

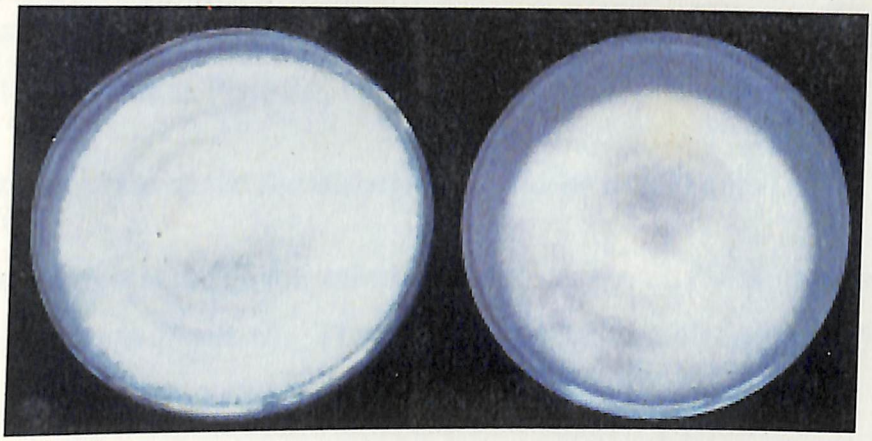


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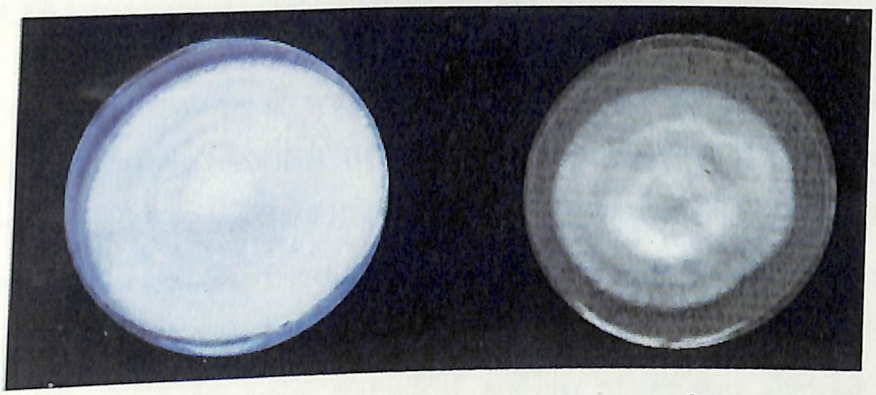
Plate 25



1 2

Plate 26

Plate 27

inoculated pots showed approximately uniform disease development. Score used for determining PD1 was given in Plate 28.

4.11.2. Preparation of talc based formulation of selected fungal antagonists.

Talc based formulation of the selected fungal antagonists was prepared in polypropylene bags (Plate 29a & b). The cfu for *T.viride* formulation was 47 cfu $g\Gamma^1$ and that of *A.terreus* formulation was 58 cfu $g\Gamma^1$ at the time of application

4.12 FIELD APPLICATION OF ECOFRIENDLY MATERIALS AND THEIR COMBINATION.

The data on field experiments were statistically analysed and are presented in Table 12a & b. In the first experiment, treatment T_{12} (combination T_2T_5 *A. terreus* and neem cake) was found to be most effective in disease suppression on 45th DAT. The treatments T_6, T_7, T_9 and T_{10} were found to be on par with T_{12}. In the second experiment, the treatment T_{12} (combination of T_2T_5 – *A. terreus* + neem cake) was found to be most effective. However statistical analysis of the data showed that treatment T_1, T_6, T_7 and T_9 were found to be on par with T_{12}.

Pooled analysis of data of two experiments showed that the treatments did not show considerable difference between the two seasons. However, there was significant difference between the different treatments irrespective of the seasons.

PDI showed that the treatment T_{12} (combination T_2T_5 – *A. terreus* + neem cake) most effective (PDI = 13.69%) in suppressing the disease at 45 DAT. The treatments T_6, T_7 and T_9 were on par with T_{12}. Treatment T_4 (SA) was least effective (PDI-31.73%) at 45 DAT. All other treatments were significantly different from the control (PDI = 57.49%). Data are presented in Table 13.

4.13. ESTIMATION OF DEFENSE RELATED ENZYMES (DRE)

The DRE were estimated after 45 days of imposition of treatment and the results are furnished in Table 14 (Fig. 5).

Table 12 a Effect of ecofriendly materials on anthracnose of thippali

(Experiment No. 1)

Treatment	PDI (DAT) *		
	15	30	45
T_1	22.39 (4.73)	20.72 (4.55)	33.06 (5.75)
T_2	23.55 (4.85)	17.90 (4.23)	30.63 (5.53)
T_3	24.57 (4.96)	23.8 (4.88)	27.02 (5.20)
T_4	34.09 (5.84)	32.42 (5.69)	27.77 (5.27)
T_5	33.83 (5.82)	28.18 (5.31)	25.85 (5.08)
T_6	19.00 (4.36)	13.02 (3.16)	14.77 (3.84)
T_7	19.16 (4.38)	20.95 (4.58)	16.66 (4.08)
T_8	28.91 (5.38)	28.61(5.35)	26.30 (5.13)
T_9	13.11 (3.62)	13.83(3.72)	18.24(4.27)
T_{10}	25.62 (5.06)	20.74 (4.55)	20.56 (4.53)
T_{11}	26.43 (5.14)	29.59(5.44)	29.45 (5.43)
T_{12}	15.15 (3.89)	14.70(3.83)	**14.06 (3.75)**
T_{13}	31.53 (5.61)	27.48 (5.24)	29.05 (5.39)
T_{14}	25.95 (5.09)	25.19 (5.02)	27.62 (5.26)
T_{15}	32.44 (5.70)	26.32 (5.13)	29.77 (5.46)
C	39.52 (6.29)	53.52 (7.31)	61.48 (7.83)
AC	33.48 (5.78)	38.62 (6.21)	37.34 (6.11)
CD	0.76	1.06	1.10

*Average of 3 replications
Figures in parenthesis are $\sqrt{x}$ transformed values

Table 12 b Effect of ecofriendly materials on anthracnose of thippali

(Experiment No. 2)

Treatment	PDI (DAT) *		
	15	30	45
T_1	22.65(4.76)	20.74(4.55)	20.63(4.54)
T_2	23.28(4.82)	19.32(4.40)	21.94(4.68)
T_3	22.95(4.79)	22.51(4.74)	21.42(4.63)
T_4	34.82(5.90)	34.79(5.90)	35.67(5.97)
T_5	34.90(5.91)	31.18(5.58)	31.87(5.65)
T_6	19.72(4.44)	13.74(3.71)	16.27(4.03)
T_7	19.43(4.41)	16.67(4.08)	17.46(4.18)
T_8	34.83(5.90)	33.80(5.81)	32.35(5.69)
T_9	14.23(3.77)	15.35(3.92)	15.43(3.93)
T_{10}	31.00(5.57)	27.34(5.23)	28.43(5.33)
T_{11}	25.84(5.08)	20.64(4.54)	21.85(4.67)
T_{12}	15.33(3.91)	11.91(3.45)	**13.30(3.65)**
T_{13}	30.36(5.51)	71.88(8.48)	26.12(5.11)
T_{14}	25.84(5.08)	23.85(4.88)	23.36(4.83)
T_{15}	33.42(5.78)	27.48(5.24)	27.80(5.27)
C	45.67(6.76)	51.81(7.18)	53.85(7.33)
AC	35.34(5.94)	38.87(6.20)	24.30(6.38)
CD	0.73	2.24	0.95

* Average of 3 replications
Figures in parenthesis are $\sqrt{x}$ transformed values.

Table 13 Pooled analysis of effect of ecofriendly materials on anthracnose of thippali across two seasons

Treatments	PDI (DAT)*								
	15			30			45		
	I	II	Mean	I	II	Mean	I	II	Mean
T_1	22.39(4.84)	22.65(4.86)	22.52(4.85)	20.73(4.66)	20.75(4.66)	20.74(4.66)	33.07(5.84)	20.65(4.65)	26.86(5.25)
T_2	23.56(4.96)	23.28(4.93)	23.42(4.95)	17.91(4.35)	19.32(4.51)	20.95(4.43)	30.65(5.63)	21.95(4.79)	26.30(5.21)
T_3	24.59(5.06)	22.97(4.90)	23.78(4.98)	23.82(4.98)	22.54(4.85)	23.18(4.92)	27.05(5.30)	21.45(4.74)	24.25(5.02)
T_4	34.09(5.92)	34.82(5.98)	34.46(5.95)	32.43(5.78)	34.79(5.98)	33.61(5.88)	27.77(5.36)	35.68(6.06)	31.73(5.71)
T_5	33.83(5.90)	34.90(6.00)	34.37(5.95)	28.18(5.40)	31.18(5.67)	29.68(5.54)	25.85(5.18)	31.87(5.73)	28.86(5.46)
T_6	19.00(4.47)	19.72(4.55)	19.36(4.51)	13.03(3.75)	13.74(3.84)	13.39(3.80)	14.81(3.98)	16.27(4.16)	15.54(4.07)
T_7	19.18(4.49)	19.44(4.52)	19.31(4.51)	20.95(4.69)	16.67(4.20)	18.81(4.44)	16.67(4.20)	17.46(4.30)	17.07(4.25)
T_8	28.92(5.47)	34.84(5.99)	31.88(5.73)	28.63(5.44)	33.81(5.90)	31.22(5.67)	26.34(5.23)	32.36(5.78)	29.35(5.50)
T_9	13.13(3.76)	14.23(3.90)	13.68(3.83)	13.89(3.86)	15.36(4.04)	14.63(3.95)	18.24(4.39)	15.44(4.05)	16.84(4.22)
T_{10}	25.62(5.16)	31.03(5.66)	28.33(5.41)	20.75(4.66)	27.35(5.32)	24.05(4.99)	20.56(4.64)	28.45(5.43)	24.51(5.04)
T_{11}	26.43(5.24)	25.86(5.18)	26.15(5.21)	29.61(5.53)	20.64(4.65)	25.13(5.09)	29.45(5.52)	21.86(4.78)	25.66(5.15)
T_{12}	15.16(4.01)	15.34(4.04)	15.25(4.03)	14.70(3.96)	11.91(3.59)	13.31(3.78)	14.06(3.88)	13.31(3.78)	13.69(3.83)
T_{13}	31.53(5.70)	30.35(5.60)	30.94(5.65)	27.49(5.34)	72.11(8.55)	49.8(6.94)	29.08(5.48)	26.15(5.21)	27.62(5.35)
T_{14}	25.95(5.19)	25.84(5.18)	25.90(5.19)	25.19(5.12)	23.85(4.99)	24.52(5.05)	27.62(5.35)	23.37(4.94)	25.50(5.14)
T_{15}	32.44(5.78)	33.43(5. 87)	32.94(5.83)	26.34(5.23)	27.48(5.34)	26.91(5.28)	29.77(5.55)	27.80(5.37)	28.79(5.46)
C	39.50(6.36)	45.66(6.83)	42.58(6.60)	53.37(7.37)	51.67(7.26)	52.52(7.32)	61.30(7.82)	53.67(7.39)	57.49(7.64)
AC	33.42(5.87)	35.32(6.03)	34.37(5.95)	38.58(6.29)	38.47(6.28)	38.53(6.29)	37.32(6.19)	40.69(6.46)	39.01(6.32)
CD	0.67	0.67	0.67	1.63	1.63	1.63	0.97	0.97	0.97

* Average of 3 replications

Figures in parantheses are $\sqrt{x+1}$ transformed values

Table 14. Effect of ecofriendly materials on the activity of Defense Related Enzymes 45 DAT on anthracnose of thippali

Treatment	Enzyme activity *		
	Change in absorbance $min\Gamma^{1}$ $g\Gamma^{1}$ fresh weight		Change in absorbance $\mu g\ g\Gamma^{1}\ min\Gamma^{1}$ fresh weight
	PO	PPO	PAL
T_1	2.18	36.85	0.49
T_2	**1.74**	37.49	0.47
T_3	8.46	**38.65**	0.48
T_4	7.26	35.17	0.42
T_5	7.49	29.00	**0.39**
T_6	10.03	29.78	0.57
T_7	6.29	33.83	**0.54**
T_8	9.30	29.78	0.47
T_9	7.45	28.45	0.48
T_{10}	12.04	24.19	0.50
T_{11}	17.23	25.67	0.45
T_{12}	24.32	**17.49**	0.43
T_{13}	27.72	18.41	0.46
T_{14}	**29.96**	24.29	0.45
T_{15}	28.65	24.99	0.42
C	1.94	31.18	0.46
AC	3.66	36.36	0.34
CD	6.37	6.11	

* Average of 3 replications, 45 DAT.

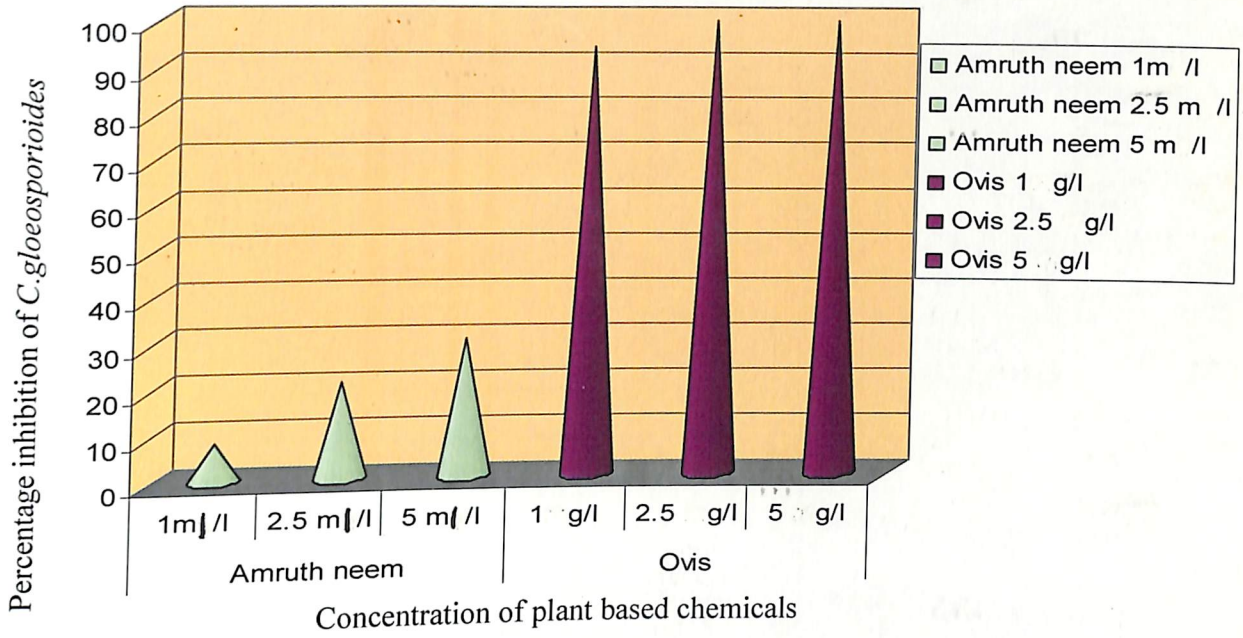
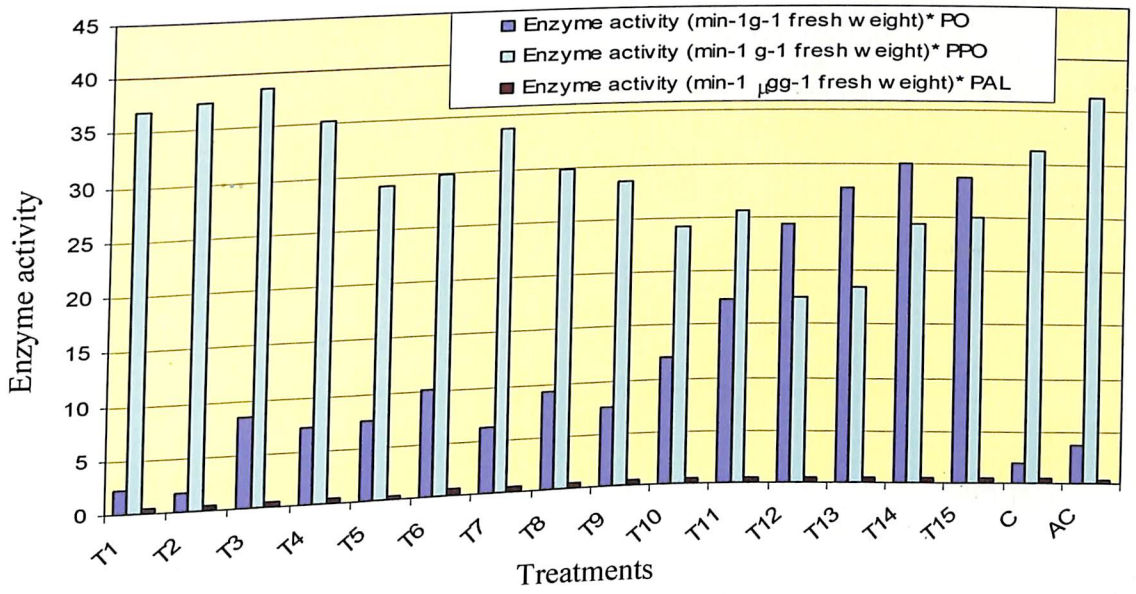


Fig. 4 *In vitro* inhibition of *C. gloeosporioides* by plant based chemicals



T1	-	*Trichoderma viride*
T2	-	*Aspergillus terreus*
T3	-	Ovis @ 1g/L
T4	-	SA @ 1g/L
T5	-	Neem cake
T6	-	*T. viride* + *A. Terreus*
T7	-	*T. viride* + Ovis
T8	-	*T. viride* + SA
T9	-	*T. viride* + Neem cake
T10	-	*A. terreus* + Ovis
T11	-	*A. terreus* + SA
T12	-	*A. terreus* + Neem cake
T13	-	Ovis + SA
T14	-	Ovis + Neem cake
T15	-	SA + Neem cake
C	-	Control (Pathogen inoculated)
AC	-	Control (Uninoculated)

Fig. 5 Activity of defense related enzymes 45 DAT with different ecofriendly materials and their combinations on the Anthracnose of thippali

Plate 28 Score chart for Anthracnose of Thippali

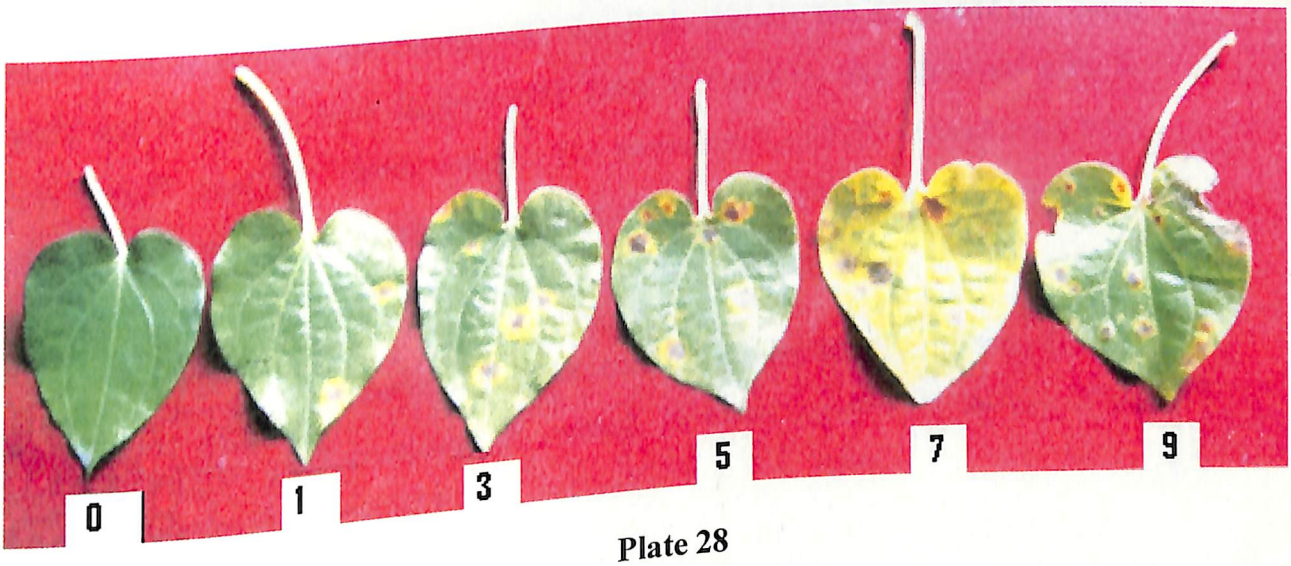


Plate 28

Among the individual treatments, Ovis reported the maximum activity of PO and PPO (8.46 and 38.46 min^{-1} g^{-1} fresh weight). The PAL activity was maximum in *T.viride* treated plants (0.49 $\mu g g^{-1}$ min^{-1}freshweight). Least PO activity (1.7 $min\Gamma^{1}g\Gamma^{1}$ fresh weight) was obtained with T_2 (*A.terreus*). The treatment T_5 showed least PPO and PAL activity (29 min^{-1} g^{-1} fresh weight and 0.39 min^{-1} g^{-1} fresh weight respectively).

Combined application of the treatments showed that maximum PO activity (29.96 $min\Gamma^{1}$ $g\Gamma^{1}$ fresh weight) was with treatment T_{14} (T_3T_5 - Ovis + neem cake). It was found to be significantly superior over control. The treatments T_7 (T_1T_3 – *T. viride* + Ovis) showed maximum PPO and PAL activity (33.83 $min\Gamma^{1}$ $g\Gamma^{1}$ fresh weight and 0.54 $\mu g g^{-1}$ min^{-1}freshweight). Least PO activity was observed with T_7 (T_1T_3 - 6.29 $min\Gamma^{1}$ $g\Gamma^{1}$ fresh weight). The treatment T_{12} (T_2T_5 showed least PPO activity (17.49 $min\Gamma^{1}$ $g\Gamma^{1}$ fresh weight) The treatment T_{15} (T_4T_5) showed least PAL activity (0.42 μg $g^{-1}$$min^{-1}$freshweight).

Analysis of DRE showed that treatments were significantly different in PO and PPO activity when compared to control. However treatments did not differ considerably in their activity of PAL.

Plate 29a Talc based formulation of *T. viride*

Plate 29b Talc based formulation of *A. terreus*

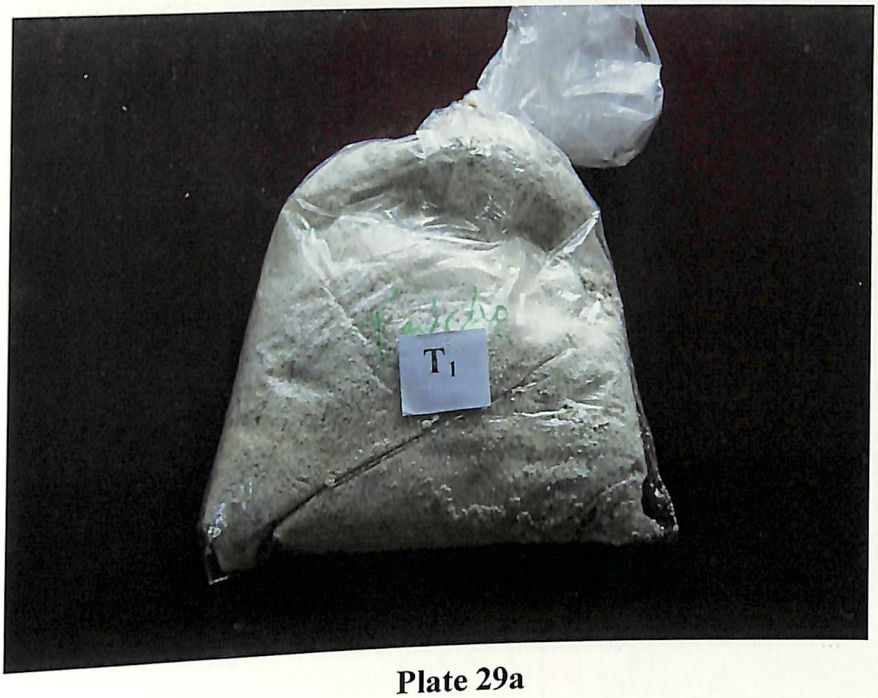


Plate 29a

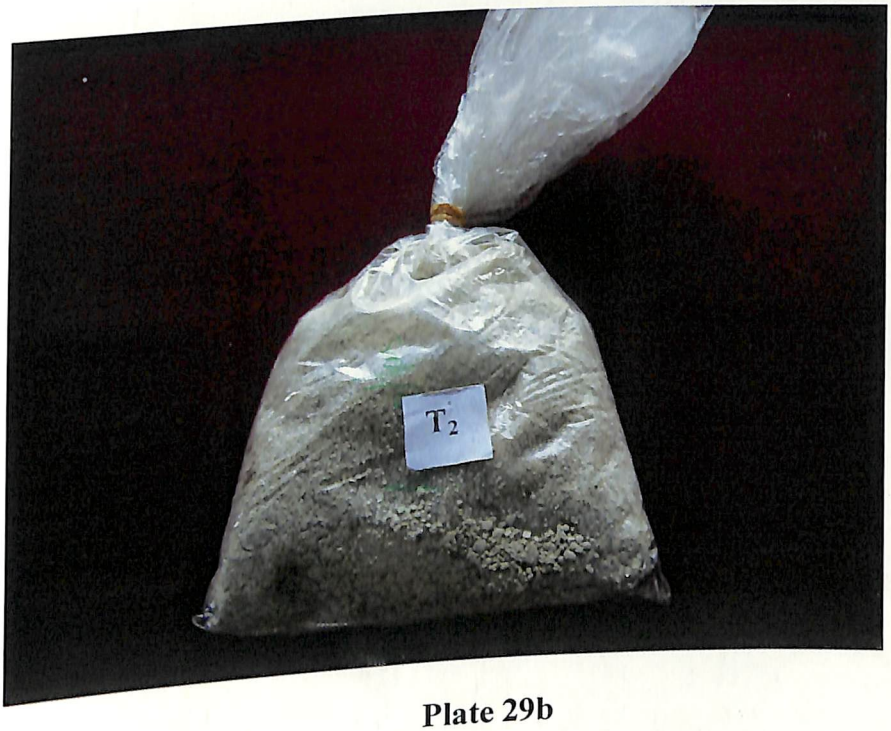


Plate 29b

Discussion

5. DISCUSSION

A survey was conducted to identify the disease affecting thippali in the medicinal gardens, College of Agriculture, Vellayani and medicinal gardens of Ayurveda Research Institute Poojappura, Thiruvananthapuram. A perusal of the literature revealed that the only reported disease on thippali was a leaf and vine rotting caused by *colletotrichum* sp. (Farooqi and Sreeramu, 2001). In the present study, only a leaf spot and blight was the major disease observed on thippali. No rotting symptom was observed in the present study through out the year. The pathogen associated was found to be *C. gloeosporioides.* Since the pathogen associated and the symptom were similar to the anthracnose of black pepper, this disease is designated as Anthracnose of Thippali.

The pathogen associated was identified as *Colletotrichum gloeosporioides* (Penz) Penz and Sacc. based on the colony and conidial morphology. Further confirmation was done by comparing the culture with *C gloeosporioides* culture available in the Department of Plant Pathology, College of Agriculture, Vellayani, Thiruvananthapuram. A scan of the literature revealed that *C. gloeosporioides* on thippali has not been reported else where. Hence this is the first report of this pathogen on this medicinal plant. *C. gloeosporioides* is found to cause disease in closely related plants of thippali belonging to the Piperacea family. This pathogen is reported to cause fungal pollu / anthracnose of black pepper (Rao, 1926). *Colletotrichum* caused anthracnose of betel vine (Chattopadhyay and Satyabrata 1990). During the study, tissue isolation of anthracnose affected betel vine from College of Agriculture, Vellayani showed that the organism was *C. gloeosporioides.*

All the three isolates of *C. gloeosporioides viz* thippali, black pepper and betel vine showed difference in their cultural and conidial morphology. Thippali isolate was a shy sporulating one. Conidial measurements of the three isolates revealed that the *C. gloeosporioides* of thippali was the smallest one. Shy sporulation and smallest conidial size of thippli isolate showed that this is entirely different from the isolates of other two sister crops. Further confirmation on the specificity of *C. gloeosporioides* of thippali has to be made by molecular

characterisation. The cultural and conidial morphology of *C. gloeosporioides* isolate is in agreement with the earlier observations (Ali and Warren, 1987; Pande *et al*, 1991; Mathur *et al*,2001; Anoop, 2002).

Cross inoculation of the three *C. gloeosporioides* also showed variations in symptom development. Black pepper and betel vine isolates produced characteristic leaf spot symptoms on cross inoculation. However thippali isolate failed to produce any symptom when cross inoculated on black pepper and betel vine. This also indicated that the thippli isolate is host specific. *C. gloeosporioides* isolate from guava could cross infect grape, egg plant and black pepper (Quimio and Quimio, 1975).

A perusal of the literature showed that *Colletotrichum* spp. is able to survive in the soil and in the crop debris, and served as the source of infections in the subsequent seasons or years (Dasgupta, 1989; Hegde *et al*, 1989). During off season, the pathogen survived in the crop debris (Dasgupta, 1989; Palarpawar and Ghurde, 1989; Wilson *et al*, 1992; Misra and Sinha, 1996; Urena – Padilla *et al*, 2001). *C. gloeosporioides* causing anthracnose of black pepper survived for 150 days in infected leaves kept under laboratory conditions and for 90 days in the soil (Anoop 2002; Anoop and Kumari, 2002). In the present investigation *C. gloeosporioides* survived in the infected leaves kept under laboratory condition for 105 days and in the soil for 150 days. Reduced survival of the pathogen under laboratory condition may be due to the unavailability of moisture and nutrition. The survival of thippali isolate was almost close to the above reports.

Biological control of plant diseases became the most sought after one, since it reduces the environmental hazards and are ecofriendly. Biological control of foliar pathogens were reported by several workers (Blakeman and Fokkema, 1982; Cristinzio, 1987; Anandraj and Sarma, 1995; Sen, 2000; Anoop, 2002). During the present investigation, native fungi isolated from phyllosphere and rhizosphere of thippali were tested for their antagonistic effect on the pathogen *Trichoderma viride* and *Aspergillus terreus* were found to be most effective under *in vitro* studies. Effects of *Trichoderma* spp. and *Aspergillus* spp. in suppressing a variety of

pathogens was extensively studied by various workers (Weindling, 1932; Elad *et al.*, 1983; Gokulapalan and Nair, 1984; Venkatasubbiah *et al*, 1984; Cristinzio, 1987; Gabje and Lanjewar, 1991; Mukherjee and Sen, 1992; Vaishnaw *et al*, 1992; Aujla and Kaur, 1993; Chet and Inbar, 1994; Suriyachandra selvan, 1997; Jubina and Girija,1997; Reghuchander *et al*, 1998; Sen, 2000; Anith and Manomohandas, 2001; Sarma and Sain, 2004; Ayana *et al.*, 2005). Anoop (2002) reported the effectivity of *T.harzianum* and *A. niger* against *C.gloeosporioides*. The present study also is in agreement with these reports.

Five resistance inducers were tested against *C.gloeosporioides* under *in vitro* conditions. SA at 1g/l was selected for field application. Efficiency of SA in inducing the resistance in plants has been studied by various scientists. White (1976) reported that application of SA resulted in plant disease resistance through the expression of PR (Pathogenesis related) genes in TMV tobacco. Bowels (1990) reported that SA induced PR gene which possessed antifungal / antibacterial properties. Gaffney *et al.* (1993) reported that SAR is obtained by the accumulation of SA through induced phenyl propenoid biosynthesis. Delaney *et al* (1995) reported that SA is an important component in the signal transduction pathway and is involved in the local and systemic resistance to pathogens. Meena *et al* (2001) also reported the same physiology with SA in the late leaf spot of groundnut. SA has a role in SAR induction by a variety of pathogens or pathogen elicitors (Ryals *et al.*, 1996). Dempsey *et al* (1999) reported that alteration in the activity of synthesis of certain enzymes was increased through SA mediated SAR and it offered defense response on generation of free radicals. No studies are available on the inhibition of *C.gloeosporioides* by SA under *in vitro*.

Plant based chemicals are becoming popular in plant disease management owing to their less hazardous nature. In the present investigation Ovis (Lantana based) compound was found to be effective against the pathogen. The extract of *L.camera* was found to be effective against *Oidium phyllanthi* and *Macrophomina phaseolina*. (Sahayarani *et al.*, 2005; Shan *et al.*, 2005)

No reports are available so far on the effect of Ovis on *C. gloeosporioides* on thippali. In the present study Ovis inhibited *C. gloeosporioides* completely at all the levels tested. Though the three levels were found to be effective, the lowest level was selected for field application since this was more economical.

One of the extensively studied organic substance and used in biological strategy for plant disease control is neem cake. Effect of neem cake for control of plant pathogens, especially soil borne inoculam was studied by numerous scientists. (Meena and Mariappan, 1993; Diyora and Khander, 1995; Lodha, 1995; Ushamalini *et al.,* 1997; Padmodaya and Reddy, 1995; Haque *et al.,* 1998; Sachin *et al.,* 2002; Rajani *et al.,* 2005). Vidyasagar and Akbar (2005) reported that 10 % neem cake was effective for controlling anthracnose of oil palm caused by *C.gloeosporioides.*

The five treatments selected viz. *T.viride* @ 10g/kg potting mixture, *A.terreus* @ 10g/kg potting mixture, Ovis (1mg/l), SA (1mg/l) and neem cake @ 10g/kg potting mixture were tested in the field. *T.viride* and *A. terreus* were applied as talc based formulation. There are numerous reports about the efficiency of talc based formulations of antagonists (Backamn and Kabana, 1975; Lewis and Papavizas, 1985; Mukopadhyay, 1987; Lewis *et al*; 1991; Anoop Sankar, 2002)

The treatment T_{12} (combination of T_2T_5 - *A. terreus* + neem cake) was the best in suppressing the disease (PDI=13.61%). Application of *A. niger* was reported to be effective against *R. solani* and *C. gloeosporioides* (Gokulapalan and Nair, 1984; Padmakumari, 1989; Anoop, 2002). Kalisena, a proprietary compound of *A. niger* was reported to be effective against a series of pathogens (Sen et al, 1995; Sen et al; 1998; Sen, 2000). Kalisena controls charcoal rot of potato and stalk rot of cauliflower (Mondal, 1998; Sarma *et al,* 2001). Combined application of *A. terreus* and Neem cake might have complemented their effect and resulted in increased disease suppression. Neelamegam (2005) reported the effectiveness of *T. viride* with neem cake for the management of damping off of tomato

The present study also indicated that apart from reducing the soil inoculum of the pathogen, it is essential to check the aerial phase of the plant by foliar application with antagonists. Therefore a combination of soil application and foliar spray was used for the delivery of the formulated product. Foliar application of the fungal antagonists have been extensively studied with respect to foliar pathogens (Tronsomo and Dennis, 1977; Trutmann *et al;* 1991; Harman, 2000; Krishnamoorthy and Gnanamanickam, 1998). Effectiveness of the foliar application of *Aspergillus* sp. was also reported (Gogoi and Royi, 1993; Saikia and Choudhary, 1993).

Soil and foliar application of talc based formulation of *A. terreus* @10 g/kg potting mixture along with foliar spray 1% suspension of talc based formulation applied twice for a period of 45 days of the experiment has effectively checked the spread of anthracnose of thippali. Maximum disease suppression was obtained at 45 DAT. However this antagonist has to be tested in large scale in the field to derive a concrete conclusion.

Statistical analysis of the data showed that *A.terreus* was found to be soundly interacting with *T.viride.* This indicated that the combined application of the two antagonists can be ensured. Development of fungicide resistant/tolerant strain, effective delivery system, safety of the antagonist towards animal fauna, their persistence in the soil etc. is to be studied before commercialising the product.

Analysis of DRE revealed that the ecofriendly materials either alone or their combination enhanced the activity of DRE. Among the individual treatments, Ovis reported the maximum activity of PO and PPO (8.46 and 38.46 $min\Gamma^{1}$ $g\Gamma^{1}$ fresh weight). Tha PAL activity was maximum in *T.viride* treated plants (0.49 µg g^{-1} min^{-1}freshweight). This indicated that *T.viride* is not only fungistatic but can also enhance the DRE. PAL enhancement with *T.viride* was reported in pea plants infected with *F. solani f. sp. pisi* (Jha and Jalali, 2005). The present study is also in agreement with the above report.

The combined application of ecofriendly materials revealed that the PO activity was higher in treatment T_{14} (T_3T_5 - Ovis + Neem cake) treated plants (29.96 $\mu g g^{-1} min^{-1}$) compared to control. Combined application of treatment T_7 (T_1T_3 - *T. viride* + Ovis) enhanced the activity of PPO and PAL (33.83 and 0.54 $\mu g\ g^{-1}$ min^{-1}freshweightμ) in the treated plants. DRE was induced in plants when they were challenged by pathogens or antagonists (Cramer *et al.*, 1985; Avdiushko *et al.*, 1993) Though the increased PAL activity was noticed, statistical analysis of the data revealed that the values were on par with control.

In the present study Ovis was found to enhance the DRE activity in general. Efficiency of Ovis for the management of the disease can be attributed to its dual quality, plant protection and DRE enhancement. This quality of Ovis can be utilized in the management of plant diseases. A perusal of reviews revealed that so far no reports are available on the effect of Ovis and its combination with antagonists in the anthracnose infected thippali plants.

Summary

6. SUMMARY

The Indian long pepper, commonly known in Malayalam as thippali *(Piper longum* L.) is an important medicinal plant. It is a reservoir of various alkaloids such as piperine, piplatin, piperolactum A, piperolactum B, piperadione, piperlonguminine etc. It is a constituent of various indigenous medicines.

A survey was conducted for a period from January to December 2005 at the medicinal garden of College of Agriculture, Vellayani and the medicinal garden of Ayurveda Research Institute, Poojappura, Thiruvananthapuram to identify, the major diseases associated with thippali. A severe leaf spot / blight that ultimately led to defoliation was the major disease observed. Isolation of the pathogen revealed that the causal agent is *C. gloeosporioides.* Koch's postulate studies proved that the pathogen is *C. gloeoporioides.* Isolation of the pathogens associated with that of anthracnose of black pepper and betel vine, the two closely related crops were also made. The studies revealed that the pathogens were *C. gloeosporioides.* Since the symptomatology and pathogen associated were similar, the disease in thippali was designated as Anthracnose.

Cross inoculation studies were carried out using the *C. gloeosporioides* isolates of thippali, black pepper and betel vine. Thippali isolate failed to produce symptoms on both black pepper and betel vine. Moreover the cultural and conidial charactors of thippali isolate was different from that of black pepper and betel vine.

The survival studies indicated that the pathogen survived under laboratory condition for 105 days, where as in the soil on infected materials it survived for 150 days. This study revealed that the infected plant materials served as the primary source of inoculum for the next season.

The rhizosphere studies recorded eight fungi and two bacteria from the healthy thippali plants. Among the fungi *A. terreus* was the predominant one. The bacteria isolated were gram negative. From the phyllosphere, three fungi and two bacteria were isolated. *Penicillium* sp. was found to be the predominant one. The

bacteria were gram positive. Antagonistic studies revealed that *T.viride* and *A.terreus* were the most effective in suppressing the pathogen.

Of the five different resistance inducers tested at three different levels, SA at 1mg/l. gave the least suppression of the pathogen under *in vitro* conditions. Hence this was selected for further studies.

Among the two plant based chemicals tested Ovis showed highest suppression of the pathogen under *in vitro* condition. Though all the levels of Ovis gave suppression of the pathogen, the lowest level (1mg/l) was selected for field application.

The four selected ecofriendly materials along with neem cake and their combinations were tested in the field for the management of the disease. PDI,45 DAT showed that T_{12} (T_2T_5 - *A. terreus* + neem cake) was found to be the best in controlling the disease. The treatment T_4 (SA) was least effective. The other treatments were significantly different from the control.

Estimation of DRE showed that PO and PPO activity was highest in T_3 (Ovis) where as PAL activity was maximum in T_1 (*T. viride*). Combination of treatments revealed that maximum PO activity was in treatment T14 (T_3T_5 - Ovis + neem cake). Treatment T_7 (T_1T_3 - *T.viride* + Ovis) showed maximum PPO activity. PAL activity was maximum in T_7 (T_1T_3 - *T. viride* + Ovis).

References

7. REFERENCES

Akinwunmi, O., Latunde - Dada and John, A.I. 2001. The plant defense activator Acibenzolar-S-Methyl in cowpea seedlings for rapid induction of resistance. *Physiol. Mol. Pl. Path.* 58: 199-208

*Alcazar, M.D., Egea, C., Espin, A. and Candela, M.E. 1995. Peroxidase isozymes in the defence response of *Capsicum annuum* L. to *Phytophthora capsici*. *Phytopathologia Plantarum* 94: 736-742

Ali, M.E.K. and Warren, H.L. 1987. Physiological races of *Colletotrichum graminicola* on sorghum. *Pl. Dis.* 71: 402-404

Alvira D' souza, Roy, K.J., Mahanty, B. and Dasgupta, B. 2001. Screening of *Trichoderma harzianum* against major fungal pathogens of betel vine. *Indian Phytopath.* 54 (3): 340-345

Amaresh, C., Anjali, A., Mandal, P.K. and Pradeep, S. 2001. Influence of salicylic acid on protein content and catalase activity in relation to systemic acquired resistance in cowpea against root rot. *Indian Phytopath.* 54: 284-287.

Amirsadegi, S., Robson, C.A., Mc Donald, A.E. and Vanlerberghe, G.C. 2006. Changes in plant mitochondrial electron transport alter cellular levels of reactive oxygen species and susceptibility to cell death signaling molecules. *Pl. Cell Physiol.* 47: 1509-1519.

Anandraj. M. and Sarma, Y.R. 1995. Biological control of black pepper diseases. *Indian Cocoa Arecanut Spices J.* 18: 22-23

Angappan, K., Dureja, P. and Sen, B. 1996. Multiprong actions of biocontrol agents, *Aspergillus niger* AN 27. Second International Crop Science Congress on Crop Productivity and Sustainability – Shaping the future, 17-24 November 1996. New Delhi, *Abstract*: 10-30

Anith, K.N. and Manomohandas, T.P. 2001. Combined application of *Trichoderma harzianum* and *Alcaligens* sp. strain AMB 8 for controlling nursery rot disease of black pepper. *Indian Phytopath.* 54 (3): 335-339

Anoop, S. 2002. Biocontrol of anthracnose of black pepper (*Piper nigrum* L.) caused by *Colletotrichum* sp. using mycoparasites. M.Sc. (Ag.) thesis, Kerala Agricultural university, Trichur, 64p.

Anoop, S. and Kumari, P.S. 2002. Survival of *Colletotrichum gloeosporioides*, the causal organism of anthracnose disease of black pepper. *J. Spices Aromatic Crops.* 11 (2): 129-131

Aujla, S.S. and Kaur, S. 1993. *In vitro* effect of antagonistic fungi on *Neovossia indica. Punjab Agric. Univ. Res. J.* 30: 160-163.

Avdiushko, S.A., Ye, X.S. and Kuc, J. 1993. Detection of several enzymatic activities in several leaf prints of cucumber plants. *Physiol. Mol. Pl.Path.* 42: 441-445

Ayana C., Das, S., Manna, K.B., Basu, A. and Raj, S. K. 2005. Different bio antagonist combinations against *Pythium* damping off of Brinjal. Second Global Conference, Plant health - Global wealth, 25-29 November 2005. Indian Society of Mycology and Plant Pathology and Maharana Pratap University of Agriculture and Technology, Udaipur, India. *Abstract*: 181.

Ayyar, T.K.R. 1921. A preliminary investigation of the pollu disease of pepper in North Malabar in 1921. *Madras Agric. Year Book*, pp. 18-31

Bestwick, C.S., Brown, I.R., Bennett, M.H. and Mansfield, J.W. 1997. Localisation of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringe* pv. *phaseolicola. Pl. Cell.* 9 (2): 209-221

Bharathi, S., Marimuthu, T. and Vanitha, S. 2005. Ecofriendly management of leaf blight disease of tomato and onion. National seminar on Emerging Trend in Plant Pathology and their Social Relevance (ETPPSR), 7-8 March 2005. Department of Plant Pathology, Faculty of Agriculture, Annamalai University. *Abstract*: 130

Biles, C.L. and Hill, J.P. 1988. Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on wheet seedling leaves. *Phytopathology* 78: 656-659.

Blakeman, J.P. and Fokkema, N.J. 1982. Potential for biologial control of plant diseases on the phylloplane. *A. Rev. Phytopath.* 20: 167-192

Bonner, J. 1950. *Plant Biochemistry*. Academic Press, New York, 537 p.

Bowels, D.J. 1990. Defense related proteins in higher plants. *A. Rev. Biochem.* 59: 873-907

Brian, P.W. and Mc Grawn, J.C. 1945. Viridin: A highly fungistatic substance produced by *Trichoderma viride. Nature.* 156: 144-145

Backman, P.A. and Kabana, R.R. 1975. A system for the growth and delivery of biological control agents to the soil. *Phytopathology* 65: 819-821

Chakraborty, P.K., Mukewar, P.M., Raj, S. and Kumar, V.S. 2002. Biochemical factors governing resistance in diploid cotton against grey mildew. *Indian Phytopath.* 55 (2): 140-146

Chattopadhyay, S.B. and Satyabrata M.1990. *Diseases of Betel vine and Spices* Second edition. Indian Council of Agricultural Research, New Delhi. 160 p.

Chen, Z., Silva, H. and Klessing, D.F. 1993. Active oxygen species in the induciton of plant systemic acquired resistance by salicylic acid. *Science.* 262 (5141): pp. 1883-1886

Chet, I. and Inbar, J.1994. Biological control of fungal pathogens. *App. Biochem. Biotech.* 48: 37-48

*Cramer, C.L., Bell, J.N., Ryder, T.B., Bailey, J.A., Suhuch, W., Boiwell, G.P., Robins, M.P., Dixon, R.A. and Lamb, C.J. 1985. Co-ordinated synthesis of phytoalexin in biosynthetic enzymes in biologically stressed cells of bean. (*Phaseolus vulgaris*) *EMBO Journal.* 4: 285-289

Cristinzio, G. 1987. Studies on biological control of *Phytophthora capsici* on pepper. *Capsicum Newsl.* 6: 65

Das, B.C. and Roy, A.K. 2000. Combined effect of press mud and *Aspergillus terreus* on management of sheath blight of rice. *Indian Pl. Path.* 18: 64-68

Dasgupta, B. 1989. Competitive saprophytic ability of *Colletotrichum capsici* causing leaf spot and stem anthracnose of betel vine. *Indian Phytopath.* 42: 431- 435

Dastur, J.F. 1935. Disease of pan (*Piper betle*) in the Central provinces. *Proc. of Indian Acad. Sci. B.* 1: 778-815

De Oliveria, V.L., De Bellei, M. and Borges, A.C. 1984. Control of white rot of garlic by antagonistic fungi under controlled environmental conditions. *Can. J. Microbiol.* 30: 884-889.

*Delaney, T.P., Friedrich, L. and Ryals, J. 1995. Arabidiopsis signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc. Natl. Acad.* USA. 92: 6602-6606

Dempsey, D.A., Jyothi, S. and Nessig, D.F. 1999. Salicylic acid and disease resistance in plants. *Crit. Rev. Pl. Sci.* 18: 547-575

Dennis, C. and Webster, I. 1971. Antagonistic properties of species group of *Trichoderma* – Production of nonvolatile antibiotics. *Trans. Br. Mycol. Soc.* 57: 25-39.

Dickerson, D.P., Pascholati, S.F., Hagerman, A.E., Butler, L.G. and Nicholson, R.L. 1984. Phenyl alanine ammonia lyase and hydroxy cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum. Pysiol. Pl. Path.* 25: 111-123

*Dickson, C.H. and Skidmore, A.M. 1976. Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans. Br. Mycol. Soc.* 66: 57-64.

Dillard, H.R. and Cobb, A.C. 1993. Survival of *Colletotrichum lindemuthianum* in bean debris in New York State. *Plant Dis.* 77: 1233- 1238

Dillard, H.R. and Cobb, A.C. 1998. Survival of *Colletotrichum cocodes* in infected tomato tissue and in soil. *Plant Dis.* 82: 235-238

Diyora, P.K. and Khander, P.R. 1995. Management of wilt of cumin (*Cuminum cuminum* L.) by organic amendments. *J. Spices and Aromatic Crops.* 4: 80-81

Dubey, S.C. and Patel, B. 2001. Evaluation of fungal antagonists against *Thanatephorus cucumeris* causing web blight of urd and mung bean. *Indian Phytopath.* 54 (2): 206-209

Eastburn, P.M. and Gublerg, W.D. 1992. Effect of soil moisture and temperature on survival of *Colletotrichum acutatum. Pl. Dis.* 76: 481-482

Elad, Y., Chet, I. and Kalan, J. 1983. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. *Phytopathology*. 73: 109-121.

Farooqi, A.A. and Sreeramu, B.S. 2001. *Cultivation of Medicinal and Aromatic Crops*. University press, Hyderabad, 518 p.

Foyer, C.H., Lopez-Delgado, H., Dat, J.F. and Scott, I.M. 1997. Hydrogen peroxide and glutathione associated mechanism of acclimatory stress tolerance and signaling. *Physiol. Pl.Path.* 100 : 241-254

Fredrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Gutrella, M., Meier, B., Dincher, S., Staub, T., Ukens, S., Metraux, J., Kessman, H. and Ryals, J. 1996. A benzothiadiazole derivative induced systemic acquired resistance in tobacco. *Pl. J.* 10: 61-70

*Friend, J. and Thronton, J.D. 1974. Caffeic acid -O- methyl transferase, phenolase and peroxidase in potato tuber tissue inoculated with *Phytophthora infestans*. *Phytopathology Z.* 81 (1): 56-64

Gabje, D.W. and Lanjewar, R.D. 1991. Antagonism of *Aspergillus niger* on some seed borne fungi of two rice cultivars. *PKV Res. J.* 15: 168-171

Gaffney, T., Fredrich, L., Vernooji, B., Negrotto, D., Ney, G., Uknes, S., Ward, E., Kessman, H. and Ryals, J. 1993. Requirements of salicylic acid for induction of systemic acquired resistance. *Science*. 261: 754-756

Gogoi, R. and Roy, A.K. 1993. Effect of foliar spraying of *Aspergillus terreus* Thom. on sheath blight and rice plant characteristics. *Int. Rice. Res. Notes*. 18: 31-32

Gokulapalan, C. and Nair, M.C. 1984. Antagonism of a few fungi and bacteria against *Rhizoctonia solani* Kühn. *Indian J. Microbiol.* 24: 57-58

Gorlach, J., Volralt, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendrop, M., Staub, T., Ward, E., Kessman, H. and Ryals, T. 1996. Benzothiadiazole, a novel class of inducer of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Pl. Cell.* 8: 629-643

Guleria, S., Sohal, B.S., Bajaj, K.L. and Mann, A.P.S. 2001. Peroxidase, superoxide dismutase isoforms and pisatin content in *Erysiphe pisi* resistant and susceptible pea cultivars. *Pl. Dis. Res.* 16 (1): 34-39.

Hammerschmidt, R., Nuycklese, M. and Kuc, J. 1982. Association of enhanced peroxides activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium. Physiol. Pl. Path.* 20: 73-82

* Haque, S.E., Zaki, M.J., Vahidy, A.A., Abdul Ghaffar. and Ghaffer, A. 1998. Effect of organic amendments on the efficiency of *Pseudomonas aeruginosa* in the control of root rot disease of sunflower. *Pakist. J. Bot.* 30 (1): 45-50

Harman, G.E. 2000. Myths and Dogmas of Biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Pl. Dis.* 84: 377-395.

Hegde, Y.R., Hegde, R.K. and Kulkarni, S. 1989. Perpetuation of *C.gloeosporioides* – the causal agent of anthracnose of arecanut. *Curr.Res.* 18: 98-100.

Howell, C.R., Hansen, L.E., Stiponovic, R.D. and Puckhaber, L.S. 2000. Induction of terpenoid synthesis in cotton roots and control of *Rhizoctonia solani* by seed treatment with *Trichoderma virens. Phytopathology* 90 : 248-252

*Huang, Y., Deverall, B.J., Tang, W.H., Wang, W. and Kul, F.W. 2000. Foliar application of Acibenzolar-S-Methyl and protection of post harvest rock melons and hami melons from diseases. *Eur. J. Pl. Path.* 106: 651-656

*Ishii, H., Tomita, Y., Horio, T., Marusaka, Y., Nakazawa, Y., Nishimura, K.O. and Iwatomo, S. 1999. Induced resistance of Acibenzolar-S-Methyl (CGA 245704) to cucumber and Japanese pear disease. *Eur. J. Pl. Path.* 105: 77-85

Jackson, A.J., Walter, D.R. and Marshall, G. 1994. Evaluation of *Penicillium chrysogenum* and its antifungal extracts as potential biocontrol agents against *Botrytis fabae* on beans. *Mycol.Res.* 98 : 1117-1126

Jane, L. and Marc, R.K. 2002. Protection against heat stress induced oxidative damage in *Arabidiopsis* involves calcium, abscisic acid, ethylene and salicylic acid. *Pl. Physiol.* 128 (2): 682-695

Jebakumar, R.S., Anandaraj, M. and Sharma, Y.R. 2001. Induction of pathogenesis related proteins and defence related enzymes in black pepper due to inoculation with *Phytophthora capsici. Indian Phytopath.* 54: 23-28

Huang, J.S. and Hsu, H.T. 2003. Induced disease resistance in plants. *Advaces in Plant Disease.* (eds. Huang-Chang Huang and Surya N. Acharya) Agriculture and Agri-Food Canada Research Centre. Canada, pp. 237-258

Jeyarajan, R., Ramakrishnan, G., Dinakaran, D. and Sridar, R. 1994. Development of products of *Trichoderma viride* and *Bacillus subtilis* for the biocontrol of root rot disease. *Biotechnology in India.* (ed. Diwedi). Bioved Research Society, Allahabad, pp. 25-36

Jha, P.K. and Jalali, B.L. 2005. Induction of defence related enzymes in pea (*Pisum sativum* L.) by *Trichoderma viride* against *Fusarium solani* f. sp. *pisi* causing root rot disease. Second Global Conference, Plant health - Global wealth, 25-29 November 2005. Indian Society of Mycology and Plant Pathology and Maharana Pratap University of Agricultureand Technology, Udaipur, India. *Abstract*: 63

Jones, D., Gorden, A.H. and Bacon, J.S.D. 1974. Co-operative action by endo- exo-β-(1,3) - glucanases from parasitic fungi on the degradation of cell wall glucans of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Biochem. J.* 140: 47-55

Jubina, P.A. and Girija, V.K. 1997. Fungal antagonists from forest soils for the control of foot rot pathogen of black pepper. 23rd Annual meeting and Symposium of MSI on fungi in forest ecosystem, 9-10 May 1997. Kerala Forest Research Institute, Peechi. *Abstract*: 14.

Kalix, S., Anfoka, G., Li, Y., Stadnic, M. and Buchenauer, H. 1996. Induced resistance in some selected crops - prospects and limitations. *Modern fungicides and antifungal compounds* (eds. Lyr, H., Russel, P.E. and Sisler, H.D.). Intercept Ltd., 505 p.

Kannayan, S. and Prasad, N.N. 1983. Suppression of seedling infection of rice due to *Rhizoctonia solani* by organic amendments. *Madras Agric. J.* 70: 209-210

Knight, H. 2000. Calcium signaling during abiotic stress in plants. *Int. Rev. Cytol.* 195: 269-324

Kosuge, T. 1969. The role of phenolics in host response to infection. *A. Rev. Phytopath.* 7: 195-222

Krishnamurthy and Gnanamanickam, S.S. 1998. Biologiocal control of rice blast by *Pseudomonas fluorescens* strain pf-7-14. *J. Biol. Control.* 13: 158-165

*Kuc, J. 2001. Concepts and directions of induced systemic resistance in plants and its applications. *European J. Pl. Path.* 107: 7-12

Laha, G.S. and Venkataramanan, S. 2001. Sheath blight management in rice with biocontrol agents. *Indian Phytopath.* 54 (4): 461-464

Lal, H.C. and Upadhyay, J.P. 2002. Biological control of leaf blight caused by *Alternaria tenuissima* (Kunzer ex. Pers.) Wiltshire in pigeon pea. *J.Biol.Control.* 16 (2): 141-144

Leon, J., Lawton, M.A. and Raskin, J. 1995. Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. *Pl. Physiol.* 108 (4): 1673-1678

Lewis, J.A. and Papavizas, G.C. 1985. A new approach to stimulate population proliferation of *Trichoderma* species and other biocontrol fungi introduced into natural soil. *Phytopathology* 74: 1240-1244.

Lewis, J.A., Papavizas, G.C. and Lumsden, R.D. 1991. A new formulation for the application of the biocontrol fungi to soil. *Biocontrol Sci. Technol.* 3: 181-183

Lizzy, Y. and Coulomb, P. J. 1991. Compartmentation of soluble peroxidase and catalase fractions during interactions between pepper and tobacco mosaic virus or *Phytophthora capsici Can. J. Bot.* 69 (8): 1853-1864

Lodha, S. 1995. Soil solarisation, summer irrigation and amendments to the control of *Fusarium oxysporum* f. sp. *cumini* and *Macrophomina phaseolina* on arid soils. *Crop Prot.* 14: 215-219

Macko, V., Woodbury, W. and Stahmann, M.A. 1968. The effect of peroxidase on the germination and growth of *Puccinia graminis* f. sp. *tritici. Phytopathology* 58: 1250-1254

Maiti, S. and Sen, L. 1982. Incidence of three major diseases of betel vine (*Piper betle* L.) in relation to weather. *Indian Phytopath.* 35: 14-17

Mandal, S., Srivastava, K.D., Agarwal, R. and Singh, D.V. 1999. Mycoparsitic action of some fungi on spot blotch pathogen (*Dreschlera sorokiniana*) of wheat. *Indian Phytopath.* 52 (1): 39-43

*Mannia, L., Sepre, A.L., Ritieni, A., Fogliane, V., Vinnole, F., Randozzo, G., Maddau, L. and Bottalice, A. 1997. A new fungal growth inhibitor from *Trichoderma viride. Tetrahedron* 53: 3135-3144

Mathivanan, N., Srinivasan, K. and Chelliah, S. 2000. Field evaluation of *Trichoderma viride* Pers. Ex. S.F. Gray and *Pseudomonas fluorescens* Migula against foliar diseases of groundnut and sunflower. *J. Biol. Control* 14 (1): 31-34

Mathur, K., Thakur, R.P. and Rao, V.P. 2001. Characterisation of *Colletotrichum graminicola* populations from a sorghum hybrid CSH 9 for morphological and pathogenic variability. *Indian Phytopath.* 54: 299-303

Mayee, C.D. and Dater, V.V. 1986. Phytopathometry. Technical bulletin –1 (Special bulletin–3), Marathwada Agricultural University, Maharashtra. pp. 146

Mayer, A.M., Harel, E. and Shaul, R.B. 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochemistry* 5: 783-789

Meena, S.S. and Muthusamy, M. 1999. Effect of organic soil amendments against rice sheath blight. *Indian Phytopath.* 52 (1): 92-93

Meena, B., Marimuthu, T. and Velazhahan, R. 2001. Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidum personatum. J. Mycol. Pl. Path.* 31 (2): 139-145

Meena, S.S. and Mariappan, V. 1993. Effect of plant products on the seed borne microflora of sorghum. *Madras Agric. J.* 80: 383-387

Mehta, R.D., Patil, K.A., Rao, K.K. and Mehta, M.H. 1995. *Trichoderma harzianum* as biological control agent in formulated product . *Indian J. Mycol. Pl. Path..* 25: 129

Misra, A.N. and Sinha, A.P. 1996. Studies on the survival of sorghum anthracnose (*Colletotrichum graminicola*) pathogen on crop debris. *Indian Phytopath.* 49: 381- 383.

Mohanbau, R., Seetharaman, K.; Ebenezer, E.G. and Sajeena, A. 2002. Efficiency of *Trichoderma viride* Pers. Gray and neem cake amendment in different soils against blackgram root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. *J.Biol.Contro.* 16 (1): 49-55

Mondal, G. 1998. *In vitro* evaluation of *Aspergillus niger* AN 27 against soil borne fungal plant pathogens and field testing against *Macrophomina phaseolina* on potato. Ph.D. thesis, Indian Agriciultural Research Institute, New Delhi. 117 p.

Mondal, G. and Sen, B. 1999. Siderophore production by *Aspergillus niger* AN 27, a biocontrol agent. *Curr. Sci.* 77: 337-338

Mukherjee, B. and Sen, C. 1992. *Aspergillus* and *Penicillium* species: Potential agents for biocontrol of *Macrophomina phaseolina. Indian Phytopath. 45: 39-43*

Mukopadhyay, AN. 1987. Biocontrol efficacy of *Trichoderma* spp. in controlling soil borne diseases. Workshop on biological control of plant diseases, 10-12 March 1987, Tamil Nadu Agricultural University, Coimbatore. *Abstract*: 29.

Nair, B.C. 2005 Management of foliar blight of Amaranthus using rhizobacteria and chemical activator Acibenzolar -S- methyl. M.Sc. (Ag.) thesis, Kerala Agricultural University, Trichur, 87 p.

Nair, R.A. 2005. Microbial antagonists and resistance inducers for the management of bacterial blight of Anthurium (*Anthurium andreannum* Linden). M.Sc. (Ag.) thesis, Kerala Agricultural University, Trichur, 64 p.

Nakkeeran, S., Sankar, P. and Jeyarajan, R. 1997. Standardisation of storage conditions to increase the shelf life of *Trichoderma* formulations. *J. Mycol. Pl. Path.* 27: 60-63

Nallathambi, P., Mohanraj, D. and Padmanabhan, P. 2000. Biocontrol of red rot caused by *Colletotrichum fulcatum* in sugar cane. *Proceedings of Indian Phytopathological Society Goldeen Jubilee. International conference on integrated plant disease management for sustainable agriculture.November 11-15, 1997.* (eds. Jain, R.K.). Indian Phytopathological society, IARI, New Delhi, pp. 315-316

Neelamegam,R. 2005. Effect of different organic matter sources and *Trichoderma viride Pers: Er.* on damping off of tomato (*Lycopersicon esculentum* L.) var. Co-1 seedlings caused by *Pythium indicum* Bal. *R. J. Biol. Control.* 19 (2): 149-156

*Oostendrop, M., Kunz, W., Dietrich, B. and Staub, T. 2001. Induced disease resistance in plants by chemicals. *European J. Pl. Path.* 107: 19-28

Padmakumari, G. 1989. Survival of *Rhizoctonia solani* Kühn. with special reference to antagonistic soil microflora. Ph.D. thesis, Kerala Agricultural University, Trichur, 124 p.

Padmodaya, B. and Reddy, H.R. 1999. Effect of organic amendments on seedling diseases of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici. J. Mycol. Pl. Path..* 29: 38-41

Palarpawar, M.Y. and Ghurde, V.R. 1989. Perpetuation and host range of *Colletotrichumm curcumae* causing leaf spot disease of turmeric. *Indian Phytopath.* 42: 576-578.

Pandey, S., Maghogho, C.K., Bandhyopadhyay, R. and Karunakar, R.I. 1991. Variation in pathogenic and cultural characteristic of sorghum isolates of *Colletotrichum graminicola* in India. *Pl. Dis.* 75: 778-783.

Pant, R. and Mukopadhyay, A.N. 2001. Integrated management of seed and seedling rot complex of soybean. *Indian Phytopath.* 54 (3): 346-350

Papavizas, G.C. 1985. *Trichoderma* and *Glyocladium*: Biology, Ecology and Potential for biocontrol. *A. Rev. Phytopath.* 23: 23-54

Papavizas, G.C., Dunn, M.T., Lewis, J.A. and Beagle-Ristaino, J. 1984. Liquid fermentation technology for experimental production of biocontrol fungi. *Phytopathology* 74: 1171-1175.

Patibanda, A.K. and Sen, B. 2005. Mechanism of *Aspergillus niger* van. Teigh. antagonism towards *Fusarium oxysporum* Schlect f.sp. *melonis* Sny. and Hans., musk melon wilt pathogen. *J. Biol. Control.* 19 (2): 115-120

Pereira, L.F., Goodwin, P.H. and Erickson, L. 2000. Peroxidase activity during susceptible and resistant interactions between cassava (*Manihot esculenta*)

and *Xanthomonas axenopodis pv. manihotis* and *Xanthomonas cassavae. J. Phytopath.* 148: 575-577

Pramod M. and Singh, K.P. 2005. Screening of some botanicals against leaf blight of wheat caused by *Bipolaris sorokiniana*. Second Global Conference, Plant health - Global wealth, 25-29 November 2005. Indian Society of Mycology and Plant Pathology and Maharana Pratap University of Agricultureand Technology, Udaipur, India. *Abstract*: 285

Prasad, R.D. and Rangeshwaran, R. 2000. Shelf life and bioefficiency of *Trichoderma harzianum* formulated in various carrier materials. *Pl. Dis. Res.* 15: 38-42

Prasad, R.D., Rangeshwaran, R. and Sreeramakumar, P. 1997. Mass production of *Trichoderma harzianum* and its shelf life in different formulations. Indian Phytopathological Society-Golden Jubilee International Conference, 10-15November 1997, India, *Abstracts*: 212

Quimio, T.H. and Quimio, A.J. 1975. Notes of Philippine grape and guava anthracnose. *Pl. Dis. Rep.* 59: 221-224

Rahayuningsih, S.A. 1990. Peroxidase activity and their relationship to resistance of pepper plant to *Phytophthora palmivora. Ind. Crop Res. J.* 3 (1): 18-22

Rajan, P.P., Sarma, Y.R. and Anandraj, M. 2002a. Management of foot rot disease of black pepper with *Trichoderma* spp. *Indian Phytopath.* 55 (1): 34-38

Rajan, P.P., Gupta, S.R., Sarma, Y.R. and Jackson, G.V.H. 2002b. Diseases of ginger and their control with *Trichoderma harzianum. Indian Phytopath.* 55 (2): 173-177

Rajani, V.V. Parakhia, A.M. and Rakholiya, K.B. 2005. Effect of soil amendments and biocontrol agents on root rot disease (*Macrophomina phaseolina*) of castor. Second Global Conference, Plant health - Global wealth, 25-29 November 2005, Indian Society of Mycology and Plant Pathology and Maharana Pratap University of Agriculture and Technology, Udaipur, India. *Abstract*: 185

Rao, Y.R. 1926. Pollu disease of pepper. *J. Madras Agric. Student Union* 14: 5-10

Rohilla, R., Singh, R.L., Singh, U.S., Singh, R., Duveiller, E. and Singh, H.B. 2001. Recent advances in the management of plant diseases using chemicals. *Indian J. Plant Path* 19 (1&2): 1-23 pp.

Ravi, S., Sabita D. and Jeyalakshmi, C. 1999. Effect of biocontrol agents on seed borne *Colletotrichum* in french bean. *Plant Dis. Res.* 14: 146-151

Reghuchander, T. Rajappan, K. and Samiyappan, R. 1998. Influence of biocontrol agents and organic amendments on soyabean root rot *Int. J. Tropical Agric.* 16: 247-252

Riddle, R.W. 1974. Slide cultures. *Mycologia* 42: 265-270.

Romero, A.K., Kaushik, C.S. and Ritchie, D.F. 2001. Resistance to bacterial spot in bell pepper induced by Acibenzolar-S-Methyl. *Pl. Dis.* 85: 185-194

Roy, A.K. 2003. Mycological problems of crude herbal drugs – Overview and challenges. *Indian Phytopath.* 56 (1): 1-13

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y. and Hunt, M.D. 1996. Systemic acquired resistance. *Pl. Cell.* 8: 1809-1819

Sachin, U., Gupta, K., Shyam, K.R. and Upamanyu, S. 2002. Innovative approaches for the management of root rot and web blight (*R. solani*) of french bean. *J. Mycol. Pl. Path.* 32 (3): 317-331

Sahayarani, S., Chitra, K., Mareeshwari., Kamalakkannan, A. and Mohan, L. 2005. Effect of plant extracts on the spore germination of *Oidium phyllanthi.* National seminar on Emergency Trends in Plant Pathology and their Social Relevance (ETPPSR), 7-8 March 2005. Faculty of Agriculture, Annamalai University, Annamalai Nagar. *Abstracts*: 107

Saikia, P. and Chowdhury, H.R. 1993. Phylloplane microflora for the control of bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae. Indian Phytopath.* 46: 218-223

Sanders, D., Brownlee, C. and Harper, J.F. 1999. Communicating with calcium. *Pl. Cell.* 22: 691-706

Sarma, Y.R., Anandraj, M. and Ramanan, K.V. 1996. Disease management in *Phytophthora* foot rot affected black pepper plantations. *Ann. Rep. IISR,* 63 p.

Sen, B. 2000. Biological control: A success story. *Indian Phytopath.* 53: 243-249

Sen,B., Mukherjee.K, Patibanda,A.K and Sharma. J. 1995 *Aspergillus niger* a potential biocontrol agent for soil borne plant pathogens. *Proceedings of Global Conference on Advances in Research on Plant Diseases and their Management, April 24-25, 1995.* (eds. Khetarpur, R.K., Gupta, K., Celia, C.V., Gour, H.N., Thakore, B.B.L. and Bhargav, H.). Society of Mycology and Plant Pathology, Rajasthan Agricultural University, Udaipur, pp. 161-169.

Sen, B., Sanjeevkumar, S., Mondal. G., Angappan, K., Mukherjee, K., Chattopadhyay, C. and Das, I.K. 1998. Kalisena; a novel biopesticide for disease free crop and better yield. *Proceedings of National Symposium on Development of Microbial pesticides and insect pest Management, November 12-13, 1998.* BARC Mumbai and Hindustan antibiotics Ltd., Pune, pp. 106-108.

Shan, M., Sanjeevkumar, K., Kaviyarasan, N. and Prabhu, S. 2005. Effect of some plant materials on the sclerotial germination of *Macrophomina phaseolina.* National seminar on Emerging Trends in Plant Pathology and their Social Relevance (ETPPSR), 7-8 March 2005, Faculty of Agriculture, Annamali University, Annamalai Nagar. *Abstracts*: 115.

Shanmugam, V. and Varma, A.S. 1999. Effect of native antagonists against *Pythium aphanidermatum,* the causal organism of ginger rhizome rot. *J. Mycol. Pl. Path.* 29: 375-379

Sharma, P., Singh, L. and Adlakha, D. 2001. Evaluation of *Trichoderma* and *Aspergillus* species against *Sclerotium sclerotiorum* (Lib.) de Bary causing rots in cauliflower. *Indian Phytopath* 54 (4): 439-451.

Sharma P. and Sain, S.K. 2004. Induction of systemic resistance in tomato and cauliflower by *Trichoderma* spp. against stalk rot pathogen, *Sclerotinia sclerotiorum* (Lib) de Bary. *J. Biol. Control,* 18 (1): 21-28

Singh, A.K. and Bhatt, M.N. 2002. Control of french bean rust through fungicides and a neem based formulation. *Indian Phytopath.* 55 (2): 241-243

Singh, B.P. and Joshi, L.K. 1973. Studies on the diseases of betel vine (*Piper betle* L.) in Jabalpur (Madhya Pradesh) a new leaf spot incited by *Cephalosporium*. *Sci. Cult.* 39: 312

Singh, J.P. and Singh, D.B. 2000. *In vitro* screening of non volatile metabolites of phyllosphere mycoflora of bringal against leaf spot fungus *Alternaria solani* for biocontrol potential. *Pl. Prot. Bull.* 49: 22-24

Sivaprasad, P., Meenakumari, K.S. and Geeji, M.T. 2005. Microbial inoculants for crop disease management . *Proceedings of National workshop on microbial inoculants for crop nutrition and health, September 29 – 30, 2005.* (eds. Sivaprasad, P., Meena Kumari, K.S.), Kerala Agricultural university, College of Agriculture, Vellayani, Thiruvananthapuram, pp. 1-10.

Spletzer, M.E. and Enyadi, A.J. 1999. Salicylic acid induced resistance to *Alternaria solani* in hydroponicaly grown tomato. *Phytopathology* 89: 722-727

*Srivastava S.K. 1987. Peroxidase and Polyphenol in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassic) Goid and their implication in disease resistance. *Phytopathology Z.* 120: 249-254

Steven, V., van Derkelen., K. James, D., Inya G., Tom, B., Stijn, M., Pieter, R., Luit, S., van Montagu, M. Marc, Z., Dirk, I. and van Breusegem, F. 2003. Role of Hydrogen Peroxide in stress tolerance in plants. *Pl. Biol.* 100 (26): 1613-1618

Sticher, L., Mauh-Mani, B. and Metraux, J.P. 1997. Systemic acquired resistance. *Ann. Rev. Phytopath.* 35: 235-270

Sunder, S., Singh, R. and Dodan, D.S. 2005. Management of bacterial blight of rice with botanical extracts and non-conventional chemicals. *Pl. Dis. Res.* 20 (1): 12-17

Surendran, M. 2005. Newer botanicals for rice sheath blight disease in Kuttanadu. UGC sponsored National seminar on Emerging Trends in Plant Pathology and their Social Relevance (ETPPSR), 7-8 March 2005, Department of Plant Pathology, Faculty of Agriculture, Annamali University, Annamalai Nagar, *Abstracts*: 112

Suriyachandraselvan, M. 1997. Studies on the charcoal rot of sunflower (*Helianthus annus* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. M.Sc. (Ag.) thesis, TNAU, Coimbatore, 295 p.

Suseelabai, R., Joseph, T. and Sarma, Y.R. 2000. Biocontrol of capsule rot of cardamom. *Proceedings of Indian Phytopathological society - Golden Jubilee. International Conference on Integrated Plant Disease Management for Sustainable Agriculture, November 11-15,1997.*(eds. Jain, R.K.). Indian Phytopathological Society, IARI, New Delhi, pp. 331-332.

Sutton, B.C. 1992. The genus *Glomerella* and its anamorph *Colletotrichum. Colletotrichum Biology, Pathology and Control.* (eds. Bailey, J. and Jager, M.). CAB International, UK, pp.1-26

Timonin, M.I. 1940. Interaction of higher plants and soil micro organisms with microbial population of seedlings of certain cultivated plants. *Can. J. Res.* 18: 307-317.

Tosi, L. and Zazzerini, A. 1994. Evaluation of some fungi and bacteria for potential control of safflower rust. *J. Phytopath.* 142: 131-140

*Tronsmo, *A.* and Dennis, C. 1977. The use of *Trichoderma* species to control strawberry fruit rots. *Neth . J. Plant Path.* 83: 449-455

*Tronsmo, A., Klemsdal, S.S., Hayes, C.K., Lorito, M. and Harman, G.E. 1993. The role of hydrolytic enzymes produced by *Trichoderma harzianum* in biological control of plant diseases. *Enzyme structures, Biochemistry, Genetics and Applications* (eds. Suominen, P. and Reinikinen, T.) Foundation for Biochemical and Industrial Fermentation Research, Helsinki, Finland, 159-168 pp.

Trutmann, P., Keane, P.J. and Merriman, P.R. 1991. Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyper parasite *Coniothyrium minitans. Biol. Control.* 1: 293-301

Tschen. J.S.M and Kuo. W.L. 1981. Antibiotic inhibition and control of *Rhizoctonia solani* by *Bacillus subtilis. Pl. Protection Bull. Taiwan.* 27: 85-103.

Umamaheswari, M.P., Muthusamy, M. and Alice, D. 2002. Evaluation of antagonists against jasmine wilt caused by *Sclerotium rolfsii. J. Biol. Control.* 16 (2): 135-140

Urena – Padilla, A.R., Mitchell, D.J. and Legard, D.E. 2001. Over summer survival of inoculum of *Colletotrichum* crown rot in buried strawberry crown tissue. *Pl. Dis.* 85: 750-754

Urs, R.R.N.V. and Dunkavy, J.M. 1974. Bacterial activity of horse radish peroxidase on *Xanthomonas phaseoli* var. *sojensis. Phytopathology* 64: 514

Ushamalini, C., Rajappan, K. and Kausalya, G. 1997. Control of cowpea wilt by non chemical means. *Pl. Dis. Res.* 12: 122-129

Vaishnav, M.U., Sabalpara, A.N. and Khandar, R.R. 1992. Mycoparasitism on sugarcane smut (*Ustilago scitaminae*) by four fungi. *J. Mycol. Pl. Path.* 22: 142-145

Venkatasubbiah, P. and Safeeulla, K.M. 1984. *Aspergillus niger* for biological control of *R. solani* on coffee seedlings. *Trop. Pest Mgmt.* 30: 401-406

*Vernooji, B., Friedrich, L., Goy, P.A., Staub, T., Kessman, H. and Ryals, J. 1995. 2, 6- dichloro isonicotinic acid induced resistance to pathogens without the accumulation of salicylic acid. *Mol. Plant-Microbe Interact.* 8: 228-234

Vidyasagar, B. and Akbar, A.F.M. 2005. Effect of neem cake on anthracnose of oil palm nurseries caused by *Colletotrichum gloeosporioides.* National seminar on Emerging Trend in Plant Pathology and their Social Relevance (ETPPSR), 7-8 March 2005. Department of Plant Pathology, Faculty of Agriculture, Annamalai University. *Abstract*: 104

Weindling, R. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology* 22: 837-845

White, R.F. 1976. Acetyl salycilic acid (aspirin) induces resistance to TMV in tobacco. *Virology* 99: 410 -412

Wilson, L.L., Madden, L.V. and Ellis, M.A. 1992. Over winter survival of *Colletotrichum acutatum* in infected strawberry fruit in Ohio. *Pl. Dis.* 76: 948-950

*Yamamoto, H., Hokin, H. and Tani, T. 1978. Peroxidase and polyphenol oxidase in relation to the crown rust resistance of oat leaves. *Phytopathology Z.* 91: 193-202

Yoshida, S. and Shirata, A. 1999. Survival of *Colletotrichum dematium* in soil and infected mulberry leaves. *Pl. Dis.* 83: 465-468

*Zeppa, G., Allergone, G., Barberi, M. and Gaurda, P.A. 1990. Variability in the production of volatile metabolites by *Trichoderma viride. An. Microbiologia Entomologia* 90: 171-176

Zhou, T. and Reeleder, R.D. 1989. Application of *Epicoccum purpurascens* spores to control white mold of snap bean. *Pl. Dis.* 73: 639-642.

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* Original not seen

Appendix

APPENDIX - I

Buffers for enzyme analysis

A) 0.1 M sodium borate buffer (pH, 8.8)

A: 0.2 M solution boric acid (12.4g in 100 ml)

B: 0.05 M solution of borax (19.05 g in 1000 ml)

50 ml of A is mixed with 30 ml of B, diluted to a total of 200 ml

B) 0.1 M sodium phosphate buffer (pH 6.5)

A: 0.2 M solution of monobasic sodium phosphate (27.8 g in 1000 ml)

B: 0.2 M solution of dibasic sodium phosphate (53.65 g of Na_2HPO_4. 7 H_2O in 1000 ml)

68.5 ml of A is mixed with 31.5 ml of B, diluted to a total of 200 ml

ETIOLOGY AND ECOFRIENDLY MANAGEMENT OF FUNGAL DISEASES OF THIPPALI
(*Piper longum* L.)

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Abstract of the
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ABSTRACT

A survey was carried out at the medicinal garden of College of Agriculture, Vellayani and medicinal garden of Ayurveda Research Institute, Poojappura, Thiruvananthapuram to study the diseases associated with *Piper longum* L. (Indian long pepper or thippali). The major disease observed was anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. Pathogenicity test proved that *C. gloeosporioides* was the pathogen associated with the disease.

Cross inoculation studies conducted using *C. gloeosporioides* isolate of thippali, black pepper and betel vine - the sister plants – showed that the thippali isolate was highly host specific. The colony and conidial morphology of the three isolates also showed considerable difference. *C. gloeosporioides* of thippali had very small conidia and the culture was also found to be shy sporulating.

Studies on the survival of *C. gloeosporioides* showed that the pathogen survived for 105 days in the infected leaves, under laboratory conditions. In the soil, it survived for 150 days.

Of the eleven fungal and four bacterial isolates tested against *C. gloeosporioides*, *T. viride* and *A.terreus* were found to be most effective under *in vitro* condition. Among the different resistance inducers tested *in vitro*, SA (1 g/l) was selected for field evaluation, as it had no direct action on the pathogen. Of the two plant based chemicals tested, Ovis reported the highest suppression of the pathogen.

These four eco friendly materials selected from the *in vitro* studies and neem cake were tested in the field to determine their effectiveness in controlling the disease. Their combinations were also studied.

Among the different eco friendly materials tested in the field, treatment T_{12} (T_2T_5 - *A. terreus* + neem cake) was found to be best in disease suppression at 45 DAT.

Estimation of DRE showed that PO and PPO activity was highest in T_3 (Ovis) where as PAL activity was maximum in T_1 (*T. viride*). Combination of treatments revealed that maximum PO activity was in treatment T_{14} (T_3T_5 - Ovis + neem cake).The PPO and PAL activity was maximum in T_7 (T_1T_3 - *T. viride* + Ovis).