EVALUATION OF BIO-FUNGICIDES IN THE CONTROL OF DRY ROOT ROT OF GREENGRAM

By

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B.Sc (Ag)

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CERTIFICATE

Mr.B.MALLAIAH has satisfactorily prosecuted the course of research and that the thesis entitled **"EVALUATION OF BIO-FUNGICIDES IN THE CONTROL OF DRY ROOT ROT OF GREENGRAM"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION OF BIO-FUNGICIDES IN THE CONTROL OF DRY ROOT ROT OF GREENGRAM"** submitted in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture** of the **Acharya N.G.Ranga Agricultural University,** Hyderabad, is a record of the bonafide research work carried out by **Mr. B.MALLAIAH** under my guidance and supervision. The subject of the thesis has been approved by the **Student's Advisory Committee.**

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. The author of the thesis has duly acknowledged all assistance and help received during the course of investigation.

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SYMBOLS AND ABBRIEVATIONS

%	:	Per cent
°C	:	Degree Celsius
@	:	At the rate of
a.i.	:	Active ingredient
cfu	:	Colony Forming Units
CD	:	Critical difference
CMRA	:	Chloroneb Mercury Rosebengal Agar Medium
cv.	:	Cultivar
DAS	:	Days after sowing
EC	:	Emulsifiable concentration
et al	:	Co-workers
Fig	:	Figure
g	:	Gram
h	:	Hours
ha	:	Hectare
i.e.,	:	That is
kg	:	Kilogram
L	:	Litre
m^2	:	Square meter
mg	:	Milli gram
ml	:	Milli litre
PDA	:	Potato dextrose agar
pH	:	Hydrogen ion concentration
ppm	:	Parts per million
SEM	:	Standard Error Mean
spp.	:	Species
t/ha	:	Tonnes per hectare
viz.,	:	Namely
W/W	:	Weight per weight
WP	:	Wettable powder

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DECLARATION

I, **Mr.B.MALLAIAH** hereby declare that the thesis entitled "**EVALUATION OF BIO-FUNGICIDES IN THE CONTROL OF DRY ROOT ROT OF GREENGRAM**" submitted to Acharya N.G.Ranga Agricultural University, Hyderabad for the degree of **Master of Science in Agriculture** is the result of original work done by me. I also declare that the material contained in this thesis has not been published earlier.

Date : 9/10/02

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ABSTRACT

Author	:	**B.MALLAIAH**
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Greengram (*Vigna radiata* L.) Wilczek is one of the important pulse crops grown in India and the crop is incited by a number of diseases. Dry root rot caused by *Rhizoctonia bataticola* L. (Taub) Butler (Pycnidial stage: *Macrophomina phaseolina*) is an important disease causing yield losses upto 25 per cent.

Survey conducted on the incidence of dry root rot in eight major greengram growing mandals of Chittoor district, A.P. indicated occurrence of dry root rot incidence in all the farmers fields surveyed and the incidence ranged from 5.7 to 12 per cent with low and high incidence in Pileru and Chinnagottigallu mandals, respectively. Among the three soil types surveyed, high (11.1%) and low (4.2%) incidences of dry root rot was recorded in sand loam and clay soils respectively. Greengram cv. ML-267 recorded high dry root rot incidence (11%) while the incidence was low (4.8%) in local cultivars.

An inoculum level of 7 per cent (w/w) was found to be optimum infection threshold level of test pathogen, *R.bataticola* with greengram cv. ML-267 in steam sterilized sandy loam soil. *In vitro* studies on the effect of certain fungal (*Trichoderma viride, T.harzianum, T.reesei,* and *Gliocladium virens*) and bacterial antagonists (*Pseudomonas fluorescens* and *Bacillus subtilis*) showed that *T.viride* is more effective in inhibiting the test pathogen (70%). Among the bacterial antagonists *P.fluorescens* was found significantly more effective in inhibiting the growth of test pathogen (63.7%).

Similarly, studies on *in vitro* effect of neem based commercial formulations *viz.*, Starneem and Neem gold. Starneem was found to be more effective in inhibiting the growth of *R.bataticola* compared to Neem gold. In the compatibility studies neem oil (Starneem) was found to be incompatible with all the fungal antagonists whereas it is compatible with bacterial antagonists. Similarly, thiram was found to be incompatible with *T.viride*.

In pot culture studies combined seed treatment with *T.viride* (4 g kg^{-1} seed). *P. fluorescens* (10 g kg^{-1} seed), neem oil (Starneem) (3 ml kg^{-1} seed) and thiram (3 g kg^{-1} seed) was found to be highly effective in increasing seedling emergence (91.3%), plant dry weight (98.6%) shoot length (73.9%) and root length (70.8%).

Seed treatment with all the four combinations was found to be superior in reducing dry root rot incidence (87.8%) and population levels of *R.bataticola* in soil compared to control.

Introduction

CHAPTER – I

INTRODUCTION

Greengram (*Vigna radiata* L.) Wilczek is one of the important pulse crops grown in India. It is an excellent source of high quality proteins, rich in potassium and phosphorus. Being a pulse crop, it fixes atmospheric nitrogen and thereby enriches soil fertility and is also used occasionally as green manure cover crop and inter crop or mixed crop with many cereals.

India is one of the leading countries in greengram production covering an area of 2.2 m.ha and production of 0.8 m.t. In Andhra Pradesh, it is cultivated in an area of 4.6 lakh ha with production of 2.1 lakh tonnes (Statistical Abstracts of Andhra Pradesh 2000). In Chittoor district, the crop is grown in an area of 244 ha with production of 135 tonnes. The average production is less than 0.5 t/ha as against their yield potential of about 1.5 to 2.0 t/ha.

Diseases caused by various plant pathogens are major contributing factors for lowering crop yields. In India, it is estimated that more than 50 per cent of the crop losses are due to soil borne plant pathogens.

Dry root rot of greengram caused by *Rhizoctonia bataticola* (Taub) Butler (Pycnidial stage: *Macrophomina phaseolina*) is one of the most destructive diseases in tropical and subtropical countries. The losses due to *R.bataticola* are estimated to be around 10.8 to 24.1 per cent in India (Kataria and Grover (1977) and Tyagi *et al.* (1988).

Management of soil borne fungal pathogens is difficult because of long-term survival and wide host range of the pathogens. These pathogens not only persist in the soil as saprophytes along with other soil organisms but also are transmitted from seed. Different fungicides are being used in the management of soil borne diseases including dry root rot of greengram. However, chemical management is uneconomical, hazardous, disturb the biological balance cause, ground water pollution, leave residues on food crops and results in development of resistance in pathogens to the chemicals and ultimately breakdown the varietal resistance.

In view of the disadvantages of these fungicides and non-availability of resistant cultivars, there is a need to develop a method which is effective, economical and ecofriendly, to reduce the dry root rot of greengram. The use of bio-control agents and botanical products as potential "bio-fungicides" may offer more environmental friendly and ecologically safe methods of protection of crop from soil borne plant pathogens.

Biological control of seed and soil borne pathogens with antagonistic fungi and bacteria has been under intensive investigation for the last many years and has been reviewed in detail by Baker and Cook (1974) and Cook and Baker (1983). Antagonists like *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma* spp. have been exploited successfully in the control of seed and soil borne fungi *viz.*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *R.bataticola*, *Aspergillus niger* and *Fusarium oxysporum*. Thus control of seed and soil borne pathogens with antagonistic microorganisms has gained considerable attention and appears to be promising as a viable supplement or alternative to chemical control and other management practices. The

biological agents applied to the seed also have the potential to colonize and protect the root from soil as well as seed borne fungal pathogens making seed application an affective delivery system (Paulitz and Fernando, 1996).

A large number of botanical products in particular, neem products (*Azadirachta indica*) have been screened and reported to be as an effective bio-fungicides against several plant pathogens *viz.*, *Sclerotium rolfsii*, *R.bataticola* (Grainge and Ahmed, 1998).

Keeping in view the importance of the greengram crop, losses caused by dry root rot pathogen and to exploit the naturally occurring bio-control agents and certain botanical products as potential bio-fungicides as an alternative to the chemicals, an attempt was made on "Evaluation of bio-fungicides in the control of dry root rot of greengram" during the course of present investigation with the following objectives.

1. To survey for the incidence of dry root rot of greengram in major greengram growing mandals of Chittoor district, Andhra Pradesh.
2. To isolate and identify the dry root rot pathogen associated with diseased greengram.
3. To test the antagonistic activity of certain bacterial and fungal bio-agents and fungitoxicity of botanical formulation (neem) against dry root rot pathogen of greengram.
4. To test the compatibility of bio-control agents with botanical formulation (neem) *in vitro*
5. To evaluate an effective antagonist and botanical formulation in control of dry root rot pathogen of greengram under green house conditions.

Review of Literature

CHAPTER – II

REVIEW OF LITERATURE

The available literature on various aspects of management of dry root rot incidence of greengram has been reviewed and presented under following headlines.

2.1 Survey
2.2 Symptomatology
2.3 Pathogenicity studies
2.4 Antagonistic studies
2.5 Compatibility studies
2.6 Management with chemicals, bio-control agents and botanical products
2.7 Integrated management of soil borne diseases

2.1 SURVEY

Greengram infected severely by *Macrophomina phaseolina* causing seed rot, and seeding blight at early stages and premature death and leaf blight at later stages.

Kataria and Grover (1977) and Tyagi *et al.* (1988) reported yield loss of 10.8 and 24.1 per cent due to *M.phaseolina* in mungbean from the states of Haryana and Rajasthan respectively.

Jaya *et al.* (1988) studied the influence of soil type on the severity of dry root rot caused by *R.bataticola* of chickpea and observed more severe incidence of the disease in sandy soils than clay soils.

Pandey and Singh (1990) reported *Fusarium oxysporum,* and *R.bataticola* on greengram crop in Allahabad. These pathogens, were found associated with the crop in an average incidence of 19.2 and 5.0 per cent respectively.

Tosi *et al.* (1997) reported on 51 varieties of sunflower at South Apollinare and Papiano (Perugia), Umbria (Italy). The predominant pathogen associated with the crop was *M.phaseolina* with 72 and 63 per cent infection at Perugia and Italy respectively.

Sahu and Jena (1997) surveyed and studied the seed microflora of 10 cultivars of greengram and isolated 20 fungal taxa belonging to 13 genera, of which *Macrophomina phaseolina* was the dominant species associated with all the cultivars tested.

Prameela Devi and Singh (1998) conducted a survey to know the virulence of *M.phaseolina* isolates of blackgram and greengram crops collected from different localities of North-South, North-East and Central India. The isolates were categorized as highly virulent, moderately virulent and weakly virulent based on disease incidence and intensity on greengram and blackgram.

Rettinassababady and Ramadoss (1999) surveyed on the incidence of root rot disease of rice fallow black gram caused by *M.phaseolina* in Karaikal region and revealed the incidence of 16 to 33 per cent. In general soils of sandy loam/sandy clay recorded higher percentage of root rot incidence than in clay soil.

Kratisharma and Tribhuwan Singh (2000) observed twenty four per cent of mungbean (*Vigna radiata)* seed samples collected from 11 districts of Rajasthan during 1996-97 showing 0.5 to 38 per cent *Rhizoctonia bataticola* infection.

2.2 SYMPTOMATOLOGY

The disease appears in different phases on mungbean. It produces seedling rot, ashy stem blight, leaf spot (blight) and root rot phases.

2.2.1 Charcoal rot phase / Seedling rot phase

Symptoms of seedling rot phase were first described on greengram by Philip *et al.* (1969). Subsequently charcoal rot symptoms were observed in other species of plants (Dhingra and Sinclair, 1977) and in mungbean (Scholefield and Griffin, 1979). They also observed that the pathogen causes seed decay and pre-and-post emergence damping off of seedlings, Ungerminated seeds exhibited rotting of radicles and plumules.

2.2.2 Leaf spot/blight

First observation was recorded on the occurrence of leaf blight phase in greengram and symptoms include brown discoloration along the veins of leaves starting with cotyledonary leaves. Affected leaves become distorted followed by leaf drop in severe cases. Circular brown spots of 0.5 to 1.0 mm in size appears on the upper surface of cotyledonary leaves and leaflets which later become greyish in centre with a definite brown margin (Philip, 1963).

2.2.3 Root Rot Phase

Philip (1963) first described the root rot infection due to *M.phaseolina* in greengram. The infection resulted in decaying of tap root as well as the secondary roots which can be uprooted easily from soil. The root tips of the affected plants become slightly moist and sticky and numerous sclerotia are generally visible on shredded roots.

2.2.4 Causal organism

The pathogen occurs in the sclerotial form with the taxonomic nomenclature as *Rhizoctonia bataticola* (Taub) Butler. The pycnidial stage of this pathogen is *M.phaseolina* (Tassi) Goid. Mc Rae (1929) reported the first occurrence of *M.phaseolina* on wilted plants of mungbean and urdbean and established the genetic similarity between *M.phaseolina and R.bataticola.* Among the several fungi reported, it is the most prominently occurring fungus on *Phaseolus* (Ramnath *et al.*, 1970). Different plant parts of the host were harbouring the pathogen and isolated the pathogen from various plant parts *viz.*, root, stem, leaf, pod and seed and the pathogens also differed in their cultural and morphological characters. (Jain *et al.*, 1973)

2.2.5 Morphology

Philip *et al.* (1969) noticed the differences in morphology of *M.phaseolina* isolates from various parts of host plants. They include the pycnidial development *in vitro* on mungbean roots infected with

Macrophomina, as black, globose or depressed and 150-200 µm in diameter. Pycnidiospores were oval or elliptical, hyaline, non-septate, thin walled with 10-24 x 6-10 µm in size. The Pycnidiospores develop into *R.bataticola* in culture at room temperature. Occurrence of numerous small black sclerotia of this pathogen *in vitro* conditions was also reported by Jain *et al.* (1973). According to him sclerotial morphology of *M.phaseolina* of urdbean was found to be different when isolated from different plant parts. The soil and seed isolate developed small sclerotia which were more pathogenic than leaf isolate which developed larger sclerotia.

2.2.6 Disease cycle

The pathogen can survive in infected seed, soil and diseased plant debris which helps in the development of the disease. The sclerotia present in the soil serve as primary source of inoculum of the disease and the pycnidiospores produced on the host are the secondary source of infection.

The pathogen has been reported to be externally and internally seed borne in both mungbean and urdbean (Moore, 1931; Philip, 1963; Kaiser *et al.*, 1968). Saprophytic survival of the pathogen was better in black soils (Pedagaonkar, 1989) and survives as sclerotia. In the field, the pathogen was found to survive on infected plant debris.

2.3 PATHOGENICITY STUDIES

Hooda and Grover (1982) studied the isolates of *M.phaseolina* obtained from different plant species and plant parts of the same host. They differed in their morphological, cultural characteristics and pathogenicity on *Vigna radiata,* young inoculum (3-5 days old) was more infective than old (3-34 days) and with increase in inoculum density disease intensity also increased.

Vishwadhar and Sarabhoy (1993) studied the variation in isolates of *R.bataticola* isolated from soybean plants collected from 11 locations at IARI. About 44 isolates were isolated from different parts of the plant and it was observed that the isolates differed with reference to colour of the colony, mycelial growth, shape and size of sclerotia. Morphological and cultural variations in isolates of *R.bataticola* isolated from different crops was also reported by other workers.

Prameela Devi and Singh (1998) reported 10 per cent inoculum density causing 71 per cent seedling mortality in greengram and black gram.

2.3.1 Biological Control

Management of plant diseases using living organisms to control the plant pathogens has gained much importance during recent years. These measures are called "Biological Control". Baker and Cook (1974) defined biological control of plant disease as "the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or

dormant state by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonists".

Considerable knowledge has been acquired on the role of naturally occurring microorganisms in regulating soil-borne pathogen populations. The activities of root or rhizosphere inhabiting microorganisms have a significant influence on plant health. There is a great deal of literature available describing many types and species of organisms antagonistic to plant pathogens (Baker and Cook, 1974; Burges, 1981; Cook and Baker, 1983; Mukerji and Jayanthi 1986; Hornby, 1990; Jairajpuri *et al.*, 1990; Stirling, 1991)

Prashanthi *et al.* (2000) evaluated certain fungal and bacterial bio-control agents as a seed and soil application against safflower root rot caused by *R.bataticola*. Both seed treatment and soil drenching increased safflower seedling percentage survival, Seed treatment was more effective than soil drenching with highest survival rate with *T.viride* (88.33%) and *P.flourescens* (86.66%).

2.4 ANTAGONISTIC STUDIES

Fungal Antagonists

Weindling (1932) reported that the culture of virulent fast growing strains of *Rhizoctonia* and *Armillaria mellea* pathogens were parasitised by *Trichoderma lignorum.*

Ghaffar (1968) reported that the fungal antagonist *T.viride* inhibited the growth of *M.phaseolina,* root rot pathogen of cotton. The hyphae of *T.viride* were found to coil round the *M.phaseolina* and parasitise.

Sarwar (1974) isolated and screened eight antagonistic fungi against *M.phaseolina,* the casual agent of root rot of castor and found that *Myrothecium* and *Trichoderma* spp were found to be effective in inhibiting the test pathogen.

Elad *et al.* (1984) studied the mode of action of *T.harzianum* against *S. rolfsii* and the results indicated that the parasitism of *T.harzianum* was due to the production of chitinase and α, β(1-3) gluconase enzyme.

Parakhia and Vaishnav (1986) reported that the growth of *R.bataticola* casual agent of root rot of chickpea was inhibited by *T.harzianum* in culture and the antagonist overgrew the pathogen.

Alagarsamy and Sivaprakasam (1988) reported *in vitro* antagonistic activity of *Trichoderma* spp against *M.phaseolina* infection in cowpea and observed that the antagonist inhibited the growth of test pathogen by 58.5 per cent.

Ikotun and Adenkunle (1990) reported that *T.harzianum* was effective antagonist, against the mycelia of *M.phaseolina* and prevented further growth *in vitro.*

Calistru *et al.* (1997) observed the antagonistic nature of *Trichoderma* spp against *Aspergillus flavus* and concluded that the activity was due to production of extracellular amylolytic, pectinolytic, proteolytic and cellulolytic enzymes.

Manoranjitham *et al.* (2001) Showed application of talc based formulation of *T.viride* and *P.fluorescens* in nursery beds before sowing of tomato seeds reduced the pre and post emergence damping off, in addition antagonists increased root length (67.5%) shoot length (74.9%) and dry matter production (52.7%).

Bacteria Antagonists

Ganesan and Gnanamanickam (1987) reported the antagonistic activity of *P.fluorescens* against mycelial growth of *S. rolfsii* by 60 per cent *in vitro*.

Elangovan and Gnanamanickam (1992) evaluated 85 strains of *P. fluorescens* against *Sclerotium oryzae,* of which 19 strains were found to be more effective with inhibition zone ranging from 6.0 to 30.0 mm.

Majundar *et al.* (1996) studied the antagonistic activity of bio-control agents viz. *T.viride, T.harzianum and B.subtilis* in vitro against *M.phaseolina,* the incitant of leaf blight of moth bean. Among the three antagonists tested *T.harzianum* was found to be highly effective in inhibiting the pathogen.

Ray and Mukerjee (1997) screened 68 isolates of micro organisms against certain soil borne fungal pathogens of which 11 were found to be inhibitory. Among them *Bacillus* strains S_{12} and S_{17} showed high antagonistic properties followed by S_{11} and S_{16} strains against *S. rolfsii.*

2.5 COMPATIBILITY STUDIES

Mukhopadhyay (1995) integrated biological agents with suitable fungicide in view of insensitivity of the bio-agents to some chemicals in chickpea for control of diseases caused by *R.solani, Pythium* spp and *Fusarium oxysporum.* The treatment was highly effective and resulted in enhanced crop performance when compared with biological or chemical treatment alone.

Shivpuri and sobti (1995) tried seven systemic and non-systemic fungicides for compatibility with *T.harzianum* under *in vitro* conditions. The studies showed that vitavax and thiram supported maximum mycelial growth of antagonist which differed significantly from check.

Sharma and Mishra (1995) studied the effect of four fungicides on the growth and spore germination of *T.harzianum* under *in vitro* conditions. Fungicides metalaxyl, chlorothalonil and captafol showed little inhibition while thiram was highly inhibitory even at lower concentrations.

Kausalya and Jeyarajan (1991) found that individual and combined application of *T.viride,* and *T.harzianum* to black gram against *M.phaseolina* infested soil increased the germination per cent.

The efficacy of seed treatment with fungal antagonist, *T.viride* in controlling the dry root rot caused by *M.phaseolina* of mungbean was evaluated by Kehri and Chandra (1991). The antagonist applied as seed coating reduced the seed mortality in two cultivars when compared to control in unsterilized soil. The antagonist also increased the dry weight of shoot, and nodules up to 31.7 and 100 per cent respectively, over control.

Kehri and Chandra (1991) reported 100 per cent reduction of dry root rot incidence by seed pelleting of *T.viride* in sterilized soil with *M.phaseolina* in green house studies.

Mukhopadhyay *et al.* (1992) found that seed treatment with *Gliocladium virens* was found effective in controlling soil borne pathogens *viz., S.rolfsii, R. solani* and *Fusarium oxysporum* in groundnut.

Raghuchander *et al.* (1993) studied the effects of different isolates of *T.viride* in the control of root rot of mungbean caused by *M.Phaseolina.* Application of bio-control agents in soil resulted in reduction of root rot incidence to the extent of 16 per cent as compared to control.

Uma Maheswari and Ramakrishna (1994) reported that seed treatment with *T.viride* reduced the root rot incidence caused by *M.phaseolina* in groundnut.

Out of two methods of application of fungal bio-control agent, *T.viride* in the control of root rot *(M.phaseolina)* of mungbean tested by seed pelleting was found to be highly effective in reducing the root rot incidence and also sclerotial number compared to row application. Moreover, rhizosphere soil showed a higher number of *Trichoderma* population in seed pelleting treatment compared to row application (Raghuchander *et al.*, 1997),

2.6.2 Bacterial Bio-control Agents

Successful use of seed treatment with *B.subtilis* as a biological agent has been reported in the case of potato seed pieces for the control of charcoal rot caused by *M.phasealina* and *Botryodiplodia solanitubersi* (Tirumalachar and O'brein 1977).

Paramjit Singh and Mehrotra (1980) studied the effect of seed treatment with bacterial antagonist *Bacillus* spp in the control of *R.bataticola* of greengram and the results indicated that the bacterial antagonist was proved to be antagonistic to the test pathogen and also effective in reducing the incidence caused by the pathogen.

Turner and Backman (1991) concluded that seed treatment with *B.subtilis* improved seedling emergence, nodulation by *Rhizobium* spp plant nutrition with reduced levels of root canker (*R.solani*) in groundnut.

Laha *et al.* (1992) tested seed treatment with certain isolates of fluorescent pseudomonas for their ability to suppress the root rot and damping off incidence in cotton (*R.solani*) and found that the disease intensity was reduced from 52.6 per cent (with non bacterised seeds) to zero per cent (with seed bacterized with isolates of F-13, F-14 and F-11) in pot experiments.

Rabindran and Vidyasekaran (1996) tested four isolates of *P.fluorescens viz.*, PFALR1, PFALR2, PFK1 and PFK2 against *R.solani* and reported that isolate PFALR2 was found to be the most effective for the control of sheath blight of rice (36%) under green house conditions.

Alice *et al.* (1996) evaluated 11 antagonists against *M.phaseolina*. Among 11, *T.harzianum* isolate 2, *T.viride* native isolate and *T.viride* commercial were effective against test pathogen,. Pot culture experiments revealed that coating jasmine cuttings with *T.viride* along with neem cake application in soil were effective in controlling root rot incidence.

Seed treatment with peat based formulation of bacterial bio-control agent, *P.fluorescens* @ 16 g/kg seed was found to be effective in controlling sheath blight of maize caused by *R.solani* (Siyakumar *et al.*, 2000).

Rangeshwaran *et al.* (2001) studied the effect of seed treatment with two bacterial antagonists *viz., P.putida* and *P.fluorescens* in control of root rot of chickpea caused by *Rhizoctonia* spp and the results indicated that seed treatment with both the antagonists significantly reduced root rot incidence of chickpea when compared to control. Among the two bacterial antagonists, *P.fluorescens* was found to be highly effective in reducing the incidence by 6.4 per cent compared to *P.putida.* Seed treatment with these two bio-control agents also resulted in significant increase in seed yield.

2.6.3 Disease Management by Chemicals

Sharma *et al.* (1975) showed that seed treatment with Thiram @ 0.325 per cent is very effective in controlling seedling blight of mungbean (*M.phaseolina*).

Illyas *et al.* (1975) reported that mycelium of *M.phaseolina,* the causal agent of charcoal rot of soybean was most sensitive and equally inhibited by three benzimidazole fungicides *viz.,* Benomyl, thiabendazole and thiophanate methyl, and also intermediately sensitive to thiram *in vitro.*

Shukla and Singh (1973) evaluated different fungicides to check root rot of sesamum caused by *M.phaseolina.* Captan treated seeds showed less mortality of plants and gave the highest yields, followed by Dithan M-45, Agrosan GN and Thiram.

Gupta and Bhardwaj (1980) used poisoned food technique to observe the inhibitory effect of various fungicides on radial growth of *R.bataticola*. Linear growth of fungus was found minimum with Benomyl and Dithane M-45 at 100 ppm concentration.

Natarajan *et al.* (1983) evaluated certain fungicides against *M.phaseolina* and observed that use of Thiram and Dithan M-45 were effective in suppressing the disease with higher yields in groundnut.

Chauhan (1986) compared efficacy of different fungicides for the control of seedling diseases of cotton due to *Rhizoctonia* spp. Maximum germination of seeds was observed in seeds treated with carbendazim followed by quintozene. Seeds treated with fungicides gave better yield of seed cotton than control.

Patel and Patel (1990) tested different fungicides *in vitro* against *M.phaseolina*, dry root rot pathogen of sesamum in which benomyl, thiram and carbendazim were found on par and were significantly superior in inhibiting the growth of the pathogen over the rest of the fungicides. Complete growth inhibition was obtained with benomyl at 0.05 per cent.

Rajpurohit (1997) evaluated 8 fungicides against stem and root rot of sesamum caused by *M.phaseolina*. Seed treatment with carbendazim, carboxin, captan and thiram were found effective in reducing the diseases.

2.6.4 Botanical Products

Lakshmanan and Mohan (1989) reported that the water extracts of Garlic, Bougainvillea and *Azadirachta* significantly inhibited the mycelial growth of *Thanatephorus cucumeris in vitro.*

Dhanapal *et al.* (1993) studied the antifungal properties of commercial neem products, neem oil against *R.solani* which found to be effective in reducing the growth of pathogen to the extent of 80-100 per cent *in vitro*. The same products were also found effective in reducing the disease incidence when applied to the seedlings in pots.

Sarvamangala *et al.* (1993) reported that leaf extract of *Azadirachta indica* was found effective in inhibiting spore germination of *Cerotelium fici* by 91.2 per cent, where as extracts of *Eucalyptus* spp and *Calotropis gigantia* proved highly toxic to *Cercospora mersiola* by inhibiting the conidial germination by 91.5 and 91.3 per cent, respectively.

Dohroo and Gupta (1995) revealed *Azadirachtin* and other limonoids were quite effective in the control of plant diseases of diverse nature. Increased populations of biological control agents were found during decomposition of the oil cake by the release of acids and other chemicals. The addition of neem cake to soil reduced the incidence of damping off, wilt, blight and rot of cotton, soybeans, coconut and ginger. The neem oil had fungicidal properties that were inhibitory to sclerotia of *Sclerotium*, *Rhizoctonia*, and *Sclerotinia.*

Jagannathan and Sivaprakasam (1996) studied the ability of neem (*Azadirachta indica*) derivatives (neem oil and neem seed kernel extract) to control sheath rot (*Sarocladium oryzae*) of rice. All treatments significantly reduced sheath rot compared to the control.

Lokhande *et al.* (1998) conducted field trials to evaluate neem oils in control of leaf spot of groundnut. The neem (*Azadirachta indica)* products (neem seed extract and neem oil) were less effective than the fungicides but all the treatments were significantly superior in comparison with the control.

Ahamad and Srivastava (2000) conducted a field trail to evaluate the activity of various plant products (0.1% palmarosa oil, 0.3% neem oil, 1.0% *Ocimum tenuiflorum* leaf extract) along with antagonistic microorganisms like *B.subtilis P.fluorescens T.viride* against dry root rot (*R.bataticola*) in chickpea cv.C235 under pot conditions and observed the reduction in disease incidence.

Rajappan *et al.* (2001) studied neem (*Azadirachta indica)* and pungam oil based emulsifiable concentrate formulations for their efficacy to inhibit the mycelial growth of the fungi like *Helminthosporium oryzae* (C*ochliobolus miyabeanus)* and *Pyricularia oryzae* causing grain discoloration on rice under *in vitro* conditions. All three formulations *viz.,* neem oil 60 per cent EC (acetic acid), neem oil 60 EC (citric acid) and neem oil + pungam oil EC (citric acid) inhibited mycelial growth of the pathogens. These formulations effectively controlled the grain discoloration of rice.

2.7 INTEGRATED DISEASE MANAGEMENT STRATEGY

Disease management plays a small but nevertheless a vital role in checking disease from flaring into epidemics. Although management of the disease can be achieved either by the use of resistant varieties, application of bio-agents, chemicals, field sanitation or the manipulation of the cultural practices. A single method alone may not avoid the danger of sustaining heavy crop losses. Now the strategy has been shifted from the use of any single method of control to the integration of different approaches of disease management. Therefore selection of suitable bio-agents with other control methods would suppress the disease incidence and consequent epidemic development.

2.7.1 Integrated control of *M.phaseolina*

Integrating biological and chemical control seems to be a very promising way of controlling pathogens with minimal interference with the biological equilibrium (Papavizas, 1973; Henis and Chet, 1975; Baker and Cook, 1982).

a. Bio agent + fungicide

Elad *et al.* (1986) reported that an integrated approach involving *T.harzianum* and PCNB or dazomet was as effective as the use of antagonist alone or better then the individual treatment for the management of dry root rot in melons and bean.

Vyas and Khare (1986) obtained good control of soybean dry root rot by combined application of *T.harzianum* and carbendazim. Vyas (1987) further confirmed that the disease could be reduced by combination of thiram and carbendazim followed by seed treatment with fungicide tolerant strain of *Trichoderma* spp or *B.subtilis* in *Macrophomina* sick soils. Simultaneous application of *T.viride* or *T.harzianum* with carbendazim treatment was effective in reducing dry root rot of soybean (Yyas, 1994).

Alagarsamy and Sivaprakasam (1988) reported seed pelleting with *T.viride* alone or in combination with carbendazim reduced seedling mortality in cowpea incited by *M.phaseolina* besides enhancing yields.

Kuruvilla Jacob (1989) observed that integration of carbendazim seed treatment with *T.harzianum* was found to be superior to either of the methods used alone in reducing root rot of urdbean.

Rajeswari *et al.* (1999) evaluated the bio-efficacy of carbendazim tolerant isolate of *T.harzianum* and exotic isolates of *T.viride* and *T.virens* against dry root rot pathogen of mungbean incited by *M.phaseolina* under glass house conditions. *T.harzianum* when applied to the soil besides being effective against the disease, increased seed germination by 96 per cent, plant height by 35.5cm and total biomass by 2.5g/plant. Integration of *T.harzianum* as soil application and seed treatment with sub-lethal doses of carbendazim significantly reduced dry root rot incidence (95.3%) over soil treatment (91.5%) and seed treatment alone (86.5%)

Rajeev Pant and Mukhopadhyay (2001) conducted field experiment on seed and seedling rot complex of soybean and explained integration of vitavax with *G.virens* and *T.harzianum* improved seedling emergence, plant stand and yield over individual treatments.

Bunker and Kusum (2001) evaluated *T.harzianum* and *T.aureoviride* individually as well as in combinations with Bavistin seed treatment in pathogen infested soil in pots on dry root rot of chilli. Seed treatment with bio-control agent was as effective as Bavistin seed treatment. Combination of two bio-control agents was better than the individuals. The integrated application with fungicide gave higher germination and reduced mortality due to disease.

b. Bio-agent + Neem oil

Singh (1993) studied the combined effects of *T.harzianum* with neem oil, neem gold on collar rot of pigeonpea caused by *S.rolfsii*. The results indicated that seed treatment with *T.harzianum* and neem oil reduced the mortality of seedling to 20 per cent as against 98 per cent in control.

Materials and Methods

CHAPTER – III

MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE

Experiments pertaining to the present research work were conducted in the Department of Plant Pathology, S.V. Agricultural College, Tirupati during 2001-2002. The pot culture experiments were conducted in green house at the Department of Plant Pathology, S.V. Agricultural College, Tirupati. The college is situated at 13° North latitude and 79°E longitude and at an altitude of 182.9 m in tropical belt of South India.

Tirupati area falls under south zone based on agroclimatic conditions of Andhra Pradesh. This zone is characterized by fairly hot summer and rainfall receiving in two spells, *viz.,* South-west (June-September) and North-East (October-January) monsoon periods. The total rainfall during south-west and north-east was 350 and 320 mm respectively.

3.2 GLASSWARE AND CHEMICALS

3.2.1 Glassware

The glassware used in the present study comprised of Borosil flasks (250, 500 and 1000 ml capacity), petriplates (90 mm diameter), test tubes (150 x 15 mm), microscopie slides and pipettes.

The glassware was thoroughly washed with a detergent followed by cleaning with tap water before placing them in cleaning solution with the following composition.

Potassium dichromate	-	60 g
Concentrated sulphuric acid	-	60 ml
Distilled water	-	1000 ml

The glassware was kept in cleaning solution for 24 h and later rinsed with distilled water for three times before use.

3.2.2 Chemicals, Equipment and Apparatus

Chemicals used were of "Analytical grade" (B.D.H. Analar; E.Merck). The pH of the medium was adjusted to the required level with N/10 NaOH or N/10 Hcl.

Stereobinocular microscope was used for observing the fungal culture *etc.* Weighments were done on a single pan electronic balance with a sensitivity of 0.01 g. Spore concentrations were enumerated and adjusted by Haemocytometer. Size of sclerotial bodies were measured by micrometry.

3.2.3 Media Used

The composition of the different media used during the course of investigation are given below.

a. Potato Dextrose Agar Medium (PDA) (Ainsworth, 1961)

Peeled potato slices	-	200 g
Dextrose	-	20 g
Agar	-	20 g
Distilled water	-	1000 ml
pH	-	6.0 to 6.5

b. Potato Dextrose Broth

Peeled potato slices	-	200 g
Dextrose	-	20 g
Distilled water	-	1000 ml
pH	-	6.0 to 6.5

c) Nutrient Agar Medium : (Allen, 1953)

Beef extract	-	3.0 g
Peptone	-	5.0 g
Glucose	-	5.0 g
Sodium chloride	-	5.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7

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

Protease peptone	-	20 g
K_2HPO_4	-	2.5 g
Glycerol	-	15 ml
$MgSO_4$ $7H_2O$	-	6 g
Agar	-	15 g
Distilled water	-	1000 ml
pH	-	7.0

e) *Trichoderma* selective medium: (Elad and Chet, 1983)

K_2HPO_4	-	0.9 g
Mg SO_4	-	0.2 g
NH_4NO_3	-	1 g
Kcl	-	0.15 g
Glucose	-	3.0 g
Agar	-	20.0 g
Metalaxyl(Ridomyl 25 WP)	-	0.3 g
Rose Bengal	-	0.15 g
Chloromphenicol	-	0.25 g
Distilled water	-	1000 ml
pH	-	7.0

f) *Rhizoctonia bataticola* (Pycnidial stage *Macrophomina phaseolina*) Selective medium: Chloroneb Mercury Rose bengal Agar Medium (CMRA) (Meyer *et al.*, 1973)

Polished rice	-	10 g
Agar Agar	-	20 g
Chloroneb	-	300 mg
$HgCl_2$	-	7 mg
Rose Bengal	-	90 mg
Streptomycin sulphate	-	40 mg

3.3 STERILIZATION OF GLASSWARE, MEDIA, SOIL AND SEED

Glassware kept in appropriate containers were sterilized in hot air oven at 160°C for 90 minutes. Media were sterilized at 15 lbs for 15 minutes in an autoclave. The soil mixture for pot culture studies was sterilized by using an auto clave at 15 lbs pressure for one hour for three consecutive days. For all the pot culture experiments earthen pots of 12 cm diameter with a capacity of 2 kg were used. Greengram seeds were surface sterilized using 2.5 percent sodium hypochlorite solution for 3 minutes followed by three consecutive washings separately with sterile water.

3.4 TEST PLANT

Greengram (*Vigna radiata* L.) Wilczek cv. ML-267 obtained from Regional Agricultural Research station, Tirupati was used for all the studies.

3.5 SURVEY

Survey was conducted in eight major greengram growing mandals of Chittoor district of Andhra Pradesh during *rabi* 2001-2002 to study the incidence of dry root rot.

3.5.1 Collection of Diseased Plant Samples

The farmers' fields were selected at random in eight major greengram growing mandals of Chittoor district of Andhra Pradesh *viz.*, Srikalahasti, Chandragiri, Pileru, Pakala, Pulicherla, Madanapalli, Chinnagottigallu and Nagari. In each mandal, three villages were selected at random and from each village one farmer field was surveyed. From each field, 4-6 places of 1 sq meter area (depending upon the area of the field) were selected at random on diagonal line of the field for taking diseased plant samples representing whole field. About 5-10 diseased plants showing symptoms of dry root rot incidence were collected from each field (Singh, 1984). The plant samples were collected in a polythene bag and tied with a rubber band, labelled and brought to the laboratory for further studies. From the diseased plant samples the test pathogen was isolated and identified using appropriate keys. (Gillman, 1957; Barnett, 1962)

3.5.2 Dry Root Rot Incidence

The diseased plants were recorded from 1 sq meter area in 4 to 6 places in each field and number of plants with dry root rot incidence was recorded and expressed in percentage following formula given by Singh (1984).

$$\text{Dry root rot incidence (\%)} = \frac{\text{No.of infected plants}}{\text{Total number of plants}} \times 100$$

3.6 LABORATORY TECHNIQUES

The general laboratory techniques followed were those described by Dingra and Sinclair (1994).

3.6.1 Test Pathogen

Isolation of test pathogen: The pathogen *Rhizoctonia bataticola* (Taub) Butler (Pycnidial stage *Macrophomina phaseolina* (Tassi) Goid) was isolated on potato dextrose agar (PDA) medium from greengram plant showing dry root rot symptoms. Bits of diseased portion along with healthy tissue from the infected root comprising small, black sclerotia were cut with a sterile blade and washed thoroughly under running tap water to get rid of foreign matter. These were surface sterilized with 1 percent mercuric chloride ($HgCl_2$) for 3 minutes followed by three washings with sterile distilled water. These bits were then transferred to PDA containing petriplates and were incubated at room temperature (28°$\pm$ 2) for 48 hours. A portion of the mycelium was

taken from fungal growth arising from incubated bits and was transferred on to a fresh PDA plate. This fungus was purified by single hyphal tip method (Rangaswami, 1958) and maintained on agar slants for further use.

3.6.2 Mass Multiplication of the Pathogen

The inoculum of the test pathogen, *Rhizoctonia bataticola* maintained on agar slants was further multiplied on sorghum grains. One hundred grams of sorghum seeds were washed thoroughly in tap water and soaked overnight in 250 ml conical flasks with addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flasks at room temperature they were shaken well to separate the sterilized grains and were inoculated with disc of 4 day old culture of *R.bataticola* and incubated at 28 $\pm$ 2°C for seven days in BOD incubator.

3.6.3 Pathogenicity Studies

The test pathogen *R.bataticola* isolated from diseased plants collected during survey was used to study the effect of different inoculum levels, so as to determine optimum infection threshold level.

A pot culture experiment was conducted in the green house to study the pathogenicity of the test pathogen at different inoculum levels by soil infestation following the procedure given by Haware (1980). Earthen pots of 12 inch diameter (capacity 2 kg) were filled with 2 kg steam sterilized soil.

The following are the characteristics of the soil used for the pathogenicity studies.

Soil structure	=	Sub angular blocky
Soil texture	=	Sandy loam
pH	=	8.0
E.C	=	0.4 m mho/cm
Organic Carbon	=	0.22 (medium)
Available nitrogen	=	197 kg/ha (medium)
Available P_2O_5	=	43 kg/ha (medium)
Available K_2O	=	164 kg/ha (medium)

3.6.4 Effect of Different Inoculum Levels on Dry Root Rot Incidence

The inoculum of test pathogen multiplied on sorghum grains was added to the steam sterilized soil @ 1,3,5,7 and 9 percent (w/w) in the pots. The soil in pots without inoculum of the pathogen served as control. Each pot was sown with 10 surface sterilized greengram seeds cv. ML-267. Six treatments with five replications for each inoculum level of test pathogen were maintained. The data on dry root rot incidence was recorded at 45 days after sowing and expressed in percentage.

3.6.5 Testing the Antagonistic Activity of Fungal and Bacterial Bio-control Agents

The antagonistic activity of fungal bio-control agents *viz., Trichoderma viride*, *T.harzianum, T.reesei,* and *Gliocladiam virens,* and bacterial bio-control agents *Pseudomonas fluorescens Bacillus subtilis* obtained from Agricultural College and Research Station, Madurai, TNAU was tested against test pathogen, *R.bataticola* following dual culture technique (Dennis and Webster, 1971)

3.6.6 Dual Culture Technique

Fungal bio-control agents

20 ml of molten PDA was poured in 90 mm petri dishes and allowed to solidify. Seven days old fungal disc of the antagonist of size 9 mm was placed at one end of media on petriplate. A 9 mm disc of test pathogen of five days old was placed at the opposite end.

Five replications along with suitable control were maintained. The plates were incubated at room temperature (28 $\pm$ 1°C) till mycelial growth in the control plates covered the entire plate. The linear growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula.

$$\% \text{ inhibition of pathogen} = \frac{\left(\text{Growth of pathogen in control plate}\right) - \left(\text{Growth of pathogen in presence of fungal antagonist}\right)}{\text{Growth of pathogen in control plate}} \times 100$$

3.6.7 Testing Antagonistic Activity of Bacterial Bio-control Agents by Dual Culture Technique

The antagonistic activity of *Pseudomonas fluorescens*, and *Bacillus subtilis* against the test pathogen *R.bataticola* was tested following dual culture technique.

A gentle superficial streak of one cm with bacterial bio-control agent was made at one end of the petriplate on PDA media by means of a sterilized inoculation loop. A nine mm PDA culture disc of the test pathogen was placed at the opposite end.

Five replications along with suitable control were maintained. The plates were incubated at room temperature (28 $\pm$ 1°C) till the mycelial growth in the control plates covered the entire plate. The linear growth of the test pathogen was measured and the percentage inhibition was calculated by adopting formula as in the case of fungal bio-control agents (3.6.6) where as bacterial antagonist was used in the place of fungal bio-control agent.

3.6.8 Evaluation of Fungicide and Neem based formulations Against Test Pathogen

The effect of a fungicide *i.e.* Thiram and two neem (*Azadirachta indica*) based formulations (Neem Gold 0.15% and Neem oil (starneem) 0.15%) were evaluated against the test pathogen *R.bataticola* following poisoned food technique (Nene and Thapliyal, 1993) to test their fungi toxicity.

3.6.9 Preparation of Fungicidal Concentration

One mg of fungicide (pure solute/active ingredient 100 per cent pure chemical) was dissolved in one liter of water to obtain 1ppm concentration. Required concentrations of the fungicide *viz.*, 25, 50, 100, 150, 200 and 300 ppm were prepared accordingly.

The effect of fungicide on the pathogen was tested following poisoned food technique (Nene and Thapliyal, 1993).

3.6.10 Poisoned Food Technique

Potato Dextrose Agar (PDA) medium was prepared with double the recommended strength. A double strength PDA medium contains double the concentration of potato, dextrose and agar except water. The fungicidal solution was prepared double the test concentrations. Ten ml of fungicidal solution was mixed thoroughly with 10 ml molten PDA medium and allowed

to solidify. Appropriate controls were maintained with sterilized water with out fungicide. The test fungal culture was cut into nine mm discs from the periphery of five days old pure culture with sterile cork borer and transferred to the center of each plate containing poisoned medium. Controls were maintained by placing fungal disc in untreated medium (*i.e.* without fungicide) in petriplate. Five replications were maintained for each treatment. The whole procedure was carried out under aseptic conditions. All the inoculated petriplates were incubated in an incubator at 28 ± 2°C. The diameter of fungal colony was measured, when the colony growth of fungus in control plates was full. The colony diameter in treated plates as compared to control was taken as measure of fungitoxicity. The percent inhibition was calculated by adopting formula as in the case of fungal bio-control agents 3.6.6 where as fungicide was used in the place of fungal bio-control agent.

3.6.11 Fungicide used *in vitro*

Common name	Trade name	Formulation	Chemical name	Concentrations in ppm
Thiram	Thiride	75% wp	Tetra Methyl Thiram Disulphide	25 50 100 150 200 300

3.6.12 Neem Based Formulations

Commercial neem based formulations of *Azadirachta indica* were used to test against the pathogen at different concentrations. The required concentrations of neem products were prepared from neem commercial formulations (*viz.*, Neem gold 0.15%, Neem oil (Starneem) 0.15%) as given in the fungicides in 3.6.9.

The fungitoxicity of neem formulations against test pathogen was assayed following poisoned food technique as described in 3.6.10 using neem product in place of fungicidal solution.

3.6.13 Details of commercial neem products

Common name	Trade name	Active ingradient	Chemical name	Concentrations ppm
Neem gold (neem oil)	Neem gold	0.15%	Neem based bio-pesticides	100 200 400 600 800 1000
Starneem (neem oil)	Starneem	0.15%	Neem based bio-pesticides	

3.6.14 Compatibility Studies

Thiram and T.viride in vitro:

The compatibility of thiram at 25, 50, 100, 150, 200 and 300 ppm concentrations with fungal antagonist *T.viride* was studied *in vitro* following poisoned food technique as described in 3.6.10.

Thiram and *Pseudomonas fluorescens*

Fungicide (thiram) at different concentrations were evaluated against *P.fluorescens* through paper disc plate method (Bandopadhyaya *et al.*, 1979). Each petriplate was poured with 10 ml nutrient agar medium and allowed to solidify. After that 5 ml warm nutrient agar medium seeded with *P.fluorescens* was poured and uniformly spread on the surface of solidified medium. Sterilized blotting paper discs of 10 mm diameter were dipped out in required concentrations of fungicidal solution and such pieces were placed in middle of each petridish on the surface of the medium pre-seeded with *P.fluorescens* in five replications. These plates were incubated at 28 $\pm$ 2 and observations in respect of inhibition zone around paper disc were recorded after one week of incubation.

Neem oil and Bio-control Agents

Fungal bio-control agents:

The compatibility between neem oil (Starneem) and fungal bio-control agents *viz., T.viride, T.harzianum, T.reesei* and *Gliocladium virens* at required concentrations of effective neem formulation, Neem oil 0.15% (Starneem) was prepared as described for fungicide in 3.6.9. And the compatibility of neem based formulations was tested following poisoned food technique (Nene and Thapliyal, 1993) described in 3.6.10 for fungicides.

Neem Oil and Bacterial Bio-control Agents

The compatibility between neem oil 0.15% starneem and Bacterial bio-control agents *Pseudomonas fluorescens* and *Bacillus subtilis* was studied as per procedure given in 3.6.14 for compatibility between fungicide and *P.fluorescens*

3.7 POT CULTURE STUDIES

The pot culture experiment was conducted to know the individual as well as combined effect of bio-control agents, fungicide, and neem based formulations against test pathogen *R.bataticola.* Earthern pots of size 12 inch diameter were used and filled with steam sterilized soil @ 2 kg/pot. The effective fungal and bacterial antagonists and neem based commercial formulations (Starneem) were selected to test against test pathogen. The standard fungicide, thiram was used as check for comparison. The experiment was conducted in a complete randomized block design with three replications and the treatmental details are as follows.

T_1 = *Pseudomonas fluorescens* (*P.f*) (10 g/kg seed)

T_2 = *Trichoderma viride* (*T.v*) (4g/kg seed)

T_3 = Neem oil (Starneem) 3 ml/kg seed

T_4 = Thiram 75 WP 3g/kg

T_5 = *P.f* + *T.v*

T_6 = *P.f* + Thiram

T_7 = *P.f* + Neem oil (Starneem)

T_8 = *T.v* + Thiram

T_9 = *T.v* + Neem oil (Starneem)

T_{10} = Thiram + Neem oil (Starneem)

T_{11} = *P.f* + *T.v* +Thiram

T_{12} = *P.f* + *T.v* + Neem oil (Starneem)

T_{13} = *P.f* + Thiram + Neem oil (Starneem)

T_{14} = *T.v* + Thiram + Neem oil (Starneem)

T_{15} = *P.f* + *T.v* + Thiram + Neem oil (Starneem)

T_{16} = Inoculated control

Sowing and Treatments

Seed treatment with Thiram

The seeds of greengram cv ML-267 were treated with thiram (75 WP) @ 3g/kg seed using gum (5ml/kg) as sticker and the treated seeds were used for sowing.

Seed treatment with T.viride/ P. fluorescens

The talc based bio-control agents *T.viride* @ 4 g/kg seed and *P.fluorescens* 10 g/kg seed were used for treating the seeds by using gum (5ml/kg) as sticker. The treated seeds were spread over a clean paper and dried in a cool shady place. The seeds were sown immediately after drying.

Combined seed treatment with Thiram, neem oil and bio-control agents

On the treatments involving combinations of thiram, neem oil, *T.viride* and *P.fluorescens*, the seeds were first treated with thiram, followed by neem oil, and then mixed with *T.viride* and *P.flurescens* at recommended doses using gum as sticker. The treated seeds were shade dried over a clean paper and used for sowing. Thiram (25 ppm) and neem oil (Starneem) (100 ppm) were found to be sub lethal doses.

Termination of Experiment

The plants were grown for a period of 65 to 70 days *i.e.*, till the period of harvest and the data on seedling emergence, dry root rot incidence (%) and plant growth characters *viz.*, shoot length, root length, shoot and root dry weights, populations levels of *R.bataticola* were recorded.

Estimation of population levels of Rhizoctonia bataticola

The population levels of test pathogen, *R.bataticola* in soils in different treatments was enumerated following serial dilution plate count technique (Johnson and Curl 1972) on Chlorneb mercury rose bengal agar (CMRA) medium.

3.8 STATISTICAL ANALYSIS

The data were statistically analysed and the data pertaining to percentage values were analyzed after converting them into transformed values (Gomez and Gomez, 1984).

Results

CHAPTER IV

RESULTS

The results pertaining to the survey on dry root rot incidence of greengram caused by *Rhizoctonia bataticola* (Taub) Butler (Pycnidial stage: *Macrophomina phaseolina*), pathogenicity studies, antagonistic studies with certain fungal and bacterial bio-control agents, fungitoxicity of neem based commercial formulations, compatibility between neem oil and bio-control agents and effectiveness of bio-control agents and neem oil as bio-fungicides in the management of dry root rot of greengram under green house conditions are presented below.

4.1 SURVEY

4.1.1 Dry Root Rot Incidence of Greengram in Major Greengram growing Mandals of Chittoor district of Andhra Pradesh

A preliminary field survey was conducted to know the incidence of dry root rot caused by *R. bataticola* (Pycnidial stage: *Macrophomina phaseolina*) in major greengram growing mandals of Chittoor district of Andhra Pradesh and the data are presented in Table 1. A total of 24 samples were collected from 24 farmers fields representing 8 major greengram growing mandals of Chittoor district, A.P.

The symptoms associated with dry root rot affected plants were leaves and stems of the affected plants turned straw coloured, became flaccid and drooped, exhibiting wilting symptoms (Plate 1). Discoloration of the basal portion of the stem, rotting of under ground plant parts were also observed. The taproot became dark, showing rotting symptoms and was devoid of most of its lateral and finer roots. Decay of the secondary roots and shredding, brittleness of the cortex of the taproot, were recorded. Minute sclerotial bodies with mycelial bits of the pathogen were seen all over the affected and shredded roots. In advanced stages drying and death of the plants was observed. The dry root rot incidence was recorded in all the farmers' fields surveyed and the incidence ranged from 2.2 to 24 per cent with maximum incidence in T. Sattevaripalem (24%) village in Chinnagottigallu mandal and minimum in Venkanaddivaripalle village in Pulicherla mandal with an average of 7.4 per cent.

Among the eight mandals surveyed, mean dry root rot incidence ranged from 5.7 to 12 per cent with a maximum incidence in Chinnagottigallu mandal (12%) followed by Pakala (9%), Pulicherla (8.1%), Srikalahasti (6.6%), Nagari (6.3%), Madanapalli (6.0%), Chendragiri (5.8%) and least in Pileru mandal (5.7%).

Table 1: Survey on incidence of dry root rot caused by *R.batalicola* in major greengram growing mandals of Chittoor district during *rabi* 2001-2002

Sl. No.	Name of the mandal and village	Sample number/ isolate	Stage of the Crop (*rabi*)	Soil type	Variety	Dry root rot incidence (%)	Mandal average
1	**Srikalahasti**						
	Ammapalem	1	Vegetative	Sandy clay loam	Local	7	
	Thondamandu	2	Vegetative	Sandy loam	Pusa Baisakhi	8	6.7
	Muchivolu	3	Vegetative	Sandy loam	Local	5	
2	**Chandragiri**						
	Thondavada	4	Flowering	Clay loam	Local	3	
	Sanambatla	5	Vegetative	Clay loam	Local	2.5	5.8
	Ithepalli	6	Vegetative	Sandy clay loam	ML-267	12	
3	**Chinnagottigallu**						
	T.Sattevaripalem	7	Vegetative	Sandy loam	ML-267	24	
	Yadanvaripalli	8	Vegetative	Clay loam	Local	3	12.0
	Rangangariadda	9	Flowering	Sandy clay loam	Pusa Baisakhi	9	
4	**Pileru**						
	Reddivaripalli	10	Vegetative	Clay loam	ML-267	7	
	Kotapalle	11	Vegetative	Clay loam	ML-267	6	5.7
	Yellamanda	12	Vegetative	Clay loam	Local	4	
5	**Pakala**						
	Mugarala	13	Flowering	Clay loam	Local	5	
	Nendragunta	14	Vegetative	Sandy loam	Local	9	9.0
	Dhanujavaripalli	15	Vegetative	Sandy loam	ML-267	13	
6	**Pulicherla**						
	Ramireddigaripalle	16	Vegetative	Sandy loam	Pusa Baisakhi	13	
	Venkanaddivaripalle	17	Vegetative	Clay loam	Local	2.2	8.1
	Kalluru	18	Flowering	Sandy clay loam	ML-267	9	
7	**Madanapalli**						
	Edigapalli	19	Flowering	Sandy clay loam	Local	5	
	Pothabolu	20	Vegetative	Sandy clay loam	Local	7	6.0
	Reddivaripalli	21	Vegetative	Clay loam	ML-267	6	
8	**Nagari**						
	Sathrawada	22	Vegetative	Clay loam	Local	5	
	Kakivedu	23	Flowering	Sandy clay loam	Pusa Baisakhi	8	6.3
	E.Kuppam	24	Vegetative	Sandy loam	Local	6	
	Total	**24**			**Average**	7.4	

Plate 1 Diseased greengram plant showing dry root rot symptoms

4.1.2 Influence of Soil Type and Cultivars on Occurrence of Dry Root Rot of Greengram

4.1.2.1 Soil type

Among the three soil types surveyed, the dry root rot incidence varied with soil type Sandy loam soils recorded the highest per cent of dry root rot incidence (11.1%) followed by sandy clay loam (8.1%) and the least incidence was recorded in clay loams (4.3%) (Table 2 and Fig.1).

4.1.2.2 Cultivars

The dry root rot incidence was recorded in all cultivars of greengram *i.e.*, ML-267, Pusa Baisakhi and Local cultivars. Greengram cv. ML-267 recorded highest incidence of dry root rot (11%) followed by Pusa Baisakhi (9.5%). The disease incidence was lowest in local cultivars (4.8%) (Table 3 and Fig.2).

4.1.2.3 Stage of the crop

The dry root rot incidence was recorded at vegetative and flowering stages. The incidence was high during vegetative stage (7.8%) compared to flowering stage (6.5%).

4.2 ISOLATION OF THE PATHOGEN

The pathogen associated with diseased plants of greengram collected from different farmers' fields during survey was isolated on PDA and purified by single hyphal tip technique and identified using appropriate key. The detailed characteristics of pathogen isolated from T.Sattevaripalem village of Chinnagottigallu mandal (isolate number-7) was studied as the dry root rot incidence rate was highest among the fields surveyed. The same isolate was used for subsequent studies in the present investigation.

Table 2: **Influence of soil types on the dry root rot incidence assocaited with greengram in major green gram growing mandals of Chittoor district A.P.**

S.No.	Soil type	Total samples	Mean per cent dry root rot incidence
1	Sandy loam	7	11.1
2	Sandy clay loam	7	8.1
3	Clay loam	10	4.3
	Total	24	

Fig. 1: Influence of soil types on the dry root rot incidence associated with greengram in major greengram growing mandals of Chittoor district, A.P.

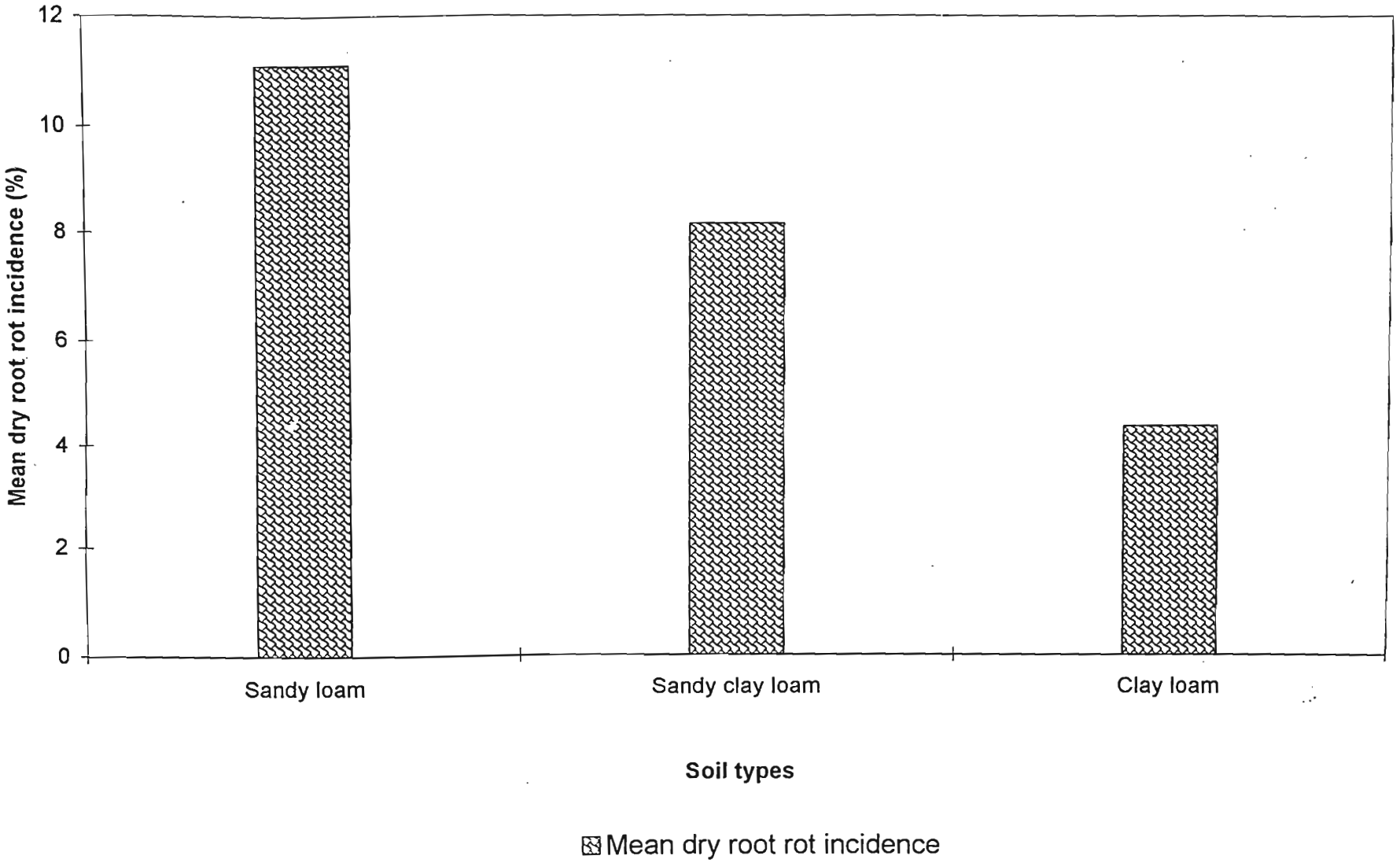
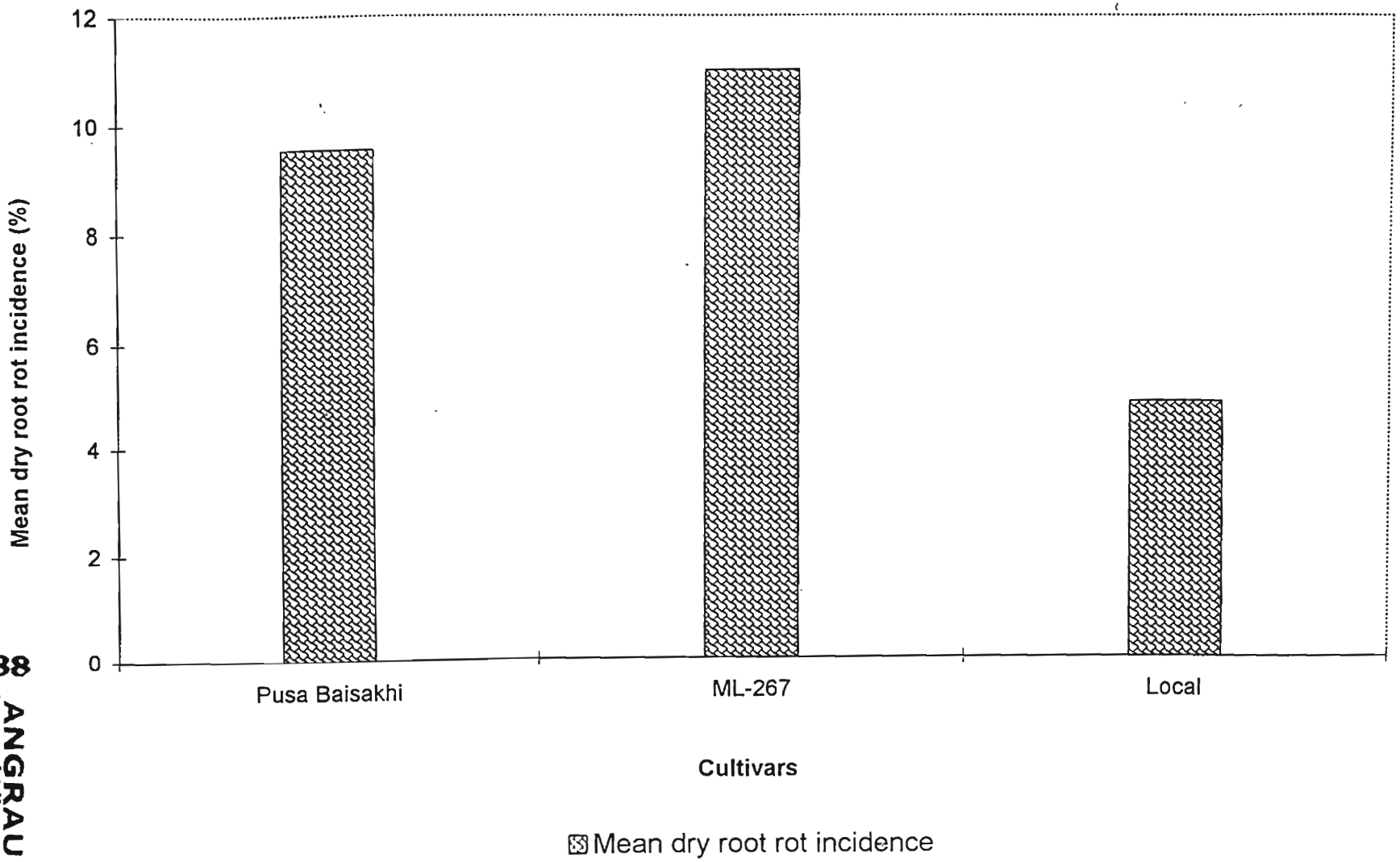


Table 3: Influence of cultivars on the dry root rot incidence assocaited with green gram in major green gram growing mandals of Chittoor district A.P.

S.No.	Cultivars	Total samples	Mean per cent dry root rot incidence
1	Pusa Baisakhi	4	9.5
2	ML-267	7	11.0
3	Local	13	4.8
	Total	24	

Fig. 2: Influence of cultivars on dry root rot incidence associated with greengram in major greengram growing mandals of Chittoor district, A.P.



4,2.1 Characteristics of the Pathogen

The growth of mycelia was observed 24 h after inoculation on PDA medium. With in 4 days, the colonies became fluffy, carbonaceous, brown to black in colour with concentric zonation covering the entire plate. With time numerous sclerotia developed through out the colony. The pycnidia were not observed on PDA medium (Plate 2).

Mycelia were hyaline to grey or brown in colour, septate, branched, dendroid and frequently ran parallel to each other with right angled hyphae. Initially the hyphae was thin and became thicker with age. Sclerotia were black, varied from spherical to irregular in shape and measured 75 to 110 µm in diameter. Based on cultural and morphological characters the pathogen was identified as *Rhizoctonia bataticola* (Plate 2 and 3).

4.3 PATHOGENICITY STUDIES

The test pathogen *Rhizoctonia bataticola* which was isolated from diseased plants was purified and mass multiplied on sorghum grains (Plate 4 and 5) and further tested at different inoculum levels so as to identify the optimum infection threshold levels of the pathogen using greengram cv. ML-267 in pot studies.

The dry root rot incidence was recorded based on the following symptoms associated with greengram cv. ML-267. During pathogenicity studies failure of germination, seedling necrosis, stunted growth, wilting and

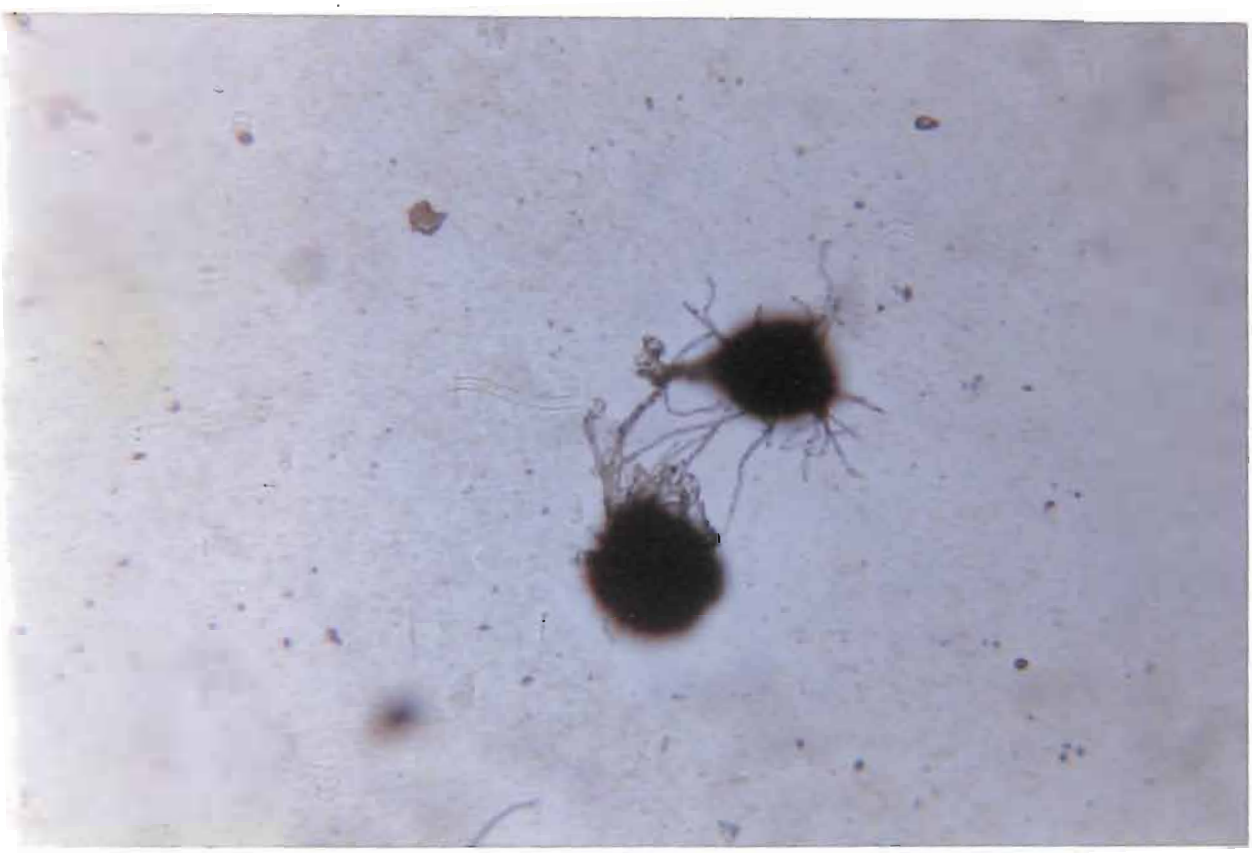


Plate 2 Sclerotia of pathogen (*Rhizoctonia bataticola*)

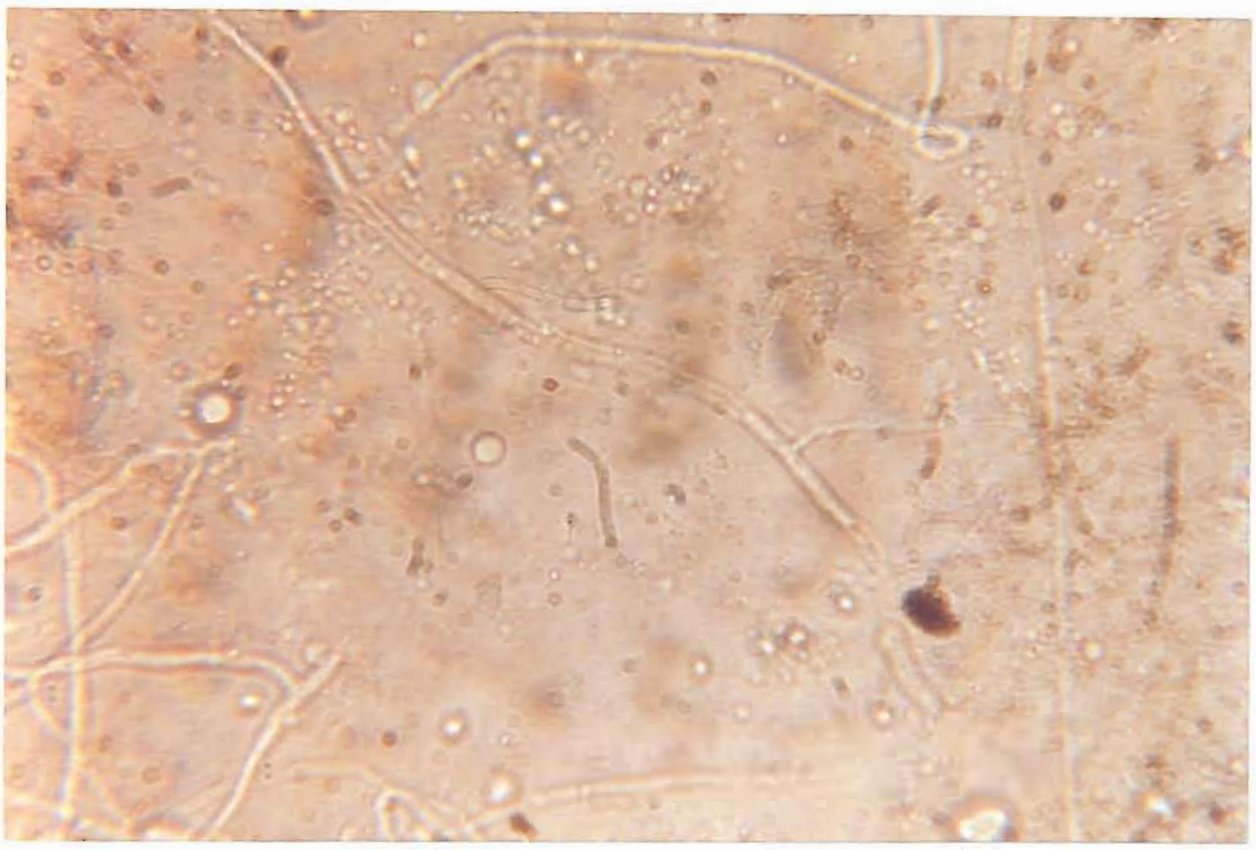


Plate 3 Mycelia of pathogen (*Rhizoctonia bataticola*)

Plate 4 Growth of *Rhizoctonia bataticola* on PDA medium

Plate 5 Mass multiplication of *Rhizoctonia bataticola* on sorghum grains

Table 4: Effect of different inoculum levels of *Rhizoctonia bataticola* on dry root rot incidence of green gram cv. ML-267

S.No.	Inoculum density per cent (w/w)	*Dry root rot incidence (%)
1	Control	0.0 (0.0)
2	1	12.5 (20.40)
3	3	25.0 (29.89)
4	5	37.5 (37.73)
5	7	78.2 (62.17)
6	9	82.7 (65.65)
	S.Em ±	2.344
	CD at 5%	6.960

* Mean of five replications

Figures in parentheses are angular transformed values

Fig 3: Effect of inoculum levels of *R.bataticola* on dry root rot incidence of greengram cv. ML-267

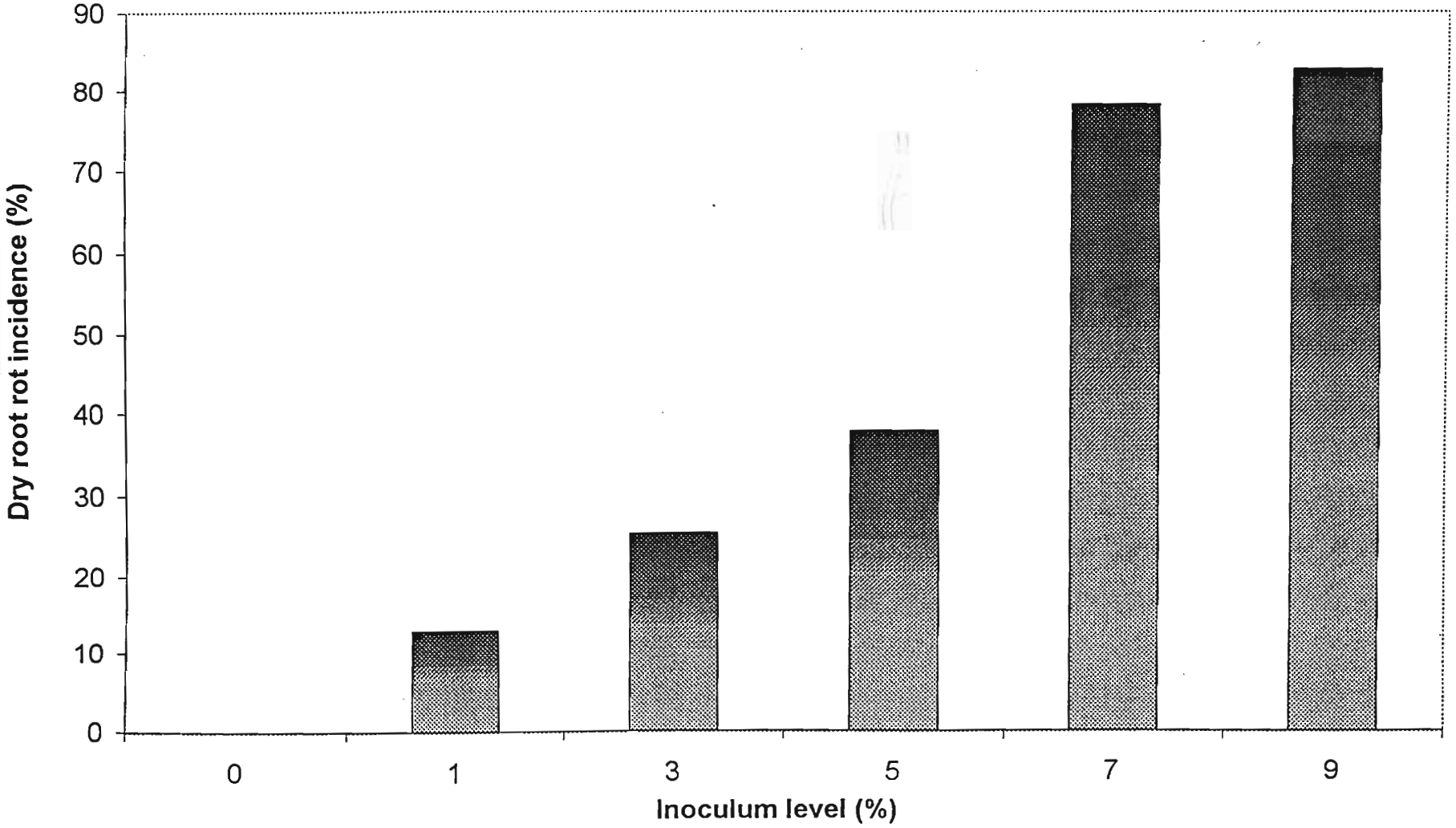


Plate 6 Effect of different inoculum levels of *R.bataticola* on dry root rot incidence of greengram cv.ML-267 in pots

drying of leaves and stem was observed. Tap roots exhibited blackening and formation of numerous black sclerotia on the infected roots. Shredding of the cortex and absence of secondary roots were observed.

The data on dry root rot incidence at different inoculum levels is presented in Table 4, Fig. 3 and Plate 6. The dry root rot incidence increased from 12.5 per cent to 82.7 per cent with an increase in inoculum density from 1 to 9 per cent. There was no significant difference in disease incidence with the inoculum level from 7 to 9 per cent. This gives an indication that infection threshold of 7 per cent is optimum for maximum dry root rot incidence.

4.4 ANTAGONISTIC STUDIES

4.4.1 *In vitro* Effect of Fungal and Bacterial Antagonists on Growth of *Rhizoctonia bataticola*

4.4.1.1 Fungal antagonists

In order to select a suitable fungal antagonist against the test pathogen *R. bataticola*, fungal antagonists *Trichoderma viride* (Plate 7), *T.harzianum* *T.reesei* and *Gliocladium virens* were tested following dual culture technique.

The data presented in Table 5 and Fig. 4 revealed that all antagonists inhibited the growth of pathogen. However fungal antagonist *T.viride* inhibited highest growth of the pathogen (70%) followed by *T. harzianum* (67%), *G. virens* (45.5%) and *T. reesei* exhibited lowest inhibition (43.1%) (Plate 8, 9 and 10).

Table 5: *In vitro* effect of bacterial and fungal antagonists on growth of *R. bataticola*

S.No.	Antagonist	*Radial growth (cm)	Inhibition over control (%)
1	*Pseudomonas fluorescens*	3.26	63.7
2	*Bacillus subtilis*	5.5	38.8
3	*Trichoderma viride*	2.7	70
4	*Trichoderma harzianum*	2.9	67
5	*Trichoderma reesei*	5.1	43.1
6	*Gliocladium virens*	4.9	45.5
7	Control	9	-
	S.Em ±	0.068	
	CD at 5%	0.196	

*Mean of five replications

Fig. 4: In vitro effect of antagonists on growth (cm) of *R.bataticola*

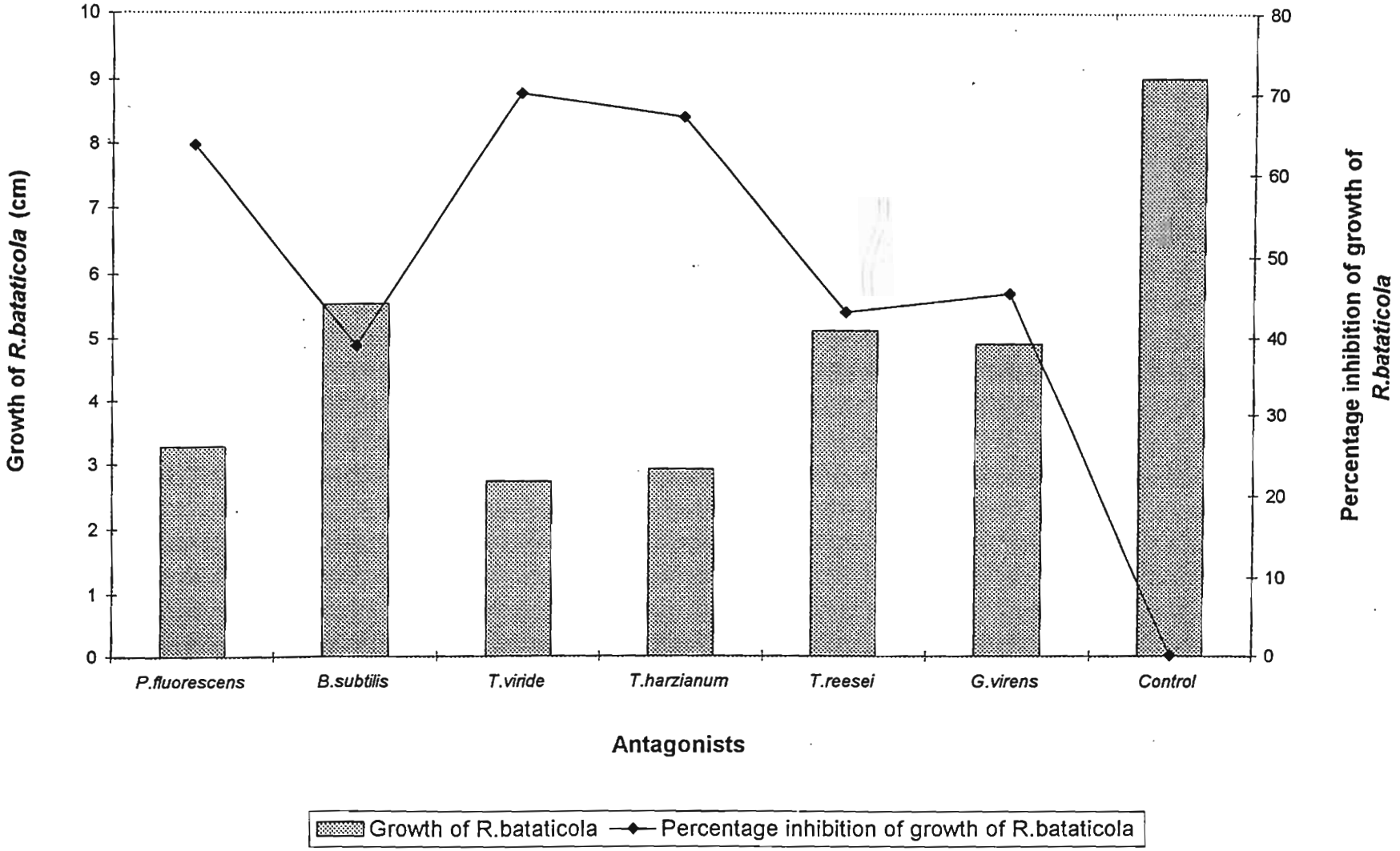


Plate 7 Growth of *Trichoderma viride* on PDA medium in petriplate

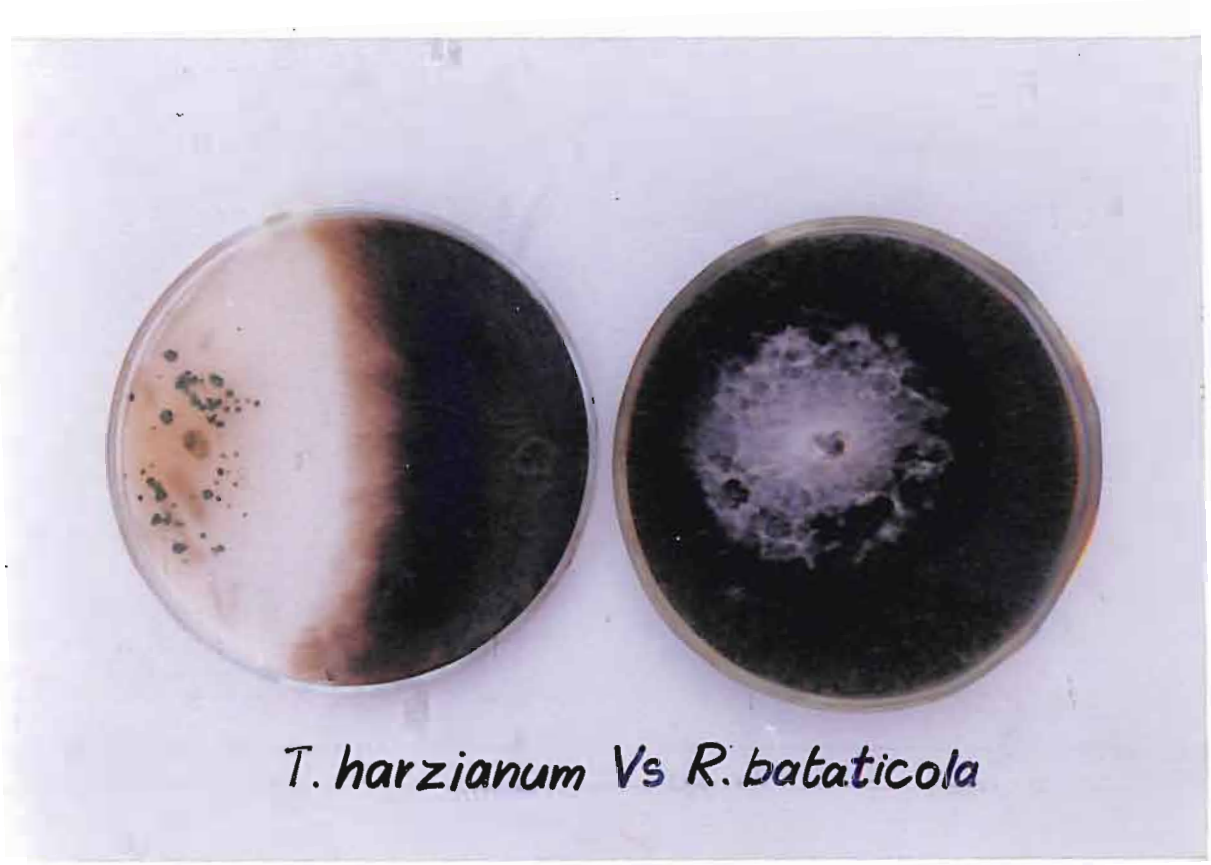


Plate 9 *In vitro* effect of *T.harzianum* on growth of *R.bataticola*

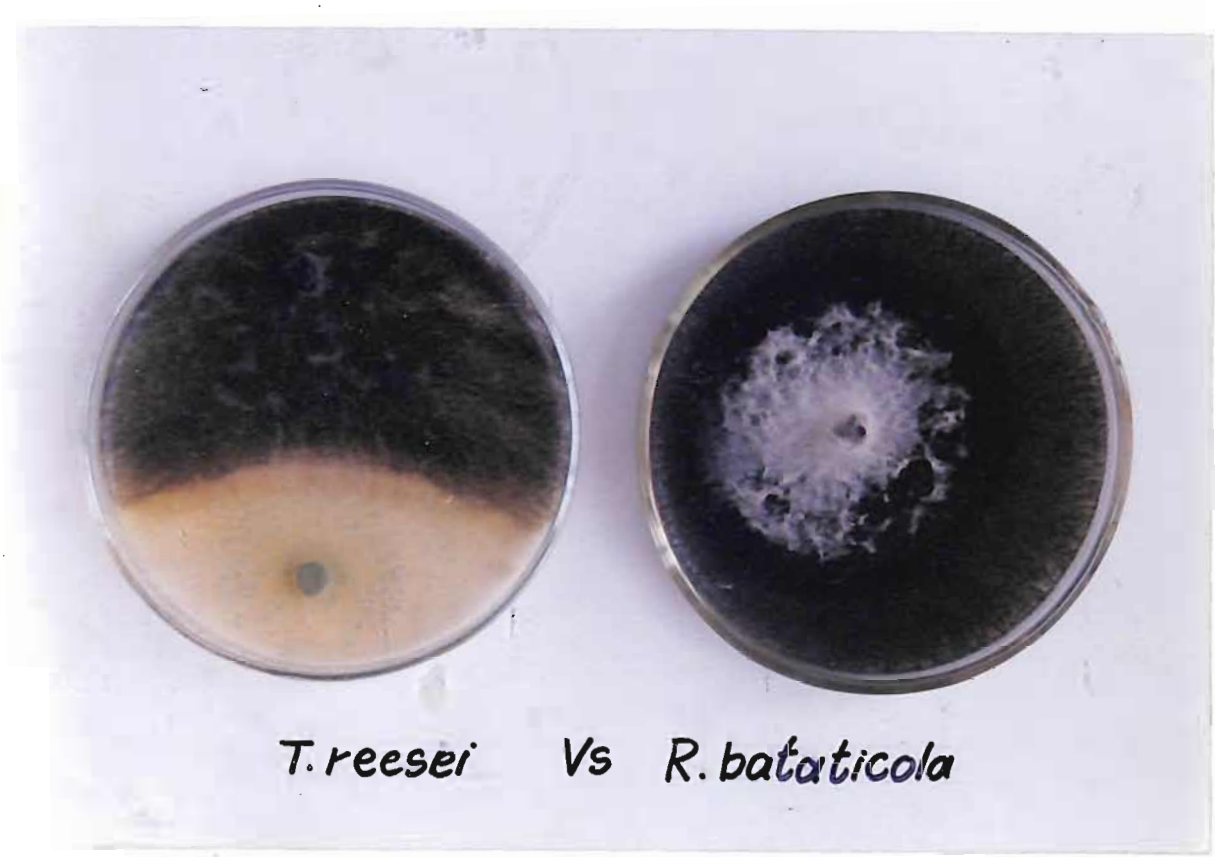


Plate 10 *In vitro* effect of *T.reesei* on growth of *R.bataticola*

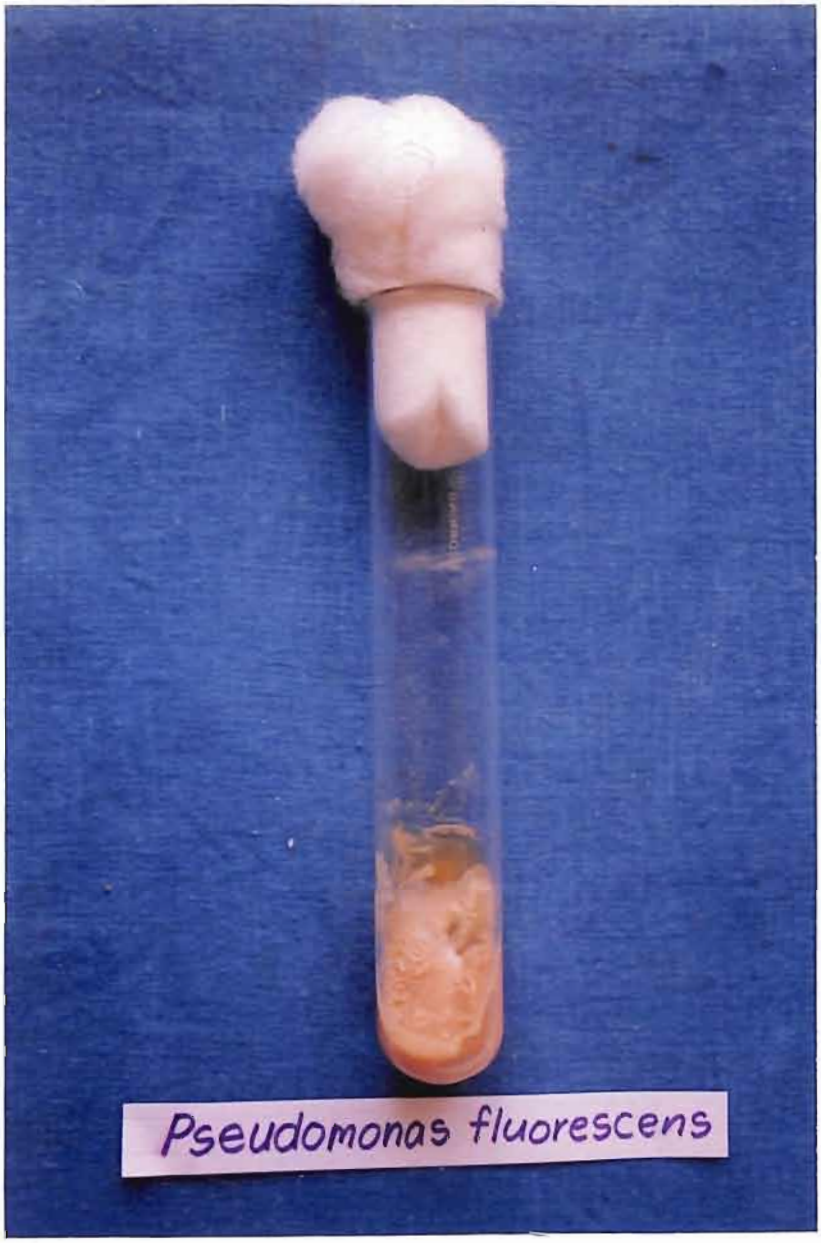


Plate 11 Growth of *Pseudomonas fluorescens on* King's B medium in slants

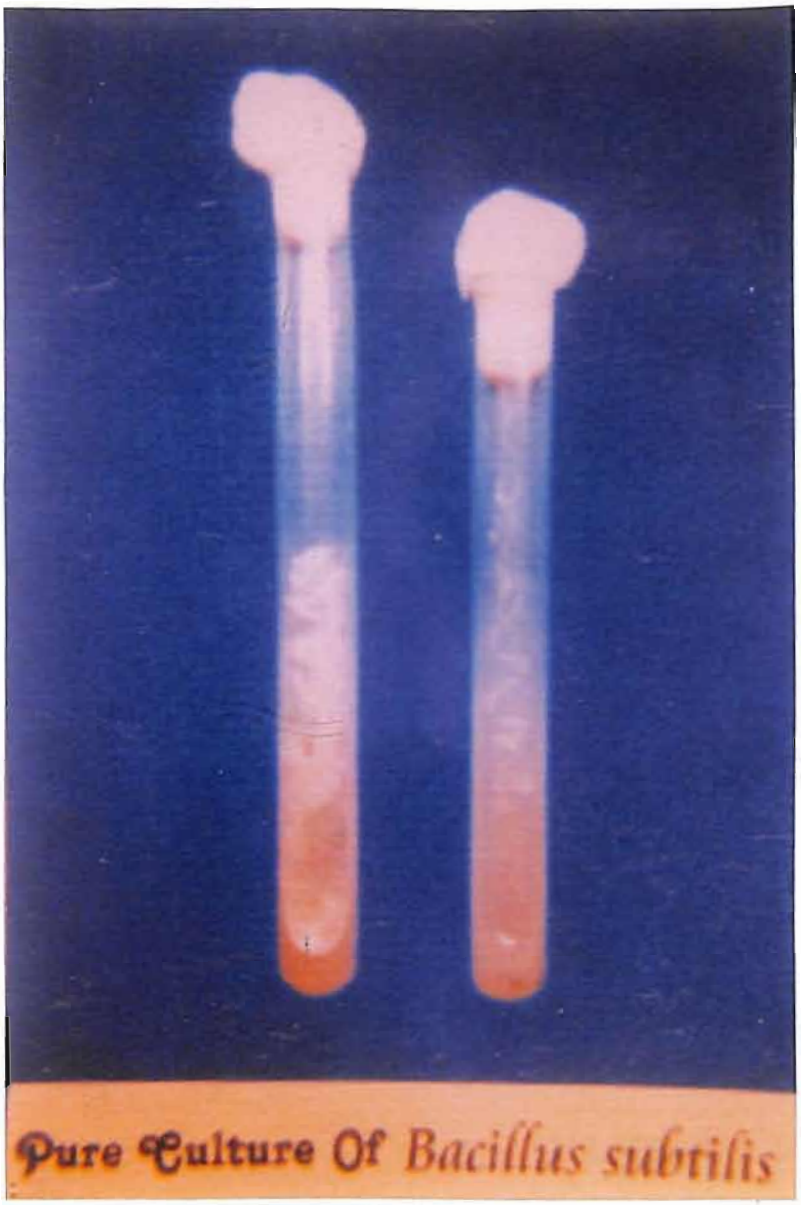


Plate 12 Growth of *Bacillus subtilis* on Nutrient Agar Medium in slants

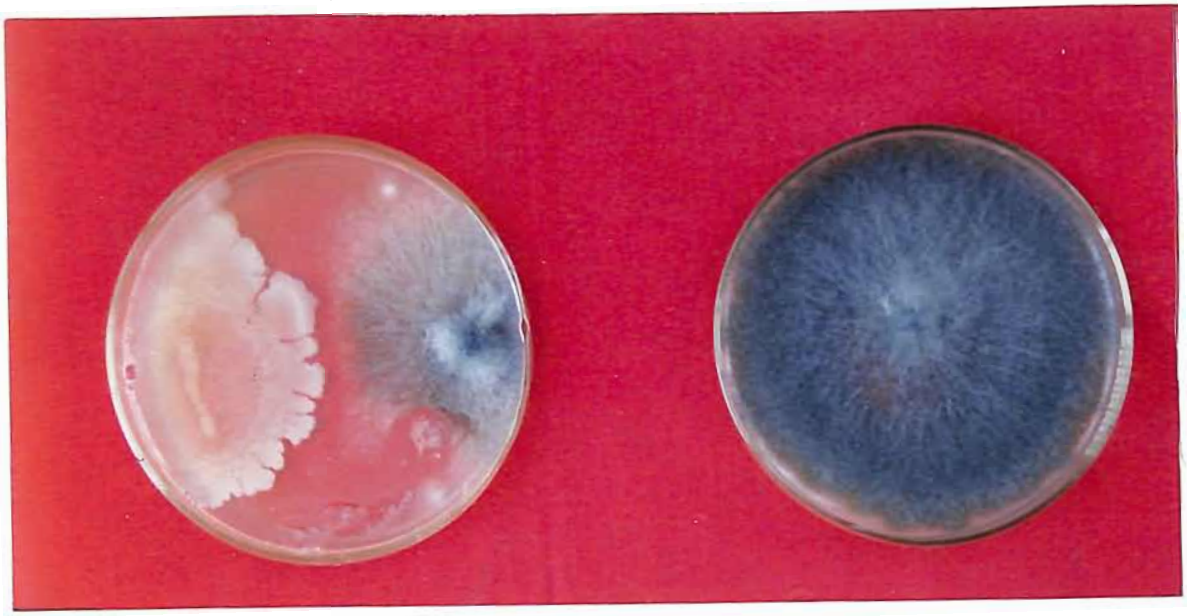


Plate 13 *In vitro* effect of *P.fluorescens* on growth of *R.bataticola*

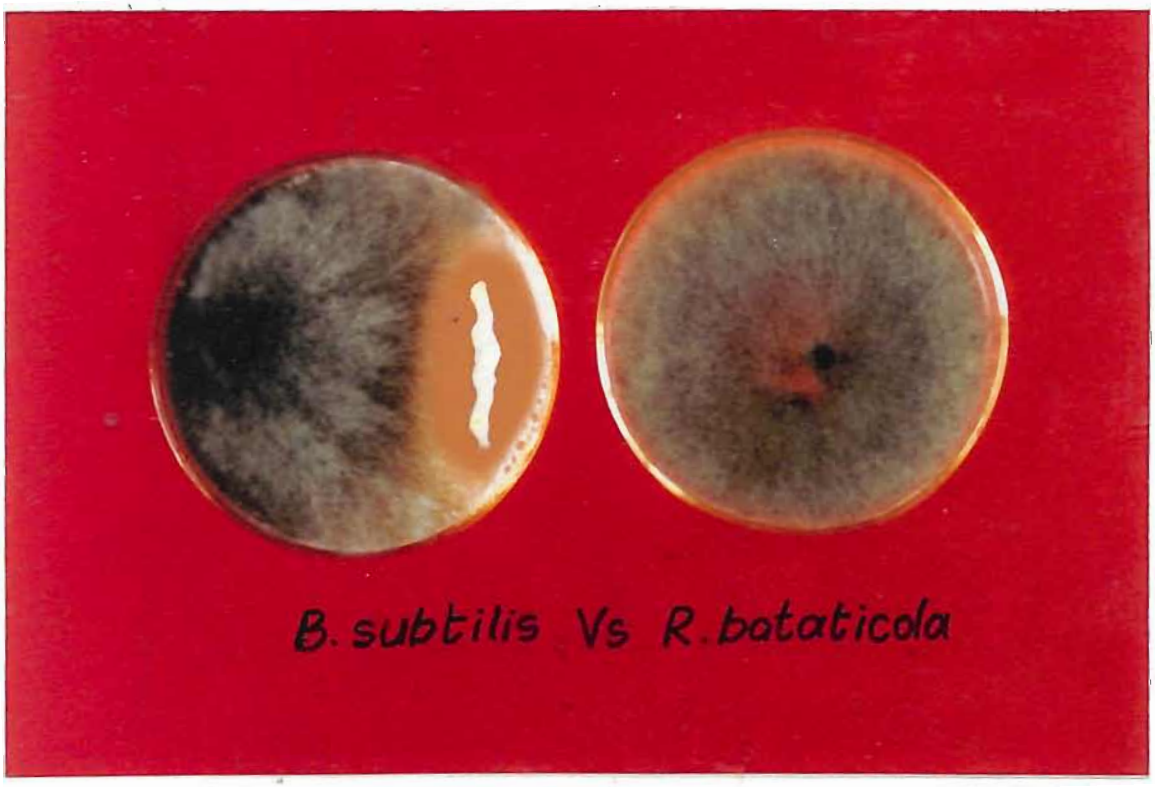


Plate 14 *In vitro* effect of *B.subtilis* on growth of *R.bataticola*

4.4.1.2 Bacterial antagonists

Similarly in order to select a suitable bacterial antagonist against *R. bataticola*, two antagonists *viz., Pseudomonas fluorescens* (Plate 11) and *Bacillus subtilis,* (Plate 12) were tested following dual culture technique. The data presented in Table 5 and Fig. 4 revealed that the bacterial antagonist *Pseudomonas fluorescens* was significantly superior (63.7%) in inhibiting the growth of the pathogen over *Bacillus subtilis* (38.8%) (Plate 13 and 14).

4.5 FUNGITOXICITY STUDIES

4.5.1 *In vitro* Effect of Neem Based Commercial Formulations against Mycelial Growth of *R. bataticola*

To know the efficacy and select a suitable neem based commercial formulation, an experiment was conducted with two neem based commercial neem oil formulations *viz.,* Starneem and Neem gold by following poisoned food technique.

The data presented in Table 6 revealed that both the neem based formulations inhibited mycelial growth of the pathogen completely at 800 and 1000 ppm concentrations. However, neem oil (Starneem) was found to be more effective in inhibiting the growth of the pathogen by 87.1 per cent at 600 ppm than neem gold with (75.1%) inhibition (Plate 15 and 16).

Table 6: Effect of neem based commercial formulations on the growth of *R. bataticola*

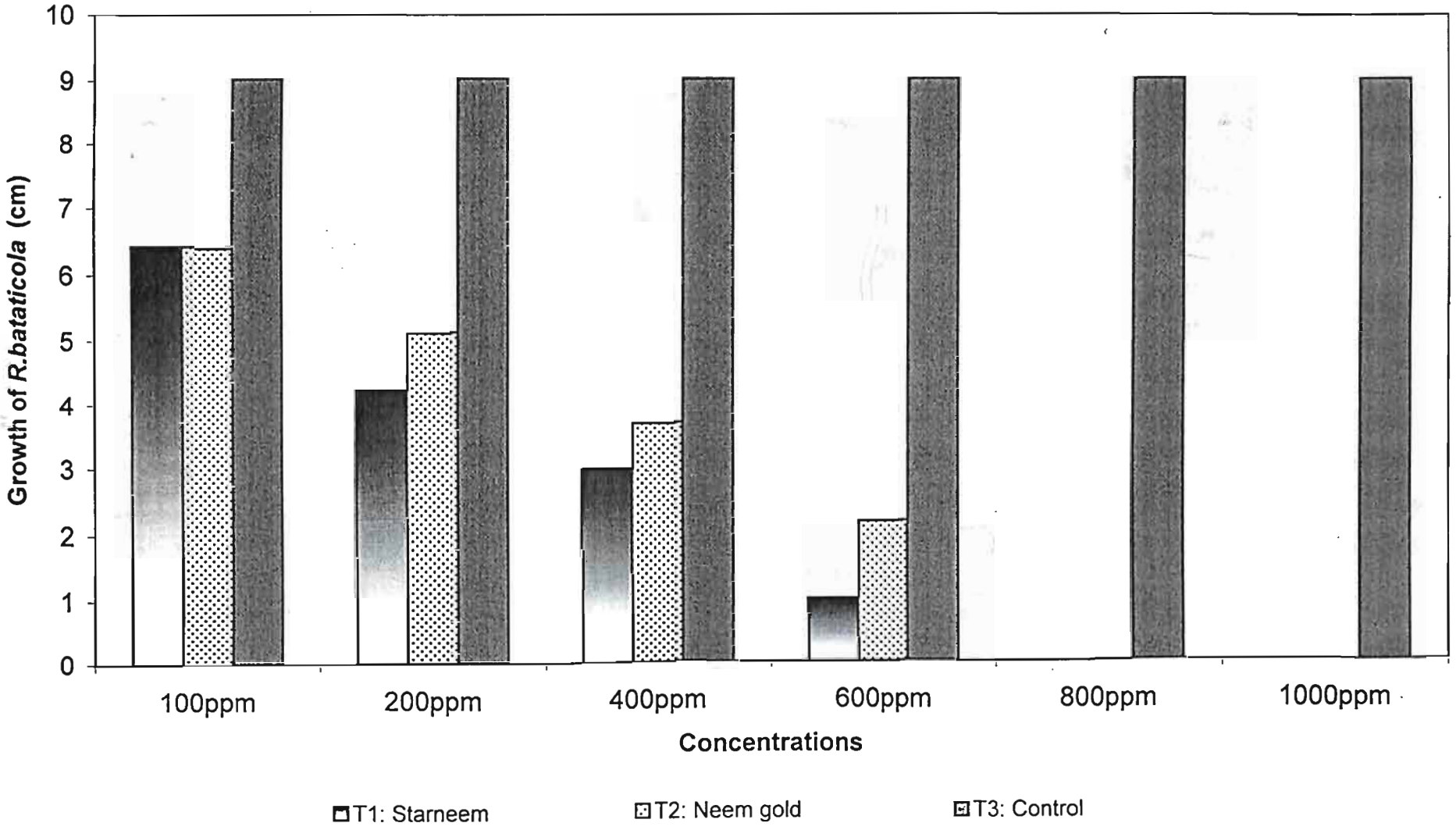
S.No.	Treatment	Concentration (ppm)	*Colony diameter of *R. bataticola* (cm)	Percentage inhibition of growth of *R. bataticola*
1	Starneem	100	6.4	28.6
		200	4.2	53.3
		400	3.0	65.7
		600	1.1	87.1
		800	0.0	100.0
		1000	0.0	100.0
2	Neem gold	100	6.4	28.6
		200	5.1	43.1
		400	3.7	58.0
		600	2.2	75.1
		800	0.0	100.0
		1000	0.0	100.0
3	Control	-	9.0	0.0

S.Em ± 0.027

CD at 5% 0.076

*Mean of five replications

Fig 5: Effect of neem based commercial formulations on the growth of *R.bataticola*



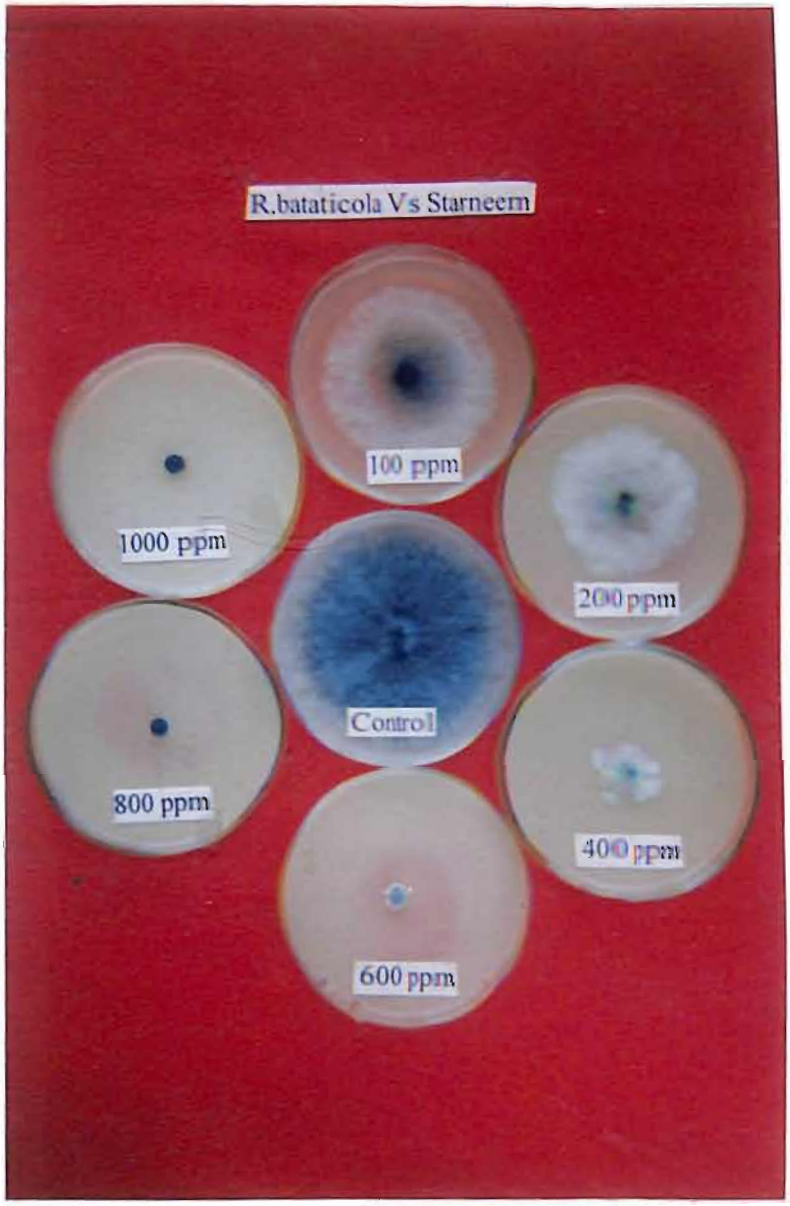


Plate 15 *In vitro* effect of Starneem on growth of *R.bataticola*

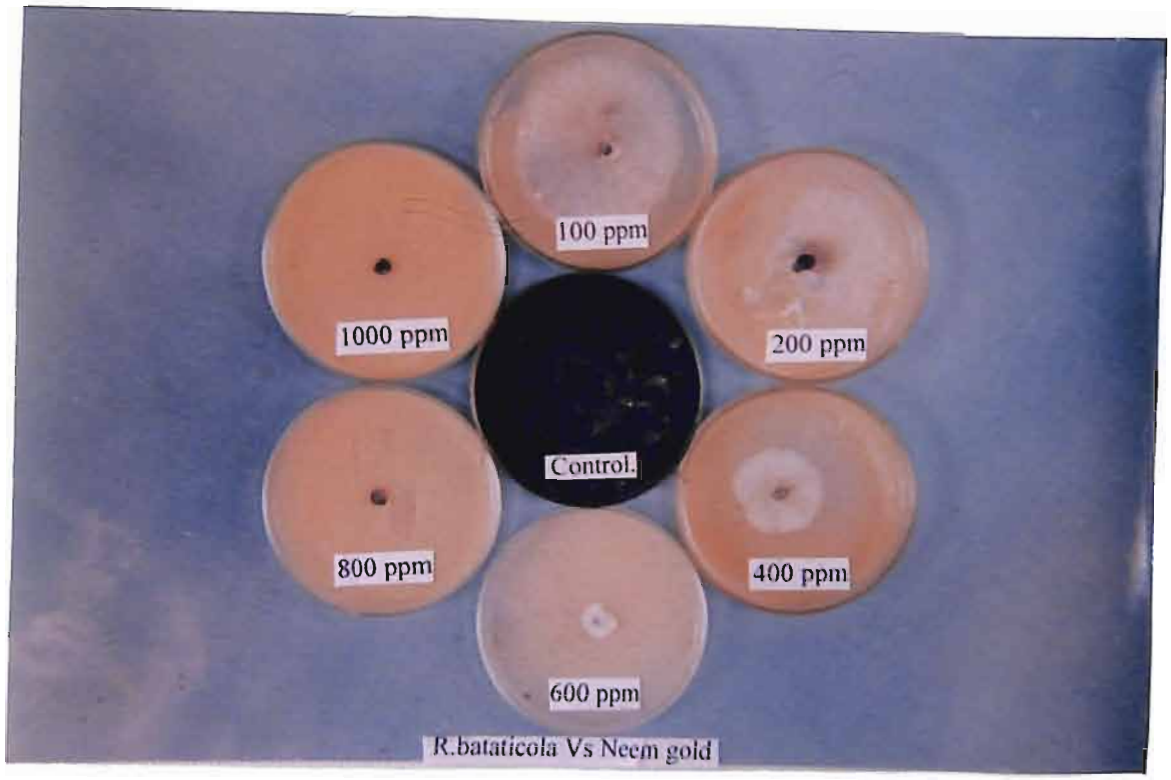


Plate 16 *In vitro* effect of Neem gold on growth of *R.bataticola*

4.5.2 *In vitro* Effect of Fungicide Thiram Against Mycelial Growth of *R. bataticola*

An experiment was conducted to know the efficacy of thiram at 25, 50, 100, 150, 200 and 300 ppm concentrations on mycelial growth of *R. bataticola in vitro*.

The data presented in Table 7 and Fig. 6 revealed that the fungicide thiram at all the concentrations *viz.*, 25, 50, 100, 150, 200 and 300ppm inhibited the growth of the test pathogen ranging from 48.2% to 100%. The pathogen was completely inhibited at 200 and 300 ppm concentrations while the inhibition was least at 25 ppm (48.2%) (Plate 17).

4.6 COMPATIBILITY STUDIES

4.6.1 Compatibility of Neem oil (Starneem) with Fungal Bio-control agents

An experiment was conducted to know the compatibility of neem oil with different fungal bio-control agents *viz., Trichoderma viride, T. harzianum, T. reesei* and *G. virens* following poisoned food technique.

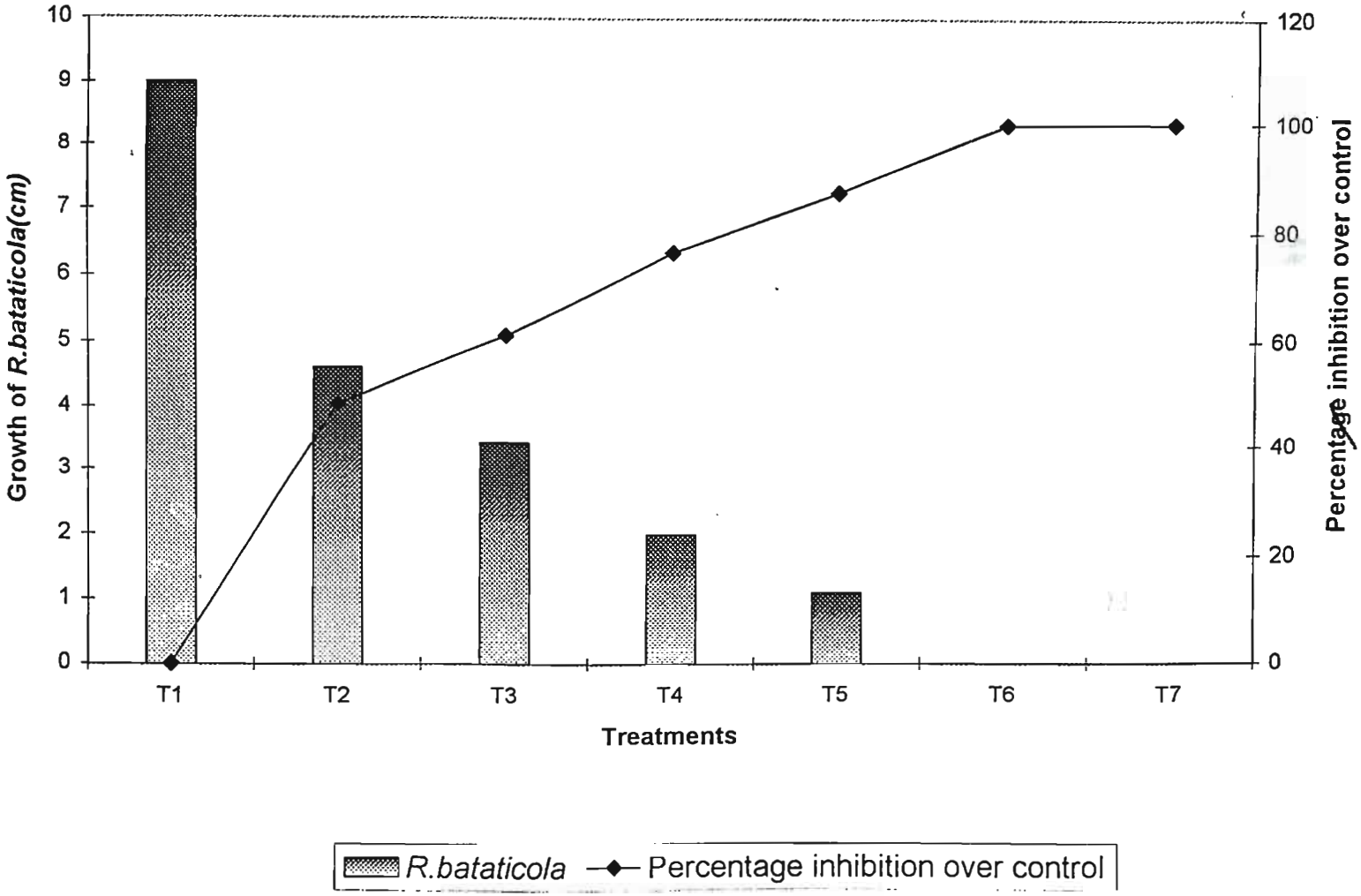
The data presented in Table 8 revealed that all the four fungal antagonists tested were inhibited by neem oil (Starneem) and the inhibition increased with increased levels of neem oil concentrations. Among the four antagonists *T. viride and G. virens* recorded less inhibition (87.7%) compared to other antagonists at 600 ppm concentrations (Plate 18 and 19).

Table 7: *In vitro* effect of Thiram against mycelial growth of *R. bataticola*

S.No.	Treatments	*Colony diameter of *R.bataticola* (cm)	Percentage inhibition over control
1	Control	9.0	0.0
2	Thiram (25 ppm)	4.6	48.2
3	Thiram (50 ppm)	3.4	61.2
4	Thiram (100 ppm)	2.0	76.8
5	Thiram (150 ppm)	1.1	87.5
6	Thiram (200 ppm)	0.0	100.0
7	Thiram (300 ppm)	0.0	100.0
	S.Em ±	0.052	
	CD at 5%	0.15	

*Mean of five replications

Fig. 6: *In vitro* effect of thiram against mycelial growth of *R.bataticola*



T1-Control
T2-Thiram 25ppm
T3-Thiram 50ppm
T4-Thiram 100ppm
T5-Thiram 150ppm
T6-Thiram 200ppm
T7-Thiram 300ppm

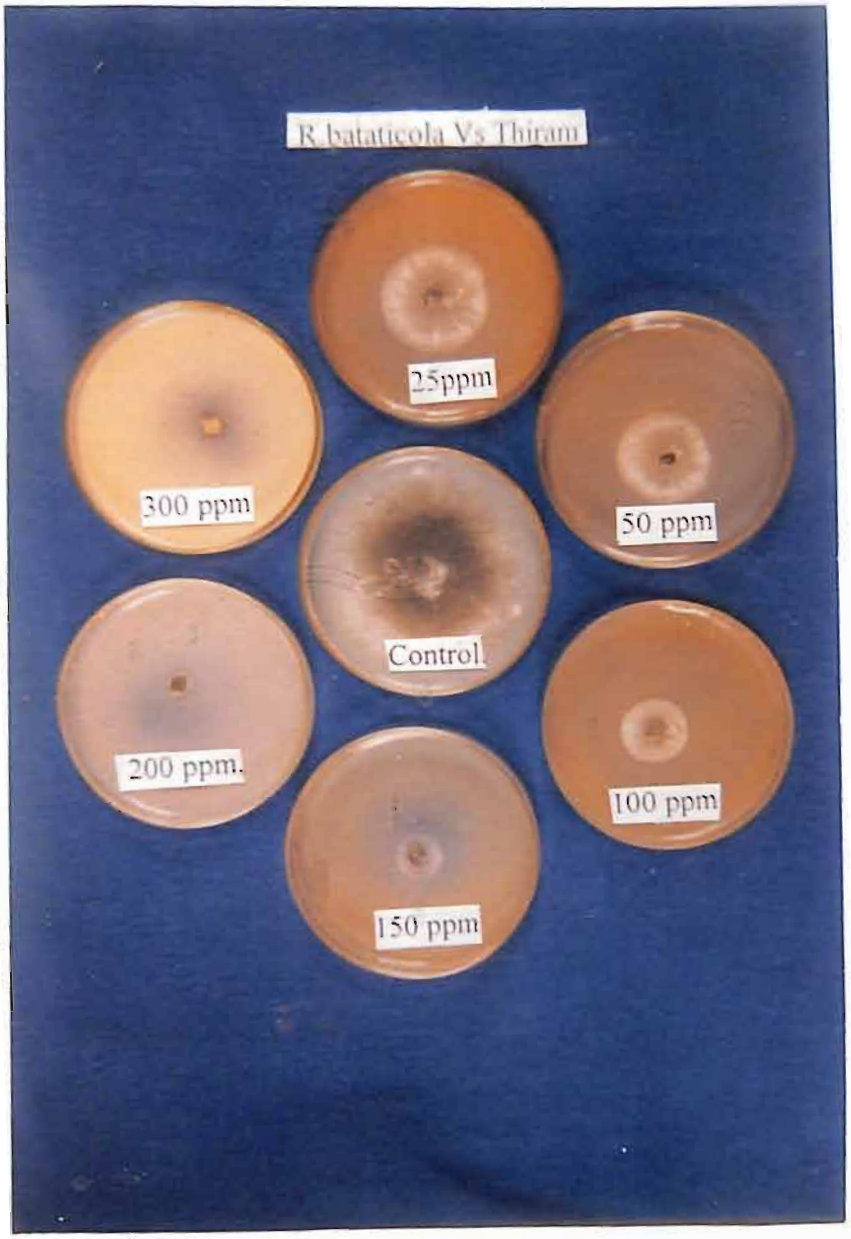


Plate 17 *In vitro* effect of Thiram on mycelial growth of *R.bataticola*

Table 8: Compatibility of neem oil (star neem) with fungal biocontrol agents

S.No.	Treatment	Concentration of neem oil(ppm)	*Colony diameter (cm)	Percentage inhibition of growth
1	*T. viride*	100	6.9	23.8
		200	3.9	56.2
		400	2.6	71.3
		600	1.1	87.7
		800	0.0	0.0
		1000	0.0	0.0
2	*T. harzianum*	100	8.5	5.5
		200	7.1	20.6
		400	2.6	70.2
		600	0.0	100.0
		800	0.0	100.0
		1000	0.0	100.0
3	*T. reesei*	100	9.0	0.0
		200	6.5	38.4
		400	5.2	42.2
		600	0.0	100.0
		800	0.0	100.0
		1000	0.0	100.0
4	*Gliocladium virens*	100	5.5	40.0
		200	2.2	75.5
		400	1.7	79.5
		600	1.1	87.7
		800	0.0	100.0
		1000	0.0	100.0
3	Control	-	9.0	0.0
S.Em ±			0.09	
CD at 5%			0.37	

*Mean of five replications

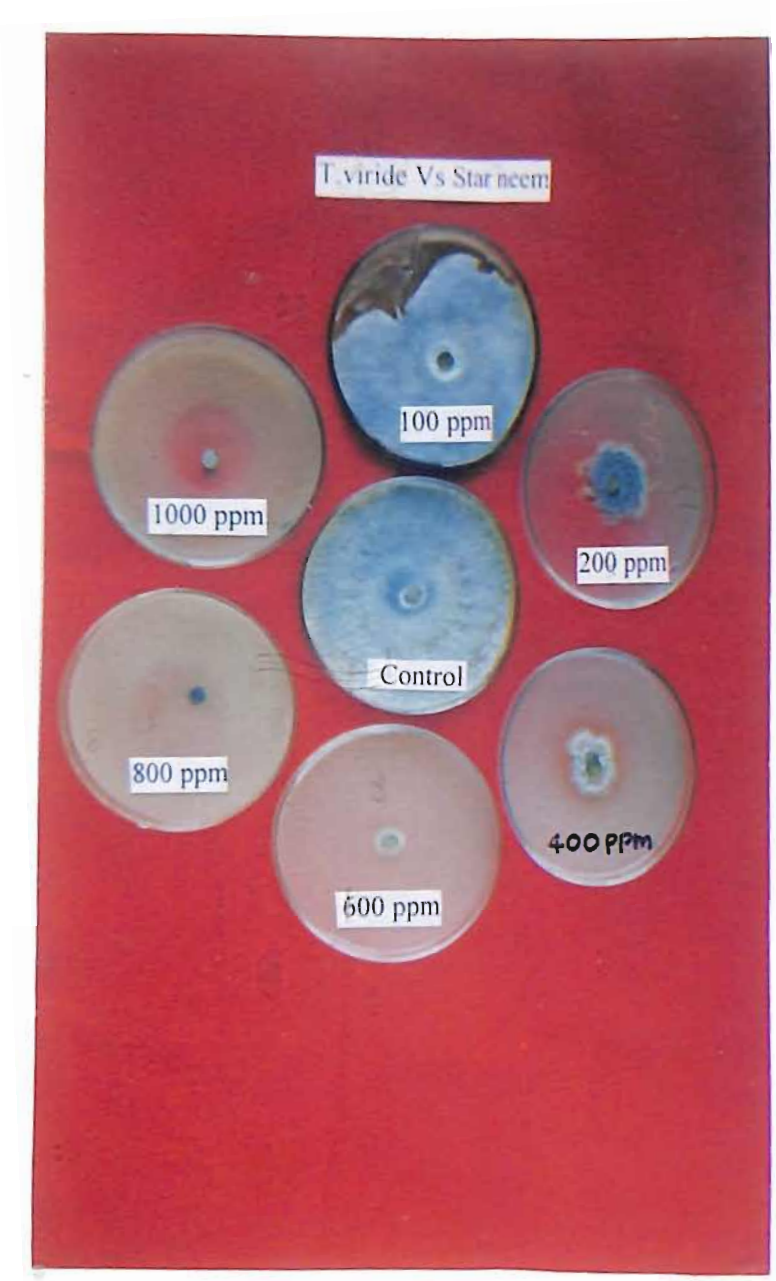


Plate 18 Compatibility between neem oil (starneem) and *T.viride*

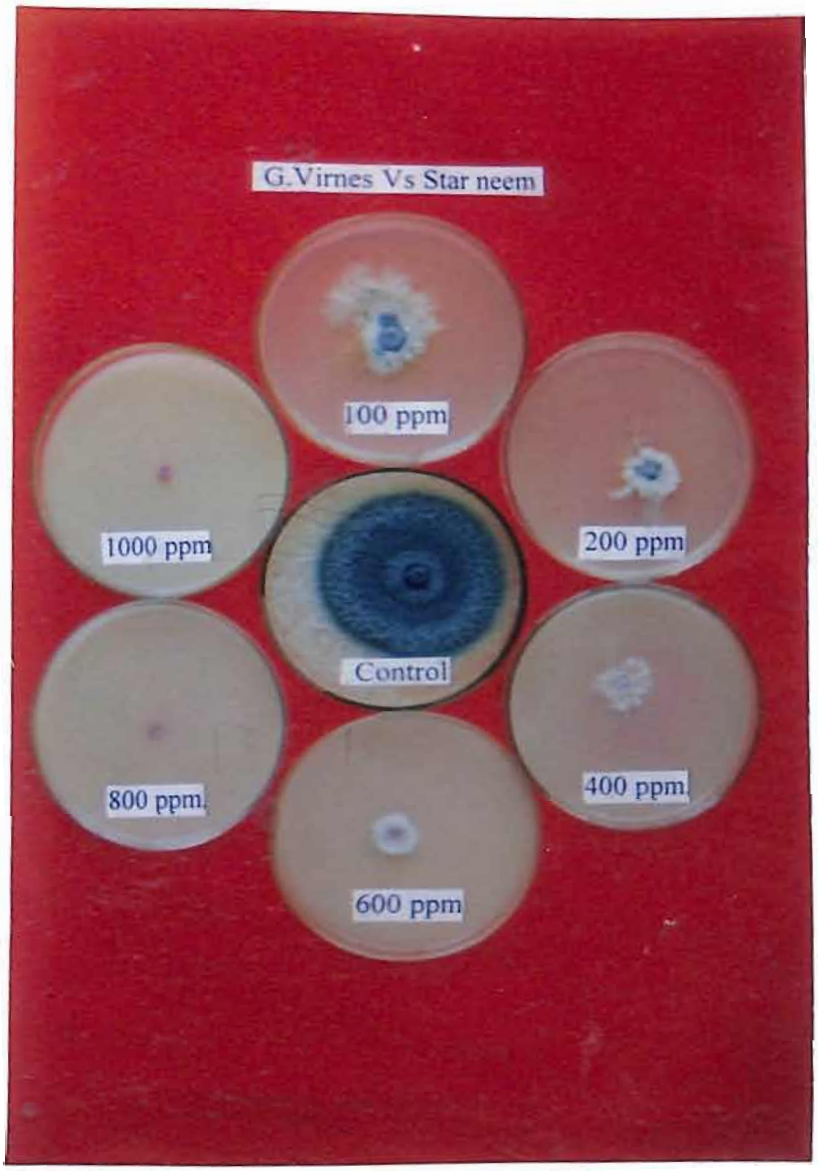


Plate 19 Compatibility between neem oil (starneem) and *G.virens*

4.6.2 Compatibility of Neem Oil (Starneem) with Bacterial Bio-Control Agents

An experiment was conducted to know the compatibility between neem oil and bacterial bio-control agents *viz., P.fluorescens* and *B.subtilis* by following paper disc plate method and they were found to be compatible at all the concentrations tested *viz.*, 100, 200, 400, 600, 800 and 1000ppm (Table 9).

4.6.3 Compatibility of Thiram with *T. viride*

The compatibility of thiram at 25, 50, 100, 150, 200 and 300 ppm concentrations on mycelial growth of *T. viride* was tested following poisoned food technique (Plate 20).

The antagonist *T.viride* was able to survive even at a higher concentration of 300 ppm with a colony diameter of 1.2 cm and the colony diameter was maximum at 25 ppm concentrations (8.53 cm). The data presented in Table 10 and Fig. 7 revealed that the fungicide thiram inhibited the growth of the antagonist *T.viride* from 3.7 to 86.4 per cent at all the concentrations tested.

4.6.4 Compatibility of Thiram with *P.fluorescens*

In order to know the compatibility of thiram at 25, 50, 100, 150, 200 and 300 ppm with *P.fluorescens,* an experiment was conducted following paper disc plate method. The results indicated that both of them were compatible at all the concentrations except at 300 ppm in which the growth of *P.fluorescens* was inhibited by 2.2 per cent (Table11).

Table 9 : Compatibility of neem oil (starneem) at different concentrations with colony growth of bacterial bio-control agents

S.No.	Name of bacterial bio-control agent	Inhibition zone (mm) at different concentrations (ppm)					
		100	200	400	600	800	1000
1	*P.fluorescens*	0	0	0	0	0	0
2	*B.subtilis*	0	0	0	0	0	0

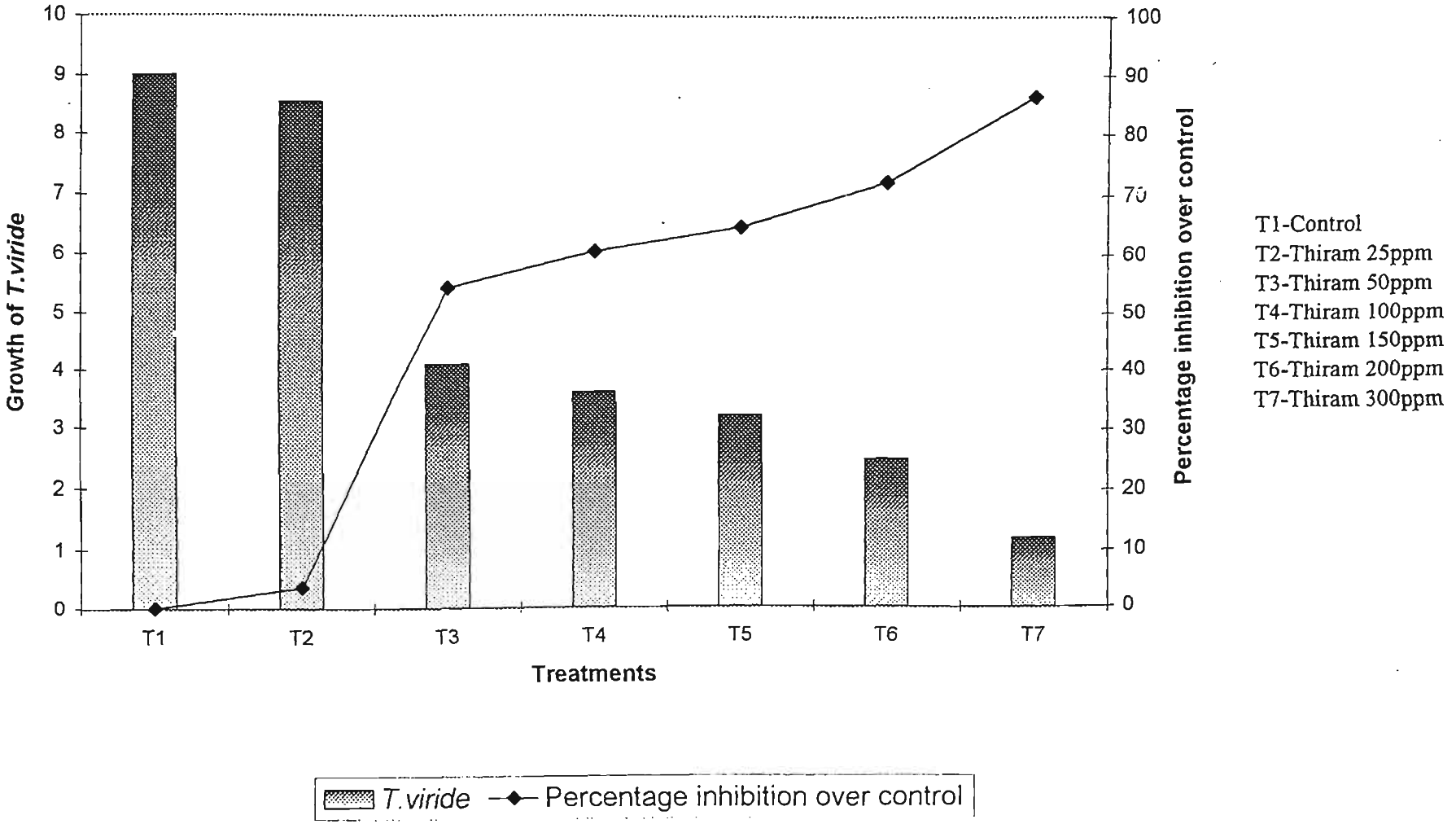
Mean of five replications

Table 10: Compatibility between Thiram and *T. viride in vitro*

S.No.	Treatments	*Colony diameter of *T. viride* (cm)	Percentage inhibition over control
1	Control	9.0	0.0
2	Thiram (25 ppm)	8.5	3.7
3	Thiram (50 ppm)	4.1	54.2
4	Thiram (100 ppm)	3.6	60.2
5	Thiram (150 ppm)	3.2	64.4
6	Thiram (200 ppm)	2.5	72.2
7	Thiram (300 ppm)	1.2	86.4
	S.Em ±	0.08	
	CD at 5%	0.472	

*Mean of five replications

Fig. 7: Compatibility between Thiram and *T.viride in vitro*



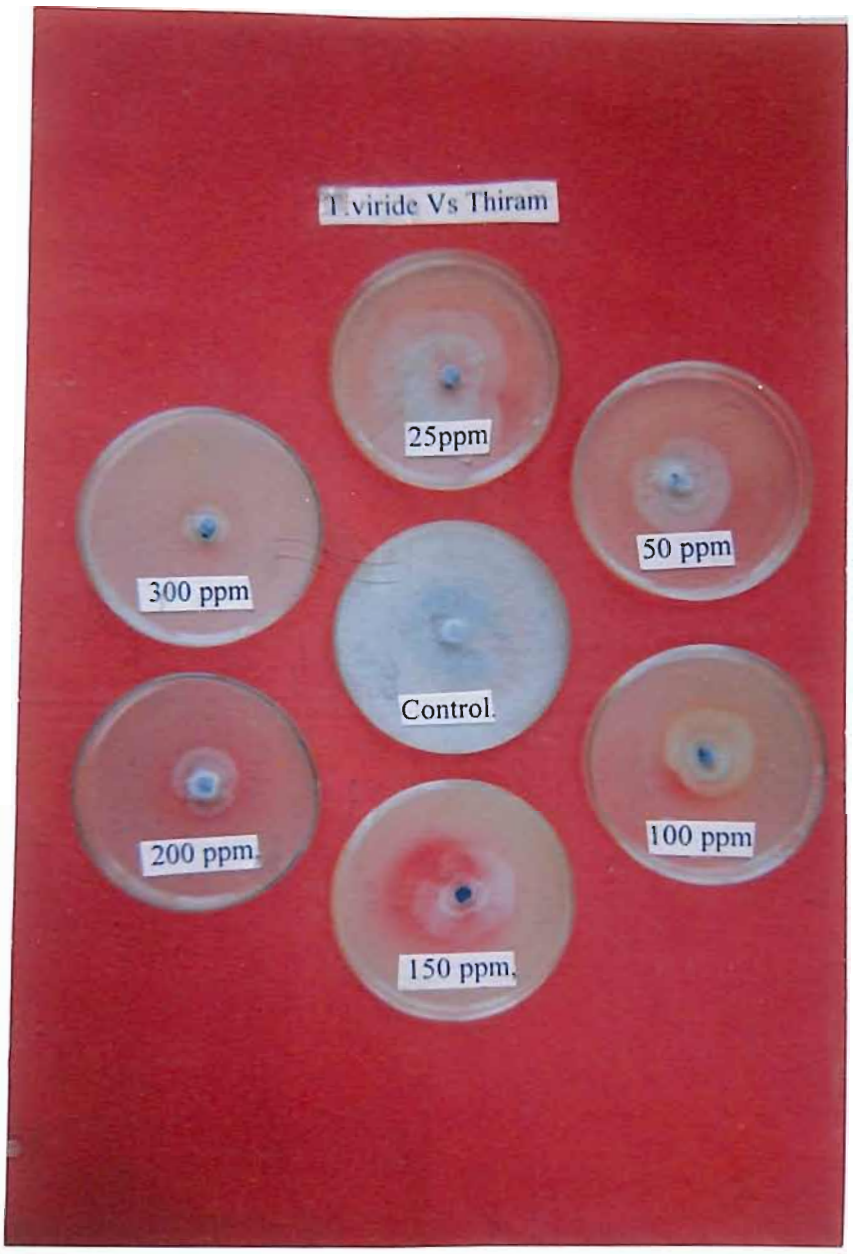


Plate 20 Compatibility between Thiram and *T.viride*

Table 11 : Compatibility of Thiram with colony growth of *P.fluorescens*

S.No.	Concentrations	Inhibition zone (mm)	Inhibition over control (%)
1	Thiram 25 ppm	0	0
2	Thiram 50 ppm	0	0
3	Thiram 100 ppm	0	0
4	Thiram 150 ppm	0	0
5	Thiram 200 ppm	0	0
6	Thiram 300 ppm	2.0	2.2
7	Control	0	0

Mean of five replications

4.7 POT CULTURE STUDIES

Effect of seed treatment with bio-control agents, neem oil (starneem) and thiram on seedling emergence, plant growth parameters and dry root rot incidence was studied in potculture with greengram cv. ML-267 in *R.bataticola* infested soil

4.7.1 Effect on Seedling Emergence

The data on germination and seedling emergence was recorded ten days after sowing. The data presented in Table 12 and Fig. 8 revealed that seedling emergence was significantly improved in all the treatments compared to control (62.7%). Seed treatment with combined treatment of *P.fluorescens* + *T.viride* + neem oil + thiram gave maximum seedling emergence (91.3%) followed by seed treatment with *P.fluorescens* + *T.viride* + thiram (84.3%) and the minimum seedling emergence was recorded with neem oil (76.2%) (Plate 21 to 24).

4.7.2 Effect on Dry Root Rot Incidence

The effect of seed treatment with bio-control agents, neem oil and thiram on dry root rot incidence was recorded at 45 DAS and the data was presented in Table 13 and Fig. 9. The results revealed that individual as well as combined seed treatment with bio-control agents, neem oil and thiram significantly reduced dry root rot incidence ranging from 10 to 34.6 per cent compared to control (77.3%). Seed treatment with *P. fluorescens* + *T. viride* +

Table12: Effect of seed treatment with biocontrol agents, neem oil (star neem) and thiram on seedling emergence of green gram cv. ML-267 in *R. bataticola* infested soil in pots

S.No.	Treatment	*Seedling emergence (%)	Percentage increase over control
1	*Pseudomonas fluorescens* (P.f) (10 g kg^{-1} seed)	78.20 (62.17)	24.6
2	*Trichoderma viride* (T.v) (4 g kg^{-1} seed)	80.10 (63.51)	27.6
3	Neem oil (Starneem) (3 ml kg^{-1} seed)	76.20 (60.82)	21.4
4	Thiram (3 g kg^{-1} seed)	80.20 (63.58)	27.8
5	*P.f* + *T.v*	84.0 (66.43)	33.9
6	*P.f* + Thiram	81.20 (64.36)	29.4
7	*P.f* + Neem oil (Starneem)	79.00 (62.73)	25.9
8	*T.v* + Thiram	83.10 (65.73)	32.4
9	*T.v* + Neem oil (Starneem)	80.47 (63.79)	28.2
10	Neem oil (Starneem) + Thiram	80.32 (63.68)	28.0
11	*P.f* + *T.v* + Thiram	84.37 (66.75)	34.4
12	*P.f.* + *T. v.* + Neem oil (Starneem)	84.20 (66.59)	34.2
13	*P.f* + Thiram + Neem oil (Starneem)	83.60 (66.12)	33.2
14	*T.v* + Thiram + Neem oil (Starneem)	83.20 (65.36)	32.7
15	*P.f* + *T.v* + Thiram + Neem oil (Starneem)	91.30 (72.88)	45.5
16	Inoculated control	62.73 (52.39)	-
	S.Em +	0.690	
	CD at 5%	1.989	

Figures in parentheses are angular transformed values
*Mean of three replications

Fig. 8: Effect of seed treatment with biocontrol agents, neem oil (starneem) and thiram on seedling emergence of greengram cv. ML-267 in *R.bataticola* infested soil in pots

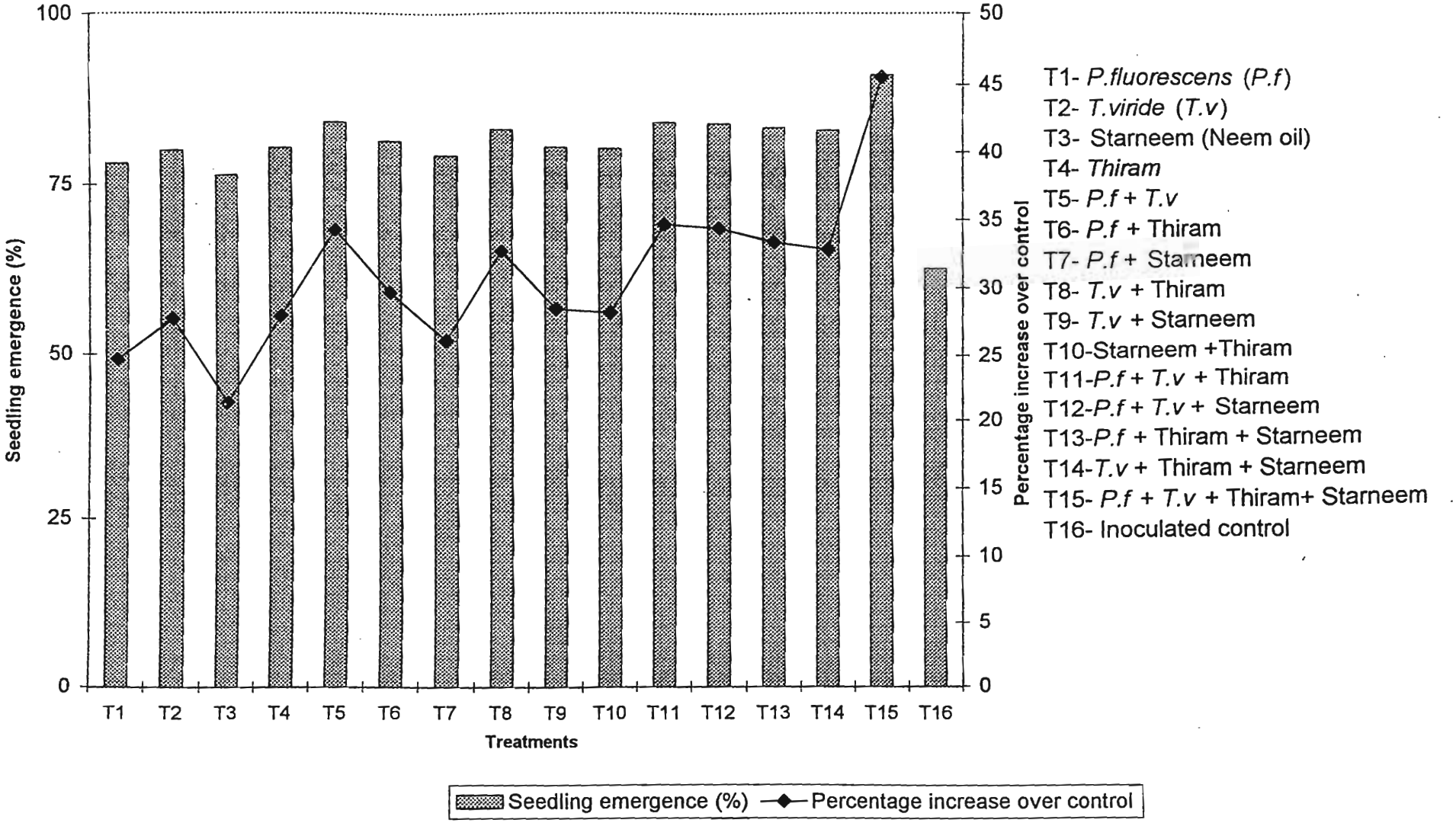


Table 13 : Effect of seed treatment with biocontrol agents, neem oil (star neem) and thiram on dry root rot incidence of green gram cv. ML-267 in *R. bataticola* infested soil in pots

S.No.	Treatment	Dry root rot incidence (%)	Percentage decrease over control
1	*Pseudomonas fluorescens* (P.f) (10 g kg^{-1} seed)	28.5 (32.8)	63.1
2	*Trichoderma viride* (T.v) (4 g kg^{-1} seed)	27.4 (31.5)	64.5
3	Neem oil (Starneem) (3 ml kg^{-1} seed)	34.5 (35.9)	55.3
4	Thiram (3 g kg^{-1} seed)	29.2 (32.6)	62.2
5	*P.f* + *T.v*	21.0 (27.2)	72.8
6	*P.f* + Thiram	23.2 (28.8)	69.9
7	*P.f* + Neem oil (Starneem)	22.4 (2.82)	71
8	*T.v* + Thiram	20.1 (26.6)	73.9
9	*T.v* + Neem oil (Starneem)	22.4 (28.2)	71
10	Neem oil (Starneem) + Thiram	27.0 (31.3)	65
11	*P.f* + *T.v* + Thiram	13.3 (21.3)	82.7
12	*P.f.* + *T. v.* + Neem oil (Starneem)	14.2 (22.6)	81.6
13	*P.f* + Thiram + Neem oil (Starneem)	14.4 (22.4)	81.3
14	*T.v* + Thiram + Neem oil (Starneem)	15.2 (23.0)	80.3
15	*P.f* + *T.v* + Thiram + Neem oil (Starneem)	9.4 (17.6)	87.8
16	Inoculated control	77.3 (61.5)	-
	S.Em +	0.290	
	CD at 5%	0.850	

Mean of three replications
Figures in parentheses are angular transformed values

Fig. 9: Effect of seed treatment with biocontrol agents, neem oil (starneem) and thiram on dry root rot incidence of greengram cv. ML-267 in *R.bataticola* infested soil in pots

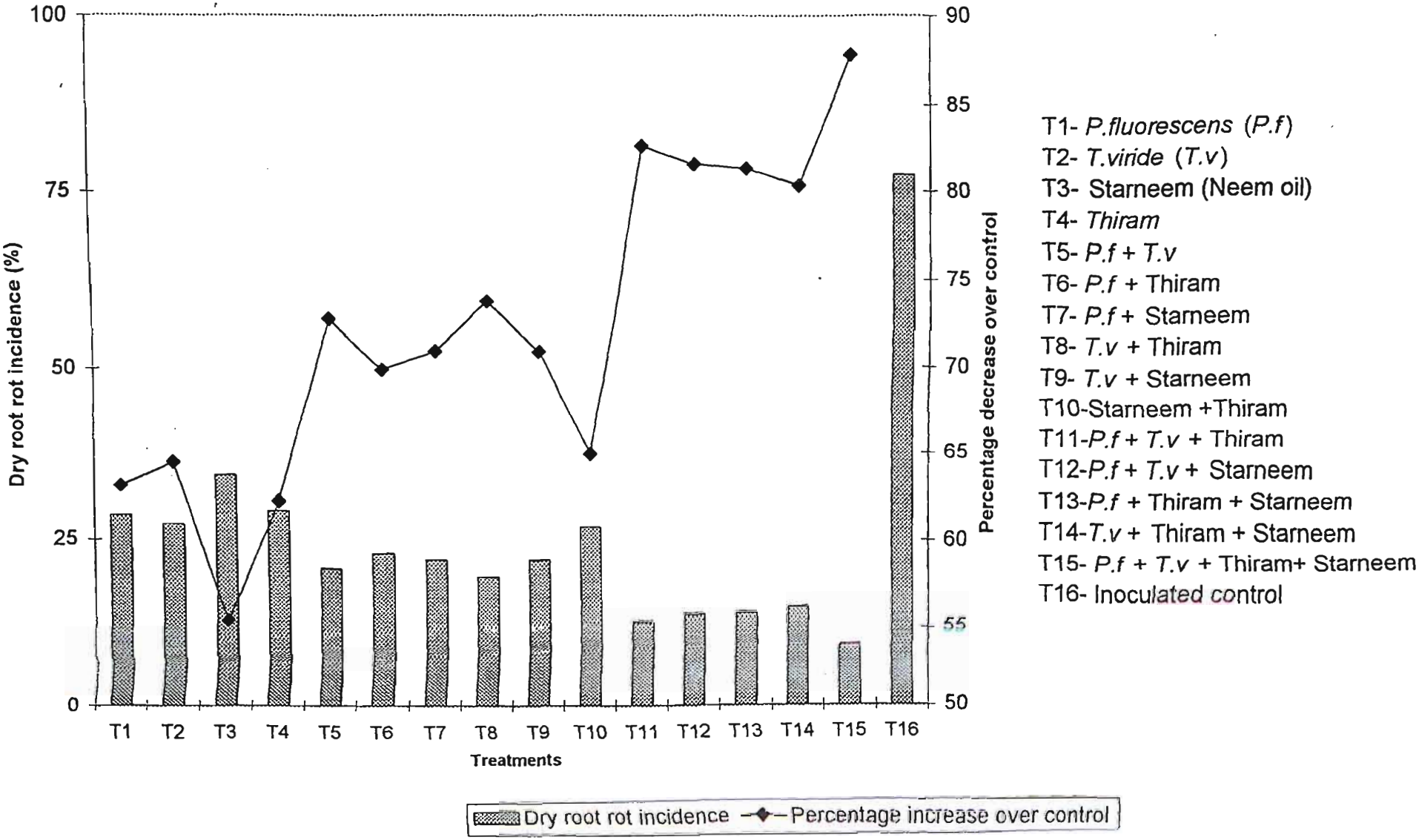


Plate 21 Effect of individual seed treatment with *P.fluorescens T.viride,* neem oil (starneem) and Thiram on dry root rot incidence of greengram cv. ML-267 in pots

Plate 22 Combined effect of any two seed treatments on dry root rot incidence of greengram cv. ML-267 in pots (T_1= *P.fluorescens*, T_2= *T.viride*, T_3=Neem oil (Starneem), T_4=Thiram)

Plate 23 Combined effect of any three treatments on dry root rot incidence of greengram cv.ML-267 in pots (T_1= *P.fluorescens*, T_2= *T.viride*, T_3=Neem oil (Starneem), T_4=Thiram)

Plate 24 Effect of combined seed treatment with *P.fluorescens, T.viride,* Neem oil (starneem) and Thiram on dry root rot incidence of greengram cv.Ml-267 in pots

neem oil + thiram recorded maximum percentage reduction of dry root rot incidence (88%) followed by *P. fluorescens* + *T. viride* + thiram (83%) while neem oil was less effective (55.3%) (Plate 21 to 24).

4.7.3 Effect on Growth Parameters

Dry weight

The results pertaining to the effect of seed treatment with bio-control agents, neem oil and thiram presented in Table 14 revealed that there was a significant increase in dry weight of plant by all the treatments when compared to control (1.49 g plant^{-1}) and the increase in dry weight ranged from (2.50 to 2.96 g plant^{-1}). Combined seed treatment with *P. fluorescens, T. viride,* neem oil and thiram recorded maximum percentage increase in dry weight (98.6%) followed by combined seed treatment with *P. fluorescens, T. viride* and thiram (96.6%) and the minimum increase was observed in seed treatment with neem oil (67.7%) compared to control plants (Plate 21 to 24).

Root length

Results in Table 14 revealed that all the treatments were found to be effective in increasing the root length of greengram plants ranging from 10.33 to 11.80 cm compared to control (6.93 cm). Combined seed treatment with *P. fluorescens, T. viride,* neem oil and thiram recorded maximum percentage increase in root length (70.8 %) followed by seed treatment with *P. fluorescens, T. viride* and thiram (68.4%) compared to control. And the least per cent increase was observed in neem oil (Starneem) treated seeds (49%).

Table 14: Effect of seed treatment with certain biocontrol agents, neem oil (star neem) and thiram on growth parameters of green gram cv.ML-267 in *R. bataticola* infested soil in pots*

S.No.	Treatment	Dry weight (g/plant)	Increase over control (%)	Root length (cm)	Increase over control (%)	Shoot length (cm)	Increase over control (%)
1	*Pseudomonas fluorescens (P.f)* (10 g kg^{-1} seed)	2.57	72.4	10.47	51.0	24.67	43.5
2	*Trichoderma viride (T.v)* (4 g kg^{-1} seed)	2.73	83.2	10.67	53.9	25.10	46.0
3	Neem oil (Starneem) (3 ml kg^{-1} seed)	2.50	67.7	10.33	49.0	24.40	41.9
4	Thiram (3 g kg^{-1} seed)	2.77	85.9	10.67	53.9	25.00	45.4
5	*P.f* + *T.v*	2.83	89.9	11.43	64.9	28.00	62.8
6	*P.f* + Thiram	2.82	89.2	11.00	58.7	27.80	61.7
7	*P.f* + Neem oil (Starneem)	2.79	87.2	10.50	51.5	27.50	59.9
8	*T.v* + Thiram	2.85	91.2	11.20	61.6	29.00	68.7
9	*T.v* + Neem oil (Starneem)	2.81	88.5	10.90	57.2	28.50	65.7
10	Neem oil (Starneem) + Thiram	2.80	87.9	10.83	56.2	27.00	57.0
11	*P.f* + *T.v* + Thiram	2.93	96.6	11.67	68.4	29.00	68.7
12	*P.f.* + *T. v.* + Neem oil (Starneem)	2.92	95.9	11.53	66.3	29.60	72.1
13	*P.f* + Thiram + Neem oil (Starneem)	2.91	95.3	11.30	63.0	29.00	68.7
14	*T.v* + Thiram + Neem oil (Starneem)	2.90	94.6	11.29	62.9	29.00	68.7
15	*P.f* + *T.v* + Thiram + Neem oil (Starneem)	2.96	98.6	11.84	70.8	29.90	73.9
16	Inoculated control	1.49		6.93		17.19	
S.Em ±		0.036		0.130		0.220	
CD at 5%		0.104		0.374		0.633	

* 45 days after sowing
Mean of three replications

Shoot length

The data presented in Table 14 revealed that all the treatments were found to be effective in increasing the shoot length of greengram cv. ML-267 ranging from 24.4 to 29.9 cm over control (17.2 cm). Seed treatment with *P. fluorescens* + *T. viride* + neem oil + thiram recorded maximum percentage of shoot length (73.9%) and the minimum percentage was observed with individual treatment with neem oil (41.9%) compared to control (Plate 23-24).

4.7.4 Effect on Population Levels of *R.bataticola*

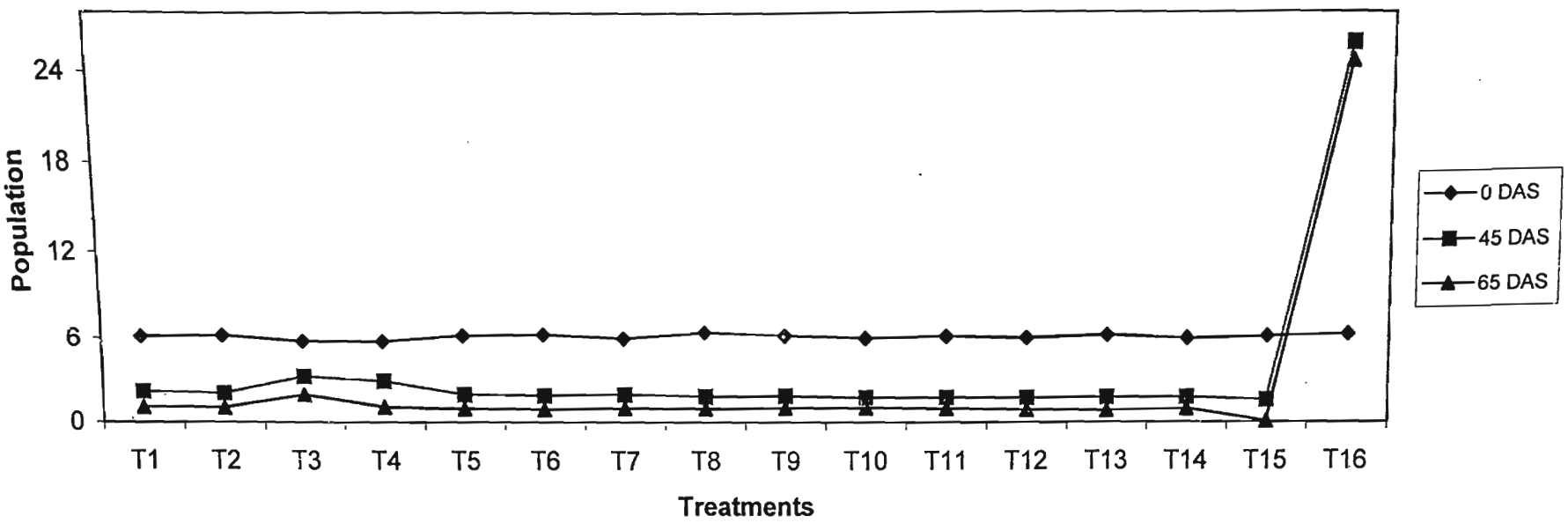
The results on population levels of *R. bataticola* recorded at 0,45 and 65 DAS associated with greengram cv. ML-267 in pots are presented in Table 15. The results indicated that individual as well as combined seed treatment with bio-control agents, neem oil (starneem) and thiram significantly reduced the population levels at 65 DAS compared to control 24.8 x 10^3 cfu/g soil. Seed treatment with combined application of bio-control agents, neem oil (starneem) and thiram was found to be highly effective in reducing the population from 6.03 x 10^3 cfu/g soil at 0 DAS to 0.60 x 10^3 cfu/g soil at 65 DAS. Whereas seed treatment with neem oil (starneem) was found to be least effective in reducing the population from 5.73 x 10^3 cfu/g soil at '0' DAS to 1.93 x 10^3 cfu/g soil at 65 DAS.

Table 15: Effect of seed treatment with biocontrol agents, neem oil (star neem) and thiram on population levels of *R. bataticola* in potted soil at different intervals associated with green gram cv. ML-267

S.No.	Treatment	Population of *R. bataticola* cfu/g soil x 10^3			Percent increase or decrese of pathogen population at 65 DAS over 0 DAS
		`0' DAS	45 DAS	65 DAS	
1	*Pseudomonas fluorescens (P.f)* (10 g kg^{-1} seed)	6.10 (2.47)	2.17 (1.63)	1.06 (1.03)	82.2(–)
2	*Trichoderma viride (T.v)* (4 g kg^{-1} seed)	6.17 (2.48)	2.04 (1.59)	1.00 (1.00)	83.7(-)
3	Neem oil (Starneem) (3 ml kg^{-1} seed)	5.73 (2.39)	3.23 (1.93)	1.93 (1.38)	66.3(-)
4	Thiram (3 g kg^{-1} seed)	5.70 (2.38)	2.90 (1.84)	1.05 (1.02)	81.6(-)
5	*P.f* + *T.v*	6.10 (2.47)	1.93 (1.56)	0.84 (0.93)	86.2(-)
6	*P.f* + Thiram	6.20 (2.49)	1.87 (1.54)	0.89 (0.94)	85.6(-)
7	*P.f* + Neem oil (Starneem)	5.91 (2.43)	1.93 (1.56)	0.97 (0.99)	83.5(-)
8	*T.v* + Thiram	6.33 (2.45)	1.80 (1.52)	0.94 (0.96)	85.2(-)
9	*T.v* + Neem oil (Starneem)	6.1 (2.45)	1.83 (1.53)	0.98 (0.99)	83.9(-)
10	Neem oil (Starneem) + Thiram	5.93 (2.43)	1.73 (1.49)	1.00 (1.00)	83.1(-)
11	*P.f* + *T.v* + Thiram	6.06 (2.46)	1.73 (1.49)	0.94 (0.97)	84.4(-)
12	*P.f.* + *T. v.* + Neem oil (Starneem)	5.94 (2.43)	1.70 (1.48)	0.85 (0.93)	85.6(-)
13	*P.f* + Thiram + Neem oil (Starneem)	6.13 (2.46)	1.73 (1.49)	0.80 (0.89)	86.9(-)
14	*T.v* + Thiram + Neem oil (Starneem)	5.88 (2.42)	1.73 (1.49)	0.92 (0.98)	84.3(-)
15	*P.f* + *T.v* + Thiram + Neem oil (Starneem)	6.03 (2.45)	1.53 (1.43)	0.60 (0.77)	90(-)
16	Inoculated control	6.13 (2.46)	26.0 (5.09	24.8 (5.03)	75.3(+)
	S.Em ±	0.024	0.043	0.0198	
	CD at 5%	NS	0.124	0.055	

Mean of three replications
Values in parentheses are square root transformed values
(+) increase (-) decrease

Fig. 10 : Effect of seed treatment with bio-control agents, neem oil (star neem) and thiram on population levels of *R.bataticola* in potted soil at different intervals associated with green gram cv. ML-267



T1- *P.fluorescens* (*P.f*)
T2- *T.viride* (*T.v*)
T3- Neem oil (Starneem)
T4- *Thiram*
T5- *P.f* + *T.v*
T6- *P.f* + Thiram
T7- *P.f* + Neem oil (Starneem)
T8- T.v + Thiram
T9- *T.v* + Neem oil (Starneem)
T10-Neem oil (Starneem) +Thiram
T11-*P.f* + *T.v* + Thiram
T12-*P.f* + *T.v* + Neem oil (Starneem)
T13-*P.f* + Thiram + neem oil (Starneem)
T14-*T.v* + Thiram + Neem oil (Starneem)
T15- *P.f* + *T.v* + Thiram+ Neem oil (Starneem)
T16- Inoculated control

Discussion

CHAPTER V

DISCUSSION

Dry root rot of greengram an important fungal disease causing yield losses upto 25 per cent became one of the major constraints in greengram growing tracts of India. The endemic and serious nature of the disease in most of the areas particularly in low rainfall and sandy loam soils of Andhra Pradesh prompted taking up investigations on "Evaluation of bio-fungicides in control of dry root rot of greengram".

The pathogen *R. bataticola* is seed borne as well as soil borne affecting many crop plants *viz.*, cereals, vegetables, pulses, oilseeds etc. and causes seed rot, seedling blight, charcoal rot of stem, foliage blight, tuber decay, dry root rot and fruit rot, etc (Raut and Ingle, 1989).

Management of these soil borne diseases is very difficult because of long term survival and wide host range of pathogen. Application of chemicals to manage the crop disease may lead to adverse effect on beneficial soil microflora and soil microfauna including the most beneficial microorganisms like endomycorrhiza (Reddy, 1997).

Among the alternative methods, using living organisms to control the plant pathogens is gaining importance during recent years. Bio-control agents may offer more environmental friendly measures compared to chemicals (Baker and Cook, 1974). These measures of controlling plant pathogens using living organisms are now called 'Biological control'.

Cook (1977) stated that biological control presents one of the greatest challenges of all times to our discipline and it requires the attention of all facts of our discipline, offers something for commercial interests as well as the public sector and to the practitioner as well as scientist conducting basic research.

Biological control offers durable, environmentally safe and cost effective alternative to chemicals. Among the bio-control agents the most important and widely exploited bio-control agents are *Trichoderma* sp., *P. fluorescens and Bacillus subtilis.*

The role of botanical products especially neem products in the bio-control of plant pathogens have been recognised, studied and made use for decades. The bio-control agents and botanical products applied to the seed also have the potential to protect the root from soil borne fungal pathogens making seed application as effective delivery system (Paulitz and Fernando, 1996).

As with many other diseases, dry root rot of greengram caused by *Rhizoctonia bataticola* continues to reserve considerable attention with regard to the development of biological control strategies. The present work was conducted to test the effect of various fungal antagonists (*Trichoderma viride, T.harzianum, T.reesei and Gliocladium virens*), bacterial antagonists (*P.fluorescens, Bacillus subtilis*) and some neem based commercial

formulations so as to develop an effective, economical potential bio-fungicides in the management of dry root rot of greengram caused by *R. bataticola* in pots under green house conditions.

SURVEY

The results on the survey conducted during *rabi* 2001 in major greengram growing mandals of Chittoor district on dry root rot incidence revealed that there was a variation in dry root rot incidence with high incidence (12%) in Chinnagottigallu mandal and low in Pileru mandal (5.6%). Kratisharma and Tribhuwan Singh (2000) also reported variation in dry root rot incidence (0.5-38%) in greengram at a survey conducted in Rajasthan. Kataria and Grover (1977), Tyagi (1988) also reported 10.8 and 24.1 per cent dry root rot incidence in Haryana and Rajasthan, respectively. The variation in dry root rot incidence could be due to influence of various ecological, physical and chemical characteristics of soil.

The soil type also influence the dry root rot incidence, a high incidence of dry root rot was observed in sandy loam (11.1%) followed by sandy clay loam (8.1%) and least in clay soils (4.3%). In light sandy soils, the incidence of dry root rot due to *R. bataticola* was reported to be high. Similar results with the same pathogen were also earlier observed by Taya *et al.* (1988) and Rettinassababady and Ramodoss (1999) in chick pea and black gram, respectively. The fungal activity is influenced by soil aeration and soil texture.

The amount of free oxygen obviously decides the activity of soil borne fungus. A critical stage for oxygen competition between plants and microorganisms arises during seed germination. Sandy soils with more number of macropores compared to clay soils can hold adequate air though they are poor in water holding capacity (Baver *et al.*, 1962). This could be the probable reason for high percentage of dry root rot incidence in sandy loams when compared to clay loams.

The dry root rot incidence was found in all the cultivars surveyed. Out of three cultivars surveyed greengram cv. ML-267 recorded a high dry root rot incidence (11%) followed by Pusa Baisakhi and least in local cultivars. Sahu and Jena (1997) also reported the incidence of dry root rot (4.8%) on all the cultivars surveyed.

ISOLATION AND IDENTIFICATION OF THE PATHOGEN

The pathogen was isolated from infected plant parts showing dry root rot symptoms on PDA medium. Pathogenicity was established by inoculation and reisolation of the pathogen from infected plant parts. The fungus produced spherical hyaline colonies which later became fluffy, carbonaceous, brown to block with numerous small black sclerotia on PDA. The sclerotia were 75 to 110 µm in size. The morphology of the pathogen observed was in accordance with the description given by Philip *et al.* (1969) in greengram.

PATHOGENICITY STUDIES

The studies on pathogenicity tests in pot culture revealed that dry root rot incidence of greengram cv. ML-267 increased from 12.5 to 82.7 per cent as the inoculum level increased from 1 to 9 per cent. The optimum infection threshold level was found to be 7 per cent inoculum causing 78 per cent incidence. Similar pathogenicity studies were conducted by earlier workers but a variation in dry root rot incidence with respective inoculum level was observed. Prameela Devi and Singh (1998) reported 10 per cent inoculum density caused 71 per cent seedling mortality in greengram and black gram.

ANTAGONISTIC STUDIES

Trichoderma sp. have been long known as effective antagonists against many soil borne pathogenic fungi and are the focus of recent research (Hadra *et al.*, 1979). *In vitro* antagonism of *Trichoderma* spp. against *M.phaseolina* has been well documented under laboratory conditions (Elad *et al.*, 1986; Deshmukh and Raut, 1992; Ramesh Sundar *et al.*, 1995, Majundar *et al.*, 1996). In present study the antagonistic activity of *T. viride, T. harzianum, T. reesei* and *Gliocladium virens* tested against test pathogen *R. bataticola* employing dual culture technique indicated a maximum percent inhibition of *R. bataticola* in the presence of *T. viride* followed by *T. harzianum*. Thus indicating *T. viride* as highly effective, which was also reported earlier by Ghaffer (1968) in cotton, Sarwar (1974) in castor, Alagarsamy and Sivaprakasam (1988), in cowpea, Parakhia and Vaishnav (1986) in chickpea on the same pathogen *M.phaseolina*.

Baker and Cook (1974) reported that there exists four forms of antagonism *i.e.*, competition, antibiosis, parasitism. The growth inhibition of *R. bataticola* could be attributed mainly due to antibiosis or hyper parasitism as opined by Baker and Cook (1974). Most fungi have chitin ß (1-3) glucanase as essential constituent in their cell wall. *Trichoderma* spp produce chitinase and ß (1-3) glucanase enzyme which degrades the cell wall leading to lysis of *Rhizoctonia* sp., as reported by Wu *et al.* (1986).

The dual culture technique was also employed to identify the potential bacterial antagonist against the test pathogen. Maximum reduction in the growth of *R. bataticola* was observed in the presence of *P. fluorescens* indicating the potential antagonism against test pathogen. Laha and Venkataraman (2001) also reported antagonistic activity of six isolates of *Pseudomonas* spp. against *R. solani in vitro*. The antagonistic activity of *P. fluorescens* was also reported on other pathogens, *Sclerotium rolfsii* (Ganesan and Gnanamanikam, 1987) and *S.oryzae* (Elangovan and Gnanamanikam, 1992).

The antagonistic activity of bacteria was attributed mainly due to production of antagonistic compounds, such as antibiotics, siderophores, ammonia, cyanide and hydrolytic enzymes (Baker, 1987). Antibiotics like phenazine-1-carboxylic acid, pyoluteorin, and the acetyl-phlorolucinols produced by strains of *P. fluorescens* were the main causes of antagonistic activity of bacteria as reported by Vincent *et al.* (1991) and Thomashow *et al.* (1991).

The role of siderophore production by biological control strains in the antagonism of phyto-pathogens has been reviewed by Loper and Buyes (1991) and Loper and Ishimaru (1991). Iron depletion of competing microbes, including phytopathogens, resulting from their lack of receptors for bacterial strain's, ferric siderophore is thought to be the mechanism of this type of antagonism (Loper and Ishimaru, 1991). The pyoverdin class of siderophore has been implicated either in plant growth promotion or disease suppression (Kloepper and Schroth, 1982; Baker and Cook, 1988 and Gutterson, 1990). Production of HCN by *P. fluorescens* strain is thought to be the another cause of antagonism (Keel *et al.*, 1990).

It has long been recognized that extra cellular hydrolytic enzymes synthesized and released by soil microbes might play a role in the antagonism of phytopathogenic fungi. In particular, numerous correlations between fungal antagonism and bacterial production of chitinase and/or ß-1, 3 glucanases have been noted (Inbar and Chet, 1991). Chitin and ß-1, 3 glucans are major constituents of many fungal cell walls and various workers have demonstrated *in vitro* lysis of fungal cell wall either by bacterial chitinase or ß 1-3 glucanse alone or by a combination of both enzymes (Shapira *et al.*, 1985). It revealed that these hydrolytic enzymes contribute to bio-control efficacy.

FUNGI TOXICITY

The poisoned food technique was employed to test the fungal toxicity of neem based commercial formulations *viz.*, Starneem and neem gold at 100, 200, 400, 600, 800 and 1000 ppm concentrations on mycelial growth of test pathogen, *R. bataticola in vitro*. Both the tested neem formulations inhibited the mycelial growth of the pathogen at all the concentrations and the complete inhibition was recorded at 800 and 1000 ppm. Dhanpal *et al.* (1993) also reported the fungi toxicity of neem oils against *R. solani in vitro*. The effectiveness of neem may be attributed due to the presence of certain antifungal ingredients, "Azadirachtin".

The poisoned food technique was also employed to test the efficacy of thiram at six concentrations on mycelial growth of *R. bataticola*. All concentrations were found inhibiting the growth of pathogen with 100 per cent inhibition at 200 and 300 ppm concentrations. Illyas *et al.* (1975) and Patel and Patel (1990) also reported inhibition of *Macrophomina phaseolina* by thiram *in vitro*. Sinha and Khare (1977) found that thiram and captan at 300 ppm inhibited the dry root rot pathogen of cowpea, to an extent of 100 per cent. Peshney *et al.* (1992) observed that thiram, captan and mancozeb were effective in inhibiting the growth of *M.phaseolina* at 200 ppm.

COMPATIBILITY STUDIES

Any bio-control agent must be effective and compatible with modern production practices so that its use can be integrated in to the production system. A novel blending technique has been reported in which the bio-control agents were used simultaneously with seed dressing fungicides, without any toxic effect on antagonists (Papavizás and Lumsden, 1980).

The effective neem based commercial formulation neem oil (starneem) was tested for its compatibility with fungal antagonists *viz., T. harzianum, T. viride, T. reesei,* and *G. virens* and found that neem oil is incompatible at 100, 200, 400, 600, 800 and 1000 ppm concentrations. However, the inhibition at 100 ppm was not statistically significant. All the fungal antagonists *in vitro* showed varying degree of percentage inhibition. However, the percentage inhibition of *T.viride* and *G.virens* with neem oil at 600 ppm concentrations was less compared to other antagonists.

Compatibility studies between neem oil and bacterial bio-control agents *viz., P.fluorescens* and *B.subtilis* indicated that they are compatible at all the tested concentrations *viz.*, 100, 200, 400, 600, 800 and 1000 ppm.

The *in vitro* studies on compatibility between *T. viride* and thiram indicated that thiram at 25, 50, 100, 150, 200 and 300 ppm concentrations found to be incompatible with the test antagonist. However, at 25 ppm the inhibition is not statistically significant. Dubey and Patel (2001) also reported

that thiram at 200 ppm was highly inhibitory to *T. viride*. Singh *et al.* (1995) also reported that thiram at 200 ppm concentrations completely inhibited the growth of *T.harzianum*.

The compatibility of *P.fluorescens* with thiram at 25, 50, 100, 150, 200 and 300 ppm concentrations indicated that both of them were compatible upto 200 ppm only but at 300 ppm the growth of *P.fluorescens* was inhibited by 2.2 per cent.

POT CULTURE STUDIES

In the present investigation application of *P.fluorescens, T.viride*, neem oil and thiram individually as well as in combinations was studied on dry root rot incidence of greengram in pot culture.

Seedling emergence

Seed treatment either alone or in combination with *T.viride*, neem oil (starneem), *P.fluorescens* and thiram improved the seedling emergence of greengram cv. ML-267 in pots under green house conditions. The maximum seedling emergence was recorded with combined application of *T.viride*, *P.fluorescens*, neem oil and thiram. Increased plant survival with integration of *Trichoderma* sp. and chemical fungicides was also reported by earlier workers. Rajeswari *et al.* (1999) reported that *T.harzianum* in combination with carbendazim improved seedling emergence of greengram. Alagarsamy and Sivaprakasam (1988) also reported that seed treatment with *T. viride* and carbendazim increased the seedling emergence by 91.8 per cent in cowpea.

Dry root rot incidence

Seed treatment with antagonists, botanical products and fungicide is a common method of application to protect the crop plants from seed and soil borne plant pathogens (Paulitz and Fernado, 1996).

In the present investigation the percentage of dry root rot incidence was significantly reduced in the treatments where fungal and bacterial antagonist, neem oil (starneem) and fungicide were used in combination. Similar results were also obtained by earlier workers when *Trichoderma* spp. was used both as seed and soil treatments in combination with fungicide (Thiram) (Manmohan Das and Sivaprakasam, 1994). Dhanapal *et al.* (1993) reported that application of commercial neem product, neem gold was found effective in reducing disease incidence by *R. solani.*

Vyas and Khare (1986) obtained least dry root rot incidence in soybean by combined application of *T.harzianum* and carbendazim. Alagarsamy and Sivaprakasam (1988) reported seed pelleting with *T. viride* alone or in combination with carbendazim reduced dry root rot incidence in cowpea. The incidence of *M.Phaseolina* in mungbean was also reduced by *T.harzianum* + carbendazim (Jayaraj and Rambadran, 1996) and *S.rolfsii* in groundnut (Muthamilan and Jeyarajan, 1996).

Integrated biological and chemical control seems to be a very promising way of controlling pathogens with minimal interference with the biological equilibrium (Baker and Cook, 1982; Henis and Chet, 1975; Papavizas, 1973) one of the most attractive ways of reducing the amount of fungicide is the integration of sublethal doses of chemicals with *Trichoderma* sp. which is resistant to high doses of chemicals (Munnecke, 1973, Elad *et al.*, 1981).

Growth parameters

The growth characters *viz.*, plant dry weight, shoot length, and root length were studied in the present investigation. The combined application of *T. viride*, *P.fluorescens*, neem oil (starneem) and thiram were found to be most effective in inducing the plant growth parameters when compared to individual treatment.

Two mechanisms have been advanced most frequently to explain the increased growth response induced by certain microflora. The first hypothesis was that enhanced growth of plants induced by antagonists might be due to biological control of plant pathogens in the soil. The other hypothesis so far, not demonstrated clearly for any biological system was that a microbial agent produced growth regulatory metabolites (Kloepper and Schroth, 1981). Thus the rate of germination and dry weight of root and shoot were increased (Windham *et al.*, 1986). Similar reports of increase in vegetative growth using

fungicide and *Trichoderma* spp. was also recorded by Rajeswari *et al.* (1999) in greengram, Manmohan Das and Sivaprakasam (1994) in chilly, Manoranjitham *et al.* (2001) in tomato and Alagarsamy and Sivaprakasam (1988) in cowpea.

Pathogen population studies

The pathogen *R. bataticola* populations were found to be decreased from 'O' to 65 DAS in all the treatments imposed. Maximum reduction in pathogen population level was observed in combined treatment with *P. fluorescens, T.viride,* neem oil (starneem) and thiram. The decrease in pathogen population may be due to increased levels of antagonists and fungitoxicity of thiram and neem oil. Where as the pathogen population was very high in control. This may be due to lack of competition with any other microbes and fungitoxicants in pots.

These present studies show that use of fungicide Thiram (3 g kg^{-1} seed) and neem oil (Starneem) (3 ml kg^{-1} seed) provides initial protection to seed and seedlings from the attack of soil and seed borne pathogen, *R.bataticola* in greengram and thereby helping in establishment and multiplication of antagonists *T.viride* (4g kg^{-1} seed) and *P.fluorescens* (10g kg^{-1} seed) which provide the protection throughout the crop growth period. Though thiram was found in compatible with *T.viride in vitro*, this effect was not found when it was applied in combination with other bacterial bio-control agents and

commercial neem product Starneem. This positive effect may be due to interaction effect of bio-control agents, fungicides and seed exudates.

It is concluded from the present studies that survey conducted on the incidence of dry root rot of greengram indicated the occurrence of disease incidence in all the greengram growing mandals of Chittoor district surveyed, ranging from 5.7 to 12%. A high incidence was recorded in sandy loam soils (11.1%) in green gram cv.ML-267. An inoculum level of 7% was found to be optimum infection threshold level of test pathogen *R.bataticola* with cv.ML-267. Among the antagonists studied fungal antagonist *T.viride* and bacterial antagonist *P.fluorescens* was found to be highly effective in inhibiting the test pathogen. Out of the two neem based commercial formulations tested Star neem was found to be more effective against test pathogen. Combined seed treatment with *P.fluorescens* (10 g kg^{-1} seed) *T.viride* (4 g kg^{-1} seed), thiram (3 g kg^{-1} seed) and Starneem (3 g kg^{-1} seed) was found to be effective in reducing dry root rot incidence population levels of the pathogen and promoting plant growth parameters in pot culture studies.

Summary

CHAPTER-VI

SUMMARY

Summary on the present investigations carried out on greengram to know the incidence of dry root rot in different mandals of Chittoor district, pathogenicity studies, antagonistic studies, fungitoxicity of neem based commercial formulations, compatibility studies and evaluation of effective bio-control agents and neem oils as bio-fungicides in the management of dry root rot of greengram under green house conditions is presented below:

Survey conducted on dry root rot incidence in eight major greengram growing mandals of Chittoor district. A.P. indicated, the disease incidence ranged from 5.6 to 12 per cent with low and high incidence in Pileru and Chinnagottigallu mandals, respectively.

Out of three soil types surveyed, sandy loams recorded high dry root rot incidence of (11.1%) whereas the incidence was low in clay soils (4.3%). Among the cultivars surveyed greengram cv. ML-267 recorded high dry root rot incidence of (11%) and the incidence was low (4.8%) in local cultivars and the pathogen *Rhizoctonia bataticola* was isolated from diseased plants collected.

An inoculum level of 7 per cent was found to be optimum infection threshold level for *R. bataticola* on greengram cv. ML-267 in steam sterilized sandy loam soil.

A fungal antagonist *Trichoderma viride* was found to be significantly more effective in suppressing the growth of *R. bataticola* (70%) *in vitro* than other antagonists tested. Similarly bacterial antagonist *Pseudomonas fluorescens* was found to be significantly more effective in inhibiting the growth of *R. bataticola* (63.7%) *in vitro*.

Out of two neem based commercial formulations tested neem oil (Starneem) was found to be more effective than neem gold in inhibiting the growth of the pathogen *in vitro*.

In compatibility studies, the neem oil (Starneem) was found incompatible with fungal antagonists *viz.*, *T. viride, T. harzianum, T. reesei* and *G. virens*. However, it was compatible with bacterial bio-control agents *viz., P. fluorescens* and *B. subtilis in vitro* and the fungicide thiram was found incompatible with *T. viride* and compatible with *P. fluorescens in vitro*.

Combined seed treatment with *T. viride* (4 g kg^{-1} seed), *P. fluorescens* (10 g kg^{-1} seed), neem oil (Starneem) (3 ml kg^{-1} seed) and thiram (3 g kg^{-1} seed) not only reduced the dry root rot incidence (87.8%) but also improved the seedling emergence, (91.3%) and growth parameters *viz.*, dry weight (98.6%), shoot length (73.9%) and root length (70.8%) and maximum reduction in the pathogen population (90.0%)

It is concluded that the combined seed treatment with *T.viride* (4 g kg^{-1} seed) *P.fluorescens* (10 g kg^{-1} seed), commercial neem product Starneem (3 ml kg^{-1} seed) and thiram (3 g kg^{-1} seed) can be used as an effective and ecofriendly practice in the management of dry root rot of greengram and can be further tested under field conditions.

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