DISEASE MANAGEMENT AND GROWTH IMPROVEMENT IN CHILLI AND TOMATO USING *TRICHODERMA* SPP. AND FLUORESCENT PSEUDOMONADS

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DECLARATION

I hereby declare that this thesis entitled '**Disease management and growth improvement in chilli and tomato using *Trichoderma* spp. and fluorescent pseudomonads**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled '**Disease management and growth improvement in chilli and tomato using *Trichoderma* spp. and fluorescent pseudomonads**' is a record of research work done independently by Ms. Rini, C.R. (2001-21-18) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Unless the LORD builds the house,
its builders labour in vain.
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Rini C.R.

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LIST OF ABBREVIATIONS

%	–	Per cent
°C	–	Degree celcius
µl	–	Microlitre
µm	–	Micrometre
@	–	At the rate of
CD	–	Critical difference
cfu	–	Colony forming units
cm	–	Centimetre
DAP	–	Days after planting
et al.	–	And others
Fig.	–	Figure
g	–	Gram
h	–	Hour
ha	–	Hectare
i.e.	–	That is
kg	–	Kilogram
L	–	Litre
min	–	Minute
ml	–	Millilitre
mm	–	Millimitre
nm	–	Nanometer
NS	–	Non-significant
ppm	–	Parts per million
rpm	–	Rotations per minute
Rs	–	Rupees
sp.	–	Species (singular)
spp.	–	Species (plural)
t	–	Tonnes
v/v	–	Volume/volume
viz.	–	Namely
w/w	–	Weight/weight

Introduction

1. INTRODUCTION

The importance of vegetables in human diet needs no overemphasis. They are reckoned as protective food for maintenance of good health and beneficial in acquiring resistance against some degenerative diseases. However the per capita consumption of vegetables in India is only 120 g, which is far below the minimum dietary requirement of 295 g/day/person as per the Recommended Dietary Allowances suggested by Indian Council of Medical Research.

Chilli and tomato are two important and popular vegetables of the family Solanaceae. Of these, chilli (*Capsicum annuum* L.) is used as an important spice cum vegetable crop valued for its pungency and flavour that imparts to food and beverages. It is grown throughout India for domestic as well as export markets. Tomato (*Lycopersicon esculentum* Mill) – another member of the family is also an important vegetable from nutritional as well as consumptional point of view. High nutritive value and multifarious uses in food industry elevate chilli and tomato to an inevitable position among vegetable crops.

Though chilli and tomato are very popular vegetables of Kerala, it is a disheartening fact that the area under cultivation in the state is very low. The main reason is the susceptibility of these crops to an array of biotic and abiotic stresses especially in the nursery stage. Many of the serious plant diseases are associated with soil borne plant pathogens, which cause root rots, crown or collar rots, damping offs, wilts, blights and fruit decay in the field. Among the major soil-borne plant pathogens, *Rhizoctonia solani* Kühn and *Fusarium* spp. are problematic world over, each having a wide host range involving all groups of plants.

Rhizoctonia root and stem rot caused by *R. solani* is a common early season disease and is most often found as a pre-emergence or post

emergence damping off of seedlings. Although it generally does not cause much damage to the mature plants, 60-70 per cent seedling losses have been reported (Bunker and Mathur, 2001). If stand losses are extensive. yield may be greatly reduced.

Wilt caused by *Fusarium* spp. is another major constraint in tomato and chilli cultivation and losses may go even upto 80 per cent in cases of severe infection (Padmodaya and Reddy, 1998). The fungus enters the root system, establishes and blocks the conducting tissues resulting in wilting of the plants and consequently a complete loss of the crop.

Although some chemicals are known to check *R. solani*, they are not always effective (Campbell, 1989). Exclusive dependence on fungicides for the control of diseases of vegetable crops is hazardous. uneconomical and not advisable due to very high residue and persistence problems, the risk of ground water pollution, death of non-target beneficial flora and evolution of fungicide resistant pathogen populations. As vegetables are the main dietary constituents, it becomes more important to find out alternative strategies to chemical control. The current concept of plant disease management involves integration of compatible, effective, economical and environmentally sound disease management methods such as biological means of control.

Biological control includes management of resident populations of organisms (the black box approach) and introduction of specific organisms (the silver bullet approach) to reduce disease. The black box approach involves the complex interactions of the suppressive soils. The silver bullet approach while simplistic, has yielded some practical solutions to plant disease problems and resulted in the development of several commercially available biopesticide products. During the past few years notable success has been made in the control of soil borne diseases through introduction of antagonistic fungi, *Trichoderma* spp. (Cook,

1993). Among the many bacterial antagonists, fluorescent pseudomonads have received much attention as an organism with great potential in biological control and plant growth promotion (Dube, 2001). Any consortium development of these biocontrol agents against *R. solani* and *Fusarium* spp. would be a matter of much interest at farmers level. Moreover there is greater demand globally for pesticide free produce which fetches premium prices.

Based on the above considerations, the present study was taken up to explore the feasibility of utilizing native species of *Trichoderma* and fluorescent pseudomonads in the management of Rhizoctonia rot and Fusarium wilt in chilli and tomato and to develop an effective formulation.

The study was taken up with the following specific objectives:

- Isolation of target pathogens (*R. solani* and *Fusarium* spp.)
- Isolation of native *Trichoderma* spp. and fluorescent pseudomonads from the rhizosphere and phyllosphere of chilli and tomato.
- *In vitro* screening of the antagonists against *R. solani* and *Fusarium* sp.
- Evaluation of the antagonists for disease suppression and growth improvement in greenhouse and under field conditions.
- Characterization and identification of promising isolates
- To study the mode of inhibition of the antagonists
- To study the multiplication and persistence of the antagonists in the rhizosphere soil
- Formulation and mass multiplication of the promising antagonists for commercial application.
- Economic analysis

Review of Literature

2. REVIEW OF LITERATURE

Among the major diseases of chilli and tomato, wilt caused by *Fusarium* and rot by *Rhizoctonia* causes considerable reduction in crop yield. Many attempts are being made for controlling these diseases and to improve the general growth of plants by suitable biocontrol agents like *Trichoderma* spp. and fluorescent pseudomonads.

2.1 BIOLOGICAL CONTROL

Cook (1987) defined biological control as the use of natural or modified organisms, genes or gene products to reduce the effects of undesirable organisms (pests) and to favour desirable organisms such as crops, trees, animals and beneficial insects and microorganisms. Dube (2001) describes biological control as a mysterious natural phenomenon which operates in disease suppressive soil and is not so easy to comprehend and imitate with success.

2.2 ETIOLOGY

2.2.1 *Rhizoctonia solani*

Rhizoctonia solani Kühn [Teleomorph. *Thanatephorus cucumeris* (Frank) Donk.] is one of the most widely distributed plant pathogens causing various maladies in crop plants such as seed decay, damping off, root and stem rot; canker, sheath blight and rot in monocots and dicots. It can remain in soil saprophytically for longer periods in the absence of host plants. It comprises of at least 12 anastomosis groups (AG-1 to AG-1 and AG-B1) of distinctive physiology and genetic composition (Ogoshi, 1987; Carling *et al.*, 1994). Isolates of *R. solani* are characterized by some shade of brown hyphal pigmentation, branching near distal septum of cells in young hyphae, constriction at the branching point, dolipore septum and multinucleate cells in young hyphae (Parmeter and Whitney, 1970). Branching of young hyphae at right angles, inclination of young hyphal

branches to the direction of growth and constriction at the point of union of main hyphae in *R. solani* were observed by Palo (1926). Colonies of AG-4 isolates (which comprises *R. solani* from *Lycopersicon esculentum*) were light brown or brown with a few, light brown, small sclerotia of size 0.3 – 1.5 mm which never showed conglomeration. Mean hyphal width is 7.4 ± 0.6 μm with 5.0 ± 1.0 numbers of nuclei per vegetative hyphal compartment (Sunder *et al.*, 2003).

R. solani is a seed as well as soil borne pathogen (Almeida *et al.*, 1980). Dry root-rot of chilli (*Capsicum annuum* and *Capsicum frutescens* L.) by *R. solani* Kühn was reported by Mathur *et al.* (1995) though its seed transmission was reported earlier (Chitkara *et al.*, 1986).

2.2.2 *Fusarium* spp.

The genus *Fusarium* described by Link in 1809 included the fungi with fusiform non septate spores, borne on a stroma. However, stroma or sporodochium are rarely developed on artificial media. Therefore, the presence of macroconidia with a foot cell is the most definite character. Microconidia are terminal and intercalary. Chlamydospores may be present or absent. Perithecial stages, if present belong to the Hyprocreales (Booth, 1971).

Fusarium oxysporum Schlecht. is a cosmopolitan fungus that exists in many pathogenic forms (Armstrong and Armstrong, 1981). It survives in soil in the form of chlamydospores and mycelia. Several pathogenic strains, designated as *Forma specialis*, exist within *F. oxysporum* and within *Forma specialis*, races have been found to exist. These *Forma specialis* cause vascular wilt (Haware, 1993).

F. solani (Mart) Sacc. is distributed world wide. It is a well known fungus causing root rot in temperate and tropical soils. In India, *F. solani* is reported to cause root rot in several crops (Haware, 1993). *F. solani* develops a blue to bluish brown discolouration on agar medium.

Microconidia develop abundantly on microconidiophores which are elongated and unbranched. Macroconidia are formed on short, multibranched conidiophores which are elongated and unbranched. Macroconidia are formed on short, multibranched conidiophores. Macroconidia are inequilaterally fusoid with several spores having the widest diameter in the penultimate cells (Booth, 1971).

2.3 BIOLOGICAL CONTROL OF SOIL-BORNE DISEASES

Antagonistic microorganisms, by their interactions with various soil-borne plant pathogens, play a major role in microbial equilibrium and serve as powerful agents for biological disease control (Baker, 1968; Papavizas and Lumsden, 1980; Cook, 1985). The interactions between biocontrol agents and plant pathogens have been studied extensively and the application of biocontrol agents in the protection of some commercially important crops is promising (Papavizas, 1985; Weller, 1988).

According to Adams (1990) the use of antagonistic organisms is a promising alternative to existing chemical management practices for soil and seed borne diseases. Several organisms such as *Trichoderma* spp. have been successfully used as biocontrol agents (Raguchander *et al.*, 1997), *Bacillus* spp. (Capper and Campbell, 1986) and *Pseudomonas* spp. (Vidhyasekaran and Muthamilan, 1995).

2.3.1 *Trichoderma* as a Potential Biocontrol Agent

Antagonists of fungal or bacterial origin have been studied well for the management of plant diseases. *Trichoderma* is commonly found in soil and decaying organic matter as a saprophytic fungus. Species of *Trichoderma* have been extensively tested and used as biocontrol agent against wider range of plant pathogens *viz.*, *Rhizoctonia*, *Fusarium*, *Sclerotium*, *Macrophomina*, *Sclerotinia*, *Pythium* spp. etc. (Papavizas, 1985; Chet, 1987; Hornby, 1990; Deacon, 1991).

The effectiveness of *T. viride* against *Rhizoctonia solani* in soil was first demonstrated by Weindling (1932). The antagonism of *T. harzianum* against *R. solani* under *in vitro* conditions was observed by various workers (Hadar *et al.*, 1979; Hazarika and Das, 1998; Mathew and Gupta, 1998). Kim and Roh (1987) also reported the antagonistic activity of *T. harzianum*, *T. viride* and *Gliocladium* spp. against *R. solani* under *in vitro* conditions. Wells *et al.* (1972) first demonstrated under field conditions that an isolate of *T. harzianum* had highly significant potential as biocontrol agent against *Sclerotium rolfsii*. The effectiveness of *T. harzianum* in the management of root rot caused by *R. solani* under glasshouse conditions was reported by Hadar *et al.* (1979), Elad *et al.* (1980) and Upmanyu *et al.* (2002), while that of *T. hamatum* has been reported by Chet and Baker (1981).

Hadar *et al.* (1979) observed that *T. harzianum* could directly attack *R. solani* and that a wheat bran culture of the fungus could control damping off of bean, tomato and egg plant seedlings caused by *R. solani*. The fermenter biomass production of *T. viride* applied as dust to the seed potatoes infected with sclerotia of *R. solani* before planting reduced disease incidence in field by 50 per cent (Beagle-Ristanio and Papavizas, 1985). Dipping of black scurf infected potato tuber in two per cent aqueous suspension of *T. viride* grown on wheat straw – wheat bran substrate significantly reduced black scurf incidence for three years in field experiments (Singh *et al.*, 1997).

Vyas and Mathur (2002) reported that *Trichoderma* spp. under *in vitro* conditions effectively inhibited the growth and sporulation of *Fusarium oxysporum* with the effect of volatile and non-volatile antibiotics produced by antagonists. *Trichoderma* spp. produce β-1,3-glucanase and chitinase enzymes. These enzymes have been indicated to play a major role in inhibiting the cell wall synthesis of *F. culmorum* (Witkowska and Maj, 2002). Gupta *et al.* (2002) reported that *T. viride* drastically inhibited the

growth of *F. oxysporum* f. sp. *ciceri, S. rolfsii* and *R. solani* under *in vitro* conditions.

Sivan and Chet (1982) have found the incorporation of *Trichoderma* spp. in field gave 60 to 83 per cent control of *Fusarium* wilt of tomato. Padmodaya and Reddy (1998) demonstrated that introduction of *T. viride* through seedling root dip and soil application recorded the highest seedling stand with least percentage of wilt incidence in tomato. Pretreatment of seeds and seedlings of tomato with *T. virens* resulted in reduction or complete suppression of *Fusarium* incited wilt (Narain and Behera, 2000). They also indicated that ten day old cell free culture filtrate of *T. virens* significantly inhibited the spore germination of *F. solani* causing wilt of tomato.

The research done by different workers on biological control of diseases using *Trichoderma* spp. in various crops is presented in the following table.

Table 1. Successful biological control of plant diseases employing *Trichoderma* spp.

Antagonist	Pathogen	Host	References
T. harzianum	*R. solani*	Bean, Tomato, Brinjal	Hadar *et al.* (1979)
T. harzianum	*R. solani* *S. rolfsii*	Bean, cotton, tomato	Elad *et al.* (1980)
T. viride	*R. solani*	Potato	Beagle-Ristanio and Papavizas (1985)
T. hamatum	*R. solani*	Sugarbeet	Lewis and Papavizas (1985)
T. harzianum *T. koningii*	*R. solani*	Rice	Zacharia (1990)
T. viride	*R. solani*	Potato	Singh *et al.* (1997)
T. virens	*R. solani*	Groundnut	Dubey (2000)
T. viride	*R. solani*	Potato, Rice	Rama *et al.* (2000)

T. harzianum	*R. solani*	Chickpea	Prasad and Rangeshwaran (2000a)
T. harzianum	*R. solani*	Chilli	Bunker and Mathur (2001)
G. virens	*R. solani*	French bean	Dubey (2002)
T. harzianum	*R. solani*	Groundnut	Devi and Reddy (2002)
T. harzianum	*R. solani* *F. oxysporum* f. sp. *ciceri*	Chickpea	Prasad *et al.* (2002c)
T. viride	*R. solani*	Cauliflower	Saikia and Gandhi (2003)
Gliocladium virens	*S. rolfsii* *R. solani* *F. oxysporum*	Chickpea	Tewari and Mukhopadhyay (2003)
Trichoderma spp.	*F. oxysporum* f. sp.*lycopersici*	Tomato	Sivan and Chet (1982)
T. viride *G. virens*	*F. solani* f. sp. *pisi*	Pea	Castejon and Oyarzun (1995)
T. viride	*F. udum*	Pigeonpea	Somasekhara *et al.* (1996)
T. harzianum	*F. udum*	Pigeonpea	Siddiqui and Mahmood (1996)
T. viride	*F. oxysporum* f. sp. *lycopersici*	Tomato	Padmodaya and Reddy (1998)
T. virens	*F. oxysporum* f. sp. *phaseoli*	French bean	Mukherjee and Tripathi (2000)
T. viride *T. harzianum*	*F. oxysporum* f. sp. *capsici*	Chilli	Naik *et al.* (2000)
T. virens	*F. solani*	Tomato	Narain and Behera (2000)
T. viride *T. virens*	*F. oxysporum* f. sp. *ciceri*	Chickpea	Jha and Singh (2000)
T. virens	*F. oxysporum* f. sp. *ciceri*	Chickpea	Tewari and Mukhopadhyay (2001)
T. harzianum	*F. oxysporum* f. sp. *gladioli*	Gladiolus	Shyama and Sunita (2003)
T. viride *T. virens*	*F. pallidoroseum*	Cowpea	Senthilkumar (2003)
T. harzianum	*F. oxysporum* f. sp. *ciceri*	Chickpea	Podder *et al.* (2004)

Biological seed treatment with suitable antagonists such as *T. harzianum* and *T. viride* either alone or in combination with sticker like carboxy methyl cellulose or $MgSO_4$ promises to be an effective and economic management practice against sheath blight of rice (Das and Hazarika, 2000). Similar suggestion was made by Tewari and Mukhopadhyay (2003) against chickpea root rot, collar rot and wilt pathogens (*R. solani*, *S. rolfsii*, *F. solani* and *F. oxysporum* f. sp. *ciceri*) in glasshouse condition. Increased seedling emergence, final plant stand and grain yield was observed when *Trichoderma* sp. was applied along with carboxymethyl cellulose in chickpea plants.

Abhimanyu *et al.* (2002) reported that combination of seed and soil treatment with *Trichoderma* was more effective in minimizing the web blight of groundnut than single application.

Seed treatment with slurry or water mixed spores of *T. viride* and *G. virens* gave the best protection to germinating seeds of urd/mung bean against *R. solani* (Dubey, 2003).

According to Pandey and Upadhyay (1999) seed coating with pure powder of *T. viride* and *T. harzianum*-c @ 0.1 per cent may be considered best for the management of *Fusarium* wilt of pigeon pea. *Trichoderma* spp. applied as seed treatment could establish well, reached high population densities, suppressed *R. solani* and *F. solani* and resulted in higher dry weight of the biomass of Guar (Jatav and Mathur, 2002).

Seedling disease and wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* was significantly reduced by *Trichoderma* spp. when these antagonists were used as seed dressers or soil inoculants or seedling root inoculants (Padmodaya and Reddy, 1998).

Prasad *et al.* (2002c) indicated that soil application of *T. harzianum* and *T. viride* one week before sowing was found to be more effective than

seed treatment in reducing wilt and wet rot of chickpea caused by *F. oxysporum* f. sp. *ciceri* and *R. solani*.

Parminder *et al.* (2002) reported that the combination of soil treatment with root dip gave better protection for all the antagonists *viz.*, *T. harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* in comparison to their single treatment as soil treatment or root dip.

2.3.2 Fluorescent Pseudomonads as Potential Biocontrol Agents

Bacteria are by far the most common type of soil microorganisms, possibly because they can grow rapidly and have the ability to utilize a wide range of substances as either carbon or nitrogen sources. The bacteria that provide some benefits to plants are found near, on or even within the roots of plants (Kloepper *et al.*, 1980a; Peer and Schippers, 1988). Among the many potential bacterial antagonists associated with the plant roots, the fluorescent pseudomonads have received much attention due to their abundance in plant rhizosphere and their ability to colonize roots of a wide range of crop plants. Fluorescent pseudomonads have been chosen because these bacteria are most widely studied for their potential to protect plants against diseases and to promote plant growth (Cook *et al.*, 1988; Munshi and Sokhi, 2000). In the past three decades, numerous strains of fluorescent pseudomonads have been isolated from the soil and plant roots by several workers (Rosales *et al.*, 1993; Rabindran and Vidhyasekharan, 1996). Several pathogens of tomato including *R. solani* and *F. oxysporum* f.sp. *lycopersici* were reported to be inhibited by fluorescent pseudomonads (Varshney and Chaube, 1999). Singh *et al.* (2002) reported that among the various antagonists tried maximum protection against root rot in chilli due to *R. solani* was observed with the application of fluorescent pseudomonads. Duijff *et al.* (1997) indicated that fluorescent pseudomonads suppressed Fusarium wilt of tomato by microbial antagonism and induced resistance and attributed

this to systemic acquired resistance associated with the synthesis and accumulation of PR proteins.

The following table exemplifies the research done by different workers on biological control of diseases using fluorescent pseudomonads in various crops.

Table 2. Successful biological control of plant diseases by application of fluorescent pseudomonads

Antagonist	Pathogen	Host	Reference
Pseudomonas fluorescens	*R. solani*	Cotton	Howell and Stipanovic (1979)
P. fluorescens	*R. solani*	Potato Tobacco Radish Cucumber	Kloepper and Schroth (1981)
P. fluorescens	*R. solani*	Rice	Mew and Rosales (1986)
P. fluorescens	*R. solani*	Pea nut	Savitry and Gnanamanickam (1987)
P. fluorescens	*R. solani*	Rice	Devi *et al.* (1989)
P. fluorescens	*R. solani*	Rice	Podile *et al.* (1990)
P. cepacia	*R. solani* *S. rolfsii* *Pythium ultimum*	Cotton Cucumber Melon	Fridlender *et al.* (1993)
P. fluorescens	*R. solani*	Rice	Rabindran (1994)
P. fluorescens	*R. solani*	Turf grass	Murakami *et al.* (1997)
P. fluorescens	*R. solani*	Cotton	Laha and Verma (1998)
P. putida	*R. solani*	Rice	Krishnamurthy and Gnanamanickam (1998)
P. fluorescens	*R. solani* *S. rolfsii* *F. oxysporum* f. sp. *lycopersici*	Tomato	Varshney and Chaube (1999)
P. fluorescens	*R. solani*	Amaranthus	Smitha (2000)

P. fluorescens	*R. solani*	Rice	Laha and Venketaraman (2001)
P. fluorescens	*R. solani*	Rice	Nandakumar *et al.* (2001a)
P. fluorescens	*R. solani*	Chilli	Singh *et al.* (2002)
P. fluorescens	*R. solani* f. sp. sasakii	Maize	Sivakumar and Sharma (2003)
P. fluorescens	*F. moniliforme*	Maize	Hebbar *et al.* (1992)
P. fluorescens	*F. oxysporum* sp. *lycopersici*	Tomato	Duijff *et al.* (1997)
P. fluorescens	*Fusarium udum*	Pigeonpea	Vidhyasekaran *et al.* (1997b)
P. fluorescens	*F. oxysporum* f. sp. *cubense*	Banana	Raguchander *et al.* (1997)
P. fluorescens	*F. oxysporum*	Radish	Marjan *et al.* (1999)
P. fluorescens strain NBR 11303	*F. oxysporum* f. sp. *ciceri*	Chickpea	Nautiya (2000)
P. fluorescens	*F. oxysporum* f. sp. *ciceri*	Chickpea	Pandey and Choube (2003)
P. fluorescens	*R. solani* *Fusarium oxysporum* *Sclerotinia sclerotiorum* *S. sclerollorum* *Macrophomina phaseolina* *Phomopsis vexans*	Brinjal Chilli Tomato	Singh *et al.* (2003)
P. fluorescens	*F. oxysporum* f. sp. *ciceri*	Chickpea	Ramandeep *et al.* (2003)

Soil bacteria having antagonistic property against soil borne plant pathogens are usually used for seed bacterization and seedling root dip (Harman, 1991; Mao *et al.*, 1997; Anith *et al.*, 1998). Seed treatment with *P. fluorescens* strain WCS 417 protected radish through induction of systemic resistance against the fungal root pathogen *F. oxysporum* f. sp. *raphani* (Hoffland *et al.*, 1996) and cucumber against *F. oxysporum* f. sp. *cucumerinum* (Liu *et al.*, 1995). The other methods of application include seedling root dip (Nayar, 1996; Maurhofer *et al.*, 1994), soil application

(Vidhyasekaran *et al.,* 1997a, b; Samiyappan, 1988) and foliar application (Chatterjee *et al.*, 1996; Mew and Rosales, 1986. Bioformulation of *P. fluorescens* strains when applied alone or in combination as seed treatment, root dipping, foliar spray and soil application significantly reduced the sheath blight incidence both under glasshouse and field conditions (Nandakumar *et al.*, 2000).

2.3.3 Combined Application of Antagonists and Synergestic Effect

Although the potential benefits in the application of a single biocontrol agent has been demonstrated in many studies, it may also partially account for the repeated inconsistent performance, because a single biocontrol agent is not likely to be active in all kinds of soil environments and agricultural ecosystems (Raupach and Kloepper, 1998). The possibility of introduction of combined treatments may provide an ever wider spectrum of disease suppression. The use of combined inoculation of fungal and bacterial antagonists have already been reported for effective disease management (Pierson and Weller, 1994; Duffy *et al.*, 1996; Xhang *et al.*, 1996).

Cook (1993) suggested repeated introduction of antagonists instead of single application while Loon *et al.* (1998) has emphasized combination of different treatments and different biological control agents for better performance.

Anith and Manomohandas (2001) reported that fungal and bacterial antagonists applied alone or in combination significantly reduced the mortality of rooted cuttings due to *Phytophthora capsici* in black pepper nursery.

Soil application of talc based formulation of *T. viride* and *P. fluorescens* either individually or in combination for reducing pre and post emergence damping off of tomato was recommended by Manoranjitham *et al.* (2001).

Bohra and Mathur (2002) obtained maximum biocontrol potentiality with combination of *Pseudomonas* and *Trichoderma* against root rot of soybean caused by *F. solani*.

Chaube and Sharma (2002) reported that *T. viride* / *T. harzianum* + *P. fluorescens* were found to be compatible and improved plant growth and suppressed seedling disease of cabbage, brinjal and tomato significantly when the treatments are combined and integrated with solarization.

Mishra *et al.* (2002) also suggested that use of mixtures of biocontrol agents would be a reliable means for management of plant diseases.

In contrary to the above findings Varshney *et al.* (2000) reported that fungal and bacterial bioagents could be incompatible and mask the effect of fungal bioagents if used together.

2.4 MECHANISM OF INHIBITION

2.4.1 *Trichoderma* spp.

An important strategy used for biological control of soil borne plant pathogens is to protect the infection court (for example, the rhizosphere and spermosphere of the crop) using biological control agents. There are three main mechanisms by which one microorganism may limit the growth of other microorganism- competition for resources, mycoparasitism and antibiosis.

Microorganisms compete with each other for carbon, nitrogen, iron and other nutrients. The competition for nutrients and space may, therefore play a vital role in antagonist - pathogens interaction. Lewis *et al.* (1990) has emphasized the role of competition in biological control of *R. solani* by *T. virens*. The correlation of the comparative growth rates, culture filtrate and volatile results suggested that the mechanism operative in antagonism were antibiosis and competition. The rapid growth might probably provide an advantage to *T. virens* over *F. oxysporum* f. sp.

gladioli in competing for nutrients and space utilization (Mishra *et al.*, 2004). The role of competition for nutrients and space in suppressing the population of *Fusarium* spp. has been explained by other researchers (Cugudda and Garibaldi, 1987; Widden and Scattolin, 1988).

Another mechanism of biocontrol is destructive mycoparasitism. This is parasitism of a pathogenic fungus by another fungus. It involves direct contact between the fungi resulting in death of the plant pathogens and nutrient absorption by the parasite. *Trichoderma* spp. though produce antibiotics and cell wall degrading enzymes, mainly act as mycoparasites on other fungi and bring about the disease control (Lewis and Papavizas, 1991). Complete suppression of *Rhizoctonia* by *Trichoderma* spp. by their overgrowth, coiling and disintegration has been reported by several workers (Weindling, 1932; Elad *et al.*, 1983; Prashanthi *et al.*, 2000).

Penetration and coiling of isolates of *Trichoderma* around *F. oxysporum* has been reported by Dennis and Webster (1971a).

Gokulapalan (1989) observed the parasitism of *T. viride* and *Penicillium oxalicum* on *R. solani* by coiling and penetration.

According to Chung and Choi (1990), the effect of *Trichoderma* spp. may be due to the direct attack and lysis of mycelium and sclerotial parasitisation of *R. bataticola*.

Dubey (2000) also reported stronger hyperparasitism of *G. virens* towards *R. solani*.

Antibiosis is defined as inhibition of the growth of one microorganism by another as a result of diffusion of an antibiotic. *G. virens* was reported to produce volatile substances inhibitory to many pathogens (Padmodaya and Reddy, 1998; Kumar and Dubey, 2001). Indra and Thirbuvanamala (2002) recorded inhibition of *Macrophomina phaseolina* with the volatile metabolites of *T. viride*, *T. pseudokoningii*, *T. longibrachiatum* and *T. harzianum*. Saikia and Gandhi (2003) reported

the production of a volatile inhibitory substance by *T. viride* against *R. solani*.

Upadhyay and Mukhopadhyay (1983) observed the inhibition of the growth of *S. rolfsii* through the non volatile substances produced by *T. harzianum*. Studies on the inhibitory effects of culture filtrates of *T. viride* against *R. solani* revealed a reduction in the mycelial weight of the pathogen which was directly correlated with the concentration of the filtrate (Khan and Hussain, 1991). Spore germination and radial growth of *F. solani* were inhibited by the cell free culture filtrate of *T. virens* (Mishra and Narain, 1992).

While studying the cytochemical aspects of chitin breakdown by parasitic action of a *Trichoderma* sp. on *F. oxysporum* f. sp. *radicis lycopersici*, rapid hydrolysis of wall bound chitin by extracellular chitinase of *Trichoderma* has been observed by Naik (2003).

However, a single mechanism of inhibition by the antagonist does not seem to be significant enough to provide the desired level of protection from the pathogens. Thus, there appears to be more than one mechanism that either individually or simultaneously may be involved in any fungus-fungus interaction.

The mechanism of inhibition by *T. harzianum* observed by Kumar and Dubey (2001) was a combination of competition for food and space, production of antibiotics and mycoparasitism, though the principle mechanism involved was mycoparasitism by coiling of antagonistic hyphae around the hyphae of pathogen and lysis. Lalitha and Raveesha (2002) also found the antagonistic action of *Trichoderma* sp. to be due to competition, hyperparasitism and antibiosis. According to Dubey and Patel (2001), antibiosis, lysis and mycoparasitism were the suggested mechanisms of biocontrol by *G. virens* against *R. solani*. Jatav and Mathur (2002) reported that *Trichoderma* spp. were found to produce volatile as well as nonvolatile antibiotics and also exhibit mycoparasitism.

Godwin-Egein and Arinzae (2001) in his study on antagonism between *T. harzianum* and *F. oxysporum* revealed that the cell wall degrading enzymes, chitinases and β-1,3-glucanases were produced by *T. harzianum* cultures and the mechanism of antagonism employed by *T. harzianum* were competition, lysis and hyperparasitism. But no antibiotic activity was detected towards *F. oxysporum* by *T. harzianum*.

2.4.2 Mechanism of Disease Suppression by Rhizobacteria

The mechanisms by which the bacterial biocontrol agents bring about the disease control include production of siderophores antifungal antibiotics, HCN, ammonia, competition for nutrients and space, colonization and induction of systemic resistance (Dowling and O'Gara, 1994). Diby *et al.* (2000) reported that fluorescent pseudomonads suppress *Phytophthora capsici*, the foot rot pathogen of black pepper through changing the microbial balance in the rhizosphere, by production of siderophores, volatile and non volatile inhibitory metabolites and Hydrogen cyanide (HCN).

Elad and Baker (1985) opined that disease suppression by fluorescent pseudomonads depends mainly on competition for nutrients and space and its ability to colonize rhizosphere (Scher *et al.*, 1988).

2.4.2.1 Production of Antibiotics

Fluorescent pseudomonads produce secondary metabolites like Phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), Oomycin-A, Pyocyamine, Pyoluteorin and Pyrrolnitrin with antibiotic activities many of which have been implicated in suppression of soil borne diseases (Hammer *et al.*, 1995; Maurhofer *et al.*, 1995; Thomashow and Weller, 1996).

Anith (1997) in his studies had drawn the conclusion that *Pseudomonas* strain EM 85 has got multiple antifungal traits like siderophore production and antifungal metabolite production. It was

reported that the antifungal property of the strain EM 85 is mainly due to the extracellular secretion of the toxic metabolite which makes the strain a better biocontrol agent. Mathre *et al.* (1999) observed the production of variety of secondary metaolites by fluorescent pseudomonads.

Gardener *et al.* (2000) also reported the production of antibiotics *viz.*, pyrrolnitrin, pyoverdine and 2,4-diacetylphloroglucinol by fluorescent pseudomonads against soil borne pathogens.

2.4.2.2 HCN Production

HCN is a representative of the class of volatile inhibitors which besides inhibiting activity of harmful organisms, can also reduce plant growth. HCN contributes to suppression of root rot of tobacco caused by *Thielaviopsis bassicola* by *P. fluorescens* strain CHAO (Voisard *et al.*, 1989). Role of hydrogen cyanide in the degradation of the toxin produced by the pathogen and subsequent suppression of the disease is reported (Borowitz *et al.*, 1992; Duffy and Defago, 1997).

2.4.2.3 Production of Lytic Enzymes

Some bacteria can parasitize fungi and kill them by secreting lytic enzymes like chitinases, β-1,3-glucanases, proteases and lipases. Fridlender *et al.* (1993) reported the production of β-1,3-glucanase by *P. cepacia.* Lim *et al.* (1991) have demonstrated that *P. stutzeri* and YPL-1 reduces disease caused by *F. solani*, mainly via laminarinase and chitinase activities. Lytic enzymes of *P. fluorescens* may participate in hydrolyzing the pathogen (*Sclerospora graminicola*) offering downy mildew resistance in susceptible cultivar as in resistant pearl millet (Poornima *et al.*, 2002).

2.4.2.4 Siderophore and Disease Suppression

The biocontrol and plant growth promoting activity of the PGPR reside in the siderophores (low molecular weight compounds with high affinity for Fe^{3+} ions) that they secrete in iron deficient soils. especially at alkaline pH regimes. Although iron is one of the most abundant metals in

the soil, its extremely high affinity for hydroxide, silicate and phosphate renders the water soluble fraction extremely low ($<10^{-17}$M). Rhizosphere is the region where there is a very high competition for nutrients among the heterogenous microflora present. Under these conditions some microorganisms secrete siderophores, which are iron binding legands and sequester iron in the ferric (Fe^{3+}) form with high affinity, making it less available to certain fungal pathogens and deleterious rhizobacteria and thus inhibiting their growth (Leong, 1986).

Siderophore production by *Pseudomonas* as mechanisms of biological control was first demonstrated by Kloepper *et al.* (1980a). It was reported that suppression of *Erwinia carotovora* by *Pseudomonas* strain B10 was mediated by siderophore produced by the biocontrol agent and the purified siderophore Pseudobactin was able to suppress the pathogen under *in vivo* conditions.

Siderophore mediated inihibition of chlamydospore germination of *Fusarium oxysporum* by fluorescent pseudomonads was demonstrated by Sneh *et al.* (1984).

Laha *et al.* (1992) observed the suppression of *R. solani* by *P. fluorescens* due to the production of siderophores.

Studies conducted by Kumar (2002) revealed that the presence of fluorescent *Pseudomonas* strain RRLJ 130 showed suppression of fusarial wilt in pigeon pea caused by *F. udum* but the siderophores isolated from this strain had no effect on disease suppression but enhanced the plant growth, whereas, the antibiotic extracted from this strain had a negative effect on growth but showed disease suppression against the fungus in field conditions.

2.4.2.5 Rhizobacteria Mediated Induced Systemic Resistance (ISR) in Plants

The disease suppressing activity of biocontrol bacteria can also operate through its action on plant by triggering plant mediated resistance mechanism called induced systemic resistance, which has been

successfully demonstrated in different crops (Liu *et al.*, 1995; Nayar, 1996; Ramamoorthy *et al.*, 2002; Sivakumar and Sharma, 2003).

2.5 PLANT GROWTH PROMOTION BY BACTERIA AND FUNGI

Plant growth promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Kloepper, 1994; Glick, 1995; Cleyet-Marcel *et al.*, 2001). Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoot growth. These organisms also provide protection against diseases by suppressing deleterious and pathogenic microorganisms (Baker and Schippers, 1987). Seed bacterization with these organisms has emerged as a powerful technology to enhance plant growth and yield besides providing protection against diseases (Dube, 1995).

Some plant growth promoting rhizobacteria like *P. fluorescens* and *P. aeruginosa* may promote plant growth by secreting hormones such as gibberelic acid (Weller, 1988). Kloepper *et al.* (1980b) attributed the enhancement of plant growth to yellow green siderophores produced by fluorescent pseudomonads. Seed or root inoculation with fluorescent pseudomonads are reported to improve growth and yield of potato (Burr *et al.*, 1978; Kloepper *et al.*, 1980c). Kumar and Dube (1992) proved the efficacy of seed bacterization with a fluorescent pseudomonad (Strain RBT 13) on the germination, growth and yield of chickpea and soybean and disease suppression in chickpea wilt caused by *F. oxysporum* f. sp. *ciceri*. Muthamilan (1994) observed increased growth rate of rice plants by seed treatment with *P. fluorescens*. Gupta *et al.* (1995) reported that inoculation of *P. fluorescens* increased seedling emergence rate, total dry weight and length of root and shoot in tomato plants. Izhar *et al.* (1999) observed that seed treatment with *P. fluorescens* enhanced the growth of cotton and reduced infection by *R. solani*. Talc based formulation of

antagonists significantly reduced the soil population of *Pythium* and increased the shoot length, root length and dry matter production of tomato seedlings (Manoranjitham *et al.*, 2000). *P. fluorescens* NBR 11303 strain was found to be highly inhibitory to *F. oxysporum* f. sp. *ciceri* on chickpea and also significantly increased the shoot length, dry weight and grain yield of chickpea (Nautiya, 2000).

Nandakumar *et al.* (2001b) used a mixture of PGPR strains against sheath blight in rice which, in addition to disease suppression promoted plant growth in terms of increased plant height, number of tillers and ultimately grain yield.

A combination of seed treatment, seedling root dipping and foliar spray with the formulation of *P. fluorescens* suppressed sheath blight disease and increased the root weight, shoot weight and also the biomass yield of rice (Heera *et al.*, 2002). Fluorescent *Pseudomonas* strain RRLJ – 130 isolated from pigeonpea rhizosphere when applied as seed inoculant produced a number of bioactive secondary metabolites and enhanced the percentage of seed germination, shoot height, root length, fresh and dry weight in chickpea and pigeonpea (Kumar, 2002).

Raji and Lekha (2003) reported that seed bacterization with talc based formulation of *P. fluorescens* enhanced the root length and height of rice plants.

The enhanced growth of tomato seedlings by *Trichoderma* treatment alone was reported by Kleifeld *et al.* (1983). Mechanisms like production of hormone like metabolites and release of nutrients from soil organic matter and making it available to plants by *Trichoderma* spp. were found to be involved in causing enhanced plant growth in different crops (Kleifeld and Chet, 1992; Altomare *et al.*, 1999; Prasad *et al.*, 2002c). Inbar *et al.* (1994) observed that *Trichoderma harzianum* treated seedlings were more developed and grew more vigorously with higher chlorophyll content and were more resistant to damping off caused by *Pythium* sp. and

R. solani. Singh *et al.* (1997) recorded that the growth of chickpea roots, shoots and leaves was enhanced in the presence of different fungal antagonists, with maximum growth in soil inoculated with *T. harzianum*. Increase in seedling and plant growth of chickpea due to soil application of *T. harzianum* prior to sowing was also reported by Sharma *et al.* (1999).

Combination of *T. viride* and *P. fluorescens* resulted in reduced damping off and also gave a better growth and highest dry matter content in tomato (Manoranjitham and Prakasam, 1999; Manoranjitham *et al.*, 2001). Similar findings were made by Ramanathan (1989) and Emayavaramban (1994) who worked on damping off disease of chilli. Karpagavalli and Ramabadran (2001) found that the application of *T. viride* and *T. harzianum* along with seed reduced the damping off incidence and improved the growth of root and shoot length and also dry matter production of tomato seedlings. Anith and Manomohandas (2001) reported that the combined application of *T. harzianum* and *Alcaligenes* also resulted in enhanced shoot weight in pepper. In addition to disease suppression, treatment with *Trichoderma harzianum* showed better yield in betelvine (Singh and Singh, 2002). Priyadarsini (2003) reported that the fungal antagonist *T. harzianum* was found successful in managing the disease and enhancing the growth of amaranthus.

2.6 SURVIVAL OF ANTAGONISTS IN SOIL

2.6.1 Fungal Antagonist

Survival and establishment of any biocontrol agent at the target site is important for successful and efficient biological control. The population density of *T. viride* and *T. harzianum* had been reported to increase about 10^4 and 10^3 folds respectively in natural soil during first three weeks when used as mycelial inoculum in wheat bran-sand culture (Lewis and Papavizas, 1984).

Saha and Sitansu Pan (1998) reported that in red laterite soil with pH 5.6, the population of *G. virens* increased upto 21 days and declined thereafter. The rate of decrease was more affected by moisture condition of the soil than that of temperature. The reason for the initial increase in population upto 21 days was attributed to germination of different spore forms and subsequent proliferation under natural condition with or without a food base. The cause of reduction in population in soil have been stated as 1) direct lysis of different spore forms 2) germination of spores followed by subsequent lysis of germtube in soil and a continued slow exhaustion of propagules in soil under natural conditions.

Anith and Manomohandas (2001) while studying the persistence of antagonistic microorganisms in the pepper rhizosphere observed that the population of introduced *T. harzianum* in the soil decreased to a level of 10^5 during the first 15 days, stabilized at the same level till 60^{th} day and declined thereafter.

2.6.2 Bacterial Antagonist

Successful root colonization holds the key to success in the life of antagonistic bacteria. Schroth and Hancock (1981) opined that seed treatment with beneficial rhizobacteria resulted in their colonization of root system at population ranging upto 10^5 per cm and persisted throughout the season. Wei *et al.* (1991) reported a slow but progressive decline in mean bacterial population with increase in time after planting. However, application of *P. aureofaciens* to potato seedlings by root dip method resulted in high population density of 10^4 to 10^5 cfu g^{-1} in both rhizosphere and roots upto eight weeks after transplanting (De la Cruz *et al.*, 1992). Peer and Schippers (1992) obtained 6 x 10^4 cfu g^{-1} of *Pseudomonas* sp. strain WOS 417r from the roots after 12 weeks of application. Wiehe and Hoflich (1995) recorded high population of *P. fluorescens* Ps IA12 during the vegetative plant development of legumes which declined with flowering. Nayar (1996) reported a rapid increase in population of

P. fluorescens upto 16 days after seed treatment and root dipping of rice plants, after which there was a decline. Seed treated *P. putida* survived on rice roots upto 60 days in sterile soil and upto 50 days in non sterile soil (Mageswari and Gnanamanickam, 1997). Yeole and Dube (2001) reported that the root colonization by introduced antibiotic resistant bacteria in chilli rhizosphere increased upto 21 days which thereafter showed a gradual decline. Anith and Manomohandas (2001) observed that the bacterial antagonist, *Alcaligenes* sp. strain AMB 8 when applied alone in the pepper rhizosphere declined to a population level of 10^6 during the first 30 days, stabilized at the same level till 60^{th} day and thereafter showed a declining trend. But when it is co-inoculated with *Trichoderma*, a similar trend was obtained till 60^{th} day and showed an increase in population thereafter.

2.7 MASS MULTIPLICATION AND FORMULATION OF ANTAGONISTS

2.7.1 *Trichoderma* spp.

Once a biocontrol agent is selected on the basis of its potentiality under *in vitro* and *in vivo* conditions, production of its biomass and suitable formulations become the major concern (Lumsden and Lewis, 1989). To improve the efficacy of microbial antagonists several formulations of biocontrol agents have been developed which are being produced commercially in many countries (Papavizas, 1985). A formulated biocontrol product with agricultural application should possess several desirable characteristics. These include ease in preparation and application, abundant viable propagules with good shelf life, sustained efficacy and acceptable cost (Lumsden *et al.*, 1995).

In India, Jeyarajan *et al.* (1994) developed a talc based formulation of *T. viride* for seed treatment to control *Macrophomina phaseolina* which incited diseases in pulses and oil seed crops. Prasad and Rangeshwaran (2000b) reported that kaolin and talc are ideal carriers of *T. harzianum* which retained more than 10^6 viable propagules per gram upto 90 days.

The minimum recommended population of fungal bioagents in any seed treatment formulation is more than 10^6 cfu g^{-1} (Jeyarajan and Angappan, 1998).

Prasad and Rangeshwaran (1999) indicated that modified wheat bran granule formulation of *Trichoderma* reduced saprophytic growth of *R. solani*. In addition to improving chickpea plant stand and reducing the saprophytic growth of *R. solani*, the application of granules of various biocontrol agents also resulted in population proliferation of biocontrol fungi. Shyama and Sunitha (2003) used wheat bran for mass multiplication of *T. harzianum* against *F. oxysporum* f. sp. *gladioli*.

The use of various grains and seeds *viz*., pregelatinized starch flour (Lewis *et al*., 1995), shelled maize cob (Gandhikumar *et al*., 2001), cassia seeds (Desai and Kulkarni, 2002) sorghum grains (Tewari and Mukhopadyay, 2003) for mass multiplication of *Trichoderma* spp. has been successfully exploited against various soil borne diseases in different crops.

The efficacy of jaggery in combination with soy or yeast, as a growth medium for the mass production of the biological control agent *T. harzianum* was determined and compared by Prasad *et al*. (2002a).

A simple method for achieving high cfu of *T. harzianum* on organic wastes for field application was developed by Sawant and Sawant (1996). Addition of *T. harzianum* into organic media like neem cake, coirpith, famyard manure (FYM) and decomposed coffee pulp supported high inoculum production and also served as nutrient additives to the crop (Saju *et al*., 2002). Gaur and Sharma (2002) suggested faecal pellets of goat, sheep and FYM as ideal substrates for mass multiplication of *Trichoderma*. Naik (2003) also reported that application of *Trichoderma* through FYM at seven days in advance helped in development and proliferation of bioagent against chilli wilt fungus. Tewari and Bhanu (2003) developed a suitable substrate combination by mixing various categories of substrates *viz*., agroindustrial cellulosic wastes, organic

manures and cereal brans along with chickpea flour and rice bran supplementation for higher conidial yield and mass multiplication of the biocontrol agent at low cost. Neem cake - cowdung mixture in the ratio 1 : 10 w/w is recommended as ideal food base for introduction of *Trichoderma* in to the soil and is well documented in Package of Practices Recommendations : Crops of Kerala Agricultural University, (KAU. 2002).

Works done by different workers on the shelf life of *Trichoderma* spp. on various substrates is presented in the following table.

Table 3. Shelf life of *Trichoderma* spp. on different substrates as reported by other workers

Trichoderma spp.	Substrate	Period	Reference
T. harzianum	Lignite and stillage	4 months	Jones *et al.* (1984)
T. koningii	Mycelial powder	3 years	Latunde - Dada (1993)
Trichoderma spp.	Talc	4 months	Sankar and Jayarajan (1996)
T. viride	Charcoal powder	3 months	Rama *et al.* (2000)
T. harzianum	Talc	3 months	Prasad and Rangeshwaran (2000b)
G. virens	CMC	3 months at room temperature; 6 months at refrigerated temperature (5°C)	Tewari and Mukhopadhyay (2001)
Trichoderma spp.	Talc	90 days	Gaur and Sharma (2002)
T. harzianum *T. viride*	Organic substrates	45 days	Sangle *et al.* (2002)
T. harzianum	Conidial powder Chlamydospore formulation	180 days 150 days	Prasad *et al.* (2002b)

2.7.2 Development of Formulation for Bacterial Antagonist

Cell suspension of the bacteria has been used for seed treatment, but the use of such suspension is impractical for large scale field application (Mew and Rosales, 1986; Capper and Higgins, 1993). The development of a powder formulation of the bacteria with a shelf life of more than eight months (Vidhyasekaran and Muthamilan, 1999) and an efficient method of application may be highly useful for large scale field application of the product and effective control of the diseases. Good control of sheath blight in rice was obtained when talc based products of fluorescent pseudomonads were applied as seed and root bacterisation and foliar spray (Rabindran, 1994; Muthamilan, 1994; Nayar, 1996). The talc based formulation containing antagonistic bacterium was effective against chickpea wilt, pigeon pea wilt, rice blast and rice sheath blight (Vidhyasekaran and Muthamilan, 1995; Rabindran and Vidhyasekaran, 1996; Vidhyasekaran *et al.*, 1997a, b). Sivakumar and Narayanaswamy (1998) reported that seed treatment with peat based formulation of *P. fluorescens* strain showed encouraging results for the control of sheath blight of rice.

According to Vidhyasekaran *et al.* (1997b) talc formulations were effective even after six months of storage, while peat formulations were effective upto 60 days of storage. The shelf life of vermiculite, lignite and kaolinite formulations was short and the unformulated bacterial suspensions could not be stored even for 10 days at which time their efficacy was completely lost. Chaluvaraju and Shetty (2002) suggested carboxymethyl cellulose as an efficient sticker for retaining the shelf life of the organisms in formulations.

A satisfactory control of banded leaf and sheath blight (*R. solani* f. sp. *sasakii* Exner) was obtained by seed treatment with peat based formulation of *P. fluorescens* (Sivakumar *et al.*, 2000). Smitha (2000) reported that a fluorescent pseudomonad (P_1) was found to survive well in talc even after

five months of storage. There was a significant reduction in the disease intensity when talc based formulation of P_1 was given as soil application followed by foliar spray. The shelf life studies indicated talc as the best carrier material for the survival of fluorescent *Pseudomonas* in which a population of 1.5 x 10^9 was recorded even after 75 days of storage (Heera *et al.*, 2002; Kaur and Thind, 2002) and a concentration of 0.8 per cent was most effective in reducing the seed mycoflora, increasing the germination and seedling vigour of rice (Sagar *et al.*, 2002).

Materials and Methods

3. MATERIALS AND METHODS

The present investigation entitled "Disease management and growth improvement in chilli and tomato using *Trichoderma* spp. and fluorescent pseudomonads" was carried out at College of Agriculture, Vellayani during the period 2001–2004. Chilli variety 'Jwalasakhi' and tomato variety 'Mukthi' were used in the study.

3.1 ISOLATION AND CHARACTERIZATION OF PATHOGENS ASSOCIATED WITH ROT/WILT DISEASES

3.1.1 Collection of Samples for Isolation

Plants showing typical symptoms of Rhizoctonia rot and Fusarium wilt were collected from the major vegetable growing tracts of Thiruvananthapuram, Ernakulam and Idukki districts.

3.1.2 Isolation of the Pathogen

Specimens of diseased samples collected from the naturally infected plants of chilli and tomato were first washed in tap water. For isolation of *Rhizoctonia* sp., infected stem regions were cut into small bits along with some healthy portion. For isolation of *Fusarium*, the roots of the wilted plants were used. The pieces were then surface sterilized in 0.1 per cent mercuric chloride ($HgCl_2$) solution for one minute and washed in three changes of sterile distilled water. The bits were then placed on potato dextrose agar (PDA) medium (Appendix I) in sterile petriplates under aseptic conditions and incubated at room temperature. After about 48 h, when the fungal growth was visible, mycelial bits were transferred to PDA slants, labelled and maintained by periodical subculturing.

The isolates thus obtained were purified by single hyphal tip method (Parmeter *et al.*, 1969) for *Rhizoctonia solani* and single spore isolation (Johnston and Booth, 1983) for *Fusarium* spp. and maintained by

periodical sub culturing on PDA slants for further studies. The pathogens were identified based on morphological characteristics.

3.1.3 Pathogenicity Test

The pathogenicity of the isolates was proved following Koch's postulates. The seedlings were grown in small plastic cups. Fifteen days old seedlings were inoculated with isolates of pathogen by placing the culture bits from five day old culture of the pathogen near the plant at soil level for *Rhizoctonia*. In the case of *Fusarium* culture bit was placed at root portions after giving pinpricks. Three replications were maintained. Based on the number of days taken for the initiation of disease after inoculation, virulence rating was done and the most virulent cultures from each pathogen (*Fusarium* and *Rhizoctonia*) were selected, from both the crops.

The selected cultures of *Fusarium* and *Rhizoctonia* were further cross inoculated between the crops, chilli and tomato and the best proven virulent culture from this were used for further studies.

3.1.4 Identification of the Pathogen

Morphological and cultural characteristics of the pathogens were studied by growing them on suitable media. *Rhizoctonia* was grown on potato dextrose agar medium. For morphological studies of *Fusarium*, potato sucrose agar (PSA) medium was used (Appendix I). Colony characters such as colour, (both upper and lower side of the medium), texture and growth pattern were recorded. Based on the morphological, cultural and microscopic characters, the selected isolates were identified and confirmed at Agharkar Research institute, Pune.

3.2 ISOLATION OF ANTAGONISTS

3.2.1 Isolation of Antagonistic Fungi

Trichoderma spp. were isolated from the rhizosphere of healthy chilli and tomato plants from various locations of important vegetable growing tracts and virgin forest soils of Kerala following standard dilution

plate technique (Johnson and Curl, 1972). Ten gram soil was weighed and mixed thoroughly in 100 ml sterile distilled water in 250 ml conical flasks to make a 10^{-1} dilution. From this it was serially diluted to get 10^{-4} dilution. One ml of this aliquot was transferred aseptically into petridishes and plated with Rose Bengal Agar (RBA) medium (Appendix I). Petridishes were then incubated at room temperature (28 ± 2°C for 48 – 72 h. Fungal colonies with white mycelium which later changed into green were examined and transferred to PDA slants. The fungal culture was purified by the single hyphal tip method (Parmeter *et al.*, 1969). The purified cultures were then stored for subsequent studies on antagonism.

3.2.2 Isolation of Fluorescent Pseudomonads

3.2.2.1 From Rhizosphere

The rhizosphere inhabiting fluorescent pseudomonads were isolated using dilution plate technique (Johnson and Curl, 1972) as described under 3.2.1 to get a dilution of 10^{-4}. The process was repeated to get 10^{-6} dilution. From this aliquot, one ml was transferred into sterile petriplates. King's Medium B (KMB) (Appendix I) was poured into this, well mixed and incubated for 24-48 h. Bacterial colony showing fluorescence was picked and maintained on KMB slant (King *et al.*, 1954).

3.2.2.2 From Phyllosphere

Endophytic bacteria from leaves were isolated following dilution plate technique. Ten grams of healthy disease free leaves of chilli and tomato were collected, surface sterilized in 0.1 per cent $HgCl_2$ solution, rinsed in three changes of sterile distilled water. It was then crushed in sterile mortar and pestle. The extract was then mixed in 100 ml sterile water to make 10^{-1} dilution. One ml of this was transferred to 99 ml of sterile water blank to make 10^{-3} dilution. One ml of this aliquot was transferred to sterile plates and plated with KMB agar. The plates were then incubated for 24-48 h. The fluorescent colony developed was picked up and maintained on KMB slant.

3.3 *IN VITRO* STUDIES ON PATHOGEN SUPPRESSION

3.3.1 Screening of *Trichoderma*

The dual culture technique outlined by Skidmore and Dickinson (1976) was followed for studying the antagonistic activity against *R. solani* and *Fusarium* sp. Agar discs of five mm dia. of *Trichoderma* and the test pathogen were placed five cm apart on opposite sides of the petriplates containing sterilized PDA. The plates were then incubated at 28 ± 2°C for observing the suppression process. Control consisted of petridishes inoculated with five mm culture discs of pathogen alone. The plates were observed from the third day of inoculation at 24 h interval for about seven days.

Colony development was observed and assessment on interactions between the organisms were made. Interaction types was assigned into four groups. Types of reactions are:

A - Homogenous – Free intermingling between pairing organisms

B - Overgrowth – Pathogen overgrown by the test fungus

C - Cessation of growth at the line of contact of the cultures

D - Aversion – A clear zone of inhibition was observed between the two organisms

The radial growth of the pathogen was also recorded. The percentage inhibition of mycelial growth was calculated using the formula (Vincent, 1927).

$$I = \frac{C - T}{C} \times 100$$ Where,

I = Inhibition of mycelial growth

C = Growth of pathogen in control plates (cm)

T = Growth of pathogen in dual culture (cm)

Among the best isolates obtained from the primary screening, two most potential isolates against each pathogen (*R. solani* and *Fusarium* sp.) were selected based on a secondary screening under *in vitro* conditions.

For this the pathogen was inoculated on sterilized PDA and incubated for seven days to obtain full growth of the pathogen. After the complete establishment of the pathogen, five mm culture discs of the antagonist was introduced over the pathogen. The plates were kept under constant observation at room temperature for nine days. Antagonism observed as sporulating *Trichoderma* over the pathogen was noted at regular intervals from fifth day onwards. Based on the observation, ratings were done using the modified Bell's (Bell *et al.*, 1982) scale (Class 1-5) developed as follows.

Class 1 – The antagonist completely overgrew the pathogen (100 per cent overgrowth)

Class 2 – The antagonist overgrew at least $^{3}/_{4}^{th}$ of pathogen surface (75 per cent overgrowth)

Class 3 – The antagonist colonized on half of the growth of the pathogen (50 per cent overgrowth)

Class 4 – The pathogen and the antagonist locked at the point of contact

Class 5 – The pathogen overgrew the mycoparasite

3.3.2 Screening of Antagonistic Bacteria

Isolates of *P. fluorescens* were obtained from the rhizosphere and phyllosphere of healthy chilli and tomato plants. Isolates from both the plants were pooled and tested for their antagonistic potential against *Rhizoctonia* and *Fusarium* sp. of chilli and tomato. Culture bits of five mm size of the pathogen were placed at the centre of the sterilized petriplates containing sterilized media. Both KMB and PDA were used in the screening process. The respective bacterial isolate was then streaked two cm away from the pathogen at the centre, in a triangular pattern. Each

treatment was replicated thrice. Inhibition zone was measured after five days of incubation.

3.3.3 Compatibility of the Selected Isolates under *in vitro* Condition

The selected isolates of *Trichoderma* and *Pseudomonas* sp. were evaluated for their compatibility under *in vitro* condition. The compatibility of *Trichoderma* spp. with *Pseudomonas* sp. was tested by a modified dual culture method. A five mm diameter fungal disc was cut out from a seven day old culture of *Trichoderma* sp. and dispensed in 2-3 ml sterile distilled water. One ml of this was plated in KMB agar so as to get a uniform fungal growth over the media. The selected *Pseudomonas* isolate was coinoculated at three points on the *Trichoderma* plated media. The culture plates were incubated and observed for the formation of inhibition zone produced by *Pseudomonas* against *Trichoderma*. Similarly to study the compatibility of *Pseudomonas* isolates with *Trichoderma*, the above method was reversed by plating the media seeded with *Pseudomonas*. Five mm disc of *Trichoderma* culture was cut out and coinoculated on the media and observed for the inhibition of bacteria by the growing fungus.

The experiment was repeated on PDA also and the observations recorded.

3.4 *IN VIVO* SCREENING OF BIOCONTROL AGENTS FOR DISEASE SUPPRESSION AND GROWTH PROMOTION

3.4.1 Greenhouse Condition

The antagonistic fungi and bacteria selected through the *in vitro* screening procedure were further evaluated for their efficiency in reducing disease and enhancing plant growth. Pot culture experiments in completely randomized design (CRD) was conducted at College of Agriculture. Vellayani with four replications in each treatment.

The experiments and treatments were as follows.

3.4.1.1 Experiment 1

Crop	:	Tomato (Mukthi)
Disease	:	Rhizoctonia rot
Period of study	:	July – October 2003

T_0 – Pathogen alone
T_1 – *Trichoderma* sp. (TR 17) + pathogen
T_2 – *Trichoderma* sp.(TR 20) + pathogen
T_3 – *Pseudomonas* sp. (P28) + pathogen
T_4 – *Pseudomonas* sp. (P51) + pathogen
T_5 – TR 17 + P28 + pathogen
T_6 – TR 20 + P28 + pathogen
T_7 – TR 17 + P51 + pathogen
T_8 – TR 20 + P51 + pathogen
T_9 – Copper oxychloride @ 0.3 % + pathogen
T_{10} – Untreated check

3.4.1.2 Experiment 2

Crop	:	Chilli (Jwalasakhi)
Disease	:	Rhizoctonia rot
Period of study	:	September – December, 2003.

Treatments were same as described under 3.4.1.1.

3.4.1.3 Experiment 3

Crop	:	Tomato (Mukthi)
Disease	:	Fusarium wilt
Period of study	:	February – May 2004

T_0 – Pathogen alone
T_1 – *Trichoderma* sp.(TR 22) + pathogen
T_2 – *Trichoderma* sp.(TR 19) + pathogen
T_3 – *Pseudomonas* sp.(P20) + pathogen
T_4 – *Pseudomonas* sp.(P28) + pathogen
T_5 – TR 22 + P20 + pathogen
T_6 – TR 19 + P20 + pathogen
T_7 – TR 22 + P28 + pathogen
T_8 – TR 19 + P28 + pathogen
T_9 – Carbendazim @ 0.1 % + pathogen
T_{10} – Untreated check

3.4.1.4 Experiment 4

Crop	:	Chilli (Jwalasakhi)
Disease	:	Fusarium wilt
Period of study	:	February – May 2004

Treatments were the same as in 3.4.1.3.

3.4.1.5 Preparation of Pathogen Inoculum

The pathogens *viz.*, *R. solani, Fusarium oxysporum* and *F. solani* for the above four experiments were mass multiplied on rice bran sand mixture using the modified method of Kousalya and Jeyarajan (1990). Rice bran was mixed with sand in the ratio 1:1 (w/w). The mixture was moistened with water sufficient enough to promote fungal growth. This mixture was taken in polypropylene bags, sealed properly and sterilized at 1.02 kg cm^{-2} for one hour. Actively growing culture disc of the pathogen was aseptically transferred into the bags and incubated at room temperature for 12 days to develop fungal growth.

3.4.1.6 Preparation of Inoculum of Antagonists

3.4.1.6.1 Talc Based Formulation of Bacterial Antagonist

Talc based formulations of selected fluorescent pseudomonad isolates were prepared following the method of Vidhyasekaran and

Muthamilan (1995). The isolates of fluorescent *Pseudomonas* sp. were multiplied in King's B broth. A loopful of each isolates was inoculated into the broth and incubated for 48 h at room temperature (28 ± 2°C). Hundred gram of talc was taken in polypropylene bags. One gram of carboxymethyl cellulose (CMC) was added to this, mixed, sealed and autoclaved for one hour at 1.02 kg cm^{-2}. To this, 40 ml of 48 h grown culture was added, mixed thoroughly and then stored at room temperature (Plate 1).

3.4.1.6. 2 Mass Multiplication of Trichoderma for Soil Application

The inoculum of the selected *Trichoderma* spp. were prepared in rice bran sand mixture (1 : 1 w/w) using the modified method of Kousalya and Jeyarajan (1990) as described under 3.4.5.

For mass introduction of the fungi in the soil, this inoculum was further multiplied in a non-axenic system *viz.*, cowdung, neem cake mixture as per the Package of Practices Recommendations : Crops (KAU, 2002), with a slight modification. Dry cowdung and neemcake were powdered and mixed in the ratio of 6 : 1 (v/v) to get a coarse texture and then moistened by sprinkling water. The rice bran based *Trichoderma* inoculum was then added @ 10 g kg^{-1} cowdung neemcake mixture. After thorough mixing, it was heaped, covered with ordinary newspaper and kept under shade for 10 days without any disturbance. After 10 days, the covering was removed and again mixed well and kept for another three days. The moisture level was maintained all through the period by sprinkling water. This organic matter enriched *Trichoderma* spp. were used for soil application in the *in vivo* experiments @ 100 g pot^{-1} (Plate 2).

3.4.1.7 Application of Antagonists

Fluorescent pseudomonads were applied as seed treatment, soil application and seedling root dip, while organic matter enriched *Trichoderma* spp. was applied as soil application.

Plate 1. Talc based formulation of fluorescent pseudomonad

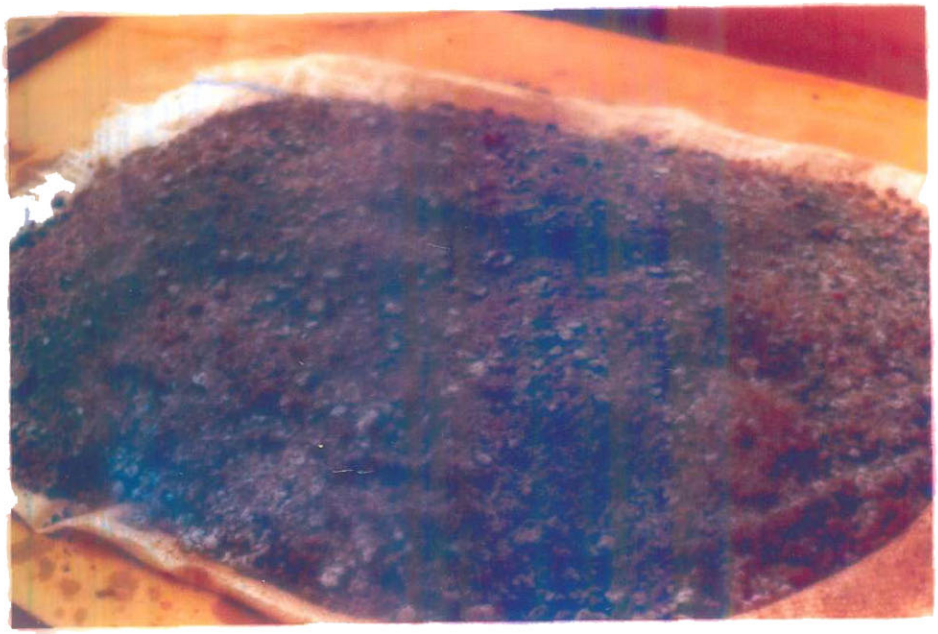


Plate 2. Organic matter based *Trichoderma* inoculum

3.4.1.7.1 Seed Treatment

Pseudomonas was streaked into sterile petriplates containing KMB agar medium and incubated for 48 h. A loopful of the bacterium was taken and suspended in sterile water to which one per cent CMC was added. Seeds were treated in this mixture, dried under shade and used for sowing in pots.

3.4.1.7.2 Soil Application

Earthen pots were filled with potting mixture (garden soil : FYM : sand – 2:1:1). The talc based *Pseudomonas* (2%) and organic matter based *Trichoderma* @ 100 g pot $^{-1}$ were applied into the respective pots and mixed thoroughly. The pots were watered regularly and kept for one week before transplanting the seedlings for better proliferation of the antagonists in the soil.

3.4.1.7.3 Seedling Root Dip

Fifteen day old seedlings were carefully uprooted and dipped in a thick slurry of *Pseudomonas* formulation for 30 min. These seedlings were then transplanted into the respective pots. Six seedlings were maintained in each pot.

3.4.1.8 Inoculation of Pathogen

Pathogens *viz*., *R. solani* and *Fusarium* spp. multiplied in rice bran sand mixture were applied at the root zone of the plants @ 5 g kg^{-1} soil after five days of seedling transplanting and thoroughly incorporated into the soil.

3.4.1.9 Application of Fungicides

Copper oxychloride (0.3 per cent) and carbendazim (0.1 per cent) was used as check against *R. solani* and *Fusarium* spp. respectively. The chemicals of each experiment was prepared by suspending the required quantity of fungicide in appropriate quantity of water. It was thoroughly

mixed and used for drenching @ 50 ml per seedling the root zone of the plants after 24 h of pathogen inoculation.

The plants were maintained as per the Package of Practices Recommendations : Crops (KAU, 2002) by giving timely application of manures, fertilizers etc.

*3.4.1.10 **Observations***

Biometric observations like shoot and root length, fresh and dry weight of shoot and root, yield, fresh and dry weight of fruits at 45 and 90 days after transplanting were taken.

Occurrence of the disease (rot and wilt) was recorded as percentage of disease incidence and its intensity was calculated using the formula suggested by James (1974).

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

The wilt intensity was calculated using score chart. The individual plants in each treatment were scored by using the disease scale of 0-3 (Chattopadhyay and Sen, 1996).

$$\text{Disease intensity (\%)} = \frac{\text{Sum total of scores}}{\text{Total number of plants}} \times \frac{100}{\text{Maximum grade}}$$

The score chart is as follows.

Grade	Description
0	Healthy plants with no infection
1	Yellowing of the lower leaves
2	Yellowing and marginal necrosis / defoliation
3	Drying and wilting / complete death of the plant

3.5 FIELD TRIALS

Based on the disease suppression and performance on plant growth under green house conditions, best biocontrol agent isolates (both fungal and bacterial) were selected and further evaluated under field conditions. The experiments were set in Randomized Block Design (RBD) at Instructional Farm, College of Agriculture, Vellayani. Four replications were maintained for each treatment.

The experiments and treatment details are as follows.

3.5.1 Experiment 1

Crop	:	Tomato (Mukthi)
Disease	:	Rhizoctonia rot
Period of study	:	November – February 2003-04
Plot size	:	3 m^2

T_0 – Pathogen alone
T_1 – *Trichoderma* sp.(TR 20) + pathogen
T_2 – *Pseudomonas* sp.(P28) + pathogen
T_3 – TR 20 + P28 + pathogen
T_4 – Copper oxychloride @ 0.3 % + pathogen
T_5 – Untreated control

3.5.2 Experiment 2

Crop	:	Chilli (Jwalasakhi)
Disease	:	Rhizoctonia rot
Period of study	:	February – May, 2004
Plot size	:	1.8 m^2

Different treatments applied were same as that detailed under 3.5.1.

3.5.3 Experiment 3

Crop	:	Tomato (Mukthi)
Disease	:	Fusarium wilt
Period of study	:	January - September 2004
Plot size	:	3 m^2

T_0 – Pathogen alone
T_1 – *Trichoderma* sp.(TR 22) + pathogen
T_2 – *Pseudomonas* sp.(P28) + pathogen
T_3 – TR22 + P28 + pathogen
T_4 – Carbendazim @ 0.1 % + pathogen
T_5 – Untreated control

3.5.4 Experiment 4

Crop	:	Chilli (Jwalasakhi)
Disease	:	Fusarium wilt
Period of study	:	June - September 2004
Plot size	:	1.8 m^2

Different treatments applied were same as that described under 3.5.3.

3.5.5 Preparation and Application of Pathogens and Antagonists' Inoculum

The respective pathogens and antagonists of each experiment were mass multiplied and inoculated as per the methods described under 3.4.1.5, 3.4.1.6 and 3.4.1.7.

3.5.6 Observations

Different observations including biometric characters, per cent disease incidence and disease intensity were recorded as per the methods described under 3.4.1.10.

3.6 CHARACTERIZATION AND IDENTIFICATION OF PROMISING ISOLATES

3.6.1 *Trichoderma* spp.

Morphological and cultural characteristics of different isolates of *Trichoderma* spp. were studied by growing them on PDA and Czepek's Dox Agar (CDA) medium (Appendix I). Five to seven day old culture was used for the study. Colony characters such as growth rate, growth pattern etc. were recorded. The mycelial and conidial characters were studied

under low and medium power objectives of compound microscope and the micromorphological characters were recorded.

3.6.2 *Fluorescent pseudomonads*

Pseudomonas sp. was identified by observing the morphological and physiological characters and by biochemical tests (Schaad, 1992). The following tests were conducted.

3.6.2.1 ***Gram Staining*** as per Harry and Paul (1975) (Appendix II)

3.6.2.2 ***Catalase Test*** (Harry and Paul, 1975)

Twenty four h old bacterial growth was scraped from a nutrient slant with a non-metallic instrument and suspended in a drop of three per cent hydrogen peroxide on a slide and examined immediately for bubbles.

3.6.2.3 ***Fluorescence Test*** (Schaad, 1992)

The King's medium B was used for detection of fluorescein, a fluorescent green or blue pigment. Colonies were examined for their fluorescence in ultra violet light with a wave length of 366 nm.

3.6.2.4 ***Growth at 4 °C and 41 °C***

This test was done in King's medium B broth. Five ml of KMB broth was dispensed into test tubes and autoclaved at 1.02 kg cm^{-2} for 20 min. The test isolate was inoculated into the broth and kept at 4°C and 41°C. The growth was measured by recording the absorbance of the inoculated broth at regular intervals.

3.6.2.5 ***Biochemical Tests*** (Schaad, 1992)

3.6.2.5.1 Levan Formation

Levan formation was detected on nutrient agar to which five per cent sucrose (w/v) was added. The presence of convex, white mucoid colonies after three to five days incubation was indicative of levan formation.

3.6.2.5.2 Gelatin Liquefaction

Nutrient gelatin medium with the following composition was used in this test.

Peptone	-	10 g
Beef extract	-	5 g
Gelatin	-	120 g
Distilled water	-	1000 ml
pH	-	7

The medium was dispensed in test tubes to a depth of 4 cm and sterilized at 1.02 kg cm^{-2} pressure for 20 min. The medium was checked for its sterile condition for two days. Forty eight hour old culture of the isolate was stab inoculated in the properly sterilized gelatin columns. The tubes were incubated and observed for liquefaction of the gelatin column at regular intervals for one month.

3.6.2.5.3 Carbon Source Utilization

Sugars *viz.*, Arabinose and Galactose and sugar alcohols *viz.*, Sorbitol and m-Inositol were employed as the carbon source in this test. The basal medium used in this test was Hayward's medium (Hayward, 1964).

Hayward's medium

Peptone	-	1 g
$NH_4H_2PO_4$	-	1 g
KCl	-	0.2 g
$MgSO_4.7H_2O$	-	0.2 g
Bromothymol blue	-	0.03 g
Agar-agar	-	3 g
Distilled water	-	1000 ml
pH	-	7.2

An aliquot of 90 ml each of the basal medium was dispensed in 250 ml conical flasks and autoclaved at 1.02 kg cm^{-2} pressure for 20 min. Ten per cent solution of sugars and sugar alcohols were prepared in sterile distilled water and filter sterilized. Ten ml each of the filtered solution was added to 90 ml aliquot of the melted medium and dispensed in sterilized test tubes to a depth of four cm. The medium was inoculated with the test isolate and incubated. Growth was compared to tubes without carbon source and observations were recorded as positive and negative. Change in colour of the medium from blue to yellow indicated positive utilization of the carbon compounds with the production of acids.

3.6.2.5.4 Utilization of Alcohol

Hayward's medium was used in this test also. Ten per cent ethanol was added to Hayward's medium to get ten per cent concentration and it was inoculated with the bacterium as described under 3.6.2.5.3.

3.6.2.5.5 Utilization of Propylene Glycol

Hayward's medium with the addition of propylene glycol to get a concentration of one per cent was used in this test.

3.7 ANALYSIS OF THE MECHANISM OF INHIBITION

3.7.1 *Trichoderma* spp.

The different mechanisms by which *Trichoderma* spp. inhibits *R. solani* were studied. For this the isolates TR17 and TR20 which performed better under *in vitro* and *in vivo* were selected. Similarly for *Fusarium* the *Trichoderma* isolates TR19 and TR22 were used.

3.7.1.1 Mycoparasitism

Hyphal interaction between the *Trichoderma* sp. and *R. solani* were observed under the microscope from the zone of interaction in dual culture plates (Dennis and Webster, 1971a). In 90 mm sterile petriplates sterile PDA was poured and allowed to solidify. Sterilized cellophane discs of 90 mm dia. were placed over this so as to lie flat on the medium

using a pair of sterile forceps. A five mm dia. agar disc containing the mycelium of the pathogen taken from an actively growing culture of the fungus was placed on one end of the petridish and five mm dia. agar disc of the test fungus was placed two cm away from it. The plates were incubated at 28 ± 2°C for 3-7 days. Direct observations were carried out after incubation period. Microscopic observations for hyphal interactions was also made by cutting one cm^2 portion of cellophane containing intermingling hyphal growth and mounting in lactophenol cotton blue. The different mechanisms of mycoparasitism such as overgrowth, hyphal coiling etc. exhibited by the *Trichoderma* spp. were recorded.

3.7.1.2 Inhibitory Metabolite Production

3.7.1.2.1 Volatile Antibiotics

Trichoderma spp. were evaluated for the production of volatile inhibitory substances in *in vitro* following the techniques described by Dennis and Webster (1971b). Five mm discs of each pathogen (*R. solani* and *Fusarium* sp.) was inoculated centrally in petriplates containing PDA medium. Sides of the petriplates were sealed and kept in polythene bags and incubated, at room temperature. After five days, the test pathogen was fresh inoculated on PDA in another set of sterilized petriplates and the lids of the antagonist inoculated plates were replaced by the culture of the pathogen. The plates were fixed with cellotape and reincubated. Three replications were maintained. Control consisted of the pathogen inoculated over the PDA dishes were also maintained. Growth of the pathogen was measured after five days of incubation and the percent inhibition was calculated using the formula:

$$I = \frac{C - T}{C} \times 100$$

Where, I = Inhibition of the mycelial growth of the pathogen

C = Growth of the pathogen in control plates

T = Growth of the pathogen in the treatment plates

3.7.1.2.2 Non-volatile / Diffusible Antibiotics

The effect of non-volatile substances produced by the *Trichoderma* isolates was determined by assaying the culture filtrate of the *Trichoderma* spp. against the pathogen (Anith, 1997). To prepare the culture filtrate of *Trichoderma* spp., the fungus was grown in potato dextrose broth for 15 days at room temperature. The mycelial mat of the fungus was removed from the broth and it was centrifuged at 10000 rpm for 15 min. The supernatent was collected and concentrated to $1/10^{th}$ of its original volume. This was then passed through bacteria proof filter (Millipore filter 0.45 μ) and was used for the studies.

Sterile molten PDA was poured into sterile petriplates. Wells of 10 mm dia. were made towards the edge in PDA plates by removing agar discs from the medium using a cork borer. The wells were then partially sealed with molten soft agar. When the agar solidified 100 μl concentrated culture filtrate was poured carefully into the well using a micropipette and allowed to percolate. Bioassay against the pathogen was performed by co-inoculating an agar disc of the pathogen on the centre of the plate. Growth of the pathogen was observed after two days of incubation.

3.7.2 *Pseudomonas* sp.

3.7.2.1 Inhibitory Metabolite Production

3.7.2.1.1 Volatile Antibiotics

The efficacy of the *Pseudomonas* sp. in inhibiting the pathogens (*R. solani* and *Fusarium* sp.) through the production of volatile antibiotics were evaluated (Dennis and Webster 1971b). Bacteria was streaked on KMB agar, incubated for 48 h at 28 ± 2°C and the lids were replaced by freshly inoculated pathogen as described under 3.7.1.2.1.

3.7.2.1.2 Non-volatile Antibiotics

A loopful of 24 h grown *Pseudomonas* was added to KMB broth and incubated for seven days. The culture filtrate was extracted and bioassay was done against the pathogen as per the procedure detailed under 3.7.1.2.2.

3.7.2.1.3 Production of HCN

Production of HCN was determined by a modified procedure described by Millar and Higgins (1970). *P. fluorescens* cultured on KMB agar supplemented with glycine @ 4.4 g L^{-1}. Filter paper discs soaked in picric acid solution (picric acid - 2.5 g and Na_2CO_3 - 12.5 g in one L water) were placed in the lid of each petridish. Dishes were sealed with parafilm and incubated for four days at room temperature (28 ± 2°C). Production of cyanide was determined by a colour shift from yellow to orange in the filter paper.

3.7.2.2 Siderophore Formation

Siderophore synthesis of the selected isolate was determined by dual culturing the antagonist and the pathogen in low iron media and observed for inhibition. Dual culture on media containing $FeCl_3$ (0.01 g L^{-1}) served as the check.

Low iron media (LIM)

Glucose	–	3.64 g
Casamino acids (10 %)	–	10 ml
$MgSO_4$. $7H_2O$ (10%)	–	2 ml
$CaCl_2$. 2 H_2O	–	0.5 ml
K_2 HPO_4	–	0.058 g
NaCl	–	0.2 g
Micronutrient solution*	–	1 ml
Water (double distilled)	–	1 L

***Micronutrient solution**

$Na_2 MoO_4$	–	5 mg
$ZnSO_4 . 7 H_2O$	–	4 mg
H_3BO_3	–	6.25 mg
$MnSO_4 . 4 H_2O$	–	5 mg
$CuSO_4 . 5 H_2O$	–	0.5 mg
Distilled water	–	100 ml

The solution was sterilized by autoclaving.

3.7.3 Genetic Analysis of the Inhibitory Characters

Genetic analysis of the inhibitory character of the best pseudomonad isolate (P28), which can suppress the rot as well as wilt diseases in chilli and tomato was carried out. The plasmid curing was done using Sodium dodecyl sulphate (SDS) inorder to ascertain whether the inhibitory property of *Pseudomonas* is a plasmid borne or a chromosome based character.

King's Medium B broth was prepared and 100 ml of the same was dispensed in five, 250 ml conical flasks. SDS required to give final concentration of 0.8, 1.0, 1.2 and 1.4 per cent was added in the flasks and sterilized. The broth was uniformly inoculated with one ml of overnight grown fluorescent pseudomonad isolate. One flask without SDS was maintained as control. The multiplication of the cells was monitored at two h intervals by measuring the optical density at 570 nm for six h using a spectrophotometer.

The SDS concentration which showed an increase in the optical density and then a sudden decrease in OD values were marked for further testing of the colonies for inhibitory properties. The SDS concentrations of 0.8, 1.0, 1.2 and 1.4 per cent at six h were serially diluted and plated on KMB agar and incubated for 48 h. Fifty colonies from 1 and 1.2 per cent

concentration were marked and tested against *Fusarium* sp. The pathogen was placed at the centre of PDA plates and marked colonies were spotted at eight points in each plate and the colonies and spots were marked properly. The plates were incubated for five days and growth of the pathogen was monitored. The pathogen overgrown colonies were considered as the one lost the inhibitory property. The pathogen growing upto the spots without formation of inhibition zone as colonies partially lost the inhibitory character and the one showing the inhibition zone as colonies retaining the inhibitory property.

3.8 MULTIPLICATION AND PERSISTENCE OF ANTAGONISTS IN THE SOIL

The isolates which were found effective in the field trials were further studied for their multiplication and rhizosphere colonization using appropriate markers.

3.8.1 Intrinsic Antibiotic Resistance of *P. fluorescens*

The intrinsic antibiotic resistance of the *Pseudomonas* isolate was tested by growing the organism on KMB agar containing different concentration of antibiotics. The commercially available antibiotics for human use were tried for the study. Antibiotics *viz.*, Ampicillin, Erythromycin, Gentamycin, Streptomycin and Tetracycline at different concentrations *viz.*, 5, 10, 50, 100, 150 and 200 ppm were tested. Stock solution of the antibiotics were prepared separately in sterile distilled water and the required quantity of the stock solution was added for each antibiotic in separate KMB agar just before plating. After solidification, the isolate was spot applied on the medium and incubated for 48 h. Medium without antibiotic served as control. The growth of *Pseudomonas* was recorded as '+' (good), 'F' (faint) and '-' (no growth). The concentration of antibiotic to which the *Pseudomonas* showed natural resistance were selected and used as marker for the population estimation.

3.8.2 Population Estimation of the Antagonists in the Rhizosphere Soil

Chilli and tomato plants were raised in pots containing potting mixture. *Trichoderma* sp. and *P. fluorescence* were applied in the soil as detailed under 3.4.7. The plants were maintained in open space under normal sunlight. The population of the introduced *Trichoderma* was estimated using *Trichoderma* selective medium (Elad and Chet, 1983) (Appendix I) at every seven days interval by dilution plate technique. *Pseudomonas* was estimated on KMB agar medium with Tetracycline (5 ppm) and Streptomycin (10 ppm) as markers at weekly interval. The plates were incubated at room temperature for 72 h and then the colonies were counted and recorded.

3.9 FORMULATION AND MASS MULTIPLICATION OF THE ANTAGONISTS

3.9.1 Substrate Development for Fungal Antagonist

For the mass multiplication of the selected *Trichoderma* spp., different substrates *viz.*, cowdung, neem cake, coirpith, sorghum grains, sawdust, rice bran, jaggery and wheat flour in different proportions were tried. The different combinations and the various treatments tested were given below.

T_1 – Cowdung + neem cake (1 : 1)
T_2 – Coirpith
T_3 – Coirpith + neem cake (1 : 1)
T_4 – Sorghum grains (pre-boiled)
T_5 – Sawdust
T_6 – Rice bran
T_7 – Cowdung + neem cake + jaggery (3 %)
T_8 – Cowdung + neem cake + jaggery + wheat flour (10 %)

The substrates were moistened by adding sufficient quantity of water to promote fungal growth. These were taken in 150 ml conical flasks and sterilized at 1.02 kg cm^{-2} for one hour. One cm^2 of actively growing culture disc of *Trichoderma* sp. was aseptically transferred into

the flasks and incubated at room temperature for 10 days to develop fungal growth. Visual observations on the fungal growth were made at constant intervals. The propagule density was estimated on PDA supplemented with Rosebengal @ 25 mg L^{-1} at 10^{th} day of inoculation by dilution plate technique. The number of fungal colonies were counted after two days of incubation.

3.9.2 Effect of Different Moisture Levels on Spore Viability of *Trichoderma* spp.

On the basis of fungal growth, the best three substrates were further evaluated at different moisture levels for their efficacy in sustaining the viable population. The selected substrates and the different treatments were as follows.

T_1 – Sorghum grains (pre boiled)

T_2 – Coirpith + neem cake (1 : 1) – 35 % moisture (original moisture)

T_3 – Coirpith + neem cake (1 : 1) – 45 % moisture

T_4 – Coirpith + neem cake (1 : 1) – 55 % moisture

T_5 – Cowdung + neem cake (1 : 1) + wheat flour (10 %) - (20 % moisture)

T_6 – Cowdung + neem cake (1 : 1) + wheat flour (10 %) - (40 % moisture)

T_7 – Cowdung + neem cake (1 : 1) + wheat flour (10 %) – (60 % moisture)

Respective moisture percentages were given to the different substrates taken in conical flasks and sterilized at 1.02 kg cm^{-2} for one h. These were then inoculated with one cm^2 discs of actively growing culture of *Trichoderma* and incubated. Visual observations on growth pattern as well as the population count were made at regular intervals. PDA - Rosebengal medium was employed for population estimation by dilution plate technique.

3.9.3 Formulation of Bacterial Antagonist

With the objective of standardizing the moisture level to get maximum shelf life of the selected *Pseudomonas* an experiment was set in the laboratory with four different carrier materials with one per cent CMC at three different moisture levels each. The population of the *Pseudomonas* in different treatments was estimated in KMB agar medium at every 15 days interval following dilution plate technique. The different carrier materials and the treatments were as follows.

T_1 – Talc (30 % moisture)
T_2 – Lignite (30 % moisture)
T_3 – Vermiculite (30 % moisture)
T_4 – $CaCO_3$ (30 % moisture)
T_5 – Talc (40 % moisture)
T_6 – Lignite (40 % moisture)
T_7 – Vermiculite (40 % moisture)
T_8 – $CaCO_3$ (40 % moisture)
T_9 – Talc (50 % moisture)
T_{10} – Lignite (50 % moisture)
T_{11} – Vermiculite (50 % moisture)
T_{12} – $CaCO_3$ (50 % moisture)

310 ECONOMIC ANALYSIS

Economics of crop production was worked out on the basis of additional input and additional income.

3.11 STATISTICAL ANALYSIS

The data generated from the different experiments were subjected to Analysis of Variance (ANOVA) technique after appropriate transformation wherever needed (Snedecor and Cochran, 1967).

Results

4. RESULTS

4.1 ISOLATION AND CHARACTERIZATION OF PATHOGENS ASSOCIATED WITH ROT / WILT DISEASES IN CHILLI AND TOMATO

4.1.1 Collection of Samples for Isolation

Plants showing typical symptoms of Rhizoctonia rot and Fusarium wilt were collected from the major vegetable growing tracts of Thiruvananthapuram, Ernakulam and Idukki districts. The list of pathogens isolated from the disease samples and their locations are presented in Table 4.

4.1.2 Isolation of the Pathogen

The pathogens *viz.*, *Rhizoctonia solani* and *Fusarium* spp. were isolated from the naturally infected host plants collected from farmer's fields. *Rhizoctonia* infections are characterized by a shrunken, reddish brown lesion or canker developing at or near the soils level. Under favourable conditions the decayed area can continue to expand into the stem and roots causing girdling of the hypocotyls or loss of roots. Wirestem is the most common and destructive phase of *Rhizoctonia* disease. The stem above and below the soil level shrivels and darkens, becoming tough and woody or wiry. Affected seedlings quickly wilt, topple over and die. Damped off plants commonly occur in circular patches in the plant beds or along sections of rows (Plate 3 and 4).

The first indication of the wilt disease caused by *Fusarium* sp. in the field is a yellowing and drooping of the lower leaves. In tomato the symptom often occurs on one side of the plant or on one shoot. As the disease progresses, successive leaves become yellow, wilt and die. The plants become stunted, enter into permanent wilting and dies with brown leaves clinging to the stem (Plate 5 and 6). When the lower portion of the stem is cut lengthwise, brown streaking is evident under the surface of the main stem.

Table 4. Pathogen isolates from different locations

Pathogen	Crop	Isolates	Locations	District	Growth characters
Rhizoctonia sp.	Tomato	R1t	Vellayani	Thiruvananthapuram	Light brown spreading mycelia with micro sclerotia. At times on subculturing it forms white thick wrinkled mycelial mat.
		R2t	Udumalpettah	Idukki	Dark brown mycelia, fine dust like sclerotia, spreading mycelium less found
		R3t	Marayoor	Idukki	Brown mycelia with fine sclerotia
	Chilli	R1c	Marayoor	Idukki	Light brown mycelia with minute sclerotia
		R2c	Marayoor	Idukki	Light brown mycelia with minute sclerotia
		R3c	Vellayani	Thiruvananthapuram	Dark mycelium with soft large sclerotia with honey dew which mainly concentrated at the periphery of the petriplates
Fusarium sp.	Tomato	F1t	Udumalpettah	Idukki	White mycelia with a purplish tinge, wet growth, white to purplish sclerotial bodies (small hard mass) found in old cultures
		F2t	Udumalpettah	Idukki	Same as above
		F3t	Udumalpettah	Idukki	Same as above
	Chilli	F1c	Kalliyoor	Thiruvananthapuram	Off-white mycelia formed in concentric rings, reddish brown / blue pigmentation in medium
		F2c	Muvattupuzha	Ernakulam	Fluffy mycelium with off-white colour formed in concentric rings, reverse medium yellow in colour
		F3c	Kazhakoottam	Thiruvananthapuram	Fluffy white mycelia, uniform growth cover the media, no colour change
		F4c	Udumalpettah	Idukki	Fluffy mycelia, dirty white / cream colour, reverse medium yellowish cream
		F5c	Marayoor	Idukki	Fluffy mycelium, reverse side no change in colour
		F6c	Palappoor	Thiruvananthapuram	Velvetty growth with pink colouration
		F7c	Vellayani	Thiruvananthapuram	White velvetty growth, no colour change

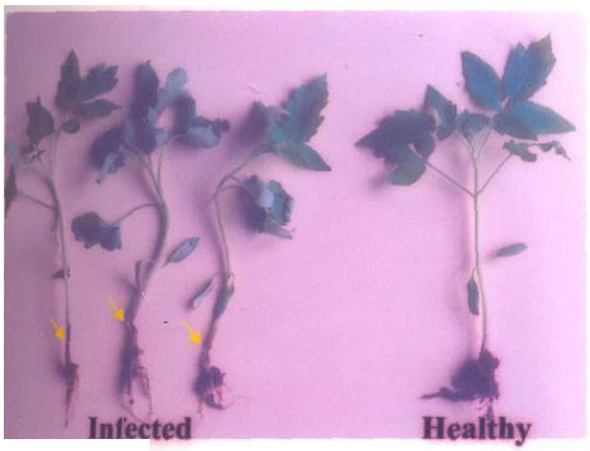


Plate 3. Symptoms of Rhizoctonia rot on tomato seedlings

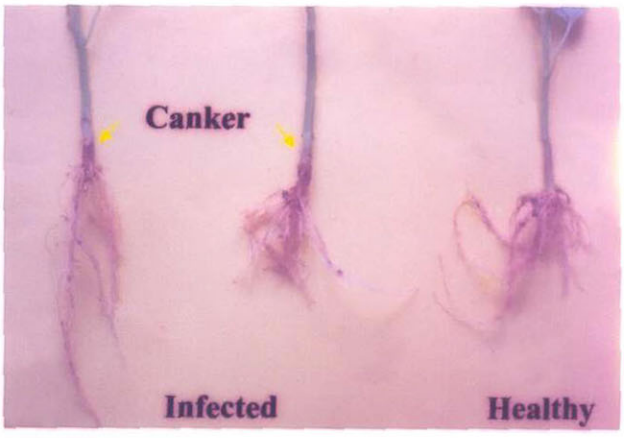


Plate 4. Symptoms of Rhizoctonia rot on chilli seedlings

Plate 5. Symptoms of Fusarium wilt in tomato plant

Plate 6. Symptoms of Fusarium wilt in chilli plant

4.1.3 Pathogenicity Test

Pathogenicity and comparative virulence of the different isolates of both *Rhizoctonia* and *Fusarium* were proved following Koch's postulates. The different isolates varied with respect to the time taken for symptom development (Table 5). Based on the time taken for the development of symptom the pathogens were grouped into four categories *viz.*, highly virulent, virulent, moderately virulent and avirulent.

4.1.3.1 R. solani

The highly virulent pathogen developed symptoms within three days of artificial inoculation while virulent isolates took three to seven days, moderately virulent above seven days and avirulent did not produce any symptoms. *Rhizoctonia* isolate R1t of tomato obtained from Vellayani was highly virulent and it took only two days for symptom expression. The isolate R2t was moderately virulent while R3t was avirulent to tomato seedlings. In the case of chilli, R2c was the most virulent one whereas the other two isolates *viz.*, R1c and R2c did not cause any infection.

4.1.3.2 Fusarium spp.

The isolates which initiated the symptoms within five days were grouped in highly virulent, six to ten days in virulent, 10–15 days in moderately virulent category and those which did not produce any symptoms were put under avirulent group. F1t isolate of *Fusarium* sp. initiated the wilt symptoms within five days was highly virulent when compared to the other isolates of *Fusarium* in tomato. Inoculation of chilli with *Fusarium* isolates obtained from the same crop showed that the isolate F4c was the best one which initiated the infection within four days of inoculation. The other isolates, except F3c and F7c which were avirulent, exhibited virulence and started infection within a range of five to ten days.

On the basis of the virulence studies R1t, R3c, F1t and F4c isolates were selected for further studies.

4.1.4 Cross Inoculation of the Pathogens between the Crops

Infectivity of the isolates R1t and R3c (*Rhizoctonia* from tomato and chilli) and F1t and F4c (*Fusarium* from tomato and chilli) were further studied by cross inoculating them between the crops. R1t when inoculated on tomato and chilli initiated the disease within three days and four days respectively, whereas R3c inoculation on tomato plants did not produce the disease. The infective capacity of R3c to start the infection even on its host (chilli) was less when compared to R1t (Table 6).

F1t and F4c failed to produce the wilt symptoms on the crop plants other than their hosts on cross inoculation.

Hence R1t, which was highly virulent in producing wilt symptoms on both the crops was selected for further studies. Similarly F1t and F4c were selected as the most virulent *Fusarium* isolates for tomato and chilli respectively.

4.1.5 Identification of Pathogen

4.1.5.1 R. solani (isolate R1t)

The pathogen had light brown to brownish mycelium which produced a few, light brown, small round sclerotia of size 0.3–1.5 mm which never showed conglomeration. Hyphal thickness ranged from 6.8–8 µm with 4-6 numbers of nuclei per vegetative hyphal compartment. Based on these microscopic examination of the morphological characters the pathogen was identified as *R. solani* (Sunder *et al.*, 2003).

Table 5. Comparative virulence of pathogen isolates

Isolates	Crop	Time taken for symptom development after inoculation (days)	Virulence rating
R1t	Tomato	2	Highly virulent
R2t	"	4	Virulent
R3t	"	-	Avirulent
R1c	Chilli	-	Avirulent
R2c	"	-	Avirulent
R3c	"	10	Moderately virulent
F1t	Tomato	5	Highly virulent
F2t	"	7	Virulent
F3t	"	-	Avirulent
F1c	Chilli	7	Virulent
F2c	"	6	Virulent
F3c	"	-	Avirulent
F4c	"	4	Highly virulent
F5c	"	7	Virulent
F6c	"	10	Virulent
F7c	"	-	Avirulent

Table 6 Infectivity of the pathogens on cross inoculation

Pathogen	Number of days taken to initiate infection	
	Tomato	Chilli
R1t	3	4
R3c	-	10
F1t	5	-
F4c	-	4

4.1.5.2 Fusarium spp.

Parameters	F1t (from tomato)	F_4c (from chilli)
Growth	Fast growing, reaching 4.5 cm dia. in four days at room temperature	Fast growing, reaching 4 cm dia. in four days at room temperature
Colony morphology	Aerial mycelium sparse to abundant and floccose becoming felted and sometimes moist	Aerial mycelium little but abundant cream coloured conidial mass formed in sporodochia
Colour	Usually with a purple or violet tinge, more intense at the stromatic agar surface	Dirty white / cream; reverse side of medium yellowish cream
Macroconidia		
Shape	Fusiform, moderately curved, pointed by both ends, basal cells pedicellate	Moderately curved with short blunt apical and indistinctly pedicellate basal cells
Septa (average)	3	3
Size (average)	25.6 x 3.8 µm	32 x 5.5 µm
Micro conidia	Abundant	Abundant
Shape	Ellipsoidal to cylindrical - straight, sometimes curved	Ellipsoidal - straight
Septa	Mostly 0-septate	Mostly 0-septate
Size	6 x 2.3 µm	10 x 2.5 µm
Chlamydospore	Terminal or intercalary in hyphae, often also in conidia	Produced singly or in pairs in terminal
	Hyaline, smooth walled	Hyaline, smooth walled
	7.5 µm dia	9.5 µm dia
Sclerotial pustules	Present, pale or deep violet	Absent

Based on the morphological, cultural and microscopic characters mentioned above F1t and F4c were tentatively identified as *Fusarium oxysporum* and *F. solani* respectively (Domsch *et al.*, 1980). These isolates were then sent to Agharkar Research Institute, Pune and confirmed their identity.

4.2 ISOLATION OF ANTAGONISTS

4.2.1 Isolation of Antagonistic Fungi

A total of 26 *Trichoderma* isolates were obtained from the rhizosphere of healthy chilli and tomato plants and virgin forest soils of Kerala. The isolates and their sources are depicted in Table 7.

4.2.2 Isolation of Antagonistic Fluorescent Pseudomonads

A total of 56 pseudomonad isolates were obtained from the rhizosphere and phyllosphere of chilli and tomato and virgin forest soils of Kerala. The isolates and their sources are presented in Table 7.

4.3 *IN VITRO* STUDIES ON PATHOGEN SUPPRESSION

4.3.1 Screening of *Trichoderma* spp. against *R. solani* and *Fusarium* sp.

In general, all the *Trichoderma* isolates showed inhibition of *R. solani* and *Fusarium* sp. to varying extents (Table 8). It was observed that 11 isolates of *Trichoderma viz.*, TR2, TR13, TR15, TR17, TR18, TR19, TR20, TR21, TR22, TR24 and TR26 were overgrown and completely suppressed *R. solani* within six days of inoculation. Maximum inhibition of 59.3 per cent followed by 58.9 per cent was observed with these isolates. These 11 isolates were selected for the secondary screening.

Among the 26 *Trichoderma* isolates screened against *Fusarium* sp., eight isolates *viz.*, TR2, TR13, TR17, TR18, TR19, TR20, TR22 and TR24 suppressed the *Fusarium* within six days of inoculation (Table 9). More than 60 per cent inhibition of the mycelial growth of the pathogen was observed with the isolates and these were selected for further screening.

Table 7. Different isolates of antagonists and their sources

Antagonists	Isolated from				
	Forest soils	Rhizosphere		Phyllosphere	
		Tomato	Chilli	Tomato	Chilli
Trichoderma isolates	TR1 TR2 TR3 TR4 TR5 TR6 TR7 TR8 TR9 TR10 TR11 TR12	TR13 TR14 TR15 TR16 TR17 TR24 TR25 TR26	TR18 TR19 TR20 TR21 TR22 TR23		
Fluorescent pseudomonads	P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11 P12	P24 P25 P26 P27 P28 P29 P30	P13 P14 P15 P16 P17 P18 P19 P20 P21 P22 P23 P51 P52 P53 P54 P55 P56	P31 P32 P33 P34 P35 P36 P37 P38 P39 P40	P41 P42 P43 P44 P45 P46 P47 P48 P49 P50

Table 8. Screening of *Trichoderma* isolates against *R. solani*

Trichoderma isolates	Radial growth (*R. solani*) (cm)	% inhibition	Type of interaction and metabolite production (days after inoculation)				
			3	4	5	6	7
TR1	2.30	48.89	A	A	A	B	B
TR2	1.85	58.89	A	A	B	B^{FS}	B^{FS}
TR3	2.40	46.67	A	A	A	A	A
TR4	2.70	40.00	A	A	A	B	B
TR5	2.40	46.67	A	A	A	A	A
TR6	2.35	47.78	C	C	B	B	B
TR7	2.30	48.89	C	C	B	B	B^{FS}
TR8	2.20	51.11	A	A	B	B	B
TR9	3.25	27.78	C+	C+	C+	C+	$C+^{FS}$
TR10	2.40	46.67	C	C	B	B	B
TR11	3.15	30.00	A	A	A	A	A
TR12	2.80	37.78	C	C	B	B	B
TR13	1.85	58.89	A	B	B^{FS}	B^{FS}	B^{FS}
TR14	2.20	51.11	A	A	B	B	B
TR15	1.85	58.89	B	B	B	B^{FS}	B^{FS}
TR16	2.20	51.11	C+	C+	C+	C+	C+
TR17	1.85	58.89	C+	C+	B+	$B+^{FS}$	$B+^{FS}$
TR18	1.85	58.89	A	B	B	B^{FS}	B^{FS}
TR19	1.83	59.33	B	B	B	B^{FS}	B^{FS}
TR20	1.83	59.33	B	B	B^{FS}	B^{FS}	B^{FS}
TR21	1.83	59.33	B	B	B	B^{FS}	B^{FS}
TR22	1.85	58.89	B	B	B	B^{FS}	B^{FS}
TR23	3.2	28.89	C	C	C	C	C
TR24	1.83	59.33	A	A	B	B^{FS}	B^{FS}
TR25	2.20	51.11	A	A	A	A	A
TR26	1.85	58.89	C+	C+	C+	$C+^{FS}$	$C+^{FS}$
Control	4.50						
CD	0.05						

A – Free intermingling between organisms
A – Pathogen overgrown by *Trichoderma* spp.
C – Cessation of growth at line of contact
+ – Presence of metabolite production
FS – Full suppression of pathogen

Table 9. Screening of *Trichoderma* isolates against *Fusarium* sp.

Trichoderma isolates	Radial growth (*Fusarium* sp.) (cm)	% inhibition	Type of interaction and metabolite production (days after inoculation)				
			3	4	5	6	7
TR1	2.10	53.33	A	A	B	B	B
TR2	1.80	60.00	A	A	B	B^{FS}	B^{FS}
TR3	2.80	37.78	A	A	A	A	B
TR4	2.70	40.00	A	A	A	B	B
TR5	2.50	44.44	C	C	C	C	C
TR6	2.60	42.22	C	B	B	B	B
TR7	2.10	53.33	C	C	B	B	B
TR8	2.00	55.56	A	A	B	B	B
TR9	2.50	44.44	C+	C+	C+	C+	C+
TR10	2.50	44.44	C	C	B	B	B
TR11	2.40	46.67	A	A	A	A	A
TR12	2.70	40.00	C	B	B	B	B
TR13	1.80	60.00	A	B	B	B^{FS}	B^{FS}
TR14	2.30	48.89	A	B	B	B	B
TR15	2.00	55.56	A	B	B	B	B
TR16	2.40	46.67	C+	C+	C+	C+	C+
TR17	1.80	60.00	C+	C+	B+	$B+^{FS}$	$B+^{FS}$
TR18	1.80	60.00	B	B	B	B^{FS}	B^{FS}
TR19	1.80	60.00	B	B	B	B^{FS}	B^{FS}
TR20	1.75	61.11	B	B	B	B^{FS}	B^{FS}
TR21	1.90	57.78	B	B	B	B	B
TR22	1.70	62.22	B	B	B	B^{FS}	B^{FS}
TR23	2.80	37.78	C	C	C	C	C
TR24	1.80	60.00	A	B	B	B^{FS}	B^{FS}
TR25	2.60	42.22	A	A	A	A	B
TR26	1.90	57.78	C+	C+	C+	C+	C+
Control	4.50						
CD	0.028						

A – Free intermingling between organisms
A – Pathogen overgrown by *Trichoderma* spp.
C – Cessation of growth at line of contact
+ – Presence of metabolite production
FS – Full suppression of pathogen

Secondary screening of *Trichoderma* isolates

In order to select the most efficient isolates of *Trichoderma* against *R. solani* and *Fusarium* sp., the isolates obtained from the primary screening were subjected to a secondary screening and ratings were done based on modified Bells' (Class 1-5) scale.

Among the 11 isolates tested against *R. solani*, TR20 and TR17 completely overgrew and suppressed the pathogen (Class-1 stage) within seven days of incubation (Table 10). All the other isolates took more time for the complete inhibition of *R. solani*. Hence TR20 and TR17 were selected for further *in vitro* studies against *R. solani* (Plate 7).

Of the eight isolates tested against *Fusarium* sp. TR19 and TR22 were found to be the most potential ones, which reached the class-1 stage of inhibition within seven days of incubation (Table 11). Other isolates except TR17, inhibited the pathogen, but it took longer period for their effective antagonism. TR17 exhibited class four type of inhibition *i.e.*, TR17 isolate could not grow over the established *Fusarium* sp. On the basis of the inhibitory potential TR19 and TR22 were selected against *Fusarium* sp. for further *in vivo* studies (Plate 8).

4.3.2 Screening of Antagonistic Bacteria

The efficiency of the 56 pseudomonad isolates obtained from the rhizosphere and phyllosphere of chilli and tomato in inhibiting the growth of *R. solani* and *Fusarium* sp. under *in vitro* condition was studied by dual culturing technique. The bacterial isolates varied in their ability to inhibit the pathogens.

The isolate P28 inhibited the growth of *R. solani* and produced the maximum inhibition zone of 14.25 mm followed by P51 which produced an inhibition zone of 14 mm when co-inoculated with it on KMB agar (Table 12 and Plate 9). On PDA, a maximum inhibition zone of 7.5 mm was produced by P11, P28 and P51 isolates of *Pseudomonas*.

Table 10. Ratings of selected *Trichoderma* isolates based on the inhibition of *R. solani*

Sl. No.	*Trichoderma* isolate	*Bell's scale (modified) (days)				
		5	6	7	8	9
1	TR2	C4	C4	C2	C1	C1
2	TR13	C4 – C3	C3	C2	C1	C1
3	TR15	C4	C4	C4	C3	C3
4	TR17	C4	C4	C1	C1	C1
5	TR18	C4	C4	C2	C2	C1
6	TR19	C4	C4	C3	C2	C2
7	TR20	C3 – C2	C2 – C1	C1	C1	C1
8	TR21	C4	C4	C4 – C3	C4 – C3	C4 – C3
9	TR22	C4	C3	C2	C1	C1
10	TR24	C4	C3	C2	C1	C1
11	TR26	C4	C4	C4	C4 – C3	C4 – C3

*Observed as sporulating *Trichoderma* sp. over the pathogen
C1 – Class 1 – The antagonist completely overgrew the pathogen (100 % over growth)
C2 – Class 2 – 75 per cent over growth
C3 – Class 3 – 50 per cent over growth
C4 – Class 4 – The pathogen and antagonist locked at the point of contact
C5 – Class 5 – Pathogen overgrew the mycoparasite

Table 11. Ratings of selected *Trichoderma* isolates based on the inhibition of *Fusarium* sp.

Sl. No.	*Trichoderma* isolate	*Bell's scale (modified) (days)				
		5	6	7	8	9
1	TR2	C4	C4	C4	C4	C2
2	TR13	C4	C4	C3 – C2	C1	C1
3	TR17	C4	C4	C4	C4	C4
4	TR18	C4	C4	C3 – C2	C1	C1
5	TR19	C3 – C2	C2	C1	C1	C1
6	TR20	C4	C4 – C3	C3	C3	C3
7	TR22	C3 – C2	C2	C1	C1	C1
8	TR24	C4	C4 – C3	C3	C3	C2

*Observed as sporulating *Trichoderma* sp. over the pathogen
C1 – Class 1 – The antagonist completely overgrew the pathogen (100 % over growth)
C2 – Class 2 – 75 per cent over growth
C3 – Class 3 – 50 per cent over growth
C4 – Class 4 – The pathogen and antagonist locked at the point of contact
C5 – Class 5 – Pathogen overgrew the mycoparasite

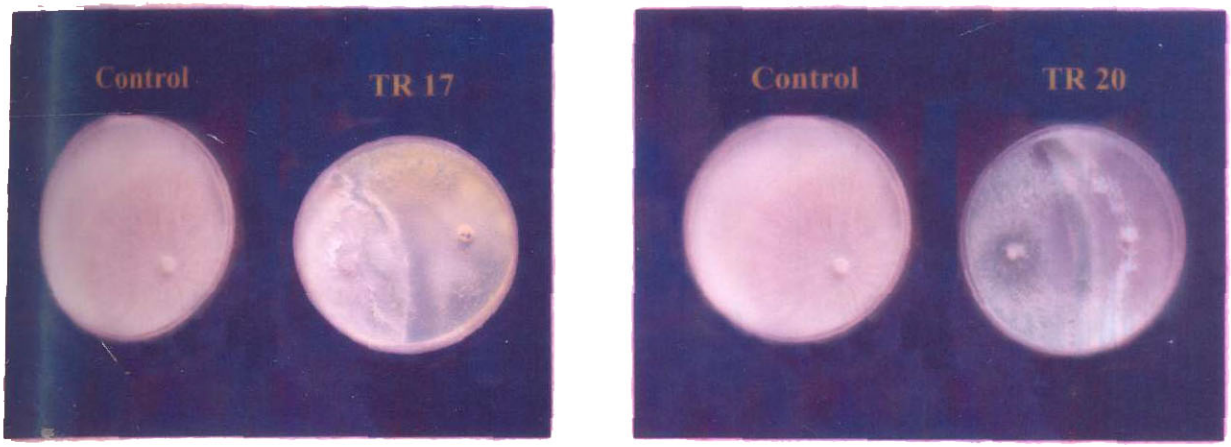


Plate 7. ***In vitro*** **inhibition of** ***R. solani*** **by** ***Trichoderma*** **sp.**

Plate 8. ***In vitro*** **inhibition of** ***Fusarium*** **sp. by** ***Trichoderma*** **sp.**

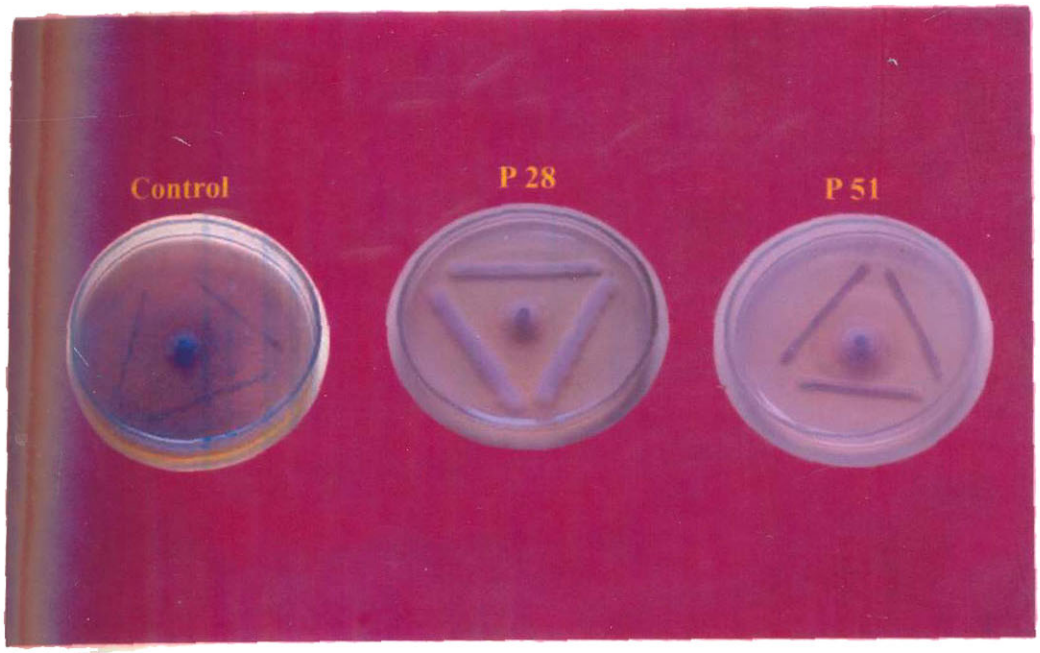


Plate 9. *In vitro* inhibition of *R. solani* by fluorescent pseudomonads

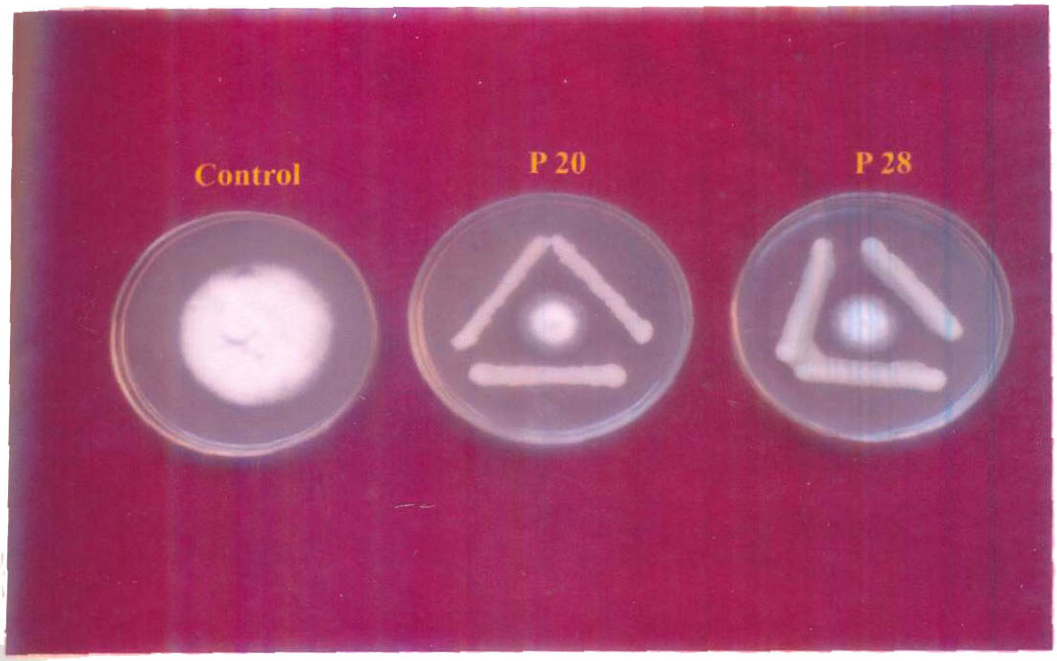


Plate 10. *In vitro* inhibition of *Fusarium* sp. by fluorescent psuedomonads

Antagonism of *Pseudomonas* against *Fusarium* on KMB agar showed that pseudomonad isolate P20 produced an inhibition zone of 11.25 mm which was the maximum among all other isolates. The next best isolate observed was P28 with an inhibition zone of 10.75 mm on KMB agar. On PDA also P20 and P28 exerted the maximum inhibition as evidenced by the formation of 7.5 and 7.25 mm of inhibition zone respectively (Table 13 and Plate 10).

It was also observed that many of the pseudomonads which inhibited the pathogen on KMB agar was found ineffective in reducing the pathogen growth, as they were overgrown by the test fungus when dual cultured on PDA medium.

Pseudomonads isolated from the phyllosphere of tomato and chilli, in general were totally ineffective and was overgrown by both the pathogens *i.e.*, *Rhizoctonia* and *Fusarium* in both the media employed.

Based on the extent of inhibition exerted on mycelial growth of the pathogen two bacterial isolates, P28 and P51 were selected against *R. solani* and P20 and P28 were selected against *Fusarium* sp. for further studies.

4.3.3 Compatibility of the Selected Isolates

The selected isolates of *Trichoderma* and *Pseudomonas* were further tested for their ability to co-exist with each other by a modified dual culture technique.

Inhibitory effects of *Pseudomonas* strains on *Trichoderma* isolates were not noticed in the KMB agar plates where *Pseudomonas* was inoculated on *Trichoderma* seeded medium *i.e.*, there was no inhibition zone around the bacterial spot. However, *Trichoderma* did not grow at the point where the bacteria was spotted. In the second experiment, where the *Trichoderma* was inoculated on the *Pseudomonas* seeded medium, bacterial growth was seen within 24 h on the medium and no growth of the

Table 12. *In vitro* inhibition of *R. solani* by fluorescent pseudomonads

Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)	
	KMB	PDA
P1	12.00 (3.60)	5.00 (2.45)
P2	8.50 (3.08)	Overgrew
P3	11.50 (3.52)	4.25 (2.29)
P4	Overgrew	Overgrew
P5	"	"
P6	"	"
P7	"	"
P8	4.50 (2.35)	"
P9	12.50 (3.65)	6.00 (2.65)
P10	Overgrew	Overgrew
P11	12.75 (3.71)	7.50 (2.92)
P12	5.25 (2.50)	2.25 (1.80)
P13	6.50 (2.74)	Overgrew
P14	9.00 (3.16)	"
P15	5.50 (2.55)	0.75 (1.32)
P16	11.25 (3.50)	Overgrew
P17	11.75 (3.57)	2.00 (1.73)
P18	13.25 (3.77)	0.00 (1.00)
P19	4.00 (2.24)	Overgrew
P20	9.50 (3.24)	1.00 (1.41)
P21	6.50 (2.74)	Overgrew
P22	13.00 (3.74)	"
P23	Overgrew	"
P24	12.25 (3.64)	2.75 (1.94)
P25	13 (3.74)	Overgrew
P26	Overgrew	3.25 (2.06)
P27	11.25 (3.50)	5.50 (2.55)
P28	**14.25** (3.91)	**7.50** (2.92)

Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)	
	KMB	PDA
P29	13.00 (3.74)	0.5 (1.22)
P30	6.00 (2.65)	Overgrew
P31	3.00 (2.00)	"
P32	4.00 (2.24)	"
P33	Overgrew	"
P34	1.00 (1.41)	0.00 (1.00)
P35	Overgrew	Overgrew
P36	"	0.00 (1.00)
P37	"	Overgrew
P38	2.75 (1.94)	2.20 (1.79)
P39	Overgrew	Overgrew
P40	"	"
P41	"	"
P42	"	1.00 (1.41)
P43	3.00 (2.00)	1.00 (1.41)
P44	0.00 (1.00)	0.00 (1.00)
P45	5.25 (2.50)	Overgrew
P46	Overgrew	"
P47	"	"
P48	0.00 (1.00)	0.00 (1.00)
P49	0.00 (1.00)	0.00 (1.00)
P50	Overgrew	Overgrew
P51	**14.00** (3.87)	**7.50** (2.92)
P52	8.00 (3.00)	1.00 (1.41)
P53	4.5 (2.35)	Overgrew
P54	8.00 (3.00)	"
P55	Overgrew	"
P56	5.75 (2.59)	1.00 (1.41)
CD	0.36	0.34

Figures in parenthesis indicate $\sqrt{x+1}$ transformation

Table 13. *In vitro* inhibition of *Fusarium* sp. by fluorescent pseudomonads

Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)	
	KMB	PDA
P1	9.50 (3.20)	6.00 (2.65)
P2	8.50 (3.08)	5.50 (2.54)
P3	10.00 (3.30)	4.75 (2.40)
P4	Overgrew	Overgrew
P5	"	"
P6	"	"
P7	"	"
P8	3.25 (2.06)	3.25 (2.06)
P9	6.50 (2.74)	4.50 (2.34)
P10	4.50 (2.35)	3.50 (2.15)
P11	9.50 (3.24)	5.00 (2.45)
P12	5.25 (2.50)	5.50 (2.54)
P13	5.00 (2.45)	5.00 (2.45)
P14	8.50 (3.08)	6.00 (2.64)
P15	5.50 (2.55)	0.00 (1.00)
P16	8.50 (3.08)	6.50 (2.74)
P17	8.50 (3.08)	5.00 (2.45)
P18	9.25 (3.20)	6.75 (2.78)
P19	3.00 (2.00)	Overgrew
P20	**11.25** (3.46)	**7.50** (2.90)
P21	4.50 (2.35)	4.50 (2.34)
P22	9.75 (3.28)	6.25 (2.70)
P23	Overgrew	Overgrew
P24	7.50 (2.90)	5.50 (2.54)
P25	7.00 (2.83)	3.25 (2.06)
P26	2.50 (1.87)	Overgrew
P27	8.00 (3.00)	6.50 (2.74)
P28	**10.75** (3.35)	**7.25** (2.87)

Pseudomonad isolates	Inhibition zone (mm) (5th day of inoculation)	
	KMB	PDA
P29	9.00 (3.16)	6.50 (2.70)
P30	6.00 (2.64)	5.00 (2.40)
P31	3.00 (2.00)	2.00 (1.70)
P32	4.00 (2.24)	3.50 (2.12)
P33	3.00 (2.00)	Overgrew
P34	1.50 (1.58)	"
P35	Overgrew	"
P36	"	"
P37	"	"
P38	"	"
P39	"	"
P40	"	"
P41	"	"
P42	"	"
P43	"	"
P44	"	"
P45	"	"
P46	"	"
P47	"	"
P48	0.00 (1.00)	"
P49	2.00 (1.73)	"
P50	Overgrew	"
P51	9.50 (3.24)	6.0 (2.60)
P52	8.00 (3.00)	5.25 (2.50)
P53	5.50 (2.55)	5.50 (2.55)
P54	8.00 (3.00)	6.00 (2.64)
P55	0.00 (1.00)	Overgrew
P56	7.50 (2.90)	4.50 (2.30)
CD	0.37	0.25

Figures in parenthesis indicate $\sqrt{x+1}$ transformation

fungus was observed for 48 h. After 48 h *Trichoderma* was found growing over the medium. The results on PDA was almost similar to that observed on KMB agar.

4.4 *IN VIVO* SCREENING OF BIOCONTROL AGENTS FOR DISEASE SUPPRESSION AND GROWTH PROMOTION

4.4.1 Greenhouse Condition

4.4.1.1 Experiment 1 : Influence of antagonists on the incidence of Rhizoctonia rot and growth improvement in tomato

Two isolates each of *Trichoderma* and *Pseudomonas* selected from the *in vitro* studies were tested individually and in combination for their effect on disease suppression and growth improvement in tomato under greenhouse condition (Plate 11). The infection initiated from the third day of pathogen inoculation and continued for 15 days. Since all the infected plants succumbed to death, per cent disease incidence was calculated based on the number of dead plants. Results obtained revealed that all the antagonists, individually and in combination caused considerable reduction in rot incidence in tomato plants which were not statistically different from each other. Among the different treatments, combined application of Pseudomonad isolate P28 with *Trichoderma*, TR17 and TR20 and *Pseudomonas* P51 with TR20 showed 100 per cent protection (0 % disease incidence), while both the *Trichoderma* sp. (TR17 and TR20) and P28 isolate of *Pseudomonas* when applied singly reduced the rot incidence by 85.4 per cent (4.17 per cent disease incidence) when compared to the pathogen check. No significant difference was observed between antagonist treatments and fungicide application in terms of per cent disease incidence (Table 14 and Fig. 1).

In general, combined application of *Trichoderma* and *Pseudomonas* proved as the best treatment in enhancing the general growth of plants. Statistical analysis showed that the various treatments did not differ

significantly with respect to characters like dry weight of root at 45 and 90 days after planting (DAP). With regard to fruit weight, same trend could be observed. However significant difference was noticed for all other parameters *viz.*, fruit yield, shoot and root length, fresh and dry weight of shoot, fresh weight of roots at both 45 and 90 DAP. Maximum yield (680 g) was obtained with combination of TR20 and P28 which was statistically on par with TR20 + P51 (630 g). These two treatments resulted in 159.05 and 140 per cent increase in yield over control. It was observed that individual application of *Trichoderma* was found superior to *Pseudomonas* application. Of the two *Trichoderma* sp. *viz.*, TR17 and TR20 maximum yield (450 g) was recorded with TR20 which was 71.43 per cent more when compared to control, whereas the individual application of both *Pseudomonas* isolates did not differ significantly with control (Table 14 and Fig. 2).

The shoot length at 45 DAP was maximum (51 cm) (Table 15) with TR20 + P51 isolates which was on par with other combination treatments. Similar trend was observed in case of fresh and dry weight of shoots at 45 DAP, where TR20 + P 51 recorded the maximum fresh weight (48.13 g) which was significantly high over other treatments. It was followed by TR17 + P28, where 38.18 g fresh weight was recorded. The dry matter content of shoot at 45 DAP was maximum with TR17 + P28 and TR20 + P51 where 4.22 and 3.82 g respectively was recorded. Individual application of *Trichoderma* and *Pseudomonas* did not differ significantly from the control with respect to shoot length, fresh and dry weight of shoot at 45 DAP (Plate 12).

Maximum elongation of the roots at 45 DAP was recorded with TR17 + P28 (28.13 cm) followed by TR17 + P51 (27.28 cm) and TR20 + P51 (25.25 cm). But no statistical difference was observed among these and control. Regarding the fresh weight of roots, all the combination treatments tried proved superior to the individual application of biocontrol agents and control.

At 90 DAP, combined application of fungal and bacterial antagonists was found best in improving the plant growth, in general when compared to individual application of these organisms. TR20 + P51 recorded the maximum plant height (92 cm), fresh (262.569 g) and dry weight of shoot (63.43 g) at 90 DAP (Table 16). Individual application of *Pseudomonas* was found superior to the individual application of *Trichoderma* in terms of shoot length, fresh and dry weight of shoot.

Maximum root length at 90 DAP was achieved with the application of TR20 + P28 (54.23 cm) followed by TR20 + P51 (53.35 cm), whereas maximum fresh weight of root was observed with TR20 + P51 (34.66 g) which was significantly higher over other treatments (Table 16).

4.4.1.2 Experiment 2 : Influence of Antagonists on the Incidence of Rhizoctonia Rot and Growth Improvement in Chilli

All the treatments tested in the greenhouse experiment were significantly superior in reducing the incidence of Rhizoctonia rot in chilli. Among the different antagonist treatments TR20 + P28 was the best in reducing the seedling rot caused by *R. solani*. Only 10 per cent disease incidence was recorded with this treatment whereas in control 30 per cent rot incidence was observed (Table 17 and Fig. 2). TR20 + P28 application was equally effective as copper oxychloride (0.3 %) which could protect the seedling by 66.7 per cent more than control. Amongst the treatments where antagonists were applied individually TR20 was most effective with 44.4 per cent increased protection and was on par with fungicide application.

Significant difference was observed between the different treatments with regard to yield. From the table (Table 17 and Fig. 2) it is clear that yield enhancement was in the combination treatment of *Trichoderma* and *Pseudomonas*. Maximum yield was recorded with TR20 + P28 (427.67 g) followed by TR17 + P51 (401 g). TR17 and TR20 when applied alone significantly increased the yield (395.33 and 360.67 g

Table 14. Effect of antagonists on the incidence of Rhizoctonia rot and yield in tomato under greenhouse condition⊕

Treatment No.	Treatments	% Disease incidence*	% variation over T_0	Yield (g plant^{-1})	% variation over T_0
T_0	Pathogen alone (control)	29.15 (32.68)	-	262.50	-
T_1	*Trichoderma* sp. (TR 17)	4.17 (11.78)	-85.40	358.75	36.67
T_2	*Trichoderma* sp. (TR 20)	4.17 (11.78)	-85.40	450.00	71.43
T_3	*Pseudomonas* sp. (P28)	4.17 (11.78)	-85.40	240.00	-8.57
T_4	*Pseudomonas* sp. (P51)	8.33 (16.78)	-71.18	218.75	-16.67
T_5	TR 17 + P 28	0.00 (11.78)	100	451.25	71.90
T_6	TR 20 + P 28	0.00 (11.78)	100	680.00	159.05
T_7	TR 17 + P 51	8.33 (16.78)	-71.18	380.00	44.76
T_8	TR 20 + P 51	0.00 (11.78)	100	630.00	140.00
T_9	Copper oxy chloride (0.3 %)	8.33 (16.78)	-71.18	235.00	-10.48
T_{10}	Untreated check	-	-	263.75	0.48
	CD (0.05)	10.73		77.35	

*Figure in parenthesis indicates angular transformation

⊕Mean of four replications

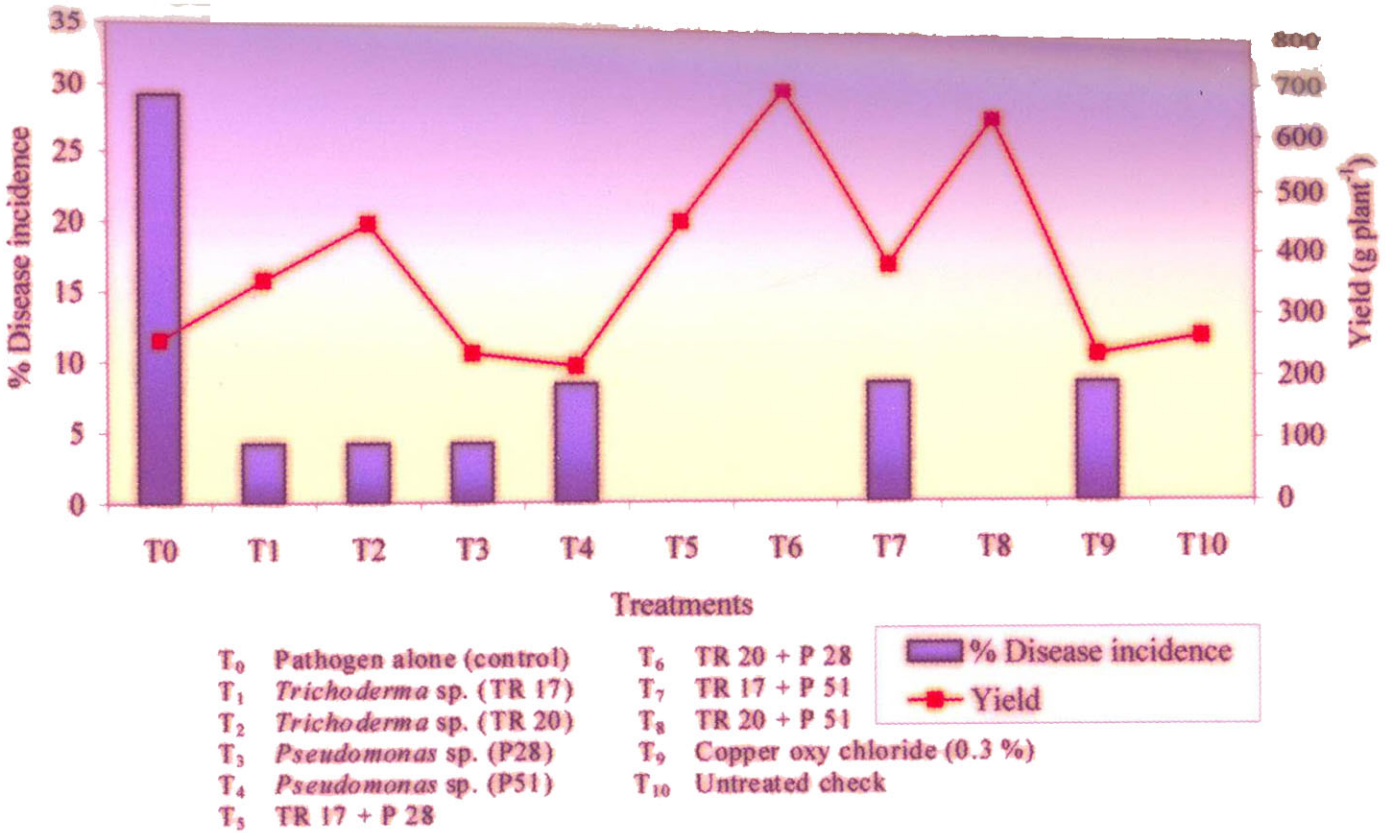
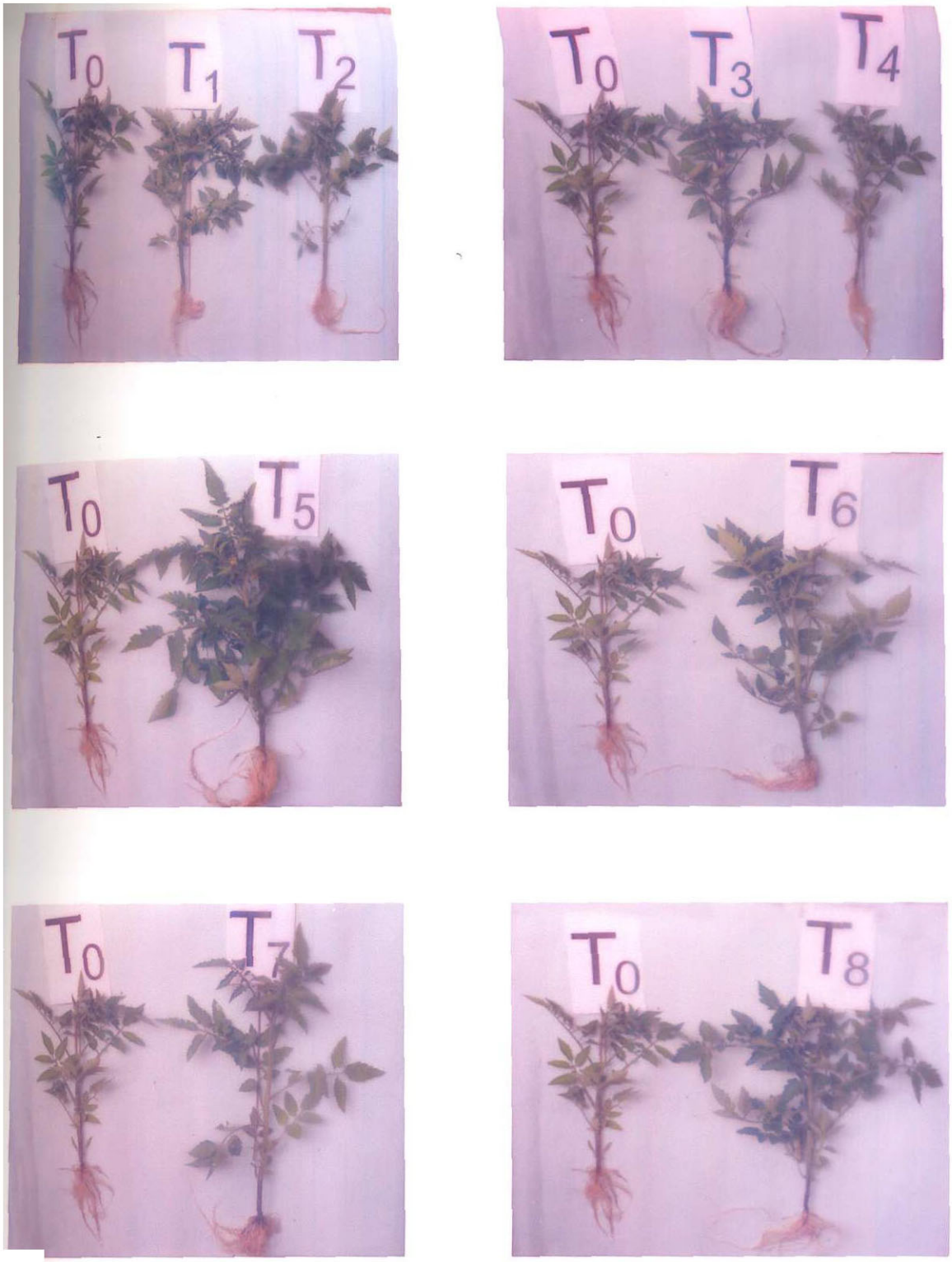


Fig. 1. Effect of antagonists on the incidence of Rhizoctonia rot and yield in tomato under greenhouse condition



T0 - Pathogen alone
T1 - *Trichoderma* sp. (TR 17) + pathogen
T2 - *Trichoderma* sp. (TR 20) + pathogen
T3 - *Pseudomonas* sp. (P 28) + pathogen
T4 - *Pseudomonas* sp. (P 51) + pathogen
T5 - TR 17 + T 28 + pathogen
T6 - TR 20 + P 28 + pathogen
T7 - TR 17 + P 51 + pathogen
T8 - TR 20 + P 51 + pathogen

Plate 11. Effect of different antagonist treatments on the growth of tomato plants infected by *R. solani*

Table 15. Effect of antagonist on different biometric characters of tomato infected with *R solani* at 45 DAP under greenhouse condition⌖

Treatment No.	Treatments	Shoot length (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)
T_0	Pathogen alone (control)	34.08	-	16.03	-	2.45	-	24.75	-	2.03	-	0.42
T_1	*Trichoderma* sp. (TR 17)	33.50	-1.70	18.73	16.84	2.28	-6.94	19.90	-19.60	1.85	-8.87	0.33
T_2	*Trichoderma* sp. (TR 20)	33.25	-2.44	18.80	17.28	2.32	-5.31	24.45	-1.21	2.10	3.45	0.38
T_3	*Pseudomonas* sp. (P28)	28.00	-17.84	10.78	-32.75	1.65	-32.65	18.80	-24.04	1.85	-8.87	0.28
T_4	*Pseudomonas* sp. (P51)	34.63	1.61	15.63	-2.50	2.13	-13.06	20.50	-17.17	1.68	-17.24	0.35
T_5	TR 17 + P 28	46.63	36.83	38.18	138.18	4.22	72.24	28.13	13.66	3.90	92.12	0.65
T_6	TR 20 + P 28	48.00	40.85	33.15	106.80	3.26	33.06	20.40	-17.58	2.60	28.08	0.47
T_7	TR 17 + P 51	45.88	34.62	30.13	87.96	3.22	31.43	27.28	10.22	3.00	47.78	0.58
T_8	TR 20 + P 51	51.00	49.65	48.13	200.25	3.82	55.92	25.28	2.14	3.33	64.04	0.55
T_9	Copper oxy chloride (0.3 %)	44.50	30.58	22.15	38.18	2.49	1.63	17.63	-28.77	2.15	5.91	0.41
T_{10}	Untreated check	47.05	38.06	26.78	67.06	3.11	26.94	19.88	-19.68	2.35	15.76	0.47
	CD (0.05)	8.30		4.22		0.71		5.08		1.05		NS

⌖Mean of four replications DAP -- Days after planting

Table 16. Effect of antagonists on different biometric characters of tomato infected with *R solani* at 90 DAP under greenhouse condition⊕

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	Fruit weight (g)
T_0	Pathogen alone (control)	68.00	-	114.52	-	33.41	-	34.33	-	21.70	-	3.10	13.55
T_1	*Trichoderma* sp. (TR 17)	68.00	0.00	151.52	32.31	46.82	40.14	40.50	17.97	24.68	13.73	4.65	12.43
T_2	*Trichoderma* sp. (TR 20)	69.75	2.57	110.28	-3.70	36.90	10.45	32.13	-6.41	18.06	-16.77	3.18	15.02
T_3	*Pseudomonas* sp. (P28)	80.75	18.75	162.33	41.75	43.01	28.73	48.25	40.55	24.86	14.56	3.79	15.20
T_4	*Pseudomonas* sp. (P51)	86.63	27.40	184.60	61.19	49.29	47.53	40.88	19.08	28.90	33.18	4.41	14.44
T_5	TR 17 + P 28	88.25	29.78	195.11	70.37	54.21	62.26	47.50	38.36	26.32	21.29	5.72	14.10
T_6	TR 20 + P 28	86.75	27.57	185.90	62.33	51.23	53.34	54.23	57.97	22.87	5.39	4.01	14.48
T_7	TR 17 + P 51	84.75	24.63	202.68	76.98	46.89	40.35	38.13	11.07	26.07	20.14	3.91	18.00
T_8	TR 20 + P 51	92.00	35.29	262.56	129.27	63.43	89.85	53.35	55.40	34.66	59.72	5.36	15.95
T_9	Copper oxy chloride (0.3 %)	83.00	22.06	162.28	41.70	40.90	22.42	33.00	-3.87	20.69	-4.65	3.18	14.05
T_{10}	Untreated check	67.25	-1.10	132.20	15.44	35.30	5.66	52.40	52.64	28.18	29.86	4.14	10.45
	CD (0.05)	6.76		26.90		7.62		8.23		5.94		NS	NS

⊕ Mean of four replications DAP – Days after planting

respectively) and was found superior to both the pseudomonads when applied individually.

The overall growth of the plants was enhanced by the application of biocontrol agents (BCAs) which was more pronounced with the combined application of *Trichoderma* and *Pseudomonas*. Maximum plant height at 45 DAP was recorded for TR17 + P28 isolates (40.37 cm) followed by TR20 + P28 (34.57 cm) (Table 18). A significant increase in fresh and dry weight of shoot at 45 DAP was recorded with TR20 + P28 (110.10 and 20.68 g) which was the maximum over all other treatments. On comparison with the control, a reduction in shoot length and fresh weight of shoot was observed at 45 DAP when the Pseudomonad isolates were applied individually. The dry matter content of shoots at 45 DAP was higher than the control. However there was no statistical difference between these treatments (Plate 13).

In the case of root growth at 45 DAP bacterial isolate P28 played a significant role. Individual application of P28, TR17 + P51, TR20 + P51 and TR20 + P28 (Treatments T_3, T_7, T_8 and T_6 respectively) increased the root length significantly, maximum (25.3 cm) being recorded for P28 (Table 18). Fresh and dry weight of the roots also showed a similar trend where the different combination of *Pseudomonas* and *Trichoderma* (treatments *viz*., T_8, T_6 and T_7) and the individual application of *Pseudomonas*, P28 performed best in enhancing the root growth.

At 90 DAP, the different treatments showed significant difference for plant height and root length. Maximum plant height was recorded for TR17 + P28 (58.67 cm) followed by TR20 + P51 (58.63 cm). whereas maximum root elongation was observed for P28 (36.7 cm) (Table 19). P28 in combination with TR20 was the next best treatment in enhancing the root elongation (36.23 cm).

A significant increase in the shoot and root weight (fresh and dry) at 90 DAP was observed with the antagonists treatment. TR20 + P28

recorded the maximum shoot weight (fresh) of 163.37 g which was statistically high over other treatments. Dry weight of the shoot was also recorded maximum (58.20 g) by TR20 + P28, immediately followed by TR17 + P28 (47.88 g) which was statistically on par with each other. Among the individual application of antagonists TR17 proved to be the best isolate in enhancing the fresh weight of shoots (117.70 g) followed by P28 (107.70 g) at 90 DAP (Table 19). But the dry matter content of shoots was more in P28 application (47.30 g) compared to other individual antagonist applications.

All the combination treatments increased the fresh weight of root at 90 DAP, maximum being recorded for TR20 + P51 (52.30 g). Root dry weight was also maximum in the combination of TR20 and P51 (16.03 g) followed by P28 (15.90 g) and TR20 + P28 (12.97 g). But statistically there was no difference between these treatments. In general, all the treatments increased the root weight at 90 DAP.

Average fresh and dry weight of fruits was maximum with TR20 (7.59 and 1.52 g) followed by TR17 + P51 (5.96 and 1.50 g). Out of the two bacterial isolates *viz.*, P28 and P51 large sized fruits as well as high fresh and dry weight of fruits were recorded with the isolate P51.

Statistical analysis revealed that application of COC (0.3%), in general caused a reduction in the overall growth of the plants.

4.4.1.3 Experiment 3: Influence of Antagonists on the Incidence of Fusarium Wilt and Growth Improvement in Tomato

Antagonistic fungi (TR19 and TR22) and fluorescent pseudomonad isolates (P20 and P28) which were effective against *Fusarium* under *in vitro* condition were evaluated for their efficiency in suppressing Fusarium wilt disease in tomato under greenhouse condition. The manifestation of wilt symptoms started within seven days of pathogen inoculation. From the table (Table 20 and Fig. 5) it is clear that wilt

Table 17. Effect of antagonists on the incidence of Rhizoctonia rot and yield in chilli under greenhouse condition

Treatment No.	Treatments	% Disease incidence*	% variation over T_0	Yield (g plant^{-1})	% variation over T_0
T_0	Pathogen alone (control)	30.00 (33.21)	-	189.33	-
T_1	*Trichoderma* sp. (TR 17)	20.00 (6.57)	-33.33	395.33	108.80
T_2	*Trichoderma* sp. (TR 20)	16.67 (24.09)	-44.43	360.67	90.50
T_3	*Pseudomonas* sp. (P 28)	20.00 (26.57)	-33.33	224.33	18.49
T_4	*Pseudomonas* sp. (P 51)	20.00 (26.57)	-33.33	195.33	3.16
T_5	TR 17 + P 28	16.67 (24.09)	-44.43	342.00	80.64
T_6	TR 20 + P 28	10.00 (18.43)	-66.67	427.67	125.89
T_7	TR 17 + P 51	20.00 (26.57)	-33.33	401.00	111.80
T_8	TR 20 + P 51	16.67 (24.09)	-44.43	373.00	97.01
T_9	Copper oxy chloride (0.3 %)	10.00 (18.43)	-66.67	161.67	-14.61
T_{10}	Untreated check	-	-	162.00	-14.44
	CD (0.05)	10.55		84.20	

*Figure in parenthesis indicates angular transformation

⁜ Mean of three replications

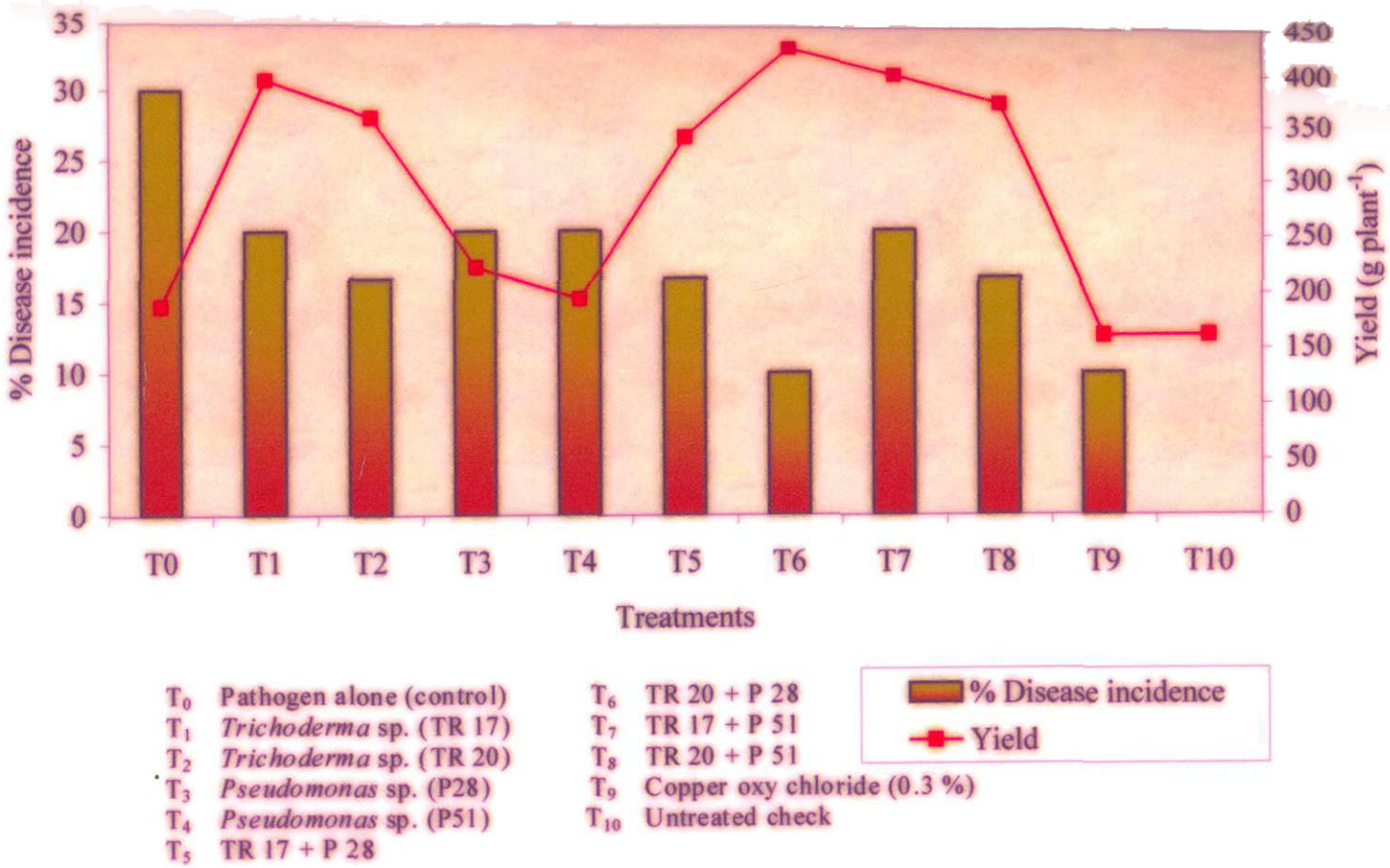


Fig. 2. Effect of antagonists on the incidence of Rhizoctonia rot and yield in chilli under greenhouse condition

Table 18. *Effect of antagonists on different biometric characters of chilli infected with R solani at 45 DAP under greenhouse condition*

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0
T_0	Pathogen alone (control)	20.43	-	45.90	-	7.93	-	15.87	-	13.30	-	2.68	-
T_1	*Trichoderma* sp. (TR 17)	30.80	50.76	67.17	46.34	13.25	67.09	14.80	-6.74	17.50	31.58	3.63	35.45
T_2	*Trichoderma* sp. (TR 20)	24.00	17.47	45.87	-0.07	9.66	21.82	18.80	18.46	19.60	47.37	3.76	40.30
T_3	*Pseudomonas* sp. (P28)	17.47	-14.49	43.67	-4.86	10.66	34.43	25.30	59.42	22.13	66.39	5.45	103.36
T_4	*Pseudomonas* sp. (P51)	19.70	-3.57	45.17	-1.59	9.38	18.28	13.67	-13.86	15.70	18.05	3.09	15.30
T_5	TR 17 + P 28	40.37	97.60	66.83	45.60	12.60	58.89	13.60	-14.30	19.97	50.15	3.98	48.51
T_6	TR 20 + P 28	34.57	69.21	110.10	139.87	20.68	160.78	22.03	38.82	24.67	85.49	4.28	59.70
T_7	TR 17 + P 51	28.60	39.99	71.23	55.19	12.71	60.28	24.80	56.27	20.93	57.37	3.91	45.90
T_8	TR 20 + P 51	27.57	34.95	66.93	45.82	11.80	48.80	23.20	46.19	25.57	92.26	4.77	77.99
T_9	Copper oxy chloride (0.3 %)	20.97	2.64	32.20	-29.85	7.11	-10.34	17.63	11.09	10.92	-17.89	2.20	-17.91
T_{10}	Untreated check	24.03	17.62	34.23	-25.42	6.89	-13.11	16.60	4.60	10.90	-18.05	2.19	-18.28
	CD (0.05)	8.23		11.73		5.03		5.68		5.11		1.42	

Mean of three replications DAP – Days after planting

Table 19. Effect of antagonists on different biometric characters of chilli infected with *R solani* at 90 DAP under greenhouse condition

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0	Fruit weight fresh (g)	% variation over T_0	Fruit weight dry (g)	% variation over T_0
T_0	Pathogen alone (control)	51.83	-	83.22	-	34.15	-	35.93	-	35.90	-	6.90	-	3.32	-	0.79	-
T_1	*Trichoderma* sp. (TR 17)	57.43	10.80	117.70	41.43	39.48	15.61	31.00	-13.72	36.50	1.67	10.04	45.51	3.60	8.43	0.85	7.59
T_2	*Trichoderma* sp. (TR 20)	49.17	-5.13	96.48	15.93	35.76	4.71	35.23	-1.95	34.04	-5.18	10.57	53.19	7.59	128.61	1.52	92.41
T_3	*Pseudomonas* sp. (P28)	54.20	4.57	107.70	29.42	47.30	38.51	36.70	2.14	38.06	6.02	15.90	130.43	3.90	17.47	0.95	20.25
T_4	*Pseudomonas* sp. (P51)	47.57	-8.22	87.38	5.00	35.41	3.69	27.57	-23.27	27.46	-23.51	8.83	27.97	5.84	75.90	1.43	81.01
T_5	TR 17 + P 28	58.67	13.20	113.22	36.05	47.88	40.20	29.17	-18.81	41.36	15.21	10.67	54.64	4.34	30.72	1.05	32.91
T_6	TR 20 + P 28	57.07	10.11	163.37	96.31	58.20	70.42	36.23	0.83	51.07	42.26	12.97	87.97	4.70	41.57	1.02	29.11
T_7	TR 17 + P 51	53.63	3.47	113.72	36.65	43.98	28.78	34.67	-3.51	42.18	17.49	10.57	53.19	5.96	79.52	1.50	89.87
T_8	TR 20 + P 51	58.63	13.12	118.72	42.66	45.52	33.29	36.17	0.67	52.30	45.68	16.03	132.32	4.79	44.28	1.21	53.16
T_9	Copper oxy chloride (0.3 %)	47.43	-8.49	74.99	-9.89	24.64	-27.85	29.33	-18.37	23.49	-34.57	4.40	-36.23	3.10	-6.63	0.77	-2.53
T_{10}	Untreated check	41.93	-19.10	63.83	-23.30	27.43	-19.68	25.83	-28.11	21.31	-40.64	8.60	24.64	4.40	32.53	1.11	40.51
	CD (0.05)	12.70		22.62		10.80		10.21		13.11		4.42		2.41		0.52	

* Mean of three replications DAP – Days after planting

T0 - Pathogen alone
T1 - *Trichoderma* sp. (TR 17) + pathogen
T2 - *Trichoderma* sp. (TR 20) + pathogen
T3 - *Pseudomonas* sp. (P 28) + pathogen
T4 - *Pseudomonas* sp. (P 51) + pathogen
T5 - TR 17 + T 28 + pathogen
T6 - TR 20 + P 28 + pathogen
T7 - TR 17 + P 51 + pathogen
T8 - TR 20 + P 51 + pathogen

Plate 12. Effect of different antagonist treatments on the growth of chilli plants infected by *R. solani*

incidence was reduced by the antagonist treatments. The least wilt incidence (35 %) was recorded with the individual application of *Trichoderma* isolate TR22 and in combination with pseudomonad isolate P20 and P28 (*i.e.*, TR22 + P20 and TR22 + P28) which was equally as good as Carbendazim (0.1 %) application. Percentage wilt intensity calculated on the basis of score chart (0-3 scale) revealed that all the treatments were effective in reducing the severity of wilt incidence under greenhouse condition. The least wilt intensity was observed with TR22 + P20 and TR22 + P28 (11.65 %) whereas in control wilt intensity recorded was 30.03 per cent. When compared to control, these two combinations could reduce the intensity by 61.20 percent and were found equally effective as Carbendazim (0.1 %) application. Individual application of the biocontrol agents (BCAs) did not show any significant difference between themselves in reducing the wilt intensity. Amongst the different antagonist treatments, TR19 + P28 was found inferior to other treatments in controlling the wilt.

The data regarding the effect of antagonists on the growth of tomato (Table 20 and Fig. 6) indicated that the combination of TR22 + P28 recorded the maximum yield (605.75 g) *i.e.*, 71.12 per cent more over control. Significant increase in yield was also observed by Carbendazim (0.1 %) (481.25 g); TR19 + P28 (437.50 g) and TR22 + P20 (432.5 g). Statistically these values did not differ much. A reduction in yield was recorded with TR19, P20, TR19 + P20 when compared to control.

Regarding the shoot and root growth, no difference was observed between the combined and individual application of biocontrol agents at 45 DAP. However, amongst the different treatments, individual application of antagonist, P20 proved to be the best with highest plant height (32.75 cm), shoot weight (fresh) (16.40 g) and shoot weight (dry) (4.22 g) which was closely followed by TR22. Maximum root growth

including root length (28.88 cm) and fresh (2.21 g) and dry (0.53 g) weight was recorded with TR22 (Table 21 and Plate 14).

Soil drenching with Carbendazim (0.1 %) was statistically on par with the BCAs and superior to control in enhancing the plant growth.

At 90 DAP, TR19 and P20 proved to be the most effective antagonists with respect to shoot and root growth. Application of TR19 enhanced the plant height to 67 cm and with P20 it was 65.50 (Table 22), whereas in treatment where pathogen (*Fusarium oxysporum*) alone was given it was only 53.25 cm. An increase in shoot and root weight was also observed with these antagonists. Fresh weight of shoot recorded due to individual application of TR19 and P20 at 90 DAP was 126.50 and 103.00 g and root weight obtained was 19.25 and 20.25 g respectively. In pathogen inoculated plants (control), the shoot and root weights recorded was 56.00 and 7.00 g respectively. Amongst the combinations of *Trichoderma* and *Pseudomonas*, combination of TR22 + P28 and TR22 + P20 was most effective with respect to other biometric characteristics than other combinations.

With the application of Carbendazim (0.1 %), 72.77 and 89.29 per cent increase in shoot and root weight was noticed when compared to control.

4.4.1.4 Experiment 4: Influence of Antagonists on the Incidence of Fusarium Wilt and Growth Improvement in Chilli

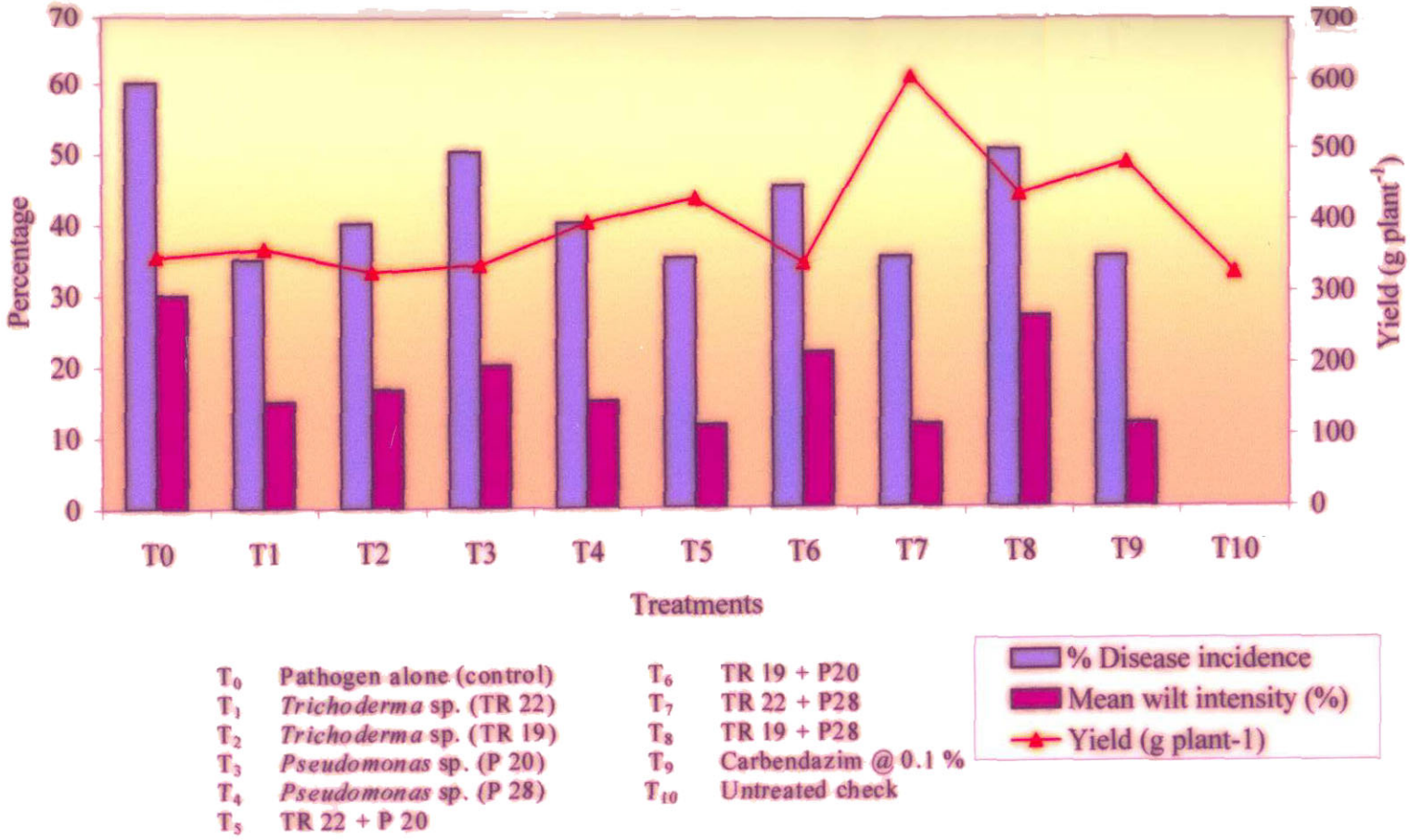
Incidence of wilt and its intensity under greenhouse conditions were reduced by different treatments (Table 23 and Fig. 7). Soil drenching with Carbendazim (0.1 %) was superior to all other treatments where only 15 per cent disease incidence was recorded, while the disease intensity was still low *i.e.*, only five per cent. When compared to the control (T_0), this was 75 and 85 percent less. In treatment where pathogen alone was inoculated (T_0) the per cent incidence and per cent intensity

Table 20. Effect of antagonists on the incidence and intensity of Fusarium wilt and yield in tomato under greenhouse condition⌖

Treatment No.	Treatments	%Disease incidence*	% variation over T_0	Mean wilt intensity (%)☒	% variation over T_0	Yield (g plant^{-1})	% Variation over T_0
T_0	Pathogen alone (control)	60.00 (50.56)	-	30.03 (5.48)	-	354.00	-
T_1	*Trichoderma* sp. (TR 22)	35.00 (36.27)	-41.67	15.00 (3.87)	-50.04	365.00	3.11
T_2	*Trichoderma* sp. (TR 19)	40.00 (39.23)	-33.33	16.68 (4.09)	-44.46	331.25	-6.43
T_3	*Pseudomonas* sp. (P 20)	50.00 (45.00)	-16.67	20.03 (4.47)	-33.31	341.25	-3.60
T_4	*Pseudomonas* sp. (P 28)	40.00 (39.23)	-33.33	14.98 (3.87)	-50.12	400.00	12.99
T_5	TR 22 + P 20	35.00 (36.27)	-41.67	11.65 (3.42)	-61.20	432.50	22.18
T_6	TR 19 + P 20	45.00 (42.13)	-25.00	21.70 (4.66)	-27.73	341.75	-3.46
T_7	TR 22 + P 28	35.00 (36.27)	-41.67	11.65 (3.42)	-61.20	605.75	71.12
T_8	TR 19 + P 28	50.00 (45.00)	-16.67	26.68 (5.16)	-11.16	437.50	23.59
T_9	Carbendazim (0.1 %)	35.00 (36.27)	-41.67	11.65 (3.42)	-61.20	481.25	35.95
T_{10}	Untreated check	-	-	-	-	327.50	-7.49
	CD (0.05)	14.34		1.27		58.98	

*Figure in parenthesis indicates angular transformation ☒ $\sqrt{x}$ transformation

⌖Mean of four replications



T_0	Pathogen alone (control)	T_6	TR 19 + P20
T_1	*Trichoderma* sp. (TR 22)	T_7	TR 22 + P28
T_2	*Trichoderma* sp. (TR 19)	T_8	TR 19 + P28
T_3	*Pseudomonas* sp. (P 20)	T_9	Carbendazim @ 0.1 %
T_4	*Pseudomonas* sp. (P 28)	T_{10}	Untreated check
T_5	TR 22 + P 20		

Fig. 3. Effect of antagonists on the incidence and intensity of Fusarium wilt and yield in tomato under greenhouse condition

Table 21. Effect of antagonists on different biometric characters of tomato infected with *F. oxysporum* at 45 DAP under greenhouse condition⊕

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0
T_0	Pathogen alone (control)	21.63	-	3.15	-	0.70	-	11.48	-	0.31	-	0.08	-
T_1	*Trichoderma* sp. (TR 22)	31.63	46.23	12.58	299.37	3.05	335.71	28.88	151.57	2.21	612.90	0.53	562.50
T_2	*Trichoderma* sp. (TR 19)	19.13	-11.56	7.30	131.75	1.76	151.43	20.13	75.35	0.75	141.94	0.20	150.00
T_3	*Pseudomonas* sp. (P 20)	32.75	51.41	16.40	420.63	4.22	502.86	22.38	94.95	2.08	570.97	0.53	562.50
T_4	*Pseudomonas* sp. (P 28)	20.83	-3.70	6.98	121.59	1.73	147.14	17.25	50.26	1.22	293.55	0.29	262.50
T_5	TR 22 + P 20	27.28	26.12	5.35	69.84	1.38	97.14	13.38	16.55	0.47	51.61	0.12	50.00
T_6	TR 19 + P 20	20.80	-3.84	2.63	-16.51	0.69	-1.43	16.35	42.42	0.54	74.19	0.14	75.00
T_7	TR 22 + P 28	19.30	-10.77	3.15	0.00	0.82	17.14	12.40	8.01	0.65	109.68	0.18	125.00
T_8	TR 19 + P 28	27.20	25.75	4.34	37.78	1.13	61.43	13.83	20.47	0.49	58.06	0.12	50.00
T_9	Carbendazim (0.1 %)	24.88	15.03	6.03	91.43	1.54	120.00	16.93	47.47	0.73	135.48	0.18	125.00
T_{10}	Untreated check	23.10	6.80	3.65	15.87	0.92	31.43	13.13	14.37	0.45	45.16	0.18	125.00
	CD (0.05)	9.51		2.77		0.98		3.83		0.69		0.22	

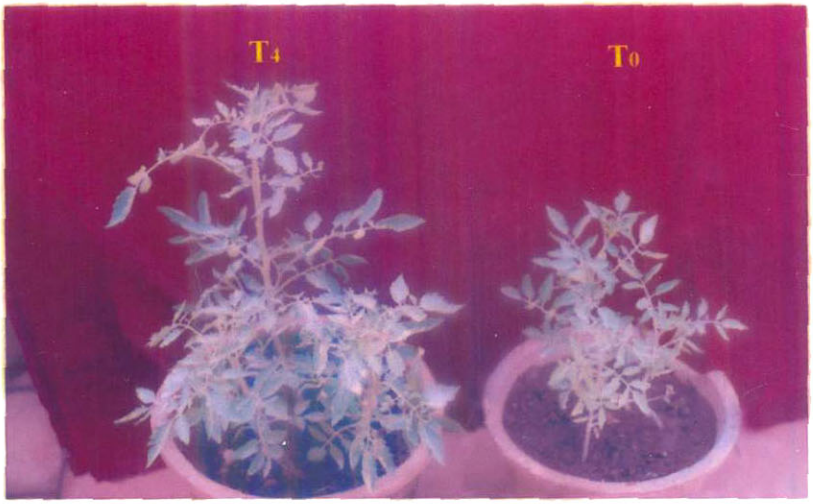
⊕ Mean of four replications DAP – Days after planting

Table 22. *Effect of antagonists on different biometric characters of tomato infected with F. oxysporum at 90 DAP under greenhouse condition*

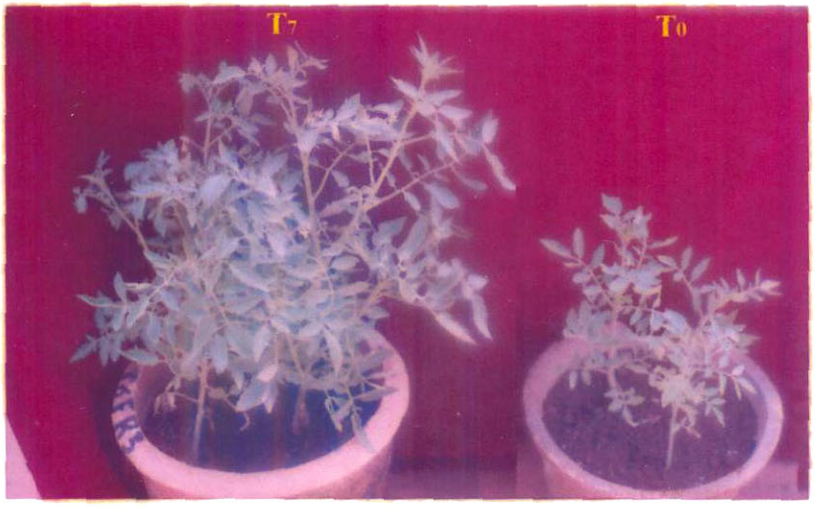
Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0	Fruit weight (g)
T_0	Pathogen alone (control)	53.25	-	56.00	-	32.50	-	26.00	7.00	-	1.31	-	12.07
T_1	*Trichoderma* sp. (TR 22)	52.50	-1.41	68.25	21.88	38.25	17.69	33.88	13.50	92.86	3.48	165.65	11.36
T_2	*Trichoderma* sp. (TR 19)	67.00	25.82	126.50	125.89	70.50	116.92	30.50	19.25	175.00	6.09	364.89	11.97
T_3	*Pseudomonas* sp. (P 20)	65.50	23.00	103.00	83.93	69.25	113.08	27.18	20.25	189.29	7.79	494.66	10.93
T_4	*Pseudomonas* sp. (P 28)	56.00	5.16	59.50	6.25	42.50	30.77	28.75	10.75	53.57	2.34	78.63	10.91
T_5	TR 22 + P 20	62.13	16.68	79.75	42.41	48.75	50.00	22.38	10.00	42.86	2.33	77.86	11.63
T_6	TR 19 + P 20	52.75	-0.94	49.00	-12.50	27.50	-15.38	27.25	7.00	0.00	1.15	-12.21	15.94
T_7	TR 22 + P 28	70.25	31.92	92.75	65.63	59.50	83.08	33.63	9.75	39.29	2.68	104.58	12.99
T_8	TR 19 + P 28	60.50	13.62	64.25	14.73	47.25	45.38	26.25	10.25	46.43	2.49	90.08	12.08
T_9	Carbendazim (0.1 %)	69.00	29.58	96.75	72.77	74.25	128.46	28.38	13.25	89.29	5.74	338.17	11.29
T_{10}	Untreated check	67.25	26.29	92.00	64.29	43.75	34.62	25.13	8.00	14.29	4.22	222.14	12.32
	CD (0.05)	8.57		29.73		17.76		NS	3.98		2.63		NS

Mean of four replications DAP – Days after planting

T_1 - TR 22 (*T. viride*) treated, T_0 - Control



T_4 - P 28 (*P. fluorescens*) treated, T_0 - Control



T_7 - TR 22 + P 28 treated, T_0 - Control

Plate 13. Effect of *Trichoderma* sp. and fluorescent pseudomonad and their combination on the growth of tomato plants infected by *F. oxysporum*

were 60 and 33.3 respectively. Significant reduction in the disease incidence was recorded with TR19 + P28 (25 %) which was statistically on par with P28 and TR22 + P28.

Amongst the biocontrol agents (BCAs) least wilt intensity was recorded with TR22 + P28 combination (10.03 %) with 69.92 per cent more protection over control. The bacterial antagonist P28 and the fungal antagonist TR22 when applied individually reduced the wilt intensity by 65.04 and 60.02 per cent respectively over control and proved better than P20 and TR19. In recording the percentage incidence and intensity of wilt TR19 was the least performing treatment. It recorded 55 per cent wilt incidence and 20 per cent wilt intensity.

Statistical analysis revealed that there was significant difference among treatments with respect to yield. All the BCAs in general improved the yield (Table 23 and Fig. 4), where the highest quantity was recorded with TR19 + P20 (322.50 g) followed by TR22 + P28 (299.50 g). Statistically these two treatments were on par. *Trichoderma* isolates TR19 and TR22 when applied alone enhanced higher yield (294.50 and 288.00 g respectively) which was on par with TR19 + P28 with 268.00 g fruits plant^{-1}. Eventhough there is no statistical difference, the isolates P28 and P20 when applied individually resulted in higher yield, compared to control. Soil drenching with Carbendazim (0.1 %) was least effective in yield maximization.

Regarding the general growth of the plant various treatments (plant height and weight) did not differ statistically at 45 DAP whereas the root length and weight showed statistical difference. But at 90 DAP significant difference was observed between the treatments with regard to shoot growth while variation among the different treatments with regard to root growth and fruit weight were statistically insignificant.

Maximum root elongation (13.03 cm) was observed in Carbendazim (0.1%) treated plants at 45 DAP (Table 24). Compared to the untreated

check, all other treatments showed a decreasing trend with regard to root growth. All the combination treatments significantly increased the fresh weight of roots at 45 DAP, maximum being recorded with TR19 + P20 (1.44 g) which was on par with Carbendazim (0.1 %) application (1.42 g). Amongst the individual application of antagonists, P28 proved most effective in increasing the root length and root weight (fresh) (9.01 cm and 0.57 g respectively). Least root weight was recorded with the pathogen control (0.35 g).

At 90 DAP, all the treatments significantly improved the shoot growth when compared to control. Combined inoculation of the antagonists (T_5, T_6, T_7 and T_8), though not statistically differ from each other, were superior to the individual applications in enhancing shoot growth and was statistically on par with the fungicide application. Regarding the shoot weight TR19 + P28 recorded the maximum fresh and dry weight of shoot (43.13 and 37.75 g respectively) immediately followed by fungicide treatment (42.88 and 32.5 g) and was superior to other treatments. Amongst the individual application of antagonists P28 recorded maximum fresh weight (33.38 g) followed by TR19 (31.15 g) whereas the dry matter content was more with TR19 (23.25 g) when compared to P28 (22.25 g) (Table 25 and Plate 14).

4.5 FIELD EVALUATION OF BIOCONTROL AGENTS (BCAS) FOR DISEASE SUPPRESSION AND GROWTH IMPROVEMENT

4.5.1 Experiment 1 : Influence of Antagonists on the Incidence of Rhizoctonia Rot and Growth Improvement in Tomato

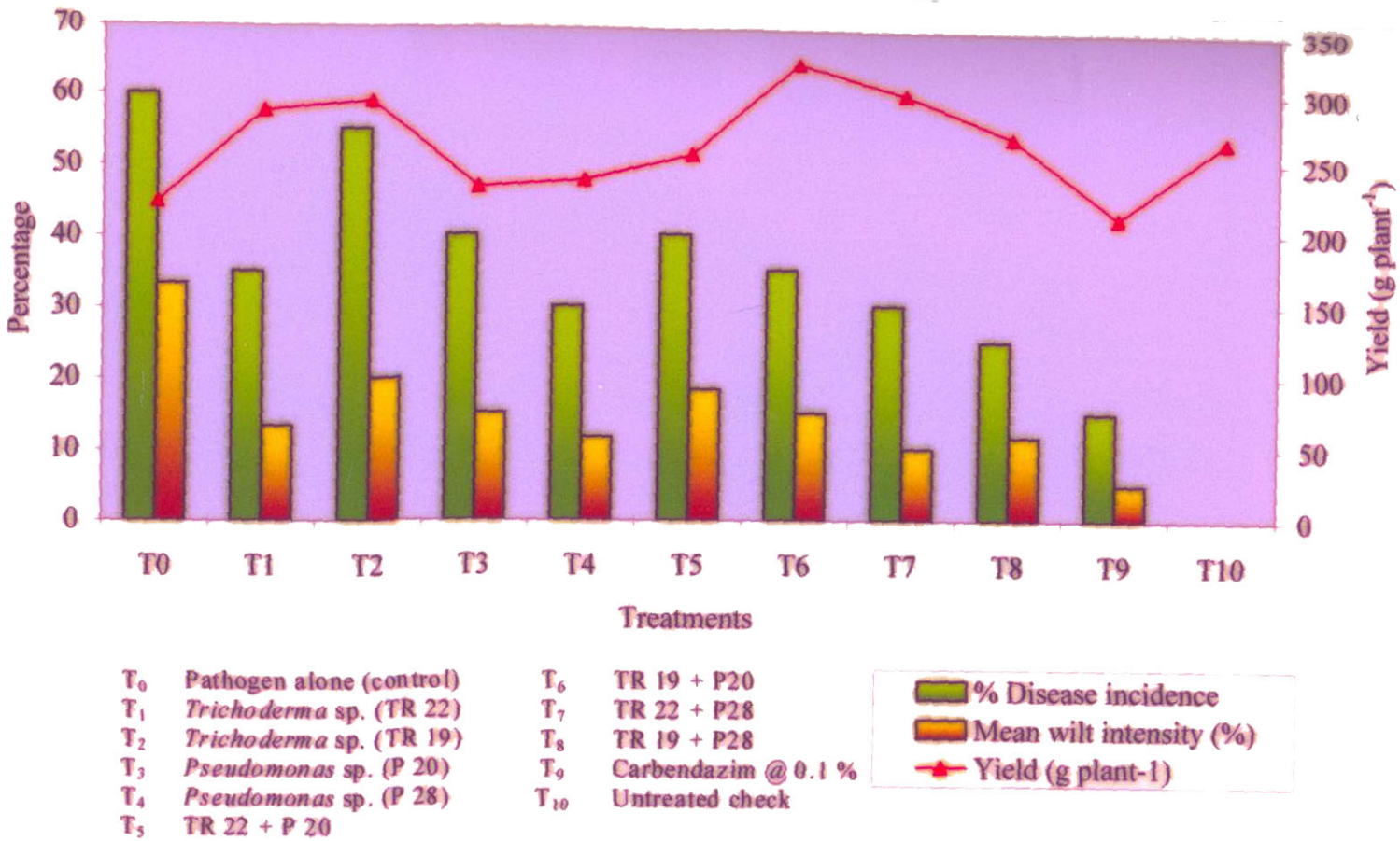
The promising isolates obtained from the preliminary *in vivo* (greenhouse) experiments were further evaluated for their efficiency on disease suppression and growth improvement under field conditions. Results of the data presented (Table 26 and Fig. 5) revealed that all the treatments tried were more or less effective in suppressing the rotting symptoms caused by *R. solani*. *Trichoderma*, TR20 and *Pseudomonas*, P28

Table 23. Effect of antagonists on the incidence and intensity of Fusarium wilt and yield in chilli under greenhouse condition

Treatment No.	Treatments	%Disease incidence*	% variation over T_0	Mean wilt intensity (%)☒	% variation over T_0	Yield (g plant^{-1})	% Variation over T_0
T_0	Pathogen alone (control)	60 (50.77)	-	33.33 (5.77)	-	224.25	-
T_1	*Trichoderma* sp. (TR 22)	35 (36.27)	-41.67	13.33 (3.65)	-60.02	288.00	28.43
T_2	*Trichoderma* sp. (TR 19)	55 (47.87)	-8.33	20.00 (4.47)	-39.98	294.50	31.33
T_3	*Pseudomonas* sp. (P 20)	40 (39.23)	-33.33	15.00 (3.87)	-54.99	233.25	4.01
T_4	*Pseudomonas* sp. (P 28)	30 (33.21)	-50.00	11.65 (3.42)	-65.04	238.00	6.13
T_5	TR 22 + P 20	40 (39.23)	-33.33	18.33 (4.28)	-45.01	256.00	14.16
T_6	TR 19 + P 20	35 (36.27)	-41.67	15.03 (3.87)	-54.91	322.50	43.81
T_7	TR 22 + P 28	30 (33.21)	-50.00	10.03 (3.16)	-69.92	299.50	33.56
T_8	TR 19 + P 28	25 (30.00)	-58.30	11.68 (3.42)	-64.97	268.00	19.51
T_9	Carbendazim (0.1 %)	15 (22.79)	-75.00	5.00 (2.23)	-85.00	211.25	-5.80
T_{10}	Untreated check	-	-	-	-	266.00	18.62
	CD (0.05)	18.07		1.98		60.22	

*Figure in parenthesis indicates angular transformation ☒ $\sqrt{x}$ transformation

Mean of four replications



T_0	Pathogen alone (control)	T_6	TR 19 + P20
T_1	*Trichoderma* sp. (TR 22)	T_7	TR 22 + P28
T_2	*Trichoderma* sp. (TR 19)	T_8	TR 19 + P28
T_3	*Pseudomonas* sp. (P 20)	T_9	Carbendazim @ 0.1 %
T_4	*Pseudomonas* sp. (P 28)	T_{10}	Untreated check
T_5	TR 22 + P 20		

Fig. 4. Effect of antagonists on the incidence and intensity of Fusarium wilt and yield in chilli under greenhouse condition

Table 24. Effect of antagonists on different biometric characters of chilli infected with F. solani at 45 DAP under greenhouse condition

Treatment No.	Treatments	Plant height (cm)	Shoot weight fresh (g)	Shoot weight dry (g)	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)
T_0	Pathogen alone (control)	15.98	3.12	0.58	6.78	-	0.35	-	0.08
T_1	*Trichoderma* sp. (TR 22)	17.25	2.70	0.65	6.03	-11.06	0.38	8.57	0.16
T_2	*Trichoderma* sp. (TR 19)	19.25	2.80	0.47	8.48	25.07	0.43	22.86	0.08
T_3	*Pseudomonas* sp. (P 20)	15.50	2.20	0.43	8.75	29.06	0.43	22.86	0.09
T_4	*Pseudomonas* sp. (P 28)	18.05	2.64	0.43	9.01	32.89	0.57	62.86	0.12
T_5	TR 22 + P 20	19.25	5.05	0.62	8.75	29.06	0.98	180.00	0.10
T_6	TR 19 + P 20	17.75	3.03	0.46	9.28	36.87	1.44	311.43	0.11
T_7	TR 22 + P 28	19.70	4.05	0.42	6.80	0.29	1.05	200.00	0.07
T_8	TR 19 + P 28	17.88	3.95	0.44	9.15	34.96	1.10	214.29	0.11
T_9	Carbendazim (0.1 %)	23.63	6.38	0.87	13.03	92.18	1.42	305.71	0.18
T_{10}	Untreated check	22.00	5.28	0.91	11.15	64.45	1.50	328.57	0.19
	CD (0.05)	NS	NS	NS	3.74		0.61		NS

Mean of four replications DAP - Days after planting

Table 25. Effect of antagonists on different biometric characters of chilli infected with *F. solani* at 90 DAP under greenhouse condition*

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	Root weight dry (g)	% variation over T_0	Fruit weight fresh (g)	Fruit weight dry (g)
T_0	Pathogen alone (control)	20.63	-	28.15	-	19.08	-	16.12	3.38	0.83	-	2.63	0.62
T_1	*Trichoderma* sp. (TR 22)	22.13	7.27	22.40	-20.43	19.18	0.52	13.23	2.30	0.47	-43.37	3.45	0.82
T_2	*Trichoderma* sp. (TR 19)	27.75	34.51	31.15	10.66	23.25	21.86	18.45	7.28	1.30	56.63	3.53	0.82
T_3	*Pseudomonas* sp. (P 20)	21.50	4.22	24.98	-11.26	21.25	11.37	13.00	3.60	0.57	-31.33	2.73	0.66
T_4	*Pseudomonas* sp. (P 28)	27.28	32.23	33.38	18.58	22.25	16.61	16.33	3.50	0.74	-10.84	2.98	0.70
T_5	TR 22 + P 20	30.50	47.84	42.25	50.09	29.50	54.61	17.85	5.60	1.02	22.89	3.80	0.91
T_6	TR 19 + P 20	34.13	65.44	39.95	41.92	28.25	48.06	16.65	6.58	1.28	54.22	2.98	0.72
T_7	TR 22 + P 28	28.87	39.94	34.10	21.14	22.50	17.92	12.65	6.13	0.58	-30.12	3.60	0.87
T_8	TR 19 + P 28	33.88	64.23	43.13	53.21	37.75	97.85	17.28	7.30	1.49	79.52	3.63	0.88
T_9	Carbendazim (0.1 %)	40.00	93.89	42.88	52.33	32.50	70.34	16.17	7.45	2.02	143.37	2.65	0.64
T_{10}	Untreated check	34.50	67.23	32.08	13.96	26.00	36.27	19.45	6.95	1.56	87.95	3.80	0.92
	CD (0.05)	8.14		9.99		6.38		NS	NS	0.73		NS	NS

* Mean of four replications DAP - Days after planting

T1 - TR 22 (*T. viride*) treated, T0 - Control

T4 - P 28 (*P. fluorescens*) treated, T0 - Control

T7 - TR 22 + P 28 treated, T0 - Control

Plate 14. Effect of *Trichoderma* sp. and Fluorescent pseudomonads and their combination on the growth of chilli plants infected by *F. solani*

reduced the disease incidence by 33.34 and 19.05 per cent respectively but their combination offered an additive effect with 57.14 per cent protection over control. Soil drenching with COC (0.3 %) was the most effective treatment in reducing the rot incidence of seedlings in such a highly heterogenous condition. Applying COC could protect the seedlings by 90.48 per cent when compared to control.

A significant increase in the yield of tomato was observed due to different treatments. Individual application of *Trichoderma* recorded the maximum yield of 855.50 g (Table 26 and Fig. 5) which was significantly superior to all other treatments. Combined application of TR20 + P28 was the next best treatment with 770.50 g but statistically it was not significant to fungicide treatment and *Pseudomonas* applications. The lowest yield of 690.75 g was recorded in the control. Among the BCAs, the yield obtained was lowest with *Pseudomonas* P28 (721.25 g).

Plant growth was also influenced by the application of BCAs. Plants treated with TR20 recorded maximum shoot length at 45 DAP (29.88 cm), followed by the combination of TR20 and P28 (28.88 cm). Statistically these two treatments did not differ significantly. Individual application also do not differ significantly with regard to shoot weight. They were statistically on par with fungicide application. Among the different treatments, the combined application of *Trichoderma* and *Pseudomonas* recorded the highest shoot weight (fresh) (142.69 g) but the dry matter content recorded maximum with P28 application (41.86 g). Least shoot weight at 45 DAP was recorded with the control and untreated check (Table 27).

Regarding the root length, maximum elongation of roots at 45 DAP (27.08 cm) was noticed with P28, which was significantly high over others. All other treatments were statistically on par with control. Root weight at 45 DAP was also maximum with P28 (17.42 g) followed by TR20 (16.00 g) and their combination (14.84 g).

At 90 DAP no significant difference was observed between antagonist application and chemical treatment with respect to plant height. However, the maximum shoot weight was recorded with the combination of TR20 + P28 (250.48 g) followed by TR20 (238.90 g) whereas the maximum dry weight of the shoot was observed with P28 (78.97) followed by TR20 (76.56 g) (Table 28).

The highest root length recorded by TR20 + P28 (46.48 cm) at 90 DAP was statistically superior to other treatments. P28 recorded next best in the series with a root length of 39.6 cm and root weight (fresh) of 32.23 g. In dry matter content TR20 recorded highest (5.24 g) followed by P28 (4.96 g).

Increased fruit weight was observed with the combined antagonistic treatment. Maximum fruit weight was recorded in TR20 + P28 application (22.83 g) followed by TR20 (22.05 g).

4.5.2 Experiment 2 : Influence of Antagonists on the Incidence of Rhizoctonia Rot and Growth Improvement in Chilli

Trichoderma TR20 and *Pseudomonas* P28 which were promising in controlling the rot disease in chilli in the greenhouse experiment were further evaluated under field condition. The treatments showed statistical difference with respect to per cent disease incidence and yield. Results of the data obtained (Table 29 and Fig. 6) showed that among the various treatments, the percentage of seedling rot incited by *R. solani* was least with TR20 application (22.98 %) which was about 42.76 per cent less when compared to control. It was followed by TR20 + P28 (34.38 %) and fungicide application (37.35 %) but they were statistically on par with control. Least protection of seedlings was recorded with P28 application.

Highest yield was recorded with TR20 + P28 application (370.25 g) (Table 29 and Fig. 6). A reduction in the yield over control was observed with fungicide application and the BCAs when applied individually.

Table 26. Effect of antagonists on the incidence of Rhizoctonia rot and yield in tomato under field condition

Treatment No.	Treatments	% Disease incidence*	% variation over T_0	Yield (g plant^{-1})	% variation over T_0
T_0	Pathogen alone (control)	65.63 (54.11)	-	690.75	-
T_1	*Trichoderma* sp. (TR 20)	43.75 (41.41)	-33.34	855.50	23.85
T_2	*Pseudomonas* sp. (P 28)	53.13 (46.79)	-19.05	721.25	4.42
T_3	TR 20 + P 28	28.13 (32.03)	-57.14	770.50	11.55
T_4	Copper oxychloride (0.3%)	6.25 (14.48)	-90.48	724.00	4.81
T_5	Untreated check	-	-	767.25	11.07
	CD (0.05)	18.23		71.62	

*Figure in parenthesis indicates angular transformation

Mean of four replications

Table 27. Effect of antagonists on different biometric characters of tomato infected with *R solani* at 45 DAP under field condition

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)
T_0	Pathogen alone (control)	22.63	-	82.26	-	22.65	-	14.48	-	8.92	-	1.75
T_1	*Trichoderma* sp. (TR 20)	29.88	32.04	107.01	30.09	40.83	80.26	15.20	4.97	16.00	79.37	2.85
T_2	*Pseudomonas* sp. (P 28)	23.43	3.54	126.75	54.08	41.86	84.81	27.08	87.02	17.42	95.29	2.90
T_3	TR 20 + P 28	28.88	27.62	142.69	73.46	34.68	53.11	17.95	23.96	14.84	66.37	2.28
T_4	Copper oxychloride (0.3%)	25.75	13.79	124.53	51.39	28.93	27.73	15.55	7.39	10.44	17.04	2.28
T_5	Untreated check	27.50	21.52	82.26	0.00	25.83	14.04	15.23	5.18	9.84	10.31	1.65
	CD (0.05)	4.23		39.9		13.62		4.67		5.91		NS

Mean of four replications DAP – Days after planting

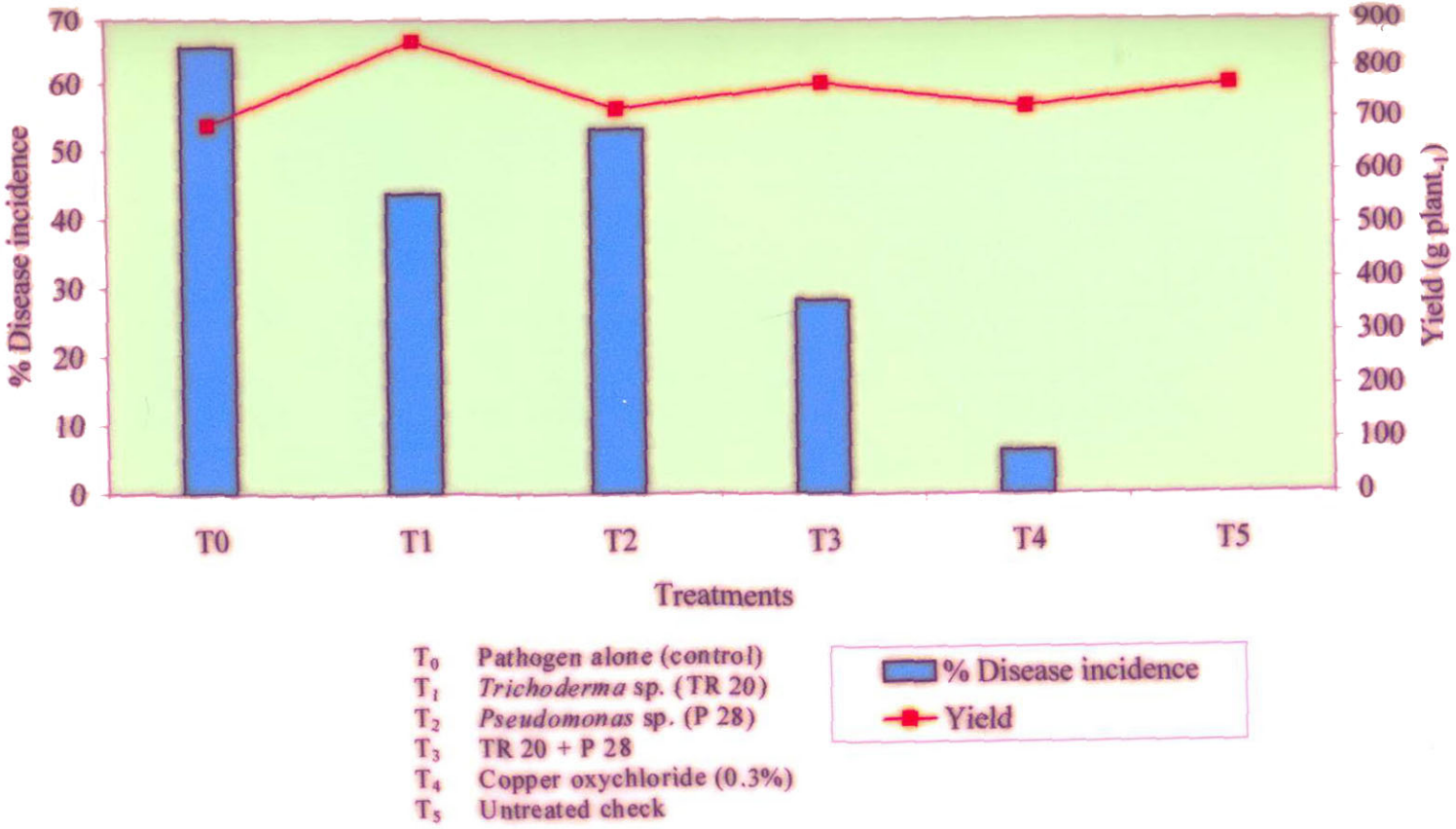


Fig. 5. Effect of antagonists on the incidence of Rhizoctonia rot and yield in tomato under field condition

Table 28. Effect of antagonists on different biometric characters of tomato infected with *R solani* at 90 DAP under field condition⁺

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0	Fruit weight (g)	% variation over T_0
T_0	Pathogen alone (control)	62.63	-	163.49	-	49.84	-	26.00	-	20.83	-	3.01	-	18.35	-
T_1	*Trichoderma* sp. (TR 20)	84.25	34.52	238.90	46.13	76.56	53.61	35.58	36.85	31.63	51.85	5.24	74.09	22.05	20.16
T_2	*Pseudomonas* sp. (P 28)	75.88	21.16	225.45	37.90	78.97	58.45	39.60	52.31	32.23	54.73	4.96	64.78	20.75	13.08
T_3	TR 20 + P 28	85.63	36.72	250.48	53.21	69.61	39.67	46.48	78.77	28.10	34.90	4.73	57.14	22.83	24.41
T_4	Copper oxychloride (0.3%)	89.25	42.50	234.60	43.50	62.41	25.22	37.25	43.27	22.25	6.82	3.33	10.63	20.65	12.53
T_5	Untreated check	86.50	38.11	181.41	10.96	52.46	5.26	35.88	38.00	24.49	17.57	4.70	56.15	17.25	-5.99
	CD (0.05)	10.25		40.57		22.17		6.17		8.14		1.43		3.33	

⁺ Mean of four replications DAP – Days after planting

Statistical analysis revealed that various parameters *viz.*, shoot length at 45 DAP, root length and weight at 45 and 90 DAP and fruit weight did not show any significant difference among treatments. However statistical difference was noticed among treatments with respect to shoot length at 90 DAP and shoot weight at 45 and 90 DAP.

Among the antagonists tried *Trichoderma* (TR20) applied plots recorded maximum shoot weight (22.79 g) at 45 DAP which was statistically on par with control (Pathogen alone) (28.50 g). All other treatments recorded a lower shoot weight (fresh) than control (Table 30). Highest dry weight of shoot was recorded with TR20 (14.25 g) which was statistically on par with untreated check (11.39 g) but was superior to all other treatments including control.

At 90 DAP, maximum elongation of the plant was noticed with TR20 application (55 cm). All other treatments showed a reduction in plant height when compared to control. The effect of combined inoculation of the antagonists was more pronounced in shoot weight where the highest weight of 145.1 g was recorded with TR20 + P28 (Table 31) followed by TR20 application (132.93 g). A similar trend was recorded for shoot dry weight where the highest weight (70.78 g) was recorded with TR20 + P28, followed by TR20 application (65.93 g). The least records of plant growth parameters were with individual application of P28 which always seemed to be less than control.

4.5.3 Experiment 3 : Influence of Antagonists on the Incidence of Fusarium Wilt and Growth Improvement in Tomato

Promising isolates obtained from the greenhouse studies were subjected to further field evaluation. In general, there was a reduction in the wilt incidence due to various treatments tried. With regard to wilt incidence and intensity, various treatments did not show any significant difference among them. However, fungicide application recorded the least wilt incidence (41.65 %) followed by the combined application of

Table 29. Effect of antagonists on the incidence of Rhizoctonia rot and yield in chilli under field condition

Treatment No.	Treatments	% Disease incidence*	% variation over T_0	Yield (g plant^{-1})	% variation over T_0
T_0	Pathogen alone (control)	40.15 (39.32)	-	330.50	-
T_1	*Trichoderma* sp. (TR 20)	22.98 (28.64)	-42.76	329.50	-0.30
T_2	*Pseudomonas* sp. (P 28)	40.23 (39.37)	0.20	273.25	-17.32
T_3	TR 20 + P 28	34.38 (35.89)	-14.37	370.25	12.03
T_4	Copper oxychloride (0.3%)	37.35 (37.67)	-6.97	305.50	-7.56
T_5	Untreated check	-	-	331.75	0.38
	CD (0.05)	10.18		39.07	

*Figure in parenthesis indicates angular transformation

Mean of four replications

Table 30. Effect of antagonists on different biometric characters of chilli infected with *R solani* at 45 DAP under field condition

Treatment No.	Treatments	Plant height (cm)	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	Root weight dry (g)
T_0	Pathogen alone (control)	23.00	28.50	-	8.23	-	10.58	2.63	0.82
T_1	*Trichoderma* sp. (TR 20)	26.70	22.79	-20.04	14.25	73.15	11.33	2.35	0.90
T_2	*Pseudomonas* sp. (P 28)	22.50	17.20	-39.65	8.45	2.67	10.48	2.11	0.73
T_3	TR 20 + P 28	25.30	16.67	-41.51	8.68	5.47	14.20	2.36	0.60
T_4	Copper oxychloride (0.3%)	19.45	15.53	-45.51	7.70	-6.44	10.13	2.18	0.60
T_5	Untreated check	21.88	18.41	-35.40	11.39	38.40	11.40	2.38	0.79
	CD (0.05)	NS	8.29		3.98		NS	NS	NS

Mean of four replications DAP – Days after planting

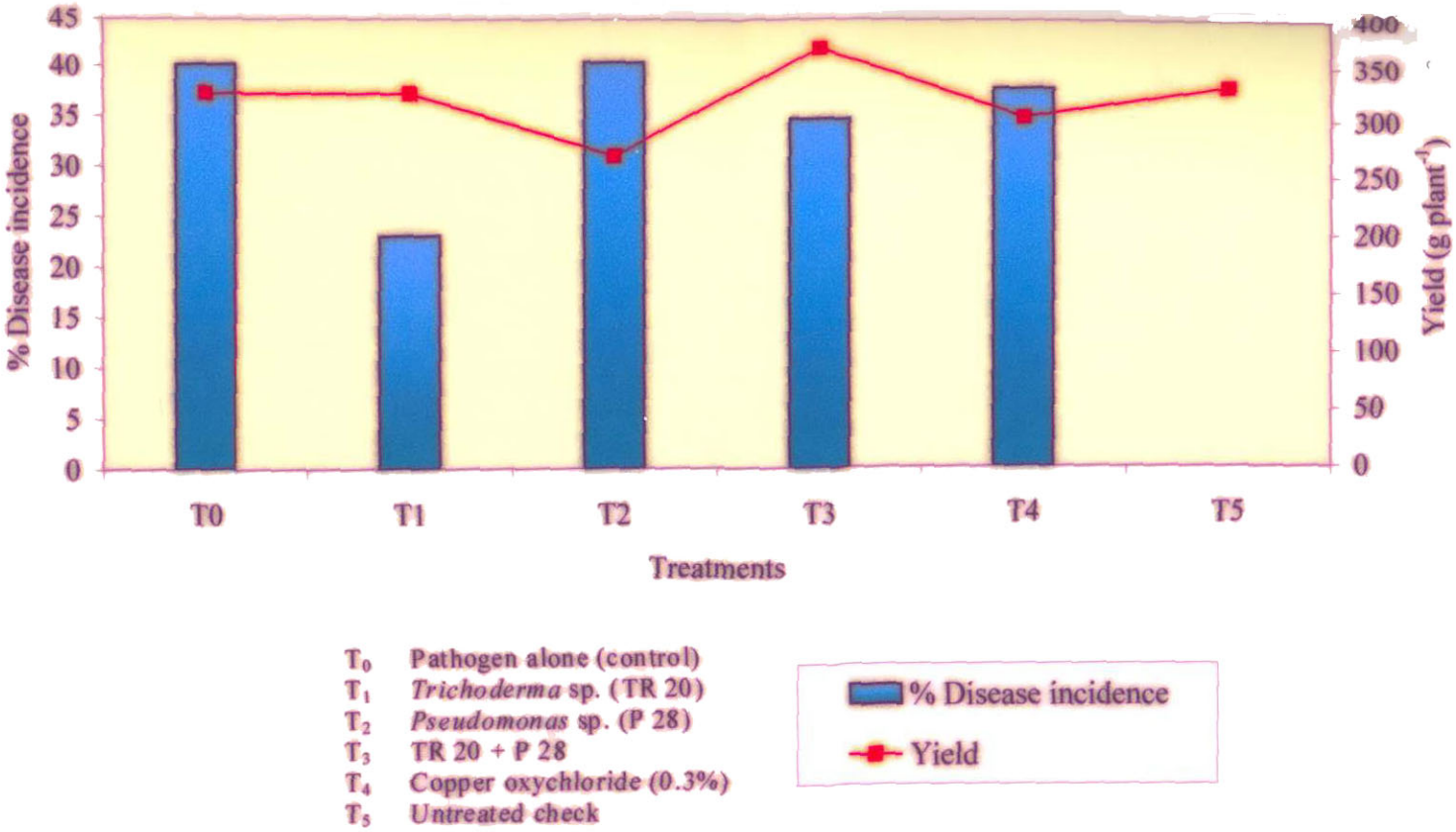


Fig. 6. Effect of antagonists on the incidence of Rhizoctonia rot and yield in chilli under field condition

Table 31. Effect of antagonists on different biometric characters of chilli infected with *R solani* at 90 DAP under field condition ⊕

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	Root weight dry (g)	Fruit weight fresh (g)	Fruit weight dry(g)
T_0	Pathogen alone (control)	52.25	-	83.43	-	53.88	-	22.48	12.98	3.35	3.77	0.86
T_1	*Trichoderma* sp. (TR 20)	55.00	5.26	132.93	59.33	65.93	22.36	21.20	11.20	4.85	5.25	1.18
T_2	*Pseudomonas* sp. (P 28)	41.50	-20.57	78.16	-6.32	36.08	-33.04	17.88	10.75	3.77	4.63	1.05
T_3	TR 20 + P 28	49.50	-5.26	145.10	73.92	70.78	31.37	27.28	11.00	5.13	5.00	1.09
T_4	Copper oxychloride (0.3%)	42.50	-18.66	77.78	-6.77	43.58	-19.12	17.53	11.09	3.30	4.56	0.95
T_5	Untreated check	42.75	-18.18	88.95	6.62	46.15	-14.35	21.58	10.95	3.78	4.73	0.99
	CD (0.05)	8.93		32.47		15.64		NS	NS	NS	NS	NS

⊕ Mean of four replications DAP – Days after planting

Trichoderma and *Pseudomonas* (TR20 + P28) (45.83 %) which was 44.49 and 38.92 per cent less when compared to control where 75.03 per cent wilt incidence was observed. A similar trend was observed in case of percentage wilt intensity also, where the disease intensity was minimum (16.68 %) with Carbendazim (0.1 %) application. The intensity of wilt in plants inoculated with fluorescent pseudomonad P28 was 22.23 per cent compared to 34.73 per cent observed in control. Inoculation with this bacterial isolate offered a protection of 35.95 per cent over control and proved superior to others (Table 32 and Fig. 7).

The growth of tomato plants was enhanced by the application of biocontrol agents. This was more pronounced with the combined inoculation of fungal and bacterial bioagents than that of their individual application. Statistical analysis showed that various treatments did not differ significantly with respect to biometric characters like yield, fruit weight, plant height and root growth at 90 DAP. Significant difference could be noticed for all other characters studied.

The maximum plant height at 45 DAP was noticed in plants inoculated with TR22 + P28. It recorded maximum plant height of 29.25 cm whereas the control (pathogen inoculated) showed a height of only 21.25 cm which was 37.65 per cent more over control. This was followed by P28 application (28.25 cm) where the plant height was statistically on par with TR22 application (27.68 cm) and fungicide treatment (27.00 cm) (Table 33). Fresh and dry weight of the shoot at 45 DAP was maximum (84.25 and 22.03 g respectively) with the combined inoculation of TR22 + P28. This was followed by TR22 application. In Carbendazim (0.1 %) applied plots the shoot weight was slightly higher than TR22 (Table 33). Among the different treatments P28 recorded least shoot weight (both fresh and dry) at 45 DAP, but was superior to control.

Combined inoculation of *Trichoderma* (TR22) and *Pseudomonas* (P28) resulted in increased root length at 45 DAP (16.88 cm). All the

treatments were statistically on par but superior to control. Plants inoculated with *Pseudomonas* (P28) had a root fresh weight of 15.93 g which was 88.97 per cent more over control. This was closely followed by the combination treatment of TR22 + P28 with 15.4 g. The dry weight of root was maximum (4.09 g) with TR22 + P28 application and minimum (2.19 g) in control plants. Bacterial antagonist P28 alone resulted in an increased root dry weight of 3.86 g, which was 76.26 per cent more over control (Table 33).

At 90 DAP, the plants inoculated with TR22 recorded with maximum fresh weight (250.25 g) and dry weight (63.80 g) of shoots. This was followed by the application of TR22 + P28 and P28 applied alone where no statistical difference was observed but they were superior to control (Table 34).

4.5.4 Experiment 4: Influence of Antagonists on the Incidence of Fusarium Wilt and Growth Improvement in Chilli

Results of the data presented in the table (Table 35 and Fig. 8) revealed that all the treatments were effective in reducing the wilt incidence of chilli under field conditions. Significantly lower incidence was observed for treatment with TR22 + P28, P28 (applied alone) and Carbendazim, 0.1 per cent. Only 41.65 per cent disease incidence was observed with P28 application whereas in control the disease incidence recorded was 79.18 per cent. Combined application of TR22 and P28 and fungicide treatment recorded lower incidence of 54.18 and 66.68 per cent respectively. Maximum reduction in disease intensity was recorded in plants treated with the combination of TR22 + P28 (20.85) which was 49.94 per cent less than control where 41.65 per cent wilt intensity was recorded. Inoculation with *Pseudomonas*, P28 alone also reduced the wilt intensity by 46.64 per cent which was on par with Carbendazim application.

Table 32. Effect of antagonists on the incidence and intensity of Fusarium wilt and yield in tomato under field condition

Treatment No.	Treatments	% Disease incidence*	% variation over T_0	Mean wilt intensity (%)☒	% variation over T_0	Yield (g plant^{-1})
T_0	Pathogen alone (control)	75.03 (60.02)	-	34.73 (5.89)	-	711.00
T_1	*Trichoderma* sp. (TR 22)	50.00 (45.00)	-33.36	26.38 (5.14)	-23.99	731.25
T_2	*Pseudomonas* sp. (P28)	50.00 (45.00)	-33.36	22.23 (4.71)	-35.95	639.75
T_3	TR 22 + P28	45.83 (42.61)	-38.92	27.78 (5.27)	-19.96	783.00
T_4	Carbendazim (0.1 %)	41.65 (40.19)	-44.49	16.68 (4.09)	-51.95	757.50
T_5	Untreated check	-	-	-	-	665.00
	CD (0.05)	15.90		1.17		NS

*Figure in parenthesis indicates angular transformation ☒ $\sqrt{x}$ transformation

*Mean of four replications

Table 33. Effect of antagonists on different biometric characters of tomato infected with *F. oxysporum* at 45 DAP under field condition*

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	% variation over T_0	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0
T_0	Pathogen alone (control)	21.25	-	50.50	-	13.95	-	10.35	-	8.43	-	2.19	-
T_1	*Trichoderma* sp. (TR 22)	27.68	30.26	68.00	34.65	17.93	28.53	16.18	56.33	12.80	51.84	3.21	46.58
T_2	*Pseudomonas* sp. (P28)	28.25	32.94	63.50	25.74	16.90	21.15	14.85	43.48	15.93	88.97	3.86	76.26
T_3	TR 22 + P28	29.25	37.65	84.25	66.83	22.03	57.92	16.88	63.09	15.40	82.68	4.09	86.76
T_4	Carbendazim (0.1 %)	27.00	27.06	71.50	41.58	18.68	33.91	14.85	43.48	12.88	52.79	3.49	59.36
T_5	Untreated check	28.00	31.76	65.50	29.70	17.14	22.87	16.75	61.84	9.63	14.23	2.56	16.89
	CD (0.05)	4.16		16.87		4.14		3.90		4.63		1.29	

*Mean of four replications DAP – Days after planting

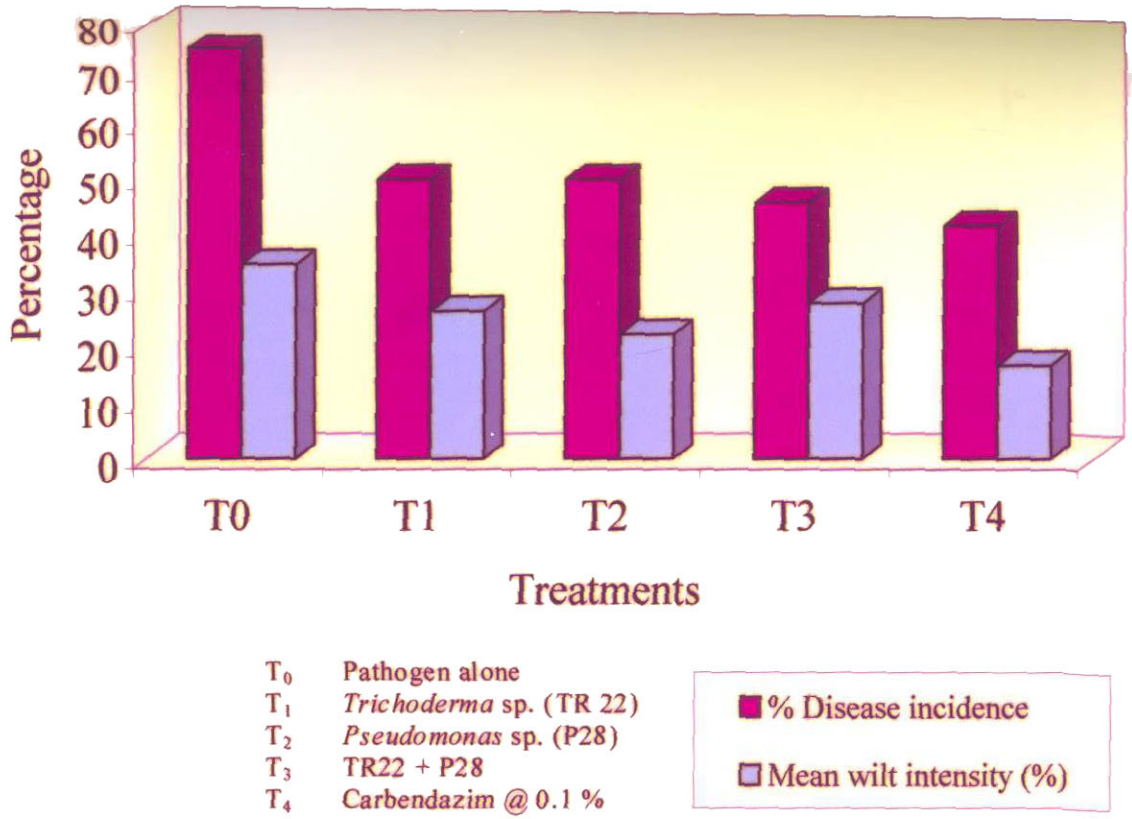


Fig. 7. Effect of antagonists on the incidence and intensity of Fusarium wilt in tomato under field condition

Table 34. Effect of antagonists on different biometric characters of tomato infected with *F. oxysporum* at 90 DAP under field condition⊕

Treatment No.	Treatments	Plant height (cm)	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	Fruit weight (g)
T_0	Pathogen alone (control)	87.00	184.75	-	46.89	-	26.25	22.10	-	4.03	16.26
T_1	*Trichoderma* sp. (TR 22)	99.50	250.25	35.45	63.80	36.06	23.63	30.58	38.37	5.03	19.18
T_2	*Pseudomonas* sp. (P28)	85.25	223.00	20.70	57.73	23.12	28.50	31.33	41.76	5.43	20.39
T_3	TR 22 + P28	91.25	227.75	23.27	57.04	21.65	28.25	30.93	39.95	5.55	21.06
T_4	Carbendazim (0.1 %)	91.25	220.75	19.49	51.53	9.90	23.75	31.90	44.34	5.25	18.33
T_5	Untreated check	90.25	201.00	8.80	51.20	9.19	22.45	21.45	-2.94	4.89	19.89
	CD (0.05)	NS	26.85		5.40		NS	7.41		NS	NS

⊕Mean of four replications DAP – Days after planting

The maximum plant height at 45 DAP was observed for the treatment with P28 alone (21.90 cm). This was followed by the combination of TR22 + P28 (21.80 cm). A significant increase in shoot length over control was observed with *Trichoderma* TR22 and Carbendazim application (Table 36). The combined inoculation of *Trichoderma* and *Pseudomonas* increased the shoot weight (fresh) (22.00 g) which was on par with the individual application of P28. However the maximum dry matter content of shoots (5.95 g) was recorded with P28 applied plots followed by TR22 (5.88 g).

Plants inoculated with P28 also showed an increase in root weight (fresh) (4.97 g) at 45 DAP, followed by the combination of TR22 + P28 (4.60 g) and the individual treatment of *Trichoderma* TR22 (4.32 g). Statistically these treatments do not differ significantly.

At 90 DAP, individual and combined application of *Trichoderma* and *Pseudomonas* showed an increase in plant height and weight. No significant difference was observed between these treatments (Table 37) but was superior to control. *Pseudomonas*, P28 applied plots recorded the maximum plant height (48.25 cm) and fresh weight (63.50 g). The dry matter content of shoot was maximum in the combined application of TR22 + P28 (26.45 g).

Fresh and dry weights of the root at 90 DAP recorded maximum in TR22 applied plots (7.43 and 2.16 g respectively) followed by TR22 + P28 application.

4.6 CHARACTERIZATION AND IDENTIFICATION OF THE PROMISING ANTAGONISTS

4.6.1 *Trichoderma* sp.

Based on the cultural, morphological and microscopic characters, *Trichoderma* isolates, TR17, TR20, TR19 and TR22 were identified as *T. pseudokoningii, T. harzianum, T. viride* and *T. viride* respectively.

Table 35. Effect of antagonists on the incidence and intensity of Fusarium wilt and yield in chilli under field condition

Treatment No.	Treatments	% Disease incidence*	% variation over T_0	Mean wilt intensity (%)☒	% variation over T_0	Yield (g plant^{-1})
T_0	Pathogen alone (control)	79.18 (62.85)	-	41.65 (6.46)	-	163.50
T_1	*Trichoderma* sp. (TR 22)	66.68 (54.74)	-15.79	31.93 (5.65)	-23.35	174.00
T_2	*Pseudomonas* sp. (P28)	41.65 (40.19)	-47.40	22.23 (4.71)	-46.64	185.75
T_3	TR 22 + P28	54.18 (47.39)	-31.58	20.85 (4.57)	-49.94	175.75
T_4	Carbendazim (0.1 %)	66.68 (54.74)	-15.79	25.00 (5.00)	-39.98	192.75
T_5	Untreated check	-	-	-	-	181.00
	CD (0.05)	17.91		1.09		NS

*Figure in parenthesis indicates angular transformation ☒ $\sqrt{x}$ transformation

⌖ Mean of four replications

Table 36. Effect of antagonists on different biometric characters of chilli infected with *F. solani* at 45 DAP under field condition⌖

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	% variation over T_0	Root weight dry (g)
T_0	Pathogen alone (control)	17.50	-	16.75	-	4.68	-	6.60	3.43	-	0.99
T_1	*Trichoderma* sp. (TR 22)	19.10	9.14	19.50	16.42	5.88	25.64	6.90	4.32	25.95	1.18
T_2	*Pseudomonas* sp. (P28)	21.90	25.14	20.25	20.90	5.95	27.14	6.70	4.97	44.90	1.56
T_3	TR 22 + P28	21.80	24.57	22.00	31.34	5.02	7.26	6.40	4.60	34.11	1.52
T_4	Carbendazim (0.1 %)	19.03	8.74	19.50	16.42	5.60	19.66	6.80	3.30	-3.79	1.17
T_5	Untreated check	18.28	4.46	17.25	2.99	4.58	-2.14	5.90	3.60	4.96	1.19
	CD (0.05)	2.88		2.29		0.69		NS	1.123		NS

⌖ Mean of four replications DAP – Days after planting

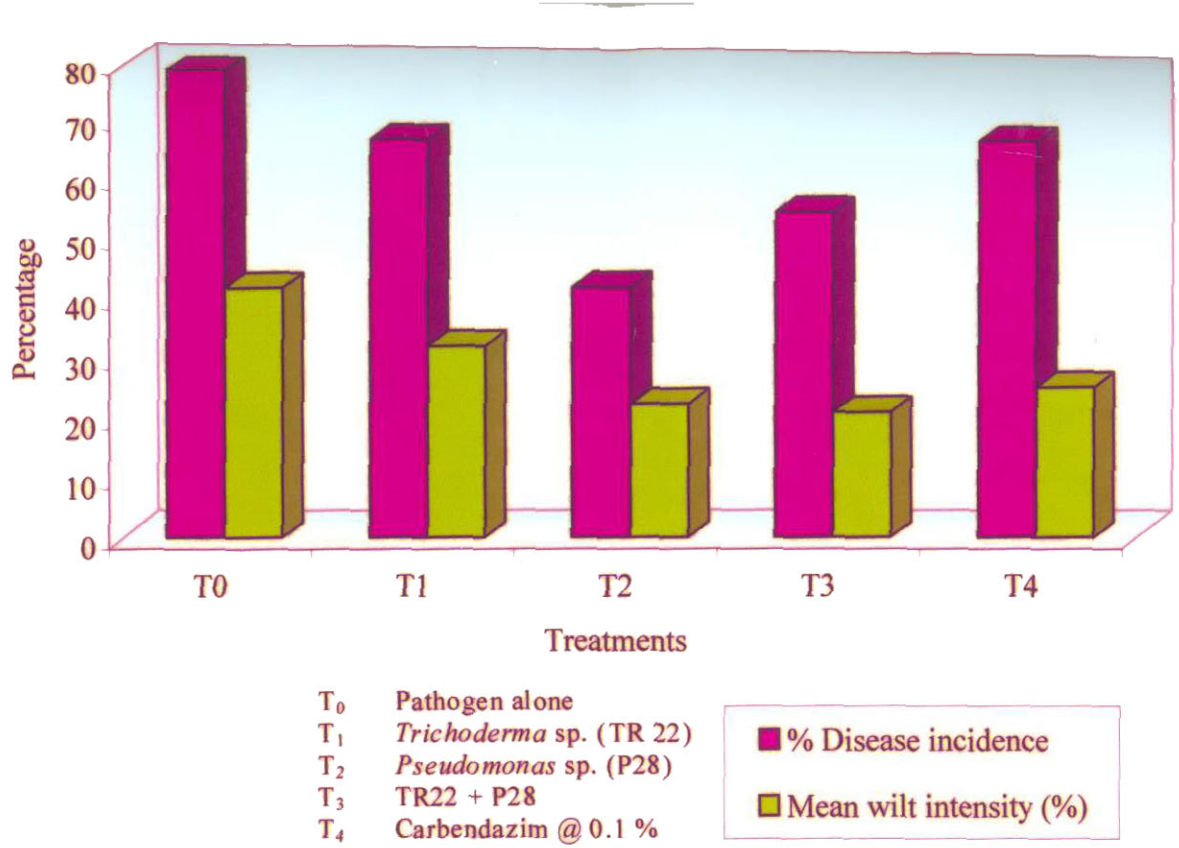


Fig. 8. Effect of antagonists on the incidence and intensity of Fusarium wilt in chilli under field condition

Table 37. Effect of antagonists on different biometric characters of chilli infected with *F. solani* at 90 DAP under field condition⊕

Treatment No.	Treatments	Plant height (cm)	% variation over T_0	Shoot weight fresh (g)	% variation over T_0	Shoot weight dry (g)	% variation over T_0	Root length (cm)	Root weight fresh (g)	% variation over T_0	Root weight dry (g)	% variation over T_0	Fruit weight fresh(g)	Fruit weight dry (g)
T_0	Pathogen alone (control)	29.95	-	23.00	-	12.02	-	10.25	5.03	-	1.25	-	4.63	1.08
T_1	*Trichoderma* sp. (TR 22)	41.00	36.89	43.25	88.04	18.10	50.58	14.25	7.43	47.71	2.16	72.80	4.79	1.15
T_2	*Pseudomonas* sp. (P28)	48.25	61.10	63.50	176.09	25.45	111.73	15.63	6.25	24.25	1.79	43.20	5.09	1.02
T_3	TR 22 + P28	47.25	57.76	55.75	142.39	26.45	120.05	14.25	6.63	31.81	1.96	56.80	4.64	1.18
T_4	Carbendazim (0.1 %)	30.00	0.17	28.25	22.83	15.73	30.87	10.63	4.23	-15.90	1.30	4.00	4.45	1.19
T_5	Untreated check	30.00	0.17	29.25	27.17	16.25	35.19	11.58	4.18	-16.90	1.26	0.80	4.60	1.03
	CD (0.05)	13.53		25.01		8.84		NS	2.21		0.57		NS	NS

⊕Mean of four replications DAP – Days after planting

Morphological Characters of *Trichoderma* sp.

***T. pseudokoningii* Rifai (Isolate TR17)**

Colonies grow very rapidly at room temperature (28 ± 2°C) covering 9 cm plates in four days on PDA and in five days in Czepek's Dox agar. The surface of the colony is smooth, translucent, sparse mycelial mat with a very poor aerial growth. The colour changes from white to greenish white to bright green; usually the reverse of the colony become yellowish. Chlamydospores are formed infrequently, mostly globose, smooth walled and hyaline. The conidiophores are 4-5 μm wide and produces few side branches. The phialides are irregularly disposed in verticals of 2-5 at the apex, solitarily and alternately, more or less lageniform, constricted at the base, swollen at the middle, narrowing into a short neck measuring 6.2 x 2.6 μm size. The phialospores are one celled, smooth walled short cylindrical or almost oblong measuring 3.7 x 1.9 μm (Plate 15).

***T. harzianum* (Isolate TR20)**

Colonies grow rapidly upto 9 cm in four days at room temperature (28 ± 2°C) on PDA and in five days in Czepek's Dox agar, smooth surfaced, watery white and sparse mycelial mat but soon develop aerial hyphae on their surface. The colour of the colony changed from whitish green to bright green with the development of conidial areas. The reverse of the colony remains uncoloured. The hyphae are septate, branched, smooth walled, colourless and 3.8 μm wide. Chlamydospores are formed intercalary or terminally. The conidiophore is much branched and form continuous ring like zones. The phialides arise in false verticals of upto five beneath the terminal phialide. They are short skittle shaped, bulged at the middle, narrower at the base, attenuated abruptly into sharp pointed neck and 7.7 x 3.14 μm size. The phialospores are produced singly and successively, accumulate at the tip of each phialide and form into globose conidial head.

Phialospores are subglobose, smooth walled, pale green singly and darker in mass and measuring 3.7 – 4.6 µm (Plate 15).

***T. viride* (Isolates TR19 and TR22)**

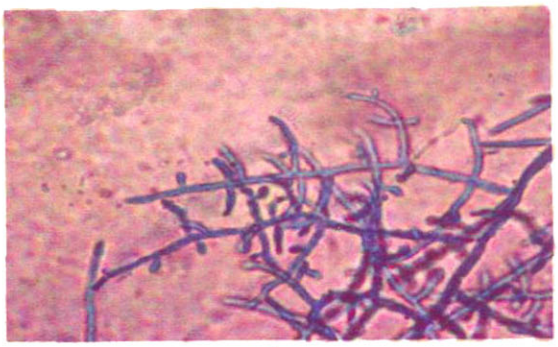
Colonies grow rapidly upto 9 cm in four days on PDA and in five days on Czepek's Dox agar at room temperature (28 ± 2°C). The mycelium is watery white, becoming hairy from the formation of loose scanty aerial mycelium which makes the colonies floccose to somewhat whitish. The colonies become green to dark green with maturity and reverse remained uncoloured. The mycelium is hyaline measuring 2.7 µm (TR19) and 3.7 µm (TR22) hyphal width and produces chlamydospores (intercalary/terminal). Conidiophores arise in compact or loose tufts which often form ring like zones. Phialides are formed in false whorls beneath each terminal phialide, generally two or three phialides arise at right angles to the bearer. Size of the phialides are 7.5 x 1.8 µm (TR19) and 5.6 x 2.37 µm (TR22). Phialospore are globose, green coloured and rugose with 3.7 (TR19) and 3.88 µm (TR22) diameter (Plate 15).

4.6.2 Fluorescent pseudomonad

The fluorescent pseudomonad isolate P28 was identified based on its morphological and physiological characters and by their biochemical traits. Based on the characters mentioned below, the bacterial isolates P28 was tentatively identified as *P. fluorescens*.

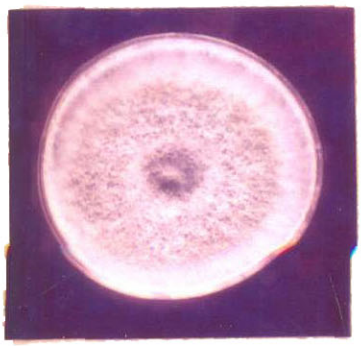
Colony morphology	-	Circular, raised with even undulating margin, colonies creamy white
Gram reaction	-	Gram negative, rods
Catalase reaction	-	Positive
Fluorescence test	-	Positive
Growth at		
4°C	-	Positive
41°C	-	Negative

a.

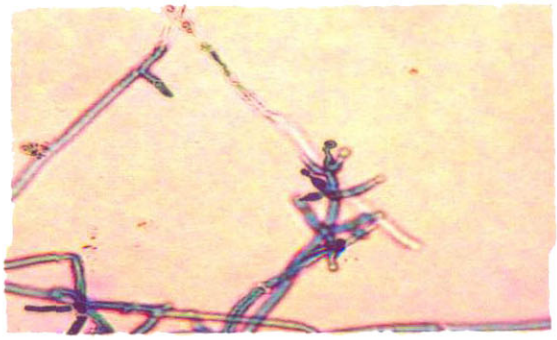


b.

Trichoderma pseudokoningii

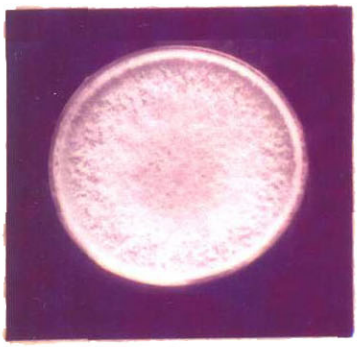


a.

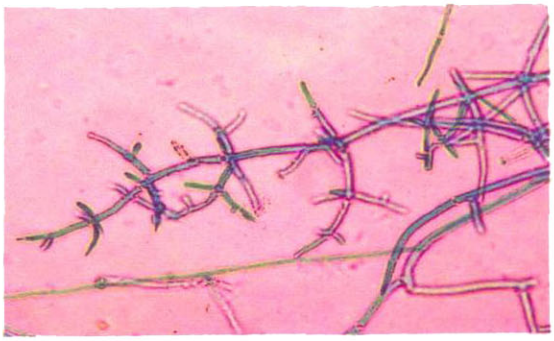


b.

Trichoderma harzianum



a.



b.

Trichoderma viride

a - Cultural character, b - Conidiophore

Plate 15. Photo micrographs of fungal cultures

Levan formation	-	Positive
Gelatin liquefaction	-	Positive
Utilization of		
Arabinose	-	Positive
Galactose	-	Positive
Sorbitol	-	Positive
m - Inositol	-	Negative
Ethyl alcohol	-	Negative
Propylene glycol	-	Negative

4.7 ANALYSIS OF THE MECHANISM OF INHIBITION

4.7.1 *Trichoderma* sp.

4.7.1.1 Mycoparasitism

Trichoderma spp. inhibited the mycelial growth of *R. solani* in dual culture. No contact between *Trichoderma* spp. (TR20 and TR22) and the pathogen was observed in the dual cultures till 24 h of incubation and during this period normal intact hyphae of pathogen and antagonists were observed without any abnormalities. Contact between *R. solani* and *T. harzianum* (TR20) was established after 36 h of incubation. At first they grew in intimate contact with the hyphae of *R. solani* and then coiled around the latter, resulting in emptying of the contents of hyphae. Ultimately host hyphae shrivelled and got killed. Other abnormalities observed with the pathogen hyphae were bulging of its cells and excessive granulation of cell contents. The antagonist completely overgrew and killed the pathogen within five days of incubation (Plate 16).

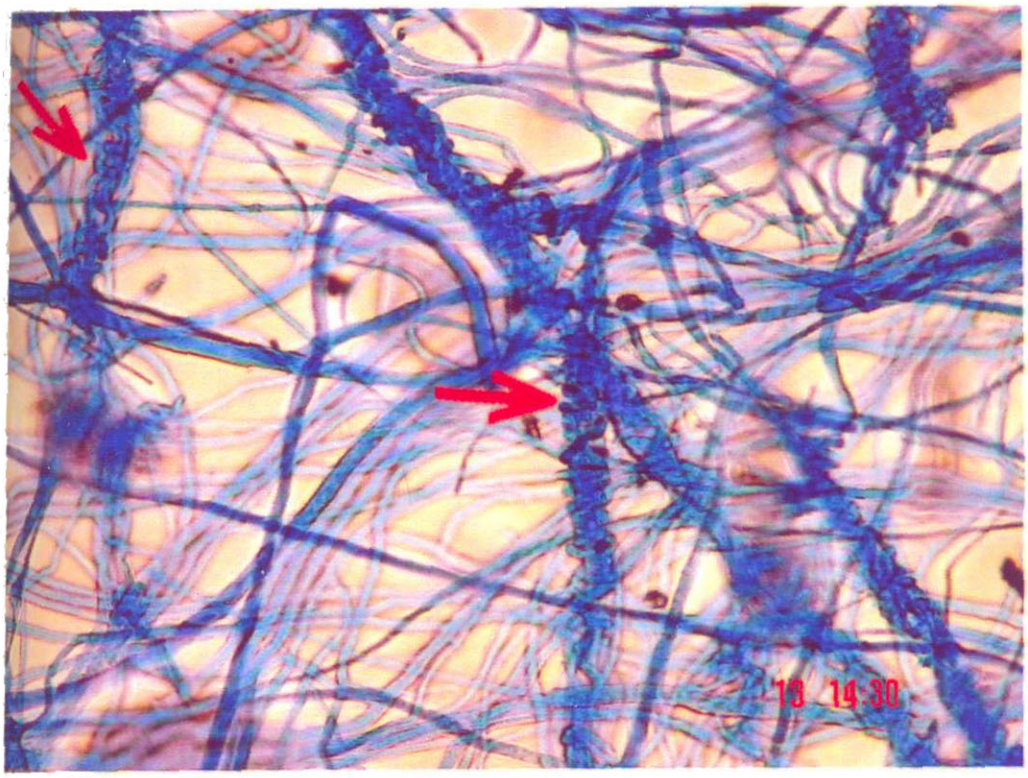


Plate 16. Overgrowth and hyphal coiling of *Trichoderma* over *R. solani*

4.7.1.2 Inhibitory Metabolite Production

4.7.1.2.1 Volatile Antibiotics

Volatile compound released from the cultures of *Trichoderma* spp. inhibited colony growth of *R. solani* and *Fusarium* sp. on PDA (Table 38). The growth of *R. solani* was 54 per cent less than control when exposed to the five day old culture of *T. pseudokoningii* while *T. harzianum* inhibited the growth of *R. solani* by 18 per cent. The growth of *Fusarium* was inhibited by 38.8 per cent by both *T. viride* isolates (TR19 and TR22) where only 5.5 cm mycelial growth was observed compared to 9 cm in control (Plate 17).

4.7.1.2.2 Non volatile / Diffusible Antibiotics

The results showed that the fungal antagonists inhibited the growth of both the pathogens *viz.*, *R. solani* and *Fusarium* sp. by the production of non volatile antifungal substances (Table 38 and Plate 18). The diffusible antibiotics in the culture filtrate of *T. harzianum* restricted the growth of *R. solani* and produced an inhibition zone of 6 mm around the wells containing the culture filtrate while *T. pseudokoningii* produced 4 mm zone of inhibition.

Non volatile antibiotics produced by both *T. viride* isolates (TR19 and TR22) inhibited the growth of *Fusarium* sp. by 2 and 2.5 mm inhibition zone.

4.7.2 *Pseudomonas* sp.

4.7.2.1 Volatile Antibiotics

Volatiles produced by *P. fluorescens* isolate P28 inhibited the growth of *R. solani* and *Fusarium* sp. by 30 and 58 per cent respectively where 6.3 and 3.7 cm growth of the respective pathogens were noticed compared to 9 cm in control (Table 38 and Plate 19).

4.7.2.2 Non Volatile Antibiotics

The culture filtrates of *P. fluorescens* isolate P28 did not exert any inhibitory effect on the mycelial growth of *R. solani* and *Fusarium* sp. The pathogen overgrew the wells on PDA containing the filtrate of the antagonist (Table 38).

4.7.2.3 Production of HCN

A negative result was obtained for the production of HCN as there was no change in colour of the filter paper from yellow.

4.7.2.4 Siderophore Formation

The possibility of siderophore production by *P. fluorescens* isolate P28 was investigated by observing the dual culture after 60 h of growth in iron deficient medium. It is observed that *P. fluorescens* isolate P28 was not able to produce an extracellular siderophore in iron deficient medium as the fungal growth was not inhibited by the *Pseudomonas* isolate in the dual culture.

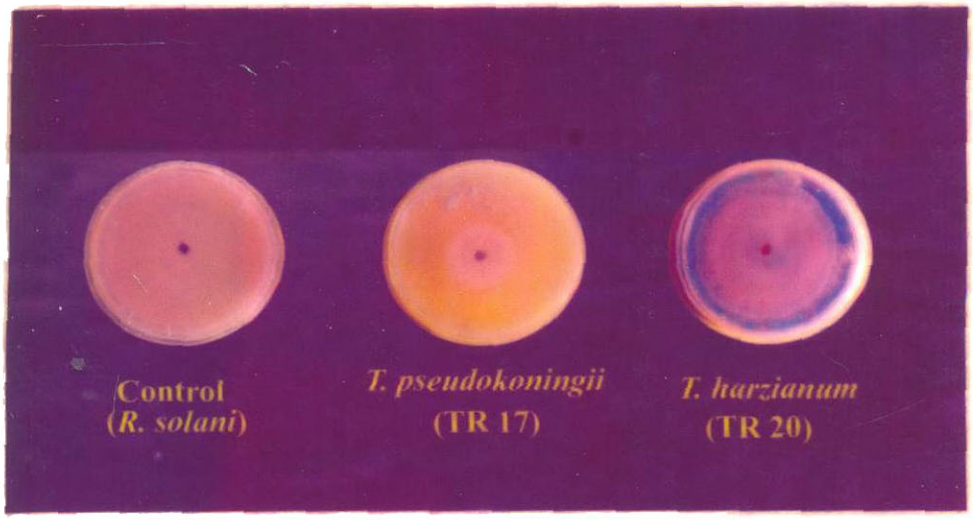
4.7.3 Genetic Analysis of the Inhibitory Character of *P. fluorescens* (P28)

Of the four concentrations of SDS *viz.*, 0.8, 1.0, 1.2 and 1.4 per cent tested, 0.8, 1.0 and 1.2 per cent recorded increase in growth upto 4 h and then a reduction in optical density was observed (Table 39).

The optical density recorded was 0.02, 0.032 and 0.022 at 2, 4 and 6 h of incubation respectively for SDS concentration of 0.8 per cent. At 1.0 per cent concentration the OD value recorded was 0.028, 0.050 and 0.011 at 2, 4 and 6 h of incubation. At 1.2 per cent SDS concentration, the OD value at second and fourth hour recorded a slight increase but at sixth hour a sharp decline in the OD was recorded. At 1.4 per cent SDS concentration the OD at 2 h was 0.028 and no further increase in OD was observed.

Table 38 *In vitro* effects of *Trichoderma* spp. and *Pseudomonas* sp. against *R. solani* and *Fusarium* sp.

Pathogen	Treatments	Volatile activity		Non volatile activity
		Growth diameter (cm)	Per cent inhibition	Inhibition zone (mm)
R. solani	*T. pseudokoningii* (TR 17)	4.20	54.00	4.00
	T. harzianum (TR 20)	7.40	18.00	6.00
	P. fluorescens (P28)	6.30	30.00	-
	Control	9.00	-	-
Fusarium sp.	*T. viride* (TR 19)	5.50	38.80	2.00
	T. viride (TR 22)	5.50	38.80	2.50
	P. fluorescens (P28)	3.70	58.00	-
	Control	9.00	-	-



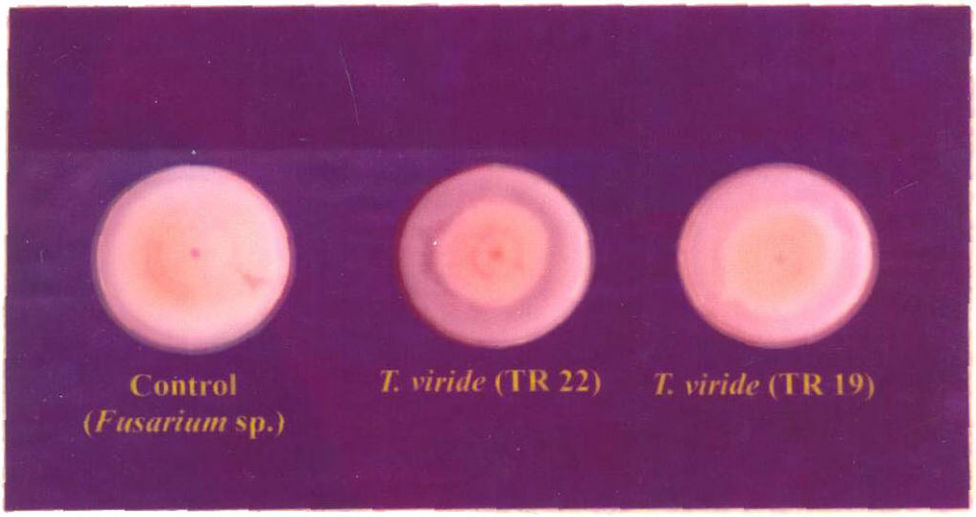


Plate 17. Inhibition of growth of *R. solani* and *Fusarium* due to the effect of volatiles produced by *Trichoderma* spp.

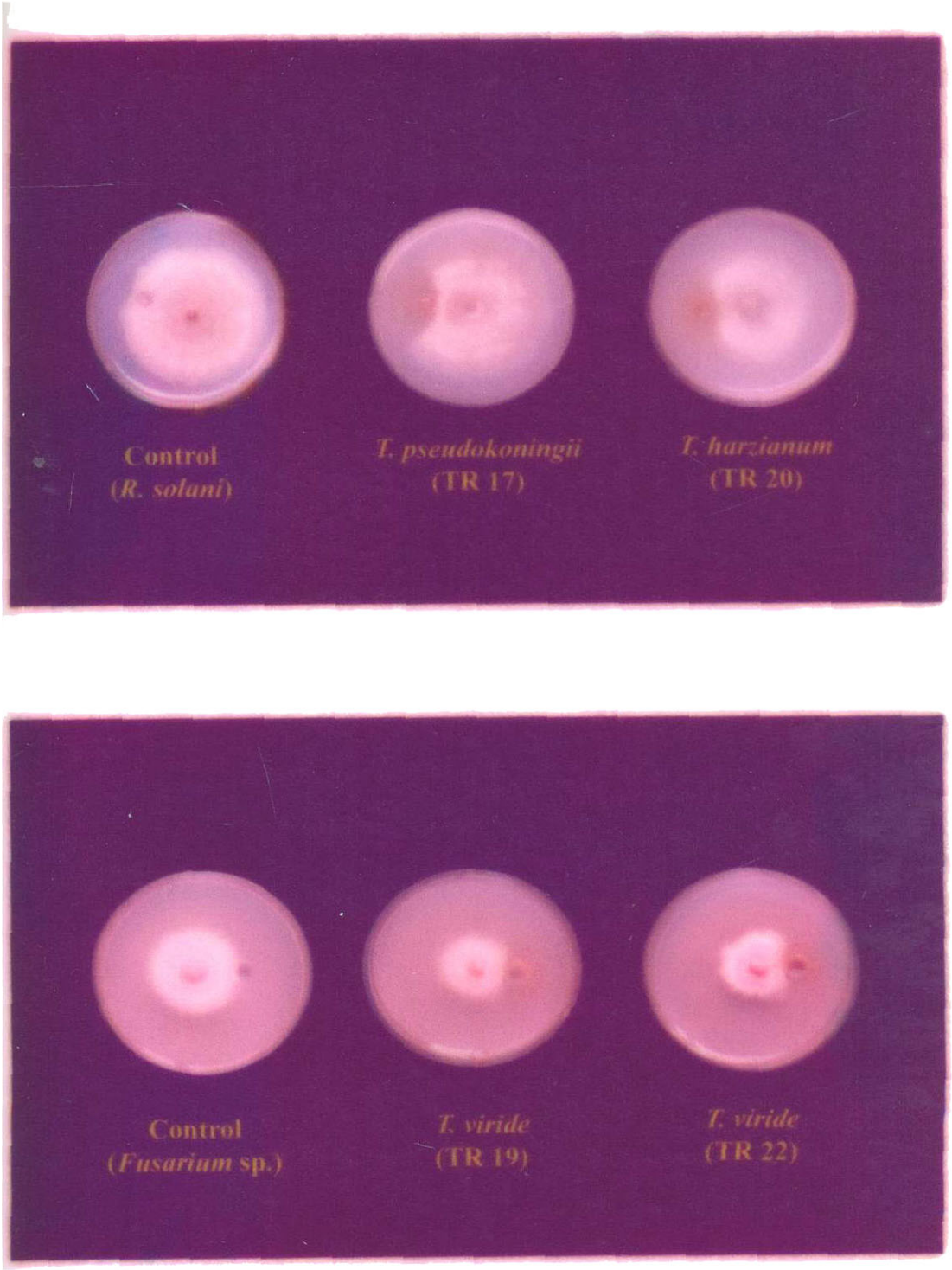
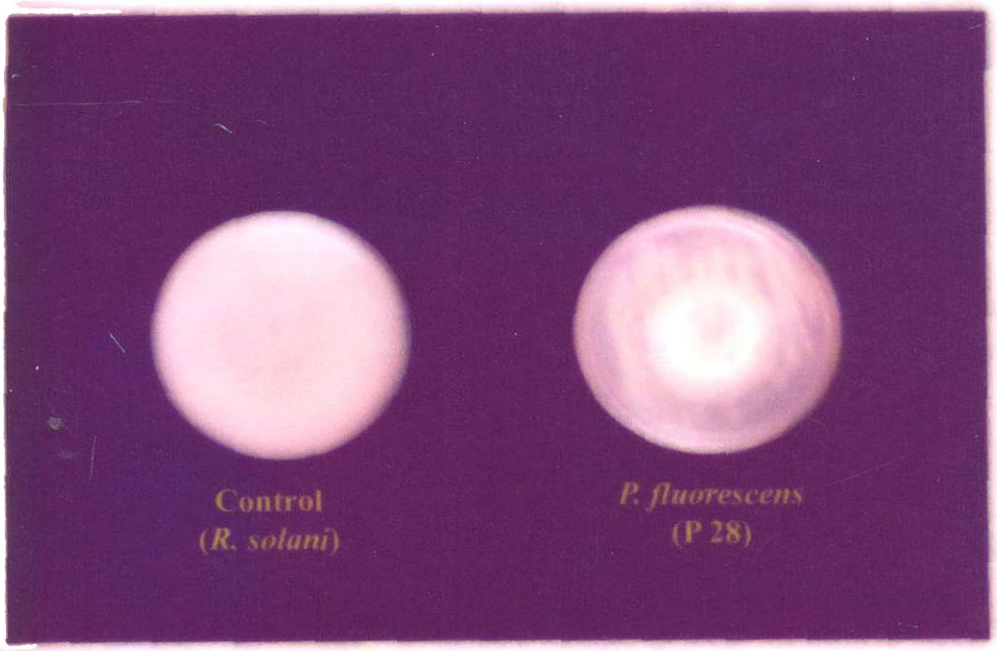


Plate 18. Inhibition of growth of *R. solani* and *Fusarium* due to the effect of non-volatile antibiotics produced by *Trichoderma* spp.



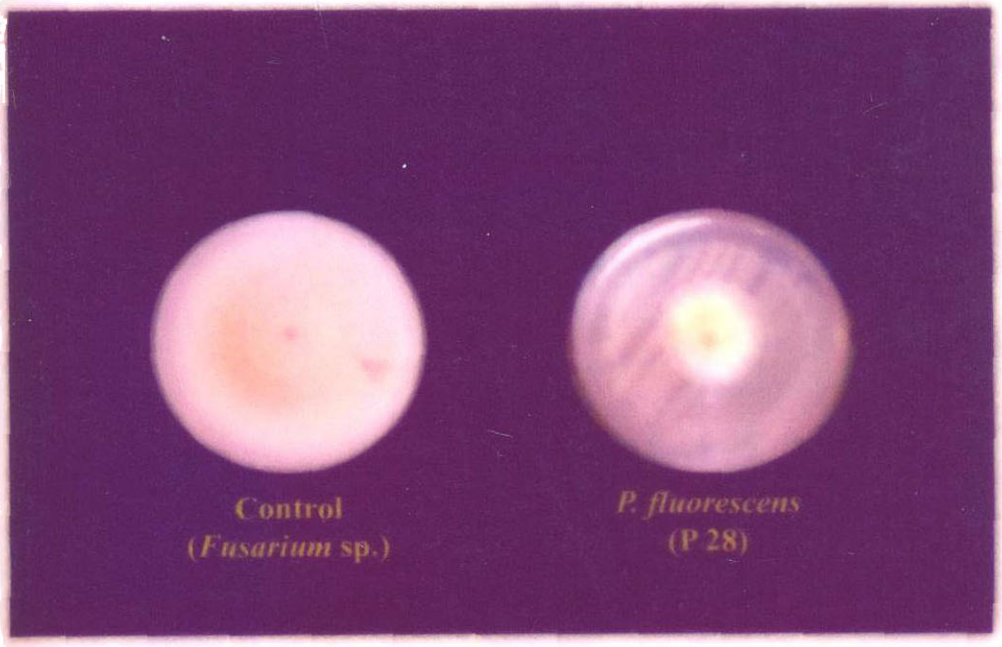


Plate 19. Inhibition of growth of *R. solani* and *Fusarium* due to the effect of volatiles produced by *P. fluorescens* (P28)

The colonies tested for antagonistic property after SDS curing showed different rates of inhibition ranging from no inhibition-to-inhibition as exhibited before curing (Table 40). The cured suspension was serially diluted and plated so as to pick up individual colonies for further testing of antagonistic property. Dilutions 10^{-5} and 10^{-7} produced isolated colonies.

A high mortality of the bacterial cells was recorded when treated with different concentrations of SDS. Maximum mortality was recorded with 1.0 per cent SDS concentrations followed by 1.2 per cent. The population recorded at these concentrations were 231 x 10^5 and 240 x 10^5 cells ml^{-1} whereas in control the population was 375 x 10^7 ml^{-1}.

Among the 50 colonies tested from 1.0 per cent SDS treated suspensions two colonies showed 100 per cent loss of antagonistic property as the pathogen overgrew these two cultures. Thirty six colonies exhibited partial loss in antagonistic property where pathogen group close to the culture but no over growth and definite inhibition zone was seen. The remaining 12 colonies showed the original antagonistic property against the pathogen. In the case of 1.2 per cent SDS concentration 50 per cent showed partial loss of inhibitory property while the remaining 50 per cent were seen unaffected.

4.8 MULTIPLICATION AND PERSISTENCE OF ANTAGONISTS IN THE RHIZOSPHERE SOIL

4.8.1 Intrinsic Antibiotic Resistance of *P. fluorescens* Isolate P28

Among the five antibiotics tested, Ampicillin was not inhibitory upto 300 ppm concentration to the bacterial isolate tested. The resistance to Erythromycin was upto 150 ppm and a faint growth appeared at 200 ppm concentration. The bacterium was resistant to Gentamycin upto 10 ppm concentration, but showed inhibition from 50 ppm onwards. The

Table 39. Effect of different concentrations of SDS on the growth of *P. fluorescens* (P28)

SDS concentration (%)	Optical density			Population at 6 h (cells ml $^{-1}$)
	2 h	4 h	6 h	
0.0	0.019	0.023	0.037	375×10^7
0.8	0.020	0.032	0.022	307×10^5
1.0	0.028	0.050	0.011	231×10^5
1.2	0.030	0.037	0.013	240×10^5
1.4	0.028	0.019	0.017	243×10^5

Table 40. Effect of SDS concentrations on the inhibitory property of *P. fluorescens* (P28)

SDS concentration (%)	Colonies tested	Inhibitory property		
		Complete loss	Partial loss	Unaffected
1.0	50	2	36	12
1.2	50	-	25	25

bacterium showed resistance to Streptomycin at 10 ppm. The least resistance was against Tetracycline (upto 5 ppm) (Table 41).

Based on the antibiotic resistance pattern exhibited by the *P. fluorescens* isolate P28, the antibiotics Tetracycline (5 ppm) and Streptomycin (10 ppm) were selected and a combination of these two antibiotics were used as marker for the persistence studies of *P. fluorescens* (P28) in the rhizosphere of both the crops.

4.8.2 Population Dynamics of *Trichoderma harzianum* (TR20), *T. viride* (TR22) and *P. fluorescens* (P28) in the Rhizosphere Soil

The multiplication and persistence of *Pseudomonas* isolate P28 and *Trichoderma harzianum* (isolate TR20) and *T. viride* (isolate TR22) incorporated in the rhizosphere of tomato and chilli were monitored at weekly intervals for a period of 35 days. The results of these studies are given in the Table 42.

In the rhizosphere of tomato, the bacterial antagonist *P. fluorescens* isolate P28 recorded an initial population of 11 x 10^6 cfu g^{-1} of rhizosphere soil. The population gradually increased and reached a density of 45 x 10^6 cfu g^{-1} at 15^{th} day and showed a decreasing trend from 21^{st} day (Fig. 9).

The population of *T. harzianum* and *T. viride* in the tomato rhizosphere showed an initial population of 37 x 10^6 and 30.5 x 10^6 cfu g^{-1} respectively. At seventh day the population increased to 47 x 10^6 and 41.5 x 10^6 cfu g^{-1} and declined thereafter (Fig. 9).

In the rhizosphere of chilli, the population of *Pseudomonas* immediately after application was 16.5 x 10^6 cfu g^{-1} of rhizosphere soil. An increase in the population at seventh day (25 x 10^6 cfu g^{-1}) was noticed which got declined to the initial level at 15^{th} day and remained almost stable till 21^{st} day of application. A notable reduction in the population was noticed thereafter (Fig. 10).

The population of *T. harzianum* and *T. viride* in the chilli hizosphere showed a similar trend as in the case of tomato rhizosphere where the initial population of 28 x 10^6 and 17.5 x 10^6 cfu g^{-1} increased to 47.5 x 10^6 and 44 x 10^6 cfu g^{-1} at seventh day. The population declined thereafter and reached 1.1 x 10^6 and 0.15 x 10^6 cfu g^{-1} at 35th day (Fig. 10).

4.9 FORMULATION AND MASS MULTIPLICATION OF ANTAGONISTS OBTAINED

The fungal (TR20 and TR22) and bacterial (P28) antagonistic isolates selected through different experiments in the present study were subjected to their mass multiplication capacity using different types of carrier materials. For fungal and bacterial antagonists separate substrates were tried for the formulation of the isolates.

4.9.1 Mass Multiplication of Fungal Antagonists (TR20 and TR22)

A study was undertaken to mass multiply the fungal antagonist, *T. harzianum* (TR20) and *T. viride* (TR22) which proved efficient in controlling the rot and wilt diseases in tomato and chilli using different substrates in various proportions. The different substrates used were cowdung + neem cake (1 : 1), coirpith, coirpith + neem cake (1 : 1) sorghum grains, saw dust, rice bran, cowdung + neem cake (1:1) + jaggery (3 %) and cowdung + neem cake (1:1) + wheat flour (10 %). Observations on nature and extent of growth was recorded for 10 days and propagule density was estimated on tenth day. The results of the experiment are given below.

4.9.1.1 T. harzianum (TR20)

Extent of Growth

There was variation in the extent of growth of fungi tested between substrates (Table 43 and Plate 20). Visible growth of *T. harzianum* was observed on sorghum grains on third day of incubation. White mycelial growth covered the entire surface of this substrate by sixth day and the

Table 41. Antibiotic resistance pattern of *P. fluorescens* (P28)

Antibiotics	Concentration (ppm)						
Ampicillin	50	100	150	200	250	300	Control
	+	+	+	+	+	+	+
Erythromycin	5	10	50	100	150	200	Control
	+	+	+	+	+	F	+
Gentamycin	5	10	50	100	150	200	Control
	+	+	-	-	-	-	+
Streptomycin	5	10	50	100	150	200	Control
	+	+	-	-	-	-	+
Tetrcycline	5	10	15	50	100	150	Control
	+	-	-	-	-	-	+

+ Good growth, F – Faint growth, - No growth

Table 42. Population dynamics of *P. fluorescens* (P28), *T. viride* (TR22) and *T. harzianum* (TR20) in the rhizosphere soil

Crop	Antagonists	Days after inoculation ($\times 10^6$ cfu g^{-1})					
		0	7	15	21	28	35
Tomato	*P. fluorescens* (P28)	11.00	30.00	45.00	32.50	13.00	1.28
	T. harzianum (TR20)	37.00	47.00	15.50	4.10	0.62	0.47
	T. viride (TR22)	30.50	41.50	12.50	7.00	3.52	0.28
Chilli	*P. fluorescens* (P28)	16.50	25.00	15.00	17.00	7.00	0.81
	T. harzianum (TR20)	28.00	47.50	25.00	11.00	4.50	1.10
	T. viride (TR22)	17.50	44.00	17.00	2.00	1.35	0.15

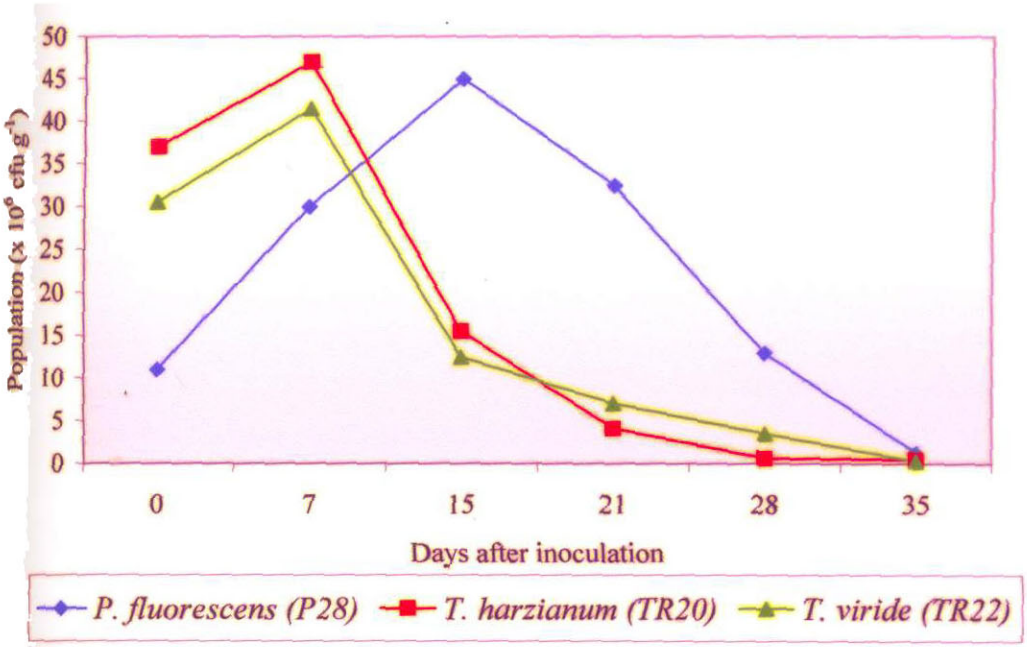


Fig. 9. Population dynamics of *P. fluorescens* (P28), *T. harzianum* (TR20) and *T. viride* (TR22) in the tomato rhizosphere

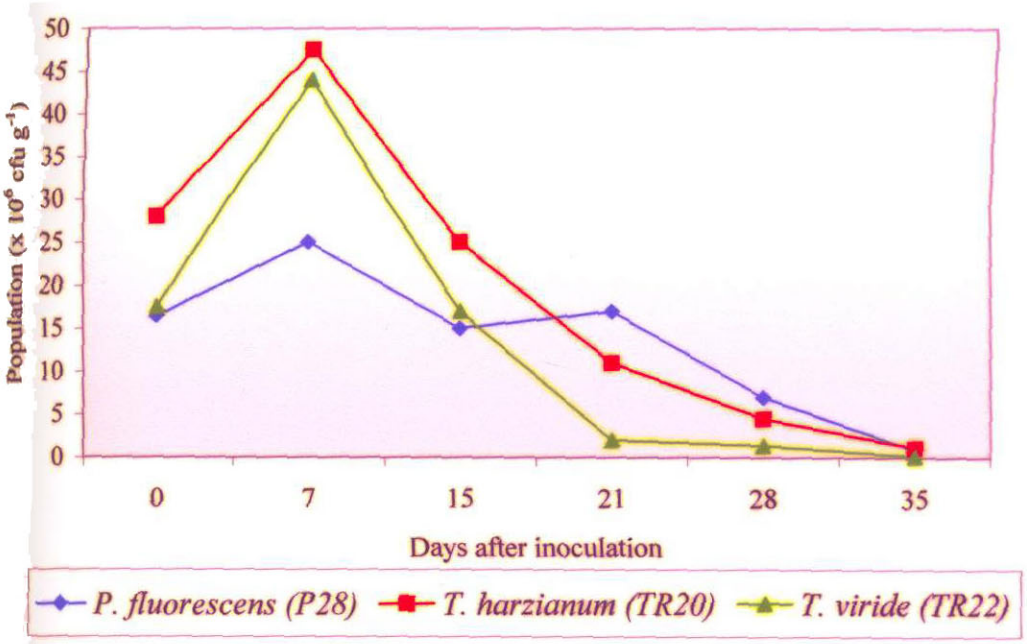


Fig. 10. Population dynamics of *P. fluorescens* (P28), *T. harzianum* (TR20) and *T. viride* (TR22) in the chilli rhizosphere

green spore mass was visible over the entire area. The mycelial growth was visible over the surface of cowdung + neem cake (1 : 1) mixture from fourth day and covered the whole surface by eighth day. But an increase in the growth rate was observed when jaggery (3 %) and wheat flour (10 %) were used as nutrient supplements with this mixture. Among these two supplements wheat flour supported the fastest growth and sporulation which got completed within six days.

No visible growth on coirpith was observed till fifth day and thereafter scanty growth was noticed covering less than 25 per cent area within eight days. But when it was used with neem cake in the ratio 1 : 1, visible growth was noticed on fourth day which covered cent per cent area by eighth day with profuse sporulation. In sawdust, the sparse mycelial growth was observed on seventh day and remained as such till tenth day. Rice bran supported good growth of *T. harzianum* as it could grow over the substrate and completed within nine days.

Propagule density

All the organic substrates tested supported good growth of *T. harzianum*. However sorghum grains supported maximum biomass production and colony forming units (156.3 x 10^8 cfu g^{-1}) (Table 45 and Fig. 11) at 10 days which was significantly superior to other substrates. A population of 23.66 x 10^8 cfu $g^{-1}$was recorded with cowdung + neem cake (1 : 1) mixture, but an increase in the growth and spore production was observed when wheat flour 10 percent (45.6 x 10^8 cfu g^{-1}) and jaggery. three per cent (34 x 10^8 cfu g^{-1}) were used as nutrient supplement in cowdung + neem cake (1 : 1) mixture. Coirpith and saw dust when used alone was the least acceptable substrates by the bioagent when compared to other treatments. But coirpith when used along with neem cake in the ratio 1: 1 supported a high population of 112.3 x 10^8 cfu g^{-1}. A population of 31 x 10^8 cfu $g^{-1}$was observed in rice bran which was statistically on par to that in cowdung + neem cake (1 : 1) mixture.

4.9.1.2 T. viride (TR 22)

Extent of growth

T. viride showed a growth pattern similar to that of *T. harzianum* on different substrates except on coirpith and sawdust where no visible growth of the former was recorded on coir pith throughout the period of study. In sawdust, no visible growth of the fungus was observed till ninth day but sparse thin mycelial run was noticed on tenth day of incubation (Table 44 and Plate 20).

Propagule density

The population trend of *T. viride* on different organic substrates was similar to that of *T. harzianum* where sorghum grains gave the maximum spore count of 138.6 × 10^8 cfu g^{-1} (Table 45 and Fig. 11) followed by coirpith + neem cake (1 : 1) mixture and cowdung + neem cake + wheat flour (10 %) (69 × 10^8 and 53.6 × 10^8 cfu g^{-1} respectively). In cowdung + neem cake mixture a population of 15.6 × 10^8 was noticed but an increase in the spore count (49.3 × 10^8) was recorded when jaggery was used as nutrient supplement. Coirpith and saw dust recorded the least population of *T. viride* (15.3 × 10^8 and 12 × 10^8 cfu g^{-1}respectively) when compared to other substrates.

4.9.2 Effect of Different Moisture Levels on Spore Viability of *Trichoderma* spp.

Based on the viable propagule density of the *Trichoderma* isolates on different substrates, most efficient substrates were further evaluated for their keeping quality at different moisture levels. By this study the shelf life of the selected isolates was ascertained. Thus *T. harzianum* and *T. viride* on different substrates *viz.*, sorghum grains, coirpith + neem cake (1 : 1) and cowdung + neem cake (1 : 1) + wheat flour (10 %) mixture were selected and evaluated at three different moisture levels each. Spore viability at 15 days interval for a period of three months was observed and the results are given.

Table 43. Effect of different substrates (organic) on the growth of *T. harzianum* (TR20)

Treatments	Days after incubation							
	3	4	5	6	7	8	9	10
Cowdung + neem cake (1 : 1)	-	+	+	++	+++ (Sporulation)	++++	++++	++++
Coirpith	-	-	-	+	+	+	+++ (Sporulation)	+++
Coirpith + Neem cake (1 : 1)	-	+	+	++ (Sporulation)	+++	++++	++++	++++
Sorghum grains	+	++ (Sporulation)	+++	++++	++++	++++	++++	++++
Sawdust	-	-	-	-	+	+	+	+
Rice bran	-	+	+	++ (Sporulation)	++	+++	++++	++++
Cowdung + neem cake (1 : 1) + jaggery (3 %)	-	+	++	+++ (Sporulation)	++++	++++	++++	++++
Cowdung + neem cake (1 : 1)+ wheat flour (10%)	+	++	+++ (Sporulation)	++++	++++	++++	++++	++++

- No visible growth
+ 25 per cent area covered
++ 50 per cent area covered
+++ 75 per cent area covered
++++ Complete growth over the substrate

Table 44. Effect of different substrates (organic) on the growth of *T. viride* (TR22)

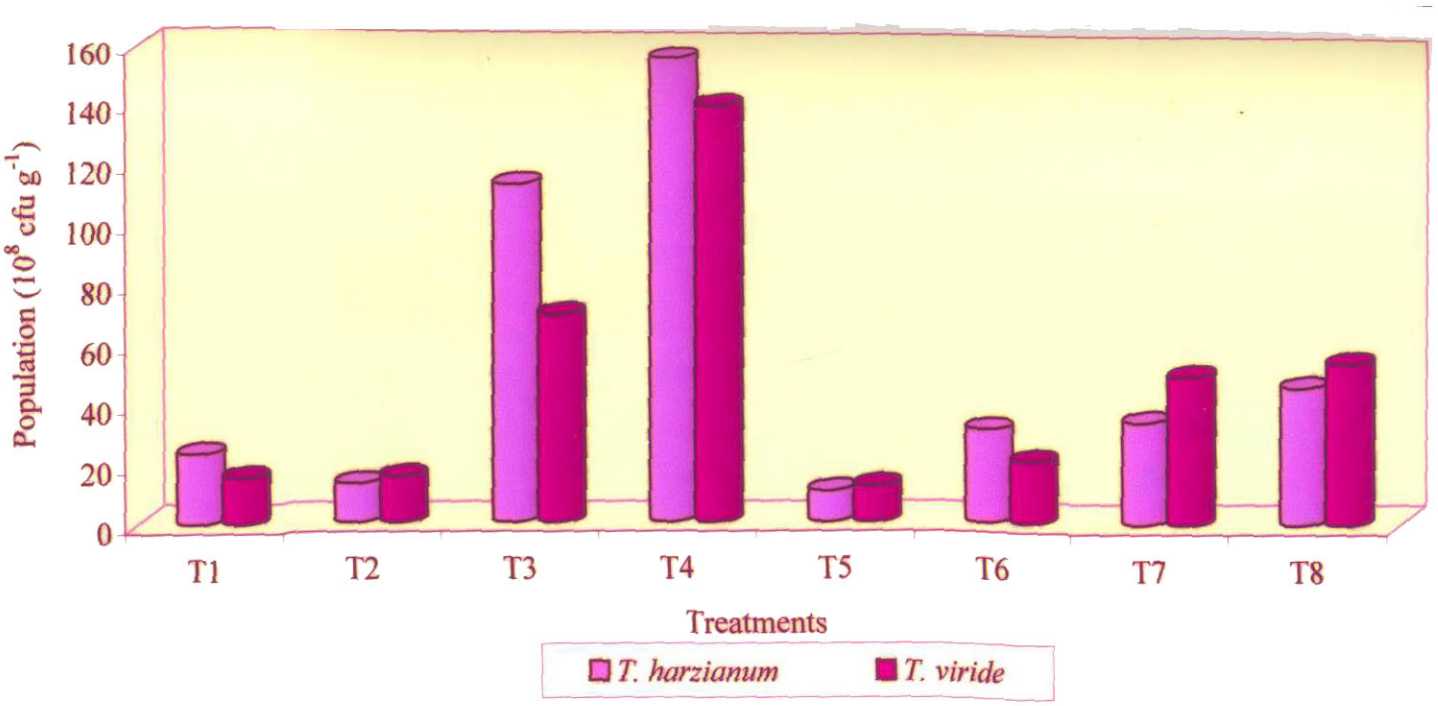
Treatments	Days after incubation							
	3	4	5	6	7	8	9	10
Cowdung + neemcake (1 : 1)	-	+	+	++ (sporulation)	+++	++++	++++	++++
Coirpith	-	-	-	-	-	-	-	-
Coirpith + Neemcake (1 : 1)	-	-	+	+	++ (sporulation)	+++	++++	++++
Sorghum grains	+	++ (sporulation)	+++	++++	++++	++++	++++	++++
Sawdust	-	-	-	-	-	-	-	+
Rice bran	-	+	++	+++	+++ (sporulation)	++++	++++	++++
Cowdung + neemcake (1 : 1) + jaggery (3 %)	-	+	++	+++ (sporulation)	++++	++++	++++	++++
Cowdung + neemcake (1 : 1)+ wheat flour (10%)	+	++	+++ (sporulation)	++++	++++	++++	++++	++++

- No visible growth
+ 25 per cent area covered
++ 50 per cent area covered
+++ 75 per cent area covered
++++ Complete growth over the substrate

Table 45. Effect of different substrates on the population of *T. harzianum* (TR20) and *T. viride* (TR22)

Treatments	At 10th day of incubation* ($\times 10^8$ cfu g^{-1})	
	T. harzianum	*T. viride*
Cowdung + neem cake (1 : 1)	23.66 (4.86)	15.60 (3.95)
Coirpith	13.00 (3.61)	15.30 (3.91)
Coirpith + Neem cake (1 : 1)	112.30 (10.59)	69.00 (8.31)
Sorghum grains	156.30 (12.50)	138.60 (11.77)
Sawdust	10.30 (3.21)	12.00 (3.46)
Rice bran	31.00 (5.57)	20.60 (4.54)
Cowdung + neem cake (1 : 1) + jaggery (3 %)	34.00 (5.83)	49.30 (7.02)
Cowdung + neem cake (1 : 1)+ wheat flour (10%)	45.60 (6.75)	53.60 (7.32)
CD (0.05)	1.042	1.149

Figures in parenthesis indicate $\sqrt{x}$ transformation
*Mean of three replications



T_1 Cowdung + neem cake (1 : 1)
T_2 Coirpith
T_3 Coirpith + Neem cake (1 : 1)
T_4 Sorghum grains
T_5 Sawdust
T_6 Rice bran
T_7 Cowdung + neem cake (1 : 1) + jaggery (3 %)
T_8 Cowdung + neem cake (1 : 1)+ wheat flour (10%)

Fig. 11. Effect of different substrates on the population of *T. harzianum* (TR20) and *T. viride* (TR22)

1 - Cowdung + neem cake (1 : 1)
2 - Coirpith
3 - Coirpith + neem cake (1 : 1)
4 - Sorghum grains
5 - Sawdust
6 - Rice bran
7 - Cowdung + neem cake + jaggery (3 %)
8 - Cowdung + neem cake + wheat flour (10 %)

Plate 20. Growth of *Trichoderma* spp. on different organic substrates

4.9.2.1 T. harzianum

Studies on the spore viability of *T. harzianum* at different moisture levels of the selected substrates revealed significant difference between the substrates in inoculum density at all the intervals tested. The spore load normally increased upto 30 days irrespective of the substrates whereas the sporulation per gram of substrate recorded decreasing rate after 30 days of incubation.

Sorghum grains recorded the maximum spore count (289 × 10^8 cfu g^{-1}) at 15 days (Table 46 and Fig. 12), which increased to 951 x 10^8 cfu g^{-1} at 30 days and thereafter showed a declining trend till the end of the study. Viability of *T. harzianum* in cowdung + neem cake + wheat flour mixture at 20 per cent and 40 per cent moisture levels recorded an increasing rate till 30 days (850 × 10^8 and 1193.3 × 10^8 cfu g^{-1} respectively), then decreased to 0.43 × 10^8 and 0.5 × 10^8 at 60 days and remained more or less stable till 90 days. As the moisture content increases (60 %), the population showed a continuous decrease after 30 days. Inoculum density in coirpith + neem cake (1 : 1) at 35 and 45 per cent moisture levels increased upto 30 days, then showed reduction till 60 days and then increased at 90 days. The maximum population at 90 days was recorded with coirpith + neem cake at 45 per cent moisture (9.5 × 10^8 cfu g^{-1}) followed by 35 per cent moisture level (3.67 × 10^8 cfu g^{-1}).

4.9.2.2 T. viride

In all the treatments tested an increase in population at 30 days and a gradual reduction was noticed thereafter. Sorghum grains which showed an initial spore count of 209 × 10^8 cfu g^{-1} increased to 846.7 × 10^8 cfu g^{-1} at 30 days. Thereafter a constant decrease in the viable spore count was noticed which finally reached 0.033 × 10^8 cfu g^{-1} by 90 days. In coirpith + neem cake (1 : 1) mixture at 55 per cent moisture levels the spore count increased at 30 days and declined afterwards upto 90 days. At 35 and 45

per cent moisture levels the population increased at 30 days, declined till 75 days and then showed a slight increase in the spore count at 90 days. In cowdung – neem cake – wheat flour mixture at all the three moisture levels, the spore load first increased at 30 days, then declined gradually till 75 days, and there was a slight difference in the spore count at 90th day of observation (Table 47 and Fig. 13).

4.9.3 Formulation of Bacterial Antagonist

Four different carriers *viz.*, talc, lignite, vermiculite and $CaCO_3$ at three different moisture levels *viz.*, 30, 40 and 50 per cent were tried to select the best carrier and the best moisture level for formulating the fluorescent pseudomonad and to increase their shelf life (Plate 21).

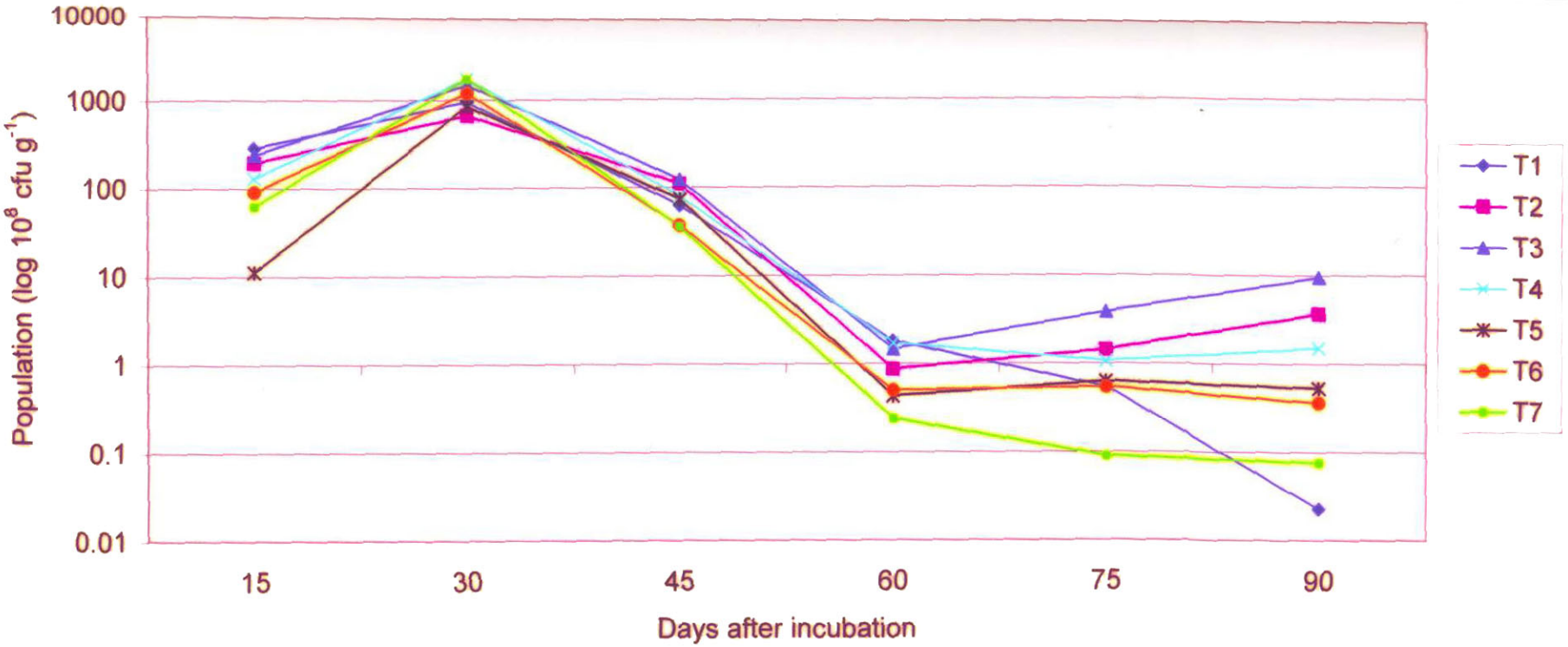
In general, an immediate rise in the population of the bacterial cells upto 15 days was observed, irrespective of the moisture levels and the substrates. After that a gradual reduction in number was noticed till the end of the study. From the mean table (Table 48 and Fig. 14), it is evident that on the day of formulation, the population of *P. fluorescens* did not differ significantly with respect to substrates and it is also obvious that on the same day of formulation, at 50 per cent moisture level, the population of bacteria was maximum followed by 40 and 30 per cent moisture levels in all the substrates tried. Formulation in talc recorded an initial mean population of 24.95×10^8 cfu g^{-1} which increased to 1137.71×10^8 cfu g^{-1} at 15 days, declined gradually and recorded 1.28×10^8 cfu g^{-1} at 90 days. In vermiculite, the initial mean population (24.95×10^8 cfu g^{-1}) rose to 304.85×10^8 cfu g^{-1} at 15 days and declined thereafter maintaining a mean count of 0.62×10^8 cfu g^{-1} at 90 days. The population in $CaCO_3$ was recorded high when compared to lignite till 60th day but at 90 days of storage, the population in both the carriers recorded more or less the same [0.44×10^8 (lignite) and 0.45×10^8 ($CaCO_3$)].

With regard to the moisture status, at 15 days, the population in different carriers with 40 per cent moisture supported maximum bacterial

Table 46. Effect of different moisture levels on the spore viability of *T. harzianum* (TR20)

Treatments	Population after days of incubation* ($\times 10^8$ cfu g^{-1})					
	15	30	45	60	75	90
Sorghum grains	289 (17)	951 (30.81)	62.67 (7.9)	1.82 (1.4)	0.54 (0.73)	0.023 (0.15)
Coirpith + Neem cake (1:1) - 35% (original) moisture	196 (13.90)	667 (25.8)	110.3 (10.5)	0.87 (0.9)	1.43 (1.19)	3.67 (1.9)
Coirpith + Neem cake (1:1) - 45%moisture	240 (15.48)	1473.3 (38.4)	123 (11.1)	1.47 (1.2)	3.83 (1.95)	9.5 (3.11)
Coirpith + Neem cake (1:1) - 55% moisture	131.7 (11.47)	1753.3 (41.9)	80.3 (8.9)	1.72 (1.31)	1.07 (1.03)	1.51 (1.23)
Cowdung + Neem cake (1:1) + Wheat flour (10%) - 20% moisture	11.3 (3.36)	850 (29.32)	73.7 (8.6)	0.43 (0.65)	0.63 (0.79)	0.53 (0.73)
Cowdung + Neem cake (1:1) + Wheat flour (10%) - 40% moisture	91.3 (9.55)	1193.3 (34.54)	37.3 (6.1)	0.5 (0.71)	0.54 (0.73)	0.36 (0.6)
Cowdung + Neem cake (1:1) + Wheat flour (10%) - 60% moisture	62.7 (7.9)	1743.3 (41.70)	36 (6)	0.24 (0.49)	0.09 (0.29)	0.076 (0.28)
CD (for treatments) (0.05)	0.409	0.927	1.010	0.085	0.179	0.229

Figure in parenthesis indicates $\sqrt{x}$ transformation
*Mean of three replications



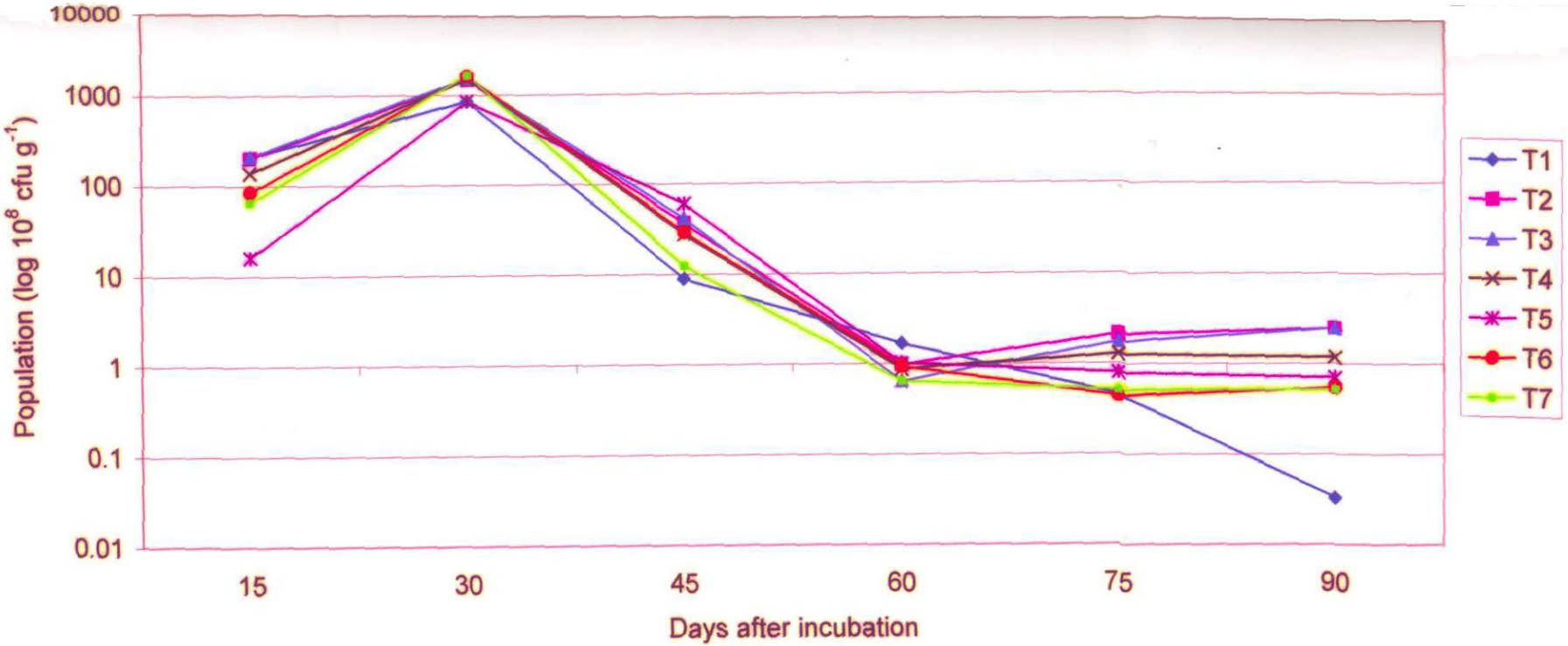
T_1 Sorghum grains
T_2 Coirpith + Neem cake (1:1) - 35% (original) moisture
T_3 Coirpith + Neem cake (1:1) -45%moisture
T_4 Coirpith + Neem cake (1:1) - 55% moisture
T_5 Cowdung + Neem cake (1:1) + Wheat flour (10%) - 20% moisture
T_6 Cowdung + Neem cake (1:1) + Wheat flour (10%) - 40% moisture
T_7 Cowdung + Neem cake (1:1) + Wheat flour (10%) - 60% moisture

Fig. 12. Effect of different moisture levels on the spore viability of *T. harzianum* (TR20) at different intervals

Table 47. Effect of different moisture levels on the spore viability of *T. viride* (TR22)

Treatments	Population after days of incubation * ($\times 10^8$ cfu g^{-1})					
	15	30	45	60	75	90
Sorghum grains	209 (14.47)	846.7 (29.06)	8.9 (2.96)	1.67 (1.27)	0.46 (0.67)	0.033 (0.18)
Coirpith + Neem cake (1:1) - 35% (original) moisture	201.6 (14.2)	1486.67 (38.5)	36.2 (6.01)	0.97 (0.99)	2.1 (1.45)	2.43 (1.56)
Coirpith + Neem cake (1:1) - 45%moisture	210.3 (14.49)	1536.7 (39.19)	41.33 (6.43)	0.64 (0.8)	1.73 (1.32)	2.45 (1.56)
Coirpith + Neem cake (1:1) - 55% moisture	138.3 (11.73)	1530 (39.11)	27.73 (5.26)	0.87 (0.93)	1.27 (1.12)	1.17 (1.08)
Cowdung + Neem cake (1:1) + Wheat flour (10%) - 20% moisture	16 (4)	846.7 (29.09)	59.67 (7.72)	1.01 (1)	0.79 (0.89)	0.7 (0.84)
Cowdung + Neem cake (1:1) + Wheat flour (10%) - 40% moisture	84.67 (9.18)	1630 (40.37)	28.97 (5.38)	0.93 (0.96)	0.44 (0.66)	0.54 (0.73)
Cowdung + Neem cake (1:1) + Wheat flour (10%) - 60% moisture	65.33 (8.07)	1680 (40.97)	12.33 (3.51)	0.64 (0.8)	0.51 (0.71)	0.52 (0.72)
CD (for treatments) (0.05)	1.034	1.160	0.374	0.077	0.101	0.090

Figure in parenthesis indicates $\sqrt{x}$ transformation
*Mean of three replications



T_1 Sorghum grains
T_2 Coirpith + Neem cake (1:1) - 35% (original) moisture
T_3 Coirpith + Neem cake (1:1) -45%moisture
T_4 Coirpith + Neem cake (1:1) - 55% moisture
T_5 Cowdung + Neem cake (1:1) + Wheat flour (10%) - 20% moisture
T_6 Cowdung + Neem cake (1:1) + Wheat flour (10%) - 40% moisture
T_7 Cowdung + Neem cake (1:1) + Wheat flour (10%) - 60% moisture

Fig. 13. Effect of different moisture levels on the spore viability of *T. viride* (TR22) at different intervals

count (463.54×10^8). Substrates kept at 30 per cent moisture recorded the least mean population *i.e.*, 392.83×10^8 cfu g^{-1}. But at 30 days the viable bacterial count was maximum in substrates with 30 per cent moisture (122.1×10^8 cfu g^{-1}). At 60 days no significant difference was noticed between the bacterial population at all the three moisture levels tried. However the mean bacterial count was found slightly higher with 40 per cent moistened substrates. The mean population at 90 days was recorded higher with 40 per cent (0.83×10^8 cfu g^{-1}) followed by 30 per cent moisture (0.74×10^8 cfu g^{-1}) (Table 48 and Fig. 15).

4.10 ECONOMIC ANALYSIS

Economic analysis was made based on the additional input and additional income. In general the net return varies depending upon different treatments. It is obvious from the table (Table 49) that a net income of Rs. 64407 ha^{-1} and 23170 ha^{-1} was obtained by the application of organic matter based *Trichoderma* and talc based *Pseudomonas* respectively in tomato crop infected by *R. solani*. But when these antagonists were used in combination net income increased to Rs. 99577 ha^{-1}, which was superior to all other treatments.

A maximum net income of Rs. 75577 ha^{-1} was obtained in *Trichoderma* + *Pseudomonas* combinations used against *Fusarium* wilt in tomato (Table 50) which was followed by the individual application of *Trichoderma* (Rs. 71607 ha^{-1}).

Results of the data presented in the table (Table 51) revealed a maximum net income of Rs. 42387 ha^{-1} in treatment where *Trichoderma* was applied individually against *R. solani* in chilli. It was followed by the combined application of *Trichoderma* and *Pseudomonas* (Rs. 34077 ha^{-1}). In chilli plants where the antagonists were applied against *Fusarium* wilt, the net income was maximum in *Pseudomonas* treated plants (Rs. 77690 ha^{-1}) which was followed by the combined application (Rs. 66077 ha^{-1}) (Table 52).

Table 48. Effect of different substrates at different moisture levels in the cell viability of *P. fluorescens* (P28)

Per cent moisture	Carrier	Mean cfu $g^{-1} \times 10^8$ (days after incubation)									
		0	Mean (substrates)	15	Mean (substrates)	30	Mean (substrates)	60	Mean (substrates)	90	Mean (substrates)
30	Talc	18.8 (4.34)	24.95 (4.99)	900 (30)	11371.71 (33.73)	301.3 (17.36)	364.43 (19.09)	15.77 (3.96)	12.04 (3.47)	1.06 (1.03)	1.28 (1.13)
	Lignite	18.97 (4.34)	25.1 (5.01)	273.67 (16.53)	212.86 (14.59)	37 (6.1)	17.47 (4.18)	2.9 (1.7)	3.31 (1.82)	0.46 (0.68)	0.44 (0.66)
	Vermiculite	18.7 (4.32)	24.95 (4.99)	297.3 (17.24)	304.85 (17.46)	166.1 (12.89)	108.58 (10.42)	37.3 (6.11)	48.58 (6.97)	1.08 (1.04)	0.62 (0.79)
	$CaCO_3$	18.3 (4.28)	24.89 (4.99)	240 (15.49)	296.87 (17.23)	61.67 (7.85)	36.72 (6.06)	12.67 (3.55)	9.06 (3.01)	0.44 (0.67)	0.45 (0.67)
Mean (moisture)		18.69 (4.32)		392.83 (19.82)		122.1 (11.05)		14.67 (3.83)		0.74 (0.86)	
40	Talc	25.27 (5.03)		1243.3 (35.26)		450.67 (21.22)		10.67 (3.24)		1.42 (1.19)	
	Lignite	25.53 (5.05)		209.3 (14.47)		9.33 (3.05)		3.67 (1.88)		0.49 (0.7)	
	Vermiculite	26.47 (4.81)		294 (17.15)		83.67 (9.15)		52.3 (7.23)		0.64 (0.8)	
	$CaCO_3$	25.47 (5.05)		369.67 (19.23)		36 (6)		10 (3.16)		0.88 (0.94)	
Mean (moisture)		25.7 (5.07)		463.54 (21.53)		97.02 (9.85)		15.05 (3.88)		0.83 (0.91)	
50	Talc	31.63 (5.62)		1290 (36.28)		350 (18.71)		10.4 (3.21)		1.32 (0.82)	
	Lignite	31.6 (5.62)		162.67 (13.08)		11.67 (3.4)		3.47 (1.86)		0.34 (0.58)	
	Vermiculite	30.43 (5.52)		323.3 (18.22)		85 (9.22)		57.3 (7.57)		0.27 (0.52)	
	$CaCO_3$	31.8 (5.64)		288 (16.7)		19 (4..35)		5.47 (2.33)		0.16 (0.4)	
Mean (moisture)		31.36 (5.6)		437.23 (20.91)		79.57 (8.92)		14.06 (3.75)		0.44 (0.66)	
CD for treatments		0.082		0.321		0.505		0.46		0.033	
Moisture		0.041		0.161		0.253		NS		0.017	
Substrates		NS		0.186		0.292		0.264		0.019	
Moisture × Substrate		0.082		0.321		0.505		0.460		0.033	

Figures in parenthesis indicate $\sqrt{x}$ transformation

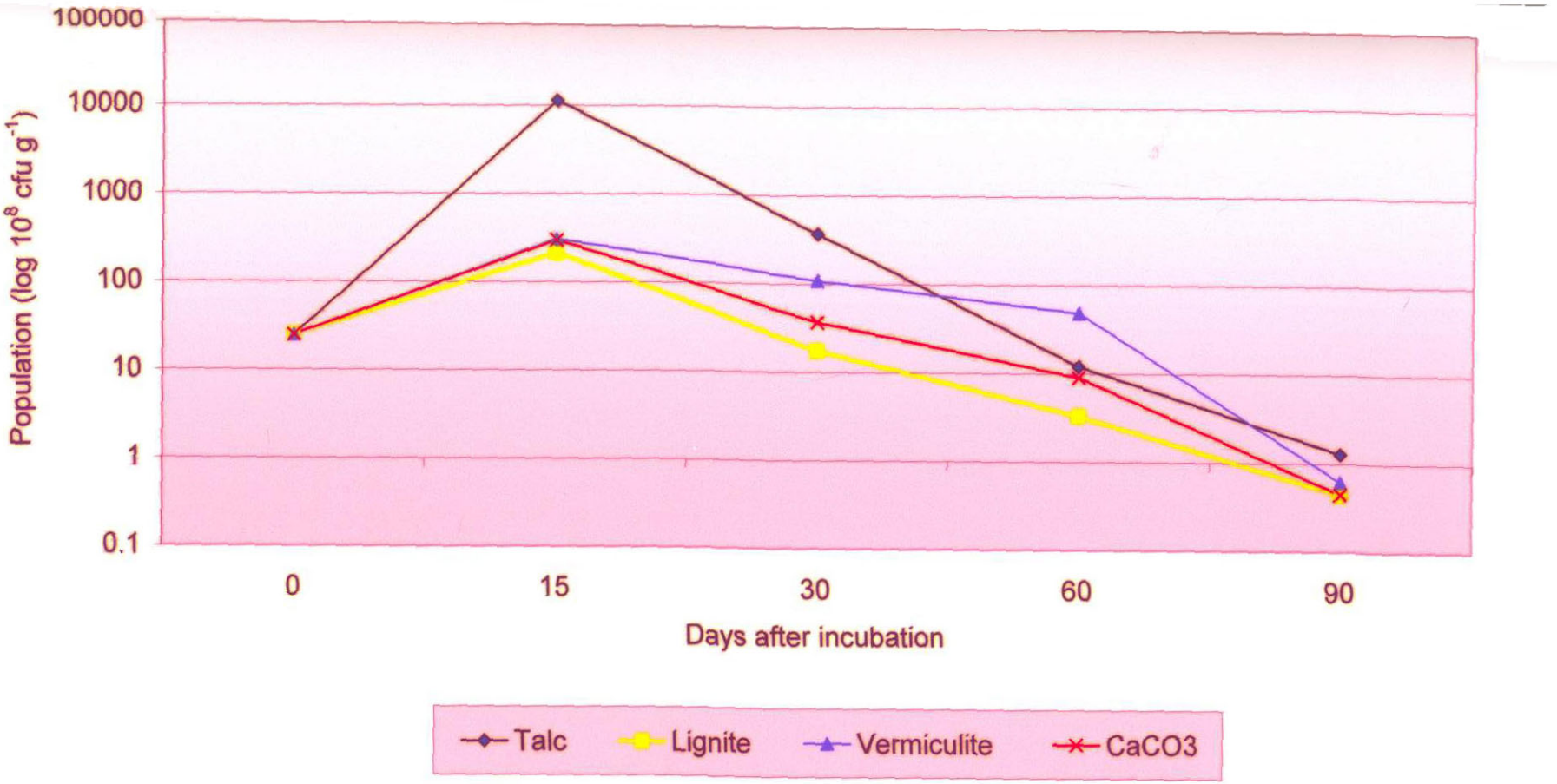


Fig. 14. Effect of different substrates on the cell viability of *P. fluorescens* (P28) at different intervals

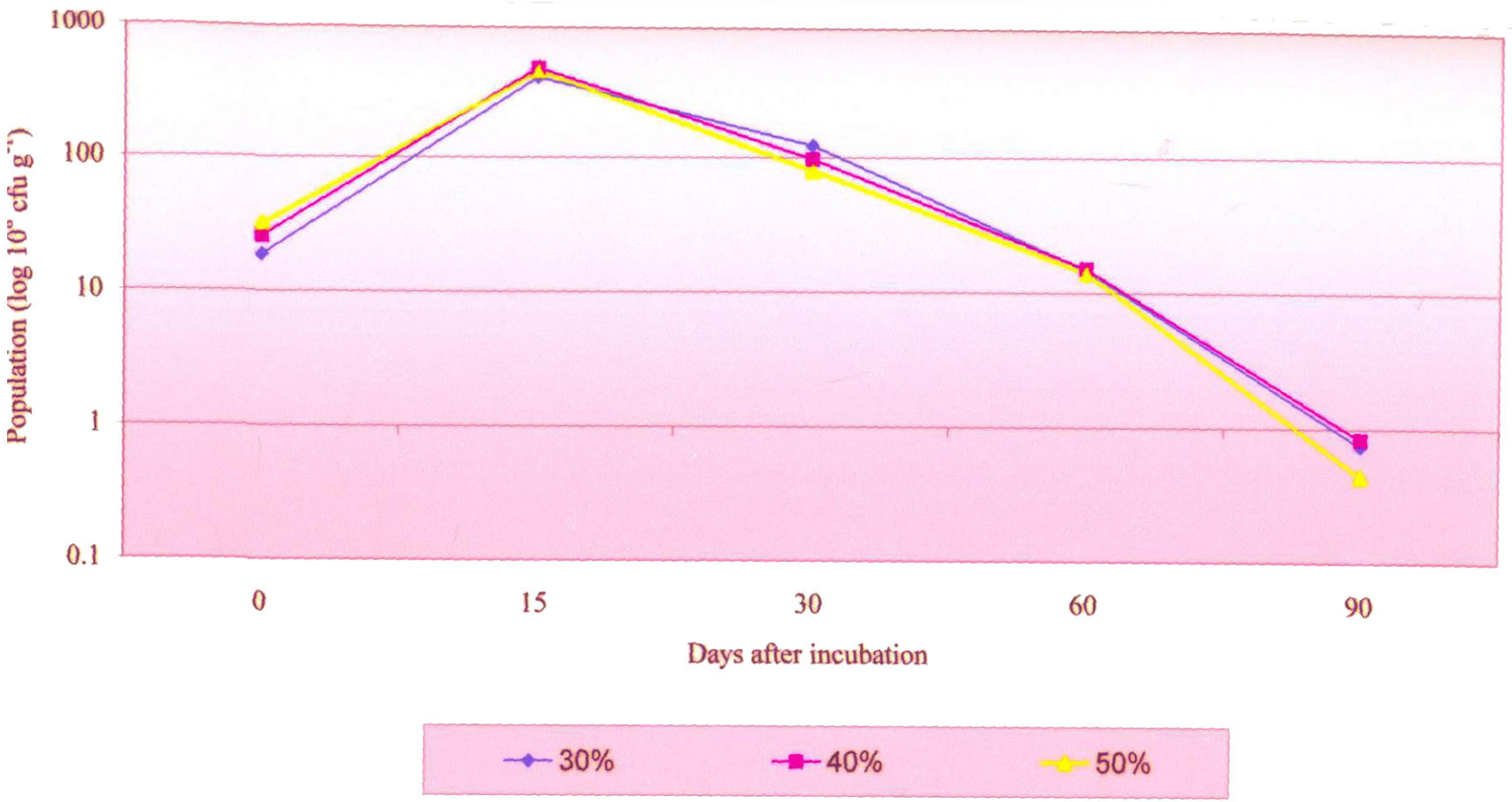


Fig. 15. Effect of different moisture levels on the cell viability of *P. fluorescens* (P28) at different intervals

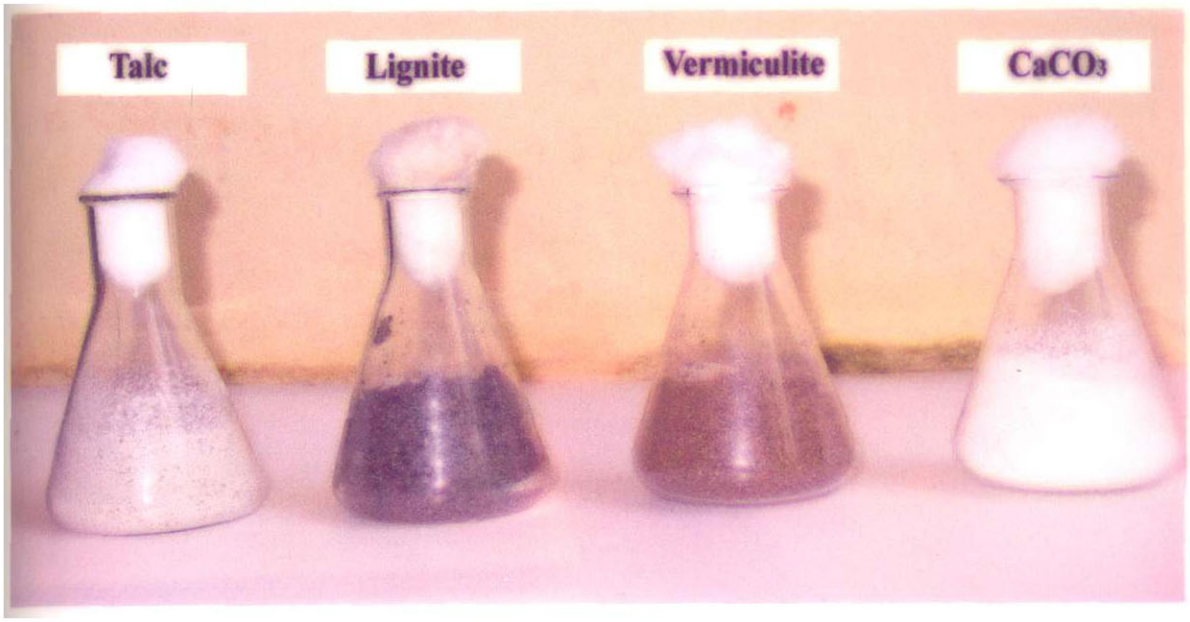


Plate 21. Formulation of P. fluorescens on different carriers

Table 49 Effect of different treatments on the economics of tomato cultivation infected by *R. solani**

Treatments	Yield (t ha^{-1})	Additional yield over control (t)	Additional cost (Rs.)	Additional income (Rs.)	Net income over control (Rs.)
Pathogen alone (Control)	6.7	-	-	-	-
Pathogen + *Trichoderma* sp.	12.3	5.6	2793	67200	64407
Pathogen + *Pseudomonas* sp.	8.9	2.2	3230	26400	23170
Pathogen + *Trichoderma* sp. + *Pseudomonas* sp.	15.5	8.8	6023	105600	99577
Pathogen + fungicide (COC)	19.2	12.5	3100	1,00,000	96900

*Not statistically analysed
Cost of 1 kg tomato in fungicide treatment = Rs. 8/-
Cost of 1 kg tomato in organic treatment = Rs. 12/-

Table 50 Effect of different treatments on the economics of tomato cultivation infected by *Fusarium oxysporum**

Treatments	Yield (t ha^{-1})	Additional yield over control (t)	Additional cost (Rs.)	Additional income (Rs.)	Net income over control (Rs.)
Pathogen alone (Control)	4.9	-	-	-	-
Pathogen + *Trichoderma* sp.	11.1	6.2	2793	74400	71607
Pathogen + *Pseudomonas* sp.	9.5	4.6	3230	55200	51970
Pathogen + *Trichoderma* sp. + *Pseudomonas* sp.	11.7	6.8	6023	81600	75577
Pathogen + fungicide (Carbendazim)	12.0	7.1	2344	56800	54456

*Not statistically analysed
Cost of 1 kg tomato in fungicide treatment = Rs. 8/-
Cost of 1 kg tomato in organic treatment = Rs. 12/-

Table 51 Effect of different treatments on the economics of chilli cultivation infected by *R. solani**

Treatments	Yield (t ha^{-1})	Additional yield over control (t)	Additional cost (Rs.)	Additional income (Rs.)	Net income over control (Rs.)
Pathogen alone (Control)	7.3	-	-	-	-
Pathogen + *Trichoderma* sp.	9.6	2.3	3613	46000	42387
Pathogen + *Pseudomonas* sp.	6.8	-0.5	4310	-10000	-14310
Pathogen + *Trichoderma* sp. + *Pseudomonas* sp.	8.9	2.1	7923	42000	34077
Pathogen + fungicide (COC)	7.7	0.4	4060	6000	1940

*Not statistically analysed
Cost of 1 kg chilli in fungicide treatment = Rs. 15/-
Cost of 1 kg chilli in organic treatment = Rs. 20/-

Table 52 Effect of different treatments on the economics of chilli cultivation infected by *Fusarium solani**

Treatments	Yield (t ha^{-1})	Additional yield over control (t)	Additional cost (Rs.)	Additional income (Rs.)	Net income over control (Rs.)
Pathogen alone (Control)	2.5	-	-	-	-
Pathogen + *Trichoderma* sp.	4.2	1.7	3613	34000	30387
Pathogen + *Pseudomonas* sp.	6.6	4.1	4310	82000	77690
Pathogen + *Trichoderma* sp. + *Pseudomonas* sp.	6.2	3.7	7923	74000	66077
Pathogen + fungicide (Carbendazim)	4.1	1.6	2740	24000	212260

*Not statistically analysed
Cost of 1 kg chilli in fungicide treatment = Rs. 15/-
Cost of 1 kg chilli in organic treatment = Rs. 20/-

Discussion

5. DISCUSSION

Seedling rot caused by *R. solani* and wilt caused by *Fusarium* sp. are two serious and challenging diseases of tomato and chilli and is a major limiting factor in the commercial cultivation of these crops. Biological management of soil borne diseases is increasingly gaining stature as a possible practical and safe approach. In the aforesaid respect here, the present investigation was carried out to assess the biocontrol potential of some rhizosphere and phyllosphere microorganisms against *R. solani* and *Fusarium* sp. and to select a suitable substrate for their commercial formulation and mass multiplication. The results pertaining to the study is discussed hereunder.

R. solani and *Fusarium* sp. causing seedling rot and wilt respectively in chilli and tomato were isolated from naturally infected plants covering the major vegetable growing tracts of Thiruvananthapuram, Ernakulam and Idukki districts in Kerala. Six isolates of *Rhizoctonia solani* and ten isolates of *Fusarium* spp. could be collected from both the crops. The different isolates of *R. solani* and *Fusarium* spp. varied in their growth characters like appearance, colour, sclerotia formation etc. when grown on PDA.

R. solani is a ubiquitous soil borne pathogen having a wide host range. Variations among the different isolates of *R. solani* in characters like hyphal thickness, colour of mycelium, sclerotial size and distribution has been reported earlier. Cultural studies by Sunder *et al.*, (2003) on 20 selected isolates of *R. solani* belonging to anastomosis group (AG) – 1, 3, 4 and 7 have revealed considerable variation within and among AG's with regard to colony colour and sclerotial characteristics. *Fusarium* sp. important as plant pathogens are present in soil both in temperate and tropical regions and are most frequently isolated. They are reputed for

their complexity and capacity for rapid change. Haware (1993) reported that in India, *Fusarium* diseases are economically important in tropical crops.

Pathogenicity of the diseases were proved by following Koch's postulates. The symptoms observed in the field under natural conditions and those observed on artificial inoculation differed slightly with *R. solani* and *Fusarium* sp. Under natural conditions, *R. solani* produces shrunken reddish brown lesions at the collar regions of the stem and expands to the root resulting in root decay which ultimately kills the plant. But on artificial inoculation, the root decay was totally absent. The collar infection causes the girdling of the stem and collapse of the seedlings. *Fusarium* wilt under natural conditions showed yellowing and drooping of the leaves starting from the lower portion. Reddish brown vascular discolouration was observed when the stem is cut lengthwise. Artificial inoculation of *Fusarium* on tomato yields the same symptoms except the vascular discolouration. In chilli, on artificial inoculation, yellowing followed by excessive leaf shedding was noticed ultimately leaving the bare stem. The root of the artificially infected chilli plants were blackened and rotten and no vascular discolouration was noticed.

On inoculation, the different isolates varied for the time taken for symptom development. The *Rhizoctonia* isolate, R1t., of tomato obtained from Vellayani was highly virulent in initiating the rot symptoms and in chilli R3c (*Rhizoctonia* isolate from chilli) was moderately virulent. When these isolates were cross inoculated between the two crops *viz*., tomato and chilli, R1t was highly potent in causing seedling rot in both the crops. Hence R1t was selected as the most virulent *Rhizoctonia* isolate for further studies in both the crops.

Among the *Fusarium* isolates of tomato and chilli, F1t of tomato and F4c of chilli was highly virulent in initiating the wilt symptoms in the respective crops. These two isolates were highly specific in causing wilt in

tomato and chilli as no infection could be initiated by these isolates in crops other than their host.

Morphological characters of highly virulent isolates were studied in detail and these isolates *viz.*, R1t, F1t and F4c were identified as *R. solani*, *F. oxysporum* and *F. solani* respectively.

In the area of biological control of plant diseases, especially soil borne diseases, introduction of *Trichoderma* and fluorescent pseudomonads gained momentum. In the present investigation, an attempt was made to isolate some of the native isolates of *Trichoderma* and fluorescent pseudomonads antagonistic to *R. solani* and *Fusarium* sp. in tomato and chilli.

A total of 26 isolates of *Trichoderma* were collected from the soil, out of which 12 isolates were obtained from virgin forest soil, eight from the rhizosphere of healthy tomato and six from the rhizosphere of chilli. Out of the total 56 isolates of fluorescent pseudomonads collected from the rhizosphere and phyllosphere of healthy tomato and chilli plants, 12 were from forest soil, seven from rhizosphere of tomato, 17 from rhizosphere of chilli and 10 each from phyllosphere of tomato and chilli.

The efficacy of *Trichoderma* isolates on suppression of *R. solani* and *Fusarium* sp. was done following dual culture technique (Skidmore and Dickinson, 1976) under *in vitro* conditions. Amongst the 26 *Trichoderma* isolates tested against *R. solani*, 11 isolates *viz.*, TR2, TR13, TR15, TR17, TR18, TR19, TR20, TR21, TR22, TR24 and TR26 were found equally effective in suppressing *R. solani*. Remaining isolates were less effective in suppressing the mycelial growth of the pathogen.

On dual culturing with *Fusarium* sp., eight isolates of *Trichoderma viz.*, TR2, TR13, TR17, TR18, TR19, TR20, TR22 and TR24 exerted almost equal effect in inhibiting the growth of the pathogen. The two pathogens *viz.*, *R. solani* and *Fusarium* sp. were overgrown by the

respective *Trichoderma* isolates and fully suppressed within six days of inoculation.

Species of *Trichoderma* have been extensively tested and used as biocontrol agents against wider range of plant pathogens *viz.*, *Rhizoctonia, Fusarium, Sclerotium, Macrophomina, Sclerotinia, Pythium* etc. (Papavizas, 1985; Chet, 1987; Hornby, 1990; Deacon, 1991). Kim and Roh (1987) reported the antagonistic activity of *T. harzianum*, *T. viride* and *Gliocladium* spp. against *R. solani* under *in vitro* conditions. The antagonistic property of *Trichoderma* spp. against *Fusarium* sp. under *in vitro* has been reported by Vyas and Mathur (2002). They observed that *Trichoderma* spp. under *in vitro* conditions effectively inhibited the growth and sporulation of *Fusarium oxysporum*, which was the effect of volatile and non-volatile antibiotics produced by the antagonists.

The antagonism exhibited by the *Trichoderma* isolates on co-inoculation with the pathogens in dual culture plate may not reflect their true antagonistic property as both the organisms are availed with sufficient nutrients and space for their initial establishment. In a highly versatile soil condition, the pathogen may be established at a high density. The inconsistent behaviour of the biocontrol agents under *in vitro* and *in vivo* conditions may partly be due to the inability of these organisms to cope with such situation. Broadbent *et al.* (1971) reported that antibiosis *in vitro* had little correlation to antibiosis in soil for controlling plant pathogens. The overgrowth of a fully established pathogen by the antagonist under *in vitro* conditions may be a good criteria for selecting an antagonist, provided the isolate shows uniform performance under *in vitro* conditions. In order to select such as efficient *Trichoderma* isolate against *R. solani* and *Fusarium* sp. for the *in vivo* experiments, a secondary *in vitro* screening was carried out with the selected *Trichoderma* isolates obtained from the initial *in vitro* screening.

In the secondary screening where the antagonists were inoculated over the full grown *R. solani* and *Fusarium* sp., none of the *Trichoderma* isolates grew over both the pathogens upto five days. The isolates TR17 and TR20 were antagonistic to *R. solani* by totally overgrowing the pathogen within seven days and were categorized in class-1 as per Bell's scale (Bell *et al.*, 1982). When compared with other isolates, they were more inhibitory to *R. solani.*

In the secondary screening where the *Trichoderma* isolates were tested against *Fusarium* sp., TR19 and TR22 were totally antagonistic to *Fusarium* sp., overgrowing the latter within seven day. These isolates were categorized in class-1 according to Bell's scale. None of the other isolates appeared effective as TR19 and TR22.

The overview of the results showed that these isolates *viz.*, (TR17 and TR20) and (TR19 and TR22) have the best antagonistic potential for *R. solani* and *Fusarium* sp. respectively and hence selected for further *in vivo* experiments.

It is well known that there is sufficient selectivity of isolates of *Trichoderma* in their antagonistic efficiency towards a particular pathogen (Papavizas and Lumsden, 1980; Cook and Baker, 1983). Saikia and Gandhi (2003) obtained clear variation in antagonistic potential among isolates of *Trichoderma* in *in vitro* screening against *R. solani.*

Bunker and Mathur (2001) studied the antagonistic potential of *T. harzianum*, *T. viride*, *T. virens* and *T. aureoviride* against *R. solani* causing dry root rot of chilli under *in vitro* conditions. Among the various isolates, *T. harzianum* was most effective in causing significant suppression of mycelial growth and sclerotial formation through production of volatile and non-volatile antibiotics and by the production of lytic enzymes like β-1,3 glucanase and chitinase (Hadar *et al.*, 1979).

Shyama and Sunita (2003) noticed differential biocontrol ability among the different antagonistic fungi including different species of *Trichoderma* against *Fusarium oxysporum*.

The trend of the results also indicated that there was not only variability amongst the isolates of *Trichoderma* with differential degree of antagonism towards a single pathogen but also towards different pathogens *viz.*, *R. solani* and *Fusarium* sp. Patel and Anahosur (2001) reported that mode of antagonism of *T. harzianum* was not necessarily similar towards all host fungi. Bell *et al.* (1982) tested antagonistic activities of 77 isolates of *Trichoderma* against six plant pathogens and recorded highly significant differences between pathogen-antagonists interactions. There are reports (Elad *et al.*, 1980 and Alvira *et al.*, 2001) which indicate that some isolates were highly antagonistic to some pathogen, yet there was a clear, isolate to isolate variability in the degrees of parasitism.

To assess the efficacy of the fluorescent pseudomonad isolates on suppression of *R. solani* and *Fusarium* sp., they were evaluated under *in vitro* condition by dual culture technique. The results revealed that inhibition potential of all the isolates differed significantly from each other. Against *R. solani*, P28 and P51 exerted the maximum inhibition as evidenced by the widest inhibition zone in both the media, while P20 and P28 were the most inhibitory isolates against *Fusarium* sp. These isolates were selected for further *in vivo* evaluation.

Several workers have studied the biocontrol potential of fluorescent pseudomonads against *R. solani* in tomato (Varshney and Chaube, 1999; Singh *et al.*, 2003) and chilli (Singh *et al.*, 2002, 2003) and *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Varshney and Chaube, 1999; Duijff *et al.*, 1997) and in chilli (Singh *et al.*, 2003).

Antagonistic activity of bacterial strains also depended on the media used. Some bacterial isolates inhibited fungal pathogens on both

PDA and KMB, whereas others were effective only on one medium. Similar findings were also observed by Hebbar *et al.* (1992). The carbon nutrient source or its concentration in the medium affecting the production of the antifungal agent, may be responsible for the difference in the antagonistic activity against *R. solani* and *Fusarium* sp. based on the inhibition on PDA and KMB.

Interestingly pseudomonads isolated from the phyllosphere of both chilli and tomato were totally ineffective in inhibiting the growth of pathogens *viz.*, *R. solani* and *Fusarium* sp. causing rot and wilt in chilli and tomato, under *in vitro* conditions.

In the recent years, attempts have been made to use mixed inocula of biocontrol agents to get satisfactory and persistent biocontrol of plant pathogens (Duffy *et al.*, 1996). However, while laying such experiments, the compatibility among the antagonists in the mixed inoculum is often not tested. In the present study, interactions between strains of fluorescent pseudomonads and *Trichoderma* spp. showing high degrees of antagonism individually against *R. solani* and *Fusarium* sp. were studied.

The results showed that both the organisms were compatible as they hardly inhibit each other in dual culture. The absence of growth of *Trichoderma* and *Pseudomonas* on the medium at the point where the other organism has been inoculated, could be due to the physical obstruction at that particular point. These observations are in accordance with the findings of Duffy *et al.* (1996) who reported that strains of fluorescent pseudomonad and *T. koningii* were compatible as their co-inoculation was significantly more suppressive to take all of wheat but contradicts the observations of Varshney *et al.* (2000) who have shown that identified strains of fluorescent pseudomonads reduced the growth of *Trichoderma* sp. under *in vitro*. Contradiction in our observation to that of Varshney *et al.* (2000) may partly be due to difference in the species and strains of *Trichoderma* and fluorescent pseudomonads investigated.

In vivo assays on comparative biocontrol ability against *R. solani* and *Fusarium* sp. revealed that the antagonists; *Trichoderma* spp. and *Pseudomonas* sp. which caused maximum inhibition in the growth of the *R. solani* and *Fusarium* sp. under *in vitro* tests also had higher biocontrol potentiality against these pathogens in soil too. Although antagonism *in vitro* does not necessarily relate to antagonism in the rhizosphere, in the present study, the observations are in accordance with that of Nandakumar *et al.* (2001b), who have shown that activity *in vitro* does correlate with activity in root zone. But the magnitude of disease reduction varied among the individual biocontrol agents (BCAs) and their combination and also between the crops. The application of organic matter enriched TR20 and TR17 one week before planting tremendously reduced the rot incidence (85.4 %) in tomato which was equally effective as fungicide treatment. In chilli, these two *Trichoderma* spp. reduced the rot incidence by 44.4 and 33.3 per cent respectively.

Various species of *Trichoderma* have been reported to have successfully inhibited the growth and development of *R. solani* (Elad *et al.*, 1980; Hadar *et al.*, 1979). Prasad *et al.* (2002c) reported that soil application of *T. harzianum* and *T. viride* one week before sowing was found very effective in reducing wilt and wet rot of chickpea caused by *R. solani*. Prasad and Rangeshwaran (2000a) also indicated that soil application of *T. harzianum* granules before sowing resulted in significantly less *Rhizoctonia* root rot incidence in chickpea. *Trichoderma* applied to the soil either through seed or soil treatments are reported to establish and proliferate in the rhizosphere (Harman *et al.*, 1980; Beagle-Ristanio and Papavizas, 1985). However when applied in the soil with an effective food base, these being natural soil inhabitants may have continued to grow better and had better access to the rhizosphere and established higher inoculum densities. Once established, being better rhizosphere competitors by virtue of their fastest growth and potential

antibiosis and/or mycoparasitism these suppressed the pathogen (Papavizas, 1985).

The present study proved the efficacy of *P. fluorescens* in reducing *Rhizoctonia* rot. Fluorescent pseudomonads are commonly found in soil and are effective colonizers of the rhizosphere of many crop plants and inhibit a number of phytopathogenic fungi (Weller, 1988). Bioformulation of *P. fluorescens* strains when applied alone or in combination as seed treatment, root dip and foliar spray and soil application significantly reduced the sheath blight incidence both under green house and field conditions (Nandakumar *et al.*, 2000).

The mechanism by which the bacterial biocontrol agents bring about the disease control include production of siderophores, antifungal antibiotics, HCN, ammonia, competition for nutrients and space, colonization and induction of systemic resistance (Dowling and O'Gara, 1994).

It is noteworthy that in tomato plants treated with the combination treatments *viz.*, TR20 + P28, TR20 + P51 and TR17 + P28 remained completely free from seedling rot caused by *R. solani*. In chilli, the maximum reduction (66.7 %) in seedling rot was in TR20 + P28 application.

The use of combined inoculation of fungal and bacterial antagonists have already been reported for effective disease management (Pierson and Weller, 1994; Duffy *et al.*, 1996). Soil application of talc based formulation of *T. viride* and *P. fluorescens* either individually or in combination for reducing pre and post emergence damping off of tomato is recommended by Manoranjitham *et al.* (2001). Anith and Manomohandas (2001) reported that *T. harzianum* and *Alcaligenes* sp. applied alone or in combination significantly reduced the mortality of rooted cuttings due to *Phytophthora capsici* in black pepper nursery. The fungal – bacterial combination in the soil may exert any of their antagonistic mechanisms

individually or more than one of them acting simultaneously in synergistic manner for better disease suppression.

In addition to suppressing the disease, the antagonist treatment greatly induces the plant growth. The growth promotion was more clearly realized in the case of *Trichoderma* + *Pseudomonas* combination, especially TR20 + P28, rather than their individual applications. It was observed even at the early stages of growth, where the treated plants were sturdy and somewhat taller than the control plants. The increase in plant height and biomass of shoot and root in the treated plants may have contributed to increased yield.

The enhanced growth of tomato seedlings by *Trichoderma* treatment alone was reported by Kleifeld *et al.* (1983). Mechanisms like production of hormone like metabolites and release of nutrients from soil or organic matter and making it available to plants by *Trichoderma* spp. were found to be involved in enhanced plant growth (Kleifeld and Chet, 1992; Altomare *et al.*, 1999). Prasad *et al.* (2002c) observed that soil application of *T. harzianum* resulted in better seedling emergence and highest vigour index which resulted in higher yields.

The results of the present study are also in agreement with Manoranjitham *et al.* (2000) who reported that talc based formulation of *Pseudomonas* significantly reduced the soil population of *Pythium* and increased the shoot length, root length and dry matter production of tomato seedlings. As certain strains improve plant growth in addition to biological control, these strains are collectively called plant growth promoting rhizobacteria designated as PGPR. The PGPR strains are reported to induce plant growth regulators like gibberllins, cytokinins and indole acetic acid (IAA) (Lifshitz *et al.*, 1987; Dubeikovsky *et al.*, 1993) which can either directly or indirectly modulate the plant growth and development.

Manoranjitham and Prakasam (1999) and Manoranjitham *et al.* (2001) reported that the combination of *T. viride* and *P. fluorescens* resulted in reduced damping off and also gave a better growth and highest dry matter content in tomato. Similar findings were also made by Ramanathan (1989) and Emayavaramban (1994) who worked on damping off disease of chilli.

It is interesting to note that *Trichoderma* sp. had a better influence on plant growth than the fluorescent pseudomonads when applied individually. Though better disease reduction and yield improvement was noticed in both the crops, the influence was more in tomato than in chilli.

In the greenhouse experiments where different antagonists were tried against Fusarium wilt, effective suppression of wilt disease was obtained. However, the combination of fungal and bacterial bioagent was distinctly better than their individual application. This improvement was evident in the incidence and intensity of wilt in both the crops evaluated.

TR22 + P28 was the best combination treatment with maximum protection to both the crops. Bohra and Mathur (2002) obtained maximum biocontrol potentiality with combination of *Pseudomonas* and *Trichoderma* against root rot of soybean caused by *F. solani.*

All the BCAs had a positive influence on yield and other growth characters. The impact of TR22 + P28 on yield was more promising. This is in accordance with Chaube and Sharma (2002) who reported that *T. viride / T. harzianum* + *P. fluorescens* were compatible and improved plant growth and suppressed seedling disease of cabbage, brinjal and tomato.

Positive impact of BCAs on disease suppression and growth enhancements was noticed in field trials. In field condition, *Trichoderma* was found more suited against *R. solani* than *Fluorescent pseudomonas.* This may be due to the direct attack and killing of *R. solani* by the

Trichoderma sp. rather than antagonism through antibiosis. Devi and Reddy (2002) reported *T. harzianum* to be the most potential antagonist among five isolates of *Trichoderma* spp., *P. fluorescens* and *Bacillus* sp. against *R. solani* causing damping off of groundnut.

In managing wilt disease, *Pseudomonas* was more effective than *Trichoderma*. It has been reported that application of *P. fluorescens* triggers / activates plants latent defense mechanisms in response to infection by pathogen. The induction of systemic resistance might have contributed in reducing the intensity of wilt in tomato and chilli. Ramamoorthy *et al.* (2002) reported that induction of defense related proteins involved in phenyl propanoid metabolic pathway and accumulation of phenolics and PR proteins might have contributed to restriction of invasion of *F. oxysporum* f. sp. *lycopersici* in tomato plants treated with *P. fluorescens*.

The growth of the plants was also enhanced by the antagonists, yet a slight unevenness was noticed in between the treatments with regard to different characters studied. The biocontrol activity of the antagonists is certainly affected by soil physical, chemical and biological factors. The erratic nature of the antagonists observed in the present field experiments may also be due to the various soil and environmental factors that affected their antagonistic potential and dominant influence of the rhizosphere microbiota over the introduced antagonists.

The characterization studies of the promising antagonists indicate that the *Trichoderma* isolates TR17 and TR20 were effective against *R. solani* are *T. pseudokoningi* and *T. harzianum* respectively, whereas the *Trichoderma* isolates TR19 and TR22 which were effective against *Fusarium* belong to *T. viride*. The fluorescent pseudomonad, isolate P28 isolated from the rhizosphere of tomato which was effective against *R. solani* and *Fusarium* sp. under *in vitro* and *in vivo* in both the crops belongs to *P. fluorescens*.

A study was undertaken to investigate the actual mechanism by which *Trichoderma* sp. and *P. fluorescens* curtails the growth and multiplication of *R. solani* and *Fusarium* sp. *in vitro*. Microscopic examination of mycelium from the zone of interaction of *T. harzianum* and *R. solani* showed extensive coiling of the mycoparasite over the host hyphae. Cell abnormalities were also found associated with the pathogen-antagonist interaction suggesting mycoparasitism as the major mechanism operative in antagonism of *T. harzianum*. Under *in vitro* conditions, the *Trichoderma* spp. overgrew and suppressed the colony of *R. solani* and *Fusarium* sp. More or less similar findings were made by Elad *et al.* (1983) and Prasanthi *et al.* (2000) who observed complete suppression of *Rhizoctonia* by *Trichoderma* spp. by their overgrowth, coiling and disintegration.

The correlation of the comparative growth and their volatile and non volatile nature suggest that competition and antibiosis is playing a substantial role in antagonism and in suppressing the population of the pathogen. The rapid growth may probably provide an advantage to *Trichoderma* spp. over the pathogen in competing for nutrients and space utilization. The role of competition for nutrients and space in suppressing the population of *Fusarium* sp. have been explained by other researchers (Cugudda and Garibaldi, 1987; Widden and Scattolin, 1988; Mishra *et al.*, 2004).

The present *in vitro* study clearly indicates that more than one mechanism is involved in the fungus – fungus interaction. The mechanism of inhibition by *T. harzianum* observed by Kumar and Dubey (2001) was a combination of competition for food and space, production of antibiotics and mycoparasitism, though the principle mechanism involved was mycoparasitism by coiling of antagonistic hyphae around the hyphae of pathogen and lysis. Jatav and Mathur (2002) also reported that

Trichoderma spp. were found to produce volatile as well as non-volatile antibiotics and also exhibit mycoparasitism.

The mode of inhibition exhibited by *Pseudomonas* isolate P28 was studied under *in vitro*. The production of volatile inhibitory substances and competition for nutrients were the mechanisms involved in the suppression of fungal pathogens. Elad and Baker (1985) opined that disease suppression by fluorescent pseudomonads depends mainly on competition for nutrients and space. Diby *et al.* (2000) reported that fluorescent pseudomonads suppress *Phytophthora capsici*, the foot rot pathogen of black pepper through changing the microbial balance in the rhizosphere, by production of siderophores, volatile and non-volatile inhibitory metabolites and HCN. In this study the culture filtrate in KMB broth which is supposed to contain the non-volatile inhibitory metabolites of the bacterial antagonist did not exert any inhibitory effect on the growth of both the pathogens tested. This may be due to the difference in medium composition and variation in the strain selected.

Production of HCN also showed a negative result indicating that cyanide production could not considered to be a significant factor in the inhibition of fungal growth by *P. fluorescens* isolate P28. Nielsen *et al.* (1998) observed cyanide production in all *P. fluorescens* biovars except biovar I and III, but with different intensities. It could be interpreted that production of HCN is also a variable inhibitory character which varies upon the strains selected.

The possibility of siderophore production by *P. fluorescens* isolate P28 was investigated and it is observed that this isolate was not able to produce an extracellular siderophore in low iron medium. The results of the present study are in agreement with Lim *et al.* (1991), who was also not able to detect an extracellular sidrophore by *P. stutzeri* YPL-1 against *F. solani* in iron deficient medium. He demonstrated that *P. stutzeri* YPL-1 reduces disease caused by *F. solani* mainly via laminarinase and chitinase

activities. Similar findings were also made by Hebbar *et al.* (1992) who reported that antibiotic production rather than the siderophores seem to be responsible for the antifungal activity of rhizobacteria including *P. fluorescens*, *P. cepacia, Bacillus, Acinetobacter* etc.

An attempt was made to study the plasmid borne nature of the antagonism of Pseudomonad isolate by subjecting it to different concentrations of SDS. Concentrations of 1.0 and 1.2 per cent showed an increase in the optical density upto 4 h of incubation and then there was a sudden fall in OD values. Perhaps these concentrations of SDS caused maximum interference during replication leading to high frequency of curing and / or mortality of cells. Hence 1.0 and 1.2 per cent were used for isolating the cured colonies. Out of these two concentrations 1.0 per cent SDS was found optimum for curing fluorescent pseudomonad isolate P28. It was seen that the pathogen tested is overgrowing the colony, indicating the loss of inhibitory property during the curing process. The colonies which were closely grown by the pathogen without inhibition indicate the partial loss of the inhibitory character. The loss in the inhibitory property may be due to the loss of plasmids during the curing process indicating the antagonistic property as a plasmid borne character. The plasmid borne nature of the inhibitory metabolite production in fluorescent pseudomonad has been reported by Sujoy *et al.* (2000) and Varghese (2001). But this study does not agree with Anith (1997) who reported that the genes responsible for the antifungal property lies in the chromosome of the strain EM85. However it requires further studies to elaborate upon this point.

In the present investigation an attempt was made to elucidate the ability of *Trichoderma* spp. and *Pseudomonas fluorescens* to multiply and persist in the rhizosphere soil of tomato and chilli. As part of studying the intrinsic antibiotic resistance of *P. fluorescens* (P28), the cultivar was exposed to different concentrations of Ampicillin, Erythromycin,

Gentamycin, Streptomycin and Tetracycline. The isolate P28 showed specific resistance against the combination of Streptomycin (10 ppm) and Tetracycline (5 ppm) whereas most of the soil bacteria exhibited relative sensitivity against this combination.

The multiplication and persistence of *P. fluorescens* (P28), *T. harzianum* (TR20) and *T. viride* (TR22) in the rhizosphere was studied using tomato and chilli as the host system under pot culture condition. Immediately after application all the antagonists showed a fairly good number of viable propagule in the rhizosphere of both the plants as is reflected by the population count at zero day. Data on subsequent population of *P. fluorescens* in the rhizosphere of tomato showed a constant increase during the first 15 days and declined thereafter. The initial increase during the first 15 days may perhaps be due to the better adaptation and utilization of the root exudates of tomato as the primary carbon source for the growth and development of this isolate in the rhizosphere as P28 was originally isolated from the tomato rhizosphere. It is known that the native isolates exhibit better multiplication and persistence in the soil. This interpretation further holds good as the population of P28 in the rhizosphere of chilli increased only till seventh day and showed a reducing trend thereafter. The fungal antagonists *T. harzianum* and *T. viride* in the rhizosphere of tomato and chilli showed an initial increase on seventh day which then declined. However, a population of more than 10^6 propagules per gram of rhizosphere soil - the minimum number required for effective biocontrol - could be maintained by the antagonists upto 28 days.

The results corroborated with the previous observations made by Nayar (1996) who reported a rapid increase in population of *P. fluorescens* upto 16 days after seed treatment and root dipping of rice plants afterwards there was a decline. Yeole and Dube (2001) reported that the root colonization by introduced antibiotic resistant bacteria in

chilli rhizosphere increased upto 21 days which thereafter showed a gradual decline.

Saha and Sitansu Pan (1998) noted an initial increase in population of *T. harzianum* upto 20-30 days rather than decrease. The reason for the initial increase in population was attributed to germination of different spore forms and subsequent proliferation under natural condition with or without a food base. The cause of reduction in population in soil have been stated as 1) direct lysis of different spore forms, 2) germination of spores followed by subsequent lysis of germtube in soil and a continued slow exhaustion of propagules in soil under natural conditions.

In contradiction, Anith and Manomohandas (2001) observed a decline in the population of the bacterial antagonist *Alcaligenes* sp. during the first 30 days of application in the pepper rhizosphere. They also reported that the population of introduced *T. harzianum* in the soil decreased to a level of 10^5 during the first 15 days.

The population densities of the antagonists in soil is governed by factors like soil moisture, temperature, depth of sampling, the host system, total soil microflora in the rhizosphere of the host plants and definitely the test antagonist strain. The contradiction of the present observation to that of Anith and Manomohandas (2001) could be due to any of the factors mentioned here. At present, information regarding survival ability of free propagules of both fluorescent pseudomonads and *Trichoderma* in general and *Trichoderma* in particular in the rhizosphere of vegetable crops are deficient.

The formulation technology of the antagonists is critical for the effective implementation of biocontrol of various crop diseases. Biomass of these bioagents used for biological control must be inexpensive and should be produced in large quantities in a short time. In the present investigation an attempt was made to select various locally available organic substrates for mass multiplication of *Trichoderma* spp. Among

the eight different solid substrates evaluated, sorghum grains, coir pith + neem cake (1 : 1) and cowdung + neem cake + wheat flour (10 %) were the most favoured substrates by both the *Trichoderma* spp. It is noted that an increase in the growth and spore production was observed when wheat flour (10 %) and jaggery (3 %) was used as nutrient supplement.

Whenever a substrate is selected for mass multiplication two things are to be taken into account *viz.*, ability of the organism to grow in the substrate and ability to withstand minimum viable propagules (10^6) for a longer period of time. For most of the biocontrol agents a minimum population of 10^6 cfu g^{-1} of substrate is required for effective management. Since the viability of the propagules is highly affected by the moisture content of the substrates, an attempt was made to find out the most suitable level of moisture in sustaining the viable population.

All the substrates tested supported 10^6 viable propagules per gram for a period of three months. Sorghum grains though showed a steady population in the beginning, recorded a continuous decline after 30 days. More or less same trend was noticed with cowdung + neem cake + wheat flour mixture. Inoculum density of coir pith : neem cake (1 : 1) mixture at all the three moisture levels *viz.*, 35 (original moisture), 45 and 55 per cent increased upto 30 days then showed a reduction upto 60 days and then increased. Saju *et al.* (2002) reported that the addition of *T. harzianum* into organic media like neem cake, coirpith, farm yard manure and decomposed coffee pulp supported high inoculum production as well as served as nutrient additive to the crop.

It is seen that huge quantity of coir pith is left as an agricultural waste which creates pollution problem to the environment. Growth and sporulation of *Trichoderma* spp. on this agro-industrial waste may solve the problem of environmental pollution as well as its mass production for augmenting biological control. Supplementation of this lignocellulosic substrate with neem cake enhanced the growth and sporulation of

Trichoderma spp. This may be due to the abundant nutrients and growth factors present in the neem cake. As a result of heavy inoculum density and exhaustion of nutrients the population naturally got declined and the further increase in population after 60 days may be due to the availability of nutrients resulting from the degradation of coir pith. *Trichoderma* sp. is known to produce cellulolytic enzymes which helps in the degradation of cellulose into simpler glucose units. This glucose would be utilized by the *Trichoderma* for its growth and proliferation. Since the biocontrol is primarily linked to the sustained increase in active propagules of the antagonists, coir pith – neem cake mixture at its original or slightly higher (45 %) moisture proved to be an ideal cheap substrate for mass multiplication and long term survival of the antagonist.

For the bacterial antagonist, powder formulation using for different substrates at three different moisture levels were evaluated for their capacity to retain maximum shelf life of the organism. A spurt in the population of the bacterium in the formulations was recorded at 15 days irrespective of the substrates and the moisture levels and declined thereafter. The sudden shift in the base medium might have resulted in the immediate build up of the inoculum. Subsequent observations indicated that the bacteria survived for a long time in the talc formulation which retained a mean population of 1.28×10^8 cfu g^{-1} even at 90 days of storage. A high bacterial population could be maintained by the formulation in vermiculite till 60 days of storage.

The results of the present study is in agreement with Heera *et al.* (2002) who reported talc as the best carrier material for the survival of fluorescent pseudomonad in which a population of 1.5×10^9 cfu g^{-1} was recorded even after 75 days of storage.

According to Vidhyasekaran *et al.* (1997a), talc formulations were effective even after six months of storage while the shelf life of vermiculite, lignite and kaolinite formulations are short.

It is also clear from the results that a moisture percentage of 40 would be optimum for maintaining the bacterium in viable state for long periods.

The economics of production were worked out and clearly indicates that the bioagents could be recommended at field level since their application resulted in pesticide free produce with high net return. The reduction in yield observed in treatment with *Pseudomonas* in chilli infected by *R. solani* may be due to the adverse climatic and soil factors that existed at the time of crop cultivation.

Summary

6. SUMMARY

Rot caused by *R. solani* and wilt caused by *Fusarium* sp. are the two serious diseases in chilli and tomato. Both the disease initiate at the nursery level and extend to the main field leading to heavy economic loss. Eventhough chemical methods of control are partially effective, high cost of chemical fungicides and hazards of environment pollution forced the farming community to adopt alternate strategy for plant disease management.

The pathogens (*R. solani* and *Fusarium* sp. from chilli and tomato) were isolated from naturally infected plants collected from the major vegetable growing tracts of Thiruvananthapuram, Ernakulam and Idukki districts of Kerala. Six isolates of *R. solani* and ten isolates of *Fusarium* spp. could be collected from both the crops. Among these isolates R1t (*R. solani* from tomato) F1t (*F. oxysporum* from tomato) and F4c (*F. solani* from chilli) were selected for the study since they incited maximum disease symptoms.

The antagonists, *Trichoderma* spp. and fluorescent pseudomonads were isolated from the rhizosphere and phyllosphere of the healthy target crops. A total of 26 native isolates of *Trichoderma* and 56 native isolates of fluorescent pseudomonads were obtained from the crops.

In vitro studies were carried out to test the effect of biocontrol agents on *R. solani* and *Fusarium* sp. During the *in vitro* screening of *Trichoderma* against *R. solani,* 11 isolates of *Trichoderma* were found equally effective in suppressing *R. solani* and eight isolates showed equal effect on *Fusarium* sp. In order to select the highly efficient *Trichoderma* isolates against *R. solani* and *Fusarium* sp. for the *in vivo* experiments, a secondary *in vitro* screening was carried out with the selected *Trichoderma* isolates obtained from the initial *in vitro* screening. In the

secondary screening TR17 and TR20 were more inihibitory to *R. solani* whereas TR19 and TR22 were the efficient isolates against *Fusarium* sp. and hence these were selected for *in vivo* experiments.

In vitro screening of fluorescent pseudomonads against *R. solani* and *Fusarium* sp. revealed that P28 and P51 exerted maximum inhibition on *R. solani*, while P20 and P28 were the most inhibitory isolates against *Fusarium* sp.

The compatibility studies of the selected biocontrol agents under *in vitro* conditions showed that the fungal and bacterial antagonists were compatible.

Pot culture experiments conducted revealed that the biocontrol agents were effective in managing the disease and enhancing the growth of tomato and chilli. The efficient *Trichoderma* isolates multiplied in cowdung – neem cake mixture and fluorescent pseudomonads formulated in talc were used in the experiments. Among the different antagonist treatments, application of TR20, TR17 and P28, one week before planting considerably reduced the seedling rot in tomato and enhanced the growth of plants. However the combination of *Trichoderma* and *Pseudomonas* were highly effective and offered 100 per cent protection to the seedling against *R. solani*. The growth and yield of the plants were also maximum with the combination treatments.

In chilli the combination of TR20 + P28 was the most effective treatment with minimum disease incidence and maximum yield.

In the greenhouse experiments, where the different antagonists were tried against Fusarium wilt, effective suppression of wilt disease in terms of incidence and intensity was obtained by different antagonist treatments in both the crops. TR22 + P28 was the best combination treatment with maximum protection to the both the crops. All the BCAs

had a positive influence on yield and other growth characters. The impact of TR22 + P28 on yield was more promising.

Under field condition, all the BCAs showed positive impact on disease suppression and growth enhancements. *Trichoderma* sp. was more effective against *R. solani* than fluorescent pseudomonads whereas the wilt disease caused by *Fusarium* sp. was more suppressed by fluorescent pseudomonads.

The promising isolates of *Trichoderma viz.*, TR17, TR20, TR19 and TR22 were identified as *T. pseudokoningii, T. harzianum, T. viride* and *T. viride* respectively and fluorescent pseudomonad (P28) was identified as *P. fluorescens* based on the characterization studies.

Competition, mycoparasitism and antibiosis were the mechanisms operating in inhibiting the pathogen in the case of *Trichoderma*. The production of volatile inhibitory substances and competition for nutrients were the mechanisms involved in the suppression of fungal pathogens by fluorescent *Pseudomonas* (P28). The culture filtrate of the bacterial antagonist did not exert any inhibitory effect on the fungal pathogens. Studies on the HCN and siderophore production also showed negative results under *in vitro* conditions indicating that these could not be considered as the inhibitory mechanism by the fluorescent *Pseudomonas* (P28).

Genetic analysis studies of the selected pseudomonad isolate (P28) indicates that plasmid curing by SDS leads to loss of inhibitory property. This clearly shows the plasmid borne nature of the inhibitory property.

The multiplication and persistence of the antagonist in the rhizosphere soil was studied. *P. fluorescens* (P28) exhibited specific resistance against Streptomycin (10 ppm) to Tetracycline (5 ppm) and this was used as the marker for *P. fluorescens* isolate (P28). The organisms

showed satisfactory multiplication and persistence in the rhizosphere of both chilli and tomato.

Among the eight different solid substrates tested for mass multiplication of *T. harzianum* and *T. viride*, sorghum grains, coirpith + neem cake (1 : 1) and cowdung + neem cake (1 : 1) + wheat flour (10 %) were the most favoured substrates for both the organisms. The shelf life studies of *T. harzianum* and *T. viride* in these substrates at different moisture levels showed that coirpith + neem cake (1:1) at its original (35 %) or slightly higher (45 %) moisture was an ideal and cheap substrate for mass multiplication and long term survival of these fungal antagonists.

Among the four different carriers used for formulating *P. fluorescens*, talc at 40 per cent moisture was the best in retaining maximum viable population for longer periods.

The economic feasibility of using bioagents in crop production was worked out and proved that application of bioagents resulted in increased plant stand with improved yield ultimately resulting in high economic return.

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*Original not seen

Appendices

APPENDIX–I

Composition of different media

Czapek's (Dox) Agar

Sucrose	:	30 g
$NaNO_3$	:	2 g
K_2HPO_4	:	1 g
$MgSO_4. 7H_2O$	:	0.5 g
KCl	:	0.5 g
$Fe SO_4$	:	0.01 g
Agar	:	20 g
Distilled water	:	1 L

King's medium B (KMB)

Peptone	:	20 g
K_2HPO_4	:	1.50 g
$MgSO_4. 7H_2O$	:	1.50 g
Glycerol	:	10 ml
Distilled water	:	1 L
pH	:	7.2

Martin's rose bengal agar

Dextrose	:	10 g
Peptone	:	5 g
KH_2PO_4	:	1 g
$MgSO_4. 7H_2O$	:	0.5
Rose Bengal	:	33 mg L^{-1}
Streptomycin	:	30 mg
Agar	:	20 g
Distilled water	:	1 L

Potato dextrose agar (PDA)

Potato	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1 L

Potato Sucrose Agar (PSA)

Potato	:	200 g
Sucrose	:	20 g
Agar	:	20 g
Distilled water	:	1 L

***Trichoderma* selective media**

Glucose	:	3 g
$MgSO_4$	:	0.2 g
K_2HPO_4	:	0.9 g
Rose Bengal	:	0.15 g
Chloramphenicol	:	0.25 g
Agar	:	20 g
Water	:	1 L

DISEASE MANAGEMENT AND GROWTH IMPROVEMENT IN CHILLI AND TOMATO USING *TRICHODERMA* SPP. AND FLUORESCENT PSEUDOMONADS

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Abstract of the
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ABSTRACT

The study entitled "Disease management and growth improvement in chilli and tomato using *Trichoderma* spp. and fluorescent pseudomonads" was undertaken at College of Agriculture, Vellayani during 2001–2004.

The pathogens *viz.*, *R. solani* and *Fusarium* sp. causing seedling rot and wilt in chilli and tomato respectively were isolated from naturally infected plants collected from major vegetable growing tracts in Kerala and their detailed symptoms were studied. Among the different isolates of *R. solani* and *Fusarium* sp. R1t (*R. solani* from tomato), F1t (*F. oxysporum* from tomato) and F4c (*F. solani* from chilli) which produced maximum disease symptoms were selected for the study.

Trichoderma spp. and fluorescent pseudomonads isolated from the rhizosphere and phyllosphere of healthy chilli and tomato plants collected from various locations of vegetable tracts and also from virgin forest soils of Kerala were tested for their antagonistic activity against the test fungi. Among the 26 native isolates of *Trichoderma* tested under *in vitro* conditions, TR17 and TR20 emerged as the efficient antagonists against *R. solani* and TR19 and TR22 against *Fusarium* sp. Out of the 56 native fluorescent pseudomonads tested the isolates P28 and P51 exerted maximum inhibition on *R. solani* and P20 and P28 were most inhibitory isolates against *Fusarium* sp.

Pot culture experiments were conducted to assess the efficacy of biocontrol agents in managing the disease and enhancing the growth of chilli and tomato. Among the different antagonist treatments combined application of *Trichoderma* and *Pseudomonas* (TR17 + P28, TR20 + P28 and TR20 + P51) was highly effective and offered 100 per cent protection to tomato seedlings against *R. solani*. The growth and yield of the plants were

also maximum with the combination treatments. In chilli also the combination of *Trichoderma* and *Pseudomonas* (TR20 and P28) was most effective with minimum disease incidence and maximum yield.

Under greenhouse conditions, the wilt caused by *Fusarium* sp. was significantly reduced by the application of biocontrol agents. The maximum reduction in incidence and intensity of the disease was observed with the combination of TR22 + P28 in both the crops. The influence of TR22 + P28 on yield was also more promising in both chilli and tomato.

The efficient isolates of antagonists obtained from the green house studies were further evaluated under field condition. Positive impact of BCAs on disease suppression and growth enhancement in chilli and tomato were noticed under field condition also leading to better economic yields based on the benefit : cost analysis done.

Based on the characterization studies the isolates, TR17, TR20, TR19 and TR22 were identified as *T. pseudokoningi*, *T. harzianum*, *T. viride* and *T. viride* respectively and P28 was identified as *P. fluorescens*.

In vitro studies on the mechanism of inhibition by the antagonists showed that competition, mycoparasitism and antibiosis were the mechanisms exhibited by the *Trichoderma* sp. against the fungal pathogens whereas fluorescent pseudomonad (P28) inhibited the pathogens through competition for nutrients and by the production of volatile antibiotics. Genetic analysis of the inhibitory character of *Pseudomonas* isolate (P28) clearly showed the plasmid borne nature of the inhibitory property.

The multiplication and persistence of the antagonists in the rhizosphere soil was studied using antibiotic markers. The organisms showed satisfactory multiplication and persistence in the rhizosphere.

Among the different solid substrates tested for mass multiplying *T. harzianum* and *T. viride*, sorghum grains, coirpith + neem cake (1 :1)

and cowdung + neem cake (1 :1) + wheat flour (10 %) were the most favoured substrates by these organisms. Among these substrates coirpith + neem cake (1 : 1) at is original (35 %) or slightly higher (45 %) moisture sustained the viable population for longer period. For formulating *P. fluorescens,* talc at 40 per cent moisture was the best carrier.

Economic feasibility of using bioagents in crop production was worked out and it was proved that application of bioagents resulted in increased plant stand with improved yield ultimately resulting in high economic return.