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**(A. KAMALAKANNAN)**

## ABSTRACT

# DEVELOPMENT OF MANAGEMENT STRATEGIES FOR THE CONTROL OF COLEUS (*Coleus forskohlii*) BRIQ. ROOT ROT CAUSED BY *Macrophomina phaseolina* (TASSI.) GOID. AND *Rhizoctonia solani* (KUHN.)


By

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A survey was undertaken in major coleus growing areas of Tamil Nadu to elicit information on coleus root rot incidence. Among the villages surveyed, Kaniyamoor village of Viluppuram district recorded the high root rot incidence (36.0%). In Salem district, maximum root rot incidence (69.0%) was recorded in Keeripatti village. Fungi *viz.*, *Macrophomina phaseolina* and *Rhizoctonia solani* were found to be associated with the root rot of coleus and produced drying of stem and root rot symptoms followed by wilting. Coleus plant at the age group of 30-120 days are affected by *M. phaseolina*, where as, plants less than 30 days infected by *R. solani*. The maximum loss of forskolin alkaloid (0.06%) was noticed in 100 per cent infected tubers. The sclerotial population of *M. phaseolina* varied from 1.8 to 278 no. / g of dry soil and the maximum population was observed in Kaniyamoor fields. Root rot infection leads to the disruption of epidermal cells, cortical parenchyma cells, corky layer formation and plugging of xylem vessels in both root and stem.

Among the biocontrol agents tested, *Trichoderma harzianum* Rifai. – Th, *T. viride* Pers. TVC5, *Pseudomonas fluorescens* Migula - PFC6 effectively reduced the mycelia growth of *M. phaseolina* to an extent of 62.5, 56.8 and 52.9 per cent over control, respectively. The same biocontrol agents significantly reduced the mycelial growth of *R. solani* to an extent of 64.3, 55.1 and 47.3 per cent over control, respectively. Aqueous extract of *Allium sativum* Lin. bulbs gave maximum reduction in the mycelial growth (76.0 %) and sclerotial production (47.9%) of *M. phaseolina.*

Wintergreen (*Gaultheria procumbens* L.) oil (WGO) and Lemon grass (*Cymbopogan citratus* (D.C.) Stapf) oil (LGO) and their 40 Emulsifiable Concentrate (EC) formulation at 0.2 per cent were highly inhibitory to the pathogens under *in vitro* condition (>90.0%). Zinc sulphate (1.0%) solution effectively reduced the mycelial growth of *M. phaseolina* (90.6%) and *R. solani* (90.5%). Zinc sulphate did not show any inhibitory effect on the growth of all the biocontrol agents tested, whereas wintergreen and lemongrass oil at 0.2 per cent concentration completely inhibited the growth of *T. harzianum*- Th and *T. viride*- TVC 5. However these oils did not inhibit growth of *P. fluorescens.*

*Trichoderma* species and *P. fluorescens* strains survived upto 120 and 90 days of storage, respectively in the $ZnSO_4$ and WGO amended talc-based formulations with the population ($10^8$ cfu/ml). The 40 EC formulation of wintergreen oil was stable at room temperature, neutral pH and 1:4 dilutions. It retained its efficacy in reducing mycelial growth up to eight months of storage at room temperature.

Application of talc-based bioformulation containing PFC6 + LGO + $ZnSO_4$, PFC6 + WGO + $ZnSO_4$, *T. harzianum* - Th + $ZnSO_4$ at the rate of 2 g/ planting hole, reduced the root rot incidence to an extent of 62.0,60.0 and 59.3 per cent over control, respectively. The fungicides Carbendazim and Propiconazole at 0.1 per cent concentration checked the root rot incidence to an extent of 74.6 and 71.8 per cent, respectively in pot culture experiment.

The soil application of Zinc sulphate (1g / planting hole) and Neem cake (5 g/ planting hole) reduced the root rot incidence of coleus. Soil application of talc based

formulation of biocontrol agents containing $ZnSO_4$ and WGO significantly increased their population in Coleus rhizosphere under field condition.

The dipping of cuttings and soil application of biocontrol agents and fungicides effectively reduced the root rot incidence with concomitant increase in the tuber yield. The maximum root rot reduction (85.1%) was observed in Carbendazim treated plot followed by Propiconazole (76.5%). The combined application of PFC6 + WGO +$ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ reduced the root rot incidence (68.7%) and recorded the maximum tuber yield (5674 Kg/ha), where as untreated control recorded maximum disease incidence of 69.3 per cent and tuber yield of 918 Kg/ ha at NeermulliKuttai field trial and similar trend was noticed in Coimbatore field trial also.

However, none of the biocontrol agent formulations and chemicals were effective in Kaniyamoor field trial. The biocontrol agent formulations and chemicals were effective in reducing coleus root rot incidence only up to moderate inoculum level of sclerotia (110 no./ g of soil), where as at higher inoculum level (278 no./ g of soil), all the biocontrol agents formulations and chemicals were ineffective.

The *Trichoderma* species inhibited the root rot pathogens through mycoparasitism and production of diffusible metabolites. *Pseudomonas fluorescens* – PFC 6 showed a higher production of lytic enzymes (Chitinase and β-1,3- glucanase), antibiotics (Phenazine, Diacetyl phloroglucinol), siderophores, hydrogen cyanide (HCN) and salicylic acid than *P. fluorescens* Pf1.

The biocontrol agents were found to induce the defense related enzymes such as Phenylalanine ammonia lyase, Peroxidase, Polyphenol oxidase, chitinase, β-1,3-glucanase and phenolics which in turn enabled the coleus plants to resist the root rot infection effectively.

## LIST OF TABLES



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…..Contd.

## LIST OF FIGURES

## LIST OF PLATES



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**CONTENTS**

# CHAPTER I

## INTRODUCTION

Coleus (*Coleus forskohlii* Briq.), is a subtropical and warm temperate species belonging to the family Labiatae naturally growing at 600 to 1800 m elevation. It grows well in loamy soil at 6.4 to 7.9 pH of arid and semi arid climatic zones (Shah, 1989). Recently farmers have started raising this crop because of its economic potential (Vishwakarma *et al.*, 1988). It is cultivated in India, Nepal, Srilanka, Africa and Thailand. In India, the annual production of Coleus root is approximately 1500 tonnes. Coleus is cultivated in 1200 and 600 ha in Viluppuram and Salem districts of Tamil Nadu, respectively. The area under cultivation is expanding every year (Rajamani and Azhakiyamanavalan, 2003).

The tuberous roots of this crop contains forskolin, a labdane diterpene (Bhat *et al.*, 1977), which has pharmacological properties namely antiglaucoma, antiplatelet aggregation, anti-inflammatory, antithrombotic (Rupp *et al.*, 1985) and a biochemical activity of adenylate cyclase stimulant increasing intracellular cyclic AMP (Metzger and Lindner, 1981; Seamon *et al.*, 1981).

Among the several limiting factors for successful cultivation, susceptibility to diseases is one of the major constraints. *C. forskohlii* is affected by many fungal and bacterial diseases such as wilt caused by *Fusarium chlamydosporum* (Shyla, 1998; Boby and Bagyaraj, 2003), Botrytis blight caused by *Botrytis cinerea*, leaf blight caused by *Coryneospora cassilicola* and *Pseudomonas cichorii* (www.hort world.com). Recently, Kamalakannan *et al.* (2003a) for the first time reported the occurrence of root rot of coleus caused by *Macrophomina phaseolina* and *Rhizoctonia solani* in Tamil Nadu.

Among the above diseases, root rot is considered to be the most important and occurrence of this disease has been increased in Tamil Nadu. Though root rot of Coleus caused by different pathogens was reported earlier, information on various aspects of root

rot caused by *M. phaseolina* and *R. solani* is meager. Having this in mind, investigations were taken up with the following objectives.

1. Survey for the occurrence of root rot of Coleus in Tamil Nadu, identification of pathogens and establish pathogenicity.
2. Studying the cytological changes and loss of alkaloid due to root rot infection.
3. Quantification of sclerotial population of root rot pathogen from soils.
4. Isolation, screening and selection of effective fungal and bacterial biocontrol agents under *in vitro* condition.
5. Screening of plant extracts, plant oils, organic amendments and fungicides against root rot pathogens.
6. Development of new bioformulations (talc based and emulsifiable concentrate formulation) by combining effective bio-control agents, plant oil and micronutrient.
7. Testing the efficacy of bioformulations, organic amendments, micronutrient and fungicides under green house and field conditions.
8. Studying the mechanisms involved in the disease resistance by bioformulations against root rot pathogens.
9. Studying the mode of action of biocontrol agents

# CHAPTER II
# REVIEW OF LITERATURE

Coleus (*Coleus forskohlii* Briq.) is cultivated mainly for their medicinal values in India. There are several production constraints among which losses due to pest and diseases are much concerned. Among the various diseases, root rot caused by *Rhizoctonia solani* and *Macrophomona phaseolina* was the most devastating disease. The root rot disease causes reduction in the tuber yield and forskolin content.

## 2.1. Coleus (*Coleus forskohlii*) Briq. and its diseases

Coleus (*Coleus forskohlii*) Briq. belonging to the family Labiatae is a subtropical and warm temperate species naturally growing at 600 to 1800 m elevation. It grows well in the loamy and sandy loam soils of 6.4 to 7.9 pH of arid and semiarid climatic zones. (Shah, 1989). It is cultivated commercially in India, Nepal, Srilanka, Africa, Burma and Thailand. In India, it is cultivated with annual production of 1500 tonnes. (Rajamani and Azhakiyamanavalan, 2003). The tuberous root of these medicinal herbs contains forskolin, a labdane diterpene (Bhat *et al.*, 1977). Coleus is affected by number of diseases such as wilt caused by *Fusarium chlamydosporum* (Shyla, 1998; Boby and Bagyaraj, 2003) and *Botrytis* blight caused by *Botrytis cinerea*, leaf spot caused by *Coryneospora cassilicola* and *Pseudomonas cichorii* (www.hortworld.com). Recently, Kamalakannan *et al.* (2003a) reported the occurrence of root rot of coleus caused by *Macrophomina phaseolina* and *Rhizoctonia solani* in Tamil Nadu.

Since very meager information related to the root of Coleus caused by *M. phaseolina* and *R. solani* is available in the literatures, the literatures of other crops root rot are reviewed hereunder.

## 2.2. Isolation of Pathogens

Malathi (1996) isolated *M. phaseolina* from the root rot infected groundnut crop collected from different locations. Among the locations, isolate collected from Paramathy was highly virulent. Merin Babu (2002) isolated a stem and stolon rot pathogen from the infected Peppermint and identified the causal agent as *Rhizoctonia solani*. Indra and Gayathri (2003) isolated root rot causal organism from the black gram plants showing

root rot symptoms and identified as *M. phaseolina* based on the morphological features. Uma maheswari *et al.* (2002) isolated a wilt pathogen of Jasmine from the infected plants collected from the various districts of Tamil Nadu and identified the pathogen as *Sclerotium rolfsii* from the morphology of sclerotia and mycelium. Santha kumari and Anoop sankar (2003) observed that the Bush pepper affected by leaf and berry blight disease and isolated the causal agent on PDA and identified it as *Colletotrichum gloeosporioides*. Gupta *et al.* (2002) collected the root samples from the guava tree showing wilt incidence and identified the pathogen as *Fusarium oxysporum* f. sp. *psidii.*

2.3. Pathogenicity

Malathi (1996) conducted pathogenicity studies on different isolates of pathogen *M. phaseolina* and found isolate 1 from Paramathy was highly virulent which showed 75 per cent inhibition of germination and 90.6 per cent root rot incidence in groundnut. Merin Babu (2002) induced stolon rot diseases in both sterilized and unsterilized soils using sand maize medium grown inoculum of *R. solani* (10g/kg of soil). She also reported that *R. solani* isolate R.s.2 (Chittepalayam) exhibited the highest degree of virulence (93.75%).

Sundaravadana (2002) proved the pathogenicity of root rot of Blackgram pathogen *M. phaseolina* grown on sand maize medium (Riker and Riker, 1933) in both sterilized soil and unsterilized soil.

2.4. Histological changes in healthy and infected leaves of crop plants

Dienelt and Lawson (1989) reported that *Xanthomonas campestris* pv. *citri* infection on leaves of *Citrus aurantifolea* lead to cell wall thickening and formation of outer wall apposition. Rey *et al.* (1998) found that the deposition of phenolics and thickening of cell wall in tomato plants infected by *Pythium oligandrum*. They also observed the thickening of xylem vessels, cytoplasmic disorganization and large number of small vacuoles. Lee *et al.* (2000) observed the colonization of *P. capsici* hyphae in the

epidermis and cortical cells of pepper plants. They also observed intercellular impregnated haustoria in the host cells.

Belanger *et al.* (2003) observed the growth of external hyphae of *Blumeria graminis* f.sp. *tritici*, the powdery mildew pathogen of wheat, on the epidermal surface and penetrated the cells by producing haustoria. They also observed thickening of plant cells in the infected tissue due to suberization. Sahayarani (2003) stated that *Phyllanthus niruri* plants exhibited several histological changes due to infection by powdery mildew pathogen. Microtome section of infected leaves showed external mycelium on the epidermal layer of both upper and lower surface, disruption of cuticle, palisade and spongy parenchyma cells, thickening of xylem vessels and the presence of haustoria inside the cuticular regions. Whereas, the healthy leaves showed an intact, regular arrangement of cuticular region and parenchyma cells.

2.5. Loss of alkaloids due to pathogen infection

Boby and Bagyaraj (2003) stated that *Coleus forskohlii* infected with *F.chlamydosporum* recorded comparatively lesser per cent of forskolin content in the roots. The forskolin content of the root decreased with the increased percentage of the wilt incidence. Merin Babu (2002) reported that *R. solani* infection in *Mentha piperita* reduced the oil content in all susceptible varieties and the extent of reduction varied with the species. *R. solani* infection led to a reduction of oil content in *M. citrata* (0.75%) and in *M. piperita* (0.752%).

2.6. Quantification of pathogen inoculum from soil

Cloud and Rupe (1991) used three selective media for enumeration of sclerotia of *M. phaseolina* present in the soil sample. Among the three media, the population of sclerotia enumerated with RB medium was as high or higher than that enumerated with MP or MSK. Sclerotia can be counted on RB medium three to 14 days after plating, compared with not more than 4 days on MP and no fewer than 7 days on MSK. Suriachandraselvan and Seetharaman (2000) estimated the population of *M. phaseolina* in sterilized and unsterilized soil by dilution plate technique using PDA medium at monthly intervals and expressed as cfu $g^{-1}$ soil.

Merin Babu (2002) estimated the population of *R. solani* from the naturally infected soil collected from various places using the method given by Ko and Hora (1971). The highest population (2.18 propagules/g of soil) was recorded in sample collected from Chettipalayam. The lowest inoculum load (0.87 propagules/g of soil) was recorded in Thondamuthur soil. Mihail and Alcorn (1982) developed method for quantitative recovery *M. phaseolina* sclerotia from soil. A similar assay was also developed by the McCain and Smith (1972).

### 2.7. *In vitro* screening of biocontrol agents against root rot pathogens

The most frequently studied fungi in relation to biological control are species of *Trichoderma.* They were antagonistic to *M. phaseolina* because of rapid growth and ability to degrade sclerotia (Punja and Grogan, 1981). The linear growth and microsclerotial production of *Rhizoctonia bataticola* were inhibited by four isolates of *T.harzianum* (Elad *et al.,* 1986). The antagonism of *T. harzianum* against *R. bataticola* was reported by Jacob (1989), Selvarajan (1990) and Malathi (1996). Antagonistic effect of *T. viride* against *M. phaseolina* was reported by many workers (Alagarsamy *et al.,* 1987; Dubey and Dwivedi, 1988; Umamaheswari, 1991; Krishnaveni, 1991). *T. harzianum* caused reduction in growth and sclerotial production of *M. phaseolina* (Nakkeeran, 1992).

Hoda Ahmed *et al.* (2000) reported that *T. harzianum, T. viride* and *B. subtilis* inhibited the mycelial growth of *R. solani, M. phaseolina* and *F. oxysporum* f. sp. *vasinfectum* under *in vitro* condition. *T. harzianum* was the most effective one followed by *T. viride* and *B. subtilis.* Pandey *et al.* (2000) used *T. viride* and *Aspergillus niger* strain V as biocontrol agents to control the *M. phaseolina.* The highest antagonistic potentiality was observed with the *T. viride* against *M. phaseolina* in dual culture.

El-Nashar *et al.* (2001) screened several *Trichoderma* species isolated from wheat rhizosphere against the pathogenic fungi *Fusarium graminearum* and

*R. solani* under *in vitro* condition. *In vitro* studies showed that *T. harzianum* caused highest reduction in mycelial growth and sclerotial production and *Bacillus subtilis* caused malformation and decay of mycelia of pathogenic fungi. Prashanthi *et al.* (2000) screened 9 antagonistic microorganisms against *R. bataticola* under *in vitro* condition. The highest inhibition zone was observed in the *Pseudomonas fluorescens* (10 mm). *T. viride* and *T. harzianum* overgrew and completely suppressed the growth of *R. bataticola*, which could be due to coiling and disintegration of hyphae of the test pathogen by *Trichoderma* spp.

Biswas and Sen (2000) screened eleven isolates of *T. harzianum* in *in vitro* condition against *M. phaseolina*. They observed the isolates T2, T6, T8 and T10 completely overgrew the pathogen within two days after contact and inhibited the pathogen growth. Gayathri and Indra (2003) screened 7 biocontrol agents against the growth of *A. niger* in dual culture method. The highest inhibition was observed in the *T.viride* followed by *T. harzianum*. The other agents *P. fluorescens* and *B. subtilis* were least effective.

In recent years, there has been much success in biological control of soil borne diseases with the use of antagonistic fluorescent Pseudomonads (Weller and Cook 1986; Vidhyasekaran and Muthamilan 1999). Several strains of *P. fluorescens, P. putida, P.cepacia and P. aeruginosa* have been successfully used for the biological control of plant diseases (Anderson and Guerra 1985; Leeman *et al.*, 1995; Rabindran and Vidhyasekaran 1996; Vidhyasekaran and Muthamilan 1999).

Meena *et al.* (2001) screened ten *P. fluorescens* strains for their inhibitory effect on mycelial growth of *M. phaseolina,* causal agent of root rot of groundnut. Among them, pf1 was the most effective one in inhibiting the fungal growth. The strain Pf1 produced an inhibition zone of 17.0 mm, whereas the strains of PfALR-7, PfSLM-1, PfMDU-2 and PfALR-10 produced inhibition zones of 12.3, 12.0, and 11.3 mm, respectively.

The antagonists, *viz*, *Trichoderma viride, T. reesei, T. lognibrachiatum, T.harzianum, Gliocladium* virens, *P. fluorescens, B. subtilis* and *Saccharomyces cerevisiae* were tested against *S. rolfsii* for their antagonistic activity in dual culture

technique under *in vitro* condition. Among the different antagonists tested, *P. fluorescens* inhibited the maximum mycelial growth (67.22%) followed by *B. subtilis* (55.56%) and *T. viride* (45.56%)(Uma Maheswari *et al.,* 2002). Rangeshwaran *et al.* (2002) isolated 25 endophytic bacteria from internal tissues of root and stem portions of chickpea, sunflower, niger, chilli and capsicum plants and were screened in dual culture on Potato Dextrose Agar (PDA) against *F. oxysporum* f. sp. *ciceri*, *F. udum*, *R. solani* and *S. rolfsii*. Maximum per cent inhibition (37.93%) of *F. oxysporum* f.sp. *ciceri* was obtained on PDA with *B. subtilis* (PDBCEN 3). Testing against *F. udum* in dual culture test revealed that *Pseudomonas* sp. (PDBCEN 8) exerted maximum (40.45%) inhibition on PDA. Against *R. solani*, maximum inhibition (44.96%) was recorded with endophyte PDBCEN 7. Per cent inhibition of *S. rolfsii* was maximum (40.93%) with *Pseudomonas* sp. (PDBCEN 6) on PDA.

Kamalakannan *et al.* (2003b) stated that out of five *P. fluorescens* and four *B.subtilis* isolates tested for their *in vitro* inhibitory effect against *R. solani*, stem and stolon rot pathogen of *Mentha piperita, P. fluorescens* isolate PFMMP and *B. subtilis* isolate BSG3 recorded highest inhibition zone of 21.67 and 8.66 mm and lowest mycelial growth of 47.0 and 56.0 mm, respectively. Elad *et al.* (2002) studied the efficacy of soil borne *B. subtilis* strains (BB-2 and Ab-27) and *T. harzianum* against five different pathogenic isolates of *R. solani*. The mycelial growth of five different isolates were inhibited at the ratio between 75 to 90 per cent and 95 to 100 per cent by using *B. subtilis* and *T. harzianum*, respectively under *in vitro* condition.

## 2.8. *In vitro* screening of Botanicals/ plant products against root rot pathogens

The aqueous extract of *Bougainvillea spectabilis* and *Azadirachta indica* were the most effective in inhibiting the mycelial growth and sclerotial germination of *R.solani*, the causal agent of collar rot of *Phaseolus aureus* (Lakshmanan *et al.*,1990). Amaresh *et al.* (2000) stated that neem seed kernel extract at 2, 5 and 10 per cent concentration effectively reduced the mycelial growth of *Alternaria helianthi* the causal agent of sunflower leaf blight. The leaf extract of *Ocimum cannum* and *Tridex*

*procumbens* also had lesser inhibitory effect. Singh and Navi (2000) observed a 100 per cent inhibition of conidial germination of *Claviceps soghi*, the causal agent of sorghum ergot, in the Garlic extract at 9 per cent concentration. All other higher concentration also exerted complete inhibition of germination. Dubey and Patel (2000) reported that 10 per cent leaf extract of Subabul (*Leucaena leucocephala*) inhibited the maximum mycelial growth of *Thanatephorus cucumeris* followed by Karanj and Bhangria extract with same inhibition over control. Gautam *et al.* (2003b) screened 24 botanicals belonging to the Compositae family against *R. solani*. Of the botanicals, *Xanthium strumarium* was significantly more effective (67.85%), followed by *Blumea mollis* (51.10%).

Guatam *et al.* (2003a) stated that leaf extract of *Chrysanthemum coronarium*, *Eclipta alba* and *Launea aspleniifolia* reduced the mycelial growth of *M. phaseolina* to an extent of 36.4, 32.1 and 31.7 per cent reduction over control, respectively under *in vitro* condition. Sameer and Bohra (2003) observed a complete inhibition of mycelial growth of *F. oxysporum var cumini* by *Boerhavia diffusa* at 100 per cent concentration. At 50 and 10 per cent concentration, they observed a mycelial growth of 4 and 15 mm, while the control recorded 79.00 mm. Al-Mughrabi (2003) reported that extracts drawn from dried and powdered flowers, stems and leaves of *Euphorbia macroclada* with some organic solvents showed antimicrobial activity against the pathogenic fungi *Verticillium dahlea, F. oxysporum, R. solani, Pythium species, Rhizopus stolonifer* and *Alternaria solani*. Butanol was the best solvent to extract antimicrobial compounds from the plant.

**2.9. *In vitro* screening of plant oils against root rot pathogens**

Chaudhary *et al.* (1995) screened the essential oils from eleven higher plants for their toxic activity against different fungal pathogens. Oil extracted from the leaves of *Chenopodium ambrosioides* at a concentration of 2000 ppm and seed oils of *Ocimum canum, Anethum graveolens* and *Pimpinella anisum* at a concentration of 3000 ppm exhibited complete mycelial inhibition of all test pathogens (*C. falcatum, F. moniliforme, R. solani, Ceratocystis paradoxa, Curvularia lunata, C. pallescens, Periconia*

*atropurpurea* and *Epicoccum nigrum). P. anisum* oil was fungicidal at 3000 ppm to *C.falcatum, R. solani* and *C. paradoxa* . Ibrahim and Al- Mihanna (2002) stated that Carnation oil (*Eugenia caryophyllus*) was the most effective in reducing the growth of *R. solani* (80.7%) followed by *P. fluorescens* strain 103 (70.3%) and 106 (69.7%). The other oils were lesser in their effectiveness in reducing the mycelial growth of the *R. solani*, the causal agent of damping-off pathogen in Eggplant.

Bellerbeck *et al.* (2001) studied the effect of Lemon grass (*Cymbopogan nardus*) oil on the growth and morphology of *A. niger*. At 800mg/lit concentration, mycelial growth was completely inhibited. 400mg/l caused 80 per cent inhibition of mycelial growth and delay in conidiation up to four days. Pitarokili *et al.* (2002) reported that the essential oil from the plant *Salvia pomifera* subspecies *calycina* showed antifungal activity on the six phytopathogenic fungi *viz.*, *F. oxysporum* f.sp. *dianthi, F. solani, F.solani* f.sp. *cucurbitae, Verticillium dahliae, S.sclerotiarum* and *R. solani.* The oil exhibited fungistatic effect at 1000 µg/l on *S. sclerotiarum* and *R. solani.*

Bouchra *et al.* (2003) reported that essential oil from *Chrysanthemum viscidehirtum* at a concentration of 150 ppm (v/v) strongly inhibited *in vitro* growth of *Penicillium digitatum, Phytophthora citrophthora* and *B. cenerea*. The other 24 oils reduced fungal development less than 69 per cent at a concentration of 250 ppm. The inhibitory activity is due to the presence of β - farnesene, limonene and many oxygenated sesquiterpenes.

**2.10. *In vitro* screening of fungicides against root rot pathogens**

Prashanthi *et al.* (2000) screened different fungicides against the root rot pathogen *R. bataticola* under poisoned food technique. He observed carbendazim (500µg/ml), Propiconazole (500µg/ml) and chlorothalonil-SC (2000 µg/ml) were found to be effective against root rot of safflower. Effect of eight fungicides at different concentrations *viz.*, 2.5, 5, 10, 25, 50 and 100 µg/ml was tested against *S. rolfsii, R. solani* and *F. oxysporum* f.sp. *phaseoli.* Bavistin was most effective against *R. solani* while contaf and bavistin (10-25 µg/ml) inhibited 100 per cent radial growth of *F. oxysporum* f. sp. *phaseoli* and *S.rolfsii.* (Mukherjee and Tripathi, 2000).

Malathi and Sabitha (2003) screened three fungicides like Capton, Carbendazim and Thiram at four different concentrations for their inhibitory effect on growth and sclerotial production of *M. phaseolina*. Among the fungicides tested, Carbendazim at 100 µg/ml completely inhibited the mycelial growth. The other concentration of Carbendazim *viz*., 50, 10 and 5 µg/ml also exerted an inhibitory effect of 85.2, 75.9 and 72.2 per cent reduction over control. The other two chemicals were lesser in their effectiveness.

2.11. Effect of Zinc sulphate on the growth of pathogens

Macro and micro nutrients were recognized to be associated with changes in the levels of disease and crop yield. Mc New (1953) discussed the effect of fertilizers on soil borne pathogens. Now it is well established that almost all essential plant nutrients have significant role in influencing the disease incidence (Huber, 1980). Sundaravadana (2002) reported that Zinc sulphate at 500 and 750 ppm completely inhibited the mycelial growth of *M. phaseolina* under i*n vitro* conditions. Zinc sulphate 50 kg / ha reduced the root rot incidence (*M. phaseolina*) on cowpea, black gram and green gram (Latha *et al.*, 1997). Pareek (1999) found that 40 kg Potassium $ha^{-1}$ and 20 kg MnSO4 $ha^{-1}$ applied together effectively reduced charcoal rot of maize. The combination of micronutrients 10 kg $ZnSO_4$ $ha^{-1}$ +2 kg $FeSo_4$ $ha^{-1}$ induced resistance by reducing the wilt by *F. udum* f.sp. *crotalariae* (71.49% reduction) on Sunnhemp (Sarkar *et al.,* 2000). Singh and Badhoric (1984) reported that Zinc, Boron and Manganese restricted the saprophytic ability of *F. udum* on pigeon pea. Zinc sulphate effectively inhibited growth of *R.bataticola* causing root rot on chickpea (Gupta, 1999). Kalim *et al.* (2003) reported that severity of root rot (*Rhizoctonia solani* and *Rhizoctonia bataticola*) was reduced by 47.8 and 44.0 per cent by the application of 20 µg $g^{-1}$ Zn as zinc sulphate, respectively. Similarly, application of 10 µg $g^{-1}$ Mn as manganese sulphate reduced the disease severity by 42.7 and 42.0 per cent in case of *R. solani* and *R. bataticola*, respectively. Reduction in disease incidence was associated with increased levels of polyphenol oxidase (PPO), and peroxidase (PO) and total phenols. Peroxidase activity was several times more as compared to PPO –

specific activity and increased markedly after infection either with *R.solani* or *R. bataticola.*

2.12. Compatibility of biocontrol agents

Soil application of NaCl 560 kg $ha^{-1}$ suppressed crown and root rot of asparagus and increased Mn reducing rhizobacteria (64% fluorescent Pseudomonads), which was reported by Elmer (1995). Application of 33 μg $ml^{-1}$ $Zn^{2+}$ improved antibiotic biosynthesis by *P. fluorescens* against Fusarium crown and root rot of tomato (Duffy and Defago, 1997). Sundaravadana (2002) reported that the growth of *T. viride* was 88 mm and 88.5 mm at 250 and 500 ppm of zinc sulphate on 72 hours of inoculation and they were on par with each other and with control (89.5 mm). Hence *T. viride* was compatible with zinc sulphate at 250 and 500 ppm. Dubey and Patel (2000) observed the compatibility in the growth of *T. viride* and *Gliocladium virens* with the 10 per cent concentration of gingelly oil cake and groundnut cake, respectively. Neem leaf extract at 10 per cent concentration promoted the growth of *G. virens.*

2.13. Formulation of biocontrol agents

Tewari and Mukhopadhyay (2001) tested different formulations of *G. virens* against chickpea wilt complex caused by *S. rolfsii*, *R. solani* and *Fusarium oxysporum* f.sp. *ciceri* in glasshouse and field condition. *G. virens* remained viable in various formulations for three months at room temperature (20-35$^{o}$C) and six months at refrigerator temperature (5$^{o}$C) . The highest spore longevity was observed in formulation with *G. vierns* + Carboxy Methyl Cellulose (CMC).

Ali *et al.* (2001) tested the survival period of *P. aeruginosa* on Mung bean seed coated with different carriers/substrates and was found best on the talc amended with CMC (or) on gum arabic. On all substrates the antagonists population were declined dramatically at 120 days after coating.

El-Nashar *et al.* (2001) tried different formulations of biocontrol agents to combat the wheat root rot. Among different formulations, application of *T. harzianum* as talc powder at the rate of 10 g / kg of soil and *B. subtilis* as bacterial suspension were

most efficient in controlling pathogenic fungi under field condition. Manjula and Podile (2001) developed a formulation supplemented with 6.5 per cent chitin of *B. subtilis* AF1 to effectively reduced the *A. niger and F. udum* caused crown rot of groundnut and wilt of Pigeon pea, respectively. *B. subtilis* AF1 talc based formulation promoted the seed germination and biomass yield of both crops. Gasoni *et al.* (1998) studied the survival potential of biocontrol bacteria in various formulations and their ability to control damping off disease caused by *R. solani*. The population size of *P. fluorescens* decreased after 30 days of storage in peat based formulation, vermiculite/clay formulations, while the bacteria survived up to 150 days in all formulations.

## 2.14. Effect of Bio control agents on the root rot incidence under green house condition

Prashanthi *et al.* (2000) reported that soil and seed treatments of *T. viride* and *P.fluorescens* recorded 80 per cent survival of seedlings and zero per cent mortality due to pre-emergence rot. The soil application of *T. viride* and *P. fluorescens* recorded 25 and 20 per cent mortality seedlings due to *R. bataticola* in pot culture condition. Kurzawinska and Gajda (2001) stated that *T. viride* and *B. polymixa* limited the growth of *R. solani*, the causal agent of tuber rot of in potato to an extent of 54 and 30 per cent over control, respectively under green house condition.

Indra and Gayathri (2003) studied the effect of different biocontrol agents like *T. viride, T. harzianum* along with *Rhizobium* species on the root rot incidence of black gram under pot culture experiment. They found that seed treatment of gypsum based *T. viride* formulation with *Rhizobium* species treated seed recorded lower disease incidence of 29.6 per cent on 60 days after sowing (DAS). However on 75 DAS, gypsum formulation and talc formulations were on par in controlling root rot incidence. Theradimani and Juliet Hepziba (2003) recorded a least charcoal incidence in sunflower in seed treatment *T. viride* at the rate of 4 g $kg^{-1}$ combined with soil application of Neem cake at the rate of 250 kg $ha^{-1}$ (19.18%) followed by *T. viride* seed treatment + soil application of Farm Yard Manure (FYM) at the rate of 12.5 tonnes $ha^{-1}$ (19.57). The *T. viride* alone recorded 33.66 per cent and its combination with soil application of FYM at the rate of 12.5 tonnes $ha^{-1}$ recorded 23.82 per cent.

Ramamoorthy *et al*. (2002a) reported that *P. fluorescens* strains isolated from different crops rhizosphere effectively reduced the *Pythium* damping of in tomato and hot pepper under greenhouse condition. Kamalakannan *et al.* (2003b) stated that soil application of talc based formulation of *P. fluorescens*, *T. viride* and *B. subtilis* effectively reduce the stem and stolon rot incidence of peppermint caused by *R. solani*. Among the different biocontrol agents, *P. fluorescens* isolate PFMMP recorded a lowest disease incidence of 23.33 per cent under pot culture condition. Elad *et al.* (2002) reported that treatment of *B. subtilis* isolates and *T. harzianum* reduced the disease caused by five different isolates of *R. solani* causal agent of root rot disease in pigeon pea at the ratio between 75.76 and 56.96 per cent, respectively under pot culture experiment Elad *et al.* (2002) tested different *B. subtilis* isolates against *R. solani, Pythium debaryanum,*
*F. oxysporum, S. sclerotiarum* and *M. phaseolina* on tomato and Cucumber under green house condition. In Tomato, the highest efficacy against *R. solani* was obtained with Bs 98, against *F. oxysporum* with Bs 30, against *M. phaseolina* with BS sal and Bs 30, where the efficacy reached 100 per cent.

## 2.15. Effect of plant extracts/ oil cakes on the disease incidence under green house condition

Hoda Ahmed *et al.* (2000) studied the effect of plant extracts on the mycelial growth of *R. solani*, *M. phaseolina* and *F. oxysporum* f.sp. *vasinfectum.* The aqueous extracts of Garlic and Onion were found to be highly inhibitory to all pathogen, while other plant extracts had no effect on the growth under pot culture experiment. Singh and Navi (2000) stated that prophylactic spray of garlic protected both unemasculated and emasculated florets of Sorghum up to 48 h after spray, whereas the control treatment recorded 47.8 per cent of ergot infection. Soil application of karanj cake (2.5%) with spraying of karanj leaf extract (2.5%), soil application of karanj cake (2.5%) with spraying of subabul leaf extract (2.5%) and only soil application of karanj cake (5%) increased the seed germination and grain yield of Urd and Mung bean and decreased the seedling mortality and disease intensity caused by *T. cucumeris* (Dubey, 2002).

Hundekar *et al.* (1998) conducted a pot culture experiment to study the effect of organic amendments on the stalk rot sorghum caused by *M. phaseolina*. Among the amendments Neem cake recorded a low disease incidence of 42 per cent followed by groundnut cake 51 per cent.

## 2.16. Management of root rot disease using biocontrol agents, organic amendments and chemical fungicides under field condition

Boby and Bagyaraj, (2003) studied the effect of biocontrol agent on the wilt disease caused by *F. chlamydosporum* under field condition. They observed that combined application of *T. viride* and *Glomus mossae* gave best result in controlling the disease. The plant treated with *T. viride* and *G. mossae* showed the disease severity index of 33.28 per cent compared to uninoculated control (85.55%).

Mukherjee *et al.* (2001) observed the effectiveness of Contaf (Hexaconazole) (0.025%) and *Gliocluadim virens* to control the wilt complex of French bean caused by *S. rolfsii, R. solani* and *F. oxysporum* f.sp. *phaseoli* under field condition. They also observed integration of fungicide and biocontrol agents resulted in higher per cent of seed germination, lower disease incidence and higher grain yield. Rangeshwaran *et al.* (2002) noted that application of talc based formulation *of P. putida* and *P. fluorescens* reduced the root rot of chickpea caused by *M. phaseolina* to an extent of 5.8 and 4.4 per cent, respectively on 60 DAS. The lowest wilt incidence of chickpea caused by *F. oxysporum* f.sp. *ciceri* was recorded in the *P. putida* treatment( 3.3%). Combined application of *P. putida* and *P. fluorescens* exhibited suppression of root rot and wilt incidence to an extent of 5.8 and 7.5 per cent, respectively.

Anitha and Tripathi (2000) evaluated an integrated disease management strategy for controlling seedling disease of Okra caused by *R. solani* and *P. aphanidermatum* using combination of verietal resistance, chemical and biocontrol agents. They found that sowing of carbendazim treated seeds of moderately resistant variety ‘Varsha Upahaar’ along with the application of *T. viride* before sowing proved to be an ideal combination in combating seedling disease of Okra.

Theradimani and Juliet Hepziba (2003) studied the effect of different organic amendments such as FYM, Neem cake and Coir waste on the charcoal rot of sunflower caused by *M. phaseolina* during 1999 and 2000. Among three amendments, soil application of Neem cake at the rate of 250 kg/ ha recorded 30.79 per cent compared to control (55.53). Muthusamy (1989) reported that the application of FYM and Neem cake either alone or in combination with *T. viride* and *T. harzianum* significantly reduced the charcoal rot/root rot of soybean caused by *M. phaseolina.* Dubey (2002) stated that soil application of karanj cake (10%) and Groundnut cake (10%) reduces the disease incidence of Web blight in groundnut to an extent of 2.1 per cent and 6.0 per cent, respectively. The groundnut cake suppressed the seed germination and cause maximum mortality of plants may be due to the presence of inhibitory substances. Siddiqui *et al.* (2001) stated that application of Neem cake at 1 per cent mixed with *Pseudomonas aeruginosa* strain IE-2 caused greatest inhibition of root knot development due to *Meloidogyne javanica* and reduce the *M. phaseolina, R. solani* and *F. solani*

Soil application of Neem cake at the rate of 150 kg per hectare effectively reduced the damping off incidence in Onion, which recorded a 55.3 per cent of total plant stand. Seed treatment with *T. harzianum* and *P. fluorescens* at the rate of 4 and 10 g /kg, respectively along with soil application of the same at the rate of 500g and 2.5 kg along with 50 kg FYM reduced the damping off incidence in Brinjal, Chilli and cabbage. (Champawat and Sharma, 2003).

2.17. Shelf life of biocontrol agents

The efficient biocontrol agents should have long shelf life without losing its efficacy. Shelf life of an antagonist in the substrate is important in commercial production. In general, viability of antagonists in different formulations is maintained well at low temperature ($5^{o}$C) than at high temperature (above $20^{o}$ C) (Papavizas *et al.*, 1984). Soybean seeds treated with conidial suspension had viable conidia up to four months (Krishnaveni, 1991). Talc based formulation of *T. viride* retained 50 and 25 per cent of its initial population after two and four months, respectively (Jayarajan *et al.*, 1994). When gingelly seeds treated with Talc powder formulation of *T. viride* retained 65 per cent of its initial population after 120 days of storage (Sankar, 1994). The population

of *T. viride* in talc based formulation for seed treatment in different periods of storage was more when stored in 10 $^{o}$ C (Vidya, 1995). Loganathan (2002) reported that talc was found to be best for the survival of *Trichoderma* spp. and *Pseudomonas* spp. The *Trichoderma* spp. survived with required colony forming units ($10^{8}$ cfu/ml) in talc formulation up to 120 days of storage. Both Pf1 and CHAO strains survived with $10^{8}$ cfu / ml up to 90 days in talc formulation and after that it declined while the decrease was much earlier in other carrier materials.

Manav and Singh (2003) tested the shelf life of *T. harzianum* for a period of 120 days under wet and dry condition. The wet formulation recorded maximum number of cfu than the dry formulation. The low temperature of 8 ± 2$^{o}$C favored maximum number of propagules (18.7) than the high temperature of 25 ± 2$^{o}$C (14.7).

**2.18. Influence of biocontrol application on its population in soil**

Boby and Bagyaraj (2003) observed an increase in *Trichoderma* population in the root zone soil from the date of planting to the date of harvest. The maximum cfu of *Trichoderma* ($8.5x10^{4}$) was obtained in the treatment *G. mossae* and *T. viride* than the other treatments. Similarly *Pseudomonas* population also increased in all treatments starting from date of planting to date of harvest. The maximum *Pseudomonas* population was in the presence of *T. viride.* Malathi (1996) estimated the populations of beneficial bacteria and fungi from the soil treated with different biocontrol agents and organic amendments. The maximum population noticed with *T. harzianum* + neem cake application (81% increase over control). The bacterial and fungal populations increased with the age of the plant and reached maximum at 50 DAS, which was not further advanced to 75 DAS.

**2.19. Level of pathogen inoculum on the efficacy of bio control agents**

Prasad *et al.* (2002) studied the efficacy of biocontrol agents *T. harzianum* on three inoculum levels of log 3.04, 4.98 and 5.34 cfu/g of soil of *F. udum.* *T. harzianum* applied as seed treatment (10 and 20 g kg of seed) and as a soil amendment 10

and 20 g/9 $m^2$ . The *Trichoderma* population increased to more than $10^8$ cfu $g^{-1}$ by 60 days in soil application, where as seed treatment recorded only $10^{4.62}$ cfu/g of soil with in 45 days after sowing and there after start to decline. At inoculum level log 3.04, soil application of *T. harzianum* at 10 and 20 g recorded 42.9 and 61.5 per cent disease control, respectively and seed treatment alone resulted in less than 30 per cent disease control. At log 4.98 and 5.34 the disease control ranged between 35.5 and 22per cent for soil treatments.

Siddiqui, *et al.* (2001) studied the effect of different level of inoculum of root knot nematode and root rot fungi *R. solani* and *M. phaseolina* in the efficacy of *P. aeruginosa* strain IE – 6 as biocontrol agents. *P. aeruginosa* showed biological control effects at lower population level (250 eggs/plant and 1 ml culture suspension of pathogen $kg^{-1}$ of soil), where as at higher population densities, (2000 eggs/plants and 3 ml/kg of soil) did not showed significant difference in the control of pathogens.

## 2.20. Mechanism of action of biocontrol agents on plant pathogenic fungi

### 2.20.1. Plant growth promotion

Kloepper and Schroth (1978) for the first time reported that specific rhizosphere bacteria applied to seeds could colonize roots and promote plant growth which were later called as plant growth promoting rhizobacteria (PGPR). Normally most of the reported PGPR strains are from *Pseudomonas* and *Bacillus* spp. The studies on mechanism of growth promotion indicated that PGPR promote plant growth directly by production of plant growth regulators or indirectly by stimulating nutrient uptake, by producing siderophores or antibiotics to protect plants from soil borne pathogens or deleterious rhizosphere organisms. These *Pseudomonas* spp. may increase plant growth by producing gibberellin-like substances (Brown, 1972), mineralizing phosphates (Kavimandan and Gaur, 1971) or by other mechanisms which are not clearly understood. Barea *et al.* (1976) observed 50 phosphate solubilizing bacteria positive for IAA, gibberllin and cytokinin production.

Seed treatment with PGPR resulted in increased yield and growth in potato under field conditions (Kloepper *et al.,* 1980). Van Peer and Schippers (1988) documented the increased root and shoot fresh weight of tomato, cucumber, lettuce and potato as a result of bacterization with *Pseudomonas* strains. Fluorescent pseudomonads increased the plant growth of rice and cotton by 27 and 40 per cent, respectively when the bacteria were applied to the seed (Sakthivel *et al.*, 1986).

Seeds treated with fluorescent pseudomonads resulted in increased number of tillers and grain yield in addition to control of sheath blight disease in rice (Mew and Rosales, 1986). An increase in germination (30 to 60%) in maize by plant growth promoting strains of *P. aeruginosa* strain 7NSK2 and *P. fluorescens* ANP 15 was observed by Hofte *et al.* (1991). Seed and soil application of PGPR strains showed significant plant growth promotion with increased runner length and increased leaf number per plant in cucumber (Wei *et al.*, 1996). Group of *Pseudomonas* spp. significantly improved the seedling emergence in the proportion of healthy seedlings in sugar beet when compared to seedlings from untreated seeds (Williams and Asher, 1996). Various fluorescent *Pseudomonas* strains improved vegetative sett germination, plant height, cane diameter, Brix values and cane weight in sugarcane (Viswanathan and Samiyappan, 1999).

Bacterization of peanut seeds with fluorescent Pseudomonads GRC2 resulted in increased seed germination, early seedling growth, fresh nodule weight, grain yield and reduced charcoal rot disease incidence caused by *M. phaseolina* (Gupta *et al.,* 2002). Similarly, application of Pf1 as seed treatment followed by soil application against root rot effectively supported higher plant growth, better native *Rhizobium* nodulation and grain yield (Jayashree *et al.,* 2000). Soil application with *T. harzianum* hastened flowering of periwinkle and increased the number of blooms per plant on *chrysanthemum* and these were directly correlated with high population density of fungus in soil (Yachung chang *et al.,* 1986). Harman (2000) reported that seed treatment with *T.harzianum* in corn increased the greenness and accumulation of nitrogen. Altomare *et al.* (1999) observed the solubilization of rock phosphate, Zn, $Mn^{4+,}$ $Fe^{3+}$ and $Cu^{3+}$ by *Trichoderma* spp. *in vitro*. Loganathan (2002) stated that both fungal and bacterial

biocontrol agents like *T. viride*, *T. harzianum* and *P. fluorescens* promoted the germination, growth and vigour index of cabbage and cauliflower.

2.20.2. Antibiosis

Many bacterial strains are known to suppress fungal growth *in vitro* by the production of one or more antifungal antibiotics that may also have activity *in vivo* (Carruthers *et al.*, 1994; Pal, 1995; Whipps, 2001). Some of these antibiotics producing strains were also shown to suppress fungal plant disease *in vitro* ((Carruthers *et al.*, 1994; Pal,1995). Several strains of *Pseudomonas* spp. and *Bacillus* spp. have been shown to produce wide array of antibiotics which includes ammonia, butyrolactones, 2-4 diacetyl phloroglucinol, HCN, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrolnitrin, tropolone, pyocyanin, iturin, ksurfactin, viscosinamide, zwittermycin A, agrocin 84 as well as several other uncharacterized moieties (Asaka and Shoda, 1996; Fujimoto *et al.*, 1995; Kerr, 1980; Maurhofer *et al.*, 1995; Mazzola *et al.*, 1995;
Milner *et al.*, 1996; Whipps, 1997).

Burkhead *et al.* (1994) reported that *P. cepacia* B37W produced pyrrolnitrin antibiotic inhibitory to *F. sambucinum.* Michereff *et al.* (1994) could correlate the *in vitro* inhibition of *Pythium* and *Rhizoctonia* by 2,4–diacetylphloroglucinol, an antibiotic produced by *P. fluorescens* PF5 and *in vivo* control of sorghum anthracnose caused by *C.graminicola*. *P. fluorescens* (Trevisan.) Migula F113 was shown to control the potato soft rot pathogen, *Erwinia caratovora* subsp. *atroseptica* (van Hall) Dye by the production of antibiotic 2,4–diacetylphloroglucinol (DAPG) (Cronin *et al.*, 1997). Some evidence was also obtained that siderophore production by *P. fluorescens* F113 may play a role in biocontrol of potato soft rot under iron – limiting conditions, but DAPG appears to be the major biocontrol determinant. Similar result was obtained by Khmel *et al.* (1998) that *Pseudomonas* species might also control crown gall disease in many dicotyledonous plants caused by *Agrobacterium tumefaciens*. Dennis and Webster (1971) first described the antagonistic properties of *Trichoderma* in terms of antibiotic production which included both non – volatiles and volatiles. *Trichodermin*, a sesquiterpenes antibiotic produced by *Trichoderma* spp. has been reported to be active

against fungi. They also produced the antibiotics named as gliotoxin and viridin (Wright, 1956). *Trichoderma* spp. produced growth inhibitory substances, which induced leakage from cells of *R. solani* with destruction of cell integrity (Howell, 1982). Pande (1985) observed retardation of growth of *M. phaseolina* by the culture filtrates of *T. viride*. The extracts of *T. viride* and *T. hamatum* have been shown to prevent the growth of *R. solani* (Lewis and Papavizas, 1987).

**2.20.3. Competition**

Several reports stress the importance of colonization of the biocontrol agents in rhizosphere and endorhizosphere regions of plant (Mc lnroy and Kloepper, 1995; Quadt–Hallman *et al.*, 1997). Suppression of damping off of peas by *Burkholderia cepacia* showed a significant relationship between population size of the biocontrol agent and the degree of disease suppression (Parke, 1990). Also suppression of take all of wheat and *Fusarium* wilt of radish was correlated with colonization of roots by *Pseudomonas* strains (Bull *et al.*, 1991; Raaijmakers *et al.*, 1997). *Trichoderma* spp. colonized substrates in soil after chemical or thermal treatment of soil (Cook and Baker, 1983). Widden and Scattolin (1988) reported *T. polysporum* and *T. viride* showing tolerance to stress conditions and enhanced competitive saprophytic ability.

2.20.4. Rhizosphere colonization

The crucial factor in the success of biological control by fluorescent pseudomonads is their ability to colonize the rhizosphere and their persistence throughout the growing season. Fluorescent Pseudomonads are root colonizers because they occur in the natural habitat of rhizosphere and thus when they are reintroduced to roots through seed or seed – piece inoculation, they colonize root surface profusely (van Loon *et al.*,1998).

Scher *et al.* (1985) reported that disease suppression by fluorescent Pseudomonads depends mainly on its ability to colonize rhizosphere. Introduction of sss gene encoding rhizosphere colonization ability into poor colonizer strain of *P fluorescens* WCS 307 increased competitive rhizosphere colonization ability in tomato root tip

resulting in increased protection against *F. oxysporum* f. sp. *radicis – lycopersici* (Dekkers *et al.*, 2000).

**2.20.5. Parasitism and lysis**

Chu and Wu (1982) reported that the hyphae of ten isolates of *T. pseudokoningii*, three of *T. longibrachiatum*, one of *T. hamatum* and two of *T. harzianum* could coil around the hyphae of *R. solani*, which consequently often lost their cell contents and collapsed. Elad *et al.* (1983) showed that *Trichoderma* spp. attaches to *S. rolfsii* and *R. solani* by hyphal coils, hooks or appressoria. Lysed sites and penetration holes were found in hyphae of the plant pathogenic fungi following removal of the parasitic hyphae. Bedlan (1988) observed the hyphae of *T. viride* parasitizing the hyphae of *R. solani* by encircling, penetrating and growing on them. Upadhyay and Mukhopadhyay (1986) observed the lysis dof mycelium and sclerotia of *S. rolfsii* by *T. harzianum* in dual culture plate. Koby *et al.* (1994) reported the introduction of Tn7 based chi A gene into *P.fluorescens* and the construct could improve the biocontrol activity against *R. solani*. Enzymes β- 1,3 – glucanase produced by *P. stuzeri* YPL – 1 could suppress *Fusarium solani* (Lim *et al.*, 1991), whereas that *P. cepacia* controlled *R. solani, S. rolfsii* and *P.ultimum* by 85, 48 and 71 per cent, respectively (Fridlender *et al.*, 1993).

**2.20.6. Induction of systemic resistance**

The biocontrol agents bring about induced systemic resistance (ISR) through fortifying the physical and mechanical strength of cell wall as well as changing physiological and biochemical reaction of host leading to synthesis of defense chemicals against challenge inoculation of pathogens. Defense reaction occurs due to accumulation of PR proteins (chitinase and β – 1,3 – glucanase), chalcone synthase, phenylalanine ammonia lyase, peroxidase, phenolics, callose, lignin and phytoalexins.

Strain of *T. harzianum* T39 when inoculated on roots or leaves controlled disease caused by *B. cinerea* on leaves spatially separated from the site of application of the biocontrol agents (De Meyer *et al.*, 1998) and the level of protection offered by T39 applied to roots was similar to that of the known bacterial ISR inducing organisms.

Bailey and Lumsden (1998) studied the ability of xylanase or elicitors from *Trichoderma* spp. to induce resistance. Howell (1991) reported the induction of terpenoid synthesis in cotton roots and control of *R. solani* by seed treatment with *T. virens.*

Loganathan (2002) reported that induction of defense related proteins and chemicals *viz*, phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, phenols, chitinase and β-1, 3- glucanase were found to be in higher levels in treatments involving bioformulation mixture containing *P. fluorescens* (Pf1) and *T. viride* or mixture of *Trichoderma* strains and chitin against fungal pathogens and root knot nematode in cabbage and cauliflower.

The reduction of wilt incidence in pigeon pea is due to the increased activity of PAL and PO. *P. fluorescens* isolate 4-92 treated chick pea plants exhibited the time course accumulation of PR Proteins like chitinase and glucanase. The level of chitinase and glucanase increased by 6.6 to 7.0 fold up to four days after post inoculation. Thereafter a little decrease in the activity of PR protein was observed (Srivastava *et al.*, 2000). Podile and Laxmi (1998) reported that seed bacterized with *B. subtilis* AF1 showed an increase in Phenyl alanine ammonia lyase (PAL) from the day 1 of challenge inoculation with the pathogen *F. oxysporum* f.sp. *udum*, while the Peroxidase (PO) activity increased from day 6. The activity of PAL and PO in untreated inoculated plants decreased beyond initial level. Kamalakannan *et al.* (2003b) observed that soil application of biocontrol agents such as *Trichoderma* species and bacterial isolates like *P.fluorescens* induced plant to synthesize more amount of PO, PPO, PAL and total phenol which act as precursor to formation of lignin and suberin materials in the plant system. Their accumulation in turn makes the plant resistance to stem and stolon rot of Peppermint.

**2.20.6.1. Peroxidase (PO)**

Peroxidases have been implicated in the regulation of plant cell elongation, phenol oxidation, polysaccharide cross – linking, IAA oxidation, cross linking of extensin monomers, oxidation of hydroxy – cinnamyl alcohols into free radical intermediates and

wound healing (Vidhyasekaran *et al.*,1997). Bradley *et al.* (1992) reported that the increased PO activity has been correlated with resistance in many species including barley, cucurbits, cotton, tobacco, wheat and rice and these enzymes are involved in the polymerization of proteins and lignin or suberin precursors into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls and movement through vessels.

Plant root colonization by PGPR was associated with PO activity (Albert and Anderson, 1987). Two peroxidase isoforms have been induced in the PGPR treated rice plants inoculated with the sheath blight pathogen, *R. solani* (Nandakumar *et al.*, 2001). These enzymes are also part of the response of plant defense to pathogens (Hammerschmidt and Kuc, 1995) and they may decrease the quality of these plants as host for insects. High-level expression of PO was reported in *P. fluorescens* Pf1 treated tomato plants challenged with *F. oxysporum f. sp. lycopersici* (Ramamoorthy *et al.*, 2002b). Induction of four isoforms of PO was observed in PGPR treated green gram plants inoculated with root rot pathogen *M. phaseolina* (Saravanakumar, 2002). Loganathan (2002) reported that two PO isoforms were induced in the *T. viride* isolates TvO, TvOL and chitin treated cabbage plants challenge inoculated with *R. solani* and *M.incognita.* Kamalakannan *et al.* (2003b) observed that soil application of biocontrol agents such as *Trichoderma* species and bacterial isolates like *P. fluorescens* induced plant to synthesize more amount of peroxidase than the untreated inoculated plants.

**2.20.6.2. Polyphenol oxidase (PPO)**

PPO usually accumulated upon wounding in plants. Biochemical approaches to understand PPO function and regulation are difficult because the quinine reaction products of PPO covalently modify and cross – link the enzyme. The increased activation of PPO could be detected in the cucumber leaf in the vicinity of lesions caused by some foliar pathogens. Moreover, PPO can be induced via Octadecaniod defense signal pathway (Constabel *et al.*, 1995). Chen *et al.* (2000) reported that PPO was stimulated by PGPR or by the pathogen, but the wounds on split roots did not influence PPO activity compared to intact control in 13 days. PGPR untreated canes after pathogen inoculation

showed comparatively lesser induction of PPO isoforms then the PGPR treated sugarcane (Viswanathan, 1999). Expression of new PPO isoform in *P. fluorescens* Pf1 treated tomato plants challenged with *F. oxysporum*. f. sp. *lycopersici* (Ramamoorthy *et al.*, 2002b).

Saravanakumar (2002) identified three isoforms *viz.,* PPO1, PPO2, and PPO3 were observed in PGPR treated plants after inoculation with *M. phaseolina* where as in control only two isoforms were noticed and these isoforms were with less intensity. Higher induction of PPO was observed in plants pretreated with Pf1 along with chitin and challenge inoculated with *M. phaseolina*.

**2.20.6.3. Phenylalanine ammonia lyase (PAL)**

The activation of the phenylpropaniod pathway in plants by environmental stimuli is one of the most known universal biochemical stress responses. PAL catalyzes the deamination of L – phenylalanine to trans – cinnamic acid, which is the first step in the biosynthesis of large class of plant natural products based on the phenylpropane skeleton, including lignin monomers as well as certain classes of phytoalexins. Induction of enzymes such as PAL and PO leading to the accumulation of phenolics and lignin can occur in response to insect and pathogen attack, exposure to oxidizing pollutants, mechanical stimulation and are though to function in the resistance of plants to damage by these stresses. PAL is the key enzyme in inducing synthesis of salicylic acid (SA), which induces systemic resistance in many plants. In rice, ZB8 PAL gene was found to be induced by the elicitor treatment in rice cells (Li *et al.*, 1993). The gene was cloned and transgenic rice plants expressing PAL ZB8 showed systemic resistance against rice pathogens (Lamb *et al.*, 1997). Seed treatment and seedling root dipping with PGPR induced early and enhanced levels of PAL in rice plants (Nayar, 1996). Plants treated with *Pseudomonas* strains initially showed higher levels of PAL compared to control (Chen *et al.*, 2000).

Loganathan (2002) reported that cabbage and cauliflower plants treated with the bioformulation of TvO, TvOL along with chitin and challenge inoculated with both *R.solani and M. incognita*, a higher induction of PAL was observed from seven days after challenge inoculation and reached a maximum at 14 days.

**2.20.6.4. Strengthening of cell wall structures**

The rapid strengthening of reaction sites of fungal and insect entry delays the infection process and allows sufficient time for the host to built up other defense reactions. Seed treatment with PGPR in bean induces the lignification of cell wall (Anderson and Guerra, 1985). *A. rhizogenes* Ri T – DNA transformed pea roots pre – inoculated with the endophytic bacterium *Bacillus pumilus* SE34 were protected against the root rot pathogen *F. oxysporum* f. sp. *pisi.* They found that cell wall were strengthened at the sites of attempted fungal penetration by opposition containing large amounts of callose and phenolic substances, effectively preventing the fungal ingress. In tomato, bacterization with same bacterial strain has brought about cell wall thickening, deposition of phenolic compounds and formation of callose resulting in restricted growth of *F. oxysporum* f. sp. *radicis – lycopersici* to the epidermal cell and outer cortex in the root system in the treated plants (M' Piga *et al.,* 1997). Similar wall appositions and papillae were observed in pea roots treated with the *P. fluorescens* 63 – 28R upon challenge inoculation with either *F. oxysporum* f. sp. *pisi* or *P. ultimum* (Benhamou *et al.*, 1996), indicating a general induction of physical defense barriers to pathogen ingress. Induction of thickening of cortical cell walls in tomato was seen after colonization of roots by *P. fluorescens* WCS417 (Duijff *et al.*, 1997).

Saravanakumar (2002) observed higher levels of accumulation of phenolics in green gram roots up to 28 days which was treated with the bioformulation mixture of Pf1 along with chitin and challenge inoculated with *M. phaseolina*. In PGPR untreated plants increased phenolic content was noticed up to 21 days. Loganathan (2002) reported that cabbage and cauliflower plants treated with the bioformulation of TvO, TvOL along with chitin and challenge inoculated with both *R. solani and M. incognita*, accumulated a

higher amount of phenol at 21 and 14 days after challenge inoculation in cabbage and cauliflower, respectively.

### 2.20.7. Production of Extracellular lytic enzymes

Several studies have shown that efficient biocontrol agents such as *Serratia marcescens* (Lee *et al.,* 1992), *P. cepacia* (Fridlender *et al.,* 1993), *P. stutzeri* (Lim *et al.,* 1991), *P. fluorescens* (Velazhahan *et al.,* 1999) secrete chitinase and B- 1,3- glucanase capable of degrading chitin and β – 1,3 glucan, respectively the major components of fungal cell walls. Laminariase and chitinase were reported to be involved in the reduction of *F. solani* infection by *P. stutzeri* VPL 1 strain (Lim *et al.,* 1991). Fridlender *et al.,* (1993) reported that *P. cepacia* reduced the infection of *Rhizoctonia solani*, *S. rolfsii* and *P. ultimum* through production of β – 1,3 – glucanase. Velazhahan *et al.* (1999) observed a significant relationship between antagonistic activity of *P. fluorescens* against *R.. solani* and their level of chitinase production. Meena *et al.* (2001) reported that observed a significant relationship between antagonistic activity of fluorescent Pseudomonads and their level of glucanase production, which suggest that production of glucanase may also be responsible for the biological control of *M. phaseolina* by fluorescent Pseudomonads.

### 2.20.8. Siderophore production

Siderophore – mediated iron deprivation of deleterious microorganisms has been considered as one of the important mechanisms by which fluorescent Pseudomonads exert their antagonistic activity and plant growth promotion (Scher and Baker, 1982). Meena *et al.* (2001) reported that the siderophore production was more in Pf ALR – 7 and Pf1 isolates. Leeman *et al.* (1996) reported that LPS of *P. fluorescens* strains WCS 417 are the major determinants of ISR under iron – replete conditions, but under iron – limited conditions, LPS of these bacteria were not involved in ISR in radish against Fusarium wilt. They also found that pyoverdin, a type of siderophore, produced by these bacteria was responsible for ISR. Application of purified pseudobactin alone, isolated from strain WCS 374, to the roots of radish induced resistance. Thus, different bacterial determinants in inducing systemic resistance in radish vary depending upon iron

availability. Induction of ISR by LPS and siderophores seems to be complementary rather than additive and full induction of resistance by one determinant masks contributions by other(s).

Yeole and Dube (2000) reported that 12 *Pseudomonas* isolates produced siderophores under iron – deficient condition and inhibited the growth of 12 test soil – borne fungal plant pathogens. This inhibition was curtailed by 24-60 per cent in presence of iron (50 µM) when siderophore production was abolished. This indicates involvement of siderophores in the antibiosis of the rhizobacterial Pseudomonads, along with certain other yet unidentified chemical(s).

2.20.9. Hydrogen cyanide (HCN) production

The production of volatile cyanide is very common among the rhizosphere Pseudomonads (Dowling and O'gara 1994). Laha *et al.* (1996) reported that the volatile metabolites of fluorescent Pseudomonads significantly suppressed the mycelial growth of Sclerotium rolfsii. Meena *et al.* (2001) reported that the volatile metabolites produced by *P. fluorescens* strain Pf1 significantly inhibited the growth of *M. phaseolina in vitro*. Among the various strains of *P. fluorescens* tested for HCN production, the strains Pf1 and Pf ALR – 7 produced more of HCN. The involvement of HCN in the suppression of plant pathogens was reported by several workers in various hosts (Voisard *et al.,* 1989; Weststeijn 1990).

2.20.10. Salicylic acid

Salicylic acid (SA) is another siderophore produced by *P. fluorescens* strain WCS 374, WCS 417r (Leeman *et al.,* 1996), CHAO (Maurhofer *et al.,* 1994) and *P. aeruginosa* 7 NSK2 (De Meyer and Hofte 1997). SA has been proposed as a systemic signal inducer or required factor in systemic acquired resistance (SAR) or induced systemic resistance (ISR) mechanisms in many plants (Malamy *et al.,* 1990). De Meyer and Hofte (1997) reported that SA – deficient mutants of *P. aeruginosa* could not induce resistance to *B.cinerea*. Chen *et al.* (1999) reported that *P. corrugata* strain 13 and *P. aureofaciens* strain 63-28, which induced systemic resistance in cucumber against *P.*

*aphanidermatum*, produced SA in culture and also induced endogenous SA in cucumber roots. Meena *et al.* (2001) stated that production of SA was maximum in *P. fluorescens* strain Pf1 followed by Pf ALR – 7 and Pf MDU 2 isolates. There was a significant relationship between inhibitory activity of *P. fluorescens* strains *in vitro* and their level of salicylic acid production. Siddiqui and Shaukat (2003) detected the production of SA in the culture filtrate of the P. aeruginosa IE – 6S strain was detected in TLC as a blue spot at an Rf – value of 0.91 after exposure to ammonia fumes. *In vitro* SA production by *P. aeruginosa* IE – 6S, determined spectrophotometrically, revealed that the bacterial inoculant cultivated in casamino acid liquid medium synthesized SA at 3.9 ±1.1 µg /ml.

2.20.11. Indole Acetic Acid (IAA) production

Ramamoorthy and Samiyappan (2001) reported that among the twenty *Pseudomonas* isolates tested, seven isolates viz., Pf1, FP7, PB2, ATR, PSV, COP and COT were found to increase plant vigour and produced higher amounts of IAA under laboratory conditions. Among these seven isolates, *P. fluorescens* isolate Pf1 effectively increased the plant vigour and produced the maximum amount of IAA in the culture medium. The same isolate also effectively inhibited the mycelial growth of the pathogen by recording an inhibition zone of 10.8mm.

2.20.12. Production of Volatile metabolites

Knox *et al.* (2000) reported that two strains of *B. subtilis* showed strong inhibition of number of pathogens on agar plate and this inhibition is due to Antifungal volatile compounds (AFV) produced by the *B. subtilis* under nitrate-amended medium at the rate of 10 mM. Sharifi – Tehrani and Omati (1999) observed a strong inhibitory effect on *P.capsici* by rhizosphere bacteria *P. fluorescens* and *B. subtilis*. The inhibitory effect might be due to the volatile and non- volatile metabolites produced by the bacteria. The production of volatile cyanide is very common among the rhizosphere pseudomonads (Dowling and O'gara 1994). Laha *et al.* (1996) reported that the volatile metabolites of fluorescent Pseudomonads significantly suppressed the mycelial growth of *S. rolfsii*. Meena *et al.* (2001) tested the volatile metabolites produced by 10 *P. fluorescens* strains. Among them, strain Pf1 significantly inhibited the growth of *M.*

*phaseolina in vitro*. The inhibition may be due to the production of more amounts of volatile metabolites.

Bunker and Mathur (2001) reported that *T. harzianum* was the most effective, in causing significant suppression of growth and sclerotia formation of *R. solani in vitro* through production of volatile and non-volatile antibiotics followed by *G. virens* and *T. aureoviride.* The activity was more diffusible (non-volatile) antibiotic than the volatile antibiotics.

# CHAPTER III
# MATERIALS AND METHODS

## 3.1. Survey

A detailed survey was conducted during 2002 (July to December) to assess the occurrence of root rot disease of *Coleus forskohlii* under farmer holdings at Salem and Vilupuram districts of Tamil Nadu (Table 3). In one village, ten farmers were selected and three plots with an area of ten square metres each were selected randomly in each farmers field. Root rot incidence was recorded and expressed as per cent infection.

$$\text{Per cent Infection} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

## 3.2. Isolation of pathogen

The root rot pathogens were isolated from the roots of the infected Coleus plants showing typical root rot symptoms by tissue segment method on Potato Dextrose Agar (PDA) medium (Annexure-I) (Rangaswami, 1972). Axenic culture of the pathogen was obtained by single hyphal tip method and maintained in PDA slants. The pathogens were identified based on their morphological characters.

## 3.3. Pathogenicity

The root rot pathogens *viz., M. phaseolina* and *R. solani* were multiplied in sand – maize medium (Riker and Riker, 1933). Sand and ground maize grains were mixed at a ratio of 19:1, moistened with water and autoclaved at 1.04 kg per $cm^2$ pressure for two hours on three successive days. The sterilized medium was inoculated with mycelial disc of *M. phaseolina* and *R. solani* and incubated at room temperature for 15 days. Sieved garden soil was sterilized in an autoclave at 1.04 kg per $cm^2$ pressure for three hours on two consecutive days. The pathogens multiplied on sand maize medium were applied at a rate of 10 g per kg of soil. Five days after inoculation of pathogens, Coleus cuttings were planted and per cent disease incidence was recorded on 45 days after planting for

*Macrophomina* rot and on 20 days after planting for *Rhizoctonia* rot. The causal organisms were reisolated from the infected plant.

### 3.4. Cytological changes due to root rot infection

#### 3.4.1. Root and stem anatomy through microtomy

##### 3.4.1.1. Sectioning

Paraffin sectioning of the stem and root was done as described by Johansen (1940). Stem and root samples were collected from healthy and infected plants of *C. forskohlii*. The samples were washed and fixed with five parts of 30 per cent formalin, five parts of glacial acetic acid and 90 parts of 70 per cent ethanol for 29 h at 4 $^{o}$ C. The samples were then transferred to 50, 60 and 70 per cent ethanol each at 1 h interval.

##### 3.4.1.2. Dehydration

The samples were gradually dehydrated in tertiary butyl alcohol (TBA) series as shown below.

| Alcohol (%) | TBA (ml) | Ethanol (95%) | Distilled water (ml) | Duration (h) |
|---|---|---|---|---|
| 50 | 1 | 4 | 5 | 2-3 |
| 70 | 2 | 5 | 3 | 12 |
| 85 | 3 | 5 | 1.5 | 12 |
| 95 | 4.5 | 5 | 1 | 12 |
| 100 | 7.5 | 2.5 | 0 | 12 |

The samples were washed in running tap water for 10-15 min and in different concentrations of ethanol *viz.*, 10, 50, 70 and 100 per cent for 5 min. Then they were washed with xylene ethanol (1:1), xylene I and xylene II for 5 min and stained with toluidine blue (1g/100 ml). The slides were mounted on DPX mountant and left undisturbed for 24 h for preservation. Finally the slides were photographed with Image Analyzer under oil immersion at a magnification of 40 X.

### 3.5. Assessment of loss of forskolin alkaloid due to root rot infection

3.5.1. Sample preparation

The Coleus root sample (5g) infected by root rot pathogen *M. phaseolina* with various level of infections *viz.*, 0, 10, 25, 50, 75 and 100 per cent were dried and powdered. The forskolin was extracted from the sample using 150 ml of benzene in a soxhlet extraction unit. Then benzene was air-dried and the residue was dissolved in 1.5 ml of benzene for estimation of forskolin.

#### 3.5.2. Forskolin estimation

Forskolin present in the roots of *C. forskohlii* was determined by High Performance Thin Layer Chromatography (HPTLC). Standard forskolin and other samples were spotted on precoated silica gel plates as narrow bands of 4 mm width at a constant rate of 8µl $sec^{-1}$ using a Camag Linomat IV model applicator under nitrogen atmosphere. A mixture of benzene and ethyl acetate (85:15v/v) was used as the mobile phase. The length of the chromatogram was 90 mm and 15 min was required for each run. The plates were sprayed with anisaldehyde sulphuric acid reagent (0.5 ml anisaldehyde +1 ml $H_2SO_4$+ 50 ml acetic acid) and heated at $110^o$ C for 5 min. Orange fluorescence observed at 366 nm was optimally detected and quantified at 315 nm using the Camag TLC scanner with CATS 3.17 software for quantification of the separated compounds in the chromatogram (Malathy and Pai, 1999).

### 3.6. Quantification of sclerotial population of *M. phaseolina* from soil

Thirty soil samples (each 1 kg) of Coleus field, representing various levels of root rot incidence and different crop rotations were collected from different villages of Vilupuram and Salem districts (Table 5). Samples were taken within crop rows to 15 cm depths and at random locations within a diamond shaped pattern over the entire field. The soil samples were stored at $25^o$ C until processed. The sclerotial populations were quantified by following the method of Cloud and Rupe (1991). Three sub samples (10g of field soil or 1 g of artificially infested soil) were taken from each soil sample to estimate the sclerotial population of *M. phaseolina*. Three additional sub samples (10 g) were placed in an oven ($105^o$ C) to determine the soil moisture percentage. Each

sub sample was blended for three times at 30 sec intervals alternated with three times 30 sec idle periods in 250 ml of 0.5 per cent sodium hyphochlorite. The soil slurry was passed through a 0.045 mm sieve and debris on the sieve was rinsed for two minutes under distilled water and then washed into a beaker with 25 ml of water. The contents of each beaker were mixed with 90 ml of selective RB medium (Annexure-I). Rifampicin was dissolved in 1ml of dimethyl sulpoxide. Rifampicin, metalaxyl and tergitol were added after the medium was autoclaved and cooled to 55$^{o}$ C and dispensed into six sterile Petri dishes. Then the plates were incubated at 30$^{o}$C for 4 days before the colonies of *M. phaseolina* were counted.

### 3.7. Isolation and maintenance of native antagonists

Soil antagonistic microbes *viz*., *Pseudomonas* spp., *Trichoderma* spp., and *Bacillus* spp., were isolated from the rhizosphere soil using King's B medium (KB) (King *et al*., 1954), *Trichoderma* special medium (TSM) (Papavizas and Lumsden, 1980) and Nutrient Agar medium (NA) (Difco Manual, 1953), respectively. Morphological identification was made through the light microscope and pure cultures were maintained on respective agar slants at 4$^{o}$ C. The biocontrol agents used in the study were given in Table1.

#### 3.7.1. *In vitro* screening of *Trichoderma* species

*In vitro* screening of *Trichoderma* species against root rot pathogens was done by dual culture method (Dennis and Webster, 1971). For testing antagonism in dual culture, 8 mm discs of antagonists and test pathogens were cut from the edge of 4 day old culture and placed 1 cm away from the edge of the PDA plate. The Petri plates were incubated at (28 $\pm$ 2 $^{o}$C). After four days of incubation, the pathogen growth and inhibition zone were recorded and the sclerotial count was taken after ten days. Per cent inhibition in mycelial growth and sclerotial number were calculated using the formula suggested by Pandey *et al.* (2000).

$$\text{Per cent Inhibition} = \frac{Dc - Dt}{} \times 100$$

**Dc**

Dc: average diameter of fungal growth in control

Dt: average diameter of fungal growth in treatment

The overgrowth of antagonists over the pathogen was measured seven days after incubation. The overgrowth and zone of inhibition was measured and expressed in mm.

### 3.7.2 *In vitro* screening of bacterial antagonists

The bacterial strains were streaked on one side of the Petri dish (1 cm away from the edge of the plate) on PDA medium and a mycelial disc (8 mm diameter) of 4 day old culture of pathogens were placed on the opposite side of the Petri dish perpendicular to the bacterial streak (Vidhyasekaran *et al.,* 1997). The plates were incubated at room temperature ($28 \pm 2\ ^{o}C$) for 4 days. After four

days of incubation, the pathogens growth and inhibition zone were recorded and the sclerotial count was taken after ten days. The per cent inhibition in mycelial growth and sclerotial number (Dhingra and Sinclair, 1978) were calculated using the formula suggested by Pandey *et al*. (2000). The zone of inhibition was measured and expressed in mm.

### 3.8. *In vitro* screening of plant extracts/plant oils

### 3.8.1. Preparation of aqueous extracts

Ten gram of fresh leaves of the plant species (Table 2) were collected and washed well with distilled water. The leaves were ground with sterile distilled water (1:1 ratio) using a pestle and mortar. The extract was filtered through a muslin cloth and the filtrate was centrifuged at 10,000 rpm for 15 minutes. The supernatant served as the standard leaf extract solution (100%) and was used for the antifungal assay.

3.8.2. Effect of plant extracts on mycelial growth and sclerotial production

The leaf extracts of the ten plant species were assayed for antifungal activity by poisoned food technique (Schmitz, 1930). Five ml of aqueous leaf extract was mixed with 50 ml of PDA medium to obtain 10 per cent concentration. Fifteen ml of the poisoned medium was poured to each Petri plate. A mycelial disc of the pathogens (8 mm diameter) taken from 4 day old culture was placed at the centre of each Petri plate

and incubated at room temperature (28 ± 2 $^{o}$ C). PDA mixed without any plant extract served as control. Three replications were maintained for each treatment. After four days of incubation, the pathogen growth in all the plates was recorded and the sclerotial count was taken after ten days. The per cent inhibition of mycelial growth and sclerotial number were calculated (Pandey *et al*., 2000).

### 3.8.3. Effect of plant oils on mycelial growth and sclerotial production

The plant oils such as Wintergreen oil (*Gaultheria procumbens* L.) and Lemon grass oil (*Cymbopogan citratus* (D.C) were assayed for antifungal activity by poisoned food technique (Schmitz, 1930). Plant oils were mixed @ 0.2, 0.1 and 0.05 ml with 100 ml of PDA medium to obtain 0.2, 0.1 and 0.05 per cent concentration. Fifteen ml of the poisoned medium was poured to each Petri plate. A mycelial disc of the pathogens (8 mm diameter) taken from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature. PDA mixed without any plant oil served as control. Three replications were maintained for each treatment. After four days of incubation, the pathogen growth in all the plates was recorded and the sclerotial count was taken after ten days. The per cent inhibition in mycelial growth and sclerotial number were calculated (Pandey *et al*., 2000).

### 3.9. Effect of Zinc sulphate on the mycelial growth and sclerotial production

The efficacy of zinc sulphate on the growth and sclerotial production of root rot pathogens was studied by poisoned food technique (Schmitz, 1930). In poisoned food technique, 1.0 and 0.5 g of zinc sulphate was mixed with 100 ml of PDA to obtain 1 and 0.5 per cent concentration of nutrient, respectively. Fifteen ml of the zinc sulphate amended medium was poured to each Petri plate. A mycelial disc of the pathogens (8 mm diameter) taken from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature (28 ± 2 $^{o}$ C). PDA mixed without any nutrient served as control. Three replications were maintained for each treatment. After four days of incubation, the pathogen growth in all the plates was recorded and the sclerotial count

was taken after ten days. Percent inhibition in mycelial growth and sclerotial number were calculated (Pandey *et al*., 2000).

### 3.10. Screening of chemical fungicides against root rot pathogens under *in vitro* condition

The chemical fungicides like Tricyclazole, Thiophenate methyl, Thiram, Carbendazim, Hexaconazole, Copper oxychloride and Propiconazole were assayed for fungicidal activity by poisoned food technique (Schmitz, 1930). The required quantity (0.2 and 0.1 gram or ml) of the above fungicides were mixed with 100 ml of PDA medium to obtain 0.2 and 0.1 per cent concentration, respectively. Fifteen ml of the poisoned medium was poured to each Petri plate. Mycelial disc of the pathogens (8 mm diameter) taken from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature. PDA mixed without any fungicide served as control. Three replications were maintained for each treatment. After four days of incubation, the radial growth of the pathogen in all the plates was recorded and the sclerotial count was taken after ten days. The per cent inhibition in mycelial growth and sclerotial number were calculated (Pandey *et al*., 2000).

### 3.11. Compatibility of biocontrol agents

#### 3.11.1. Compatibility of biocontrol agents with plant oils

The plant oils like Wintergreen oil and Lemongrass oil were assayed for their compatibility with biocontrol agents by poisoned food technique. Plant oils (0.2 ml) were mixed with 100 ml of PDA medium to obtain 0.2 per cent concentration. Fifteen ml of the poisoned medium was poured to each Petri plate. Mycelial disc of *T. viride* isolate TVC5 and *T. harzianum* (8 mm diameter) taken from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature. To study the compatibility of the bacterial antagonist, bacterial strains were streaked on the Petri dish. PDA mixed without any plant oil served as control. Three replications were maintained for each treatment. The growth of bacterial and fungal antagonists was recorded on $2^{nd}$ and $4^{th}$ day of incubation at room temperature, respectively and expressed as compatible (+) or incompatible (-).

#### 3.11.2. Compatibility of biocontrol agents with Zinc sulphate

The compatibility of zinc sulphate with biocontrol agents was assayed by poisoned food technique. One and 0.5 g of zinc sulphate was mixed with 100 ml of PDA

to obtain 1 and 0.5 per cent concentration of nutrient. Fifteen ml of the nutrient amended medium was poured to each Petri plate. A mycelial disc of *T. viride* isolate TVC5 and *T. harzianum* (8 mm diameter) from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature (28 ± 2$^{o}$C). To study the compatibility of the bacterial antagonist, bacterial strains were streaked on the Petri dish. PDA mixed without zinc sulphate served as control. Three replications were maintained for each treatment. The growth of bacterial and fungal antagonists was recorded on 2$^{nd}$ and 4$^{th}$ day of incubation at room temperature, respectively and expressed as compatible (+) or incompatible

(-).

3.12. Development of bioformulations

3.12.1. Development of talc based formulation using biocontrol agents, plant oils and zinc sulphate

A talc-based formulation was developed based on the compatibility between biocontrol agents, plant oils and zinc sulphate. The bacterial biocontrol agents like PfC6 and Pf1 were grown in King's B broth for 48 h as shake culture by incubating in an Infors AG shaker at 150 rpm at room temperature (28 ± 2$^{o}$C). Ten gram of Corboxy methyl cellulose (CMC) was added to 1 kg of talc and mixed well. The pH of the media was adjusted to 7.0 by adding calcium carbonate. The talc was autoclaved for two consecutive days for about 30 minutes. Then zinc sulphate was mixed with autoclaved talc at the rate of 5 g kg $^{-1}$. The plant oils were mixed with 400 ml of bacterial suspension containing 9x 10$^{8}$ cfu ml $^{-1}$ at the rate of 0.2 per cent. The bacterial suspension containing plant oils were added to one kg of carrier and mixed well under sterile condition. The materials were packed in polythene bags,

sealed and incubated at room temperature (28 ± 2$^{o}$C) (Vidhyasekaran and Muthamilan, 1995).

**3.12.2. Development of talc based formulation using *Trichoderma* spp. and Zinc sulphate**

A talc-based formulation was developed based on the compatibility between *Trichoderma* spp and zinc sulphate. Each species of *Trichoderma* was grown in yeast molasses medium (Annexure-I). The medium was sterilized in 250 ml conical flasks,

inoculated with 8 mm disc of actively growing *Trichoderma* spp. and incubated at room temperature for 14 days. Five gram of CMC was added to a kg of talc and mixed well. The talc was autoclaved for two consecutive days for about 30 minutes. Then zinc sulphate was mixed with autoclaved talc at the rate of 5 g $kg^{-1}$. Five hundred ml of yeast molasses medium containing *Trichoderma* species were added to one kg of carrier and mixed well under sterile condition. The materials were shade dried and packed in polythene bags, sealed and incubated at room temperature (28 ± $2^{o}$ C). (Vidhyasekaran and Muthamilan, 1995).

### 3.12.3. Development of Emulsifiable Concentrate (EC) formulations from plant oils

The plant oils such as Wintergreen oil and Lemongrass oil, which proved their antifungal efficacy under *in vitro* condition, were used for developing the EC formulations. Three emulsifiable concentrate formulations *viz*. 20, 30 and 40 EC were developed using the above plant oils. The EC formulations were developed by mixing recommended quantities of plant oils, emulsifying agent (Unitox), stabilizing agent (Epichlorohydrin) and solvent (Cyclohexanone). The different EC formulations were named as follows. The EC formulations (20, 30 and 40 EC) of wintergreen oil and lemon grass oil were designated as formulation A, B, C, D, E and F, respectively.

#### 3.12.3.1 Effect of EC formulation of plant oils on the mycelial growth of pathogens under *in vitro* condition

The effect of EC formulation of wintergreen oil and lemongrass oil were assayed for their antifungal activity by poisoned food technique. The formulation (0.2 ml) was mixed with 100 ml of PDA medium to obtain 0.2 per cent concentration. Fifteen ml of the poisoned medium was poured to each Petri plate. A mycelial disc of the pathogens (8 mm diameter) taken from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature. PDA mixed without any formulation served as control. Three replications were maintained for each treatment. After four days of incubation, the pathogen growth in all the plates was recorded and the sclerotial count was taken after ten days. The per cent inhibition in mycelial growth and sclerotial number were calculated using the formula suggested by Pandey *et al*. (2000).

### 3.12.3.2. Volatile effect of plant oils and EC formulation on the mycelial growth of pathogens under *in vitro* condition

The volatile effect of plant oils and EC formulation on the mycelial growth of root rot pathogens *M. phaseolina* and *R. solani* was studied by the paired Petri dish technique (Laha *et al*., 1996). The plant oils and EC formulation (0.2ml) were mixed with 100 ml of PDA medium to obtain 0.2 per cent concentration. To one set of Petri plates, 15 ml of the poisoned medium was poured. A mycelial disc of 8 mm diameter cut from a 4 day old culture of pathogens was placed at the centre of another set of PDA plates. Then the PDA plates inoculated with the pathogens (upper) were paired with the PDA plate containing plant oils and EC formulations (lower), sealed with parafilm and incubated at $28^{o}$ C. The diameter of the fungal growth was measured 5 days after incubation. PDA plates inoculated with the fungus alone and paired with PDA plate without biocontrol agents served as control. Three replications were maintained for each treatment.

### 3.12.3.3. Effect of dilution of EC formulations on the mycelial growth of root rot pathogens

Based on the results obtained with the EC formulations under *in vitro* conditions, 0.2 per cent concentration of 40 EC was used for further investigations. The product was diluted with sterile distilled water in the ratio of 1:2, 1:4, 1:6, 1:8 and 1:10. The diluted products (0.2ml) were mixed with 100 ml of PDA medium to obtain 0.2 per cent concentration. Fifteen ml of the poisoned medium was poured to each Petri plate. A mycelial disc of the pathogens (8 mm diameter) taken from a 4 day old culture was placed at the centre of each Petri plate and incubated at room temperature. PDA mixed without any formulation served as control. Three replications were maintained for each treatment. After four days of incubation, the pathogen growth in all the plates was recorded and the sclerotial count was taken after ten days. Per cent inhibition of mycelial growth and sclerotial number were calculated using the formula suggested by Pandey *et al*. (2000).

### 3.12.3.4. Stability of EC formulation at different pH

The stability of the WGO 40 EC (0.2 %) was determined at different pH *viz.* 4.0, 7.0 and 9.0. Acidic pH was obtained with 1N HCl and alkalinity was obtained with 1N NaOH. The stability for antifungal action was tested by adopting the poisoned food technique. Three replications were maintained for each treatment. The plates were incubated at room temperature and the pH at which maximum growth reduction occurred was recorded.

**3.12.3.5. Stability of EC formulation at different temperatures**

The WGO 40 EC formulation was maintained at different temperatures *viz*., 30°C, 60°C, 80°C and 100°C for a period of 15 minutes. The products were then tested for stability using poisoned food technique. Three replications were maintained for each treatment. The temperature at which the products exhibited maximum growth inhibition was recorded as the stable temperature.

**3.12.3.6. Stability of EC formulation on storage**

The stability of the WGO 40 EC formulation (0.2%) under storage condition was determined for eight months. The EC formulation was placed in a 100 ml screwcap plastic container and stored at room temperature ($28 \pm 2\ ^{o}C$). The samples were drawn at periodical intervals *viz.,* 0, 60, 120, 180 and 240 days after storage and their efficacy in inhibiting the mycelial growth of *M. phaseolina* and *R. solani* was assessed.

3.12.3.7. Effect of talc based bioformulations and EC formulations on Coleus root rot incidence under greenhouse condition

Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 h for two consecutive days and filled in pots. The talc based bioformulations were applied to the planting hole at the rate of 2g per hole and EC formulations were applied as soil drench at the rate of 100 ml of 40 EC (0.2%) per plant. The culture of *M. phaseolina* and *R. solani,* mass multiplied in sand maize medium (sand and Maize powder at the ratio of 19: 1) was incorporated with potting medium at the rate of 10g per kg of soil. The Coleus cuttings were planted in the holes. The pathogen alone inoculated

served as control. Three replications (Three pots per replication) were maintained and the pots were arranged in a randomized manner. The root rot incidence of *M. phaseolina* and *R. solani* was recorded on 45 and 20 days after planting, respectively and expressed as percentage of disease incidence.

### 3.13. Effect of organic amendments and zinc sulphate against Coleus root rot under pot culture experiment

A pot culture experiment was laid out in completely randomized design to test the efficacy of organic amendments in controlling the root rot of Coleus. Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for one h for two consecutive days. The treatment consists of gingelly, cotton, neem, groundnut, coir pith compost and farmyard manure. The amendments were applied basally at the rate of 10 g kg $^{-1}$of the soil and allowed to wither for 10 days. Zinc sulphate was applied basally at the rate of one gram per planting hole. The virulent strain of *M. phaseolina* and *R. solani*, mass multiplied in sand maize medium (sand and maize powder at the ratio of 19: 1) was incorporated in the soil at the rate of 10 g per kg of soil. The Coleus cuttings were planted in the inoculated pots. Three replications (three pots per replication) were maintained and the pots were arranged in a randomized manner. The root rot incidence of *M. phaseolina* and *R. solani* was recorded on 45 and 20 days after planting, respectively and expressed as percentage of disease incidence.

### 3.14. Effect of fungicides on Coleus root rot under pot culture experiment

A pot culture experiment was laid out in completely randomized design to test the efficacy of fungicides in controlling the root rot of Coleus. Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 h for two consecutive days. The virulent strain of *M. phaseolina* and *R. solani*, mass multiplied in sand maize medium (sand and maize powder at the ratio of 19: 1) was incorporated in the soil at the rate of 10g per kg of soil. The Coleus cuttings were planted in the inoculated pots. The treatment consist of soil drenching of 0.1 per cent Triazole, Thiophenate methyl, Carbendazim, Hexaconazole and Propiconazole. Three replications (Three pots per replication) were maintained and the pots were arranged in a randomized manner. The

root rot incidence of *M. phaseolina* and *R. solani* was recorded on 45 and 20 days after planting, respectively and expressed as percentage of disease incidence.

3.15. Shelf life of biocontrol agents

The shelf life of the polybag stored talc based formulations of biocontrol agents was studied by the serial dilution technique (Pramer and Schmidt, 1956). One gram of samples was collected from each formulation periodically at 0, 30, 60, 90 and 120 days of storage. The sample was mixed with 10 ml of sterile distilled water ($10^{-1}$dilution). From this, 1 ml of the suspension was pipetted out and transferred to 9 ml of sterile distilled water ($10^{-2}$ dilution). Similarly $10^{-3}$ to $10^{-8}$ dilutions were prepared. From the final dilution, 1 ml aliquot was pipetted out into sterilized Petri plates and incubated at room temperature (28 $\pm$ $2^{o}$ C). The number of colony forming units of bacteria and *Trichoderma* spp. were counted on 3rd and 5 days after plating and expressed as number of cfu $g^{-1}$ of formulation.

3.16. Rhizosphere population of antagonists

The antagonists population in the rhizosphere of Coleus was estimated at 0, 45 90 and 120 days after planting. For this purpose, three plants in each treatment were taken. *Trichoderma* spp. and *P. fluorescens* population were estimated by using special media *viz.*, *Trichoderma* special medium (Elad and Chet, 1983) and modified King's B medium (King *et al.*, 1954), respectively. The antagonists population in the rhizosphere region was estimated by the method of Papavizas and Davey (1961). The plants were pulled out gently with roots intact and tapped against the palm to remove the adhering soil. The root portions (3-5cm) were cut from the tip from different radii of the plant and transferred to 100 ml sterile water. After thorough shaking, the antagonist population in the suspension was assayed by dilution plate technique (Pramer and Schmidt, 1956). The *Trichoderma* and *P. fluorescens* populations were estimated using$10^{-3}$ and $10^{-4}$ dilutions, respectively. From the final dilution, 1 ml aliquot was pipetted out into sterilized Petri plates and incubated at room temperature (28 $\pm$ $2^{o}$ C). The number of colony forming units of bacteria and *Trichoderma* spp were counted on 3 and 5 days after plating. The weight of

rhizosphere soil was calculated by removing the roots from the flask and evaporating the water. Then the soil residue was dried to constant weight in an oven at 105$^{o}$C and weighed. The population was expressed on dry weight basis.

*3.17. Assay of defense related proteins and chemicals induced by bioformulations*

3.17.1. Sample collection

For all the experiments, root samples were collected from treated, pathogen inoculated and uninoculated control at 7 days interval starting from 0 to 28 days after inoculation of the pathogen.

3.17. 2. Enzyme extraction

One gram of root sample was homogenized with 2ml of 0.1 M sodium citrate buffer (pH 5.0) at 4$^{o}$C. The homogenate was centrifuged for 20 min at 10,000 rpm. The supernatant was used as crude enzyme extract for assaying the chitinase activity. The enzyme extracted in 0.1 M sodium phosphate buffer was used for estimation of Peroxidase (PO), Polyphenol oxidase (PPO) and Phenylalanine Ammonia- Lyase (PAL).

Protein content in the extract was determined by the method of Bradford (1976). Ten milligram of Coomassie brilliant blue G-250 was dissolved in 4.7 ml of absolute alcohol and 10 ml of concentrated phosphoric acid and the volume was made up to 100 ml with distilled water. A sample of 50 µl was added to 950 µl of dye solution and the mixture was incubated for 5 minutes at room temperature. Bovine serum albumin was used as the standard. The absorbance was recorded at 595 nm in GS5703 AT Spectrophotometer.

3.17.3. Estimation of Phenylalanine Ammonia- Lyase (PAL) activity

Plant samples (1 g) were homogenized in 2 ml of ice cold 0.1 M sodium borate buffer at pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone. The extract was filtered through cheesecloth and the filtrate was

centrifuged at 16,000 g for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm (Dickerson *et al.,* 1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L - phenylalanine in the same buffer for 30 min at 30$^{o}$C. The amount of transcinnamic acid synthesized was calculated (Dickerson *et al.,* 1984). Enzyme activity was expressed as nmol transcinnamic acid $min^{-1}$ $g^{-1}$ tissue.

**3.17.4. Assay of peroxidase (PO)**

Plant samples (1 g) were homogenized in 2 ml of 0.1M-phosphate buffer, pH 7.0 at 4$^{o}$C. The homogenate was centrifuged at 16,000 g at 4$^{o}$C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent $H_2O_2$. The reaction mixture was incubated at room temperature (28 $\pm$ 2$^{o}$C). The change in absorbance at 420 nm was recorded at 30 second interval for 3 min. The enzyme activity was expressed as change in absorbance $min^{-1}$ $g^{-1}$ tissue (Hammerschmidt *et al.,* 1982).

3.17.5. Assay of polyphenol oxidase (PPO)

Plant samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16,000 g for 15 min at $4^{o}$ C. The supernatant was used as enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5ml of 0.1 M sodium phosphate buffer (pH 6.5). To initiate the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as change in absorbance at 495 nm $min^{-1}$ $g^{-1}$ tissue (Mayer *et al.,* 1965).

3.17.6. Assay of chitinase

The colorimetric assay of chitinase was carried out as per the method of Boller and Mauch (1998). Reagents used were colloidal chitin, snail gut enzyme, dimethyl amino benzaldehyde (DMAB) and buffer (Annexure-II). The reaction mixture consists of 10 µl of 0.1 M sodium acetate buffer (pH 4.0), 0.4 ml of enzyme solution and 0.1 ml of colloidal chitin (10mg). After incubation for 2 h at $37^{o}$ C, the reaction was arrested by centrifugation at 10,000 rpm for 3 minutes. An aliquot of the supernatant (0.3 ml) was pipetted into a glass reagent tube containing 30 µl of 1 M potassium phosphate buffer (pH 7.0) and incubated with 20 µl of 3 per cent (w/v) snail gut enzyme for one hour. After one hour, the reaction mixture was brought to pH 8.9 by the addition of 70 µl of 0.1 M sodium borate buffer (pH 9.8). The mixture was incubated in a boiling water bath for 3 min and then rapidly cooled in ice water bath. After addition of 2 ml of DMAB, the mixture was incubated for 20 minutes at $37^{o}$C and the absorbance was measured at 585 nm immediately. N- acetylglucosamine (GlcNac) was used as a standard. The enzyme activity was expressed as nmoles GlcNac equivalents $min^{-1}$ $g^{-1}$ of fresh weight.

3.17.7. Assay of β-1, 3- glucanase

β-1,3-glucanase activity was assayed by the laminarin dinitrosalicylic acid method (Pan *et al.,* 1991). The reaction mixture consisted of 62.5µl of 4 per cent laminarin and 62.5µl of enzyme extract. The reaction was carried out at $40^{o}$C for 10 minutes. The reaction was then stopped by adding 375 µl of dinitrosalicylic acid and heating for 5 minutes on a boiling water, vortexed and its absorbance was measured at 500 nm. The enzyme activity was expressed as µg glucose released $min^{-1}$ $mg^{-1}$ of protein.

3.17.8. Estimation of phenol

Root samples (1 g) were homogenized in 10 ml of 80 per cent methanol and agitated for 15 min at 70$^{o}$ C (Zieslin and Ben-Zaken, 1993). One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) and the solution was kept at 25$^{o}$ C. The absorbance of the developed blue colour was measured using a Spectrophotometer at 725 nm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol $g^{-1}$ tissue.

**3.18. Native gel electrophoresis**

**3.18.1. Peroxidase (PO)**

To study the expression pattern of different isoforms of peroxidases in different treatments, activity gel electrophoresis was carried out for native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent acrylamide concentration and stacking gel of 4 per cent acrylamide concentration were prepared (Annexure-III). After electrophoresis, the gels were incubated in the solution containing 0.15 per cent benzidine in 6 per cent $NH_4Cl$ for 30 min in dark. Then $H_2O_2$ (30 %) was added drop wise with constant shaking till the bands appear. After staining, the gel was washed with distilled water and photographed (Sindhu *et al*., 1984).

*3.18.2. Polyphenol Oxidase (PPO)*

After native gel electrophoresis, the gel was equilibrated for 30 min in 0.1 per cent p-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7.0) followed by 10 mM catechol in the same buffer. A gentle shaking followed by the addition of catechol resulted in the appearance of dark brown discrete protein bands.

**3.19. Mode of action of biocontrol agents**

**3.19.1. Mode of action of *Trichoderma* species**

**3.19.1.1. Mycoparasitism**

Mycoparasitism of *Trichoderma* species on the *M. phaseolina* and *R. solani* were studied as per the procedure given by Bunker and Mathur (2001). The dual cultures were grown on cellophane discs (9cm) placed atop of PDA medium in the lower lid of Petri

dish. The control plates were inoculated by opposing the two discs of culture of *R. solani* and *M. phaseolina*. The plates were incubated at 28$^{o}$C. After 5 days of incubation, 1x1 cm$^{2}$ of the cellophane was cut from the sites of interaction between biocontrol agent and pathogen and mounted on the microscopic slides in aniline –blue- lactophenol stain and examined under microscope.

### 3.19.1.2. Effect of volatile metabolites of *Trichoderma* spp. on the mycelial growth of root rot pathogens

Effect of volatile metabolites produced by *Trichoderma* species on the growth of root rot pathogens *M. phaseolina* and *R. solani* was studied by the paired Petri dish technique (Laha *et al*., 1996). An eight mm diameter mycelial disc of 4 day old culture of *Trichoderma* species was placed on PDA medium in a Petri dish. In another set, 8 mm diameter mycelial disc cut from a 4 day old culture of pathogen was placed at the centre of PDA plate. Then the PDA plate inoculated with the pathogen (upper) was paired with the PDA plate containing *Trichoderma* species (lower) and sealed with Parafilm and incubated at 28$^{o}$ C. PDA plates inoculated with the fungus alone and paired with PDA plate without biocontrol agents served as control. Three replications were maintained in each treatment. The diameter of the fungal growth was measured five days after incubation and expressed as per cent inhibition over control.

### 3.19.1.3. Effect of diffusible metabolites of *Trichoderma* species on the mycelial growth of root rot pathogens

The effect of diffusible metabolites produced by the *Trichoderma* species on the growth of the pathogen was studied by the method given by Dennis and Webster (1971). The culture of the *Trichoderma* species was grown on a sterilized cellophane paper disc of 9 cm placed atop of PDA medium. The plates were incubated at 28$^{o}$ C. On 3 $^{rd}$ day, the cellophane disc along with the growth of each antagonist was gently and aseptically removed and in the centre of each plate 8mm disc of pathogens from actively growing culture was placed and then the plates were again incubated. For control, the pathogen

was similarly inoculated on the plates where it was grown earlier. The diameter of the fungal growth was measured 5 days after incubation

### 3.19.2. Mode of action of *Pseudomonas fluorescens*

#### 3.19.2.1. Production of extracellular lytic enzyme by *P. fluorescens*

##### 3.19.2.1.1. Assay of chitinase

For the preparation of crude chitinase, *P. fluorescens* strains were cultured at room temperature for 48 h on a rotary shaker in 250 ml flasks containing 50 ml of Chitin – Peptone medium (Annexure- I). The cultures were centrifuged at 12,000 g for 20 min at 4$^{o}$C and the supernatant was used as enzyme source. Colloidal chitin was prepared from crab shell chitin according to the method of Berger and Reynolds (1958). The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 1 mol per litre sodium acetate buffer (pH 5.3) and 0.5 ml of colloidal chitin (0.1%) and incubated at 50$^{o}$C for 4 h. Chitinase activity was determined by measuring the release of reducing sugars by the method of Nelson (1944). One unit of chitinase was determined as one nmol of GlcNAc released per min per milligram of protein. Protein content in all the samples was determined according to Bradford (1976) method using bovine serum albumin as a standard.

##### 3.19.2.1.2. Assay of β-1, 3 glucanase

*Pseudomonas fluorescens* strains were grown at 30$^{o}$C for 96 h on a medium containing laminarin (0.2%) (from *Laminaria digitata*; Sigma, USA) (Lim *et al*., 1991). The cultures were centrifuged at 12,000 g for 20 min at 4$^{o}$C and the supernatant was used as enzyme source. The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 0.1mol per litre phosphate buffer (pH 5.5) and 0.5 ml of laminarin (0.2%) (Lim *et al*., 1991). The reaction was carried out at 40$^{o}$C for 2 h. β-1, 3 glucanase activity was determined as 1 nmol of glucose released per minute per mg of protein. Protein content in all the samples was determined according to Bradford (1976) method using bovine serum albumin as a standard.

### 3.19.2.2. Siderophore production

#### 3.19.2.2.1. Quantitative assay

The *P. fluorescens* strains were grown in KB broth for 3 days and centrifuged at 2000 rpm for 10 min. The pH of supernatant was adjusted to 2.0 with HCl and equal quantity of ethyl acetate was added in a separating funnel, mixed well and ethyl acetate fraction was collected. This process was repeated three times to bring the entire quantity of siderophore from the supernatant. The ethyl acetate fractions were pooled, air-dried and dissolved in 5 ml of ethanol (50%). Five ml of ethyl acetate fraction was mixed with 5 ml of Hathway's reagent (1.0 ml of 0.1 M $FeCl_3$ in 0.1 N HCl to 100 ml of distilled water + 1.0 ml of potassium ferricyanide). The absorbance for dihydroxy phenol was read at 700 nm (Reeves *et al*., 1983). A standard curve was prepared using dihydroxy benzoic acid .The quantity of siderophore synthesized was expressed as $\mu g\ ml^{-1}$ of culture filtrate.

#### 3.19.2.2.2. Qualitative assay

Production of siderophores by *P. fluorescens* strains were performed by plate assay. The tertiary complex Chrome azural S (CAS) / $Fe^{3+}$ / hexadecyl trimethyl ammonium bromide served as an indicator. Forty eight hour old culture of the strains was streaked on to the Succinate medium (Annexure-I) amended with indicator dye. The resultant dark blue liquid was observed for the formation of bright zone with yellowish fluorescent colour in the dark colored medium indicating siderophore production. The result was scored either positive or negative to this test (Schwyn and Neilands, 1987).

### 3.19.2.3. Hydrogen cyanide (HCN) production

#### 3.19.2.3.1. Qualitative assay

Production of HCN was determined by using modified procedure of Millar and Higgins (1970). *P. fluorescens* strains were grown on Trypticase Soy Agar (TSA) ( Annexure-I). Filter paper soaked in picric acid solution (2.5 g of picric acid; 12.5 g of $Na_2CO_3$, 1000 ml of distilled water) was placed in the lid of each Petri dish. Dishes were sealed with parafilm and incubated at 28$^oC$ for 48 h. A change in colour of the

filter paper discs from yellow to light brown, brown or reddish brown was recorded as an indication of weak, moderate or strong in producing HCN by each strain, respectively.

**3.19.2.3.2. Quantitative assay**

*Pseudomonas fluorescens* strains were grown on Trypticase Soy broth (TSB)(Annexure-I). Filter paper was cut into uniform strips of 10 cm long and 0.5 cm wide saturated with alkaline picrate solution and placed inside the conical flasks in a hanging position. After incubation at 28 ± 2 $^{0}$C for 48 h, the sodium picrate in the filter paper was reduced to a reddish compound in proportion to the amount of hydrocyanic acid evolved. The colour was eluted by placing the filter paper in a clean test tube containing 10 ml of distilled water and absorbance was measured at 625 nm (Sadasivam and Manickam, 1992).

**3.19.2.4. Salicylic acid (SA) production**

Salicylic acid production of the *P. fluorescens* strains was determined as per the method described by Meyer and Abdallah (1978). The strains were grown in the standard succinate medium (Annexure-I) at 28 ± 2$^{O}$C for 48 h. Cells were collected by centrifugation at 6000 g for 5 min and were resuspended in 1 ml of 0.1 M phosphate buffer. Four ml of cell free culture filtrate was acidified with 1 N HCl to pH 2.0 and SA was extracted in $CHCl_3$ (2×2ml). Four ml of water and 5 µl of 2M $FeCl_3$ was added to the pooled $CHCl_3$ phases. The absorbance of the purple iron-SA complex, which was developed in the aqueous phase was read at 527 nm. A standard curve was prepared with SA dissolved in succinate medium and quality of SA produced was expressed as µg ml$^{-1}$ (Meyer *et al*., 1992).

**3.19.2.5. Antibiotic production by fluorescent pseudomonads**

**3.19.2.5.1. Extraction of phenazine**

*Pseudomonas fluorescens* strains were grown at 28$^{0}$C in a Pigment Production Medium (PPM) (Annexure-I). The cultures were grown in PPM broth for 5 days, centrifuged at 5000xg. The pH of the supernatant was adjusted to 2.0 with HCl and extracted with an equal volume of benzene. The benzene layer formed was evaporated in a water bath and the residues were resuspended in 0.1N NaOH (Rossales *et al*., 1991).

**3.19.2.5.2. Extraction of 2,4 Diacetylphloroglucinol**

*Pseudomonas fluorescens* strains were grown in 5 ml of PPM broth for 4 days on a rotary shaker at $30^0$C. The fermentation broth was centrifuged at 3500 rpm for 5 min in a tabletop centrifuge and the supernatant was collected. It was adjusted to pH 2.0 with 1N HCl and extracted with an equal volume of ethyl acetate. The ethyl acetate extracts were reduced to dryness *in vacuo*. The residues were dissolved in methanol (Rossales *et al*., 1991).

The crude residues of Phenazine and 2,4 DAPG were evaluated at 0.1, 0.2 and 0.5 per cent concentration for their inhibitory effect on the mycelial growth of *M. phaseolina* and *R. solani* by poisoned food technique.

**3.19.2.6. Indole Acetic Acid (IAA) production**

Tripticase Soy Broth (TSB) (Annexure-I) with tryptophan as a precursor (100 µg / ml was inoculated with *Pseudomonas* strains and incubated on a rotary shaker for 30 h. Supernatants from the cultures were collected after centrifugation at 2000 rpm for 10 min. To one ml of cell free culture filtrate, 2 ml Salkowsky reagent (1 ml of 0.5 M $FeCl_3$ on 50 ml of 35% $HClO_4$) was added and incubated at 28 ± 2°C for 30 min. The absorbance was measured at 530 nm. A standard curve was prepared with IAA and quantity of IAA produced was expressed as µg $ml^{-1}$ (Gorden and Paleg, 1957).

**3.19.2.7. Effect of volatile metabolites of *P. fluorescens* on the mycelial growth of root rot pathogens**

Effect of volatile metabolites produced by *P. fluorescens* and *Trichoderma* species on the growth of root rot pathogens, *M. phaseolina* and *R. solani* was studied by the paired Petri dish technique (Laha *et al*., 1996). Two days old fresh cultures of *P. fluorescens* strains were uniformly streaked on PDA medium in a Petri dish. In another set, 8 mm diameter mycelial disc cut from a 4 day old culture of pathogen was placed at the centre of PDA plate. Then the PDA plate inoculated with the pathogens (upper) was paired with the PDA plate containing *P. fluorescens* (lower) and sealed with parafilm and

incubated at 28$^{o}$ C. PDA plates inoculated with the fungus alone and paired with PDA plate without biocontrol agents served as control. Three replications were maintained in each treatment. The diameter of the fungal growth was measured 5 days after incubation and expressed as per cent inhibition over control.

### 3.19.2.8. Effect of diffusible metabolites of *P. fluorescens* on the mycelial growth of root rot pathogens

The effect of diffusible metabolites produced by the *P. fluorescens* strains on the pathogen on the growth was studied by the method given by Yeole and Dube (2000). This study was carried out on PDA plates at pH 7.2. A loopful of *P. fluorescens* strain suspension was spot inoculated on the periphery of the Petri dishes, 2.0 cm inside at equidistant places and incubated at 28$^{o}$C for 48 h. A mycelial disc of 8 mm of the test fungus cut from actively growing culture was placed in the centre and allowed to grow. PDA plate inoculated with fungal pathogens alone served as control. The diameter of the fungal growth was measured 5 days after incubation.

## 3.20. Management of Coleus root rot using bioformulation, organic amendments and chemicals under field condition

During the year 2003, three field trials at farmer's holdings of Neermullikuttai village (Salem district), Kaniyamoor village (Vilupuram district) and Field No. 37 D of Tamil Nadu Agricultural University, Coimbatore were conducted to control Coleus root rot using bioformulations, soil amendments and chemicals. The treatments included in the trials were as follows.

| Treatment Number | Treatment details | Method of application | Dosage |
|---|---|---|---|
| $T_1$ | *P. fluorescens*- PFC6 | Dipping + Soil application | 2 g / hole |
| $T_2$ | *P. fluorescens*- Pf1 | Dipping + Soil application | 2 g / hole |
| $T_3$ | PFC6+ WGO+ Zn $SO_4$ | Dipping + Soil application | 2 g / hole |

…..Contd.

| Treatment Number | Treatment details | Method of application | Dosage |
|---|---|---|---|
| $T_4$ | PF1+ WGO+ Zn $SO_4$ | Dipping + Soil application | 2 g / hole |
| $T_5$ | *T. harzianum*- Th + $ZnSO_4$ | Dipping + Soil application | 2 g / hole |
| $T_6$ | *T. viride* – TVC5+ $ZnSO_4$ | Dipping + Soil application | 2 g / hole |
| $T_7$ | Neem cake | Soil application | 5 g / hole |
| $T_8$ | Zinc sulphate | Soil application | 1 g / hole |
| $T_9$ | EC- C | Soil Drenching | 0.2 % |
| $T_{10}$ | EC- F | Soil Drenching | 0.2% |
| $T_{11}$ | Carbendazim | Soil Drenching | 0.1 % |
| $T_{12}$ | Propiconazole | Soil Drenching | 0.1% |
| $T_{13}$ | $T_3 + T_4$ | Dipping + Soil application | 2 g / hole |
| $T_{14}$ | $T_5 + T_6$ | Dipping + Soil application | 2 g / hole |
| $T_{15}$ | Control | - | - |

All the treatments were applied by dipping of cuttings, soil application/ soil drenching at the time of planting. Subsequent applications of the treatments were done on 30 and 60 days after planting. Two replications were maintained for each treatment. The trials were laid out in a randomized block design (RBD). The root rot incidence was recorded on 45 and 90 days after planting and expressed as per cent infection. The growth parameters *viz.*, plant height, Number of branches, tuber length, tuber diameter and yield were recorded in all the treatments.

*3.21. Statistical Analysis*

All the experiments were repeated once with similar results. The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute, Biometrics Unit, The Philippines.

# CHAPTER IV
# EXPERIMENTAL RESULTS

## 4.1. Survey

The Kaniyamoor village in Villupuram district recorded the highest incidence of root rot disease (86.0%) (Plate 1a). Namashivayapuram and V.P. Agaram villages recorded 77.8 and 70.6 per cent of root rot incidence, respectively. The per cent of root rot incidence was comparatively lesser in other villages of Villupuram district.

In Salem district, maximum root rot incidence was recorded in Keeripattti (69.0%) followed by Mankuttai (64.2%) and Paithur (60%). Neermullikuttai village recorded the lowest incidence of root rot (37.45%) (Table 3; Plate1b).

## 4.2. Isolation of pathogen and pathogenicity test

Root rot pathogens were isolated from the infected plants and identified as *Macrophomina phaseolina* and *Rhizoctonia solani* based on their mycelial characters and sclerotial structures (Plate 2a,b,c,d and 3a,bi,ii). Pathogenicity studies indicated that there was a significant increase in the root rot incidence and decrease in plant growth compared to healthy plants. In pathogen inoculated plants typical root rot symptoms such as wilting of plants, drying of stem and branches and rotting of root portions, appeared on 30- 45 days after planting (DAP) in case of *M. phaseolina* (Plate 3c) and on 20- 30 DAP in case of *R. solani* (Plate 3d).

## 4.3. Cytological changes due to root rot pathogen infection

Root rot infection leads to several histological changes in the infected root and stem. Microtomy sectioning of healthy root and stem showed regular arrangement of epidermal cells, cortical parenchyma cells, conducting vessels and pith cells (Plate 4ai, ii).

In the infected root, thickening of cell wall was pronounced and cytoplasm was dense and granular. The epidermal cells and cortical parenchyma were collapsed and larger cavities were also seen. Infected cells became black and both inter and intra cellular mycelium was present in the infected cells. The xylem vessels were thickened

and both proto and metaxylem plugged with mycelium. Large numbers of small vacuoles were observed in the cytoplasm (Plate 4b i).

In the infected stem, epidermal cell layer and cortical parenchyma cells were damaged and larger cavities were seen. Plugging of xylem vessels with fungal mycelium and sieve tubes malformation were also seen (Plate 4b ii).

### 4.4. Loss of Forskolin alkaloid due to root rot infection

The effect root rot infection on the forskolin alkaloid content of coleus root was studied and results revealed that forskolin content was decreased with increasing level of infection. The maximum forskolin content (0.42%) was observed in healthy uninfected root. This was followed by 10 and 25 per cent infection recorded 0.19 and 0.17 per cent, respectively. The forskolin content of the 50 and 75 per cent infected roots were comparatively lesser than the healthy roots. Completely decayed roots (100% infection) contained the lowest forskolin content of 0.06% (Table 4; Plate 4c; Fig.1).

### 4.5. Quantification of sclerotial population of *M. phaseolina* from naturally infected soil

Sclerotial population of *M. phaseolina* from 32 soil sample collected from 14 villages, were quantified using RB medium and the results are presented in Table5. All the soil samples recorded varying level of sclerotial populations. However the maximum sclerotial population of 278.0 number of sclerotia /g of soil was observed in the Kaniyamoor village of Villupuram district. This was followed by Namashivayapuram-I (236.0No/g), Thenchettinathur-II (200.1 No/g), Namachivayapuram-II (173.0) V.P.agaram-I (157.0 No./g), Kumbakottai-I (160.5 No./g) and Sankarapuram-II (153.0 No./g of soil). The other soil samples recorded sclerotial population ranging from 145.0 to 3.8 No./g of soil. However, the least population (1.3 No/g of soil) in sample I of Thenchettianthur village was noticed.

In Salem district, the maximum population of sclerotia was observed in sample-I of Keeripatti (128.0 No./g). This was followed by Paithur-II (100 No/ g), sample-II of

Mankuttai (88.3 No./g) and sample- I of Neermullikuttai (62.5 No./g) of soil. The other soil samples recorded a population of 30.1 to 17.7 No./g of dry soil. The minimum number of sclerotial population was seen in sample-I of Valapadi Village (9.1 No./g of soil). In Coimbatore, sample I and sample II recorded sclerotial population of 110.0 and 125.0 $No.g^{-1}$ of dry soil, respectively (Table 5; Plate 5a).

In general, the maximum sclerotial population was estimated in the field with crop rotation of coleus - coleus - coleus. Crop rotation with other host crops like groundnut, maize, sorghum also recorded the higher inoculum load in the soil. The crop rotation with non-host crops like paddy, sunhemp and sugarcane recorded low level of inoculum. Sandy loam soil type recorded maximum population of sclerotial followed by loamy soil and silty loam. Fine silty loam recorded a low number of sclerotial population than others.

### 4.6. *In vitro* screening of biocontrol agents

#### 4.6.1. Screening of *Trichoderma* species against *M. phaseolina*

Six isolates of *Trichoderma viride* Pers. and one isolate of *Trichoderma harzianum* Rifai. were tested for their efficacy in inhibiting the mycelial growth and sclerotial production of *M. phaseolina* . Among the *Trichoderma* isolates tested, *T. harzianum*-Th recorded maximum inhibition of mycelial growth (62.5%) and scletorial production (49.6%) over control, respectively. This was followed by *T. viride* isolates viz., TVC5, TVC1 and TVC6 that recorded 56.8, 50.2 and 52.5 per cent reduction over control, respectively. The inhibitory effect of TVC5, TVC1 and TVC6 on sclerotial production was found to be 42.5, 39.2 and 37.9 per cent reduction over control, respectively. However the other *T. viride* isolates showed lesser inhibitory effect.

The maximum inhibition zone of 14.0 mm was observed in *T. harzianum* – Th. This was followed by TVC6, TVC5 and TVC2 which showed an inhibition zone of 11.0,

9.0 and 8.7 mm, respectively. The other isolates recorded an inhibition zone ranging from 5 to 0.7 mm. The maximum mean mycelial over growth was recorded in *T. harzianum* -Th (56.7 mm). The *T. viride* isolates TVC5 and TVC6 recorded 42.7 and 40.7 mm, respectively. However, the other isolates showed lesser mycelial over growth (Table 6; Plate 6a).

### 4.6.2. Screening of *Trichoderma* spp. against *R. solani*

Among the *Trichoderma* spp. screened, *T. harzianum* - Th recorded maximum inhibition of mycelial growth (64.3%) and of sclerotial production (54.6%) with an inhibition zone of 18.0 mm and mean mycelial over growth of 44.7 mm. *T. viride* isolates TVC5, TVC3, TVC4, TVC1 and TVC6 showed reduction of 62.4, 56.2, 55.6, 55.4 and 55.1 per cent over control in mycelial growth, respectively.

Among the *T. viride* isolates, TVC5 recorded maximum inhibition of sclerotial production (53.1%). The other isolates TVC4, TVC3 and TVC1 were on par in their effectiveness. TVC5 recorded an inhibition zone of 16.0 mm followed by TVC2 (14.0 mm), TVC3 (13.7 mm), TVC4 (13.3 mm) and TVC1 (12.7 mm), whereas TVC6 recorded minimum inhibition zone of 11.0 mm.

The maximum mycelial over growth was recorded in TVC5 (49.3 mm) and TVC3 (48.0 mm). The other isolates recorded comparatively lesser mycelial over growth (Table 7; Plate 6b).

### 4.6.3. Screening of *Pseudomonas fluorescens* Migula. isolates against *M. phaseolina*

The maximum inhibition of mycelial growth (52.9%) was recorded in *P. fluorescens* isolate PFC6. This was followed by PFC4 (40.7%), PFC3 (31.3%) and PFC7 (12.7%). The other isolates were less effective in their effectiveness. PFC6 reduced sclerotial production to an extent of 62.8 per cent over control. The other isolates such as PFC4 and PFC3, PFC5 and Pf1 recorded 56.3, 49.9, 46.3 and 46.3 per cent inhibition in sclerotial production, respectively, whereas the other isolates were less effective.

The maximum inhibition zone of 21.3 mm was recorded in PFC6. This was followed by PFC4 (10.0 mm), PFC3 (4.6 mm). The other isolates were recorded less than 1.0 mm inhibition zone (Table 8; Plate7a, b).

### 4.6.4. Screening of *P. fluorescens* isolates against *R. solani*

Among the eight isolates screened, PFC6 recorded maximum inhibition of mycelial growth (47.3%). This was followed by PFC 5 (42.9%), PFC 1 (37.0%) and PFC 4 (36.5%). The other isolates were found to be less effective. The maximum inhibition of sclerotial production was observed in PFC6 (58.3%). This was followed by Pf1 (49.0%), PFC7 (48.2%) and PFC3 (47.4%), whereas the other isolates were less effective.

PFC6 recorded highest inhibition zone of 21.7 mm. Pf1 and PFC4 also recorded an inhibition zone of 17.0 mm and 10.3 mm, respectively. However, the other isolates showed lesser inhibition zone (Table 9).

### 4.6.5. Screening of *Bacillus subtilis* isolates against *M. phaseolina*

Among the ten *B. subtilis* Webster. isolates screened, BSC5 showed maximum inhibitory effect on mycelial growth (23.6%). The other isolates BSC7, BSC1, BSC10 and BSC8 recorded an inhibition of 22.47, 21.3, 21.2 and 19.7 per cent reduction over control, respectively. The other isolates showed lesser inhibitory effect with a range of 16.3 to 4.9 per cent. (Table 10; Plate 8).

The maximum inhibition of sclerotial production was observed in BSC5 (37.9%). BSC7 and BSC8 showed the sclerotial inhibition of 28.9 and 27.6 per cent, respectively. The other isolates were less effective in inhibiting sclerotial production. All the isolates showed zone of inhibition. However, maximum inhibition zone (11.00 mm) was observed in BSC5 and BSC10. The isolates BSC7 and BSC8 also showed an inhibition zone of 10.67 and 9.67 mm, respectively. All other isolates were produced lesser inhibition zone (Table 10).

### 4.6.6. Screening of *B. subtilis* isolates against *R. solani*

The *B. subtilis* isolate BSC7 recorded maximum inhibition (49.0%) of mycelial growth of *R. solani*. BSC8 was on par in its effectiveness to inhibit the mycelial growth (46.7%). This was followed by BSC10 (47.8%), BSC5 (44.3%) and BSC1 (39.7%). The other isolates also showed lesser inhibitory effect of mycelial growth of *R. solani*.

The maximum inhibition of sclerotial production (48.9%) was observed in BSC7. This was followed by BSC8 (45.4%), BSC10 (44.0%) and BSC5 (40.4%). The other isolates recorded inhibition ranging from 38.3 to 27.9 per cent reduction over control.

The isolate BSC 7 showed maximum inhibition zone of 10.67 mm followed by BSC 8 (10.33 mm). The *B. subtilis* isolate BSC 5, BSC 10 and BSC 6 observed with 9.67, 8.67 and 5.00 mm inhibition zone. However, the other *B. subtilis* isolates were less effective in producing inhibition zone (Table 11).

## 4.7. *In vitro* screening of plant extracts

### 4.7.1. Screening of plant extracts against *M. phaseolina*

All the plant extracts preparation was inhibitory to the mycelial growth. Among the eight plant species used, *Allium sativum* executed maximum reduction of mycelial growth (76.0%). The leaf extracts from *Lawsonia inermis* and *Vitex negundo* recorded 32.9 and 17.1 per cent reduction over control, respectively. The remaining plant extracts inhibited the growth of *M. phaseolina* to an extent of 14.4 to 3.7 per cent over control.

The maximum inhibition of sclerotial production was observed in *A. sativum* (47.9%) followed by *L. inermis* (37.9%), *V. negundo* (23.2%) and *Azadirachta indica* (16.4%). The other plant extracts showed lesser inhibitory effect on the sclerotial production of *M. phaseolina* (Table 12; Plate 9a).

### 4.7.2. Screening of plant extracts against *R. solani*

Among the eight plant extracts used, *A. sativum* @ 10 per cent concentration exerted 97.2 and 92.3 per cent reduction in mycelial growth and sclerotial production, respectively over control. The leaf extract from *L. inermis* and *A. indica* also recorded

40.5 and 16.5 per cent reduction in mycelial growth and 38.7 and 22.0 per cent reduction in sclerotial production over control, respectively. The remaining plant species were less effective in reducing both mycelial growth and sclerotial production (Table 13; Plate 9b).

### 4.8. Effect of plant oils on mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*

Winter green oil (WGO) and Lemon grass oil (LGO) were posted for their effect on mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* at three different concentrations. Among them, WGO at 0.1 and 0.2 per cent and LGO at three different concentrations *viz.,* 0.05,0.1 and 0.2 recorded 91.0 per cent inhibition of mycelial growth of *M. phaseolina* over control, whereas WGO at 0.05 per cent showed only 4.5 per cent inhibition.

The maximum inhibition (91.1%) of *R. solani* mycelial growth was observed in WGO and LGO at 0.2 per cent concentration. This was followed by LGO (0.1%) and WGO (0.1%), which showed an inhibition of 28.4 per cent. The WGO and LGO at 0.05 per cent concentration were less effective.

The sclerotial production of *M. phaseolina* was inhibited by WGO and LGO at all concentrations tested. However, the maximum inhibition was observed at 0.2 per cent concentration of WGO and LGO (95.6%). The sclerotial production of *R. solani* was effectively inhibited at 0.2 per cent concentration of WGO and LGO (99.7%). This was followed by WGO at 0.1 per cent (37.5%) and LGO at 0.1 per cent (27.1%). The other concentrations were less effective (Table 14; Plate 10a and 10b).

### 4.9. Effect of $ZnSO_4$ on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*

The effect of $ZnSO_4$ on the mycelial growth of *M. phaseolina* was studied with four different concentrations as indicated in materials and method. Among four concentrations used, 0.75 and 1.0 per cent of $ZnSO_4$ recorded maximum inhibitory effect (90.6%) on mycelial growth and sclerotial production (96.3). $ZnSO_4$ at 0.5 per cent concentration showed 87.9 per cent inhibition of mycelial growth and 67.0 per cent

inhibition of sclerotial production. The minimum concentration 0.25 per cent showed less effective against *M. phaseolina* ( Plate11).

The $ZnSO_4$ at 0.50, 0.75 and 1.0 per cent concentration were on par in inhibitory mycelial growth and sclerotial production of *R. solani*. However 1.0 per cent concentration showed the highest inhibitory effect (90.5%). A minimum concentration of 0.25 per cent inhibited mycelial growth and sclerotial production to an extent of 54.7 and 52.2 per cent over control (Table 15).

### 4.10. Effect of fungicides on the mycelial growth and sclerotial production of root rot pathogens

#### 4.10.1. Effect of fungicides on the mycelial growth and sclerotial production of of *M. phaseolina*

The fungicides at 0.1 and 0.2 per cent concentrations were tested for their effect on mycelial growth and sclerotial production of *M. phaseolina* under *in vitro* condition. Carbendazim, Tricyclazole, Thiram, Hexaconazole, Thiophenate methyl and Propiconazole completely inhibited the mycelial growth and sclerotial production of *M. phaseolina* at both concentrations. However no inhibitory effect was observed in Copperoxychloride at 0.1 and 0.2 per cent concentration (Table 16; Plate 12b).

#### 4.10.2. Effect of fungicides on the mycelial growth and sclerotial production of *R. solani*

Carbendazim, Tricyclazole, Hexaconazole and Propiconazole completely inhibited the mycelial growth at 0.2 and 0.1 per cent concentration. Thiram and Copper oxychloride and Thiophenate methyl at 0.1 per cent concentration showed 73.9 and 58.1 and 85.2 per cent inhibition, respectively. At 0.2 per cent concentration, thiophenate methyl also completely inhibited the mycelial growth. The Copper oxychloride and Thiram showed 74.3 and 83.1 per cent inhibition at 0.2 per cent concentration.

The sclerotial production was completely inhibited by Carbendazim, Tricyclazole, Hexaconazole and Propiconazole. The other fungicides also exhibited a inhibition of 96.0 to 96.5 per cent over control (Table 17; Plate 12a).

### 4.11. Compatibility of biocontrol agents with plant oils and $ZnSO_4$

Compatibility of two effective isolates of *P. fluorescens viz.,* PFC6 and Pf1 and two *Trichoderma* species such as *T. harzianum* - Th and *T. viride* -TVC5 with plant oils WGO and LGO at 0.2 per cent concentration along with 0.5 per cent concentration of $ZnSO_4$ was done. The isolates PFC6 and Pf1 were compatible with WGO and LGO at 0.2 per cent concentration. Both these isolates showed no growth retardation with $ZnSO_4$ at 0.5 per cent concentration. *Trichoderma* species such as *T. harzianum*-Th and *T. viride*-TVC5 were incompatible with WGO and LGO and compatible with $ZnSO_4$ at 0.5 per cent. However, $ZnSO_4$ at 1.0 per cent concentration completely inhibited the growth of both *Pseudomonas* and *Trichoderma* species tested (Table 18; Plate 13a; 13b).

### 4.12. Effect of EC formulation on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*

The effect of EC formulation of WGO and LGO was tested at 0.1and 0.2 per cent concentration on the mycelial growth and sclerotial production. Both WGO and LGO inhibited the mycelial growth of *M. phaseolina* at 0.1 and 0.2 per cent concentration. The LGO 40 EC showed maximum inhibition of mycelial growth with 79.0 and 91.2 per cent over control at 0.1 and 0.2 per cent, respectively. This was followed by WGO 40 EC with mycelial inhibition of 65.6 and 90.9 per cent over control at 0.1 and 0.2 per cent respectively.

The sclerotial production was completely inhibited (99.7%) at 0.2 per cent concentration of WGO 40 EC and LGO 40 EC followed by 30 EC of LGO and WGO

recorded 39.5 and 34.7 per cent reduction over control, respectively. The 20 EC of both LGO and WGO had lesser inhibitory effect on sclerotial production (Table 19).

The maximum inhibition of mycelial growth of *R. solani* was observed at 0.2 per cent concentration of LGO 40 EC (90.1%) followed by 0.2 per cent of WGO 40 EC (89.8%). The other formulations of LGO and WGO with 20 and 30 EC were less effective.

Both WGO and LGO (40 EC) at 0.2 per cent concentration completely inhibited the sclerotial production of *R. solani*. This was followed by 30 EC LGO (88.2%) and 30 EC of WGO (84.8 %). 20 EC formulations were less effective even at 0.2 per cent concentration (Table 20).

### 4.13. Volatile effect of plant oils and EC formulation on mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*

Based on the efficacy of plant oils and its EC formulation under *in vitro* condition, 0.2 per cent of WGO, LGO, WGO 40 EC and LGO 40 EC were selected for studying its volatile effect on the mycelial growth and sclerotial production. The maximum inhibitory effect of mycelial growth of *M. phaseolina* was observed in LGO (90.5%) followed by WGO (88.1%). The EC formulation of WGO and LGO had inhibitory effect of 85.7 and 88.1 per cent over control, respectively.

Both plant oils and their EC formulation inhibited the mycelial growth of *R. solani.* However, maximum inhibition was observed in WGO (90.7%) and LGO (90.7%) followed by LGO 40 EC (86.1) and WGO 40 EC (85.3%).

Sclerotial production of *M. phaseolina was* completely inhibited by LGO (100%) followed by WGO (88.0%). However, the EC formulation of LGO and WGO had comparatively lesser effect of 77.6 and 76.0 per cent over control, respectively.

Plant oils *viz.,* WGO, LGO and LGO 40 EC at 0.2 per cent concentration completely suppressed the production of sclerotia of *R. solani*. The 40 EC of WGO recorded 86.7 per cent inhibition over control (Table 21).

4.14. Stability of EC formulation at dilution, temperature, pH and storage

Among the five dilutions tested for the stability of WGO 40 EC formulation, up to 1:4 dilution recorded relatively more effective in reducing mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*. The other dilutions were less effective (Table 22).

The WGO 40 EC was relatively more effective in reducing mycelial growth of *M. phaseolina* and *R. solani* at all the three pH tested. However the maximum inhibitory effect was recorded at neutral pH (7.0) followed by alkaline (9.0) and acid (4.0) (Table 23).

Exposure of EC formulation at low temperature ($20^{o}$C) recorded maximum inhibitory effect (91.0%) on mycelial growth of *M. phaseolina* and *R. solani*. Room temperature storage of EC formulation also had similar effect of $20^{o}$C. When the temperature was increased from 40 to $80^{o}$C, the efficacy was reduced (Table 24).

The EC formulation retained the antifungal activity even after eight months. There was not much difference between fresh EC formulation and
formulation stored for 240 days, which recorded an inhibition per cent of 91.0 and 89.8, respectively on *M. phaseolina* and 91.1 per cent and 87.7 per cent inhibition on *R. solani* (Table 25).

### 4.15. Pot culture experiments

#### 4.15.1. Effect of biocontrol agents on the root rot incidence caused by *M. phaseolina* and *R. solani*

The effect of soil application of seven biocontrol agents in reducing root rot incidence of Coleus was tested in pot culture experiment and results are furnished in Table 26. All the biocontrol agents reduced the root rot incidence caused by *M. phaseolina* compared to control. Among the biocontrol agents tested, PFC6, *T. harzianum* - Th and Pf1 were relatively more effective and recorded maximum reduction

of root rot incidence when compared to other biocontrol agents. They recorded a reduced root rot infection over control to an extent of 61.4, 56.4 and 53.8 per cent, respectively. The remaining biocontrol agents were also effective, but were relatively less inhibitory when compared to PFC6, *T. harzianum* - Th and Pf1.

In reducing the root rot caused by *R. solani, P. fluorescens* isolate PFC6, *T. harzianum* -Th and Pf1 showed relatively more disease reduction than the control and other biocontrol agents with the inhibitory effect of 64.1, 61.5 and 54.7 per cent, respectively. The other biocontrol agents showed disease reduction ranging from 47.4 to 30.4 per cent over control (Table 26).

4.15.2. Effect of formulation of biocontrol agents and plant oils on incidence the root rot under green house condition

The talc based formulations of biocontrol agents and EC formulations of plant oils were tested for their efficacy in reducing root rot incidence and results furnished in Table 27. Among the different formulations tested, maximum disease reduction was observed in PFC6 + LGO + $ZnSO_4$ (62.0%). This was followed by PFC6 + WGO + $ZnSO_4$ (60.0%), *T. harzianum*-Th + $ZnSO_4$ (59.3%), and Pf1 + $ZnSO_4$ + LGO (57.3%). The other talc based and EC formulations were less effective in reducing root rot incidence caused by *M. phaseolina*.

The root rot caused by *R. solani* was reduced by soil application of talc based formulation of biocontrol agents and EC formulations of plant oils. The maximum disease reduction was observed in PFC6 + $ZnSO_4$ + WGO (67.5%). Talc based formulation of PFC6 + $ZnSO_4$ + LGO and Th + $ZnSO_4$ were on par in their efficacy in reducing root rot incidence (65.0%). The other formulations were less effective (Table 27) (Plate14a,b).

**4.15.3. Effect of organic amendments and $ZnSO_4$ on the incidence of coleus root rot caused by *M. phaseolina* and *R. solani* under greenhouse condition**

In view of the fact that organic amendments serve as food bases for the multiplication of antagonists, an experiment was conducted to study the effect of soil

application of organic amendments. The results revealed that all the treatments recorded disease reduction from 12.8 to 58.1 per cent in case of *M. phaseolina* and 15.8 to 61.9 per cent in case of *R. solani* root rot. However, the maximum reduction of root rot caused by *M. phaseolina* was observed in $ZnSO_4$ (59.3%) followed by neem cake (58.1%), coir pith compost (53.9%) and farm yard manure (FYM) (52.3%). The other amendments were less effective in reducing root rot incidence. Zinc sulphate, Neem cake and coir pith compost were relatively more effective in reducing root rot caused by *R. solani*. They recorded the inhibition of 61.9, 55.5 and 52.3 per cent over control, respectively. The other amendments were relatively less effective in reducing root rot incidence caused by *R. solani* (Table 28).

### 4.15.4. Effect of fungicides on root rot incidence of coleus caused by *M. phaseolina* and *R. solani*

Based on the *in vitro* efficacy of fungicides in reducing mycelial growth and sclerotial production, six fungicides were selected to test their efficacy under pot culture experiment. All the fungicides were effective in reducing root rot incidence caused by *M. phaseolina* and *R. solani*. However, maximum reduction of root rot incidence of *M. phaseolina* (74.6) and *R. solani* (70.0%) was recorded in carbendazim. This was followed by Propiconazole (71.8 and 65%), Hexaconazole (60.6 and 56.3%) and Thiophenate methyl 7.70 and 52.5 per cent reduction over control, respectively. Thiram and Tricyclazole showed lesser effect in reducing root rot incidence (Table 29; Plate 15a,b).

### 4.16. Shelf life of biocontrol agents in different talc based formulation

*Trichoderma* species and *P. fluorescens* isolates were tested for their shelf life in talc based formulations. The maximum population of *T. harzianum* ($3.5 \times 10^8$ cfu/ml), T. *viride* - TVC5 ($4.2 \times 10^8$ cfu/ml), PFC6 ($4.9 \times 10^8$ cfu/ml) and Pf1 ($4.9 \times 10^8$ cfu/ml) were recorded on 0 days after storage. As the days after storage increased, population of all biocontrol agents decreased. However *Trichoderma* species survived with required colony forming units ($10^8$ cfu/ml) in the formulation up to 120 days after storage. Similar trend was also observed in case of *P. fluorescens* isolates. Both PFC6

and Pf1 isolates survived up to 90 days in talc formulations and after that the declined (Table 30).

### 4.17. Rhizosphere population of biocontrol agents under field condition

#### 4.17.1. Rhizosphere population of *Trichoderma* spp. - Neermullikuttai

The survival ability of antagonists plays an important role in soil borne disease management. Influence of various treatments on *Trichoderma* population in the rhizosphere region was enumerated at different intervals after planting. *Trichoderma* populations were significantly increased with increasing in age of the crop from 45 to 120 days after planting. At 0 DAP, *Trichoderma* populations were 2.1 to 2.7 x $10^3$ cfu $g^{-1}$ of soil, whereas at 45 DAP, the maximum population was recorded in *T. harzianum* + $ZnSO_4$ treated plots (18.0 x $10^3$ cfu $g^{-1}$), followed by *T. viride* isolate TVC5+ $ZnSO_4$ (14.0 x $10^3$ cfu $g^{-1}$). All the other treatments recorded *Trichoderma* population ranging from 6.5 to 2.6. Similar trend was observed in 90 and 120 DAP. Plots treated with chemical fungicides and EC formulations recorded lowest population of *Trichoderma* than the control (Table 31).

#### 4.17.2. Rhizosphere population of *P. fluorescens* - Neermullikuttai

The population of *P. fluorescens* in the rhizosphere region significantly increased with increase in age of the plant from 45 to 120 days after planting. At 0 DAP, the low level of *P. fluorescens* population in the range of 2.2 to 27 x $10^4$ cfu $g^{-1}$ was observed in different treatments, on 45 and 120 days after planting, the *Pseudomonas* population increased in all treatments compared to 0 DAP. However, the maximum population was recorded in PFC6 + WGO+ $ZnSO_4$ treated plots (69 and 88 x $10^4$ cfu $g^{-1}$) followed by mixture of PFC6 + WGO + $ZnSO_4$ and Pf1 WGO + $ZnSO_4$ and Pf1+ WGO + $ZnSO_4$ (65 and 82 x $10^4$ cfu $g^{-1}$) treated plot even after 90 and 120 days after planting. The plots treated with *P. fluorescens* based formulation recorded relatively more population than the others. The plots treated with chemical fungicides and EC formulation recorded relatively low level of *P. fluorescens* populations (Table 32).

#### 4.17.3. Rhizosphere population of *T. viride* and *P. fluorescens* - Kaniyamoor

*Trichoderma* population in rhizosphere region was estimated up to 90 DAP in Kaniyamoor field trial since the root rot incidence reached more than 90 per cent on 90 DAP. *Trichoderma* population increased with increase in days after planting. A low level of population (1.0 to 1.4 x $10^3$ cfu /g) of *Trichoderma* was recorded on 0 DAP. The population level increased from 45 and 90 DAP in all the treatments (Table 33). However, the population was relatively more in application of *T. harzianum*- Th +$ZnSO_4$, *T. viride* TVC5 +$ZnSO_4$, and their combination on 90 DAP (20.0, 18.0 and 19.7 x $10^3$ cfu $g^{-1}$, respectively).

Similar trend was observed in the population of *P. fluorescens*. The minimum population was observed in 0 DAP. The population increased with increase in DAP. The maximum population was observed on 90 DAP. PFC6 + WGO + $ZnSO_4$ recorded maximum population (52 x $10^4$ cfu $g^{-1}$) followed by Pf1 + WGO + $ZnSO_4$ (50 x $10^4$ cfu $g^{-1}$), mixture of PFC6 + $ZnSO_4$ + WGO and Pf1 + $ZnSO_4$ + WGO (48.0 x $10^4$ cfu $g^{-1}$) and PFC6 (47 x $10^4$ cfu $g^{-1}$). The other treatments recorded less population of *P. fluorescens* (Table 34).

### 4.17.4. Rhizosphere population of *Trichoderma* species and *P. fluorescens* - Coimbatore

In Coimbatore field trial, biocontrol agents population increased with increase in age of the crop from 45 to 120 DAP. The maximum population was observed on 120 DAP. Among the various treatments, application of *Trichoderma* based formulation recorded relatively more number of *Trichoderma* populations than the others. *T. harzianum*-Th+ $ZnSO_4$, *T. viride*- TVC5+$ZnSO_4$ and their combined application recorded a population of 25.5, 24 and 24.2 x $10^4$ cfu $g^{-1}$, respectively. The other treatments showed lesser population of *Trichoderma* species. A similar trend was noticed in case of *P. fluorescens* population. The maximum population of *P. fluorescens* was observed on 120 DAP in PFC6 + WGO + $ZnSO_4$ (80.9 x $10^4$ cfu $g^{-1}$) followed by PFC6 (78.0 x $10^4$ cfu $g^{-1}$), Pf1 + WGO +$ZnSO_4$ (75 x $10^4$ cfu $g^{-1}$) and mixture of PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ (74.0 x $10^4$ $g^{-1}$) (Table 35 and 36).

4.18. Management of Coleus root rot incidence under field conditions

4.18.1. Field trail I- Neermullikuttai village

The results of field trial at Neermullikuttai village of Salem district (Table 37; Plate 16) revealed that the plots treated with carbendazim recorded maximum reduction of root rot incidence to an extent of 94.3 and 85.1 per cent on 45 and 90 days after planting, respectively. This was followed by Propiconazole, which recorded 91.4 and 76.5 per cent reduction on 45 and 90 DAP. Among the bioformulations, mixture of *T. harzianum*-Th+$ZnSO_4$ and TVC5+$ZnSO_4$ reduce root rot incidence to an extent of 68.7 per cent followed by mixture of PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ (62.0%), *T. harzianum* (60.1%) and PFC6 + WGO + $ZnSO_4$ (53.8%) on 90 days after planting. The other bioformulation reduced root rot incidence ranging from 47.2 to 18.5 per cent. The soil amendments such as neem cake and $ZnSO_4$ reduced root rot incidence to an extent of 29.9 and 25.2 per cent on 90 DAP, respectively. The two EC formulations *viz.*, EC-C and EC-F were least effective in reducing root rot incidence (20.6 and 18.2 per cent reduction, respectively). The control plots are recorded with a maximum root rot incidence of 35.0 and 69.3 per cent on 45 and 90 days after planting.

The maximum yield of 5674 kg $ha^{-1}$ was obtained with bioformulation mixture containing PFC6+ WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ and carbendazim (5674 kg $ha^{-1}$) followed by PFC6+WGO+$ZnSO_4$ (5361 kg $ha^{-1}$), Propiconazole (5109 kg $ha^{-1}$), mixture containing *T. harzianum*-Th+$ZnSO_4$ and TVC5+ $ZnSO_4$ (4891 kg $ha^{-1}$) and *T. harzianum* (4320 kg $ha^{-1}$). The other treatments also significantly increase the yield of coleus to control. The control plot recorded with lowest yield of 918 kg $ha^{-1}$.

All the plant growth parameter such as plant height, number of branches, tuber length and tuber diameter significantly increased in all treatments. However, maximum increase was obtained with bioformulation mixture PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ followed by PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ (Table 38; Plate 17).

**4.18.2. Field trail II - Kaniyamoor Village**

The results of field trial at Kaniyamoor village (Table 39; Plate 18) revealed that the bioformulations and its mixtures, soil amendments and EC formulations were effective in reducing the root rot incidence up to 45 DAP. The maximum reduction of root rot was observed on 45 DAP in carbendazim and Propiconazole, respectively recorded 56.8 and 54.4 percent reduction over control.

This was followed by *T. harzianum* +$ZnSO_4$ and TVC5+$ZnSO_4$ application (52.2%), mixture of PFC6 + WGO+ $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ (48.8%), *T. harzianum* + $ZnSO_4$ (48.8%) and PFC6 + WGO + $ZnSO_4$ (45.5%). The other treatments were less effective in reducing root rot incidence.

On 90 DAP, most of the treatments were failed to reduce the root rot incidence above 10 per cent over control. The maximum inhibition of 19.2 per cent reduction was observed in carbendazim followed by Propiconazole (9.6%) combined application of *T. harzianum*-Th + $ZnSO_4$ and TVC5+ $ZnSO_4$ also recorded 10.2 per cent inhibition over control. The other treatments recorded less than 10 per cent reduction over control (Table 39).

The yield and other growth parameters were not recorded in Kaniyamoor field trial since there was a 100 per cent infection on 100 DAP followed by collapse and decay of branches and roots.

**4.18.3. Field trail III - Coimbatore**

In Coimbatore field trial, all the treatments significantly reduced the root rot incidence when compared to control on 45 and 90 DAP. Among the fourteen different treatments, carbendazim and Propiconazole reduced the root rot incidence to an extent of 80.5 and 76.6 per cent inhibitions over control, respectively on 90 DAP. Among bioformulation, mixture of *T. harzianum* + $ZnSO_4$ and TVC5 + $ZnSO_4$ showed maximum

reduction of root rot incidence (63.6%) followed by *T. harzianum* + $ZnSO_4$ (58%). The other treatments were lesser effective in reducing root rot incidence (Table 40; Plate19).

The maximum yield of 5452 kg $ha^{-1}$ was recorded in bioformulation mixture of *T. harzianum*-Th + $ZnSO_4$ and TVC5+ $ZnSO_4$. The other formulation such as PFC6 + WGO + $ZnSO_4$, PFC6, Pf1, Pf1 + WGO + $ZnSO_4$ and combination of PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ were on par in their yield. The other treatments recorded lesser yield than the above treatments. The control recorded the lowest yield of 781 kg $ha^{-1}$.

All the growth parameters significantly increased in all treatments than the control. The maximum increase of growth parameters were observed in bioformulation PFC6 + WGO + $ZnSO_4$ followed by mixture of the PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$, PFC6 and Pf1. The others were less effective in increasing the plant growth parameters (Table 41).

## 4.19. Induction of defense related proteins and chemicals against root rot pathogens

### 4.19.1. Phenylalanine ammonia lyase (PAL)

Soil application of all bioformulations induced the synthesis of PAL. Treatment with bioformulation containing PFC6 + WGO + $ZnSO_4$ followed by challenge inoculated with *M. phaseolina* induced the plant to synthesize higher level of PAL. The enzyme activity reached a maximum level on 21 days after challenge inoculation and thereafter declined with a decreasing rate than the inoculated control. The bioformulation containing Pf1 + WGO + $ZnSO_4$ recorded higher level of PAL when challenge inoculated with *M. phaseolina*. The other bioformulations were less effective in inducing PAL synthesis. The inoculated control showed reduction of PAL activity starting from seventh day and then decreased to a lower level than the uninoculated plants at 28 days after inoculation (Fig. 2).

A similar trend was observed in case of *R. solani*. The bioformulation mixture containing PFC6 + WGO + $ZnSO_4$ and Pf1+WGO+$ZnSO_4$ recorded higher level of PAL activity throughout the study than the inoculated control. The other bioformulation were less effective in inducing synthesis of PAL. Pathogen alone inoculated plants showed reduction of PAL from seven days after inoculation and decreased beyond initial level and decreased below the level of uninoculated control at 28 days after inoculation (Fig.3).

4.19.2. Peroxidase (PO)

Peroxidase activity was measured in the roots of *M. phaseolina*, *R. solani* inoculated and bioformulations pretreated plants. Among the various bioformulations used, treatment of PfC6+WGO+$ZnSO_4$ followed by challenge inoculation with root rot pathogens showed higher induction of peroxidase and the induction reached a maximum level on 21 days after challenge inoculation. The activity of the enzyme thereafter declined with a decreasing rate than the inoculated control. Plants treated with bioformulation Pf1 +WGO + $ZnSO_4$ also recorded a higher level of PO activity throughout study period than the other treatments. The inoculated control showed reduction of PO activity starting from $7^{th}$ day and then decreased to lower level than uninoculated control on 28 days after inoculation (Fig.4,5).

Native PAGE analysis revealed four isoforms of PO (PO1, PO2, PO3 and PO4) were observed in the plants pretreated with bioformulation mixture containing PFC6 + WGO + $ZnSO_4$, Pf1+WGO + $ZnSO_4$, *T. harzianum*- Th + $ZnSO_4$ and TVC6 + $ZnSO_4$ and challenge inoculated with *M. phaseolina*. The induction was very prominent in bioformulations PFC6 + WGO + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ (Plate 20a).

In case of *R. solani*, two PO isoforms (PO1 and PO2) were observed in all four bioformulation pretreated and challenge inoculated plants. However, induction was prominent in bioformulation PFC6 + WGO + $ZnSO_4$, Pf1+WGO+$ZnSO_4$ and *T. harzianum* + $ZnSO_4$ (Plate 20b).

4.19.3. Polyphenol oxidase (PPO)

All bioformulations induced the plants to synthesize the PPO. The induction was noticed from 7 to 21 DAI and thereafter decreased with decreasing rate. However, the maximum induction was observed in the plants pre-treated with bioformulation PFC6 + WGO + $ZnSO_4$ and challenge inoculated with root rot pathogens *viz., M. phaseolina* and *R. solani*. This was followed by bioformulation containing *T. harzianum* + $ZnSO_4$.The rests of the bioformulations were comparatively less effective in inducing PPO activity. However, the inoculated control showed a reduction of PPO activity from seven DAI as reached below initial level and below uninoculated Control on 28 DAI (Fig. 6 and 7).

Native PAGE analysis revealed that three isoforms of PPO (PPO 1, PPO 2, PPO 3 and PPO 4) was observed in all four bioformulations pretreated and challenge inoculated with *M. phaseolina*. Plants treated with bioformulation alone did not show any additional PPO isoforms (Plate 21a). In the plants pre-treated with bioformulations and challenge inoculated with *R. solani* observed four isoforms (PPO 1, PPO 2, PPO 4). The plants pretreated with Pf1 + WGO + $ZnSO_4$ and challenge inoculated with *R. solani* observed only three PPO isoforms. The plants pretreated with bioformulation alone did not express any additional isoforms. The isoforms trending patterns were prominent in PFC6 + WGO + $ZnSO_4$ and *T. harzianum*+ $ZnSO_4$ pretreated plants and challenge inoculated with *R. solani* (Plate 21b).

**4.19.4. Chitinase**

Induction of chitinase in coleus root by soil application of bioformulations against root rot pathogens *M. phaseolina* and *R. solani* was studied. All bioformulations significantly enhance the chitinase activity after challenge inoculation with root rot pathogens. The maximum chitinase activity was noticed on 21 DAP in PFC6 + WGO + $ZnSO_4$ pre treated plants challenge inoculated with root rot pathogens. This was follow by Pf1 + WGO + $ZnSO_4$ pre-treated plants challenge inoculated with root rot pathogens. The other bioformulations pre-treatment showed relatively less effective in induction of chitinase. In the inoculated control, chitinase induction was observed up to 7 DAP after that the enzyme activity declines and reached below initial level on 28 DAI (Fig.8 and 9).

**4.19.5. β-1,3 glucanase**

The effect of soil application of four bioformulations in the induction of β-1,3-glucanse activity in coleus roots against root rot pathogens *M. phaseolina* and *R. solani* were studied. All bioformulation treatment enhanced the β-1,3-glucanase activity in coleus roots than the inoculated control. However higher level of induction was observed in bioformulations pretreated and challenge inoculated with root rot pathogens. The maximum induction was noticed in PFC6 + WGO + $ZnSO_4$ pretreated plants challenge inoculated with root rot pathogens followed by Pf1 + WGO + $ZnSO_4$. The other bio formulations were lesser in their effectiveness to induce β-1, 3-glucanse activity. The root rot pathogen inoculated control showed a decrease in enzyme activity starting from seventh day of inoculation to 28 DAI (Fig. 10 and 11).

### 4.19.6. Total phenol

Higher level of phenolics accumulation was observed in coleus roots pretreated with bioformulation PFC6 + WGO + $ZnSO_4$ and challenge inoculated with root rot pathogens *viz.*, *M. phaseolina* and *R. solani* throughout the study period. However, the maximum phenolics accumulation was observed on 21 DAI. The bioformulation *T. harzianum* + $ZnSO_4$ and Pf1 + WGO + $ZnSO_4$ pretreated plants also recorded higher level of phenolics than inoculated control. In inoculated control, phenolics accumulation increased upto 7 DAI and after that decreased with increasing rate and reached below initial level on 28 DAI (Fig.12 and 13).

## 4.20. Mode of action of biocontrol agents

### 4.20.1. Mode of action of *Trichoderma* species

#### 4.20.1.1. Mycoparasitism

*Trichoderma* species were able to grow over pathogen and caused hyphal coiling, hyphal abnormalities reduction in sclerotial production, lysis of hyphae and sclerotia. Plate 22 showed hyphal coiling ,hyphal malformation inhibition of sclerotial production caused by *T. harzianum* - Th isolate.

#### 4.20.1.2. Volatile metabolite production of *Trichoderma* species

The Volatile metabolite *T. harzianum* - *Th* and *T. viride* - TVC5 reduced the mycelial growth of *M. phaseolina* to an extent of 20.0 and 7.6 per cent and 0.49 and 0.11 per cent over control, respectively on 3 and 7 DAI. The sclerotial production of *M. phaseolina* was inhibited to an extent of 15.2 and 19.0 per cent over control, respectively (Table 42). The mycelial growth of *R. solani* was inhibited by *T. harzianum* and *T. viride* to an extent of 7.8 and 4.5 per cent over control, respectively on 3 DAI. After seven days of inoculation, 6.1 and 4.2 per cent inhibition was observed by *T. harzianum* and *T. viride*. The sclerotial production of *R. solan*i was inhibited to an extent of 40.6 and 35.9 per cent, respectively by volatile metabolites of *T. harzianum* and *T. viride* TVC5 (Table 43).

#### 4.20.1.3. Effect of diffusible metabolites of *Trichoderma* species

*Trichoderma* species such as *T. harzianum* - Th and *T. viride*-TVC5 effectively inhibited the mycelial growth of *M. phaseolina* to an extent of 50.0 and 41.1 per cent over control, respectively on 3 DAI and 52.7 and 44.4 per cent over control, respectively on 7 DAI. The maximum inhibition of sclerotial production was observed in *T. harzianum* (26.9%) followed by TVC5 (15.3%) (Table 44).

A similar trend was observed in case of *R. solani*. *T. harzianum* - Th recorded maximum inhibition of mycelial growth on both 3 DAI (78.5%) and 7 DAI (55.4%). *T. viride* isolate TVC5 showed 74.8 and 18.2 per cent inhibition over control on 3 and 7 DAI, respectively. *T. harzianum* reduced the sclerotial production of *R. solani* to an extent of 69.2 followed by *T. viride* TVC5 (49.2%) (Table 45).

### 4.20.2. Mode of action of *P. fluorescens*

#### 4.20.2.1. Production of extra cellular lytic enzymes

Various modes of action of *P. fluorescens* was studied with effective isolate PFC6 and compared with a standard isolate Pf1. Between two isolates tested for chitinase production, PFC6 recorded higher level of chitinase activity (4.36 nmol min/ mg/ of protein) compared to Pf1 isolate (3.15 μmol/ min/ ng of protein). β-1,3-glucanse

activity was found to be higher in PFC6 (45.3) than Pf1 (40 μmol $min^{-1}$ protein) (Figure 14a,b).

#### 4.20.2.2. Siderophore production

Siderophore production has been considered as one of the important mechanisms by which fluorescent pseudomonads exert their antagonistic activity. Siderophore production of PFC6 and Pf1 were tested by qualitatively and quantitatively. The qualitative analysis revealed that PFC6 isolate showed strong production of siderophore than Pf1 isolate, which recorded moderate production (Plate 23). The quantitative analysis revealed that the maximum production of siderophore was observed in PFC6 (10 μmol benzoic acid $ml^{-1}$ followed by Pf1 (9.0 μmol benzoic acid / ml) (Fig. 14a,b).

#### 4.20.2.3. HCN production

HCN production of *P. fluorescens* isolates were analysed qualitatively and quantitatively. PFC6 isolate recorded a strong production of HCN followed by Pf1, which showed weak HCN production (Plate 24). The quantitative analysis revealed that PFC6 recorded maximum production of 79.4 μg / ml, while Pf1 recorded 45.1 μg / ml (Fig.14a ,b).

#### 4.20.2.4. Salicylic acid Production

Salicylic acid (SA) content of *P. fluorescens* isolates were analysed and expressed as μg $ml^{-1}$. Between two *P. fluorescens* isolates, PFC6 recorded maximum content of SA (45.3) followed by Pf1 (27.2) (Fig.14a ,b).

#### 4.20.2.5. Effect of phenazine and 2,4-Diacetyl Phloroglucinol (DAPG) on the mycelial growth of *M. phaseolina* and *R. solani*

Among the various concentration of crude extract of phenazine and 2,4- DAPG tested, 0.5 per cent concentration of DAPG recorded maximum inhibition of mycelial growth of *M. phaseolina* (70.4%). This was followed by 0.5per cent of phenazine (64.7%), 0.2 per cent of DAPG (57.9%) and 0.2 per cent of phenazine (53.4%). The lowest concentration of 0.1 per cent recorded less inhibitory effect on mycelial growth. A similar trend was observed in the inhibition of mycelial growth of *R. solani*. The maximum inhibition was observed in 0.5 per cent concentration of DAPG (65.0%) followed by 0.5 per cent of phenazine (58.1%). This was followed by 0.2per cent of DAPG and phenazine recorded an inhibitory effect of mycelial growth 53.5 and 50 per cent over control, respectively. The other concentration of phenazine and DAPG was less effective (Table 46).

**4.20.2.6. IAA**

The Indole acetic acid (IAA) production was maximum in Pf1 (240 μg/ml) followed by PFC6 (21.0 μg/ml) (Fig.14a ,b).

**4.20.2.7. Production of volatile metabolites by *P. fluorescens***

The effect of volatile metabolites on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* was studied. Volatile metabolites of PFC6 and Pf1 reduced the mycelial growth of *M. phaseolina* to an extent of 11.1 and 6.15 per cent reduction over control, respectively on 3 DAI. On 7 DAI, 3.0 and 1.67 per cent inhibition was observed in PFC6 and Pf1, respectively. The sclerotial production of *M. phaseolina* was inhibited to an extent of 20.0 and 12.69 per cent over control by PFC6 and Pf1, respectively (Table 42).

The mycelial growth of *R. solani* was inhibited to an extent of 3.0 and 3.5 per cent over control on 3 DAI, respectively in PFC6 and Pf1. However, on 7 DAI, PFC6 and Pf1 recorded the inhibition level of 6.6 and 3.2 per cent over control, respectively. The sclerotial production of *R. solani* was inhibited to an extent of 39.0 and 32.8 by PFC6 and Pf1 (Table 43).

**4.20.2.8. Production of diffusible metabolites**

Diffusible metabolites produced by PFC6 and Pf1 effectively reduced the mycelial growth of *M. phaseolina* and *R. solani* on both 3and 7 DAI. The PFC6 and Pf1 reduced the mycelial growth of *M. phaseolina* to an extent of 55.4 and 48.0 per cent over control, respectively on 7 DAI. They also recorded 46.1 and 36.9 per cent reduction in sclerotial production (Table 44;Plate 25).

The maximum inhibition of mycelial growth of *R. solani* was recorded in PFC6 at 3 DAI (80.5%) followed by 7 DAI (57.4%). Pf1 also reduced the mycelial growth to an extent of 66.4 and 26.9, respectively on 3 and 7 DAI. The above two isolates were also effective in reducing the sclerotial production of (76.9 and 53.0% reduction over control, respectively) (Table 45).

# CHAPTER V
# DISCUSSION

In recent years, root rot disease of Coleus caused by *Macrophomina phaseolina* and *Rhizoctonia solani* has assumed serious proportions in most of the villages of Villupuram and Salem districts of Tamil Nadu and also a major constraint in coleus production. The pathogen attacks the plant at all stages of crop growth. Though the occurrence of coleus root rot has been reported recently in Tamil Nadu, information on many aspects including management strategies has been lacking. Hence, the present investigation was undertaken to establish etiology and to work out management practices.

## Survey

Survey report revealed that the occurrence of disease incidence was more in Villupuram district with sandy loam type of soil compared to Salem district and the incidence was more pronounced 60 DAP (Table3; Plate1). Similar findings were reported earlier. Malathi (1996) reported that maximum incidence of groundnut root rot in sandy and sandy loam soil 60 DAS. Maximum root rot incidence in groundnut was recorded in sandy loam soil than in clay soil. Maximum root rot infection was observed at flowering and pod setting stages in soybean (Muthusamy, 1989; Krishnaveni, 1991) and pigeon pea (Nakkeeran, 1992). Merin Babu (2002) reported that incidence of stolon rot of mint was prevalent in all the areas surveyed and the incidence ranged from 16.48 to 36.81 per cent.

The severity of root rot incidence ranged from 16.6-86.0 per cent in Villupuram district (Plate1a) and 48.2- 69.0 per cent in Salem district (Plate1b). The maximum root rot incidence was noticed 60 DAP in sandy loam soil (Table 3).

## Pathogen and Pathogenicity

The pathogens responsible for the root rot of Coleus were isolated and identified as *M. phaseolina* (Plate 2a,b,c,d) and *R. solani* (Plate 3a, bi, ii). *M. phaseolina* was isolated from the infected plant (above 30 DAP) (Plate 3c) and *R. solani* from seedling stage (less than 30 DAP) (Plate 3di, ii, iii). Plants affected by root rot disease showed

typical root rot symptoms such as wilting of plants, drying of stem and branches and rotting of root portions in case of *Macrophomina* root rot and seedling rot symptom in case of *Rhizoctonia* root rot. Shyla (1998) reported Coleus root rot caused by *Fusarium chlamydosporum* is the most important disease, occurring in severe form. *Macrophomina phaseolina* was isolated from root rot infected groundnut (Malathi, 1996), pigeonpea (Nakkeeran, 1992), soyabean (Krishnaveni, 1991) and blackgram (Sundaravadana, 2002). *R. solani* was isolated from infected mint plant (Merin Babu, 2002), cabbage and cauliflower (Loganathan, 2002). Kamalakannan *et al.* (2003a) reported the occurrence of root rot in *C. forskohlii* caused by *M. phaseolina* and *R. solani* for the first time in India.

**Cytological changes and loss of alkaloid due to root rot infection**

Pathogen infection in crop plants leads to several histological changes that may vary with different type of cells. Belanger *et al.* (2003) observed external growth of hyphae of *Blumeria graminis* f. sp. *tritici* on the epidermal surface and penetrated cells had haustoria and cell wall thickening due to suberization. Lee *et al.* (2000) observed the colonization of *Phytophthora capsici* in the epidermal and cortical cells of pepper plants. They also observed intercellular hyphae and haustoria in host cells. Rey *et al.* (1998) found that deposition of phenolics in the cell wall of tomato plants infected with *Pythium oligandrum* led to cell wall thickening. They also observed deposition of electron dense material on the secondary cell wall, pith membrane and xylem vessels thickening and presence of large number of small vacuoles.

Dienelt and Lawson (1989) observed cell wall thickening and formation of outer wall opposition in the leaves of *Citrus aurantifolia* due to the infection of *Xanthomonas campestris* pv. *citri*. Sahayarani (2003) reported that the powdery mildew pathogen infection of *Phyllanthus niruri* leaves led to thickening of cell wall and granulation of cytoplasm and presence of cavities in palisade parenchyma cells with fungal structure.

In the present study, *M. phaseolina* infection in *Coleus* root and stem caused disruption of epidermal layer and cortical parenchyma cells. The presences of inter and intra cellular mycelium and thickening and plugging of xylem vessels were also seen (Plate 4bi, ii).

Forskolin is an important alkaloid present in the roots of the coleus. The content of the forskolin varied between 0.07 to 0.5 per cent in healthy coleus root. Root rot infection led to reduction of forskolin content. Boby and Bagyaraj (2003) reported that root rot caused by *Fusarium chlamydosporum* reduced the forskolin content of coleus roots. *R. solani* infection caused a reduction of essential oil content (0.75%) in *M. citrata* and (0.752%) in *M. pipaita.* Sahayarani (2003) reported that the percentage of extractives recovered from healthy plant was more than the infected roots with different grades.

In the present, Forskolin content decreased with increased level of infection. At low level of infection, reduction was less compared to higher level of infection (Table 4; Plate 4c; Fig.1).

**Quantification of sclerotial population of *M. phaseolina***

The efficacy of biocontrol agents in controlling soil borne diseases depends on the inoculum density of soil borne pathogens. Hence, quantification of inoculum density of pathogen present in soil is very important in biological control. The results of the present study revealed that maximum sclerotial population of *M. phaseolina* was present in sandy loam soil with the crop rotation of coleus-coleus-coleus at Kaniyamoor village of Villupuram districts. Higher population of *M. phaseolina* was observed in sandy loam and loamy soil with the cropping system of groundnut, coleus, sorghum and maize. The lower population was detected in the silty loam and fine silty loam soils with paddy, sunnhemp and tapioca cropping system (Table 5; Plate 5a). Cloud and Rupe (1991) reported that loamy and sandy loam soil with previous crop soybean (or) corn recorded relatively high *M. phaseolina* sclerotial population than silty loam and fine silty loam soil with rice and wheat crops. The sclerotial population was estimated in several crop soils by several authors (Meyer *et al.*, 1973; Mihail and Alcorn ,1982; Tsao, 1970 and Malathi ,1996).

***In vitro* screening of biocontrol agents against root rot pathogens**

The mycoparasitic potential of *Trichoderma* species is well established (Dennis and Webster, 1971). This trait has often been utilized as means for *in vitro* screening of biocontrol agents (Elad *et al.,* 1983). In the present study, *T. harzianum* - Th showed maximum inhibition of mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*. (Table 6, 7; Plate 6a,b). The inhibitory effect of *Trichoderma* species on the *M. phaseolina* and *R. solani* may be due to the production of antibiotics (Dennis and Webster, 1971), competition (Cook and Baker, 1983), hyperparasitism and lysis (Chu and Wu, 1982; Elad *et al*., 1983; Bedlan , 1988 ; Papavizas and Lumsden, 1980; Abd-E1 Moity *et al.,* 1982 and Papavizas, 1985).

In recent years, there has been much success in biological control of soil borne diseases with the use of antagonistic fluorescent pseudomonads (Weller and Cook, 1986; Vidhyasekaran and Muthamilan, 1999). Several strains of *P. fluorescens, P. putida, P. cepacia* and *P. aeruginosa* have been successfully used for biological control of plant diseases (Anderson and Guerra, 1985; Rabindran and Vidhyasekaran, 1996). In the present study, varied level of inhibition of mycelial growth and sclerotial production of both *M. phaseolina* and *R. solani* were observed. However, the maximum inhibition was observed in PFC6 strain (Table 8, 9; Plate7a,b).

This inhibitory effect may be due to production of antibiotics (Howie and Suslow, 1991), production of lytic enzymes (Meena *et al.,* 2001), production of siderophores (Loper, 1988), production of hydrogen cyanide (HCN) (Ahl *et al.,* 1986), competition for substrates (Elad and Baker, 1985) and induced systemic resistance (Van Peer *et al.,* 1991).

*Bacillus* species is known to inhibit the number of plant pathogens include *Fusarium udum* (Podile and Dube, 1985), *Sclerotinia sclerotiorum, F. oxysporum* (Schmiedeknecht *et al.,* 2001).

In the present study, *B. subtilis* isolates BSC5, BSC7 and BSC8 effectively reduced the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* (Table 10, 11; Plate 8).

Effect of plant extracts and plant oils on the mycelial growth and sclerotial production of root rot pathogens

Studies conducted on the use of plant extracts and plant oils have opened a new avenue for the control of plant pathogens. Besides, being safe and non-phytotoxic, the plant extracts and plant oils are known to be effective against various plant pathogens (Sharma and Bohra, 2003; Gautam *et al.*, 2003a and b).

In the present study, extract from *Allium sativum* has showed the highest inhibitory effect on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* (Table 12,13; Plate 9a,b). Singh and Navi (2000) reported that Garlic (*A. sativum*) extract (16%) completely inhibited the conidial germination of *Claviceps sorghi* and also recorded maximum germination of pollen grain and reduced the ergot severity in sorghum. This inhibitory effect might be due to the allicin compound, which possess anti fungal and anti-insecticidal properties (Dalvi and Solunkhe, 1993).

Wintergreen oil and lemongrass oil possess antifungal activity against a wide range of pathogens. Bellerberck *et al.* (2001) reported that lemongrass oil completely inhibited the mycelial growth of *Aspergillus niger* at the concentration of 800 mg $lit^{-1}$, whereas 80 per cent inhibition of mycelial growth and a delay in conidiation were found at 400 mg $lit^{-1}$ concentration. This oil also caused structural modification such as reduced hyphal diameter and hyphal wall. Sahayarani (2003) stated that wintergreen oil at 0.2 per cent decreased the conidial germination of *Oidium phyllanthi*, the powdery mildew pathogen of *Phyllanthus niruri*. Ribnicky *et al.* (1998) reported that wintergreen plant extract containing

Gaultherin, an active compound that reduced the growth of *Botrytis cinerea, Sclerotinia sclerotiorum* and *Pseudomonas syringae* pv *lachrymans*.

In the present study, wintergreen and lemongrass oil at 0.1 and 0.2 per cent concentrations effectively inhibited the mycelial growth and sclerotial production of root rot pathogens *M. phaseolina* and *R. solani* (Table 14; Plate 10a,b).

**Effect of zinc sulphate on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani***

Micronutrients play an important role in the inhibition of mycelial growth of root rot pathogens under *in vitro* conditions. The result of the present study revealed that $ZnSO_4$ at one per cent concentration completely inhibited the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani.* The other lower concentrations were also effective than the control.

Sundaravadana (2002) reported that $ZnSO_4$ at 500 and 750 ppm completely inhibited the mycelial growth of *M. phaseolina,* root rot pathogens of blackgram and cotton. Gupta (1999) reported that $ZnSO_4$ inhibited the growth of *M. phaseolina* causing root rot of chickpea. The inhibitory effect of $ZnSO_4$ at 500 ppm on the mycelial growth of *R. solani* causing sheath blight of rice was reported by Lakpale *et al.* (1997) (Table 15; Plate11).

***In vitro* screening of fungicides against *M. phaseolina* and *R. solani***

Studies on *in vitro* evaluation of fungicides revealed that carbendazim, propiconazole, hexaconazole and tricylazole at 0.1 per cent completely inhibited the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* (Table 16,17; Plate 12a,b). Earlier workers have pointed out the efficacy of carbendazim against *R. solani* both under *in vitro* and *in vivo*. Mukherjee and Tripathi (2000) stated that bavistin, propiconazole and hexaconazole at 10 $\mu g\ ml^{-1}$ concentration completely inhibited the mycelial growth of *R. solani* under *in vitro* condition.

Kanakamahalakshmi *et al.* (1998) stated that carbendazim, thiram, thiophanate-methyl and captan were equally and highly effective at 1500 ppm which inhibited the mycelial growth of *M. phaseolina* completely under *in vitro* condition. Similar results were reported by several authors in various crops (Anitha and Tripathi, 2001; Prasanthi *et al.,* 2000; Malathi and Sabitha, 2003).

**Compatibility of biocontrol agents with plant oils and $ZnSO_4$ and development of bioformulations**

Compatability studies of biocontrol agents with plant oils and $ZnSO_4$ is essential for the development of new bioformulations. The present study revealed that *P. fluorescens* strains were compatible with plant oils (WGO and LGO) at 0.2 per cent concentration and $ZnSO_4$ at 0.5% concentration, where as *Trichoderma* spp.were incompatible with WGO and LGO at all the concentrations tested and compatible with 0.5 per cent of $ZnSO_4$. But the above concentrations completely inhibited the growth of *M. phaseolina* and *R. solani* (Table 18; Plate 13a,b). Sundaravadana (2002) reported that *T. viride* was compatible with $ZnSO_4$ at 250 and 500 ppm concentration. Duffy and Defago (1997) stated that application of 33 μg of zinc $ml^{-1}$ improved the antibiotic potential of *P. fluorescens* against *Fusarium* crown rot of tomato. *Trichoderma viride* and *Gliocladium virens* were compatible with 10 per cent concentration of gingelly, groundnut, oilcakes and neem leaf extracts (Dubey and Patel, 2000).

In most of the research, to date, biocontrol agents are applied singly to combat the growth of the pathogens. Although the potential benefits of a single biocontrol agent application has been demonstrated in many studies, it may also partially account for the reported inconsistent performance because a single
biocontrol agent is not likely to be active in all kinds of soil environment and all agricultural ecosystems (Raupach and Kloepper, 1998). These have resulted in inadequate colonization, limited tolerance to changes in environmental conditions and fluctuations in production of antifungal metabolites (Weller and Thomashow, 1994; Dowling and O' Gara, 1994). Several approaches have been used to overcome these problems including combined application of two (or) more biocontrol strains to enhance

the level and consistency in disease control (Pierson and Weller, 1994; Schisler *et al.*, 1997; Raupach and Kloepper, 1998).

A perusal of literature revealed that combination of plant oils, $ZnSO_4$ and biocontrol agents has not been reported earlier from India (or) abroad. In the present study, a talc based bioformulation was prepared by combining either WGO (or) LGO at 0.2 per cent and 0.5 per cent $ZnSO_4$ along with *P. fluorescens* isolates. Another talc based bioformulation containing *Trichoderma* species and $ZnSO_4$ (0.5%) has also been formulated.

Development of EC formulations and their effect on mycelial growth and sclerotial production of root rot pathogens

The last two decades have witnessed a tremendous interest in the investigation of botanicals as sources of disease control strategies and as possible alternatives to chemical fungicides (Narasimhan *et al.,* 1995). In spite of the wide recognition that many plants possess antibiotic properties, only a handful of products directly obtained from plants are in use in developed countries. Efforts are underway to find alternatives to fungicides due to the constant use of fungitoxic chemicals and their consequence on the surroundings. Even today Indian farmers are using leaf, seed kernel (or) cake extracts (or) plant oils as such for the plant disease control. Preparation of these materials is time consuming and moreover, oils as such cannot be stored for a long period because of the development of rancidity, leading to reduction in their efficacy. However, EC (emulsifiable concentrate) formulations can be kept for a considerable length of time and further such preparation would be easily miscible in water before using. Though few workers have formulated oils obtained from certain plants, no attempts have been made to develop EC formulations using wintergreen oil and lemongrass oil.

Neem and pungam oil based emulsifiable concentrate formulations were developed by Narasimhan *et al.* (1998) *viz.,* Neem oil 60 EC (citric acid), Neem oil 60 EC (acetic acid) and Neem oil + Pungam oil 60 EC (citric acid). The formulations were tested against two fungal diseases of rice, namely sheath rot (Narasimhan *et al.,* 1998)

and grain discolouration (Rajappan *et al.*, 2001). Rajappan *et al.* (1994) developed neem oil based EC formulation NO 60 EC (acetic acid) and dust formulation of neem oil, neem seed kernel extract, neem cake, pungam oil and pungam cake. The efficacy of the formulation was tested against green gram powdery mildew. Anusha (2003) developed 60EC formulation from *Lantana camara* leaf extracts in 2 and 4 per cent concentrations.

Reports suggested that emulsifiable concentrate was the most desirable type of formulation for botanicals. In the present investigation, 20, 30 and 40 EC formulations were developed from wintergreen oil and lemongrass oil.

Testing the efficacy of EC formulations under *in vitro* condition is very important to identify appropriate concentration to control the pathogens. Narasimhan *et al.* (1998) tested the efficacy of different EC formulations at 3 per cent concentration against *Sarocladium oryzae* and found that neem oil 60 EC (A) showed higher inhibitory effect on the mycelial growth *in vitro*. Anusha (2003) tested the efficacy of 60 EC formulation prepared from *L. camera* against *R. solani* and found that 2 and 4 per cent of 60 EC formulation effectively inhibited the mycelial growth under *in vitro* condition.

The volatile effect of WGO and LGO and 40 EC of each oil at 0.2 per cent concentration were studied and the results revealed that both plant oils and its EC formulations effectively checked the growth and sclerotial production of *M. phaseolina* and *R. solani* (Table 21). Though several reports are available for supporting the inhibitory effect of plant oils and perusal of literature revealed that volatile effect of WGO and LGO and their EC formulation has not been studied and this is the first report.

In the present study, 40 EC formulation of WGO and LGO at 0.2 per cent completely inhibited the mycelial growth of *M. phaseolina* and *R. solani* (Table 19 and 20).

**Stability of EC formulations**

The EC formulations prepared during the present study were tested for their stability in different dilution, pH level and temperatures and period of storage. The 40 EC formulation of WGO was standardized for optimal dilution and tested for antifungal activity. Dilution of EC formulation with distilled water upto 1:4 ratio proved to retain the antifungal activity and further dilutions were ineffective (Table 22). Anusha (2003) reported that upto 1:4 dilution of EC formulation of *L. camara* retained the antifungal activity, beyond which the efficacy was reduced.

The maximum antifungal activity was observed at neutral pH (7.0) and the same was reduced at acidic and alkaline conditions *viz.*, 4.0 and 9.0 pH levels (Table 23). Similar findings were reported by Anusha ((2003) and Manickam and Rajappan (1999). They reported the minimum antifungal activity observed at pH 4.0 and activity was maximum at pH 7.0 and again reduced at pH 9.0. The results of this study on effect of temperature on the inhibitory activity of EC formulation revealed that the maximum inhibitory activity was observed at $20^0$C. However even at a high temperature of $80^0$C, the EC formulation exhibited certain level of inhibition on the mycelial growth (Table 24). Anusha (2003) reported that the maximum inhibition of mycelial growth of *R. solani* was observed at $30^0$C and the activity decreased with increase in temperature. Manickam and Rajappan (1999) reported that the maximum inhibitory effect of AVP from *Crotons sparsiflorus* was observed at $60^0$C and the high temperature reduced the inhibition per cent.

The inhibitory effect of 40 EC formulation of WGO has retained even up to eight months of storage. After eight months of storage, the EC formulation recorded 89.9 and 87.7 per cent reduction over control on the mycelial growth of *M. phaseolina* and *R. solani*, respectively (Table 25). Narasimhan *et al.* (1998) tested the shelf life of three neem oil based EC formulations and found that the activity was retained even after nine months of storage.

**Effect of soil application of biocontrol agents on the root rot of coleus**

Soil application of biocontrol agents *viz., Trichoderma viride, T. harzianum, P. fluorescens* and *subtilis* effectively reduced root rot caused by soil borne pathogens in

several crops (Malathi, 1996; Radjacommare, 2000; Saravanakumar, 2002 and Vidhyasekaran and Muthamilan, 1995). The inhibitory effect of *Trichoderma* species might be due to direct mycoparasitism in addition to competition for nutrients (Elad *et al.,* 1983; Henis and Papavizas, 1983). The *P. fluorescens* strains reduced the root rot infection through several mechanisms including production of lytic enzymes (Velazhahan *et al.,* 1999), Siderophores (Scher and Baker, 1982), salicylic acid (Klessig and Malamy, 1994) and hydrogen cyanide (Bakker and Schippers, 1987). *B. subtilis* strains known to inhibit several soil borne diseases such as Fusarial wilt of red gram (Podile and Dube, 1985) and *R. solani* damping off of peppermint (Kamalakannan *et al.*, 2003b).

In the present study, soil application of talc based formulation of bio-control agents such as *Trichoderma* sp., *P. fluorescens* reduced the root rot caused by *M. phaseolina* and *R. solani* under pot culture experiment (Table 26).

Effect of bioformulations and EC formulations root rot incidence under pot culture experiment

The results of the present study revealed that the bioformulation containing PFC6 + WGO + $ZnSO_4$ exerted maximum reduction of root rot incidence caused by both *M. phaseolina* and *R. solani* under pot culture experiment (Table 27; Plate14a, b). The other formulations constitute PFC6, Pf1 and *T. harzianum* –Th also effectively reduced the root rot infection. Loganathan (2002) reported that fungal bacterial formulation mixture (TVMNT7 + Pf1) and fungal strain mixture (TVO + TVOL + *T. harzianum*) effectively checked the club root, root knot complex and damping off-root knot and head rot complex of cabbage, respectively. Saravanakumar (2002) studied the effect of different bioformulations against root rot of black gram caused by *M. phaseolina* and bioformulation mixture consists of Pf1 + neem oil exerted 62.78 per cent reduction over control. Seed treatment with *T. viride* and *P. fluorescens* followed by soil application of $ZnSO_4$ recorded lower root rot incidence of blackgram *viz.,* 17.73 and 19.07 per cent, respectively under pot culture experiment (Sundaravadana, 2002). Multiple strain mixture of microbial agents has been employed with some success against several plant pathogens in previous studies. These include mixture of fungi (Paulitz *et al.,* 1990; Budge *et al.,*

1995; Schisler *et al.,* 1997), mixture of bacteria (Pierson and Weller, 1994; Singh *et al.,* 1999). With this knowledge, the biocontrol agents which performed well *in vitro* against root rot pathogen were combined.

Efficacy of EC formulation in reducing the disease incidence was studied by several authors. Narasimhan *et al.* (1998) reported that neem oil NO 60 EC (A) reduced the rice sheath rot incidence and increased the yield compared to control. Yesuraja *et al.* (1995) evaluated different neem products against the management of fungal diseases of rice under pot culture condition. In the present study, the 40 EC formulations of WGO and LGO at 0.2 per cent concentration reduced the root rot incidence caused by both *M. phaseolina* and *R. solani* under pot culture experiment.

**Effect of organic amendments and $ZnSO_4$ on the coleus root rot incidence under pot culture experiment**

Organic amendments are recommended as biological means to reduce the incidence of several soil borne diseases. It is the cheapest, practicable and effective method of disease control. Roy (1989) reported that the activity of *R. solani* in organic amendmended soil was temporarily checked which was due to increase in $CO_2$ and decrease in N content of soil. Hundekar *et al.* (1998) reported that stalk rot of sorghum effectively checked by neem cake and cotton cake application. Similar findings were reported by Rathore (2000), Dubey (2002), Dubey (1998) and Dubey and Patil (2000). In the present study, application of neem cake effectively checked the root rot incidence caused by *M. phaseolina* and *R. solani*.

Soil application of $ZnSO_4$ significantly reduced the root rot incidence followed by combined application of *T. viride* + $ZnSO_4$ and *P. fluorescens* + $ZnSO_4$ both under pot culture and field condition (Sundaravadana, 2002). Latha *et al.* (1997) reported that application of $ZnSO_4$ at 50 kg $ha^{-1}$ reduced the root rot incidence of blackgram and greengram.

In the present study, $ZnSO_4$ application reduced root incidence caused by *M. phaseolina* and *R. solani* (Table 28).

The $ZnSO_4$ application increased the defense related proteins such as peroxidase, Polyphenol oxidase and phenol content which inturn results in reduced root rot incidence (Sundaravadana, 2002).

Effect of fungicides on the root rot incidence of coleus under pot culture experiment

Chemical fungicides are effective in controlling the root rot incidence of various crops. The inhibitory effect of fungicides against different isolates of *M. phaseolina* was reviewed by Ramadoss and Sivaprakasam (1987), Patel and Patel (1990) and Malathi (1996). The results of the present study revealed that carbendazim and propiconazole at 0.1 per cent concentration reduced the coleus root rot caused by *M. phaseolina* and *R. solani* (Table 29; Plate 15a,b). The effect of carbendazim and propiconazole on disease suppression was reviewed by Prasanthi *et al.* (2000), Mukerjee and Tripathi (2000) and Malathi and Sabitha (2003).

**Shelf life of biocontrol agents in different bioformulations**

The results of the present study revealed that the *Trichoderma* species survived with the required colony forming units (cfu) ($10^8$ cfu $ml^{-1}$) in the bioformulation upto 120 days of storage and *P. fluorescens* strains survived up to 90 days of storage with $10^8$ cfu $ml^{-1}$ (Table 30).

Loganathan (2002) reported that talc based formulation was the best over other carriers tested and retained required cfu until 120 and 90 days in *Trichoderma* and *Pseudomonas fluorescens*, respectively. The superiority of talc as a carrier material was reviewed by Vidhyasekaran and Muthamilan (1999), Ramakrishnan *et al.* (1994), Ali *et al.* (2001) and Saravanakumar (2002).

**Field experiments**

**Rhizosphere population of biocontrol agents**

Reduced disease incidence by soil application of biocontrol agents can be attributed to protection of infection court by rhizosphere colonization of antagonists. The results of the present study showed significant increase in rhizosphere population of *Trichoderma* species and *P. fluorescens* up to 120 DAP and reached maximum level in both Neermullikuttai and Coimbatore field trials ( Table 31, 32,35 and 36).

In Kaniyamoor field trial also, the population of *T. viride* and *P. fluorescens* was increased up to 90 DAP and the maximum population was observed in *T. harzianum* and PFC6 + WGO + $ZnSO_4$ treated plots (Table 33, 34).

Malathi (1996) reported that seed treatment with *Trichoderma* species showed significant increase of *Trichoderma* population in rhizosphere population. Several authors reported the similar findings (Papavizas, 1982; Ahamad and Baker, 1987; Muthamilan, 1989; Sankar, 1994; El-Nashar *et al.*, 2001; Anitha and Tripathi, 2000).

Soil application of *P. fluorescens* increased the rhizosphere population of *Pseudomonas fluorescens.* Fluorescent pseudomonads, which constitute 20 per cent of the total bacterial population are expected to give better protection against soil-borne pathogens. They had high affinity for aminoacid exudates and probably this might have contributed to their high rhizosphere competence (Baker and Chet, 1982).

Association of fluorescent pseudomonads with reduction in survival and activity of *M. phaseolina* has been reported by Arora *et al.* (1992), Mihail (1983) and Samiyappan (1988).

**Management of root rot of coleus under field condition**

In the field experiment conducted at Neermullikuttai village, Salem district (Table 37, 38; Plate16, 17) and field No.37D of Tamil Nadu Agricultural University, Coimbatore (Table 40,41; Plate19), carbendazim and propiconazole treated plots recorded the lowest root rot incidence. The talc based bioformulation and their mixture treated plots showed significantly less disease incidence and correspondingly resulted in

enhanced yield. However, application of 1:1 mixture of *T. viride* - TVC5 +$ZnSO_4$ and *T. harzianum* – Th + $ZnSO_4$ and PFC6 + WGO+ $ZnSO_4$ and Pf1 + WGO+ $ZnSO_4$ showed maximum inhibition of root rot incidence even up to 90 DAP.

Similarly talc based formulation was found to be effective in the control of chickpea wilt (Vidhyasekaran and Muthamilan, 1995), pigeonpea wilt (Vidhyasekaran *et al.*, 1997), rice sheath blight (Radja Commare, 2000; Nandakumar *et al.*, 2001), groundnut root rot (Malathi, 1996), blackgram root rot (Saravanakumar, 2002; Sundaravadana, 2002). In addition to the disease control, bioformulation and their mixtures enhanced the plant growth in terms of increased seedling emergence (Dunne *et al.*, 1998), plant height (Raupach and Kloepper, 1998) and yield (Malathi, 1996; Saravanakumar, 2002; Loganathan, 2002).

In the field experiments conducted at Kaniyamoor village of Villupuram district, the chemicals, bioformulation and their mixture showed upto 50 per cent inhibition of root rot on 45 DAP, whereas on 90 DAP, all the treatments recorded more than 80 per cent root rot incidence and none of the treatments were found effective (Table 39; Plate 18). The least efficacy of biocontrol in disease reduction might be due to high level of inoculum density present in the soil (278 No./g of dry soil). Prasad *et al.* (2002) studied the efficacy of biocontrol agents *T. harzianum* on three inoculum levels of log 3.04, 4.98 and 5.34 cfu/g of soil of *F. udum*. At inoculum level log 3.04, soil application of *T. harzianum* at 10 and 20 g recorded 42.9 and 61.5 per cent disease control, respectively and seed treatment alone resulted in less than 30 per cent disease control. As the inoculum density increases from log 3.04 to 4.98, the per cent disease control (35.5%) was decreased. At higher inoculum level (log5.34) *T. harzianum* recorded minimum inhibition of wilt incidence (22%). Siddiqui, *et al.* (2001) reported that *P. aeruginosa* strain IE – 6 showed biological control effects at lower inoculum level of *R. solani* and *M. phaseolina* (1 ml culture suspension of pathogen / kg of soil), where as at at higher inoculum level (3 ml / kg of soil) did not showed significant difference in the control of pathogens.

Induced defense related proteins by bioformulations against root rot disease

Enhancement of defensive capacity of the plants against a broad spectrum of pathogens and pests is acquired after appropriate stimulation. The resulting elevated resistance due to biotic agents (e.g. PGPR) is referred to as ISR whereas that by other than biocontrol agents is called SAR (Vanloon *et al.,* 1997). The acquired resistance in plants in the study was mainly focussed on induced defense related proteins and chemicals *viz.*, phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), chitinase, β-1,3-glucanase and phenols.

**Induction of enzymes in phenyl propanoid pathway**

PAL plays an important role in the biosynthesis of various defense chemicals in phenylpropanoid metabolism (Daayf *et al.,* 1997). De Mayer *et al.* (1998) reported that rhizosphere colonization of *P. aeruginosa* 7 NSK2 activated PAL in bean roots and increased the SA levels in leaves. In the present study, increased activity of PAL was recorded in the plants directed with bioformulation and challenge inoculated with the root rot pathogens. The time required to activate the defense mechanisms is important for the suppression of pathogen, earlier and higher level expression of defense related proteins and accumulation of chemicals at the infection site certainly prevent the colonization of pathogen in coleus plants treated with bioformulations. The PAL activity reached seven days after inoculation of *M. phaseolina* and thereafter declined at a decreasing rate than inoculated control.

In coleus plants pretreated with bioformulation containing PFC6 + WGO + $ZnSO_4$, the PAL activity increased upto seven days after challenge inoculation with *M. phaseolina*. In plants pretreated with PFC6+WGO+$ZnSO_4$ and challenge inoculated with *R. solani*, a higher induction of PAL was observed from seven days after challenge inoculation that reached maximum level on 14 days (Fig. 2,3).

Induction of PAL by fluorescent pseudomonads was reported in cucumber against *P.aphanidermatum* (Chen *et al.,* 2000), bean against *Botrytis cinerea* (Zdor and Anderson, 1992) and in cabbage and cauliflower against *R. solani* (Loganathan, 2002).

**Peroxidase (PO)**

Increased peroxidase (PO) has been showed in a number of interactions involving plant pathogenic fungi, bacteria and viruses (Dalisay and Kuc, 1995; Chen *et al.,* 2000; Nandakumar *et al.,* 2001). Peroxidases have been implicated in a number of physiological functions that may contribute to resistance including exudation of hydroxyl cinnamyl alcohol into free radical intermediates (Gross, 1980), phenol oxidation (Schmidt and Feucht, 1980), polysaccharide cross linking (Fry, 1986), cross linking of extension monomers (Everdeen *et al.,* 1988) and lignification (Walter, 1992) and also correlated with deposition of phenolic materials into plant cell wall during resistant interactions (Graham and Graham, 1991). Accumulation of peroxidase has been correlated with induced systemic resistance in several plants (Hammerschmidt *et al.,* 1982; Chen *et al.,* 2000; Dalisay and Kuc, 1995).

In the present study, plants treated with the bioformulations and challenged with the pathogens showed higher induction of peroxidase (Fig. 4,5).

Induction of four isoforms *viz.,* PO1, PO2, PO3 and PO4 in coleus occurred in treatments with bioformulations PFC6 + WGO + $ZnSO_4$, Pf1+ WGO + $ZnSO_4$, *T. harzianum*-Th + $ZnSO_4$ and *T. viride*-TVC5 + $ZnSO_4$ and challenge inoculated with *M. phaseolina*. The induction of isoforms were more prominent in the treatment involving PFC6 + WGO + $ZnSO_4$ (Plate 20a).

Two isoforms PO1 and PO2 were observed in plants treated with all bioformulations treated and challenge inoculated plants. However prominent induction was observed in PFC6, Pf1 and *T. harzianum* amended with WGO and $ZnSO_4$ (Plate 20b).

**Polyphenol oxidase (PPO)**

The results of the present study revealed that the application of bioformulations induced PPO. Induction was higher in plants treated with PFC6 + WGO + $ZnSO_4$ and challenged with the pathogens *M. phaseolina* and *R. solani* (Fig. 6,7). Four isoforms

(PPO1, PPO 2, PPO 3 and PPO 4) were induced in plants treated with all bioformulations and challenge inoculated with *M. phaseolina* (Plate 21a). Four isoforms (PPO1, PPO2, PPO3and PPO4) were induced in plants pretreated with bioformulation PFC6+WGO+$ZnSO_4$ and *T. harzianum* + $ZnSO_4$ and challenged with *R. solani* (Plate 21b). However, only three isoforms were induced in Pf1+WGO+$ZnSO_4$ pretreated plants. Earlier, Radjacommare (2000) reported that *P. fluorescens* induced PPO isozymes in rice against *R. solani*. Chen *et al.* (2000) reported that various rhizobacteria and *P. aphanidermatum* induced PPO activity in cucumber root tissues. Loganathan (2002) reported that application of bioformulation mixture induce the several isoforms of PPO in cabbage and cauliflower against *Plasmodiophora brassicae, Sclerotinia sclerotiorum* and *R. solani*.

**Chitinase and β-1, 3-glucanase**

Pathogenesis related (PR) proteins are host coded proteins induced by different types of pathogens and abiotic stresses (Van Loon, 1997). Synthesis and accumulation of PR proteins have been reported to play an important role in plant defense. Chitinases and β-1, 3-glucanase (which are classified under PR-3 and PR-2 groups of PR proteins, respectively) have been reported to be associated with plant resistance against fungal pathogens (Maurhofer *et al.,* 1994; Van Loon, 1998). Normally fungal cells contain chitin and glucan as their cell wall constituent. The main mode of antagonistic activity of microbes in production of lytic enzymes (Chitinases and β-1, 3-glucanase) which act on cell wall of organisms which have chitin (or) glucan as their cell wall component (Singh *et al.,* 1999) and also through induced systemic resistance in plants. In the present study, invariably in all experiments, elevated levels of chitinase and β-1, 3-glucanase were observed in plants treated with bioformulation and challenged with root rot pathogens.

In plants treated with bioformulation PFC6 + WGO + $ZnSO_4$ and challenged with root rot pathogens recorded higher induction of chitinase and glycanase activity. The activity of lytic enzymes reached maximum on 21 days after challenge inoculation (Fig. 8, 9, 10 and 11). Induction of the hydrolytic enzymes was also reported in pea against *P. ultimum* and *F. oxysporum* f.sp. *pisi* (Benhamou *et al.,* 1996). The enzymatic

degradation of the fungal cell wall by hydrolytic enzymes may release non-specific elicitors (Ham *et al.*, 1991; Ren and West, 1992), which in turn elicit various defense reactions. The fungal cell wall elicitors have been reported to elicit various defense reactions in green gram (Ramanathan *et al.,* 2000). Induction of chitinase in sugarcane against red rot disease was reported by Viswanathan and Samiyappan (1999).

**Phenolics**

Phenolic compounds enhance the mechanical strength of host cell wall and also inhibit the invading pathogenic organisms. Seed treatment with *P. fluorescens* 63 induced the accumulation of phenolics in tomato root tissue (M'piga *et al.,* 1997). The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganisation and loss of protoplasmic content. Accumulation of phenolics by prior application of *P. fluorescens* has been reported against *P. ultimum* and *F. oxysporum* f.sp. *pisi* (Benhaumou *et al.,* 1996) and against *R. solani* in peppermint (Kamalakannan *et al.,* 2003b).

In the present study also, higher level of phenolic accumulation occurred in coleus plant pretreated with bioformulation and challenged with *M. phaseolina* and *R. solani* (Fig. 12,13). Similar findings were reported in rice against *R. solani* (Radjacommare, 2000), cabbage and cauliflower against *R. solani* (Loganathan, 2002), black gram against *M. phaseolina* (Saravanakumar, 2002) and in groundnut against *M. phaseolina* (Malathi, 1996).

Thus, induction of defense related proteins and chemicals occur in plants against the root rot pathogens due to application of bioformulation. Earlier and enhanced levels of such proteins and chemicals might have prevented the colonization of the fungus in the crops.

**Mode of action of biocontrol agents**

***Trichoderma* species**

It is well established that antagonism of *Trichoderma* is due to its different mechanism of action *viz.*, competition, hyperparasitism and antibiosis, which have been

proved in the present investigation. Competition of antagonist for space and nutrients generally occurs under *in vivo* condition.

As a mycoparasite, *Trichoderma* spp. grew over the pathogen and caused hyphal coiling, hyphal abnormalities, reduction in sclerotial production, lysis of hyphae and sclerotia (Plate 22). Elad *et al.* (1983) reported the parasitism of *T. harzianum* on *R. solani*. Henis and Papavizas (1983) found that *T. harzianum* degraded the sclerotia by lysing the medullar tissue. *T. viride* and *T. harzianum* were mycoparasites of *M. phaseolina* and caused lysis of the pathogen (Singh *et al.,* 1990; Deshmukh and Rant, 1992). *T. harzianum* caused hyphal coiling and sclerotial lysis (Nakkeeran, 1992; Malathi, 1996).

`The effect of volatile and diffusible metabolities of *Trichoderma* species on the mycelial growth of *M. phaseolina* was studied by Malathi (1996), Pande (1985), Mathur and Bhat Nagar (1994), Deb (1990), Umamaheswari (1991), Tamimi and Hutchinson (1975) and Dennis and Webster (1971). Mukherjee and Tripathi (2000) reported that volatile compounds of *Gliocladium virens* had lesser inhibitory effect than the diffusible compounds on the mycelial growth of *R. solani*, *S. rolfsii* and *F.oxysporum*. f.sp. *phaseoli*.

The results of the present study revealed that the volatile compounds produced by *Trichoderma* species showed lesser efficacies against mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*, whereas diffusible compounds of *Trichoderma* species effectively inhibited the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* (Table 44, 45).

Pseudomonas fluorescens

Several studies have shown that efficient biocontrol agents such as *P. cepacia* (Fridlender *et al.,* 1993), *P. stutzeri* (Lim *et al.,* 1991), *P. fluorescens* (Velazhahan *et al.,* 1999) secrete chitinase and β-1,3-glucanase capable of degrading chitin and β-1,3-glucan, respectively, the major components of fungal cell wall. Laminariase and chitinase were reported to be involved in the reduction of *Fusarium*

*solani* infection by *P. stutzeri* VPL 1 strain (Lim *et al.,* 1991). Fridlender *et al.* (1993) reported that *P. cepacia* reduced the infection of *R.solani*. *Sclerotium rolfsii* and *Pythium ultimum* through production of β-1,3-glucanase. Velazhahan *et al.* (1999) observed a significant relationship between antagonistic activity of *P. fluorescens* against *R. solani* and their level of chitinase production. Meena *et al.* (2001) also stated that *P. fluorescens* isolates produced chitinase and β-1,3-glucanase which degrade the chitin and glucan part of *M. phaseolina* cell wall. The results of the present study indicated that there was a relationship between antagonistic activity of PFC6 and Pf1 and their level of chitinase and glucanase production, which suggests that higher production of chitinase and glucanase by PFC6, may also be now possible for its higher biological control against *M. phaseolina* (Fig. 14a, b).

Siderophore mediated iron deprivation of deleterious microorganisms has been considered as one of the important mechanism by which fluorescent pseudomonads exert their antagonistic activity and plant growth promotion (Scher and Baker, 1982). Several authors studied the role of siderophores in disease control against *P. ultimum* (Loper, 1988; Paulitz and Loper, 1991). *R. solani*, *Fusarium solani* and *S. rolfsii* (Yole and Dube, 2000), *F. oxysporum* f.sp. *raphani* (Leeman *et al.,* 1996) and *M. phaseolina* (Meena *et al.,* 2001).The results of the present study revealed that both PFC6 and Pf1 strains of *P. fluorescens* produced siderophores. However, the maximum production was observed in PFC6 (Fig. 14a, b; Plate 23).

The production of volatile cyanide is very common among the rhizosphere pseudomonads (Bakker and Schippers, 1987; Dowling and O'Gara, 1994). Laha *et al.* (1996) reported that the volatile metabolites of fluorescent pseudomonads significantly suppressed the mycelial growth of *C. rolfsii*. Meena *et al.* (2001) compared the HCN production by several strains of *P. fluorescens* and their efficacy in controlling root rot of groundnut caused by *M. phaseolina*. In the present study, both qualitative and quantitative estimation revealed that PFC6 showed strong production of HCN than Pf1 (Fig. 14a, b; Plate 24).

Salicylic acid (SA) is an endogenous regulator of localized and systemic acquired resistance in many plants. When plants become infected, the level of SA increases to combat the infection. The exogenous application of SA in healthy plants induced the expression of the same set of defense related genes that was induced in infected plants (Klessig and Malamy, 1994). Role of SA producing *P. aeruginosa* in disease suppression was studied by Buysens *et al.* (1996). SA production has also been reported in *P. fluorescens* WCS 374, WCS 4178 (Leeman *et al.,* 1996) and CHAO (Maurhofer *et al.,* 1994). Inoculation of roots of chickpea with *P. fluorescens* strain 4-92 (or) with synthetic O- acetyl salicylic acid induced systemic resistance against the charcoal rot fungus *M. phaseolina* (Srivastava *et al.,* 2000) and against groundnut root rot (Meena *et al.,* 2001).

In the present study, the *P. fluorescens* isolates *viz.,* PFC6 and Pf1 produced Salicylic acid. However, SA production was more in PFC6 than in Pf1 (Fig. 14a, b).

Fluorescent Pseudomonads produce secondary metabolites with antibiotic activities many of which have been implicated in suppression of soil borne diseases like phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (DAPG) (Thomashow and Weller, 1988). De La Fuente (2000) reported that the suppression of *P. ultimum* and *R. solani* was due to phenazine antibiotics produced by *P. fluorescens* strain UP 148.

In the present study, phenazine and DAPG antibiotics were inhibitory to mycelial growth of *M. phaseolina* and *R. solani*. However, the maximum inhibition of mycelial growth was observed at 0.5 per cent of DAPG and phenazine (Table 46).

Fluorescent pseudomonads produce plant growth promoting substances such as auxin and gibberellin and enhance the plant growth and yield in addition to direct antagonism against plant pathogens (Dubeikovsky *et al.,* 1993). Ramamoorthy and Samiyappan (2001) reported that Pf1 recorded the maximum production of IAA (160.4 µg/ml) and effectively inhibited the mycelial growth of *Colletotrichum capsici*. Karthika Devi (2004) studied the IAA production of five *P. fluorescens* isolates and PFKS -3

recorded maximum IAA production and this isolate effectively inhibited the mycelial growth of *M. phaseolina* and *R. solani*.

The observations of the present study are in agreement with the results of the earlier worker who suggested the positive correlation between IAA production and inhibitory effect on fungal growth by *P. fluorescens* isolates (Fig. 14a, b).

Inhibition of mycelial growth of fungus by the volatile metabolites produced by the *P. fluorescens* isolates has been studied by several authors (Bakker and Schippers, 1987; Latha *et al.,* 1994; Meena *et al.,* 2001). The results of the present study also in agreement with earlier workers in relation to effect of diffusible compounds of *P. fluorescens* isolates. However, the volatile metabolites had lesser inhibitory effect on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* (Table 42,43,44 and 45; Plate 25).

# CHAPTER VI

## SUMMARY

1. A survey was conducted in major coleus growing areas of Tamil Nadu for the occurrence of root rot. Kaniyamoor village of Villupuram and Keeripatti village of Salem district recorded the highest root rot incidence 86 and 69 per cent, respectively.
2. Root rot pathogens identified as *Macrophomina phaseolina* and *Rhizoctonia solani* based on morphological characters.
3 Root rot caused by *M. phaseolina* affect the plants between 30-120 days after planting (DAP) and *R. solani* infection appeared 20 - 30 DAP.
4. Root rot infection leads to thickening of cell wall, dense and granular cytoplasm, disruption of epidermal and cortical parenchyma with plugging of proto and meta xylem by fungal mycelium at cell level.
5. The maximum reduction of forskolin content (0.06%) was noticed in 100 per cent infection. The forskolin content was found to be decreased with increasing level of infection.
6. The maximum sclerotial population of *M. phaseolina* per gram of soil was recorded at Kaniyamoor – II (278 No.) and minimum population at Thenchettianthur –I (1.3 No.) in Villupuram district. In Salem district, Keeripatti and Valappadi villages recorded maximum (128 No) and minimum (9.1 No.) of sclerotial population, respectively.
7. *Trichoderma harzianum*-Th (62.5%), *T.viride*-TVC5 (56.8%), *Pseudomonas fluorescens* strains PFC6 (52.9%) and Pf1(13.7%) and *B. subtilis* strains BSC7 (22.4%) and BSC8 ( 19.7%) effectively inhibited the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani*.
8. *Trichoderma harzianum*-Th (64.3%), *T. viride*-TVC5 (62.4%), *P. fluorescens* strains PFC6 (47.3 %) and Pf1(18.7%) and *B. subtilis* strains BSC7 (49.0%) and BSC8 (47.2%) effectively inhibited the mycelial growth and sclerotial production of *R. solani*.
9 *Allium sativum* bulb extract executed maximum reduction of mycelial growth and

sclerotial production of *M. phaseolina* (76.0%) and *R. solani* (97.2%).

10. More than 90 per cent inhibition of mycelial growth and sclerotial production of *M. phaseolina* and *R. solani* were recorded at 0.2 per cent concentration of Wintergreen oil (WGO), Lemon grass oil (LGO), WGO 40 EC and LGO 40 EC formulations.
11. WGO, LGO and their EC formulations had volatile effect, which inhibited the mycelial growth (>90%) and sclerotial production (100%) of *M. phaseolina* and *R. solani*.
12. The highest inhibitory effect on mycelial growth (>90%) and sclerotial production (>90%) of *M. phaseolina* and *R. solani* was recorded in per cent $ZnSO_4$ under *in vitro*.
13. Carbendazim, Tricyclazole, Hexaconazole and Propiconazole completely inhibited the mycelial growth sclerotial production of *M. phaseolina* and *R. solani* at 0.1 per cent concentration by recording more than 90 per cent.
14. All the biocontrol agents were compatible with 0.5 per cent $ZnSO_4$. *P. fluorescens* isolates PFC6 and Pf1 were compatible with 0.2 per cent WGO and LGO whereas *Trichoderma* species were incompatible with WGO and LGO.
15. Application of talc based bioformulation containing PFC6+ LGO + $ZnSO_4$(62.0%), PFC6+ WGO + $ZnSO_4$ (53.3%)and *T. harzianum* – Th + $ZnSO_4$ (59.3%) at the rate of 2 g / hole effectively reduce the root rot incidence caused by both *M. phaseolina*. A similar trend was observed against *R. solani* under green house condition.

16. Among the soil amendments, $ZnSO_4$ (1g/hole) and Neem cake (5g/hole) effectively reduced the root rot incidence of *M. phaseolina* to an extent of 59.3 and 58.1 per cent, respectively.
17. $ZnSO_4$ (1g/ hole) and Neem cake (5g/hole) effectively reduced the root rot incidence of *R. solani* to an extent of 61.9 and 55.5 per cent, respectively.
18. Soil drenching of 0.1 per cent carbendazim and propiconazole effectively reduced the root rot incidence (>70%).
19. In the talc based bioformulations, the *Trichoderma* species and *P. fluorescens* strains survived up to 120 and 90 days after storage, respectively with required population ($10^8$ cfu /ml).
20. The WGO 40 EC formulation stable at room temperature, neutral pH and 1:4 dilution and refined its efficacy of even eight months after storage.
21. Among the three field trials conducted in various level of inoculum, at low (30.1 No./g) and medium level of inoculum (110 No./g), the bioformulations were effectively controlled the root rot incidence and increased the yield, where as at high inoculum level (278 No./g of soil) formulations were not effective.
22. Dipping of cuttings and soil drenching of 0.1% carbendazim and propiconazole reduced the root rot incidence to an extent of 94.3 and 91.4 per cent and yield of 5604 and 5109 kg $ha^{-1}$, respectively.
23. Among the bioformulations, combined application of PFC6+WGO + $ZnSO_4$ and Pf1+WGO+ $ZnSO_4$ reduced the root rot incidence ( 77.1%) with an increased yield (5674 $kgha^{-1}$).
24. Bioformulations treated plants significantly enhance the plant growth parameter such as plant height, No. of branches, tuber diameter and tuber length than control.

25. The maximum rhizosphere population of *Trichoderma* ($20x10^3$ cfu/ml) and *P. fluorescens* ($82 \times 10^4$ cfu/ml) were recorded on 120 Days after planting in the respective biocontrol agents treated plots.

26. Induction of defense related proteins and chemicals *viz.,* phenyl alaline ammonia lyase, peroxidase, polyphenol oxidase, phenols, chitinase and β,1-3 glucanase were found to be in higher levels in treatments involving bioformulation containing PFC6 + WGO + $ZnSO_4$, Pf1 + WGO + $ZnSO_4$, *T. harzianum* -Th + $ZnSO_4$ and *T. viride* - TVC5 + $ZnSO_4$ against root rot pathogens in coleus.

27. The reduction of root rot by *Trichoderma* spp. is due to mycoparasitism and inhibitory effect of volatile and diffusible metabolites produced by *Trichoderma* species.

28. The efficacy of *P. fluorescens* against root rot pathogens is due to production of lytic enzymes, siderophores, hydrogen cyanide salicylic acid, Indole acetic and acid antibiotics phenazine and 2,4-Diacetyl phloroglucinol.

## ANNEXURE-I

**Media**

**Potato Dextrose Agar (PDA)** (Riker and Riker, 1933)

| | | |
|---|---|---|
| Peeled potato | : | 250.0 g |
| Dextrose | : | 20.0 g |
| Agar | : | 15.0 g |
| Distilled water | : | 1000ml |
| pH | : | 6.5 |

**RB medium (Cloud and Rupe, 1991)**

| | | |
|---|---|---|
| Difco Potato Agar | : | 39 mg |
| Rifampicin | : | 100 mg |
| Metalaxyl | : | (224 mg a.i.) (Ridomil 2E-G) |
| Tergitol NP-10 | : | 1ml |
| Distilled water | : | 1000 ml |

**King's B medium** (Kings *et al*., 1954)

| | | |
|---|---|---|
| Peptone | : | 20.0 g |
| Dipotassium hydrogen phosphate | : | 1.5 g |
| Magnesium sulphate | : | 1.5 g |
| Glycerol | : | 10.0 ml |
| Agar | : | 15 g |
| Distilled water | : | 1000 ml |

***Trichoderma* special medium (TSM)** (Papavizas and Lumsden, 1980)

| | | |
|---|---|---|
| $MgSO_4$ | : | 0.2g |

| | | |
|---|---|---|
| $K_2HPO_4$- | : | 0.9g |
| KCl | : | 0.15g |
| $NH_4NO_3$ | : | 1.05g |
| Glucose | : | 3.0g |
| Agar agar | : | 15g |
| Distilled water | : | 1000 ml |
| Rose Bengal | : | 0.15g |
| Chloromphenicol | : | 0.25g |
| Metalaxyl | : | 0.3g |

**Chitin – peptone medium** (Lim *et al*., 1991)

| | | |
|---|---|---|
| Glucose | : | 0.5% |
| Peptone | : | 0.2% |
| Colloidal chitin | : | 0.2% (from crab shells, sigma) |
| K2HPO4 | : | 0.1% |
| $MgSO_4.7\ H_2O$ | : | 0.05% |
| NaCl | : | 0.05% |
| pH | : | 6.8 |

**Succinate medium** (Schwyn and Neilands, 1987)

| | | |
|---|---|---|
| Succinic acid | : | 4.0 g |
| $K_2HPO4$ | : | 6.0g |
| $KH_2PO4$ | : | 3.0 g |
| $NH_2SO_4$ | : | 1.0 g |
| $MgSO_47H_2O$ | : | 0.2 g |
| Distilled water | : | 1000 ml ( pH 7.0) |

**Trypticase Soy Agar (TSA)** (Millar and Higgins, 1970)

| | | |
|---|---|---|
| Animal peptone | : | 5.0 g |
| Soya peptone | : | 5.0 g |

| | | |
|---|---|---|
| NaCl | : | 5.0 g |
| Glycine | : | 4.4 g |
| Distilled water | : | 1000 ml |

**Nutrient agar medium (NA) ((Difco Manual, 1953)**

| | | |
|---|---|---|
| Peptone | : | 5.0 g |
| Beef extract | : | 3.0 g |
| Nacl | : | 5.0 g |
| Agar | : | 20.0 g |
| Distilled water | : | 1000 ml |

**Production Medium** (Rossales *et al*., 1991)

| | | |
|---|---|---|
| Peptone | : | 20g |
| Glycerol | : | 20g |
| NaCl | : | 5g |
| $KNO_3$ | : | 1g |
| Distilled Water | : | 1litre |
| pH | : | 7.2 |

**Yeast molasses medium** (Papavizas *et al*., 1984)

| | | |
|---|---|---|
| Molasses | : | 30g |
| Active dry yeast granules | : | 5.0g |
| Distilled water | : | 1000 ml |

## ANNEXURE-II

## REAGENTS USED FOR CHITINASE ASSAY

Preparation of colloidal chitin

Colloidal chitin for chitinase assay was prepared from crab shell chitin (Sigma, USA) by the method described by Berger and Reynolds (1958). The chitin (1 g) was mixed with acetone to form paste. Then it was ground in a mortar by slowly adding 5-10 volumes of conc. HCl while maintaining the temperature at 5-10 °C. After grinding, the material was filtered through glass wool and poured into 50% aqueous ethanol with vigorous stirring to precipitate the chitin in a highly dispersed state. The colloid was sedimented and resuspended in water several times to remove excess acid and ethanol and then dialyzed against distilled water. Chitin content was determined by drying the sample in vacuum and adjusted with distilled water to get a final concentration of 10 mg/ml (dry w/v) and stored at 4 °C.

**Snail gut enzyme (3%)**

300 mg desalted snail gut enzyme (Helicase) in 10 ml distilled water (Boller *et al*., 1983).

**Para dimethyl aminobenzaldehyde (DMAB) reagent**

8 g DMAB in 70 ml glacial acetic acid and 10 ml conc. HCl (10 N). It was diluted to 9 times with glacial acetic acid just before use.

**Potassium tetra borate buffer (0.8 M, pH 9.2)**

Aqueous solution of potassium tetra borate (0.8 M) was prepared and pH was adjusted to 9.2 with KOH.

## ANNEXURE-III

**Native gel electrophoresis** (Sindhu *et al*., 1984).)

**Separating gel** (8%) 7.5 ml

| | | |
|---|---|---|
| Acrylamide stock solution | : | 2.00 ml |
| Distilled water | : | 3.625 ml |
| 1.5 M Tris buffer pH 8.8 | : | 1.875 ml |
| 10% APS (Ammonium per sulphate) | : | 25 µl |
| TEMED (N, N, N', N'-tetra methyl ethylene diamine) | : | 5 µl |

**Stacking gel** (4%) 5.0 ml

| | | |
|---|---|---|
| Acrylamide stock solution | : | 0.65 ml |
| Distilled water | : | 3.25 ml |
| 1.5 M Tris buffer pH 6.8 | : | 1.25 ml |
| 10% APS (Ammonium per sulphate) | : | 25 µl |
| TEMED (N, N, N', N'-tetra methyl ethylene diamine) | : | 5 µl |

Separating gel was casted as per the above composition. Ammonium persulphate and TEMED were added-just prior to pouring the gel. The solution was overlaid carefully with a film of distilled water. After polymerisation, the water layer was removed and stacking gel was poured. The comb was placed between the plates, at the top portion of the plate. The comb was carefully removed from the wells after polymerization of the stacking gel. The dialyzed/lyophilized culture filtrates of *Trichoderma* mutants were mixed with sample buffer and heated to 90°C for 5 min and cooled at 5°C. These samples were then carefully loaded into the wells and the electrode buffer was poured. Gels were run at a constant current of 20 mA till the dye front reached the bottom of the gel. Protein molecular weight marker, which comprised of myosin 220.0 kDa, phosphorilase b.97.4 kDa, bovine serum albumin 66.0 kDa. ovalbumin 46.0 kDa, carbonic anhydrase 30.0 kDa, trypsin inhibitor 21.5 kDa and lysozyme 14.3kDa were used in this experiment.

**Table 6.** ***In vitro*** **screening of** ***Trichoderma*** **species against** ***M. phaseolina***

| S.No. | *Trichoderma* species | Mean mycelial growth* | | Sclerotial production* | |
|---|---|---|---|---|---|
| | | (mm) | % of inhibition | Mean number | % reduction over control |
| 1. | *T. harzianum* - Th | 32.3[a] | 62.5 [a] (52.23) | 77.3[a] | 49.67 [a] (44.81) |
| 2. | *T. viride* - TVC1 | 43.0[c] | 50.2 [c] (45.17) | 93.3[bc] | 39.25[bc] (38.78) |
| 3. | *T. viride* - TVC2 | 46.0[d] | 46.7 [d] (43.11) | 99.0[d] | 35.50 [d] (36.56) |
| 4. | *T. viride* - TVC3 | 50.0[e] | 42.1 [e] (40.45) | 111.6[e] | 27.34 [e] (31.50) |
| 5. | *T. viride* - TVC4 | 52.3[e] | 39.4 [e] (38.87) | 114.3[e] | 25.58 [e] (30.30) |
| 6. | *T. viride* - TVC5 | 37.3[b] | 56.8 [b] (48.90) | 88.3[b] | 42.5 [b] (40.63) |
| 7. | *T. viride* - TVC6 | 41.0[c] | 52.5 [c] (46.43) | 95.3[cd] | 37.95 [cd] (38.05) |
| 8. | Control | 86.3[f] | - | 153.6[f] | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 7.** ***In vitro*** **screening of** ***Trichoderma*** **species against** ***R. solani***

| S.No. | *Trichoderma* species | Mycelial growth (mm)* | Per cent inhibition over control | Sclerotial production (mean number)* | Per cent inhibition over control | Inhibition zone (mm)* | Over growth of antagonist (mm)* |
|---|---|---|---|---|---|---|---|
| 1. | *T. harzianum* - Th | 32.0[a] | 64.3 [a] (53.3) | 43.3[a] | 54.6 [a] (47.6) | 18.0[a] | 44.7[b] |
| 2. | *T. viride* - TVC1 | 40.0[b] | 55.4 [b] (48.1) | 47.3[a] | 46.8 [b] (43.1) | 12.7[c] | 39.7[c] |
| 3. | *T. viride* - TVC2 | 48.3[c] | 46.1 [c] (42.7) | 54.3[b] | 38.8 [c] (38.5) | 14.0[b] | 39.3[c] |
| 4. | *T. viride* - TVC3 | 39.3[b] | 56.2 [b] (48.5) | 43.6[a] | 50.9 [ab] (45.5) | 13.7[b] | 48.0[a] |
| 5. | *T. viride* - TVC4 | 40.0[b] | 55.6 [b] (48.2) | 46.6[a] | 47.5 [b] (43.5) | 13.3[b] | 44.0[b] |
| 6. | *T. viride* - TVC5 | 33.6[a] | 62.4 [a] (52.1) | 43.3[a] | 53.1 [a] (46.8) | 16.0[ab] | 49.3[a] |
| 7. | *T. viride* - TVC6 | 40.3[b] | 55.1 [b] (47.9) | 52.3[b] | 41.1 [c] (39.8) | 11.0[d] | 43.7[b] |
| 8. | Control | 89.7[d] | - | 89.0[c] | - | - | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 8.** ***In vitro*** **screening of** ***P. fluorescens*** **strains against** ***M. phaseolina***

| S.No. | *P. fluorescens* strains | Mycelial growth (mm)* | Per cent inhibition over control | Sclerotial production (mean number)* | Per cent inhibition over control | Inhibition zone (mm)* |
|---|---|---|---|---|---|---|
| 1. | *P. fluorescens* - PFC1 | 76.3[f] | 6.96 [f] (15.3) | 91.0[e] | 43.2 [e] (41.0) | 0.3[d] |
| 2. | *P. fluorescens* – PFC2 | 79.0[g] | 3.64 [g] (11.0) | 99.0[f] | 38.2 [f] (38.1) | 0.7[d] |
| 3. | *P. fluorescens* – PFC3 | 56.3[c] | 31.37 [c] (34.0) | 80.3[c] | 49.9 [c] (44.9) | 4.6[c] |
| 4. | *P. fluorescens* – PFC4 | 48.6[b] | 40.76 [b] (39.6) | 70.0[b] | 56.3 [b] (48.6) | 10.0[b] |
| 5. | *P. fluorescens* – PFC5 | 79.6[g] | 2.89 [h] (9.7) | 86.0[d] | 46.3 [d] (42.8) | 0.3[d] |
| 6. | *P. fluorescens* – PFC6 | 38.7[a] | 52.9 [a] (36.6) | 59.6[a] | 62.8 [a] (52.4) | 21.3[a] |
| 7. | *P. fluorescens* - PFC7 | 71.6[d] | 12.7 [d] (20.9) | 103.0[g] | 35.7 [g] (36.6) | 0.3[d] |
| 8. | *P. fluorescens* - Pf1 | 70.7[e] | 13.7 [e] (21.8) | 86.0[d] | 46.3 [d] (42.8) | 0.3[d] |
| 9. | Control | 82.0[h] | - | 160.3[h] | - | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 9.** ***In vitro*** **screening of** ***P. fluorescens*** **strains against** ***R. solani***

| S.No. | *P. fluorescens* strains | Mycelial growth (mm)* | Per cent inhibition over control | Sclerotial production (mean number)* | Per cent inhibition over control | Inhibition zone (mm)* |
|---|---|---|---|---|---|---|
| 1. | *P. fluorescens* - PFC1 | 53.7$^{c}$ | 36.3 $^{c}$ (37.0) | 560$^{c}$ | 40.7 $^{cd}$ (39.6) | 0.67$^{e}$ |
| 2. | *P. fluorescens* – PFC2 | 69.7$^{ef}$ | 17.0 $^{e}$ (24.3) | 57.3$^{c}$ | 39.3 $^{d}$ (38.8) | 0.67$^{e}$ |
| 3. | *P. fluorescens* – PFC3 | 72.0$^{f}$ | 14.3 $^{f}$ (22.2) | 49.6$^{b}$ | 47.4 $^{bc}$ (43.5) | 5.0$^{d}$ |
| 4. | *P. fluorescens* – PFC4 | 54.3$^{c}$ | 35.4 $^{c}$ (36.5) | 59.0$^{c}$ | 37.5 $^{d}$ (37.7) | 10.3$^{c}$ |
| 5. | *P. fluorescens* – PFC5 | 48.3$^{b}$ | 42.9$^{b}$ (40.9) | 60.0$^{c}$ | 36.5$^{d}$ (37.1) | 0.67$^{e}$ |
| 6. | *P. fluorescens* – PFC6 | 45.0$^{a}$ | 47.3 $^{a}$ (43.4) | 39.3 | 58.3 $^{a}$ (49.8) | 21.7$^{a}$ |
| 7. | *P. fluorescens* - PFC7 | 70.3$^{ef}$ | 17.1 $^{e}$ (24.4) | 50.3$^{b}$ | 48.2 $^{b}$ (43.9) | 4.3$^{d}$ |
| 8. | *P. fluorescens* - Pf1 | 68.7$^{d}$ | 18.7 $^{d}$ (25.6) | 47.3$^{b}$ | 49.0 $^{b}$ (44.4) | 17.0$^{b}$ |
| 9. | Control | 84.0$^{g}$ | - | 94.6$^{d}$ | - | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 10.** ***In vitro*** **screening of** ***B. subtilis*** **strains against** ***M. phaseolina***

| S.No. | *B. subtilis* strains | Mycelial growth (mm)* | Inhibition over control (%) | Inhibition zone (mm)* | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|---|
| 1. | *B. subtilis* - BSC1 | 64.6$^{bc}$ | 21.3 $^{c}$ (27.5) | 4.33$^{c}$ | 145$^{ef}$ | 12.9 $^{e}$ (21.0) |
| 2. | *B. subtilis* - BSC2 | 78.0$^{f}$ | 4.9 $^{i}$ (1.27) | 0.67$^{e}$ | 155$^{fg}$ | 6.96 $^{f}$ (15.2) |
| 3. | *B. subtilis* - BSC3 | 77.3$^{f}$ | 5.8 $^{h}$ (14.1) | 1.0$^{e}$ | 157.6$^{gh}$ | 5.4 $^{g}$ (13.4) |
| 4. | *B. subtilis* - BSC4 | 75.0$^{e}$ | 8.6 $^{g}$ (17.1) | 2.33$^{d}$ | 146.0$^{ef}$ | 12.3 $^{e}$ (20.5) |
| 5. | *B. subtilis* - BSC5 | 62.7$^{a}$ | 23.6 $^{a}$ (29.0) | 11.0$^{a}$ | 103.3$^{a}$ | 37.9 $^{a}$ (38.0) |
| 6. | *B. subtilis* - BSC6 | 68.7$^{d}$ | 6.3 $^{e}$ (23.8) | 5.0$^{bc}$ | 136.6$^{de}$ | 18.0 $^{d}$ (25.1) |
| 7. | *B. subtilis* - BSC7 | 63.7$^{ab}$ | 22.4 $^{b}$ (28.3) | 10.67$^{a}$ | 118.3$^{b}$ | 28.9 $^{b}$ (32.5) |
| 8. | *B. subtilis* - BSC8 | 66.0$^{c}$ | 19.7 $^{d}$ (26.3) | 9.67$^{a}$ | 120.6$^{bc}$ | 27.6 $^{b}$ (31.6) |
| 9. | *B. subtilis* - BSC9 | 74.7$^{e}$ | 8.9 $^{f}$ (17.3) | 6.67$^{b}$ | 129.6$^{cd}$ | 22.2 $^{c}$ (28.1) |
| 10. | *B. subtilis* - BSC10 | 64.7$^{bc}$ | 21.2 $^{c}$ (27.4) | 11.0$^{a}$ | 141.6$^{e}$ | 15.0 $^{d}$ (24.7) |
| 11. | Control | 82.0 | - | - | 106.6$^{h}$ | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 11.** ***In vitro*** **screening of** ***B. subtilis*** **strains against** ***R. solani***

| S.No. | *B. subtilis* strains | Mycelial growth (mm)* | Inhibition over control (%) | Inhibition zone (mm)* | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|---|
| 1. | *B. subtilis* - BSC1 | 52.0$^{cd}$ | 39.7 $^{d}$ (38.8) | 3.67$^{cd}$ | 62.3$^{ef}$ | 33.7 $^{ef}$ (35.40) |
| 2. | *B. subtilis* - BSC2 | 57.7$^{c}$ | 33.1$^{f}$ (35.4) | 0.66$^{e}$ | 67.6$^{g}$ | 28.0 $^{g}$ (31.89) |
| 3. | *B. subtilis* - BSC3 | 58.7$^{e}$ | 31.9 $^{g}$ (34.2) | 1.33$^{e}$ | 64.6$^{fg}$ | 31.3 $^{fg}$ (33.91) |
| 4. | *B. subtilis* - BSC4 | 54.7$^{d}$ | 36.6 $^{e}$ (37.3) | 2.67$^{e}$ | 62.7$^{ef}$ | 33.3 $^{efg}$ (35.24) |
| 5. | *B. subtilis* - BSC5 | 48.0$^{b}$ | 44.3 $^{b}$ (41.3) | 9.67$^{a}$ | 56.0$^{cd}$ | 40.4 $^{bcd}$ (39.30) |
| 6. | *B. subtilis* - BSC6 | 51.3$^{c}$ | 40.5 $^{c}$ (39.9) | 5.0$^{cb}$ | 60.0$^{de}$ | 36.1 $^{def}$ (36.85) |
| 7. | *B. subtilis* - BSC7 | 44.0$^{a}$ | 49.0 $^{a}$ (43.8) | 10.67$^{a}$ | 48.0$^{a}$ | 48.9 $^{a}$ (44.37) |
| 8. | *B. subtilis* - BSC8 | 46.0$^{ab}$ | 47.2 $^{a}$ (43.4) | 10.33$^{a}$ | 51.3$^{ab}$ | 45.4 $^{ab}$ (42.30) |
| 9. | *B. subtilis* - BSC9 | 57.7$^{e}$ | 33.1$^{fgh}$ (34.8) | 6.33$^{b}$ | 58.8$^{d}$ | 38.3 $^{cde}$ (38.26) |
| 10. | *B. subtilis* - BSC10 | 46.6$^{ab}$ | 47.8 $^{b}$ (42.1) | 8.67$^{a}$ | 52.6$^{bc}$ | 44.0$^{abc}$ (41.40) |
| 11. | Control | 86.3$^{f}$ | - | - | 94.0$^{h}$ | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 12. Effect of plant extracts on the mycelial growth and sclerotial production of *M. phaseolina***

| S.No. | Plant species | Mycelial growth (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|
| 1. | **A. vasica** | 85.3[d] | 4.1 [d] (11.6) | 126.6[d] | 8.0 [de] (16.2) |
| 2. | **A. sativum** | 21.3[a] | 76.0 [a] (60.7) | 71.6[a] | 47.9 [a] (43.8) |
| 3. | **A. paniculata** | 75.3[c] | 14.4 [c] (22.3) | 104.3[c] | 24.2 [bc] (29.4) |
| 4. | **A. indica** | 83.0[d] | 6.7 [d] (14.6) | 115.0[c] | 16.4 [cd] (23.9) |
| 5. | **C. barbatus** | 86.0[d] | 5.0 [d] (12.9) | 131.0[d] | 4.8 [e] (10.4) |
| 6. | **L. camera** | 85.7[d] | 3.7 [d] (10.7) | 130.0[d] | 5.5 [e] (10.7) |
| 7. | **L. inermis** | 59.7[b] | 32.9 [b] (34.9) | 85.3[b] | 37.9 [ab] (38.0) |
| 8. | **V. negundo** | 73.7[c] | 17.1[c] (24.4) | 105.6[c] | 23.2 [bc] (28.7) |
| 9. | ***Control*** | 89.0[d] | - | 137.6[d] | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 13. Effect of plant extracts on the mycelial growth and sclerotial production of *R. solani***

| S.No. | Plant species | Mycelial growth (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|
| 1. | **A. vasica** | 88.0[d] | 1.70 [f] (7.6) | 91.3[def] | 8.7 [de] (16.7) |
| 2. | **A. sativum** | 1.33[a] | 97.2 [a] (80.8) | 7.7[a] | 92.3 [a] (75.0) |
| 3. | **A. paniculata** | 78.0[c] | 12.8 [d] (20.9) | 82.6 [cd] | 17.4 [cd] (23.9) |
| 4. | **A. indica** | 75.0[c] | 16.5 [c] (23.9) | 78.0[c] | 22.0 [c] (27.3) |
| 5. | **C. barbatus** | 86.7[d] | 3.00[e] (10.0) | 93.0[ef] | 7.0[e] (14.7) |
| 6. | **L. camera** | 87.3[d] | 2.5 [ef] (9.0) | 90.0[de] | 10.0 [de] (17.7) |
| 7. | **L. inermis** | 53.0[b] | 40.5 [b] (39.5) | 61.3[b] | 38.7 [b] (38.2) |
| 8. | **V. negundo** | 86.7[d] | 3.1 [e] (10.1) | 61.3[b] | 10.7 [de] (18.6) |
| 9. | ***Control*** | 89.3[e] | - | 100[f] | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 14. Effect of plant oils on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani***

| S.No. | Plant oils (%) | Mycelial growth of *M.p.* (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) | Mycelial growth of *R. solani* (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | WGO (0.05) | 85.0[b] | 4.5[b] (12.3) | 130.0[b] | 5.6 [b] (13.8) | 83.3[d] | 7.13 [c] (15.4) | 82.0[d] | 14.5 [d] (22.3) |
| 2. | WGO (0.1) | 8.0[a] | 91.0[a] (72.8) | 5.0[a] | 96.3 [a] (77.8) | 64.2[b] | 28.4 [b] (32.1) | 60.0[b] | 37.5 [b] (37.7) |
| 3. | WGO (0.2) | 8.0[a] | 91.0 [a] (72.8) | 6.0[a] | 95.6 [a] (76.3) | 8.0[a] | 91.1 [a] (73.4) | 0.3[a] | 99.7 [a] (86.8) |
| 4. | LGO (0.05) | 8.0[a] | 91.0 [a] (72.8) | 6.0[a] | 95.6 [a] (76.3) | 83.3[c] | 7.13 [c] (15.4) | 85.0[d] | 11.5 [d] (19.8) |
| 5. | LGO (0.1) | 8.0[a] | 91.0 [a] (72.8) | 5.0[a] | 96.3 [a] (77.8) | 64.2[b] | 28.4 [b] (32.1) | 70.0[c] | 27.1 [c] (31.3) |
| 6. | LGO (0.2) | 8.0[a] | 91.0 [a] (72.8) | 6.0[a] | 95.6 [a] (76.3) | 8.0[a] | 91.1 [a] (73.4) | 0.3[a] | 99.7 [a] (86.8) |
| 7. | Control | 89.0[c] | - | 138.0[c] | - | 89.7[d] | - | 96.0[f] | - |

M.p= *Macrophomina phaseolina;*WGO – Wintergreen oil ; LGO – Lemongrass oil

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 15. Effect of $ZnSO_4$ on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani***

| S.No. | $ZnSO_4$ concentration (%) | Mycelial growth of *M.p.* (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) | Mycelial growth of *R. solani* (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1. | 0.25 | 29.0[c] | 67.3 [c] (55.8) | 128.0[c] | 5.8 [c] (14.3) | 39.7[b] | 54.7 [c] (47.7) | 43.0[b] | 52.2 [b] (42.2) |
| 2. | 0.50 | 10.7[b] | 87.9 [b] (69.6) | 45.0[b] | 67.0 [b] (54.5) | 10.0[a] | 88.5 [b] (70.2) | 6.0[a] | 93.3 [a] (75.0) |
| 3. | 0.75 | 8.3[a] | 90.6 [a] (72.5) | 5.0[a] | 96.3 [a] (78.9) | 8.3[a] | 90.5 [a] (72.0) | 5.0[a] | 94.4 [a] (76.3) |
| 4. | 1.0 | 8.3[a] | 90.6 [a] (72.5) | 5.0[a] | 96.3 [a] (78.9) | 8.3[a] | 90.5 [a] (72.05) | 5.0[a] | 94.4 [a] (76.3) |
| 5. | Control | 88.7[d] | - | 136.0[d] | - | 87.7[c] | - | 90.0[c] | - |

*M.p - Macrophomina phaseolina*

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 16. Effect of fungicides on the mycelial growth and sclerotial production of *M. phaseolina***

| S.No. | Fungicides | Mycelial growth at 0.1% (mm)* | Inhibition over control (%) | Mycelial growth at 0.2% (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|---|---|
| 1. | Carbendazim | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.0[a] | 99.8 [a] (87.4) |
| 2. | Tricyclazole | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.00[a] | 99.8 [a] (87.4) |
| 3. | Copper oxychloride | 89.7[c] | 0.0 [b] (1.9) | 86.6[b] | 3.2 [b] (10.2) | 138[c] | 4.8 [c] (12.6) |
| 4. | Thiram | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.00[a] | 99.7 [a] (86.8) |
| 5. | Hexaconazole | 0.70[a] | 99.2 [a] (84.8) | 0.33[a] | 99.6 [a] (86.3) | 15.0[b] | 89.6 [b] (87.4) |
| 6. | Thiophenite methyl | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.0[a] | 99.8 [a] (87.4) |
| 7. | Propiconazole | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.00[a] | 99.8 [a] (87.4) |
| 8. | Control | 89.7[c] | - | 89.5[c] | - | 145[d] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 17. Effect of fungicides on the mycelial growth and sclerotial production of *R. solani***

| S. No. | Fungicides | Mycelial growth at 0.1% (mm)* | Inhibition over control (%) | Mycelial growth at 0.2% (mm)* | Inhibition over control (%) | Sclerotial production (mean number)* | Inhibition over control (%) |
|---|---|---|---|---|---|---|---|
| 1. | Carbendazim | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.0[a] | 99.8 [a] (87.4) |
| 2. | Tricyclazole | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.00[a] | 99.8 [a] (87.4) |
| 3. | Copper oxychloride | 37.0[d] | 58.1 [d] (49.6) | 22.67[c] | 74.38 [c] (59.6) | 5.0[b] | 96.0[b] (79.2) |
| 4. | Thiram | 23.0[c] | 73.9 [c] (59.2) | 15.0[b] | 83.1 [b] (65.7) | 4.0[b] | 96.8 [b] (85.0) |
| 5. | Hexaconazole | 0.33[a] | 99.6 [a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.00[a] | 99.8 [a] (87.4) |
| 6. | Thiophenite methyl | 13.0[b] | 85.2 [b] (67.3) | 0.33[a] | 99.6 [a] (86.3) | 4.0[b] | 96.8 [b] (85.0) |
| 7. | Propiconazole | 0.33[a] | 99.6[a] (86.3) | 0.33[a] | 99.6 [a] (86.3) | 0.00[a] | 99.8 [a] (87.4) |
| 8. | Control | 88.3[e] | - | 88.5[d] | - | 126.0[e] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 18. Compatibility of biocontrol agents with plant oils and $ZnSO_4$**

| S.No. | Biocontrol agents | Compatibility (+) or (-) | | | |
|---|---|---|---|---|---|
| | | WGO (0.2%) | LGO (0.2%) | $ZnSO_4$ (1%) | $ZnSO_4$ (0.5%) |
| 1. | *P. fluorescens* - PFC6 | + | + | - | + |
| 2. | *P. fluorescens* - Pf1 | + | + | - | + |
| 3. | *T. harzianum* – Th | - | - | - | + |
| 4. | *T. viride* - TVC5 | - | - | - | + |
| 5. | *P. fluorescens* - PFC6 (Control) | + | + | + | + |
| 6. | *P. fluorescens* - Pf1 (Control) | + | + | + | + |
| 7. | *T. harzianum* – Th (Control) | + | + | + | + |
| 8. | *T. viride* - TVC5 (Control) | + | + | + | + |

+ Compatible; - Incompatible

**Table 19. Effect of EC formulations on the mycelial growth and sclerotial production of *M. phaseolina***

| S.No. | EC formulation | Mycelial growth* (0.1%) | | (0.2%) | | Sclerotial production* (Mean number) | Inhibition over control (%) |
|---|---|---|---|---|---|---|---|
| | | (mm) | % inhibition | (mm) | % inhibition | | |
| 1. | WGO 20 EC | 70.3$^{e}$ | 19.5 $^{e}$ (26.2) | 47.6$^{c}$ | 45.9 $^{bc}$ (42.6) | 121.6$^{d}$ | 14.5 $^{c}$ (22.3) |
| 2. | WGO 30 EC | 58.0$^{d}$ | 33.6 $^{d}$ (35.4) | 39.0$^{b}$ | 55.7 $^{b}$ (48.2) | 93.0$^{c}$ | 34.7 $^{b}$ (36.0) |
| 3. | WGO 40 EC | 30.0$^{b}$ | 65.6 $^{b}$ (54.1) | 8.0$^{a}$ | 90.9 $^{a}$ (73.1) | 0.3$^{a}$ | 99.7 $^{a}$ (84.2) |
| 4. | LGO 20 EC | 74.7$^{f}$ | 14.4 $^{f}$ (22.9) | 49.9$^{c}$ | 43.3 $^{c}$ (41.1) | 135.0$^{e}$ | 5.1 $^{d}$ (13.0) |
| 5. | LGO 30 EC | 45.6$^{c}$ | 47.8 $^{c}$ (43.7) | 41.0$^{b}$ | 53.4 $^{bc}$ (46.9) | 86.0$^{b}$ | 39.5 $^{b}$ (44.2) |
| 6. | LGO 40 EC | 18.3$^{a}$ | 79.0$^{a}$ (62.7) | 7.7$^{a}$ | 91.2 (72.8)$^{a}$ | 0.3$^{a}$ | 99.7 $^{a}$ (84.2) |
| 7. | Control | 87.3g | - | 88.0$^{d}$ | - | 142.3 | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 20. Effect of EC formulations on the mycelial growth and sclerotial production of *R. solani***

| S.No. | EC formulation | Mycelial growth* | | | | Sclerotial production* | |
|---|---|---|---|---|---|---|---|
| | | (0.1%) | | (0.2%) | | (Mean number) | Inhibition over control (%) |
| | | (mm) | Inhibition over control | (mm) | Inhibition over control | | |
| 1. | WGO 20 EC | 75.7[e] | 14.9 [e] (22.6) | 37.0[d] | 58.0 [c] (48.2) | 43.3[d] | 54.9 [d] (47.8) |
| 2. | WGO 30 EC | 50.0[d] | 43.8 [d] (64.2) | 19.0[b] | 78.4 [b] (61.5) | 14.6[c] | 84.8 [c] (67.0) |
| 3. | WGO 40 EC | 17.0[a] | 80.9[a] (76.4) | 9.0[a] | 89.8 [a] (71.2) | 0.0[a] | 100 [a] (89.3) |
| 4. | LGO 20 EC | 78.3[e] | 12.0 [e] (20.2) | 44.0[e] | 50.1 [c] (43.3) | 52.6[e] | 45.2 [e] (42.2) |
| 5. | LGO 30 EC | 46.0[c] | 48.3 [c] (44.0) | 21.3[c] | 75.8 [b] (59.6) | 11.3[b] | 88.2 [b] (69.9) |
| 6. | LGO 40 EC | 28.3[b] | 68.2 [b] (55.6) | 8.7[a] | 90.1 [a] (71.1) | 0.0[a] | 100 [a] (89.3) |
| 7. | Control | 89.0[f] | - | 88.3[f] | - | 96.0[f] | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 21. Volatile effect of WGO, LGO and their EC formulation on the mycelial growth and sclerotial production of *M. phaseolina* and *R. solani***

| S.No. | Plant oils / EC formulation | Mycelial growth (mm)* | | Inhibition over control (%) | | Sclerotial production (mean number)* | | Inhibition (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | ***M. p.*** | ***R.s.*** | ***M. p.*** | ***R.s.*** | ***M. p.*** | ***R.s.*** | ***M. p.*** | ***R.s.*** |
| 1. | WGO (0.2%) | 10.0[b] | 8.0[a] | 88.1[b] (69.8) | 90.7[a] (72.2) | 15.0[b] | 0.0[a] | 88.0[b] (69.7) | 100[a] (89.5) |
| 2. | LGO (0.2%) | 8.0[a] | 8.0[a] | 90.5[a] (72.0) | 90.7[a] (72.2) | 0.0[a] | 0.0[a] | 100.0[a] (89.4) | 100.0[a] (89.5) |
| 3. | WGO 40 EC (0.2%) | 12.0[c] | 15.0[c] | 85.7[c] (67.8) | 85.3[b] (67.4) | 30.0[d] | 12.0[b] | 76.0[c] (60.7) | 86.7[b] (68.2) |
| 4. | LGO 40 EC (0.2%) | 10.0[b] | 12.0[b] | 88.1[b] (69.8) | 86.1[b] (68.1) | 28.0[c] | 0.0[a] | 77.6[c] (61.7) | 100.0[a] (89.5) |
| 5. | Control | 84.0[d] | 86.0[d] | - | - | 125.0[e] | 90.0[c] | - | - |

*M. p.- M. phaseolina; R. s- R. solani*
*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 22. Effect of dilution of WGO 40 EC formulation on mycelial growth and sclerotial production of *M. phaseolina* and *R. solani***

| S.No. | Dilution | Mycelial growth of *M.p.* (mm)* | Inhibition over control (%) | Mycelial growth of *R .s.* (mm)* | Inhibition over control (%) | Sclerotial production ( mean number)* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *M. p.* | Inhibition over control (%) | *R. s* | Inhibition over control (%) |
| 1. | 1:2 | 28.3[a] | 68.5 [a] (55.9) | 18.2[a] | 79.7 [a] (63.3) | 120[a] | 13.6 [a] (21.6) | 14.0[a] | 84.4 [a] (66.9) |
| 2. | 1:4 | 49.0[b] | 45.5 [b] (42.4) | 40.3[b] | 55.2 [b] (47.9) | 134[b] | 3.6 [b] (10.9) | 29.0[b] | 67.7 [b] (55.3) |
| 3. | 1:6 | 80.0[c] | 11.1 [c] (19.4) | 73.7[c] | 18.1 [c] (25.1) | 136[b] | 2.1 [c] (8.3) | 65.0[c] | 27.7 [c] (31.7) |
| 4. | 1:8 | 88.0[d] | 2.2 [d] (8.5) | 86.0[d] | 4.4 [d] (12.1) | 136[b] | 2.1 [c] (8.3) | 76.0[d] | 15.5 [d] (23.1) |
| 5. | 1:10 | 89.0[d] | 1.1 [e] (6.0) | 88.0[d] | 2.2 [e] (8.5) | 138[b] | 0.7 [d] (4.7) | 76.5[d] | 15.0 [d] (22.7 |
| 6. | Control | 90.0[d] | - | 90.0[d] | - | 139[d] | - | 90[e] | - |

*M. p.- M. phaseolina; R. s- R. solani*
*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 24. Stability of WGO 40 EC formulation at different temperatures**

| S.No. | Temperature | M. phaseolina | | R. solani | |
|---|---|---|---|---|---|
| | | Mycelial growth (mm) | Inhibition (%) | Mycelial growth (mm) | Inhibition (%) |
| 1. | $20^0$C | 8.0[a] | 91.0 [a] (73.3) | 8.0[a] | 90.9 (73.1)[a] |
| 2. | $40^0$C | 14.5[b] | 83.7 [b] (65.3) | 17.3[b] | 80.3 (63.7)[b] |
| 3. | $60^0$C | 30.6[c] | 65.6 [c] (54.1) | 38.1[c] | 56.7 (48.8)[c] |
| 4. | $80^0$C | 63.0[d] | 29.2 (32.7)[d] | 73.0[d] | 17.0 (24.3)[d] |
| 5. | Room temperature ($37 \pm 2^0$C) | 8.0[a] | 91.0 [a] (73.0) | 8.0[a] | 90.0 (72.9)[a] |
| 6. | Control | 89.0[e] | - | 88.0[e] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 26. Effect of biocontrol agents on the root rot incidence of coleus caused by *M. phaseolina* and *R. solani* under pot culture experiment**

| S.No. | Biocontrol agents | M. phaseolina | | R. solani | |
|---|---|---|---|---|---|
| | | Root rot (%) | % reduction over control | Root rot (%) | % reduction over control |
| 1. | **T. harzianum** | 34.6[b] (36.02) | 56.4[f] (48.6) | 30.0[b] (33.2) | 61.5[f] (51.64) |
| 2. | TVC5 | 44.3[f] (41.72) | 44.1[d] (41.6) | 41.0[d] (39.81) | 47.4[d] (43.56) |
| 3. | PFC6 | 30.6[a] (33.58) | 61.4[g] (51.6) | 28.0[a] (31.94) | 64.1[g] (53.19) |
| 4. | PFC4 | 55.3[f] (48.04) | 30.2[b] (33.3) | 50.0[f] (44.99) | 35.9[b] (36.80) |
| 5. | Pf1 | 36.6[c] (37.27) | 53.8[e] (47.2) | 35.3[c] (36.26) | 59.7[e] (47.69) |
| 6. | BSC7 | 48.3[e] (44.02) | 39.1[e] (38.7) | 43.3[e] (40.97) | 44.5[c] (41.84) |
| 7. | BSC8 | 58.0[g] (49.60) | 26.9[a] (31.2) | 54.3[g] (47.29) | 30.4[a] (33.45) |
| 8. | Control | 79.3[h] (62.94) | - | 78.0[h] (62.03) | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 27. Effect of formulations of biocontrol agents and plant oils on the root rot incidence of coleus under pot culture experiment**

| S.No. | Formulations | M. phaseolina | | R. solani | |
|---|---|---|---|---|---|
| | | Root rot (%) | % reduction over control | Root rot (%) | % reduction over control |
| 1. | **T. harzianum + *ZnSO₄*** | 30.5 [bc] (38.52) | 59.3 [f] (50.36) | 28.0 [b] (31.94) | 65.0 [g] (53.73) |
| 2. | TVC5 + $ZnSO_4$ | 35.1 [d] (36.33) | 53.2 [d] (46.83) | 40.0 [e] (39.23) | 50.0 [d] (44.99) |
| 3. | PFC6 + WGO + $ZnSO_4$ | 30.0 [ab] (33.20) | 60.0 [f] (50.76) | 26.0 [a] (30.65) | 67.5 [h] (55.24) |
| 4. | PFC6 + LGO+ $ZnSO_4$ | 28.5 [a] (32.26) | 62.0 [g] (51.94) | 28.0 [b] (31.94) | 65.0 [g] (53.73) |
| 5. | Pf 1 + WGO+ $ZnSO_4$ | 35.0 [d] (36.26) | 53.3 [d] (46.89) | 34.0 [d] (35.66) | 57.5 [e] (49.31) |
| 6. | Pf1 + LGO+ $ZnSO_4$ | 32.0 [e] (34.44) | 57.3 [e] (49.19) | 31.0 [c] (33.83) | 61.2 [f] (51.47) |
| 7. | EC - C | 49.3 [g] (44.59) | 34.2 [a] (35.78) | 52.0 [g] (46.14) | 35.0 [b] (36.26) |
| 8. | EC - F | 45.2 [f] (42.24) | 39.7 [b] (39.05) | 58.0 [h] (49.60) | 27.5 [a] (31.62) |
| 9. | EC - H | 40.2 [e] (42.24) | 46.4 [c] (42.43) | 45.0 [f] (42.12) | 43.8 [c] (41.43) |
| 10. | Control | 75.0 [h] (39.30) | - | 80.0 [i] (63.43) | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 28. Effect of organic amendments and $ZnSO_4$ on the coleus root rot caused by *M. phaseolina* and *R. solani* under pot culture experiment.**

| S.No. | Organic amendments | M. phaseolina | | R. solani | |
|---|---|---|---|---|---|
| | | Root rot (%) | % reduction over control | Root rot (%) | % reduction over control |
| 1. | ***Gingelly cake*** | 49.3 [c] (44.59) | 42.7 [c] (40.80) | 44.3 [d] (41.72) | 47.3 [b] (43.45) |
| 2. | Cotton cake | 58.3 [d] (49.77) | 32.2 [b] (34.57) | 41.6 [c] (40.16) | 50.5 [c] (45.28) |
| 3. | Neem cake | 36.0 [a] (36.86) | 58.1 [e] (49.66) | 37.3 [b] (37.70) | 55.5 [d] (48.15) |
| 4. | Groundnut cake | 75.0 [e] (60.00) | 12.8 [a] (20.95) | 70.7 [e] (57.23) | 15.8 [a] (23.41) |
| 5. | Coir pith compost | 39.6 [b] (38.99) | 53.9 [d] (47.23) | 40.0 [c] (39.23) | 52.3 [c] (46.31) |
| 6. | Farm yard manure | 41.0 [d] (39.8) | 52.30 [d] (46.31) | 38.0 [b] (38.05) | 54.7 [d] (47.69) |
| 7. | Zinc sulphate | 35.0 [a] (36.26) | 59.3 [e] (50.36) | 32.0 [a] (34.44) | 61.9 [e] (51.88) |
| 8. | Control | 86.0 [f] (68.03) | - | 84.0 [f] (66.42) | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 29. Effect of fungicides on the root rot incidence of coleus under pot culture experiment**

| S.No. | Fungicides | M. phaseolina | | R. solani | |
|---|---|---|---|---|---|
| | | Root rot (%) | % reduction over control | Root rot (%) | % reduction over control |
| 1. | ***Carbendazim*** | 18.0 [a] (25.09) | 74.6 [f] (59.73) | 24.0 [a] (29.33) | 70.0 [f] (56.79) |
| 2. | Hexaconazole | 28.0 [c] (31.94) | 60.6 [d] (51.12) | 35.0 [c] (36.26) | 56.3 [d] (48.61) |
| 3. | Propiconazole | 20.0 [b] (26.56) | 71.8 [e] (57.92) | 28.0 [b] (31.94) | 65.0 [e] (53.73) |
| 4. | Thiophenitic methyl | 30.0 [d] (33.20) | 57.7 [c] (49.42) | 38.0 [d] (38.05) | 52.5 [c] (46.43) |
| 5. | Tricycloazole | 35.0 [e] (36.26) | 50.7 [b] (45.40) | 41.0 [c] (39.81) | 48.8 [b] (44.31) |
| 6. | Thiram | 40.0 [f] (39.23) | 43.7 [a] (41.38) | 46.0 [f] (42.70) | 42.5 [a] (40.68) |
| 7. | Control | 71.0 [g] (57.41) | - | 80.0 [g] (63.43) | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 30. Shelf life of biocontrol agents in the talc based bioformulations**

| S.No. | Days after storage | *Population of* **Trichoderma** *species (10$^8$ cfu/ml)* | | *Population of* **P.fluorescens** *(10$^8$ cfu/ml)* | |
|---|---|---|---|---|---|
| | | **T. harzianum** | TVC5 | PFC6 | Pf1 |
| 1. | ***0*** | 3.51$^a$ | 4.20$^a$ | 4.90$^a$ | 4.90$^a$ |
| 2. | 30 | 2.70$^b$ | 3.50$^b$ | 3.91$^b$ | 3.85$^b$ |
| 3. | 60 | 2.10$^c$ | 2.70$^c$ | 1.98$^c$ | 1.90$^c$ |
| 4. | 90 | 1.75$^d$ | 1.80$^d$ | 1.30$^d$ | 1.21$^d$ |
| 5. | 120 | 1.20$^c$ | 1.30$^e$ | 0.98$^e$ | 0.93$^e$ |

*Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 46. Effect of phenazine and DAPG on the mycelial growth of *M. phaseolina* and *R. solani***

| S.No. | Treatments | *M. phaseolina* | | *R. solani* | |
|---|---|---|---|---|---|
| | | Mycelial growth (mm) | Inhibition over control (%) | Mycelial growth (mm) | Inhibition over control (%) |
| 1. | Phenazine (0.1%) | 63.0[d] | 28.4 [d] (32.2) | 69.0[d] | 19.7 [d] (26.3) |
| 2. | Phenazine (0.2%) | 41.0[c] | 53.4 [c] (46.9) | 43.0[c] | 50.0 [c] (44.9) |
| 3. | Phenazine (0.5%) | 31.0[ab] | 64.7 [ab] (53.6) | 36.0[b] | 58.1 [b] (49.7) |
| 4. | DAPG (0.1%) | 70.0[e] | 20.45 [e] (26.9) | 76.0[e] | 11.6 [e] (19.9) |
| 5. | DAPG (0.2%) | 37.0[bc] | 57.9 [bc] (49.6) | 40.0[bc] | 53.5 [bc] (47.0) |
| 6. | DAPG (0.5%) | 26.0[a] | 70.4 [a] (57.0) | 29.0[a] | 65.0 [a] (53.7) |
| 7. | Control | 88.0[f] | - | 86.0[f] | - |

*Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 42. Effect of volatile compounds of biocontrol agents on mycelial growth and sclerotial production of *M. phaseolina***

| S.No. | Biocontrol agents | Mycelial growth | | | | Sclerotial production | |
|---|---|---|---|---|---|---|---|
| | | 3 DAI | | 7 DAI | | Mean number | Inhibition over control (%) |
| | | (mm) | Inhibition (%) | (mm) | Inhibition (%) | | |
| 1. | *T. harzianum* – Th | 52.0[a] | 20.0 [a] (26.5) | 89.6[c] | 0.44 [d] (3.8) | 106.8[c] | 15.23 [c] (22.9) |
| 2. | *T. viride* – TVC5 | 60.0[c] | 7.69 [c] (16.0) | 89.9[c] | 0.11 [c] (1.9) | 102.0[b] | 19.0 [b] (25.8) |
| 3. | *P. fluorescens* – PFC6 | 56.0[b] | 11.1 [b] (19.5) | 87.3[a] | 3.0 [a] (9.9) | 100.0[a] | 20.6 [a] (26.9) |
| 4. | *P. fluorescens* – Pf1 | 61.0[d] | 6.15 [d] (14.4) | 88.5[b] | 1.67 [b] (7.4) | 110.0[d] | 12.69 [d] (20.8) |
| 5. | Control | 65.0[e] | - | 90.0[d] | - | 126.0[e] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 43. Effect of volatile compounds of biocontrol agents on the mycelial growth and sclerotial production of *R. solani*.**

| S.No. | Biocontrol agents | Mycelial growth | | | | Sclerotial production | |
|---|---|---|---|---|---|---|---|
| | | 3 DAI | | 7 DAI | | No./Plate | Inhibition over control (%) |
| | | (mm) | Inhibition (%) | (mm) | Inhibition (%) | | |
| 1. | *T. harzianum* – Th | 55.3[c] | 7.8 [a] (16.2) | 84.5[b] | 6.1 [c] (14.2) | 38.0[a] | 40.6 [a] (39.5) |
| 2. | *T. viride* – TVC5 | 57.3[a] | 4.5 [b] (12.2) | 86.2[c] | 4.2 [d] (11.8) | 41.0[ab] | 35.9 [c] (36.8) |
| 3. | *P. fluorescens* – PFC6 | 58.2[b] | 3.0 [d] (9.9) | 82.6[a] | 8.2 [a] (16.6) | 39.0[ab] | 39.0 [b] (38.6) |
| 4. | *P. fluorescens* – Pf1 | 57.9[a] | 3.5 [c] (10.8) | 84.0[b] | 6.6 [b] (14.9) | 43.0[b] | 32.8 [d] (44.9) |
| 5. | Control | 60.0[d] | - | 90.0[d] | - | 64.0[c] | - |

**Table 44. Effect of diffusible metabolites of the biocontrol agents on the mycelial growth and sclerotial production of *M. phaseolina***

| S.No. | Biocontrol agents | Mycelial growth | | | | Sclerotial production | |
|---|---|---|---|---|---|---|---|
| | | 3 DAI | | 7 DAI | | Mean number | Inhibition over control (%) |
| | | (mm) | Inhibition (%) | (mm) | Inhibition (%) | | |
| 1. | *T. harzianum* – Th | 30.0[b] | 50.0 [b] (45.0) | 42.5[b] | 52.7 [b] (46.5) | 95.0 [c] | 26.9 [c] (31.2) |
| 2. | *T. viride* – TVC5 | 35.3[c] | 41.1 [c] (39.8) | 50.0[d] | 44.4 [d] (41.7) | 110.0 [d] | 15.3 [d] (23.0) |
| 3. | *P. fluorescens* – PFC6 | 26.0[a] | 56.7 [a] (48.8) | 40.0[a] | 55.4 [a] (48.1) | 70.0 [a] | 46.1 [a] (42.7) |
| 4. | *P. fluorescens* – Pf1 | 29.5[b] | 50.8 [b] (45.4) | 46.8[c] | 48.0 [c] (43.8) | 82.0 [b] | 36.9 [b] (37.3) |
| 5. | Control | 60.0[d] | - | 90.0[e] | - | 130.0[e] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 45. Effect of diffusible metabolites of the biocontrol agents on the mycelial growth and sclerotial production of *R. solani*.**

| S.No. | Biocontrol agents | Mycelial growth | | | | Sclerotial production | |
|---|---|---|---|---|---|---|---|
| | | 3 DAI | | 7 DAI | | Mean number | Inhibition over control (%) |
| | | (mm) | Inhibition (%) | (mm) | Inhibition (%) | | |
| 1. | *T. harzianum* – Th | 15.0[b] | 78.5 [b] (62.4) | 40.0[b] | 55.4 [b] (48.1) | 20.0[b] | 69.2 [b] (56.3) |
| 2. | *T. viride* – TVC5 | 17.6[c] | 74.8 [c] (59.9) | 73.3[d] | 18.2 [d] (25.2) | 33.0[d] | 49.2 [d] (44.5) |
| 3. | *P. fluorescens* – PFC6 | 13.6[a] | 80.5 [a] (63.9) | 38.2[a] | 57.4 [a] (49.2) | 15.0[a] | 76.9 [a] (61.3) |
| 4. | *P. fluorescens* – Pf1 | 23.5[d] | 66.4 [d] (54.6) | 65.5[c] | 26.9 [c] (31.2) | 30.5[c] | 53.0 [c] (46.7) |
| 5. | Control | 70.0[e] | - | 89.7[e] | - | 65.0[e] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 25. Shelf life of WGO 40 EC formulation at room temperature**

| S.No. | Storage period ( days) | Mycelial growth of *M. phaseolina* * | | Mycelial growth of *R. solani** | |
|---|---|---|---|---|---|
| | | Radial growth (mm) | Inhibition per cent (%) | Radial growth (mm) | Inhibition per cent (%) |
| 1 | 0 | 8.0[a] | 91.0[a] (72.55) | 8.0[a] | 91.1[a] (72.65) |
| 2. | 60 | 8.5[a] | 90.4[a] (71.96) | 10.3[b] | 88.5[b] (70.18) |
| 3. | 120 | 8.7[a] | 90.2[a] (71.77) | 10.3[b] | 88.5[b] (70.18) |
| 4. | 180 | 9.0[a] | 89.8[a] (71.38) | 10.5[b] | 88.3[b] (70.00) |
| 5. | 240 | 9.0[a] | 89.8[a] (72.55) | 11.0[b] | 87.7[b] (69.47) |
| 6. | Control | 89.0[b] | - | 90.0[c] | - |

*Mean of three replications
Values in parentheses are arcsine transformed
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 1.** List of biocontrol agents used

| S. No. | Biocontrol agents | Crop rhizosphere | Location |
|---|---|---|---|
| 1. | *T. viride* - TVC1 | Coleus | Keeripatti |
| 2. | *T. viride* - TVC2 | Coleus | Keeripatti |
| 3. | *T. viride* - TVC3 | Maize | Paithur |
| 4. | *T. viride* - TVC4 | Groundnut | Kaniyamoor |
| 5. | *T. viride* - TVC5 | Coleus | V.P. agaram |
| 6. | *T. viride* - TVC6 | Coleus | Namashivayapuram |
| 7. | *T. harzianum* - Th | Coleus | Namashivayapuram |
| 8. | *P. fluorescens*- PFC1 | Groundnut | Keeripatti |
| 9. | *P. fluorescens* - PFC2 | Coleus | Paithur |
| 10. | *P. fluorescens* - PFC3 | Sorghum | Mankini |
| 11. | *P. fluorescens* - PFC4 | Groundnut | Melur |
| 12. | *P. fluorescens* - PFC5 | Coleus | Inthili |
| 13. | *P. fluorescens* - PFC6 | Coleus | Kaniyamoor |
| 14. | *P. fluorescens* - PFC7 | Tapioca | Erikadu |
| 15. | *B. subtilis* - BSC1 | Coleus | Keeripatti |
| 16. | *B. subtilis* - BSC2 | Groundnut | Keeripatti |
| 17. | *B. subtilis* - BSC3 | Maize | Paithur |
| 18. | *B. subtilis* - BSC4 | Tapioca | Erikadu |
| 19. | *B. subtilis* - BSC5 | Groundnut | Kaniyamoor |
| 20. | *B. subtilis* - BSC6 | Tapioca | Namashivapuram |
| 21. | *B. subtilis* - BSC7 | Coleus | Mankuttai |
| 22. | *B. subtilis* - BSC8 | Coleus | Inthili |
| 23. | *B. subtilis* - BSC9 | Maize | V.P. agaram |
| 24. | *B. subtilis* - BSC10 | Coleus | Melur |

**Table 2 . Plant species and Plant oils tested for their antifungal activity**

| S.No. | Scientific Name | Common name | Parts used | Family |
|---|---|---|---|---|
| 1. | Adathoda vasica *Medic.* | Adathodai | Leaf | Acanthaceae |
| 2. | Allium sativum *Linn.* | Garlic | Bulb | Liliaceae |
| 3. | Andrograpis paniculata *Wall.ex.Ness.* | Indian chiretta | Leaf | Acanthaceae |
| 4. | Azadirachta indica *A. Juss* | Neem | Leaf | Meliaceae |
| 5. | Coleus barbatus *Briq.* | Coleus | Leaf | Labiatae |
| 6. | Cymbopogan citrates *(D.C.) stapf* | Lemon grass | Oil | Graminae |
| 7. | Gaultheria procumbens *Linn.* | Wintergreen | Oil | Ericaceae |
| 8. | Lantana camera *Linn.* | Wild sage | Leaf | Verbinaceae |
| 9. | Lawsonia inermis *Linn.* | Henna | Leaf | Lythraceae |
| 10. | Vitex negundo *Linn.* | Notchi | Leaf | Verbinaceae |

**Table 3. Survey for Coleus root rot disease in Viluppuram and Salem districts of Tamil Nadu.**

| S.No | Village | Age of the crop | Root rot incidence (%) |
|---|---|---|---|
| | **Viluppuram district** | | |
| 1. | Kaniyamoor | 105 | 86.0 [a] (68.1) |
| 2. | Namashivayapuram | 90 | 77.8 [ab] (62.4) |
| 3. | V.P. agaram | 95 | 70.6 [bc] (57.8) |
| 4. | Thenchettianthur | 65 | 35.8 [f] (36.5) |
| 5. | Melur | 70 | 16.6 [g] (23.8) |
| 6. | Inthili | 85 | 59.0 [cde] (50.5) |
| | **Salem district** | | |
| 7. | Paithur | 80 | 60.0 [cde] (50.8) |
| 8. | Mankuttai | 120 | 64.2 [cd] (53.3) |
| 9. | Keeripatti | 100 | 69.0 [bc] (56.3) |
| 10. | Erikadu | 50 | 48.2 [ef] (43.9) |
| 11. | Mankini | 65 | 52.0 [de] (46.1) |
| 12. | Neermullikuttai | 24 | 37.4 [f] (37.6) |

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 23.** *Stability of WGO 40 EC at different pH*

| S.No. | pH level | *M. phaseolina** | | *R. solani ** | |
|---|---|---|---|---|---|
| | | Mycelial growth (mm) | Inhibition over control (%) | Mycelial growth (mm) | Inhibition over control |
| 1. | 4.0 | 18.0[b] | 79.7 [b] (63.3) | 14.0[c] | 84.4 [c] (66.7) |
| 2. | 7.0 | 8.0[a] | 91.0 [a] (72.9) | 8.0[a] | 91.1 [a] (72.6) |
| 3. | 9.0 | 10.3[a] | 88.4 [ab] (70.5) | 12.0[b] | 86.6 [b] (68.5) |
| 4. | Control (Untreated) | 89.0[c] | - | 90.0[d] | - |

* Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 31. Rhizosphere populations of *Trichoderma* species – Neermullikuttai**

| S.No. | Treatments | *Trichoderma* population * ($10^3$ cfu /g of soil) | | | |
|---|---|---|---|---|---|
| | | Days after planting | | | |
| | | 0 | 45 | 90 | 120 |
| 1. | PFC6 | $2.60^{ab}$ | $3.5^{f}$ | $4.2^{c}$ | $4.8^{ef}$ |
| 2. | Pf1 | $2.31^{de}$ | $3.8^{e}$ | $4.3^{ab}$ | $4.9^{e}$ |
| 3. | PFC6 + WGO + $ZnSO_4$ | $2.42^{bcd}$ | $3.3^{g}$ | $4.1^{c}$ | $4.5^{g}$ |
| 4. | Pf1 + WGO + $ZnSO_4$ | $2.16^{ef}$ | $3.0^{h}$ | $4.0^{c}$ | $4.7^{f}$ |
| 5. | *T. harzianum* –Th + $ZnSO_4$ | $2.58^{abc}$ | $18.0^{a}$ | $22.0^{a}$ | $26.0^{b}$ |
| 6. | *T. viride* - TVC5 + $ZnSO_4$ | $2.6^{ab}$ | $14.0^{b}$ | $23.0^{a}$ | $28.0^{a}$ |
| 7. | Neem cake | $2.30^{de}$ | $6.5^{c}$ | $8.30^{bc}$ | $10.0^{c}$ |
| 8. | Zinc sulphate | $2.70^{a}$ | $4.3^{d}$ | $5.8^{c}$ | $6.2^{d}$ |
| 9. | EC – C | $2.1^{f}$ | $2.6^{j}$ | $2.9^{c}$ | $3.2^{j}$ |
| 10. | EC – F | $2.4^{cd}$ | $2.9^{hi}$ | $3.0^{c}$ | $3.5^{i}$ |
| 11. | Carbendazim | $2.5^{bc}$ | $2.9^{hi}$ | $3.1^{c}$ | $3.5^{i}$ |
| 12. | Propiconazole | $2.4^{cd}$ | $2.7^{j}$ | $3.0^{c}$ | $3.5^{i}$ |
| 13. | $T_3 + T_4$ | $2.50^{bc}$ | $3.2^{g}$ | $3.7^{c}$ | $4.0^{h}$ |
| 14. | $T_5 + T_6$ | $2.56^{abc}$ | $17.0^{a}$ | $21.9^{a}$ | $24.7^{b}$ |
| 15. | Control | $2.5^{bc}$ | $2.75^{ij}$ | $4.0^{c}$ | $4.2^{h}$ |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 32. Rhizosphere populations of *P. fluorescens*- Neermullikuttai**

| S.No. | Treatments | *P. fluorescens* population* ($10^4$ cfu/g of soil) | | | |
|---|---|---|---|---|---|
| | | Days after planting | | | |
| | | 0 | 45 | 90 | 120 |
| 1. | PFC6 | $26.0^{b}$ | $45.0^{d}$ | $62.0^{d}$ | $80.0^{c}$ |
| 2. | Pf1 | $24.0^{f}$ | $40.0^{f}$ | $60.0^{f}$ | $75.0^{d}$ |
| 3. | PFC6 + WGO + $ZnSO_4$ | $22.0^{h}$ | $48.00^{b}$ | $69.0^{a}$ | $88.0^{a}$ |
| 4. | Pf1 + WGO + $ZnSO_4$ | $25.0^{d}$ | $43.0^{e}$ | $54.0^{c}$ | $76.0^{d}$ |
| 5. | T. harzianum - *Th* + *$ZnSO_4$* | $25.4^{c}$ | $31.3^{h}$ | $41.0^{I}$ | $56.0^{f}$ |
| 6. | T. viride - *TVC5* + *$ZnSO_4$* | $25.1^{d}$ | $29.6^{k}$ | $40.5^{j}$ | $55.0^{f}$ |
| 7. | *Neem cake* | $24.3^{e}$ | $36.5^{g}$ | $50.6^{g}$ | $59.8^{e}$ |
| 8. | *Zinc sulphate* | $22.0^{h}$ | $30.2^{I}$ | $47.8^{h}$ | $60.5^{e}$ |
| 9. | *EC-C* | $25.5^{c}$ | $28.0^{m}$ | $35.0^{k}$ | $43.0^{g}$ |
| 10. | *EC-F* | $23.8^{g}$ | $27.0^{n}$ | $34.0^{l}$ | $40.5^{h}$ |
| 11. | *Carbendazim* | $26.0^{b}$ | $29.0^{l}$ | $32.0^{m}$ | $40.0^{h}$ |
| 12. | *Propiconazole* | $27.0^{a}$ | $30.0^{j}$ | $32.0^{m}$ | $34.9^{I}$ |
| 13. | $T_3 + T_4$ | $24.0^{f}$ | $49.0^{a}$ | $65.0^{b}$ | $82.0^{b}$ |
| 14. | $T_5 + T_6$ | $25.0^{d}$ | $28.0^{m}$ | $35.5^{k}$ | $52.0^{f}$ |
| 15. | *Control* | $26.1^{b}$ | $29.5^{k}$ | $30.2^{n}$ | $32.5^{j}$ |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 35. Rhizosphere populations of *Trichoderma* species – Coimbatore**

| S.No. | Treatments | *Trichoderma* population* ($10^3$ cfu/g of soil) | | | |
|---|---|---|---|---|---|
| | | Days after planting | | | |
| | | 0 | 45 | 90 | 120 |
| 1. | PFC6 | $2.10^{de}$ | $3.4^{e}$ | $3.98^{ef}$ | $4.7^{e}$ |
| 2. | Pf1 | $2.0^{e}$ | $3.6^{de}$ | $4.0^{ef}$ | $4.9^{e}$ |
| 3. | PFC6 + WGO + $ZnSO_4$ | $2.30^{bcd}$ | $3.2^{e}$ | $4.10^{e}$ | $4.6^{e}$ |
| 4. | Pf1 + WGO + $ZnSO_4$ | $2.20^{b-e}$ | $3.1^{e}$ | $4.05^{e}$ | $4.5^{ef}$ |
| 5. | T. harzianum - *Th* + *$ZnSO_4$* | $2.25^{bcd}$ | $16.0^{a}$ | $21.0^{a}$ | $25.5^{a}$ |
| 6. | T. viride - *TVC5* + *$ZnSO_4$* | $2.38^{bc}$ | $12.0^{b}$ | $20.0^{b}$ | $24.0^{b}$ |
| 7. | *Neem cake* | $2.40^{b}$ | $6.0^{c}$ | $8.0^{c}$ | $9.6^{c}$ |
| 8. | *Zinc sulphate* | $2.60^{a}$ | $4.0^{d}$ | $5.5^{d}$ | $5.9^{d}$ |
| 9. | *EC-C* | $2.31^{bcd}$ | $2.42^{f}$ | $3.0^{hi}$ | $3.0^{I}$ |
| 10. | *EC-F* | $2.28^{bcd}$ | $2.39^{f}$ | $2.95^{i}$ | $3.4^{ghi}$ |
| 11. | *Carbendazim* | $2.20^{b-e}$ | $2.50^{f}$ | $3.2^{f-i}$ | $3.6^{f-I}$ |
| 12. | *Propiconazole* | $2.16^{cde}$ | $2.36^{f}$ | $3.0^{hi}$ | $3.2^{hi}$ |
| 13. | *$T_3$* + *$T_4$* | $2.18^{b-e}$ | $3.30^{e}$ | $3.8^{e-h}$ | $4.0^{e-h}$ |
| 14. | *$T_5$* + *$T_6$* | $2.02^{e}$ | $13.20^{b}$ | $23.9^{a}$ | $24.2^{b}$ |
| 15. | *Control* | $2.3^{bcd}$ | $3.05^{e}$ | $3.10^{ghi}$ | $4.0^{e-h}$ |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 36. Rhizosphere populations of *P. fluorescens* – Coimbatore**

| S.No. | Treatments | *P. fluorescens* population* ($10^4$ cfu/g of soil) | | | |
|---|---|---|---|---|---|
| | | Days after planting | | | |
| | | 0 | 45 | 90 | 120 |
| 1. | PFC6 | 24.0[a] | 40.0[c] | 59.5[bc] | 78.0[ab] |
| 2. | Pf1 | 25.0[a] | 36.0[d] | 56.0[c] | 70.0[c] |
| 3. | PFC6 + WGO + $ZnSO_4$ | 24.5[a] | 46.0[a] | 69.2[a] | 80.0[a] |
| 4. | Pf1 + WGO + $ZnSO_4$ | 25.1[a] | 42.0[bc] | 60.1[bc] | 75.0[abc] |
| 5. | T. harzianum - *Th* + *$ZnSO_4$* | 23.8[a] | 30.2[e] | 40.0[e] | 49.0[e] |
| 6. | T. viride - *TVC5* + *$ZnSO_4$* | 25.5[a] | 28.0[ef] | 36.0[ef] | 48.2[e] |
| 7. | *Neem cake* | 23.5[a] | 34.5[d] | 48.0[d] | 60.0[d] |
| 8. | *Zinc sulphate* | 24.1[a] | 30.0[e] | 45.3[d] | 55.0[d] |
| 9. | *EC-C* | 23.9[a] | 25.0[f] | 34.0[fg] | 39.1[f] |
| 10. | *EC-F* | 24.0[a] | 27.5[ef] | 30.0[g] | 40.2[f] |
| 11. | *Carbendazim* | 25.2[a] | 29.0[e] | 30.0[g] | 38.0[fg] |
| 12. | *Propiconazole* | 23.8[a] | 29.3[e] | 30.0[g] | 33.0[g] |
| 13. | *$T_3$* + *$T_4$* | 23.5[a] | 48.0[a] | 64.0[b] | 74.0[bc] |
| 14. | *$T_5$* + *$T_6$* | 23.9[a] | 28.2[ef] | 35.0[f] | 47.5[e] |
| 15. | *Control* | 24.0[d] | 30.0[e] | 32.0[fg] | 35.0[fg] |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 4. Loss of forskolin alkaloid due to root rot infection**

| S.No. | Tuber Infection (%) | Forskolin content (%) * |
|---|---|---|
| 1. | 0 | 0.42 (3.71) [a] |
| 2. | 10 | 0.19 (2.49) [b] |
| 3. | 25 | 0.17 (2.36) [b] |
| 4. | 50 | 0.13 (2.06) [c] |
| 5. | 75 | 0.08 (1.61) [d] |
| 6. | 100 | 0.06 (1.40) [e] |

* Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 38. Effect of bioformulations on the growth parameters and Yield of Coleus- Neermullikuttai***

| Sl. No. | Treatments | Yield (kg/ha) | Plant height (cm) | No. of branches (No.) | Tuber length (cm) | Tuber diameter (mm) |
|---|---|---|---|---|---|---|
| 1. | PFC6 | 3980$^{de}$ | 52.0$^{bc}$ | 25.0$^{bc}$ | 28.0$^{ef}$ | 22.0$^{bc}$ |
| 2. | Pf1 | 2914$^{fg}$ | 46.0$^{de}$ | 23.0$^{cd}$ | 26.0$^{cde}$ | 21.5$^{bc}$ |
| 3. | PFC6 + WGO + $ZnSO_4$ | 5361$^{ab}$ | 56.0$^{ab}$ | 26.0$^{ab}$ | 29.0$^{f}$ | 23.0$^{b}$ |
| 4. | Pf1 + WGO + $ZnSO_4$ | 3702$^{e}$ | 48.0$^{cd}$ | 24.0$^{bcd}$ | 27.5$^{def}$ | 22.0$^{bc}$ |
| 5. | T. harzianum- *Th* + *$ZnSO_4$* | 4320$^{d}$ | 45.0$^{de}$ | 22.0$^{de}$ | 27.0$^{c-f}$ | 20.5$^{cd}$ |
| 6. | T. viride- *TVC5* + *$ZnSO_4$* | 3210$^{f}$ | 42.0$^{ef}$ | 20.5$^{ef}$ | 26.0$^{cde}$ | 20.0$^{cde}$ |
| 7. | *Neem cake* | 3165$^{fg}$ | 40.0$^{fg}$ | 19.8$^{f}$ | 25.0$^{cd}$ | 18.0$^{ef}$ |
| 8. | *Zinc sulphate* | 3020$^{fg}$ | 38.0$^{fg}$ | 19.4$^{f}$ | 24.5$^{c}$ | 18.5$^{de}$ |
| 9. | *EC-C* | 2783$^{gh}$ | 36.0$^{gh}$ | 14.0$^{g}$ | 22.0$^{b}$ | 16.0$^{g}$ |
| 10. | *EC-F* | 2510$^{h}$ | 36.5$^{g}$ | 14.7$^{g}$ | 22.0$^{b}$ | 16.2$^{fg}$ |
| 11. | *Carbendazim* | 5604$^{a}$ | 52.0$^{bc}$ | 24.0$^{bcd}$ | 27.0$^{c-f}$ | 20.0$^{cde}$ |
| 12. | *Propiconazole* | 5109$^{bc}$ | 50.5$^{c}$ | 23.0$^{cd}$ | 27.5$^{def}$ | 20.5$^{cd}$ |
| 13. | $T_3$ + $T_4$ | 5674$^{a}$ | 59.0$^{a}$ | 28.0$^{a}$ | 32.0$^{g}$ | 25.0$^{a}$ |
| 14. | $T_5$ + $T_6$ | 4891$^{c}$ | 48.0$^{cd}$ | 23.5$^{cd}$ | 28.5$^{ef}$ | 21.0$^{bc}$ |
| 15. | *Control* | 918.0$^{i}$ | 32.0$^{h}$ | 10.0$^{h}$ | 16.5$^{a}$ | 14.0$^{h}$ |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 41. Effect of bioformulations on the growth parameters and Yield of Coleus –Coimbatore***

| Sl. No. | Treatments | Yield (kg/ha) | Plant height (cm) | No. of branches (No.) | Tuber length (cm) | Tuber diameter (mm) |
|---|---|---|---|---|---|---|
| 1. | PFC6 | 3630$^{bc}$ | 42.0$^{cd}$ | 20.0$^{bc}$ | 22.0$^{c}$ | 19.0$^{b}$ |
| 2. | *Pf1* | 2514$^{bc}$ | 38.0$^{def}$ | 17.0$^{d}$ | 20.0$^{cde}$ | 18.0$^{bc}$ |
| 3. | *PFC6+WGO+$ZnSO_4$* | 5032$^{b}$ | 46.0$^{b}$ | 21.00$^{ab}$ | 29.0$^{a}$ | 21.0$^{a}$ |
| 4. | *Pf1+WGO+$ZnSO_4$* | 3329$^{bc}$ | 40.0$^{de}$ | 19.0$^{c}$ | 21.0$^{cd}$ | 17.0$^{c}$ |
| 5. | T. harzianum- *Th + $ZnSO_4$* | 4050$^{cd}$ | 38.0$^{def}$ | 17.0$^{d}$ | 20.0$^{cde}$ | 17.0$^{c}$ |
| 6. | T. viride -*TVC5+ $ZnSO_4$* | 2963$^{cde}$ | 40.0$^{de}$ | 15.0$^{e}$ | 20.0$^{cde}$ | 14.0$^{d}$ |
| 7. | *Neem cake* | 2827$^{ef}$ | 39.0$^{de}$ | 15.0$^{e}$ | 20.0$^{cde}$ | 15.0$^{d}$ |
| 8. | *Zinc sulphate* | 2657$^{de}$ | 37.0$^{efg}$ | 14.0$^{e}$ | 19.0$^{de}$ | 15.0$^{d}$ |
| 9. | *EC-C* | 2475$^{g}$ | 34.0$^{g}$ | 14.0$^{e}$ | 16.0$^{f}$ | 15.0$^{d}$ |
| 10. | *EC-F* | 2382$^{fg}$ | 35.0$^{fg}$ | 14.0$^{e}$ | 15.0$^{f}$ | 14.0$^{d}$ |
| 11. | *Carbendazim* | 5042$^{cde}$ | 46.0$^{b}$ | 20.0$^{bc}$ | 25.0$^{b}$ | 18.0$^{bc}$ |
| 12. | *Propiconazole* | 4707$^{cd}$ | 45.0$^{bc}$ | 21.0$^{ab}$ | 24.0$^{b}$ | 17.0$^{c}$ |
| 13. | *$T_3$+$T_4$* | 5452.3$^{a}$ | 50.0$^{a}$ | 22.0$^{a}$ | 30.0$^{a}$ | 22.0$^{a}$ |
| 14. | *$T_5$+$T_6$* | 4366.0$^{bc}$ | 39.0$^{de}$ | 17.0$^{d}$ | 20.0$^{cde}$ | 18.0$^{bc}$ |
| 15. | *Control* | 781.0$^{h}$ | 30.0$^{h}$ | 12.0$^{f}$ | 18.0$^{e}$ | 14.0$^{d}$ |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 5. Quantification of sclerotial population of *M. Phaseolina* in soil samples collected at different locations**

| S.No | Location | Crop rotation | Soil type* | Sclerotia** (Number/g dry soil) |
|---|---|---|---|---|
| 1. | Kaniyamoor –I | *Coleus - Groundnut – Coleus* | L | 161.0$^{e}$ |
| 2. | Kaniyamoor –II | Coleus - Coleus - Coleus | SL | 278.0$^{a}$ |
| 3. | Namashivayapuram-I | Coleus - Coleus - Coleus | SL | 236.0$^{b}$ |
| 4. | Namashivayapuram-II | Groundnut - Coleus – Coleus | SL | 173.0$^{d}$ |
| 5. | V.P. agaram –I | Sorghum - Groundnut – Coleus | L | 157.0$^{e}$ |
| 6. | V.P. agaram –II | Paddy - Sunnhemp - Coleus | SiL | 24.6$^{o}$ |
| 7. | Melur –I | Sorghum - Sunnhemp – Coleus | SiL | 33.5$^{m}$ |
| 8. | Melur - II | Paddy - Sunnhemp - Coleus | SL | 28.3$^{no}$ |
| 9. | Melur –III | Tapioca - Tapioca - Coleus | FSiL | 4.4$^{r}$ |
| 10. | Melur – IV | Sunnhemp - Tapioca - Coleus | SiL | 9.6$^{a}$ |
| 11. | Melur – V | Paddy - Tapioca - Coleus | SiL | 5.0$^{r}$ |
| 12. | Inthili –I | Paddy - Paddy - Coleus | FSiL | 3.8$^{r}$ |
| 13. | Inthili –II | Groundnut - Coleus – Coleus | SL | 145.0 |
| 14. | Kumbakottai –I | Groundnut - Coleus – Coleus | SL | 160.5$^{e}$ |
| 15. | Kumbakottai –II | Maize - Groundnut - Coleus | **SL** | 130.0$^{hi}$ |
| 16. | Thenchettiathur –I | Sunnhemp - Paddy - Coleus | FSiL | 1.3$^{s}$ |
| 17. | Thenchettiathur –II | Groundnut - Coleus - Maize | L | 200.1$^{c}$ |
| 18. | Sankarapuram- I | Groundnut - Coleus - Coleus | L | 138.1$^{gh}$ |
| 19. | Sankarapuram- II | Coleus - Coleus - Coleus | L | 153.0$^{ef}$ |
| 20. | Sankarapuram- III | Paddy - Paddy - Coleus | FSiL | 1.8$^{s}$ |
| 21. | Paithur –I | Paddy - Paddy - Coleus | SiL | 18.1$^{p}$ |
| 22. | Paithur-II | Groundnut - Coleus - Coleus | SiL | 100.7$^{j}$ |
| 23. | Mankuttai- I | Paddy - Maize - Coleus | SiL | 20.1$^{p}$ |
| 24. | Mankuttai- II | Groundnut-Groundnut-Coleus | SL | 88.3$^{k}$ |
| 25. | Keeripatti | Maize - Groundnut - Coleus | SL | 128.0$^{hi}$ |
| 26. | Erikadu | Sunnhemp - Paddy - Coleus | FSiL | 17.7$^{p}$ |
| 27. | Neermuttukuttai- I | Sorghum - Sorghum - Groundnut | SiL | 62.5$^{l}$ |
| 28. | Neermuttukuttai- II | Maize - Sorghum - Coleus | SiL | 30.1$^{mn}$ |
| 29. | Valapadi-I | Paddy - Sugarcane - Coleus | FSiL | 9.1$^{q}$ |
| 30. | Valapadi- II | Maize -Groundnut - Coleus | FSiL | 20.0$^{p}$ |
| 31. | Coimbatore – I | Sorghum - Chickpea - Greengram | L | 110.0$^{l}$ |
| 32. | Coimbatore- II | Groundnut - Groundnut - Coleus | L | 125.0$^{i}$ |

* L- Loamy; SiL – Silty Loam; FSiL- Fine Silty Loam; SL- Sandy Loam

** Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 40 . Effect of bioformulations on the Root rot incidence of Coleus under field condition – Coimbatore**

| S. No | Treatments | 45 DAP* | | 90 DAP* | |
|---|---|---|---|---|---|
| | | Root rot (%) | Inhibition over control (%) | Root rot (%) | Inhibition over control (%) |
| 1. | PFC6 | 30.0$^{g}$ (33.2) | 43.4$^{fg}$ (41.1) | 43.0$^{de}$(41.0) | 44.1$^{de}$ (41.6) |
| 2. | *Pf1* | 33.0$^{h}$ (35.0) | 38.7$^{gh}$ (37.8) | 48.0$^{fg}$(43.9) | 37.6$^{f}$ (37.8) |
| 3. | *PFC6+WGO+ $ZnSO_4$* | 24.0$^{f}$ (29.3) | 54.7$^{e}$ (47.7) | 39.1 $^{d}$ (38.7) | 49.2$^{d}$ (44.5) |
| 4. | *Pf1+WGO+$ZnSO_4$* | 28.0$^{g}$ (31.9) | 47.1$^{f}$ (43.3) | 46.0 $^{ef}$(42.7) | 40.2$^{ef}$ (39.3) |
| 5. | T. harzianum-*Th+ $ZnSO_4$* | 18.0$^{d}$ (25.0) | 66.0$^{d}$ (54.3) | 32.3 $^{c}$ (34.6) | 58.0$^{c}$ (49.6) |
| 6. | T.viride-*$TVC_5$+ $ZnSO_4$* | 35.0$^{h}$ (36.3) | 33.9$^{h}$ (35.6) | 49.0 $^{fg}$(44.4) | 36.3$^{fg}$ (37.0) |
| 7. | *Neem cake* | 39.5$^{i}$ (38.9) | 25.5$^{i}$ (30.3) | 52.0 $^{g}$ (46.1) | 32.4$^{g}$ (34.6) |
| 8. | *Zinc sulphate* | 41.0$^{ij}$ (39.8) | 22.6$^{ij}$ (28.3) | 58.0 $^{h}$ (49.6) | 24.6$^{h}$ (29.7) |
| 9. | *EC-C* | 43.7$^{jk}$ (41.3) | 17.5$^{jk}$ (24.2) | 62.1$^{i}$ (52.0) | 19.3$^{i}$ (26.1) |
| 10. | *EC-F* | 45.0$^{k}$ (42.1) | 15.09$^{k}$ (22.8) | 65.0 $^{j}$ (53.7) | 15.5$^{j}$ (23.1) |
| 11. | *Carbendazim* | 5.0$^{a}$ (12.9) | 90.56$^{a}$ (72.5) | 15.0 $^{a}$ (22.7) | 80.5$^{a}$ (63.8) |
| 12. | *Propiconazole* | 8.0$^{b}$ (16.4) | 84.9$^{b}$ (67.2) | 18.0 $^{a}$ (25.0) | 76.6$^{a}$ (61.1) |
| 13. | *$T_3$+ $T_4$* | 21.0$^{e}$ (27.2) | 60.37$^{de}$ (50.9) | 35.0 $^{c}$ (36.2) | 54.5$^{c}$ (47.5) |
| 14. | *$T_5$+ $T_6$* | 12.0$^{c}$ (20.2) | 77.3$^{c}$ (66.6) | 28.0 $^{b}$ (31.9) | 63.6$^{b}$ (52.9) |
| 15. | *Control* | 53.0$^{l}$ (46.7) | - | 78.0 $^{j}$ (62.0) | - |

* Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 37. Effect of bioformulations on the Root rot incidence of Coleus under field condition – Neermullikuttai**

| S.No | Treatments | 45 DAP* | | 90 DAP* | |
|---|---|---|---|---|---|
| | | Root rot (%) | Inhibition over control (%) | Root rot (%) | Inhibition over control (%) |
| 1. | PFC6 | 15.0 (22.8)$^{g}$ | 57.1 (49.0)$^{ef}$ | 40.7 (39.6)$^{g}$ | 41.3 (39.9)$^{g}$ |
| 2. | *Pf1* | 17.0 (24.3)$^{h}$ | 51.4 (45.8)$^{fg}$ | 42.5 (40.7)$^{g}$ | 38.6 (33.4)$^{gh}$ |
| 3. | *PFC6+WGO + $ZnSO_4$* | 12.0 (20.3)$^{f}$ | 65.7 (54.1)$^{de}$ | 32.0 (34.4)$^{e}$ | 53.8 (47.1)$^{e}$ |
| 4. | *Pf1+WGO+ $ZnSO_4$* | 18.5 (25.4)$^{i}$ | 47.1 (43.3)$^{fg}$ | 36.6 (37.3)$^{f}$ | 47.2 (43.3)$^{f}$ |
| 5. | T. harzianum-*Th+ $ZnSO_4$* | 10.0 (18.4)$^{e}$ | 71.4 (57.7)$^{cd}$ | 27.7 (31.7)$^{d}$ | 60.1 (50.3)$^{d}$ |
| 6. | T.viride-*$TVC_5$ + $ZnSO_4$* | 20.0 (26.6)$^{i}$ | 42.8 (40.8)$^{g}$ | 46.1 (42.8)$^{h}$ | 33.5 (35.3)$^{hi}$ |
| 7. | *Neem cake* | 24.0 (29.3)$^{j}$ | 31.4 (34.0)$^{h}$ | 48.6 (44.2)$^{g}$ | 29.9 (33.1)$^{ij}$ |
| 8. | *Zinc sulphate* | 26.0 (30.7)$^{k}$ | 25.7 (30.4)$^{h}$ | 51.7 (46.0)$^{j}$ | 25.4(30.2)$^{j}$ |
| 9. | *EC-C* | 31.0 (33.8)$^{l}$ | 11.4 (14.7)$^{i}$ | 55.0 (47.9)$^{k}$ | 20.6 (27.0)$^{k}$ |
| 10. | *EC-F* | 32.0 (34.4)$^{l}$ | 8.5 (17.0)$^{i}$ | 56.6 (48.8)$^{k}$ | 18.2 (25.2)$^{k}$ |
| 11. | *Carbendazim* | 2.0 (8.1)$^{a}$ | 94.3 (78.3)$^{a}$ | 10.3 (18.7)$^{a}$ | 85.1 (67.4)$^{a}$ |
| 12. | *Propiconazole* | 3.0 (10.0)$^{b}$ | 91.4 (72.4)$^{ab}$ | 16.3 (23.8)$^{b}$ | 76.5 (61.0)$^{b}$ |
| 13. | *$T_3$+$T_4$* | 8.0 (16.4)$^{d}$ | 77.1 (61.4)$^{c}$ | 26.3 (30.8)$^{d}$ | 62.0 (51.9)$^{d}$ |
| 14. | *$T_5$+$T_6$* | 5.0 (12.9)$^{c}$ | 85.7 (68.1)$^{b}$ | 21.7 (27.7)$^{c}$ | 68.7 (56.0)$^{c}$ |
| 15. | *Control* | 35.0 (36.3)$^{m}$ | - | 69.3 (56.4)$^{l}$ | - |

* Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT.

**Table 39. Effect of bioformulations on the Root rot incidence of Coleus under field condition - Kaniyamoor**

| S.No | Treatments | 45 DAP* | | 90 DAP* | |
|---|---|---|---|---|---|
| | | Root rot (%) | Inhibition over control (%) | Root rot (%) | Inhibition over control (%) |
| 1. | PFC6 | 50.0 (45.0)$^{cd}$ | 43.1(41.0)$^{ef}$ | 95.3 (77.5)$^{c}$ | 4.6 (12.3)$^{e}$ |
| 2. | *Pf1* | 58.0 (46.1)$^{d}$ | 34.0 (35.6)$^{g}$ | 98.3 (82.6)$^{d}$ | 1.6 (7.2)$^{g}$ |
| 3. | *PFC6+WGO + $ZnSO_4$* | 48.0 (43.8)$^{cd}$ | 45.5 (42.4)$^{de}$ | 90.6 (72.2)$^{b}$ | 9.3 (17.5)$^{cd}$ |
| 4. | *Pf1+WGO+$ZnSO_4$* | 52.0 (49.6)$^{e}$ | 40.9 (39.7)$^{f}$ | 95.6 (78.0)$^{c}$ | 4.3 (11.9)$^{e}$ |
| 5. | T. harzianum- *Th* + *$ZnSO_4$* | 45.0 (42.1)$^{bc}$ | 48.8 (44.3)$^{cd}$ | 91.3 (72.9)$^{b}$ | 8.6 (17.0)$^{d}$ |
| 6. | T.viride-*$TVC_5$* + *$ZnSO_4$* | 64.0 (53.1)$^{f}$ | 27.2 (31.4)$^{h}$ | 96.0 (78.7)$^{c}$ | 3.6 (10.9)$^{f}$ |
| 7. | *Neem cake* | 68.0 (53.5)$^{fg}$ | 22.7 (28.4)$^{i}$ | 96.3 (79.2)$^{c}$ | 3.6 (10.9)$^{f}$ |
| 8. | *Zinc sulphate* | 70.0 (56.8)$^{g}$ | 20.4 (26.8)$^{i}$ | 98.3 (82.6)$^{d}$ | 1.6 (7.2)$^{g}$ |
| 9. | *EC-C* | 76.0 (60.7)$^{h}$ | 13.6 (21.7)$^{j}$ | 98.3 (82.6)$^{d}$ | 1.6 (7.2)$^{g}$ |
| 10. | *EC-F* | 80.0 (63.4)$^{h}$ | 10.0 (18.4)$^{k}$ | 95.8 (77.5)$^{c}$ | 4.6 (12.3)$^{e}$ |
| 11. | *Carbendazim* | 38.0 (38.1)$^{a}$ | 56.8 (48.9)$^{a}$ | 80.67 (63.9)$^{a}$ | 19.2 (25.9)$^{a}$ |
| 12. | *Propiconazole* | 40.0 (39.2)$^{ab}$ | 54.4 (47.5)$^{ab}$ | 90.3 (71.9)$^{b}$ | 9.6 (18.0)$^{bc}$ |
| 13. | *$T_3$ + $T_4$* | 45.0 (42.1)$^{bc}$ | 48.8 (44.3)$^{cd}$ | 95.7 (78.0)$^{c}$ | 4.2 (11.8)$^{e}$ |
| 14. | *$T_5$ + $T_6$* | 42.0 (40.4)$^{ab}$ | 52.2 (46.3)$^{bc}$ | 89.7 (71.2)$^{b}$ | 10.2 (18.6)$^{b}$ |
| 15. | *Control* | 88.0 (69.8)$^{i}$ | - | 99.9 (89.3)$^{e}$ | - |

* Mean of three replications

Values in parentheses are arcsine transformed

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 34. Rhizosphere populations of *P. fluorescens* – Kaniyamoor**

| S.No. | Treatments | *P. fluorescens* population* ($10^4$ cfu/g of soil) | | |
|---|---|---|---|---|
| | | Days after planting | | |
| | | 0 | 45 | 90 |
| 1. | PFC6 | $12.0^{f}$ | $36.0^{bc}$ | $47.0^{bc}$ |
| 2. | Pf1 | $15.0^{b-e}$ | $30.0^{d}$ | $45.0^{cd}$ |
| 3. | PFC6 + WGO + $ZnSO_4$ | $16.0^{abc}$ | $39.2^{a}$ | $52.0^{a}$ |
| 4. | Pf1 + WGO + $ZnSO_4$ | $17.2^{a}$ | $35.2^{c}$ | $50.0^{ab}$ |
| 5. | T. harzianum- *Th+ $ZnSO_4$* | $14.5^{cde}$ | $25.5^{g}$ | $38.0^{fg}$ |
| 6. | T.viride-*$TVC_5$+ $ZnSO_4$* | $15.0^{b-e}$ | $21.0^{fg}$ | $32.0^{ef}$ |
| 7. | *Neem cake* | $15.8^{abc}$ | $26.7^{e}$ | $40.0^{g}$ |
| 8. | *Zinc sulphate* | $13.5^{e}$ | $23.5^{f}$ | $36.0^{h}$ |
| 9. | *EC-C* | $13.8^{de}$ | $16.3^{I}$ | $26.0^{ij}$ |
| 10. | *EC-F* | $14.0^{de}$ | $16.9^{hi}$ | $26.5^{i}$ |
| 11. | *Carbendazim* | $16.4^{ab}$ | $19.0^{ghi}$ | $28.0^{ij}$ |
| 12. | *Propiconazole* | $15.2^{abc}$ | $18.1^{ghi}$ | $27.0^{ij}$ |
| 13. | *$T_3$ + $T_4$* | $13.8^{de}$ | $39.0^{a}$ | $48.0^{bc}$ |
| 14. | *$T_5$ + $T_6$* | $14.7^{cde}$ | $21.0^{fg}$ | $31.8^{ef}$ |
| 15. | *Control* | $15.1^{bcd}$ | $19.5^{gh}$ | $30.1^{hi}$ |

* Mean of three replications
In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Table 33. Rhizosphere populations of *Trichoderma* species – Kaniyamoor**

| S.No. | Treatments | *Trichoderma* population* ($10^3$ cfu/g of soil) | | |
|---|---|---|---|---|
| | | Days after planting | | |
| | | 0 | 45 | 90 |
| 1. | PFC6 | $1.30^{a}$ | $2.80^{def}$ | $4.0^{e}$ |
| 2. | Pf1 | $1.32^{a}$ | $3.0^{d}$ | $4.1^{e}$ |
| 3. | PFC6 + WGO + $ZnSO_4$ | $1.38^{a}$ | $2.60^{d-g}$ | $4.0^{e}$ |
| 4. | Pf1 + WGO + $ZnSO_4$ | $1.40^{a}$ | $2.52^{d-h}$ | $3.9^{e}$ |
| 5. | T. harzianum- *Th*+ $ZnSO_4$ | $1.10^{b}$ | $14.0^{a}$ | $20.0^{a}$ |
| 6. | T.viride-$TVC_5$+ $ZnSO_4$ | $1.40^{a}$ | $12.5^{b}$ | $18.0^{c}$ |
| 7. | *Neem cake* | $1.0^{b}$ | $4.2^{c}$ | $7.0^{d}$ |
| 8. | *Zinc sulphate* | $1.10^{b}$ | $4.0^{c}$ | $5.2^{b}$ |
| 9. | *EC-C* | $1.35^{a}$ | $2.3^{fgh}$ | $2.7^{fgh}$ |
| 10. | *EC-F* | $1.32^{a}$ | $2.4^{e-h}$ | $2.8^{gh}$ |
| 11. | *Carbendazim* | $1.10^{b}$ | $2.15^{gh}$ | $2.9^{gh}$ |
| 12. | *Propiconazole* | $1.36^{a}$ | $2.0^{h}$ | $2.5^{h}$ |
| 13. | $T_3$ + $T_4$ | $1.35^{a}$ | $2.87^{de}$ | $3.6^{efg}$ |
| 14. | $T_5$ + $T_6$ | $1.40^{a}$ | $12.7^{b}$ | $19.7^{a}$ |
| 15. | *Control* | $1.30^{a}$ | $2.60^{d-g}$ | $3.8^{ef}$ |

* Mean of three replications

In a column, means followed by a common letter (s) are not significantly different (P=0.05) by DMRT

**Fig 14a. Biocontrol determinants of *P.fluorescens*-PFC6**

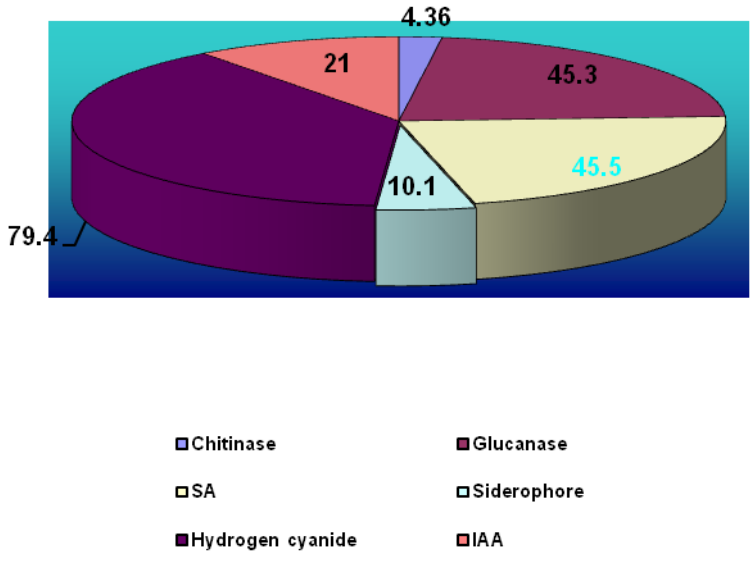


**Fig 14b. Biocontrol determinants of *P. fluorescens* -Pf1**

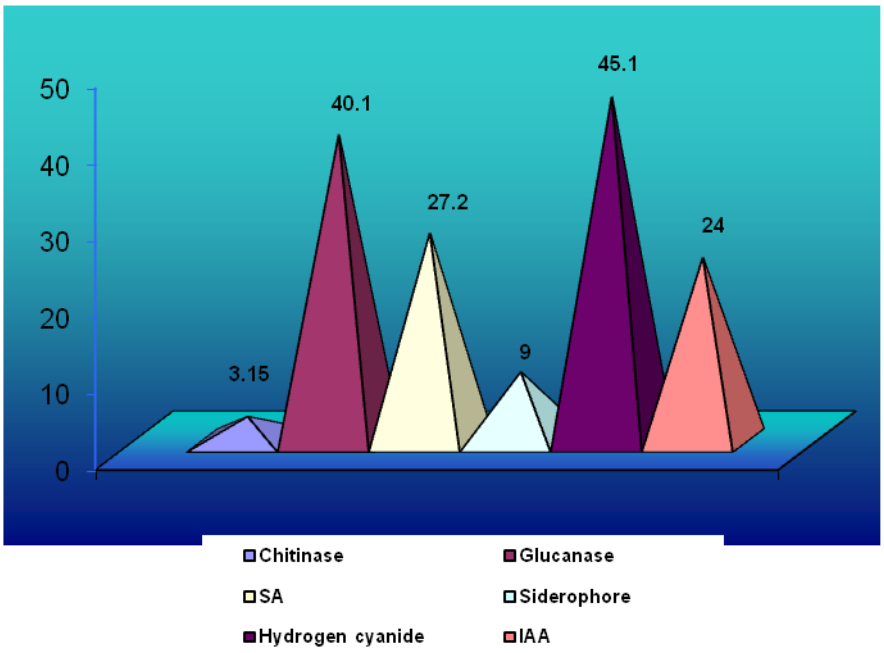


**Fig 12. Induction of phenol by bioformulations against *M.phaseolina***

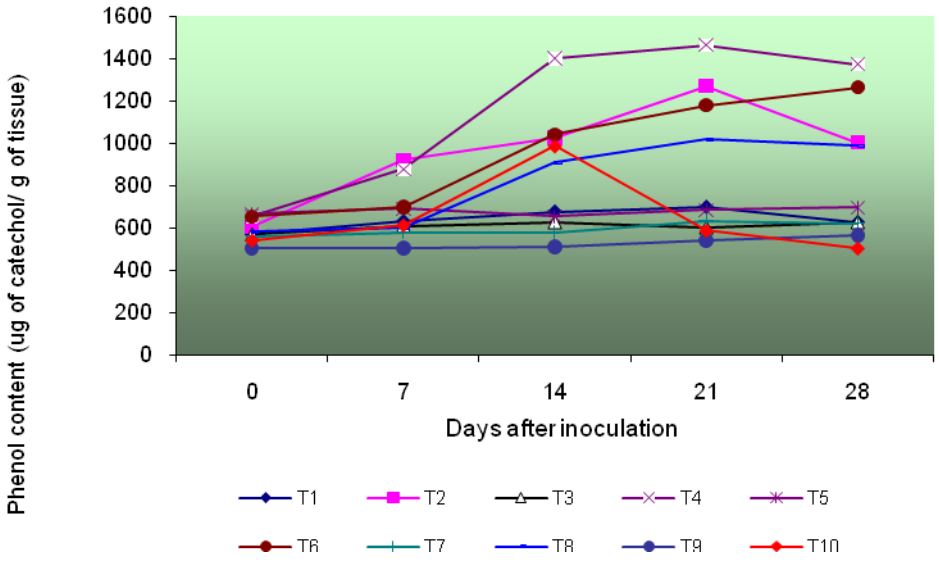


**Fig 13. Induction of phenol by bioformulations against *R. solani***

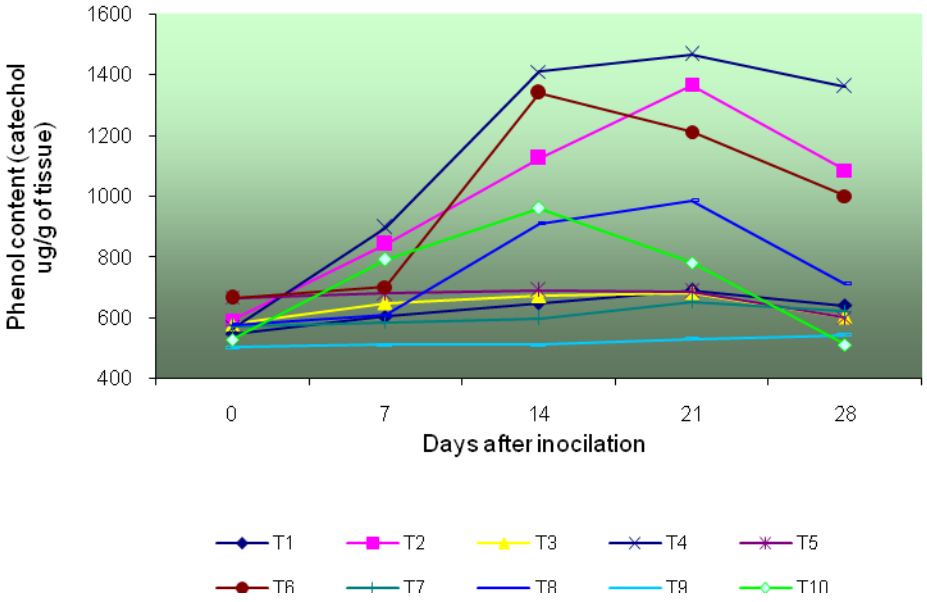



**Fig 10. Induction of B-1,3-glucanase activity by bioformulations against *M. phaseolina***

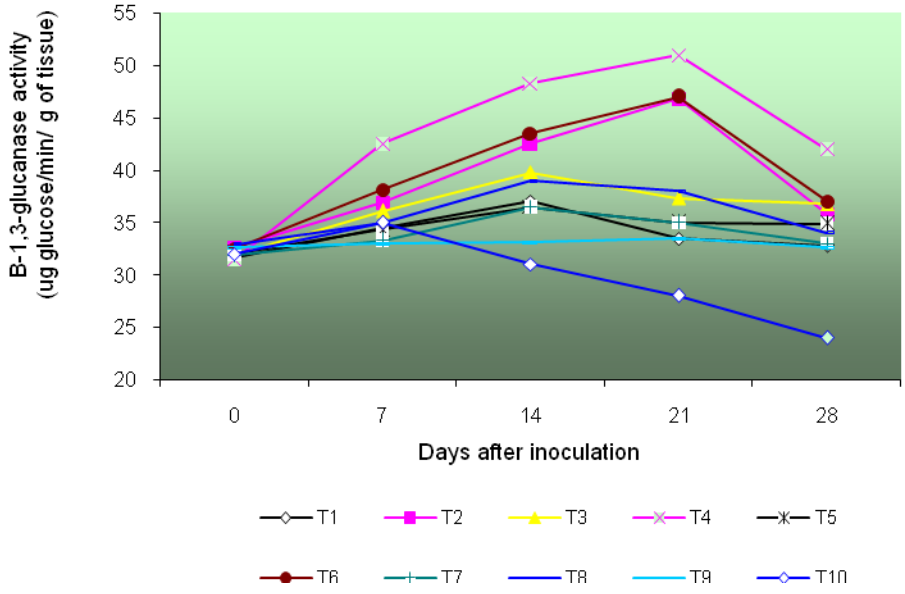


**Fig 11. Induction of B- 1,3- glucanase activity by bioformulations against *R.solani***

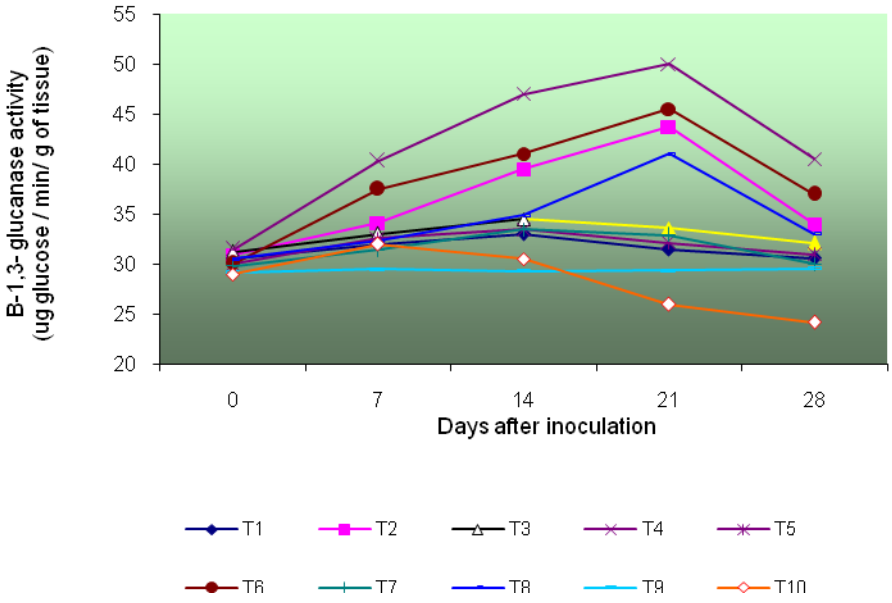


**Fig 8. Induction of chitinase by bioformulations against *M. phaseolina***

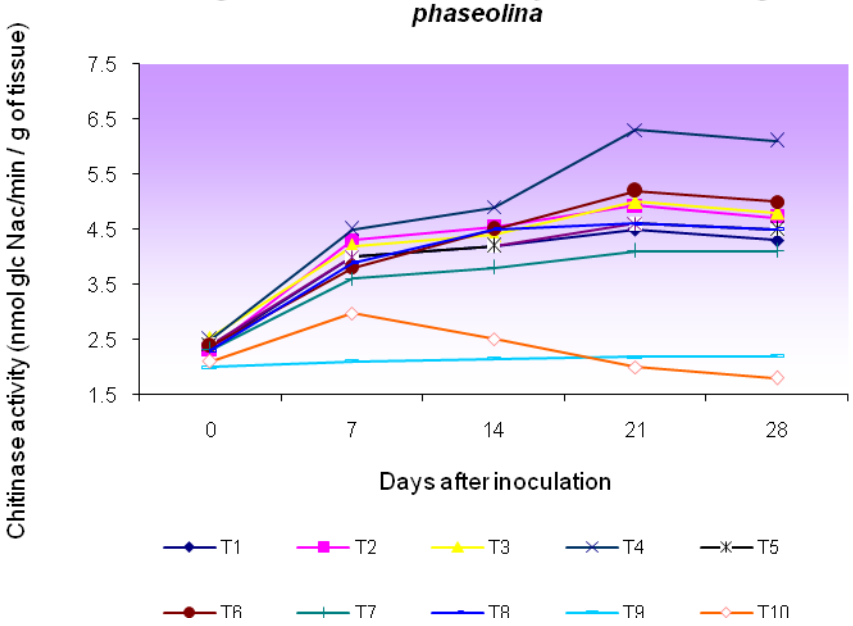


**Fig 9. Induction of chitinase activity by bioformulations against *R. solani***

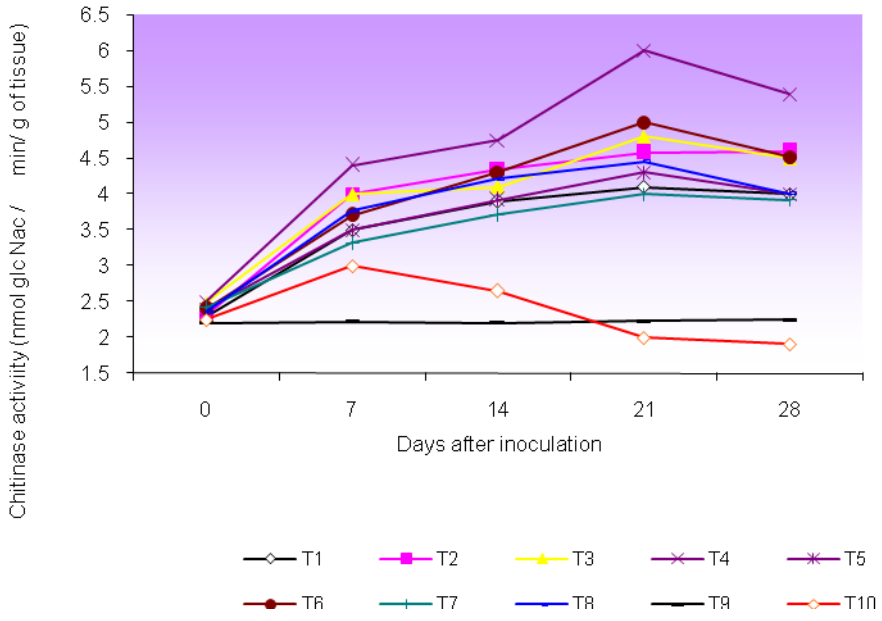


**Fig 6. Induction of PPO activity by bioformulations against *M. phaseolina***

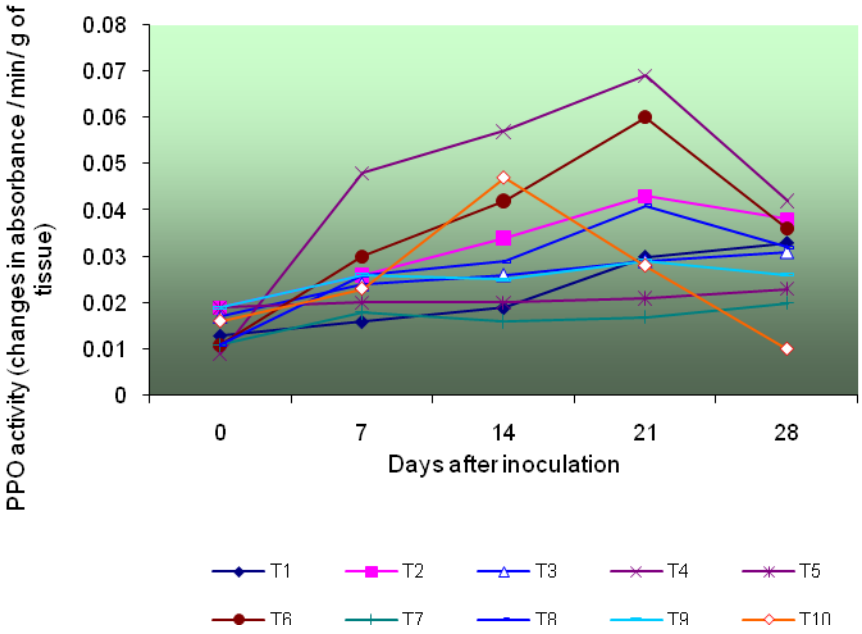


**Fig 7. Induction of PPO activity by bioformulations against *R. solani***

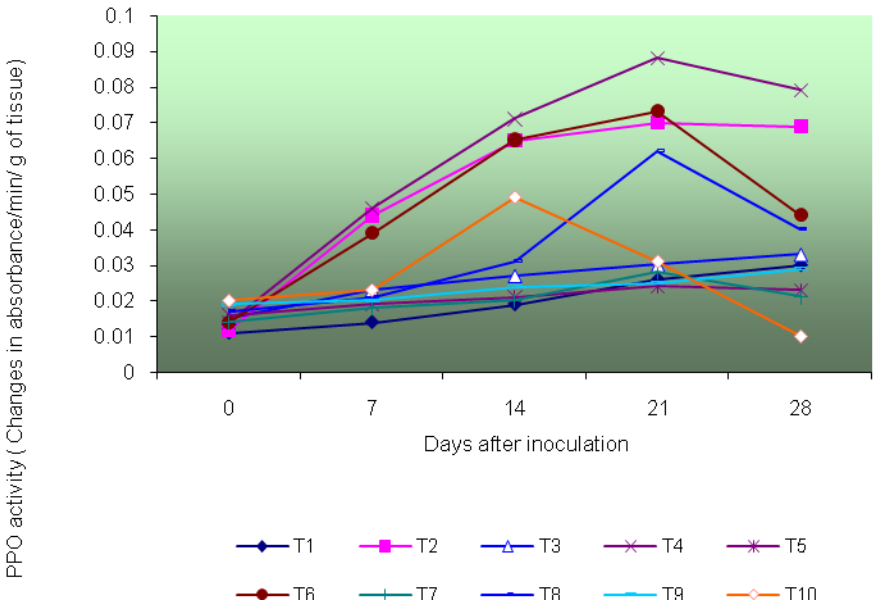



**Fig 4. Induction of peroxidase activityby bioformulations against *M. phaseolina***

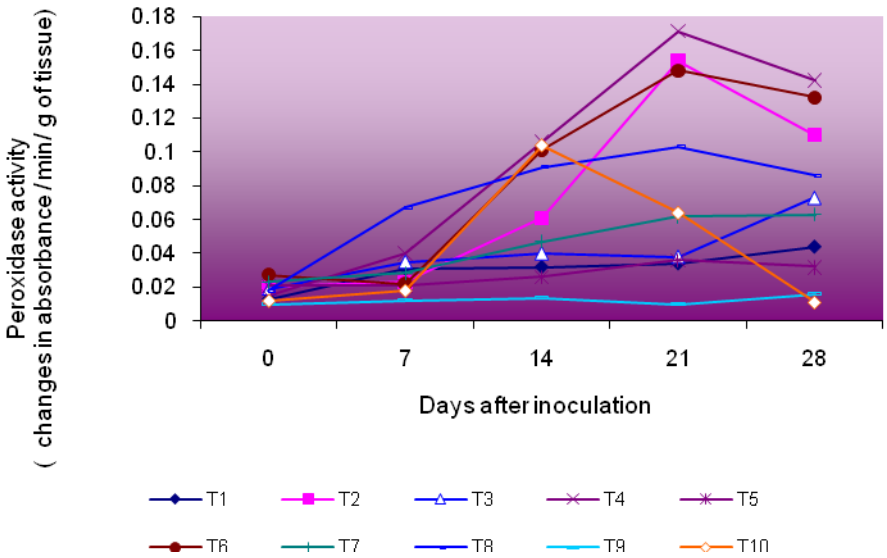


**Fig 5. Inductuion peroxidase activity by bioformulationsagainst *R. solani***

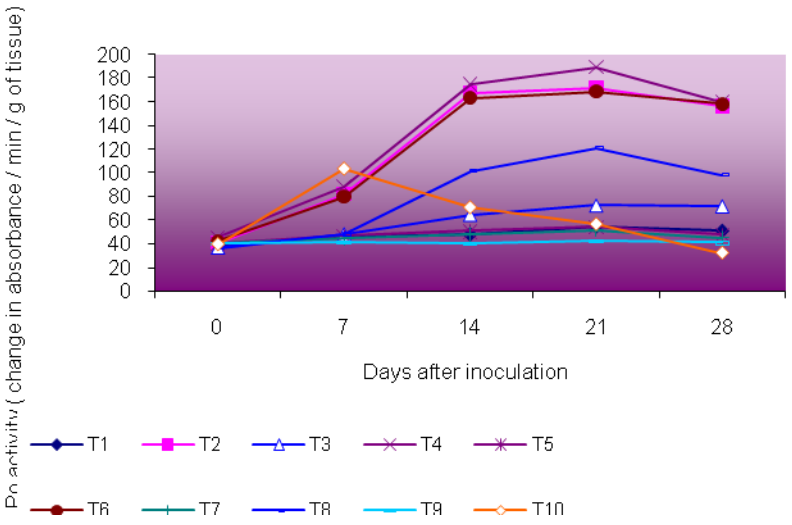



**Fig 2. Induction of PAL activity by bioformulations against *M. phaseolina***

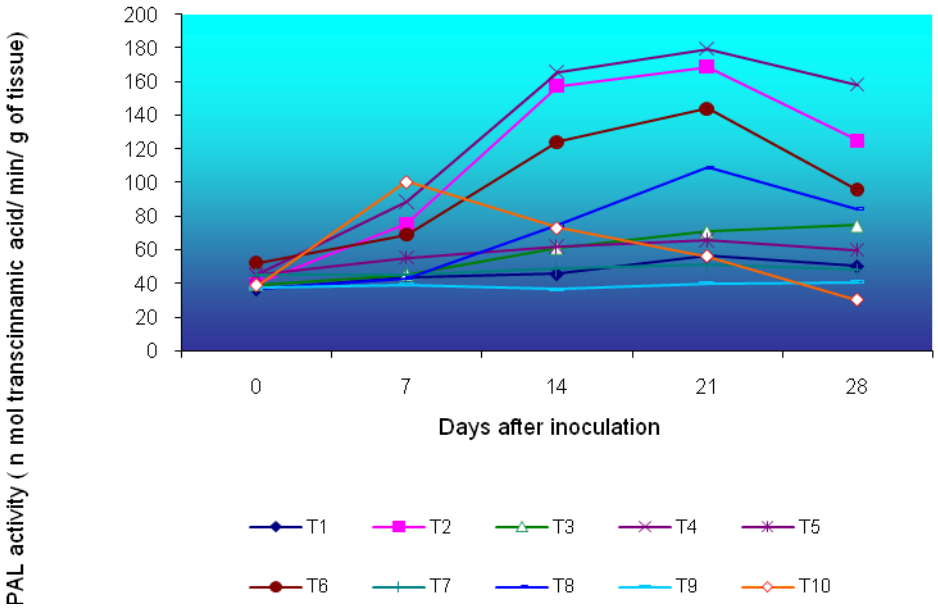


**Fig 3. Induction of PAL activity by bioformulations against *R. solani***

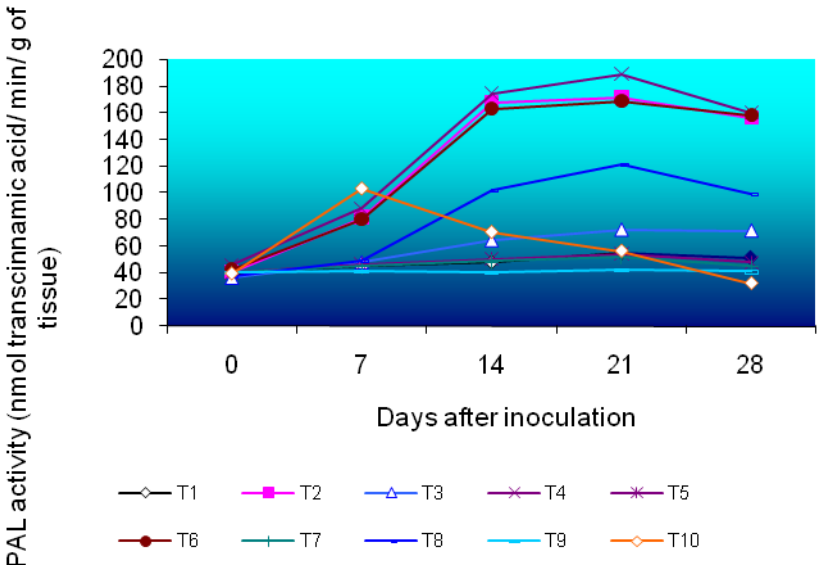
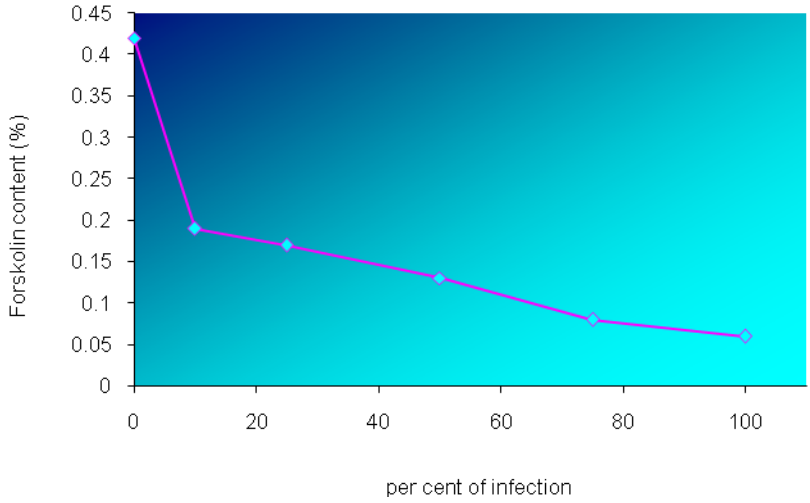


**Fig.1. Loss off Forskolin alkaloid due to root rot infection**



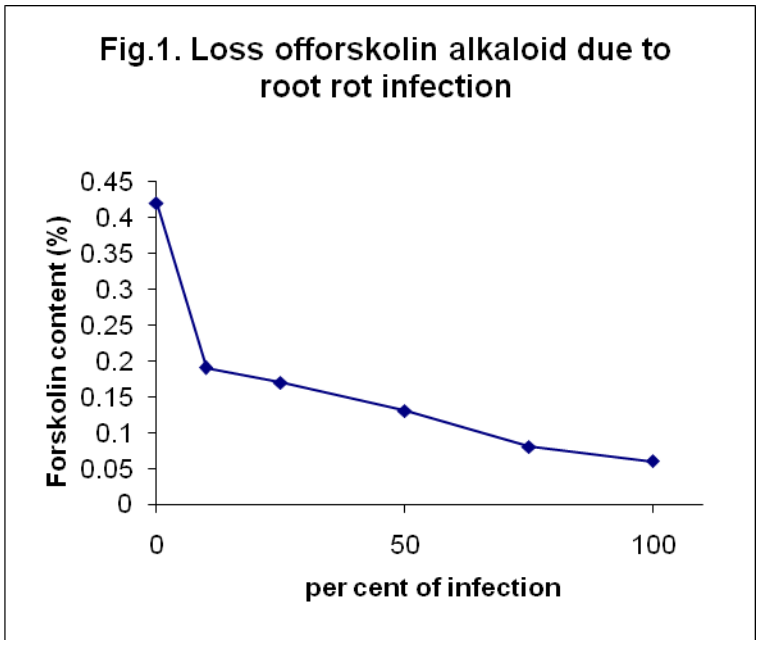



**Fig 7. Induction of chitinase by bioformulations against *M. phaseolina***

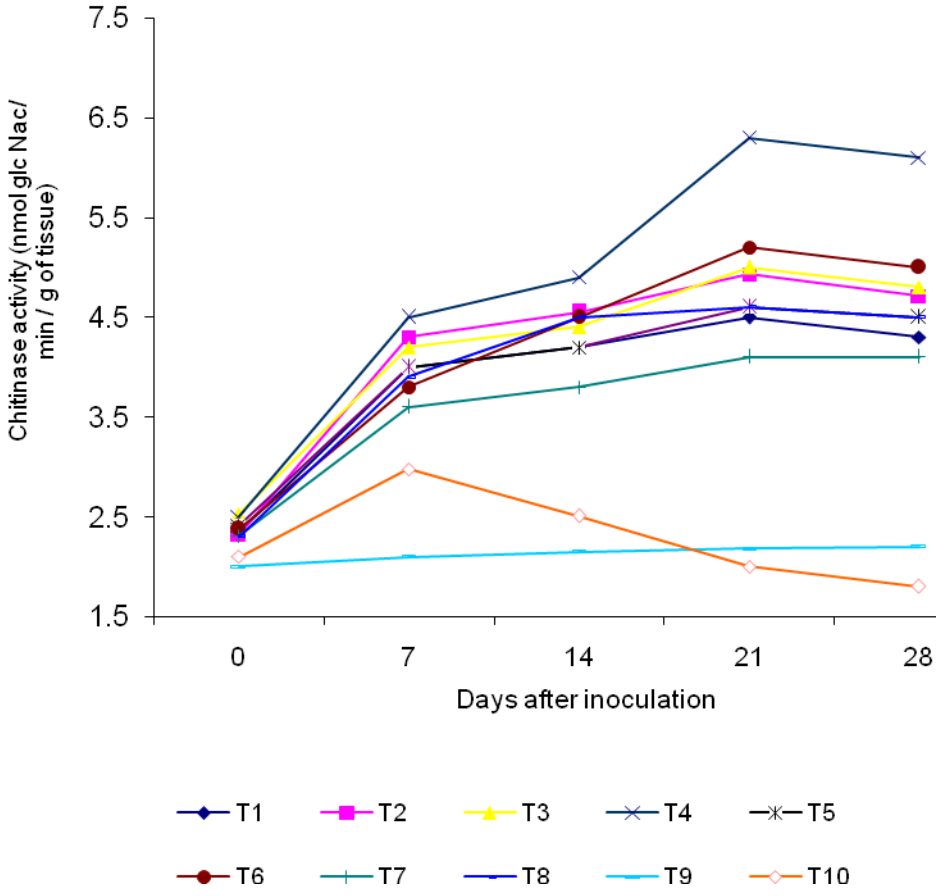


Fig 9. Induction of B-1,3-glucanase activity by bioformulations against *M. phaseolina*

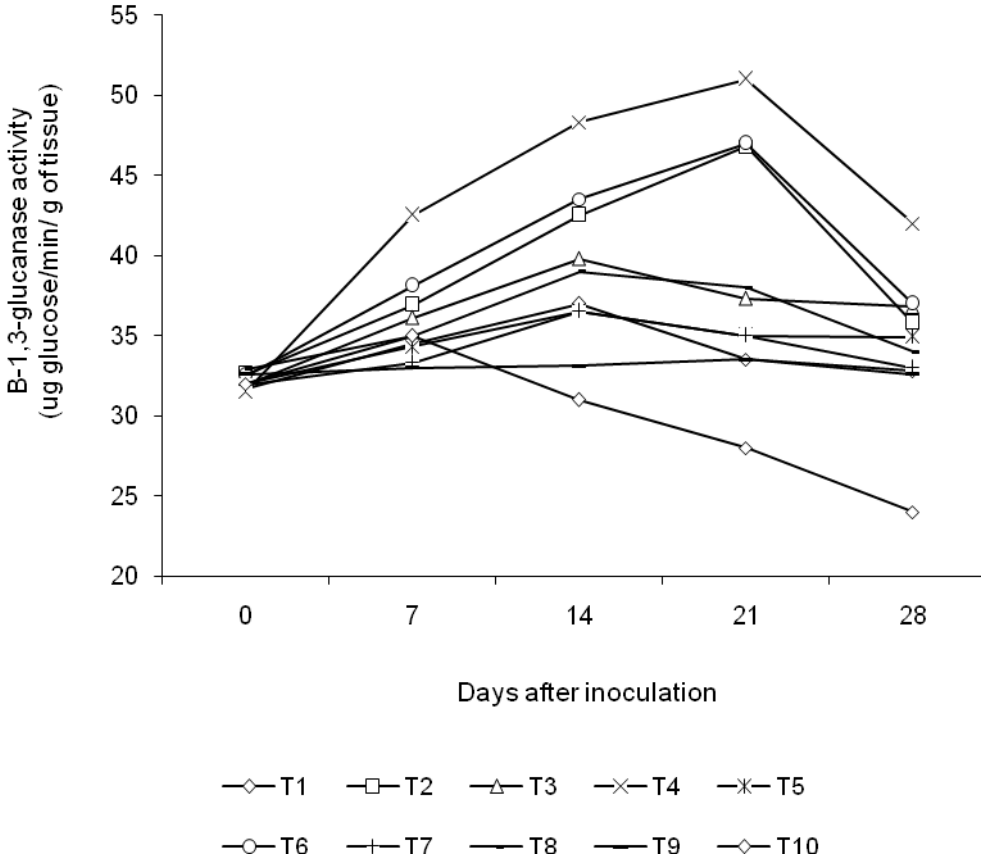
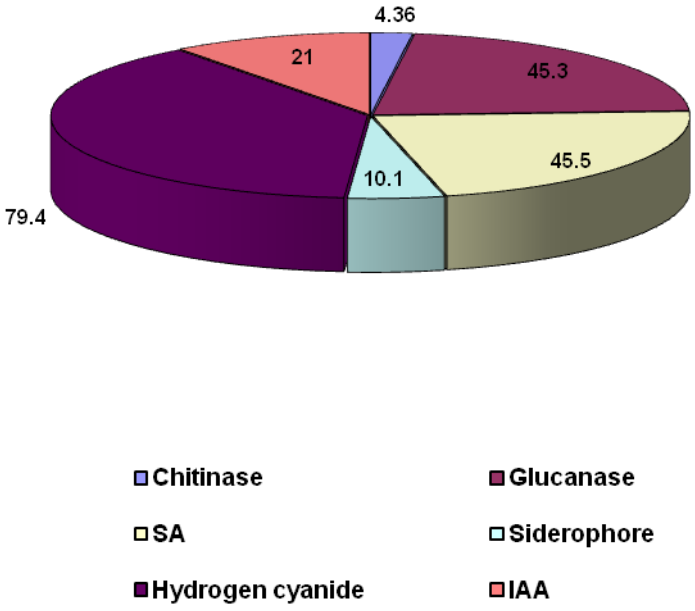


**Fig 13a. Biocontrol determinants of *P.fluorescens*-PFC6**



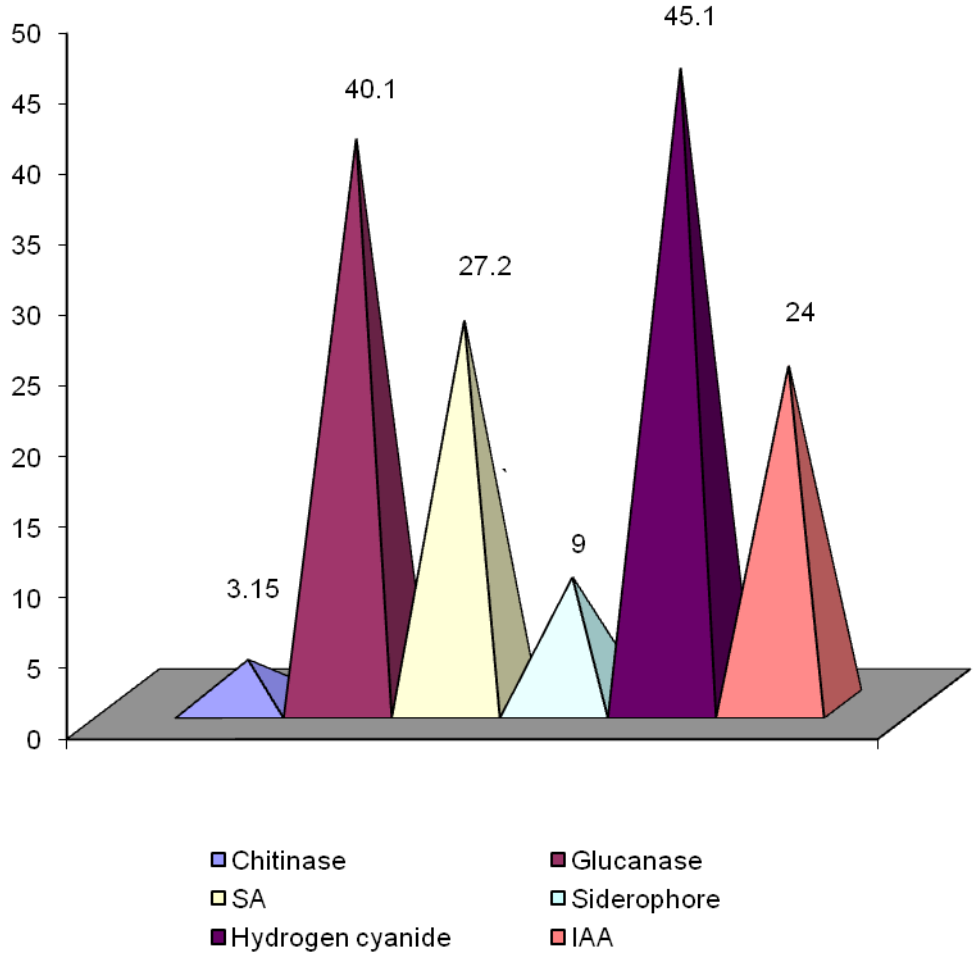
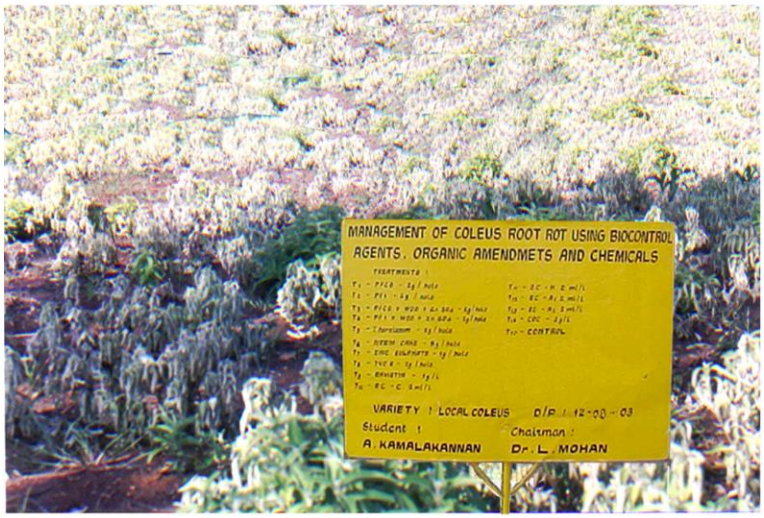
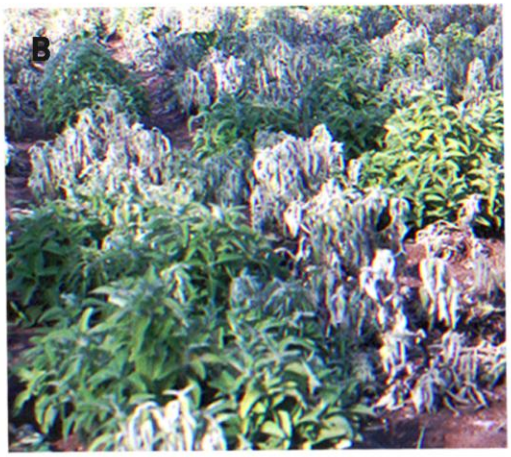

50
45
40
35
30
25
20
15
10
5
0
3.15
40.1
27.2
9
45.1
24
Chitinase
Glucanase
SA
Siderophore
Hydrogen cyanide
IAA

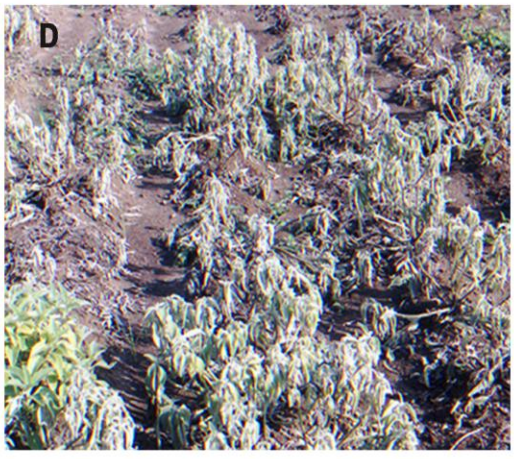
Plate 18. Management of coleus root rot under field condition -Kaniyamoor









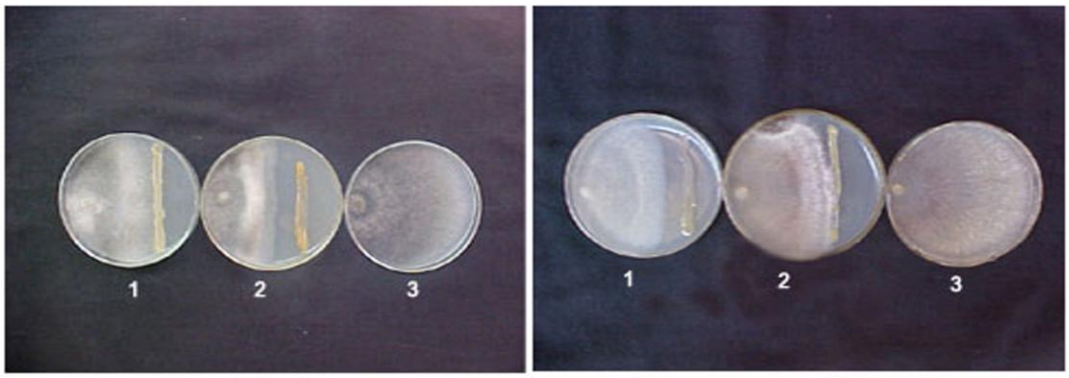



A. Carbendazim B. Th + ZnSO4 C. PFC6 + WGO + ZnSO4 D. Control

Plate 7a. Cultures of *Pseudomonas fluorescens*

PFC6 Pf1

Plate 7b. Effect of *P. fluorescens* strains against *M. phaseolina* and *R. solani*




*M. phaseolina* *R. solani*

1. Pf 1 2.PFC6 3.Control 1. PFC6 2. Pf1 3.Control

Plate 4c. Different levels of root rot infection (%)

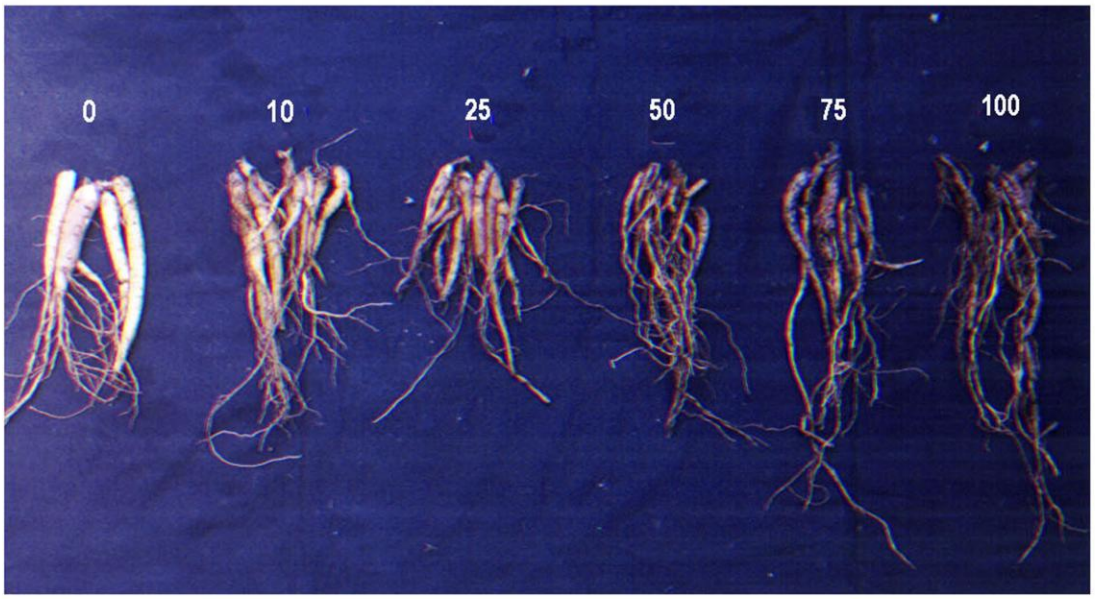
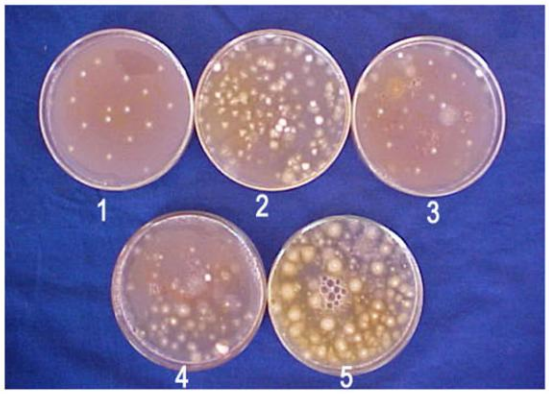
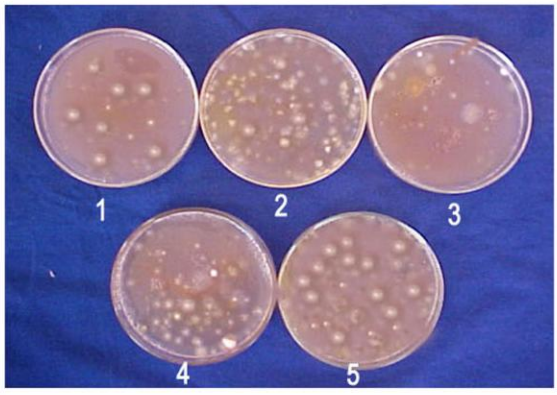



Plate 5. Sclerotial population of *M. phaseolina*




1. Melur -II
2. Kumbakottai - I
3. Sangarapuram - III
4. Inthili - II
5. Kaniyamoor - II




1. Paithur - I
2. Keeripatti - I
3. Valapadi - I
4. Paithur - II
5. Coimbatore - II

Plate 3d i. Root rot symptom of *R. solani*


1. infected 2. Healthy

Plate 3d ii. Rotting of collar region and roots

Plate 3a. *Rhizoctonia solani* culture

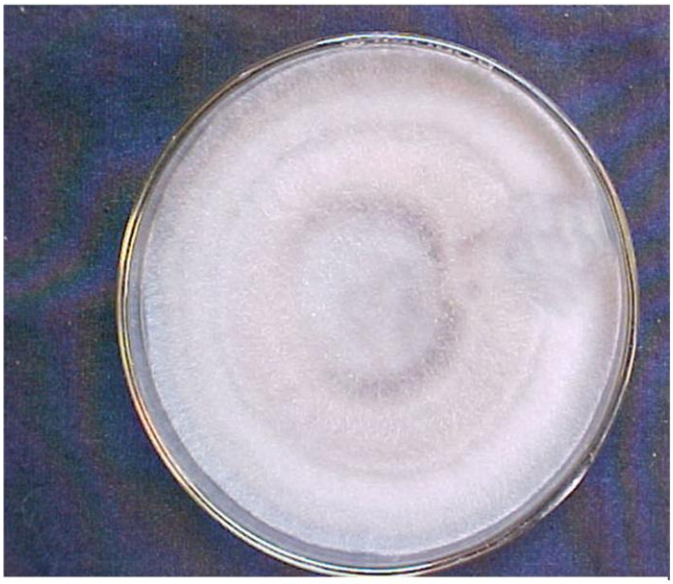


Plate 3b i. Spongy sclerotia

Plate 3b ii. *R. solani* mycelium

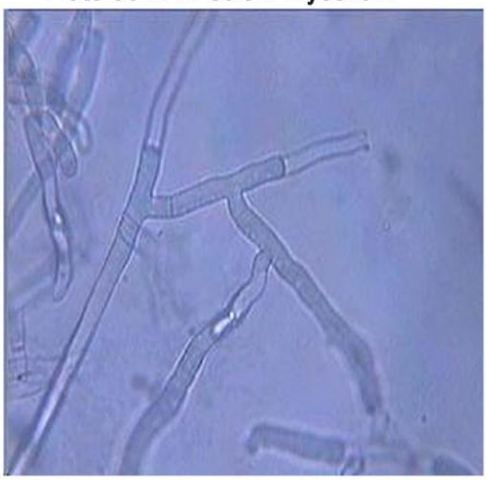


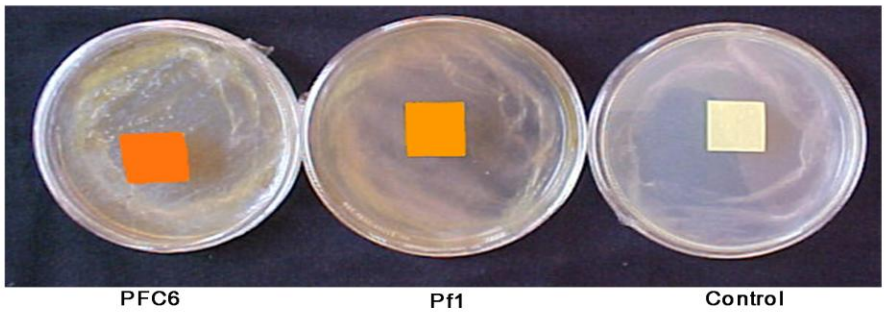
Plate 1a. Root rot incidence in Kaniyamoor village (Villupuram district)

Plate 1b. Root rot incidence in Neermullikuttai village (Salem district)

**Plate 23. Siderophore production by *P. fluorescens* strains**

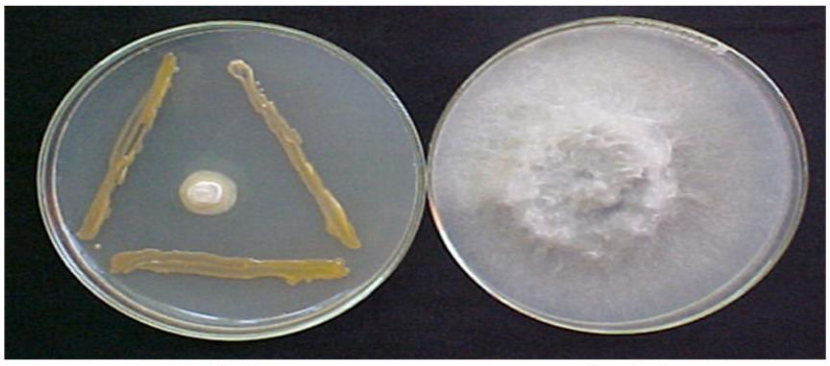
PFC6 Pf1 Control

**Plate 24. HCN production by *P. fluorescens* strains**



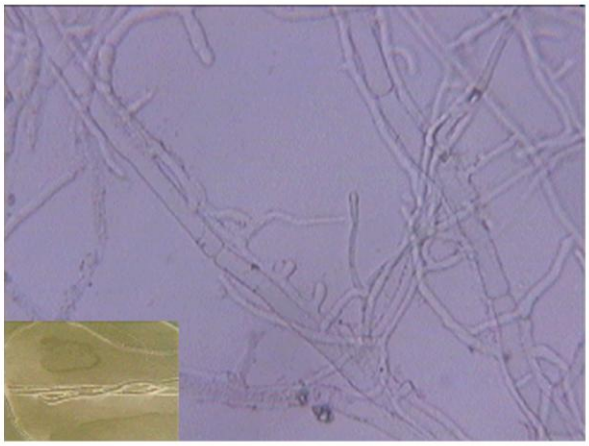
PFC6 Pf1 Control

**Plate 25. Effect of diffusible metabolites of PFC6 on growth of *M. phaseolina***

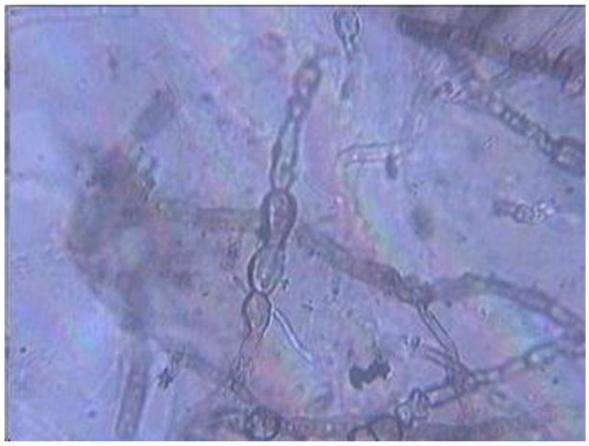


PFC6 Control

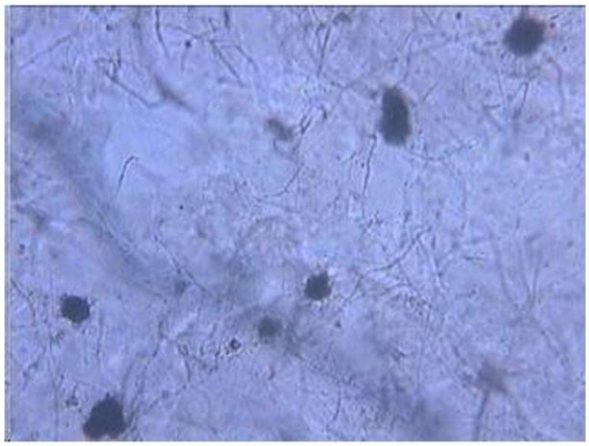
Plate 22. Mycoparasitism of *T. harzianum*



Hyphal coiling

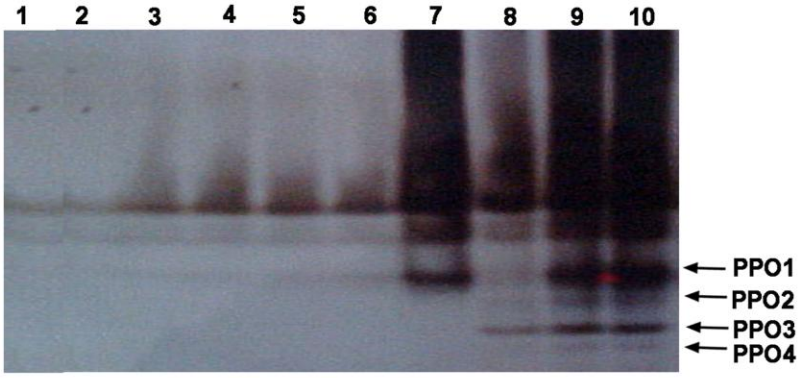


Hyphal malformation



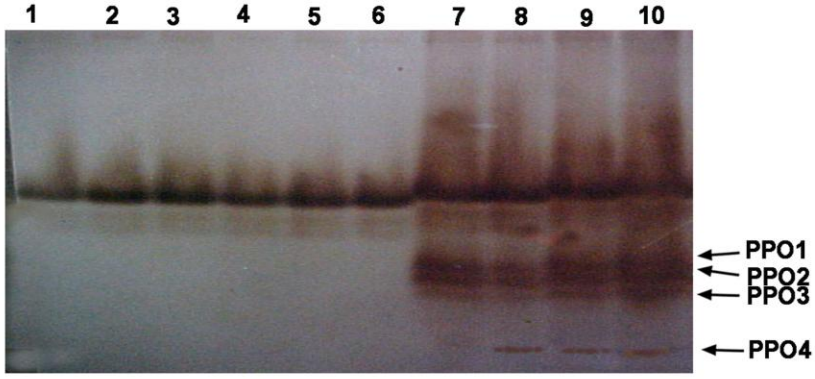
Inhibition of sclerotial production

Plate 21a. Native-PAGE profile of polyphenol oxidase isoforms induced by bioformulations in coleus on 21st day after challenge inoculation with or without *M. phaseolina.*



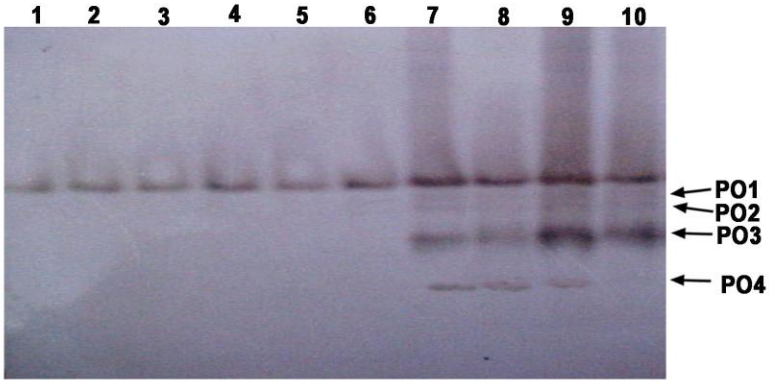

Lane 1, healthy control; Lane 2, control inoculated with *M. phaseolina*; Lane 3, Th + ZnSO4; Lane 4, TVC5 + ZnSO4; Lane 5, PFC6 + WGO + ZnSO4; Lane 6, Pf1 + WGO + ZnSO4; Lane 7, Th + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 8, TVC5 + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 9, PFC6 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 10, Pf1 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*.

Plate 21b. Native-PAGE profile of polyphenol oxidase isoforms induced by bioformulations in coleus on 21st day after challenge inoculation with or without *R. solani.*



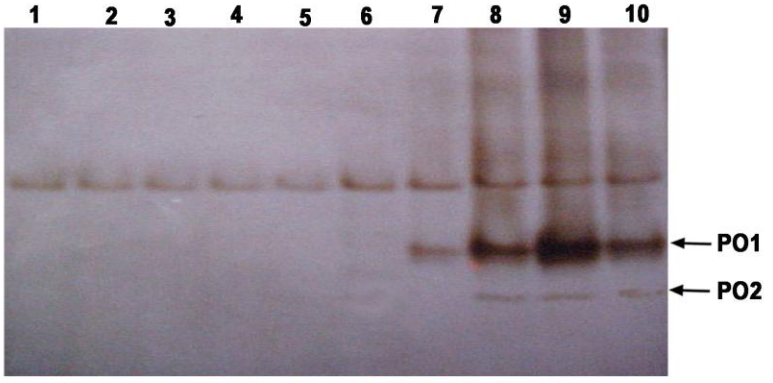

Lane 1, healthy control; Lane 2, control inoculated with *M. phaseolina*; Lane 3, Th + ZnSO4; Lane 4, TVC5 + ZnSO4; Lane 5, PFC6 + WGO + ZnSO4; Lane 6, Pf1 + WGO + ZnSO4; Lane 7, Th + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 8, TVC5 + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 9, PFC6 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 10, Pf1 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*.

**Plate 20a. Native-PAGE profile of peroxidase isoforms induced by bioformulations in coleus on 21st day after challenge inoculation with or without *M. phaseolina***




Lane 1, healthy control; Lane 2, control inoculated with *M. phaseolina*; Lane 3, Th + ZnSO4; Lane 4, TVC5 + ZnSO4; Lane 5, PFC6 + WGO + ZnSO4; Lane 6, Pf1 + WGO + ZnSO4; Lane 7, Th + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 8, TVC5 + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 9, PFC6 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 10, Pf1 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*.

**Plate 20b. Native-PAGE profile of peroxidase isoforms induced by bioformulations in coleus on 21st day after challenge inoculation with or without *R. solani***



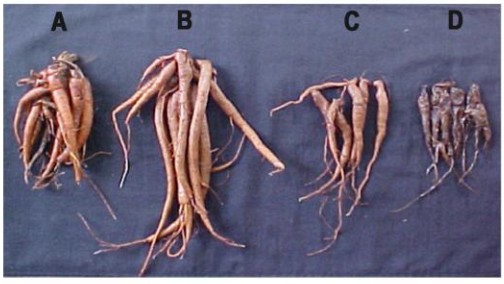

Lane 1, healthy control; Lane 2, control inoculated with *M. phaseolina*; Lane 3, Th + ZnSO4; Lane 4, TVC5 + ZnSO4; Lane 5, PFC6 + WGO + ZnSO4; Lane 6, Pf1 + WGO + ZnSO4; Lane 7, Th + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 8, TVC5 + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 9, PFC6 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*; Lane 10, Pf1 + WGO + ZnSO4 treated and inoculated with *M. phaseolina*.

Plate 16. Management of coleus root rot under field condition - Neermullikuttai



A. Carbendazim B. Th + ZnSO4 C. PFC6 + WGO + ZnSO4 D. Control

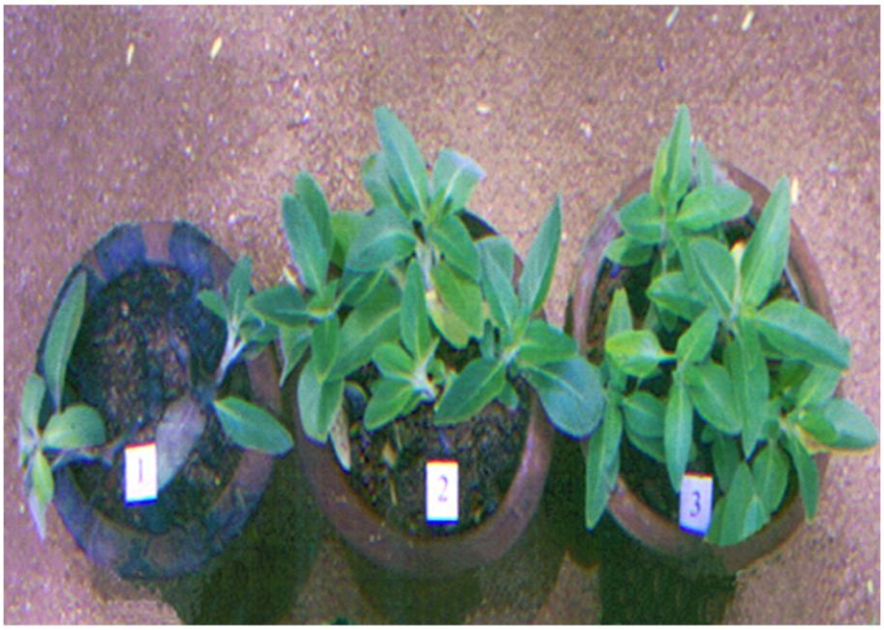
Plate 17. Effect of bioformulation on the yield of Coleus




A. Carbendazim B. PFC6 + WGO + ZnSO4 C. Th + ZnSO4 D. Control

Plate 15a. Effect of fungicides on the Macrophomina root rot


Plate 15b. Effect of fungicides on the Rhizoctonia root rot




1. Control 2. Carbendazim 3. Propiconazole

**Plate 14a. Effect of bioformulations on the Macrophomina root rot**


1. Control 2. Th + $ZnSO_4$ 3. PFC6 + WGO + $ZnSO_4$ 4. Carbendazim

**Plate 14b. Effect of bioformulations on the Rhizoctonia root rot**


1. Control 2. Pf1 + WGO + $ZnSO_4$ 3. PFC6 + WGO + $ZnSO_4$
4. TVC5 + $ZnSO_4$ 5. Th + $ZnSO_4$ 6. Carbendazim

Plate 8. Effect of *B. subtilis* strains on the mycelial growth of *M. phaseolina*

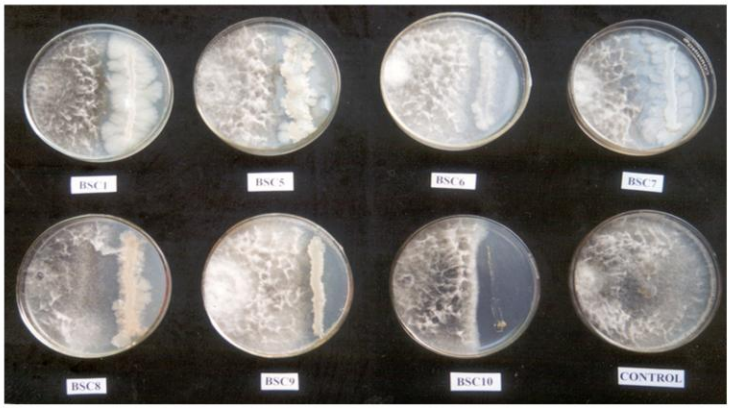
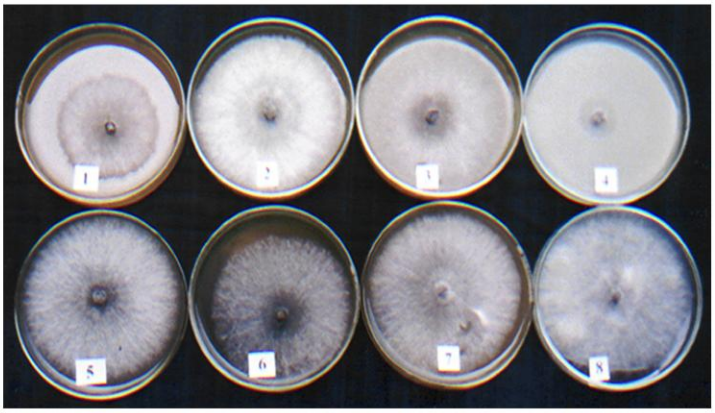
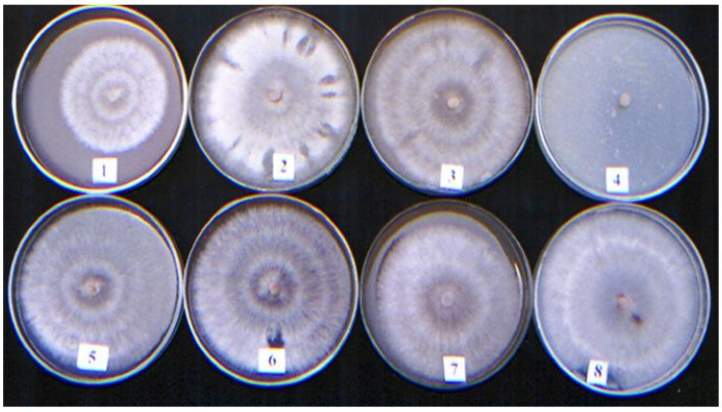



Plate 9a. Effect of plant extracts on the mycelial growth of *M. phaseolina*




1. *L. inermis* 2. *L. camera* 3. *A. paniculata* 4. *A. sativum* 5. *C. barbatus*
6. *V. negunda* 7. *A. indica* 8. Control

Plate 9b. Effect of plant extracts on the mycelial growth of *R. solani*




1. *L. inermis* 2. *L. camera* 3. *A. paniculata* 4. *A. sativum* 5. *C. barbatus*
6. *V. negunda* 7. *A. indica* 8. Control

Plate 6a. Effect of *Trichoderma* spp. on the mycelial growth of *M. phaseolina*

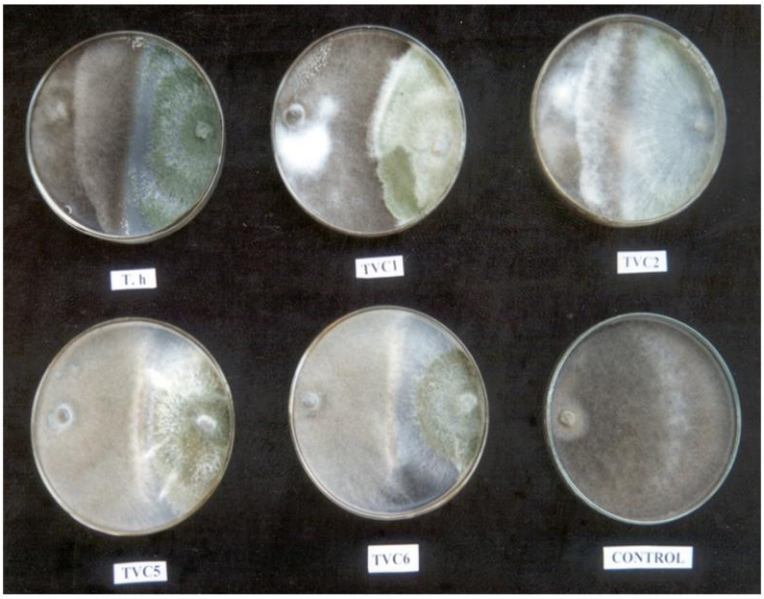



Plate 6b. Effect of *Trichoderma* spp. on the mycelial growth of *R. solani*

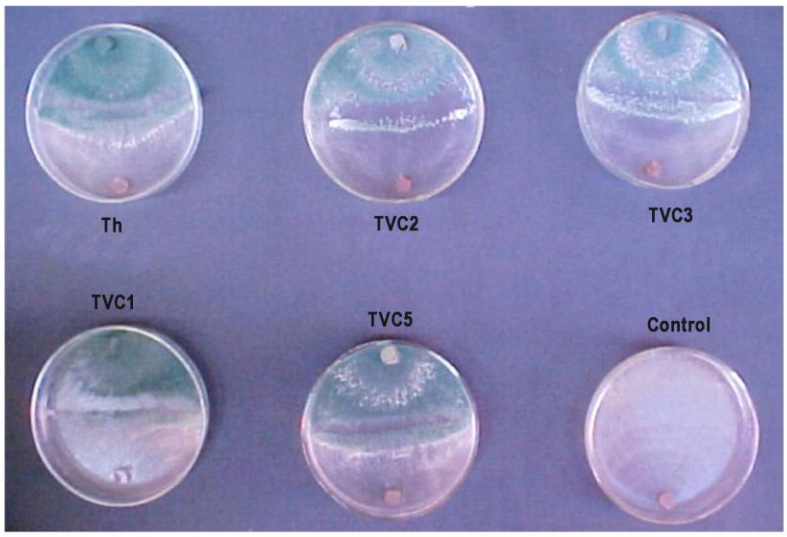
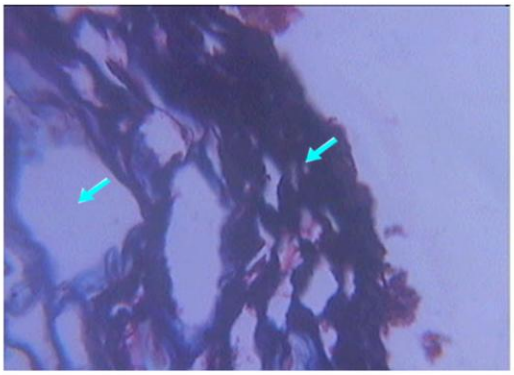
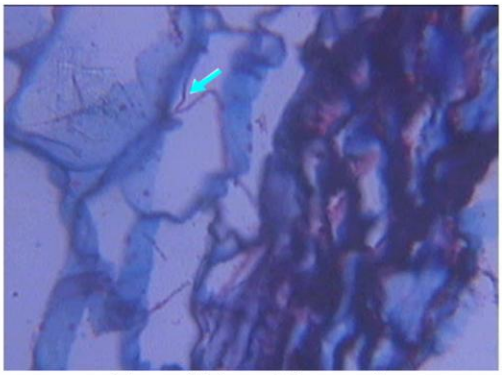


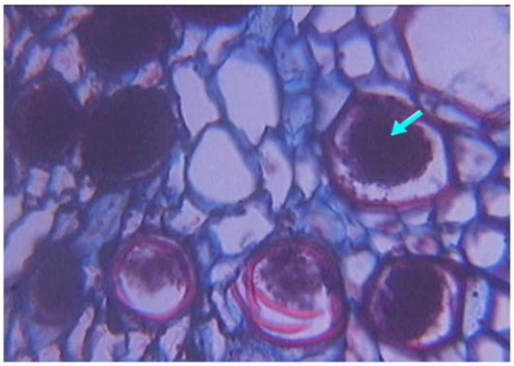
Plate 4b. Cross section of infected roots of Coleus



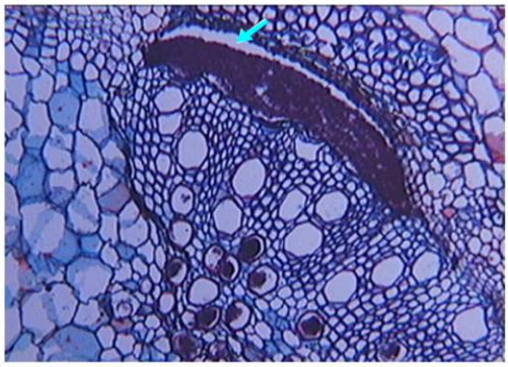
Damaged epidermal layer and larger cavities



Inter and intra cellular mycelium

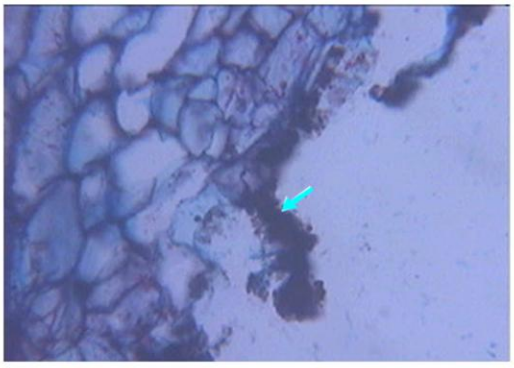


Blockage of proto and meta xylem

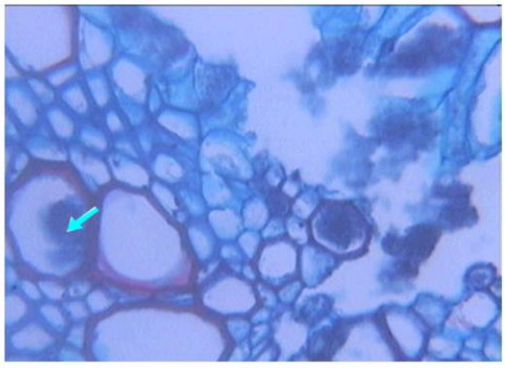


Formation of corky layer

Plate 4b. ii. Cross section of infected stem of Coleus

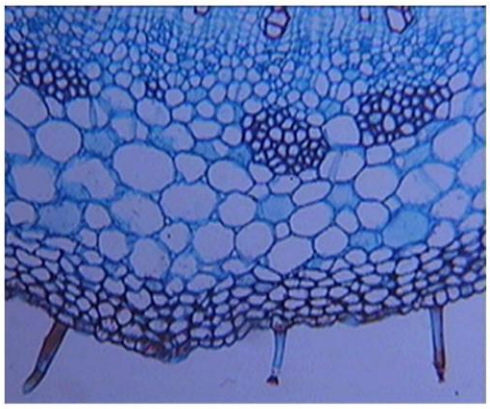


Distruption of epidermal layer

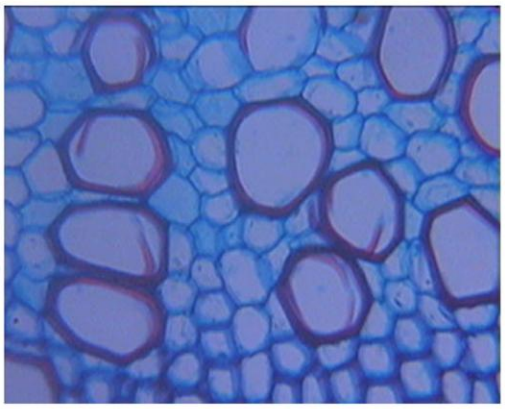


Blockage of xylem vessels

Plate 4a i. Cross section of healthy root of Coleus

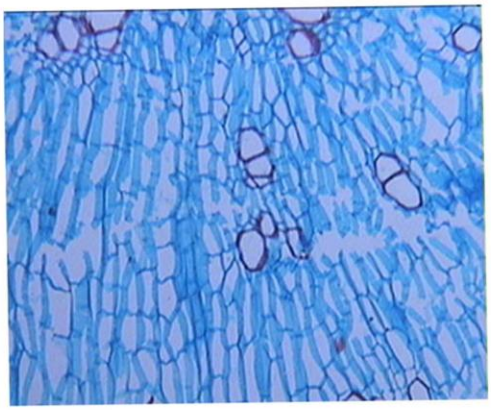


Intact epidetmal layer and cortical parenchyma cells

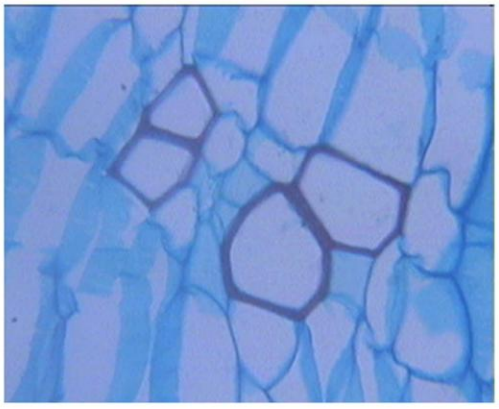


Healthy proto and meta xylem

Plate 4a ii. Cross section of healthy stem of Coleus



Intact cortical parenchyma and seive tubes



Opened xylem vessels

Plate 3c. Symptoms of *M. phaseolina* root rot in Coleus

i. Wilting of plants

ii. Drying of stem and branches

iii. Collapsed infected plant

iv. Rotting of roots

Plate 2a. *Macrophomina phaseolina* culture

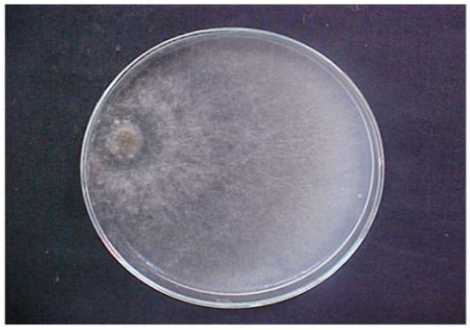


Plate 2b. Mycelium of *M. phaseolina*

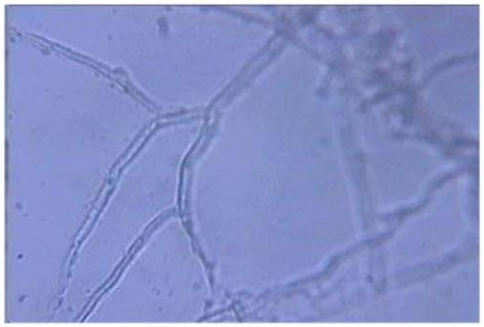


Plate 2c. Microsclerotia of *M. phaseolina*

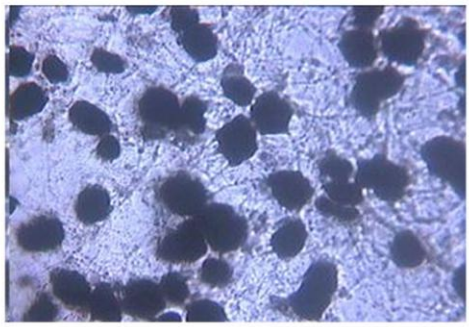


Plate 2d. Sclerotium with mycelial appendages

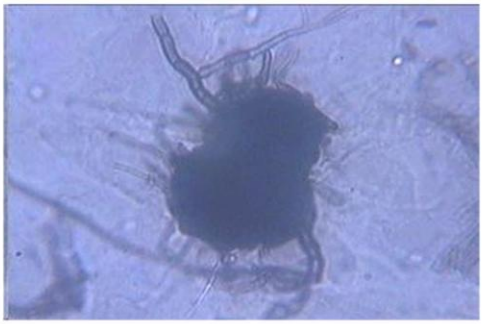


Plate 13a. Compatibility of *P. fluorescens* and *Trichoderma* sp. with $ZnSO_4$

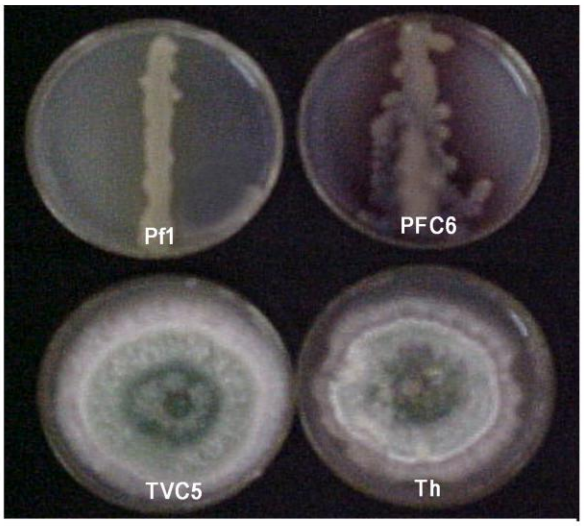



Plate 13b. Compatibility of *P. fluorescens* and *Trichoderma* sp. with WGO

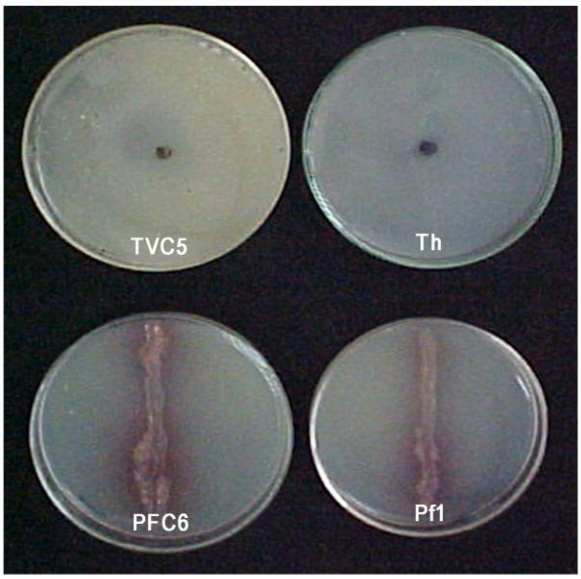
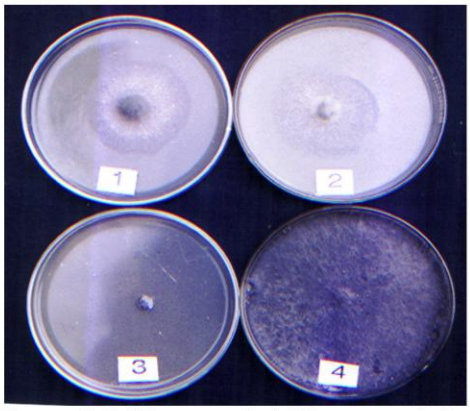
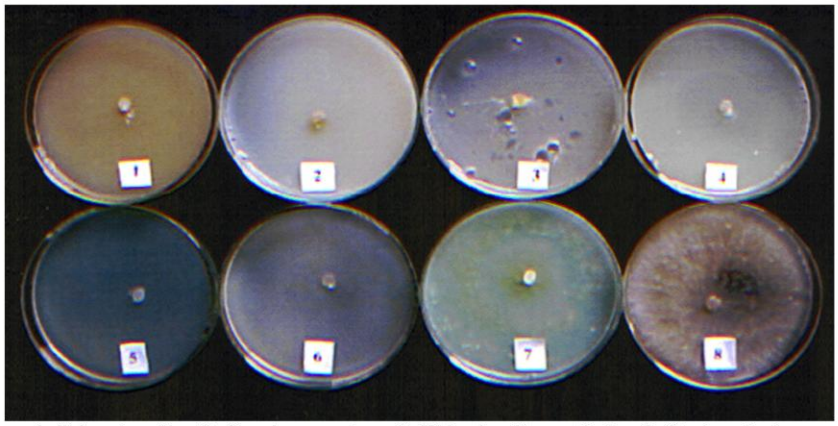


Plate 11. Effect of zinc sulphate on the mycelial growth of *M. phaseolina*



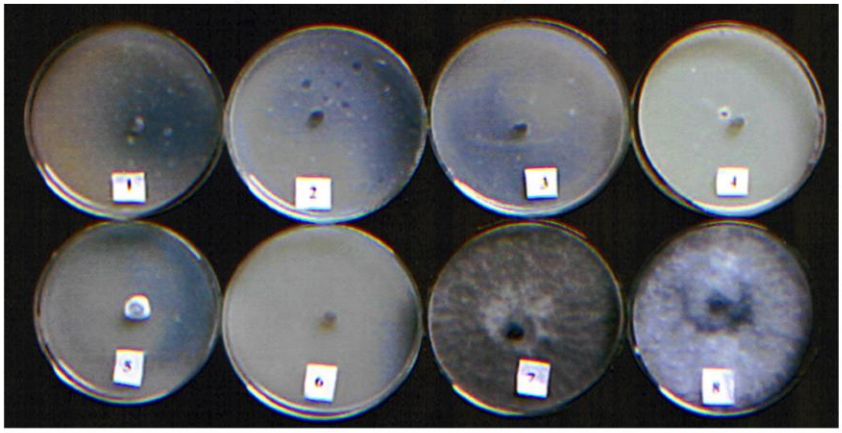
1. 0.25% 2. 0.5% 3. 1.0% 4. Control

Plate 12a. Effect of fungicides on the mycelial growth of *R. solani*



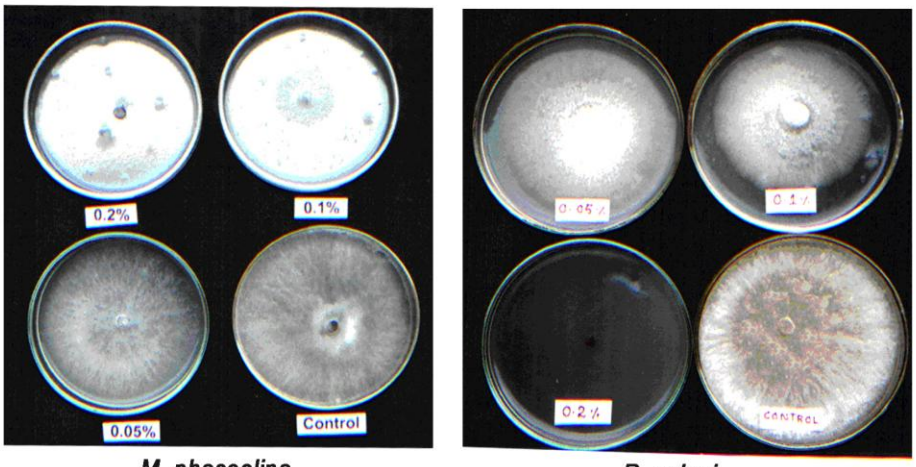
1. Tricyclazole 2. Propiconazole 3. Thiophenite methyl 4. Carbendazim
5. Hexaconazole 6. Thiram 7. COC 8. Control

Plate 12b. Effect of fungicides on the mycelial growth of *M. phaseolina*



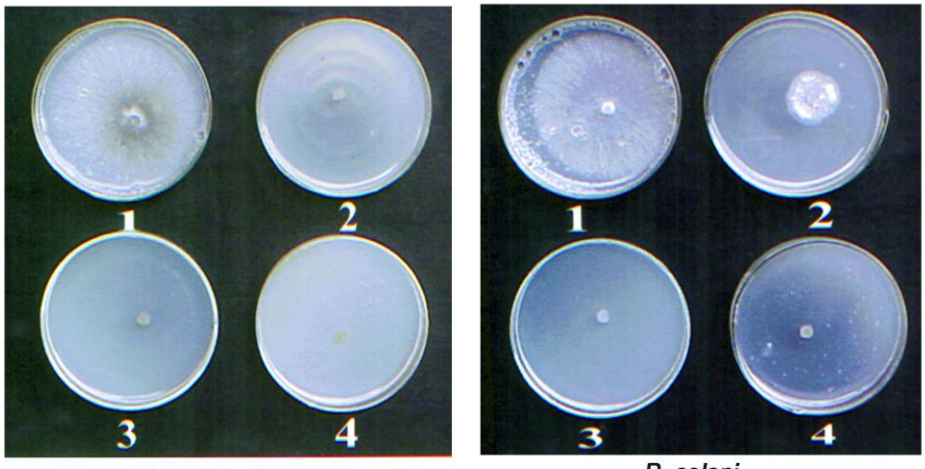
1. Tricyclazole 2. Propiconazole 3. Thiophenite methyl 4. Carbendazim
5. Hexaconazole 6. Thiram 7. COC 8. Control

Plate 10a. Effect of wintergreen oil on the growth of *M. phaseolina* and *R. solani*




*M. phaseolina* *R. solani*

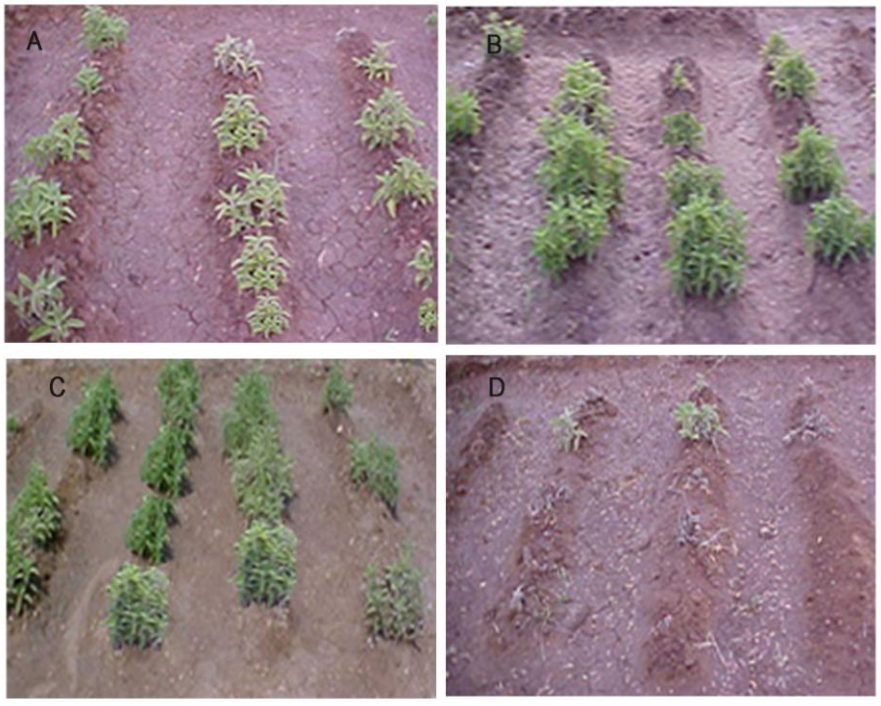
Plate 10b. Effect of lemon grass oil on the growth of *M. phaseolina* and *R. solani*




*M. phaseolina* *R. solani*

1. Control 2. 0.05% 3. 0.1% 4. 0.2%

Plate 19. Management of coleus root rot under field condition - Coimbatore


A. Carbendazim B. Th + ZnSO4 C. PFC6 + WGO + ZnSO4 D. Control