BIOLOGICAL CONTROL OF FOOT ROT OF BLACK PEPPER (*Piper nigrum* L. Walp) WITH ANTAGONISTIC BACTERIA FROM RHIZOSPHERE

By

JUBINA. P. A.

Thesis

Submitted in partial fulfilment of the requirement

for the degree

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF AGRICULTURE

Vellayani, Thiruvananthapuram

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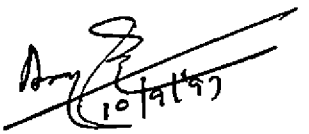

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DECLARATION

I hereby declare that this thesis entitled "**Biological control of foot rot of black pepper (*Piper nigrum* L. Walp) with antagonistic bacteria from rhizosphere**", 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 to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

College of Agriculture
Vellayani
9-09-1997

JUBINA. P.A.

CERTIFICATE

Certified that the thesis entitled "**Biological control of foot rot of black pepper (*Piper nigrum* L. Walp) with antagonistic bacteria from rhizosphere**", is a record of research work done independently by **Kum. Jubina. P.A.** (Admission No.95-11-31) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

1. INTRODUCTION

Black pepper (*Piper nigrum* L. Walp), the most important and ancient spice crop grown in India, is a major foreign exchange earner for the country. During the year 1995-96, black pepper alone accounted for about 24.3 per cent of the export earnings of India. As per the official statistics, the production of pepper in India during 1994-95 was 53110 tonnes from an area of 195050 ha and Kerala alone accounted for 97.6 per cent and 97.93 per cent in area and production, respectively.

Productivity of black pepper in India is only 290 kg ha^{-1} as against the 2925 kg ha^{-1} in Malaysia. One of the major reasons attributed to this low productivity is the high incidence of the devastating disease, foot rot or quick wilt, especially in the state of Kerala. The incitant of the disease, *Phytophthora capsici*, is a soil borne fungus, endemic to the pepper growing tracts. The disease has become rampant all over the state owing to heavy rainfall and high humidity which are highly congenial for the incidence and spread of the disease. Lack of disease resistant varieties, high cost and inadequate

protection by fungicides have been indicated as the major setback in tackling this peril (Sulaiman, 1994). The application of plant protection chemicals also endanger the environment and micro and macro fauna. Hence there is an urgent need for cheaper disease management strategies which are ecofriendly, sustainable, yet effective. In this context, biological control appears to be the main strategy for protecting the crop and also for promoting and preserving the environmental equilibrium.

Bacteria have been used world wide to provide good protection against several fungal pathogens in greenhouse and under field conditions (Weller, 1988). Efforts in this direction for combating foot rot of black pepper using bacterial antagonists are very limited. Hence, the present study was undertaken with the following objectives:

1) Isolation of bacteria from soil and rhizosphere of pepper plants.

2) Testing bacterial isolates for antagonism to *P. capsici in vitro* and in excised pepper shoots.

3) Selection of bacterial isolates with ability to suppress foot rot in black pepper.

4) Testing the efficacy of cell free filtrates of bacterial antagonists in suppressing the pathogen *in vitro*.

5) Testing the efficacy of cell free filtrates in disease suppression in black pepper.

6) Assessing the effect of selected bacterial antagonists on growth promotion of black pepper.

7) Assessment of the cellulolytic characteristics of bacterial antagonists.

8) Preliminary characterisation of selected bacterial antagonists.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Phytophthora foot rot or quick wilt is one of the most destructive diseases of black pepper causing severe economic losses. On a global scale, this dreaded disease accounts for an annual loss of about US $ 4.5 - 7.5 million (de Ward, 1979) and is a major constraint in pepper production. Almost all pepper growing areas in India are subject to severe crop losses by this devastating disease which poses a serious threat to the very existence of pepper vines. In Kerala, the major producer of black pepper in India, foot rot incidence of 3.7 and 9.4 per cent accounting to an annual loss of 119 and 905 metric tonnes of black pepper has been reported from Kozhikode and Kannur districts respectively (Balakrishnan *et al.*, 1986, Anandaraj *et al.*, 1988).

The sudden collapse and death of pepper vines was first reported from Lampung, Indonesia in 1885 (Chattopadhyay and Maiti, 1990). The disease is known in India since 1902 when Barber (1902, 1903 and 1905) and later, Butler (1906, 1918) investigated the disease in Wynad. However, they could not conclusively establish the etiology of the disease. The first report of '*Phytophthora* wilt' of black pepper in Kerala was by Samraj and Jose (1966) adopting the identification as

Phytophthora palmivora var. *parasitica*. The identity and nomenclature of *Phytophthora* isolates from black pepper remained controversial for several years until Tsao and Alizadeh (1988) confirmed the causal organism of foot rot of black pepper as *Phytophthora capsici*.

Cultural and chemical methods have been advocated to manage foot rot disease of black pepper (KAU, 1993). However, only little success have been obtained with the use of fungicides (Ramachandran *et al.*, 1991). Sarma *et al.* (1991) therefore have emphasised the need for biological control by manipulating the microbial status of soil suppressive to the pathogen.

Antagonism to *P. capsici* by fungi has been reported by several workers. Cristinzio (1987) reported the antagonistic property of *Trichoderma* spp. and *Chaetomium* spp. against *P. capsici*. Anandaraj and Sarma (1995) observed that *Trichoderma* spp. and *Gliocladium virens* were effective against *P. capsici* under field conditions.

An integrated approach to management of foot rot of black pepper involving the addition of fungal antagonists like *G. virens* and *Trichoderma* spp. to soil along with foliar sprays of Bordeaux mixture, metalaxyl or phosphoric acid has been proposed by Anandaraj and Sarma (1994). An IDM with *Trichoderma harzianum* as a component for the management of foot rot reduced

the disease incidence from 25 per cent to 15 per cent in the field (Sarma *et al.*, 1996a). Combination of fungal antagonists *Aspergillus* sp. and *Penicillium* sp. was also found to delay foliar infection in black pepper by *P. capsici* (Jubina and Girija, 1997).

Sarma (1994) reported increased plant growth and reduced root infection of black pepper by *P. capsici* on application of Vesicular Arbuscular Mycorrhiza (VAM). The benefits of VAM in reducing foot rot of black pepper was also reported by Sivaprasad (1996). A native isolate of *Glomus monospermum*, IS6 offered protection from this disease both in the greenhouse and field conditions. Enhanced crop protection against *P. capsici* resulted from dual inoculation of VAM and the fungal antagonist, *Trichoderma* sp. (Sivaprasad, 1997).

Bacteria as Biocontrol Agents

Several rhizosphere bacteria have been proven to be effective as biocontrol agents. Weller (1988) listed bacteria belonging to several genera showing antagonism against several phytopathogens. Three genera of bacteria viz., *Agrobacterium*, *Bacillus* and *Pseudomonas* have been widely tested in both laboratory and field conditions.

The first bacterium used successfully on a commercial scale for biological control was *Agrobacterium radiobacter*

(Strain K 84). Kerr (1972) used the avirulent strain for the control of crown gall of peach caused by *Agrobacterium tumefaciens* which reduced infection to 31 per cent compared to 79 per cent in control. Since then several workers have reported the use of *A. radiobacter* as biocontrol agent (Alconero, 1980; Kerr, 1980 and Moore, 1979). A modified strain of *A. radiobacter*, K 1026, is available in Australia under the trade name No Gall™ for the control of grape crown gall (Maloy, 1993).

Bacillus spp. proved to be potential candidates for biological control of plant pathogens because of its ability to form endospores resistant to heat and desiccation and to produce highly stable antibiotics detrimental to several pathogens (Baker and Cook, 1974; Handelsman *et al.*, 1990; Osburn *et al.*, 1995 and Weller, 1988). *Bacillus subtilis* strain A13 isolated from the surface of lysed mycelium of *Sclerotium rolfsii* (Broadbent *et al.*, 1971) has been sold under the name QUANTUM-4000 since 1983 (Weller 1988).

Bacillus spp. are known to exhibit *in vitro* antagonism to several plant pathogenic fungi. Gokulapalan and Nair (1984) reported the antagonistic effect of *Bacillus* sp. on *Rhizoctonia solani*. Khot *et al.* (1988) observed that in liquid medium, *B. subtilis* reduced the mycelial dry weight of all eight plant pathogenic fungi tested viz., *Alternaria alternata*,

Curvularia lunata, *Fusarium oxysporum* f. sp. *udum*, *Fusarium* sp., *Drechslera australiensis*, *Macrophomina phaseolina*, *R. solani* and *S. rolfsii*. Four isolates of *B. subtilis* inhibited the growth of fungal pathogens like *Rhizoctonia zeae*, *Fusarium moniliforme*, *M. phaseolina* and *Diplodia zeae* (Adetuyi and Olowoyo, 1993). Thomas *et al.* (1994) found that *B. subtilis* caused growth inhibition of *Pythium vexans* and *R. solani*, causing rhizome rot of small cardamom.

Bacillus spp. have also been proven to control diseases in a wide variety of plants. The efficacy of *B. subtilis* in the control of root rot caused by *Macrophomina phaseolina* in legumes was reported by Singh and Mehrothra (1980) in chickpea and Jharia and Khare (1986) in soybean. Greenhouse experiments conducted by Tschen (1987) revealed the efficacy of *B. subtilis* applied as seed treatment in reducing black scurf of potato caused by *R. solani*. Soil application of *B. subtilis* resulted in disintegration of sclerotia of the pathogen, *Claviceps purpurea* infecting *Pennisetum americanum* (Mahadevamurthy *et al.*, 1988). *B. subtilis* was found to inhibit sclerotial growth of *R. solani* and reduced sheath blight incidence in rice (Padmakumary, 1989). Hwang *et al.* (1993) reported that *Bacillus cereus* strain alf. 87A reduced the incidence and severity of basal foot rot and end rot caused by *Sclerotinia sclerotiorum* in pea plants when applied at pod development stage. Krebs *et al.* (1993) reported that

application of *B. subtilis* in combination with the fungicide zineb reduced the infection by *F. oxysporum* f.sp. *dianthi* in carnation plants. *B. subtilis* and *Enterobacter cloacae* isolated from wheat and cotton respectively were found to reduce summer patch disease in Kentucky blue grass caused by *Magnaporthe poae* in field trials conducted during 1991 and 1994 (Thompsun *et al.*, 1996).

Pseudomonas spp, especially *P. fluorescens*, have been widely reported to exert biocontrol of pathogens through various mechanisms (Defago *et al.*, 1990; Schippers *et al.*, 1987; Thomashow and Weller, 1990 and Young *et al.*, 1991).

Wong and Baker (1984) found that pseudomonads isolated from a Fusarium suppressive soil showed *in vitro* antagonism to wheat take all pathogen, *Ophiobolus*, pathogenic to turf grass. Sakthivel *et al.* (1986) observed that several strains of *P. fluorescens* isolated from the rhizosphere exhibited antagonism to several plant pathogens like *F. oxysporum* f. sp. *cubense*, *R. solani*, *Acrocylindrium oryzae* and *Xanthomonas campestris* pv. *oryzae*. Andreoli *et al.* (1993) reported that fluorescent *Pseudomonas* isolated from rhizosphere of sunflower plants inhibited *S. sclerotiorum* and promoted lysis of the fungal mycelium and inhibited ascospore germination in liquid culture medium by production of an antifungal substance. *Pseudomonas cepacia* strain UPR 5C, which was a potent antagonist of

M. phaseolina, inhibited the growth of other fungal pathogens of *Phaseolus vulgaris* (Sanchez *et al*. 1994).

Weller and Cook (1983) observed that *P. fluorescens* strain 2-79 isolated from a suppressive soil reduced take all of wheat caused by *Gaeumannomyces graminis* in both greenhouse and field trials and increased yield of wheat. Ganesan and Gnanamanickam (1987) reported inhibition of mycelial growth and reduced germination of sclerotium of *S. rolfsii* by native strains of *P. fluorescens in vitro*. They also obtained good control of the pathogen in peanuts under greenhouse conditions. Seed treatment of chickpea with two strains of *P. fluorescens* suppressed seed rot and pre-emergence damping off caused by *Pythium ultimum* (Kaiser *et al*., 1989). Laha (1991) investigated the role of fluorescent pseudomonads in managing cotton diseases and found that seed bacterisation resulted in reduction in disease caused by *R. solani*. The bacteria also also suppressed other pathogens like *P. ultimum*, *Rhizopus* sp., *M. phaseolina* and *R. solani in vitro*.

George (1995) reported that two isolates of *P. fluorescens* reduced sheath rot caused by *Sarocladium oryzae* in rice under field conditions. Application of *P. fluorescens* to pear plants followed by inoculation of *Stemphylium vesicarium* causing brown spot disease resulted in 57 per cent less disease incidence and 88 per cent less severity than untreated control

(Montesinos *et al.*, 1996). Rice plants could be protected against blast, brownspot, blight and bacterial blight pathogens by the application of *P. fluorescens* (Nayar, 1996).

Biological control of *Phytophthora* spp.

Soil inhabiting bacteria exert inhibitory effects on species of *Phytophthora*. Malajczuk (1983) has reported a positive correlation between lysis of hyphae of *Phytophthora* spp. and populations of *Pseudomonas*, *Bacillus* and *Streptomyces* spp.

Utkhede (1984) tested and found that 21 isolates of *B. subtilis* obtained from sclerotia of local and exotic strains of *Sclerotium cepivorum* were antagonistic to *Phytophthora cactorum*, the incitant of crown rot of apple. Narula and Mehrotra (1987) obtained 37-43 per cent reduction in leaf blight in colocasia caused by *Phytophthora colocasiae* on inoculating with antagonistic bacterial isolates. Treatment of roots with a bacterial antagonist resulted in 60 per cent reduction of root infection by *Phytophthora cinnamomi* in avocado seedlings after 6 weeks of inoculation (Maas and Kotze, 1990).

Duvenhage *et al.* (1991) reported high populations of aerobic, spore forming bacteria and actinomycetes in soil sample collected from healthy avocado trees in *P. cinnamomi* infested orchards and found these to be highly antagonistic to the pathogen. Utkhede and Smith (1991) found that six strains of *B. subtilis* and one strain of *Enterobacter aerogenes* were

effective in reducing the percentage incidence of *Phytophthora* crown and root rot of apples. Galindo (1992) reported antagonism of *P. fluorescens* to *Phytophthora palmivora*, the causal agent of black pod of cocoa and found that the disease control effected by the bacterium was superior to that of copper oxide or chlorothalonil.

Sauer and Ziller (1992) observed that bacterial antagonists inhibited the growth of *P. cactorum* and gave good control of collar rot disease of apples. Upadhyay and Jayaswal (1992) compared the *in vitro* interaction between *P. cepacia* and several phytopathogenic fungi including *P. megasperma* var. *sojae* and found that the bacteria inhibited the sporulation and caused mycelial deformities in all the pathogens. Treatment with bacterial antagonists, *Bacillus azotoformans* and *B. megaterium*, reduced root rot and root colonization by *P. cinnamomi* in avocado seedlings (Duvenhage and Kotze, 1993).

Application of *E. aerogenes* in combination with the fungicide metalaxyl reduced crown and root rot symptoms in apple trees caused by *P. cactorum* (Levesque *et al.*, 1993). Myatt *et al.* (1993) opined that *P. cepacia* and *P. fluorescens* were effective in inhibiting *P. megasperma* f. sp. *medicaginis*, the incitant of root rot of chickpea, both in the laboratory and field. Sarathchandra *et al.* (1993) reported the inhibition of growth of *P. nicotianae* var *nicotianae* and four other fungal

pathogens by fluorescent pseudomonads. Metalaxyl alternated with the biocontrol agent *E. aerogenes* was found to be effective in affording long term control of crown and root rot of apple caused by *P. cactorum* under field conditions (Utkhede and Smith, 1993).

B. subtilis when used as seed treatment showed antagonism to *P. nicotianae* var. *parasitica* and five other damping off fungi in *Casuarina equisetifolia* (Pena *et al.*, 1994). Marchi and Utkhede (1994) reported the ability of *E. aerogenes* which is active against *P. cactorum*, to inhibit several other soil fungi and bacteria.

Fernando and Linderman (1995) reported reduced mycelial growth and sporangial production of *Phytophthora vignae* by bacteria isolated from cowpea fields through production of inhibitory substances. Of these, three bacteria, *Brevibacterium linens*, *Bacillus thuringiensis* and *B. pumilis* suppressed the disease under greenhouse when applied as seed or soil treatment. Treatment with *Pseudomonas aureofaciens* PA 147-2 afforded protection to asparagus seedlings against *P. megasperma* var *sojae* as indicated by the reduced disease incidence and severity (Carruthers *et al.*, 1995). Berger *et al.* (1996) obtained biological control of damping off caused by *Phytophthora* and *Pythium* spp. in *Astilbe*, *Photinia* and *Hemerocallis* in glasshouses which was as good as treatment with fungicide metalaxyl.

Promising results were obtained on the biological control of *P. capsici* causing blight of chillies (*Capsicum annuum*). Cho (1987) found that application of the antagonists - *P. cepacea*, *B. polymyxa* and *Bacillus* sp. together with soil amendments of organic compost and calcium fertilizers could effect biological control of *P. capsici* in chillies. Of the several microorganisms isolated from rhizosphere and non rhizosphere soils by Jee *et al.* (1988), *T. harzianum*, *P. cepacia* and *B. polymyxa* markedly inhibited the mycelial growth and zoosporangial germination of *P. capsici* in solid or liquid medium. They obtained significant decrease in *Phytophthora* blight of chillies using combinations of *T. harzianum* and *P. cepacia*. Kim *et al.* (1988) obtained significant control of blight of chillies using *Bacillus* str. AC-1, both in greenhouse and in laboratory.

Okamoto and Isaka (1989) reported inhibition of germination of zoosporangia and cytospores of *P. capsici* by four isolates of bacteria. All the four isolates reduced seedling damping off in cucumber in pot trials while two isolates reduced disease incidence in seedlings cultivated by hydroponics. *P. cepacia* was more effective than *T. harzianum* in suppressing *Phytophthora* blight in *C. annuum* (Kim *et al.*, 1990). Lee *et al.* (1990) observed a delay of 30-79 days in the incidence of *P. capsici* when chilli seedlings were transplanted into field with pot soil formulated with *P. cepacia*. Okamoto *et al.* (1991)

reported antagonism of *Serratia marcescens* to *P. capsici* and consequently caused reduction of damping off of cucumber seedlings. Sarma *et al.* (1996b) found that rhizosphere bacteria, especially the 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.

Role of culture filtrates of bacterial antagonists

Cell free culture filtrates or extracts can be used to study the role of antibiosis in biological control. Baker *et al.* (1982) reported the reduction of number of uredia produced by *Uromyces phaseoli* in bean plants sprayed with culture filtrates of *B. subtilis*. On the treated leaves, the culture filtrates reduced uredospore germination by more than 95 per cent and the germtubes that developed were abnormal. Complete inhibition of *P. cactorum in vitro* by a 40 per cent autoclaved extract of bacterial isolate B8 was reported by Utkhede and Guance (1983). The application of autoclaved culture filtrates 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). Similarly Cubeta *et al.* (1985) reported the effect of autoclaved culture filtrates of *B. subtilis* in suppressing *Phomopsis* infection of soybean in the field.

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. Cell free culture filtrates of *B. subtilis*, *B. pumilis* and *P. fluorescens* suppressed the growth of *Septoria tritici*, causal agent of blotch disease in wheat (Mehdizadegan and Gough, 1987). Application of culture filtrates of *Bacillus licheniformis*, *P. aeruginosa* and *Streptomyces diastaticus* to barley plants reduced mortality rates caused by *S. rolfsii* to 40, 53.3 and 46.6 per cent respectively (Singh and Dwivedi, 1987). Tschen (1987) observed inhibition of lesion development by *R. solani* in potatoes by culture filtrates of the bacterial antagonist *B. subtilis*. Fravel (1988) discussed the role of antibiosis in plant disease control. Gilbert *et al.*(1991) reported that the lysis of zoospores of *P. megasperma* f.sp. *medicaginis* and *P. cactorum* by culture filtrates of *Bacillus cereus* depended on the pH and calcium and ammonia content of the growth medium.

Reduction in incidence of anthracnose in lucerne caused by *Colletotrichum trifolii* from 56 per cent to 16 per cent and severity from 2 to 1.2 was reported by Douville and Boland (1992) by treatment of seedlings with cell free culture filtrates of *B. subtilis*. Badel and Kelemu (1994) showed that cell free culture filtrates of *B. subtilis* significantly reduced mycelial

growth, germination and conidial production of *Colletotrichum gloeosporioides in vitro*. The inhibitory effect of the crude preparation on *Thanatephorus cucumeris*, *R. solani*, *Colletotrichum lindemuthianum*, *Glomerella cingulata*, *P. capsici* and *Pyricularia oryzae* was comparable to or better than that obtained with benomyl at 530 ug/ml. 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 (Rabindran, 1994). Nowak - Thompson *et al.* (1994) found that the antibiotic 2,4 - diacetylphloroglucinol, isolated from culture filtrates of *P. fluorescens*, inhibited the growth of the phytopathogens *P. ultimum*, *R. solani* and *Erwinia carotovora* sub sp. *atroseptica* Silo-Suh *et al.* (1994) reported suppression of damping off of lucern caused by *P. medicaginis* by culture filtrates of *B. cereus* UW85. The antibiotics present in the culture filtrates, termed Antibiotic B and zwittermicin A, reversibly inhibited the growth of the pathogen. Montesinos *et al.* (1996) detected antifungal activity of cell free culture filtrates of *P. fluorescens* and *Erwinia herbicola* against *S. vesicarium*, the causal agent of brown spot of pear.

Production of hydrolytic enzymes

Hydrolytic enzymes produced and released by soil microorganisms might have a role in suppression of phytopathogenic fungi (Lam and Gaffney, 1993). These enzymes may help

in the utilization of complex carbohydrates like cellulose, hemicellulose and pectin present in the root tip mucilages facilitating efficient colonization of plant roots by these microorganisms (Weller, 1988). Ahmad and Baker (1987 a,b) have reported the increased competetive saprophytic ability and rhizosphere competence of *T. harzianum* mutants with increased cellulase production.

There are several reports of positive correlation between production of hydrolytic enzymes by the antagonists and biological control. Production of chitinase or β-1,3-glucanase by bacteria resulted in the lysis of fungal cell walls in which the major constituents are chitin and β-1,3-glucan (Fiske *et al.*, 1990 and Shapira *et al.*, 1989). Lim *et al.* (1991) reported that biocontrol of root rot caused by *F. solani* by the bacterial antagonist *Pseudomonas stutzeri* was mainly by the production of the enzymes laminarinase and chitinase. Production of β-1, 3-glucanase by *P. cepacia* was responsible for the reduction in number of plants infected by *R. solani*, *S. rolfsii* and *P. ultimum* (Fridlender *et al.*, 1993).

However, there are also reports of lack of correlation between *in vitro* production of lytic enzymes and biocontrol. Mavingui and Heulin (1994) did not find any correlation between chitinase activity of *B. polymyxa* and inhibition of the pathogen *G. tritici*. Similarly, Thara and Gnanamanickam (1994) could not

observe any correlation between cellulase activity of the bacterial antagonists and their ability to suppress *R. solani* in nursery or field plots.

Plant Growth Promotion by Rhizobacteria

Plant growth promoting rhizobacteria (PGPR) are thought to improve plant growth not only by increasing nutrient uptake and providing roots with phytohormones and solubilized iron but also by suppressing either major or minor pathogens of plant (Cook and Rovera, 1976, Defago *et al.*, 1990; Kloepper *et al.*, 1980; Schippers *et al.* 1987 and Weller, 1988) through an array of mechanisms.

B. subtilis was reported to increase the yield of carrots by 48 per cent and oats by 40 per cent (Merriman *et al.* 1974a,b). Broadbent *et al.* (1977) reported that application of *Bacillus* spp. to steamed soil increased seedling growth of many plant species when the nutrient content in the soil was low. Seed treatment with flourescent pseudomonads are reported to improve growth in potato, sugarbeet and radish (Burr *et al.*, 1978; Kloepper and Schroth, 1978 and Suslow and Schroth, 1982).

Several PGPR have been demonstrated to promote plant growth through reduction of plant diseases. Rhizosphere bacteria, belonging to *Pseudomonas* spp, *Bacillus* sp. and *Flavobacterium* sp. applied as seed dressing increased the

seedling emergence in sunflower by 28 per cent in the presence of the pathogen *Corticium rolfsii* (Hebbar *et al.*, 1991). Howie and Buslow (1991) reported a 70% reduction of *Pythium* infection in cotton and about 50 per cent increase in emergence of seedlings by an antibiotic producing *P. fluorescens*. Adetuyi and Olowoyo (1993) coated maize seeds with *B. subtilis* antagonistic to several phytopathogenic fungi and obtained increased germination and seedling growth.

Sakthivel *et al.* (1986) found that seed bacterization of rice and cotton with fluorescent pseudomonads antagonistic to several plant pathogens *in vitro* increased plant growth by 12-27 per cent and 8-40 per cent respectively. Gamaliel and Katan (1993) reported increased growth of tomato plants in non solarized soils as a result of root colonisation by flourescent pseudomonads and inhibition of *Penicillium pinophilum*, a minor pathogen. Harris *et al.* (1994) obtained several bacteria with ability to control damping off caused by *Pythium ultimum* var. *sporangiferum* and to increase growth of *C. annuum*. 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 the presence of the pathogen.

Kaiser *et al*. (1989) reported increased emergence and yield of chickpea in soils naturally infected with *P. ultimum* when fluorescent pseudomonads were applied as seed treatment. Kumar and Dube (1992) obtained increased seed germination, growth and yield of chickpea and soybean and reduced incidence of chickpea wilt caused by *F. oxysporum* f.sp. *ciceris* through seed bacterization. Seed treatment of ground nut with *B. subtilis* improved seed germination and emergence, increased nodulation by *Rhizobium* spp, reduced root cankers caused by *R.solani*, increased root and plant growth and enhanced yield (Turner and Backman, 1991).

Application of *P. fluorescens* 2-79 alone or in combination with strain 13-79 to wheat as seed treatment suppressed take all disease and increased yield by 17 per cent in experimental plots and 11 per cent in commercial seed tests (Weller, 1988). *P. fluorescens* and *Bacillus* spp. reduced wilt and tuber rot in potato caused by *P. solanacearum* and increased tuber yield by more than 100 per cent (Shekhawat *et al*., 1992). Gagne *et al*. (1993) demonstrated the effect of some PGPR on fruit yields of green house tomato. Osburn *et al*. (1995) reported significantly high yield of soybean cultivar susceptible to *P. sojae* in all the five growing seasons by suppression of the disease.

Young *et al.* (1991) found a good correlation between production of plant growth regulators and biocontrol activity of *Pseudomonas* and *Serratia* strains in suppression of disease caused by *F. solani*.

PGPR can also reduce incidence and severity of disease by inducing plant resistance to pathogen attack. Leeman *et al.* (1995) obtained control of *Fusarium* wilt of radish by foliar application of *P. fluorescens*. Field trials in 1992-93 by Wei *et al.* (1996) with PGPR strains viz. *P. putida*, *Serratia marcescens*, *Flavomonas oryzihabitans* and *Bacillus pumilis* showed that the bacteria increased runner length, number of leaves and yield of cucumbers. These strains also induced systemic resistance in plants against *Colletotrichum orbiculare* and reduced the angular leaf spot caused by the pathogen. Hoffland *et al.* (1996) demonstrated that pretreatment of radish with *P. fluorescens* induced systemic resistance against the root pathogen *F. oxysporum* f.sp. *raphani* in plants and also protected them from infection by foliar pathogens *Pseudomonas syringae* pv. *tomato*, *Alternaria brassicola* and *F. oxysporum*.

Survival of Rhizobacteria

Root colonization and persistence of the introduced antagonist in the plant rhizosphere are considered essential for successful biological control. The influence of rhizosphere

colonization by beneficial bacteria on plant health has been reported (Kloepper and Schroth, 1981; Berger *et al.*, 1996). Schroth and Hancock (1981) opined that seed treatment with beneficial rhizobacteria resulted in their colonization of root system at population ranging upto 10^5 per cm and persisted throughout the season. High population of fluorescent pseudomonads was obtained from roots of wheat infected with *G. graminis* var. *tritici* (Weller, 1983). Xu and Gross (1986) reported that planting of potato seed pieces treated with *P. putida* resulted in population of the antagonist ranging from 10^4 to 10^5 cfu per gram of roots, while that of the pathogen *Erwinia carotovora* was reduced considerably. Soil application of a carrier based preparation of *Bacillus* strain AC-1 antagonistic to *P. capsici* resulted in high population of the antagonist in the rhizosphere of chilli (Kim *et al.*, 1989). Fernando and Linderman (1995) reported that a rifampicin resistant mutant of *Brevibacterium linens* established in the rhizosphere of cowpea at high population density of 6 log cfu g^{-1} soil after two weeks of application.

Population of antagonistic bacteria vary with the stage of crop growth, method of inoculation and progress of time after inoculation. High population of fluorescent pseudomonads in the rhizosphere have been recorded during early stages of plant growth which was found to decline later probably due to the

inability of the plant to support the bacteria (Miller *et al.* 1989). Wei *et al.* (1991) reported a slow but progressive decline in mean bacterial population with increase in time after planting. However, application of *P. aureofaciens* to potato seedlings by root dip method resulted in high population density of 10^4 to 10^5 cfu g^{-1} in both rhizosphere and roots upto 8 weeks after transplanting (De la Cruz *et al.*, 1992). Van Peer and Schippers (1992) obtained 6 x 10^4 cfu g^{-1} of *Pseudomonas* sp strain WCS 417r from the roots after 12 weeks of application. Maurhofer *et al.* (1994) recovered relatively high counts of resistant mutants of *Pseudomonas* from tobacco root 42 days after planting. Sanchez *et al.* (1994) reported a decline in the population of bacterial antagonist *P. cepacia*, with distance from the base of the roots after 10 days of treating seeds. Wiehe and Hoflich (1995) recorded high population of *P. fluorescens* PsIA12 during the vegetative plant development of legumes which declined with flowering. Nayar (1996) reported a rapid increase in population of *P. fluorescens* upto 16 days after seed treatment and root dipping of rice plants, after which there was a decline.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Isolation of bacteria and pathogen

3.1.1 Collection of soil samples and vermicompost

Soil samples were collected from undisturbed forest region, rhizosphere of wild pepper plants, tolerant pepper plants in sick plantations, disease free pepper plantations and from pepper plantations where organic farming is practiced. To collect rhizosphere soils from pepper plantations, the soil in one metre diameter around the plant was carefully dug out to expose a portion of root system. The soil adhering to the root system was collected in polythene bags. For collecting rhizosphere soils of wild pepper plants, the plants with roots were carefully dug out and shaken gently to remove excess soil. The root system along with the adhering soil was separated from the plant and taken in polybags. The list of samples collected and other details is given in Table 1.

Table 1 Details of samples collected for isolation of bacteria

Location	District	Elevation (m above MSL)	Description
Kulathupuzha	Kollam	800	Undisturbed forest soil
Narimala Karadimala Sirenthry Thondakkulam Damsite Punnamala	Palakkad	1100	Rhizosphere of wild pepper plants growing in Silent Valley forest

Location	District	Elevation	Description
Marayur	Idukki	1200-1500	Pepper plantation where organic farming is practiced
Pampadumpara	Idukki	1100	Rhizosphere of tolerant plants in sick pepper plantations
Karunapuram	Idukki	895	
Panniyur	Kannur	90	
Chowa	Kannur	40	
Kannur	Kannur	10	
Ambalavayal	Wynad	914	
Kottayam	Kottayam	30-40	
Palaruvi	Thiruvananthapuram	700-900	Rhizosphere of pepper plants in disease free plantations
Yeroor	Kollam	450-600	

Vermicompost prepared by composting farm waste for 45 days using the exotic species of earthworm, *Eudrillus eugineae* was collected from the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Vellayani.

3.1.2 Isolation of Bacteria

Isolation of bacteria from soil and vermicompost was done using the serial dilution plate technique (Waksman, 1922). One gram of the sample was transferred aseptically into a 250 ml

Erlenmeyer flask containing 100 ml of sterile water and shaken well in a mechanical shaker for 20 min. One ml of this suspension was transferred to another flask containing 99 ml. of sterile water using a sterile pipette so as to get a dilution of 10^{-4}. The process was repeated to get 10^{-6} dilution. One ml aliquots from these two dilutions were transferred to sterile petriplates. Melted and cooled soil extract agar medium (Appendix I) at 45°C was poured at the rate of 20 ml per dish and rotated gently for thorough mixing. The petridishes were incubated at 28±1°C for 96 h.

Observation was recorded on colony counts in the plates with 10^{-4} dilution and expressed as number of colony forming units (cfu) per gram of soil. Colonies formed after incubation period were transferred to nutrient agar (Appendix I) slants based on their differences in colour and shape.

The isolates were maintained by periodic subculturing on nutrient agar slants.

3.1.3 Isolation of *Phytophthora capsici*

The foot rot pathogen, *Phytophthora capsici*, was isolated from naturally infected pepper plants collected from Wynad district. The leaf bits with both healthy and infected portions were surface sterilized with 0.1 per cent mercuric

chloride solution for one minute and then repeatedly washed in two to three changes of sterile water. These bits were placed on carrot agar (Appendix I) in sterile petridishes and incubated at room temperature (28±1°C) for 72 h. When the fungal growth was visible, mycelial bits were transferred to carrot agar slants and purified by frequent transfer of hyphal tip. The identification of *P. capsici* was confirmed by microscopic observation of morphological and cultural characters.

The pathogenicity of the isolate obtained was proved by following Koch's postulates. Leaf and stem of rooted pepper cuttings of the variety Karimunda was inoculated by placing mycelial discs of *P. capsici* cut from five day old culture grown on carrot agar. Humidity was provided by placing moist cotton over it. The inoculated plants were incubated under high humidity. Adequate number of controls were also maintained. Observations were recorded on the development of typical symptoms of foot rot. The pathogen was reisolated from the artificially produced lesions and compared with the original isolate. This isolate maintained on carrot agar slants was used throughout the course of study.

3.2 Procurement of proven biocontrol organisms

A culture of *Trichoderma harzianum* tested and found effective in controlling *P. capsici* was obtained from Indian Institute of Spices Research, Kozhikode for including as a

treatment for comparison in the experiment on biocontrol of foot rot. A standard culture of *Pseudomonas fluorescens* was also obtained from the Department of Plant Pathology, TNAU, Coimbatore for maintaining as an additional check.

3.3 Preliminary screening of bacterial isolates for antagonism to *P. capsici*

3.3.1 *In vitro* studies

3.3.1.1. Mass screening of bacterial isolates

The bacteria isolated from the dilution plates were tested for their antagonism to *P. capsici* by cross culture method (Henis *et al.*, 1978). Twenty four hour old cultures of the bacteria were used for inoculation. Discs of six mm diameter cut from five day old culture of *P. capsici* grown on carrot agar were used as pathogen inoculum.

Bacterial isolates were spotted at the rate of four isolates per petridish containing carrot agar at four equidistant points near the periphery and at distance of 3 cm from the centre. Two days later, the actively growing test pathogen was introduced at the centre of the petridish. Three replicates were maintained and the dishes were incubated at 28±1°C for five days. The dishes were visually examined for inhibition of growth of the pathogen by the bacterial isolates.

3.3.1.2 Screening of selected cultures

3.3.1.2a *In vitro* inhibition of mycelial growth

The bacterial isolates selected after initial screening for antagonism to *P. capsici* were tested further by dual culture method (Utkhede and Rahe, 1983). The bacterial cultures were singly streaked at a spacing of three cm from six mm diameter mycelial discs of the pathogen placed on carrot agar in standard petridishes (nine cm diameter). Standard cultures of *P. fluorescens* was dual-cultured with the pathogen as described above. To study the antagonism offered by *T. harzianum*, six mm diameter discs of the fungus and the pathogen cut from five day old cultures were placed three cm apart on carrot agar. Carrot agar plates inoculated with the pathogen alone, served as control. Three replications were maintained for each treatment. The dishes were incubated at 28±1°C for five days and observation was made on the radial growth of the pathogen. The percentage inhibition of mycelial growth was also calculated using the formula,

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

I = inhibition of mycelial growth

C = Growth of the pathogen in control plates (cm)

T = Growth of the pathogen in dual culture (cm)

3.3.1.2b *In vitro* inhibition of sporangial production

To study the inhibition of sporangial production, dual culturing of the pathogen and selected cultures was done as described in the section 3.3.1.2a. Mycelial discs of six mm diameter were cut from the growth region of the pathogen nearest to the antagonists from dishes containing the paired cultures. Discs cut from carrot agar plates with *P. capsici* alone served as control. The discs were placed on slides and gently heated over the flame to remove the agar. The remaining mycelium in each slide was stained with lactophenol cotton blue (Appendix II) and observed under the microscope. The number of sporangia present per microscopic field was counted. The average of four microscopic fields for each replication was compared with that of control and percentage inhibition of sporangial production calculated.

3.3.2 *In vivo* screening of selected cultures in excised pepper shoots

The bacterial isolates selected for antagonism to *P. capsici* by *in vitro* studies were tested for their efficacy in checking foot rot disease in excised pepper shoots.

3.3.2.1 Preparation of excised pepper shoots

Pepper shoots of variety Karimunda which is highly susceptible to foot rot disease were used for the experiment.

Twenty five cm long shoots with mature leaves were collected from five year old pepper vines. The excised pepper shoots were cut afresh and dipped in water taken in test tubes to which three drops of 50 ppm Kinetin solution were added.

3.3.2.2 Preparation of bacterial culture for inoculation

The bacterial isolates selected by mass screening and *Pseudomonas fluorescens* were multiplied by transferring a loopful of the cultures in sterilized nutrient broth (Appendix I) taken in 250 ml Erlenmeyer flasks and incubating at 28±1°C for five days. At the end of incubation period the cell concentration in each flask was adjusted to 10^8 cfu ml^{-1} by adding sterilized nutrient broth.

3.3.2.3 Preparation of *Trichoderma harzianum* inoculum

T. harzianum was inoculated into sterile petridishes containing potato dextrose agar (PDA) (Appendix I) medium and incubated for five days. The spores of the fungus were scraped from the surface of the medium and suspended in sterile water to which 0.1 per cent carboxy methyl cellulose (CMC) was added.

3.3.2.4 Preparation of pathogen inoculum

The test pathogen, *P. capsici* was inoculated on carrot agar in sterile petridishes and allowed to grow for five days at 28±1°C. Discs cut from the fungal growth were used as inoculum.

3.3.2.5 *In vivo* testing for biocontrol

The excised pepper shoots were sprayed with the bacterial isoltes and *T. harzianum* separately. The leaves were immediately inoculated with six mm diameter discs of the test pathogen. Humidity was provided by placing moist cotton on it. Excised pepper shoots inoculated with the pathogen alone served as control. Three replications were maintained for each treatment. High relative humidity was continuously provided by placing the shoots in polythene cages containing petriplates filled with water. Observations were recorded on the time of initiation of symptoms, the rate of lesion development (cm day^{-1}) and lesion size (cm) on the fourth day of inoculation.

The bacterial isolates which were found effective in suppressing the pathogen *in vitro* and *in vivo*, were selected for further studies.

3.4 Screening of bacterial isolates for disease suppression

The selected bacterial isolates were evaluated for their efficacy in reducing foot rot disease of black pepper. A pot culture experiment in CRD with seven replications was conducted at the College of Agriculture, Vellayani. The treatments were as follows:

T1	= B3 + P	T4	= B6 + P	T7	= B13 + P	Tr	= Tr + P
T2	= B4 + P	T5	= B7 + P	T8	= B14 + P	C	= P
T3	= B5 + P	T6	= B12 + P	Pf	= Pf + P		

where B - bacterial isolate antagonistic to *P. capsici in vitro*

P - *Phytophthora capsici*

Pf - *Pseudomonas fluorescens*

Tr - *Trichoderma harzianum*

Three months old rooted pepper cuttings of the variety Karimunda raised singly in polybags were used for the study.

3.4.1 Preparation of inoculum of antagonists

3.4.1.1 Bacterial antagonists

Inoculum of bacterial antagonists were prepared as described in Section 3.3.2.2.

3.4.1.2 Inoculum of *T. harzianum*

T. harzianum was mass multiplied in wheat bran - sand mixture using modified method of Lewis and Papavizas (1984). Wheat bran was mixed with sand in the ratio 1:9 (v/v) and tap water was added to sufficiently moisten the mixture. It was dispensed in Erlenmeyer flasks (250 ml) and autoclaved at 1.05 kg cm^{-2} pressure for one hour. The sterilized medium was inoculated with mycelial discs of *T. harzianum* previously grown on PDA and incubated at 28±1°C for five days.

ii) Foliar infection - The incidence and intensity of foliar infection was calculated at 12 days after inoculating with the pathogen.

(a) The incidence of foliar infection was scored based on percentage of leaves infected by assigning scores of 0 - 4.

0 - no leaves infected

1 - upto 25% leaves showing infection

2 - 26-50% leaves showing infection

3 - 51-75% leaves showing infection

4 - > 75% leaves showing infection

(b) The intensity of foliar infection was calculated using score chart (Plate 1). The individual leaves in each plant were scored by assigning scores of 0-4 where

0 - no infection

1 - lesions covering upto 25% leaf area

2 - lesions covering 26-50% leaf area

3 - lesions covering 51-75% leaf area

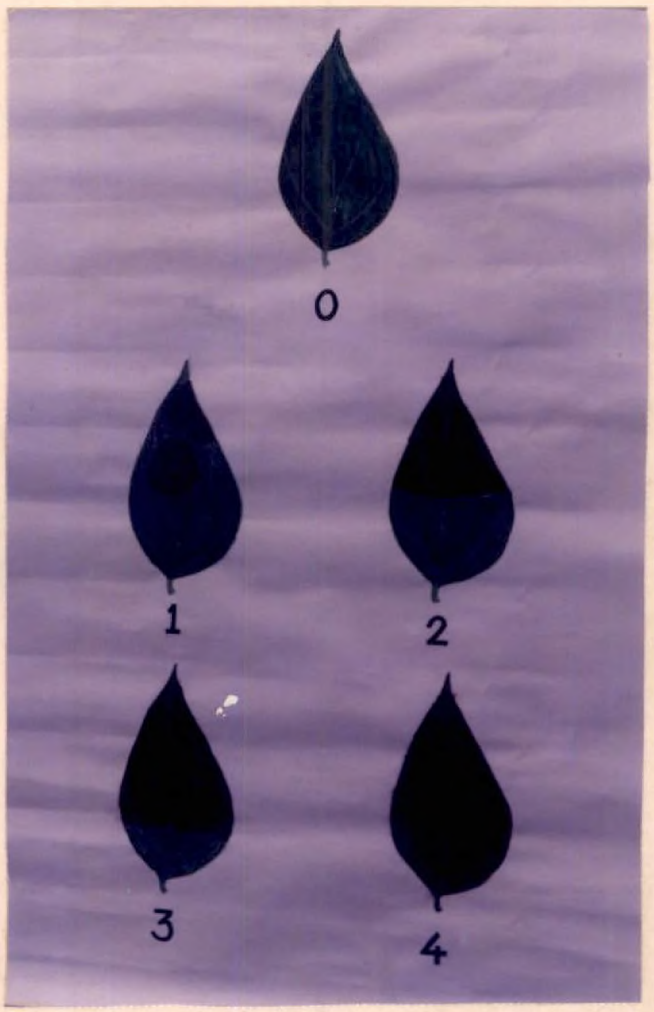
4 - lesions covering > 75% leaf area

Disease index was calculated using the formula suggested by Mayee and Datar, 1986.

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

Plate 1.

Score chart for foliar infection by *P. capsici*


0
1
2
3
4

(iii) The number of rooted cuttings killed were counted and percentage mortality of plants in each treatment was calculated by the formula,

$$\% \text{ mortality} = \frac{\text{Number of plants killed in the treatment}}{\text{Number of plants in the treatment}} \times 100$$

The percentage mortality of plants in the control was also calculated. The observation was made at 15, 30, 60 and 90 days after inoculating with the pathogen. Observation was also recorded on the 17th day of inoculation when all the plants in the untreated control were killed.

3.5 Assessment of the effect of culture filtrates of antagonists on the pathogen

The culture filtrates of the selected bacterial isolates were tested for the effect on the pathogen *in vitro* and *in vivo*.

3.5.1 Preparation of culture filtrates of bacterial antagonists

Bacteria were grown in nutrient broth by transferring a loopful of the cultures in 200 ml of the sterilized medium taken in Erlenmeyer flasks and incubating at 28±1°C for seven days. The bacterial suspensions were centrifuged at 10000 rpm at 20°C for 15 min. The supernatent were collected and filtered using bacteria proof filter to obtain the partially purified culture filtrates.

3.4.2 Preparation of pathogen inoculum

The pathogen inoculum was raised on oats-sand mixture. Oats mixed with sand in the ratio 1:9 (v/v) was sufficiently moistened by adding tap water. The mixture was taken in Erlenmeyer flasks and autoclaved for one hour at a pressure of 1.05 kg cm^{-2}. The medium in the flasks were inoculated with six mm diameter mycelial discs of the pathogen and incubated at room temperature (28±1°C) for 10 days.

3.4.3 Inoculation of antagonists and pathogen

The bacterial antagonists were applied to rooted pepper cuttings as soil drench as well as spray on foliage and stem at the rate of 30 ml per polybag. The inoculum of *T. harzianum* was added to soil at the rate of 15 g per polybag. The rooted cuttings were inoculated with the pathogen, *P. capsici*, after 24 h of application of the bacterial or fungal antagonist by mixing 3 g of the pathogen inoculum with soil per polybag.

3.4.4 Observations recorded

The following observations were recorded:

i) Rate of lesion development - The lesion size (cm) on the stem was measured and rate of lesion development was calculated by dividing the lesion size with the number of days taken for the lesion to reach the size from the day of symptom initiation. The rate of lesion development was expressed in cm day^{-1}.

3.5.2 Preparation of culture filtrates of *Trichoderma harzianum*

Six mm disc of *T. harzianum* was inoculated in 200 ml Czapek's broth (Appendix I) taken in Erlenmeyer flask and incubated for seven days. Culture filtrate was collected by filtering through Whatman No.1 filter paper.

3.5.3 *In vitro* studies

3.5.3.1 Effect of culture filtrates on mycelial growth of the pathogen

Two ml. of partially purified culture filtrates of the antagonists were added to flasks containing 50 ml autoclaved (1.05 kg cm^{-2} for 20 min) carrot agar melted, and cooled to 45°C. They were thoroughly mixed by gently swirling the flasks and poured aseptically into sterilized petridishes. Six mm diameter mycelial disc of five day old culture of the pathogen was placed in the centre of each plate. Carrot agar plates without culture filtrates and inoculated with the pathogen served as control. Three replicates were maintained for each treatment. The plates were incubated at 28±1°C for five days and the mean diameter of the growth of the pathogen at the end of incubation period was recorded.

3.5.3.2 Effect of culture filtrates on sporangial production *in vitro*

The pathogen *P. capsici* was grown on carrot agar containing culture filtrates as described in section 3.5.3.1.

Mycelial discs (six mm diameter) of the pathogen cut from these dishes were observed under the microscope after preparing the slides and staining with lactophenol cotton blue. Mycelial discs cut from the pathogen grown on carrot agar plates served as control. The number of sporangia produced per microscopic field were counted and the average of four microscopic fields for each of the three replications in each treatment was compared with that of control.

3.5.4 Efficacy of culture filtrates in reducing foot rot

3.5.4.1 Studies on excised pepper shoots

Excised pepper shoots were prepared as described in section 3.3.2.1. Culture of pathogen prepared as in 3.3.2.4 and the filtrates of antagonists prepared as per section 3.5.1 were inoculated on excised pepper shoots as described in section 3.3.2.5. Observations were made on the time of initiation of symptoms, rate of lesion development (cm day^{-1}) and lesion size (cm) on the fourth day of inoculation.

3.5.4.2 Studies on rooted pepper cuttings

A pot culture experiment was conducted at the College of Agriculture, Vellayani to study the efficacy of culture filtrates in reducing foot rot disease in rooted pepper cuttings.

The details of the experiment are as follows.

Design	-	CRD
Variety	-	Karimunda
Replications	-	Seven

Treatments

F1	= fB3 + P	F5	= fB7 + P	Pf	= fPf + P
F2	= fB4 + P	F6	= fB12 + P	Tr	= fTr + P
F3	= fB5 + P	F7	= fB13 + P	C	= P
F4	= fB6 + P	F8	= fB14 + P		

where B - bacterial isolate

f - culture filtrate

P - *Phytophthora capsici*

Tr - *Trichoderma harzianum*

Pf - *Pseudomonas fluorescens*

Three months old rooted pepper cuttings raised singly in polybags were used for the experiment.

3.5.4.2a Preparation of culture filtrates of antagonists

(i) *Culture filtrates of bacterial antagonists*

Culture filtrates of bacterial antagonists were prepared as described in section 3.5.1.

(ii) Culture filtrates of *T. harzianum* was prepared as described in section 3.5.2.

3.5.4.2b Preparation of pathogen inoculum

P. capsici was mass cultured in oats - sand mixtures as described in Section 3.4.2.

3.5.4.2c Application of culture filtrates and pathogen to rooted pepper cuttings

The culture filtrate of the antagonists were applied to rooted pepper cuttings as sprays to foliage and stem and soil drench at the rate of 30 ml per polybag. The pathogen raised in Oats-sand mixture was applied by mixing three g of the pathogen inoculum with soil in each polybag after 24 h of applying the culture filtrates.

3.5.4.2d Observations recorded

(i) Rate of lesion development:

The lesion size (cm) on the stem was measured and rate of lesion development was calculated and expressed in cm day^{-1}.

(ii) The incidence and intensity of foliar infection on the 12^{th} day of inoculation with the pathogen, was calculated as described in section 3.4.4.

(iii) The number of rooted cuttings killed were counted at 15, 27 (when all the plants were killed in the control), 30, 60 and 90 days after inoculation with the pathogen. The percentage mortality in each treatment and control was calculated.

3.6 Influence of bacterial isolates on growth of pepper

The bacterial isolates selected for studies on foot rot suppression in rooted pepper cuttings were assessed for their effect on growth of black pepper. *P. fluorescens* and *T. harzianum* were also included as additional checks. An experiment in CRD with three replications, was laid using three months old rooted pepper cuttings of Karimunda variety, obtained from the Instructional Farm, College of Agriculture, Vellayani. One cutting was maintained in each polybag. The bacterial and fungal antagonists were mass multiplied as described in section 3.4.1. The bacterial isolates and *P. fluorescens* were applied to rooted cuttings as soil drench and foliar spray, at the rate of 30 ml per polybag. *T. harzianum* was applied to soil at the rate of 15 g per polybag. Rooted cuttings without the antagonists served as control. The cuttings were grown in shade for three months with irrigation at regular intervals. Observations were made on the following:

(i) *Shoot length*

The length (cm) of the shoot from soil level to the tip of each plant was measured at monthly intervals.

(ii) *Number of leaves*

The number of leaves in each plant were counted at monthly intervals.

(iii) *Root length*

After three months of inoculation with the antagonists, the cuttings were carefully depotted from the polybags. The roots were washed gently in tapwater to remove all adhering soil particles and blot dried. The length of roots were then measured in cm.

(iv) *Root volume*

The root system was separated from each cutting and immersed in a known volume of water taken in a measuring cylinder. The final volume was noted and root volume (ml) was determined by finding out the difference between the initial and final volume.

(v) *Fresh weight*

The fresh weight (g) of the plants were taken in a Mettler Single Pan Balance.

(vi) *Dry weight*

The plants were dried at 60°C in a drying oven to a constant weight for determining the dry weight (g).

3.7 Assessment for cellulolytic property

3.7.1 Estimation of cellulase

Cellulase enzyme activity of the selected bacterial isolates was studied by reducing sugar estimation using

dinitrosaliaylic acid (DNS) method (Miller, 1959). Protein content of the samples were estimated (Lowry, 1951) and the enzyme activity expressed as ug of glucose released calculated from the standard curve, per mg of protein in unit time.

3.7.1a Preparation of standard graph

A standard graph of glucose was prepared using different concentrations of glucose ranging from 10-100 ppm.

3.7.1b Preparation of enzyme extract

Bacteria were inoculated into flasks containing 50 ml of nutrient broth modified with one per cent carboxymethyl cellulose and incubated for 72 h. These suspensions were filtered and used as enzyme samples.

Six mm diameter mycelial disc of *T. harzianum* was inoculated into flask containing 50 ml of Czapeks broth modified with one per cent CMC and incubated for 72 h. Culture filtrate obtained by filtering through Whatman No. 1 filter paper was used as enzyme extract.

3.7.1c Enzyme assay

The reaction mixture consisting of one ml of the enzyme extract mixed with 4.5 ml CMC was incubated at 55°C for 15 minutes. DNS reagent (0.5 ml) was then added to the enzyme substrate mixture and heated in boiling waterbath for 5 minutes.

One ml. potassium sodium tartrate was added to the warm mixture and final volume was made to 10 ml using distilled water. The transmittance of light was measured at 540 nm in spectrophotometer. The glucose released was measured using the standard graph and enzyme activity expressed as μg glucose min^{-1} mg^{-1} protein.

3.7.2 Estimation of protein

3.7.2a Preparation of standard graph

A standard graph of serum albumin was prepared using different concentrations of albumin.

3.7.2b Preparation of sample

Sample for protein estimation was prepared as described in Section 3.7.1b.

3.7.2c Protein estimation

Five ml of alkaline copper solution was added to 0.5 ml of the sample made upto 1 ml using distilled water. The mixture was allowed to stand for 10 min and then mixed with 0.5 ml. of Folin - Ciocalteau reagent. The blue colour, developed after the reaction in the dark for 30 minutes, was measured in an Elico colorimeter (model. CL 157) using red filter and the amount of protein present in the sample was measured using the standard curve and expressed as mg ml^{-1} sample.

3.8 Survival of antagonists in the rhizosphere

Serial dilution and plate method (Waksman, 1922) was used for the enumeration of antagonists surviving in the rhizosphere. The rhizosphere soils of rooted pepper cuttings treated with antagonists were collected after three months of application. Dilutions of 10^{-4} were prepared and bacteria and *T. harzianum* were isolated in soil extract agar and Rose bengal streptomycin agar (Appendix I) respectively. Colony counts of the organisms were taken and population expressed as cfu g^{-1} of soil.

3.9 Statistical analysis

The data of the various experiments were analysed for significance using analysis of variance (ANOVA).

3.10 Characterisation of bacteria

Preliminary tests for the identification of the bacterial isolates tried for biocontrol of *P. capsici* was conducted in the laboratory. These included:-

1. Colony characteristics

Colony characteristics were studied by plating bacteria on nutrient agar. The bacterial cultures were streaked on nutrient agar poured in petriplates and incubated at 28±1°C. After 24 h, observations were made on shape, elevation, margin of colonies, optical feature and texture of colonies.

2. Growth on Agar slants

Bacteria were streaked on nutrient agar slants and incubated for 24 h under aerobic conditions. Observations were made on the amount of growth, margin and shape.

3. Gram staining

The smear of the test bacterium was prepared and fixed on a clean microscope slide. It was stained with crystal violet stain (Appendix II) for 30 seconds. The excess stain was removed by rinsing with tap water. The smear was then flooded with Gram's iodine (Appendix II) and allowed to react for 30 seconds, after which it was again rinsed off with water. The preparation was then decolourised with 95 per cent ethanol, rinsed with water and counterstained with safranin (Appendix II) for 30 seconds. The smear was rinsed with water, blotted dry and examined under oil immersion objective of a microscope.

4. Spore staining

The smear of the test bacterium was prepared and fixed on a clean glass slide. It was stained with malachite green solution (Appendix II) and allowed to react in the cold for 30-60 seconds and then heated to steam for 30 seconds. The smear was rinsed with water and stained with aqueous solution of safranin for 30 seconds. The smear was then rinsed with water, blotted dry and observed under oil immersion objective of the microscope.

Catalase Test

The bacterial cultures were grown on nutrient agar slants for 48 h at 28±1°C. 0.5 ml of 10 per cent hydrogen peroxide was added to each test tube and observed for effervescence of oxygen which indicated catalase activity.

6 Motility Test

The motility of bacterial cultures were tested by stab culture method in semisolid nutrient agar (Appendix I). Test tubes containing sterile melted nutrient agar was cooled in an upright position and inoculated by thrusting the inoculation needle containing the bacterial culture through the centre of the medium. The test tubes were incubated at 28±1°C and observed for the nature of growth from the line of inoculation.

RESULTS

RESULTS

4.1 Isolation of bacteria and pathogen

4.1.1 Collection of samples and isolation of bacteria

Soil samples were collected from the rhizosphere of wild pepper plants, tolerant plants in sick plantations, disease free pepper plantations, pepper plantations where organic farming is practiced and from undisturbed forest area. Bacteria were isolated from these soils and also from vermicompost using serial dilution and plate method. The population of bacteria in the different samples is presented in Table 2.

In general, a significant difference in the population of bacteria could be noticed among the various soils and vermicompost (Fig. 1). The highest population of bacteria was obtained from rhizosphere soil of tolerant plants in diseased pepper plantations of Pepper Research Station, Panniyur (168×10^4 cfu g^{-1}) followed by rhizosphere soil of wild pepper plants in Punnamala region of Silent Valley forests (117×10^4 cfu g^{-1}). The least population of 13.67×10^4 cfu g^{-1} was recorded from rhizosphere soils collected from pepper plantations in Chowa.

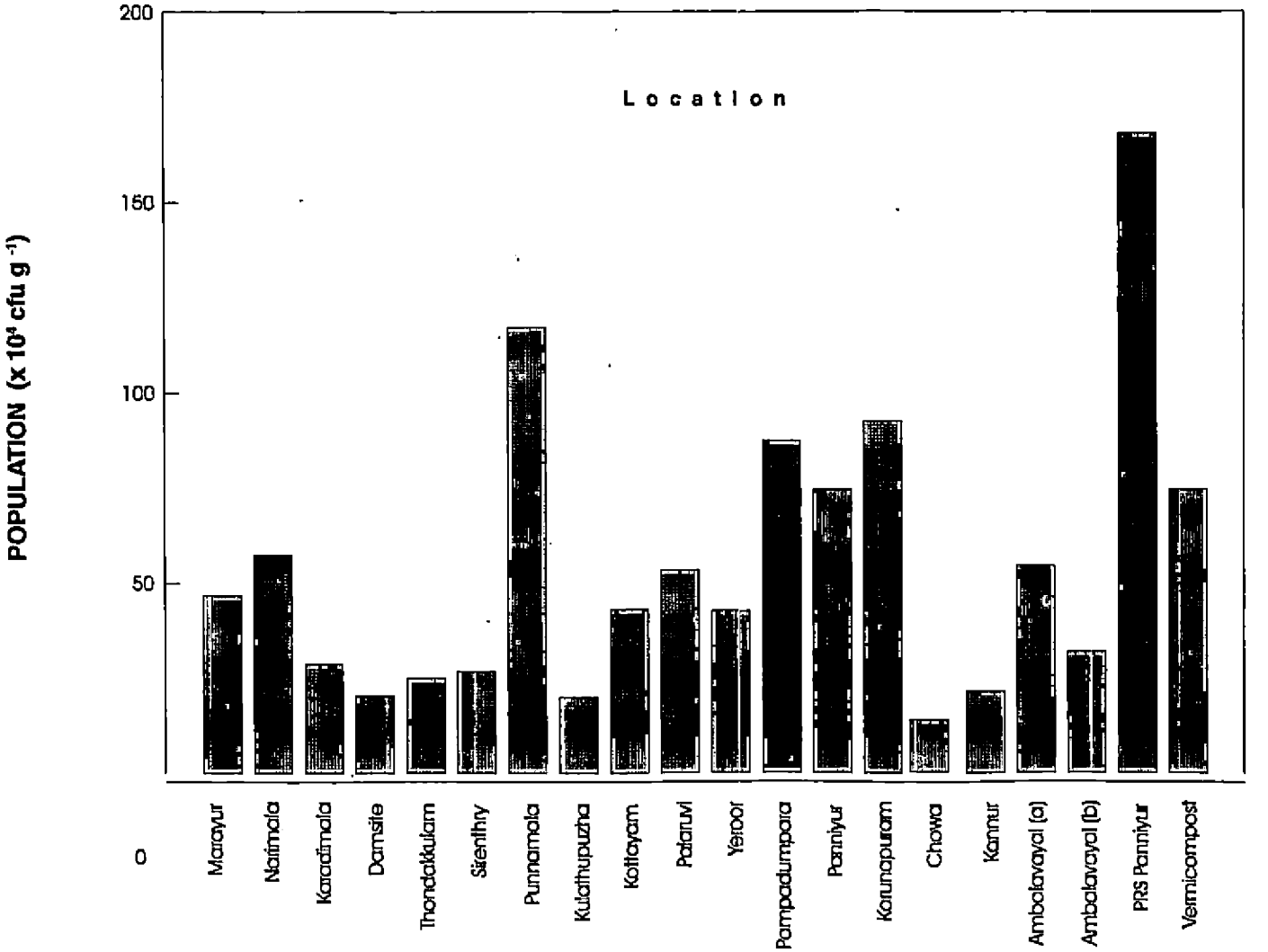
4.1.2 Isolation of the pathogen

The pathogen, *Phytophthora capsici* was isolated from naturally infected pepper plant and was maintained by periodic

Table 2 Population of bacteria in rhizosphere soils and vermi-compost

No.	Location	Description	Population (10^4 cfu g^{-1})
R1	Marayur	Pepper plantations where organic farming is practiced	46.67
R2	Narimala	Rhizosphere of wild pepper plants in Silet Valley forest	57.33
R3	Karadimala		28.67
R4	Damsite		20.33
R5	Thondakkulam		25.00
R6	Sirenthry		26.67
R7	Punnamala		117.00
R8	Kulathupuzha	Undisturbed forest soil	20.00
R9	Kottayam	Rhizosphere of pepper plants in disease free plantations	43.00
R10	Palaruvi		53.33
R11	Yeroor		42.67
R12	Pampadumpara	Rhizosphere of healthy plants in diseased pepper plantations	87.33
R13	Panniyur		74.33
R14	Karunapuram		92.33
R15	Chowa		13.67
R16	Kannur		21.33
R17	Ambalavayal (a)		54.33
R18	Ambalavayal (b)		32.00
R19	Pepper Research Station, Panniyur		168.00
R20	Vermicompost		74.33
CD(5%)			5.65

Fig. 1. Population of bacteria in rhizosphere soils and vermicompost



subculturing on carrot agar slants. The fungus formed white colonies with sparse but coarse aerial mycelium on carrot agar plates. The isolate was found to completely cover the medium in a 9 cm diameter petridish in 5-7 days. The sporangial shape varied from lemon, spherical to irregular.

Pathogenicity of the isolate was tested in rooted cuttings of the pepper variety, Karimunda. On artificial inoculation of the leaves, pale water soaked lesions appeared within 48 h which later turned dark brown. The lesions with fimbriate margins gradually enlarged covering large areas of the leaf resulting in defoliation. Inoculation of the stem resulted in water soaked lesion which appeared within three days and turned dark brown as the infection progressed. The leaves further lost turgidity with subsequent defoliation. The entire plants were killed within one week of inoculation. The symptoms were typical of foot rot of black pepper (Plate 2).

4.2 *In vitro* studies

4.2.1 Mass screening of isolates

The bacterial isolates obtained by serial dilution were screened for antagonism to *P. capsici in vitro*. The inhibition of growth of the pathogen by the different isolates was visually examined. Out of the 194 bacterial isolates, fifteen that showed antagonism to the pathogen were selected for further study.

Plate 2.

Symptoms of *Phytophthora* foot rot under artificial inoculation

a. Foliar infection

b. Stem infection

a.

b.

These were serially numbered from B1 to B15. The intensity of antagonism offered by the fifteen selected isolates is presented in Table 3.

Isolates B1, B3, B7, B14 and B15 showed high inhibition to growth of *P. capsici* whlie B2 caused only slight inhibition of the pathogen. All the other isolates gave moderate inhibition of the growth of the fungus.

Table 3 Antagonism offered by bacterial isolates selected through mass screening

Isolate No.	Inhibition of growth of pathogen
B1	+++
B2	+
B3	+++
B4	++
B5	++
B6	++
B7	+++
B8	++
B9	++
B10	++
B11	++
B12	++
B13	++
B14	+++
B15	+++

+++ = high inhibition
++ = medium inhibition
+ = slight inhibition

4.2.2 Screening of selected cultures

4.2.2.1 *In vitro* inhibition of mycelial growth

The efficacy of the 15 selected isolates in inhibiting the growth of the pathogen *in vitro* was studied by dual cultures on carrot agar medium. The bacterial isolates varied in their ability to inhibit *P. capsici* (Table 4).

B14 produced the largest inhibition zone (2.63 cm) which corresponds to 65.80% inhibition of mycelial growth over control. This was significantly higher than that of all the other bacterial isolates (Plates 3, 4, 5). B1 exhibited an inhibition zone of 1.93 cm which was statistically on par with the inhibition shown by the isolates B7, B3, B15, B11, B4, B6 and B10. The percentage inhibition of mycelial growth by B1 was 48.33 per cent. The least inhibition of 0.13 cm was shown by B2, which gave only 4.09 per cent reduction of mycelial growth of the pathogen when compared to the control and was significantly different from that of all other isolates.

Trichoderma harzianum which was included for comparison of the antagonism offered by bacterial isolates, completely overgrew the pathogen and restriced its growth. *Pseudomonas fluorescens* exhibited an inhibition zone of 1.57 cm giving 39.16 per cent inhibition of mycelial growth.

4.2.2.2 Inhibition of sporongial production *in vitro*

The effect of antagonists on production of sporangia by the pathogen was studied and the results are presented in

Table 4 *In vitro* effect of bacterial isolates on mycelial growth of *P. capsici*

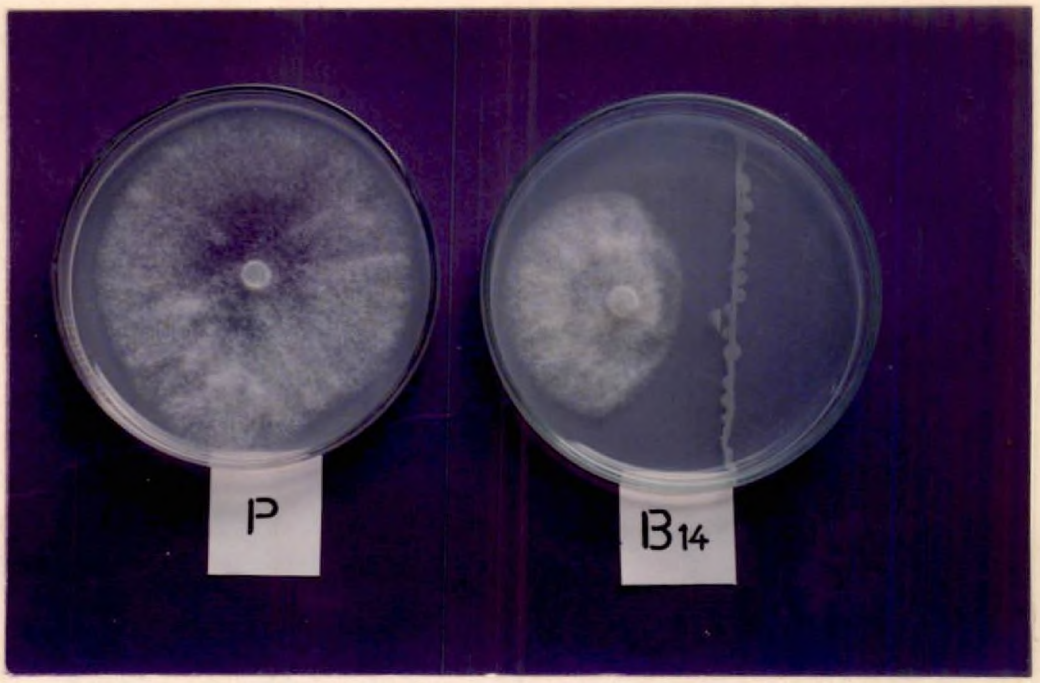
Isolate No.	Inhibition zone (cm)	Percentage inhibition of mycelial growth
B1	1.93 (1.39)	48.33 (44.03)
B2	0.13 (0.36)	4.09 (10.45)
B3	1.73 (1.31)	43.21 (41.14)
B4	1.66 (1.29)	41.51 (40.16)
B5	1.46 (1.21)	36.45 (37.20)
B6	1.60 (1.26)	39.92 (39.20)
B7	1.83 (1.35)	45.83 (42.59)
B8	1.47 (1.21)	36.63 (37.24)
B9	1.53 (1.24)	38.21 (38.21)
B10	1.57 (1.25)	39.16 (38.73)
B11	1.66 (1.29)	41.56 (40.17)
B12	1.43 (1.20)	35.79 (36.74)
B13	1.40 (1.18)	35.00 (36.26)
B14	2.63 (1.62)	65.80 (54.23)
B15	1.70 (1.30)	42.48 (40.67)
Pf*	1.57 (1.25)	39.16 (38.73)
Tr*	Overgrowth	
CD (5%)	0.17	0.70

Figures in parentheses represent $\sqrt{x}$ and angular transformed values for inhibition zone and per cent inhibition of mycelial growth respectively.
* included as additional check.

Plate 3

In vitro inhibition of *P. capsici* by bacterial antagonists

P - Control


P
B14

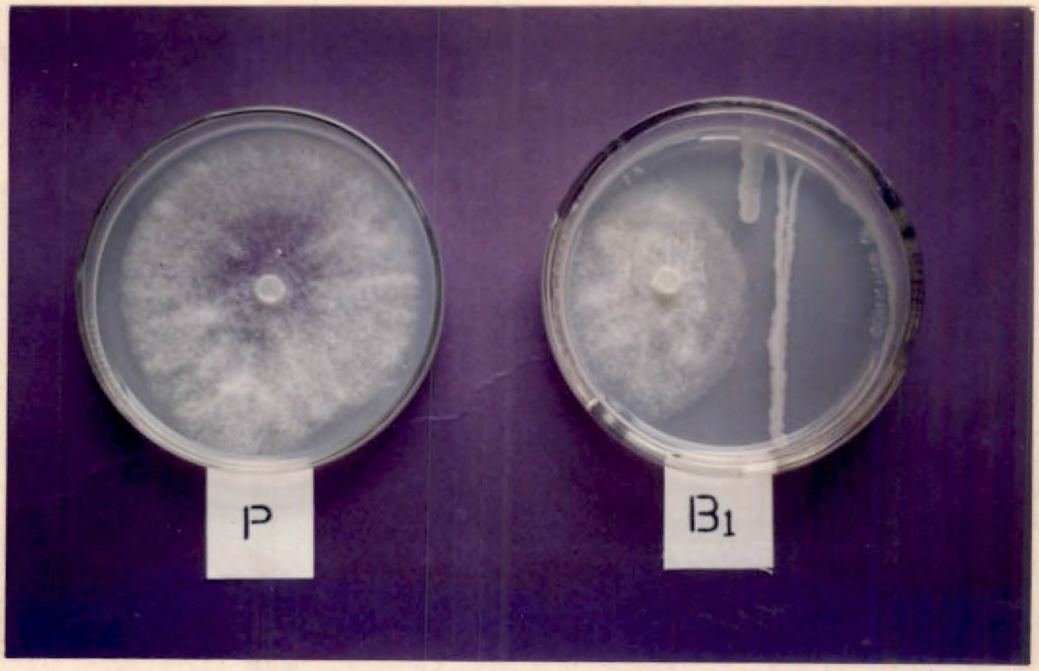

P
B1

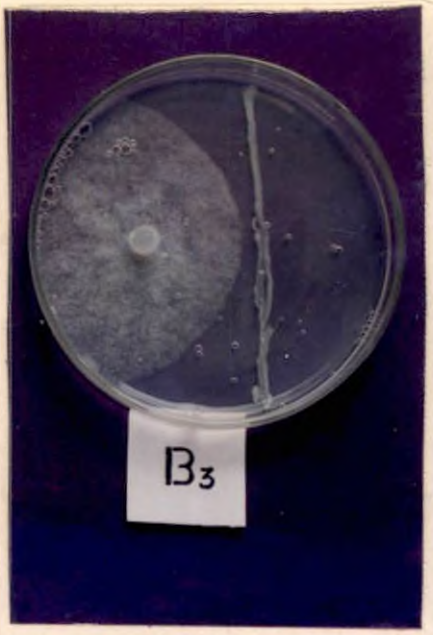
Plate 4.

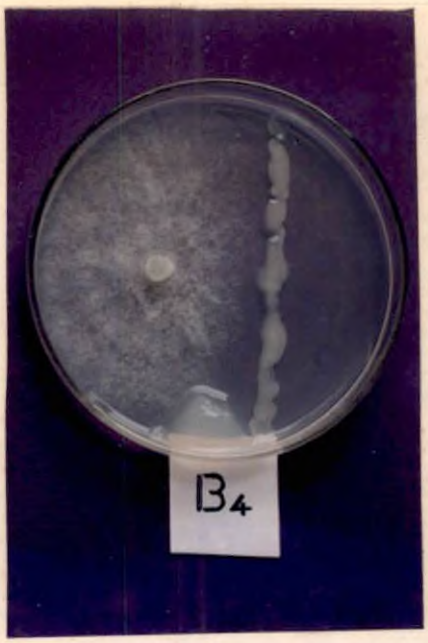
In vitro inhibition of *P. capsici* by bacterial antagonists

P - Control

Pf - *P. fluorescens*

Tr - *T. harzianum*

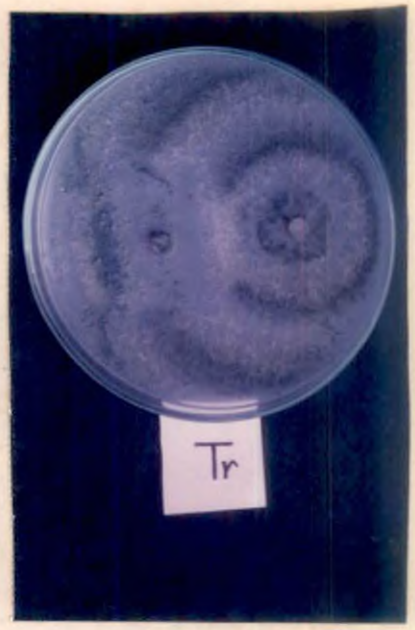

B3


B4

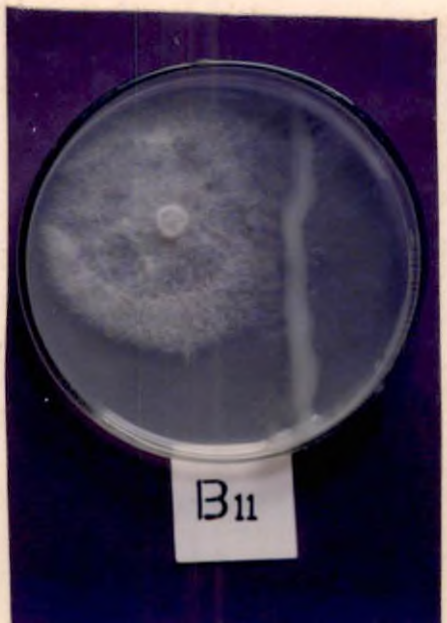

B6


P.f.


P


Tr


B7

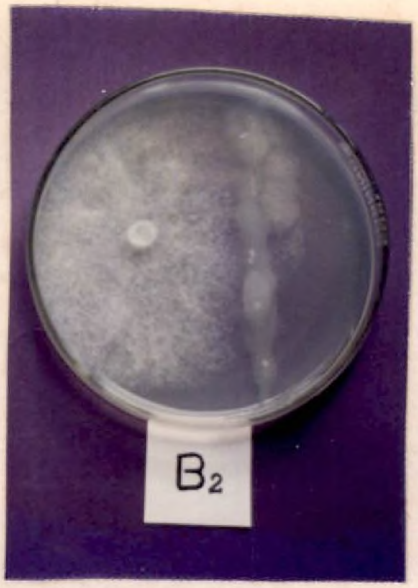

B11


B15

Plate 5.

In vitro inhibition of *P. capsici* by bacterial antagonists

P - Control

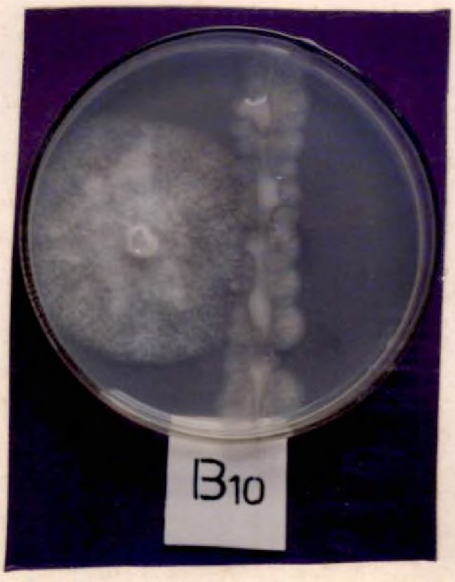

B2


B5


B8


B9


P


B10


B12


B13

Table 5. In general, all the bacterial isolates were found to reduce sporangial production. However, *T. harzianum* was the most effective giving 100 per cent inhibition.

Among the bacterial isolates, B7, B9 and B13 offered the highest inhibition of 95.48 per cent which was statistically on par with *T. harzianum*. Isolates B5 and B8 also produced good inhibition of sporangial production (93.31%). B10 was the least effective with an inhibition of 16.36 per cent.

4.2.2.3 Antagonism to *P. capsici* on excised pepper shoot

Treatment with the antagonists was found to influence the time of symptom initiation, rate of lesion development and size of lesions (Table 6). The isolates B5, B7 and B13 were found to be the most effective in delaying symptom initiation. The treatments B3, B10, B12, B14 and *P. fluorescens* delayed the lesion development to varying extent. B1, B8, B9, B11 and B15 were not effective in delaying symptom development. Inoculation with *T. harzianum* caused the symptom to appear slightly earlier to that in control.

The effect of bacterial isolates on lesion size on the fourth day of inoculation of leaves was studied (Plate 6, 7). Of the different isolates tested, B5, B7 and B13 were the most effective since no lesions were produced on application of these antagonists. B6 produced a lesion size of 0.42 cm which was

Table 5 *In vitro* effect of bacterial isolates on sporangial production by *P. capsici*

Treatments	Percentage inhibition of sporangial production
B1	83.64 (66.12)
B2	86.99 (68.83)
B3	86.99 (68.83)
B4	83.64 (66.12)
B5	93.31 (74.98)
B6	90.00 (71.54)
B7	95.48 (77.69)
B8	93.31 (74.98)
B9	95.48 (77.69)
B10	16.36 (23.85)
B11	76.82 (69.20)
B12	86.99 (68.83)
B13	95.48 (77.69)
B14	83.64 (66.12)
B15	66.74 (54.76)
Pf*	95.48 (77.69)
Tr*	100.00 (90.00)
CD (5%)	12.77

Figures in parentheses represent angular transformed values
* included as additional checks.

Table 6 Effect of bacterial antagonists on the onset and development of symptoms by *P. capsici* on excised pepper shoots

Treatments	Days for symptom initiation	Onset of disease after 10 days (+/-)	Lesion size (cm)	Rate of lesion development ($cm.day^{-1}$)
B1	2	+	2.15 (1.77)	1.78 (1.44)
B2	1.67	+	1.60 (1.61)	0.70 (1.30)
B3	3	+	0.85 (1.36)	0.85 (1.36)
B4	2.33	+	1.78 (1.67)	1.11 (1.45)
B5	-	-	0 (1)	0 (1)
B6	4	+	0.42 (1.19)	0.42 (1.19)
B7	-	-	0 (1)	0 (1)
B8	2	+	1.91 (1.71)	0.96 (1.40)
B9	2	+	2.55 (1.88)	1.28 (1.51)
B10	2	+	2.35 (1.83)	1.18 (1.47)
B11	1.33	+	2.67 (1.91)	1.00 (1.42)
B12	3	+	1.13 (1.46)	0.92 (1.39)
B13	-	-	0 (1)	0 (1)
B14	3	+	1.14 (1.46)	1.14 (1.46)
B15	2	+	2.13 (1.77)	1.07 (1.44)
Pf*	3	+	0.39 (1.18)	0.26 (1.12)
Tr*	1.5	+	1.35 (1.53)	0.61 (1.27)
C	2	+	1.79 (1.67)	0.90 (1.38)
CD (5%)	1.03 0.94 0.84		0.34	0.26

Figures in parenthesis represent $\sqrt{x+1}$ transformed values
* included as additional check

Plate 6.

Interaction of bacterial antagonists with *P. capsici* on excised pepper shoots

C - Control

Pf - *P. fluorescens*

Tr - *T. harzianum*

T5 - Isolate B5

T7 - Isolate B7

T6 - Isolate B6

T13 - Isolate B13

T14 - Isolate B14

T12 - Isolate B12

T3 - Isolate B3

T4 - Isolate B4


T6
T7
T8
T9
C
Pf
F


T4
T10
T5
T4
C
Pf
F

Plate 7.

Interaction of bacterial antagonists with *P. capsici* on excised pepper shoots

C - Control

Pf - *P. fluorescens*

Tr - *T. harzianum*

T15 - Isolate B15

T2 - Isolate B2

T1 - Isolate B1

T8 - Isolate B8

T10 - Isolate B10

T9 - Isolate B9

T11 - Isolate B11


T₁₅
T₂
T₂
T₈
C
Pf
Tf


T₁₀
T₉
T₅
C
Pf
Tf

significantly less than in untreated control. All the other isolates either produced results similar to untreated control or increased the lesion size.

The performance of Pf was statistically on par with the best three bacterial isolates, B5, B13 and B7. With Pf, the lesion size was only 0.39 cm which was also comparable to B6, B3, B12 and B14 and was significantly smaller than the lesion of 1.79 cm in the untreated control. Treatment with *T. harzianum* produced a lesion size of 1.35 cm which was statistically on par with untreated control as well as with the bacterial isolates B14, B12, B3 and B6.

A comparison was made on the effect of different isolates on the rate of lesion development. The isolates B5 , B7 and B13 were very efficient in checking infection in excised shoots. Among the treatments in which lesions developed, the application of *P. fluorescens* and isolate B6 were found to be effective in suppressing the lesion development. They produced only a rate of 0.26 cm day^{-1} and 0.42 cm day^{-1} respectively which were statistically comparable to that of B5, B7 and B13. However, they were on par with the rate of 0.90 cm day^{-1} in the untreated control. All the other isolates and *T. harzianum* were not efficient in reducing the rate of progress of lesion development and were statistically on par with that of control.

4.3 Selection of bacterial isolates for foot rot suppression

The fifteen bacterial isolates were compared for their ability to inhibit *P. capsici in vitro* and in excised pepper shoots. The performance of the fifteen isolates is presented in Table 7 and Fig. 2.

The bacterial isolates that scored rank of 1 or 2 in at least three of the five characters were selected for further studies. Thus eight isolates viz. B3, B4, B5, B6, B7, B12, B13 and B14 were selected.

4.4 Screening of bacterial isolates for disease suppression

The comparative efficacy of the selected bacterial isolates, *P. fluorescens* and *T. harzianum* in reducing foot rot was studied by applying the antagonists to rooted pepper cuttings and then inoculating the pathogen to soil. The effect of application of the antagonist on the incidence and spread of foot rot and foliar blighting of black pepper is presented in Tables 8a, 8b and 8c.

A comparison of rate of lesion development showed that the treatments T3 and T7 were very effective compared to control in delaying the progress of the disease (Table 8a). The rates of lesion development in these treatments were only 1.03 and 1.50 cm day^{-1} respectively while it was 2.68 cm day^{-1} in the control.

Table 7 Summary of performance of bacterial isolates *in vitro* and on excised shoots against *P. capsici*

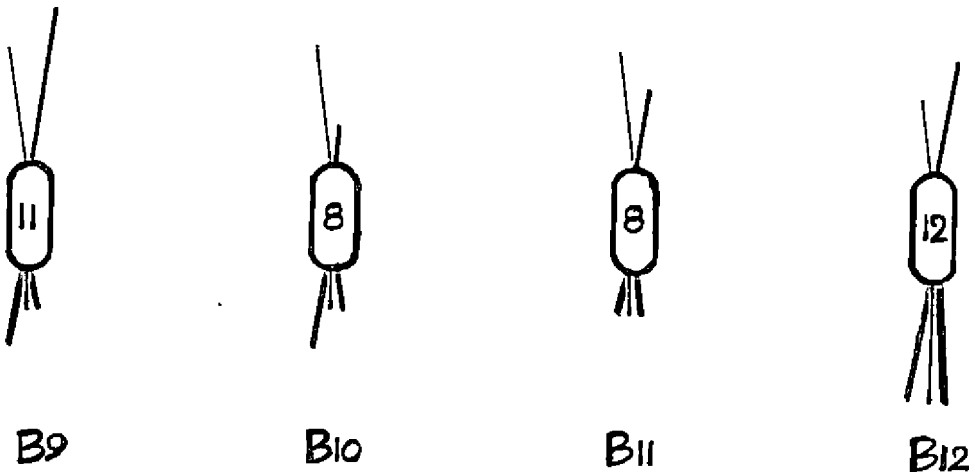
	Characters	Rank			
		1	2	3	4
In vitro studies	inhibition of mycelial growth	B14, B1	B7, B3, B15, B11, B4, B6, B10, B9	B8, B5, B12, B13	B2
	inhibition of sporangial production	B7, B9, B13	B8, B5, B6, B3, B12, B2, B4, B14, B1	B11, B15	B10
studies in excised pepper shoots	delaying onset of disease	B5, B7, B13, B6, B14	B12, B3, B4	B10, B1, B8, B15, B9	B2, B11
	reducing lesion size	B5, B13, B7, B6	B3, B12, B14	B2, B4	B8, B15, B1, B10, B9, B11
	decreasing rate of lesion development	B5, B13, B7, B6	B2, B3		B12, B8, B11, B15, B1, B4, B14, B10, B9

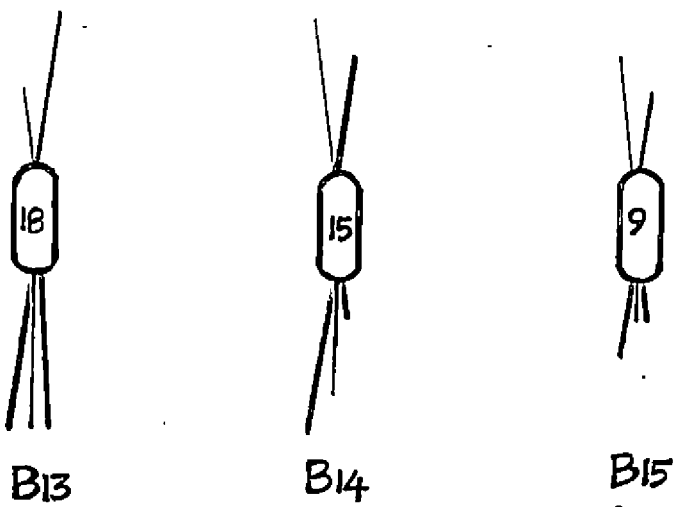
1 - effective
2 - statistically comparable to the effective isolates
3 - less effective and not comparable to the effective isolates
4 - least/not effective

Fig. 2. Performance of bacterial isolates against *P. capsici in vitro* and in excised pepper shoots.

Rank	1	2	3	4
Value	4	3	2	1
Scale	2.0 cm	1.5 cm	1.0 cm	0.5 cm

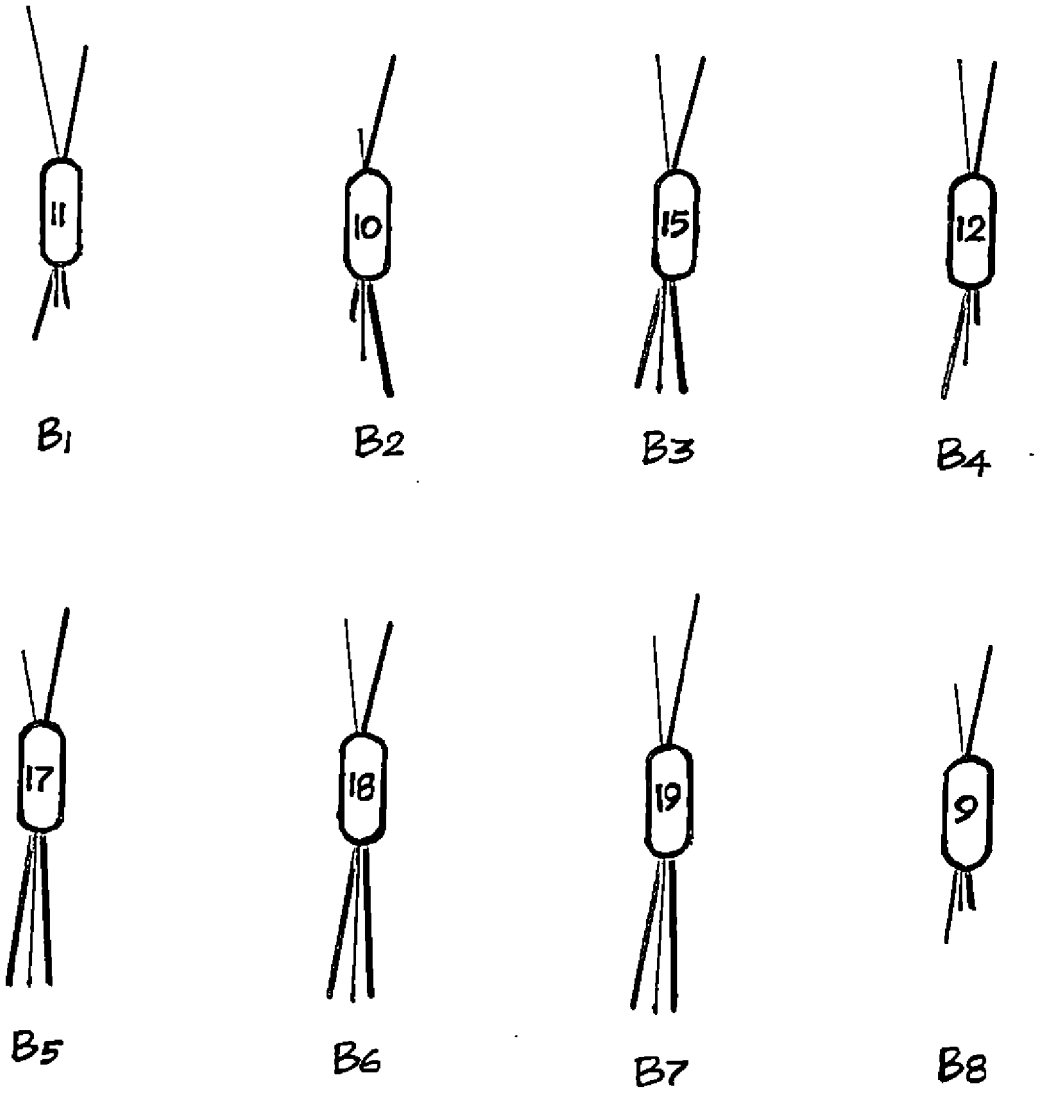
Fig. 2.





— inhibition of mycelial growth
— inhibition of sporangial production
— delaying onset of disease
— reducing lesion size
— decreasing rate of lesion development

Fig. 2.



— inhibition of mycelial growth
— inhibition of sporangial production
— delaying onset of disease
— reducing lesion size
— decreasing rate of lesion development

Table 8a Effect of bacterial antagonists on the rate of development of foot rot disease in black pepper

Treatment	Bacterial isolate Number	Rate of lesion development ($cm\ day^{-1}$)
T1	B3	2.04 (1.74)
T2	B4	2.49 (1.87)
T3	B5	1.03 (1.43)
T4	B6	3.02 (2.00)
T5	B7	1.76 (1.66)
T6	B12	3.44 (2.11)
T7	B13	1.50 (1.58)
T8	B14	2.76 (1.94)
Pf*		2.98 (2.00)
Tr*		2.69 (1.92)
C		2.68 (1.92)
CD (5%)		0.32

Figures in parentheses represent $\sqrt{x+1}$ transformed values.
* included as additional check

T6, T1 and T2 also showed reduced rates of progress of lesions, viz. 1.75, 2.04 and 2.49 cm day^{-1} respectively, but statistically on par with control. Tr and Pf included as additional check and the other bacterial treatments produced results comparable to untreated control.

Scoring for incidence and intensity of foliar infection was done on the 12th day after inoculation with the pathogen. Mean scores of foliar disease incidence were compared (Table 8b and Fig. 3). T7, T3 and T5 were superior to control in reducing foliar infection and gave a mean score of 0.39, 0.46 and 0.50 respectively as compared to 1.86 in control. All the other treatments including Tr and Pf also reduced the number of leaves infected but were statistically on par with the control. However, the effects of Pf and Tr were also on par with the best performing treatments, T3, T5 and T7.

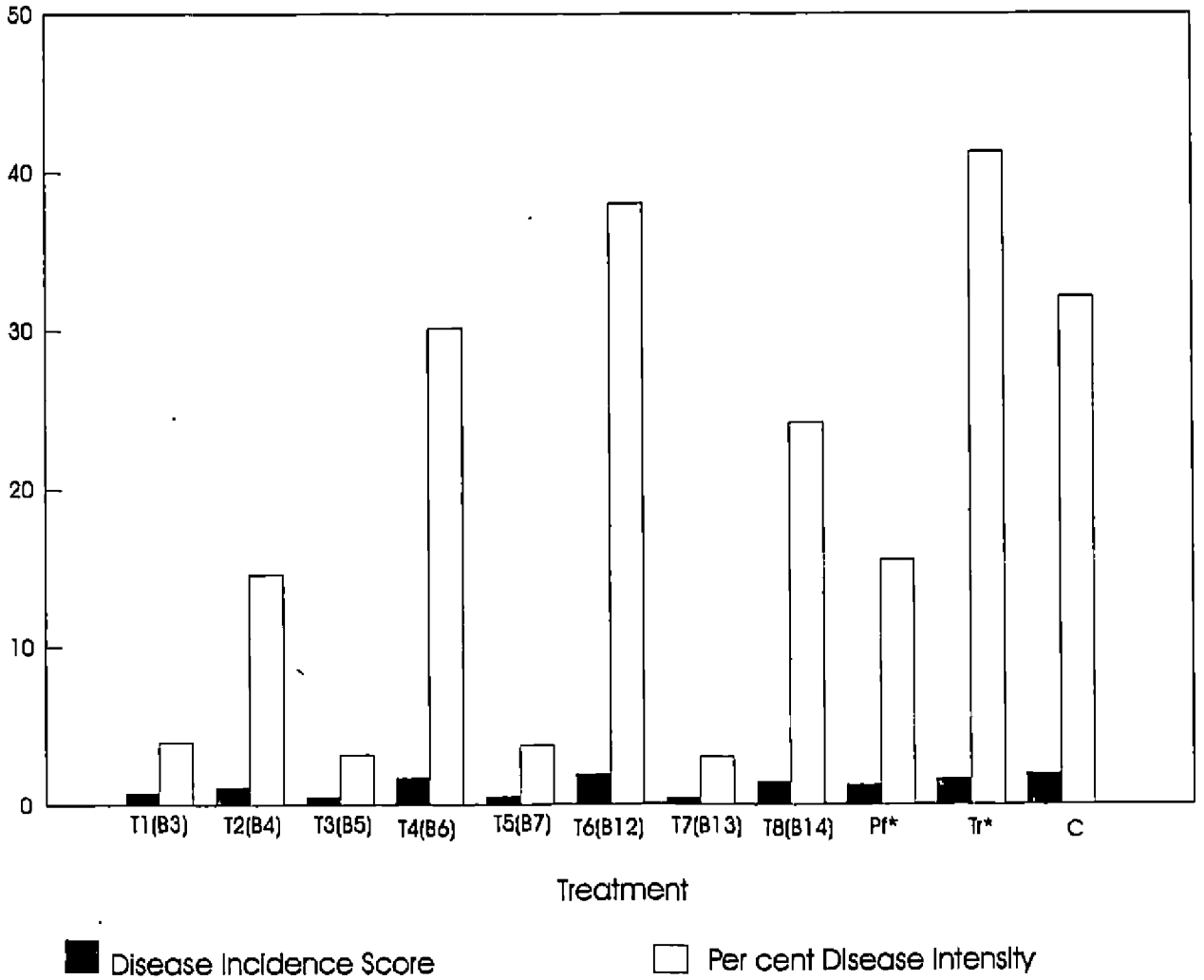
The bacterial antagonists, in general, reduced the intensity of foliar infection by *P. capsici* (Table 8b and Fig.3). The intensity of foliar infection was as high as 32.12 per cent in untreated control. However, the percentage intensity of infection of leaves in all the treatments were statistically comparable to that in untreated control (32.12%). T7 was the most effective treatment which reduced foliar disease intensity to 3.03 per cent followed by T3, T5 and T1 (3.17, 3.71 and 3.94% respectively). Pf and all the bacterial treatments except T6

Table 8b Effect of bacterial antagonists on incidence and intensity of foliar infection by *P. capsici*

Treatment	Bacterial isolate Number	Disease incidence score	Per cent Disease intensity
T1	B3	0.71 (1.31)	3.94 (11.44)
T2	B4	1.09 (1.45)	14.55 (22.41)
T3	B5	0.46 (1.21)	3.17 (10.25)
T4	B6	1.64 (1.63)	30.13 (33.28)
T5	B7	0.50 (1.22)	3.71 (11.09)
T6	B12	1.84 (1.69)	38.04 (38.07)
T7	B13	0.39 (1.18)	3.03 (10.02)
T8	B14	1.34 (1.53)	24.12 (29.40)
Pf*		1.24 (1.50)	15.49 (23.16)
Tr*		1.56 (1.60)	41.27 (39.96)
C		1.86 (1.69)	32.12 (34.51)
CD (5%)		0.45	26.38

Figures in parenthesis represent $\sqrt{x+1}$ and angular transformed values for incidence and intensity, respectively
* included as additional check

Fig. 3. Effect of bacterial antagonists on incidence and intensity of foliar infection by *P. capsici*



reduced foliar disease intensity to lesser extent. T6 and T1 resulted in increased disease intensity. The highest value of PDI was recorded with Tr which was significantly higher than that in T7, T3, T5 and T1.

In general, a positive trend was noticed between the application of antagonists and disease suppression (Table 8c and Fig. 4) (Plates 8, 9). While 71.43 per cent plants were killed after 15 days of inoculation in the untreated control, on the treatment with isolate B5, ie. treatment T3, the percentage mortality was only 14.29 per cent. In the treatments T2 and T4, only 28.57 per cent plants were killed while T1, T5, T7 and Tr and Pf maintained as additional checks gave 42.86 per cent death of vines. Among the various treatments, T6 and T8 were least effective in protecting the plants and produced mortality as high as 57.14 per cent. However, the treatments were superior to the untreated control. Observation on the 17th day after inoculation showed 100 per cent mortality of vines in the control. The best treatments were T2, T3 and T5 which showed only 42.86 per cent death of the plants. The treatments T7 and T8 showed 57.14 per cent mortality while treatment T_1 showed 71.43 per cent killing of vines. The control offered by Pf was similar to that by the best treatments, viz. T2, T3 and T5 while Tr offered little protection giving 71.43% mortality. The treatment T6 was least effective as it also gave 100 per cent mortality of plants.

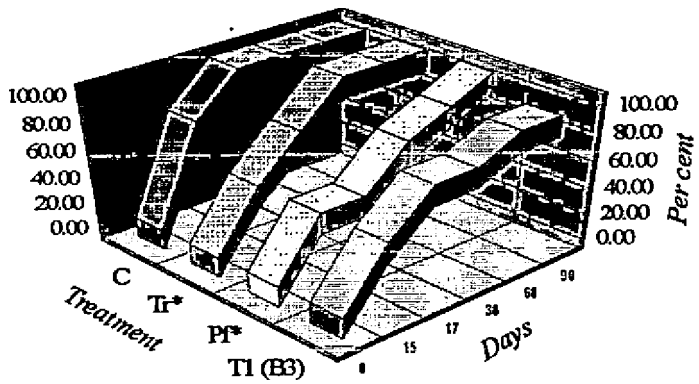
Table 8c Efficacy of bacterial antagonists in the management of foot rot of black pepper

Treatment	Bacterial isolate number	% mortality of plants days after inoculation				
		15	17	30	60	90
T1	B3	42.86	71.43	71.43	85.71	85.71
T2	B4	28.57	42.86	85.71	85.71	100.00
T3	B5	14.29	42.86	71.43	71.43	71.43
T4	B6	28.57	85.71	85.71	85.71	100.00
T5	B7	42.86	42.86	71.43	71.43	71.43
T6	B12	57.14	100.00	100.00	100.00	100.00
T7	B13	42.86	57.14	57.14	57.14	57.14
T8	B14	57.14	57.14	85.71	100.00	100.00
Pf*		42.86	42.86	71.43	85.71	100.00
Tr*		42.86	71.43	100.00	100.00	100.00
C		71.43	100.00	100.00	100.00	100.00

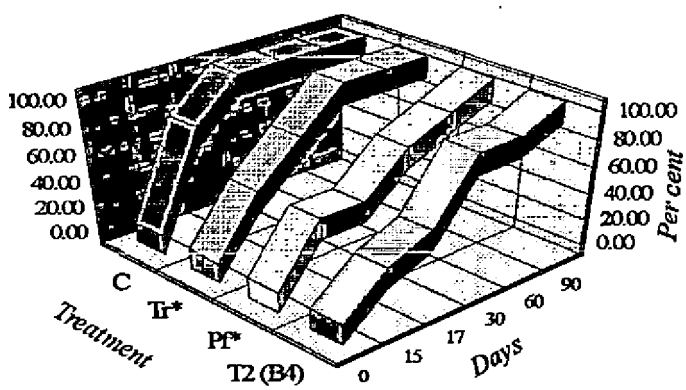
* included as additional check

Fig. 4. Effect of bacterial antagonists on percentage mortality of black pepper plants due to foot rot

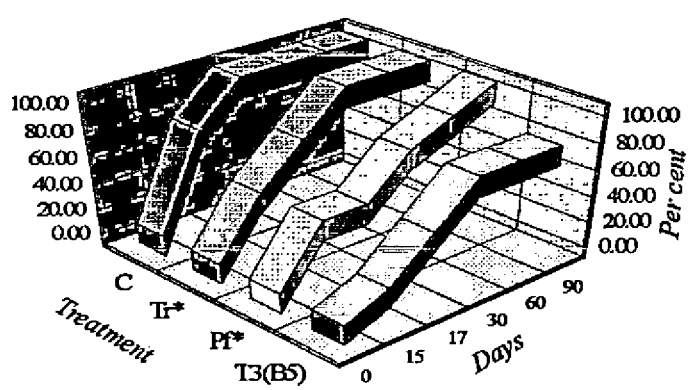
Treatment T1 (B3)



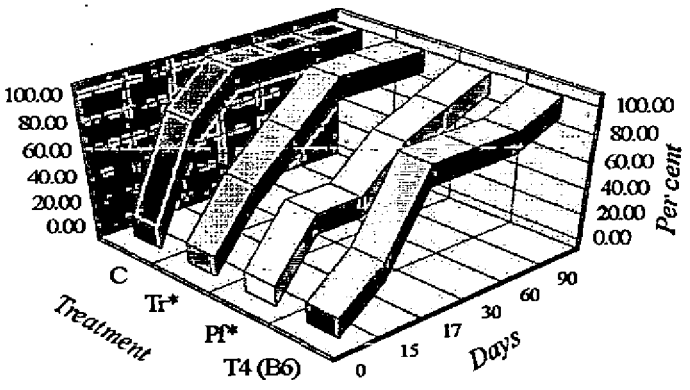
Treatment T2 (B4)



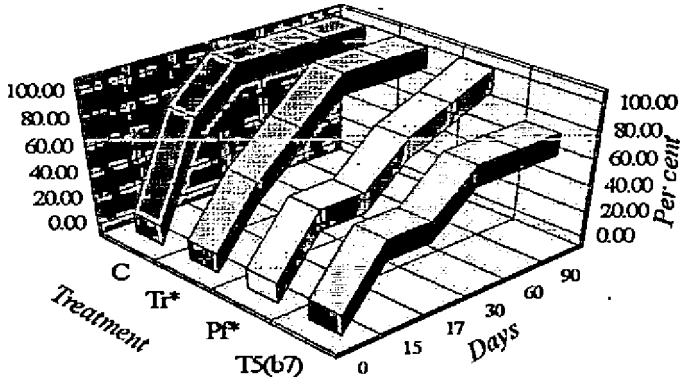
Treatment T3 (B5)



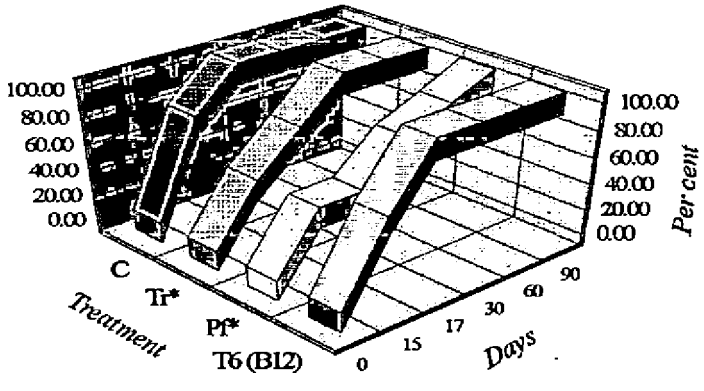
Treatment T4 (B6)



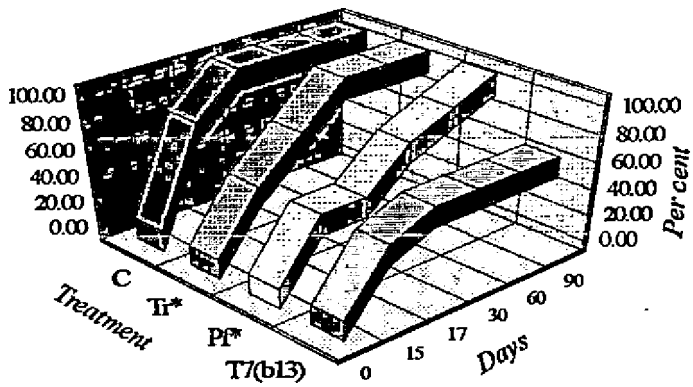
Treatment T5(B7)



Treatment T6 (B12)



Treatment T7 (B13)



Treatment T8 (B14)

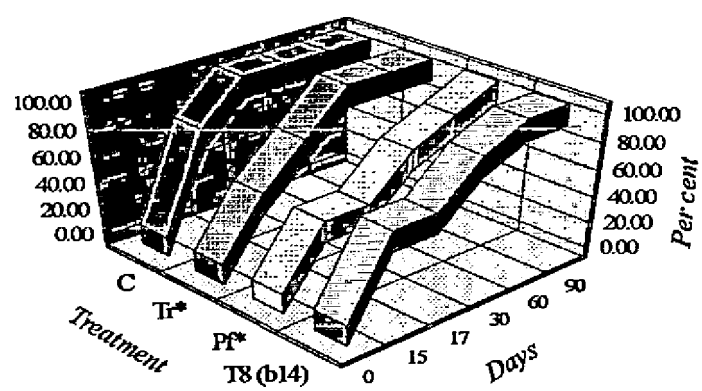


Plate 8.

Suppression of foot rot by bacterial antagonists

C - Control

Pf - *P. fluorescens*

Tr - *T. harzianum*

T3 - Isolate B5

T5 - Isolate B7


T3
P.f.
Tr
C

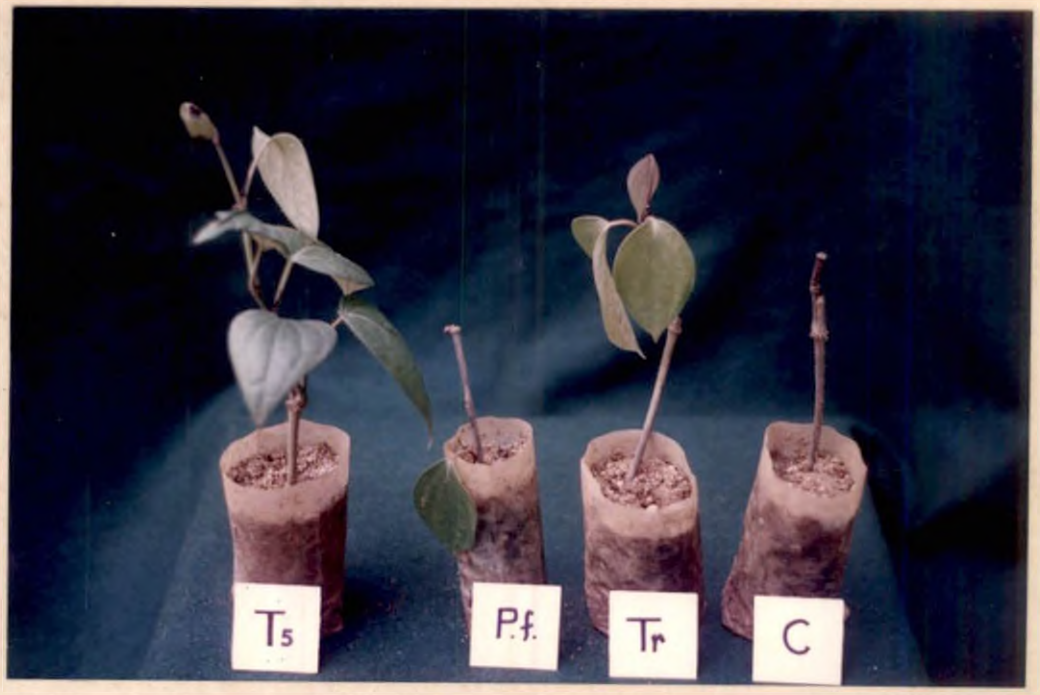

T5
P.f.
Tr
C

Plate 9.

Suppression of foot rot by bacterial antagonists

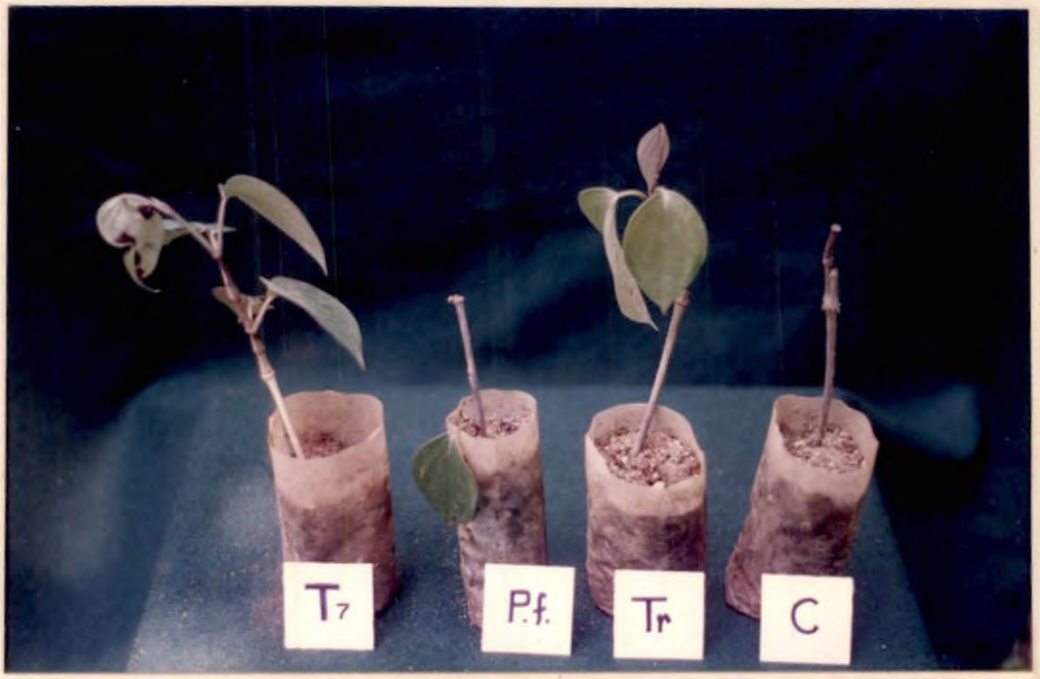
C - Control

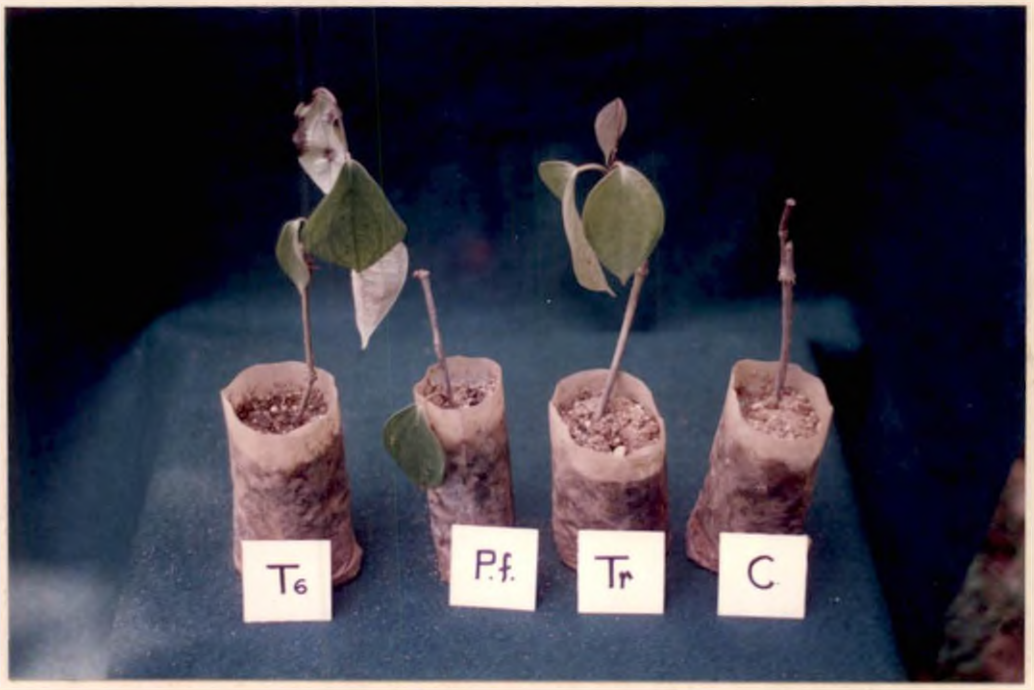
Pf - *P. fluorescens*

Tr - *T. harzianum*

T7 - Isolate B13

T6 - Isolate B12


T7
P.f.
Tr
C


T6
P.f.
Tr
C

Mortality of plants at 30, 60 and 90 days after inoculation with the pathogen was also recorded. The treatment T7 (isolate B13) was very effective in checking foot rot since it showed no further death of plants after 17 days of inoculation. The percentage death of plants in this treatment remained 57.14 per cent. This was followed by treatments T3 (isolate B5) and T5 (isolate B7) which showed 71.43 per cent mortality after 30 days of inoculation and no death of plants thereafter. In the treatment T2 (isolate B4) which showed the least percentage of death (42.86) at 17 days after inoculation, the mortality sharply increased to 85.71 per cent at 30 days after inoculation and showed 100 per cent death of vines at 90 days. A similar trend was noticed with the treatment T4 (isolate B6). Pf showed a gradual death of plants with percentage mortality of 71.43, 85.71 and 100 per cent after 30, 60 and 90 days after inoculation, respectively. In the treatment Tr all the plants were killed after 30 days of inoculation.

4.5 Assessment of culture filtrates of antagonists

4.5.1 *In vitro* studies

4.5.1.1 Effect on mycelial growth of the pathogen

The culture filtrate of the antagonists showed great variation on their effect on radial growth of the pathogen (Table 9 and Fig. 5). Tr and F1 were very effective in reducing

Table 9 *In vitro* effect of cell free filtrates of bacterial antagonists on mycelial growth of *P. capsici*

Treatment	Bacterial isolate No.	Radial growth of mycelium (cm)
F1	B3	6.33 (2.52)
F2	B4	8.25 (2.87)
F3	B5	7.12 (2.67)
F4	B6	8.36 (2.89)
F5	B7	8.75 (2.96)
F6	B12	8.57 (2.93)
F7	B13	6.53 (2.56)
F8	B14	7.18 (2.68)
Pf*		6.62 (2.57)
Tr*		6.27 (2.50)
C		6.68 (2.59)
CD (5%)		0.06

Figures in parentheses represent $\sqrt{x}$ transformed values
* included as additional check

Fig. 5. In vitro effect of cell free filtrates of bacterial antagonists on mycelial growth of *P. capsici*

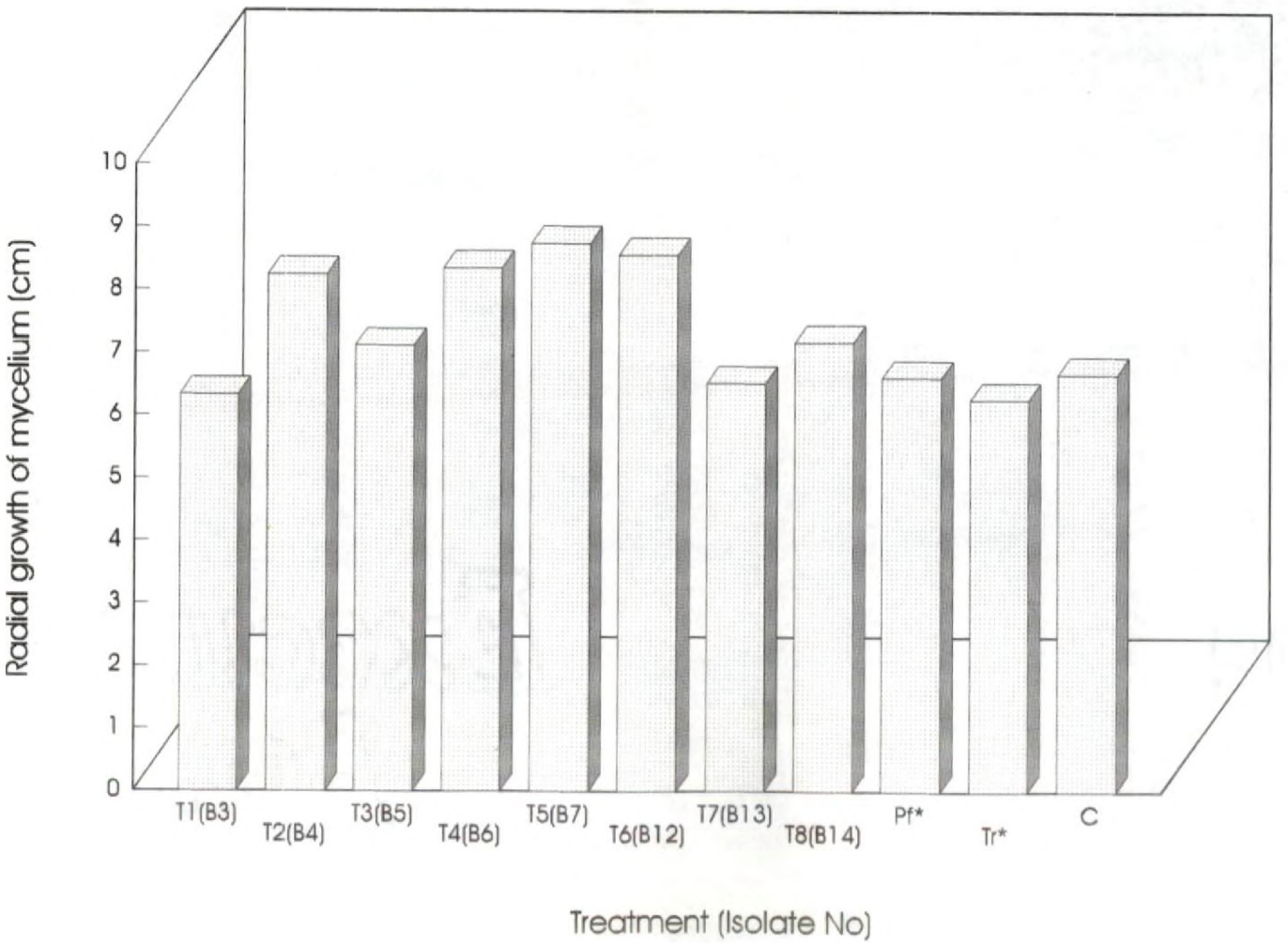


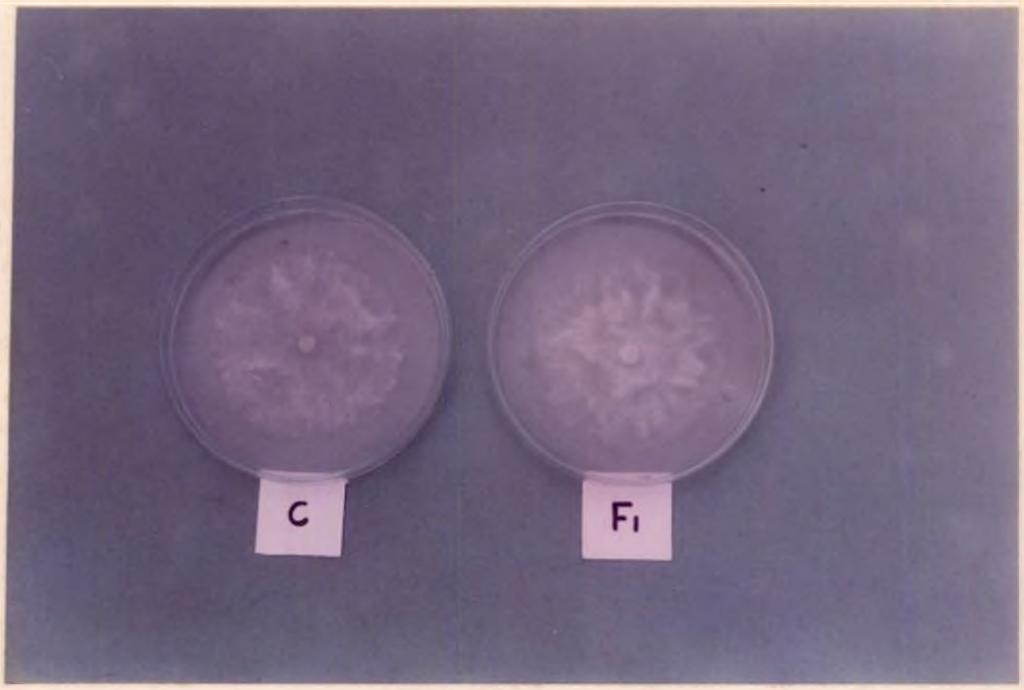
Plate 10.

In vitro inhibition of *P. capsici* by cell free filtrates of bacterial antagonists

C - Control

F1 - Filtrate of isolate B3

Tr - Filtrate of *T. harzianum*


C
F1

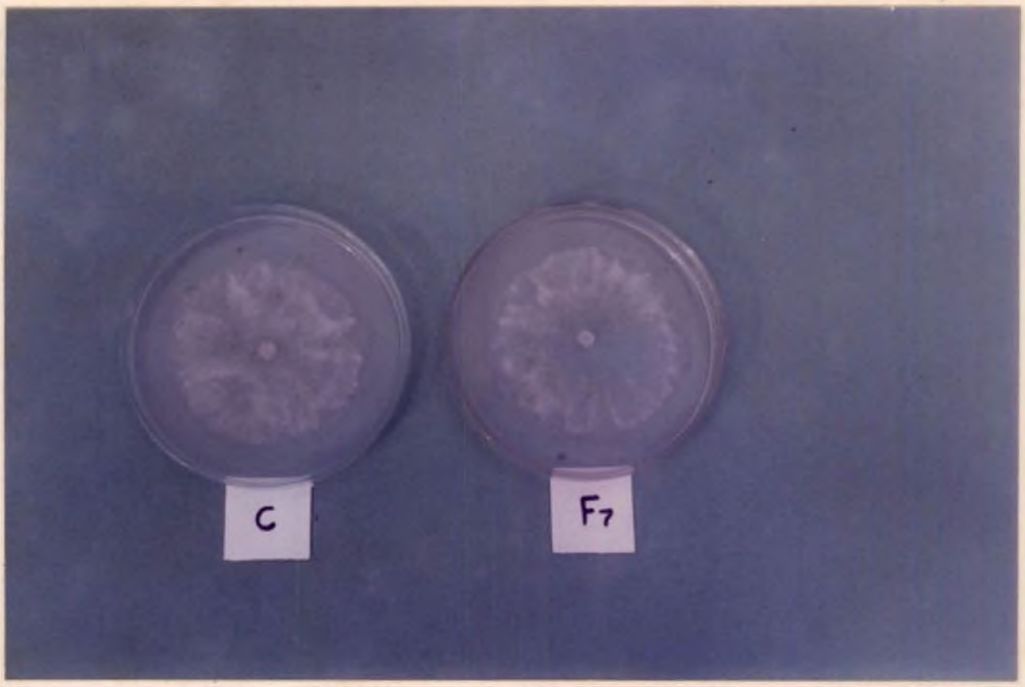

C
F7

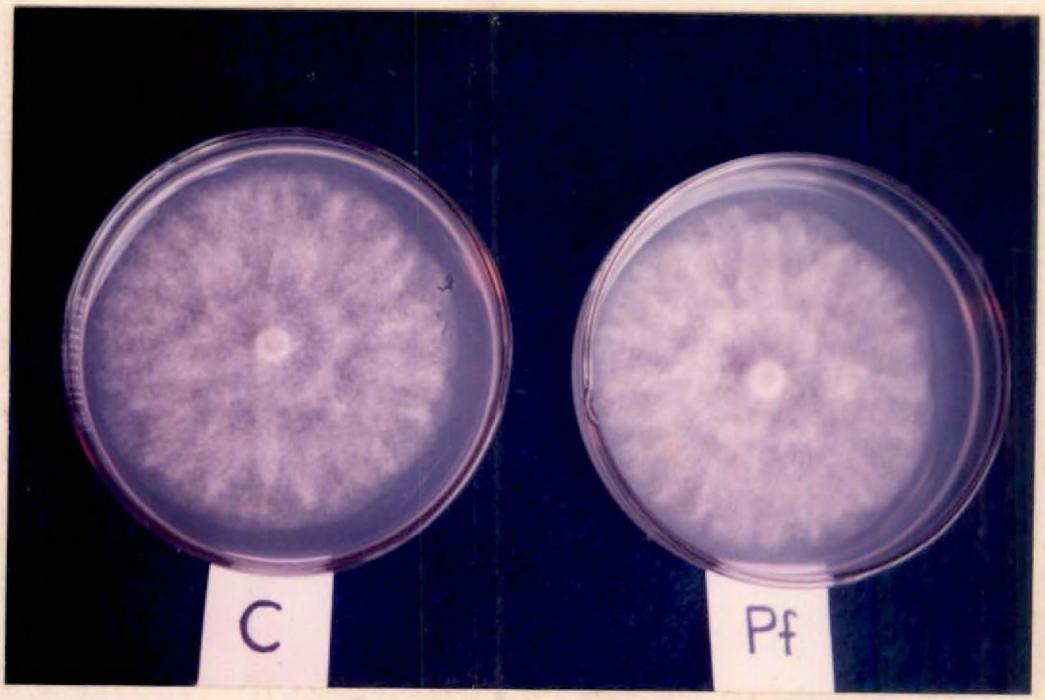
Plate 11.

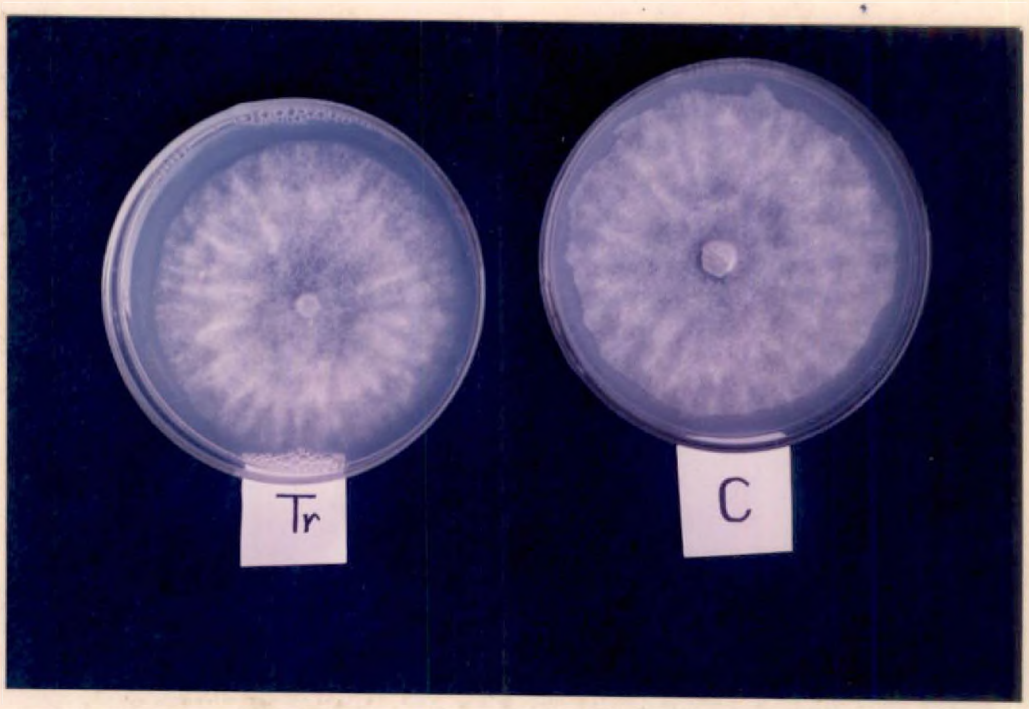
In vitro inhibition of *P. capsici* by cell free filtrates of bacterial antagonists

C - Control

F7 - Filtrate of isolate B13

Pf - Filtrate of *P. fluorescens*


C
Pf


Tr
C

mycelial growth of the pathogen (6.27 and 6.33 cm, respectively) which were significantly lower than the 6.68 cm in the control (Plate 10). F7 and Pf also reduced the growth of the pathogen (6.53 cm) but was statistically on par with control (Plate 11). On the other hand, the culture filtrates of the other bacterial antagonists produced statistically significant increase over control.

4.5.1.2 Effect on sporangial production

The effect of the different culture filtrates on production of sporangia by the pathogen was compared. In general, all the filtrates inhibited sporangial production of *P. capsici* but showed great variability (Table 10 and Fig. 6).

F1 and F7 were found to be highly efficient giving an inhibition of 68.84 per cent and 56.29 per cent respectively. Pf and Tr which served as additional checks were also effective showing 59.42 per cent inhibition of sporangial production and were statistically on par with F1.

4.5.2 Efficacy of culture filtrates in reducing foot rot

4.5.2.1 Studies on excised pepper shoots

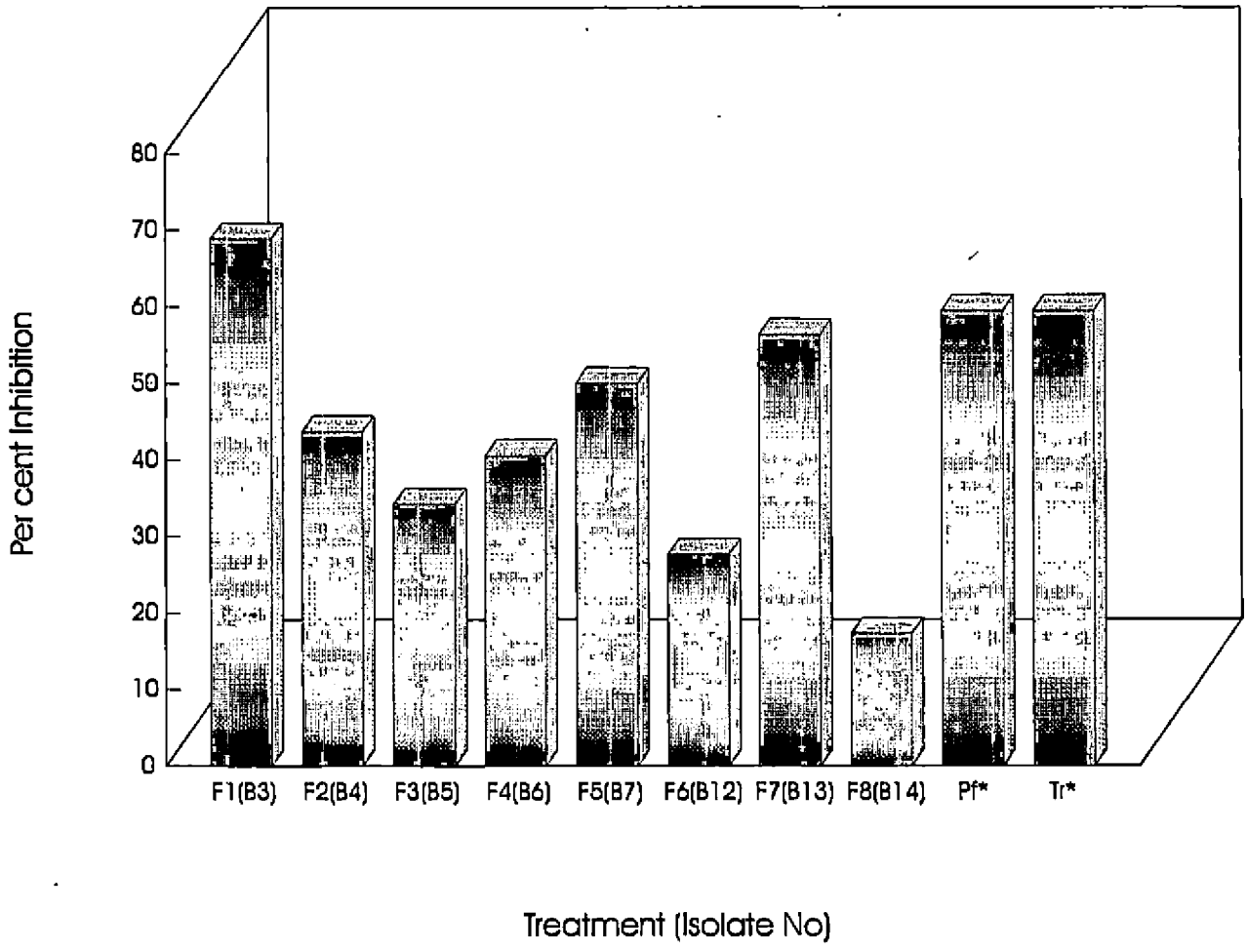
The culture filtrates of the antagonists were sprayed on excised pepper shoots and simultaneously inoculated with the pathogen to study their efficacy on reducing foot rot. The results are presented in Table 11.

Table10 *In vitro* effect of cell free filtrates of bacterial antagonists on sporangial production by *P. capsici*

Treatment	Bacterial isolate No.	Per cent inhibition
F1	B3	68.84 (56.05)
F2	B4	43.69 (41.36)
F3	B5	34.19 (35.77)
F4	B6	40.53 (39.52)
F5	B7	50.01 (44.99)
F6	B12	27.62 (31.70)
F7	B13	56.29 (48.60)
F8	B14	17.29 (24.56)
Pf*		59.42 (50.41)
Tr*		59.42 (50.41)
CD (5%)		9.64

Figures in parentheses represent angular transformed values
* included as additional check

Fig. 6. In vitro effect of cell free filtrates of bacterial antagonists on sporangial production by *P. capsici*



The filtrates differed in their efficacy in delaying symptom initiation. F_1 and F_7 were the most effective treatments in delaying lesion initiation. In these treatments, symptoms appeared only three days after inoculation. The treatment Pf also produced similar effect. The performance of all the other tretments were found to be statistically on par with control.

Observations on lesion size on the fourth day of inoculation revealed that in treatment F1 the lesion size was reduced considerably (1.48 cm) and was found to be significantly less than in the untreated control (3.16 cm). F3, F4, F7, F8, Tr and Pf also showed reduced lesion size but were on par with control. All these treatments were statistically comparable to F1 also (Plate 12) (Fig. 7).

All the treatments were statistically on par with control with respect to their effect on rate of lesion development (Table 11 and Fig. 7). F8 was the most effective treatment giving 0.95 cm day^{-1} followed by F8 and F1 which resulted in a rate of 1.35 and 1.48 cm day^{-1}, respectively. Pf showed the least effect and produced a high rate of 2.25 cm day^{-1} while Tr showed a lower rate of 1.72 cm day^{-1} with regard to lesion development.

4.5.2.2 Studies on rooted pepper cuttings

The efficacy of the culture filtrates of the selected antagonists in checking foot rot was studied. The results of the study are presented in Tables 12a, 12b and 12c.

Table 11 Effect of cell free filtrates of bacterial antagonists on the onset and development of symptoms by *P. capsici* on excised pepper shoots

Treatments	Bacterial Isolate No.	Days for symptom initiation	Lesion size (cm)	Rate of lesion development $cm.day^{-1}$
F1	B3	3	1.48 (1.58)	1.48 (1.58)
F2	B4	2	3.54 (2.13)	1.77 (1.67)
F3	B5	2	1.85 (1.69)	0.95 (1.40)
F4	B6	2.67	2.57 (1.89)	2.01 (1.74)
F5	B7	2.33	3.44 (2.11)	2.21 (1.79)
F6	B12	2	3.57 (2.14)	1.79 (1.67)
F7	B13	3	1.80 (1.67)	1.80 (1.67)
F8	B14	1.67	3.08 (2.02)	1.35 (1.53)
Pf*		3	2.25 (1.80)	2.25 (1.80)
Tr*		2.33	2.64 (1.91)	1.72 (1.65)
C		2	3.16 (2.04)	1.59 (1.61)
CD (5%)		0.76 0.69 0.62	0.47	0.38

Figures in parenthesis represent $\sqrt{x+1}$ transformed values
* included as additional check

Fig. 7. Effect of cell free filtrates of bacterial antagonists on the size and rate of development of lesions by *P. capsici* on excised pepper shoots

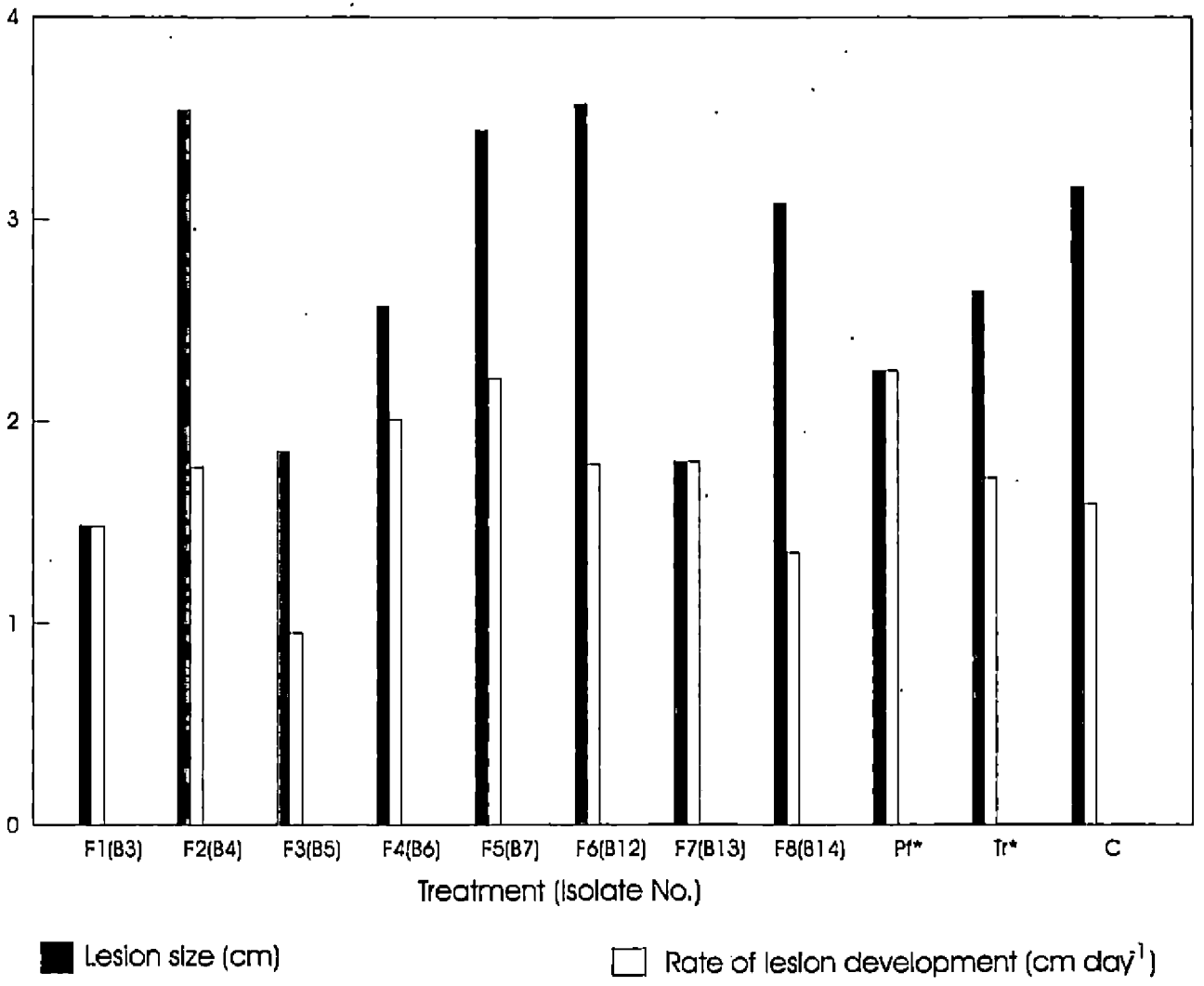


Plate 12

Interaction of cell free filtrates of bacterial antagonists with *P. capsici* on excised pepper shoots

C - Control

Pf - Filtrate of *P. fluorescens*

Tr - Filtrate of *T. harzianum*

F1 - Filtrate of isolate B3

F4 - Filtrate of isolate B6

F7 - Filtrate of isolate B13

F3 - Filtrate of isolate B5

F5 - Filtrate of isolate B7

F8 - Filtrate of isolate B14

F6 - Filtrate of isolate B12

F2 - Filtrate of isolate B4


F1
F4
F7
F3
C
Ps
Tr


F5
F8
F6
F2
C
Ps
Tr

Studies on the rates of lesion development in the stem showed F7 to be highly effective in delaying spread of the disease (Table 12a). The rate of lesion development was only 1.68 cm day^{-1} which was significant lower than that in the untreated control (3.49 cm day^{-1}). This was followed by the treatment Pf where the progress of lesions was only 2.02 cm day^{-1}. All the other treatments except F6 and Tr reduced the rate of lesion development but were found to be on par with control. F6 and Tr tended to increase the rate of progress of the disease which were 3.99 and 3.01 cm day^{-1}, respectively.

Scoring was done to compare the efficacy of different treatments in reducing foliar infection (Table 12b). All the treatments showed reduced incidence of foliar infection compared to control (Fig. 8). Pf included as additional check gave the lowest score of 0.12 as compared to 1.58 in untreated control. Among the fitrates of bacterial isolates, F3 proved to be very effective showing a low score of 0.35 which was on par with Pf and significantly lower than that in control. All the other treatments gave scores which were statistically on par with control. Tr, even though comparable to Pf, was less effective in reducing foliar infection with a mean score of 1.04.

Culture filtrates of all the antagonists tested, were found to decrease the intensity of infection in the foliage (Table 12b and Fig. 8). Pf was the most effective in reducing

Table 12a Effect of cell free filtrates of bacterial antagonists on the rate of development of foot rot disease in black pepper

Treatments	Bacterial Isolate No.	Rate of lesion development ($cm.day^{-1}$)
F1	B3	2.56 (1.89)
F2	B4	3.02 (2.01)
F3	B5	3.26 (2.06)
F4	B6	2.74 (1.93)
F5	B7	2.41 (1.85)
F6	B12	4.00 (2.23)
F7	B13	1.68 (1.64)
F8	B14	2.17 (1.78)
Pf*		2.02 (1.74)
Tr*		3.02 (2.00)
C		3.49 (2.12)
CD (5%)		0.37

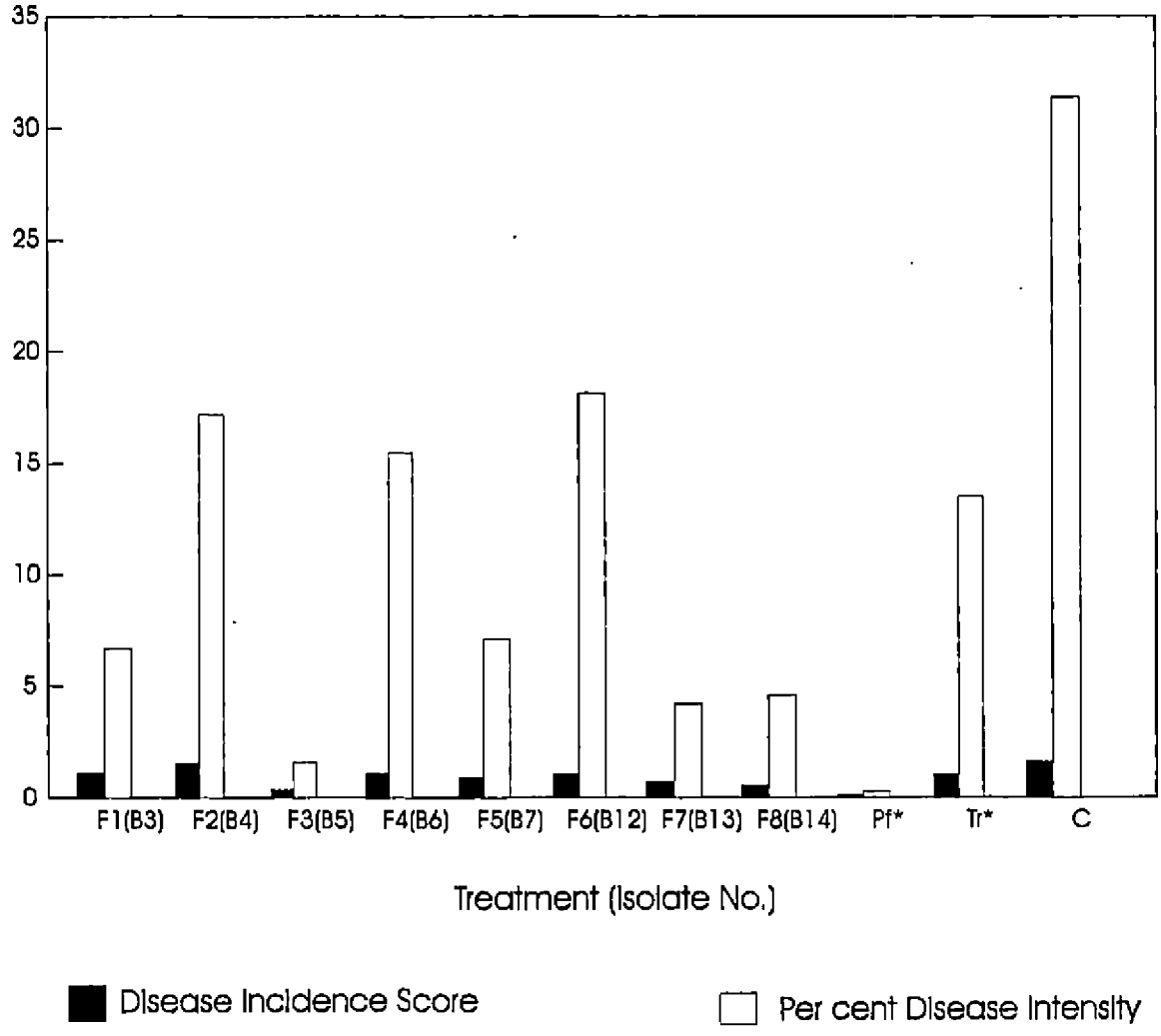
Figures in parenthesis represent $\sqrt{x+1}$ transformed values
* included as additional check

Table 12b Effect of cell free filtrates of bacterial antagonists on incidence and intensity of foliar infection by *P. capsici*

Treatments	Bacterial Isolate No.	Disease incidence score	Per cent Disease Intensity
F1	B3	1.10 (1.45)	6.70 (15.00)
F2	B4	1.55 (1.60)	17.19 (24.48)
F3	B5	0.35 (1.16)	1.59 (7.24)
F4	B6	1.09 (1.45)	15.44 (23.13)
F5	B7	0.87 (1.37)	7.14 (15.49)
F6	B12	1.05 (1.43)	18.15 (25.21)
F7	B13	0.71 (1.31)	4.19 (11.80)
F8	B14	0.56 (1.25)	4.57 (12.34)
Pf*		0.12 (1.06)	0.27 (2.96)
Tr*		1.04 (1.43)	13.48 (21.54)
C		1.58 (1.61)	31.39 (34.06)
CD (5%)		0.39	17.66

Figures in parenthesis represent $\sqrt{x+1}$ and angular transformed values for incidence and intensity respectively
* included as additional check

Fig. 8 Effect of cell free filtrates of bacterial antagonists on incidence and intensity of foliar infection by *P. capsici*



foliar infection with percentage disease intensity of 0.27 per cent as compared to 31.39 per cent in the untreated control. This was followed by F3 exhibiting only 1.59 per cent infection and was statistically comparable to Pf. The treatments F1, F5, F7 and F8 also caused significant reduction of foliar infection and were on par with Pf. F2, F4, F6 and Tr were not as efficient as Pf and resulted in reduced foliar infection which were statistically comparable to control.

Observations on the incidence of foot rot in pepper rooted cuttings taken at 15 days after inoculation, revealed that the culture filtrates exerted a significant influence (Table 12c and Fig. 9) (Plates 13, 14). In the treatments F1, F3, F4, F7, F8 and Pf no death of plants was recorded while a 42.86 per cent mortality was noticed in the untreated control. This was followed by the treatments F2, F5 and Tr where there was 14.29 per cent death of plants. The treatment F6 showed a high mortality of 57.14 per cent which was higher than that in the control.

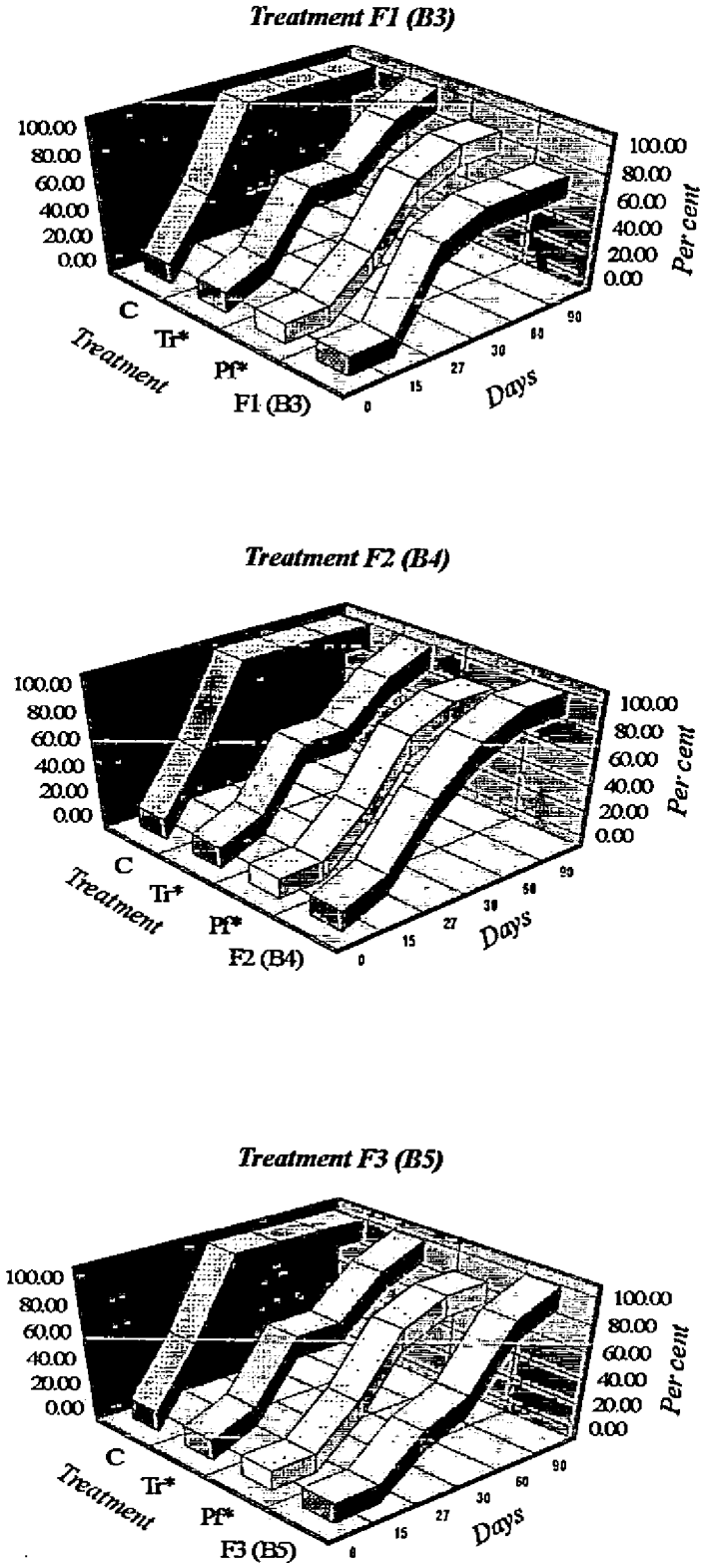
After 27 days of inoculation, when all the plants were killed in the untreated control, F3 and Pf were effective in checking foot rot as it showed only 28.57 per cent death of plants. This was followed by F7 in which 42.86 per cent plants were killed. In treatments F1, F2, F5, F8 and Tr, 57.14 per cent mortality was observed.

Table 12c Efficacy of cell free filtrates of bacterial antagonists in the management of foot rot of black pepper

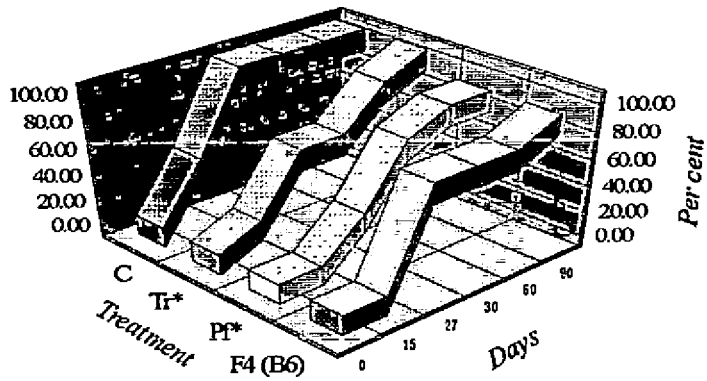
Treatments	Bacterial isolate No.	Percentage mortality of plants				
		days after inoculation				
		15	27	30	60	90
F1	B3	0	57.14	71.43	71.43	85.71
F2	B4	14.29	57.14	85.71	100.00	100.00
F3	B5	0	28.57	42.86	85.71	100.00
F4	B6	0	71.43	71.43	71.43	85.71
F5	B7	14.29	57.14	71.43	71.43	85.71
F6	B12	57.14	100.00	100.00	100.00	100.00
F7	B13	0	42.86	42.86	42.86	71.43
F8	B14	0	57.14	57.14	85.71	100.00
Pf*		0	28.57	71.43	85.71	85.71
Tr*		14.29	57.14	57.14	85.71	100.00
C		42.86	100.00	100.00	100.00	100.00

* Included as additional check

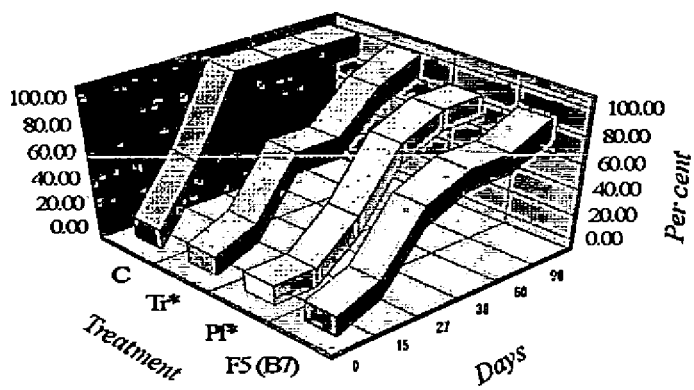
Fig. 9. Effect of cell free filtrates of bacterial antagonists on percentage mortality of black pepper plants due to foot rot disease



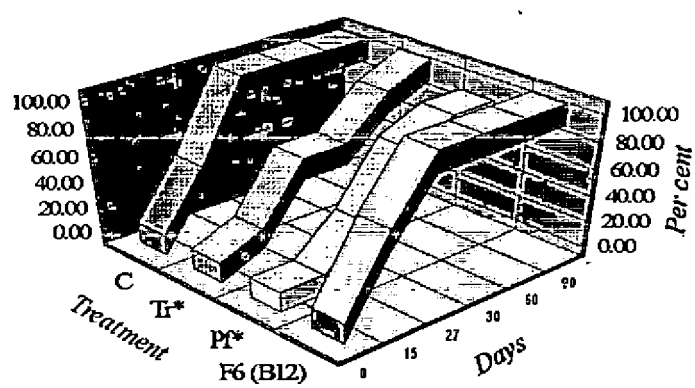
Treatment F4 (B6)



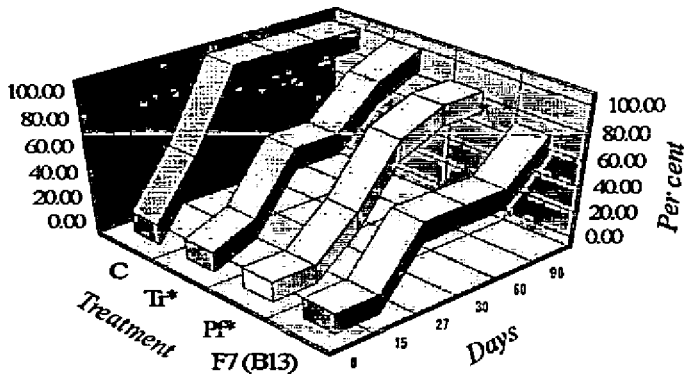
Treatment F5 (B7)



Treatment F6 (B12)



Treatment F7 (B13)



Treatment F8 (B14)

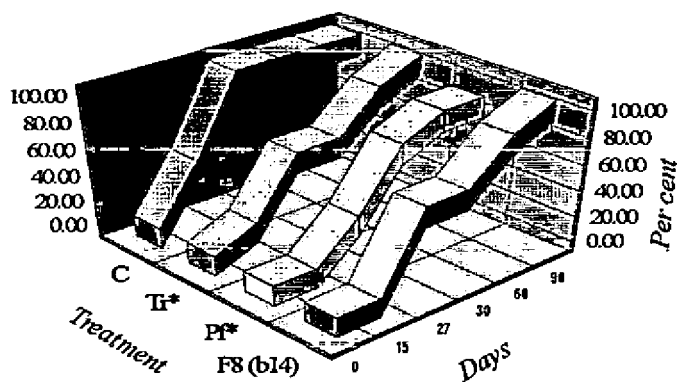


Plate 13.

Suppression of foot rot by cell free filtrates of bacterial antagonists

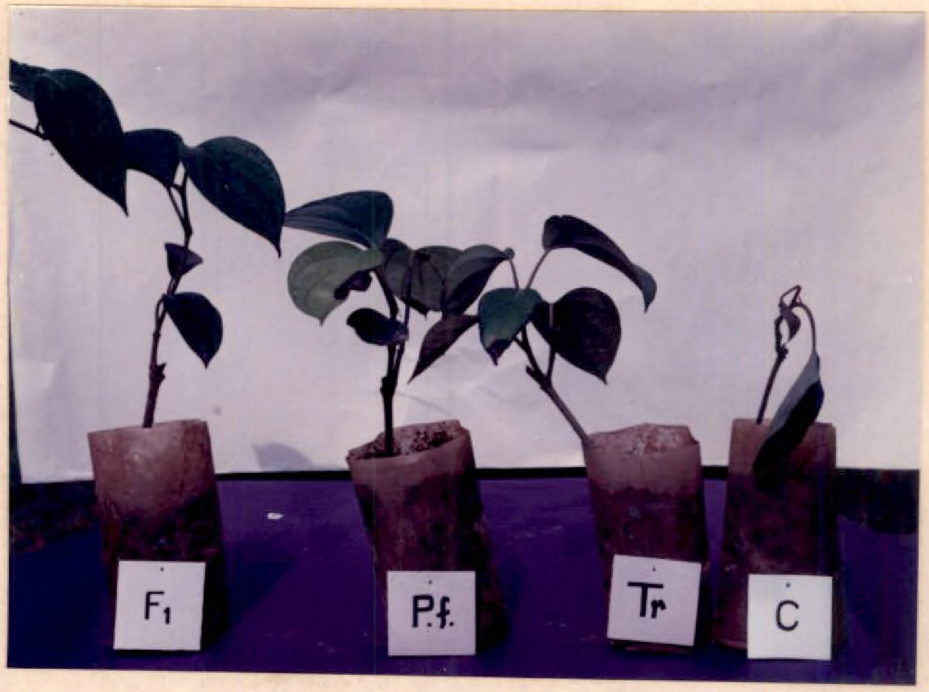
C - Control

Pf - Filtrate of *P. fluorescens*

Tr - Filtrate of *T. harzianum*

F1 - Filtrate of isolate B3

F3 - Filtrate of isolate B5


F1
P.f.
Tr
C

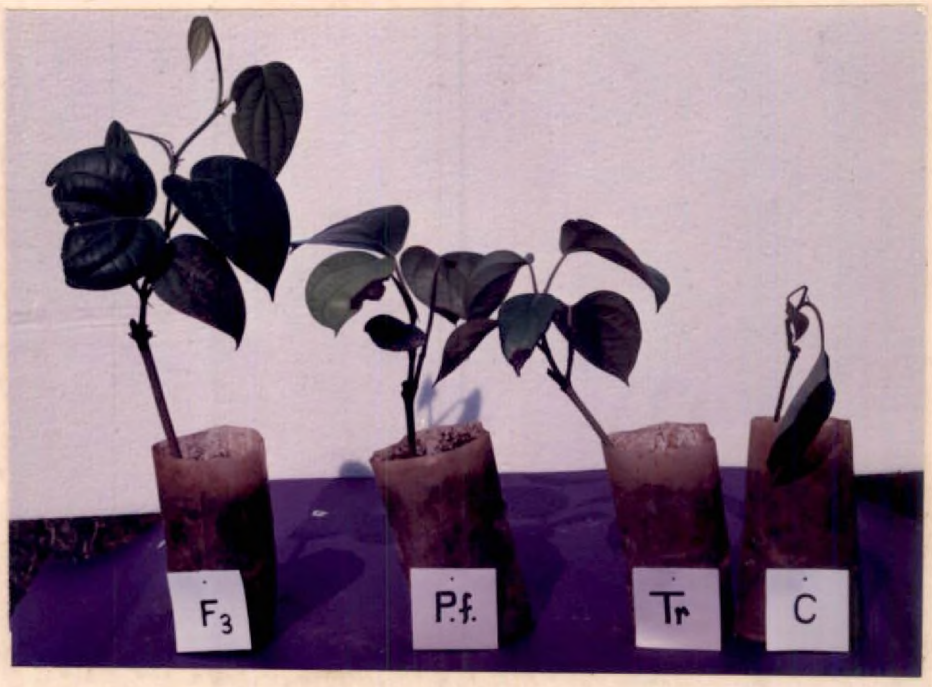

F3
P.f.
Tr
C

Plate 14

Suppression of foot rot by cell free filtrates of bacterial antagonists

C - Control

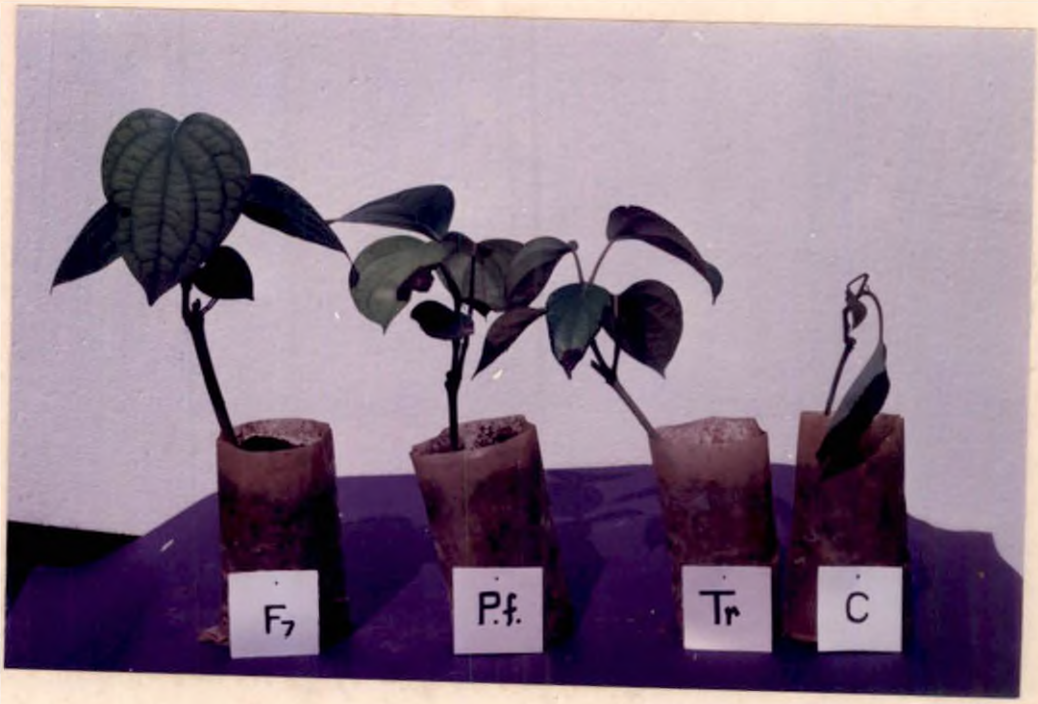
Pf - Filtrate of *P. fluorescens*

Tr - Filtrate of *T. harzianum*

F5 - Filtrate of isolate B7

F7 - Filtrate of isolate B13


F5
P.f.
Tr
C


F7
P.f.
Tr
C

The death of plants at 30, 60 and 90 days after inoculation of the pathogen was also recorded. In F7 (isolate B13), the percentage mortality remained at 42.86 per cent till 60 days after which it increased to 71.43 per cent at 90 days after inoculation. In treatments F1 and F5 (isolates B3 and B7) and Pf, 71.43 per cent plants were killed after 30 days of inoculation which increased to 85.71 per cent at 90 days. F3 which gave a low mortality of 28.57 per cent at 27 days after inoculation, showed a gradual rise in death of plants from 42.86 per cent at 30 days to 85.71 per cent at 60 days and 100 per cent by 90 days after inoculation. A similar trend was noticed with Tr, except at the initial period of observation.

4.6 Plant Growth Promotion by Bacteria

The shoot length and leaf number of pepper rooted cuttings were taken at regular intervals for studying the effect of antagonists on growth pepper (Table 13). In general, the application of bacterial antagonists seemed to promote growth of pepper.

Effect of the treatments on shoot length was recorded at 30, 60 and 90 days after application of antagonists. The bacterial antagonists were found to produce increased shoot length over untreated control.

Table 13 Effect of application of bacterial antagonists on shoot length and leaf number in rooted cuttings of black pepper

Treatments	Bacterial isolate No.	Shoot length (cm) days after inoculation			Leaf number days after inoculation		
		30	60	90	30	60	90
T1	B3	23.63	38.60	54.30	6.67	8.67	10.33
T2	B4	32.27	62.50	94.57	7.67	10.67	13.67
T3	B5	35.93	58.97	74.17	7.00	9.33	10.67
T4	B6	29.63	48.17	56.90	6.67	8.67	10.33
T5	B7	29.83	64.77	82.73	8.00	12.00	15.00
T6	B12	31.47	57.93	80.73	7.33	10.67	12.67
T7	B13	25.40	43.23	58.00	5.67	7.33	7.67
T8	B14	27.40	55.57	84.53	5.67	8.67	9.67
Pf*		23.77	38.27	48.30	5.00	7.33	8.67
Tr*		25.53	39.73	50.20	5.67	7.00	7.67
C		16.73	24.53	41.50	2.67	5.00	6.67
CD (5%)		15.31	31.23	41.32	3.13	4.97	5.92

* Included as additional check

The highest shoot length at 30 days after application of antagonists was observed in the case of T3 and T2 (35.9 cm and 32.27 cm respectively) which was significantly different from control (16.73 cm). All the other treatments showed increased shoot length but were on par with control.

After 60 days of application of antagonists to pepper plants, T5, T2, T3 and T6 showed significantly increased shoot length compared to 24.53 cm in the control. T5 gave the maximum shoot length of 64.77 cm followed by 62.5 cm, 58.97 cm and 57.93 cm in T2, T3 and T6 respectively. However after 90 days of application of antagonists, T2, T8 and T5 proved to be effective in increasing shoot length (Fig. 10). T2 was the most efficient and produced 94.57 cm plant height (Plate 15) followed by 84.53 cm in T8 and 82.73 in T5 which were significantly greater than plant height of 41.5 cm in the untreated control.

Observations taken at the different intervals showed that Pf and Tr were not efficient in increasing shoot length. Pf was less effective than Tr with 23.77 cm, 38.27 cm and 48.3 cm at 30, 60 and 90 days respectively while it was 25.53 cm, 39.73 cm and 50.2 cm respectively in Tr at these days.

A comparison of number of leaves showed that T5, T2, T6, T3 and T1 (8, 7.67, 7.33, 7 and 6.67 respectively) were significantly different from control which had an average of 2.67

leaves at 30 days after application of antagonists. However observation at 60 and 90 days revealed that in T5, T2 and T6 the plants produced significantly high number of leaves than in control (5 and 6.67 respectively). T5 was the most effective in increasing leaf number which was 12 and 15, respectively, after 60 and 90 days of application (Plate 15). The leaf numbers were as low as 5, 7.33 and 8.67 in Pf and 5.67, 7 and 7.67 in Tr at 30, 60 and 90 days respectively.

Observations on root length, root volume, fresh weight and dry weight were also made at 3 months after application of antagonists (Table 14 and Fig. 10).

The maximum root length was found in the treatment T5 (25.67 cm) which was significantly higher than in control (16.67 cm) (Plate 15). T2, T3, T4 and T8 also increased root length but was comparable with control. All the other treatments gave values of root length less than that of control.

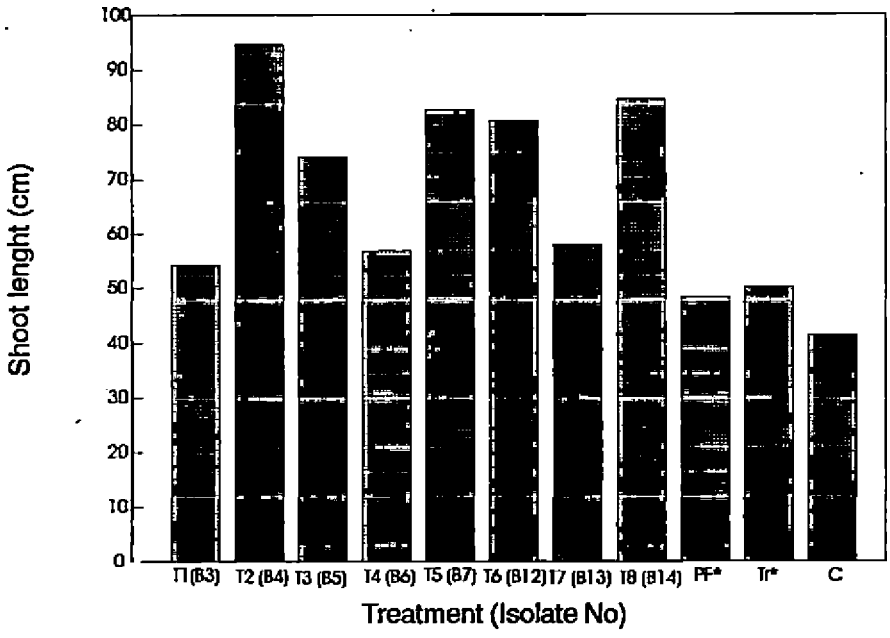
T5 showed the maximum root volume (4.33 ml) which was significantly higher than that of control plants (1.67 ml). Root volumes in T3 (2.83 ml) and T4 and T1 (2.33 ml were greater than but statistically on par with control. The other bacterial treatments did not produce any effect on root volume. Pf and Tr also did not give any positive effect and recorded only 1.33 ml and 0.67 ml of of root volumes, respectively.

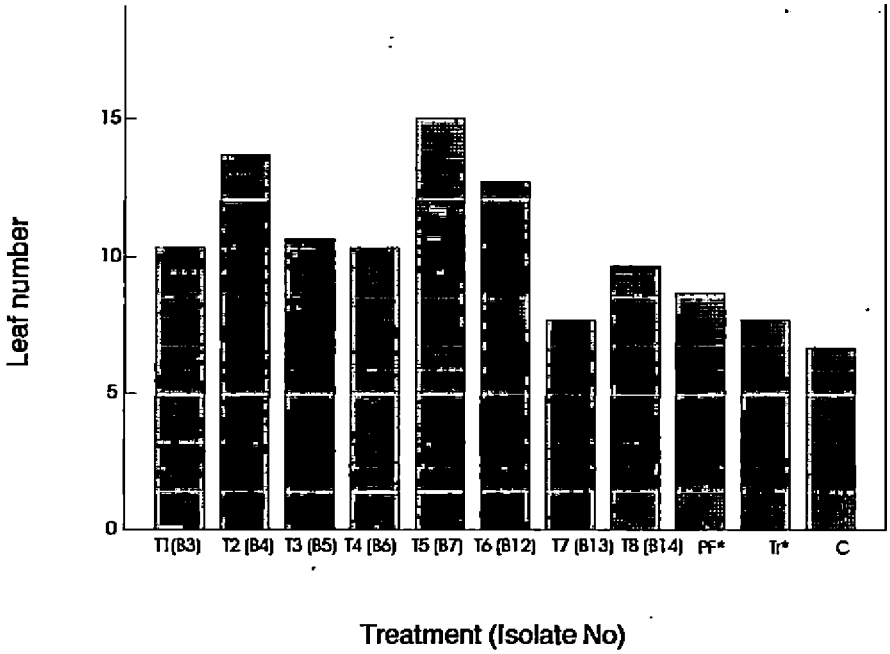
Table 14 Growth of rooted cuttings of black pepper on inoculation with bacterial antagonists

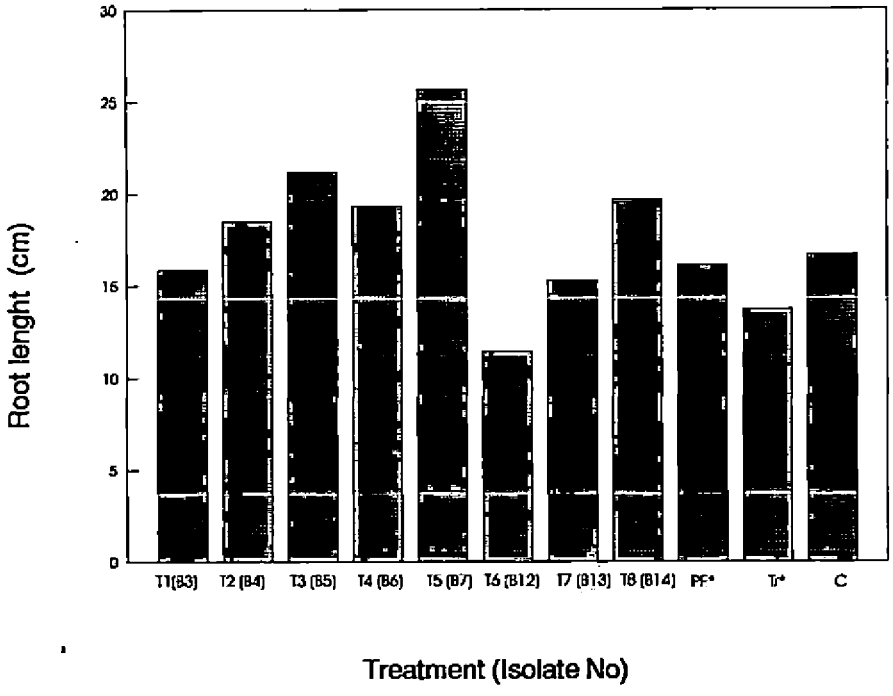
Treatment	Bacterial Isolate No.	Shoot length (cm)	Leaf number	Root length (cm)	Root volume (ml)	Fresh weight (g)	Dry weight (g)
T1	B3	54.30	10.33	15.87	2.33	16.00	2.77
T2	B4	94.57	13.67	18.53	1.67	21.70	3.50
T3	B5	74.17	10.67	21.20	2.83	17.25	3.00
T4	B6	56.90	10.33	19.37	2.33	15.17	2.27
T5	B7	82.73	15.00	25.67	4.33	21.63	3.80
T6	B12	80.73	12.67	11.47	1.67	17.73	3.51
T7	B13	58.00	7.67	15.27	0.83	11.43	2.12
T8	B14	84.53	9.67	19.70	1.67	17.52	3.19
Pf*		48.30	8.67	16.13	1.33	16.10	2.37
Tr*		50.20	7.67	13.77	0.67	13.20	2.46
C		41.50	6.67	16.67	1.67	12.03	1.74
CD(5%)		41.32	5.92	12.85	2.00	6.27	1.36

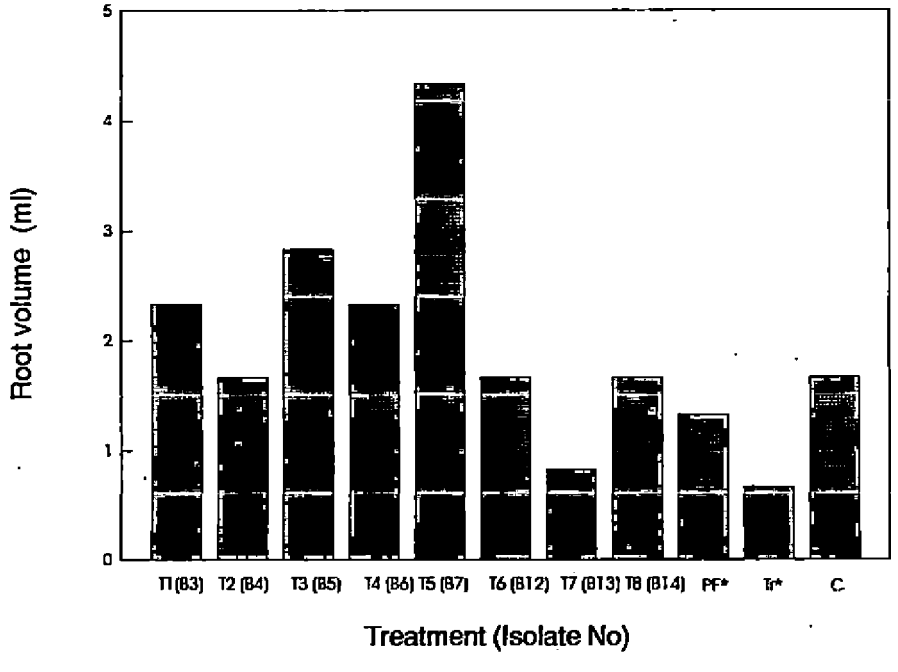
* Included as additional check

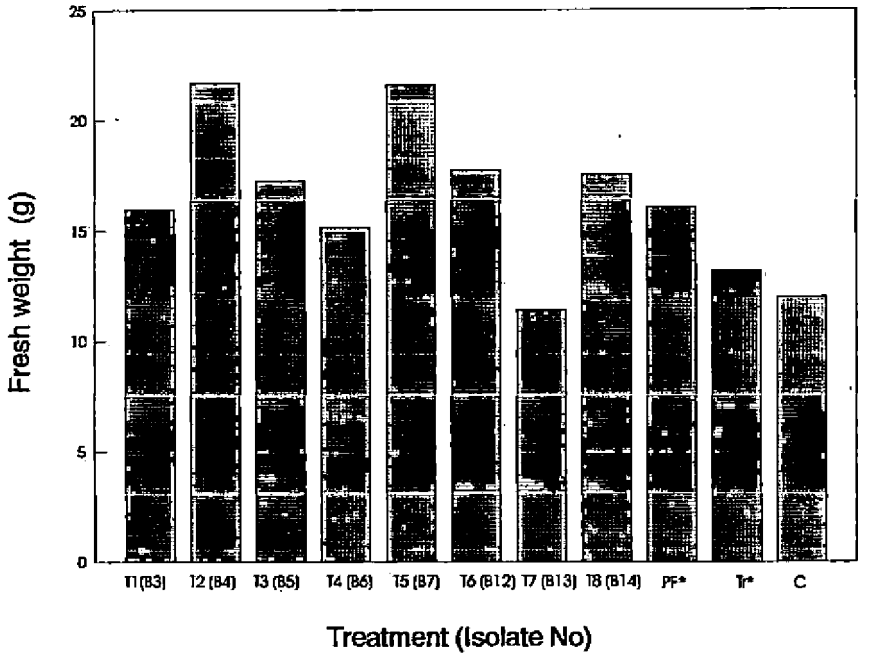
Fig. 10. Effect of bacterial antagonists on growth of rooted cuttings of black pepper






Root lenght (cm)
30
25
20
15
10
5
0
T1(B3)
T2 (B4)
T3 (B5)
T4 (B6)
T5 (B7)
T6 (B12)
T7 (B13)
T8 (B14)
PF*
Tr*
C
Treatment (Isolate No)


Root volume (ml)
5
4
3
2
1
0
T1 (B3)
T2 (B4)
T3 (B5)
T4 (B6)
T5 (B7)
T6 (B12)
T7 (B13)
T8 (B14)
PF*
Tr*
C
Treatment (Isolate No)


Fresh weight (g)
25
20
15
10
5
0
T1 (B3)
T2 (B4)
T3 (B5)
T4 (B6)
T5 (B7)
T6 (B12)
T7 (B13)
T8 (B14)
PF*
Ir*
C
Treatment (Isolate No)

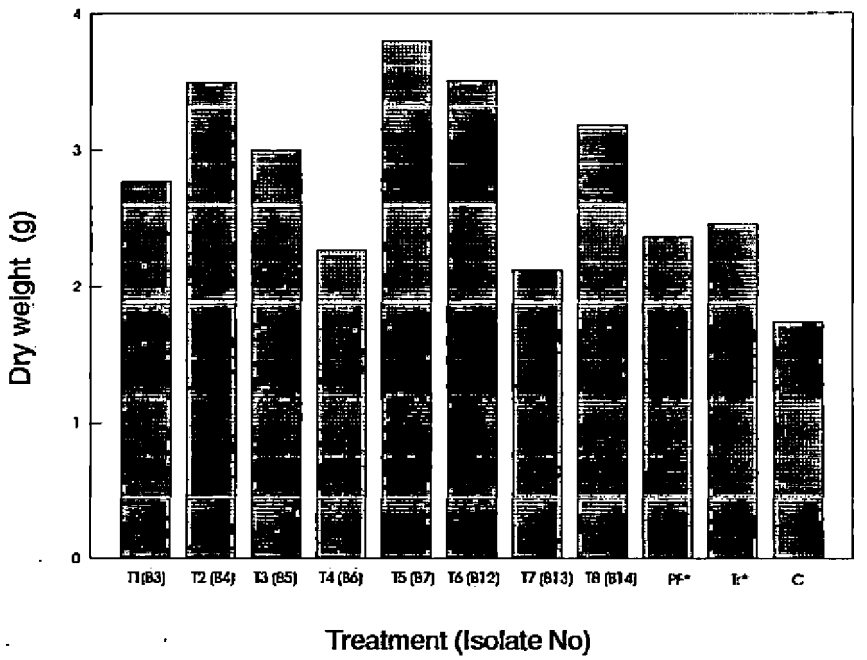

Dry weight (g)
4
3
2
1
0
T1 (B3)
T2 (B4)
T3 (B5)
T4 (B6)
T5 (B7)
T6 (B12)
T7 (B13)
T8 (B14)
PF*
Ir*
C
Treatment (Isolate No)

Plate 15.

Growth promotion of pepper plants by bacterial antagonists of *P. capsici*

C - Control

Pf - *P. fluorescens*

Tr - *T. harzianum*

T2 - Isolate B4

T5 - Isolate B7


C
Pf
Tr
T2


C
Tr
Pf
T5

Application of bacterial antagonists seemed to increase the fresh weight of plants (Table 14). The highest value for mean fresh weight (21.7 g) was recorded in T2, which was significantly higher than in control plants (12.03 g). Treatment T5 and T6 also showed significantly higher fresh weights (21.63 g and 17.73 g respectively) over untreated control. In all the other treatments except T7, fresh weight of plants were greater than, but statistically comparable to control. Pf gave an increase in fresh weight (16.1 g) over control while Tr was less effective with fresh weight of 15.2 g. However, both these treatments were on par with control.

A comparison of dry weights of plants showed all treatments to be efficient (Table 14). The plants in T5 had the maximum dry weight (3.8 g) followed by treatments T6 and T2 which were 3.51 g and 3.5 g respectively. The mean dry weights of plants in Tr and Pf were also greater than that of control. The dry weight of plants recorded an average of 2.46 g in Tr and 2.37 g in Pf which were statistically comparable to 1.74 g in control.

4.7 Assessment for cellulolytic properties

4.7.a Estimation of cellulase enzyme

Estimation of cellulase enzyme of selected antagonists using reducing sugar method revealed that only few

Table 15 *In vitro* production of cellulolytic enzyme by bacterial antagonists

Bacterial Isolate No.	Cellulase activity (μg glucose min^{-1} mg^{-1} protein)
B3	0
B4	10.57
B5	0
B6	12.45
B7	0
B12	0
B13	1.40
B14	10.64
Pf	4.58
Tr	0
CD (5%)	1.08

isolates were capable of producing the enzyme (Table 15). Only four out of the eight isolates showed cellulase activity. B6 produced the highest enzyme activity (12.45 µg glucose min^{-1} mg^{-1} protein) followed by B14, B4 and B13. Pf also showed cellulolytic activity of 4.58 µg glucose min^{-1} mg^{-1} protein. However, enzyme activity was not detected in Tr. B3, B5, B7 and B12 also did not produce cellulase.

4.8 Survival of antagonists

The population of different antagonists in the rhizosphere of pepper plants after three months of application showed considerable variation (Table 16). Tr showed the highest survival (100.67 x 10^4 cfu g^{-1} of rhizosphere soil). This was followed by T8 and T6 giving 54.66 and 44.17 x 10^4 cfu g^{-1} of soil. In the above treatments the survival of antagonists were significantly higher than the other treatments. The lowest count of 7.58 x 10^4 cfu g^{-1} of soil was recorded in T4. In the other treatments the population ranged from 33.96 x 10^4 cfu g^{-1} in T2 to 23.59 x 10^4 cfu g^{-1} in T3. Pf showed a population of 25.63 x 10^4 cfu g^{-1} soil.

4.9 Preliminary characterisation of bacterial antagonists

Some of the characters like growth on nutrient agar plates, agar slants, cell shape, grams reaction, and sporulation were studied. The results of the study are presented in Table 17.

Table 16 Survival of antagonists in rhizhosphere of pepper plants

Treatment	Bacterial Isolate No.	Population (10^4 cfu g^{-1})
T1	B3	24.22
T2	B4	33.96
T3	B5	23.59
T4	B6	7.58
T5	B7	24.54
T6	B12	44.17
T7	B13	32.30
T8	B14	54.66
Pf*		25.63
Tr*		100.66
CD (5%)		0.55

* Included as additional check

All the bacterial antagonists were gram positve sporulating rods. The effective bacterial antagonists, B5, B7 and B13 were found to have similar growth pattern. These isolates formed fast growing colonies on nutrient agar plates which were rhizoid, flat and dry with lobate margins. Characters like catalase activity and motility were also studied with these three isolates and were found positive confirming the identity to the genus *Bacillus* (Table 18).

Table 17 Characteristics of bacterial antagonists

Isolate No.	Colony characters on nutrient agar plate					Growth on nutrient agar slants			Cell shape	Grams reaction	Sporulation
	Shape	Margin	Elevation	Optical feature	Texture	Form	Margin	Amount			
B3	rhizoid	lobate	flat	translucent	dry	filiform	uniform	moderate	Rod	+	+
B4	circular	entire	convex	translucent	slimy	filiform	uniform	moderate	Rod	+	+
B5	rhizoid	lobate	flat	translucent	dry	beaded	irregular	moderate	Rod	+	+
B6	circular	curled	raised	opaque	butter like	filiform	uniform	abundant	Rod	+	+
B7	rhizoid	lobate	flat	translucent	dry	beaded	irregular	moderate	Rod	+	+
B12	circular	curled	raised	opaque	dry	filiform	uniform	abundant	Rod	+	+
B13	rhizoid	lobate	flat	translucent	dry	beaded	irregular	moderate	Rod	+	+
B14	punctiform	entire	convex	translucent	slimy	filiform	uniform	moderate	Rod	+	+

Table 18 Characters of selected bacterial antagonists that confirm its identity to the genus *Bacillus**

Characters	Isolates		
	B5	B7	B13
Cell shape	rod	rod	rod
Grams reaction	+	+	+
Sporulation	+	+	+
Motility	+	+	+
Catalase activity	+	+	+
Growth under aerobic conditions	+	+	+

* Source: Holt, J.G. (ed.) Shorter Bergey's Manual of Determinative Bacteriology

DISCUSSION

5 DISCUSSION

A search for potential antagonistic bacteria for the management of *P. capsici*, the incitant of the devastating foot rot disease of black pepper, was carried out in the present investigation. Bacteria are common inhabitants in the rhizosphere and contribute to the control of many deleterious fungal diseases (Weller, 1988). *Bacillus*, *Pseudomonas* etc. are some of the promising bacterial genera that pose threat to a wide array of soil borne pathogens like *Pythium*, *Phytophthora* etc. (Jee *et al.*, 1988; Lee *et al.*, 1990 and Sanchez *et al.*, 1994). Considering the enormous literature available on the success of laboratory and field experiments on biocontrol with bacteria, there appears to be scope for exploring the potentiality of this group of antagonists for development as commercially feasible products for the management of *P. capsici*.

To quote Cook and Baker (1983), "the starting point for biological control with rhizosphere bacteria is often the isolation and characterization of bacteria from root environment where disease is lacking such as suppressive soils or from healthy plants in diseased fields". Accordingly bacteria were isolated from the rhizosphere of wild pepper plants, healthy plants in diseased pepper plantations, pepper plants in healthy plantations, pepper plantations where organic farming is

practiced, undisturbed forest soils and vermicompost. Comparison of population of bacteria in the different soils and vermicompost showed high variability in bacterial counts (Table 2). High bacterial population was recorded from the rhizosphere soils of healthy plants in sick pepper plantations of Pepper Research Station, Panniyur and from the virgin forest soils of the Punnamala region of Silent Valley. Variability in the population of bacteria in the rhizosphere soils of healthy avocado trees growing in orchards heavily infested with *P. cinnamomi* has been recorded by Duvenhage *et al.* (1991).

Of the total of 194 rhizosphere bacteria isolated during the present study, only fifteen isolates were found to be effective in causing *in vitro* inhibition of the pathogen and these were included for detailed studies *in vitro* and in excised pepper shoots. The effective antagonistic population accounted only for 0.08 per cent of the total flora isolated. Similar low percentage recovery of antagonists have been frequently encountered in screening of bacterial antagonists for other plant pathogens. Montesinos *et al.* (1996) reported only 7 per cent recovery of antagonists against *Stemphylium vesicarium* whereas only 0.5 per cent of rhizosphere bacteria were found antagonistic to *Colletotrichum lagenarium* (Leben, 1964).

In vitro antagonism of bacteria usually present a clue to their capacity for suppression of the pathogen *in vivo*. The

isolates B14 and B1 showed good inhibition of mycelial growth of *P. capsici* (Table 4) whereas the isolates B5, B7, B8, B9 and B13 were inhibitory to the production of sporangia by the pathogen (Table 5). B5, B7 and B13 were consistently effective in suppressing the onset and development of symptoms in excised shoots (Table 6). The performance of the other isolates showed variable results. B1, B9 and *T. harzianum* that rated high in *in vitro* inhibition of the pathogen showed less impact on suppression of symptom on detached shoots. On the other hand, isolate B3 and B6 with lesser level of inhibition of mycelial growth and sporulation were effective in disease suppression on excised shoots. The effect of *Pseudomonas fluorescens* included as an additional check was also in a similar manner. Lack of correlation between *in vitro* antibiosis and biological control was observed during the screening of bacterial antagonists against take all pathogen in wheat and turf grass (Wong and Baker, 1984) and *Stemphylium vesicarium* on pear (Montesinos *et al.*, 1996). On the other hand, several workers have reported positive correlation between *in vitro* antibiosis and biocontrol (Alconero, 1980; Galindo, 1992 and Utkhede, 1984). The bacterial isolates that performed high to medium in inhibition of pathogen *in vitro* and in disease suppression on detached pepper shoots were selected for further studies on biocontrol of foot rot pathogen *in vivo* (Table 7). The isolates B3, B4, B5, B6, B7, B12, B13 and B14 were thus selected.

The *in vivo* experiments on biocontrol using the above isolates showed that the isolates B3, B5, B7 and B13 were effective in reducing foot rot to varying intensity. Incidently, the origin of these isolates happened to be the suppressive soils of Kulathupuzha and Silent Valley. Silent Valley forest ecosystem is the centre of orgin of black pepper and is a treasure house of several wild species of *Piper*. Wild plants harbour diverse microflora helping the plants to survive diseases and adversities of nature (Nayar, 1997).

In pot culture experiment, the bacterial isolates B5 and B13 were very effective in reducing the rate of spread of lesions. As compared to untreated control, the treatments with B5 and B13 lowered the rate of spread of disease to 1.03 and 1.5 cm day^{-1} respectively (Table 8a). Berger *et al*. (1996) obtained significant control of *Phytophthora* and *Pythium* damping off in garden plants by application of a strain of *Bacillus subtilis*. Suprisingly, the bacterial isolates B5, B7 and B13 isolated from the rhizosphere, were also effective in reducing foliar blighting caused by the foot rot pathogen: *P. capsici* is a soil borne pathogen where secondary infection is mediated through water borne zoospores or wind borne sporangia. In spite of being allochthonous, the bacteria have emerged successful in offering foliar protection. Similar cases of successful biocontrol of foliar diseases using rhizosphere antagonists have been reported

earlier too. Using *P. fluorescens* isolated from the root system of corn plants, Montesinos *et al.* (1996) could get efficient biocontrol of brown spot of pear.

Successful biocontrol agents should provide protection to the crop for extended periods after application. An isolate like B4 which offered immense decrease in disease incidence at the early stages, but breaking down and giving access to increase in disease, does not appear ideal for use in biocontrol. The results on decrease in percentage mortality show that the bacterial isolates such as B13 and B7 causing an initial medium level (42.86%) of mortality were effective in providing protection to the pepper plants throughout the further period of observation (Table 8c). After 90 days of inoculation, the mortality was 57.14 per cent and 71.43 per cent due to inoculation of the isolates B13 and B7, respectively. The lowest initial mortality was recorded due to inoculation of isolate B5 (14.29%) which showed a progressive increase and reached 71.43 per cent in 30 days. Thereafter no further increase in death of plants was noticed. Bacterial antagonists have been found to reduce mortality of plants by *P. capsici* infection in different crops (Jee *et al.*, 1988; Kim *et al.*, 1988; Lee *et al.*, 1990 and Okamoto *et al.*, 1991).

T. harzianum and *P. fluorescens* included as additional checks were not as effective as the isolates B5, B7 and B13. The

initial disease suppression offered by *P. fluorescens*, decreased gradually as time after application increased. The increased availability of ferric iron in acid soils and presence of certain clay minerals inhibit the antagonistic activity of *P. fluorescens* which mainly centres around production of siderophores (Defago *et al.*, 1990 and Schippers *et al.*, 1987). *T. harzianum* did not offer much protection and all the plants were killed one month after inoculation of the pathogen. An IDM programme which included *T. harzianum* as a component caused a marginal decrease of only 10 per cent of foot rot disease in black pepper (Sarma *et al.*, 1996a). Bacterial antagonist was reported to be superior to *T. harzianum* in suppressing blight in chilli caused by *P. capsici* in the field (Kim *et al.*, 1990).

The cell free culture filtrate of B3 was effective in inhibiting mycelial growth and sporangial production by the pathogen *in vitro* (Tables 9 and 10). Effect of filtrates of *T. harzianum* was also similar to B3. The effects of the filtrates of B13 and *P. fluorescens* were in a similar manner with significant inhibition of sporangial production and no appreciable reduction in mycelial growth. Inhibition of plant pathogenic fungi by bacteria was found related to the *in vitro* production of inhibitory metabolites (Nowak-Thompson *et al.*, 1994 and Tschen, 1987). Badel and Kelemu (1994) noticed significant inhibition of mycelial growth, sporulation and spore germination

of *Colletotrichum gloeosporioides* by culture filtrates of *B. subtilis*. In excised pepper shoots, filtrates of B3 and B13 were found to cause delay in the initiation of symptoms and reduction in lesion size, but did not alter the rate of lesion development (Table 11). This may be interpreted to be the result of metabolites affecting the initial stages of pathogenesis, as evidenced by the *in vitro* inhibition of mycelial growth and sporangial production by these isolates. The *in vitro* production of antibiotics by bacteria against *Stemphylium vesicarium* was related to antagonism on leaves of pear (Montesinos *et al.*, 1996). However, *P. fluorescens* and *T. harzianum* produced only a delay in the initiation of the lesions, after which there was a rapid increase in the disease. Lack of correlation between *in vitro* antibiosis and biocontrol with these organisms have been reported earlier also (Papavizas and Lewis, 1983 and Wong and Baker, 1984).

Culture filtrates of antagonists, except B12 and *T. harzianum* were effective in reducing development of disease in rooted cuttings. Filtrates of B13 showed significant reduction in the rate of progress of lesion and foliar disease intensity (Tables 12 a and 12 b). However, application of culture filtrates of B5 afforded better protection of the foliage. Culture filtrates of *P. fluorescens* also reduced the rate of progress of the disease and incidence and intensity of foliar

infection. The foliar protection offered by the filtrates of *P. fluorescens* was greater than that by any of the other bacterial isolates. *B. subtilis* suppressed infection of soybean stems by *Phomopsis* sp. in the field (Cubeta *et al.*, 1995). Treatment of seeds and seedlings with filtrates of antagonistic organisms were effective in the control of various diseases (Douville and Boland, 1992; Silo-suh *et al.*, 1994 and Tschen, 1987).

The filtrate of B13 also offered good protection of pepper rooted cuttings against foot rot. On application of the filtrate of this isolate, the mortality remained as low as 42.86 per cent for 60 days (Table 12 c). Filtrates of B3, B7 and Pf also reduced the disease. However, the efficacy of culture filtrates in protecting pepper plants gradually decreased with progress of time. The filtrates of *T. harzianum* did not offer much protection and there was a steady increase in the disease. Singh and Dwivedi (1987) reported reduced mortality of 40 and 53.3 per cent in barley plants by *Sclerotium rolfsii* on application of culture filtrates of *Bacillus licheniformis* and *Pseudomonas aeruginosa* respectively.

The effectiveness of culture filtrates in biocontrol poses immense potentiality for development of a commerically viable product which can be formulated and used as an ecofriendly fungicide. The use of living antagonist have been subjected to a

great deal of criticism from the point of view that its continued use may change the role of the antagonist into an aggressive pathogen. Use of culture filtrate appears safer from this angle and thus the results on its effectiveness in controlling *P. capsici* has immense potentialities.

From the present observations, it is evident that cell suspensions of antagonistic bacteria, even though caused an initial level of mortality of plants, afforded good protection for a considerably longer time. The culture filtrates when applied afforded an initial good protection which slowly declined with lapse of time. Even at high inoculum densities, the percentage infection of apple shoots by *Erwinia herbicola* was less in treatments with live bacteria than with culture filtrates (Campbell, 1989). The mechanism of operation of biosuppression of *P. capsici* is probably through antibiosis initially. The induction and release of metabolites may involve some time which may account for the initial time lag in affording protection. Once elucidated, it exerts a definite continuing influence on the population of the pathogen which may be the reason for its protective capacity for long spells. On the contrary, the filtrates are rich in antimicrobial compounds and as such the application affords immediate protection. With passage of time, the metabolites may get denatured by soil microorganisms or get fixed to clay particles (Lam and Gaffney, 1993) and hence the effect seem to be overwhelmed by the pathogen.

From the present investigation, it is evident that isolate B7 and B13 are effective in suppressing foot rot incidence both as whole cell suspensions and cell free filtrates. B5 in both the forms was effective in checking foliar infection. Suitable combination of live cells and culture filtrates of effective bacteria could be used to provide protection against soil borne and aerial inoculum of the pathogen. However further investigation is needed in this direction.

Beneficial rhizobacteria that promote plant growth has recently been designated as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1980 and Schippers *et al.*, 1987). According to Weller (1988) the recognition of the potential of such bacteria in hindering root pathogens has led to a new system of approach for biocontrol of such pathogens. About 2-5 per cent of bacteria isolated from root system subsequently proved to cause a positive growth response. In the present investigation also all the bacterial antagonists tended to promote the growth of pepper plants in the absence of the pathogen. The isolate B7 induced a significant increase of all the growth parameters studied, ie., shoot length, leaf number, root length and volume, fresh and dry weight of plants. Isolate B4 also promoted growth by way of increase of plant height, leaf number, fresh and dry weight of plants. Increased plant growth and yield by inoculation of crop plants with bacteria have been reported by

several workers (Broadbent *et al.*, 1977; Burr *et al.*, 1978 and Merriman *et al.*, 1974a,b). *P.fluorescens* and *T.harzianum* included as additional checks in the experiment did not have any effect on growth promotion of pepper plants. Weller and Cook (1983) could obtain good control of take all pathogen in wheat using *P. fluorescens* but could not observe any growth promoting effect. On the other hand, Carruthers *et al.* (1994) observed direct growth stimulation due to inoculation of *P. fluorescens*.

Results of the above study assign a double role as biocontrol agent and plant growth promoter for the isolate B7. Utkhede and Smith (1991) could obtain reduction in crown rot caused by *P. cactorum* and increased trunk diameter in apples by application of *B. subtilis*. Similar results were reported with application of the bacterial antagonist, *Enterobacter aerogenes* in apples (Levesque *et al.*, 1993 and Utkhede and Smith, 1994). Several other workers have also highlighted the role of PGPR in reducing plant diseases (Carruthers *et al.*, 1994; Harris *et al.* 1994 and Wei *et al.*, 1996).

Assay on cellulolytic property showed that B13 produced small quantity of the hydrolytic enzyme, cellulase. The elaboration of this enzyme could be considered as an additional weapon in the arsenal of this antagonist in fighting against *P. capsici*. Cellulose a β (1-4) linked glucan is a constituent in the cell wall of *Phytophthora* (Arnonson, 1965). The above

observation accounts for the effect of culture filtrates in disease suppression. Fravel (1988) reported that the production of cell wall degrading enzyme by the antagonists could be involved simultaneously in parasitism and antibiosis. Positive correlation between production of cell wall degrading enzymes and biological control has been reported (Lim *et al.*, 1991). The isolates B4, B6, B14 and *P. fluorescens* also produced cellulase but did not offer significant protection of pepper shoots from infection by *P. capsici*. Lack of correlation between *in vitro* production of enzymes and biocontrol *in vivo* has been observed earlier also (Mavingui and Heulin, 1994 and Thara and Gnandmanickam, 1994). *T. harzianum* was not found to produce cellulase. The elaboration of several water soluble enzymes by *Trichoderma* spp. have been however, reported earlier (Lewis and Papavizas, 1987).

Successful colonization and survival of antagonists in the rhizosphere is essential for controlling root pathogens. In the present study, bacterial antagonists showed a high population of 10^4-10^5 cfu g^{-1} soil. Thompson *et al.* (1996) demonstrated that a population of bacterial antagonist of 10^4 - 10^5 cfu g^{-1} sample were present in the rhizosphere after two weeks of application and offered to reduce the summer patch disease of Kentucky blue grass. The highest survival of 10^6 cfu g^{-1} rhizosphere soil was recorded in the case of *T. harzianum*. However, it did not have any effect on disease suppression.

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Attempt on preliminary characterisation of isolates revealed that all the successful antagonists were gram positive, sporulating rods. The ability to form endospores in the rhizosphere is a major factor boosting up the bicontrol activity of the bacterial isolates. Berger *et al*. (1996) correlated the ability to form endospores and sporulating cells in the rhizosphere to biocontrol activity by the bacterial antagonists. In gram positive, sporulating bacteria like *B. subtilis*, the primary mode of action is thought to be through the production of antibiotics, most of which are usually formed at the onset of sporulation (Handelsman *et al*., 1990). The effective bacterial isolates were found to be motile and produced catalase which confirmed its identity to the genus *Bacillus*. *Bacillus* spp. are known to be potential candidates for biocontrol of several plant pathogens (Mahadevamurthy *et al*. 1988 and Weller, 1988).

In the light of the above observation, it becomes clear that the bacterial isolates B5, B7 and B13 were effective in the suppression of foot rot disease. They outyielded the performance of other bacterial isolates and were far superior to the application of *T. harzianum* and *P. fluorescens* in many respects. The successful biocontrol with bacteria has been attributed to their capacity to produce antibiotics, competition for substrates, colonization and survival (Weller, 1988). The isolate B13 appeared to be the most ideal biocontrol agent being endowed with the capacity to reduce mortality of pepper plants

and extent of foliar blighting and to offer high residual systemic protection against *P. capsici*. It was effective both as live cells and cell free culture filtrates. It was also equipped with the ability to produce small quantities of the hydrolytic enzyme, cellulase. Further good survival was afforded by its capacity to form endospores. The isolate B5 was also effective in reducing both foliar and stem infection. The isolate B7 was found to have a dual role; disease suppression and growth promotion.

Such elite isolates of bacterial antagonists seem to have immense importance in nursery establishment in black pepper by warding off pathogens and boosting up crop growth. Development of disease free, vigorously growing pepper rooted cuttings is the stepping stone to successful management of foot rot pathogen. The selected bacterial isolates have to be further tested in the field to derive any lucid conclusions. Further work on elucidation of the exact mechanism of biocontrol, interaction effects of different antagonists, their spectrum of activity and development of fungicide resistant/tolerant strain and effective delivery systems would also be necessary. Even if the potent bacterial antagonist evolved through the present study cannot be taken as the sole means of canopy/stem protection from the foot rot pathogen in a perennial crop like pepper, they could find a worthy place as an important component of integrated disease management (IDM).

SUMMARY

SUMMARY

The present investigation was conducted with a view to isolate rhizobacteria with the potential to control foot rot, a devastating disease affecting black pepper caused by the soil borne fungus, *Phytophthora capsici*. The salient findings of the study are presented below.

1) Bacteria were isolated from the rhizosphere soils of wild pepper plants, healthy plants in diseased pepper plantations, pepper plants in disease free plantations, pepper plantations where organic farming is practiced, undisturbed forest soils and vermicompost. The population of bacteria in the different soils and vermicompost varied considerably, ranging from 13.67×10^4 to 168×10^4 cfu g^{-1}. High population of bacteria were recorded from the rhizosphere soils of healthy plants in diseased pepper plantations of Pepper Research Station, Panniyur and wild pepper plants of Punnamala region of Silent Valley forest.

2) *In vitro* screening revealed that only 15 out of the total 194 isolates exhibited antagonism to *P. capsici*.

3) In dual culture studies, isolates B14 and B1 were very effective in reducing mycelial growth of the pathogen offering 65.8 per cent and 48.33 per cent inhibition, respectively.

4) Inhibition of sporangial production by the pathogen was the highest with isolates B7, B9 and B13 (95.48%) followed by B5 and B8 (93.31%).

5) Three isolates, B5, B7 and B13 gave good suppression of infection by *P. capsici* on excised pepper shoots. Lesions did not develop in the leaves even after 10 days of inoculation of the pathogen on applying these isolates. Isolate B3 and B6 also reduced the disease in excised shoots by delaying the onset of disease and reducing size and rate of development of lesions.

6) In pot culture trial using rooted cuttings of the susceptible pepper variety, Karimunda, the three isolates viz., B5, B7 and B13 were very effective in reducing foliar blighting and mortality of plants due to foot rot and gave prolonged protection. B13 was the most effective in reducing death of vines to 57.14 per cent after 90 days of inoculation with the pathogen. B5 and B13 reduced the rate of spread of the disease (1.03 and 1.5 cm day^{-1}, respectively). The three effective isolates were obtained from suppressive soils of Kulathupuzha and Silent Valley.

7) The bacterial isolates, in general, were found to be more effective than the standard culture of *T. harzianum* procured from IISR, Kozhikode, *in vivo* though the latter showed significant inhibition of the pathogen *in vitro*. The isolates

B5, B7 and B13 were found to be far superior to *T. harzianum* in disease suppression.

8) The protection offered by *Pseudomonas fluorescens*, procured from TNAU, Coimbatore, was comparable to the best three bacterial isolates initially, yet, mortality of pepper plants steadily progressed and reached 100 per cent in 90 days after inoculation.

9) The cell free filtrates of the isolates B3 inhibited mycelial growth and sporangial production by the pathogen while that of the isolate B13 produced significant reduction in sporangial production *in vitro*.

10) The filtrates of B3 and B13 also delayed symptom initiation and reduced lesion size in excised pepper shoots.

11) In pot culture trial, the filtrate of B13 reduced rate of progress of the disease and foliar disease intensity in rooted pepper cuttings. Filtrates of B5 offered better foliar protection.

12) Mortality of pepper plants was lowest with application of filtrates of B13. Cell free filtrates of B3 and B7 also tended to reduce the death of vines.

13) Filtrates of *P. fluorescens* gave the maximum protection to foliage from infection by *P. capsici*. The application of

filtrates of *P. fluorescens* gave significant initial reduction in the disease after which mortality rate increased and reached level almost similar to that in B3 and B7.

14) Filtrates of *T. harzianum* did not offer much protection and there was a steady increase in the disease.

15) Isolate B7 was found to enhance growth of pepper plants in the absence of the pathogen. The isolate, thus had the dual function of disease suppression and growth promotion. Isolate B4 also promoted growth in pepper plants but did not offer biocontrol of the pathogen. *P. fluorescens* and *T. harzianum* did not have any growth promoting effect.

16) The isolate B13 was found to produce small quantities of cellulase.

17) All the bacterial antagonists and *P. fluorescens* showed a high population of 10^4-10^5 cfu g^{-1} in the rhizosphere of treated pepper plants. The highest survival of 10^6 cfu g^{-1} soil was recorded with *T. harzianum*.

18) All the effective bacterial antagonists were gram positive, motile, aerobic and sporulating rods with the ability to produce catalase. The bacterial isolates were thus found to conform to the genus *Bacillus*.

In the light of the above findings, the following conclusions are drawn:

The isolate B13 proved to be an ideal candidate for biocontrol of foot rot since

a) it provided prolonged protection to pepper plants from *P. capsici* (b) reduced foliar blighting (c) cell free filtrates also reduced mortality of plants due to foot rot (d) it produced small quantities of lytic enzyme, cellulase (e) it had good rhizosphere colonization and survival (f) it had the ability to form endospores and thus the capacity to tide over unfavourable conditions.

The isolate B5 as both cell suspension and cell free filtrates gave good foliar protection.

The isolate B7 had the dual function of biocontrol and growth promotion.

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* Originals not seen

APPENDICES

Appendix - I

Soil extract agar

Glucose	-	1.0 g
Dipotassium phosphate	-	0.5 g
Soil extract	-	100 ml
Agar	-	15.0 g
Distilled water	-	1 l

Soil extract prepared by steaming 1 kg garden soil in 1 l water in an autoclave for 30 min at 1.05 kg cm^{-2}.

Nutrient agar

Beef extract	-	3.0 g
Peptone	-	5.0 g
Sodium chloride	-	5.0 g
Agar	-	15.0 g
Distilled water	-	1 l

Carrot agar

Carrot agar	-	200 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Distilled water	-	1 l

Nutrient broth

Beef extract	-	3.0 g
Peptone	-	5.0 g
Sodium chloride	-	5.0 g
Distilled water	-	1 l

Potato dextrose agar

Potato	-	200 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Distilled water	-	1 l

Czapeks broth

Sodium nitrate	-	2.0 g
Dipotassium phosphate	-	1.0 g
Magnesium sulphate	-	0.5 g
Potassium chloride	-	0.5 g
Ferrous sulphate	-	0.01 g
Sucrose	-	30.0 g
Distilled water	-	1 l

Rosebengal streptomycin agar

Dextrose	-	10 g
Peptone	-	5 g
Potassium dihydrogen phosphate	-	1 g

Magnesium sulphate	-	0.5 g
Rosebengal	-	1 part in 30,000 parts of the medium
Agar	-	20 g
Distilled water	-	1 l

Semisolid nutrient agar

Beef extract	-	3.0 g
Peptone	-	5.0 g
Sodium chloride	-	5.0 g
Agar	-	7.0 g
Distilled water	-	1 l

Appendix - II

Lactophenol - cotton blue

Anhydrous lactophenol	-	67.0 ml
Distilled water	-	20.0 ml
Cotton blue	-	0.1 g

Anhydrous lactophenol prepared by dissolving 20 g phenol in 16 ml lactic acid and 31 ml glycerol.

Crystal violet

One volume saturated alcohol solution of crystal violet in four volumes of one per cent aqueous ammonium oxalate.

Gram's iodine

Iodine crystals	-	1.0 g
Potassium iodide	-	2.0 g
Distilled water	-	300 ml

Safranin

Ten ml saturated solution of safranin in 100 ml distilled water.

Malachite green

Malachite green	-	5 g
Distilled water	-	100 ml

BIOLOGICAL CONTROL OF FOOT ROT OF BLACK PEPPER (*Piper nigrum* L. Walp) WITH ANTAGONISTIC BACTERIA FROM RHIZOSPHERE

By

JUBINA. P. A.

Abstract of the Thesis

Submitted in partial fulfilment of the requirement

for the degree

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Department of Plant Pathology

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Vellayani, Thiruvananthapuram

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ABSTRACT

An attempt was made at the Department of Plant Pathology, College of Agriculture, Vellayani to screen and identify antagonistic soil bacteria with potentiality to suppress *Phytophthora capsici*, the incitant of foot rot or quick wilt of black pepper. Bacteria were isolated from the rhizosphere of wild pepper plants, pepper plants in disease free plantations, healthy plants in diseased pepper plantations, pepper plantations where organic farming is practiced, undisturbed forest soils and vermicompost. Population of bacteria in the various soils and vermicompost showed great variability ranging from 13.67×10^4 to 168×10^4 cfu g^{-1}. High population of bacteria was recorded from the rhizosphere soils of healthy plants in diseased pepper plantation of Pepper Research Station, Panniyur and wild pepper plants in virgin forest of Punnamala region of Silent Valley.

Of the 194 isolates, only 15 isolates showed antagonism to *P. capsici in vitro*. Three isolates B5, B7 and B13 were highly effective in *in vitro* inhibition of sporangial production by the pathogen and disease suppression in rooted cuttings of the susceptible pepper variety, Karimunda. Isolate B13 was found to be the most ideal candidate with the ability to reduce mortality of pepper plants and foliar blighting and to provide continued

protection for a longer period. The isolate was effective as cell free filtrates also and was found to produce small quantities of the enzyme, cellulase. Isolate B5 also reduced the disease in pepper plants. The live cells and cell free filtrate of this isolate afforded good foliar protection against *P. capsici*. The isolate B7 was found to have a dual role : disease suppression and growth promotion. All these isolates had good survival in the rhizosphere of pepper plants with population of 10^4 to 10^5 cfu g^{-1} soil. The effective isolates were gram positive, aerobic, sporulating, rods with ability to produce catalase and were found to conform to genus *Bacillus*.

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