INDUCTION OF DEFENSE RELATED PROTEINS BY *PSEUDOMONAS FLUORESCENS* FORMULATION AGAINST MAJOR FUNGAL PATHOGENS OF SOME SOLANACEOUS VEGETABLE CROPS

Thesis submitted in part fulfilment of the requirements for the degree of Doctor of Philosophy (Agriculture) in Plant Pathology to the Tamil Nadu Agricultural University, Coimbatore

by

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CERTIFICATE

This is to certify that the thesis entitled "**INDUCTION OF DEFENSE RELATED PROTEINS BY *PSEUDOMONAS FLUORESCENS* FORMULATION AGAINST MAJOR FUNGAL PATHOGENS OF SOME SOLANACEOUS VEGETABLE CROPS**" submitted in part fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) IN PLANT PATHOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a record of **bona fide** research work carried out by **Mr. V. RAMAMOORTHY** under my supervision and guidance. The part of the research program has been awarded by **Indian Society of Mycology and Plant Pathology** and **Indian Phytopathological Society, Southern Zone** and published in leading journals of National and International journals.

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Abstract

ABSTRACT

"INDUCTION OF DEFENSE RELATED PROTEINS *BY PSEUDOMONAS FLUORESCENS* FORMULATION AGAINST MAJOR FUNGAL PATHOGENS OF SOME SOLANACEOUS VEGETABLE CROPS"

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Studies were taken up on induction of defense-related proteins by *Pseudomonas fluorescens* and development of suitable bacterial formulation for the management of major fungal diseases of tomato, chilli and brinjal.

Among twenty strains characterized, two strains *viz.*, PFATR and KKM1 were identified *as Pseudomonas putida* and the remaining 18 strains belonged to *P. fluorescens*. Among these strains, *P. fluorescens* Pf1 was found to increase the vigour index of tomato, chilli and brinjal and also inhibited the mycelial growth of *Pythium aphanidermatum* under *in vitro* conditions and suppressed the damping-off incidence under greenhouse conditions. *P. fluorescens* Pf1 also showed maximum inhibitory effect on *Fusarium oxysporum* f. sp. *lycopersici*, *F. solani* and *Colletotrichum capsici in vitro* and effectively reduced wilt incidence in tomato and brinjal and fruit rot incidence in chilli under greenhouse conditions. *P. fluorescens* Pf1 produced various inhibitory metabolites such as 2, 4-diacetyl phloroglucinol, siderophores, hydrogen cyanide, salicylic acid, β-1,3-glucanase and chitinase.

Pre-treatment of tomato and chilli plants with *P. fluorescens* Pf1 induced activities of phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), β-1,3-glucanase and chitinase and increased accumulation of phenolics in response to infection by pathogens. Expression of PO isoforms (PO1 to PO5) and PPO isoforms (PPO1 to PPO3) was found at higher levels in *P. fluorescens* Pf1 treated tomato seedlings in response to infection by *P. aphanidermatum*. Similarly, in chilli, expression of PO isoform (PO1) and PPO isoform (PPO1) was very clear in *P. fluorescens* Pf1-treated seedlings in response to infection by *P. aphanidermatum*. In addition, induction of 41 kDa chitinase was noticed in *P. fluorescens*-treated tomato plants in response to infection by *P. aphanidermatum*.

Induction of new PO isoform, PO1 and PPO isoform, PPO1 and higher level expression of PPO2 was noticed in *P. fluorescens* Pf1-treated tomato plants in response to infection by *F. oxysporum* f. sp. *lycopersici*. Western blot analysis revealed that induction of 45 and 46 kDa chitinase isoforms was noticed in *P. fluorescens* Pf1-treated tomato plants in response to infection by *F. oxysporum* f. sp. *lycopersici*. Expression of 33 kDa thaumatin like proteins (TLP) was observed in all induction treatments *viz.*, bacterized plants challenged with *F. oxysporum* f. sp. *lycopersici*, plants treated with *P. fluorescens* alone and plants inoculated with *F. oxysporum* f. sp. *lycopersici* except in untreated control plants. Induction of PO isoform PO1 and PPO isoform PPO1 was noticed in *P. fluorescens*-treated chilli plants in response to infection by *C. capsici*. Induction of 42 kDa chitinase was noticed at higher levels in all induction treatments.

Incorporation of chitin in the growth medium recorded the maximum population of *P. fluorescens* Pf1. Among the various carrier materials tested for the development of formulations, talc+gypsum containing *P. fluorescens* Pf1 multiplied in chitin amended

medium supported the maximum survival of *P. fluorescens* Pf1. Seed treatment with talc+gypsum based formulation containing *P. fluorescens* Pf1 multiplied in chitin amended medium was effective for the management of damping-off of tomato, chilli and brinjal both under glasshouse conditions and field conditions. Seed and soil application of the same formulation was also found effective for the management of wilt in tomato and brinjal and fruit rot of chilli under field conditions. Moreover, increased yield was noticed in *Pseudomonas* formulation treated plants. Thus, the present study indicates that besides direct antagonism through the production of inhibitory metabolites and plant growth promoting activity, inducing resistance mechanisms by *P. fluorescens* Pf1 in plants in response to infection by pathogens is a novel strategy in the management of major fungal diseases of solanaceous vegetable crops.

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Introduction

CHAPTER I

INTRODUCTION

Vegetable forms the most important component of a balanced diet for human beings. Our country is the world's second largest producer of vegetable next to China. However, the per capita consumption in India is only about 140g which is far below the minimum dietary requirement of 280g per day per person. Solanaceous vegetable crops such as tomato, chillies (hotpepper) and brinjal (eggplant) are the major vegetable crops grown all over the country. In India, tomato, chilli and brinjal are grown in an area of 4.6, 9.6 and 4.9 lakh ha with the annual production of 82.7, 9.5 and 78.8 lakh tonnes respectively. In Tamil Nadu, they are cultivated in 25120, 80240 and 8120 ha with an annual production of 3.76, 0.56 and 1.62 lakh tonnes respectively (Anon, 1999). Chilli is an important spice cum vegetable crop.

Solanaceous vegetable crops are affected by several fungal, bacterial and viral diseases. Among the various fungal diseases, damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. brings about complete devastation of the crops in the nursery stage (Sivan *et al.*, 1984; Krishnamoorthy and Bhaskaran, 1990). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hans. in tomato and *Fusarium solani* (Mart.) Sacc. in brinjal are considered as potential threats to cultivation of these crops. Fruit rot of chilli caused by *Colletotrichum capsici* (Syd.) Butler and

Bisby is the most destructive disease of the crop resulting in loss in quantity and quality of produce (Madhukar and Reddy, 1991).

Fungicides are commonly used for the management of plant diseases, however the use of fungicides has created several problems mainly development of fungicide resistant strains, environmental pollution and human health hazards. Hence, the use of fungicides for the management of plant diseases is not a panacea. Biological control is one of the alternative methods to chemical control and it is safe, effective, economical and eco-friendly method for the management of plant diseases. There are growing awareness about the use of biocontrol agents such as *Trichoderma* spp. and fluorescent pseudomonads in the recent past to control diseases in different crops (Papavizas and Lewis, 1989; Whipps and Lumsden, 1991; Vidhyasekaran and Muthamilan, 1999; Zehnder *et al.,* 2001). Research work conducted in India proved that these biocontrol agents have been employed successfully to manage the plant diseases (Jeyarajan *et al.*, 1994; Vidhyasekaran and Muthamilan, 1995; 1999; Vidhyasekaran *et al.,* 1997a, b).

Fluorescent pseudomonads are non-pathogenic, root-colonizing rhizobacteria present on the root surface of many plants. In 1970's, Cook and Rovira (1976) reported the direct antagonism of fluorescent pseudomoands against soil-borne pathogens. Subsequently the efficacy of fluorescent pseudomonads against various plant pathogens was reported by research workers in different countries (Howie and Suslow, 1991; Maurhofer *et al.*, 1995; Vidhyasekaran *et al.,* 1997a, b). In the last decade, another dimension of fluorescent pseudomonads in disease control by induction of systemic

resistance in the host plant was found (Van Peer *et al.*, 1991; Wei *et al.*, 1991). This plant-mediated defense reaction due to prior application of fluorescent pseudomonads in response to infection by pathogen is a novel approach in the management of plant diseases (Van Loon *et al.*, 1998). This is referred as inducing systemic resistance (ISR) (Kloepper *et al.*, 1992). Seed treatment and soil application of fluorescent pseudomonads trigger the host plant to produce signal molecules which is translocated to the infection site where signal transduction takes place leading to the synthesis of various defense-related genes encoding proteins which in turn arrest further invasion of the pathogen (Kloepper *et al.*, 1992; Ton *et al.*, 2001). In addition to direct antagonism and induced systemic resistance, certain fluorescent pseudomonads exert beneficial effects on plant by increasing the germination and growth. Thus they are called as plant growth promoting rhizobacteria (PGPR).

Rhizobacteria-mediated induced resistance was found to be effective against *P. aphanidermatum* in cucumber in which rhizobacteria remained spatially separated from the challenging pathogen (Zhou and Paulitz, 1994). It has also been reported that induction of systemic resistance by fluorescent pseudomonads has been effective against several pests and diseases attacking the crop (Hoffland *et al.*, 1996; Ramamoorthy *et al.*, 2001; Zehnder *et al.*, 1999; 2001). The broad-spectrum systemic resistance induced by selected strains of fluorescent pseudomonads is gaining importance in crop protection (Hoffland *et al.*, 1996; 1997; Zehnder *et al.*, 2001). Use of single strain having broad-spectrum activity against multiple pests is also beneficial.

With this background, the present study was undertaken with the following objectives.

1. To characterize and evaluate the efficacy of fluorescent pseudomonads on plant growth promotion and inhibition of major fungal pathogens of solanaceous vegetable crops under laboratory conditions.

2. To evaluate the performance of selected fluorescent pseudomonads for the management of major diseases of solanaceous vegetable crops under greenhouse conditions.

3. To study the induction of defense-related proteins induced by promising isolate of fluorescent pseudomonads in response to infection by major fungal pathogens attacking the solanaceous vegetable crops.

4. To evaluate the suitable carriers for the development of bio-formulations.

5. To evaluate efficacy of bioformulations for the management of major fungal diseases of solanaceous vegetable crops under field conditions.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Solanaceous vegetable crops mainly tomato, chilli and brinjal are predominantly grown in all parts of India. There are several production constraints among which losses due to pests and diseases are much concerned. Among the various diseases, damping-off is the most devastating disease in the nursery stage. In the main field, Fusarium wilt is more serious. With regard to chilli, fruit rot is the prominent disease which causes reduction in the market value of the produce.

2. 1. Importance of the diseases

2. 1. 1. Seedling disease

Pythium aphanidermatum is a serious soil-borne pathogen inciting damping-off disease in solanaceous vegetable crops such as tomato, chilli and brinjal. Occurrence of damping-off disease in solanaceous vegetable crops leads to complete devastation of seedlings in the nursery stage itself (Krishnamoorthy and Bhaskaran, 1990; Sivan *et al.*, 1984). Quick germination of sporangia of *P. aphanidermatum* in 1.5 to 2.5 h (Nelson, 1987; Osburn *et al.*, 1989) followed by immediate infection in seeds or roots and rotting of tissues make management of the disease very difficult (Whipps and Lumsden, 1991). Sowmini Rajagopalan (1961) reported that 20 days old chilli seedlings were infected by various isolates of *Pythium* pathogen. Several isolates of *P. aphanidermatum* isolated from infected chilli seedlings caused 90 per cent damping-off incidence (Devadoss, 1971; Emayavaramban, 1994; Manoranjitham, 1997).

2. 1. 2. Wilt

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* and fusarium wilt of brinjal caused by *Fusarium solani* are also important soil-borne diseases. *Fusarium* spp. greatly reduces yield in the field due to high incidence of the wilt disease. Fusarium wilt of tomato is recognized as one of the most typical wilt diseases thoroughly studied all over the world (Beckman, 1987). Butler (1918) first reported this disease in Pusa (Bihar) in India. The wilt incidence was reported to be the maximum of 25 per cent in Tamil Nadu in various cultivars of tomato (Kapoor, 1988). Lukyanenko (1991) stated that fusarium wilt of tomato is the most devastating disease resulting in 10-50 per cent crop loss.

2. 1. 3. Anthracnose and fruit rot

Anthracnose and fruit rot caused by *Colletotrichum capsici* is a common and destructive disease in chilli. Bansal and Grover (1969) reported that the fruit rot of chilli caused 10-35 per cent yield loss in Haryana. Thind and Jhooty (1985) reported that the prevalence of the disease ranged from 66 to 84 per cent in Punjab. Infection of the pathogen leads to the low levels of sugars and pungency and reduces the market value of the product (Subbaraja, 1981; Madhukar and Reddy, 1991). Rajavel (2000) found that the pathogen caused up to 50 per cent yield loss in Tamil Nadu.

2. 2. Symptomatology

2. 2. 1. Damping-off

P. aphanidermatum causes seed-rot and pre-and post-emergence damping-off in tomato, chilli and brinjal. Seeds may rot before sprouting (seed-rot). A seedling rots after sprouting but before emerging from the soil is referred as pre-emergence damping-off. In post-emergence damping-off, the pathogen attacks the collar regions of emerged seedlings resulting in girdling and toppling of seedlings (Singh, 1987) (Plate 1).

2. 2. 2. Wilt

F. oxysporum f. sp. *lycopersici* and *F. solani* cause wilt disease in tomato and brinjal respectively. Infection occurs from soil-borne inoculum via roots and pathogen spreads rapidly through the host xylem and induces dark brown discolouration extending from the roots up to the leaf petioles. Leaves turn yellow and wilt and curling downward towards the stem (Plate 2). Eventually the plant wilts and dies (Singh, 1987).

2. 2. 3. Fruit rot

Anthracnose of chilli occurs in two forms such as die back and fruit rot. In die back, the pathogen attacks during flowering stage of the crop. The disease appears as necrosis of tender twigs from tip backwards. In fruit rot, fruits turning red colour are more frequently affected. Small black circular spots appear on infected fruits which later become more or less elliptical. Numerous pycnidia are scattered on the infected parts and in advanced stage the affected fruit turns into dirty grey color which is unfit for consumption (Plate 3). The fungus is seed-borne (Padaganur and Naik, 1991; Patil *et al.*, 1993).

Plate 1. Damping-off of tomato

Plate 2. Fusarium wilt of tomato

Plate 3. Fruit rot of Chilli

Plate 1. Damping-off of tomato

Plate 2. Fusarium wilt of tomato

Plate 3. Fruit rot of chilli

2. 3. Characterization of fluorescent pseudomonads

Fluorescent pseudomonads are saprophytic root-colonizing bacteria. Fluorescent pseudomonads associated with plants include *P. fluorescens*, *P. putida, P. aeruginosa* and *P. aureofaciens*. Of these, *P. aeruginosa* forms a tight cluster and grow above 41°C (Hildebrand *et al.*, 1992). Most of the plant associated *Pseudomonas* spp. belong to *P. fluorescens* and *P. putida* complex. There was no clear distinction between *P. fluorescens* and *P. putida* (Sheath *et al.*, 1981). However, these two species are identified based on trehalose utilization and gelatin liquefaction. In this, *P. fluorescens* exhibits positive for both the tests whereas *P. putida* shows negative response (Hildebrand *et al.,* 1992). The species of fluorescent pseudomonads are again grouped in different biovars and subgroups based on similarity in biochemical tests (Champion, 1980; Barrett *et al.*, 1986). Many of the fluorescent pseudomonads, predominantly *P. fluorescens*, were isolated from suppressive soil for the management of soil-borne diseases (Weller and Cook, 1986; Mills *et al.*, 1989; Stephens *et al.,* 1993; Ongena *et al.,* 1999).

2. 4. Plant growth promoting activity of fluorescent pseudomonads

As certain fluorescent pseudomonads increase plant growth and yield by production of various plant growth-promoting substances they are collectively called as plant growth promoting rhizobacteria (PGPR) (Dubeikovsky *et al.*, 1993). Production of various plant growth promoting substances such as indole acetic acid (IAA) and gibberellin by fluorescent pseudomonads are attributed in increasing plant growth and yield (Dubeikovsky *et al.* 1993; Gupta *et al.*, 2000).

Seed bacterization with specific rhizobacteria in potato and radish colonized the root surface and increased the plant growth and these bacteria are first termed as PGPR (Kloepper and Schroth, 1978; 1981). Seed treatment in rice and cotton with *P. fluorescens* resulted in increased plant growth and vigour (Lin *et al.*, 1992). Schippers *et al.* (1987) found that an increase in fresh weight of root and shoot of tomato, cucumber, lettuce and potato was noticed as a result of bacterization with *Pseudomonas* strains. Kloepper *et al.* (1988) reported that seed treatment with beneficial rhizobacteria in canola resulted in enhanced plant growth and yield. Increased germination of sugarbeet by seed treatment with *Pseudomonas* strains was noticed when compared with untreated seeds (Williams and Asher, 1996). Increase in plant growth and yield was noticed by seed and foliar application of *P. fluorescens* in groundnut (Meena, 2000), rice (Nandakumar *et al.*, 2001) and sugarcane (Viswanathan and Samiyappan, 2001b).

2. 5. Fluorescent pseudomonads as potential biocontrol agents

The role of fluorescent pseudomonads in the biological control of soil-borne diseases was reported as far back as 1976 by Cook and Rovira (1976). Several fluorescent pseudomonads have been isolated from different crops and their biocontrol activity against soil-borne and foliar pathogens has been reported by several workers (Hebber *et al.*, 1991; Vidhyasekaran *et al.*, 1997a, b; Vidhyasekaran and Muthamilan, 1999; Nandakumar *et al.*, 2001; Viswanathan and Samiyappan, 2001b).

The control of various *Pythium* fungi by specific fluorescent pseudomonads has been well documented (Howie and Suslow, 1991; Maurhofer *et al.*, 1995; Dunne *et al.*, 1998). Howell and Stipanovic (1980) isolated fluorescent pseudomonads which were

found to be effective in inhibiting *Pythium ultimum* infecting the cotton seedling. Weststeijn (1990) found that soil application of fluorescent pseudomonads suppressed the infection of *P. ultimum* in tulip. Fluorescent pseudomonads isolated from suppressive soil were able to induce suppressiveness in conducive soil for crown and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Lemanceau and Alabouvette, 1991). In cotton, seedling disease caused by *Rhizoctonia solani* and *P. ultimum* was suppressed by application of *Pseudomonas* spp. (Hagedorn *et al.*, 1993). Similarly, application of endophytic bacteria by stem injection reduced the root rot caused by *R. solani* and vascular wilt caused by *F. oxysporum* f. sp. *vasinfectum* (Chen *et al.*, 1995). Seed treatment with *P. fluorescens* strain 63-28 restricted the growth of *P. ultimum* (Benhamou *et al.*, 1996a, b) and *F. oxysporum* f. sp. *lycopersici* in tomato (M'Piga *et al.*, 1997). Seed treatment and soil application of *P. fluorescens* reduced root rot of blackgram caused by *Macrophomina phaseolina* (Jayashree *et al.*, 2000; Shanmugam *et al.*, 2001) and panama wilt of banana caused by *F. oxysporum* f. sp. *cubense* (Raguchander *et al.*, 1997).

Fluorescent pseudomonads are also found to survive in phyllosphere (Wilson *et al.*, 1992) and reduce the infection of foliar pathogens. *P. cepacia* was effective for the management of Cercospora leaf spot of groundnut and Alternaria leaf spot of sunflower (Blakeman and Fokkema, 1982). In mulberry, application of *Pseudomonas maltophila* reduced the leaf spot caused by *Cercospora moricola* (Sukumar and Ramalingam, 1986). Seed and foliar application of *P. fluorescens* reduced sheath blight of rice caused by *R. solani* (Vidhyasekaran and Muthamilan, 1999; Nandakumar *et al.*, 2001). In groundnut, seed treatment and foliar application of *P. fluorescens* reduced the late leaf

spot caused by *Cercosporidium personatum* (Meena *et al.*, 2000a).

2. 6. Mode of action of fluorescent pseudomonads

Fluorescent pseudomonads suppress the pathogens by various modes of actions. They suppress the pathogen directly by antagonism through the production of various secondary metabolites (Haas *et al.*, 2000) or indirectly by inducing plant-mediated defense reactions (Van Loon *et al.*, 1998).

2. 6. 1. Rhizosphere colonization

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

Scher *et al.* (1985) reported that disease suppression by fluorescent pseudomonad depends mainly on its ability to colonize rhizosphere. Introduction of sss gene encoding rhizosphere colonization ability into poor colonizer strain of *P. fluorescens* WCS 307 increased competitive rhizosphere colonization ability in tomato root tip resulting in increased protection against *F. oxysporum* f. sp. *radicis-lycopersici* (Dekkers *et al.*, 2000).

2. 6. 2. Antibiosis

Fluorescent pseudomonads suppress the pathogens by antibiosis through the

production of various antibiotic substances such as 2,4-diacetyl phloroglucinol, phenazine-1-carboxylic acid, oomycin A, oxychlororaphine, pyoluteorin, pyrrolnitrin and pyocyanine (Howie and Suslow, 1991; Rosales *et al.*, 1995). In wheat, suppression of take all of wheat caused by *Gaeumannomyces graminis* var *tritici* was mainly due to the production of phenazine-1-carboxylic acid (Thamashow and Weller, 1988). Howie and Suslow (1991) reported that reduction in seedling inoculum of *P. ultimum* by *P. fluorescens* Hv37R2 in cotton was mainly attributed to the production of antibiotic oomycin A. Oomycin A was found to reduce the secondary inoculum of *P. ultimum*. Similarly, suppression of black root rot caused *by Thielaviopsis basicola* and take-all of wheat by *P. fluorescens* strain CHAO was mainly due to the production of 2,4-diacetyl phloroglucinol (Keel *et al.*, 1990). This antibiotic was also inhibitory to *R. solani* and *P. ultimum in vitro*. Application of purified 2,4-diacetyl phloroglucinol inhibited the infection of *Pythium* in sugarbeet (Vincent *et al.*, 1991). *P. fluorescens* strain 5-2/4 produced the maximum amount of 2,4-diacetyl phloroglucinol and protected tomato seedling from damping-off incidence caused by *P. ultimum* when compared to wild strain (Hultberg *et al.*, 2000). Insertion of PCA gene which encodes for the synthesis of phenazine-1-carboxylic acid (PCA) in wild-type *P. fluorescens* significantly improved its ability to reduce damping-off disease of pea seedlings caused by *P. ultimum* and level of PCA biosynthesis was correlated with biocontrol efficacy and the persistence of bacterial colonies in soil ecosystem (Timms-Wilson *et al.*, 2000).

2. 6. 3. Siderophores

Siderophores are extracellular, low molecular weight substances which selectively

complex iron with high affinity. Fluorescent pseudomonads produce siderophores such as pseudobactin and pyoverdine which chelate the iron available in the soil and make it unavailable to pathogen thus the pathogen dies for want of iron (Kloepper *et al.*, 1980; Lemanceau *et al.*, 1992).

Loper (1988) reported that fluorescent siderophore production by *P. fluorescens* contributed to its antagonistic mode of action against *P. ultimum* attacking cotton seedlings whereas the siderophore minus strain did not inhibit the pathogen. Implication of pseudobactin production by fluorescent pseudomonads in biological control was well-demonstrated (Weller, 1988; Loper and Buyer, 1991). Pyoverdine production by *P. fluorescens and P. putida* was found to be associated with inhibition of *Phytophthora parasitica in vitro* (Yang *et al.*, 1994).

2. 6. 4. Lytic enzymes

Lytic enzyme production by biocontrol agents is an another factor which is associated with parasitization of fungal hyphae. Fluorescent pseudomonads also produce lytic enzymes such as chitinases and β-1,3-glucanases which degrade chitin and glucan fragments of fungal cell wall respectively.

Serratia marcescens which was found to possess chitinolytic activity was able to release N-acetyl D-glucosamine from cell walls of *Sclerotium rolfsii*. When *S. rolfsii* was sprayed with partially purified chitinase from *S. marcescens*, rapid and extensive bursting of the hyphal tips was observed. This chitinase preparation was effective in reducing disease incidence caused by *S. rolfsii* in bean and *R. solani* in cotton under greenhouse

conditions (Chet *et al.*, 1990). Lytic enzyme production by *P. fluorescens* was involved in the ultrastructural and morphological changes of mycelium of *Verticillium dahliae* and inhibition of mycelial growth (Berg and Ballin, 1994). Positive correlation was observed in chitinase production by isolates of *P. fluorescens* and suppression of sheath blight incidence in rice caused by *R. solani* (Velazhahan *et al.*, 1999). Culture filtrate of *P. fluorescens* grown in chitin-amended medium which is substrate for the production of chitinase significantly inhibited the mycelial growth *of Colletotrichum falcatum* causing red rot of sugarcane *in vitro* (Viswanathan and Samiyappan, 2001a).

2. 6. 5. Hydrogen cyanide production

Production of hydrogen cyanide (HCN) by certain strains of fluorescent pseudomonads has also been involved in the suppression of soil-borne pathogens (Voisard *et al.*, 1989). Production of HCN by *P. fluorescens* strain CHAO was implicated in suppression of black root rot of tobacco (Stutz *et al.*, 1986) and take-all of wheat (Defago *et al.*, 1990). HCN production by *P. fluorescens* inhibited the mycelial growth of *Pythium in vitro* (Weststeijn, 1990).

2. 7. Induced resistance

In addition to direct antagonism and plant growth promotion, certain isolates of fluorescent pseudomonads, interestingly bring about induction of systemic resistance against infection by pathogen (Chen *et al.*, 2000; Pieterse *et al.*, 2001; Ramamoorthy *et al.*, 2001; Zehnder *et al.*, 2001).

2. 7. 1. Concepts and inducing agents

Plants have been endowed with various latent defense mechanisms. Inducing resistance by activation of latent defense mechanism is thought to be a new approach in plant disease management. Induced resistance is defined as enhancement of plant's defensive capacity against broad-spectrum of pathogens and pests that is acquired after appropriate stimulation (Hammerschmidt and Kuc, 1995; Kloepper *et al.*, 1992). The phenomenon of induced resistance has been variously described as systemic acquired resistance (SAR) and induced systemic resistance (ISR). In this, the term systemic resistance refers the point that protection is not only confined to the treated parts but also extends into untreated plant parts and often newly developing plant parts. Induction of systemic resistance by fluorescent pseudomonads is referred as ISR whereas that by other agencies is called as SAR (Van Loon *et al.*, 1998; Pieterse *et al.*, 2001). However, induced resistance is used as general term which refers to all types of defense reactions (including locally and systemically induced resistance) (Hammerschmidt *et al.*, 2001).

The biotic inducers of systemic resistance include virulent pathogens, non-pathogens, elicitors of fungal cell wall metabolites and rhizobacteria (Van Loon *et al.*, 1998). Abiotic agents are chemicals such as salicylic acid (SA), ethylene, dichloro-isonicotinic acid, benzo thiadiazole-7-carbothioic acid-S-methyl (BTH) (Gorlach *et al.*, 1996; Sticher *et al.*, 1997; Oostendorp *et al.*, 2001), β-amino-butyric acid (BABA) (Jakab *et al.*, 2001) and Oxycom™ which consisted of combination of plant nutrients and an active oxygen generator (Kim *et al.*, 2001).

Utilization of pathogenic organisms as inducing agents has not been successful under field conditions. Generally, the duration of protection is less following induction of pathogen and prior inoculation of pathogen may provide a source of secondary inoculum (Wei *et al.,* 1991). The virulent pathogen might suppress or not activate the expression of SAR during successful infection (Daly, 1972; Gras *et al.,* 1979; Heath, 1982). But the use of PGPR as an inducer of systemic resistance in crop plants against different pathogens has been demonstrated under field conditions (Wei *et al.,* 1991; 1996; Viswanathan and Samiyappan, 2001b; Zehnder *et al.*, 2001). The utilization of natural fluorescent pseudomonads strains as inducers of plant defense responses may increase the chances of their applicability and offers a practical way to immunize the crop plants.

2. 7. 2. Induction of systemic resistance by fluorescent pseudomonads

The induction of systemic resistance by selected strains of fluorescent pseudomonads in crop plants is an additional mechanism by which these strains protect crops systemically against pests and diseases (Murphy *et al.,* 2000; Pieterse *et al.*, 2001; Ramamoorthy *et al.*, 2001; Zehnder *et al.*, 2001).

2. 7. 2. 1. Induced resistance against damping-off

Earlier it was reported that application of fluorescent pseudomonads suppressed the soil-borne pathogens mainly by direct antagonism (Weller and Cook, 1986). Later during 1991 it was found that certain strains of *P. fluorescens* induced plant-mediated defense mechanisms against infection of soil-borne pathogens (Van Peer *et al.*, 1991). ISR by fluorescent pseudomonads was earlier investigated by following split root system

in carnation and cucumber (Van Peer *et al.*, 1991; Zhou and Paulitz, 1994) in which the root was divided into two halves. In one half of the root, bacterium was applied and in another half the root, pathogen was inoculated and induction of phenolic compounds by bacterium was observed in both halves of roots.

Seed bacterization with *P. fluorescens* strain 63-28 induced a set of plant defense reactions against *P. ultimum* and *F. oxysporum* f. sp. *pisi* in pea (Benhamou *et al.*, 1996a; b). Application of resistance inducing chemicals such as 5-nitrosalicylic acid, dichloro-isonicotinic acid, amino-isobutyric acid and lichenin in combination with *P. fluorescens* enhanced the systemic resistance against *R. solani* causing pre- and post-emergence damping-off in cucumber and bean (Kataria *et al.*, 1997). Systemic resistance induced by fluorescent pseudomonads impairs pre- and post-infection of *P. aphanidermatum* causing root rot and crown rot in cucumber (Chen *et al.*, 1999; 2000). *P. putida* isolate BTP1 and its siderophore producing strains did not inhibit the mycelial growth of *P. aphanidermatum in vitro* but reduced the root rot disease incidence under field conditions indicating that the antibiosis and siderophore production was not the only mechanism of biocontrol of root rot of cucumber. Whereas, application of this isolate induced the host defense mechanisms in cucumber in response to infection by *P. aphanidermatum* (Ongena *et al.*, 1999; 2000). This implies that induced resistance plays a predominant role in suppression of *Pythium* infection.

2. 7. 2. 2. Induction of resistance against fusarium wilt

In tomato, fluorescent pseudomonads suppressed the fusarium wilt and disease suppression was related to both microbial antagonism and induced resistance (Duijff *et*

al., 1998). In carnation, application of *Pseudomonas* sp. strain WCS 417r has protected the plants systemically against fusarium wilt caused by *F. oxysporum* f. sp. *dianthi* (Van Peer *et al.*, 1991). Induction of systemic resistance by *P. putida* strain 89B-27 and *Serratia marcescens* strain 90-166 has reduced the fusarium wilt of cucumber incited by *F. oxysporum* f.sp. *cucumerinum* (Liu *et al.*, 1995a). Similarly, pretreatment with *P. fluorescens* protects radish through induction of systemic resistance against fungal root pathogen, *F. oxysporum* f. sp. *raphani* (Hoffland *et al.*, 1996; Leeman *et al.*, 1996). Culture filtrate of *B. subtilis* strain FZB-G was found to induce systemic resistance in tomato against *F. oxysporum* f. sp. *lycopersici* causing wilt disease (Gupta *et al.*, 2000).

2. 7. 2. 3. Induction of systemic resistance against foliar pathogens

PGPR strains applied as seed treatment resulted in significant reduction in anthracnose disease caused by *Colletotrichum orbiculare* in cucumber (Wei *et al.*, 1991; 1996). In pea, seed bacterization with *P. fluorescens* and *P. aeruginosa* in combination with aerial spray of either bacterial cell suspension or Neemazal, a product of neem induced resistance and systemically protected the pea plants against *Erysiphe pisi* causing powdery mildew disease (Singh *et al.*, 2000). In rice, seed treatment followed by root dipping and foliar spray with *P. fluorescens* showed higher induction of ISR against sheath blight pathogen, *R. solani* (Vidhyasekaran and Muthamilan, 1999; Nandakumar *et al.*, 2001). Seed treatment with *Bacillus pumilus* (SE 34) and *Serratia marcescens* strain 90-166 induced systemic resistance against fusiform rust incited by *Cronartium quercuum* f. sp. *fusiforme* in loblolly pine (Enebak and Carey, 2000). In cucumber, PGPR strains with or without application of methyl bromide showed a higher level of induced

resistance against foliar diseases *viz.*, angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* and anthracnose caused by *C. orbiculare* (Zehnder *et al.*, 2000). In groundnut, seed treatment and foliar spray with *P. fluorescens* Pf1 induced systemic resistance against late leaf spot by inducing various defense mechanisms in the host plant (Meena *et al.*, 2000b). Similarly, in sugarcane Viswanathan and Samiyappan (2001b) reported PGPR-mediated ISR against red rot disease.

2. 8. Spectrum of protection by fluorescent pseudomonads

Fluorescent pseudomonads mediated ISR has broad-spectrum activity and has long lasting effect against several fungal, bacterial and viral diseases and insect and nematode pests (Wei *et al.*, 1991; 1996; Hoffland *et al.*, 1996; 1997; Kuc, 2001; Zehnder *et al.*, 2001).

Induction of systemic resistance by *P. putida* strain 89 B-27 and *S. marcescens* strain 90-166 against anthracnose of cucumber was established by Wei *et al.* (1991). Later studies showed that the same PGPR strains induced systemic protection against angular leaf spot caused by *P. syringae* pv. *lachrymans* (Liu *et al.*, 1993a), fusarium wilt incited by *F. oxysporum* f.sp. *cucumerinum* (Liu *et al.*, 1993b) and cucurbit wilt caused by *Erwinia tracheiphila* (Kloepper *et al.*, 1993). In addition, the same bacterial strain has also been effective in controlling striped cucumber beetle *Acalyma vittatum* and spotted cucumber beetle *Diabrotica undecimpunctata howardi* (Zehnder *et al.*, 1997). Parallel experiments conducted by Hoffland *et al.* (1996) revealed that seed treatment with *P. fluorescens* strain WCS 417 has protected radish through induction of systemic resistance not only against the fungal root pathogen *Fusarium oxysporum* f. sp. *raphani*,

but also against the avirulent bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* and fungal leaf pathogens *Alternaria brassicola* and *F. oxysporum.* This implies that same PGPR strain can induce resistance against multiple pathogens in the same crop. Similarly, *P. fluorescens* Pf1 induced resistance against various pathogens *viz., R. solani* (Radja Commare, 2000; Nandakumar *et al.*, 2001) and *Xanthomonas oryzae* pv. *oryzae* in rice (Vidhyasekaran *et al.*, 2001), tomato spotted wilt virus in tomato (Kandan, 2000) and *C. falcatum* in sugarcane (Viswanathan and Samiyappan, 2001b). In groundnut, application of *P. fluorescens* Pf1 induced resistance against leaf spot caused by *C. personatum* (Meena *et al.*, 2000a) and rust caused by *Puccinia arachidis* (Meena *et al.*, 2000b). In tomato, application of same PGPR strains induced resistance against cucumber mosaic virus (CMV) and tomato mottle virus (ToMoV) (Zehnder *et al.*, 2001). The broad-spectrum of PGPR-mediated ISR is more rewarding than narrow spectrum of disease protection. Hence selecting a suitable strain having potential to induce systemic resistance against multiple pathogens and pests is the most important task in the delivery of microbial agents to the field.

2.9. Induction of defense-related proteins and chemicals by fluorescent pseudomonads

Fluorescent pseudomonads bring about ISR through fortifying the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reaction of the host leading to the synthesis of defense proteins and chemicals against the challenging pathogen.

2. 9. 1. Defense enzymes and chemicals involved in phenyl propanoid pathway

2. 9. 1. 1. Phenylalanine ammonia lyase

Phenylalanine ammonia lyase (PAL) is the first enzyme involved in phenyl propanoid pathway and plays a key role in the biosynthesis of phenolics and phytoalexins (Bashan *et al.*, 1985; Beaudoin-Eagan and Thorpe, 1985). Inhibition of PAL by α-amino-oxy-β-phenylpropionic acid (AOPP) reduced the localized accumulation of phenolics and suppressed the resistance that was induced by benzo thiadiazole-7-carbothioic acid-S-methyl ester against *Blumeria graminis* f. sp. *tritici* in wheat indicating importance of PAL in disease resistance (Stadnik and Buchenauer, 2000).

An increase in the level of mRNAs encoding for PAL was recorded in the early stage of interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992). When cucumber roots were treated with *Pseudomonas corrugata* 13 or *P. aureofaciens* 63-28, PAL activity was stimulated in root tissues in two days and this activated accumulation lasted for 16 days after bacterization (Chen *et al.*, 2000). Meena *et al.* (2000a) reported that foliar spray of *P. fluorescens* induced the activity of PAL in groundnut leaves.

2. 9. 1. 2. Peroxidase

Peroxidase (PO) catalyzes the last step in the biosynthesis of lignin and other oxidative phenols (Bruce and West, 1989). PO is associated with disease resistance in plants (Hammerschmidt *et al.*, 1982).

Increased PO and chalcone synthase activity (which lead to the synthesis of

phytoalexin) were recorded in interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992). Similarly, enhanced levels of PO were noticed in fluorescent pseudomonads-treated sugarcane in response to infection by *C. falcatum* (Viswanathan and Samiyappan, 1999). In groundnut, increased activity of PO was observed due to application of *P. fluorescens* and PO isoforms were expressed at higher levels (Meena, 2000). In cucumber, increased activity of PO was noticed in plants treated with *P. corrugata* strain 13 and induction of isoperoxidases by this bacterium may play an important role in ISR (Chen *et al.*, 2000). Two peroxidase isoforms have been induced in the fluorescent pseudomonads treated rice plants challenged with *R. solani* (Nandakumar *et al.*, 2001).

2. 9. 1. 3. Polyphenol oxidase

Polyphenol oxidase (PPO) catalyses the biosynthesis of oxidative phenols (Avdiushko *et al.*, 1993). Activity of PPO was stimulated by root application of *P. corrugata* 13 *and P. aureofaciens* in cucumber roots in response to infection by *P. aphanidermatum* and correlated in disease resistance (Chen *et al.*, 2000). Induction of higher PPO activity was noticed in rice against sheath blight pathogen and leaffolder attack (Radja Commare, 2000). Similarly increased activity of PPO was observed in tomato in response to infection by tomato spotted wilt virus (Kandan, 2000).

2. 9. 1. 4. Defense chemicals

Phenolics are fungitoxic in nature and increase the physical and mechanical strength of the host cell wall. Lignin is a phenolic polymer which is difficult to be

breached by pathogens and has been implicated in plant defense against pests and diseases (Nicholson and Hammerschmidt, 1992).

Seed treatment with rhizobacteria in bean induces the lignification of cell wall (Anderson and Guerra, 1985). Application of *P. fluorescens* strain WCS 417r brought about accumulation of phytoalexins in response to infection by *F. oxysporum* f. sp. *dianthi* (Van Peer *et al.*, 1991). Treatment of pea seeds with *P. fluorescens* strain 63-28 resulted in formation of structural barriers, *viz.*, cell wall apposition (papillae) and deposition of newly formed callose and accumulation of phenolic compounds at the site of penetration of invading hyphae of *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou *et al.*, 1996a). Similarly, seed treatment using *B. pumilus* strain SE 34 has also induced strengthening of cell walls in tomato against *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou *et al.*, 1998). M'Piga *et al.* (1997) reported that application of *P. fluorescens* strain 63-28 brought about cell wall thickening, deposition of phenolic compounds and formation of callose resulting in restricted growth *of F. oxysporum* f. sp. *radicis-lycopersici*. Such rapid defense reactions at the site of fungal entry delays the infection process and allows sufficient time for the host to buildup other defense reactions to restrict pathogen growth. Similarly, seed treatment and soil application of *P. fluorescens* induced synthesis of phenolic compounds in groundnut in response to infection by *C. personatum* (Meena *et al.*, 2000a). Application of *Serratia plymuthica* restricted the colonization of *P. ultimum* to the outermost root tissues and restriction of the fungal growth was positively correlated with the deposition of callose enriched cell wall appositions at the sites of pathogen penetration and accumulation of phenolic material

which is highly fungitoxic in nature. Thus, *S. plymuthica* sensitizes susceptible plants to react more rapidly and efficiently to the pathogen attack through the formation of physical and chemical barriers at sites of fungal attack (Benhamou *et al.*, 2000).

2. 9. 2. Pathogenesis-related proteins

Pathogenesis-related proteins are designated as PRs and are defined as proteins coded for by the host plant but induced specifically in pathological or related situations. They are not only accumulated locally in the infected leaves but also induced systemically associated with the development of systemic induced resistance against further infection by pathogens (Van Loon *et al.*, 1994).

Originally, five main classes of PRs (PR1-5) were characterized by both biochemical and molecular techniques in tobacco (Bol *et al.*, 1990; Van Loon *et al.*, 1987). In 1994, nomenclature for PRs was proposed based on their grouping into families sharing amino acid sequences, serological relationship and/or biological functions (Van Loon *et al.*, 1994). Later, Van Loon and Van Strien (1999) classified 14 classes of PRs (PR1-14).

Some of PRs such as chitinases (PR-3) (Legrand *et al.*, 1987) and β-1,3-glucanase (PR-2) (Kauffmann *et al.*, 1987) were identified with potential antifungal activity which degrade the chitin and glucan fragments of fungal cell wall and release chitin oligomers and glucan fragments which act as elicitors that in turn elicit various defense mechanisms in plants. Thaumatin-like proteins belonging to PR5 family permeabilize fungal membrane (Vigers *et al.*, 1991). PR-6 are proteinase inhibitors implicated in defense

against insects and other herbivores, micro-organisms and nematodes (Ryan, 1990). PR-7 acts as an endoproteinase. Lysis of fungal cell walls often requires degradation of cell wall proteins in addition to hydrolysis of chitin and glucan (Haran *et al.*, 1996; Goldman and Goldman, 1998) and thus it is hypothesized that PR-7 serves as an accessory to antifungal action. PR-9 family of peroxidases is likely to function in strengthening plant cell walls by catalyzing lignin deposition in reaction to microbial attack (Van Loon and Van Strien, 1999). PR-10 family is structurally related to ribonucleases and is effective against viruses (Moiseyev *et al.*, 1997). PR-12 type defensins, PR-13 type thionins and PR-14 type LTPs exhibit antifungal and antibacterial activity (Broekaert *et al.*, 1997; Christensen *et al.*, 1998). Collective function of several PRs may be effective in inhibiting pathogen growth, multiplication and/or spread of pathogen and be responsible for the state of induced resistance (Ryals *et al.*, 1996; Van Loon, 1997).

Induced resistance by PGPR is associated with the accumulation of PR proteins (Maurhofer *et al.*, 1994; Benhamou *et al.*, 1996a; M' Piga *et al.*, 1997; Viswanathan and Samiyappan, 1999; Nandakumar *et al.*, 2001). Colonization of bean root by fluorescent bacteria was correlated with induction of PR proteins and systemic resistance against *B. cinerea* (Zdor and Anderson, 1992). Maurhofer *et al.* (1994) reported that ISR by *P. fluorescens* strain CHAO against tobacco necrosis virus (TNV) in tobacco was associated with accumulation of PR proteins namely β-1,3 glucanases and endochitinases. They also established that ineffective strain P3 did not accumulate PR proteins indicating involvement of PR proteins in induction of resistance. In pea, seed bacterization with *P. fluorescens* strain 63-28 has signalled the host to produce hydrolytic

enzymes such as chitinases and β-1,3 glucanases. These host lytic-enzymes accumulate at the site of penetration of the fungus, *F. oxysporum* f. sp. *pisi* resulting in the degradation of fungal cell wall (Benhamou *et al.,* 1996b). Inoculation of tomato plants with same strain has similarly induced the production of plant chitinases upon challenge inoculation with the wilt pathogen, *F. oxysporum* f.sp. *radicis-lycopersici* (M' Piga *et al.*,, 1997). Similarly, enhanced levels of chitinase and peroxidase were noticed and induction of two new chitinase isoforms was found in PGPR-mediated ISR against *C. falcatum* in sugarcane, (Viswanathan and Samiyappan, 1999). Meena *et al.* (2000b) found that application of *P. fluorescens* in groundnut leaves induced 30 kDa glucanase and 23 kDa Thaumatin-like protein (TLP) when compared to untreated leaves. In rice, ISR has been correlated with two-fold increase in activity of pathogenesis-related peroxidase and chitinase proteins (Nandakumar *et al.*, 2001).

Conversely, certain studies indicate that ISR by fluorescent pseudomonads is not associated with induction of PR proteins (Pieterse *et al.*, 1996; 1998; Van Loon *et al.*, 1998). In radish plant expressing ISR elicited by *P. fluorescens* strain WCS417r against *F. oxysporum* f. sp. *raphani,* PR proteins are not accumulated (Hoffland *et al.,* 1995; 1996). In *Arabidopsis,* ISR induced by *P. fluorescens* strain WCS 417r is not associated with PR gene activation (Pieterse *et al.,* 1996). Similarly, *P. fluorescens* strains WCS 417r and WCS 358r have induced protection in both wild type *Arabidopsis* and salicylic acid-non accumulating NahG- *Arabidopsis* (plants transgened with NahG-gene encoding salicylic acid hydroxylase which converts SA to catechol) without activating PR gene expression (Van Wees *et al.,* 1997).

2. 10. Bacterial determinants in elicitation of induced resistance

There are several bacterial determinants involved in the induction of systemic resistance by PGPR the most important being lipopolysaccharides present in the outer membrane of bacterial cells, siderophore and salicylic acid production (Van Peer and Schippers, 1992; Leeman *et al.*, 1996; De Meyer and Hofte, 1997).

2. 10. 1. Lipopolysaccharides

Lipopolysaccharides (LPS) present in outer membrane of PGPR are the major determinants of ISR in certain PGPR strains. LPS of *P. fluorescens* strain WCS 417 have induced systemic resistance in carnation against fusarium wilt caused by *F. oxysporum* f. sp. *dianthi* (Van Peer and Schippers, 1992). Similarly, LPS of *P. fluorescens* strains WCS 374 and WCS 417 have induced systemic resistance in radish against *F. oxysporum* f. sp. *raphani* (Leeman *et al.,* 1995). They also established that mutant of *P. fluorescens* strain WCS 417, lacking the O-antigenic side chain of the LPS, has not induced resistance in radish indicating that O-antigen side chain of the LPS might have served as a signal or trigger in the induction of defense mechanism in plants. In contrast, LPS of *P. putida* strain WCS 358 lacking O-antigen side chain do not induce systemic resistance in radish. In another study, LPS of WCS 417r and mutant of WCS 417r lacking O-antigen side chain of LPS elicit defense mechanism in *Arabidopsis* (Van Wees *et al.,* 1997). This indicates that ISR by LPS of PGPR varies with different host plant and lipopolysaccharide is not the only trait in determining the ISR. Other traits of PGPR are also involved in ISR.

2. 10. 2. Siderophores

Siderophores are produced by PGPR under iron-limited conditions. Leeman *et al.* (1996) reported that LPS of *P. fluorescens* strains WCS 374 and WCS 417 were the major determinants of ISR under iron-replete conditions but under iron-limited conditions, LPS of these bacteria were not involved in ISR in radish against fusarium wilt. They also found that pyoverdine-type pseudobactin, siderophore, produced by these bacteria was responsible for ISR. Application of purified pseudobactin alone, isolated from strain WCS 374, to the roots of radish induced resistance. Thus, bacterial determinants in inducing systemic resistance in radish vary depending upon iron availability. It was also found that induction of ISR by LPS and siderophores seems to be complementary rather than additive and full induction of resistance by one determinant masks contributions by other(s). Recently, Press *et al.* (2001) reported that ISR by *Serratia marcescens* against *C. orbiculare* was associated with biosynthesis of siderophore.

2. 10. 3. Salicylic acid

White (1979) observed that treatment with salicylic acid (SA) decreased the disease development caused by tobacco mosaic virus in the tobacco cultivar Xanthi-nc. Salicylic acid is an important systemic signalling molecule in induction of resistance. This hypothesis was based on the observation that the endogenous level of SA increase locally and systemically in phloem infected cucumber before the expression of SAR indicating role of SA acting as a signal for SAR (Malamy *et al.*, 1990; Metraux, 2001). Kessmann *et al.* (1994) and Schneider *et al.* (1996) reviewed induction of systemic

resistance by the application of SA. Certain PGPR strains are capable of producing SA and responsible for the induction of ISR in plants (Maurhofer *et al.*, 1994). Exogenous application of salicylic acid induced systemic resistance in pea against *Erysiphe pisi* (Frey and Carver, 1998).

Salicylic acid production by *P. aeruginosa* 7NSK2 is essential for induction of resistance to *B. cinerea*. SA-negative mutants of the same strain have lost their ability to induce systemic resistance in bean (De Meyer and Hofte, 1997). Introduction of pch A and pch B gene which encode for the synthesis of SA in *P. fluorescens* strain P3, renders this strain capable of salicylic acid production and significantly improved its ability to induce systemic resistance in tobacco against TNV. *P. fluorescens* CHAO naturally produces SA under conditions of iron limitation and induces ISR in tobacco against same pathogen (Maurhofer *et al.*, 1998). In cucumber application of *P. corrugata* strain 13 and *P. aureofaciens* resulted in endogenous accumulation of more SA which induced systemic resistance against *P. aphanidermatum*, whereas exogenous application of SA did not. Thus endogenous accumulation of SA in response to treatment with fluorescent pseudomonads might be involved in the ISR (Chen *et al.*, 1999). To support this theory, De Meyer *et al.* (1999) reported that induced resistance by *P. aeruginosa* 7NSK2 was exclusively depends on the production of SA by this strain. They found that treatment of nahG-transformed tobacco with this strain induced systemic resistance and this induced resistance depended on in planta accumulation of SA.

In contrast to these examples, SA-negative mutants of *S. marcescens* strain 90-166 induces the same level of resistance in cucumber as wild strain of *S. marcescens* (SA

producing strain) do in cucumber and tobacco. In another study, SA producing strain of *S. marcescens* 90-166 induces resistance both in wild type tobacco and NahG-tobacco (Press *et al.*, 1997). Similarly, *P. fluorescens* strains WCS 417r and WCS 358r, which produce SA, induce resistance in both wild type *Arabidopsis* and SA-non accumulating NahG-gene transformed *Arabidopsis* plants (Van Wees *et al.*, 1997). This suggests that ISR induced by *P. fluorescens* strain WCS 417r and WCS 358r is independent of SA production in *Arabidopsis*. Expression of ISR by *P. fluorescens* strain WCS 417r requires ethylene-dependent signaling pathway but not a SA-dependent signaling at the site of application (Knoester *et al.*, 1999).

2. 10. 4. Indole acetic acid

In addition to LPS, siderophores and SA, production of indole acetic acid (IAA) by rhizobacteria was also implicated in ISR (Gupta *et al.*, 2000). Culture filtrate of *B. subtilis* strain FZB-G collected from transition growth phase (10-20 h post-inoculation) induced systemic resistance in tomato seedlings against *F. oxysporum* f. sp. *radicis-lycopersici* besides promoting the root growth of seedlings whereas that from stationary growth phase (72 h post-inoculation) did not. On fractionation of culture filtrate from transition phase, one of its fractions (Tr-C) stimulated root growth and induced systemic resistance in tomato seedlings against fusarium wilt. This fraction (Tr-C) was found to behave more like an auxin precursor, indole-3-pyruvic acid which has been implicated in ISR. This study indicates that precursor of phytohormones produced by PGPR might be involved in ISR (Gupta *et al.*, 2000).

All these experiments show that different determinants of PGPR are involved in

the induction of systemic resistance and ISR by these bacterial determinants varies with iron limiting conditions, bacterial strains, host plants and their cultivars.

2. 11. Role of chitin and Zinc in disease management

2. 11. 1. Role of chitin as a growth factor for fluorescent pseudomonads and in biological control

Chitin is a carbon source/substrate for the growth of chitinolytic bacteria and increased chitinase production was observed when bacteria were grown in chitin amended medium (Gooday, 1990). Chitosan, a non-toxic polymer obtained from the chitin of crustacean shell wastes is not only the inhibitor of fungal growth but also activates genes of defense responses in plants (Hadwiger *et al.*, 1986; Benhamou and Theriaut, 1992; Lafontaine and Benhamou, 1996). Chitin oligomers which are released during degradation of chitin substrate by chitinolytic bacteria are also found to elicit plant defense reactions (Benhamou and Theriaut, 1992). Incorporation of chitin in King's medium B (KMB) supported the multiplication *of P. fluorescens* when compared to the medium without incorporation of chitin and enhanced activity of chitinase was observed in chitin amended growth medium (Viswanathan and Samiyappan, 2001a).

Chitin and chitosan are found to reduce soil-borne disease incidence (Lafontaine and Benhamou, 1996; Benhamou *et al.*, 1998). Tomato plants treated with chitosan enhanced protection against crown and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Benhamou and Theriaut, 1992; Lafontaine and Benhamou, 1996). Treatment of cucumber plants with chitosan reduced the root rot disease caused by *P. aphanidermatum* (Ghauoth *et al.*, 1994). Chitosan at 1000 ppm reduced the

germination of uredospores of *Puccinia graminis*, the incitant of wheat leaf rust. Treatment of groundnut leaves with chitosan before challenge inoculation with the pathogen reduced the rust disease (Sathiyabama and Balasubramanian, 1998). Induction of resistance by combination of *B. pumilus* strain SE 34 and chitosan was more prominent when they are applied separately indicating that chitosan increase the bio-control potential of rhizobacteria (Benhamou *et al.*, 1998).

2. 11. 2. Zinc improves the biocontrol activity of fluorescent pseudomonads

Zinc sulphate acts as a growth factor and increases production of antibiotics phenazine-1-carboxylic acid produced by *P. fluorescens* strain 2-79 which is an important biocontrol agent of take-all of wheat caused by *G. graminis* var *tritici* (Slininger and Jackson, 1992). Amendment of zinc EDTA in the hydroponic nutrient solution in the soilless rockwool culture did not reduce crown and root rot of tomato caused *by F. oxysporum* f. sp. *radicis-lycopersici* when used alone but reduced the disease by 25 per cent in the presence of *P. fluorescens* CHAO. Zn at low concentration abolished the production of fusaric acid (phytotoxin and a pathogenic factor) and increased the biocontrol activity of *P. fluorescens* CHAO. Fusaric acid even at low concentration repressed the production of antibiotics such as 2,4-diacetyl phloroglucinol and pyoluteorin produced by the bacterial culture which indicated that fusaric acid affected biosynthesis of antibiotics (Duffy and Defago, 1997). In fluorescent pseudomonads, secondary metabolites production was regulated by gac S and gac A regulatory genes. Spontaneous mutation due to genetic instability in either gene blocked the biosynthesis of antimicrobial compounds such as HCN, 2,4-diacetyl phloroglucinol, pyoluteorin and

pyrrolnitrin. It was found that addition of zinc improved the genetic stability of *P. fluorescens* resulting in normal production of secondary metabolites (Duffy and Defago, 2000).

2. 12. Formulation development using different carrier materials and delivery

An important area of microbiological research with regard to biocontrol is the development of formulations that would preserve microbial activity for long period enough to enable delivery of an effective product for field level application. *P. fluorescens* can be applied in the form of bacterial suspension (Mew and Rosales,1986; Thompson,1996) and as powder formulation (Kloepper and Schroth, 1981; Vidhyasekaran *et al.,* 1997a, b). It is desirable from the consumer's perspective to formulate and package PGPR in ways similar to chemical pesticides.

Mass multiplication of PGPR in a suitable medium and development of a powder formulation was first carried out in 1980. A dried powder formulation of PGPR is especially important for seed treatment and soil application. The survival of PGPR in a dried formulation and the effectiveness of methylcellulose in a powder formulation for coating sugarbeet seed has been well documented (Suslow, 1980). A talc-based powder formulation of PGPR has been developed for inoculation of potato seed pieces (Kloepper and Schroth, 1981). They also tested the stability and efficacy of the product under field conditions and found that potato seed treatment with powder formulation produced a higher level of root colonization by PGPR than aqueous preparations. Similarly, the suitability of different carriers for the development of stable formulation has been tested and found that in talc-based and peat-based formulations, the bacterial population has

been stable upto 240 days of storage period (Vidhyasekaran and Muthamilan, 1995). Seed treatment followed by soil application of talc-based powder formulation has effectively checked chickpea wilt and pigeonpea wilt under field conditions and has increased the yield (Vidhyasekaran and Muthamilan 1995; Vidhyasekaran *et al.*, 1997b). The similar formulation was also effective for the management of rice sheath blight (Vidhyasekaran and Muthamilan, 1999). A peat-based formulation has also effectively controlled the rice sheath blight disease under field conditions (Rabindran and Vidhyasekaran, 1996). Thus PGPR can be formulated and delivered effectively to the field for systemic protection of crop plants.

Recently PGPR formulation using chitin as a carrier was formulated for application in the transplant soil-mix system for developing suppressive plants (Zehnder *et al.*, 2001). The use of LS213, a commercial formulation containing formulated spores of *Bacillus subtilis* strain GB03, *Bacillus amyloliquefaciens* strain IN 937a and chitosan as a carrier significantly increased the growth of tomato, cucumber, tobacco and pepper in addition to protection against bacterial spot and late blight of tomato, angular leaf spot of cucumber and blue mold of tobacco (Reddy *et al.*, 1999). Application of LS213 formulation showed significant protection against nematode damage and anthracnose in cucumber and bacterial spot in tomato (Kenny *et al.*, 1999). Moreover, the same bio-formulation enhanced pine seedling root and shoot growth (Enebak and Reddy, 1999).

The methods of application of formulated product include seed treatment (Rosales and Mew, 1997), root-dip (Maurhofer *et al.*, 1994), sett treatment in sugarcane (Viswanathan and Samiyappan, 1999), sucker treatment in banana (Raguchander *et al.*,

1997), soil application (Vidhyasekaran *et al.,* 1997a, b) and foliar application (Mew and Rosales, 1986; Chatterjee *et al.,* 1996). Combinations of different methods of application could be more effective in disease management than a single method of application (Vidhyasekaran *et al.,* 1997a, b; Vidhyasekaran and Muthamilan, 1999; Meena *et al.,* 2000a; Nandakumar *et al.*, 2001).

2. 13. ISR under field conditions

In most of the cases, ISR has been studied mainly in the laboratory and green house conditions. Though studies on ISR by fluorescent pseudomonads were successful under controlled conditions or growth chamber conditions, performance and consistency of ISR under practical agricultural conditions and in non-sterile field soils should be tested. Some reports indicate that ISR by PGPR can protect the crop plants under field conditions (Wei *et al.*, 1991; 1996; Zehnder *et al.*, 2001; Viswanathan and Samiyappan, 2001b).

In a field trial, PGPR strains *viz.*, *P. putida* strain 89B-27, *S. marcescens* strain 90-166 and *Flavomonas oryzihabitans* strain INR-5 have caused systemic protection against angular leaf spot and bacterial wilt besides increasing the plant growth (Kloepper *et al .,* 1993). Similarly, application of PGPR strains either as seed treatment alone or as seed treatment plus soil drenching at the time of transplanting has protected the cucumber plants inoculated with *P. syringae* pv. *lachrymans,* the angular leaf spot pathogen and reduced the level of anthracnose disease in the field (Wei *et al.*, 1996). In sugarcane, application of PGPR as sett treatment induced systemic resistance against *C. falcatum* in addition to enhanced sett germination, tillering and growth of the cane both under

controlled conditions as well as field conditions (Viswanathan and Samiyappan, 2001b). Seed treatment followed by foliar spray of *P. fluorescens* isolate Pf1 induced systemic resistance against late leaf spot caused by *C. personatum* in groundnut (Meena *et al.*, 2000a). In rice, seed treatment and root-dipping of rice seedling with *P. fluorescens* Pf1 reduced rice sheath blight disease incidence and improved the grain yield under field conditions (Nandakumar *et al.*, 2001). Seed treatment and soil application of PGPR in field grown tomatoes showed a reduction in the development of symptoms of viral diseases such as CMV and ToMoV and often a reduction in the incidence of viral infection and an increase in tomato yield (Zehnder *et al.*, 2001). Moreover, application of PGPR strain induced systemic resistance against cucumber beetles which are vectors of bacterial wilt incidence (Zehnder *et al.*, 2001). These studies clearly established that PGPR-mediated ISR and plant growth promotion are operative under field conditions.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3. 1. Plant material

Seeds of tomato cv. Co3, chilli cv. Co1 and brinjal cv. Co2 were used in all experiments. Seeds were obtained from Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

3. 2. Pathogen

Various isolates of *Pythium aphanidermatum* were isolated from naturally infected tomato, chilli and brinjal using PDA medium. Pure culture of the pathogen was obtained by single hyphal tip method (Rangaswami, 1972). The isolates were mass multiplied in sand-maize medium at 19:1 w/w (sand : maize) (Riker and Riker, 1936). Pathogenicity of the isolates was tested by artificially inoculating the fungus at 19:1 w/w (soil : sand-maize inoculum) in the potting soil (red soil : sand : cowdung manure at 1:1:1w/w/w). The potting soil incorporated with the fungus was filled in pots and seeds of tomato, chilli and brinjal were sown at 20 seeds per pot separately. The virulent isolate was selected based on the per cent damping-off incidence in tomato, chilli and brinjal under greenhouse conditions and used for further studies.

Fusarium oxysporum f. sp. *lycopersici* and *Fusarium solani* were isolated from the naturally infected tomato and brinjal plants respectively and pathogenicity was

proved as described above. The pathogen inoculum was mass multiplied in sand-maize medium as described above and used for the greenhouse study.

Colletotrichum capsici (isolated from the naturally infected chilli plants and tested the pathogenicity) causing fruit rot of chilli was maintained on potato dextrose agar (PDA) slants. The pathogen inoculum was prepared by culturing the fungus on PDA medium for 7 days in Petri dish. The conidial suspension was prepared by pouring 20 ml of sterile distilled water in each Petri dish. The conidia were harvested by stirring with glass rod. The concentration of conidia was adjusted to 1000 conidia ml^{-1}.

3. 3. Fluorescent pseudomonads

3. 3. 1. Maintenance of cultures

Twenty fluorescent pseudomonads cultures obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore were used in the study. The cultures were maintained on King's medium B (KMB) agar slants (protease peptone - 20.0 g; dipotassium hydrogen phosphate - 1.5 g; magnesium sulphate - 1.5 g; glycerol - 10.0 ml; agar - 15.0 g; distilled water - 1.0 l; pH - 7.2) (King *et al.*, 1954) (Table-1).

3. 3. 2. Characterization of Fluorescent pseudomonads

Twenty strains of fluorescent pseudomonads were characterized based on biochemical tests. The following tests, *viz.*, production of fluorescent pigments (King *et al.*, 1954), gelatin liquefaction (Aneja, 1993), nitrate reduction (Ayers *et al.*, 1919), arginine dihydrolase (Thornley, 1960), levan formation, growth at 4 °C and 41 °C and different carbon source utilization (Hildebrand *et al.*, 1992) were carried out. Results of

Table 1. Fluorescent pseudomonads isolated from the rhizosphere soil of different crops

Sl No	*Pseudomonas* strains	Host plants
1	Pf1	Urdbean
2	FP7	Rice
3	PFNL	Chilli
4	PfKO	Pepper
5	PfATR	Tapioca
6	PFNA	Banana
7	EP1	Sugarcane
8	PCU	Banana
9	PFSA	Maize
10	PFAR	Groundnut
11	PSV	Cotton
12	VPT4	Sugarcane
13	KKM1	Sugarcane
14	ARR1	Sugarcane
15	VPT10	Sugarcane
16	PB2	Rice
17	ARR1G	Sugarcane
18	ARR2	Sugarcane
19	PFCOT	Tomato
20	PFCOP	Rice

these tests were scored either as positive or as negative. The data analysis was made by using cluster analysis –NTSYPC (Numerical Taxonomy and Multivariate analysis system) version-2 program. The raw data were first converted into binary data and then analyzed for cluster analysis.

3. 4. Efficacy of fluorescent pseudomonads on plant growth under laboratory conditions

3. 4. 1. Preparation of bacterial inoculum

Twenty strains of fluorescent pseudomonads were grown in conical flasks (250 ml) containing 100 ml of KMB broth for 48 h on a rotary shaker (150 rev min^{-1}) at 28±2°C. Cells were removed by centrifugation at 8000 g for 10 min at 4°C and washed in sterile water. The pellet was resuspended in small amount of sterile distilled water and then diluted with adequate amount of sterile distilled water until to obtain bacterial colonies of 10^8 cfu ml^{-1} as measured spectrophotometrically (OD_{595}=0.3) (Thompson, 1996).

3. 4. 2. Seed bacterization

Seeds of tomato, chilli and brinjal were surface sterilized with 2 per cent sodium hypochlorite for 30 s and then rinsed in sterile distilled water and dried overnight under a sterile air stream. Ten millilitre of bacterial inoculum containing 10^8 cfu ml^{-1} was taken in a Petri dish. To this, 100 mg of carboxymethylcellulose was added as an adhesive material. One gram of seeds were soaked in 10 ml of bacterial suspension for 2 h and dried overnight in a sterile Petri dish.

3. 4. 3. Plant growth-promotion

Plant growth-promoting activity of fluorescent pseudomonads was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). Seed bacterization was done as described in 3. 4. 2. Twenty seeds were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip and gently pressed. The polythene sheet along with seeds were then rolled and incubated in growth chamber for 15 days. Three replications were maintained for each treatment. The germination percentage of seeds was calculated. The root length and shoot length of individual seedlings were also measured.

The vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973)

Vigour Index = (Mean root length + Mean shoot length) x Germination (%)

3. 5. Screening fluorescent pseudomonads against major fungal pathogens

3. 5. 1. *In vitro* testing of fluorescent pseudomonads on inhibition of mycelial growth of *P. aphanidermatum*

Twenty fluorescent pseudomonads strains were tested for inhibition of mycelial growth of *P. aphanidermatum* in dual culture technique. The bacterial culture was streaked at one side of Petri dish (1 cm from the edge of the plate) plated with PDA medium and mycelial disc (8 mm diameter) of seven days old culture of *P. aphanidermatum* was placed on the opposite side in the Petri dish perpendicular to the

bacterial streak (Dennis and Webster, 1971). The plates were incubated at room temperature (28±2°C) for four days. The zone of inhibition was measured in millimetre.

3. 5. 2. Efficacy of selected fluorescent pseudomonads against damping-off disease of solanaceous vegetable crops under greenhouse conditions

Seven fluorescent pseudomonads strains such as *P. fluorescens* Pf1, FP7, PSV, PB2, PFCOT, PFCOP and *P. putida* PFATR were selected based on plant growth promotion and *in vitro* inhibition of mycelial growth of *P. aphanidermatum.* These strains were tested for the suppression of damping-off incidence in tomato, chilli and brinjal under greenhouse conditions.

Potting soil (red soil:sand:cowdung manure at 1:1:1 w/w/w) was autoclave sterilized for two hour in two consecutive days. The virulent isolate of *P. aphanidermatum* mass multiplied in the sand-maize medium as described in 3. 2. was mixed with the sterilized potting soil. Bacterized seeds of tomato, chilli and brinjal as described in 3.4.2 were sown in small pots (pot size: 15 cm height; 10 cm diameter) at 20 seeds per pot. Ridomil as seed-treatment at 6 g kg^{-1} seed was included as standard treatment for comparison. Seeds treated with distilled water served as control. Pathogen inoculated and pathogen un-inoculated control (healthy) were maintained. Watering was done regularly and damping-off disease incidence was recorded 20 days after sowing. The disease incidence was assessed using the following formula

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of seeds sown}} \times 100$$

Number of infected plants = Total number of seeds sown – Number of plants stand

The plant growth was measured at 20 days after sowing by selecting five plants randomly in each pot. Four pots per replication were maintained. There were three replications and the experiment was conducted in completely randomized design (CRD).

3. 5. 3. Testing the selected strains of fluorescent pseudomonads for inhibition of mycelical growth of *F. oxysporum* f. sp. *lycopersici*, *C. capsici* and *F. solani in vitro*

Seven fluorescent pseudomonads strains as mentioned in 3.5.2 were tested for inhibition of *F. oxysporum* f. sp. *lycopersici*, *C. capsici* and *F. solani in vitro* as described earlier (3.5.1). The inhibition zone was measured in millimetre.

3. 5. 4. Efficacy of fluorescent pseudomonads against fusarium wilt of tomato and brinjal under greenhouse conditions

Selected fluorescent pseudomonads as mentioned in 3.5.2 were tested for the management of fusarium wilt of tomato and brinjal under greenhouse conditions. Potting soil was autoclave-sterilized for two hours in two consecutive days and was filled in pots. Bacterized seeds were sown. Carbendazim as seed treatment at 2 g kg^{-1} seed and 0.1 % as soil drenching at 10 days after transplanting was included for comparison. Untreated seeds served as control. Thirty-day-old seedlings of tomato and forty day old seedlings of brinjal were transplanted (4 seedlings pot^{-1}) in pots (size: 50 cm height; 35 cm diameter) filled with potting soil. Ten days after transplanting, soil application with 10 ml of bacterial suspension (10^8 cfu ml^{-1}) was done and one day after soil application of bacteria, the pathogen mass multiplied in sand-maize medium was

inoculated as described in 3. 2. Wilt incidence was recorded 30 days after transplanting using the formula

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The experimental design was followed as described in 3. 5. 2

3. 5. 5. Efficacy of fluorescent pseudomonads against fruit rot of chilli under greenhouse conditions

Bacterized seeds of chilli were sown in the pots filled with potting soil. Carbendazim as seed-treatment at 2 g kg^{-1} seed and 0.1 % as foliar spray at 45 days after transplanting was included as standard chemical treatment for comparison. Seeds treated with distilled water served as control. Forty five-day-old seedlings were transplanted at 4 seedlings pot^{-1} in pots containing potting soil. Forty five days after transplanting, soil application with 10 ml of bacterial suspension containing 10^8 cfu ml^{-1} was done and one day after bacterial application, pathogen was inoculated by spraying 20 ml of conidial suspension containing 10^3 conidia ml^{-1} into each pot. The experimental design was followed as described in 3. 5. 2.

The per cent disease index was calculated by using the formula given by Bansal and Grover (1969).

$$\text{Per cent Disease Index} = \frac{\text{Sum of individual ratings}}{\text{Number of fruits assessed}} \times \frac{100}{\text{Maximum disease category}}$$

Sl. No	Category value	Grade
1	No disease	0
2	1-5 per cent fruit infected	1
3	6-25 per cent fruit infected	2
4	26-50 per cent fruit infected	3
5	51-100 per cent fruit infected	4

3. 6. Production of Bacterial metabolites

3. 6. 1. Production of 2, 4- diacetylphloroglucinol

The bacterial culture was grown in 5 ml of pigment production medium (peptone-20 g; glycerol-20 ml; NaCl-5 g; KNO_3-1 g; distilled water-1 l; pH 7.2) for four days on a rotary shaker at room temperature (28±2°C). The broth was centrifuged at 1000 ·g for 5 min and the supernatant was collected. It was acidified to pH 2 with 1 N HCl and was then extracted with an equal volume of ethyl acetate (Rosales *et al.*, 1995). The ethyl acetate extract was reduced to dryness *in vacuo*. The residue was dissolved in methanol. Twenty microliter sample was chromotographed on thin layer chromatography plates (TLC) coated with silica gel with a thickness of 250 µm layer and developed in acetonitrile/methanol/water (1:1:1) as a solvent. Finally, the plates were sprayed with dinitrosalicylicacid. R_f values of the spots were compared with synthetic 2,4-diacetyl phloroglucinol.

3. 6. 2. Siderophore production

Production of siderophore by fluorescent pseudomonads was assayed by plate assay method as described by Schwyn and Neilands (1987). The ternary complex chromeazurol S (CAS) /Fe^{3+}/hexadecyltrimethylammonium bromide served as an indicator. Forty eight-hour-old culture of fluorescent pseudomonads was streaked onto the succinate medium amended with the indicator and incubated for three days. Formation of bright zone with yellowish fluorescent color in the dark-blue colored medium was the indication of production of siderophore. The result was scored either as positive or negative to this test.

3. 6. 3. Hydrogen cyanide production

Bacterial culture was streaked onto the triptic soya agar medium. The filter paper disc of 1. 5 cm diameter was soaked in the picric acid solution (picric acid-2.5 g; Na_2CO_3-12.5 g and distilled water 1.0 l) and placed in the upper lid of each petri dish (Miller and Higgins,1970). Dishes were sealed with parafilm and incubated for four days. Hydrogen cyanide (HCN) production was assessed by the presence of a colored zone around the bacteria and the yellow color of the filter paper turning brown to reddish brown. Reactions were scored as weak (yellow to light brown), moderate (brown) and strong (reddish brown).

3. 6. 5. Salicylic acid production

The fluorescent pseudomonads were grown in the standard succinate medium (Succinic acid- 4.0 g; K_2HPO_4-3.0 g; $(NH_4)_2SO_47H_2O$- 0.2 g; distilled water 1.0 l; pH

7.0) for 48 h at room temperature (28±2°C) (Meyer and Abdallah, 1978). The cell-free culture supernatant was used to measure salicylic acid. The supernatant (4 ml) was acidified with 1 N HCl to pH 2.0 and salicylic acid was extracted in $CHCl_3$ (2 x 2 ml). Five micro litre of 2 M $FeCl_3$ and 4 ml of water were added to the pooled $CHCl_3$ phase. The absorbance of the purple iron-salicylic acid complex, which developed in the aqueous phase, was measured at 527 ηm (Meyer *et al.*, 1992). Salicylic acid was measured according to a standard curve drawn with salicylic acid dissolved in succinate medium and treated as described above. The quantity of salicylic acid was expressed as $\mu g\ ml^{-1}$ of the culture filtrate.

3. 6. 6. Production of Indole acetic acid by fluorescent pseudomonads

Production of Indole acetic acid (IAA) by different fluorescent pseudomonads in the medium was assessed by following the method described by Gorden and Paleg (1957). The fluorescent pseudomonads were grown in tripticase soya broth (animal peptone-15.0 g; soyapeptone-5.0 g; glycine-4.4 g; distilled water-1.0 l) with tryptophan (100 $\mu g\ ml^{-1}$) for 30 h. One millilitre of cell free culture medium was reacted with 2 ml of Salkowsky reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) and incubated at 28±2°C for 30 min. The absorbance was read at 530 ηm. A standard curve was prepared using IAA and presence of IAA in the culture medium was quantified as $\mu g\ ml^{-1}$.

3. 6. 7. Production of extracellular β-1,3-glucanase

a. Production of β-1,3-glucanase in the culture medium

For the production of β-1,3-glucanase, fluorescent pseudomonads were grown at

room temperature (28±2°C) for 72 h on a rotary shaker in 50 ml peptone medium containing 0.5 % glucose; 0.2 % peptone; 0.2 % laminarin; 0.1 % K_2HPO_4; 0.05 % $MgSO_47H_2O$ and 0.05% NaCl. The cultures were centrifuged at 16000 g at 4°C. The supernatant was fractionated by ammonium sulphate precipitation and then dialyzed against 0.1 M phosphate buffer (pH 7) at 4°C for 24 h (Lim *et al.*, 1991).

b. Determination of protein

Protein was determined by the method of Bradford (1976). Ten mg of Coomassie brilliant blue G-250 was dissolved in 4.7 ml of absolute alcohol and 10 ml of concentrated phosphoric acid and the volume was made up to 100 ml with distilled water. Sample (50 µl) was added to 950 µl of dye solution and the mixture was incubated for 5 min at room temperature. The absorbance was recorded at 595 ηm in a spectrophotometer (Hitachi 200-20). Bovine serum albumin was used as the standard.

c. Assay of β-1,3-glucanase

The activity of β-1,3-glucanase was determined by measuring the release of reducing sugars by using laminarin as a substrate and glucose as a standard. The reaction mixture consisted of 0.25 ml of dialyzed enzyme solution, 0.3 ml of 1 M sodium acetate buffer (pH 5.3) and 0.5 ml of 4 % laminarin (Pan *et al.*, 1991). The reaction was carried out at 40°C for 2 h. The reaction was stopped by adding 375 µl of dinitrosalicylic acid and heating for 5 min on a boiling water, vortexed and its absorbance was measured at 500 ηm. Protein content was measured according to Bradford (1976) using bovine

serum albumin as a standard as described in 3. 6. 7 b. The specific activity of β-1,3-glucanase was expressed as μg glucose released min^{-1} mg^{-1} protein.

3. 6. 8. Production of chitinase

a. Preparation of colloidal chitin

Colloidal chitin was prepared by treating 1 g of crab-shell chitin powder with acetone to form a paste, then slowly adding 20 ml of concentrated hydrochloric acid (HCl) while grinding in a mortar with the temperature maintained at 5° C. After several minutes, the syrupy liquid was filtered through glass wool and poured into vigorously stirred 50% aqueous ethanol to precipitate the chitin in a highly dispersed state. The residue was sedimented and resuspended in distilled water several times to remove excess acid and alcohol and then dialysed against tap water. Chitin content of the suspension was determined by drying a sample *in vacuo* and adjusted with distilled water to a final concentration of 10 mg ml^{-1} (dry weight/volume) and stored at 5°C (Berger and Reynolds, 1958).

b. Preparation of snail gut enzyme

Six hundred mg of the commercial lyophilized snail gut enzyme (Helicase, Sepracor, France) was dissolved in 10 ml of 20 mM potassium chloride (KCl) and chromatographed on a Sephadex G-25 column (38x1.5 cm) using a 10 mM KCl solution, containing 1 mM EDTA and adjusted to pH 6.8, for equilibration and elution. The first 20 ml eluted after the void volume was collected (Boller and Mauch, 1988).

c. Preparation of ρ-dimethylaminobenzaldehyde (DMAB) reagent

The DMAB reagent was prepared by the procedure described by Reissig *et al.* (1959). Stock solution of DMAB was prepared by mixing 8 g of DMAB in 70 ml of glaciel acetic acid along with 10 ml of concentrated HCl. One volume of stock solution was mixed with 9 volumes of glacial acetic acid immediately before use.

d. Assay of chitinase

Fluorescent pseudomonads strains were cultured at 30°C for 72 h on a rotary shaker in 250 ml flasks containing 50 ml chitin-peptone medium (pH 6.8) containing glucose-0.5%, peptone-0.2%, colloidal chitin-0.2% (from crab shells; Sigma), K_2HPO_4-0.1%, $MgSO_47H_2O$-0.05% and NaCl-0.05% (Lim *et al.*, 1991) for the preparation of crude chitinase. The cultures were centrifuged at 16000 g for 20 min at 4°C and the supernatant was used. Chitanase activity was determined by following the method Boller and Mauch (1988). The reaction mixture contained 0.4 ml of enzyme solution, 0.3 ml of 1 M sodium acetate buffer (pH 5.3) and 0.1 ml of colloidal chitin (0.1%). The reaction mixture was incubated at 37°C for 2 h. The reaction mixture was centrifuged and supernatant was pipetted into a test tube. To this 20 µl desalted snail gut enzyme (Helicase) was added. Finally, the mixture was incubated with 2 ml of dimethyl amino benzaldehyde (DMAB) for 20 min at 37°C and the absorbance was measured at 585 ηm. Chitinase activity was determined as ηmol GlcNAc min^{-1} mg^{-1} protein.

3. 7. Induction of defense-related proteins and chemicals by *P. fluorescens* Pf1

Induction of defense enzymes and phenolic compounds by *P. fluorescens* Pf1 was

assessed in response to infection by *P. aphanidermatum* in tomato and chilli, *F. oxysporum* f. sp. *lycopersici* in tomato and *C. capsici* in chilli.

3. 7. 1. Induction of defense mechanisms against *P. aphanidermatum* –experimental design

P. fluorescens Pf1 was used in the induction of defense reactions in tomato and chilli against *P. aphanidermatum.* The experiment consisted of following treatments.

1. Seeds treated with *P. fluorescens* Pf1
2. Seeds treated with *P. fluorescens* Pf1 and challenge inoculated with *P. aphanidermatum* at 15 days after sowing (50 g sand-maize medium containing 10^3 cfu g^{-1} medium in each pot)
3. Plants without prior treatment with *P. fluorescens* Pf1 inoculated with pathogen at 15 days after sowing
4. Seeds treated with distilled water (Control)

Seeds were sown in pots filled with sterilized potting soil at 20 seeds per pot.

Plants were challenge inoculated with *P. aphanidermatum* at 15 days after sowing.

Three replications were maintained in each treatment; each replicate consisted of eight pots. The experiments were conducted in completely randomized design (CRD) method on a greenhouse bench. The humidity in the greenhouse was maintained at around RH 70%. The temperature was adjusted to 26°C (day) / 20°C (night).

3. 7. 2. Induction of defense mechanism against *F. oxysporum* f. sp. *lycopersici*- experimental design

P. fluorescens Pf1 was further used in the induction of defense reactions in tomato against *F. oxysporum* f. sp. *lycopersici*. Bacterized seeds were sown in pots filled with sterilized potting soil. Thirty-day-old seedlings were transplanted in pots at 4 seedlings per pot. Ten days after transplanting, soil application with bacterial suspension (10 ml of suspension containing 10^8 cfu ml^{-1}) was done as described earlier (3. 5. 4). Bacterized plants were divided into two treatments. In the first treatment, bacterized plants were challenge inoculated with *F. oxysporum* f. sp. *lycopersici* as described in 3. 2. at one day after soil application of bacterial suspension and in the second treatment, bacterized plants were not challenged with the pathogen. Plants without prior treatment of *P. fluorescens* Pf1 were inoculated with the pathogen. The plants neither treated with bacterial suspension nor inoculated with the pathogen were kept as control. Three replications were maintained in each treatment; each replicate consisted of eight pots and in each pot four plants were maintained. The experimental design was followed as described in 3. 7. 2.

3. 7. 3. Induction of defense mechanism against *C. capsici*- experimental design

P. fluorescens Pf1 was used in the induction of defense reactions in chilli against *C. capsici*. Fortyfive-day-old seedlings were transplanted in pots at 4 seedlings pot^{-1}. Bacterized seeds were sown in pots filled with sterilized potting soil. Forty five days after transplanting, soil application with bacterial suspension (10 ml of suspension containing 10^8cfu ml^{-1}) was done as described earlier. One day after application of bacterial

suspension, one set of bacterized plants was challenge inoculated with *C. capsici* (20 ml of conidial suspension containing 1000 conidia ml^{-1}) and another set of bacterized plants was not challenged with the pathogen. Plants without prior treatment of *P. fluorescens* Pf1 were inoculated with the pathogen. The plants neither treated with bacterial suspension nor inoculated with the pathogen were kept as control. The experimental design was followed as described in 3. 7. 2.

3. 7. 4. Sample collection for biochemical analysis

Induction of defense proteins was analyzed in the plant parts where the pathogen incite infection. For studying, induction of defense mechanism against soil-borne pathogens *viz., P. aphanidermatum* and *F. oxysporum* f. sp. l*ycopersici,* root samples were collected and that against *C. capsici*, leaf samples were collected.

For collection of root samples, plants were carefully uprooted without causing any damage to root tissues at different time intervals (0, 1, 2, 3, 4, 5, 7 and 10 days after the pathogen inoculation). Four plants were sampled from each replication of the treatment separately (treatments were mentioned in the experimental design) and were maintained separately for biochemical analysis. Fresh roots were washed in running tap water and homogenized with liquid nitrogen in a pre-chilled mortar and pestle. The homogenized root tissues were stored in deep freezer (-70° C) until used for biochemical analysis.

In chilli, leaf samples were collected at various time intervals after pathogen inoculation and washed in running tap water and homogenized with liquid nitrogen in a

pre-chilled mortar and pestle. The homogenized leaf tissues were stored in deep freezer (-70° C) until used for biochemical analysis.

3. 7. 4. 1. Estimation of PAL activity

Root/leaf samples (1 g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone. The extract was filtered through cheesecloth and the filtrate was centrifuged at 16000 g for 15 min. The supernatant was used as enzyme source. Phenylalanine ammonia lyase activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 ηm as described by Dickerson *et al.* (1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M^{-1} (Dickerson *et al.*, 1984). Enzyme activity was expressed as ηmol trans-cinnamic acid min^{-1} mg^{-1} protein.

3. 7. 4. 2. Assay of PO

Root/leaf samples (1 g) were homogenized in 2 ml of 0.1 M phosphate buffer, pH 7.0 at 4°C. The homogenate was centrifuged at 16000 g at 4°C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28±2°C). The changes in absorbance at 420 ηm were

recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg^{-1} protein (Hammerschmidt *et al.*, 1982).

3. 7. 4. 3. Assay of PPO

Poly phenol oxidase activity was determined as per the procedure given by Mayer *et al.* (1965). Root/leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16000 g for 15 min at 4°C. The supernatant was used as enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 ηm min^{-1} mg^{-1} protein.

3. 7. 4. 4. Estimation of Phenol

One gram of root samples/leaf samples were homogenized in 10 ml of 80 % methanol and agitated for 15 min at 70°C (Zieslin and Ben-Zaken, 1993). One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) and the solution was kept at 25°C. The absorbance of the developed blue color was measured using a spectrophotometer at 725 ηm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol mg^{-1} protein.

3. 7. 4. 5. Assay of β-1,3-glucanase

The β-1,3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method (Pan *et al.*, 1991). Root/leaf samples (1 g) were extracted with 2 ml of 0.05 M

sodium acetate buffer (pH 5.0) and centrifuged at 16000 g for 15 min at 4°C. The supernatant was used in the enzyme assay. The reaction mixture consisted of 62.5 µl of 4% laminarin and 62.5 µl of enzyme extract. The reaction was carried out as described earlier (3. 6. 7. c). The enzyme activity was expressed as µg glucose released min^{-1} mg^{-1} protein.

3. 7. 4. 6. Assay of chitinase

Root/leaf samples (1 g) were homogenized in 2 ml of 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged at 16000 g for 15 min at 4°C and the supernatant was used in the enzyme assay. The colorimetric assay of chitinase was carried out as per the method described by Boller and Mauch (1988). Colloidal chitin was prepared according to Berger and Reynolds (1958) from crab shell chitin (Sigma, USA) as described earlier (3.6.8a). The reaction mixture consisted of 10 µl of 1 M sodium acetate buffer (pH 4.0), 0.4 ml of enzyme extract and 0.1 ml of colloidal chitin (10 mg). The reaction was carried out as described earlier (3. 6. 8. d). The enzyme activity was expressed as ηmol GlcNAc min^{-1} mg^{-1} protein.

3. 7. 4. 7. Native- polyacrylamide gel electrophoresis analysis

The isoform profile of PO and PPO were examined by discontinuous native polyacrylamide gel electrophoresis (native-PAGE) (Laemmli, 1970). Root/leaf samples showing maximum activity of PO and PPO in spectrophotometer assay were used for analysis. Induction of defense reactions against *P. aphanidermatum* in tomato and chilli, the activity of PO and PPO was maximum at 3rd day after challenge inoculation. Root

samples collected at 3rd after challenge inoculation of *P. aphanidermatum* were used for PO and PPO isozyme analysis. With regard to PO and PPO isozyme analysis in tomato against *F. oxysporum* f. sp. *lycopersici,* roots samples were collected at 4th day after the pathogen challenge for PO and at 5th day after the pathogen challenge for PPO analysis during that time the activities of PO and PPO were maximum respectively. In chilli, induction of PO and PPO was maximum at 4 day after challenge inoculation and leaf samples collected at 4th day after challenge inoculation were used for this study. The protein extract was prepared by homogenizing 1 g of root/leaf samples in 2 ml of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 16000 g for 20 min at 4°C. The protein content of the sample was determined by the Bradford method (Bradford, 1976) as described in 3. 6. 7 b. Samples (50 µg protein) were loaded into 8% polyacrylamide gels (Annexure I). After electrophoresis, PO isoforms were visualized by soaking the gels in staining solution containing 0.05% benzidine (Sigma, USA) and 0.03% H_2O_2 in acetate buffer (20 mM, pH 4.2) (Nadlony and Sequira, 1980). For assessing PPO isoforms profile, the gels were equilibrated for 30 min in 0.1% ρ-phenylene diamine followed by addition of 10 mM catechol in the same buffer (Jayaraman *et al.*, 1987).

3. 7. 4. 8. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

One g of powdered root/leaf sample was extracted with one ml of 0.1M sodium phosphate buffer (pH 7.0) under 4 °C. The homogenate was centrifuged for 20 min at 16000 g. The supernatant was used for the Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). The protein content of the sample was determined by Bradford method (Bradford, 1976).

Hundred µg of protein from treatments was taken and mixed with 10 µl of sample buffer in microfuge tube, boiled for 4 min and incubated at 4°C for 30 min. Then the samples containing equal amount of proteins were loaded into the wells of polyacrylamide gels (Annexure II) (Sigma-Aldrich Techware system, Sigma, USA). The medium range molecular weight markers (Genei, Bangalore, India) were used. Electrophoresis was carried out at constant voltage of 75 volts. The gels were stained with 0.2% Coomassie brilliant blue (R 250) solution. Based on the R_m value of each protein band stained, the molecular weight was calculated.

3. 7. 4. 9. Western blotting

Induction of isoforms of chitinase and TLP was studied by Western blotting. Root/leaf samples were collected 4th day after the pathogen inoculation. Protein extract was prepared by homogenizing 1 g of root/leaf samples with 1 ml of 0.1 M phosphate buffer, pH 7.0. Protein samples (250 µg) were separated on 12 % SDS-PAGE analysis under constant voltage of 75 volts. (Laemmli, 1970) using Sigma-Aldrich Techware system (Sigma, USA) with molecular weight markers as described in 3. 7. 4. 8. (Genei, Bangalore, India). After separation of proteins on SDS-PAGE, the proteins were electroblotted onto 0.45 µm nitrocellulose membrane (Sigma, USA) as described by Gallagher *et al.* (1995). The membrane was blocked for 1.5 h at room temperature (28±2°C) in Tris-buffered saline (TBS) containing 0.05 % Tween and 2 % gelatin (Annexure III). The membranes were then incubated for 3 h in the diluted primary antibody (barley chitinase antiserum and bean TLP antiserum were obtained as generous

gift from Dr. S. Muthukrishnan, Dept. of Biochemistry, Kansas State University, Manhattan, KS 66506, USA). The membrane was then incubated in secondary antibody for 3 h. Affinity purified goat anti-rabbit immunoglobulin (IgG) alkaline phosphatase conjugate (Sigma, USA) was used as secondary antibody at a dilution of 1:7000. The membrane was then washed thrice in TBS for 10 to 15 min each time. Immunological reaction was visualized by soaking the membranes in alkaline phosphatase color development reagents (Genei, Bangalore, India). Immediately after color development the membranes were washed in distilled water and dried. Apparent molecular weight of isoforms was determined with molecular weight markers.

3. 8. Testing the talc-based formulation of *P. fluorescens* Pf1 against damping-off of tomato, chilli and brinjal under greenhouse conditions

3. 8. 1. Development of bio-formulation of *P. fluorescens*

Pot culture experiment proved that P. *fluorescens* Pf1 was found effective for the management of damping-off of tomato, chilli and brinjal, wilt in tomato and brinjal and fruit rot in chilli. This strain was formulated by using talc as carrier material which is commonly used for the development of formulation at present in Tamil Nadu Agricultural University, Coimbatore.

Talc-based bio-formulation was developed as described by Vidhyasekaran and Muthamilan (1995). Ten gram of carboxymethylcellulose was mixed with 1 kg of talc powder and the pH was adjusted to 7.0 by adding calcium carbonate. The mixture was then autoclave-sterilized for 30 min in two consecutive days. The bacterial culture was grown in King's medium B (KMB) for 48 h. Four-hundred millilitre of bacterial

inoculum was added to 1 kg of the talc mixture and mixed well under sterile conditions. The product was dried under shade to bring the moisture content less than 20 per cent. The formulation was packed in polythene bags, sealed and kept under room temperature.

3. 8. 2. Talc-based formulation of *P. fluorescens* Pf1 against damping-off disease under greenhouse conditions

Pot culture experiment was conducted as described in 3. 5. 2. Seeds of tomato, chilli and brinjal were treated with the bio-formulation of *P. fluorescens* Pf1 at 10 g kg^{-1} seed and sown in pots at 20 seeds per pot. As a standard treatment, seeds were treated with ridomil at 6 g kg^{-1} seed. Seeds treated with sterile distilled water served as control. Both pathogen-inoculated control and pathogen-uninoculated control were maintained. Disease incidence and plant growth were recorded as described in 3. 5. 2.

3. 9. Development of new bio-formulation using different carrier materials.

3. 9. 1. Chitin amendment at different concentrations on growth and multiplication of *P. fluorescens* in KMB

Colloidal chitin was incorporated in King's medium B (KMB) at 1 and 0.5% level. The medium was autoclave-sterilized for 30 min. *P. fluorescens* Pf1 was inoculated in the medium and grown for 3 days. KMB without chitin amendment was included as a control treatment. Colonies of *P. fluorescens* Pf1 were assessed at 24, 48 and 72 h after inoculation by serial dilution plate technique.

3. 9. 2. Population dynamics of *P. fluorescens* Pf1 in different carriers

P. fluorescens Pf1 was mass multiplied in KMB with and without chitin at one per cent (incorporation of one per cent chitin in KMB showed the increased growth and

multiplication of *P. fluorescens* Pf1). The culture was inoculated in the medium and grown for 48 h. Different carrier materials *viz.*, talc, gypsum, lignite and combinations such as talc+gypsum, talc+lignite and gypsum+lignite at 1:1 w/w were used for formulation development. *P. fluorescens* Pf1 was grown in KMB with chitin and mixed with different carrier materials. A parallel experiment was carried out by multiplying *P. fluorescens* Pf1 in KMB without chitin amendment and mixed with different carriers. The pH was adjusted to 7.0 by addition of CaCO3. The formulation was prepared as described in 3. 8. 1.

The survival of *P. fluorescens* Pf1 in different carrier materials was assessed during different time intervals of storage period *viz.*, 0, 15, 30, 45, 60, 75 and 90 days by serial dilution plate technique using KMB and well developed colonies were counted.

3. 9. 3. Efficacy of different formulations of *P. fluorescens* Pf1 against damping-off incited by *P. aphanidermatum* under greenhouse conditions

Efficacy of different formulations with different carriers was tested for the management of damping-off disease of tomato, chilli and brinjal. *P. fluorescens* culture mass multiplied in KMB amended with and without chitin was incorporated in various carrier materials and their efficacy were tested. The experiment included following treatments.

1. Talc
2. Gypsum
3. Lignite

4. Talc+gypsum

5. Talc+lignite

6. Gypsum+lignite

7. Ridomil

8. Control (Pathogen inoculated)

9. Control (Pathogen uninoculated)

Seeds of tomato, chilli and brinjal were treated with various formulations at 10 g kg^{-1} seed. Seeds were sown in potting soil at 20 seeds per pot. Pot culture experiment was conducted and disease incidence was assessed as described in 3. 5. 2.

3. 9. 4. Assessing the efficacy of selected formulations with or without incorporation of Zinc for the management of damping-off incidence under field conditions

From earlier studies, some formulations were screened for testing the efficacy with addition of Zinc in the formulations. The experiment included following treatments.

T1-Talc containing Pf1 multiplied without chitin

T2-Talc containing Pf1 multiplied with chitin

T3- Talc+Zinc 1% containing Pf1 multiplied with chitin

T4- Talc+gypsum containing Pf1 multiplied without chitin

T5- Talc+gypsum containing Pf1 multiplied with chitin

T_6- Talc+gypsum+Zinc1% containing Pf1 multiplied with chitin

T7-Talc

T8-Gypsum

T9-Zinc

T10-Ridomil

T11-Control

Two field experiments (one at Agricultural Research Station, Aliyar Nagar and another at Tamil Nadu Agricultural University, Coimbatore where the damping-off disease is endemic) were conducted. Seeds treated with formulation at 10 g kg^{-1} seed were sown in rows in the flat nursery bed. As a standard treatment, seeds were treated with ridomil at 6 g kg^{-1} seed. Seeds were also treated with the talc, gypsum and Zinc alone to assess their effect on disease incidence. Seeds treated with distilled water served as control. The experiment was laid out in randomized block design (RBD) with three replications. The damping-off disease incidence was recorded at 20 days after sowing as described in 3. 5. 2.

3. 9. 5. Assessing the efficacy of selected formulations with or without incorporation of Zinc for the management of fusarium wilt of tomato and brinjal under field conditions

Three field experiments were conducted for assessing the efficacy of *Pseudomonas* bio-formulation against wilt incidence in tomato and brinjal. The first experiment was conducted at Agricultural College and Research Institute, Madurai in 1999-2000, the second and third experiments were conducted at farmer's field,

Aruppukottai during 1999-2000 and 2000-2001 respectively. The same treatments as mentioned in 3. 9. 4, except ridomil which was substituted with carbendazim as standard fungicide for fusarium wilt, were included in this experiment. Seeds treated with formulations at 10 g kg^{-1} seed were sown in nursery bed. Thirty-day-old tomato seedlings were transplanted in the field. Sixty six plants were maintained (30 x 60 cm spacing) in each plot (size: 4 x 3 m). Forty days old brinjal seedlings were transplanted (75 x 45 cm spacing) in the main field. Thirty plants were maintained in each plot (size: 4 x 3 m). Ten days after transplanting, soil application of formulation was made at 2.5 kg ha^{-1}. As a standard treatment, seeds were treated with carbendazim at 2 g kg^{-1} seed followed by soil drenching 0.1 % at 10 days after transplanting. The experiments were laid out in RBD with three replications. The disease incidence was recorded as described in 3. 5. 4.

3. 9. 6. Efficacy of different formulations of *P. fluorescens* Pf1 against fruit rot of chilli under field conditions

Two field experiments were conducted for assessing the efficacy of *Pseudomonas* bio-formulation against fruit rot incidence in chilli. Both the experiments were conducted at farmer's field, Aruppukottai during 1999-2000 and during 2000-2001. The same treatments as mentioned in fusarium wilt (3. 9. 5) were followed in this experiment. Seeds treated with formulation at 10 g kg^{-1} seed were sown in nursery bed. Forty five day old seedlings were transplanted in the field. Sixty six plants were maintained (30 x 60 cm spacing) in each plot (4 x 3 m). Forty five days after transplanting, soil application with formulation was made at 2.5 kg ha^{-1}. As a standard treatment, seeds were treated with carbendazim at 2 g kg^{-1} seed followed by foliar application at 0.1% at 45 days after

transplanting. The experiments were laid out in RBD with three replications. The disease incidence was recorded as described in 3.5.5.

Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines. Cluster analysis of fluorescent pseudomonads was done by NTSYPC-version 2 program.

Experimental Results

CHAPTER IV

EXPERIMENTAL RESULTS

4. 1. Characterization of fluorescent pseudomonads

Twenty strains of fluorescent pseudomonads were found to be fluorescent on KMB and all the tested strains did not grow at 41 °C indicating that they are not belonging to *P. aeruginosa*. Most of the bacterial strains showed positive response for gelatin liquefaction and trehalose utilization tests except two strains *viz.*, PFATR and KKM1 (Table-2). Hence, these two strains were grouped under *P. putida* group. The remaining 18 strains were classified into *P. fluorescens*. The strains were again grouped into different biovars based on other primary characters of fluorescent pseudomonads and utilization of various carbon sources. Based on that, strain KKM1 was grouped into *P. putida* biovar I and the strain PFATR was grouped into *P. putida* biovar II. Among the 18 strains of *P. fluorescens,* 16 strains showed positive to both gelatin and trehalose utilization tests. The two strains *viz.*, ARR2 and VPT 10 showed positive to either one of these two tests. ARR2 showed positive to gelatin liquefaction and negative to trehalose utilization test whereas the strain VPT10 showed negative to gelatin liquefaction and positive to trehalose utilization test. Though these two strains showed positive to one test and negative to other test, they were tentatively grouped in *P. fluorescens*. Based on the similarity in the biochemical tests, the strains *viz.*, Pf1, PB2, ARR1 and PFNL were grouped into *P. fluorescens* biovar I; FP7, PFAR, ARR1G, PSV, VPT4 and VPT10 were assigned under *P. fluorescens* biovar II; PFKO belonged to *P. fluorescens* biovar III;

PFSA was classified under *P. fluorescens* biovar IV and ARR2, PFNA, EP1, PCU, PFCOT and PFCOP were grouped under *P. fluorescens* biovar V. (Table-2).

4. 2. Cluster analysis of bacterial strains

Twenty strains of fluorescent pseudomonads were clustered into six major clusters based on the similarity in the biochemical tests. All the four strains of *P. fluorescens* belonging to the biovar I *viz.*, Pf1, PB2, ARR1 and PFNL were grouped under one cluster (C1). *P. putida* strian PFATR biovar II related to this cluster. This strain remained singly in this cluster. Six strains of biovar II were assigned to two different clusters. Strains *viz.*, PFAR and VPT10 related closely and were assigned into cluster-C3. Other strains *viz.*, FP7, PSV, VPT10 and ARRIG were assigned to another cluster-C4. Among the six strains of *P. fluorescens* belonging to biovar V, four strains *viz.*, EP1, PFCOP, PCU and PFCOT related closely and were grouped into cluster-C2. The remaining two strians *viz.*, PFNA and ARR2 were assorted under distance-cluster-C6. *P. putida* strain KKM1 biovar I was assigned separately in cluster-C5. Strains of biovar III and IV *viz.*, PFKO and PFSA were assigned to clusters namely C6 and C3 respectively (Fig. 1).

4. 3. Efficacy of fluorescent pseudomonads on plant growth promotion

P. fluorescens strains Pf1, FP7 and *P. putida* strain PFATR were found to increase shoot length, root length and germination percentage of tomato, chilli and brinjal in roll towel method resulting in higher vigour index when compared to other fluorescent pseudomonads strains. *P. fluorescens* strain PB2 increased vigour index in tomato and

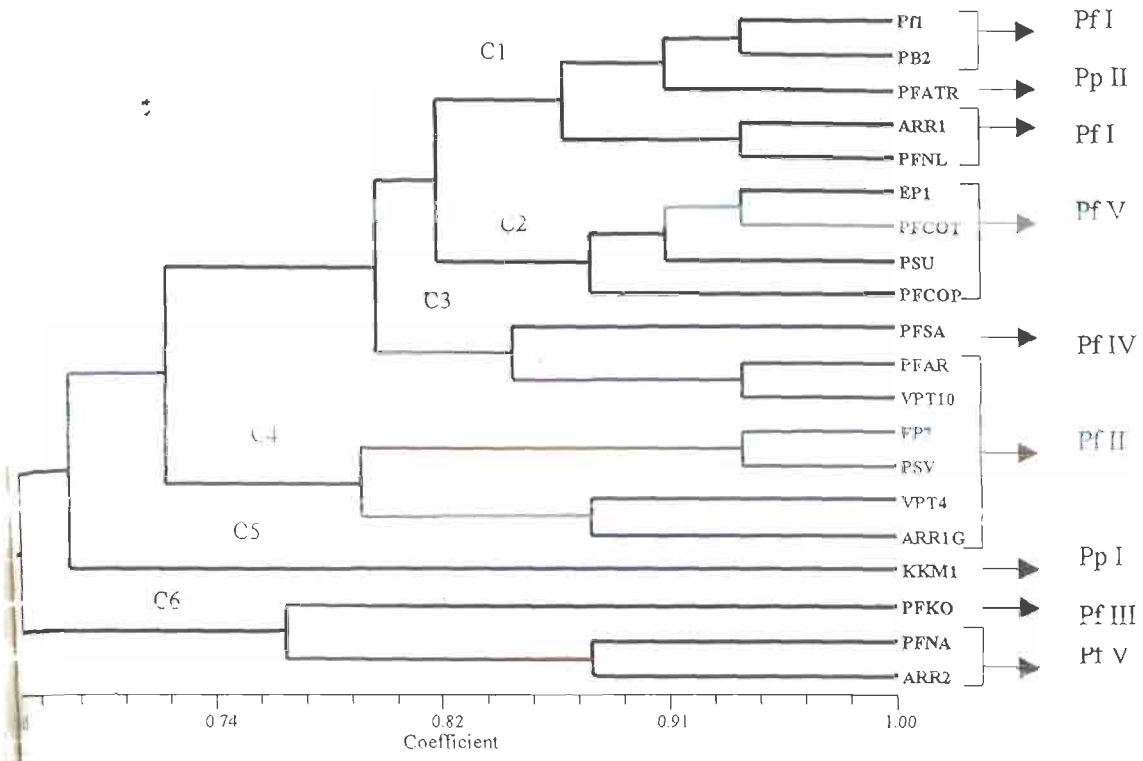


Fig. 1. Dendrogram showing relatedness among the 20 isolates of fluorescent pseudomonads

Pf-*P. fluorescens*

Pp-*P. putida*

I, II, III, IV and V- biovars

brinjal whereas *P. fluorescens* strain PSV showed the maximum vigour index in brinjal (Table 3, 4 and 5).

4. 4. Efficacy of fluorescent pseudomonads in inhibiting the mycelial growth of *P. aphanidermatum*

Among the twenty fluorescent pseudomonads strains tested for their efficacy in inhibiting the mycelial growth, five strains *viz.*, *P. fluorescens* Pf1, PFCOT, FP7, PFCOP and PB2 showed higher inhibitory effect on mycelial growth of *P. aphanidermatum*. Among these five strains, *P. fluorescens* Pf1 exhibited the maximum inhibition of mycelial growth *in vitro* by recording a inhibition zone of 13.67 mm. It was followed by *P. fluorescens* strain FP7 which recorded the inhibition zone of 11.67 mm (Table-6) (Plate 4).

Based on the *in vitro* inhibition of mycelial growth against *P. aphanidermatum* and plant growth promotion, seven strains *viz.*, *P. fluorescens* strains *viz.*, Pf1, FP7, PSV, PB2, PFCOT and PFCOP and *P. putida* strain PFATR were selected for evaluating their efficacy against damping-off in tomato, chilli and brinjal, fusarium wilt in tomato and brinjal and fruit rot of chilli.

4. 5. Efficacy of fluorescent pseudomonads against damping-off disease

Among the seven fluorescent pseudomonads tested for their efficacy in controlling damping-off disease in tomato, chilli and brinjal. *P. fluorescens* Pf1 showed the lowest disease incidence of 35.55, 31.11 and 42.22 per cent in tomato, chilli and brinjal respectively and increased the plant growth by recording the plant height of 16.52, 15.10 and 15.70 cm in tomato, chilli and brinjal respectively under greenhouse conditions. The biocontrol efficacy of *P. fluorescens* Pf1 was comparable with that of

Table 3. Plant growth promoting activity of fluorescent pseudomonads in tomato

Pseudomonas strains	Shoot length (cm)	Root length (cm)	Germination (%)	Vigour index
Pf1	5.03^{ef}	10.53^{j}	94.67^{e} (76.83)	1473.1^{jk}
FP7	5.23^{g}	9.37^{g}	97.33^{f} (80.64)	1421.0^{i}
PFNL	4.80^{cd}	9.49^{g}	93.33^{de} (75.20)	1333.7^{h}
PFKO	4.50^{b}	7.60^{b}	85.33^{ab} (67.48)	1032.5^{b}
PFATR	5.10^{fg}	10.79^{k}	94.67^{e} (76.83)	1504.3^{kl}
PFNA	5.20^{fg}	8.70^{e}	85.33^{ab} (67.48)	1186.1^{e}
EP1	5.27^{g}	9.12^{f}	85.33^{ab} (67.48)	1227.9^{f}
PCU	4.78^{cd}	8.44^{d}	89.33^{bcd} (71.01)	1180.9^{de}
PFSA	4.81^{cd}	8.79^{e}	90.67^{bcde} (72.29)	1233.1^{f}
PFAR	4.93^{de}	8.37^{d}	81.33^{a} (64.43)	1081.7^{c}
PSV	4.77^{cd}	8.10^{c}	89.33^{bcd} (71.01)	1149.7^{d}
VPT4	5.12^{fg}	8.78^{e}	93.33^{de} (75.20)	1297.3^{g}
KKM1	4.82 ^{cd}	9.43^{g}	86.67^{abc} (68.63)	1235.0^{f}
ARR1	4.70^{c}	10.77^{k}	88.00^{bcd} (69.74)	1361.4^{h}
VPT10	4.70^{c}	10.70^{hi}	88.00^{bcd} (69.74)	1335.2^{h}
PB2	5.10^{fg}	11.27^{l}	92.00^{cde} (73.59)	1506.0^{l}
ARR1G	4.79^{cd}	9.49^{g}	86.67^{abc} (68.63)	1237.6^{f}
ARR2	5.23^{g}	9.52^{g}	85.33^{ab} (67.48)	1258.6^{f}
PFCOT	5.14^{fg}	10.10^{h}	94.67^{e} (76.83)	1442.8^{ij}
PFCOP	5.05^{ef}	10.47^{i}	93.33^{de} (75.20)	1448.5^{ij}
Control	3.77^{a}	5.65^{a}	80.00^{a} (63.44)	753.6^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 4. Plant growth promoting activity of fluorescent pseudomonads in chilli

Pseudomonas strains	Shoot length (cm)	Root length (cm)	Germination (%)	Vigour index
Pf1	5.57^{g}	9.90^{j}	94.67^{e}(76.83)	1464.5^{j}
FP7	4.60^{de}	8.20^{efg}	86.67^{bcd}(68.63)	1109.4^{fg}
PFNL	4.20^{b}	8.07^{de}	86.67^{bcd}(68.63)	1063.4^{de}
PFKO	4.40^{bcd}	8.13^{def}	86.67^{bcd}(68.63)	1086.0^{ef}
PFATR	5.20^{f}	9.93^{i}	90.67^{d}(72.29)	1371.8^{i}
PFNA	4.70^{e}	8.57^{h}	90.67^{d}(72.29)	1203.2^{h}
EP1	4.57^{de}	8.10^{de}	89.33^{cd}(71.01)	1131.8^{g}
PCU	4.23^{b}	7.70^{b}	82.67^{ab}(65.43)	986.3^{b}
PFSA	4.27^{bc}	7.93^{cd}	82.67^{ab}(65.43)	1008.6^{bc}
PFAR	4.23^{b}	8.37^{g}	90.67^{d}(72.29)	1142.4^{g}
PSV	4.20^{b}	8.33^{fg}	90.67^{d}(72.29)	1136.1^{g}
VPT4	4.53^{de}	8.03^{de}	90.67^{d}(72.29)	1138.8^{g}
KKM1	4.23^{b}	8.03^{de}	85.33^{bc}(67.53)	1046.2^{d}
ARR1	4.47^{cd}	8.07^{de}	90.67^{d}(72.29)	1137.0^{g}
VPT10	4.27^{bc}	8.17^{efg}	90.67^{d}(72.29)	1128.0^{g}
PB2	4.50^{de}	8.03^{de}	94.67^{e}(76.83)	1186.2^{h}
ARR1G	4.23^{b}	7.80^{bc}	90.67^{d}(72.29)	1090.8^{ef}
ARR2	4.57^{de}	8.07^{de}	90.67^{d}(72.29)	1146.1^{g}
PFCOT	4.57^{de}	8.10^{de}	90.67^{d}(72.29)	1148.8^{g}
PFCOP	4.23^{b}	7.80^{bc}	85.33^{bc}(67.53)	1026.5^{cd}
Control	3.13^{a}	6.83^{a}	78.67^{a}(62.51)	783.6^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 5. Plant growth promoting activity of fluorescent pseudomonads in brinjal

Pseudomonas strains	Shoot length (cm)	Root length (cm)	Germination (%)	Vigour index
Pf1	5.10^{de}	10.80^{kl}	93.33^{de} (75.20)	1484.0^{k}
FP7	5.20^{e}	10.80^{kl}	94.67^{e} (76.83)	1514.7^{l}
PFNL	4.80^{bc}	10.07^{g}	89.33^{c} (71.01)	1328.3^{g}
PFKO	5.07^{de}	8.10^{d}	86.67^{bc} (68.63)	1141.1^{d}
PFATR	5.03^{de}	10.73^{kl}	93.33^{de} (75.20)	1470.9^{jk}
PFNA	5.07^{de}	7.57^{b}	86.67^{bc} (68.63)	1095.5^{c}
EP1	4.93^{cd}	10.13^{gh}	86.67^{bc} (68.63)	1305.3^{fg}
PCU	4.73^{b}	9.43^{e}	82.67^{ab} (65.43)	1170.6^{e}
PFSA	4.83^{bc}	10.07^{g}	86.67^{bc} (68.62)	1291.3^{f}
PFAR	4.77^{bc}	7.83^{c}	82.67^{ab} (65.43)	1041.6^{b}
PSV	5.40^{f}	10.83^{kl}	94.67^{e} (76.83)	1536.5^{l}
VPT4	5.10^{de}	10.57^{ij}	90.67^{cd} (72.29)	1420.8^{i}
KKM1	5.03^{de}	10.27^{h}	89.33^{c} (71.01)	1366.8^{h}
ARR1	5.07^{de}	10.13^{gh}	86.67^{bc} (68.63)	1317.3^{fg}
VPT10	5.03^{de}	10.87^{l}	86.67^{bc} (68.63)	1378.0^{h}
PB2	5.07^{de}	10.47^{i}	93.33^{de} (75.20)	1450.3^{ij}
ARR1G	5.07^{de}	10.27^{h}	86.67^{bc} (68.63)	1329.0^{g}
ARR2	4.67^{b}	9.73^{f}	82.67^{ab} (65.43)	1190.4^{e}
PFCOT	5.17^{e}	10.73^{kl}	93.33^{de} (75.20)	1484.0^{k}
PFCOP	5.13^{e}	10.67^{jk}	93.33^{de} (75.20)	1474.7^{jk}
Control	3.50^{a}	6.90^{a}	81.33^{a} (64.43)	845.9^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 6. *In vitro* inhibition of *P. aphanidermatum* by fluorescent pseudomonads

Pseudomonas strains	Inhibition zone (mm)
Pf1	13.67^{h}
FP7	11.67^{fg}
PFNL	0.0^{a}
PFKO	5.67^{c}
PFATR	3.3^{b}
PFNA	4.33^{b}
EP1	3.33^{b}
PCU	0.0^{a}
PFSA	3.33^{b}
PFAR	6.67^{cd}
PSV	0.0^{a}
VPT4	4.33^{b}
KKM1	7.33^{d}
ARR1	0.0^{a}
VPT10	7.33^{d}
PB2	10.33^{e}
ARR1G	7.67^{d}
ARR2	0.0^{a}
PGCOT	12.0^{g}
PFCOP	10.67^{ef}
Control	0.0^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Table 7. Efficacy of fluorescent pseudomonads for the management of damping-off disease in solanaceous vegetable crops

Pseudomonas strains	Tomato		Chilli		Brinjal	
	DI* (%)	Plant height (cm)	DI* (%)	Plant height (cm)	DI* (%)	Plant height (cm)
Pf1	35.55^{b} (36.58)	16.52^{f}	31.11^{b} (33.87)	15.10^{f}	42.22^{c} (40.52)	15.70^{f}
FP7	53.33^{cd} (46.91)	15.33^{e}	55.55^{d} (48.20)	14.51^{ef}	48.89^{c} (44.36)	14.91^{f}
PFATR	62.23^{d} (52.13)	14.61^{cd}	42.22^{c} (40.52)	13.60^{bc}	46.67^{c} (43.09)	15.33^{f}
PSV	57.77^{d} (49.52)	15.13^{de}	37.77^{bc} (37.91)	14.93^{f}	53.34^{cd} (46.97)	14.11^{de}
PB2	48.89^{c} (44.36)	14.89^{de}	68.98^{e} (56.13)	13.92^{cd}	64.45^{de} (53.41)	13.80^{cd}
PFCOT	51.18^{c} (45.68)	15.00^{de}	57.77^{d} (49.52)	14.10^{d}	68.89^{e} (56.13)	13.60^{c}
PFCOP	55.53^{cd} (48.20)	14.31^{bc}	68.89^{e} (56.10)	14.12^{d}	62.22^{de} (52.09)	13.50^{c}
Ridomil	31.11^{b} (33.87)	14.11^{b}	28.89^{b} (32.48)	13.20^{b}	31.00^{b} (33.80)	13.11^{b}
Control (pathogen-inoculated)	71.11^{e} (57.63)	8.09^{a}	75.55^{e} (60.41)	7.71^{a}	73.33^{de} (59.03)	7.14^{a}
Control (pathogen-uninoculated)	2.26^{a} (5.84)	14.12^{b}	2.26^{a} (5.84)	14.33^{de}	2.26^{a} (5.84)	14.41^{e}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

DI –Disease incidence (%)

Table 8. Efficacy of fluorescent pseudomonads on inhibition of mycelial growth of pathogens *in vitro*

Pseudomonas strains	Inhibition zone (mm)		
	F. oxysporum f. sp. *lycopersici*	*F. solani*	*C. capsici*
Pf1	18.33^{f}	14.67^{d}	10.67^{g}
FP7	15.33^{e}	16.00^{d}	10.00^{f}
PFATR	7.00^{b}	7.33^{b}	5.33^{b}
PSV	11.67^{cd}	11.33^{c}	6.00^{c}
PB2	11.33^{cd}	10.33^{c}	7.33^{d}
PFCOP	9.33^{bc}	9.00^{bc}	8.67^{e}
PFCOT	12.67^{d}	10.67^{c}	8.33^{e}
Control	0.00^{a}	0.00^{a}	0.00^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Plate 4. *In vitro* inhibition of *P. aphanidermatum* by *P. fluorescens* Pf1

Plate 5. *In vitro* inhibition of *F. oxysporum* f. sp. *lycopersici* by *P fluorescens* Pf1

Plate 6. *In vitro* inhibition of *C. capsici* by *P. fluorescens* Pf1

Plate 4

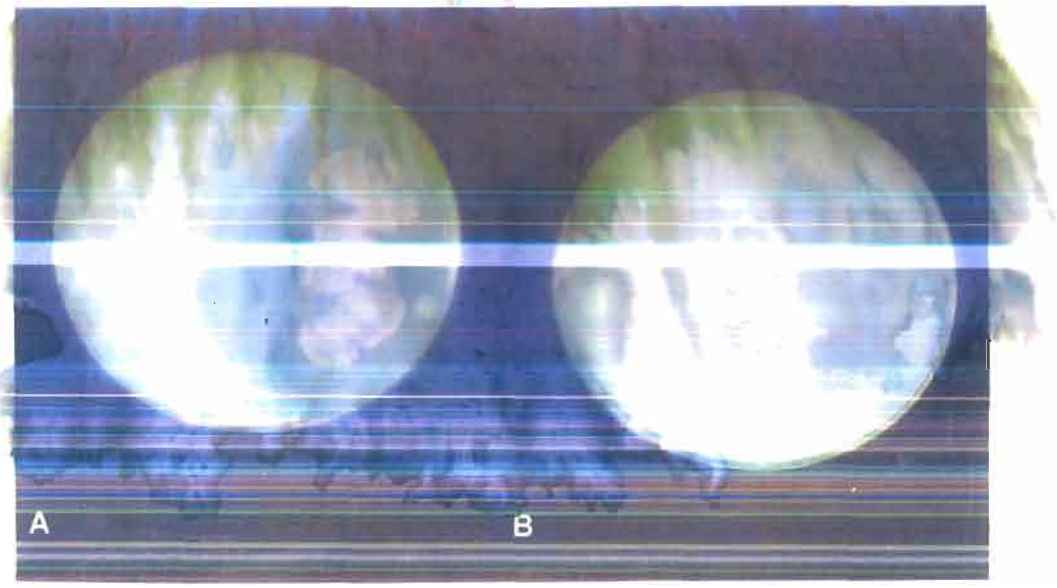


Plate 5

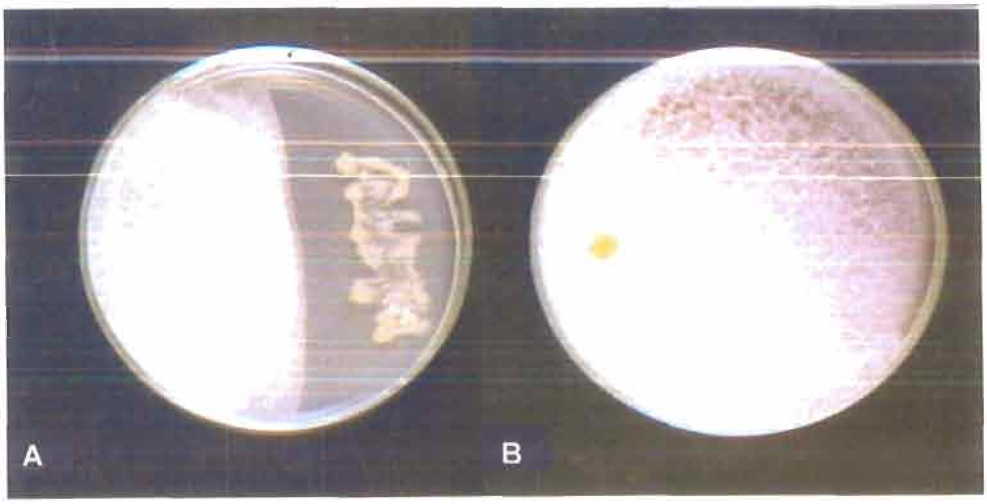
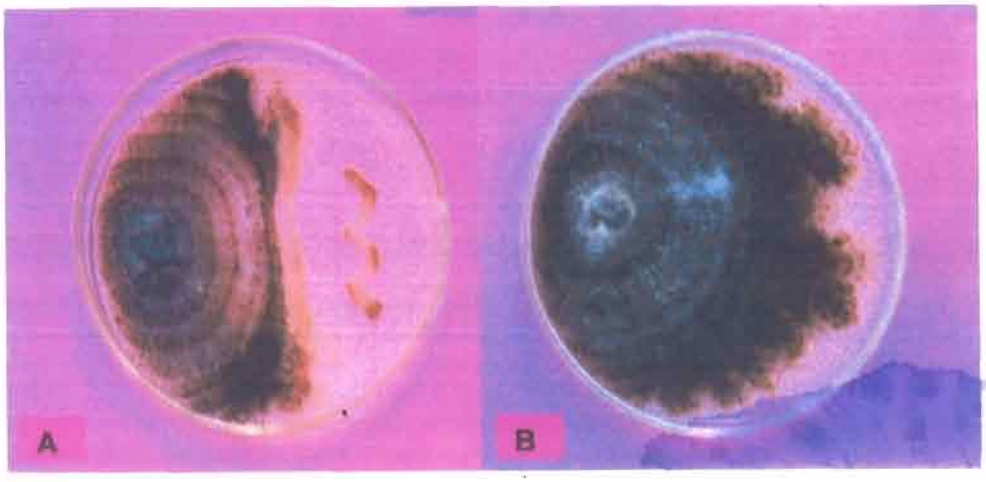


Plate 6



ridomil which recorded the disease incidence of 31.11, 28.89 and 31.00 per cent in tomato, chilli and brinjal respectively (Table-7).

4. 6. Testing the selected strains of fluorescent pseudomonads against *F. oxysporum* f. sp. *lycopersici*, *F. solani* and *C. capsici in vitro*

Among these seven strains tested, *P. fluorescens* Pf1 effectively inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* by recording an inhibition zone of 18.33 mm (Plate 5). *P. fluorescens* strain FP7 also effectively inhibited the mycelial growth of *F. oxysporum* f. sp. *lycopersici* by recording a inhibition zone of 15.33 mm (Table 8).

With regard to inhibition of mycelial growth of *F. solani*, *P. fluorescens* strains FP7 and Pf1 showed greater inhibition effect by recording inhibition zone of 16.00 and 14.67 mm respectively when compared with other fluorescent pseudomonads (Table 8).

Similarly *P. fluorescens* strains Pf1 and FP7 effectively inhibited the mycelial growth of *C. capsici* by recording an inhibition zone of 10.67 and 10.00 mm respectively (Table 8; Plate 6).

4. 7. Efficacy of fluorescent pseudomonads against fusarium wilt of tomato under greenhouse conditions

The data presented in Table 9 showed that among the different strains tested, *P. fluorescens* Pf1 reduced the disease incidence under greenhouse conditions by recording the disease incidence of 18.63 per cent and disease protection was comparable with fungicide, carbendazim which recorded the disease incidence of 17.00 per cent. Other strains *viz.*, *P. fluorescens* FP7 and *P. putida* PFATR reduced the disease incidence considerably. In addition to disease protection, *P. fluorescens* Pf1 increased plant growth

Table 9. Effect of fluorescent pseudomonads for the management of fusarium wilt of tomato and on plant growth under greenhouse conditions

Pseudomonas strains	Disease incidence (%)	Plant height (cm)
Pf1	18.63[b] (25.57)	54.50[f]
FP7	23.38[c] (28.92)	50.83[e]
PB2	35.38[d] (36.46)	50.53[e]
PFATR	26.00[c] (30.65)	48.47[cd]
PSV	34.68[d] (36.08)	46.60[c]
PFCOP	40.72[e] (39.65)	49.80[de]
PFCOT	47.02[f] (43.29)	47.47[c]
Carbendazim	17.00[b] (24.34)	43.40[b]
Pathogen inoculated	78.03[g] (62.05)	35.97[a]
Pathogen uninoculated	1.08[a] (5.96)	46.63[c]

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

Data are mean of three replications

Figures in parentheses indicate arcsine transformed values

to the maximum level (54.50 cm) when compared with fungicide treatment which recorded the plant height of 43.40 cm.

4. 8. Efficacy of fluorescent pseudomonads against fusarium wilt of brinjal under greenhouse conditions

In brinjal, seed treatment and soil application of *P. fluorescens* Pf1 was found to reduce the fusarium wilt of brinjal effectively with the minimum wilt incidence of 12.84 per cent when compared to other strains. The efficacy was comparable with that of carbendazim treatment which recorded the disease incidence of 8.34 per cent. In addition, *P. fluorescens* Pf1 also increased the plant growth to the maximum level by recording the plant height of 46.00 cm when compared with other strains or carbendazim treatment or untreated plants (Table 10).

4. 9. Efficacy of fluorescent pseudomonads against fruit rot of chilli under greenhouse conditions

The data presented in Table 11 revealed that fruit rot incidence was effectively checked by seed treatment and soil application of *P. fluorescens* Pf1 which recorded the disease incidence of 16.12 per cent under greenhouse conditions. The strain also increased the plant growth by recording a plant height of 48.32 cm. *P fluorescens* strain FP7 also reduced the fruit rot incidence to 18.16 per cent disease incidence and increased the plant growth (44.91 cm) and comparable with that of carbendazim treatment which recorded the disease incidence of 18.82 per cent with the plant height of 43.53 cm.

4. 10. Bacterial metabolites production

4. 10. 1. Diacetyl phloroglucinol

Except *P. putida* strain PFATR, all other strain grown on pigment production

Table 10. Efficacy of fluorescent pseudomonads for the management of fusarium wilt of brinjal and on plant growth promotion under greenhouse conditions

Pseudomonas strains	Disease incidence (%)	Plant growth (cm)
Pf1	12.84^{bc} (20.87)	46.00^{f}
FP7	19.09^{de} (25.87)	43.50^{e}
PB2	13.88^{cd} (21.83)	45.63^{f}
ATR	23.88^{ef} (29.30)	40.87^{d}
PSV	25.00^{ef} (29.97)	42.83^{e}
COP	30.21^{fg} (33.34)	38.87^{c}
COT	34.38^{g} (35.88)	36.00^{b}
Carbendazim	8.34^{b} (16.72)	39.63^{c}
Control (pathogen inoculated)	67.71^{h} (55.38)	32.63^{a}
Pathogen uninoculated	1.08^{a} (4.19)	40.67^{d}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 11. Effect of fluorescent pseudomonads for the management of fruit rot of chilli and on plant growth under greenhouse conditions.

Pseudomonas strains	Per cent Disease index	Plant height (cm)
Pf1	16.12^{b} (23.67)	48.32^{g}
FP7	18.16^{c} (25.22)	44.91^{d}
PB2	23.41^{e} (28.94)	44.37^{d}
ATR	26.26^{f} (30.83)	47.13^{f}
PSV	23.11^{e} (28.73)	42.12^{b}
COP	28.10^{f} (32.01)	45.48^{e}
COT	21.17^{d} (27.39)	45.81^{e}
Carbendazim	18.82^{c} (25.71)	43.53^{c}
Control (pathogen inoculated)	53.84^{g} (47.20)	36.49^{a}
Pathogen uninoculated	7.03^{a} (15.38)	44.50^{d}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Plate 7. Siderophore production by fluorescent pseudomonads

1. *P. fluorescens* Pf1
2. *P. fluorescens* FP7

Plate 8. 2, 4- diacetyl phloroglucinol production by fluorescent pseudomonads

1. *P. fluorescens* Pf1
2. *P. fluorescens* FP7
3. *P. putida* PFATR
4. Synthetic 2, 4- diacetyl phloroglucinol

Plate 9. HCN production by *P. fluorescens* Pf1

1. *P. fluorescens* Pf1
2. Control

Plate 7

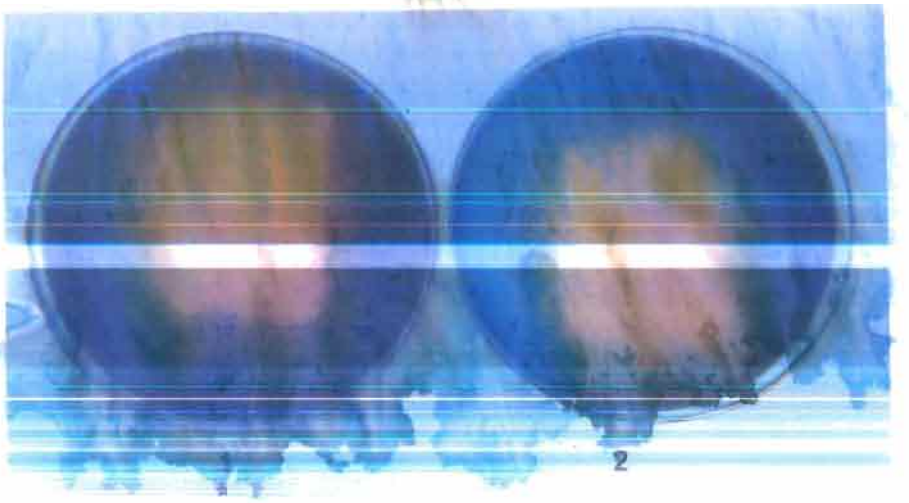


Plate 8

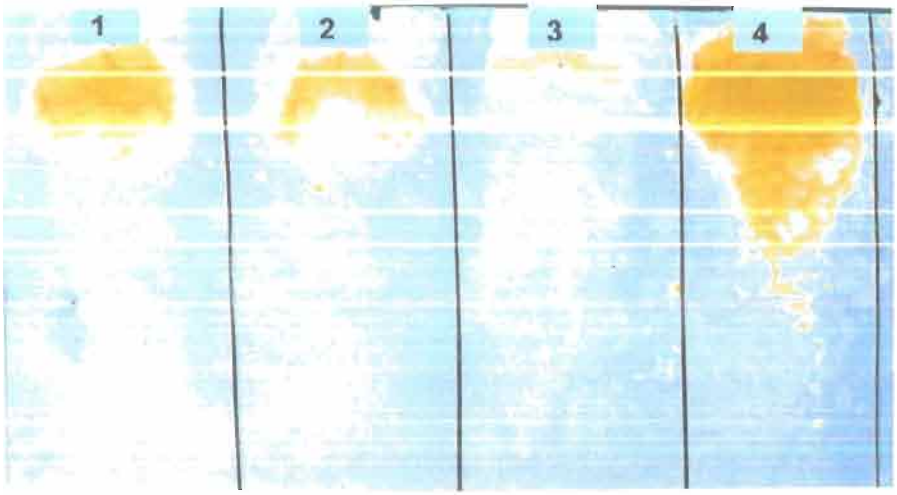
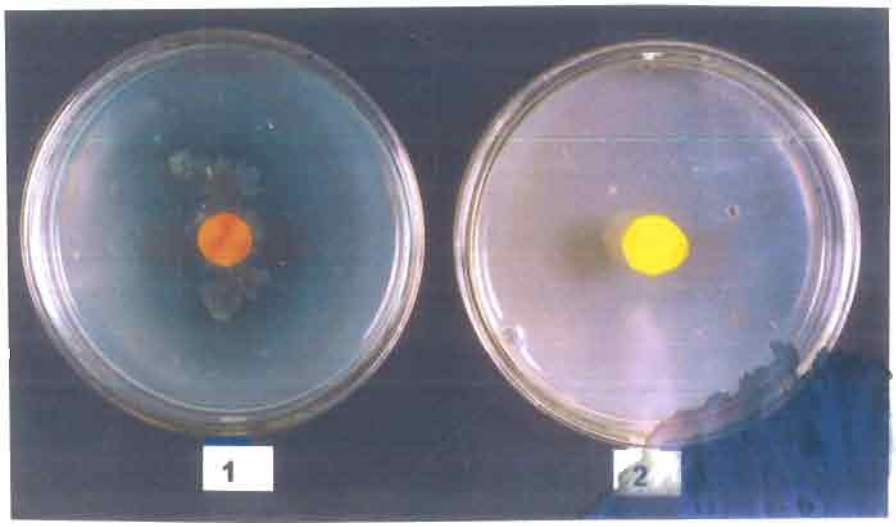


Plate 9



medium produced 2,4-diacetyl phloroglucinol. A distinct orange-colored spot at R_f value ranging from 0.86 to 0.88 was developed immediately after spraying with dinitrosalicylic acid on the thin layer chromatography (TLC) plates. Synthetic 2,4-diacetyl phloroglucinol which was included as standard chemical for the comparison produced dark orange-colored spot at R_f value of 0.88 (Table 12; Plate 7).

4. 10. 2. Siderophore

All the tested strains formed yellowish fluorescent colored halos around the colonies on dark-blue colored agar plates indicating positive reaction to the siderophore production. The diameter of the halos was uniform in all the plates streaked with different strains. The siderophore strongly chelated the iron from the dye, chromoazurol/Fe^{3+}/hexacyltrimethylammonium bromide thus its color turned from dark-blue to yellowish fluorescent color (Table 12; Plate 8).

4. 10. 3. Hydrogen cyanide production

P. fluorescens strains *viz.*, Pf1, FP7, PB2, PFCOP and *P. putida* strain PFATR produced hydrogen cyanide (HCN) and among these, *P. fluorescens* strain Pf1 and PFCOP strongly changed the yellow color of the filter paper to dark brown when compared to other strain indicating higher level of HCN production. *P. fluorescens* strain PB2 is a weak producer of HCN. *P. fluorescens* strain FP7 and *P. putida* strain PFATR produced at moderate level of HCN. *P. fluorescens* strains PSV and PFCOT showed negative reaction to this test (Table 12; Plate 9).

4. 10. 4. Salicylic acid

Among the seven strains of fluorescent pseudomonads, *P. fluorescens* strain FP7

produced the maximum amounts of salicylic acid (30.58 μg ml^{-1}) followed by *P. fluorescens* strain PFCOP (28.75 μg ml^{-1}). *P. fluorescens* strain Pf1 produced lower amounts of salicylic acid. *P. putida* strain PFATR produced the minimum amounts of SA (12.61 ug ml^{-1}) (Table 12).

4. 10. 5. Indole acetic acid

Among the various strains tested for the production of indole acetic acid (IAA), *P. fluorescens* Pf1 produced the maximum amount of IAA (160.4 μg ml^{-1} of the culture filtrate followed by *P. putida* strain PFATR recording 143.4 μg ml^{-1} of the culture filtrate (Table 12).

4. 10. 6. β-1, 3-glucanase

Among the various strains tested, *P. fluorescens* Pf1 produced the maximum amount of β-1, 3-glucanase in the laminarin-amended medium. *P. putida* strain PFATR also recorded the higher production of β-1, 3-glucanase followed by *P.fluorescens* strains FP7 and PSV when compared to the other strains *viz.*, *P. fluorescens* PB2, PFCOP and PFCOT (Fig. 2)

4. 10. 7. Chitinase

Experiment on production of chitinase by fluorescent pseudomonads in chitin amended KMB revealed that *P. fluorescens* strain FP7 produced the maximum amount of chitinase when compared with other strains. *P. putida* PFATR produced the least amount of chitinase (Fig. 3)

Table 12. Secondary metabolites production by fluorescent pseudomonads

Pseudomonas strains	2,4-diacetyl phloroglucinol	Siderophore	HCN**	Salicylic acid ($\mu g\ ml^{-1}$)	IAA ($\mu g\ ml^{-1}$)
Pf1	+ (0. 86)*	+	strong	22.57^{c}	160.4^{f}
FP7	+ (0. 88)	+	moderate	30.58^{e}	110.5^{c}
PB2	+ (0. 86)	+	weak	19.30^{bc}	82.6^{b}
PFATR	-	+	moderate	12.61^{a}	143.4^{e}
PSV	+ (0. 88)	+	none	16.24^{b}	125.5^{d}
PFCOP	+ (0.88)	+	strong	28.75^{de}	88.6^{b}
PFCOT	+ (0.86)	+	none	26.40^{d}	49.7^{a}

+ indicates positive to the test

- indicates negative to the test

*Values in parentheses indicates the R_f value

The synthetic phloroglucinol recorded R_f value of 0.88

** HCN production was scored as

Weak = light brown

Moderate = brown

Strong = reddish-brown

None = yellow

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Fig. 2. Production of extracellular β-1,3-glucanase by fluorescent pseudomonads in laminarin-amended KMB medium

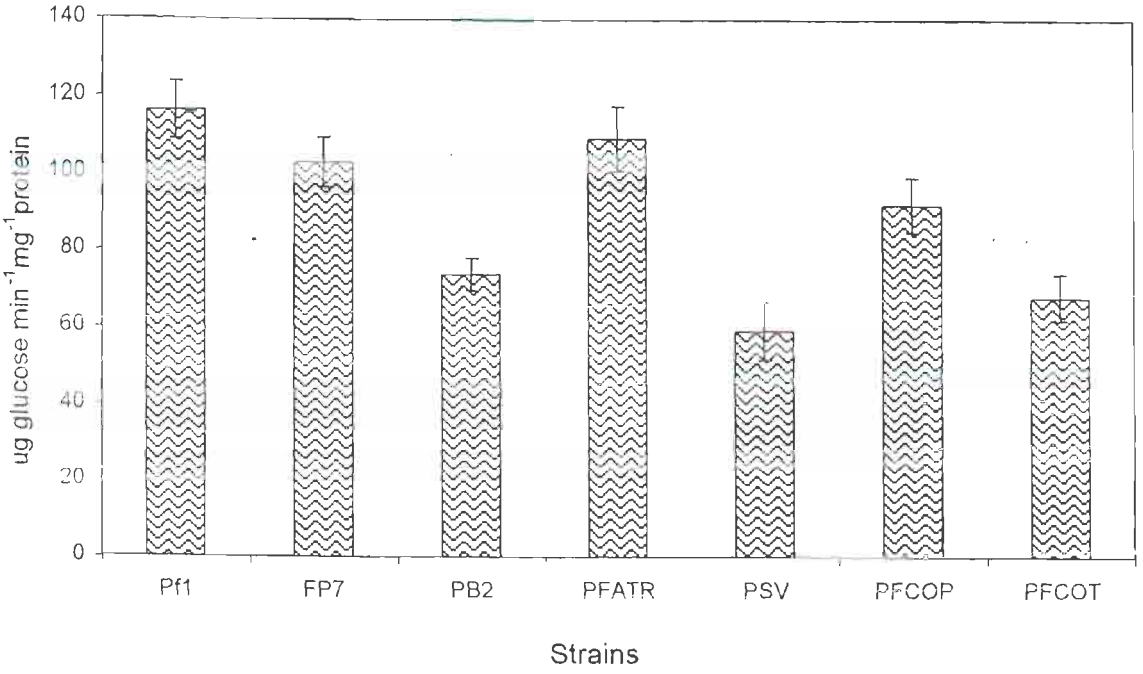
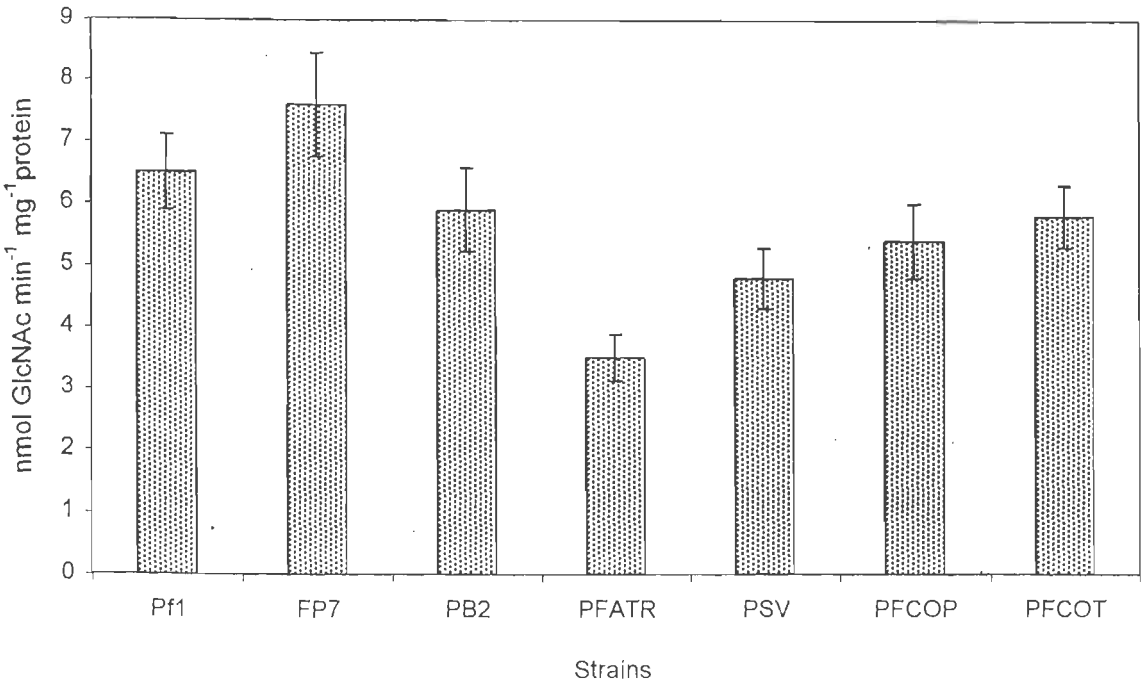


Fig. 3. Production of extracellular chitinase by fluorescent pseudomonads in chitin amended KMB medium



4. 11. Induction of defense-related proteins and chemicals by *P. fluorescens* Pf1

4. 11. 1. Induction of defense mechanisms by *P. fluorescens* Pf1 against *P. aphanidermatum*

4. 11. 1. 1. Phenylalanine ammonia lyase

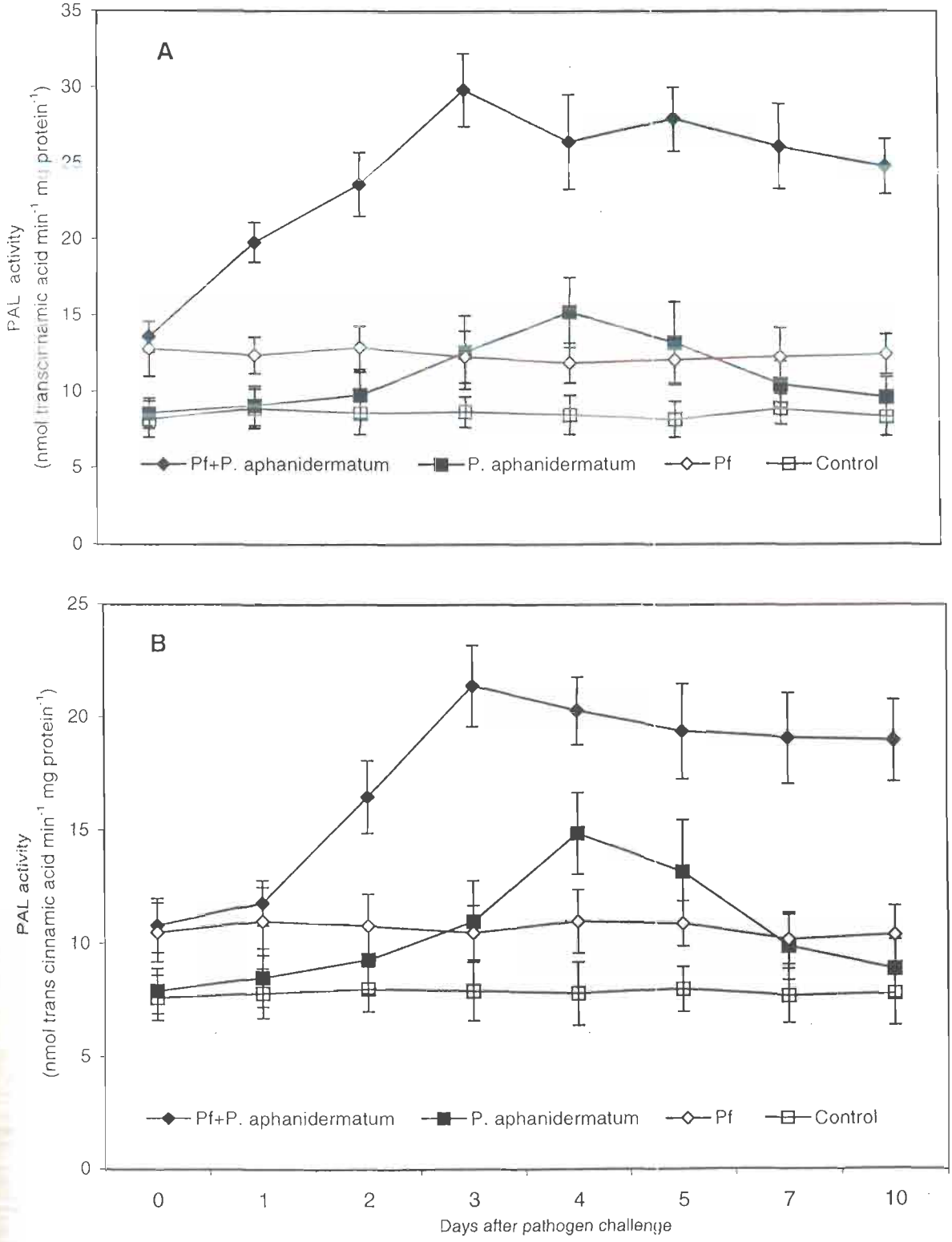
In tomato and chilli, seed treatment with *P. fluorescens* Pf1 induced the plants to synthesize phenylalanine ammonia lyase (PAL) whereas an additional increase in the synthesis was observed in *P. fluorescens* Pf1-pretreated plants challenge inoculated with *P. aphanidermatum*. The activity reached the maximum level at 3rd day after challenge inoculation and thereafter the activity remained at higher levels throughout the experimental period of 10 days. In plants inoculated with *P. aphanidermatum* alone, increased activity of PAL was observed for a period of 2-4 days thereafter declined drastically in both tomato and chilli (Fig. 4 A & B).

4. 11. 1. 2. Peroxidase

The increased activities of peroxidase (PO) were observed at very early in *Pseudomonas*-pretreated tomato and chilli plants challenge inoculated with *P. aphanidermatum* and remained at higher levels throughout the experimental period. The activity reached maximum levels at 3rd day after challenge inoculation (Fig. 5 A & B).

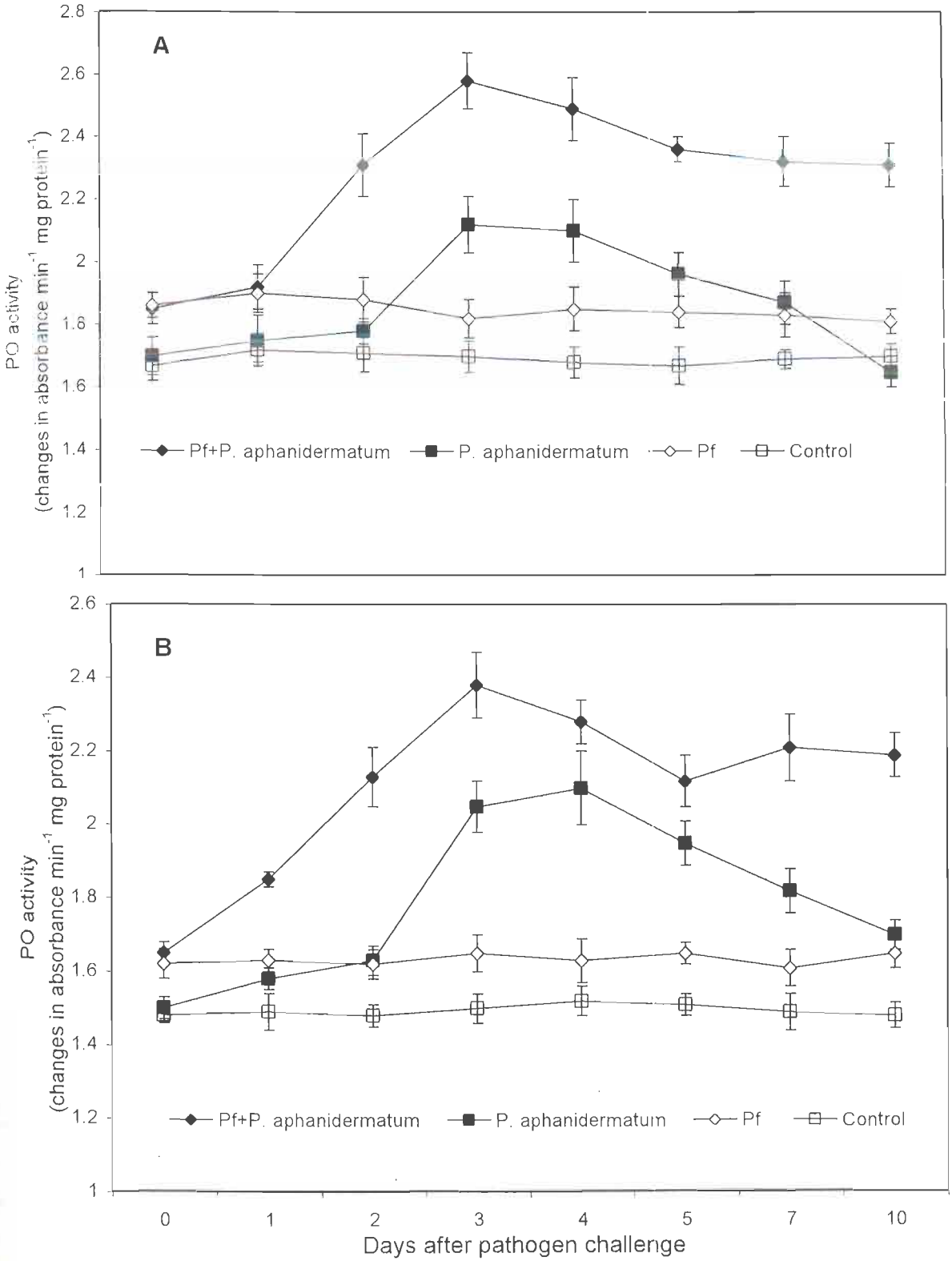
In bacterized tomato plants challenged with *P. aphanidermatum*, the expression of PO isoforms designated as PO1, PO2, PO3, PO4 and PO5 was more prominent and the expression of PO1 was higher when compared with untreated plants and also plants treated with *P. aphanidermatum* alone or plants treated with *P. fluorescens* alone

Fig. 4. Influence of seed treatment with *P. fluorescens* Pf1 on PAL activity in tomato (A) and chilli (B) challenged with or without *P. aphanidermatum*



Vertical bar indicates standard deviation of three replications

Fig. 5.Changes in PO activity by seed treatment with *P. fluorescens* Pf1 in tomato (A) and hotpepper (B) challenged with or without *P. aphanidermatum*



Vertical bars indicate standard deviations of three replications

Plate 10. PO isoform profile in tomato (A) and chilli (B) induced by *P. fluorescens* Pf1 challenged with or without *P. aphanidermatum*

Lane 1. Seed treatment with *P. fluorescens* Pf1 alone

Lane 2. *P. fluorescens* Pf1-treated plants challenged with *P. aphanidermatum*

Lane 3. Plants inoculated with *P. aphanidermatum*

Lane 4. Control plants

Plate 10A

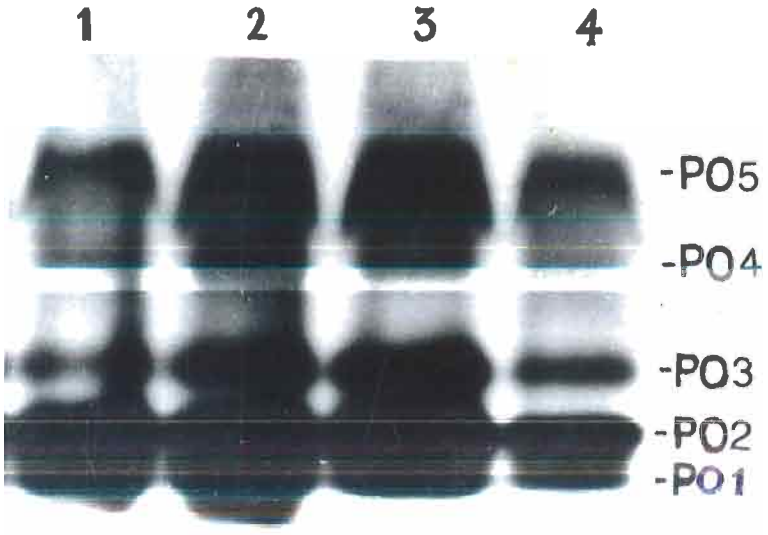
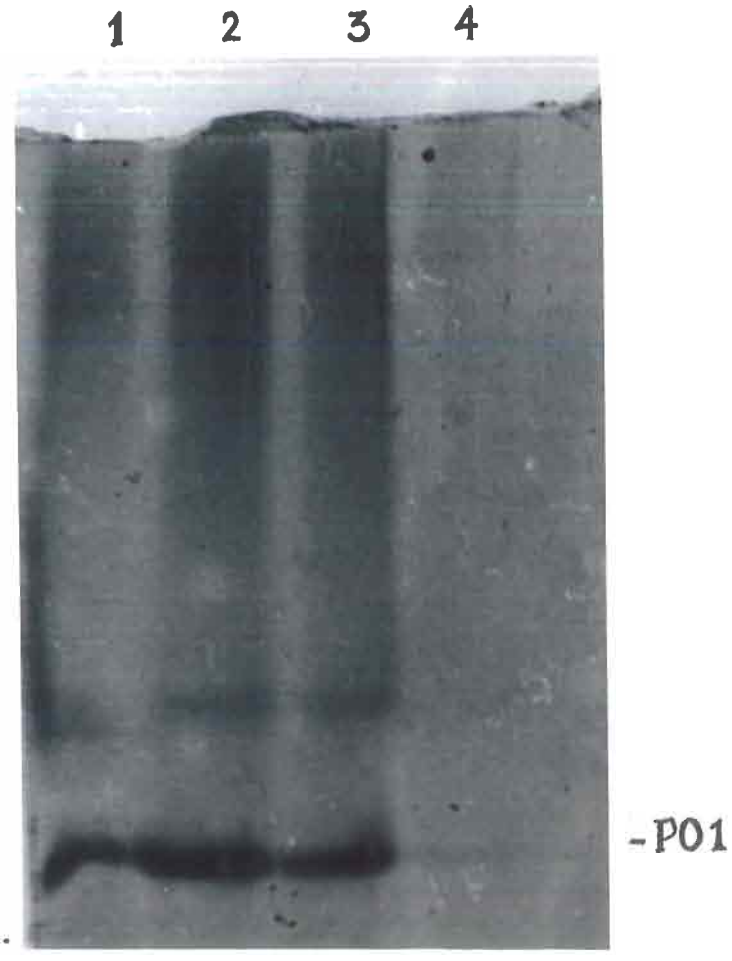


Plate 10B



(Plate 10A). Plants inoculated with *P. aphanidermatum* alone also had higher level expression of PO2-PO5 isoforms. In chilli plants only one PO isoform (PO1) was noticed in all induction treatments except untreated plants in which the expression of PO1 was very weak. However, higher level expression of PO1 was observed in *P. fluorescens* Pf1 treated chilli plants challenge inoculated with *P. aphanidermatum* (Plate 10B).

4. 11. 1. 3. Polyphenol oxidase

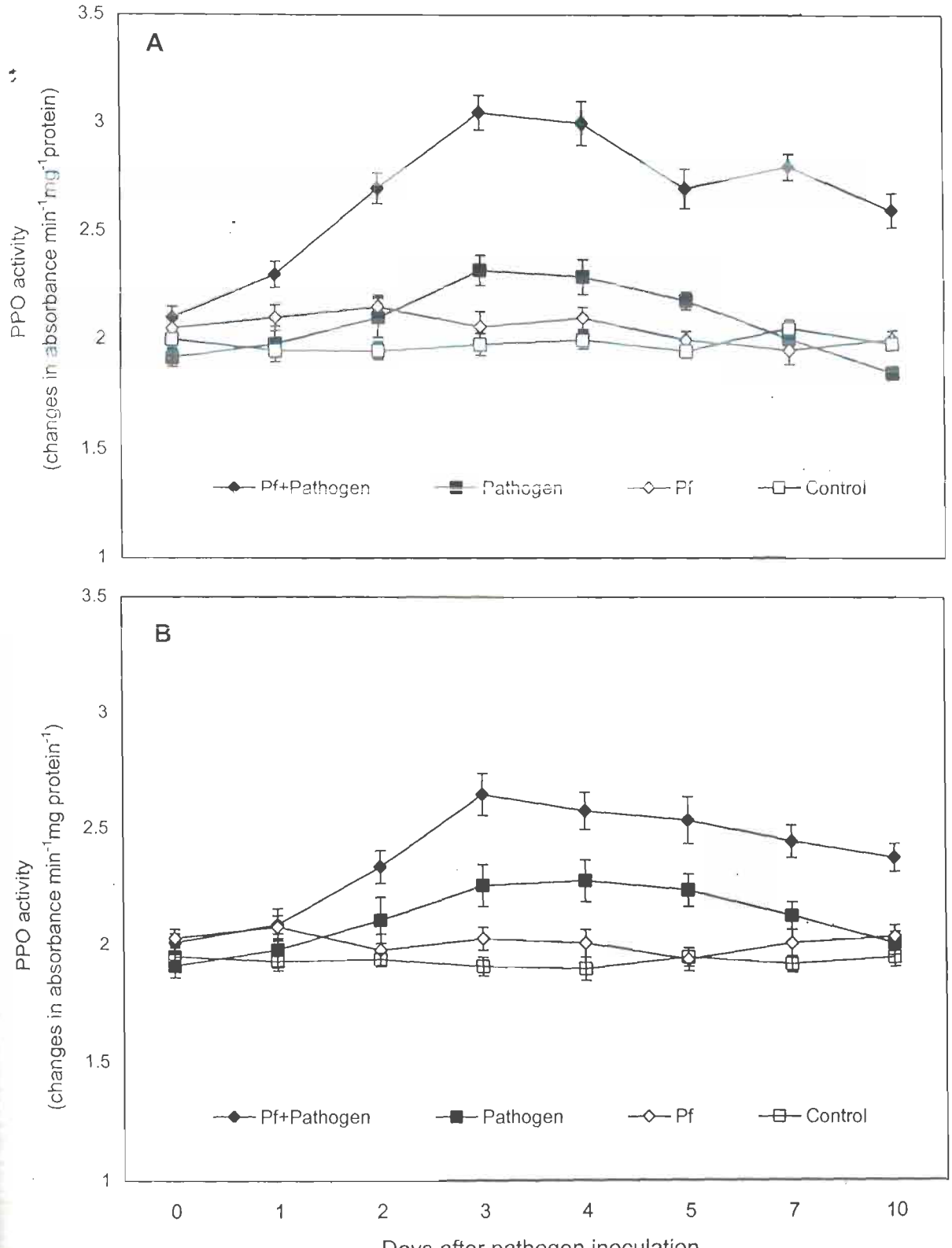
Similar to peroxidase activity, higher and increased activities of polyphenol oxidase (PPO) were noticed in *P. fluorescens* Pf1 treated tomato and chilli plants challenged with *P. aphanidermatum* when compared to other induction treatments and also untreated control plants (Fig. 6 A & B).

Three PPO isoforms designated as PPO1, PPO2 and PPO3 were prominently noticed in tomato plants treated with *P. fluorescens* and challenge inoculated with *P. aphanidermatum* (Plate 11A). In chilli, only one PPO isoform (PPO1) was expressed at higher level in all the induction treatments. In untreated plants, this constitutive PPO1 was expressed at a weak level (Plate 11B).

4. 11. 1. 4. Phenols

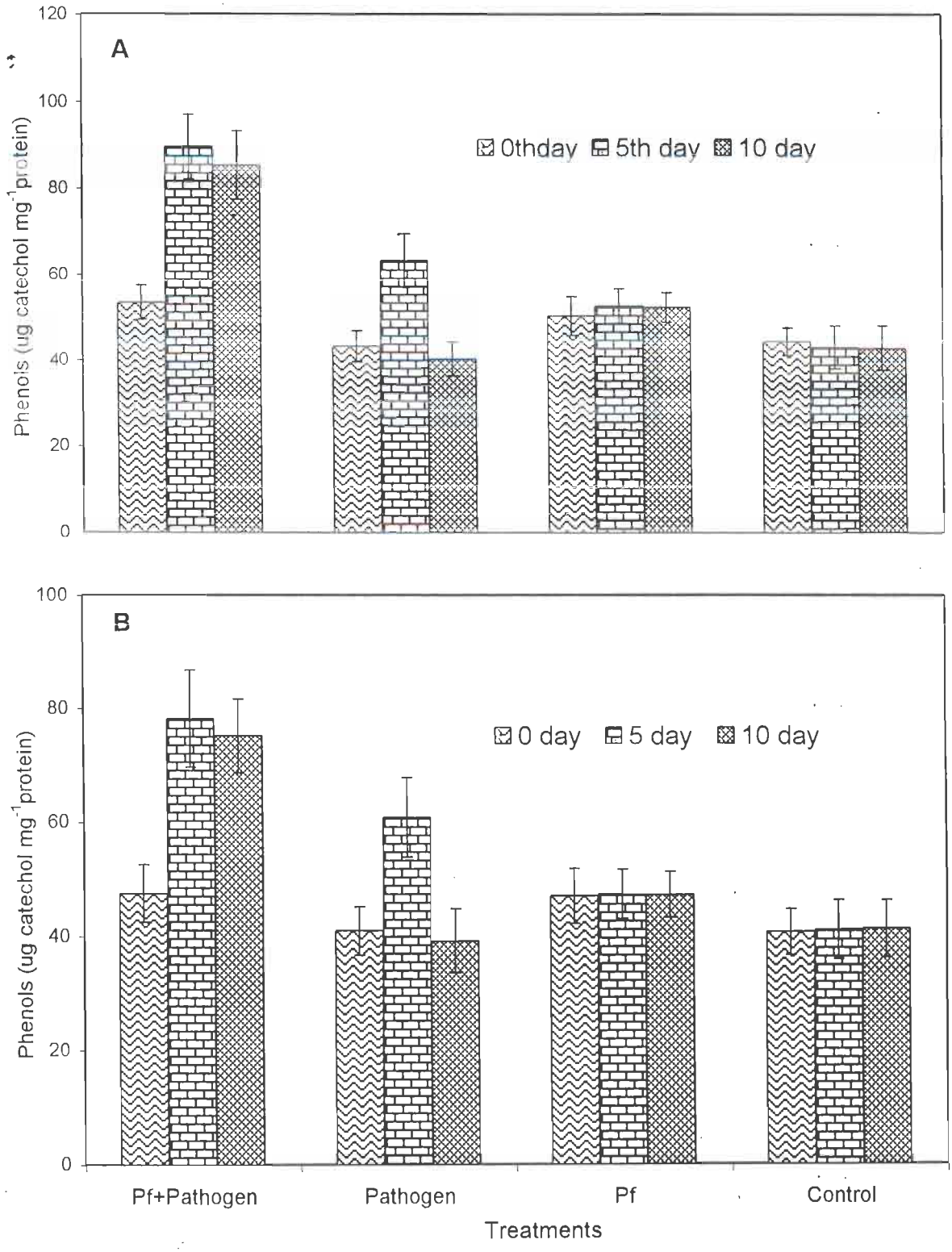
Higher level accumulation of phenolics was observed in bacterized roots challenge inoculated with *P. aphanidermatum* both at 5^{th} and 10^{th} day after challenge inoculation. In plants inoculated with *P. aphanidermatum* alone increased phenolic content was noticed at 5^{th} day whereas at 10^{th} day it was drastically reduced (Fig. 7 A & B).

Fig. 6. Changes in PPO activity by seed treatment with *P. fluorescens* Pf1 in tomato (A) and chilli (B) challenged with or without *P. aphanidermatum*



Vertical bars indicate standard deviations of three replications

Fig. 7. Accumulation of phenolics by seed treatment with *P. fluorescens* Pf1 in tomato (A) and chilli (B) challenged with or without *P. aphanidermatum*



Vertical bars indicate standard deviations of three replications

Plate 11. PPO isoform profile in tomato (A) and chilli (B) induced by *P. fluorescens* Pf1 challenged with or without *P. aphanidermatum*

Lane 1. Seed treatment with *P. fluorescens* Pf1 alone

Lane 2. *P. fluorescens* Pf1-treated plants challenged with *P. aphanidermatum*

Lane 3. Plants inoculated with *P. aphanidermatum*

Lane 4. Control plants

Plate 11a

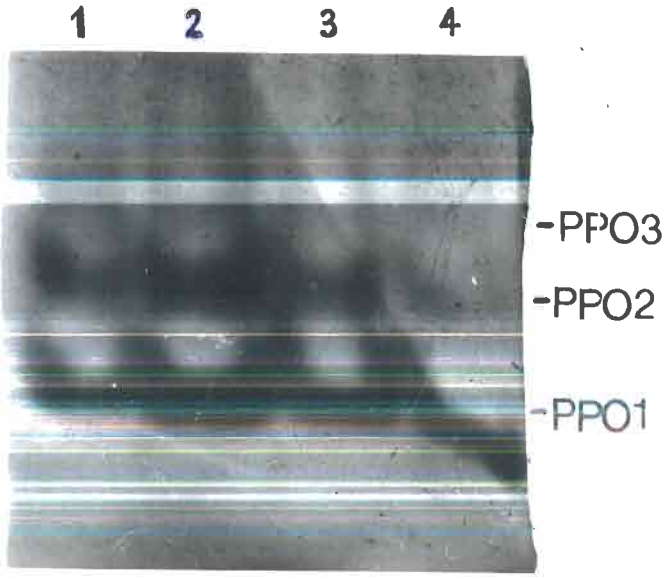
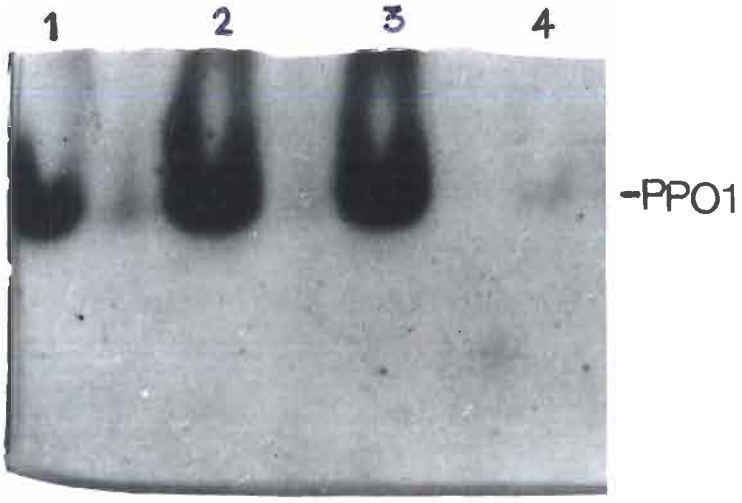


Plate 11b



4. 11. 1. 5. β-1, 3-glucanase

The activities of β-1, 3-glucanase was found at higher level in the *P. fluorescens* pretreated plants and challenged with *P. aphnidermatum.* The activity reached maximum level at 5th day after challenge inoculation in tomato and 4th day in chilli. In plants inoculated with *P. aphanidermatum* the increased activity was lasted only for a period of 2-4 days thereafter declined drastically (Fig. 8 A & B).

4. 11. 1. 6. Chitinase

Similar to other enzymes earlier and higher activities of chitinase were observed in *P. fluorescens* treated tomato and chilli plants challenge inoculated with *P aphanidermatum* (Fig. 9 A & B).

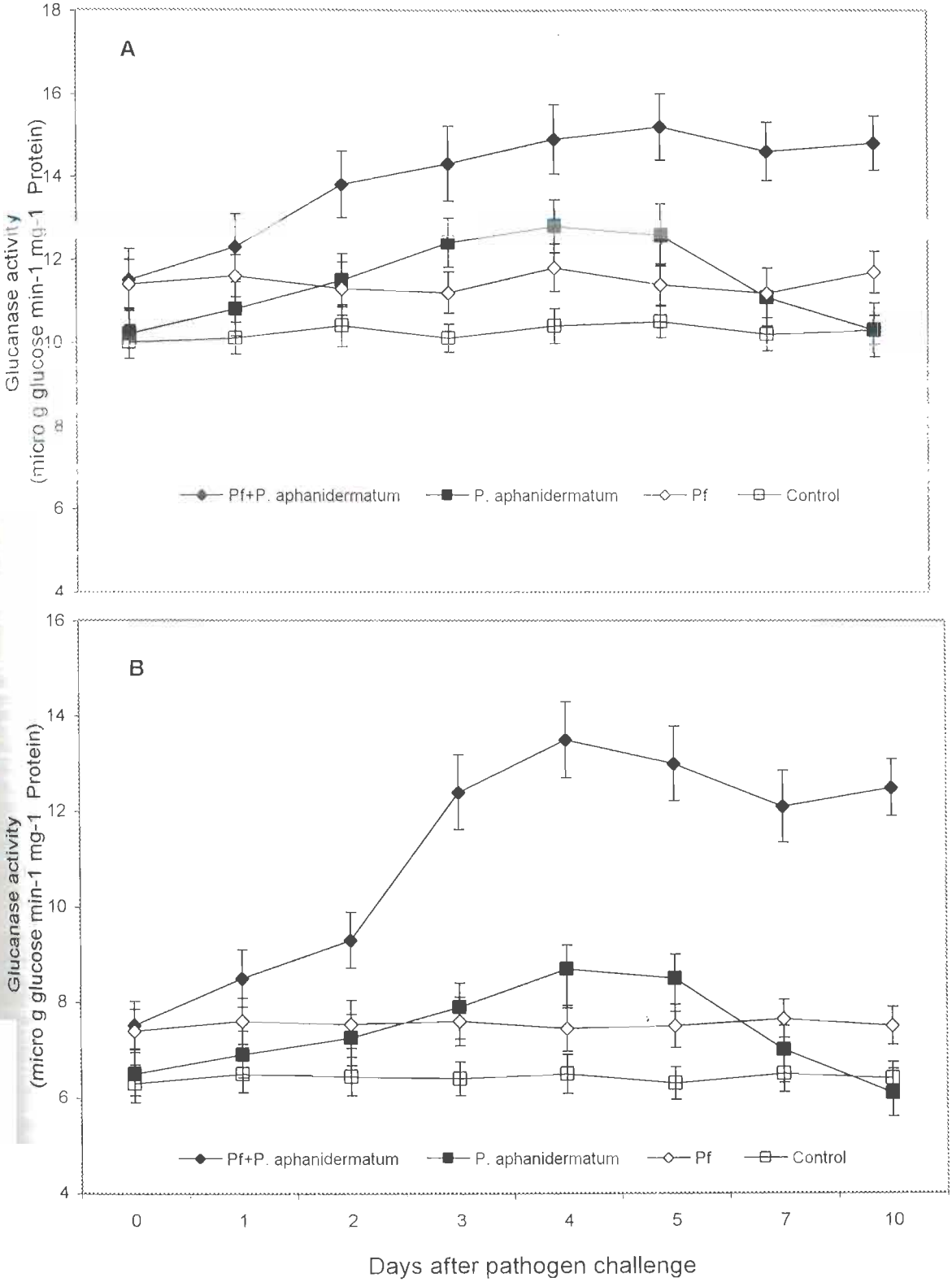
Western blot analysis on induction of chitinase protein showed that a new chitinase isoform with the molecular weight of 41 kDa was induced in *P. fluorescens*-treated tomato plants challenge inoculated with *P. aphanidermatum*. The expression of chitinase was also noticed in the *P. aphanidermatum*-inoculated plants (at weak level) but not in plants treated with *P. fluorescens* alone and untreated control plants (Plate 12).

4. 11. 2. Induction of defense mechanisms by *P. fluorescens* Pf1 against *F. oxysporum* f. sp. *lycopersici*

4. 11. 2. 1. Phenylalanine ammonia lyase

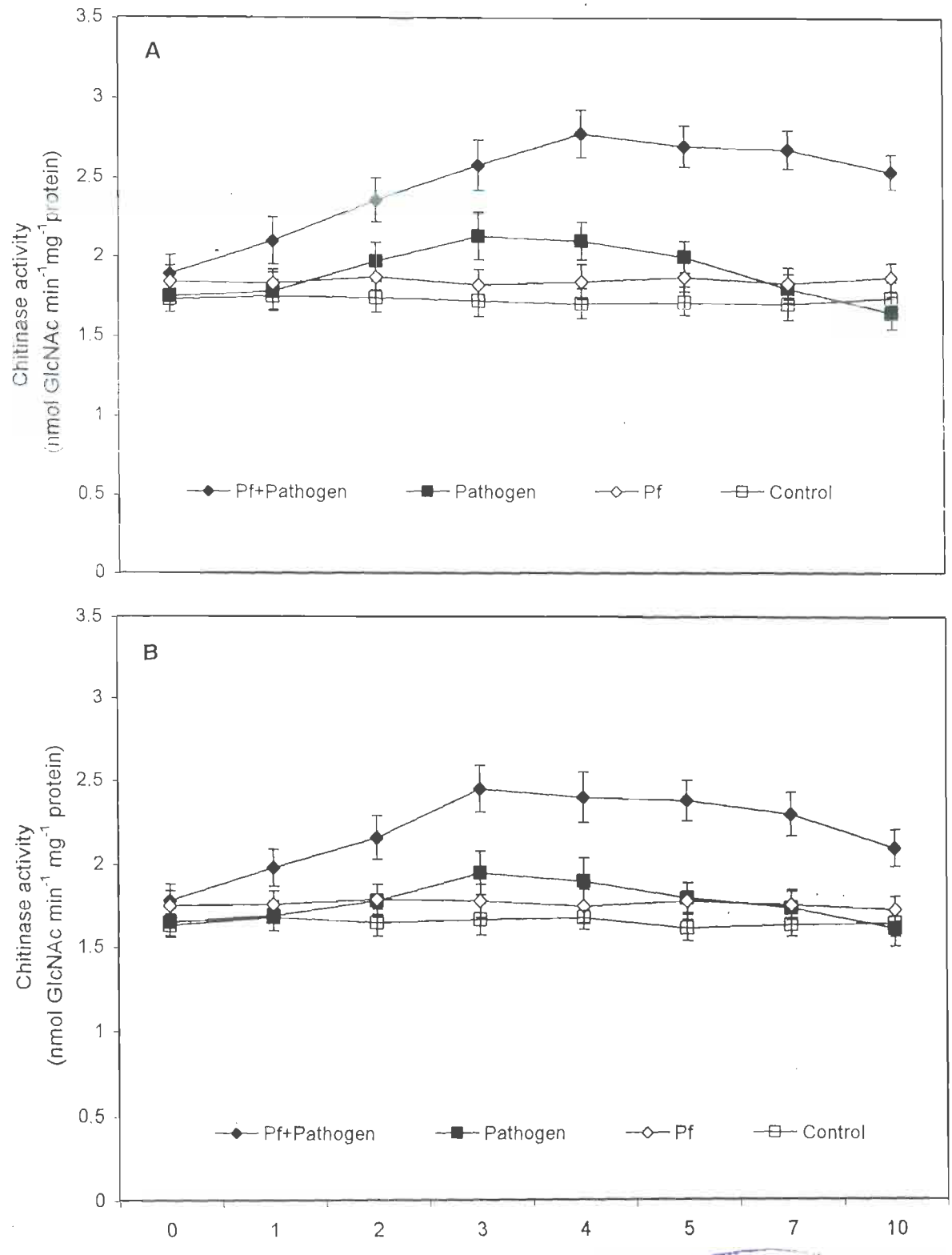
Upon challenge inoculation in bacterized tomato root tissues, PAL activity started to increase one day after inoculation of *F. oxysporum* f. sp. *lycopersici* and reached maximum at 4th day. Similarly, tomato roots inoculated with *F. oxysporum* f. sp. *lycopersici* alone recorded increased activity of PAL but the

Fig. 8 Induction of B-1,3-glucanase by *P. fluorescens* isolate Pf1 in tomato (A) and chilli (B) roots challenged with or without the pathogen *P. aphanidermatum*



Vertical bars indicates standard deviation of three replications

Fig. 9. Induction of chitinase by *P. fluorescens* Pf1 in tomato (A) and chilli (B) challenged with or without *P. aphanidermatum*



Vertical bars indicate standard deviations of three replications

Plate 12. Western blot analysis for chitinase isoform induced by *P. fluorescens* Pf1 in tomato challenged with or without *P. aphanidermatum*

Lane 1. Marker

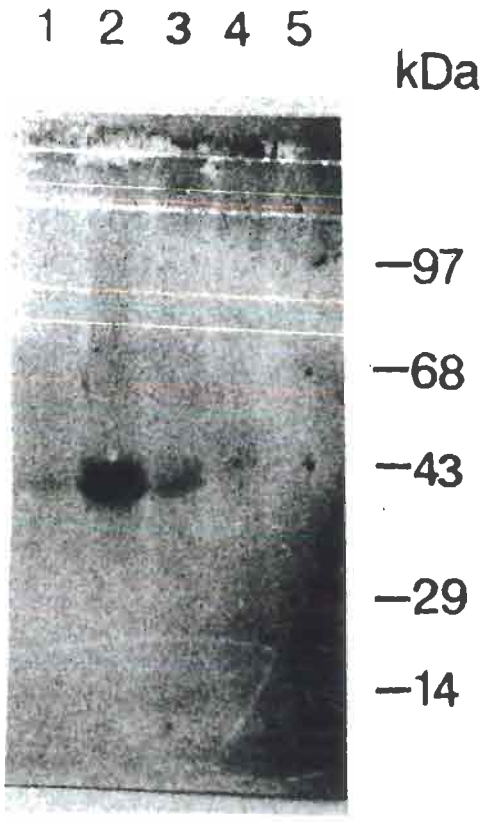
Lane 2. Plants treated with *P. fluorescens* Pf1 alone

Lane 3. *P. fluorescens* Pf1-treated plants challenged with *P. aphanidermatum*

Lane 4. Plants inoculated with *P. aphanidermatum*

Lane 5. Control plants

Plate 12



induction of activity was observed for 2-4 days, thereafter declined drastically. Tomato roots treated with *P. fluorescens* Pf1 alone also had significantly higher PAL activity compared to untreated control but activity was less compared to challenge inoculated plants (Fig. 10).

4. 11. 2. 2. Peroxidase

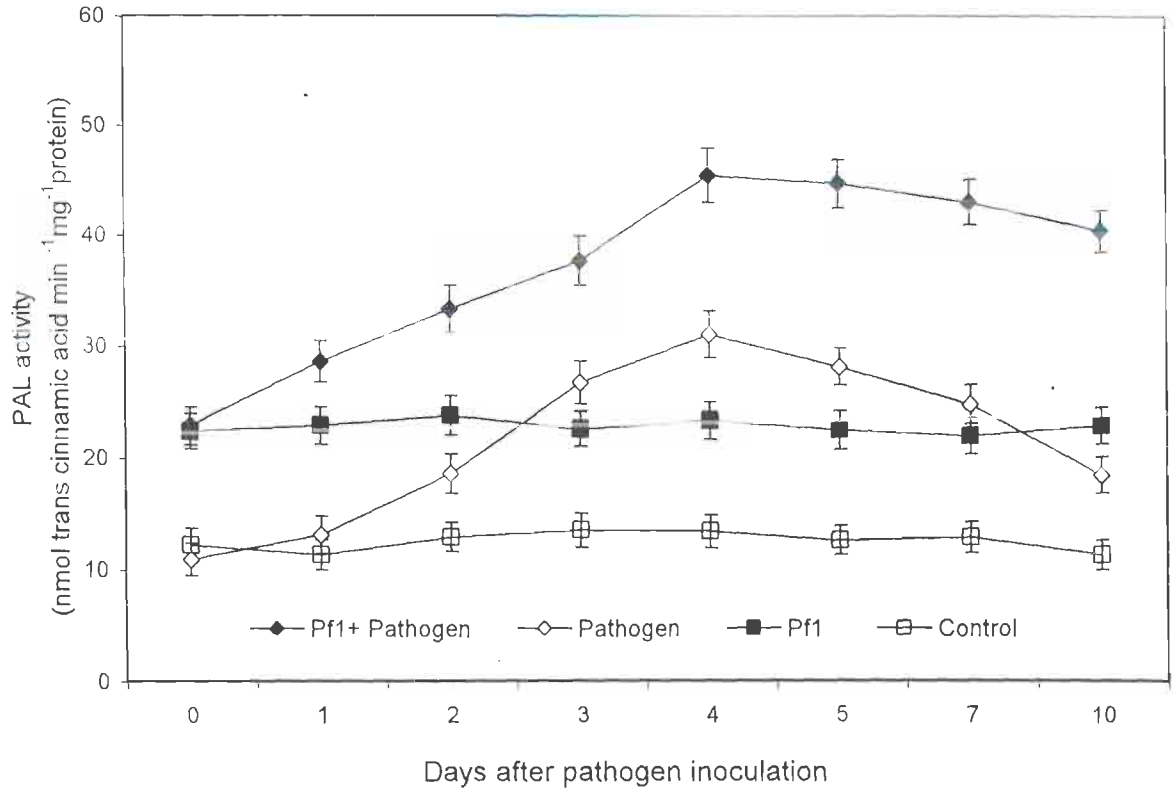
Peroxidase activity also increased in *P. fluorescens* Pf1-treated root tissues challenged with *F. oxysporum* f. sp. *lycopersici*. The maximum PO activity was observed at 4th day after challenge inoculation and the activity was maintained at higher levels throughout the experimental period. Plants inoculated with *F. oxysporum* f. sp. *lycopersici* alone had comparatively less PO activity. Peroxidase activity in roots treated with *P. fluorescens* alone remained unchanged during the experimental period but compared to control, the activity was higher (Fig. 11).

Native-PAGE analysis revealed that seven PO isoforms, PO1, PO2, PO3, PO4, PO5, PO6 and PO7 were observed in *P. fluorescens* Pf1-treated root tissues challenged with *F. oxysporum* f. sp. *lycopersici*. The expression of isoform PO1 was more prominent in *P. fluorescens* Pf1 treated plants challenged with *F. oxysporum* f. sp. *lycopersici* compared to other treatments. Moreover, other constitutive isoforms (PO2-PO7) were expressed at higher levels compared to untreated control (Plate 13).

4. 11. 2. 3. Polyphenol oxidase

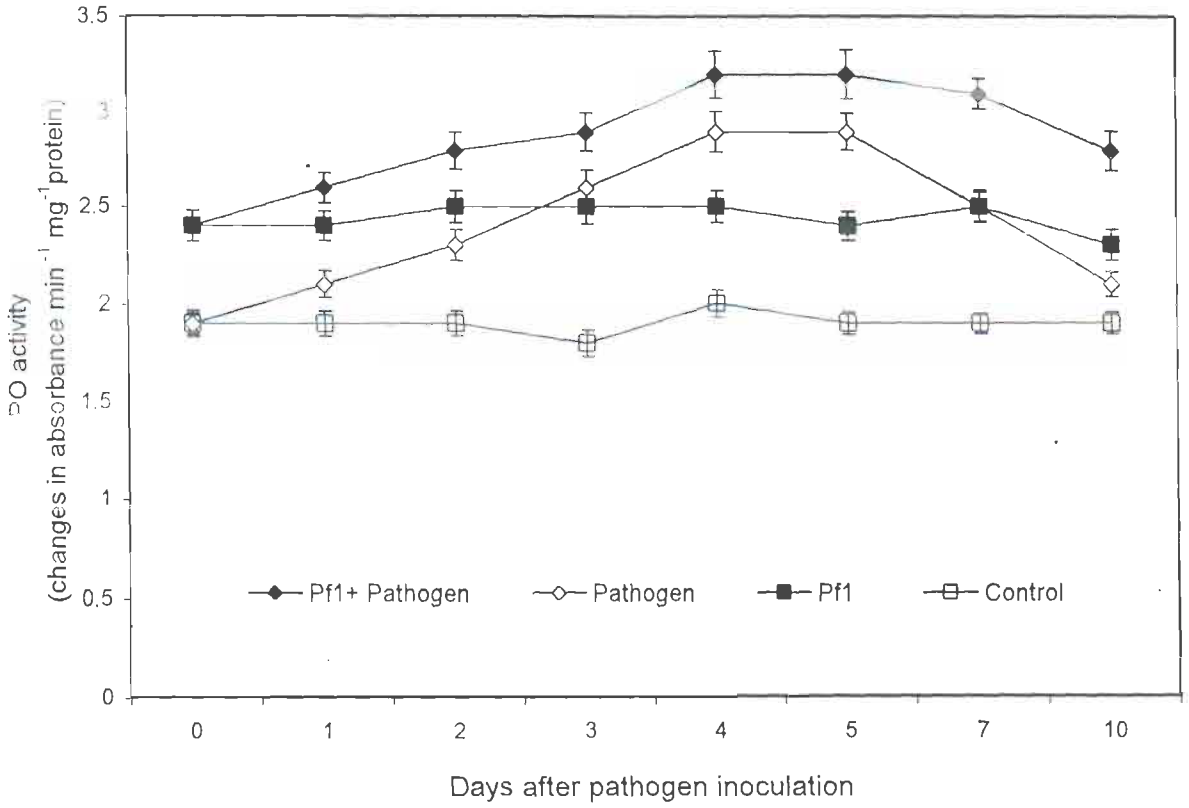
A similar pattern of increased activity of PPO was observed in bacterized tomato plants challenged with *F. oxysporum* f. sp. *lycopersici* and the activity reached maximum at 5th day (Fig. 12).

Fig. 10. Induction of PAL activity by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*



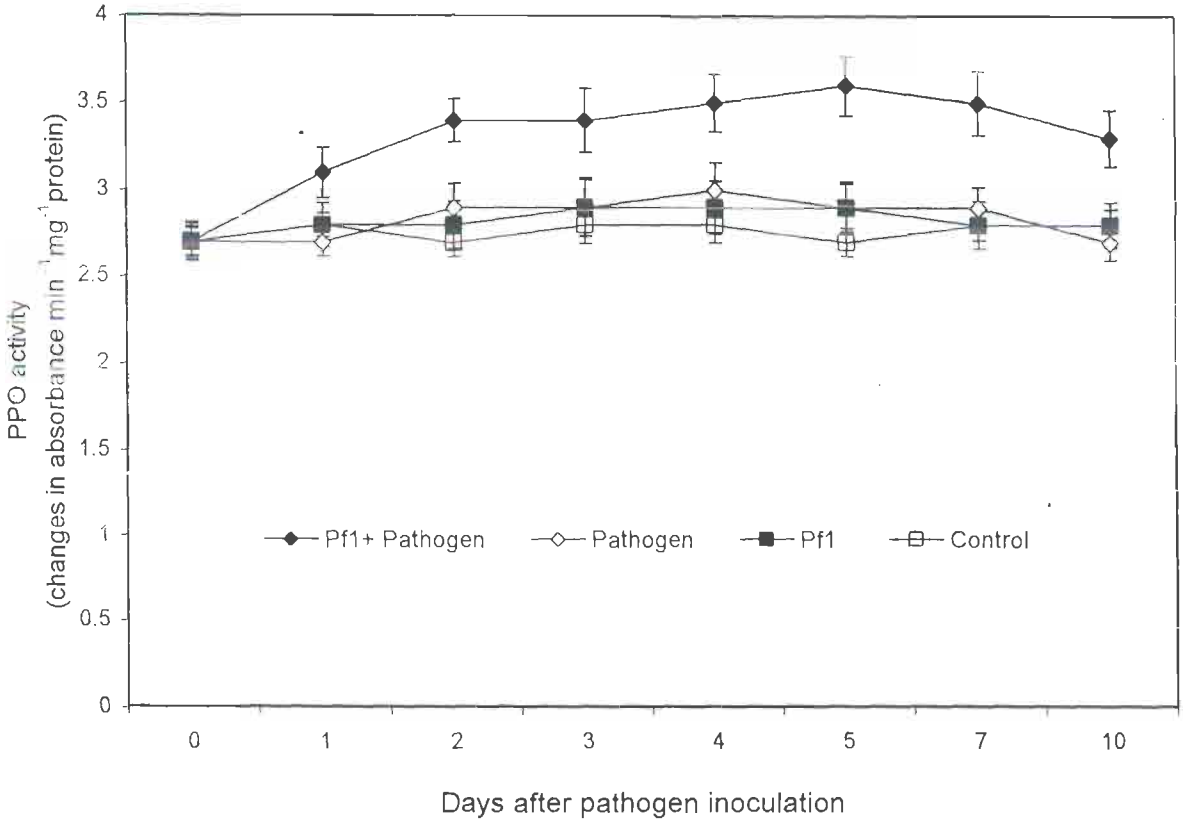
Vertical bars indicate standard deviations of three replications

Fig. 11. Changes in PO activity induced by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*



Vertical bars indicate standard deviations of three replications

Fig. 12.Changes in PPO activity induced by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f. sp. *lycopersici*



Vertical bars indicate standard deviations of three replications

Plate 13. Native-PAGE analysis for PO isoform profile induced by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*

Lane 1. Plants treated with *P. fluorescens* Pf1 alone

Lane 2. *P. fluorescens* Pf1-treated plants challenged with *F. oxysporum* f. sp. *lycopersici*

Lane 3. Plants inoculated with *F. oxysporum* f. sp. *lycopersici*

Lane 4. Control plants

Plate 13

Plate 14. Native-PAGE analysis for PPO isoform profile induced by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*

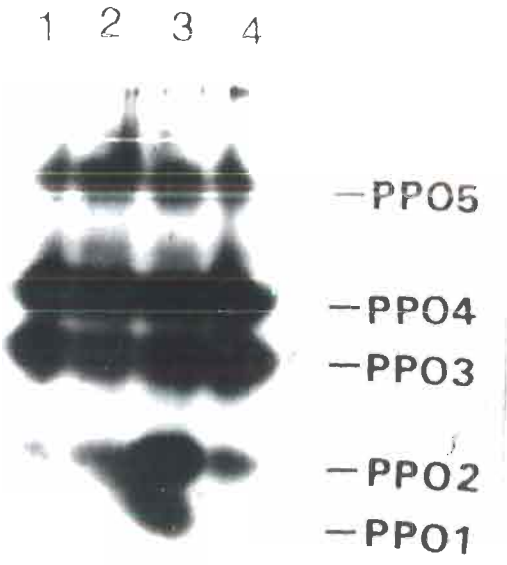
Lane 1. Control plants

Lane 2. Plants inoculated with *F. oxysporum* f. sp. *lycopersici*

Lane 3. *P. fluorescens* Pf1-treated plants challenged with *F. oxysporum* f. sp. *lycopersici*

Lane 4. Plants treated with *P. fluorescens* Pf1 alone

Plate 14



Five PPO isoforms, PPO1, PPO2, PPO3, PPO4 and PPO5 were observed in tomato root tissues treated with *P. fluorescens* Pf1 challenged with *F. oxysporum* f. sp. *lycopersici*. PPO1 was a newly induced isoform and PPO2 was expressed prominently in tomato root tissues by this treatment. In other treatments, four constitutive PPO isoforms, PPO2, PPO3, PPO4 and PPO5 were observed (Plate 14).

4. 11. 2. 4. Phenols

Increased accumulation of phenolics was observed in bacterized tomato roots challenge inoculated with *F. oxysporum* f. sp. *lycopersici* even at 10^{th} day. Plants inoculated with *F. oxysporum* f. sp. *lycopersici* alone also recorded increased accumulation of phenolics at 5^{th} day whereas at 10^{th} day the accumulation drastically declined. There was no marked change of phenols in plants treated with *P. fluorescens* alone during the time course of experimental period (Fig. 13).

4. 11. 2. 5. β-1,3-glucanase

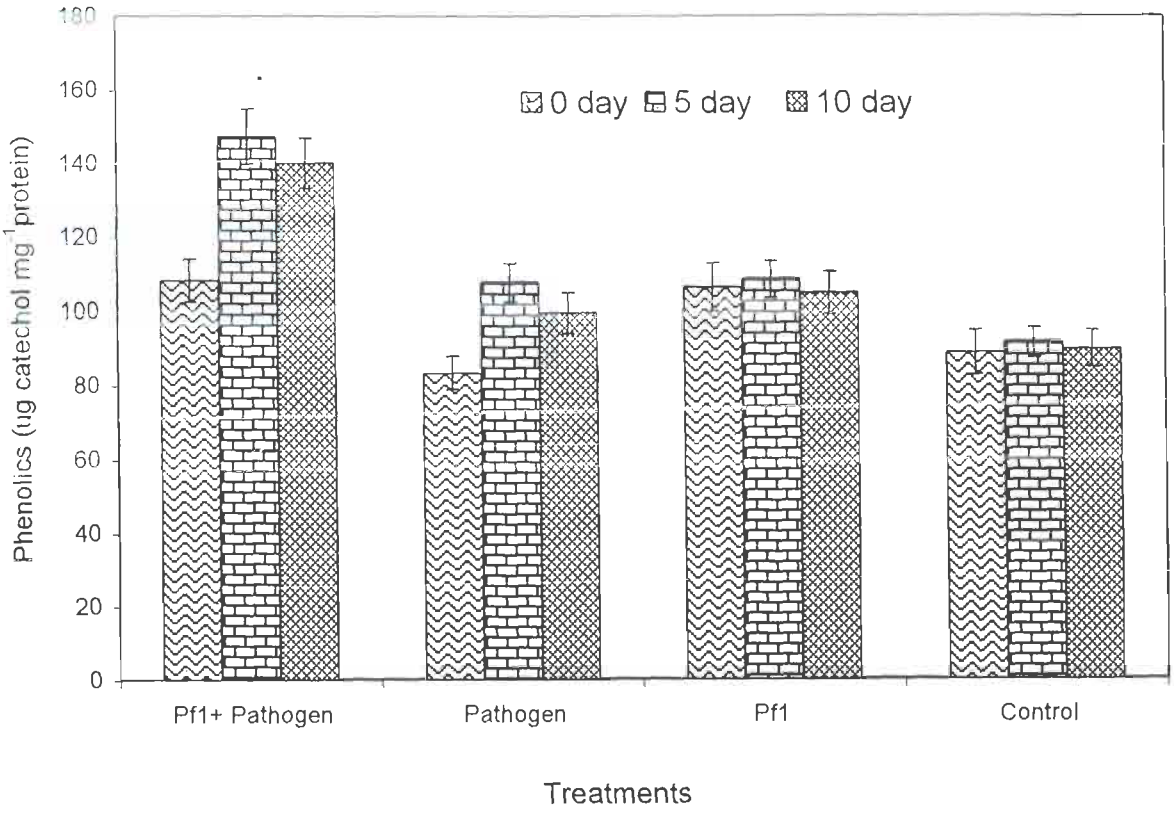
The activities of β-1,3-glucanase (Fig. 14) were maximum in bacterized root tissues challenged with *F. oxysporum* f. sp. *lycopersici* and higher activities were observed at 3-5 days after challenge inoculation.

4. 11, 2. 6. Chitinase

Higher activities of chitinase were noticed in *P. fluorescens* Pf1 treated plants challenged with *F. oxysporum* f. sp. *lycopersi* when compared with other treatments (Fig. 15).

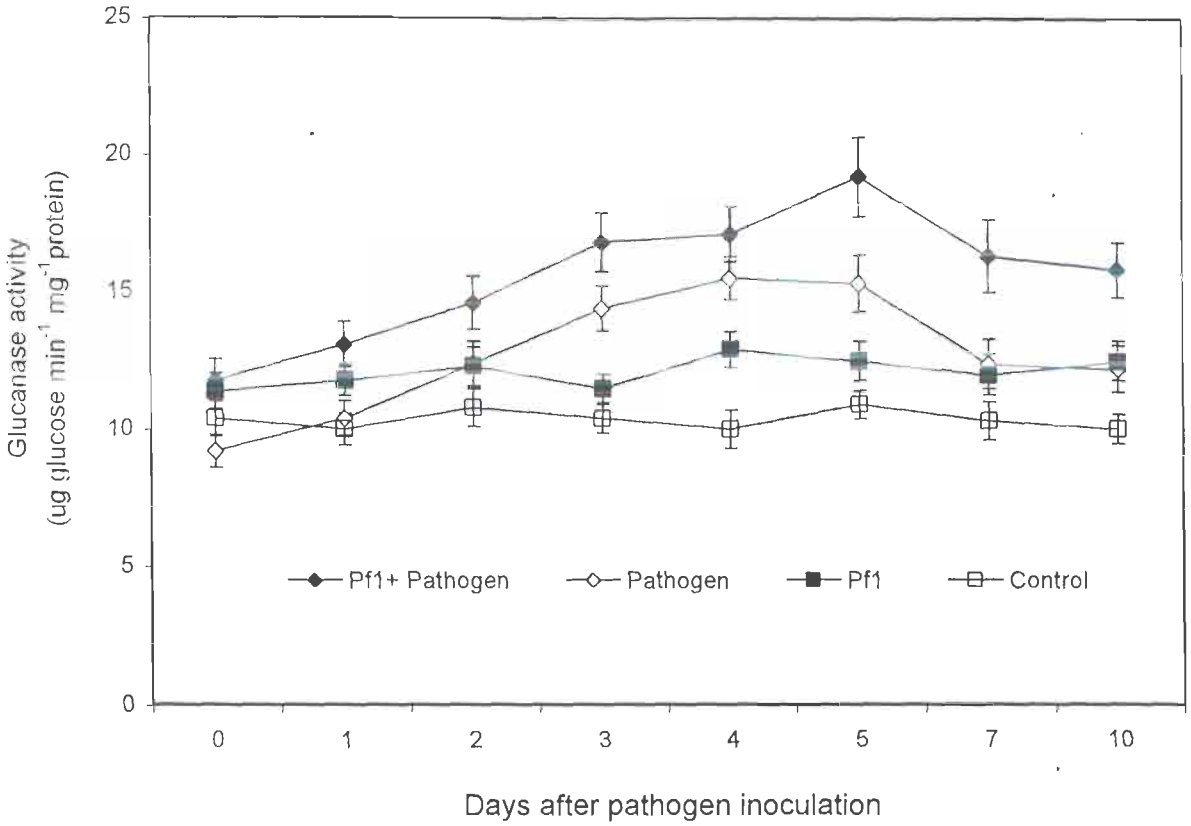
Generally, the enzyme activities were the maximum in bacterized roots challenged with the pathogen and induction of these enzyme activities was observed one

Fig. 13. Accumulation of phenolics by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxyporum* f. sp. *lycopersici*



Vertical bars indicate standard deviations of three replications

Fig. 14. Induction of β-1,3-glucanase activity by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f. sp. *lycopersici*



Vertical bars indicate standard deviations of three replications

day after challenge inoculation in bacterized plants. In plants inoculated with the pathogen alone, the enzyme activity increased initially but later declined drastically.

Chitinase isoform Chi2 with molecular weight of 46 kDa was induced in bacterized plants challenged with *F. oxysporum* f. sp. *lycopersici*. Induction of Chi2 isoform was at a low level in other treatments. The expression of both Chi1 (45 kDa) and Chi2 (46 kDa) isoforms were prominent in the bacterized plants challenged with *F. oxysporum* f. sp. *lycopersici* whereas in other two induction treatments namely plants treated with *P. fluorescens* Pf1 alone and plants inoculated with *F. oxysporum* f. sp. *lycopersici* only Chi1 was expressed prominently compared to the untreated control plants (Plate 15).

4. 11. 2. 7. Thaumatin-like proteins

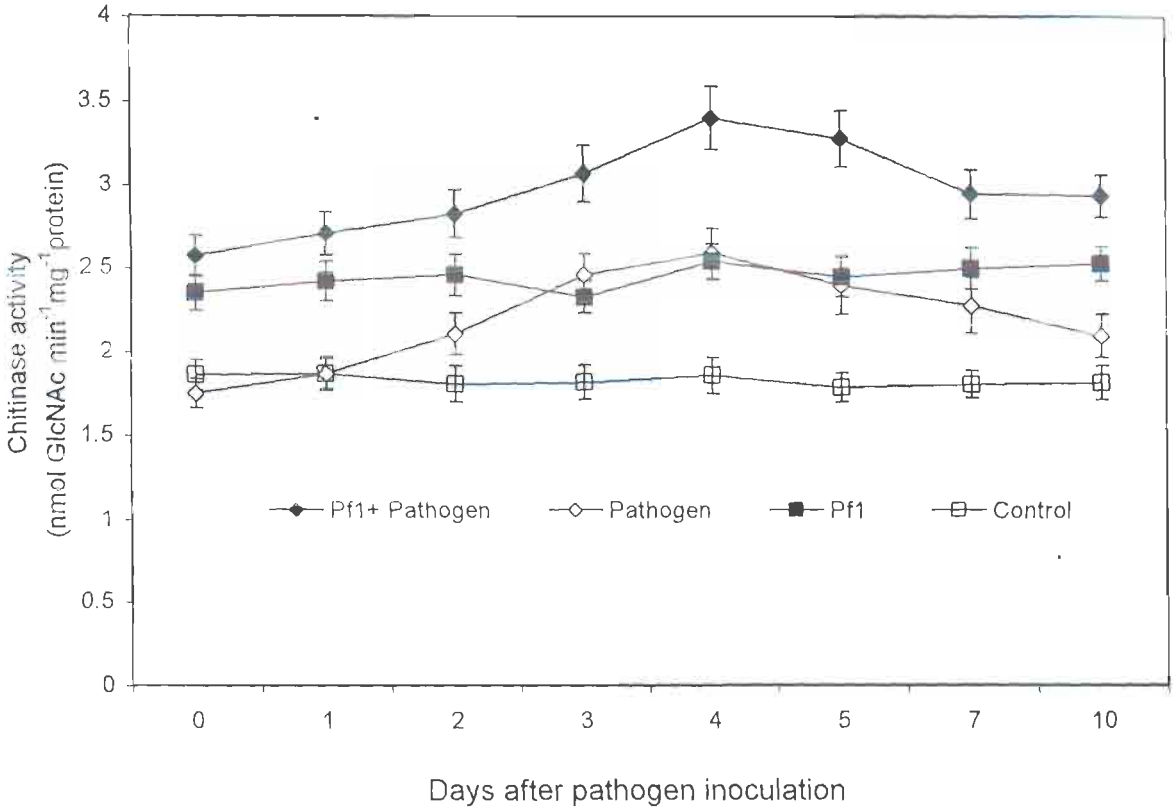
Western blot analysis of Thaumatin-like proteins (TLP) revealed that 33 kDa molecular weight TLP isoform was induced in all the induction treatments in tomato at similar level except in untreated control plants (Plate 16).

4. 11. 3. Induction of defense mechanism by *P. fluorescens* Pf1 against *C. capsici*-

4. 11. 3. 1. Phenylalanine ammonia lyase

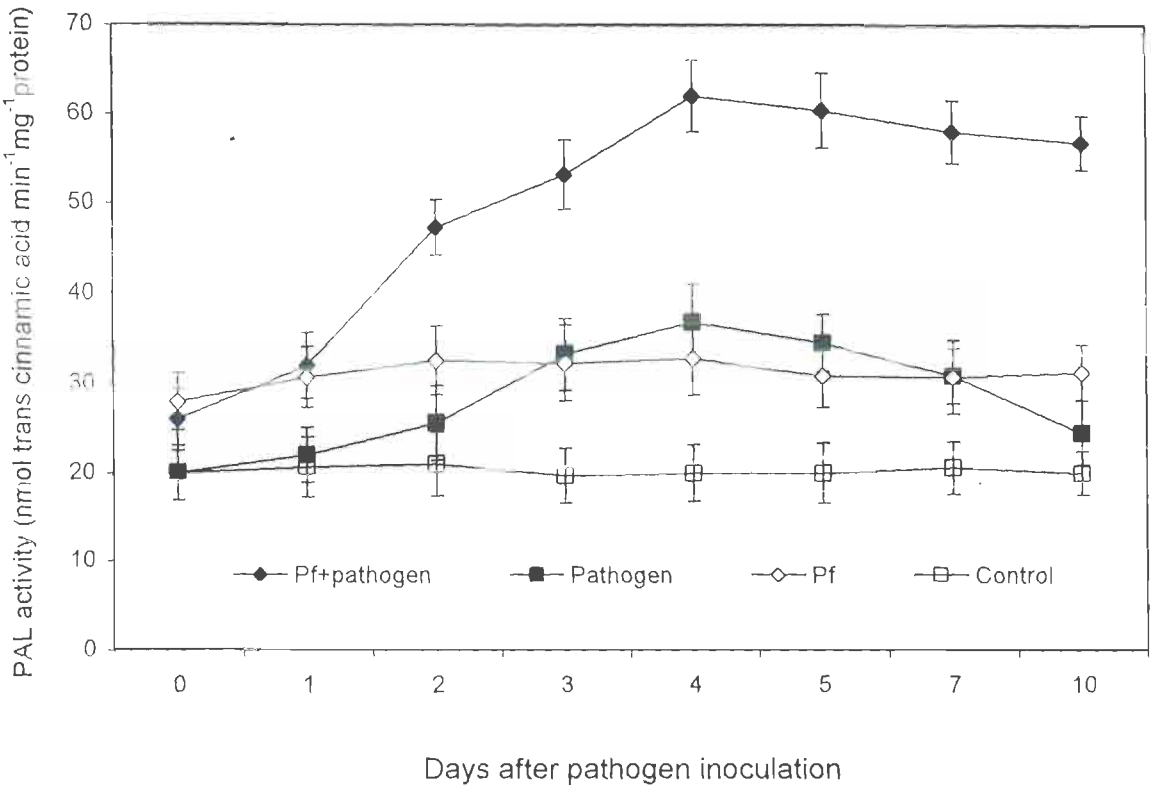
Studies on induction of defense mechanisms by *P. fluorescens* Pf1 against *C. capsici* in chilli revealed that seed treatment with *P. fluorescens* Pf1 increased the PAL activity. The activity started to increase at first day and reached the maximum levels at 5th day thereafter remained at higher level throughout the experimental period of 10 days (Fig. 16).

Fig. 15. Induction of chitinase by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*



Vertical bars indicate standard deviations of three replications

Fig. 16. Induction of PAL activity by *P. fluorescens* Pf1in chilli challenged with or without *C. capsici*



Vertical bars indicate standard deviations of three replications

Plate 15. Western blot analysis for chitinase isoform induced by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*

Lane 1. Marker

Lane 2. Plants treated with *P. fluorescens* Pf1 alone

Lane 3. *P. fluorescens* Pf1-treated plants challenged with *F. oxysporum* f. sp. *lycopersici*

Lane 4. Plants inoculated with *F. oxysporum* f. sp. *lycopersici*

Lane 5. Control plants

Plate 15

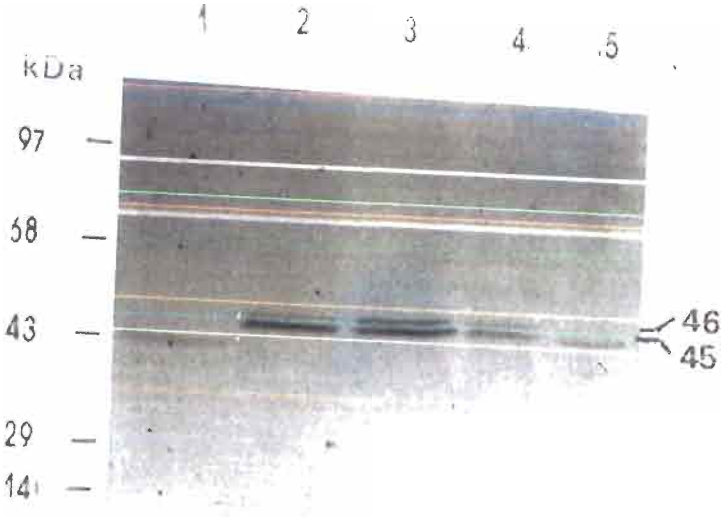


Plate 16. Western blot analysis for TLP isoform induced by *P. fluorescens* Pf1 in tomato challenged with or without *F. oxysporum* f.sp. *lycopersici*

Lane 1. Marker

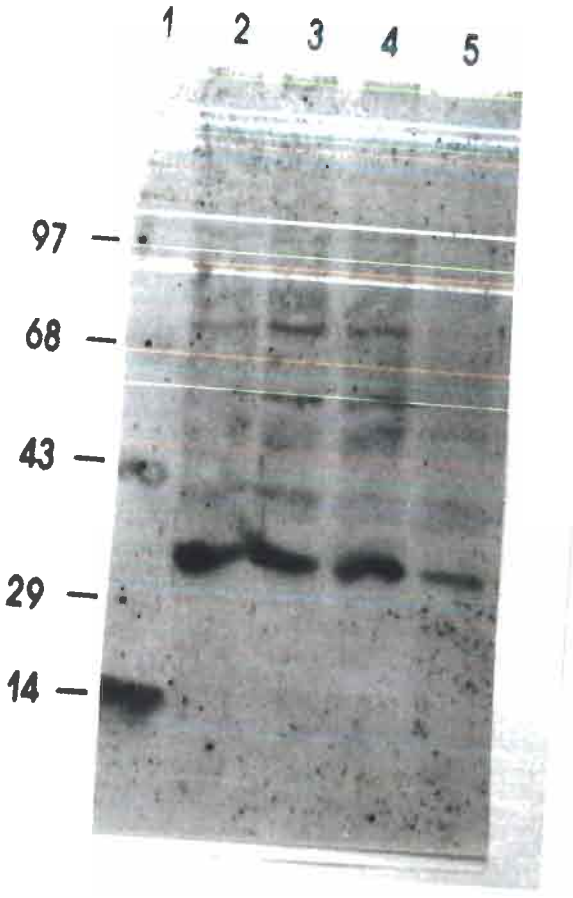
Lane 2. Plants treated with *P. fluorescens* Pf1 alone

Lane 3. *P. fluorescens* Pf1-treated plants challenged with *F. oxysporum* f. sp. *lycopersici*

Lane 4. Plants inoculated with *F. oxysporum* f. sp. *lycopersici*

Lane 5. Control plants

Plate 16



4. 11. 3. 2. Peroxidase

In *P. fluorescens* Pf1-treated plants challenged with *C. capsici,* higher levels of PO activity was noticed. The maximum PO activity was observed at 4th day after challenge inoculation and the activity was maintained at higher levels throughout the experimental period. (Fig. 17). Native PAGE studies revealed that the expression of PO isoform PO1 was prominent in *P. fluorescens* Pf1 treated plants challenged with *C. capsici.* Plants inoculated with *C. capsici* alone showed comparatively lesser level expression of PO1 isozyme (Plate 17).

4. 11. 3. 3. Polyphenol oxidase

Increased activity of PPO was observed in bacterized plants challenged with *C. capsici* (Fig. 18). Isozyme studies indicated that the expression of PPO1 isoform was noticed very clearly in *P. fluorescens* treated plants challenged with *C. capsici* when compared with other treatments (Plate 18).

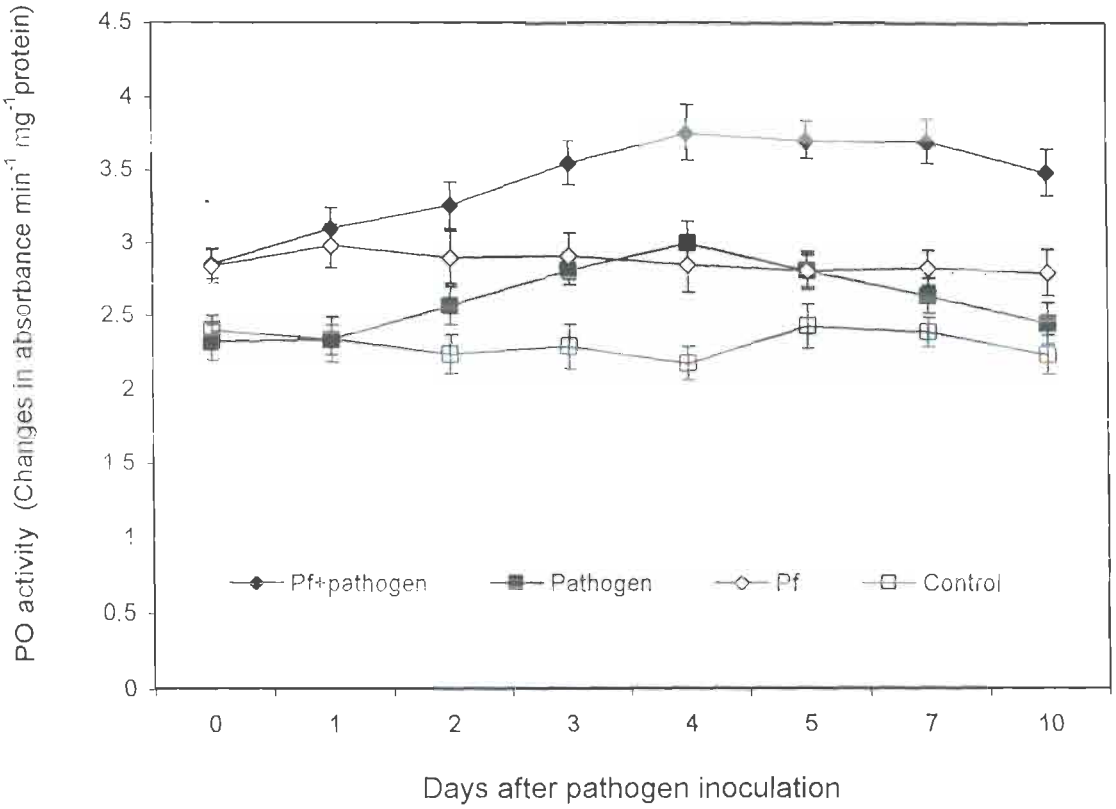
4. 11. 3. 4. Phenols

Higher accumulation of phenolics was observed in bacterized tomato roots challenge inoculated with *C. capsici* even at 10th day when compared with other induction treatments and also untreated plants. (Fig. 19).

4. 11. 3. 5. β-1,3-glucanase

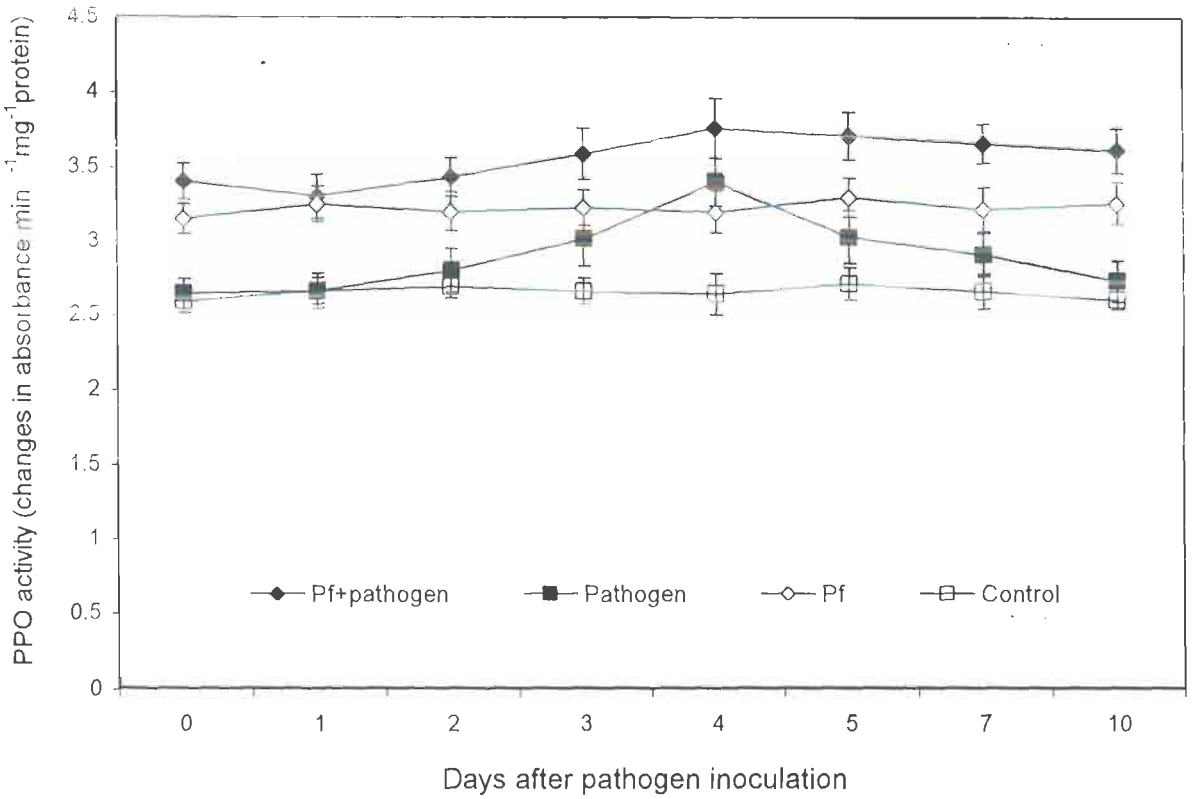
The activities of β-1,3-glucanase (Fig. 20) were higher in bacterized plants challenged with pathogen and higher activities were observed at 3-5 days after challenge

Fig.17. Changes in PO activity induced by *P. fluorescens* Pf1 in chilli challenged with or without *C. capsici*



Vertical bars indicate standard deviations of three replications

Fig. 18. Changes in PPO activity induced by *P. fluorescens* Pf1 in chilli challenged with or without *C. capsici*



Vertical bars indicate standard deviations of three replications

Plate 17. PO isoform profile in chilli induced by *P. fluorescens* Pf1 challenged with or without *C. capsici*

Lane 1. Seed treatment with *P. fluorescens* Pf1 alone

Lane 2. *P. fluorescens* Pf1-treated plants challenged with *C. capsici*

Lane 3. Plants inoculated with *C. capsici*

Lane 4. Control plants

Plate 17

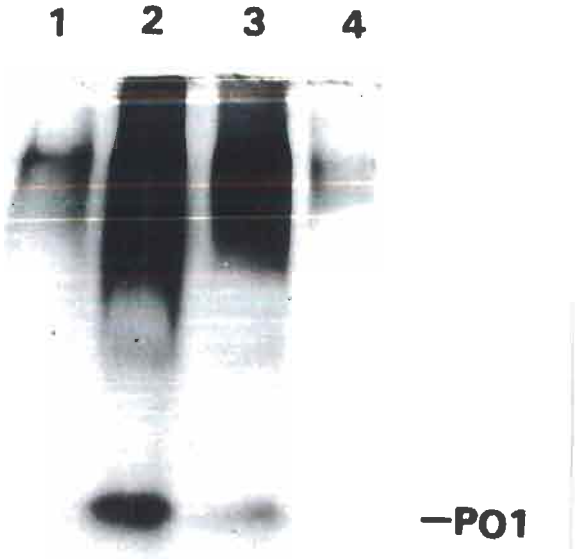


Plate 18. PPO isoform profile chilli induced by *P. fluorescens* Pf1 challenged with or without *C. capsici*

Lane 1. Seed treatment with *P. fluorescens* Pf1 alone

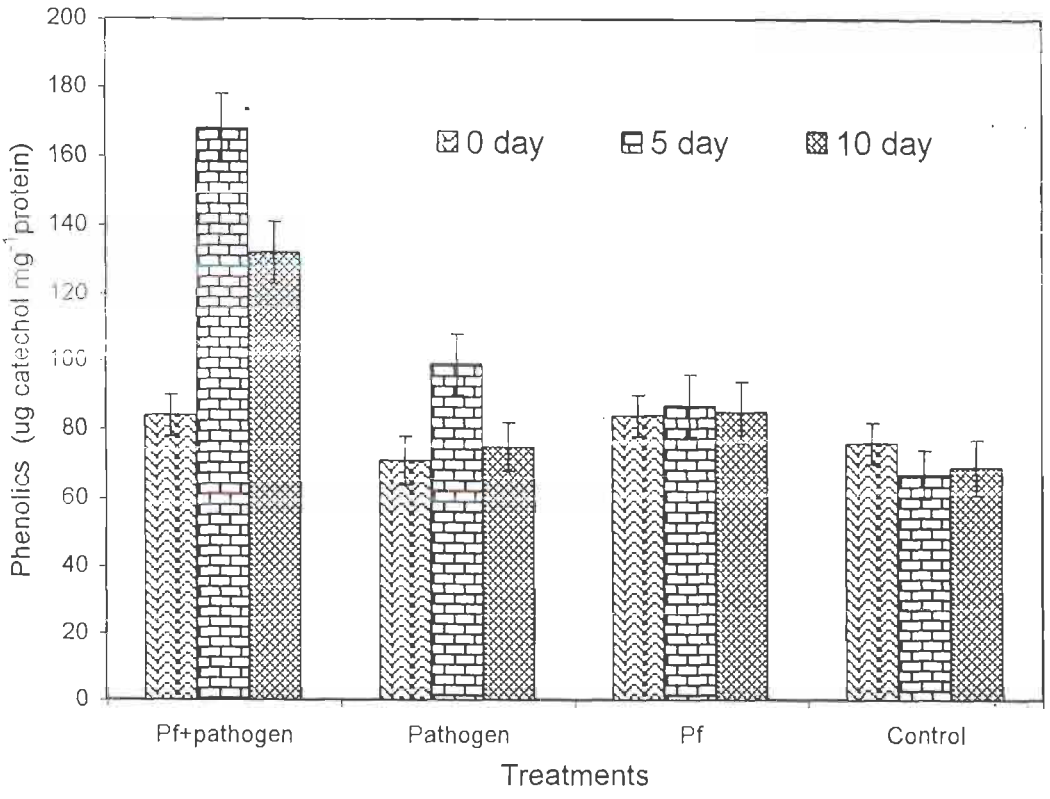
Lane 2. *P. fluorescens* Pf1-treated plants challenged with *C. capsici*

Lane 3. Plants inoculated with *C. capsici*

Lane 4. Control plants

Plate 18

Fig.19. Accumulation of phenolics by *P. fluorescens* Pf1 in chilli challenged with or without *C. capsici*



Vertical bars indicate standard deviations of three replications

inoculation and remained at higher levels throughout the experimental period.

4. 11. 3. 6. Chitinase

Similarly increased activities of chitinase was observed in *P. fluorescens* treated chilli plants challenged with *C. capsici* (Fig. 21). Studies on induction of chitinase isozymes by western blot showed that 42 kDa molecular weight protein was expressed at higher level due *P. fluorescens* treatment in response to infection by *C. capsici* (Plate 19).

4. 12. Efficacy of talc-based formulation of *P. fluorescens* Pf1 against damping-off of solanaceous vegetable crops under greenhouse conditions

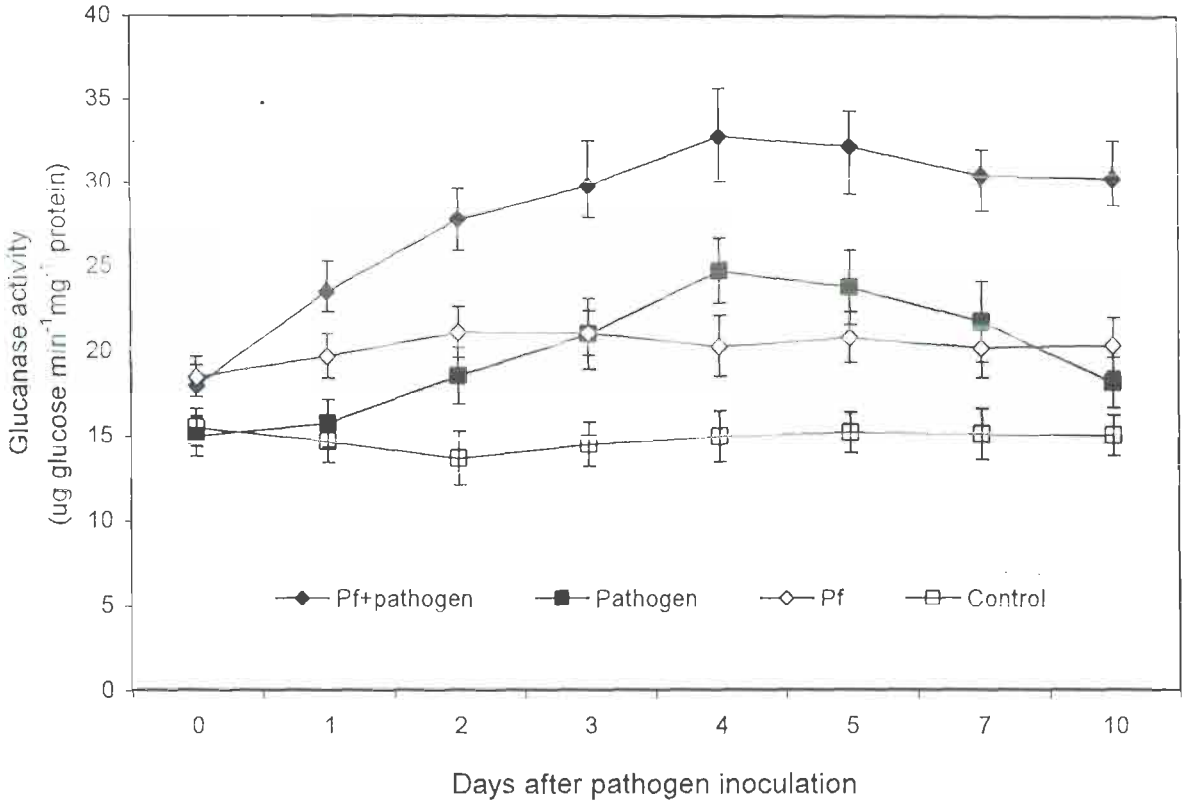
The promising strain *P. fluorescens* Pf1 which is versatile in nature having multiple modes of biocontrol mechanisms in biological control was used for developing talc-based formulation for easy delivery and its efficacy was tested for the management of damping-off of tomato, chilli and brinjal. The formulation was found to reduce the damping-off incidence by recording the disease incidence of 33.75, 35.83 and 39.58 per cent in tomato, chilli and brinjal respectively and the efficacy was comparable with that of ridomil used for comparison which recorded the disease incidence of 30.83, 32.08 and 35.42 per cent in tomato, chilli and brinjal respectively. The formulation was also found to increase the plant growth to the maximum level when compared to the ridomil treatment (Table 13).

4. 13. Development of new bioformulation for the management of major diseases of solanaceous vegetables

4. 13. 1. Chitin amendment at different concentrations on growth and multiplication of *P. fluorescens* in KMB

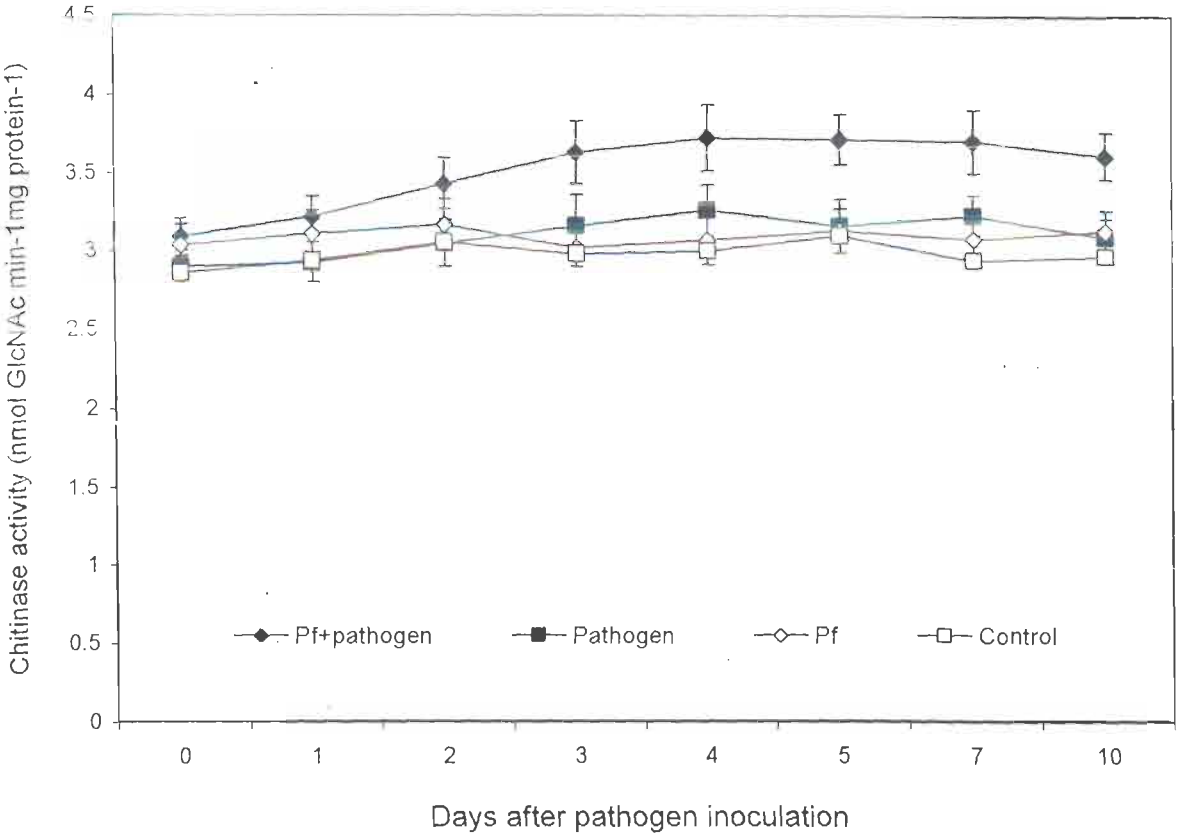
With regard to the development of new formulation of *P. fluorescens*, initially the

Fig. 20. Induction of β-1,3-glucanase activity by *P. fluorescens* Pf1 in chilli challenged with or without *C. capsici*



Vertical bars indicate standard deviations of three replications

Fig.21. Induction of chitinase activity by *P. fluorescens* Pf1 in chilli challenged with or without *C. capsici*



Vertical bars indicate standard deviations of three replications

Plate 19. Western blot analysis for chitinase isoform induced by *P. fluorescens* Pf1 in tomato challenged with or without *C. capsici*

Lane 1. Marker

Lane 2. Plants treated with *P. fluorescens* Pf1 alone

Lane 3. *P. fluorescens* Pf1-treated plants challenged with *C. capsici*

Lane 4. Plants inoculated with *C. capsici*

Lane 5. Control plants

Plate 19

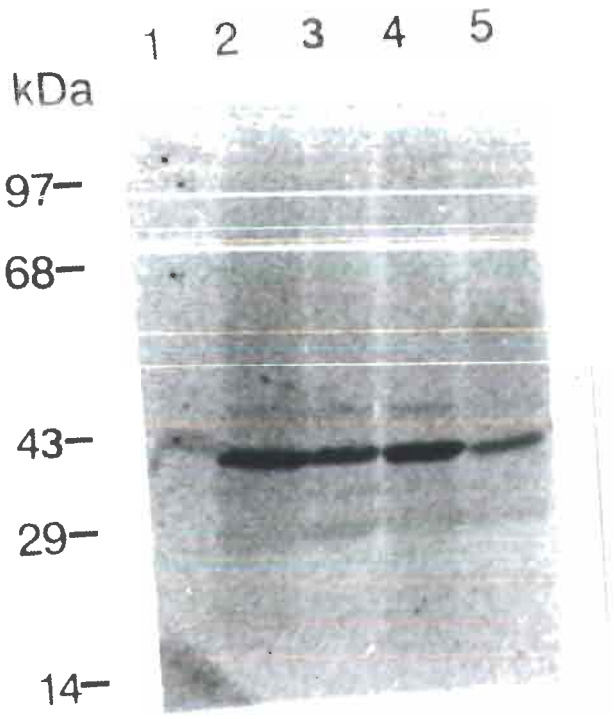


Table 13. Effect of talc-based formulation of *P. fluorescens* Pf1 for the management of damping-off disease of solanaceous vegetable crops under greenhouse conditions

Treatments	Tomato		Chilli		Brinjal	
	DI* (%)	Plant height (cm)	DI* (%)	Plant height (cm)	DI* (%)	Plant height (cm)
Seed treatment	33.75[b] (35.51)	16.58[c]	35.83[b] (36.76)	15.00[b]	39.58[b] (38.98)	14.85[b]
Ridomil	30.83[b] (33.72)	15.10[b]	32.08[b] (34.49)	14.53[b]	35.42[b] (36.51)	14.00[b]
Control (Pathogen inoculated)	80.00[c] (63.50)	11.23[a]	82.67[c] (65.43)	10.52[a]	83.33[c] (65.97)	10.4
Control (Pathogen uninoculated)	2.92[a] (9.48)	15.00[b]	3.30[a] (10.17)	14.60[b]	4.17[a] (11.65)	13.80[b]

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of five replications

Figures in parentheses indicate arcsine transformed values

*DI –Disease incidence (%)

effect of chitin on growth and multiplication of *P. fluorescens* Pf1 was tested by adding the colloidal chitin in the KMB. The data presented in Table 14 revealed that incorporation of chitin increased the growth and multiplication of *P. fluorescens*. Amending colloidal chitin in the medium at one per cent level increased the multiplication of *P. fluorescens* when compared to medium amended with the 0.5 per cent level of chitin. The population of *P. fluorescens* reached maximum (11.00×10^9) at 48 h after inoculation in KMB amended with chitin at one per cent level.

4. 13. 2. Population dynamics of *P. fluorescens* Pf1 in different carriers

Suitability of different carriers *viz*., talc, gypsum and lignite and their combination was evaluated for the development of formulation of *P. fluorescens* Pf1. Population levels of *P. fluorescens* Pf1 was assessed during different time intervals of storage period.

The data presented in Tables 15 and 16 revealed that the survival of *P. fluorescens* Pf1 decreased during the storage period invariably in all the carrier materials. However, higher levels of population was noticed when *P.fluorescens* Pf1 multiplied in chitin amended KMB was mixed with different carriers compared with culture grown in KMB without chitin amendment. The maximum population of 10.67×10^8 cfu g^{-1} product was noticed when *P. fluorescens* Pf1 mass multiplied in KMB amended with chitin was mixed in the formulation containing talc+gypsum used as a carrier at 90 days of storage period. The formulation containing talc alone recorded the population of 7.00×10^8 and 4.33×10^8 cfu g^{-1} product when it was mixed with *P. fluorescens* Pf1 mass multiplied in chitin amended and non-amended medium respectively at 90 days of storage period.

Table 14. Influence of chitin amendment on growth and multiplication of *P. fluorescens* Pf1 in the KMB medium

Treatments	Population of *P. fluorescens*		
	24 hr(x 10^7 cfu ml^{-1})	48 hr(x 10^9 cfu ml^{-1})	72 hr (x 10^9 cfu ml^{-1})
KB + 1 % Chitin	13.40^c (8.12)	11.00^c (10.04)	10.17^c (10.01)
KB + 0.5 % Chitin	8.43^b (7.93)	7.33^b (9.87)	7.00^b (9.85)
KB alone	3.98^a (7.60)	5.83^a (9.77)	4.33^a (9.64)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of six replications

Figures in parentheses indicate log transformed values

Table 15. Population dynamics of *P. fluorescens* Pf1 in different carriers and at different days of storage periods (mass multiplied in chitin amended KMB medium)

Treatments	Population of *P. fluorescens* at different days of storage period (cfu x 10^8)						
	0	15	30	45	60	75	90
Talc	19.67^{b} (9.29)	14.67^{bc} (9.17)	13.33^{bc} (9.12)	13.00^{cd} (9.11)	11.33^{bc} (9.05)	9.33^{b} (8.97)	7.00^{ab} (8.85)
Gypsum	14.00^{a} (9.15)	10.33^{a} (9.01)	7.67^{a} (8.88)	8.00^{a} (8.90)	6.00^{a} (8.78)	6.00^{a} (8.78)	4.33^{a} (8.64)
Lignite	20.33^{b} (9.31)	14.33^{b} (9.16)	11.00^{b} (9.04)	10.33^{b} (9.01)	10.33^{b} (9.01)	10.33^{b} (9.01)	4.67^{a} (8.67)
Talc+Gypsum	22.00^{b} (9.34)	17.33^{d} (9.24)	14.33^{c} (9.16)	13.67^{d} (9.14)	13.67^{e} (9.14)	13.67^{d} (9.14)	10.67^{c} (9.03)
Talc+Lignite	20.33^{b} (9.31)	16.00^{cd} (9.20)	14.67^{c} (9.17)	13.00^{cd} (9.11)	13.00^{de} (9.11)	13.00^{cd} (9.11)	7.67^{b} (8.88)
Lignite+Gypsum	20.33^{b} (9.31)	14.67^{bc} (9.17)	13.33^{bc} (9.12)	12.00^{c} (9.08)	12.00^{cd} (9.08)	11.00^{bc} (9.04)	6.67^{ab} (8.82)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate log transformed values

Table 16. Population dynamics of *P. fluorescens* Pf1 in different carriers and at different storage periods (mass multiplied with out chitin amended KMB medium)

Treatments	Population of *P. fluorescens* at different days of storage period (cfu x 10^8)						
	0	15	30	45	60	75	90
Talc	19.00^{bcd} (9.28)	14.33^{b} (9.16)	13.33^{c} (9.12)	10.00^{b} (9.00)	9.33^{bc} (8.97)	5.67^{a} (8.75)	4.33^{ab} (8.64)
Gypsum	12.00^{a} (9.08)	10.00^{a} (9.00)	7.00^{a} (8.84)	7.33^{a} (8.87)	6.00^{a} (8.78)	5.33^{a} (8.72)	3.33^{a} (8.52)
Lignite	20.33^{d} (9.31)	14.00^{b} (9.15)	8.67^{b} (8.95)	8.00^{a} (8.90)	8.00^{b} (8.90)	8.00^{ab} (8.90)	4.33^{ab} (8.64)
Talc+Gypsum	19.67^{cd} (9.29)	16.67^{c} (9.22)	13.00^{c} (9.11)	12.33^{c} (9.09)	12.33^{e} (9.09)	11.00^{b} (9.04)	9.67^{c} (8.99)
Talc+Lignite	18.00^{b} (9.26)	13.00^{b} (9.11)	13.00^{c} (9.11)	12.00^{c} (9.08)	11.00^{de} (9.04)	10.67^{b} (9.03)	6.67^{b} (8.82)
Lignite+Gypsum	18.67^{bc} (9.27)	16.67^{c} (9.22)	12.33^{c} (9.09)	11.00^{bc} (9.04)	10.00^{cd} (9.00)	9.33^{ab} (8.97)	6.33^{b} (8.80)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate log transformed values

4. 13. 3. Efficacy of *P. fluorescens* formulation for the management of damping-off under greenhouse conditions

There was no significant difference in the suppression of damping-off of tomato, chilli and brinjal among the formulations when they were applied at 0^{th} day of storage period compared with ridomil as a standard treatment. All the formulations containing different carriers effectively reduced the disease incidence when applied at 0^{th} day of storage period. Generally, formulations mixed with *P. fluorescens* Pf1 mass multiplied in KMB amended with chitin recorded less disease incidence when compared with formulation mixed with *P. fluorescens* Pf1 multiplied in KMB without chitin amendment.

During the time course of storage period the formulation containing talc+gypsum as a carrier mixed with *P. fluorescens* Pf1 mass multiplied in KMB amended with chitin showed maximum biocontrol activity even after 75 days of storage period by recording the disease incidence of 38.67, 30.67 and 32.00 per cent in tomato, chilli and brinjal respectively and the efficacy was comparable with ridomil used as a standard treatment for comparison. When compared to fungicide treatment, this formulation was effective up to 90 days of storage period in chilli and 75 days in tomato and brinjal whereas other formulations were effective only when they were used up to 30 days of storage period. Formulations mixed with *P. fluorescens* Pf1 mass multiplied in KMB without chitin amendment showed comparatively less biocontrol efficacy when compared to formulation containing the respective carriers mixed with *P. fluorescens* multiplied in KMB with chitin amendment (Tables 17-22).

Table 17. Efficacy of carriers mixed with *P. fluorescens* Pf1 mass multiplied in chitin amended medium for the management of damping-off of tomato under greenhouse conditions

Carriers	Disease incidence (%) on different days of storage period						
	0	15	30	45	60	75	90
Talc	26.67^{b} (31.04)	33.33^{bcde} (35.26)	37.33^{de} (37.66)	41.33^{cd} (40.01)	45.33^{c} (42.32)	46.67^{cd} (43.09)	48.00^{d} (43.85)
Gypsum	33.33^{b} (35.26)	37.33^{de} (37.66)	40.00^{de} (39.22)	42.67^{cd} (40.80)	48.00^{c} (43.85)	50.67^{d} (45.38)	54.67^{e} (47.68)
Lignite	34.67^{b} (36.06)	38.67^{e} (38.44)	41.33^{e} (40.00)	41.33^{cd} (40.01)	50.67^{c} (45.38)	50.67^{d} (45.38)	57.33^{e} (49.22)
Talc+gypsum	25.33^{b} (30.21)	28.00^{b} (31.91)	30.67^{bc} (33.62)	34.67^{bc} (36.06)	36.00^{b} (36.85)	38.67^{bc} (38.44)	41.33^{c} (40.00)
Talc+lignite	33.33^{b} (35.26)	32.00^{bcd} (34.42)	34.67^{cd} (36.06)	41.33^{cd} (40.01)	46.67^{c} (43.09)	45.33^{cd} (42.32)	54.67^{e} (47.68)
Gypsum+ lignite	32.00^{b} (34.42)	34.67^{cde} (36.06)	38.67^{de} (38.44)	44.00^{d} (41.55)	46.67^{c} (43.09)	48.00^{d} (43.85)	54.67^{e} (47.68)
Ridomil	34.67^{b} (36.06)	30.67^{bc} (33.62)	29.33^{b} (32.78)	30.67^{b} (33.62)	33.33^{b} (35.26)	32.00^{b} (34.42)	32.00^{b} (34.42)
Control (pathogen inoculated)	80.00^{c} (63.51)	80.00^{f} (63.51)	82.67^{f} (65.43)	84.00^{e} (66.53)	77.33^{d} (61.59)	78.67^{e} (62.51)	81.33^{f} (64.43)
Control (pathogen uninoculated)	4.00^{a} (9.51)	5.33^{a} (13.17)	6.67^{a} (14.80)	2.67^{a} (7.88)	4.00^{a} (9.51)	2.67^{a} (7.69)	4.00^{a} (11.54)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 18. Efficacy of carriers mixed with *P. fluorescens* Pf1 mass multiplied in KMB medium without chitin amendment for the management of damping-off of tomato under greenhouse conditions

Carriers	Disease incidence (%) on different days of storage period						
	0	15	30	45	60	75	90
Talc	29.33^{b} (32.78)	37.33^{b} (37.66)	41.33^{cd} (40.01)	44.00^{bc} (41.55)	49.33^{d} (44.62)	48.00^{c} (43.85)	49.33^{d} (44.62)
Gypsum	34.67^{b} (36.06)	40.00^{b} (39.22)	41.33^{cd} (40.01)	42.67^{bc} (40.78)	49.33^{d} (44.62)	53.33^{cd} (46.91)	56.00^{e} (48.45)
Lignite	30.67^{b} (33.55)	37.33^{b} (37.66)	42.67^{d} (40.78)	46.67^{c} (43.09)	50.67^{d} (45.38)	56.00^{d} (48.45)	57.33^{e} (49.22)
Talc + gypsum	33.33^{b} (35.26)	34.67^{b} (36.06)	32.00^{b} (34.42)	37.33^{bc} (37.66)	38.67^{c} (38.44)	37.33^{b} (37.65)	42.67^{c} (40.78)
Talc + lignite	32.00^{b} (34.42)	33.33^{b} (35.26)	34.67^{bc} (36.06)	41.33^{bc} (40.01)	48.00^{d} (43.85)	52.00^{cd} (46.15)	58.67^{e} (49.99)
Gypsum + lignite	32.00^{b} (34.42)	36.00^{b} (36.85)	41.33^{cd} (40.01)	44.00^{bc} (41.55)	50.67^{d} (45.38)	54.67^{d} (47.68)	60.00^{e} (50.78)
Ridomil	32.00^{b} (34.42)	32.00^{b} (34.42)	33.33^{b} (35.26)	34.67^{b} (36.06)	30.67^{b} (33.62)	34.67^{b} (36.06)	34.67^{b} (36.06)
Control (pathogen inoculated)	80.00^{c} (63.50)	76.00^{c} (60.72)	81.33^{e} (64.43)	77.33^{d} (61.59)	77.33^{e} (61.59)	82.67^{e} (65.43)	84.00^{f} (66.52)
Control (pathogen uninoculated)	4.00^{a} (9.51)	2.67^{a} (7.88)	2.67^{a} (7.88)	4.00^{a} (9.51)	2.27^{a} (7.88)	5.33^{a} (13.17)	4.00^{a} (11.54)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 19. Efficacy of carriers mixed with *P. fluorescens* Pf1 mass multiplied in chitin amended medium for the management of damping-off of chilli under greenhouse conditions

Carriers	Disease incidence (%) on different days of storage period						
	0	15	30	45	60	75	90
Talc	22.67^{b} (28.41)	26.67^{b} (31.08)	30.67^{b} (33.61)	30.67^{bc} (33.62)	34.67^{bc} (36.06)	37.33^{c} (37.66)	37.33^{c} (37.66)
Gypsum	24.00^{b} (29.28)	25.33^{b} (30.21)	28.00^{b} (31.91)	32.00^{bc} (34.42)	37.33^{c} (37.66)	40.00^{c} (39.22)	38.67^{c} (38.44)
Lignite	25.33^{b} (30.20)	25.33^{b} (30.21)	32.00^{b} (34.42)	33.33^{bc} (35.26)	36.00^{c} (36.85)	40.00^{c} (39.22)	41.33^{c} (40.00)
Talc + gypsum	22.67^{b} (28.41)	25.00^{b} (29.98)	26.67^{b} (31.08)	26.67^{b} (31.08)	29.33^{b} (32.78)	30.67^{b} (33.62)	32.00^{b} (34.42)
Talc + lignite	21.33^{b} (27.49)	26.67^{b} (31.08)	33.33^{b} (35.26)	30.67^{bc} (33.62)	33.33^{bc} (35.26)	36.00^{bc} (36.85)	38.67^{c} (38.44)
Gypsum + lignite	26.67^{b} (31.08)	25.33^{b} (30.20)	30.67^{b} (33.62)	36.00^{c} (36.85)	36.00^{c} (36.85)	38.67^{c} (38.44)	37.33^{c} (37.66)
Ridomil	28.00^{b} (31.91)	26.67^{b} (31.08)	29.33^{b} (32.79)	28.00^{b} (31.91)	33.33^{bc} (35.23)	30.67^{b} (33.62)	32.00^{b} (34.42)
Control (pathogen inoculated)	70.67^{c} (57.22)	68.00^{c} (55.58)	72.00^{c} (58.09)	76.00^{d} (60.72)	68.00^{d} (55.58)	70.67^{d} (57.22)	71.33^{d} (57.65)
Control (pathogen uninoculated)	4.00^{a} (9.51)	5.33^{a} (13.17)	8.00^{a} (16.08)	8.00^{a} (16.08)	6.67^{a} (14.80)	6.67^{a} (14.80)	9.33^{a} (17.71)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 20. Efficacy of carriers mixed with *P. fluorescens* Pf1 mass multiplied in KMB medium without chitin amendment for the management of damping-off of chilli under greenhouse conditions

Carriers	Disease incidence (%) on different days of storage period						
	0	15	30	45	60	75	90
Talc	28.00^{bc} (31.91)	26.67^{b} (31.08)	32.00^{b} (34.42)	33.33^{b} (35.26)	38.67^{cd} (38.44)	44.00^{de} (41.55)	46.67^{de} (43.09)
Gypsum	26.67^{bc} (31.08)	28.00^{b} (31.91)	32.00^{b} (34.42)	34.67^{b} (36.06)	40.00^{d} (39.22)	46.67^{e} (43.09)	46.67^{de} (43.09)
Lignite	26.67^{bc} (31.08)	29.33^{b} (32.78)	34.67^{b} (36.06)	37.33^{b} (37.66)	36.00^{bcd} (36.85)	46.67^{e} (43.09)	49.33^{e} (44.61)
Talc + gypsum	25.33^{bc} (30.21)	25.33^{b} (30.21)	28.00^{b} (31.91)	32.00^{b} (34.42)	32.00^{bc} (34.42)	36.00^{c} (36.85)	36.00^{c} (36.85)
Talc + lignite	28.00^{bc} (31.91)	26.67^{b} (31.08)	29.33^{b} (32.78)	36.00^{b} (36.85)	40.00^{d} (39.22)	38.67^{cd} (38.44)	42.67^{d} (40.78)
Gypsum + lignite	24.00^{b} (29.28)	28.00^{b} (31.91)	33.33^{b} (35.26)	34.67^{b} (36.03)	36.00^{bcd} (36.85)	44.00^{de} (41.55)	46.67^{de} (43.09)
Ridomil	30.67^{c} (33.62)	28.00^{b} (31.91)	30.67^{b} (33.62)	32.00^{b} (34.42)	30.67^{b} (33.62)	28.00^{b} (31.91)	30.67^{b} (33.62)
Control (pathogen inoculated)	72.00^{d} (58.09)	80.00^{c} (63.51)	76.00^{c} (60.72)	74.67^{c} (59.79)	76.00^{e} (60.72)	70.67^{f} (57.22)	80.00^{f} (63.51)
Control (Pathogen uninoculated)	5.33^{a} (13.17)	6.67^{a} (14.80)	5.33^{a} (13.17)	6.67^{a} (14.80)	6.67^{a} (14.80)	8.00^{a} (16.08)	10.67^{a} (18.99)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 21. Efficacy of carriers mixed with *P. fluorescens* Pf1 mass multiplied in chitin amended medium for the management of damping-off of brinjal under greenhouse conditions

Carriers	Disease incidence (%) on different days of storage period						
	0	15	30	45	60	75	90
Talc	26.67^{b} (31.08)	28.00^{bc} (31.91)	32.00^{bcd} (34.42)	35.33^{de} 36.46)	40.00^{cd} (39.22)	46.67^{c} (43.09)	48.00^{de} (43.85)
Gypsum	24.00^{b} 29.28)	24.00^{b} (29.28)	30.67^{bcd} (33.62)	32.00^{cd} (34.42)	38.67^{c} (38.44)	42.67^{c} (40.78)	46.67^{d} (43.08)
Lignite	26.67^{b} (31.08)	28.00^{bc} (31.91)	33.33^{cd} (35.26)	38.67^{e} (38.44)	42.67^{cd} (40.78)	48.00^{c} (43.85)	50.67^{de} (45.38)
Talc+Gypsum	22.67^{b} (28.41)	24.00^{b} (29.28)	25.33^{b} (30.21)	26.67^{bc} (31.08)	29.33^{b} (32.78)	32.00^{b} (34.22)	36.00^{c} (36.85)
Talc+Lignite	26.67^{b} (31.08)	30.67^{bc} (33.61)	34.67^{d} (36.06)	38.67^{e} (38.44)	46.67^{d} (43.78)	48.00^{c} (43.85)	54.67^{e} (47.68)
Gypsum+Lignite	22.67^{b} 28.41)	32.00^{c} 34.42)	36.00^{d} (36.85)	40.00^{e} (39.22)	42.67^{cd} (40.78)	46.67^{c} (43.09)	52.00^{de} (46.15)
Ridomil	26.67^{b} (31.08)	24.00^{b} (29.28)	26.67^{bc} (31.08)	24.00^{b} (29.28)	26.67^{b} (31.08)	24.00^{b} (29.28)	26.67^{b} (31.08)
Pathogen inoculated control	70.67^{c} (57.22)	68.00^{c} (55.58)	72.00^{e} (58.09)	76.00^{f} (60.72)	72.00^{e} (40.78)	68.00^{d} (55.58)	72.00^{f} (58.09)
Pathogen uninoculated control	5.33^{a} (13.17)	5.33^{a} (13.17)	8.00^{a} (16.08)	9.33^{a} (17.71)	8.00^{a} (16.08)	4.00^{a} (9.51)	8.00^{a} (16.08)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Table 22. Efficacy of carriers mixed with *P. fluorescens* Pf1 mass multiplied in KMB medium without chitin amendment for the management of damping-off of brinjal under greenhouse conditions

Carriers	Disease incidence (%) on different days of storage period						
	0	15	30	45	60	75	90
Talc	22.67^{b} (28.41)	25.33^{b} (30.21)	28.00^{bc} (31.91)	33.33^{c} (35.26)	34.67^{c} (36.06)	40.00^{cd} (39.22)	46.67^{cd} (43.09)
Gypsum	21.33^{b} (27.49)	24.00^{b} (29.28)	26.67^{bc} (31.08)	30.67^{c} (33.62)	32.00^{c} (34.42)	38.67^{cd} (38.44)	42.67^{cd} (40.78)
Lignite	24.00^{b} (29.28)	24.00^{b} (29.28)	30.67^{c} (33.62)	33.33^{c} (35.26)	36.00^{c} (36.85)	42.67^{d} (40.78)	48.00^{d} (43.85)
Talc+Gypsum	21.33^{b} (27.49)	22.67^{b} (28.41)	22.67^{b} (28.41)	24.00^{b} (29.28)	24.00^{b} (29.28)	26.67^{b} (31.08)	28.00^{b} (31.91)
Talc+Lignite	21.33^{b} (27.49)	26.67^{b} (31.08)	28.00^{bc} (31.91)	34.67^{c} (36.06)	32.00^{c} (34.42)	36.00^{c} (36.85)	40.00^{c} (39.22)
Gypsum+Lignite	22.67^{b} (28.41)	25.33^{b} (30.21)	32.00^{c} (34.42)	32.00^{c} (34.42)	34.67^{c} (36.06)	40.00^{cd} (39.22)	44.00^{cd} (41.55)
Ridomil	22.67^{b} (28.41)	24.00^{b} (29.28)	26.67^{bc} (26.49)	20.00^{b} (26.49)	24.00^{b} (29.28)	26.67^{b} (31.08)	24.00^{b} (29.28)
Pathogen inoculated control	68.00^{c} (55.58)	72.00^{c} (58.09)	68.00^{d} (57.22)	70.67^{d} (57.22)	72.00^{d} (58.09)	76.00^{e} (60.72)	72.00^{e} (58.09)
Pathogen uninoculated control	6.67^{a} (14.80)	5.33^{a} (13.17)	5.33^{a} (4.23)	1.34^{a} (4.23)	6.67^{a} (14.80)	5.33^{a} (13.17)	6.67^{a} (14.80)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

4. 13. 4. Efficacy of different formulations of *P. fluorescens* Pf1 against damping-off incidence under field conditions

Based on the greenhouse studies, the formulation consisting of talc and talc+gypsum as carrier mixed with *P. fluorescens* mass multiplied in KMB with or without chitin were further evaluated under field conditions. Zinc was added to the formulation at one per cent level to test the effect of Zinc on improving the biocontrol efficacy of the *P. fluorescens* Pf1.

The data present in Table 23 revealed that seed treatment with talc+gypsum-based formulation of *P. fluorescens* mass multiplied in chitin amended KMB gave better control of damping-off incidence of tomato, chilli and brinjal in both the field studies and its efficacy was comparable with that of the ridomil treatment. Incorporation of Zinc in formulation did not show any significant control of damping-off incidence when compared with formulations consisting same carriers without Zinc. Application of talc alone, gypsum alone and Zinc alone had no effect on damping-off disease incidence (Plates 20 to 22).

4. 13. 5. Efficacy of different formulations of *P. fluorescens* Pf1 against wilt incidence under field conditions

Formulations tested for the management of damping-off disease were also evaluated for the management of fusarium wilt of tomato and brinjal under field conditions under two locations in different seasons. The data presented in Tables 24-26 revealed that the formulation containing talc+gypsum as carriers showed better performance when compared with formulation containing talc alone. In formulations

Table 23. Efficacy of *P. fluorescens* formulations against damping-off disease in solanaceous vegetables under field conditions

Treatments	Disease incidence (%)					
	Trial I			Trial II		
	Tomato	Chilli	Brinjal	Tomato	Chilli	Brinjal
T_1	34.33^{d} (35.86)	39.33^{cd} (38.84)	49.00^{d} (44.43)	35.00^{bc} (36.26)	35.33^{d} (36.46)	41.00^{cd} (39.81)
T_2	34.67^{d} (36.07)	39.33^{cd} (38.84)	43.00^{d} (40.97)	32.33^{b} (34.65)	33.33^{cd} (35.26)	38.00^{b} (38.05)
T_3	28.33^{bc} (32.16)	41.67^{d} (40.20)	45.00^{bc} (42.13)	36.33^{c} (37.05)	33.67^{cd} (35.46)	40.67^{cd} (39.62)
T_4	33.33^{d} (35.25)	36.67^{bc} (37.27)	45.67^{c} (42.51)	37.33^{c} (37.66)	31.33^{bc} (34.04)	41.67^{d} (40.20)
T_5	22.33^{a} (28.18)	35.00^{b} (36.27)	32.67^{a} (34.86)	35.33^{bc} (36.46)	30.00^{ab} (33.21)	38.67^{bc} (38.45)
T_6	30.00^{c} (33.19)	36.33^{bc} (37.06)	33.67^{a} (35.47)	35.67^{bc} (36.66)	29.67^{ab} (32.99)	39.67^{bcd} (39.03)
T_7	64.67^{e} (53.53)	62.33^{e} (52.14)	70.33^{f} (57.01)	68.67^{e} (55.97)	68.00^{e} (55.56)	64.67^{e} (53.53)
T_8	62.67^{e} (52.34)	65.00^{ef} (53.73)	65.33^{e} (53.95)	61.33^{d} (51.56)	65.00^{e} (53.73)	66.67^{ef} (54.74)
T_9	64.67^{e} (53.53)	65.67^{f} (54.14)	63.33^{e} (52.74)	60.67^{d} (51.16)	66.33^{e} (54.54)	68.67^{fg} (55.96)
T_{10}	27.33^{b} (31.52)	30.33^{a} (33.41)	45.33^{bc} (42.32)	28.67^{a} (32.37)	28.33^{a} (32.16)	35.67^{a} (36.67)
T_{11}	64.00^{e} (53.14)	62.33^{e} (52.15)	63.33^{e} (52.74)	68.33^{e} (55.76)	67.67^{e} (55.35)	70.33^{g} (57.01)

Trial was conducted at Tamil Nadu Agricultural University, Coimbatore
Trial II was conducted at Regional Research Station at Aliyar Nagar
Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT.
The data are mean of three replications
Figures in parentheses indicate arcsine transformed values.

T_1-Talc containing Pf1 multiplied without chitin

T_2-Talc containing Pf1 multiplied with chitin

T_3- Talc +Zinc 1% containing Pf1 multiplied with chitin

T_4- Talc+ gypsum containing Pf1 multiplied without chitin

T_5- Talc+ gypsum containing Pf1 multiplied with chitin

T_6- Talc+gypsum+Zinc1% containing Pf1 multiplied with chitin

T_7-Talc

T_8-Gypsum

T_9-Zinc

T_{10}-Ridomil

T_{11}-Control

Table 24. Efficacy of *P. fluorescens* Pf1 formulation for the management of fusarium wilt of tomato and brinjal during 1999 at Aruppukottai under field conditions

Treatments	Tomato		Brinjal	
	Disease incidence (%)	Yield (t/ha)	Disease incidence (%)	Yield (t/ha)
T_1	12.15^{ab} (20.38)	20.57^{b}	12.15^{bc} (20.36)	25.57^{b}
T_2	14.14^{b} (22.08)	20.43^{b}	10.00^{ab} (18.27)	24.57^{b}
T_3	11.11^{a} (19.43)	21.30^{bc}	14.44^{c} (22.31)	25.17^{b}
T_4	10.10^{a} (18.45)	22.73^{bc}	13.33^{c} (21.32)	25.90^{b}
T_5	9.60^{a} (17.97)	23.83^{c}	8.89^{a} (17.28)	26.00^{b}
T_6	9.60^{a} (17.97)	23.57^{c}	10.00^{ab} (18.27)	25.77^{b}
T_7	44.95^{cd} (42.10)	17.87^{a}	21.11^{d} (27.34)	22.00^{a}
T_8	47.98^{d} (43.84)	17.03^{a}	18.89^{d} (25.74)	21.67^{a}
T_9	43.43^{c} (41.23)	16.80^{a}	20.00^{d} (26.51)	21.53^{a}
T_{10}	11.11^{a} (19.46)	20.47^{b}	12.22^{bc} (20.42)	24.67^{b}
T_{11}	48.48^{d} (44.13)	17.13^{a}	21.11^{d} (27.34)	21.90^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

T_1-Talc containing Pf1 multiplied without chitin

T_2-Talc containing Pf1 multiplied with chitin

T_3- Talc+Zinc 1% containing Pf1 multiplied with chitin

T_4- Talc+gypsum containing Pf1 multiplied without chitin

T_5- Talc+ gypsum containing Pf1 multiplied with chitin

T_6- Talc+gypsum+Zinc1% containing Pf1 multiplied with chitin

T_7-Talc

T_8-Gypsum

T_9-Zinc

T_{10}-Carbendazim

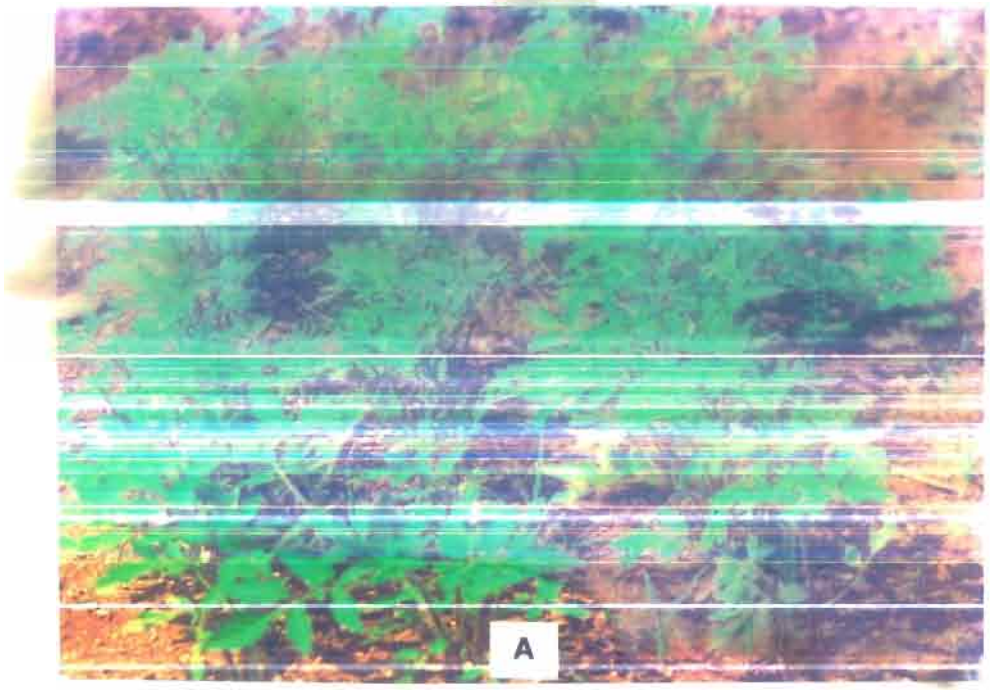
T_{11}-Control

Plate 20. Effect of *Pseudomonas fluorescens* Pf1 formulation for the management of damping-off of tomato under field conditions

A. Pf1 treated (T_5)

B. Control

Plate 20



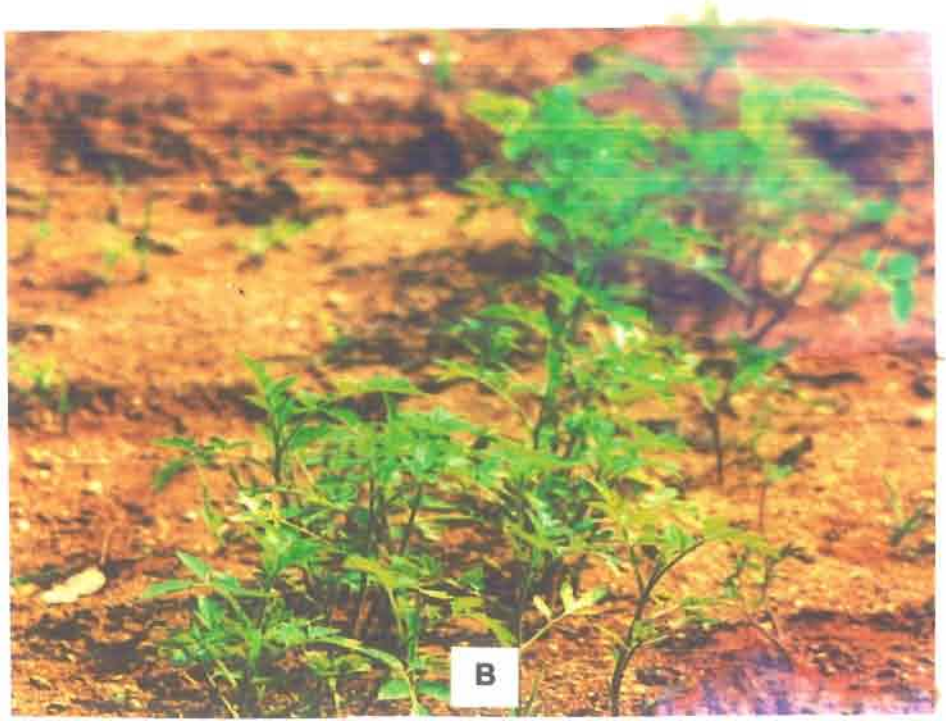


Plate 21. Effect of *Pseudomonas fluorescens* Pf1 formulation for the management of damping-off of chilli under field conditions

A. Pf1 treated (T_5)

B. Control

Plate 21

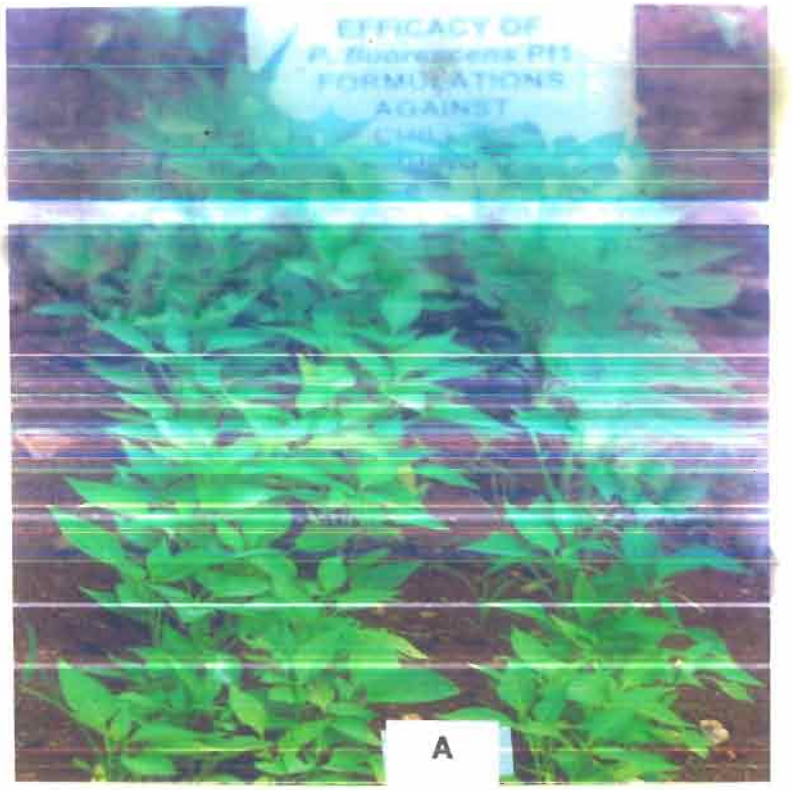


Plate 22. Effect of *Pseudomonas fluorescens* Pf1 formulation for the management of damping-off of brinjal under field conditions

A. Pf1 treated (T_5)

B. Control

Plate 22

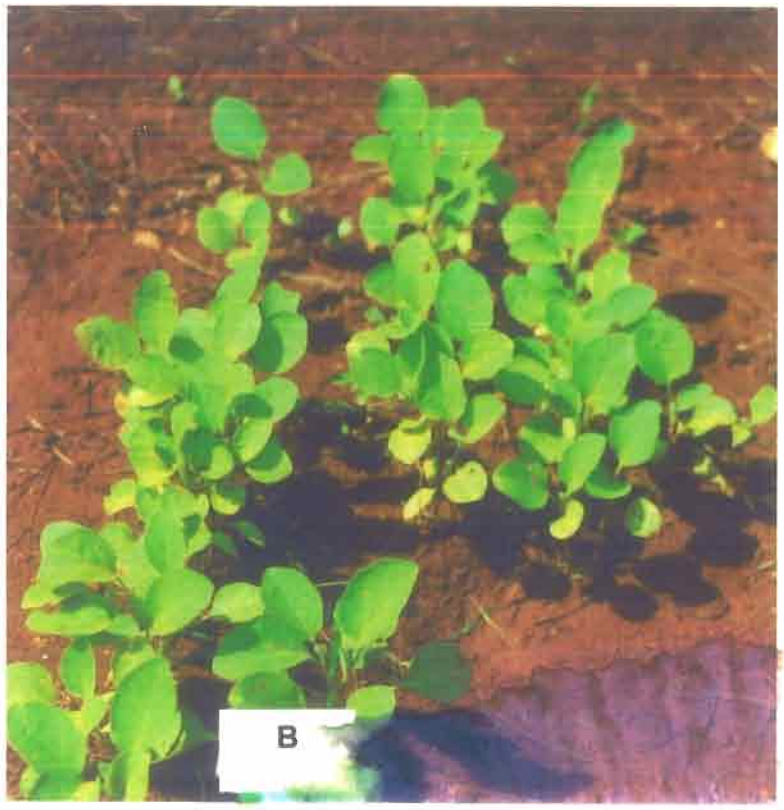


Table 25. Efficacy of *P. fluorescens* Pf1 formulation for the management of Fusarium wilt of tomato and brinjal during 1999 at Madurai under field conditions

Treatments	Disease incidence (%)	
	Tomato	Brinjal
T_1	16.67^{c} (24.08)	14.44^{bc} (22.31)
T_2	16.67^{c} (24.08)	16.67^{c} (23.80)
T_3	15.15^{b} (22.89)	13.33^{abc} (21.15)
T_4	13.64^{a} (21.59)	14.44^{bc} (22.31)
T_5	13.64^{a} (21.65)	12.22^{abc} (20.42)
T_6	12.63^{a} (20.81)	10.00^{a} (18.27)
T_7	51.52^{d} (45.87)	33.44^{d} (35.33)
T_8	53.06^{d} (46.74)	36.66^{d} (37.23)
T_9	51.52^{d} (45.87)	35.56^{d} (36.60)
T_{10}	13.64^{a} (21.65)	11.11^{ab} (19.43)
T_{11}	53.03^{d} (46.74)	37.78^{d} (37.92)

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Treatment specification is given in Table 24

Table 26. Efficacy of *P. fluorescens* Pf1 formulation for the management of fusarium wilt of tomato and brinjal during 2000-2001 at Aruppukottai under field conditions

Treatments	Tomato		Brinjal	
	Disease incidence (%)	Yield (t ha^{-1})	Disease incidence (%)	Yield (t ha^{-1})
T_1	21.21^{bc} (27.38)	19.97^{b}	15.56^{b} (23.20)	24.00^{b}
T_2	24.24^{c} (29.49)	20.03^{b}	14.44^{b} (22.31)	25.70^{c}
T_3	19.19^{ab} (25.96)	21.00^{c}	15.56^{b} (23.20)	25.93^{c}
T_4	17.17^{a} (24.45)	20.50^{bc}	14.44^{b} (22.31)	24.10^{b}
T_5	16.16^{a} (23.70)	22.50^{d}	13.44^{b} (21.41)	26.30^{c}
T_6	17.17^{a} (24.48)	22.50^{d}	12.22^{ab} (20.42)	26.40^{c}
T_7	47.98^{d} (43.84)	16.70^{a}	43.33^{cd} (41.16)	21.93^{a}
T_8	47.45^{d} (43.73)	16.53^{a}	40.00^{c} (39.22)	21.53^{a}
T_9	45.45^{d} (42.39)	17.23^{a}	46.66^{d} (43.08)	22.07^{a}
T_{10}	18.69^{ab} (25.59)	20.60^{bc}	10.00^{a} (18.27)	23.80^{b}
T_{11}	48.99^{d} (44.42)	17.23^{a}	47.77^{d} (43.22)	22.23^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Treatment specification is given in Table 24

Plate 23. Effect of *Pseudomonas fluorescens* Pf1 formulation for the management of fusarium wilt of tomato under field conditions

A. Pf1 treated (T_5)

B. Control

Plate 23

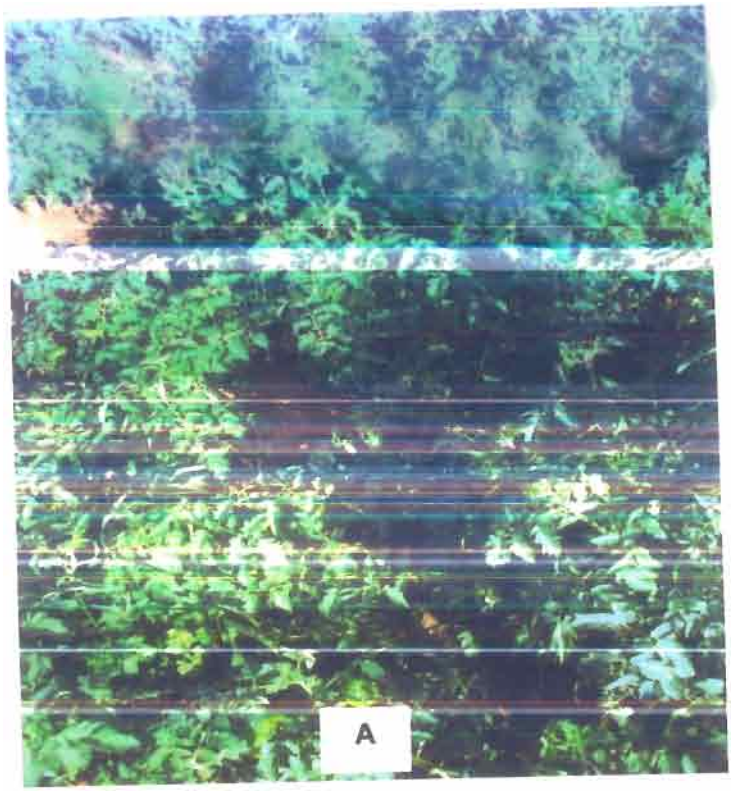
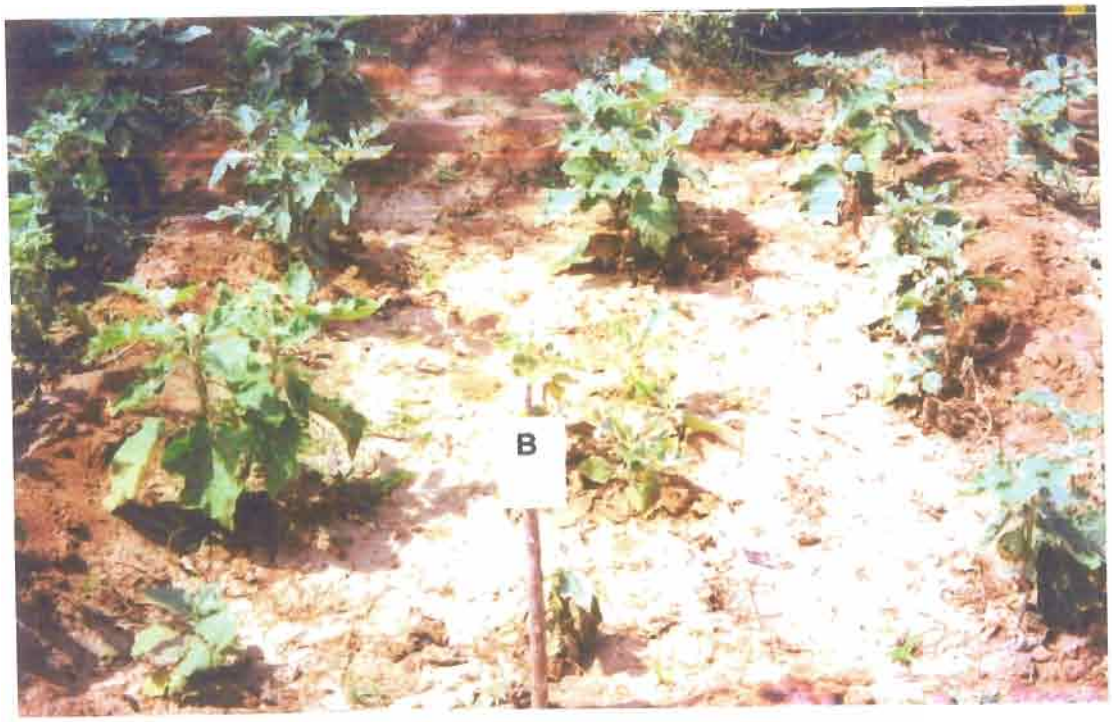


Plate 24. Effect of *Pseudomonas fluorescens* Pf1 formulation for the management of fusarium wilt of brinjal under field conditions

A. Pf1 treated (T_5)

B. Control

Plate 24



containing talc+gypsum mixed with *P. fluorescens* mixed with *P. fluorescens* mass multiplied in chitin amended medium showed the minimum wilt incidence in tomato and brinjal. The addition of Zinc in the bioformulation did not have any significant effect on the biocontrol activity of *P. fluorescens* (Plate 23 and 24).

4. 13. 6. Efficacy of different formulations of *P. fluorescens* Pf1 against fruit rot incidence in chilli under field conditions

The data presented in Tables 27 and 28 revealed that among the various formulations tested for the management of fruit rot of chilli, formulation containing talc+gypsum as a carrier with incorporation of *P. fluorescens* mass multiplied in chitin amended KMB showed less disease incidence when compared to the formulation containing other carriers. Similarly addition of Zinc neither increased the biocontrol efficacy of the formulation nor showed any negative effect on the biocontrol activity (Plate 25).

Table 27. Efficacy of *P. fluorescens* Pf1formulation for the management of fruit rot of chilli during 1999 at Aruppukottai under field conditions

Treatments	Disease incidence (%)		Yield
	135 DAS	165 DAS	(t ha^{-1})
T_1	9.62^{a}(18.07)	20.29^{c}(26.75)	22.50^{bc}
T_2	10.66^{a}(19.04)	19.11^{abc}(25.90)	21.33^{b}
T_3	10.07^{a}(18.48)	18.52^{abc}(25.48)	21.63^{b}
T_4	10.07^{a}(18.48)	17.01^{a}(24.34)	22.43^{bc}
T_5	9.18^{a}(17.63)	16.89^{a}(24.24)	23.40^{c}
T_6	9.33^{a}(17.77)	17.77^{ab}(24.93)	23.18^{c}
T_7	18.07^{b}(25.15)	28.59^{d}(32.32)	16.40^{a}
T_8	18.81^{b}(25.70)	30.66^{de}(33.62)	16.55^{a}
T_9	18.51^{b}(25.47)	31.26^{e}(33.99)	16.73^{a}
T_{10}	9.33^{a}(17.78)	19.85^{bc}(26.45)	21.22^{b}
T_{11}	18.81^{b}(25.70)	31.85^{e}(34.35)	15.40^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Treatment specification is given in Table 24

DAS-Days after sowing

Table 28. Efficacy of *P. fluorescens* formulation for the management of fruit rot of chilli during 2000 Aruppukottai under field conditions

Treatments	Fruit rot incidence (%)		Yield
	135 DAS	165 DAS	(t ha^{-1})
T_1	12.59^{b}(20.77)	32.15^{cd}(34.54)	20.81^{bcd}
T_2	14.51^{c}(22.83)	30.96^{bcd}(33.80)	20.63^{bc}
T_3	10.66^{a}(19.04)	30.81^{bc}(33.72)	21.43^{d}
T_4	10.07^{a}(18.47)	28.88^{ab}(32.51)	21.35^{cd}
T_5	10.22^{a}(18.62)	28.15^{a}(32.03)	21.60^{d}
T_6	10.66^{a}(19.04)	28.88^{ab}(32.50)	21.52^{d}
T_7	21.77^{d}(27.81)	46.96^{e}(43.26)	16.85^{a}
T_8	21.18^{d}(27.39)	49.48^{f}(44.70)	17.18^{a}
T_9	20.74^{d}(27.08)	49.77^{f}(44.87)	16.83^{a}
T_{10}	9.03^{a}(17.49)	33.18^{d}(35.17)	20.33^{b}
T_{11}	21.92^{d}(27.91)	49.78^{f}(44.87)	17.12^{a}

Data followed by a common letter within a column are not significantly different (p=0.05) by DMRT

The data are mean of three replications

Figures in parentheses indicate arcsine transformed values

Treatment specification is given in Table 24

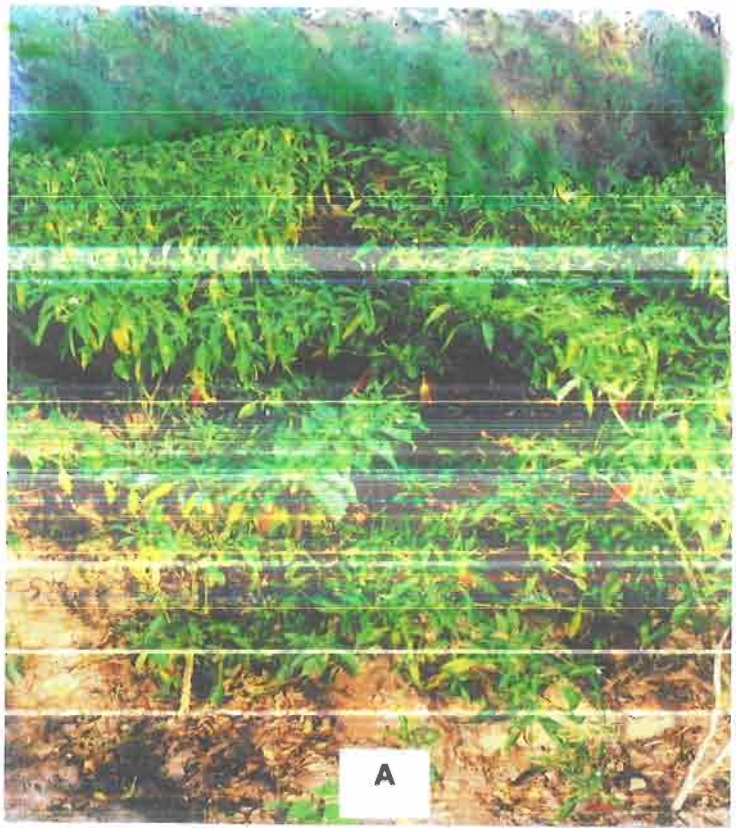
DAS-Days after sowing

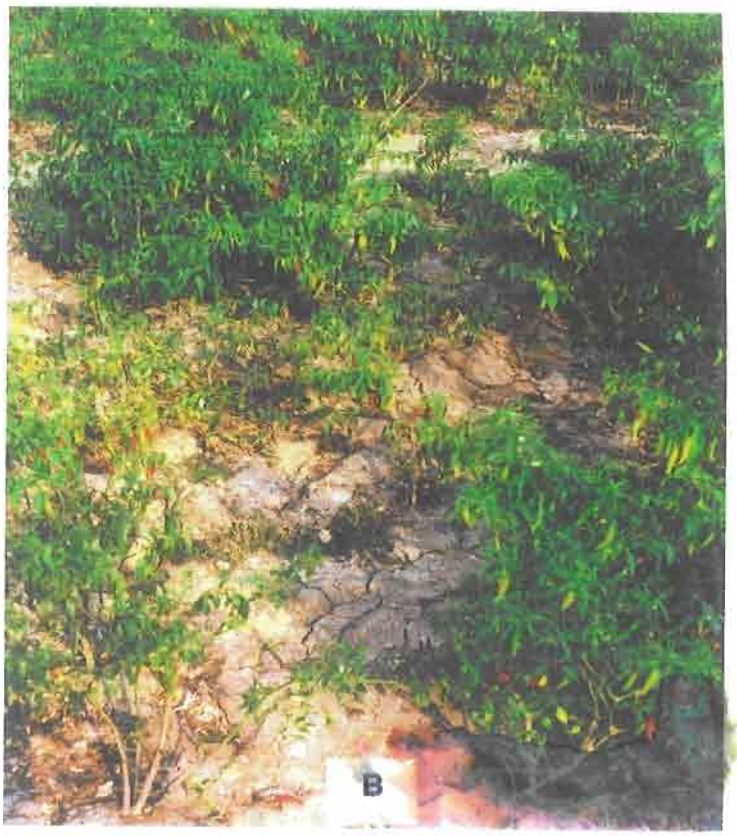
Plate 25. Effect of *Pseudomonas fluorescens* Pf1 formulation for the management of fruit rot of chilli under field conditions

A. Pf1 treated (T_5)

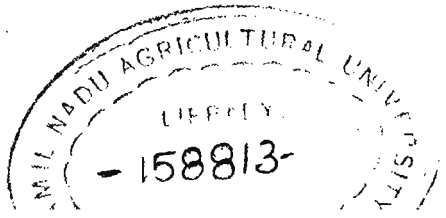
B. Control

Plate 25





Discussion



CHAPTER V

DISCUSSION

Several studies have demonstrated the biocontrol activity of various rhizobacteria against many soil-borne pathogens, including *Aphanomyces* spp., *Pythium* spp., *Fusarium oxysporum, F. solani, Gaeumannomyces graminis* var *tritici*, *Phytophthora* spp., *Sclerotium rolfsii* and *Thielaviopsis basicola* (Schippers *et al.*, 1987; Weller, 1988; Vidhyasekaran *et al.*, 1997a). Direct antagonism of fluorescent pseudomonads against soil-borne pathogens has been earlier reported as a possible mode of action in biological control. During 1980's, it was reported that fluorescent pseudomonads protected the host plants against plant pathogens through mechanisms other than direct antagonism (Voisard *et al.*, 1989). The first evidence on ISR by fluorescent pseudomonads was reported in cucumber against anthracnose (Wei *et al.*, 1991), carnation against fusarium wilt (Van Peer *et al.*, 1991) and bean against halo blight (Alstrom, 1991). The mechanism behind the induced resistance by fluorescent pseudomonads was not clearly explained. Moreover, ISR by rhizobacteria is relatively new area of research with regard to crop protection.

Characterization and biocontrol activity of fluorescent pseudomonads

Several fluorescent pseudomonads found antagonistic to soil-borne pathogens were isolated from suppressive soils of various crops (Weller, 1983; Viswanathan and Samiyappan, 2001b). Fluorescent pseudomonads are known to aggressively colonize roots therefore they are efficient competitors and persist throughout the crop season

(Kloepper and Schroth, 1981). Among the various species of fluorescent pseudomonads, *P. fluorescens* appears commonly in rhizosphere of various crop plants in different regions of Tamil Nadu (Samiyappan *et al.*, 1999; Viswanathan, 1999; Meena, 2000). However, biocontrol efficacy of the different strains vary due to inherent potential of the strains to suppress the pathogen (Sakthivel *et al.*, 1986; Rosales *et al.*, 1993).

The present study indicates that the majority of the strains isolated from rhizosphere of different crops belonged to *P. fluorescens* group. Only two strains KKM1 and PFATR belonged to *P. putida*. The dendrogram drawn based on similarity in primary characters of fluorescent pseudomonads and carbon source utilization tests revealed that the strains belonging to the same biovar showed maximum similarity and were clustered together. The strains of *P. fluorescens* belonging to biovar I showed more than 90 per cent similarity. Similarly the four strains of biovar V were more closely related. *P. putida* strain KKM1 did not cluster very closely with strains of *P. fluorescens* (Fig 1). Majority of the fluorescent pseudomonads was found to be *P. fluorescens* biovar V in rhizosphere soil of wheat in Australia (Sands and Rovira, 1971). Rosales *et al.* (1993) reported that some of the strains within each genus of bacteria isolated from rice rhizosphere could be differentiated phenotypically and through protein profile studies. Variation was observed in each dominant group of fluorescent pseudomonads. Such variation could be influenced by soil type, cropping patterns, water availability and other environmental factors.

After isolating and identifying the fluorescent pseudomonads, selecting an effective strain is the first and foremost important step in biological control. *P. fluorescens* Pf1 showed the maximum inhibitory effect on mycelial growth. In

addition, *P. fluorescens* Pf1 produced fairly higher amount of IAA and increased the vigour index. Similarly, *P. putida* strain PFATR and *P. fluorescens* strains FP7 and PSV increased the plant vigour and produced the maximum amount of IAA although they were not effective in inhibiting the mycelial growth of the pathogen under *in vitro* conditions. Earlier Radja Commare (2000) reported that seed bacterization with *P. fluorescens* Pf1 increased the seedling vigour index. Similarly the same strain also increased the plant vigour in groundnut (Meena, 2000) and rice (Nandakumar, 1998). As certain strains improve plant growth in addition to biological control, these strains are collectively called as plant growth promoting rhizobacteria (Liu *et al.*, 1995 a, b, c).

In agriculture, improving the crop production is the main objective by increasing crop germination, growth and yield parameters. The use of fluorescent pseudomonads for increasing the yield and crop protection is an attractive approach in the modern system of sustainable agriculture. Mishra and Sinha (2000) found that seed treatment and seedling root dip of *P. fluorescens* increased the germination, root and shoot length of rice. These studies further imply that quantum of chemical fertilizers recommended for the crop could be reduced by applying such plant growth promoting rhizobacteria. Fluorescent pseudomonads having antagonistic activity and increasing the plant growth would be certainly promising in evaluating the suitable strains in biological control (Viswanathan and Samiyappan, 2001b).

In controlled environmental conditions, *P. fluorescens* Pf1 which is effective in inhibiting the mycelial growth of the pathogen consistently reduced the damping-off disease incidence in addition to increasing the plant growth in tomato, chilli and brinjal.

Rhizobacteria produce certain plant growth hormones such as auxins, gibberellin and cytokinins although they are not directly implicated as a metabolite in disease control, they stimulate root-elongation and plant growth (Dubeikovsky *et al.* 1993). In the present study, it was found that production of IAA by fluorescent pseudomonads was positively correlated with plant growth-promotion. Gamliel and Katan (1993) reported that increased plant growth promotion by fluorescent pseudomonads was also associated with the suppression of deleterious microorganisms. The present study clearly indicates that increased plant growth was not only associated with production of plant growth hormones by fluorescent pseudomonads in most cases but also by the suppression of *Pythium* infection in tomato, chilli and brinjal seedlings. Suppression of damping off and root rot caused by various *Pythium* spp. with fluorescent pseudomonads was reported in various crops such as cotton (Abdelzaher and Elnaghy, 1998), sugarbeet (Moenne-Loccoz *et al.*, 1999; Timms-Wilson *et al.*, 2000), cucumber (Mc Cullagh *et al.*, 1996), pea (Heungens and Parke, 2001) and tomato (Hultberg *et al.*, 2000). Schulz and Werner (1998) reported that application of *P. fluorescens* increased the emergence of cucumber seedlings grown in *P. ultimum* infested soil under greenhouse conditions and the biomass of the plants and dry weight of shoot and root were not affected. Similarly, *Burkholderia cepacia* reduced the damping-off and root rot of pea by suppressing the mycelial growth of *P. aphanidermatum* and oogonia formation by *Aphanomyces euteiches* (Heungens and Parke, 2001).

Similarly, *P. fluorescens* Pf1 was found to be effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *lycopersici*, *F. solani* and *C. capsici*. The present study also

showed that *P. fluorescens* Pf1 was found to reduce not only the incidence of damping-off but also the incidence of fusarium wilt of tomato and brinjal and fruit rot of chilli under greenhouse conditions when compared to other strains and also increased the plant growth.

Suppression of fusarium wilt by fluorescent pseudomonads was reported in sesame (Lee *et al.*, 1995), radish (Leeman *et al.*, 1996), watermelon (Larkin *et al.*, 1993), chickpea (Vidhyasekaran and Muthamilan, 1995) and tomato (Duijff *et al.*, 1998). Various strains of *Bacillus* spp. and fluorescent pseudomonads showed strong antagonism against *F. oxysporum* f. sp. *ciceris* (Lee *et al.*, 1995; Landa *et al.*, 1997; Vidhyasekaran and Muthamilan, 1995). Seed and soil application of selected fluorescent pseudomonads suppressed the fusarium wilt of chickpea (Landa *et al.*, 1997; Vidhyasekaran and Muthamilan, 1995). Larkin and Fravel (1998) reported that *P. fluorescens* isolated from rhizosphere soils of tomato significantly reduced the fusarium wilt of tomato.

Bacterial metabolites production

Secondary metabolite production by fluorescent pseudomonads has been implicated in the direct antagonism against several soil-borne pathogens and also inducing systemic resistance. Implication of antibiotic production for the management of soil-borne diseases has been well established (Maurhofer *et al.*, 1995; Raaijmakers *et al.*, 1997). Phloroglucinol has been implicated for the management of take-all of wheat (Raaijmakers *et al.*, 1997). Oomycin A, antibiotic produced by *P. fluorescens* isolate Hv37aR2 was attributed for the suppression of damping-off incidence caused by

P. ultimum in cotton (Howie and Suslow 1991). In the present study, except *P. putida* strain ATR all other strains produced 2,4-diacetyl phloroglucinol. Though *P. putida* strain ATR did not produce 2,4-diacetyl phloroglucinol it significantly reduced the damping-off incidence. Thus the present study indicates that production of 2,4-diacetyl phloroglucinol by *P.putida* strain ATR is having lesser role in the biological control of damping-off incidence.

Fluorescent siderophore, which has a very high affinity for ferric-iron, is secreted under iron-limited conditions. The resulting ferric-siderophore complex is unavailable to other organisms, but the producing strain can utilize siderophore-iron complex *via* a very specific receptor in its outer cell membrane (Buyer and Leong, 1986). Thus, fluorescent *Pseudomonas* strains restrict the growth of deleterious bacteria and fungi at the plant-root surface. Siderophore-mediated disease suppression by fluorescent pseudomonads has been well-documented (Loper and Buyer, 1991). However, in the present study, all strains including ineffective strains of fluorescent pseudomonads were invariably capable of producing siderophores. Thus siderophore production by the fluorescent pseudomonads might have played a minor role in suppression of damping-off and wilt incidence. In support of this finding, Callen *et al.* (1990) reported that siderophore production by fluorescent pseudomonads was not an essential component for protection of corn seed against damping-off disease. Involvement of HCN produced by fluorescent pseudomonads for the management of *T. basicola* inciting black root-rot of tobacco was reported by Voisard *et al.* (1989). However, in the present study, it has been found that both effective and ineffective strains produced HCN indicating that HCN production by

fluorescent pseudomonads plays a minor role in suppression of damping-off and wilt incidence. *P. fluorescens* strains *viz.*, Pf1 produced appreciable level of salicylic acid. Further studies with mutant strain of Pf1 for these traits may throw more information on the involvement of antibiotics, siderophore, HCN and SA against disease suppression.

The effective antagonistic strains produced higher amount of β-1,3-glucanase and chitinase. Though study on the implication of lytic enzymes on suppression of damping-off and wilt diseases is rare, involvement of β-1,3-glucanase and chitinase which degrade the fungal cell wall containing especially glucans in *Pythium* spp. and glucan and chitin in *Fusarium* and *C. capsici* could not be excluded. Upon action by these enzymes, the lysis of cell occurs first resulting in the leakage of inner content of the fungal cell leading to the death of the fungal cell. Secondly chitin oligomers and glucan fragments released from the fungal cell wall act as an elicitor which elicits various defense mechanisms in the plants (Frindlender *et al.*, 1993). Ji and Kuc (1996) found that β-1,3-glucanase strongly inhibited spore production. Mitchell and Hurvitz (1965) reported that certain lytic rhizobacteria suppressed propagules of *P. debaryanum.* In this study, production of β-1,3-glucanase and chitinase by fluorescent pseudomonads was positively correlated with suppression of disease incidence. Similarly Velazhahan *et al.* (1999) and Viswanathan and Samiyappan (2001a) reported that biocontrol-efficacy of the pseudomonads was positively correlated with their ability in lytic enzyme production.

Moreover, the present study also indicates that the effective strain *P. fluorescens* Pf1 produced several metabolites at higher level and it has been assumed that this strain might have suppressed the pathogens by more than one mode of biocontrol actions

Induction of defense mechanisms

Besides direct antagonistic activity by the production of various bacterial metabolites, induction of systemic resistance by fluorescent pseudomonads against diseases has been established as a new mechanism by which the plants defend themselves from pathogen attack (Van Peer *et al.* 1991; Van Loon *et al.*, 1998). Prior application of fluorescent pseudomonads as seed-treatment induces various defense mechanisms in the plants (Chen *et al.*, 2000).

Plants have endogenous defense mechanisms that can be induced in response to attack by insects and pathogens (Bostock *et al.*, 2001; Heil, 2001). It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant's own defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy. Induced resistance by inducing agents in several crops is associated with enhancement of lignification and also increased activities of enzymes involved in phenylpropanoid pathway and PR protein synthesis (Boller and Mauch, 1988; Hammerschmidt and Kuc, 1995). Recent studies implies that prior application of fluorescent pseudomonads strengthen host cell wall structures resulting in restriction of pathogen invasion in plant tissue (Benhamou *et al.*, 2000; Chen *et al.*, 2000). However, there is a little information available on plant-mediated defense reactions induced by fluorescent pseudomonads in plants against pathogen invasion.

Induction of enzymes of phenylpropanoid pathway

Phenylalanine ammonia lyase plays an important role in the biosynthesis of various defense chemicals in phenylpropanoid metabolism (Daayf *et al.*, 1997). PAL activity could be induced in plant-pathogen interactions and fungal elicitor treatment (Ramanathan *et al.*, 2000). De Meyer *et al.* (1999) reported that rhizosphere colonization by *P. aeruginosa* 7NSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves. In the present study, increased activity of PAL was recorded in *P. fluorescens* Pf1-treated plants challenged with the pathogens. The time required to activate the defense mechanisms is important for the suppression of pathogen. Earlier and higher level of expression of defense enzymes and accumulation of chemicals at the infection site certainly prevent the fungal mycelial colonization. In tomato and chilli seedlings, the activity of PAL reached maximum at 3rd day after *P. aphanidermatum* inoculation while in grownup tomato and chilli plants the activity reached maximum at 4th day after challenge inoculation with *F. oxysporum* f. sp. *lycopersici* and *C. capsici* respectively. The activity of PAL was maintained at higher level throughout the experimental period. In plants inoculated with the pathogen alone the activity declined drastically 4 days after challenge inoculation. Invasion of root tissues by the pathogen might have resulted in decreased activity of PAL whereas earlier and increased activity of PAL due to *P. fluorescens* Pf1 treatment might have prevented the fungal invasion and thus the activity maintained at higher levels during the experimental period. *P. fluorescens* Pf1 also induced the PAL activity in rice (Meena *et al.*, 1999; Radja commare, 2000) and groundnut (Meena *et al.*, 2000a). Induction of PAL by fluorescent

pseudomonads was reported in cucumber against *P. aphanidermatum* (Chen *et al.*, 2000) and bean against *Botrytis cinerea* (Zdor and Anderson, 1992).

Peroxidase represents another component of an early response in plants to pathogen attack and plays a key role in the biosynthesis of lignin which limit the extent of pathogen spread (Bruce and West, 1989). The products of this enzyme in the presence of hydrogen donor and hydrogen peroxidase have antimicrobial activity and even antiviral activity (Van Loon and Callow, 1983). Increased activity of cell wall bound peroxidases has been elicited in different plants such as cucumber (Chen *et al.*, 2000), rice (Reimers *et al.*, 1992), tomato (Mohan *et al.*, 1993) and tobacco (Ahl Goy *et al.*, 1992) due to pathogen infection. In bean, rhizosphere colonization of various bacteria induced the peroxidase activity (Zdor and Anderson, 1992). In the present study, earlier and increased peroxidase activity has been recorded in *P. fluorescens* Pf1-treated plants challenged with the pathogen. Chen *et al.* (2000) reported the higher PO activity in cucumber roots treated with *P. corrugata* challenged with *P. aphanidermatum*. The present study also indicates that expression of five PO isoforms, PO1-PO5 in tomato seedlings and one PO isoform, PO1 in chilli seedlings was more prominent in *P. fluorescens* Pf1 treated plants challenge inoculated with *P. aphanidermatum*. In grownup tomato plants, PO1 isoform was prominently expressed in *P. fluorescens* Pf1-treated root tissues against *F. oxysporum* f. sp. *lycopersici* in addition to higher level expression of PO2-PO7. This unique isoform (PO1) induced by *P. fluorescens* Pf1 might have contributed to induced defense in tomato root tissue against invasion by *F. oxysporum* f. sp. *lycopersici*.

Similar to other enzymes, PPO activity was increased by *P. fluorescens* Pf1 against the challenged pathogens. Expression of four PPO isoforms, PPO1, PPO2, PPO3 and PPO4 was very clear in bacterized tomato seedlings challenge inoculated with *P. aphanidermatum*. Similarly, in bacterized chilli seedling expression of PPO1 isoform was at higher level. In grownup tomato plants, a new PPO1 isoform, a unique PPO isoform and a higher level expression of PPO2 isoform were observed in *P. fluorescens* Pf1-induced root tissues against *F. oxysporum* f. sp. *lycopersici* infection. The induced PPO1 isoform and a higher level expression of PPO2 isoform might have also been implicated in induced defense responses against the pathogen invasion. In chilli, expression of PPO1 isoform was more prominent in bacterized plant challenged with *C. capsici* compared to plants inoculated with *C. capsici* alone or plants treated with *P. fluorescens* alone. Earlier, Radja Commare (2000) reported that same strain induced PPO isozyme in rice against *R. solani*. Similarly, Meena *et al.* (2000a) reported that *P. fluorescens* induced the activities of PPO in response to infection by *C. personatum* in groundnut. Chen *et al.* (2000) reported that various rhizobacteria and *P. aphanidermatum* induced the PPO activity in cucumber root tissues.

The phenolic compounds may contribute to enhance the mechanical strength of host cell wall and may also inhibit the fungal growth, as phenolics are fungitoxic in nature. Seed treatment with *P. fluorescens* 63-28 induced the accumulation of phenolics in tomato root tissues (M'Piga *et al.*, 1997). The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Accumulation of phenolics by prior

application of *P. fluorescens* in pea has been reported against *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou *et al.*, 1996a). The present study also indicates that the higher level accumulation of phenolics was observed in *P. fluorescens* Pf1-treated tomato and chilli plants challenged with pathogen. Similar findings were reported in rice against *R. solani* (Meena *et al.*, 1999; Radja commare, 2000), sugarcane *against C. falcatum* (Viswanathan and Samiyappan, 1999) and in groundnut against *C .personatum* (Meena *et al.*, 2000a). Benhamou *et al.* (2000) reported that an endophytic bacterium, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum.*

Thus induction of defense enzymes involved in phenylpropanoid pathway by *P. fluorescens* Pf1 in tomato and chilli against pathogen infection leads to induced protection by synthesizing various defense compounds. Earlier and enhanced levels of PAL, PO and PPO suppressed the further colonization of soil-borne pathogens in crop plants.

Pathogenesis-related proteins

PR-proteins are host-coded proteins induced by different types of pathogens and abiotic stresses (Van Loon, 1997). Synthesis and accumulation of PR-proteins have been reported to play an important role in plant defense (Maurhofer *et al.*, 1994; Van Loon, 1997). Over expression of cloned rice TLP gene in transgenic rice enhances resistance to *Rhizoctonia solani* causing sheath blight disease (Datta *et al.*, 1999). Colonization of bean roots by rhizobacteria was correlated with induction of PR proteins resulting in induced systemic resistance against *B. cinerea* (Zdor and Anderson, 1992). Similarly in

due to application of *P. fluorescens* isolate CHAO in response to infection by tobacco necrosis virus (TNV). Induction of these hydrolytic enzymes was also reported in pea against *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou *et al.*, 1996a, b) and in tomato against *F. oxysporum* f. sp. *radicis-lycopersici* (M'Piga *et al.*, 1997). In the present study, immunoblot analysis revealed that induction of chitinase with the molecular weight of 41 kDa was noticed in the tomato seedlings in response to infection by *P. aphanidermatum*. In grownup tomato plants, 45 and 46 kDa chitinase isoforms were observed in *P. fluorescens* pretreated tomato plants in response to infection by *F. oxysporum* f. sp. *lycopersici*. In addition, expression of 33 kDa TLP was noticed in tomato root tissues in response to infection by *F. oxyporum* f. sp. *lycopersici*. In chilli, induction of 42 kDa chitinase was observed in *P. fluorescens* treated plants challenge inoculated with *C. capsici*. Moreover the activity of β-1,3-glucanase was higher in the bacterized plants challenged with the pathogen. The enzymatic degradation of the fungal cell wall by hydrolytic enzymes may release non-specific elicitors (Ham *et al.*, 1991; Ren and West, 1992) which in turn elicit various defense reactions. The fungal cell wall elicitors have been reported to elicit various defense reactions in greengram (Ramanathan *et al.*, 2000). Earlier, Viswanathan and Samiyappan (1999) reported that ISR by fluorescent pseudomonads which was associated with induction of chitinase was the promising technology for the management of red rot of sugarcane. Meena *et al.* (2000b) reported that induction of PR proteins due to application of *P. fluorescens* was mainly contributed in the suppression of late leaf spot of groundnut. However, in contrast to these results, Pieterse *et al.* (1998) and Van Wees *et al.* (1999) reported that ISR by

rhizobacteria is independent of PR-proteins accumulation in *Arabidopsis*. Similarly, in radish ISR induced by *P. fluorescens* is not associated with PR proteins accumulation (Steijl *et al.*, 1999).

However implication of chitinase enzyme induced due to bacterization of tomato plants against *P. aphanidermatum* which lack chitin in their cell walls is not yet known. Similar type of results was reported by Maurhofer *et al.* (1994) in tobacco in which application of fluorescent pseudomonads induced the accumulation of β-1,3-glucanase, chitinase and other defense proteins though the role of these compounds against tobacco necrosis virus is unlikely. These studies clearly indicate that several enzymes and chemicals are induced during induction of resistance by fluorescent pseudomonads and all the compounds are not equally effective against various pathogens.

It is also clear that ISR by fluorescent pseudomonads is non-specific, that is ISR by fluorescent pseudomonads is effective not only to the single pathogen but also effective against various pathogens attacking one and the same crop and also other crops. Van Loon *et al.* (1998) and Zehnder *et al.* (2001) have reported non-specificity of ISR mediated by fluorescent pseudomonads against various pests and diseases. In the present study, seed treatment and soil application of single strain of *P. fluorescens* induced resistance against seedling disease (damping-off) and wilt both in tomato and chilli and also against fruit rot incidence by inducing systemic resistance. Zehnder *et al.* (2001) reported that application of PGPR induced systemic resistance against various viruses and insects that transmit the viruses and also cucumber bacterial wilt.

In the present study, it was also clearly observed that several defense-related proteins and chemicals were induced by *P. fluorescens* Pf1 against challenging pathogen. Induction of several defense enzymes by fluorescent pseudomonads was noticed by Zdor and Anderson (1992), Maurhofer *et al.* (1994), Nandakumar *et al.* (2001) and Viswanathan and Samiyappan (1999). Increase in lysozyme, PO, PPO and PAL correlated with the induction of ISR by various biotic and abiotic agents was reported by Ye *et al.* (1992) and Schneider and Ullrich (1994). The present study clearly indicates that there is no specificity in the induction of multiple defense-related genes encoding proteins and chemicals in ISR mediated by *P. fluorescens* Pf1. Recent studies conducted by Ton *et al.* (2001) showed that ISR by fluorescent pseudomonads is controlled by specific gene locus in *Arabidopsis*. Thus it is hypothesized that ISR mediated by fluorescent pseudomonads is related to multigenic/polygenic/ horizontal resistance. It was also well known that multigenic resistance is considered to be non-specific, that is multigenic resistant plants resist a variety of pathogens and also pathogenic races (Tuzun, 2001). Because of that, ISR functions in non-specific manner against several pathogens and even insect and nematode pests. However, much studies in this line would further strengthen the concept of multigenic resistance induced by fluorescent pseudomonads and also elucidate whether the genes involved in multigenic resistance are also implicated in the ISR-mediated by fluorescent pseudomonads.

Interestingly, the secondary metabolites of fluorescent pseudomonads are mostly involved in the induction of resistance (Van Loon *et al.*, 1998; Haas *et al.* 2000). Among the different secondary metabolites of fluorescent pseudomonads, siderophores and

salicylic acid are implicated in the induction of systemic resistance (Leeman *et al.*, 1996; Maurhofer *et al.*, 1998). De Meyer and Hofte (1997) have reported the role of salicylic acid produced by *P. aeruginosa* 7NSK2 as a determinant of inducing systemic resistance. Similarly, *P. fluorescens* isolate CHAO produces salicylic acid and induces resistance against the same pathogen (Maurhofer *et al.*, 1998). In contrast, certain studies indicate that production of salicylic acid by fluorescent pseudomonads is not involved in the induction of resistance in the plant. Salicylic acid-negative mutants of *Serratia marcescens* strain 90-166 lacking salicylic acid production induce the resistance as wild strains (salicylic acid producing strain) do (Press *et al.*, 1997). However, in the present study the promising strain *P. fluorescens* Pf1 produced both siderophores and SA. The involvement of these traits of *P. fluorescens* could be confirmed by genetic engineering of the strain with and without particular trait.

From the present study it was found that *P. fluorescens* Pf1 not only induced resistance against seedling disease but also against wilt and fruit rot diseases. Thus induction of resistance against multiple pathogens by single strain is more rewarding than using different fungicides for the management of these diseases. The same strain also induced systemic resistance in rice against sheath blight (Meena *et al.*, 1999; Vidhyasekaran and Muthamilan, 1999; Radja Commare, 2000; Nandakumar *et al.*, 2001), bacterial blight (Vidhyasekaran *et al.*, 2001) and rice leaf tip nematode (Swarnakumari *et al.*, 1999). The present study also indicates that ISR by *P. fluorescens* Pf1 has broad-spectrum resistance against damping-off, wilt and fruit rot in vegetable crops. Similarly,

ISR by PGPR had broad-spectrum effect against multiple pests and diseases attacking cucumber (Zehnder *et al.*, 1997; 2001).

Moreover, fluorescent pseudomonads are potential rhizosphere colonizers and survive and persist for a long period. Thus induction of resistance lasts for a very long period (Van Loon *et.al.*, 1998), whereas, induction of resistance by chemical inducer appears for a short period. Thus chemical treatments need to be applied at 1-2 week intervals (Kuc, 1984).

In a nutshell, the present study implies that earlier and higher accumulation of enzymes involved in phenylpropanoid metabolism and PR-proteins has been implicated in inducing resistance against pathogen infection in bacterized tomato and chilli plants. The plant-pathogen interactions have also triggered the activities of defense enzymes initially but later the activities drastically declined when the pathogen colonized the root tissues. Accumulation of phenolics, PAL, PO, PPO, β-1,3-glucanase and chitinase and induction/expression of PO and PPO isoforms and chitinase and TLP isoforms in *P. fluorescens*-treated tomato and chilli plants might have collectively contributed to induced broad-spectrum resistance.

Formulation development

The application of fluorescent pseudomonads-mediated systemic resistance and plant growth promotion requires a delivery system which is practical on a large scale field level. Chitin amendment increased the growth and multiplication of chitinolytic microflora (Culbreath *et al.*, 1986; Bell *et al.*, 1998). In the present study, incorporation of chitin at one per cent level in the KMB increases the growth and multiplication of

P. fluorescens Pf1. Viswanathan and Samiyappan (2001a) reported that when chitin was substituted with glycerol as a carbon source it resulted in enhanced growth and multiplication of fluorescent pseudomonads. Moreover they found that fluorescent pseudomonads grown on chitin amended medium showed enhanced antifungal activity against *C. falcatum*. The chitinase enzyme produced in the chitin-amended medium might be implicated in the enhanced biocontrol activity of fluorescent pseudomonads. Yuen *et al.* (2001) also found that incorporation of chitin in the medium increased bacterial population when compared to the non-amended medium.

Mixing a biocontrol agent with suitable carrier which supports the survival of microbial colonies quite a long period for field application is the final step in the process of commercialization of biocontrol agent. Suslow (1980) first developed the formulation of *P. fluorescens* using talc as a carrier. Seed treatment with bacterial cell suspension was found effective in controlling several diseases (Kaiser *et al.*, 1989; Parke *et al.*, 1991). However, for commercial and field application, this methodology would be impractical due to difficulties in handling, transport and storage. Vidhyasekaran and Muthamilan (1995) found that the colonies of *P. fluroescens* in the suspension culture drastically declined when stored for a period of 10 days whereas the population was maintained when the culture was mixed in the carrier materials. The bacteria survived well in the talc and gypsum-based formulations for a period of more than four months. Kloepper and Schroth (1981) found that population of PGPR did not decline in the talc mixture with 20 per cent xanthan gum upto a storage period of two months. In the present study, the formulation containing talc+gypsum as a carrier material mixed with *P. fluorescens* Pf1

mass multiplied in KMB amended with chitin supported the maximum colonies of *P. fluorescens*.

Talc-based formulation was effective for the control of chickpea wilt (Vidhyasekaran and Muthamilan, 1995), pigeonpea wilt (Vidhyasekaran *et al.*, 1997b), rice sheath blight (Vidhyasekaran and Muthamilan, 1999; Radja commare, 2000; Nandakumar *et al.*, 2001) and red rot of sugarcane (Viswanathan and Samiyappan, 2001b). In the present study, formulation containing talc+gypsum mixed with chitin-amended broth culture was found to reduce damping-off, wilt and fruit rot of chilli. The maximum survival of *P. fluorescens* Pf1 in this formulation containing unspent chitin might have been implicated in the enhanced biocontrol activity whereas other formulations showed comparatively less efficacy. The reduction in the biocontrol efficacy was due to decreased population level in these formulations. Similarly biocontrol efficacy of *P. fluorescens* in controlling damping-off of sugar beet was related to its survival and colonization (Moenne-Loccoz *et al.*, 1999). Hagedorn *et al.* (1993) developed peat-based formulation of fluorescent pseudomonads for the management of seedling diseases in cotton. Rabindran and Vidhyasekaran (1996) reported that peat-based formulation was effective for the management of rice sheath blight. Gypsum, as a carrier material for the development of formulation, supported the maximum population of *T. viride* compared to talc based formulation and was found effective for the management of root rot of pulses (Renganathan *et al.*, 1995). Formulation containing *Bacillus subtilis* and *B. amyloliquefaciens* and chitosan as a carrier was found to be effective for protection of tomato crop against soil-borne pathogens (Reddy *et al.*, 1999).

Induction of systemic resistance by fluorescent pseudomonads was reported under field conditions (Wei *et al.*, 1991; 1996; Zehnder *et al.*, 2001). Seed treatment and soil application of *P. fluorescens* was found to induce systemic resistance in cucumber against anthracnose disease (Wei *et al.*, 1991; 1996). Earlier, from our laboratory, it was reported that application of talc-based formulation of *P. fluorescens* induced systemic resistance against rice sheath blight (Vidhyasekaran and Muthamilan, 1999; Radja Commare, 2000; Nandakumar *et al.*, 2001), rice bacterial blight (Vidhyasekaran *et al.*, 2001) and rice leaf folder (Radja Commare, 2000), sugarcane red rot (Viswanathan and Samiyappan, 2001) and leaf spot and rust of groundnut (Meena *et al.*, 2000a, b). In the present study talc+gypsum formulation induced systemic resistance against pathogen infection in solanaceous vegetable crops in addition to direct antagonism. The biocontrol efficacy of talc+gypsum based formulation was found better than talc based formulation. Thus this formulation can be used for the management of vegetable crop diseases.

Moreover, increased yield was noticed in all the field experiments in *P. fluorescens* treated plants when compared to the control plants and fungicide treated plants. Increased yield was due to plant growth promoting activity of *P. fluorescens* Pf1 and reduction of disease incidence. Several studies indicated that application of *P. fluorescens* Pf1 increased the plant growth and yield in rice (Vidhyasekaran and Muthamilan, 1999; Radja Commare, 2000; Nandakumar *et al.*, 2001), groundnut (Meena *et al.*, 2000a) and sugarcane (Viswanathan and Samiyappan, 2001b).

Though fungicides are effective for the management of plant diseases, due to uneasiness in their application, high cost, environmental risk, human health hazards and

development of fungicide resistance strains, their use is being discouraged. Whereas *P. fluorescens* Pf1 is easily applied as seed treatment and as soil application by mixing with organic substrate. Once applied in the soil, fluorescent pseudomonads survive and persist for a long period and protect the plants from pathogen attack (Van Loon *et al.*, 1998). Broad-spectrum of ISR and induction of muligenic defense system encoding diverse proteins by *P. fluorescens* is another advantage in which there will not be any development of resistance strains. From the present study it is concluded that management of vegetable diseases by using talc+gypsum based bio-formulation is highly effective, economical and eco-friendly and it can be fit into integrated pest management strategy.

Summary

CHAPTER VI

SUMMARY

The work was carried out with the objective to exploit the potential of effective fluorescent pseudomonad strain in inducing various defense-related genes encoding proteins and chemicals for the management of major fungal diseases of solanaceous vegetable crops. The effective strain was formulated using different carrier materials for field application. A summary of the salient findings of the research work is presented

1. Twenty strains of fluorescent pseudomonads were characterized and identified. Among these, two strains such as PFATR and KKM1 belonged to *P. putida* and the remaining 18 strains belonged to *P. fluorescens*. Strains of *P. fluorescens* were again grouped into five different biovars and *P. putida* strains were grouped into two biovars based on biochemical tests.
2. Fluorescent pseudomonads were evaluated for plant growth promotion on tomato, chilli and brinjal. Seed bacterization with *P. fluorescens* strains Pf1, FP7 and *P. putida* strain PFATR enhanced shoot length, root length and germination percentage resulting in increased vigour index in tomato, chilli and brinjal. *P. fluorescens* strain PB2 increased the vigour index in tomato and brinjal. *P. fluorescens* strain PSV increased the vigour index in brinjal.
3. Among the 20 strains of fluorescent pseudomonads tested under *in vitro* conditions for the inhibition of mycelial growth of *P. aphanidermatum*, *P. fluorescens* strains Pf1, FP7, PFCOT, PFCOP and PB2 inhibited the mycelial growth of *P. aphanidermatum*.

4. Based on plant growth promotion and maximum inhibitory effect on *P. aphanidermatum*, seven strains *viz.*, *P. fluorescens* strains Pf1, FP7, PB2, PFCOP, PFCOT and PSV and *P. putida* strain PFATR were selected to assess the efficacy of these strains for the suppression of seedling disease in tomato, chilli and brinjal. Among these strains, *P. fluorescens* Pf1 was found effective for the suppression of damping-off incidence in tomato, chilli and brinjal.
5. These seven strains were further evaluated for the management of field diseases such as fusarium wilt of tomato and brinjal and fruit rot of chilli. Among these strains, *P. fluorescens* Pf1 showed maximum inhibitory effect on *F. oxysporum* f. sp. *lycopersici, F. solani* and *C. capsici in vitro*. Under greenhouse conditions also, *P. fluorescens* Pf1 effectively reduced the wilt incidence both in tomato and brinjal and also suppressed the fruit rot incidence in chilli.
6. Studies on the production of inhibitory metabolites by fluorescent pseudomonads showed that *P. fluorescens* Pf1 produced 2, 4-diacetyl phloroglucinol, siderophore, hydrogen cyanide, salicylic acid and β-1,3-glucanase. *P. fluorescens* FP7 produced the maximum amount of chitinase followed by *P. fluorescens* strain Pf1.
7. The promising strain *P. fluorescens* Pf1 was used for the induction of defense mechanisms in tomato and chilli. Increased activities of PAL, PO, PPO, β-1,3-glucanase and chitinase enzymes and higher level accumulation of phenolic was noticed in bacterized tomato and chilli seedlings against infection by *P. aphanidermatum*. Expression of five PO and three PPO isoforms in bacterized tomato seedlings was more prominent in response to infection by *P. aphanidermatum*. Similarly, the expression of PO1 and PPO1 isoforms was at

higher levels in bacterized chilli seedlings in response to infection by *P. aphanidermatum*.

8. Prominent expression of a new chitinase isoform with the molecular weight of 41 kDa was noticed in bacterized tomato plants against infection by *P. aphanidermatum*. Whereas in *P. aphanidermatum* inoculated plants, a mild expression of this isoform was found. Plant treated with *P. fluorescens* alone and uninoculated plants, this chitinase isoform was not found.
9. In grownup tomato plants, increased activities of PAL, PO, PPO, β-1,3-glucanase and chitinase and higher level accumulation of phenolic compounds were noticed in *P. fluorescens* Pf1-treated plants challenge inoculated with *F. oxysporum* f. sp. *lycopersici* when compared with other induction treatments and also in untreated plants. Induction of new PO1 and PPO1 isoforms and higher level expression of PPO2 was noticed in *P. fluorescens* Pf1-treated tomato plants in response to infection by *F. oxysporum* f. sp. *lycopersici*.
10. Induction of two chitinase isoforms with molecular weight of 45 and 46 kDa was noticed in bacterized tomato plants in response to infection by *F. oxysporum* f. sp. *lycopersici*. However plants treated with *P. fluorescens* Pf1 alone or inoculated with *F. oxysporum* f. sp. *lycopersici* showed only 45 kDa isoform. Induction of 33 kDa TLP was noticed in all induction treatments except in untreated control plants.
11. Similarly, earlier and increased activities of PAL, PO, PPO, β-1,3-glucanase and chitinase were noticed in *P. fluorescens*-treated chilli plants challenge inoculated with *C. capsici*. Expression of PO1 and PPO1 isoforms was more prominent in *P. fluorescens* Pf1-treated chilli plants in response to infection by *C. capsici*.

Moreover induction of 42 kDa molecular weight chitinase was more prominent in all induction treatments.

12. Studies on bio-formulation development revealed that *P. fluorescens* Pf1 multiplied in KMB amended with one per cent chitin recorded the maximum population level at 48 hr of incubation period when compared with non-amended KMB.
13. Colonies of *P. fluorescens* Pf1 were survived better in more number in talc+gypsum as carrier up to 90 days of storage period as compared to other carrier materials.
14. Among the various formulations tested, talc+gypsum containing *P. fluorescens* Pf1 mass multiplied in chitin amended medium suppressed the damping off disease in tomato, chilli and brinjal to the maximum level under greenhouse conditions.
15. Evaluation of selected formulations with or without addition of Zinc showed that talc+gypsum containing *P. fluorescens* Pf1 multiplied in chitin amended medium showed maximum biocontrol efficacy against damping off incidence both under greenhouse and field conditions. Incorporation of Zinc did not improve the biocontrol efficacy of *P. fluorescens* Pf1.
16. Similarly, the same formulation was found effective for the management of fusarium wilt of toamto and brinjal and fruit rot of chilli under field conditions.
17. In addition to disease suppression, increased fruit yield was noticed in *Pseudomonas* formulation treated tomato, chilli and brinjal.

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Annexure

ANNEXURE I

Native-PAGE

Acrylamide stock

Acrylamide	: 30.00 g
Bisacrylamide	: 0.8 g
Distilled water	: 100 ml

Separating gel (8%)

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

Stacking gel (4%)

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

Sample buffer

Glycerol	: 20% (v/v)
Tris buffer, pH 6.8	: 0.125 M
Na_2 EDTA	: 5 mM
Bromophenol blue	: 0.1% (w/v)
2-mercaptoethanol	: 1% (v/v)

ANNEXURE II

SDS-PAGE

Separating gel (12%)

Acrylamide stock solution	: 3.00 ml
Distilled water	: 2.50 ml
1.5 M Tris buffer pH 8.8	: 1.875 ml
10% SDS (Sodium dodecyl sulphate)	: 75 µl
10% APS (Ammonium per sulphate)	: 38 µl
TEMED (N, N, N', N'-tetra methyl ethylene diamine)	: 5 µl

Stacking gel (4%)

Acrylamide stock solution	: 0.65 ml
Distilled water	: 3.25 ml
1.5 M Tris buffer pH 6.8	: 1.25 ml
10% SDS (Sodium dodecyl sulphate)	: 50 µl
10% APS (Ammonium per sulphate)	: 25 µl
TEMED (N, N, N', N'-tetra methyl ethylene diamine)	: 5 µl

Sample buffer

Glycerol	: 20% (v/v)
Tris buffer, pH 6.8	: 0.125 M
Na_2 EDTA	: 5 mM
SDS	: 20% (w/v)
Bromophenol blue	: 0.1% (w/v)
2-mercaptoethanol	: 1% (v/v)

Electrode buffer

Glycine	: 4.320 g
Tris base	: 0.900 g
SDS	: 0.300 g

(Contents dissolved in 200 ml distilled water and the volume were made up to 300 ml with distilled water).

Staining solution

Coomassie brilliant blue R-250	: 200 mg
Methanol	: 40 ml
Glacial acetic acid	: 10 ml
Distilled water	: 50 ml

Destaining solution

Methanol	: 40 ml
Glacial acetic acid	: 10 ml
Distilled water	: 50 ml

ANNEXURE III

WESTERN BLOTTING

Anode buffer I

Tris base	: 18.17 g
Methanol	: 100 ml
Distilled water	: 350 ml
pH	: 10.4

Anode buffer II

Tris base	: 1.51 g
Methanol	: 100 ml
Distilled water	: 500 ml
pH	: 10.4

Cathode buffer

Tris base	: 1.51 g
6-amino hexanic acid	: 2.62 g
Methanol	: 100 ml
Distilled water	: 500 ml
pH	: 9.4

Tris buffered saline (TBS): 10x stock

100 mM Tris base	: 6.05 g
1.4 M NaCl	: 40.91 g
Distilled water	: 500 ml
pH	: 7.9

(Contents dissolved in 200 ml distilled water, pH adjusted with NaOH/HCl and the volume was made up to 500 ml with distilled water in all the buffers and stock).

Tris buffered saline-Tween (TBS-T): 1x stock

10x TBS stock	: 100 ml
Tween 20	: 0.5 ml
Distilled water	: 899.5 ml

AWARDS/PRIZES

P. R. VERMA award for Ph. D. Students presented by Indian Society of Mycology and Plant Pathology for the best paper presentation at Kakatiya University, Warangal, Andra Pradesh.

NARASHIMHAN award (Second Prize) presented by indian Phytopathological Society, Southern Zone for the best paper presentation at IIHR, Bangalore, Karnataka

ARTICLES PUBLISHED

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

V. Ramamoorthy, T. Raguchander and R. Samiyappan. 2001. Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluoresc*ens Pfl and *Fusarium oxysporum* f. sp. *lycopersici* in tomato. *Plant and Soil* (Accepted)

V. Ramamoorthy and R. Samiyappan. 2001. Induction of defense-related genes in Pseudomonas fluorescens treated chilli plants in response to infection by Colletotrichum capsici. *Journal of Mycology and Plant Pathology* (accepted)