MANAGEMENT OF PHYTOPHTHORA DISEASE OF BLACK PEPPER (*Piper nigrum* L. WALP) USING PLANT GROWTH PROMOTING MICROBIAL INOCULANTS

BEENA S. NAIR

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Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

DECLARATION

I hereby declare that this thesis entitled "**Management of Phytophthora disease of black pepper (*Piper nigrum* L. Walp) using plant growth promoting microbial inoculants**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
23-01-2004.

B Nair
BEENA S. NAIR
(2001-11-20)

CERTIFICATE

Certified that the thesis entitled **"Management of Phytophthora disease of black pepper (*Piper nigrum* L. Walp) using plant growth promoting microbial inoculants"**, is a record of research work done independently by Ms. **Beena. S. Nair** (2001-11-20) under my guidance and supervision and that it has not previously formed the basis for the award any degree, fellowship or associateship to her.

Vellayani,
23-1-2004

Dr. K.N. ANITH
(Chairman, Advisory Committee)
Assistant Professor (Microbiology),
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

APPROVED BY:

CHAIRMAN

Dr. K.N. ANITH,
Chairman, Advisory Committee,
Assistant Professor (Microbiology),
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695522.

23.1.04

MEMBERS

Dr. C.K. PEETHAMBARAN,
Professor and Head,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695522.

23.1.04

Dr. V.K. GIRIJA,
Associate Professor,
Department of Plant Pathology,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695522.

23/1/04

Dr. K.B. SONI,
Assistant Professor,
Plant Molecular Biology and
Biotechnology Centre,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695522.

28.1.04

EXTERNAL EXAMINER

Dr. VINAYAK HEGDE
Senior Scientist,
CTCRI, Sreekaryam,
Thiruvananthapuram

23/1/04

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BNair

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To

Achan, Amma,
Biju chettan and Bindu

...for their undemanding
love and concern

CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF PLATES

LIST OF PLATES CONTINUED

LIST OF APPENDICES

LIST OF ABBREVIATIONS

%	per cent
@	At the rate of
µl	Micro litre
µm	Micro metre
^{0}C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungus
CD	Critical Difference
cm	Centimetre
COC	Copper oxychloride
CRD	Completely Randomised Design
et al	And others
Fig.	Figure
g	Gram
h	Hour
ha	Hectare
i.e.	that is
kg	Kilogram
kg/ha	Kilo gram per hectare
l	Litre
MAP	Months After Planting
min	Minutes
ml	Milli litre
mm	Milli metre
ppm	Parts per million
rpm	Rotations per minute
sp.	species
t	tonnes
t/ha	Tonnes per hectare
v/v	volume/volume
VAM	Vesicular Arbuscular Mycorrhiza
var.	variety
viz	namely
w/w	weight / weight

INTRODUCTION

1. INTRODUCTION

India had a long and glorious association with spice trade right from the ancient times. Black pepper is the most widely traded of all the spices and is an important foreign exchange earner of our country. Black pepper is native to the tropical, evergreen forests of Western Ghats of South Western India, commonly known as the Malabar coast in olden times. For centuries, black pepper cultivation has contributed substantially to the economic uplift and stability of the farmers in all pepper growing countries. In India, pepper is the mainstay of large number of farmers. mainly in Kerala where the cultivation is more or less restricted to the rural households.

The production of black pepper in our country in the year 2002 was 8.0000 tonnes. Out of the total production. Kerala's contribution was 60929 t from an area of 2.02 lakh hectares (FIB, 2002). However. it is paradogical to note that in spite of highly favourable climate, improved varieties and hi-tech production technologies, productivity of black pepper in the state is very low *i.e.*, 306 kg/ha as compared to other pepper producing countries (3 to 4 t/ha). One of the major reasons attributed to this low productivity is the incidence of foot rot disease incited by *Phytophthora capsici* Leonian. This disease affects all stages of the crop. right from the nursery to the main field. The pathogen often reaches the main field through the infected planting material and rooting medium. It is thus essential to ensure that the cuttings transplanted to the main field are disease free for successful management of the disease.

Development of management strategies against this disease would help to regain India's glory as a major exporter of spices, which has dwindled in recent years. The efficacy of currently used agrochemicals in combating this disease is variable and often not cost effective. Moreover, chemical control though effective is undesirable as they pollute the environment and the residue left in the product are hazardous to human health. As spices are export-oriented crops, the development of Integrated Disease Management System (IDMS) in which biological control is a major component has become a thrust area of research which in turn would minimize pesticide residues and environmental pollution.

Now-a-days there is an increasing trend worldwide to popularize ecofriendly agriculture. This is mainly due to the growing demand for 'organic' farm produce both in our country as well as abroad. Organic pepper is gaining great momentum in the world market and such products fetch a premium and competitive price in the market.

Presently microbial antagonists are increasingly being used as plant growth promoters and for the management of plant diseases including those caused by soil-borne plant pathogens (Cook and Baker, 1983; Weller and Cook, 1986; Chet, 1987; Lewis and Papavizas, 1991; Ramamoorthy *et al.*, 2002). In the light of these achievements in the field of biological control, the present study was planned to investigate the effect of plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads and *Bacillus* sp and the root entophytic fungus, *Piriformospora indica* on the suppression of Phytophthora disease of black pepper in the nursery. With the above objective, two strains each of fluorescent pseudomonads and *Bacillus* and an endosymbiotic fungus *P. indica* were compared with and without artificial inoculation with *P. capsici* the wilt inducing pathogen of black pepper. The treatments were further compared with the Package of Practices (POP) recommendations suggested by Kerala Agricultural University for the management of this disease. In this context, the following experiments were conducted in the present investigation.

1. Mycelial growth inhibition of *P. capsici* under *in vitro* conditions by two strains each of fluorescent pseudomonads and *Bacillus* sp and root endophytic fungus *P. indica*.

2. Testing the plant growth promoting ability of the biocontrol agents.

3. Testing the efficacy of the biocontrol agents in suppressing Phytophthora disease in the nursery.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The major production constraint of black pepper in India as well as in other pepper producing countries is the incidence of foot rot or quick wilt disease caused by *Phytophthora capsici* Leonian. In India, the disease is known to occur in Kerala, Karnataka, Tamil Nadu and Assam (Sarkar *et al.*, 1985). Samraj and Jose (1966) reported that foot rot is the most important disease causing severe loss to pepper gardens in Kerala. The disease usually occurs during the South West Monsoon period in India.

In India, the disease was known as early as 1902 when severe vine deaths were noticed in Wayanad region of erstwhile Madras state (Menon. 1949). This was investigated by Barber (1902, 1903, 1905) and Butler (1906, 1918). The first report of sudden collapse and death of pepper vines came from Lampung, Indonesia in 1885 (Chattopadhyay amd Maiti, 1990). The first authentic record of Phytophthora wilt of black pepper in Kerala was made by Samraj and Jose (1966). Based on Muller's identification of *Phytophthora*, they identified the causative agent as *Phytophthora palmivora* var. *piperina*. Later the black pepper isolate from India was identified as *P. palmivora* Morphological Form 4 (MF_4) on the basis of their long, pedicellate and caducous sporangia (Sarma *et al.*, 1982; Tsao *et al.*, 1985a). Tsao and Alizadeh (1988) after a detailed study of a large number of *Phytophthora* isolates from pepper throughout the world, finally identified the causal organism as *P. capsici*.. The name of the disease incited by the pathogen was changed from quick wilt to Phytophthora foot rot in 1988 (Nair and Sarma, 1988).

2.1 PATHOGEN

Detailed morphological description of *Phytophthora* affecting pepper was made by Tsao and Tummakate (1977). According to them, the sporangia of *Phytophthora* causing foot rot in Thailand were long, pedicillate often exceeding 100 μm in length and were of 48 x 22 μm in size with a L/B ratio of 2.2. Tsao *et al.* (1985b) reported that the sporangium shape of Indonesian pepper isolates varied from ovoid to obovoid and ellipsoid in most isolates. Santhakumari (1987) isolated *Phytophthora* from foot rot affected black pepper and found that the sporangia were ellipsoid to spherical, papillate, caducous with a size of 27 to 54 μm. Mammootty *et al.* (1991) conducted detailed studies on the morphological characters of six black pepper isolates of *Phytophthora* collected from Kerala and found that the breadth of the hyphae ranged from 4 to 6 μm and there was a wide variability in length of sporangiophore. According to Sarma *et al.* (1991), umbelloid ontogeny was noticed in all the isolates collected from Kerala and Karnataka and found that sporangia were obovoid, pyriform with a tapering base or fusiform and were highly variable with an average L/B ratio of 1.7:1 to 2.8:1. Mchau and Coffey (1995) conducted detailed morphological study of 113 isolates of *P. capsici* from different parts of the world and noted that the shapes of sporangia varied greatly between isolates and even within a single isolate. According to Mehrotra and Aggarwal (2001), the sporangiophores were either of umbelloid or sympodial type and the sporangia were ellipsoidal with tapering base, ovoid or even fusiform with pedicel length of 6.7 to 12.5 μm. The growth of *P. capsici* on Carrot Agar medium was characterized by petalloid type colonies with maximum growth at 25-30 ^{0}C with chlamydospore production frequently occurring in cultures (Alizadeh and Tsao, 1985; Uchida and Aragaki, 1985; Ristaino, 1990; Mchau and Coffey, 1994). Hemmes (1983) reported that the diameter of the oospore varied from 20.7 to 29.9 μm and zoospores were basically ovoid in shape.

2.2 SYMPTOMATOLOGY

Three types of symptoms *viz.*, leaf rot, collar rot and root rot are generally observed in a foot rot infected plant. Muller (1936) observed greyish brown lesions up to 5 cm in diameter near the tip and margin of the lower leaves and noticed a few drops of yellowish fluid from the under side of the lesions. Samraj and Jose (1966) found small irregular black patches on the leaves, later enlarged in size and covered the bulk of the leaf area. Holliday and Mowat (1963) and Turner (1969a, 1969b) from Sarawak and Nambiar and Sarma (1976) from India, observed fimbriate lesions during continuously humid conditions and concentrically zoned lesions under alternating wet and dry conditions.

A detailed symptomatological study of the collar rot type of infection was conducted by Muller (1936). He reported that disease cortex rapidly turned from dark watery green to black. He also observed that the external symptoms were visible only after complete decaying and disintegration of internal tissues. In his observations, the infection was usually noticed at a height of 30 cm from the base. Samraj and Jose (1966) observed that infection of vine was more common at a height of 25 cm above the ground level and it rarely occurred at any other region of the vine. The affected tissues became soft and decayed. The diseased leaves turned pale, flaccid and fell off.

In the case of root rot, the degree of damage depends upon the number of roots infected and the extent of rotting. Holliday and Mowat (1957, 1963) reported that the infection started from fine roots of the plant. Once the cortex got infected, the disease spread to the main roots and then to the underground stem. When the stem became infected, visible symptoms appeared on the above ground parts of the plant, *i.e.*, a cessation of the growth of terminal shoots, wilting, rapid yellowing and shedding of the leaves and small shoots. Detailed symptomatology was also studied by Sarma and Nambiar (1982), Manomohandas and Abicheeran(1986), Anandaraj *et al.* (1991) and Sarma *et al.* (1991).

The disease affected all parts of the plant at all stages of the crop (Sarma *et al.*, 1994). Black pepper is exclusively propagated by raising rooted cuttings from runner shoots. Anandaraj and Sarma (1995) reported that infection by *P. capsici* in the nursery results in either wilting of the cuttings or rotting of leaves and the disease is carried to the main field through the infected planting material and rooting medium.

2.3 MANAGEMENT OF FOOT ROT DISEASE

Nambiar and Sarma (1976) stressed the importance of adopting phytosanitary measures under field conditions to reduce the inoculum potential of the pathogen in the soil to check disease severity. Sarma *et al.* (1988) opined that Integrated Disease Management (IDM) involving chemical, cultural and biological methods besides host resistance is perhaps the most ideal strategy to combat foot rot disease.

The importance of nursery hygiene in preventing the spread and intensification of Phytophthora diseases has been highlighted by several workers. Even when nursery stock is destined for areas where pathogens are already present, disease control is greater if the plant is free of the pathogen at the time of establishment (Sarma and Anandaraj, 2000). They also opined that IDM with a greater thrust on hygienic cultural practices in the nursery could reduce the disease.

In the main field, pre-monsoon painting of the collar region of the vines with Bordeaux Paste besides spraying the foliage and drenching the soil with one per cent Bordeaux Mixture once during May–June (pre- monsoon) and again in July-August (post- monsoon) was found to be effective in controlling the disease (Mammootty *et al.*, 1979; Sasikumaran *et al.*, 1981; Sarma and Nambiar, 1982 and Sarma and Ramachandran, 1984). Among the three systemic fungicides such as metalaxyl (Ridomil), fosetyl- Al (Alliette) and ethazole (Terrazole) used as foliar spray and soil drench, metalaxyl-ziram and fosetyl-Al were found to be highly effective in checking the disease incidence under field conditions (Ramachandran *et al.*, 1982 and Ramachandran and Sarma, 1985).

2.4 BIOCONTROL AGENTS

Biocontrol of plant pathogens is one of the most promising options other than use of resistant variety. crop rotation and chemical pesticide application for the management of plant diseases especially soil-borne diseases (Weller, 1988; Becker and Schwinn, 1993; Mukhopadhyay and Mukherjee, 1996; Punja, 1997). Plant Growth Promoting Rhizobacteria (PGPR) are a group of potential biocontrol agents against many soil-borne plant pathogens (Bowen and Rovira, 1999).

2.4.1 Fluorescent Pseudomonads as Biocontrol Agents

Among the different PGPR, fluorescent pseudomonads have emerged as the largest and potentially the most promising group for plant disease control and growth promotion (Kloepper and Schroth, 1978; Suslow and Schroth, 1982). This is achieved through their general biological activities that include competition for space and nutrients, production of antibiotics and growth regulators, volatile and antimicrobial substances and compounds such as iron chelating siderophores and hydrocyanic acid (Kloepper *et al.*, 1980; Chet *et al.*, 1990; O'Sullivan and O'Gara, 1992; Dowling and O' Gara, 1994; Rosales *et al.*, 1995; Dave and Dube, 2000; Mondal *et al.*, 2000; Ines *et al.*, 2001).

Several workers have reported the *in vitro* suppression of fungal pathogens by fluorescent pseudomonads. Gutterson *et al.* (1986) reported that *P. fluorescens* strain HV37a inhibited growth of the fungus, *Pythium ultimum* under *in vitro* conditions. Ganesan and Gnanamanickam (1987) showed that strains of *P. fluorescens* could suppress the root and stem rot pathogen of peanut, *Sclerotium rolfsii*, by restricting the mycelial growth. They also observed that 99 per cent of the plants inoculated with the biocontrol bacteria were protected from infection. Myatt *et al.* (1993) opined that *Pseudomonas cepacia* and *P. fluorescens* were effective in inhibiting *Phytophthora megasperma* f.sp. *medicaginis*, the incitant of root rot of chickpea, both under laboratory and field conditions. Thara and Gnanamanickam (1994) reported that strains of *P. fluorescens* and *P. putida* suppressed the rice sheath blight fungus, *Rhizoctonia solani* both under laboratory and field conditions. Tambong *et al.* (2001) reported that *P. aeruginosa* PNAI could control *Pythium myriotylum* causing root rot of cocoyam *in vitro* through the production of phenazine-1-carboxylic acid and phenazine-1-carboxamides.

Reduction of fungal damage to plants by fluorescent pseudomonads through the inhibition of growth of one or more phytopathogenic fungi has been reported by Howell and Stipanovic (1979), Kloepper *et al.* (1980) and Scher and Baker (1982). Howie and Suslow (1991) reported a 70 per cent reduction of *Pythium* infection in cotton and about 50 per cent increase in emergence of seedlings by an antibiotic producing *P. fluorescens*. Galindo (1992) reported antagonism of *P. fluorescens* to *P. palmivora*, the causal agent of black pod rot of cocoa and found that the disease control affected by the bacterium was superior to that of copper oxide or chlorothalonil. Carruthers *et al.* (1995) obtained not only reduced root infection by *P. megasperma* var *sojae* in asparagus, by inoculation with *P. aureofaciens*, but also increased root length and plant weight in presence of the pathogen. Inoculation of Fusarium wilt affected cucumber plants with a PGPR isolate *P. putida* strain 89B27 delayed disease symptom development and reduced the number of dead plants compared to the nonbacterized control plants (Liu *et al.*, 1995). Sarma *et al.* (1996 a) found that fluorescent pseudomonads were effective in checking the growth of *P. capsici* and in suppressing the expression of foot rot symptoms in black pepper under controlled conditions. Imran *et al.* (1999) based on their work, reported that *P. aeruginosa* alone or in combination with urea @ 0.15 g/kg soil or potash @ 0.1 g/kg reduced root rot infection in mungbean roots. Smitha (2000) identified an isolate of fluorescent pseudomonad, P1 as the best bacterial antagonist against *R. solani* inciting foliar blight of amaranthus. Ramamoorthy *et al.* (2002) reported that

P. fluorescens isolate Pf_1 and *P. putida* isolates PFATR and KKM1 inhibited mycelial growth of *Pythium aphanidermatum* and promoted plant growth in tomato and chilly due to higher accumulation of Phenylalanine Ammonia Lyase, Peroxidase and Polyphenol Oxidase in the treated plants.

Several reports suggest that root colonization by bacterial biocontrol agents plays an important role in determining the biocontrol efficiency (Weller, 1988; O'Sullivan and O'Gara, 1992; Dowling and O' Gara, 1994 and Bowen and Rovira, 1999). Anith *et al.* (2002) reported that *Pseudomonas* isolates PN-015 and PN-026 colonized the planting material of black pepper and afforded protection from *P. capsici* induced nursery wilt. Anith and Manomohandas (2001) observed that *Trichoderma harzianum* and *Alcaligenes* sp strain AMB8 applied alone or in combination significantly reduced nursery rot of black pepper and these bioagents survived on the stem cuttings of black pepper used as planting material.

Cell free culture filtrates or extracts can also be used to study the role of antibiosis in biological control. Rabindran (1994) reported that culture filtrates of *P. fluorescens* Pf ALR2 completely inhibited the germination and reduced the virulence of sclerotia of *R. solani*, the causal agent of rice sheath blight.

Induced Systemic Resistance (ISR) is a phenomenon where by resistance to infectious diseases is systemically induced by localized infection or treatment with microbial components or products or by a diverse group of structurally unrelated organic and inorganic compounds (Kuc, 2001). Besides suppression of plant diseases by many mechanisms, fluorescent pseudomonads induce systemic resistance in plants against attack by a wide range of pathogens (Whipps, 1997; Raupach and Kloepper, 2000; Ramamoorthy, *et al.*, 2001; Viswanathan and Samiyappan, 2002). Ellis *et al.* (1996) observed that *P. fluorescens* strain WCS417 induced systemic resistance against *F. oxysporum* in several plant species without inducing synthesis of PR proteins. Yan *et al.* (2002) found that two strains of PGPR, *Bacillus pumilus* SE34 and *P. fluorescens* 89B61 elicited systemic protection against the late blight pathogen *P. infestans* on tomato. The bacterial antagonists reduced disease severity and it was seen that the germination of sporangia and zoospores on the surface of tomato plants was significantly reduced.

2.4.2 *Bacillus* as Biocontrol Agents

Bacilli are endospore formers and thus possess ability to resist adverse environmental conditions. They are easily cultivable and amenable to formulation technologies.

Gokulapalan and Nair (1984) reported the antagonistic effect of *Bacillus* sp on *R. solani*. Utkhede (1984) tested and found that 21 isolates of *B. subtilis* obtained from local and exotic strains of *Sclerotium cepirorum* were antagonistic to *Phytophthora cactorum*, the incitant of crown rot of apple. *B. subtilis* was found to inhibit sclerotial growth of *R. solani* and reduced sheath blight incidence in rice (Padmakumary, 1989) Rosales *et al.* (1993) observed that *B. subtilis*, *B. talerosporus* and *B. pumilus* inhibited the mycelial growth of eight fungal pathogens of rice. Thomas *et al.* (1994) found that *B. subtilis* caused growth inhibition of *Pythium vexans* and *R. solani* causing rhizome rot of small cardamom. In a study conducted by Berger *et al.* (1996) in fogging glass houses under high humidity conditions, it was seen that *B. subtilis* Cot1 prevented Phytophthora and Pythium damping off in Astilbe, Photinia and Hemerocallis micro plantlets and conventional Brassica seedlings. Jubina and Girija (1998) reported that *Bacillus* isolate B7 from the rhizosphere of black pepper plants showed a dual function of disease suppression and growth promotion in plants challenged with *P. capsici*. Further the *Bacillus* isolates B5, B13 and B7 were found to be superior to *T. harzianum* and *P. fluorescens* in controlling foot rot under *in vitro* and *in vivo* conditions. Zheng and Sinclair (2000) observed that *B. megaterium* strain B153-2-2 is a potential biocontrol agent against *R. solani* isolate

2B12 (ISG–2B) causing root rot of soybean. Shirokov *et al.* (2002) reported that *Bacillus* sp strain 739 inhibited the growth of *Helminthosporium sativum*, a facultative phytopathogen by the production of thermolabile peptides.

Growth promoting ability of several *Bacillus* isolates has been reported. Merriman *et al.* (1974) reported that *B. subtilis* increased the yield of carrots by 48 per cent and oats by 40 per cent. Seed treatment of groundnut with *B. subtilis* improved seed germination and emergence, increased nodulation by *Rhizobium* spp.; increased root and plant growth, enhanced yield and reduced root cankers caused by *R. solani*, (Turner and Backman, 1991). Sailaja *et al.* (1998) reported that *B. subtilis* AF1 could induce resistance against *Aspergillus niger* in groundnut plants by the production of lipoxygenases.

Vasudeva and Chakravarthi (1954) reported that culture filtrate of *B. subtilis* was antagonistic to the growth of *R. solani*, *R. bataticola*, *Alternaria solani* and several other pathogens. Complete *in vitro* inhibition of *Phytophthora cactorum* by an autoclaved extract of *Bacillus* isolate B8 was reported by Utkhede and Guance (1983). The application of autoclaved culture filtrate of *B. subtilis* three times a week, controlled bean rust in the field and was superior to weekly application of mancozeb (Baker *et al.*, 1985). Podile and Dube (1987) observed that *Phytophthora drechsleri* f. sp. *cajani* did not grow in a concentrated cell free culture filtrate of *B. subtilis* isolate. Tschen (1987) observed inhibition of lesion development by *R. solani* in potatoes by culture filtrate of the bacterial antagonist *B. subtilis*. Culture and culture filtrate of *B. cereus* UW85 was reported to suppress *Phytophthora nicotianae* in tobacco seedlings (Handelsman *et al.*, 1991), *Sclerotinia minor* in peanuts (Phipps, 1992), *Pythium aphanidermatum* causing fruit rot in cucumber (Smith *et al.*, 1993) and *P. medicaginis* causing damping off of alfalfa (Silosuh *et al.*, 1994).

2.4.3 *Trichoderma* as Biocontrol Agents

The most exhaustively researched microorganism as a biocontrol agent is *Trichoderma* spp.

Cristinzio (1987) reported the antagonistic property of *Trichoderma* and *Chaetomium* spp. against *P. capsici* under *in vitro* conditions. Tsao *et al.* (1988) found that soil application of these antagonists to sick soils was effective for the biocontrol of *Phytophthora* spp. Anandaraj and Sarma (1994a, 1995) recommended an integrated approach for the management of foot rot of black pepper involving the application of fungal antagonists such as *Gliocladium virens* and *Trichoderma* spp. to soil along with foliar spray of Bordeaux Mixture, metalaxyl or potassium phosphonate. Such management practices with *T. harzianum* were found to reduce disease incidence from 25 per cent to 15 per cent under field conditions (Sarma *et al.*, 1996b).

Combination of *T. harzianum* and *Gliocladium virens* applied @ 30 g/pot (Rajan and Sarma, 2000) and *T. harzianum* and *Alcaligenes* sp applied alone or in combination (Anith and Manomohandas, 2001) were also effective in reducing the incidence of *P. capsici* induced foot rot and nursery rot disease in black pepper. Ganesan *et al.* (2000) found that the application of *Trichoderma* sp significantly increased root dry weight in pepper cuttings when compared to untreated control. Such a beneficial effect was attributed to enhanced nutrient uptake by inoculated plants. Anith and Manomohandas (2001) reported that the combined application of *T. harzianum* and *Alcaligenes* sp also resulted in enhanced shoot weight in black pepper. Rajan *et al.* (2002) reported that there was reduction in the incidence of foot rot for a period of three years when *T. virens* and *T. harzianum* were applied. Divya (2002) reported that percentage of foliar infection by *P. capsici* on black pepper plants and disease index were minimum when *T. harzianum* was used as biocontrol agent.

2.4.4 Arbuscular Mycorrhizal Fungi (AMF) as Biocontrol Agents

The beneficial effect of arbuscular mycorrhizal association on plant growth in black pepper was reported by Manjunath and Bagyaraj (1982). Ramesh (1982) and Sivaprasad (1995). Bopaiah and Khader (1989) reported the growth promoting activities of Vesicular Arbuscular Mycorrhiza (VAM). The role of VAM in nutrient uptake, growth promotion and tolerance to biotic stress in crop plants was reported by Ewald (1991). Anandaraj *et al.* (1993) observed that the use of endomycorrhiza as a biocontrol agent significantly reduced foot rot incidence in black pepper. Incorporation of VAM propagules in the nursery mixture enhanced the rooting and growth of black pepper cuttings and increased biomass production (Anandaraj and Sarma, 1994a). Anandaraj and Sarma (1994b) suggested that the suppressive effect of AMF was due to enhanced root regeneration, nutrient uptake and altered host physiology in mycorrhizal plants. Further it was observed that some isolates of *Glomus* were more effective in growth promotion of Karimunda varieties than Panniyur-1 and Kottanadan. Similar observation was made by Sarma *et al.* (1996a). They also reported considerable reduction in root rot, foliar yellowing and defoliation in black pepper due to mycorrhizal symbiosis under field conditions. Sivaprasad *et al.* (1995) observed a negative correlation between percentage of mycorrhizal infection and spore count and foot rot incidence in black pepper mycorrhizosphere.

Anandaraj *et al.* (1996) emphasized the need for using native isolates of arbuscular mycorrhizal fungi for reducing the incidence of foot rot in black pepper. According to Sivaprasad *et al.* (1997) and Robert (1998) plants mycorrhized with *Glomus monosporum* and challenged with *P. capsici* survived up to 40 days. Further in the inoculated plants there was an increase in biomass production. Similar result was reported by Thanuja and Hegde (2001) in black pepper after inoculation with *G. fasciculatum*.

Divya (2002) reported that the mortality of foot rot affected vines was minimum when a combined application of *T. harzianum* and *G. fasciculatum* was given. It was also reported that fresh and dry weight of shoots and roots were maximum in the treatments with *G. fasciculatum*.

2.4.5 *Piriformospora indica* as Biocontrol Agent

Piriformospora indica Varma, Verma. Kost. Rexer and Franken sp. nov. is a newly described axenically cultivable plant growth promoting endosymbiont, which mimics the capabilities of AMF (Plates 3 and 4).

Cytological analysis revealed that *P. indica* belongs to Hymenomycetes of Basidiomycotina and molecular data based on 18S rRNA sequences indicated that it is related to *R. solani* (Verma *et al.*. 1998). *P. indica* was found to penetrate the rhizoids of protocorm of terrestrial orchids and developed morphological structures as seen in orchid mycorrhiza (Blechert *et al.*, 1999). Varma *et al.* (1999) found that the fungus colonised the cortex of plant roots and developed inter and intracellular vesicles, hyphal coils and pear shaped chlamydospores.

Varma *et al.* (1999) observed that *P. indica* tremendously improved the growth and overall biomass production of different plants such as herbaceous monocots and dicots, trees including medicinal plants like *Bacopa monnieri, Artemisia annua* and several economically important crops. Sahay and Varma (1999) reported that regenerated plantlets of tobacco inoculated with *P. indica* subjected to two different biological hardening techniques showed 88 to 94 per cent survival as compared to 62 per cent survival of uninoculated controls under similar conditions. Positive growth response has been reported in medicinal plants, *Spilanthus calva* and *Withania somnifera* to inoculation with *P. indica* under field conditions (Rai *et al.*, 2001).

Culture filtrate of the mycelium containing exudates of *P. indica* (hormones, enzymes, proteins etc) exerted a growth promoting effect as was exemplified by the increase in root length, shoot length and biomass of plants such as maize, *Bacopa monnieri* and tobacco (Varma *et al.*, 2001). Further they also observed that the mycelium and culture filtrate exerted inhibitory effect on *Gaeumannomyces graminis*, the pathogen causing 'takeall' disease of wheat.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study on "Management of Phytophthora disease of black pepper (*Piper nigrum* L. Walp) using plant growth promoting microbial inoculants" was conducted at the College of Agriculture, Vellayani during 2002-2003 to investigate the effect of plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads and *Bacillus* spp. and the root endophytic fungus, *Piriformospora indica* on the suppression of Phytophthora disease of black pepper in the nursery.

3.1 PATHOGEN

3.1.1 Isolation

The pathogen was isolated from foot rot affected black pepper plants (var. Karimunda) collected from the pepper gardens of the College of Agriculture, Vellayani. Infected leaves were cut into small pieces and were initially surface sterilized with 0.1 per cent mercuric chloride solution. The bits were then washed thrice with sterile water and aseptically transferred to sterile Carrot Agar medium (CA) (Appendix-I.A) supplemented with 30 ppm of Sporidex (Cephalexin, Ranbaxy Laboratories Ltd, New Delhi). The Petri plates were then incubated at $28 \pm 2\ ^{0}C$ for three days. Visible growth of the mycelium from infected parts were aseptically transferred to sterile Carrot Agar slants and then maintained in a refrigerator for further studies.

3.1.2 Pathogenicity Testing

Pathogenicity of the fungus was tested by artificial inoculation on leaves of three month old rooted cuttings of black pepper var. Karimunda maintained in poly bags. For this, the isolate was initially grown on CA in sterile Petri dishes for five days at $28 \pm 2\ ^{0}C$. Sporangial production was induced by incubating the above culture in a refrigerator for 24 h. Five mm diameter mycelial discs cut from this culture were used for inoculating leaves of healthy black pepper plants. Inoculation was given on the third and fourth leaves from the top. Mycelial discs were placed on the under surface of the leaves after giving mild pinpricks over which moist cotton was placed. The inoculated plants were watered regularly to ensure high humidity. Uninoculated plants which were given pinpricks alone served as control. Observations were recorded on the development of typical symptoms of *P. capsici* infection.

3.1.3 Identification

The cultural and morphological characters of the pathogen *viz*., width of mycelium and shape and size of sporangium were studied and compared with that of the type culture of *P. capsici* maintained in the Department of Plant Pathology, College of Agriculture, Vellayani. Based on this, the identification was made.

3.2 BIOCONTROL AGENTS

The biocontrol agents selected for the study were two strains each of Plant Growth Promoting Rhizobacteria (PGPR) belonging to fluorescent pseudomonads (*Pseudomonas putida* strain 89B61 and *Pseudomonas fluorescens* strain RCL3R4) and *Bacillus* spp. (strains BY-1 and BY-2) and a root endophytic fungus, *Piriformospora indica*. The pseudomonad strain, *P. putida* 89B61 and the two *Bacillus* strains were procured from the Department of Entomology and Plant Pathology, College of Agriculture, Auburn University, Auburn, Alabama, U.S.A. *P. fluorescens* strain RCL3R4 was obtained from the College of Agriculture Vellayani. The culture of *P. indica* was procured from the School of Life Sciences, Jawaharlal Nehru University, New Delhi. The talc based formulation of *Trichoderma harzianum* and the soil based granular inoculum of the Arbuscular Mycorrhizal Fungus (AMF), *Glomus fasciculatum* found effective in controlling *P. capsici* were obtained from the Plant Pathology Department, College of Agriculture, Vellayani and included as a treatment for comparison of the effectiveness of other biocontrol agents.

3.3 *IN VITRO* STUDIES

3.3.1 Studies on Inhibition of Mycelial Growth of *P. capsici* by Biocontrol Agents in Dual Culture

3.3.1.1 Inhibition by P. indica

Dual culture plate method devised by Skidmore and Dickinson (1976) with slight modification was used for the study. Five mm mycelial discs of *P. capsici* from four day old culture grown on CA and five mm mycelial discs of *P. indica* from six day old culture grown on Potato Dextrose Agar (PDA) were used. The mycelial discs were kept five cm apart on PDA supplemented with 30 ppm of Sporidex @100 µl /100 ml medium in 9 cm diameter Petri dishes and incubated at $28 \pm 2\ ^{0}C$ for four days. Three replications were maintained. Petri plate inoculated with mycelial disc of the pathogen alone served as control. Observation was made on the radial growth of the pathogen and the antagonist and percentage inhibition was calculated using the following formula developed by Vincent, 1927.

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

where, I - inhibition of mycelial growth of pathogen

C - radial growth of pathogen in control plates (cm)

T - radial growth of pathogen in treated plates (cm)

3.3.1.2 Inhibition by Rhizobacteria

The dual culture studies using fluorescent pseudomonads and *Bacillus* spp. were done on PDA and CA.

3.3.1.2.1 Dual Culturing with Fluorescent Pseudomonads

Two strains *viz.*, *P. putida* 89B61 and *P. fluorescens* RCL3R4 were used for the growth inhibition studies.

A heavy inoculum from actively growing 24 h old cultures of individual bacterial strains was streaked aseptically as a line of 2 cm length separately on medium contained in Petri dishes at a distance of five cm. Simultaneously a mycelial disc of five mm diameter was cut out from four day old culture of *P. capsici* grown on CA and kept at the centre of the Petri plate inoculated with the bacterial culture. The plates were then incubated at 28 ± 2 ^{0}C for four days and observed for the zone of inhibition of growth of the pathogen. Three replications each were maintained. Plates inoculated with pathogen alone served as control.

3.3.1.2.2 Dual Culturing with *Bacillus* Strains

Two strains of *Bacillus viz.*, BY-1 and BY-2 were used for the study.

Dual culture inhibition study was done as described in section 3.3.1.2.1.

3.3.2 Inhibition of Mycelial Growth of *P. capsici* by Culture Filtrates of Biocontrol Agents

3.3.2.1 Inhibition by Culture Filtrate of P. indica

Owing to the good growth of both the fungi on PDA compared to other media, PDA was selected as the medium for the study.

3.3.2.1.1 Preparation of Culture Filtrate

To prepare the culture filtrate of *P. indica*, five mm mycelial disc of the fungus was inoculated into 100 ml Potato Dextrose Broth (PDB) taken in 250 ml conical flask. After two weeks of incubation at 28 ± 2 ^{0}C, the mycelial mat of the fungus was filtered out using Sartorius filter paper grade 292 under aseptic conditions.

3.3.2.1.2 Inhibition of Mycelial Growth

The culture filtrate collected was passed through a sterile bacteriological filter (0.2μm, Fisherbrand, Cat.No.09-719C). Wells of five mm diameter were cut using a cork borer at a distance of 2 cm from the periphery in PDA plates supplemented with Sporidex 30 ppm @ 100 μl/100 ml of the medium. The wells were then partially sealed with 75 μl of molten soft agar. Then 100 μl of the filtrate was added carefully into the wells using a micropipette and was allowed to percolate. Five mm disc of *P. capsici* was placed at the centre of the Petri plates. Plates were incubated at 28 ± 2 ^{0}C and three replications were maintained. Wells in the control plates were filled with PDB instead of culture filtrate. Inhibition of mycelial growth of the pathogen was recorded after four days.

3.3.2.2 Inhibition by Culture Filtrates of Rhizobacteria

3.3.2.2.1 Inhibition by Culture Filtrates of Fluorescent Pseudomonads

Single colonies from 24 h old cultures of the two pseudomonad strains were inoculated separately into 10 ml King's B Broth taken in sterile glass vials and incubated at 28 ± 2 ^{0}C for 24 h. The bacterial cells were then separated from the broth by centrifugation at 15,000 rpm for 15 min at 4 ^{0}C in a Hettich Zentrifugen MIKRO 24-48 R model centrifuge.

The supernatant was collected and passed through bacteriological filters (0.2μm, Fisherbrand, Cat.No.09-719C). Wells of five mm diameter were cut using a cork borer at a distance of 2 cm from the periphery in PDA plates supplemented with Sporidex 30 ppm @ 100 μl/100ml of the medium. The wells were then partially sealed with 75 μl of molten soft agar. Then 100 μl of each culture filtrate was added carefully into both the wells in separate Petri plates using a micropipette and was allowed to percolate. Five mm disc of *P. capsici* were placed at the centre of the plate. Three replications were maintained. Wells in the control plates were filled with King's B Broth instead of culture filtrates. Growth of the pathogen was observed four days after incubation at 28 ± 2 ^{0}C.

3.3.2.2.2 Inhibition by Culture Filtrates of *Bacillus* Strains

The method followed to test the effect of culture filtrates of the two *Bacillus* strains on *P. capsici* was same as that described in section 3.3.2.2.1. However, Nutrient Broth was used instead of King's B Broth for cultivation of bacterial strains.

3.4 *IN VIVO* STUDIES

3.4.1 Plant Growth Promotion by PGPR Strains and *P. indica*

Four different rhizobacterial strains and root endophytic fungus, *P. indica* were tested for their growth promotion activity on runner shoot cuttings of black pepper var. Karimunda.

Design: CRD

Treatments: 8

Replications: 8

3.4.1.1 Treatments

T_1 - Cut end dip with *Pseudomonas putida* strain 89B61

T_2 - Cut end dip with *Pseudomonas flourescens* strain RCL3R4

T_3 - Cut end dip with spore suspension of *Piriformospora indica*

T_4 - Inoculation with two week old mycelial mat of *Piriformospora indica* @ one per cent (w/w) of the rooting medium

T_5 - Cut end dip with *Bacillus* sp strain BY-1

T_6 - Cut end dip with *Bacillus* sp strain BY-2

T_7 - Cut end dip with 1000 ppm Indole-3-Butyric Acid (IBA) as per the Package of Practices recommendations of Kerala Agricultural University (KAU, 2002)

T_8 - Uninoculated control

Potting mixture containing sand and soil in the ratio 1:1 was prepared and filled in polythene bags of 150 gauge thickness and size 35 x 17 cm @ 1 kg each. Disease free three node cuttings of black pepper (approximately 25 cm length) procured from the Instructional Farm of the college were used for planting.

3.4.1.2 Preparation and Application of Inoculum of PGPR Strains

Inoculum of the four bacterial isolates were prepared by collecting the cells from agar plates heavily cross streaked and incubated for two days at 28 ± 2 ^{0}C. The cells of individual bacterial isolates harvested by scraping with a glass spreader from two plates each were then suspended in 100 ml of sterile water. The fluorescent pseudomonad strains were grown on King's B Agar (Appendix I.B) and the *Bacillus* strains were grown on Nutrient Agar media (Appendix I.C). The basal cut ends of the runner shoots were dipped in the bacterial suspension for thirty min and then planted.

3.4.1.3 Preparation and Application of Spore Suspension of P. indica

Five mm mycelial discs of *P. indica* were inoculated into 100 ml PDB taken in 250 ml conical flasks. After two weeks of incubation at 28 ± 2 ^{0}C, the mycelial mat of the fungus was filtered out using Sartorius filter paper grade 292. The basal portion (5cm) of the cuttings were then dipped in 100 ml of the freshly prepared spore suspension for thirty min and planted.

3.4.1.4 Preparation and Application of Mycelial Mat of P. indica

Five mm mycelial discs of *P. indica* were inoculated into 100 ml PDB taken in 250 ml conical flasks. After two weeks of incubation at 28 ± 2^{0}C, the mycelial mat of the fungus was filtered out using Sartorius filter paper grade 292. For the treatment of the cuttings with mycelial mat of *P. indica*, initially the freshly collected hyphal mat obtained after filtration was repeatedly washed with sterile water. Then one per cent (w/w) mixture of the hyphae with sterile sand was prepared by adding and mixing thoroughly one g fresh weight of the hyphae with 100 g sterile moistened sand. Freshly prepared inoculum was added into the planting hole @ 10 g before planting the cuttings.

3.4.1.5 Preparation and Application of IBA

For the treatment using IBA, 1000 ppm solution was prepared in sterile water. The lower portion of cuttings was dipped in this solution for 45 seconds and then planted (KAU, 2002). This served as a positive check for the rooting studies.

The cuttings planted without the application of any of the growth promoting agents served as control.

All the plants were maintained under shade and watered regularly and biometric observations were recorded.

3.4.1.6 Observations

The observations were taken three months after planting.

3.4.1.6.1 Number of Leaves

The Number of leaves of the plants was counted three months after planting.

3.4.1.6.2 Number of Roots

After three months of planting, the cuttings were carefully removed from the poly bags with roots intact. The roots were then washed gently in tap water to remove all adhering soil particles and blot dried. The number of primary roots was counted.

3.4.1.6.3 Fresh Weight of Shoots

The fresh weight of shoots (g) was taken in an electronic single pan balance immediately after uprooting the plants.

3.4.1.6.4 Dry Weight of Shoots

The dry weight of shoots (g) was taken after drying the samples to a constant weight at 60 ^{0}C in a drying oven.

3.4.1.6.5 Fresh Weight of Newly Formed Roots

The fresh weight of newly formed roots (g) was taken in an electronic single pan balance immediately after uprooting the plants

3.4.1.6.6 Dry Weight of Newly Formed Roots

The dry weight of newly formed roots (g) was taken after drying the samples to a constant weight at 60 ^{0}C in a drying oven.

3.4.1.6.7 Fresh Weight of Newly Formed Leaves

The fresh weight of newly formed leaves (g) was taken in an electronic single pan balance immediately after uprooting the plants.

3.4.1.6.8 Dry Weight of Newly Formed Leaves

The dry weight of newly formed leaves (g) was taken after drying the samples to a constant weight at 60 ^{0}C in a drying oven.

3.4.1.6.9 Root Colonization by *P. indica*

The cuttings that were treated with *P. indica* were carefully removed from the poly bags with roots intact. The roots were then washed gently in tap water to remove all adhering soil particles and blot dried. Root colonization was determined by staining the newly formed roots using 0.05 per cent trypan blue (Appendix II.A) (Phillips and Hayman, 1970). Twenty five root bits of approximately one cm length each were examined for this purpose. The root bits were initially softened by immersing them in 10 per cent potassium hydroxide solution overnight. The alkali was poured off and the root samples were rinsed with acidified water (two per cent HCl) for ten min. The acid was decanted and the root bits were stained in 0.05 per cent trypan blue prepared in lactophenol. The excess stain was poured off and the root bits were destained with fresh lactophenol (Appendix II.B). Root bits were then arranged on a glass slide and gently pressed with a long coverslip. These were then observed under the microscope for the presence of pear shaped chlamydospores and mycelium of the fungus (Varma *et al.*, 2001).

3.4.2 Biological Control of Phytophthora Disease in the Nursery

The efficiency of four different rhizobacterial strains and the root endophytic fungus *P. indica* in suppressing the Phytophthora disease in the nursery was investigated by studying the extent of disease control achieved in black pepper var. Karimunda inoculated with the pathogen.

Design	:	CRD
Treatments	:	10
Replications	:	8

3.4.2.1 Treatments

T_1 - Pseudomonad strain 89 B61 + *P. capsici.*

T_2 - Pseudomonad strain RCL 3R4 + *P. capsici.*

T_3 - Spore suspension of *Piriformospora indica* + *P. capsici.*

T_4 - Mycelial mat inoculation of *P. indica* + *P. capsici*

T_5 - *Bacillus* strain BY-1 + *P. capsici.*

T_6 - *Bacillus* strain BY-2 + *P. capsici*

T_7 - Chemical control with 0.20 per cent copper oxy chloride (COC) drenched at 15 days intervals + *P. capsici*

T_8 - AMF (*Glomus fasciculatum*) + *Trichoderma harzianum* + *P. capsici*

T_9 - *P. capsici* inoculated control

T_{10} - Uninoculated control

3.4.2.2 Planting of Black Pepper Cuttings

Rooted cuttings of black pepper var. Karimunda were initially raised in potting mixture containing soil, cow dung and sand in the ratio of 2:1:1 and maintained for three months. After the establishment of the cuttings, these were transferred singly to polythene bags of 150 gauge thickness and 35 x 17 cm size containing sand: soil (1:1) medium @1 kg/bag.

3.4.2.3 Inoculation with Mycelial Mat of P. indica

In this experiment, a one per cent mixture of mycelial mat of the fungus in sand was provided at the rooting zone. For this, a potting mixture consisting of sand and soil in the ratio 1:1 was added first up to one-third height of the polythene bags. Ten g of the inoculum mixture was then layered over it. Plantlets were then kept in such a way that there is constant contact between the roots and the inoculum. The remaining part was then filled with the potting mixture. Pathogen was inoculated after three weeks.

3.4.2.4 Inoculation with Spore Suspension of P. indica

The culture filtrate of *P. indica* was drenched into the rooting medium @ 20 ml /bag.

3.4.2.5 Inoculation with Rhizobacteria

The four PGPR strains were drenched as bacterial cell suspension @ 20 ml/bag. The method followed for the preparation of bacterial cell suspension was same as that described under section 3.4.1.2.

3.4.2.6 Inoculation with T. harzianum and AMF

In the plants treated with *T. harzianum* and AMF, a talc based formulation of *T. harzianum* and a granular soil based inoculum of AMF was used. Inoculation with AMF was given at the time of transfer of the rooted cuttings to the polythene bags. *T. harzianum* was applied by mixing it with the soil around the plants @ 25 g/bag at the time of pathogen inoculation.

3.4.2.7 Application of COC

COC was drenched @ 200 ml/bag at 15 days interval. Initial application of the chemical was given at the time of pathogen inoculation.

3.4.2.8 Inoculum Preparation and Application of the Pathogen

The pathogen was raised aseptically in sterile sand-oat meal mixture for soil application. For this oats was mixed with sand in the ratio 1:9(v/v) and was sufficiently moistened by adding tap water. Two hundred and fifty grams of this mixture was taken in one litre flasks and sterilized in an autoclave at 1.05 kg cm^{-2} for 20 min at 121.5 ^{0}C for two consecutive days. The flasks were then inoculated with five mm mycelial discs of the pathogen grown on sterile CA and incubated at room temperature for seven days for mycelial proliferation. The inoculum was applied @ 10 g/bag after raking the soil around the plants. The application of pathogen was given simultaneously with biocontrol agents for all the treatments except mycelial mat inoculation with *P. indica*.

Pathogen was inoculated in the same way in the control plants. Uninoculated control plants were also maintained.

All the plants were maintained under high humidity by regular watering and sprinkling with water to ensure conducive conditions for proper disease development.

3.4.2.9 Observations

3.4.2.9.1 Disease Incidence

The number of wilted plants was counted after 40 days of pathogen inoculation. Wilt incidence was expressed as percentage using the following formula.

$$\text{Percentage wilt incidence} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \times 100$$

3.4.2.9.2 Number of Leaves

The Number of leaves of the plants was counted.

3.4.2.9.3 Fresh Weight of Shoots

The fresh weight of shoots (g) was taken in an electronic single pan balance immediately after uprooting the plants.

3.4.2.9.4 Dry Weight of Shoots

The dry weight of shoots (g) was taken after drying the samples to a constant weight at 60^0 C in a drying oven.

3.4.2.9.5 Fresh Weight of Roots

The fresh weight of newly formed roots (g) was taken in an electronic single pan balance immediately after uprooting the plants

3.4.2.9.6 Dry weight of roots

The dry weight of newly formed roots (g) was taken after drying the samples to a constant weight at 60^0 C in a drying oven.

3.4.2.9.7 Fresh Weight of Leaves

The fresh weight of newly formed leaves (g) was taken in an electronic single pan balance immediately after uprooting the plants.

3.4.2.9.8 Dry Weight of Leaves

The dry weight of newly formed leaves (g) was taken after drying the samples to a constant weight at 60^0 C in a drying oven.

3.4.2.9.9 Root Colonization by *P. indica*

The cuttings were carefully removed from the poly bags with roots intact. The roots were then washed gently in tap water to remove all adhering soil particles and blot dried. Root colonization was determined using the method described under 3.4.1.6.9.

3.5 STATISTICAL ANALYSIS

The statistical analysis of the data was done by the methods described by Snedecor and Cochran (1967). Data obtained from the pot culture experiment were tabulated, suitably transformed and subjected to statistical analysis.

RESULTS

4. RESULTS

4.1 PATHOGEN

4.1.1 Isolation

The pathogen was isolated from foot rot affected black pepper plants (var. Karimunda) collected from the pepper gardens of College of Agriculture, Vellayani, and was maintained by periodic subculturing on Carrot Agar slants.

4.1.2 Pathogenicity Testing

The pathogenicity of *P. capsici* was tested on three month old rooted cuttings of black pepper var. Karimunda. On artificial inoculation of the leaves, pale water soaked lesions appeared within 48 h which later turned dark brown to black in colour (Plates 5 and 6). These lesions with fimbriate margins gradually coalesced covering large area of the leaf resulting in defoliation. Finally the entire plant was defoliated and completely dried in about three weeks. The pathogen was then reisolated from the infected leaves.

4.1.3 Identification

On CA, the isolate produced petalloid type colonies which covered a Petri dish of 9 cm diameter in five to seven days (Plate 2). The width of the mycelium was in the range of 3.3 to 5.8 µm while the size of individual sporangium varied from 29.7 to 52.8 x 16.5 to 23.1µm. The sporangia were either papillate or spherical to irregular in shape (Plate 1). The observed characters were compared with that of the type culture available in the Department of Plant Pathology, College of Agriculture, Vellayani and based on the ability of the isolate to produce typical symptoms of foot rot disease in black pepper, the pathogen was identified as *Phytophthora capsici* Leonian.

Plate 1 *Phytophthora capsici* – the incitant of nursery wilt of black pepper (300 x)

Plate 2 Growth of *P. capsici* in Carrot Agar

Plate 3 Chlamydospores of *Piriformospora indica* (480 x)

Plate 4 Growth of *P. indica* in PDA

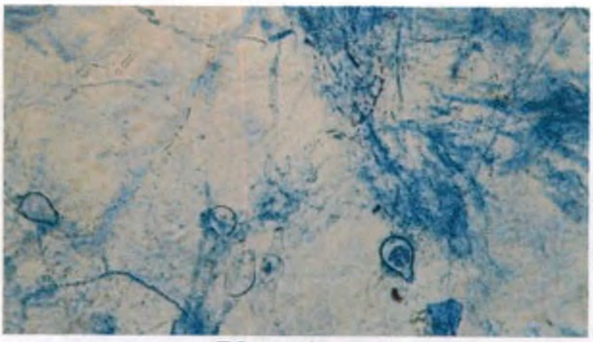
Plate 1

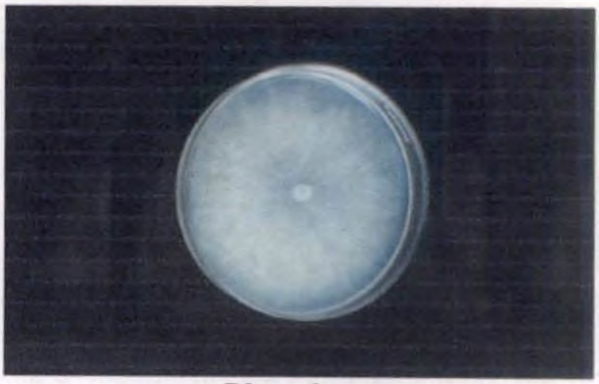
Plate 2

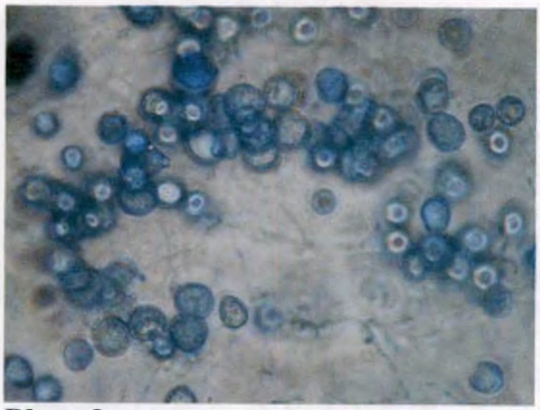
Plate 3

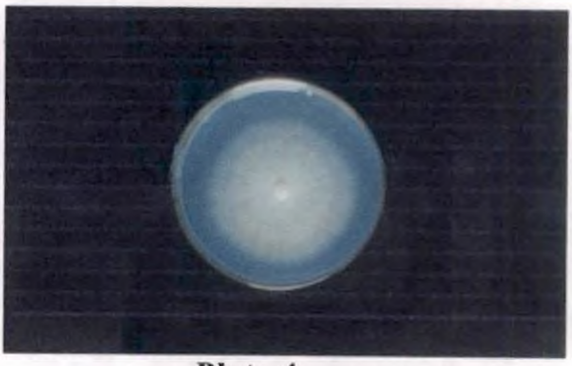
Plate 4

Plate 5 Symptoms of *P. capsici* infection in black pepper plants var. Karimunda on artificial inoculation

Plate 6 A view of foot rot affected black pepper plants in the nursery

Plate 5

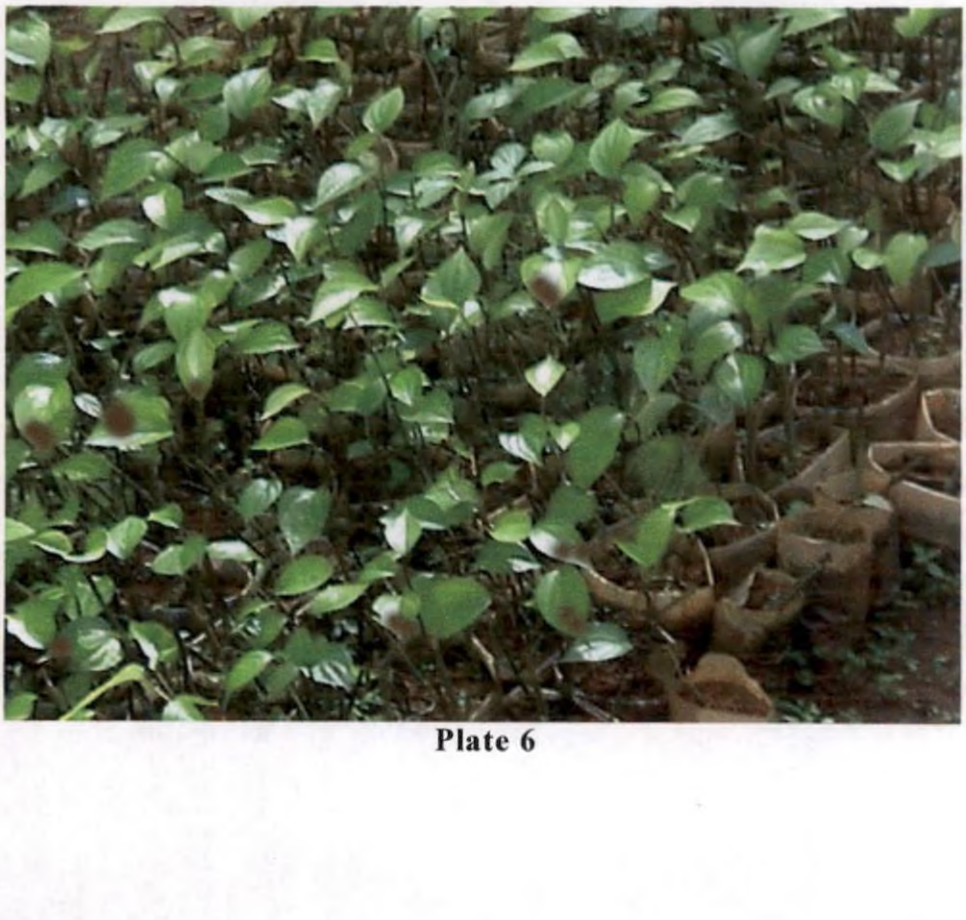

Plate 6

Table1 Inhibition* of mycelial growth of *Phytophthora capsici* in dual culture plate assay by PGPR strains

Treatments	Zone of inhibition (cm)**	
	Media	
	PDA	Carrot Agar
Pseudomonas putida 89B61	0.48 (1.22)	0.20 (1.10)
Pseudomonas fluorescens RCL3R4	1.18 (1.48)	0.35 (1.16)
Bacillus sp (strain BY-1)	0.23 (1.11)	0.13 (1.06)
Bacillus sp (strain BY-2)	0.20 (1.10)	0.13 (1.06)
Control	0.00 (1.00)	0.00 (1.00)
SE	0.019	0.010
CD (0.05)	0.060	0.030

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of three replications

4.2 *IN VITRO* STUDIES

4.2.1 Studies on Inhibition of Mycelial Growth of *P. capsici* by Biocontrol Agents in Dual Culture

4.2.1.1 Inhibition by Piriformospora indica

Four days after dual culturing with *P. indica*, percentage inhibition of mycelial growth of the pathogen was recorded. Initially, a slight inhibition of mycelial growth was observed (Plate 7). However, later the pathogen was found to overgrow the antagonist.

4.2.1.2 Inhibition by Rhizobacteria

4.2.1.2.1 Dual Culturing with Fluorescent Pseudomonads

Two strains of fluorescent pseudomonads *viz*., *Pseudomonas. putida* 89B61 and *Pseudomonas fluorescens* RCL3R4 were dual cultured against *P. capsici* on PDA and Carrot Agar media (Table 1). The performances of the two strains were significantly different on PDA with a mean inhibition zone of 0.48 and 1.18 cm, respectively for 89B61 and RCL3R4 when compared to the control (Plates 9 and 10). The highest inhibition was observed with the strain RCL3R4.

Similar result was observed on dual culturing on Carrot Agar medium where the strain RCL3R4 recorded an inhibition zone of 0.35 cm while an inhibition zone of 0.20 cm was recorded with 89B61 (Table 1 and Plate 11).

4.2.1.2.2 Dual Culturing with *Bacillus* Strains

No significant variation was observed between *Bacillus* strains, BY-1 and BY-2 on both the media. *Bacillus* strains BY-1 and BY-2 produced inhibition zones of 0.23 and 0.20 cm respectively on PDA (Table 1 and Plates 12, 14). Both the strains produced an inhibition zone of 0.13 cm on CA (Table 1 and Plates 13, 15).

Plate 7 Dual culture plate assay of *P. indica* against *P. capsici*

1- *P. indica*

2- *P. capsici* x *P. indica*

3- *P. capsici*

Plate 8 Dual culture plate assay of culture filtrate of *P. indica* against *P. capsici*

1- *P. indica* treated plate

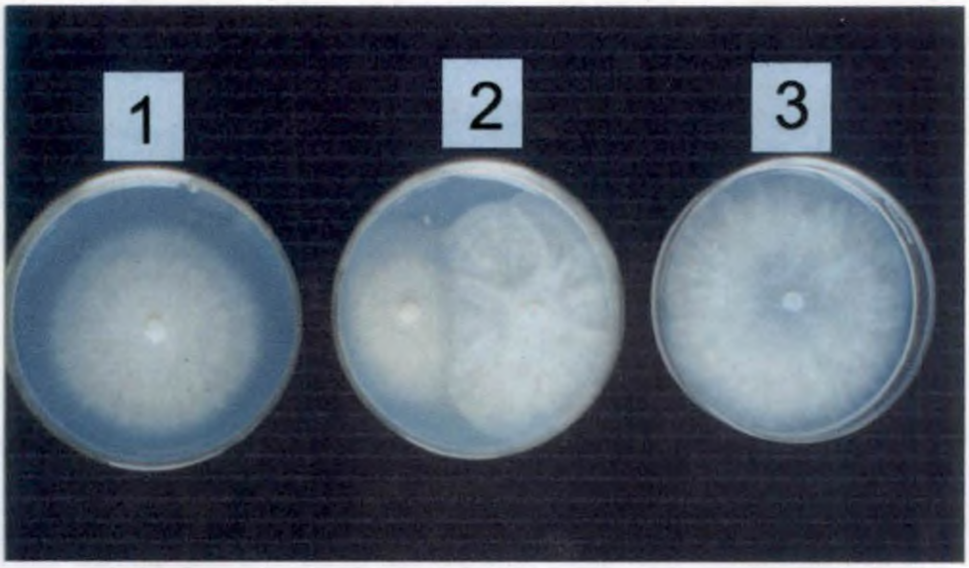

Plate 7

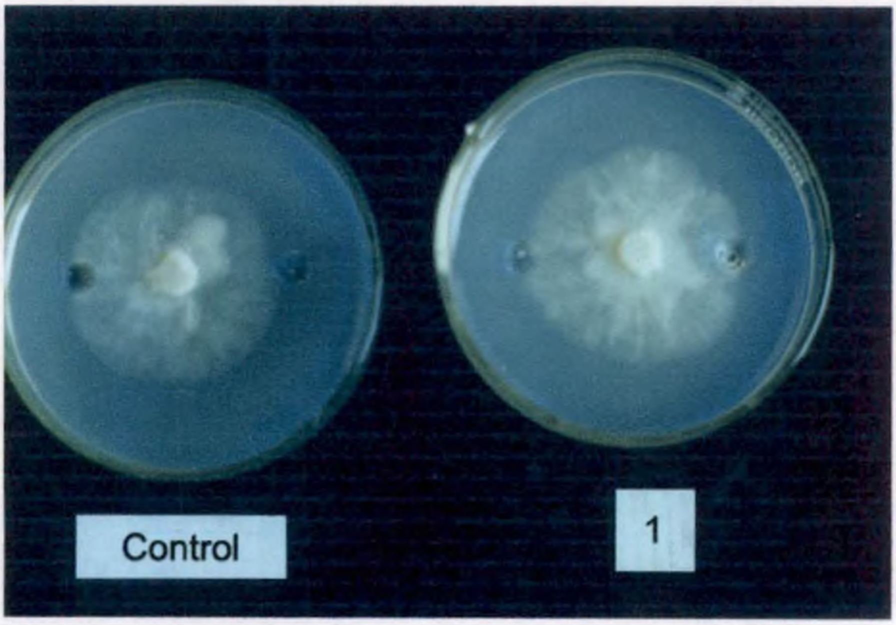

Plate 8

Plate 9 *In vitro* **inhibition of** *P. capsici* **by** *Pseudomonas putida* **strain 89B61 on PDA**

Plate 10 *In vitro* **inhibition of** *P. capsici* **by** *Pseudomonas fluorescens* **strain RCL3R4 on PDA**

Plate 11 *In vitro* **inhibition of** *P. capsici* **by** *Pseudomonas fluorescens* **strain RCL3R4 on Carrot Agar**

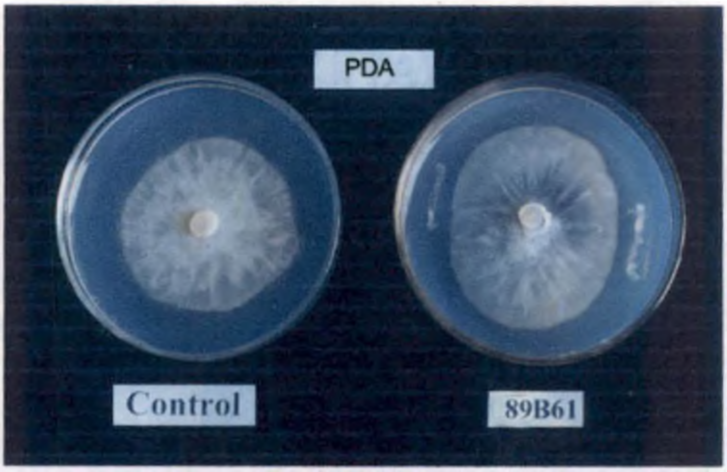

Plate 9

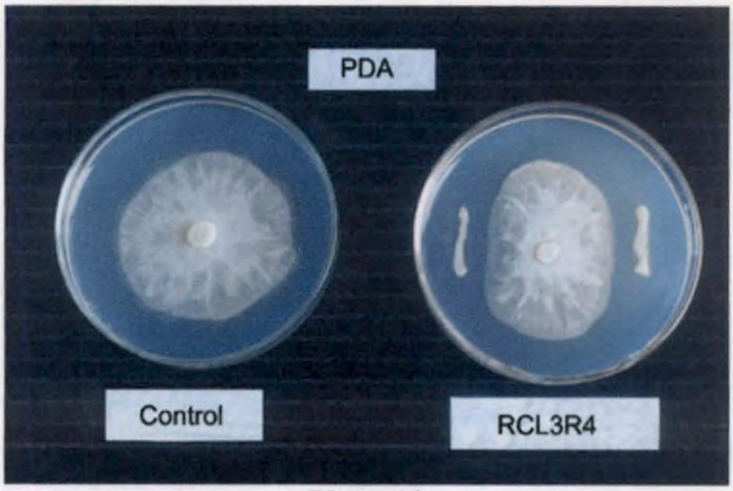

Plate 10

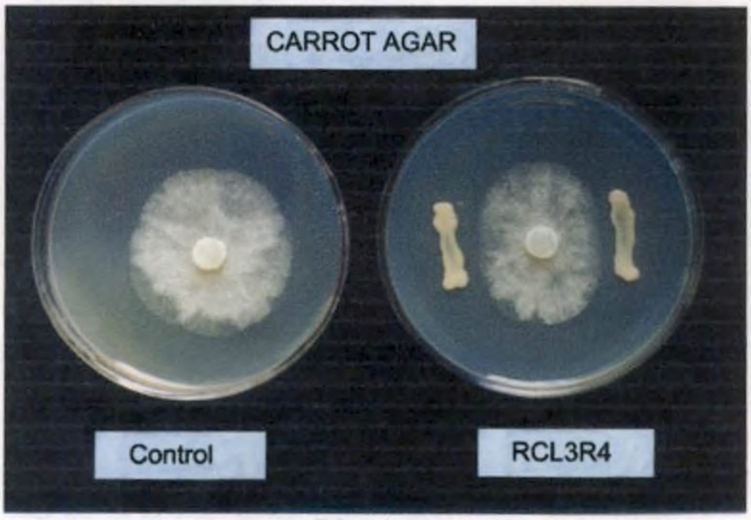

Plate 11

Plate 12 *In vitro* inhibition of *P. capsici* by *Bacillus* strain BY-1 on PDA

Plate 13 *In vitro* inhibition of *P. capsici* by *Bacillus* strain BY-1 on Carrot Agar

Plate 14 *In vitro* inhibition of *P. capsici* by *Bacillus* strain BY-2 on PDA

Plate 15 *In vitro* inhibition of *P. capsici* by *Bacillus* strain BY-2 on Carrot Agar

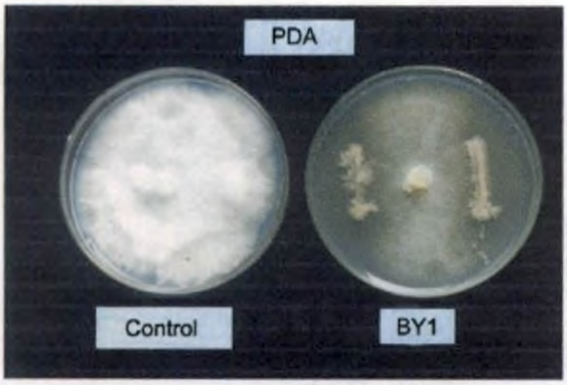

Plate 12

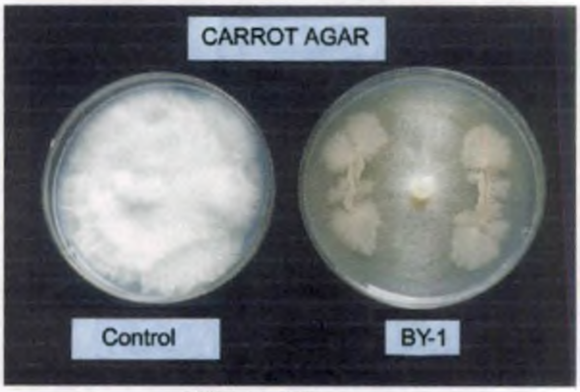

Plate 13

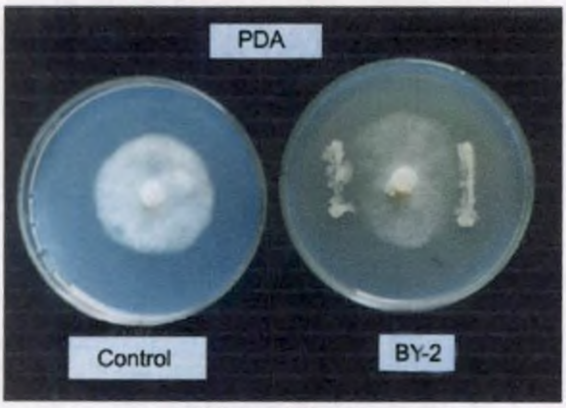

Plate 14

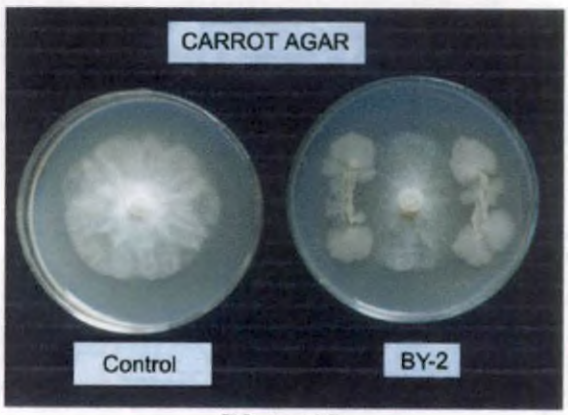

Plate 15

Plate 16 **Dual culture plate assay of culture filtrate of *P. putida* strain 89B61 against *P. capsici***

Plate 17 **Dual culture plate assay of culture filtrate of *P. fluorescens* strain RCL3R4 against *P. capsici***

Plate 18 **Dual culture plate assay of culture filtrate of *Bacillus* strain BY-1 against *P. capsici***

Plate 19 **Dual culture plate assay of culture filtrate of *Bacillus* strain BY-2 against *P. capsici***

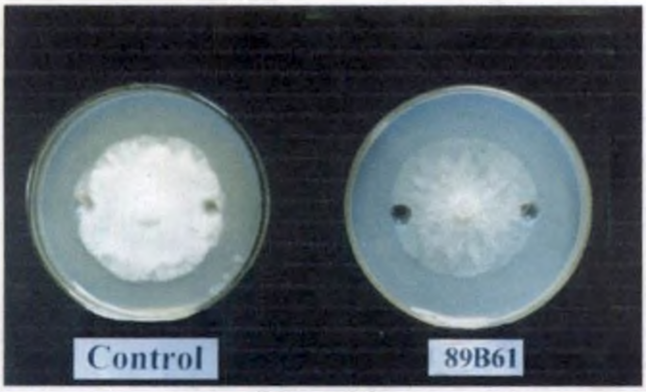

Plate 16

Plate 17

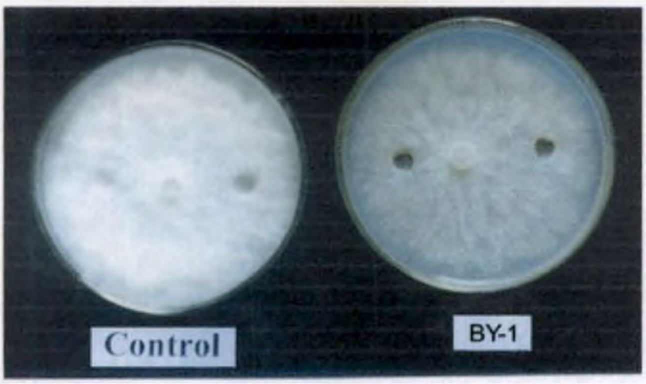

Plate 18

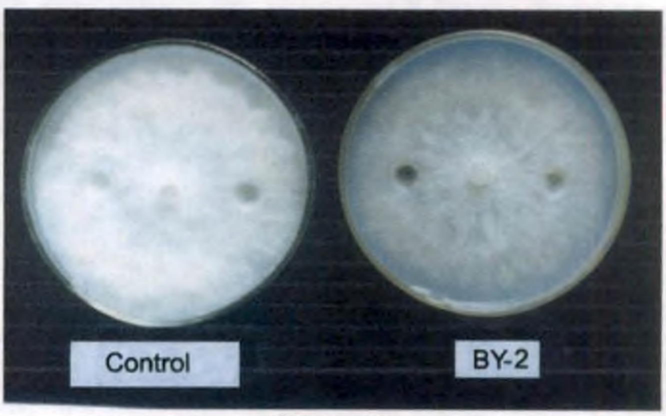

Plate 19

4.2.2 Inhibition of Mycelial Growth of *P. capsici* by Culture Filtrates of Biocontrol Agents

4.2.2.1 Inhibition by Culture Filtrate of P. indica

The culture filtrate of *P. indica* showed no inhibition on the mycelial growth of *P. capsici.* The pathogen overgrew the wells filled with the culture filtrate of *P. indica* similar to that in the control plates on PDA medium (Plate 8).

4.2.2.2 Inhibition by Culture Filtrates of Rhizobacteria

4.2.2.2.1 Inhibition by Culture Filtrates of Fluorescent Pseudomonads

The culture filtrates of both strains of fluorescent pseudomonads showed no inhibition on the mycelial growth of the pathogen (Plates 16 and 17).

4.2.2.2.2 Inhibition by Culture Filtrate of *Bacillus* strains

The culture filtrates of both strains of *Bacillus* showed no inhibition on the mycelial growth of the pathogen (Plates 18 and 19).

4.3 *IN VIVO* STUDIES

4.3.1 Plant Growth Promotion by PGPR Strains and *P. indica*

The following observations on plant growth were recorded three months after planting black pepper cuttings. Influence of various treatments on growth of black pepper cuttings are presented in Plates 20, 22, 24, 26, 28, 30 and 32.

4.3.1.1 Number of Leaves

The influence of different growth promoting agents on leaf production of black pepper cuttings was not significantly variable from that of the control (Table 2). The mean number of leaves recorded in the treatment with the fluorescent pseudomonad, *P. putida* 89B61 was 2.34 as

Table 2 Effect of PGPR and *Piriformospora indica* on number of leaves and roots produced in black pepper cuttings*

Treatments	Number of leaves/plant**	Percentage variation over control	Number of roots/plant**	Percentage variation over control
Pseudomonas putida 89B61	2.34 (1.83)	19.39	6.47 (2.73)	82.77
Pseudomonas fluorescens RCL3R4	2.06 (1.75)	5.10	3.60 (2.14)	1.69
Piriformospora indica (spore suspension)	2.07 (1.75)	5.61	5.06 (2.46)	42.94
Piriformospora indica (mycelial mat)	1.68 (1.64)	-14.29	4.63 (2.37)	30.71
Bacillus sp (strain BY-1)	2.32 (1.82)	15.52	6.48 (2.73)	83.05
Bacillus sp (strain BY-2)	2.27 (1.81)	15.82	3.55 (2.13)	0.28
IBA @ 1000ppm	2.20 (1.79)	12.24	8.61 (3.10)	143.22
Control	1.96 (1.72)		3.54 (2.13)	
SE	0.114		0.261	
CD (0.05)	0.323		0.738	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 3 Group means of number of leaves and roots produced in black pepper cuttings treated with PGPR and *Piriformospora indica**

Treatments	Number of leaves/plant	Number of roots/plant
Fluorescent pseudomonads (89B61 and RCL3R4)	2.2 (1.79)	5.04 (2.44)
Piriformospora indica (mycelial mat and spore suspension)	1.88 (1.70)	4.85 (2.42)
Bacillus sp (strains BY-1 and BY-2)	2.30 (1.82)	5.02 (2.43)
IBA + Control	2.08 (1.76)	6.08 (2.62)
CD	0.228	0.522

*Values in parenthesis are after $\sqrt{x+1}$ transformation

Plate 20 **Effect of *P. putida* strain 89B61 on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_1 – Treatment with *P. putida* strain 89B61

Plate 21 **Effect of *P. putida* strain 89B61 on shoot and root growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_1 – Treatment with *P. putida* strain 89B61

Plate 22 **Effect of *P. fluorescens* strain RCL3R4 on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_2 - Treatment with *P. fluorescens* strain RCL3R4

Plate 23 **Effect of *P. fluorescens* strain RCL3R4 on shoot and root growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_2- Treatment with *P. fluorescens* strain RCL3R4

Plate 24 **Effect of spore suspension of *P. indica* on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_3 - Treatment with spore suspension of *P. indica*

Plate 25 **Effect of spore suspension of *P. indica* on shoot and root growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_3 - Treatment with spore suspension of *P. indica*

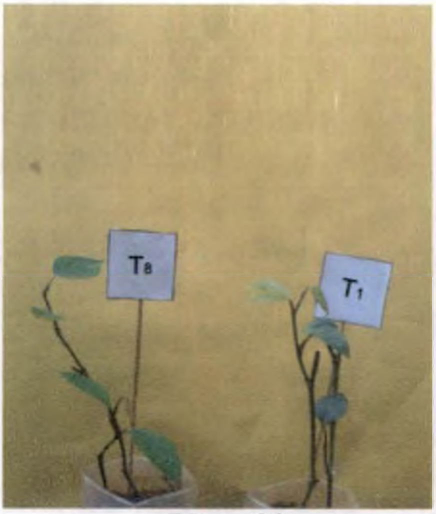

Plate 20

Plate 21

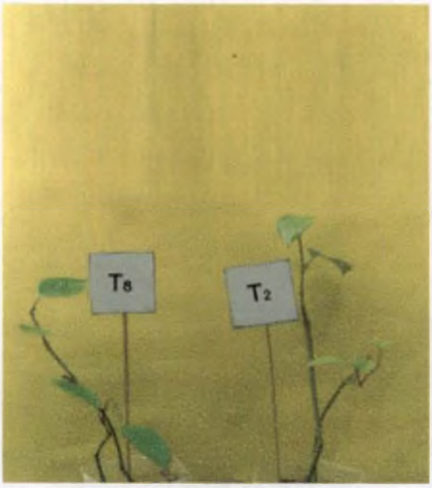

Plate 22

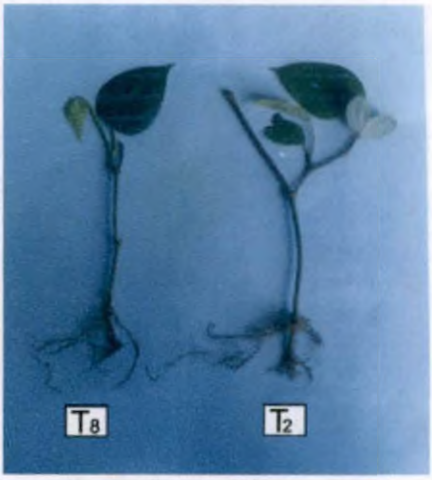

Plate 23

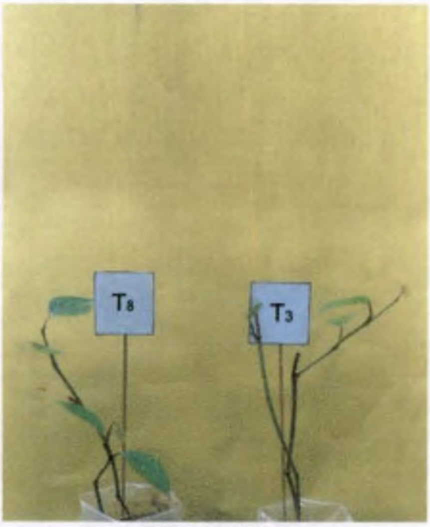

Plate 24

Plate 25

Plate 26 **Effect of mycelial mat of *P. indica* on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_4 - Treatment with mycelial mat of *P. indica*

Plate 27 Effect of mycelial mat of *P. indica* on shoot and root growth of black pepper cuttings three months after planting

T_8 – Untreated control

T_4 - Treatment with mycelial mat of *P. indica*

Plate 28 **Effect of *Bacillus* strain BY-1 on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_5 - Treatment with *Bacillus* strain BY-1

Plate 29 **Effect of *Bacillus* strain BY-1 on shoot and root growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_5 - Treatment with *Bacillus* strain BY-1

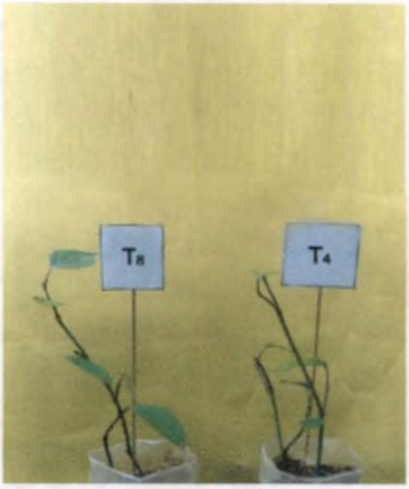

Plate 26

Plate 27

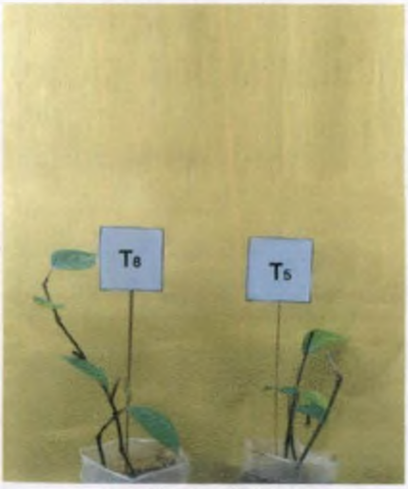

Plate 28

Plate 29

Plate 30 **Effect of *Bacillus* strain BY-2 on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_6 - Treatment with *Bacillus* strain BY-2

Plate 31 **Effect of *Bacillus* strain BY-2 on shoot and root growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_6 - Treatment with *Bacillus* strain BY-2

Plate 32 **Effect of IBA @ 1000 ppm on growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_7 - Treatment with IBA @ 1000 ppm

Plate 33 **Effect of IBA @ 1000 ppm on shoot and root growth of black pepper cuttings three months after planting**

T_8 – Untreated control

T_7 - Treatment with IBA @ 1000 ppm

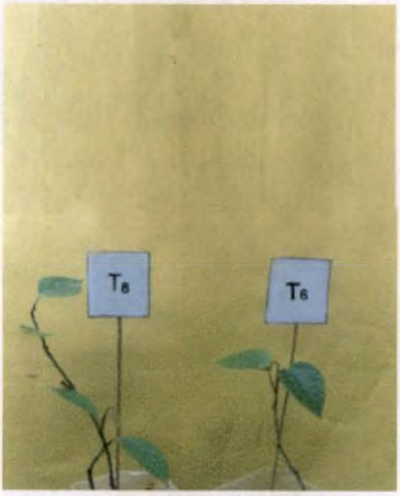

Plate 30

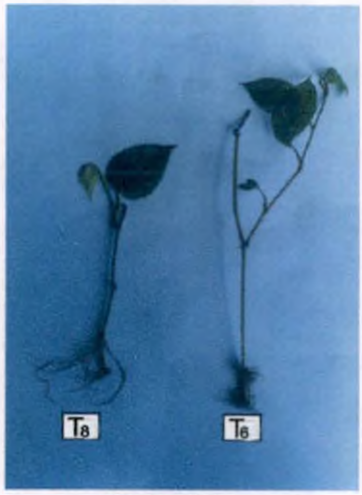

Plate 31

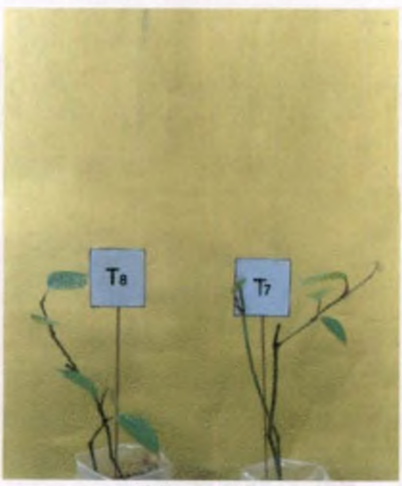

Plate 32

Plate 33

against 1.68 and 1.96 in the plants treated with mycelial mat of *P. indica* and control respectively.

The treatment with *P. putida* strain 89B61 recorded an increase of 19.39 percentage over the control (Table 2). The *Bacillus* strains BY-1 and BY-2 exhibited 15.52 and 15.82 percentage increase over the control. The treatments using mycelial mat of *P. indica* recorded a decrease of 14.29 percentage over the control with respect to number of leaves.

In order to find out the influence of fluorescent pseudomonads, *Bacillus* and *P. indica* on the growth of pepper cuttings, the treatments were grouped into four categories viz., Group I –two strains of fluorescent pseudomonads (*P. putida* 89B61 and *P. fluorescens* RCL3R4), Group II - two strains of *Bacillus* (BY-1 and BY-2), Group III-treatments with spore suspension and mycelial mat of *P. indica* and Group IV-IBA @ 1000 ppm and control maintained for comparison with the above three groups.

The treatments did not show any significant variation when grouped into four categories (Table 3). The plants treated with the *Bacillus* strains recorded the maximum leaf number (2.30) as against 2.08 in Group IV.

4.3.1.2 Number of Roots

The effect of different biocontrol agents on the number of roots produced in black pepper cuttings var. Karimunda was not statistically significant when compared to that of the untreated control and IBA treated check (Table 2). The mean number of roots observed in the treatment with IBA @ 1000 ppm was 8.61 as against 3.54 in the control. Among the treatments with various growth promoting agents, the maximum number of roots (6.48) was recorded in the cuttings treated with BY-1. The least number of roots was observed in the plants treated with BY-2 which recorded a mean value of 3.55.

The plants treated with *P. putida* 89B61 recorded 82.77 percentage increase in the number of roots over the control (Table 2). The treatments using *Bacillus* strain BY-1 and IBA@ 1000 ppm recorded an increase of 83.05 and 143.22 percentage respectively over the control.

Though the treatments were not significantly variable when grouped, fluorescent pseudomonads recorded the highest number of roots (5.04) (Table 3).

4.3.1.3 Fresh Weight of Shoots

There was an increase in the fresh weight of shoots in the treatments with *P. putida* 89B61 and *P. indica* spore suspension with mean shoot weight of 5.54 and 4.62 g respectively (Table 4). However, these treatments were not statistically significant when compared to the control which recorded an average fresh shoot weight of 4.08 g. The lowest shoot weight (1.27 g) was observed in the treatment with mycelial mat of *P. indica* (Plates 21, 23, 25, 27, 29, 31 and 33).

The treatments with *P. putida* 89B61 and spore suspension of *P. indica* recorded an increase of 35.78 and 13.24 percentage respectively in shoot fresh weight over the control (Table 4). The treatments with *P. fluorescens* RCL3R4, *Bacillus* strains, BY-1 and BY-2 mycelial mat of *P. indica* and IBA @ 1000 ppm recorded decrease in shoot weight over the control.

When grouped into four, the treatments were not statistically significant (Table 5). The fluorescent pseudomonad strains recorded the highest shoot fresh weight of 4.77 g whereas the treatments with *Bacillus* and *P. indica* recorded fresh shoot weight of 3.11 and 2.95 g respectively.

4.3.1.4 Dry Weight of Shoots

The treatments with *P. putida* 89B61 and spore suspension of *P. indica* on shoot dry weight which recorded an average shoot dry weight

Table 4 Effect of PGPR and *Piriformospora indica* on fresh and dry weight of shoots in black pepper cuttings*

Treatments	Fresh weight of shoots / plant (g)**	Percentage variation over control	Dry weight of shoots / plant (g)**	Percentage variation over control
Pseudomonas putida 89B61	5.54 (2.56)	35.78	1.32 (1.52)	21.10
Pseudomonas fluorescens RCL3R4	4.00 (2.24)	-1.96	1.03 (1.42)	-5.50
Piriformospora indica (spore suspension)	4.62 (2.37)	13.24	1.12 (1.45)	2.75
Piriformospora indica (mycelial mat)	1.27 (1.51)	-68.87	0.39 (1.18)	-64.22
Bacillus sp (strain BY-1)	3.00 (2.00)	-26.47	0.48 (1.21)	-55.96
Bacillus sp (strain BY-2)	3.22 (2.06)	-21.08	0.76 (1.33)	-30.27
IBA @ 1000ppm	2.84 (1.96)	-30.39	0.73 (1.31)	-33.03
Control	4.08 (2.25)		1.09 (1.45)	
SE	0.111		0.042	
CD (0.05)	0.314		0.120	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 5 Group means of fresh and dry weight of shoots in black pepper cuttings treated with PGPR and *Piriformospora indica**

Treatments	Fresh weight of shoots /plant (g)	Dry weight of shoots /plant (g)
Fluorescent pseudomonads (89B61 and RCL3R4)	4.77 (2.40)	1.18 (1.47)
Piriformospora indica (mycelial mat and spore suspension)	2.95 (1.94)	0.76 (1.32)
Bacillus sp (strains BY-1 and BY-2)	3.11 (2.03)	0.62 (1.27)
IBA + Control	3.46 (2.11)	0.91 (1.38)
CD (0.05)	0.222	8.464

*Values in parenthesis are after $\sqrt{x+1}$ transformation

Table 6 Effect of PGPR and *Piriformospora indica* on fresh and dry weight of roots in black pepper cuttings*

Treatments	Fresh weight of roots /plant (g)**	Percentage variation over control	Dry weight of roots / plant (g)**	Percentage variation over control
Pseudomonas putida 89B61	0.11 (1.05)	10.00	0.05 (1.02)	25.00
Pseudomonas fluorescens RCL3R4	0.06 (1.03)	-40.00	0.04 (1.02)	0.00
Piriformospora indica (spore suspension)	0.16 (1.08)	60.00	0.11 (1.05)	175.00
Piriformospora indica (mycelial mat)	0.10 (1.05)	0.00	0.08 (1.04)	100.00
Bacillus sp (strain BY-1)	0.13 (1.06)	30.00	0.05 (1.02)	25.00
Bacillus sp (strain BY-2)	0.05 (1.03)	-50.00	0.03 (1.01)	-25.00
IBA @ 1000ppm	0.09 (1.05)	-10.00	0.06 (1.03)	50.00
Control	0.10 (1.05)		0.04 (1.02)	
SE	0.023		0.014	
CD (0.05)	0.656		0.040	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 7 Group means of fresh and dry weight of roots in black pepper cuttings treated with PGPR and *Piriformospora indica**

Treatments	Fresh weight of roots /plant (g)	Dry weight of roots /plant (g)
Fluorescent pseudomonads (89B61 and RCL3R4)	0.09 (1.04)	0.05 (1.02)
Piriformospora indica (mycelial mat and spore suspension)	0.13 (1.06)	0.10 (1.05)
Bacillus sp (strains BY-1 and BY-2)	0.09 (1.04)	0.04 (1.02)
IBA + Control	0.10 (1.04)	0.05 (1.02)
CD (0.05)	0.046	0.029

*Values in parenthesis are after $\sqrt{x+1}$ transformation

of 1.32 and 1.12 g respectively and were statistically on par with the control (1.09 g) (Table 4). The lowest dry weight of 0.39 g was recorded in the cuttings treated with mycelial mat of *P. indica*.

Treatments with *P. putida* 89B61 and spore suspension of *P. indica* recorded an increase of 21.10 and 2.75 percentage over the control with respect to dry weight of shoots (Table 4). All the other treatments showed percentage decrease over the control.

The four groups of treatments were statistically on par (Table 5). However, the fluorescent pseudomonads when grouped together recorded the highest shoot dry weight (1.18g) while the group of treatments with *P. indica* and *Bacillus* recorded average group means of 0.76 and 0.62g respectively.

4.3.1.5 Fresh Weight of Newly Formed Roots

No significant difference was observed with respect to the fresh weight of roots between the cuttings treated with the various growth promoting agents as against the control (Table 6). The highest mean fresh root weight (0.11 g) was recorded in the treatment with *P. putida* 89B61. Cuttings treated with *Bacillus* strain BY-2 recorded the lowest mean root fresh weight of 0.05 g (Plates 21, 23, 25, 27, 29, 31 and 33).

The treatments with spore suspension of *P. indica* and *Bacillus* strain BY-1 recorded an increase of 60.00 and 30.00 percentage respectively over the control (Table 6).

There was no statistically significant difference between the four groups of treatments (Table 7). The treatments with *P. indica* recorded the maximum root fresh weight of 0.13g whereas both fluorescent pseudomonads and Bacilli recorded a root fresh weight of 0.09g.

4.3.1.6 Dry Weight of Newly Formed Roots

No significant variation was observed in the dry weight of roots in treatments with various growth promoting agents as against the control

Table 8 Effect of PGPR and *Piriformospora indica* on fresh and dry weight of leaves in black pepper cuttings*

Treatments	Fresh weight of leaves / plant (g)**	Percentage variation over control	Dry weight of leaves / plant (g)**	Percentage variation over control
Pseudomonas putida 89B61	0.60 (1.27)	3.45	0.14 (1.07)	7.69
Pseudomonas fluorescens RCL3R4	0.55 (1.24)	-5.17	0.10 (1.05)	-23.08
Piriformospora. indica (spore suspension)	0.84 (1.36)	44.83	0.33 (1.15)	153.85
Piriformospora. indica (mycelial mat)	0.43 (1.20)	-25.86	0.18 (1.09)	38.46
Bacillus sp (strain BY-1)	0.76 (1.33)	31.03	0.14 (1.07)	7.69
Bacillus sp (strain BY-2)	0.43 (1.20)	-25.86	0.15 (1.07)	15.38
IBA @ 1000ppm	0.64 (1.28)	10.34	0.14 (1.07)	7.69
Control	0.58 (1.26)		0.13 (1.06)	
SE	0.055		0.	
CD (0.05)	0.155		0.075	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 9 Group means of fresh and dry weight of leaves in black pepper cuttings treated with PGPR and *Piriformospora indica**

Treatments	Fresh weight of leaves (g) /plant	Dry weight of leaves (g) /plant
Fluorescent pseudomonads (89B61 and RCL3R4)	0.58 (1.26)	0.12 (1.06)
Piriformospora. indica (mycelial mat and spore suspension)	0.64 (1.28)	0.26 (1.12)
Bacillus sp (strains BY-1 and BY-2)	0.60 (1.26)	0.15 (1.07)
IBA + Control	0.61 (1.27)	0.14 (1.07)
CD (0.05)	0.110	0.053

*Values in parenthesis are after $\sqrt{x+1}$ transformation

(0.04 g) (Table 6). The highest dry weight was recorded in the treatment with spore suspension of *P. indica* (0.11 g) whereas the treatment with *Bacillus* strain BY-2 recorded the lowest root dry weight of 0.03 g.

The treatments with spore suspension and mycelial mat of *P. indica* recorded an increase of 175.00 and 100.00 percentage respectively over the control with respect to root dry weight (Table 6). The *Bacillus* strain BY-1 also produced an increase of 25.00 percentage over the control.

Though the four groups of treatments were statistically on par, the treatments with *P. indica* recorded the maximum root dry weight of 0.10g which was marginally higher when compared to the other three groups (Table 7).

4.3.1.7 Fresh Weight of Newly Formed Leaves

Effect of treatments with various growth promoting agents on the fresh weight of leaves were not statistically variable as against the control which recorded mean leaf fresh weight of 0.58 g (Table 8). Treatment with *P. indica* spore suspension recorded the highest leaf fresh weight of 0.84 g, whereas the lowest leaf weight (0.43 g) was recorded in the treatments with mycelial mat of *P. indica* and *Bacillus* strain BY-2.

The plants treated with spore suspension of *P. indica* recorded an average increase of 44.83 percentage over the control with respect to leaf fresh weight (Table 8). The *Bacillus* strain BY-1 also produced an increase of 31.03 percentage over the control.

There was no statistically significant difference between the four groups of treatments (Table 9). The treatments with *P. indica* recorded the maximum leaf fresh weight of 0.64g. The lowest leaf fresh weight (0.58) was recorded in the treatments with fluorescent pseudomonads when grouped.

4.3.1.8 Dry Weight of Newly Formed Leaves

No significant difference was observed in the dry weight of leaves between the treatments with various growth promoting agents as against the control which recorded a leaf dry weight of 0.13 g (Table 8). The highest dry weight of 0.33 g was recorded in the treatment with spore suspension of *P. indica*. The least dry weight of 0.10 g was recorded in the treatment with *P. fluorescens* RCL3R4.

The treatments with spore suspension and mycelial mat of *P. indica* recorded an average increase of 153.85 and 38.46 percentage respectively over the control (Table 8). The *Bacillus* strains, BY-1 and BY-2 produced an increase of 7.69 and 15.38 percentage over the control with respect to dry weight of leaves.

The effects of the four groups of treatments were statistically on par (Table 9). The treatments with *P. indica* recorded the highest leaf dry weight of 0.26g. The treatments with fluorescent pseudomonads and *Bacilli* recorded an average leaf dry weight of 0.12 and 0.15g respectively.

4.3.1.9 Root Colonization by P. indica

The roots of black pepper cuttings were not colonized by *P. indica* when treated with spore suspension or mycelial mat. No mycelium or chlamydospores of the fungus were observed on microscopic examination after staining the roots with trypan blue.

4.3.2 Biological Control of Phytophthora Disease in the Nursery

Observations were recorded 40 days after inoculation with the pathogen as well as the antagonists.

4.3.2.1 Disease Incidence

The plants treated with spore suspension of *P. indica* exhibited the highest wilt percentage of 93.22 per cent which was more than that in the

Table 10 Effect of biocontrol agents and COC on percentage wilt of black pepper plants in the nursery*

Treatments	Percentage wilt**
Pseudomonas putida 89B61	0.00 (0.00)
Pseudomonas fluorescens RCL3R4	6.70 (14.99)
Piriformospora indica (spore suspension)	93.22 (74.99)
Piriformospora indica (mycelial mat)	0.00 (0.00)
Bacillus sp (strain BY-1)	0.00 (0.00)
Bacillus sp (strain BY-2)	0.00 (0.00)
COC @ 0.20 %	0.00 (0.00)
Trichoderma harzianum + *Glomus fasciculatum*	0.00 (0.00)
Inoculated control	37.06 (37.48)
Uninoculated control	0.00 (0.00)
SE	7.116
CD (0.05)	22.422

* Values in parenthesis are after arc sine transformation
**Mean of 8 replications having one plant each

Table 11 Effect of biocontrol agents and COC on number of leaves in black pepper inoculated with *Phytophthora capsici**

Treatments	Number of leaves/plant**	Percentage variation over uninoculated control
Pseudomonas putida 89B61	7.43 (2.90)	17.19
Pseudomonas fluorescens RCL3R4	5.83 (2.61)	-8.04
Piriformospora indica (spore suspension)	0.51 (1.23)	-91.96
Piriformospora indica (mycelial mat)	6.91 (2.81)	8.99
Bacillus sp (strain BY-1)	8.21 (3.03)	29.50
Bacillus sp (strain BY-2)	9.11 (3.18)	43.69
COC @ 0.20 %	7.94 (2.99)	25.24
Trichoderma harzianum + *Glomus fasciculatum*	7.22 (2.87)	13.88
Inoculated control	4.30 (2.30)	-32.18
Uninoculated control	6.34 (2.71)	
SE	0.201	
CD (0.05)	0.568	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 12 Group means of number of leaves produced in black pepper inoculated with *Phytophthora capsici* and treated with biocontrol agents and COC*

Treatments	Number of leaves/plant
Fluorescent pseudomonads 89B61 and RCL3R4	6.63 (2.76)
Piriformospora indica (spore suspension and mycelial mat)	3.71 (2.02)
Bacillus sp (strains BY-1 and BY-2)	8.66 (3.11)
POP recommendations	7.58 (2.93)
Control (inoculated + uninoculated)	5.32 (2.51
CD (0.05)	0.402

*Values in parenthesis are after $\sqrt{x+1}$ transformation

inoculated control (37.48) (Table 10). The plants treated with the fluorescent pseudomonad RCL3R4 recorded a wilt percentage of 6.70. All other treatments were highly effective and checked the infection completely.

4.3.2.2 Number of Leaves

There was significant difference between the various treatments with respect to the number of leaves (Table 11). The treatments with *Bacillus* strains BY-2 and BY-1 recorded mean values of 9.11 and 8.21 as against 6.34 in the control. The treatment with *P. fluorescens* strain RCL3R4 with an average leaf number of 5.83 was inferior to the untreated control. The inoculated control recorded an average leaf number of 4.30.

The treatments with the *Bacillus* strains BY-1 and BY-2 recorded an increase of 29.50 and 43.69 percentage respectively over the control whereas an increase of 17.19 and 8.99 percentage over the control was recorded in the treatment with *P. putida* strain 89B61 and mycelial mat inoculation with *P. indica* respectively (Table 11).

The *Bacillus* strains when taken as a group performed better with an average leaf number of 8.66 (Table 12). However, the treatments with the endophytic fungus, *P. indica* with a group mean of 3.71 were inferior to control.

4.3.2.3 Fresh Weight of Shoots

In presence of the pathogen, the treatment with *Bacillus* strain BY-2 recorded the maximum shoot weight (4.34 g) as against the control with an average shoot fresh weight of 3.07 g (Table 13). The lowest shoot fresh weight (2.79 g) was recorded in the treatment with *Bacillus* strain BY-1.

The treatment with the *Bacillus* strain BY-1 recorded a decrease of 9.12 percentage over the control (Table 13). The plants treated with the biocontrol bacteria *Bacillus* strain BY-2 and *P. putida* 89B61 recorded an

Table 13 Effect of biocontrol agents and COC on fresh weight and dry weight of shoots in black pepper inoculated with *Phytophthora capsici**

Treatments	Fresh weight of shoots / plant (g)**	Percentage variation over uninoculated control	Dry weight of shoots / plant (g)**	Percentage variation over uninoculated control
Pseudomonas putida 89B61	3.64 (2.15)	18.57	0.94 (1.39)	-16.81
Pseudomonas fluorescens RCL3R4	3.26 (2.06)	6.19	0.81 (1.35)	-28.32
Piriformospora indica (spore suspension)	0.22 (1.11)	-92.83	0.07 (1.03)	-93.81
Piriformospora. indica (mycelial mat)	3.22 (2.05)	4.89	1.48 (1.58)	30.97
Bacillus sp BY-1	2.79 (1.95)	-9.12	0.09 (1.44)	-92.04
Bacillus sp BY-2	4.34 (2.31)	41.37	1.69 (1.64)	49.56
COC @ 0.20 %	3.98 (2.23)	29.64	1.62 (1.62)	43.36
Trichoderma harzianum + *Glomus fasciculatum*	3.57 (2.14)	16.29	1.00 (1.42)	-11.50
Inoculated control	1.16 (1.47)	-62.21	0.38 (1.17)	-66.37
Uninoculated control	3.07 (2.02)		1.13 (1.46)	
SE	0.138		0.090	
CD (0.05)	0.391		0.256	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 14 Group means of fresh and dry weight of shoots in black pepper inoculated with *Phytophthora capsici** and treated with biocontrol agents and COC

Treatments	Fresh weight of shoots (g) /plant	Dry weight of shoots (g) /plant
Fluorescent pseudomonads (89B61 and RCL3R4)	3.45 (2.11)	0.88 (1.37)
Piriformospora indica (spore suspension and mycelial mat)	1.72 (1.58)	0.78 (1.31)
Bacillus sp (strains BY-1 and BY-2)	3.57 (2.13)	1.39 (1.54)
POP recommendations	3.78 (2.19)	1.31 (1.52)
Control (inoculated + uninoculated	2.12 (1.74)	0.78 (1.32)

*Values in parenthesis are after $\sqrt{x+1}$ transformation

increase of 41.37 and 18.57 percentage respectively over the control with respect to fresh weight of shoots. The plants treated with 0.20 percentage COC recorded an increase of 29.64 percentage over the control.

Application of 0.20 per cent COC and combined application of *T. harzianum* and AMF when grouped together recorded the highest average shoot weight of 3.78 g (Table 14). However, the group of treatments with *P. indica* recorded the lowest group mean of 1.72 g.

4.3.2.4 Dry Weight of Shoots

Significant variation was observed in the dry weight of shoots between the treatments with the *Bacillus* strain BY-2 (1.69 g) and *P. fluorescens* RCL3R4 (0.81 g) (Table 13). The lowest shoot dry weight of 0.07 g was recorded in the treatment with spore suspension of *P. indica*. The treatments with COC @ 0.20 per cent and mycelial mat of *P. indica* with average shoot dry weight values of 1.62 and 1.48 g respectively were superior to the untreated control which recorded an average shoot dry weight of 1.13 g.

The plants treated with the *Bacillus* strain BY-2 recorded the highest percentage increase (49.56) over the control (Table 13). Both strains of fluorescent pseudomonads recorded percentage decrease over the control with respect to dry weight of shoots. The dry weight of shoots in plants which were given combined application of *T. harzianum* and AMF recorded 11.50 percentage decrease over the control.

The *Bacillus* group with an average shoot dry weight of 1.39 g performed better when compared to other groups of biocontrol agents (Table 14). *P. indica* group of treatments recorded the lowest shoot dry weight of 0.78 g.

4.3.2.5 Fresh Weight of Roots

No significant difference was noticed in the treatments with various biocontrol agents with respect to the fresh weight of roots compared to

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Table 15 Effect of biocontrol agents and COC on fresh weight and dry weight of roots in black pepper inoculated with *Phytophthora capsici**

Treatments	Fresh weight of roots / plant (g)**	Percentage variation over uninoculated control	Dry weight of roots /plant (g)**	Percentage variation over uninoculated control
Pseudomonas putida 89B61	1.22 (1.49)	46.99	0.33 (1.15)	-13.16
Pseudomonas fluorescens RCL3R4	0.80 (1.34)	-3.61	0.37 (1.17)	-2.63
Piriformospora indica (spore suspension)	1.25 (1.00)	50.60	0.01 (1.00)	-97.37
Piriformospora indica (mycelial mat)	1.02 (1.42)	22.89	0.47 (1.21)	23.68
Bacillus sp (strain BY-1)	1.03 (1.42)	24.10	0.55 (1.25)	44.74
Bacillus sp (strain BY-2)	0.98 (1.41)	18.07	0.56 (1.25)	47.37
COC @ 0.20 %	1.23 (1.49)	48.19	0.51 (1.23)	34.21
Trichoderma harzianum + *Glomus fasciculatum*	0.95 (1.40)	14.46	0.48 (1.22)	26.32
Inoculated control	0.44 (1.20)	-46.99	0.12 (1.06)	-68.42
Uninoculated control	0.83 (1.35)		0.38 (1.18)	
SE	0.062		0.028	
CD (0.05)	5.88		0.080	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 16 Group means of fresh and dry weight of roots in black pepper inoculated with *Phytophthora capsici* and treated with biocontrol agents and COC*

Treatments	Fresh weight of roots /plant (g)	Dry weight of roots /plant (g)
Fluorescent pseudomonads (89B61 and RCL3R4)	1.01 (1.42)	0.35 (1.16)
Piriformospora indica (spore suspension and mycelial mat)	1.14 (1.21)	0.24 (1.11)
Bacillus sp (strains BY-1 and BY-2)	1.01 (1.42)	0.56 (1.25)
POP recommendations	1.09 (1.45)	0.50 (1.22)
Control (inoculated + uninoculated)	0.64 (1.28)	0.25 (1.12)
CD (0.05)	0.123	0.057

*Values in parenthesis are after $\sqrt{x+1}$ transformation

control (0.83 g) (Table 15). The highest root fresh weight (1.23 g) was obtained in the treatment with COC @ 0.20 per cent.

In the case of fresh weight of roots, only the plants treated with *P. fluorescens* strain RCL3R4 recorded a decrease of 3.61 percentage over uninoculated control (Table 15).

The performance of *P. indica* group of treatments with a mean of fresh root weight of 1.14 g was superior to all other groups (Table 16).

4.3.2.6 Dry Weight of Roots

The treatments with the *Bacillus* strains BY-1 and BY-2, COC @ 0.20 per cent, combined application of *T. harzianum* and AMF and mycelial mat of *P. indica* were higher than uninoculated control with an average root dry weight of 0.38 g though the above treatments were statistically on par (Table 15). The lowest root dry weight was recorded in the treatment with spore suspension of *P. indica* with a root dry weight of 0.01 g.

The plants treated with the *Bacillus* strains BY-1 and BY-2 and mycelial mat of *P. indica* recorded an increase of 44.74, 47.37 and 23.68 percentage respectively over the control (Table 15).

The lowest group mean was recorded in the group of treatments with *P. indica* which recorded an average root dry weight of 0.24 g while the *Bacillus* group performed the best with a group mean of 0.56 g (Table 16).

4.3.2.7 Fresh Weight of Leaves

The treatment with the *Bacillus* strain BY-2 recorded the highest leaf fresh weight of 4.71 g (Table 17). Application of spore suspension of *P. indica* recorded a mean fresh weight of 0.23 g and was the lowest. When *T. harzianum* and AMF were applied together a mean fresh weight of 4.36 g was recorded which was on par with the other treatments such as

Table 17 Effect of biocontrol agents and COC on fresh weight and dry weight of leaves in black pepper inoculated with *Phytophthora capsici**

Treatments	Fresh weight of leaves / plant (g)**	Percentage variation over uninoculated control	Dry weight of leaves / plant (g) **	Percentage variation over uninoculated control
Pseudomonas putida 89B61	3.46 (2.11)	-24.45	0.98 (1.41)	-20.97
Pseudomonas fluorescens RCL3R4	3.21 (2.05)	-29.91	1.23 (1.49)	-0.81
Piriformospora. indica (spore suspension)	0.23 (1.11)	-94.98	0.07 (1.03)	-94.35
Piriformospora. indica (mycelial mat)	2.62 (1.90	-42.79	1.05 (1.43)	-15.32
Bacillus sp (strain BY-1)	4.29) (2.30	-6.33	1.86 (1.69)	50.00
Bacillus sp (strain BY-2)	4.71) (2.39	2.84	2.43 (1.85)	95.97
COC @ 0.20 %	4.19) (2.28)	-8.52	2.51 (1.87)	102.42
Trichoderma harzianum + *Glomus fasciculatum*	4.36 (2.32)	-4.80	1.89 (1.70)	52.42
Inoculated control	1.70 (1.64)	-62.88	0.42 (1.19)	-66.13
Uninoculated control	4.58 (2.36)		1.24 (1.50)	
SE	0.150		0.107	
CD (0.05)	0.425		0.302	

*Values in parenthesis are after $\sqrt{x+1}$ transformation

**Mean of 8 replications having one plant each

Table 18 Group means of fresh and dry weight of leaves in black pepper inoculated with *Phytophthora capsici* and treated with biocontrol agents and COC*

Treatments	Fresh weight of leaves /plant (g)	Dry weight of leaves/plant (g)
Fluorescent pseudomonads 89B61 and RCL3R4	3.34 (2.08)	1.11 (1.45)
Piriformospora indica (spore suspension and mycelial mat)	1.43 (1.51)	0.56 (1.23)
Bacillus sp (strains BY-1 and BY-2)	4.50 (2.34)	2.15 (1.77)
POP recommendations	4.28 (2.30)	2.20 (1.79)
Control (inoculated + uninoculated)	3.14 (2.00)	0.83 (1.34)
CD (0.05)	0.300	0.213

*Values in parenthesis are after $\sqrt{x+1}$ transformation

Bacillus BY-1, COC @ 0.20 per cent, *P. putida* 89B61 and *P. fluorescens* RCL3R4.

All the treatments except that with Bacillus strain BY-2 recorded a percentage decrease over the control (Table 17).

The lowest group mean was recorded in the group of treatments with *P. indica* which recorded an average leaf fresh weight of 1.43 g while the *Bacilli* group performed the best with a group mean of 4.5 g (Table 18).

4.3.2.8 Dry Weight of Leaves

There was significant difference between the treatments with the various biocontrol agents as compared to the untreated control which recorded an average leaf dry weight of 1.24 g (Table 17). The treatment with COC @ 0.20 per cent with a mean dry weight of 2.51 g was highly significant. Treatment with *Bacillus* strain BY-2 with an average leaf dry weight of 2.43 g and combined application of *T. harzianum* and AMF which recorded an average value of 1.89 were superior to the uninoculated control. Plants treated with spore suspension of *P. indica* recorded the lowest dry weight of 0.56 g.

The treatments with the *Bacillus* strains BY-1 and BY-2, 0.20 percentage COC, combined application of *T. harzianum* and AMF recorded an increase of 50.00, 95.97, 102.42 and 52.42 percentage respectively over the control whereas all the other treatments were inferior to control (Table 17).

The group of treatments involving application of 0.20 percentage COC and combined application of *T.harzianum* and AMF with an average leaf dry weight of 2.20 g was statistically on par with the treatments using *Bacillus* which recorded a group average of 2.15 g (Table 18).

4.3.2.9 Root Colonization by P. indica

The roots of black pepper cuttings were not colonized by *P. indica* when treated with spore suspension or mycelial mat. No mycelium or chlamydospores of the fungus were observed on microscopic examination after staining the roots with trypan blue.

DISCUSSION

5. DISCUSSION

Black pepper is an important agricultural produce of Kerala that contributes substantially to the foreign exchange earning of our country. Even though black pepper produced in our country is highly priced in the international market due to its better pungency and aroma, the productivity is abysmally low when compared to other countries. One of the major reasons attributed to this low productivity is the severe infestation of foot rot disease incited by *Phytopthora capsici*, which has been spreading at an alarming rate. Very often the pathogen which gets established in the nursery is carried to the main field through the infected planting material and rooting medium. It is thus, essential to ensure that the rooted cuttings transplanted to the main field are disease free for successful management of foot rot. None of the cultivated or wild varieties of black pepper show resistance to the disease (Sulaiman, 1994). Cultural practices including phytosanitation and repeated prophylactic application of copper fungicides are the recommended measures for the management of this disease (KAU, 2002).

Considering the high cost and health hazards that would arise by the extensive and indiscriminate use of chemicals in agriculture, biological control appears to be the most promising alternative for plant disease and pest management. Microbial antagonists are increasingly being used as plant growth promoters and for the management of plant diseases including those caused by soil borne plant pathogens (Cook and Baker, 1983; Weller and Cook, 1986; Chet, 1987; Lewis and Papavizas, 1991; Ramamoorthy *et al.*, 2002). The present study was carried out for assessing the potential of plant growth promoting microbial inoculants for the biological control of nursery wilt of black pepper caused by *P. capsici*.

The microbial inoculants used for the present investigation were fluorescent pseudomonads, *Bacillus* sp and a plant growth promoting endosymbiotic fungus, *Piriformospora indica*. Bacterial antagonists such as fluorescent pseudomonads and *Bacillus* spp. have been reported to be efficient biocontrol agents for many soil borne plant diseases (O'Sullivan and O'Gara, 1992; Dowling and O'Gara, 1994; Sanchez *et al.*, 1994; Berger *et al.*, 1996; Zheng and Sinclair, 2000). The use of mycoinoculants such as *Trichoderma* spp and Arbuscular Mycorrhizal Fungi (AMF) as specific biocontrol agents is a recommended practice for foot rot management. But the inoculum production of AMF presents a very serious problem as these fungi are obligate symbionts and could not be cultured axenically under *in vitro* conditions. Absence of pure culture technique for mass production is the greatest bottleneck in the use, application and commercialization of mycorrhiza. *P. indica* is reported to improve the growth and overall biomass production of different plants including medicinal plants (Rai *et al.*, 2001). This endosymbiotic fungus can be axenically cultured on a variety of synthetic media and is reported to produce inter and intra cellular vesicles, hyphal coils and chlamydospores similar to AMF (Verma *et al.*, 1998). The ability of the fungus to inhibit soil borne plant pathogens has also been reported (Varma *et al.*, 2001).

There are several reports on the successful use of biocontrol agents in seed propagated crops. Black pepper is a vegetatively propagated crop and very little work has been done on biocontrol of diseases in vegetatively propagated crops except potato (Chambers and Scott, 1995; Schippers, 1988). On the basis of the reports on the successful use of bacterial antagonists and *P. indica* in controlling various soil- borne plant pathogens both under laboratory and field conditions, these were included in the present study to explore their potential in plant growth promotion and foot rot suppression in black pepper. *P. capsici* inoculated control, uninoculated healthy control, chemical control (0.20 per cent copper oxychloride (COC) drenching at 15 days intervals) and dual application of

Trichoderma harzianum and an AMF, *Glomus fasciculatum* were maintained for comparison.

In vitro antagonism of bacteria and fungi against plant pathogens usually provide an indication of their capacity for suppression of the pathogen *in vivo*. *In vitro* dual culture assay was undertaken as a preliminary screening technique for testing the antagonistic effect of the biocontrol agents. Proven biocontrol agents procured from different sources were used for the *in vitro* screening studies. Two strains of fluorescent pseudomonads *viz.*, *P. putida* 89B61 and *P. fluorescens* RCL3R4, two strains of *Bacillus viz.*, BY-1 and BY-2 and the endosymbiotic fungus, *P. indica* were dual cultured against *P. capsici*. The *in vitro* dual culture assay using all the bacterial isolates was performed on PDA and CA. The fluorescent pseudomonad strains exhibited better inhibition of mycelial growth of *P. capsici* on both the media when compared to the *Bacillus* strains. Among the two fluorescent pseudomonad strains used, the performance of the strain RCL3R4 was rated high. Although RCL3R4 showed high inhibition of fungal growth in dual culture, it exhibited less ability in suppression of the disease in the *in vivo* test. Reports on lack of positive correlation between *in vitro* antagonism and biological control has been presented by many workers (Baker, 1968; Schroth and Hancock, 1981; Wong and Baker, 1984; Anith *et al.*, 2003).

The *Bacillus* strains BY-1 and BY-2 that showed less antagonistic effect against the pathogen under *in vitro* conditions exhibited better disease suppression *in vivo*. Similar results were reported by Jubina and Girija (1998) with a *Bacillus* isolate B13 which showed poor inhibition of *P. capsici* in dual culture and high disease suppression in the *in vivo* biocontrol assay. The poor performance of the *Bacillus* strains *in vitro* may be due to lack of production of specific antimicrobial metabolites against the pathogen on the test Agar. The culture filtrates of all the four bacterial isolates showed no inhibitory effect on the mycelial growth of *P. capsici*. During the process of screening of soil micro organisms for biocontrol activity, or plant growth promotion, the media used to culture the test microorganisms themselves may influence the test result (Dickie and Bell, 1995; Kloepper *et al*., 1987; Whipps, 1987; Whipps and Magan, 1987). Borowicz and Omer (2000) reported that the growth promoting activities and antagonism of certain fluorescent pseudomonad isolates in cucumber plants were curtailed when cultured in King's B Broth (KBB) as against a commendable improvement when cultured in Tryptic Soy Broth or a Mineral Media Broth. Similar results were obtained by them under *in vitro* conditions on King's B Agar plates against *Pythium* sp and *Rhizoctonia solani*. In the present study, *in vitro* dual culture assay using the culture filtrates of the bacterial isolates against *P. capsici*, KBB and Nutrient Broth were used as the culture medium for fluorescent pseudomanads and *Bacillus* respectively. Testing of culture filtrates obtained by growing these bacteria on different other media would reveal the effect of media on the production of antagonistic principles or metabolites by them.

The *Bacillus* strains BY-1 and BY-2 showed high degree of disease suppression *in vivo*, though the isolates were grown on Nutrient Agar prior to the preparation of bacterial cell suspension. As reported by Borowicz and Omer (2000), the *in vivo* bacterial performance is also affected by the physical status of the media (solid or liquid) and the differences in the production of various metabolites in the presence of various media components. Further, under *in vivo* conditions the production of antimicrobial metabolites, root colonization and induced systemic resistance which could stimulate the plant's defense mechanism may provide selective advantage to the producing strains in the highly competitive environment of the plant rhizosphere (van Peer *et al*., 1991; Yan *et al*., 2002). The treatments with the *Bacillus* strains, BY-1 and BY-2 respectively recorded an increase of 29.50 and 43.69 percentage in the leaf number when compared to the uninoculated control. The maximum shoot fresh weight, leaf fresh weight, shoot dry weight, leaf dry weight and root dry weight were obtained in the plants treated with the *Bacillus* strain BY-2 even in the presence of the pathogen. The strains BY-1 and BY-2 also showed suppression of *Phytophthora* induced wilt in the nursery. Significant control of Phytophthora and Pythium damping off in garden plants was obtained by Berger *et al*. (1996) by the application of a strain of *B. subtilis*.

In the dual culture assay conducted *in vitro* using *P. indica*, though there was a slight inhibition of mycelial growth initially, later the pathogen overgrew the antagonist and no inhibition of mycelial growth was observed when *P. capsici* was dual cultured using the culture filtrate of *P. indica*. The lack of inhibition by mycelium and culture filtrate could be attributed to the absence of specific cell wall degrading enzymes or metabolites. The cuttings treated with the spore suspension of *P. indica* showed a survival rate of only 12.50 percentage in soil artificially inoculated with *P. capsici*. No colonization of the black pepper roots by *P. indica* was observed even in the plants that survived the pathogen inoculation. The biocontrol agent might not have received enough time to establish in the rhizosphere region as the pathogen and spore suspension of *P. indica* were inoculated simultaneously. When mycelial mat of *P. indica* was inoculated one month before challenging with the pathogen, no disease could be observed. It is thus evident that this endosymbiotic fungus requires more time to get established in the rhizosphere region and bring about disease suppression. Therefore, direct antagonism by the biocontrol agent which might have survived in the applied soil on the growth of the pathogen, could have played a role in disease suppression. Further, the treatment with mycelial mat of *P. indica* recorded an increase of 27.50 and 23.68 percentage over the untreated control in root fresh weight and root dry weight respectively. This revealed that the fungus has some mechanism that induces root production and proliferation.

The combined inoculation of *T. harzianum* and *G. fascicualtum* and treatment with 0.2 percentage COC exhibited better disease suppression *in vivo* when compared to the inoculated control. The dual inoculation treatment recorded an increase of 16.29, 48.19, 52.42 and 26.32 over the untreated control with respect to shoot fresh weight, root fresh weight, leaf dry weight and root dry weight respectively (Fig. 3 and 4). *T. harzianum* is reported to have antagonistic activity against *P. capsici* infecting black pepper (Anandaraj and Sarma, 1994a ; Ganesan *et al.*, 2000). *Trichoderma* spp., though produce antibiotics and cell wall degrading enzymes mainly act as mycoparsites and bring about the disease control (Lewis and Papavizas, 1991). Application of endomycorrhiza was also reported to reduce foot rot incidence in black pepper and increase rooting and biomass production (Anandaraj *et al.*, 1993; Sivaprasad *et al.*, 1997). Anandaraj and Sarma (1994b) suggested that the suppressive effect of AMF was due to enhanced root regeneration, nutrient uptake and altered host physiology in mycorrhizal plants.

The plants treated with COC also recorded an increase of 29.86, 43.36, 48.19 and 34.21 percentage respectively with respect to shoot fresh weight, shoot dry weight, root fresh weight and root dry weight over the uninoculated control. Further, the plants exhibited no wilt when compared to the pathogen inoculated control which recorded a wilt percentage of 37.06. Anith *et al.* (2002) observed that black pepper plants treated with 0.2 percentage COC which were maintained as comparison with treatments using other biocontrol agents exhibited a wilt percentage of 27.08 as against 97.83 percentage in the inoculated control.

Several of the biocontrol bacteria fall in the category of Plant Growth Promoting Rhizobacteria (PGPR). Besides biocontrol they also influence plant growth in a positive manner (Kloepper and Schroth, 1978; Whipps, 1997; Bowen and Rovira, 1999). The *Bacillus* strains showed less impact on growth promotion studies whereas the fluorescent pseudomonad strains, especially *Pseudomonas putida* 89B61 recorded the highest shoot fresh weight, number of leaves, root fresh weight, and shoot dry weight (Fig. 1 and 2). Carruthers *et al.* (1995) obtained increased root length and plant weight in asparagus plants on inoculation with a fluorescent pseudomonad in presence of the pathogen, *Phytophthora megaspermae* var. *sojae*.

The treatments with *P. indica* gave surprisingly variable results with respect to the growth promotion in black pepper. The plants treated with spore suspension of *P. indica* recorded an increase of 13.24 and 44.83 percentage in shoot fresh weight and leaf fresh weight respectively over the control in the absence of the pathogen. The plants treated with mycelial mat of *P. indica* recorded an increase of 30.71, 100.00 and 38.46 percentage over the control with respect to number of roots, dry weight of roots and dry weight of leaves respectively which revealed the potential of the fungus as a growth promoting agent.

The present study revealed that both bacterial and fungal antagonists could be used for effective management of nursery wilt of black pepper. The development of mixed formulations of compatible PGPR strains and fungal antagonists that may have an additive effect in the biocontrol activity when used as a consortium assumes importance at this point. Combined inoculation of bacterial and fungal antagonists have already been reported for effective disease management (Pierson and Weller, 1994; Duffy *et al.*, 1996; Xhang *et al.*, 1996; Nagtzaam *et al.*, 1998; Anandaraj *et al.*, 2003). Anith and Manomohandas (2001) reported that combined application of *T. harzianum* and *Alcaligenes* sp strain AMB8 reduced the mortality of black pepper cuttings in the nursery to 7.41 percentage as against 74.01 percentage mortality in the inoculated control while, inoculation with bacteria or *Trichoderma* sp. alone resulted in 18.52 and 14.81 percentage mortality respectively. Further investigations are required for testing the compatibility of bacterial and

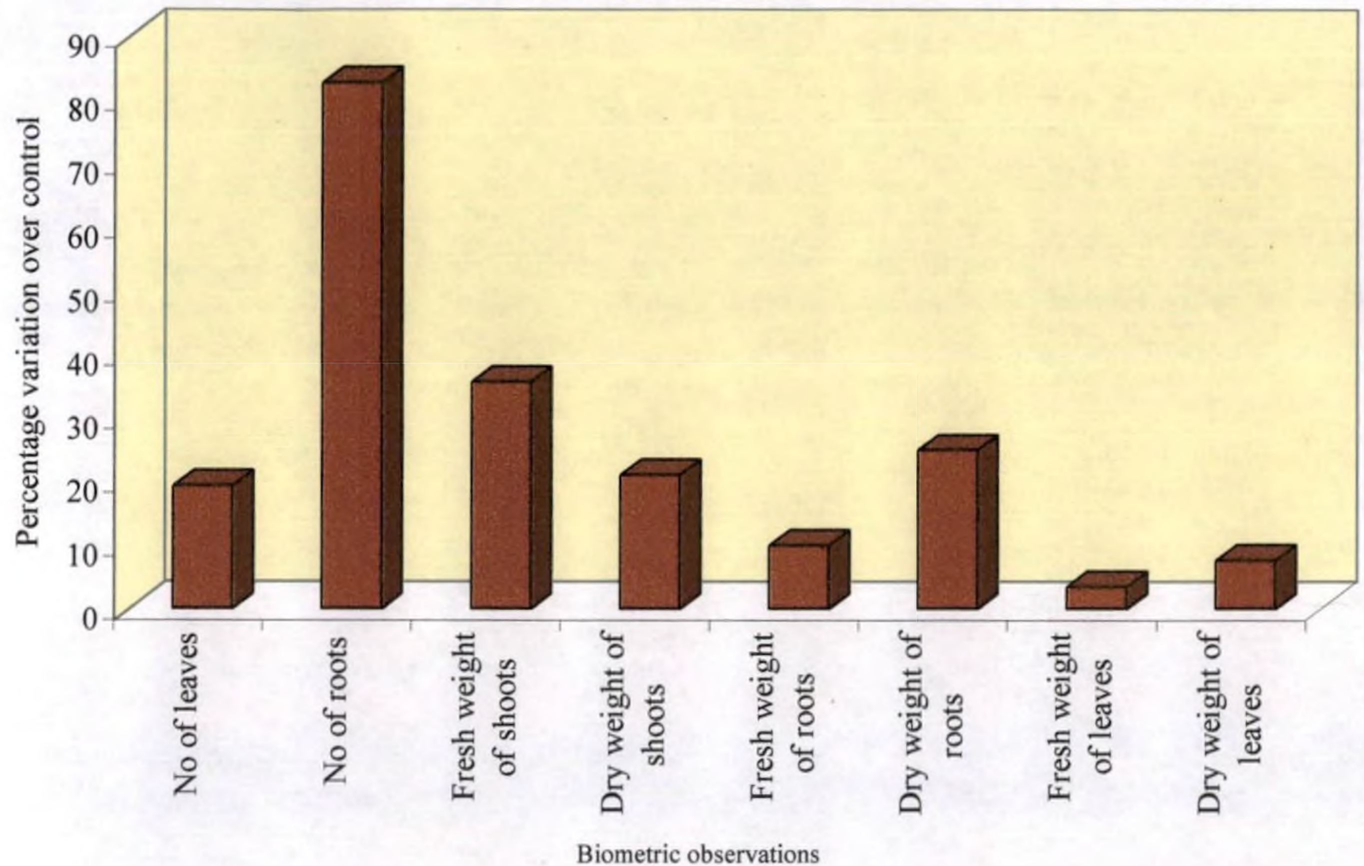
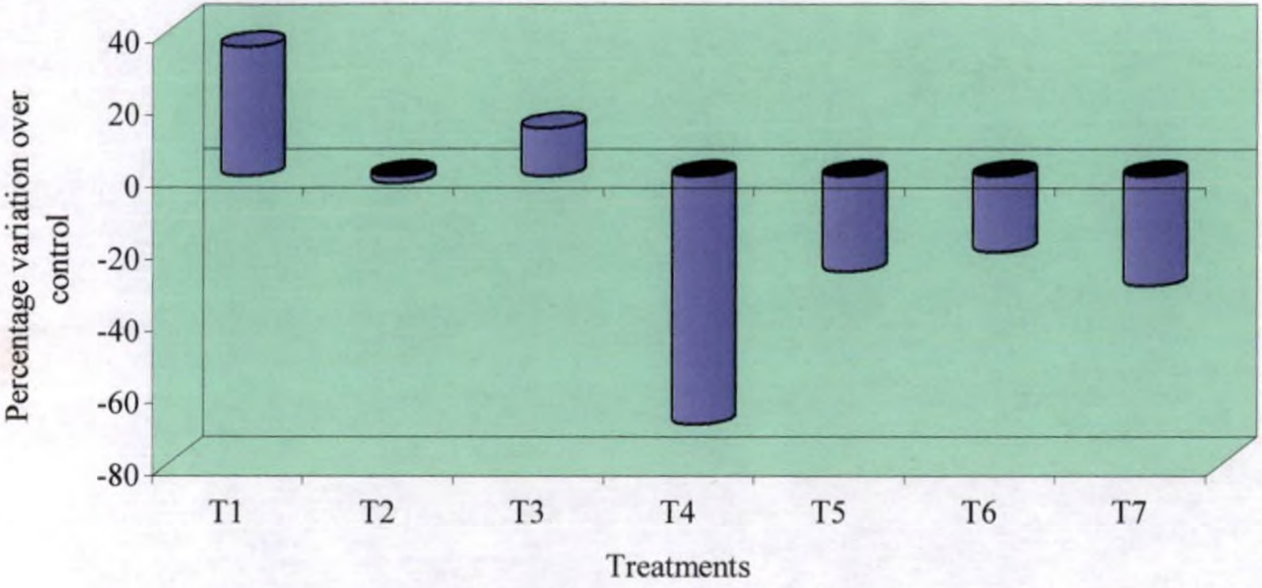


Fig. 1 Effect of *Pseudomonas putida* strain 89B61 on growth of black pepper cuttings



T1 *Pseudomonas putida* 89B61
T2 *Pseudomonas fluorescens* RCL3R4
T3 *Piriformospora indica* (spore suspension)
T4 *Piriformospora indica* (mycelial mat)
T5 *Bacillus* (strain BY-1)
T6 *Bacillus* (strain BY-2)
T7 IBA @ 1000ppm

Fig. 2 Effect of different biocontrol agents on fresh weight of shoots of black pepper cuttings

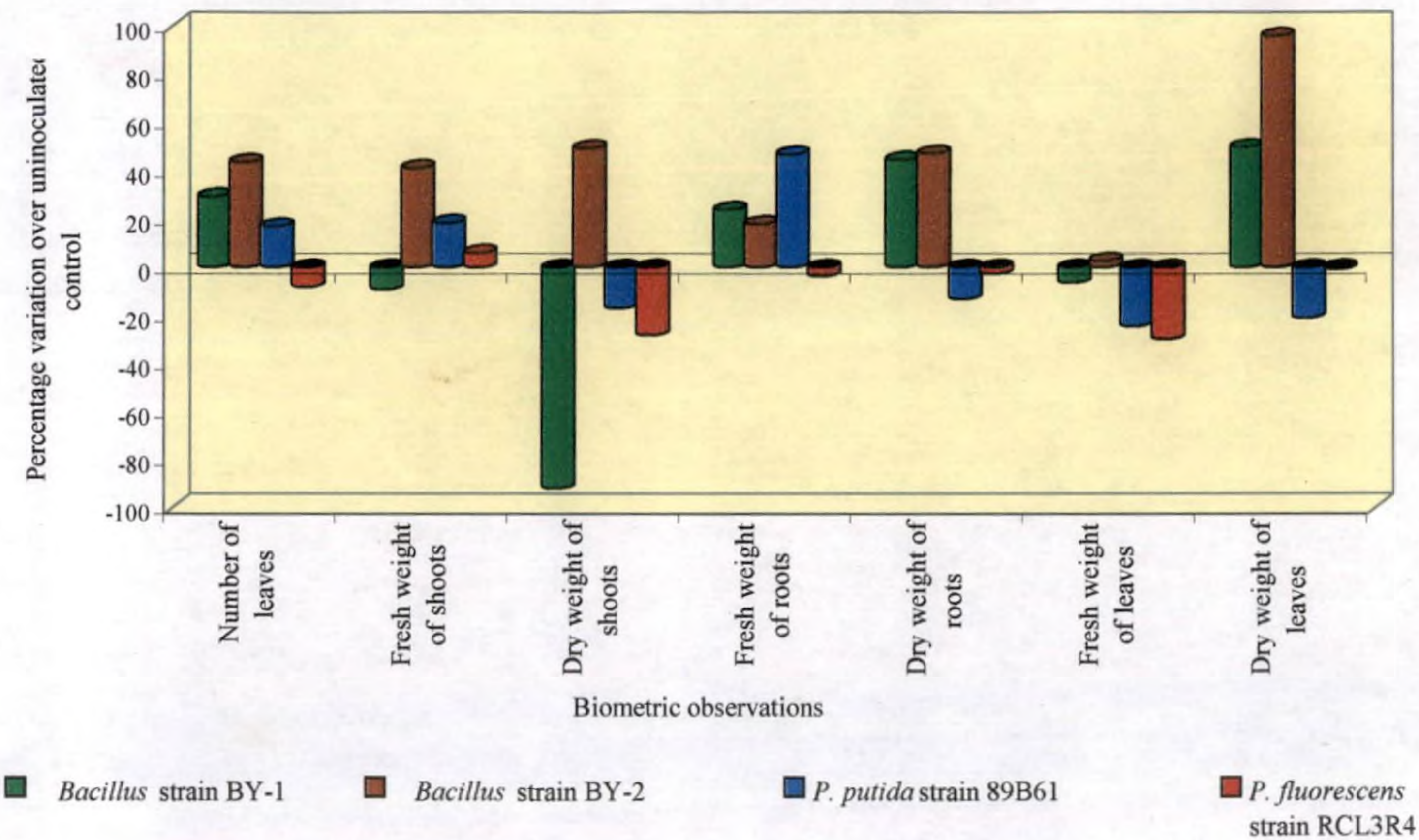


Fig. 3 Comparison of the effects of Bacillus strains (BY-1 and BY-2) and the fluorescent pseudomonads (*Pseuodomonas putida* strain 89B61 and *Pseuodomonas fluorescens* strain RCL3R4 on different characters in black pepper inoculated with *Phytophthora capsici*

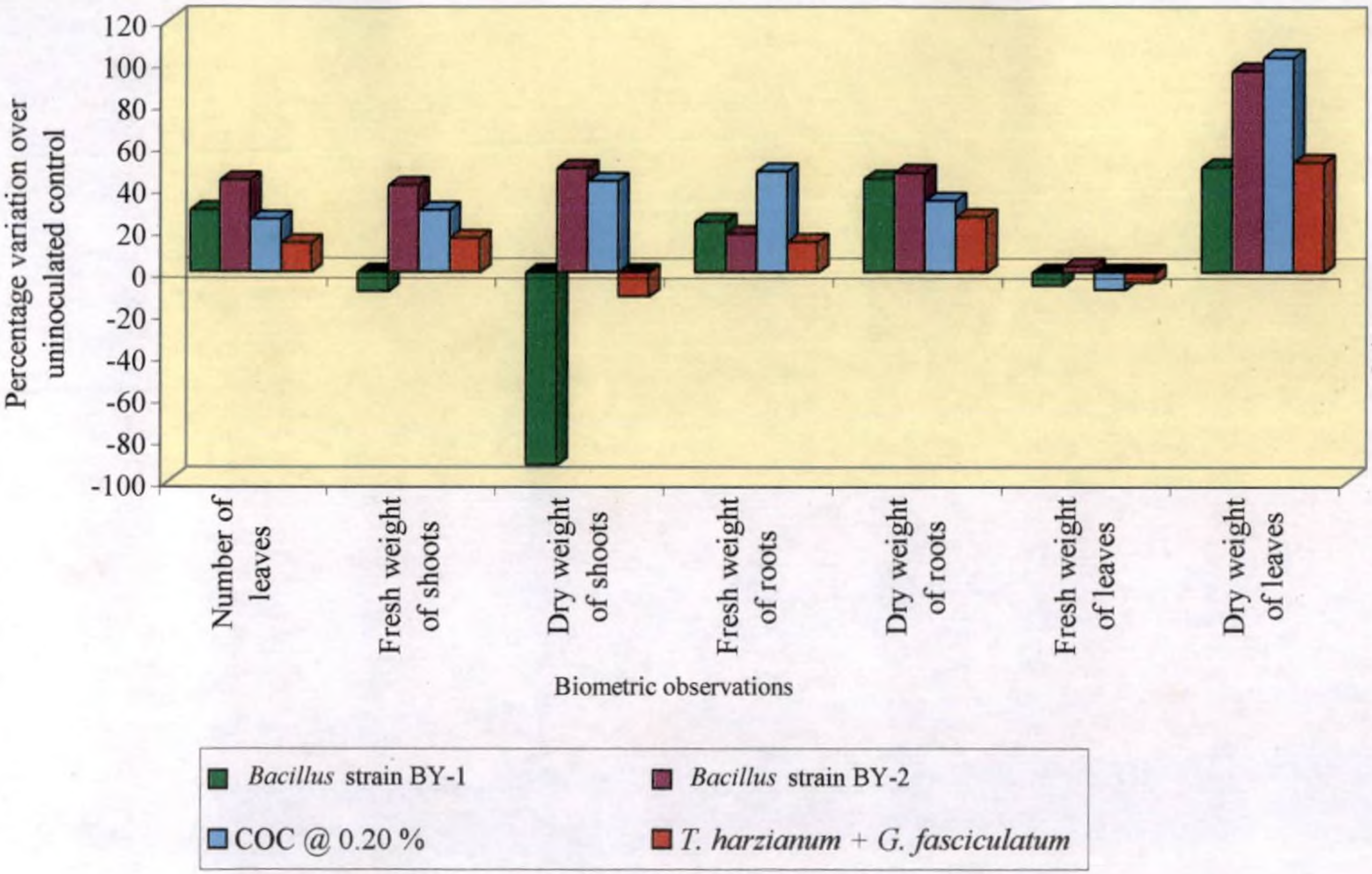


Fig. 4 Comparison of the effects of *Bacillus* strains (BY-1 and BY-2) and the POP recommendations on different characters in black pepper inoculated with *Phytophthora capsici*

fungal antagonists for *in vitro* antibiosis as well as survival and performance under field conditions.

The study showed that treatment with *P. indica* can increase root production and proliferation. Better root proliferation will help in improved water absorption. *P. indica* was isolated from desert soils and therefore can be used as a promising bioagent for improving the drought tolerance of crop plants. Further studies are required to confirm whether *P. indica* is desiccation tolerant or the plants inoculated with this endosymbiont are better adapted to dry conditions. If the tests prove successful, it would help in developing drought tolerant agriculturally important plants especially for the arid zones in rainfed agrosystems.

The possibility of replacing chemical control measures for plant diseases with biological alternatives is an exciting and challenging objective in sustainable agriculture. In this context, the use of biorationals is worth mentioning as they can enhance crop productivity without the deterioration of soil health.

6. SUMMARY

The study, "Management of Phytophthora disease of black pepper (*Piper nigrum* L. Walp) using plant growth promoting microbial inoculants" was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani to investigate the effect of plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads and *Bacillus* spp. and the root endophytic fungus, *Piriformospora indica* on the suppression of nursery wilt of black pepper incited by *Phytophthora capsici*.

The pathogen was isolated from foot rot affected black pepper plants (var. Karimunda) plants collected from the pepper gardens of College of Agriculture, Vellayani and pathogenicity was proved. It was identified as *Phytophthora capsici* Leonian based on the morphological and cultural characters besides its ability to induce typical symptoms of foot rot disease in black pepper plants var. Karimunda.

In vitro dual culture assay was undertaken as a preliminary screening technique for testing the antagonistic effect of the biocontrol agents. Proven biocontrol agents procured from different sources were used for the *in vitro* screening studies. Two strains of fluorescent pseudomonads *viz*., *Pseudomonas putida* strain 89B61 and *P. fluorescens* strain RCL3R4, two strains of *Bacillus viz*., BY-1 and BY-2 and the endosymbiotic fungus, *P. indica* were dual cultured against *P. capsici*. The *in vitro* dual culture assay using all the bacterial isolates was performed on PDA and Carrot Agar. The fluorescent pseudomonad strains exhibited better inhibition of mycelial growth of *P. capsici* on both the media when compared to the *Bacillus* strains. Among the two fluorescent pseudomonad strains used, the performance of the strain RCL3R4 was rated high. In the dual culture assay conducted *in vitro* using *P. indica*,

SUMMARY

though a slight inhibition of mycelial growth was observed initially, later the pathogen overgrew the antagonist.

The four different rhizobacterial strains and root endophytic fungus, *P. indica* were tested *in vivo* for their growth promotion activity and disease suppression on runner shoot cuttings of black pepper var. Karimunda. In the experiment conducted to test the potential of various plant growth promoting microbial inoculants in suppressing nursery wilt, the plants treated with spore suspension of *P. indica* exhibited the highest wilt percentage of 93.22 per cent which was more than that in the inoculated control (37.48). The plants treated with the fluorescent pseudomonad RCL3R4 recorded a wilt percentage of 6.70. The *Bacillus* strains BY-1 and BY-2 that showed less antagonistic effect against the pathogen under *in vitro* conditions exhibited better disease suppression *in vivo*. The maximum shoot fresh weight, shoot dry weight, leaf fresh weight, leaf dry weight and root dry weight were obtained in the plants treated with the *Bacillus* strain BY-2 even in the presence of the pathogen.

The *Bacillus* strains showed less impact on growth promotion studies whereas the fluorescent pseudomonad strains, especially *Pseudomonas putida* 89B61 recorded the highest shoot fresh weight, shoot dry weight, number of leaves and root fresh weight in the absence of the pathogen. The combined inoculation of *Trichoderma harzianum* and *Glomus fasciculatum* and treatment with 0.20 per cent COC exhibited better disease suppression *in vivo* when compared to the inoculated control. The dual inoculation treatment recorded an increase of 16.29, 48.19, 52.42 and 26.32 over the untreated control with respect to shoot fresh weight, root fresh weight, leaf dry weight and root dry weight respectively. The plants treated with 0.20 per cent COC also recorded an increase of 29.86, 43.36, 48.19 and 34.21 percentage respectively with respect to shoot fresh weight, shoot dry weight, root fresh weight and root dry weight over the uninoculated control.

The treatments with both spore suspension and mycelial mat of *P. indica* exhibited growth promotion while the mycelial mat treatment in the presence of pathogen exhibited high rate of disease suppression *in vivo*. Further, the treatment with mycelial mat of *P. indica* recorded an increase of 27.50 and 23.68 percentage over the untreated control in root fresh weight and root dry weight respectively in presence of the pathogen. This revealed that the fungus has some mechanism that induces root production and proliferation.

REFERENCES

7. REFERENCES

*Alizadeh, A and Tsao, P.H. 1985. Effect of light on sporangium formation, morphology, ontogeny and caducity of *Phytophthora capsici* and *Phytophthora palmivora* MF_4 isolates from black pepper and other hosts. *Trans. Br. Mycol. Soc.* 85 : 47-69

Anandaraj, M. and Sarma, Y.R. 1994a. Biological control of black pepper diseases. *Indian Cocoa Arecanut Spices J.* 18 : 22-23

Anandaraj, M. and Sarma, Y.R. 1994b. Effect of vesicular arbuscular mycrorrhizae on rooting of black pepper. *J. Spices Aromatic Crops* 3 : 39-42

Anandaraj, M. and Sarma, Y.R. 1995. Diseases of black pepper (*Piper nigrum*) and their management. *J. Spices Aromatic Crops* 4 : 17-23

Anandaraj, M., Diby, P., Jisha, P.J., Kumar, A., Saju, K.A., Thankam, C.K. and Sarma, Y.R. 2003. Potential of a consortium of Plant Growth Promoting Rhizobacteria and *Trichoderma* for effective nursery management in Black pepper (*Piper nigrum* L.). *Proceedings of the Sixth International Workshop on Plant Growth Promoting Rhizobacteria. October 5-10.* (eds. Reddy,M.S., Anandaraj, M., Eapen,S.J., Sarma, Y.R Kloepper, J.W.). Indian Institute of Spices Research, Kozhikode, p. 8-11

Anandaraj, M., Ramachandran, N. and Sarma, Y.R. 1991. Epidemiology of foot rot disease of black pepper (*Piper nigrum* L.) in India. *Proceedings of the International Pepper Community Workshop on Joint Research for the Control of Black Pepper Disease, October 27-28, 1988, Panaji, Goa* (eds. Sarma, Y.R. and Premkumar, T.). National Research Centre for Spices, Marikunnu, pp. 114-135

Anandaraj. M., Ramana, K.V. and Sarma, Y.R. 1993. Suppressive effects of VAM on root pathogens of black pepper, a component of Western Ghats forest ecosystem. *IVFRO Symposium*, Kerala Forest Research Institute, Peechi, pp., 64-72

Anandaraj, M., Venugopal, M.N. and Sarma, Y.R. 1996. *Biological Control of Diseases of Spice Crops*. Annual Report 1995-96, Indian Institute of Spices Research, Calicut, Kerala, p. 71

Anith, K.N. and Manomohandas, T.P. 2001. Combined application of *Trichoderma harzianum* and *Alcaligenes* sp. strain AMB8 for controlling nursery rot disease of black pepper. *Indian Phytopath.* 54 : 335-339

Anith, K.N., Radhakrishnan, N.V. and Manomohandas, T.P. 2002. Management of nursery wilt of black pepper (*Piper nigrum* L.) with antagonistic bacteria. *Curr. Sci.* 83 : 561-562

Anith, K.N., Radhakrishnan, N.V. and Manomohandas, T.P. 2003. Screening of antagonistic bacteria for biological control of nursery wilt of black pepper (*Piper nigrum*). *Microbiol. Res.* 158 : 91-97

Baker, C.J., Stavely, J.R. and Mock, N. 1985. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. *Plant Dis.* 69 : 770-772

Baker, R. 1968. Mechanisms of biological control of soil borne plant pathogens. *Annu. Rev. Phytopathol.* 6 : 263-294

*Barber, C.A. 1902. *Annual Report for 1901-1902*. Department of Agriculture, Madras

*Barber, C.A. 1903. Pepper disease in the Wynad. *Trop. Agric.* (Colombo) 22 : 805-807

*Barber, C.A. 1905. The government pepper farm in Malabar. *Trop. Agric.* (Colombo) 25 : 564

Becker, J.O. and Schwinn, F.J. 1993. Control of soil-borne pathogens with living bacteria and fungi : status and outlook. *Pesticide Sci.* 37 : 355-363

Berger, F., Li, H, White, D., Frazer, R. and Leifert, C. 1996. Effect of pathogen inoculum, antagonist density and plant species on biological control of Phytophthora and Pythium damping off by *Bacillus subtilis* Cot1 in high humidity fogging glasshouses. *Phytopathology* 86 : 428-433

*Blechert, O., Kost, G., Hassel, A., Rexer, K.H. and Varma, A. 1999. First remarks on the symbiotic interactions between *Piriformospora indica* and terrestrial orchids. *Mycorrhizae : Structure, function, molecular biology and biotechnology.* Second edition (eds. Varma, A. and Hock, B.). Springer-Verlag, Berlin, Heidelberg, New York, pp. 683-688

Bopaiah, B.M. and Khader, K.B. 1989. Effect of biofertilizers on growth of black pepper (*Piper nigrum*). *Indian J. agric. Sci.* 59 : 682-683

Borowicz, J.J. and Omer, S.Z. 2000. Influence of rhizobacterial culture media on plant growth and on inhibition of fungal pathogens. *BioControl* 45 : 355-371

Bowen, G.D. and Rovira, A.D. 1999. The rhizosphere and its management to improve plant growth. *Adv. Agron.* 66 : 1-102

Butler, E.J. 1906. The wilt disease of pigeon pea and pepper. *Agric. J. India.* 1 : 25-36

Butler, E.J. 1918. *Fungi and disease in plants.* Thacker and Spink, Calcutta, p. 598

Carruthers, F.L., Thomas, S.T., Conner, A.J. and Mahanty, H.K. 1995. The significance of antibiotic production by *Pseudomonas aureofaciens* PA147-2 for biocontrol of *Phytophthora mogasperma* root rot of asparagus. *Plant and Soil* 170 : 339-344

Chambers, A.M. and Scott, E.S. 1995. *In vitro* antagonism of *Phytophthora cinnamomi* and *P. citricola* by isolates of *Trichoderma* spp. and *Gliocladium virens*. *Phytopathology* 143 : 471-477

Chattopadhyay, S.B. and Maiti, S. 1990. *Diseases of Betelvine and Spices*. Indian Council of Agricultural Research, New Delhi, p. 33

Chet, A., Ordentlich, R., Shapira, R. and Oppenheim, A. 1990. Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria. *Plant Soil* 129 : 85-92

Chet, I. 1987. *Innovative Approaches to Plant Disease Control*. John Wiley and Sons, New York, USA, p. 372

Cook, R.J. and Baker, K.F. 1983. *The Nature and Process of Biological Control of Plant Pathogens*. American Phytopathological Society, St. Paul, MN, USA, p. 589

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

Dave, B.P. and Dube, H.C. 2000. Detection and chemical characterization of siderophores of rhizobacterial fluorescent *Pseudomonas*. *Indian Phytopath.* 53 : 97-98

Dickie, G.A. and Bell, C.A. 1995. A full factorial analysis of nine factors influencing *in vitro* antagonistic screens for potential biocontrol agents. *Can. J. Microbiol.* 41 : 284-293

Divya, S. 2002. Management of foot rot of black pepper (*Piper nigrum* L.) with mycoinoculant enriched Vermicompost. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 110

Dowling, D.N. and O'Gara, F. 1994. Metabolites of *Pseudomonas* involved in biological control of plant diseases. *TIBTECH* 12 : 133-141

Duffy, B.K., Simon, A. and Weller, D.M. 1996. Combination of *Trichoderma koeningi* and fluorescent pseudomonads for controlling take all on wheat. *Phytopathology* 86 : 188-194

Ellis, H., Johanna, H. and John, A. 1996. Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas species*. *Phytopathology* 86 : 757-762

*Ewald, S. 1991. *Vesicular arbuscular mycorrhizae management in tropical agrosystems.* Technical Co-operation Federal Republic of Germany, Friedland, Bremar, pp. 372

FIB. 2002. *Farm Guide.* Farm Information Bureau, Government of Kerala, Thiruvananthapuram, p. 96

*Galindo, J.J. 1992. Prospects for biological control of black pod of cocoa. *Cocoa Pest and Disease Management in South East Asia and Australia* (eds. Kreree, P.J. and Putter, C.A.J.). Rome, Italy, FAO 1992 – FAO Plant Production and Protection. Paper No. 112

Ganesan, G., Ravishankar, H. and Bhargava, B.S. 2000. Mass multiplication of *Trichoderma harzianum*. *Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture, Vol. I., November 11-15, 1997* (ed. Jain, R.K.). Indian Phytopathological Society, New Delhi, pp. 333-334

Ganesan, P. and Gnanamanickam, S.S. 1987. Biological control of *Sclerotium rolfsii* Sacc. in peanut by inoculation with *Pseudomonas fluorescens*. *Soil Biol. Biochem.* 19 : 35-38

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

Gutterson, N.I., Layton, T.J., Ziegle, J.S. and Warren, G.J. 1986. Molecular cloning of genetic determinants for inhibition of fungal growth by a fluorescent pseudomonad. *J. Bacteriol.* 165 : 696-703

Handelsman, J., Nesmith. W.C. and Raffel. S.J. 1991. Microassay for biological and chemical control of infection of tobacco by *Phytophthora parasitica* var *nicotianae*. *Curr. Microbiol.* 22 : 317-319

*Hemmes, D.E. 1983. Cytology of *Phytophthora*. *Phytophthora its Biology, Taxonomy, Ecology and Pathology* (eds. Erwin, D.C., Bartnicks, Garcia, S. and Tsao, P.H.). American Phytopathological Society, St. Paul, Minnesota, pp. 9-40

Holliday, P. and Mowat, W.P. 1957. A root disease of *Piper nigrum* L. in Sarawak caused by a species of *Phytophthora*. *Nature* 179 : 543-544

Holliday, P. and Mowat, W.P. 1963. Foot rot of *Piper nigrum* L. (*P. palmivora*). *Phytopathology Paper No. 5*. Commonwealth Mycol. Inst., Kew, Surrey, p. 62

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

Howie, W.J. and Suslow, T.V. 1991. Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. *Mol. Plant Microbe Interact.* 4 : 393-399

Imran, S.A., Haque, E.S., Ghaffar, A. 1999. Effects of *Pseudomonas aeruginosa* and chemical fertilizers on root rot and root knot diseases of mungbean. *Pakist. J. Nematol.* 17 : 77-86

Ines, E.G., Russel, K.H., Louise, M.N. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J. Microbiol.* 47 : 404-411

Jubina, P.A. and Girija, V.K. 1998. Antagonistic rhizobacteria for management of *Phytophthora capsici*, the incitant of foot rot of black pepper. *J. Mycol. Pl. Pathol.* 28 : 147-153

KAU. 2002. *Package of Practices Recommendations, 'Crops'*, 2002. Directorate of Extension. Kerala Agricultural University, Thrissur, p.278

Kloepper. J.W. and Schroth, M.N. 1978. Plant growth promoting rhizobacteria on radishes. *Proceedings of Seventh International Conference on Plant Pathogenic Bacteria, Vol. 11*, pp. 879-882

Kloepper. J.W., Hume, D.J., Scher, F.M., Singleton, C., Tipping, B., Laliberte, K., Frauley, K., Kutchaw, T., Simonson, C., Lifshitz, R., Zaliska, I. and Lee, L. 1987. Plant growth promoting rhizobacteria on Canola (Rapeseed). *Plant Dis.* 72 : 42-46

Kloepper, J.W., Leong, J., Teintze, M. and Schroth, M.N. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* : 286 : 885-886

Kuc, J. 2001. Concepts and direction of induced systemic resistance in plants and its application. *European J. Plant Pathol.* 107 : 7-12

Lewis, J.A. and Papavizas, G.C. 1991. Biological control of plant diseases, the approach for tomorrow. *Crop Protec.* 10 : 95-104

Liu, L., Kloepper, J.W. and Tuzun, S. 1995. Induction of systemic resistance in cucumber against Fusarium wilt by PGPR. *Phytopathology* 85 : 695-698

Mammootty, K.P., Abicheeran and Peethambaran, C.K. 1979. Studies on the control of quick wilt disease of pepper. *Indian Phytopath.* 32 : 159-160

Mammootty, K.P., Manomohandas, T.P., Sasikumaran, S., Nair, U.P.K. and Abicheeran. 1991. Some aspects on epidemiology of foot rot (quick wilt) disease of black pepper. *Proceedings of the International Pepper Community Workshop on Joint Research for the Control of Black Pepper Diseases, October 27-29, 1988, Panaji, Goa* (eds. Sarma, Y.R. and Premkumar, T.). National Research Centre for Spices, Marikunnu, pp. 102-113

Manjunath, A. and Bagyaraj. D.J. 1982. Vesicular arbuscular mycorrhizae in three plantation crops and cultivars of field bean. *Curr. Sci.* 51 : 707-708

Manomohandas, T.P. and Abicheeran. 1986. Infectiveity of *Phytophthora* species on cash crops in Kerala. *Agric. Res. J. Kerala* 24 (1) : 7-13

Mchau, G.R.A. and Coffey, M.D. 1994. Isozyme diversity in *Phytophthora palmivora* : evidence for a Southeast Asian centre of origin. *Mycol. Res.* 98 : 1035-1043

Mchau, G.R.A. and Coffey, M.D. 1995. Evidences for the existence of two sub populations in *Phytophthora capsici* and a redescription of the species. *Mycol. Res.* 99 : 89-102

Mehrotra, R.S. and Aggarwal, A. 2001. *Phytophthora Diseases in India.* Bishen Singh Mahendrapal Singh, Dehra Dun, p. 322

Menon, K.K. 1949. Survey of 'pollu' (hollow berry disease) and root disease of pepper. *Indian J. agric. Sci.* 119 : 89-136

Merriman, P.R., Price, R.R., Kollmorgen, J.F., Piggot, T. and Ridge, E.H. 1974. Effect of seed inoculation with *Bacillus* subtilis and *Streptomyces griseus* on the growth of cereals and carrots. *Aust. J. agric. Res.* 25 : 219-226

Mondal, K.K., Singh, R.P., Dureja, P. and Verma, J.P. 2000. Secondary metabolites of cotton rhizobacteria in the suppression of bacterial blight of cotton. *Indian Phytopath.* 53 : 22-27

Mukhopadhyay, A.N. and Mukherjee, P.K. 1996. Fungi as fungicides. *Int. J. Trop. Plant Dis.* 14 : 1-17

*Muller, H.R.A. 1936. The *Phytophthora* foot rot of pepper in the Dutch East India (in Dutch). *Meded. Inst. Pl. Ziekh. Batavia.* 88 : 73

Myatt, P.M., Dart, P.J. and Hayward, A.C. 1993. Potential for biocontrol of Phytophthora root rot of chickpea by antagonistic root associated bacteria. *Aust. J. agric. Res.* 44 : 773-784

Nagtzaam, M.P.M., Bollen, G.J. and Termorshizen, A.J. 1998. Efficacy of *Talaromyus flavus* alone or in combination with other antagonists in controlling *Verticillium dahliae* in growth chamber experiments. *Phytopathology* 146 : 165-173

Nair, M.K. and Sarma, Y.R. 1988. International Pepper Community Workshop on Joint Research for the Control of Black Pepper Diseases. October 27-29, 1988 at Goa. A Report. *J. Plant. Crops* 16 : 146-149

Nambiar, K.K.N. and Sarma, Y.R. 1976. Quick wilt (foot rot) disease of pepper. *Arecanut Spices Bull.* 7 : 89-91

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

Padmakumary, G. 1989. Survival of *Rhizoctonia solani* Kuhn. with special reference to antagonistic soil microflora. Ph.D thesis, Kerala Agricultural University, Thrissur, p. 186

Phipps, P.M. 1992. Evaluation of biological agents for control of Sclerotinia blight of peanut. *Plant Dis.* 7 : 60

Pierson, E.A. and Weller, D.M. 1994. Use of mixtures of fluorescent pseudomonads to suppress take all and improve the growth of wheat. *Phytopathology* 84 :940-947

Podile, A.R. and Dube, H.C. 1987. Antagonism of *Bacillus subtilis* to *Phytophthora drechsleri* f. sp. *cajani*. *Indian Phytopath.* 40 : 503-506

Punja. Z.K. 1997. Comparative efficacy of bacteria, fungi and yeasts as biological control agents for diseases of vegetative crops. *Can. J. Plant Pathol.* 19 : 315-323

Rabindran, R. 1994. Biological control of rice sheath blight caused by *Rhizoctonia solani* and blast caused by *Pyricularia oryzae* using *Pseudomonas fluorescens*. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, p. 181

Rai, M., Acharya, D., Singh, A. and Varma, A. 2001. Positive growth responses of medicinal plants, *Spilanthus calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza* 11 : 123-128

Rajan, P.P. and Sarma, Y.R. 2000. *Verticillum tenerum* a potential biocontrol agent of *Phytophthora capsici*, the foot rot pathogen of black pepper. *Proceedings of the International Conference on Integrated Plant Disease Management for Sustainable Agriculture*, Vol. I., November 11-15, 1997 (ed. Jain, R.K.). Indian Phytopathological Society, New Delhi, pp. 337-338

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

Ramachandran, N. and Sarma, Y.R. 1985. Efficacy of three systemic fungicides in controlling *Phytophthora* infection of black pepper. *Indian Phytopath.* 38 : 160-162

Ramachandran, N., Sarma, Y.R. and Nambiar, K.K.N. 1982. Effect of two Systemic fungicides, Aliette and Ridomil on *Phytophthora palmivora* isolate of *Piper nigrum*. *Proceedings of the Workshop on Phytophthora Diseases of Tropical Cultivated Plants* (ed. Nambiar, K.K.N.), 1980. Central Plantation Crops Research

Ramamoorthy, V., Raguchander, T. and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. *European J. Plant Pathol.* 108 : 429-441

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protec.* 20 : 1-11

Ramesh, C.R. 1982. Root infection and population density of a vesicular arbuscualr mycorrhizal fungi in a coconut based multistoried cropping system. *Proceedings PLACROSYM.* Central Plantation Crops Research Institute, Kasaragod, Kerala, pp. 548-554

Raupach, G.S. and Kloepper, J.W. 2000. Biocontrol of cucumber diseases in the field by plant growth promoting rhizobacteria with and without methyl bromide fumigation. *Plant Dis.* 84 : 1073-1075

Ristaino, J.B. 1990. Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* 80 : 1253-1259

Robert, C.P., 1998. Management of foot rot of black pepper (*Piper nigrum* L.) with vesicular arbuscular mycorrhizae and antagonists. Ph.D thesis, Kerala Agricultural University, Thrissur, p. 227

Rosales, A.M., Thomashow, L.S., Cook, R.J. and Mew, T.W. 1995. Isolation and identification of antifungal metaboilites produced by rice associated antagonistic *Pseudomonas* sp. *Phytopathology* 85 : 1028-1032

Rosales, A.M., Vantomme, R., Swings, J., Ley, J.D. and Mew T.W. 1993. Identification of some bacteria from paddy antagonistic to several rice fungal pathogens. *Phytopathology* 83 : 189-208

Sahay, N.S. and Varma, A. 1999. *Piriformospora indica* : a new biological hardening tool for micropropagated plants. *FEMS Microbiol. Lett.* 181 : 297-302

Sailaja, P.R., Podile, A.R. and Reddanna, P. 1998. Biocontrol strain of *Bacillus subtilis* AF1 rapidly induces lipozygenase in groundnut (*Arachis hypogaea* L.) compared to crown rot pathogen *Aspergillus niger*. *European J. Plant Pathol.* 104 : 125-132

Samraj, J. and Jose, P.C. 1966. A Phytophthora Wilt of Pepper. *Sci. Cult.* 32 : 90-92

*Sanchez, A., Badel, E.R. and Schroder, E.C. 1994. *Pseudomonas cepacia*, a potential biofungicide for root rot pathogen of beans. *Journal of Agriculture of the University of Puerto Rico* 78 : 55-57

Santhakumari, P. 1987. Studies on Phytophthora diseases of plantation crops. Ph.D. thesis, University of Agricultural Sciences, Dharwad, p. 139

Sarkar, B.B., Choudary, D.M., Nath, S.C., Bhattacharjee, N.R. and Deb Barma, T.C. 1985. Wilt disease of pepper and its control in Tripura. *Indian Cocoa Arecanut Spices J.* 8 : 63-66

Sarma, Y.R. and Anandaraj, M. 1996. *Phytophthora* foot rot of black pepper. *Management of Threatening Plant Diseases of National Importance* (eds. Agnihotri, V.P., Sarbhoy, A.K. and Singh, D.V.). Malhothra Publications, New Delhi, pp. 237-248

Sarma, Y.R. and Anandaraj, M. 2000. Diseases of spice crops and their management. *Indian J. Arecanut Spices Medicinal Plants* 2 : 6-20

Sarma, Y.R. and Nambiar, K.K.N. 1982. Foot rot disease of black pepper (*Piper nigrum* L.). *Proceedings of the Workshop on Phytophthora Disease of Tropical Cultivated Plants* (ed. Nambiar, K.K.N.) 1980. Central Plantation Crops Research Institute, Kasaragod, Kerala, pp. 209-224

Sarma, Y.R. and Ramachandran, N. 1984. *Quick Wilt Disease of Black Pepper*. Central Plantation Crops Research Institute, Extension Folder No. 5

Sarma, Y.R., Anandaraj, M. and Rajan, P.P. 1994. *Phytophthora* : A threat to black pepper : Present status and future strategies of disease management. *Spice India* 7 : 10-13

Sarma, Y.R., Anandaraj, M. and Ramana, K.V. 1996b. Disease Management in *Phytophthora Foot Rot Affected Black Pepper Plantations.* Annual Report, Indian Institute of Spices Research. Calicut, pp. 63

Sarma, Y.R., Anandaraj, M. and Venugopal, M.N. 1996a. Biological control of diseases of spices. *Biological Control in Spices* (eds. Anandaraj, M. and Peter, K.V.). Indian Institute of Spices Research, Calicut, Kerala, pp. 1-19

Sarma, Y.R., Ramachandaran, N. and Anandaraj, M. 1991. Black pepper diseases in India. *Proceedings of the International Pepper Community Workshop on Joint Research for the Control of Black Pepper Diseases, October 27-29, 1988, Panaji, Goa* (eds. Sarma, Y.R. and Premkumar, T.). National Research Centre for Spices, Marikunnu, pp. 27-29

Sarma, Y.R., Ramachandarn, N. and Anandaraj, M. 1988. Integrated disease management of quick wilt (foot root) of black pepper (*Piper nigrum* L.) caused by *Phytophthora palmivora* MF_4. Proceedings of the Workshop on Strategies on the Management of Root Disease in Plantation Crops, January 18-19, 1988. Central Coffee Research Institute, Coffee Research Station. *J. Coffee Res.* 18 : 61-67

Sarma, Y.R., Ramachandran, N. and Nambiar, K.K.N. 1982. Morphology of black pepper *Phytophthora* isolates from India. *Proceedings of the Workshop on Phytophthora Disease of Tropical Cultivated Plants* (ed. Nambiar. K.K.N.). 1980. Central Plantation crops Research Institute, Kasaragod, Kerala, pp. 232-236

Sasikumaran, S.. Mammootty. K.P.. Abicheeran and Sukumara Pillay. V. 1981. Field trials for the control of quick wilt (foot rot) disease of pepper. *Third International Symposium on Plant Pathology, December 14-18, 1981.* Indian Agricultural Research Institute, New Delhi. *Abstract* : 128

Scher, F.M. and Baker, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppresiveness to Fusarium wilt pathogens. *Phytopathology* 72 : 1567-1573

Schippers, B. 1988. Biological control of pathogens with rhizobacteria. *Philos. Trans. R. Soc.* London 318 : 283-293

Schroth, M.N. and Hancock, J.G. 1981. Selected topics in biological control. *Annu. Rev. Microbiol.* 35 : 453-476

Shirokov, A.V., Loginov, O.N., Melent'ev, A.I. and Aktuganov, G.E. 2002. Protein and peptide factors from *Bacillus* sp. 739 inhibit the growth of phytopathogenic fungi. *Appl. Biochem. Microbiol.* 38 : 139-143

Silosuh, L.A., Lethbridge, B.J., Raffel, S.J., He, H., Clardy, J. and Handelsman, J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus Cereus* UW85. *Appl. Environ. Microbiol.* 60 : 2023-2030

Sivaprasad, P. 1995. *Management of Root Diseases of Important Spice Crops of Kerala with VAM.* DBT project final report. Kerala Agricultural University, Thrissur, p. 195

Sarma, Y.R., Ramachandran, N. and Nambiar, K.K.N. 1982. Morphology of black pepper *Phytophthora* isolates from India. *Proceedings of the Workshop on Phytophthora Disease of Tropical Cultivated Plants* (ed. Nambiar. K.K.N.). 1980. Central Plantation crops Research Institute, Kasaragod, Kerala, pp. 232-236

Sasikumaran, S.. Mammootty. K.P.. Abicheeran and Sukumara Pillay. V. 1981. Field trials for the control of quick wilt (foot rot) disease of pepper. *Third International Symposium on Plant Pathology, December 14-18, 1981.* Indian Agricultural Research Institute, New Delhi. *Abstract* : 128

Scher, F.M. and Baker, R. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppresiveness to Fusarium wilt pathogens. *Phytopathology* 72 : 1567-1573

Schippers, B. 1988. Biological control of pathogens with rhizobacteria. *Philos. Trans. R. Soc.* London 318 : 283-293

Schroth, M.N. and Hancock, J.G. 1981. Selected topics in biological control. *Annu. Rev. Microbiol.* 35 : 453-476

Shirokov, A.V., Loginov, O.N., Melent'ev, A.I. and Aktuganov, G.E. 2002. Protein and peptide factors from *Bacillus* sp. 739 inhibit the growth of phytopathogenic fungi. *Appl. Biochem. Microbiol.* 38 : 139-143

Silosuh, J.A., Lethbridge, B.J., Raffel, S.J., He, H., Clardy, J. and Handelsman, J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus Cereus* UW85. *Appl. Environ. Microbiol.* 60 : 2023-2030

Sivaprasad, P. 1995. *Management of Root Diseases of Important Spice Crops of Kerala with VAM.* DBT project final report. Kerala Agricultural University, Thrissur, p. 195

Sivaprasad, P., Joseph, P.J. and Balakrishnan. S. 1997. Management of foot-rot of black pepper with Arbuscular Mycorrhizal Fungi (AMF). International conference on Integrated Plant Disease Management for Sustainable Agriculture. New Delhi. *Abstract* : 205

Sivaprasad, P., Robert, C.P., Vijayan, M. and Joseph, P.J. 1995. Vesicular arbuscular mycorrhizal colonization in relation to foot rot disease intensity in black pepper. *Mycorrhizae : Biofertilizers for the Future* (eds. Adholeya, A. and Singh, S.). Proceedings of Third National Conference on Mycorrhizae. Tata Energy Research Institute, New Delhi, pp. 137-146

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

Smith, K.P., Havey, M.J. and Handelsman, J. 1993. Suppression of cottony leak of cucumber with *Bacillus cereus* strain UW 85. *Plant Dis.* 77 :139-142

Smitha, K.P. 2000. Management of foliar blight of amaranthus (*Amaranthus tricolor*) caused by *Rhizoctonia solani* Kuhn using microbial antagonists. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p. 73

Snedecor, G.W. and Cochran, W.G. 1967. *Statistical Methods*. Sixth edition. Oxford and IBH Publishing Co., New Delhi, p. 69

Sulaiman, R. 1994. A diagnostic study on pepper production and productivity in Kerala. Ph.D. thesis, Indian Agricultural Research Institute, New Delhi, p. 164

Suslow, T.V. and Schroth, M.N. 1982. Rhizobacteria on sugarbeets : effects of seed application and root colonization on yield. *Phytopathology* 72 :199-206

Tambong, J.T. and Hafte, M. 2001. Phenazines are involved in biocontrol of *Pythium myriotylum* on cocoyam by *Pseudomonas aeruginosa* PNA1. *European J. Plant Pathol.* 107 : 511-521

Thanuja, T.V. and Hedge, R.V. 2001. Improvement of root growth of black pepper cuttings by vesicular arbuscular mycorrhizal fungi under different environmental conditions. *Proceedings of Thirteenth Kerala Science Congress,* January 24-25, 2001, Thrissur, pp. 401-403

Thara, K.V. and Gnanamanickam, S.S. 1994. Biological control of rice sheath blight in India : Lack of correlation between chitinase production by bacterial antagonists and sheath blight suppression. *Plant and Soil* 160 : 277-280

Thomas, J., Vijayan, A.K., Suseela Bai, R. and Naidu, R. 1994. Biological control of rhizome rot disease of small cardamom (*Elettaria cardamomum* Maton). *Crop. Diseases-Innovative Techniques and Management* (eds. Sivaprakasam, K. and Seetharaman, K.). Kalyani Publishers, New Delhi, pp. 189-197

*Tsao, P.H. and Alizadeh, A. 1988. Recent advances in the taxonomy and nomenclature of the so called *Phytophthora palmivora* MF_4 occurring on cocoa and other tropical crops. *Proceedings of the Tenth International Cocoa Research Conference, May 17-23, 1987.* Santo Domingo, Dominican Republic, pp. 441-445

Tsao, P.H. and Tummakate, A. 1977. The identity of *Phytophthora* species from black pepper in Thailand. *Mycologia* 69 : 631-637

*Tsao, P.H., Kasim, R. and Mustika, I. 1985b. Morpholgy and identity of black pepper. *Phytophthora isolates in Inodonesia. FAO Plant Prot. Bull.* 33 : 61-66

Tsao, P.H., Sarma, Y.R., Kasim, R., Mustika. I. and Kuch. T.K. 1985a. Variation in *Phytophthora palmivora* MF_4 (*P. capsici*) isolates from black pepper in India, Indonesia and Malaysia. *Phytopathology* 75 : 1315-1316

*Tsao, P.H., Sztejnberg, Shou, R. and Fanag, G. 1988. Biological control of *Phytophthora* root rots with antagonistic fungi. *Proceedings of Fifth International Congress on Plant Pathology*, Kyoto, Japan, pp. 85-89

Tschen, J.S.M. 1987. Control of *Rhizoctonia solani* by *Bacillus subtilis*. Transactions of the Mycological Society of Japan 28 : 483-493

*Turner, G.J. 1969a. *Phytophthora palmivora* from *Piper betle* in Sarawak. *Trans. Br. Mycol. Soc.* 52 : 411-418

*Turner, G.J. 1969b. Leaf lesions associated with foot rot of *Piper nigrum* and *Piper betle* caused by *Phytophthora palmivora*. *Trans. Br. Mycol. Soc.* 53 : 407-415

Turner, J.T. and Backman, P.A. 1991.Factors relating to peanut yield increases after seed treatment with *Bacillus subtilis*. *Plant Dis.* 75: 347-353

Uchida, J.Y. and Aragaki, M. 1985. Occurrence of chalmydospores in *Phytophthora capsici*. *Mycologia* 77 : 832-835

Uthkede, R.S. and Guance, A.P. 1983. Inhibition of *Phytophthora cactorum* by a bacterial antagonist. *Can. J. Bot.* 61 : 3343-3348

Utkhede, R.S. 1984. Antagonism of isolates of *Bacillus subtilis* to *Phytophthora capsici*. *Can. J. Bot.* 62 : 1032-1035

van Peer, R., Niemann, G.J. and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of Carnation by *Pseudomonas* sp. strain WCS417M. *Phytopathology* 81 : 728-734

Varma, A., Singh. A.. Sudha. Sahay, N.S. and Sharma. J.. Roy. A., Kumari, M.. Thakran. S.. Deka, D., Bharti. K.. Hurek. T.. Blechert. O.. Rexer. K.H.. Kost. G.. Hahn. A.. Maier. W.. Walter. M.. Strack, D. and Kranner. I. 2001. *Piriformospora indica* : An axenically cultturable mycorrhizal like endosymbiotic fungus. *The Mycota IX Fungal Associations* (ed. Hock. B.). Springer-Verleg, Berlin, Heidelberg, New York, pp. 125-150

Varma, A., Verma, S., Sudha, Sahay, N.S., Butehorn, B. and Franken, P. 1999. *Piriformospora indica*, a cultivable plant growth promoting root endophyte with similarities to arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* 65 : 2741-2744

Vasudeva. R.S. and Chakravarthi. B.P. 1954. The antibiotic action of *Bacilus sublitis* in relation to certain parasitic fungi with special reference to *Alternaria solani* (Ell. And Mart) Jones and Grout. *Ann. Appl. Biol.* 41 : 612-618

Verma, S., Varma, A., Rexer, K.H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Butehorn, B. and Franken, P. 1998. *Priformospora indica* gen et. sp. nov., a new root colonizing fungus. *Mycologia* 90 : 896-903

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159 : 850

*Viswanathan, R. and Samiyappan, R. 2002. Role of oxidative enzymes in the plant growth promoting rhizobacteria (PGPR) mediated induced systemic resistance in sugarcane against *Colletotrichum falcatum*. *Zeitschrift-fuer-Pflanzenkrankheiten-und-Pflanzenschutz.* 109 : 88-100

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopath.* 26 : 379-407

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

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

Whipps, J.B. 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. *The New Phytologist.* 107 : 127-142

Whipps, J.M. 1997. Developments in biological control of soilborne plant pathogens. *Adv. Bot. Res.* 26 : 1-34

Whipps, J.M. and Magan, N. 1987. Effects of nutrient status and water potential of media on fungal growth and antagonistic-pathogen interactions. *OEPP/EPPO Bull.* 17 : 581-591

Wong, P.T.W. and Baker, R. 1984. Suppression of wheat take all and ophiobolus patch by fluorescent pseudomonads from a Fusarium suppressive soil. *Soil Biol. Biochem.* 16 : 347-403

Xhang, L., Howell, C.R. and Starr, J.I. 1996. Suppression of *Fusarium* colonization of cotton roots and Fusarium wilt by seed treatment with *Gliocladium virens* and *Bacillus subtilis*. *Biocontrol. Sci. Tech.* 6 : 175-187

Yan, Z., Reddy, M.S., Rye, M.C., Inroy, J.A., Wilson, M. and Kloepper, J.W. 2002. Induced systemic protection against tomato late blight elicited by plant growth promoting rhizobacteria. *BioControl* 92 : 1329-1333

Yang, C.H., Menge, J.A. and Cooksey, D.A. 1993. Role of copper resistance in competitive survival of *Pseudomonas fluorescens* in soil. *Appl. Enviorn. Microbiol.* 59 : 580-584

172204

Yang, C.H., Menge, J.A. and Cooksey, D.A. 1994. Mutations affecting hyphal colonization and pyoverdine production in pseudomonads antagonistic toward *Phytophthora parasitca.* *Appl. Environ. Microbiol.* 60 : 473-481

Zheng, X.Y. and Sinclair, J.B. 2000. The effects of traits of *Bacillus megaterium* on seed and root colonization and their correlation with the suppression of Rhizoctonia root rot of soybean. *BioControl* 45 : 223-243

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*Original not seen

APPENDICES

APPENDIX-I

Composition of different media

A. Carrot Agar

Carrot	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml

B. King's B

Peptone	-	20 g
Magnesium sulphate	-	1.50 g
Dipotassium phosphate	-	1.50 g
Glycerol	-	10 ml
Agar	-	20 g
Distilled water	-	1000 ml

C. Nutrient Agar

Beef extract	-	3 g
Peptone	-	5 g
Sodium chloride	-	5 g
Agar	-	20 g
Distilled water	-	1000 ml

APPENDIX – II

Preparation of stain

A. Trypan blue stain

Trypan blue	-	0.05 g
Lactophenol	-	100 ml

B. Lactophenol

Lactic acid	-	20 ml
Phenol	-	20 ml
Glycerol	-	40 ml
Water	-	20 ml

MANAGEMENT OF PHYTOPHTHORA DISEASE OF BLACK PEPPER (*Piper nigrum* L. WALP) USING PLANT GROWTH PROMOTING MICROBIAL INOCULANTS

BEENA S. NAIR

Abstract of the
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Department of Plant Pathology
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM 695522

ABSTRACT

The study, "Management of Phytophthora disease of black pepper (*Piper nigrum* L. Walp) using plant growth promoting microbial inoculants" was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani to investigate the effect of plant growth promoting rhizobacterial strains belonging to fluorescent pseudomonads and *Bacillus* spp. and the root endophytic fungus, *Piriformospora indica* on the suppression of nursery wilt of black pepper incited by *Phytophthora capsici.* Two strains of fluorescent pseudomonads *viz.*, *Pseudomonas putida* strain 89B61 and *P. fluorescens* strain RCL3R4, two strains of *Bacillus viz.*, BY-1 and BY-2 and *P. indica* were included in the study to explore their potential in plant growth promotion and disease suppression. *P. capsici* inoculated control, uninoculated healthy control, chemical control (0.20 per cent COC drenched at 15 days intervals) and combined application of *Trichoderma harzianum* and an AMF, *Glomus fasciculatum* were maintained for comparison. In the dual culture assay, conducted *in vitro* for preliminary screening, though *P. indica* exhibited a slight mycelial growth inhibition initially, the pathogen later overgrew the antagonist. Among the bacteria, fluorescent pseudomonad strains exhibited better mycelial growth inhibition on both PDA and Carrot Agar. The influence of different biocontrol agents on growth promotion of the black pepper cuttings was not statistically significant. However, the fluorescent pseudomonad, *P. putida* strain 89B61 exhibited maximum growth promotion. In the experiment conducted to test the potential of various plant growth promoting microbial inoculants in suppressing nursery wilt, the plants treated with spore suspension of *P. indica* exhibited the highest wilt percentage of 93.22 per cent which was more than that in the inoculated control (37.48). The plants treated with the fluorescent pseudomonad, *P. fluorescens* strain RCL3R4 recorded a wilt

percentage of 6.70. All other treatments were highly effective and checked the infection completely. The *Bacillus* strain, BY-2 exhibited better disease suppression *in vivo*. The fluorescent pseudomonad, *P. putida* strain 89B61 also showed disease suppression *in vivo*, which indicated that it has the dual function of plant growth promotion and disease suppression.