"STUDIES ON THE MANAGEMENT OF DAMPING-OFF DISEASE INCITED BY *Pythium aphanidermatum* (Edson) Fitzp. IN TOBACCO NURSERIES"

By

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON THE MANAGEMENT OF DAMPING-OFF DISEASE INCITED BY *Pythium aphanidermatum* (EDSON) FITZP. IN TOBACCO NURSERIES"** 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 **Miss V. HARITHA** under our 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 by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, **Miss V. HARITHA,** hereby declare that the thesis entitled **"STUDIES ON THE MANAGEMENT OF DAMPING-OFF DISEASE INCITED BY *Pythium aphanidermatum* (Edson) Fitzp. IN TOBACCO NURSERIES"** submitted to Acharya N.G. Ranga Agricultural University, Hyderabad for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that the materials contained in this thesis has not been published earlier.

Date :

(V. HARITHA)

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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	Percentage
@	-	At the rate of
a.i.	-	Active ingredient
AICRP	-	All India Co-ordinated Research Project.
CFU	-	Colony forming units
cm	-	centimeter
DAI	-	Days after incubation
DAS	-	Days after sowing
Fig.	-	Figure
g	-	gram
h	-	Hour
ha	-	Hectare
i.e.,	-	That is
kg	-	kilogram
l	-	liter
m	-	Meter
mg	-	Milli gram
µg	-	Micro gram
ml	-	milliliter
mm	-	millimeter
no.	-	Number
ppm	-	parts per million
viz.	-	Namely
°C	-	degree celsius
psi	-	pressure per square inch

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ABSTRACT

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Title of the thesis	:	**"STUDIES ON THE MANAGEMENT OF DAMPING-OFF DISEASE INCITED BY *Pythium aphanidermatum* (Edson) Ftizp. IN TOBACCO NURSERIES"**
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Tobacco is one of the world's leading non food commercial crops, which was introduced into India by Portuguese about 400 years back. It is valued in the world trade mainly for leaf. Certainly, no other crop has exerted so much influence upon the political, economic, social and cultural life of all nations that have been concerned with its production, commerce, manufacture and use.

Among the diseases of tobacco, damping-off, incited by *Pythium aphanidermatum* is most important. This pathogen severely attacks the crop at pre-emergence and post-emergence stages in nurseries. The pathogen is soil borne in nature and hence the management is very difficult by using cultural,

physical, biological and chemical methods individually. Hence, in this experiment all the above methods were integrated to see the effectiveness.

In *in vitro* conditions *Trichoderma* isolate TT_bT-1 has shown 94.44 per cent inhibition over control regarding radial growth of the pathogen. It was effective than other soil microflora. All the *Trichoderma* isolates (TT_bT-1 to TT_bT-13) have given more than 70 per cent inhibition of radial growth of the pathogen over control. Bacterial isolates shown more than 50 per cent inhibition over control and out of five fungicides, metalaxyl MZ (Ridomil MZ 72% WP) has shown more per cent inhibition on radial growth of the pathogen over control even from 50 ppm and it has shown less inhibition of radial growth and sporulation of *Trichoderma* sp. even at 1000 ppm.

In field conditions due to soil solarization with clear plastic polyethylene sheet the temperature was increased to 43.83^{o}C where as in un solarized soils it was recorded as 33.63^{o}C and the soil moisture content recorded was 4.10 and 10.10 mm in unsolarized and solarized soils, respectively. The weeds were reduced by 93.41 per cent in solarized plots. Soil mycoflora also reduced by more than 50 per cent in solarized plots.

In the management of damping-off of tobacco in nurseries, the effective bio-agent, the effective fungicide, soil solarization and neem cake were applied individually and in combination. Among all the treatments, the integrated use of all the above has shown the highest germination percentage (85.50), the lowest per cent disease incidence (5.54), lesser disease growth rate (3.20), more fresh weight (1.25 g) and more dry weight (1.09) than the individual treatments.

CHAPTER – I

INTRODUCTION

Tobacco (*Nicotiana tabaccum* L.) one of the world's leading non food crops, was introduced in to India by Portuguese about 400 years back. The narcotic qualities of tobacco was discovered by Christopher Columbus in 1942 in his course of voyage from Persia to Spain and he introduced the seeds of tobacco in Europe.

Now the tobacco is an important commercial crop in view of revenue generation, export earnings and employment. It is valued in the world trade mainly for leaf. Certainly, no other crops has exerted so much influence upon the political, economic, social and cultural life of all nations that have been concerned with its production, commerce, manufacture and use.

The area under tobacco in India is 4 lakh ha. which accounts for 0.23 per cent of total arable land, and the production is 600 million kg. India is the 2nd largest producer and 4th largest exporter of tobacco (Anon., 2002) and in Andhra Pradesh the area under tobacco is 0.52 lakh ha, and the production is 0.88 lakh tonnes (Anon., 2000-2001).About 96 per cent of cigarette tobacco is produced in Andhra Pradesh and the rest in Mysore, Bihar, and Punjab. In Andhra Pradesh, Guntur, Krishna, East Godavari and West Godavari are the main tobacco producing districts. Now a days the oriental tobacco is introduced into Chittoor district by realizing the need for aromatic tobaccos, considering the suitability of climate and soils.

Main problem in commercial cultivation of tobacco is, occurrence of various diseases at different stages of growth. Among them damping-off disease is more important, which occurs at nursery stage. The disease is incited by *Pythium aphanidermatum*, a soil borne oomycete fungus.

Ramakrishna Iyer (1929) reported for the first time from India, *P. aphanidermatum* causing damping-off of seedlings in nurseries. The disease is more common in nurseries where the seedlings are grown crowded and usually appears immediately after the onset of rains (Rangaswamy, 1975)

The pathogen has a wide host range, among which tobacco crop is more important. Mainly the pathogen is a soil inhabitant and is responsible for damping-off at pre-emergence stage killing the germinating seeds and young seedlings before they emerge out of the soil surface and at post-emergence stage, attacking the young seedlings at or below ground level resulting in death of seedlings.

Damping off is one of the most dreadful diseases and is particularly severe in densely crowded nurseries and it appears immediately with the monsoon showers at which time the weather is most conducive for its commencement and spread. Excess of moisture, lack of sunlight, growing of nursery in heavy soils and presence of weeds favour the development of this disease. In the nursery, the disease starts in patches and in due course spreads to the entire lot.

Since the disease mostly affects nursery, proper management at this stage is very critical to ensure a healthy crop in the main field. Any negligence in the management of this disease at this juncture, may totally jeopardize the cultivation of this crop as it literally wipes out the entire nursery.

Crop losses inflicted by soil borne pathogens continue to increase and become a limiting factor in stabilizing or maximizing crop yields on a world-wide basis. The control measures available today including fungicides is not enough to the realistic elimination of soil borne plant pathogens. The ill effects of escalated use of potentially hazardous pesticides like environmental pollution, increased cost of application and pathogen resistance lead to a drastic shift in the management strategies towards biological control of plant pathogens as an alternative to or a part of IPM system for disease control (Baker and Snyder, 1965).

Economical control of plant disease is rarely achieved by a single procedure. It must be supported by use of planting material that is free of the pathogen, by tillage methods that are un-favourable to the pathogen, favourable to the host and or the use of antagonists, by sanitation or use of fungicides, and especially by host plant resistance. Such integrated control is based on the fact that different methods work best at different times and places or under different conditions, each compensating to some extent for the deficiencies of the others (Cook and Baker, 1983).

In general, control of the soil borne disease is difficult. Several management methods like physical, chemical and biological proved to be effective against soil borne pathogens. Chemical control of these soil borne pathogens is uneconomical and not advisable owing to risk of ground water pollution, death of non-target beneficial flora and evolution of fungicide resistant variants of pathogen. Hence, it is advisable to adopt integrated disease management. Present studies were undertaken in respect of integrated disease management with the following objectives:-

OBJECTIVES:

1. Isolate the damping-off pathogen associated with the infected tobacco seedlings.
2. To isolate different soil microflora from the rhizosphere soil (fungi and bacteria) and to identify the potential antagonist against pathogen *in vitro.*
3. *In vitro* screening of fungicides against pathogen
4. To test the compatibility of effective bio-agent with the fungicides.
5. Integrated management of damping-off disease by using effective bio-agent, effective fungicide, soil amendment (neem cake) and soil solarization under field conditions.

CHAPTER - II

REVIEW OF LITERATURE

A brief review of literature on the pathogen and its management has been presented under the following headings.

2.1 The pathogen

2.2 Symptomatology

2.3 Management studies

2.3.1 Biological control

2.3.2 Chemical control

2.3.3 Compatibility of fungicides with bio-agents

2.3.4 Soil solarization

2.3.5 Organic amendments

2.3.6 Integrated disease management

2.1 THE PATHOGEN

The growth of *P.aphanidermatum* was poor at temperatures below 8^{o}C and above 46^{o}C and the growth was optimum at 34 to 36^{o} C (Takahashi, 1952).

According to Luna and Hine (1964), the saprophytic growth of *P. aphanidermatum* in soil was optimum at soil temperatures from 28 to 31^{o}C. They also found that the incorporation of sugarcane residues with different

C/N ratios to soil increased microbial activity but did not reduce the damping-off potential of the fungus in tomato nursery.

Kendrick and Wilbur (1965) observed significant pre-emergence seedling death of lima beans when inoculated with oospores of *Pythium irregulare* @ 500 oospores/g soil.

Damping-off caused by *Pythium aphanidermatum* (Edson) Fitz. is a major seedling problem in sugarbeet cultivation (Rama, 1981).

Agnihotri and Sinha (1986) reported that tomato crop was highly susceptible to damping-off at seedling stage in the nursery beds and the maximum damping-off incidence was recorded at a temperature of 30^{o}C.

Patel *et al*. (1987) reported that among the seed bed diseases of tobacco, damping off caused by *Pythium aphanidermatum* (Eds.)Fitz. and *Cercospora* leaf spot caused by *Cercospora nicotianae* Ell & Eve. are the most serious endemic diseases. The former, under optimal conditions, wiped-off the tobacco nurseries resulting in complete loss.

Zhang *et al.* (1990) studied the effect of seasonal fluctuations of pathogenic *Pythium* spp. in relation to damping-off of vegetable seedlings and proved that populations of these pathogens were affected by soil temperature, soil moisture and rainfall during crop growth. Further, they observed that. *P spinosum* was abundant during winter and spring on solanaceous and cucurbitaceous vegetables while *P. aphanidermatum* peaked in summer.

Devaki and Shankar Bhat(1992) reported that damping-off of tobacco is a severe nursery disease incited mainly by *P.aphanidermatum*, typical of warmer regions. Oospores and chlamydospores in the soil act as initial sources of infection. Oospores are constitutively dormant while embedded in host tissue. They showed that *P.aphanidermatum* from *Phaseolus vulgaris* was shown to have a similar pathogenicity as *P.aphanidermatum* on tobacco. They suggested that oospores embedded in the tissue of affected *P.vulgaris* pods in the soil act as an inoculum source for development of damping-off in tobacco.

Patel *et al*. (1993) reported that damping-off is the most common and serious fungal disease in tobacco nursery caused by *P.aphanidermatum* (Edson) Fitz. It causes 80 to 100 per cent death of seedlings under favourable conditions of disease development.

The fungus, *Pythium aphanidermatum* (Edson) Fitz. is more common in both summer and winter grown tomato causing losses to an extent of 50 per cent (Bisht *et al.*,1997)

Neelamegam and Govindarajulu (2002) reported that tomato is affected by various diseases and the most serious one is the damping-off caused by several species of *Pythium*, which accounts for severe crop loss due to wide host range, soil borne nature and prolonged survival of propagules in soil by saprophytic growth and by resistant resting structures which persist for many years (Hendrix and Campbell, 1973).

2.2 SYMPTOMATOLOGY

P. aphanidermatum is a serious pathogen causing damping-off of seedlings, root rots and fruit rots of many crops.

2.2.1 Damping-off

In the pre-emergence phase, the young seedling is killed before it reaches the surface of the soil and in the post-emergence phase, the disease is characterized by toppling over of the infected seedlings at any time after their emergence from the ground until the stems have hardened enough to resist the invasion of the pathogen. Damping-off symptoms due to tissue maceration is usually due to the pectolytic and cellulolytic enzymes produced by the pathogen (Muthuswamy *et al*., 1974).

2.2.2 Root rot and stem rot

These two are the phases which affect many plants after they have become established. The fibrous root system is attacked and rootlets are softened and killed, the above ground effects consisting of stunting, sudden wilting or death. When infection occurs near soil line on stems in which secondary thickening is well established, cortical lesions develop which may girdle the stem to cause stunting or death (Walker, 1969).

2.2.3 Soft rot

It is the phase which affects the fleshy organs of many plants, particularly the vegetables. Here, the pathogen incites decay which is important in storage, transit and market. Cucurbit fruits, cabbage heads and potato tubers are examples of host substrates which are affected. Infection takes place through wounds or where fruits are in contact with wet soil and progress is usually rapid. The tissues become water soaked, lose cohesion and liquid is exuded much as in bacterial soft rot. The fungus is intracellular and may be inconspicuous in the early stages of decay, but it eventually produces fluffy white mycelium on the surface of decayed tissue (Walker, 1969).

2.3 MANAGEMENT STUDIES

2.3.1 Biological control

Seed treatment with antagonist is the cheapest method for delivery of fungal antagonists to the rhizosphere of crop plants to be protected from the soil borne disease for the success beyond the suppression of seed rots and damping-off of young seedlings. The antagonist applied to seeds must be able to multiply in the soil (or) rhizosphere to inhibit a given pathogen by competition, mycoparasitism or antibiosis. The ideal seed inoculum must be effective antagonist and aggressive spermosphere and rhizosphere colonizers (Papavizas, 1985).

Seed treatment with biological agents is considered as a means to substantially increase the value of the seed and to improve plant growth and productivity. The bio-agents proliferate on the seed coat of germinating seed and colonize the additional plant parts such as roots and collar region. The treatment is quite inexpensive and eco-friendly as compared to other methods of disease control and successfully exploited for the control of a wide range of seed as well as soil borne diseases (Mukhopadhyay, 1995).

The potential use of fungal antagonists as bio-control agent of plant diseases was suggested more than 50 years ago by Weindling (1932) who was the first to demonstrate the parasitic activity of *Trichoderma* spp. against *Rhizoctonia solani*.

Wright (1956) reported that seed treatment with spores of *Trichoderma viride, Penicillium nigricans, P. frequentans* and *P. goldiwski* controlled the disease symptoms caused by *Pythium* to some extent in case of white mustard.

In recent years, *Trichoderma* spp. have proved to be potential bio-agents for controlling several soil borne diseases under both greenhouse and field conditions (Elad *et al.,* 1981; Cook and Baker, 1983; Papavizas, 1988; Mukherjee *et al.,* 1989).

Sivan *et al.* (1984) reported that wheat bran plus peat mixture (1:1, v/v) was the most efficient of the raw plant material substrates for growing a new isolate of *Trichoderma harzianum* (T-315). The bran/peat preparation of *T* *harzianum*, applied to either soil or rooting mixture, effectively controlled damping-off induced by *Pythium aphanidermatum* in peas, cucumbers, tomatoes, peppers and gypsophila. Disease reduction up to 85 per cent was obtained in tomatoes. *Trichoderma harzianum* applied in a seed coating mixture containing $5x10^9$ conidia/ml was as effective in sandy soil as the broad cast application of wheat bran peat preparation.

Chang *et al.* (1986) observed an increase in dry weights of tomato, pepper and cucumber seedlings over control when *Trichoderma harzianum* was applied to soil at the rate of 10^5 conidia/g of soil.

Mukhopadhyay *et al.* (1986) studied the antagonistic activities of an isolate of *Trichoderma harzianum* rifai against tobacco isolate of *Pythium aphanidermatum in vitro. Trichoderma harzianum* directly attacked the hyphae of *P. aphanidermatum* causing lysis. Under glasshouse conditions, incorporation of inoculum of *T. harzianum* (prepared in wheat bran saw dust medium) in artificially infested soil significantly reduced damping-off.

Windham *et al.* (1986) reported that addition of *Trichoderma* spp. to autoclaved soil increased rate of emergence of tomato and tobacco seedlings over that of controls. Eight weeks after planting, root and shoot dry weights of tomato and tobacco were increased 213 to 275 and 259 to 318 per cent, respectively, over the controls.

Sharif *et al.* (1988) used *T. harzianum, Pencillium stipitatum* as bio-control agents and found that these antagonistic agents were equivalent to that of metalaxyl (Ridomil) in controlling *Pythium aphanidermatum* in cucumber.

Mukherjee *et al.* (1989) studied the mycoparasitism and bio-control potential of *T. harziamum* either alone or in conjunction with fungicidal seed treatment to control *Pythium* damping-off of cauliflower and reported that integration of fungicidal seed treatment with application of *T. harzianum* improved the degree of disease control than individual applications.

Krishnamoorthy and Bhaskaran (1990) conducted a pot culture experiment to find out the efficacy of antagonist in the control of damping-off disease of tomato caused by *Pythium indicum* Balakrishnan. Soil inoculation with *Trichoderma viride* Pers, *T. harzianum* rifai and *Laetisaria arvalis* gave good control of the pathogen and the treated pots recorded 78.2, 70.9 and 72.2 per cent seed germination, respectively, as against 19.3 per cent in the control. Seed treatment with above bio-agents was also found to be equally good. Improved seedling vigour was noticed in the treatments with antagonists than with fungicides and results obtained were comparable to soil drenching with fungicides like femaminosulf (0.060 g a.i. /lit) copper oxy chloride (1.125 g a.i. /lit) and seed treatment with captan or thiram (0.075 g a.i./lit).

Devaki *et al.* (1992) reported that *Trichoderma harzianum* rendered *Pythium aphanidermatum* and *P. myriotylum* non-viable in Petri dish dual culture. The *Pythium* mycelia from such cultures showed natural fluorescence in the regions of interactions, indicating their death. Non-volatile and volatile fungicidal activities were detected in *T. harzianum* culture. Lytic activity of β-(1,3) glucanase was detected on the cell walls of the *Pythium* spp. There was a significant decrease in the disease incidence when *T. harzianum* was incorporated into sterile soil, where as the effect was insignificant in natural soil.

Rajibkumar De and Mukhopadhyay (1994) reported that *Gliocladium virens* strongly antagonized *P.aphanidermatum*, incitant of tomato damping-off. Seed coating and soil amendment with *G.virens* separately resulted in 128.94, 162.38 and 107.89, 108.92 per cent increase in seedling stand over check in pre and post emergence phases, respectively, in the nursery.

Jayarajan and Ramakrishnan (1995) reported that the use of *Trichoderma viride* as bio-control agent is cheaper than fungicidal seed treatment in controlling damping-off. Application of the bio-control agent at 4g/kg seed had given cost benefit ratio of 1:400 in ginger, 1:20 in chickpea 1:360 in cotton and 1:50 in groundnut.

Lumsden (1995) reported that a strain of the fungus *Gliocladium virens*, GL-21 effectively suppressed damping-off diseases caused by two unrelated fungal pathogens, *Pythium ultimum* and *Rhizoctonia solani*.

Mehta *et al*. (1995) reported the potential use of *Trichoderma harzianum* as bio-control agent against various members of soil borne plant pathogens such as *Fusarium udum, Pythium aphanidermatum, R. solani and S. rolfsii*.

Dinakaran and Ramakrishnan (1996) conducted studies on the control of tomato damping-off with *Trichoderma viride* and reported that seed treatment with *T. viride* at 4g/kg seed significantly reduced disease incidence of *Pythium indicum* and increased seedling emergence of tomato.

Shanmugam and Varma (1998) reported that diversified group of microflora consisting of nine species of fungi belonging to four genera *Rhizopus, Aspergillus, Trichoderma* and *Eupencillium,* one actinomycete, *Streptomyces* sp. and four species of bacteria distinguished based on their colony characters, shape and gram reaction as B_1, B_2, B_3, and B_4, were obtained. Antagonistic properties of the isolated microorganisms against *Pythium aphanidermatum* incitant of ginger rhizome rot were determined by dual culture method. *A. niger* caused inhibition at distance and disintegration of pathogen while *A. fumigatus, A. flavus* and *T. viride* caused die-back and disintegration of pathogen. Of these, *T. viride* was found an efficient antagonist of *Pythium aphanidermatum*.

Sreeramulu *et al*. (1998) conducted an experiment on the efficiency of VA-mycorrhiza, *Glomus fasciculatum* and *Trichoderma harzianum* in controlling damping-off and black shank disease of tobacco seedlings under nursery conditions in comparison with the recommended fungicides viz., copper oxy chloride (blitox 50 WP) (0.2%) and metalaxyl (ridomil MZ 72 WP) (0.2%). Results showed that the dual inoculation of *G. fasciculatum* and *T. harzianum* was more effective in controlling both the diseases than the individual inoculation, and resulted in better germination count and improved the plant growth parameters.

Beena and Sarma (2000) tested combined activity of five species of *Trichoderma* and five isolates of fluorescent pseudomonads in various combinations on rhizome rot of ginger caused by *P. aphanidermatum.* The data obtained revealed that a reduced disease incidence of 0 to 35 per cent was obtained in all the treated plants when compared to 50 per cent in control.

Manoranjitham *et al.* (2000) reported that seed treatment with *T. viride* (4 g kg^{-1}) + *P. fluorescens* (5g kg^{-1}) showed 7.00 and 12.50 per cent pre and post-emergence damping-off, respectively, against 27.50 and 54.75 per cent in control. This treatment also increased the shoot length, root length and dry matter production of chilli seedlings and reduced the population of *P. aphanidermatum* from 16.75×10^2 cfu g^{-1} at the time of sowing to 13.41×10^2 cfu g^{-1} at 20 days after sowing compared to 17.50×10^2 cfu g^{-1} and 17.08×10^2 cfu g^{-1} in control.

Manoranjitham *et al.* (2001) reported that pre-sowing soil application of talc based formulation of *Trichoderma viride* and *Pseudomonas fluorescens* reduced tomato damping-off caused by *Pythium aphanidermatum* in nursery beds.

2.3.2 Chemical control

Once pathogens become established on crops especially perennials and reach damaging levels, chemical control is needed. Pests and pathogens may be killed by exposing them to toxic substances. Toxic chemicals lethal to all forms are used in Agriculture only as sterilants for eradication of pests and pathogens from soil. Chemical control has to be followed with each out break. It is however, very quick in action and still the method by which best results were obtained for mortality of disease out breaks (Hill and Waller, 1990).

Nagarajan and Reddy (1980) indicated that the promising fungicide for the control of both damping-off and leaf blight of tobacco was metalaxyl (Ridomil 25 WP) @ 0.1, 0.2 and 0.3 per cent concentrations. Next best were Bordeaux mixture (0.4%), difolatan (0.2%) and fytolan (0.2%).

In the *In vitro* studies, Jain *et al.* (1984) found that metalaxyl completely inhibited the growth of *Pythium aphanidermatum* at 100 ppm.

Reddy and Nagarajan (1985) reported that three sprays of metalaxyl at 0.05 per cent, 0.1 per cent and 0.2 per cent concentration was the most effective in giving very high percentage control of the disease. Seed treatment with apron 35SD or soil treatment with metalaxyl (ridomil 5G) each followed by 2 sprays of metalaxyl (Ridomil 25 WP) @ 0.1 per cent also afforded good protection to the seedlings against the damping-off disease of tobacco.

The pre and post emergence damping-off in cucumber could be effectively controlled through seed dressing with protective fungicides like captan at the rate of 5g/kg seed and soil drenching with a combination of metalaxyl + zineb at 10 to 20 days after sowing (Deshpande and Bochow, 1986).

According to Patel *et al.* (1987) soil drench with metalaxyl (1.0 kg/ha) was as effective as Bordeaux mixture (6:3:100) @ in controlling *Pythium aphanidermatum* the causal agent of damping-off disease in tobacco nursery.

In continuation, Patel *et al.* (1988) reported the efficacy of metalaxyl (700 g/ha) + ziram (1:6) when applied as soil drench at the rate of 5.6 l/ha in controlling tobacco damping-off as effectively as bordeaux mixture (0.6%) besides improving the number of transplantable tobacco seedlings.

The effectiveness of metalaxyl in selectively inhibiting tomato damping-off agent *P. aphanidermatum* when metalaxyl (Ridomil), vitavax (Carboxin), thiram and homai (thiophanate methyl + thiram) were used as seed treatments and soil drenches was proved by Taha *et al.* (1988)

Shenoi and Abdul Wajid (1992) suggested two alternative schedules of matalaxyl (Ridomil MZ 72 WP) for overall management of three diseases viz., damping-off, blight and black shank in tobacco nurseries, i.e., first, by per-sowing drench of fungicide to the soil @ 0.1 per cent concentration followed by a foliar spray @ 0.2 per cent concentration at 30 DAS and second by only foliar sprays commencing early at 20 DAS, to be followed at 35 DAS and at 50 DAS if required @ 0.2 per cent concentration.

Narayana Bhat and Srivastava (2003) reported that copper oxy chloride (Blitox 50 WP), mancozeb (Indofil M 45) and hexaconazole (Contaf 5 E) have completely inhibited the growth of *P. aphanidermatum* in *in vitro* at 500 and 1000 ppm concentration, where as mancozeb inhibited the growth the pathogen even at 250 ppm concentration.

2.3.3 Compatibility of *Trichoderma* spp with different fungicides.

Mukhopadhyay *et al.* (1986) tested the effect of metalaxyl on radial growth of *T. harzianum* at 0.1, 1, 10 and 100 ppm concentrations. There was no effect of metalaxyl on the growth and sporulation of *T. harzianum* up to 100 ppm concentration.

Sawant and Mukhopadhyay (1990) observed that the radial growth of *Trichoderma harzianum* was not inhibited by metalaxyl at concentration of 50 ppm while 19 and 30 per cent inhibition was observed at 500 and 1000 ppm , respectively.

Goutam Mondal *et al.* (1995) reported that *T. viride* was compatible with the formulations of neem products like antifeedant (hexane insoluble) and neem oil (hexane soluble).

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

Singh *et al.* (1995) screened isolates of *Trichoderma* spp. against fungicides like captafol, mancozeb and thiram. The growth of the isolates was not inhibited at 500 ppm concentration with captofol and mancozeb but thiram at 200 ppm concentration completely inhibited the growth.

Shanmugam and Varma (1998) reported that mancozeb (Indofil M-45) had comparatively low rate of inhibition of antagonists, when compared to bordeaux mixture, fytolan and femisan. The per cent inhibition was 4.4 in the case of *T. viride*, 7.8 in *A. niger,* 36.7 in *A. flavus* and 62.2 in *A. fumigatus.*

Ramarethinam *et al.* (2001) reported that the mancozeb (75% WP) and copper oxy chloride (88% W/W) at the concentrations of 100 ppm and 500 ppm did not inhibit the growth of *T. viride* to any statistically significant level; however, at a concentration of 1000 ppm, copper oxy chloride completely inhibited the growth of *T. viride*. Fungicides like carbendazim (50%WP), hexaconazole(5%EC), propiconazole (25%) and a weedicide metalachlor(50% EC) completely inhibited the growth of *T. viride* even at 100 ppm concentration in *in vitro*.

Sharma *et al.* (2001) tested the sensitivity of *Trichoderma harzianum* against eight fungicides. Among them two are systemic and six are non-systemic. Among systemic fungicides metalaxyl was seven times higher than carbendazim as 2578 µg ml^{-1} of concentration of metalaxyl and 354 µg ml of carbendazim inhibited 90% (ED_{90}) growth of the fungus in *in vitro*. Metalaxyl (0.1%) and carbendazim (0.0065%) seem safe tolerance limit (ED_{50}) for the bio-agent. Among six non-systemic fungicides capatafol and chlorothalonil even at 2317 µg ml^{-1} and 2037 µg ml^{-1} were tolerable (ED_{90}) by the fungus. The concentrations i.e. 169, 375 and 625 µg ml^{-1} of captain, captafol and chlorothalonil seemed to be a safe tolerance limit for the bio-control agent. Mancozeb and copper oxy chloride inhibited maximum growth (ED_{90}) at 785 and 805 µg ml^{-1} but ED_{50} value of these were 231 µg ml^{-1} and 356 µg ml^{-1} only. However thiram inhibited the growth the maximum (ED_{90}) at 145 µg ml^{-1} concentration only and 25 µg ml^{-1} seemed to be safe tolerance limit.

Umamaheswari *et al.* (2002) tested the the tolerance of 8 *Trichoderma* isolates to 6 fungicides. They reported that out of 8 isolates, isolate CIAH-225 and CIAH-240 could tolerate up to 500ppm of copper oxy chloride. Isolate CIAH-225, CIAH-151 and CIAH-149 were absolutely resistant at 250ppm of this fungicide. In presence of mancozeb, isolate CIAH-175 showed better colonization with intensive synthesis of secondary metabolites even at 1000ppm. This isolate was also resistant to metalaxyl + mancozeb (500ppm). Isolate CIAH-186 showed excellent growth and conidiogeneses at 1000ppm of wettable sulphur. This isolate was highly sensitive to thiophanate methyl. The sporulation of isolate CIAH-175 has been changed to yellow instead of green under the influence of metalaxyl +mancozeb and mancozeb.

Narayana Bhat and srivastava (2003) reported that mancozeb (Indofil M45) was fungistatic against *T.viride* at 500ppm. Where as copper oxy chloride (Blitox 50WP) inhibited the growth of *Trichoderma* spp. at 1000ppm and hexaconazole (Contaf 5E) is highly inhibitory to *Trichoderma* spp. even from 250ppm.

2.3.4 Soil solarization

The solar heating of soil is a soil disinfestation method for disease control. It aims to eradicate or reduce the inoculum existing in the soil prior to planting. Katan *et al.* (1976) developed a solar heating approach for soil disinfestation by mulching the soil with transparent polyethylene sheet in the hot season prior to planting

Various terms are used to describe this method: solar heating, soil solarization, plastic or polyethylene tarping and polyethylene or plastic mulching of soil. Since the method involved repeated daily heating at relatively mild temperatures, the term solar pasteurization was suggested by Katan (1980) was also justified.

Soil solarization was a non-chemical method for controlling soil borne plant diseases and weeds. It is based on soil heating using transparent polyethylene sheet for 4 weeks or more when temperatures are high (Katan, 1995).

The earliest experiments on solar heating by polyethylene mulching for the control of soil borne disease were carried out in Israel during 1973 and 1974 and the results were published by Katan *et al.* (1976).

In general, it is reported that saprophytic bacteria and actinomycetes survive much better than fungi in heated soils (Olsen and Baker, 1968, Bollen, 1974).

Besides reducing the soil borne pathogens, soil solarization also results in effective weed control lasting in some cases for a whole year or even longer (Katan *et al.,* 1976, 1980, 1983; Grinstein *et al.,* 1979b; Egley, 1983; Hartz *et al.*,1985).

Pullman *et al.* (1979) evaluated the effect of polyethylene mulching on soil populations of four plant pathogenic fungi, viz., *Verticillium dahliae, Pythium* spp. *Rhizoctonia solani* and *Thielaviopsis basicola.* They reported that *Rhizoctonia solani* was not detected in tarped soil in the upper 15 cm after two weeks. *V. dahliae* and *T. basicola* were eradicated to a depth of 46 cm after 4 weeks of mulching. *Pythium* spp were eliminated in the upper 30 cm after 4 weeks of tarping. Most of the soil samples were found free from *Pythium* spp.

In general, most of the annual and perennial weeds (*Amaranthus, Chenopodium, Cynodon, Digitaria, Eleusine* and *Phalaris*) were effectively controlled by soil solarization. Many weeds belong to the family graminae were found to be sensitive to soil solarization (Katan, 1981).

Rubin and Benjamin (1981) reported that nut grass (*Cyperus rotundus* L.) was partially controlled by soil solarization.

Stapleton and Devay (1982) reported the effect of soil solarization on populations of selected microorganisms. Population densities of *Agrobacterium* spp. fluorescent pseudomonads, gram-positive bacteria and fungi were greatly reduced in solarized soils. Actinomycetes and thermophilic fungi attained higher population densities following soil solarization.

Abdul Wajid *et al.* (1995) reported that mulched soil at 5 and 10 cm depths had 4.4 to 26.3 per cent higher temperature than bare soil. The temperature recorded at 5 cm depth was 54^{o}C. Bio-assay tests proved the efficacy of soil solarization against damping-off caused by *Pythium aphanidermatum* in tobacco. Besides improvement in germination of the seeds, the mean disease incidence in solarized soil was only 3.0 to 6.0 per cent 35 DAS as against 50.0 to 81.5 per cent in bare soil.

Harendar Raj *et al*. (1997) reported that soil solarization with transparent polythene mulch (25 µm) was effective to control the pathogens causing damping-off disease. Soil mulching with the polythene resulted in an increase of 13.5^{o}C temperature at eight cm soil depth with an average maximum temperature of 49.7^{o}C. Soil solarization for 40 days killed the *Pythium* spp. and *Fusarium* spp. upto 30 cm soil depth.

Sudha *et al*. (1998) reported that soil solarization with transparent polyethylene sheet for 30 days effectively controlled the weeds, increased the dry matter production of seedlings and C: B ratio in tobacco nursery.

Coelho *et al.* (1999) found that the soil temperature in soil solarized plots reached to a maximum of 47^{o}C at 10 cm depth compared to 41^{o}C at 25 cm. Soil solarization with a clear gas impermeable film was found to be effective in reducing populations of *Phytophora nicotianae* at 10 cm depth but had no effect on population of *P. capsici* at depth of 25 cm after solarization.

Reddy *et al*. (2001) conducted an experiment in Andhra Pradesh, India, during April-May 1997-99 to study the effect of soil solarization for the control of *Meloidogyne incognita* and *P.aphanidermatum* pathogen complex of tomato in the nursery. Soil solarization with clear transparent polythene sheet for 6 weeks during hot summer months showed increase in soil temperature to 48^{o} C and conservation of soil moisture to 4.5 per cent when compared to unsolarized control. Increased soil temperature coupled with soil moisture resulted in a significant reduction in population densities of *M.incognita* (85.8%), *P.aphanidermatum* (91.2%), weeds (91.2%) and increased dry weight of seedlings by 93.3 per cent. Though the physical and chemical characteristics of soil remained unchanged, the availability of soil nutrients increased by soil solarization.

Chaube *et al.* (2002) reported that the polyethylene mulching increased daily soil temperature by 10 to15^{o}C. There was little loss of the initial moisture content of the soil. Soil pH did not change significantly. However, the electrical conductivity of soil increased significantly. The increase over the un-mulched soil was about 300 per cent. Total nitrogen too increased. It was 0.247 per cent (Total Nitrogen) in solarized soil compared to 0.165 per cent in unmulched soil integrated management.

Kusum Mathur *et al*. (2002) conducted an experiment on ginger rhizome rot. In their experiment, solarization was done for 20 days in the month of June. The maximum temperature recorded in this period was 59^{o} C on the surface of mulched soil, which was 15^{o} C higher than that at the non-mulched surface.

Pandey and Pandey (2002) reported that with thin and transparent polythene, the seedling stand of tomato and chilli, and reduction in weeds was better than thick and black polythene. The temperature difference between atmosphere and nursery beds was recorded 6 to 10^{0}C under different moisture regimes. All the dicot weeds were eliminated up to 15 days after the removal of polythene while maximum reduction was noticed in *Echinocloa* spp. followed by *Cyperus rotundus*. The extent of damping-off reduction was 32.4 to 95.6 per cent over control treatment.

2.3.5 Organic amendments:

Organic amendments in general were reported to influence severity of soil borne diseases by increasing the biological buffering capacity of soil or by reducing pathogen numbers during anaerobic decomposition of organic matter and/or affecting nitrification which influences the form of nitrogen predominating in the soil (Huber and Watson, 1970).

Organic amendments were reported to be quite helpful in reducing crop diseases caused by soil borne pathogens (Stova, 1962; Latham and Watson, 1967; Wajidkhan *et al.,* 1974; Mehrotra, 1994).

Addition of organic matter is one of the effective methods of bio-control. There is considerable evidence that addition of amendments actually decreases the inoculum density of soil borne pathogens and consequently decreasing the diseases (Sivaprakasam, 1990).

Amendments in the soil in the form of crop residues, green manure, oil cakes, compost, farmyard manure etc., are known to decrease the propagules and also the inoculum density of pathogens leading to reduction of disease. Failure or success of amendments for disease management depends on several factors viz., stage of maturity of crop plant residues, degree of decomposition, nutrients, soil C:N ratio, biological properties at the time of application, type of amendments, quantity and quality of amendment. Further work and standardization under the field conditions is required to manipulate the biological balance in favour of antagonist and to the detriment of plant pathogen (Sivaprakasam, 1990).

Krishnamoorthy and Bhaskaran (1991) studied the effect of organic amendments on the biological control of damping-off disease in tomato and concluded that maximum seedling emergence and concomitant reduction in *Pythium* population was obtained in neem cake amended pots.

Madduleti and Moses (1995) have made attempts to manage damping off of tomato, chilli and brinjal with eucalyptus leaf, neem cake and neem leaf soil amendments and three neem based products viz., gujneem, repelin and nimibicidin in soil inoculated with *Pythium aphanidermatum* and *P. debaryanum* separately, and in mixture in sterile soil and in nursery. Partially decomposed eucalyptus leaf was found superior to neem cake and neem leaf in reducing both pre and post-emergence damping-off of all the three crops. Neem cake and neem leaf were more effective when used after decomposition than un-decomposition.

Narayanaswamy *et al.* (1998) reported that minimum mortality of seedlings was observed in tobacco plots in which damping-off disease incited by *P. aphanidermatum* is in severe conditions where neem cake @ 1 kg/m^2 was applied.

2.3.6 Integrated disease management

Integrated control is a flexible, multidimentional approach to disease control, utilizing a range of control components such as biological, cultural and chemical strategies needed to hold diseases below economic thresholds without damaging the agro-ecosystem (Papavizas and Lewis, 1988). Integrated disease management has been reported to be quite effective for control of soil-borne plant pathogens (Upadhyay and Rai, 1989).

Papavizas *et al.* (1982) and Abdel moity *et al.* (1982) have shown that new biotypes of *T. harzianum* can be selected for tolerance or resistance to fungicide, such as chlorothalonil, iprodione and benomyl. Particularly significant was the observation that fungicide resistant biotypes were commonly superior to the parents as bio-control agents.

Kraft and Papavizas (1983) recorded a maximum yield of peas under field conditions when the seeds of the cultivar (Dark skin perfection) susceptible to *Pythium ultimum* were treated with metalaxy and *T. harzianum.*

The positive effect of integrated control of *Pythium* was observed by integrated use of fungicide and bio-control agent. Application of *T. harzianum* preparation combined with the fungicide prothiocarb to a rooting mixture infested with *P. aphanidermatum* resulted in a synergistic effect, reducing disease incidence in gypsophila cuttings better than either treatment alone (Sivan *et al.,* 1984). These results were similar to those obtained with cucumber grown in *Pythium* infested soil. Integration of Dexon with the seed coating of *T. harzianum* reduced the percentage of diseased plants from 53 to 9, which was superior to either treatment alone (Elad *et al.,* 1983).

Al-Sammaria *et al.* (1986) reported that integration of soil solarization with the use of ridomil and rizole reduced the population levels of *Meloidogyne* spp., *Rhozoctonia solani* and *Pythium aphanidermatum* and increased root and shoot growth of cucumber.

Mukhopadhyay *et al.* (1986) reported that use of metalaxyl (3% slurry) treated seeds coupled with application of *T. harzianum* (50g/kg soil) inoculum gave an excellent control of the damping-off disease in tobacco.

Krishnamoorthy (1987) reported the higher germination percentage of tomato seeds in the treatment comprising the soil amendment with neem cake (25t/ha) + seed treatment with *Trichoderma viride.* He also reported that seed treatment resulted in higher population build up of *T. viride* in soil. The reduction in damping-off of tomato might be due to the reduction in the population of *Pythium aphanidermatum.*

Sawant and Mukhopadhyay (1990) reported that combined use of *T. harzianum* and metalaxyl (0.1%) resulted in reduction of damping-off incidence by 84 to 100 per cent in sugarbeet.

Narendrappa *et al.* (1992) recommended an effective management of seed bed diseases, caused by *Pythium aphanidermatum* and *Phytophthora parasitica* var. *nicotianae* in tobacco using combined applications of organic amendments and reduced rates of fungicides. Application of press mud to the beds 2 weeks before sowing and copper oxy chloride (0.2%) one day before sowing gave the highest count of healthy seedlings.

Shenoi *et al.* (1993) reported that application of neem cake 400g/m^2 + solarization for six weeks + metalaxyl MZ (Ridomil MZ) + foliar sprays of 0.2% at 25 and 40 DAS has significantly reduced the damping-off disease of tobacco even at 40 DAS (99 per cent). Where as at 25 and 30 DAS 100 per cent reduction was recorded.

The antagonistic effect of *Gliocladium virens* on *Pythium aphnidermatum*, the incitant of tomato damping-off and the additive effect derived when the antagonist was applied in combination with seed dressing fungicides like apron, captaf and thiram over the individual applications in controlling the disease was reported by De and Mukhopadhyay (1994).

Mani and Marimuthu (1994) reported that damping-off of chillies caused by *P. aphanidermatum* could be effectively reduced when decomposed coir pith was used in combination with *T. harzianum*.

Abdul wajid *et al.* (1995) proposed the best schedule for integrated disease management in FCV tobacco nurseries. It was solarization for six weeks from February to March of the soil amended with neem cake @ 400 g/m^2, foliar spray of metalaxy (Ridomil MZ) @ 0.2% at 30 days after sowing which gave 81.3 to 95.5 per cent control of disease and yielded maximum healthy transplants.

Patel *et al.* (1996) reported that an integrated schedule of covering soil with clear plastic sheet (100 µm) for two months during hot summer and use of metalaxyl MZ @ 2.16 kg/ha twice yielded the maximum number of transplantable seedlings in bidi tobacco.

Soil application of neem cake (900g/28x6 m^2) and potential bio-control agent, *Trichoderma harzianum* was found to most effective in managing pre- and post-emergence damping-off of tomato caused by *Pythium aphanidermatum* and improving the seedling vigour (Vijaya krishna kumar, 1997).

Patel and Patel (1998) reported that soil solarization using clear low density polyethylene plastic (25 µm) for 15 days during summer followed by two drenchings of metalaxyl (MZ) (2.16 kg/ha) and one drenching of chloropyriphos (0.04%) and dazomet (30g/m^2) was effective in controlling damping-off incidence, root-knot disease and weeds upto 59 days after seedling with increased number of transplants in bidi tobacco nursery.

The incidence of damping-off of chilli (*P. aphanidermatum*) was significantly reduced when the seeds were treated with *T. viride* and *T.*

harzianum and soil was amended with FYM or neem cake @ 20 t/ha 15 days before sowing (Kartikeyan *et al.,* 1999).

Anitha and Tripathi (2000) conducted experiments to evaluate an integrated disease management strategy for controlling the seedling diseases of okra caused by *Rhizoctonia solani* and *Pythium aphanidermatum* by using a combination of varietal resistance, chemical control and biological control. Seed treatment with carbendazim (2g/kg seed) followed by soil application of *Trichoderma viride* (24 g/kg soil) was proved effective as disease incidence recorded were 5 and 18.33 per cent, respectively, in Varsha Upahar and Pusa Sawani compared with 45 and 62 per cent incidence observed in control.

Karthikeyan *et al.* (2001) reported that the damping-off incidence of aubergine cv.CO 2 was reduced when the seeds were treated with antagonistic organisms and soil was amended with the organic amendments. The dry weight of shoot and root of okra were higher in *T. viride* + farmyard manure treatment, followed by *T. viride* + neem cake treatment.

Chaube and Jyotsna Sharma (2002) reported that integration of solarization with bio-control agents (*Trichoderma harzianum*, *T.viride* and *Pseudomonas fluorescens*) increased soil suppressiveness and improved plant growth significantly. Solarization alone reduced damping-off of cabbage, brinjal and tomato by over 47 per cent but after integration, the disease suppression increased upto 76 per cent. *T. harzianum* + *Pseudomonas* *fluorescens* or *T. viride* + *P. fluorescens* applied before solarization was less effective but their introduction after mulching, was highly effective. Population estimation of microflora in rhizosphere at different plant growth stages revealed that antagonists introduced after solarization were pioneer colonizers of roots.

Deepa and Chaube (2002) reported that soil solarization reduced damping-off of cauliflower by over 40.1 per cent. Integration with chemicals and bio-agent as seed treatment with apron, *T. harzianum* and *Pseudomonas fluorescens* was distinctly superior.

Narayanaswamy *et al.* (2002) reported that integration of soil solarization for 4 weeks along with incorporation of neem cake @400g/m^2 before sowing in addition to the need based spray of ridomil MZ 72 WP @0.2 per cent at 30 DAS has effectively controlled the damping-off disease (up to 82%) in tobacco. This schedule has also significantly increased the number of healthy transplants (304/m^2). Apart from this, there was significant increase of seedlings emergence and vigour index besides reduction in weeds. The economics worked out for this integrated schedule revealed the ICBR of 1:3.84.

Lakra (2003) reported that pre-emergence damping-off of potato was 35.9, 27.1 and 4.8 per cent when seeds were treated with *B. subtilis, T. viride* and captan @ 4g/kg, respectively, as against more than 40 per cent in control.

Incidence of post-emergence damping-off was minimum (5.5%) when seed and soil were treated with captan (4g/kg seed and 5g/m^2 respectively) followed by seed + soil treatment with *T. viride* @ 4g/kg seed and 5g/m^2 (13.5%) and seed and soil treatment with *B. subtilis* @ 4g/kg seed and 5g/m^2 (22.2%). The post emergence damping-off in control was 42.6 per cent.

CHAPTER-III

MATERIALS AND METHODS

3.1 EXPERIMENTAL SITE:

The laboratory and field experiment studies were conducted in the All India Co-ordinated Research Project on Tropical Fruits (Citrus) S.V. Agricultural College Campus, Tirupati, Chittor (Dt), Andhra Pradesh. This site is located at 13^{o} North latitude and 79^{o} East longitude and at an altitude of 182.9 m in tropical belt of South India.

3.2 CLIMATE

According to agroclimatic conditions of Andhra Pradesh, Tirupati area comes under semi-arid Tropical Southern zone. This zone receives rainfall in two spell, viz., South-West (June-September) and North-East (October-January) monsoon periods. The temperatures of this zone were recorded as more than 40^{o} C (hot summer).

3.3 GLASSWARE:

Petri plates (Borosil, Corning), Borosil flasks (250, 500 and 1000µl capacity), test tubes, microscopic slides and pipettes (1,5 and 10 ml) were used in the research programme.

3.3.1 Cleaning of glassware:

The glassware was thoroughly washed with soap solution and tap water before placing them in cleaning solution of the following composition.

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

After 24 h the glassware was removed from cleaning solution and washed thoroughly with tap water and then rinsed with distilled water before use.

3.4 CHEMICALS AND EQUIPMENTS:

Chemicals used were of ‘Analytical Grade’ (B.D.H. Analar; E. Merck of foreign make). Weighments were done on a single pan electronic balance with a sensitivity of 0.01 g. Soil thermometer was used to record soil temperatures during soil solarization period.

3.5 Media Used:

The composition of different media used in research programme is given below.

a) Potato dextrose agar medium (PDA)

Peeled potato slices	-	200 g
Dextrose	-	20 g

Agar	-	20g
Distilled water	-	1000 ml

b) Corn meal agar medium (CMA)

Corn meal	-	30 g
Agar	-	20 g
Distilled water	-	1000 ml

c) Rose Bengal agar medium (Martin, 1950):

Glucose	-	3.0 g
Peptone	-	5 g
K_2HPO_4	-	1 g
Mg SO_4	-	0.3 g
Rose Bengal	-	0.03 g
Agar	-	20 g
Distilled water	-	1lit
pH (6.0)		

d) *Trichoderma* Selective medium (Elad and Chet, 1983):

Mg SO_4	-	0.2 g
K_2HPO_4	-	0.9 g
KCl	-	0.15 g
NH_4 NO_3	-	1.0 g

Glucose	-	3.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
Rose Bengal	-	0.15 g
Chloramphenicol	-	0.25 g
Metalaxyl	-	0.3 g
pH (6.5)		

e) *Pseudomonas fluorescens selective medium (* King *et al.*, 1954)

Peptone	-	20 g
K_2HPO_4	-	2.5 g
Glycerol	-	15 ml
$MgSO_4$	-	6 g
Agar	-	15 g
Cycloheximide	-	75 mg
Ampicillin	-	50 mg
Chloromphenicol	-	12.5 mg
Distilled water	-	1000 ml

f) *Bacillus subtilis* medium (Mundt and Hinkle, 1976).

Yeast extract	-	3.0 g
Glucose	-	5.0 g
Cycloheximide	-	2.0 g
Agar	-	17.0 g
Distilled water	-	1000 ml
pH (7.0)		

g) Mass multiplication of pathogen (*P. aphanidermatum)*

P. aphanidermatum was mass multiplied on sand-maize medium (Krishnamoorthy and Bhaskaran, 1990) consisting of, Sand -90 g, Maize meal -10 g, Water-20 ml. In a 250 ml Conical flask 100g of this substrate was taken and water was added and autoclaved at 15 lbs psi for 1 h. Then 5-7 mm discs of five day old culture of *P. aphanidermatum* was added and incubated at room temperature.

h) Mass multiplication of *Trichoderma* spp.

The substrate used for mass multiplication of the *Trichoderma* was wheat bran. The substrate was powdered, sieved through 60 mesh, added with appropriate water and autoclaved at 15 lbs psi for 1 h on two successive days. Then 2 to 3 discs of seven day old culture of *Trichoderma* were added to a bag containing 50 g of substrate and incubated at room temperature.

3.6 COLLECTION OF INFECTED PLANTS AND SOIL SAMPLES

The infected plants were collected from the tobacco growing area near Kalikiri of Chittoor district. The plants showing damping-off symptoms were carefully uprooted and preserved in the polythene bags. The soil samples were collected from rhizosphere of tobacco seedlings in different fields to isolate the rhizosphere fungi and bacteria.

3.7 ISOLATION OF *Pythium aphanidermatum* FROM INFECTED PLANTS

The fungus was isolated from the collar region of the infected plants on PDA medium (Rajib Kumar De and Mukhopadhyay, 1994) and CMA medium after surface sterilization of infected plants with 0.1% $Hgcl_2$. The fungus was identified as *Pythium aphanidermatum* based on cultural and morphological characters.

3.8 ISOLATION OF SOIL MICROFLORA (FUNGI AND BACTERIA)

The soil microflora was isolated by serial dilution pour plate technique (Johnson and Curl, 1972) using selective media. Fungi were isolated on Rose Bengal agar medium (Martin, 1950) and bacteria on specific media i.e. for *Pesudomonas fluorescens* selective medium is Kings B medium and for *Bacillus subtilis* Mundt and Hinkle medium. The dilution used for fungi is 10^{-4} and for bacteria 10^{-6}.

3.9 IDENTIFICATION OF POTENTIAL BIO-AGENT *in vitro*

The antagonistic activity of the fungal and bacterial bio-agents against the test pathogen *P. aphanidermatum* was tested by dual culture technique (Dennis and Webster, 1971; Morton and Stroube, 1955). In this technique each bio-agent and the test pathogen were grown on the same plate to test the antagonistic activity. Fungal disc of the antagonist of size 7 mm was placed at one end of the media in the Petri plate and on the opposite side 7 mm disc of the test pathogen was kept in the same plate. The plates were incubated at room temperature for 4 days and observed for inhibition zone. The percentage of inhibition was calculated by adopting the following formula.

$$\%\ \text{Inhibition of}\ \textit{P. aphanidermatum} = \frac{\text{Growth}\ \textit{P. aphanidermatum}\ \text{in control plate} - \text{Growth of}\ \textit{P. aphanidermatum}\ \text{in the presence of bio-agent}}{\text{Growth of}\ \textit{P. aphanidermatum}\ \text{in control plate}} \times 100$$

3.10 IDENTIFICATION OF EFFECTIVE FUNGICIDE ON *P. aphanidermatum in vitro*

The effect of fungicides viz., mancozeb (75% WP), copper oxy chloride (50% WP), metalaxyl MZ (72% WP), hexaconozole (5% EC) and chlorothalonil (75% WP) at concentrations of 50, 100, 250, 500 and 1000 ppm were evaluated for their efficacy on *P. aphanidermatum* by using poisoned food technique (Nene and Thapliyal, 1993).

In this technique first PDA medium was prepared double the recommended strength and sterilized in autoclave. A double strength PDA medium contains double the concentration of potato, dextrose and agar except water. For each fungicide, fungicidal solution was prepared double the test concentration. Ten ml of fungicidal solution was mixed thoroughly with 10ml molten PDA medium and poured into a sterilized Petri plate under aseptic conditions and allowed for 5-10 minutes to solidify. Appropriate controls were maintained using distilled water. From seven day old culture plates 7 mm discs were cut from outer margin of vigorously growing fungus with sterilized cork borer and transferred to the center of the plates containing fungicidal medium. Controls were maintained by placing fungal discs in untreated plates. Three replications were maintained for each treatment. The Petri plates were then incubated in an incubator at 26 ± 1^{o} C. The diameter of fungal colony was measured when the growth of the fungus in control completely filled the Petri plates. The colony diameter in treated plates, compared with control, was taken as measure of fungitoxicity. The per cent inhibition was calculated by the following formula.

$$\text{\% Inhibition of } \textit{P. aphanidermatum} = \frac{\text{Diameter of colony in control plate} - \text{Diameter of colony in treated plate}}{\text{Diameter of colony in control plate}} \times 100$$

3.11 COMPATIBILITY OF EFFECTIVE BIO-AGENT WITH DIFFERENT FUNGICIDES *in vitro*

To test the compatibility of fungicides like mancozeb, copper oxy chloride, metalaxyl MZ, hexaconazole and chlorothalonil at 50, 100, 250, 500 and 1000 ppm concentrations with bio-agent, poisoned food technique with solid medium was performed as described in identification of effective fungicide against pathogen *in vitro*.

3.12 FIELD EXPERIMENT

The field experiment was conducted at AICRP on Tropical Fruits (Citrus), Tirupati. The experimental site was ploughed thoroughly and plots of 0.5x0.5 m^2 were prepared before imposing treatments.

3.12.1 Experimental layout

The following treatments were imposed in a randomized block design (RBD). Three replications for each treatment were maintained.

Treatments:

T_1 - Soil solarization

T_2 - Seed treatment with potent native bio-agent

T_3 - T_2 + Soil application of potent native bio-agent

T_4 - Seed treatment with effective fungicide

T_5 - T_4 + Soil drenching with effective fungicide

T_6 - Neem cake (1kg /m^2)

T_7	-	$T_1 + T_2$
T_8	-	$T_1 + T_3$
T_9	-	$T_1 + T_4$
T_{10}	-	$T_1 + T_5$
T_{11}	-	$T_1 + T_6$
T_{12}	-	$T_1 + T_3 + T_6$
T_{13}	-	$T_1 + T_5 + T_6$
T_{14}	-	$T_1 + T_3 + T_5 + T_6$
T_{15}	-	Uninoculated control
T_{16}	-	Inoculated control

3.12.2 Soil solarization

Soil solarization was done by following the procedure given by Katan *et al* (1976). Clear polythene sheet of 400 guage was used for solarization.

3.12.3 Method of soil solarization

First the land was thoroughly ploughed and then leveled to minimize protrusions to prevent tearing of polythene sheeting. Plot was irrigated one day prior to laying of polythene sheet. The four edges of polythene sheet were inserted in the furrows and burried. After burying, the soil around the edges was compacted so as to prevent escape of heated air and soil moisture (Plate 1).

3.12.4 Soil temperature

Soil temperature was recorded daily at 14.00 h at a depth of 10 cm by inserting soil thermometer in solarized and unsolarized plots. Thermometer was then removed and the holes formed on the polythene sheet were sealed with cellophane tape immediately (Plate 2).

3.12.5 Soil moisture

The amount of soil moisture was measured gravimetrically from the soil samples collected at 10 cm depth comparing the soil weights before and after drying at 100^{o} C for 72 h until two consecutive weights were same. Pre-solarization sampling was done 24 h after irrigation followed by the post – solarization sampling soon after removing the polythene sheet. The moisture per cent in the soil sample was calculated using the following formula.

$$\text{Moisture per cent} = \frac{\text{Wet weight of the soil} - \text{Dry weight of the soil}}{\text{Dry weight of the soil}} \times 100$$

3.12.6 Soil mycoflora

Soil mycoflora was isolated from both solarized and unsolarized soil samples by using serial dilution technique.

3.12.7 Weed growth

Pertaining to weed growth the following data was recorded from both solarized and unsolarized plots.

1) Number of weeds

2) Fresh weight of weeds per plot (g)

3) Dry weight of weeds per plot (g)

3.12.8 Termination of polythene sheet mulching

Six weeks after the commencement of solarization experiment, the covered polythene sheets were removed from beds. The weeds which remained in the area during this period were identified and their counts were taken from individual beds. Soil samples were collected separately from solarized and un-solarized beds for estimation of soil mycoflora and moisture content.

3.12.9 Preparation of field after solarization

The field was prepared after removing the polythene sheet. The weeds were cleared off from all the beds. Then the beds were set neatly.

3.12.10 Soil application of *Pythium aphanidermatum*

The pathogen was applied to the soil @ 1kg / m^2 plot which was mass multiplied on sand maize medium. This pathogen was applied to the solarized beds 1 week before sowing except in un-inoculated treatment.

3.12.11 Soil application of *Trichoderma* spp.

The effective bio-agent *Trichoderma* spp. (TT_bT – 1) multiplied on wheat bran was applied to soil @ 100g/m^2.

3.12.12 Soil drenching with effective fungicide

The effective fungicide, metalaxyl MZ (72% WP) was used to drench the soil @ 3g/litre/m^2.

3.12.13 Soil application of neem cake

The soil was amended with neem cake @ 1 kg/m^2 one week before sowing.

In the integrated management, soil drenching with fungicide was performed after application of pathogen, bio-agent and neem cake. After application of all these in the field, it was left fallow for one week and the beds were irrigated frequently. Then sowing was done with the seeds of tobacco variety, Izmir @ 200 mg/m^2.

3.12.14 Seed treatment with effective bio-agent and fungicide

Seed treatment with bio-agent *Trichoderma* spp. was done @ 4g/kg seed and with fungicide, metalaxyl MZ @ 3g/kg seed. In both cases dry seed treatment was followed.

3.12.15 Termination of the field experiment

The plants were grown for a period of 40 days. The various observations recorded during the period of investigation were:

1) Germination percentage was calculated using the following formula.

$$\text{Per cent germination} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds sown}} \times 100$$

2) Plant parameters like fresh weight and dry weight of shoots and roots; length of shoots and roots were taken at 10, 20, 30 and 40 DAS.

3) Disease incidence at different intervals i.e., 10, 20, 30, and 40 DAS was also recorded. Per cent disease incidence (PDI) was calculated by using the following formula.

$$PDI = \frac{\text{Seedling stand in un-inoculated soil} - \text{Seedling stand in treatment}}{\text{Seedling stand in un-inoculated soil}} \times 100$$

4) Disease growth rate was also recorded (y = a+bx).

FIELD LAYOUT

R_1		R_2		R_3	
T_1	T_{10}	T_{16}	T_{11}	T_7	T_1
T_7	T_{13}	T_{10}	T_9	T_2	T_8
T_2	T_{11}	T_{14}	T_2	T_9	T_3
T_8	T_{16}	T_{15}	T_8	T_{11}	T_{12}
T_3	T_{12}	T_{13}	T_3	T_4	T_5
T_9	T_{15}	T_{12}	T_7	T_{10}	T_{14}
T_4	T_6	T_5	T_4	T_6	T_{16}
T_5	T_{14}	T_6	T_1	T_{15}	T_{13}

Plot size : 0.5 x 0.5 m^2

Space between plots : 30 cm

No. of replications : 3

Fig-2: *In vitro* efficacy of fungicides against *Pythium aphanidermatum*

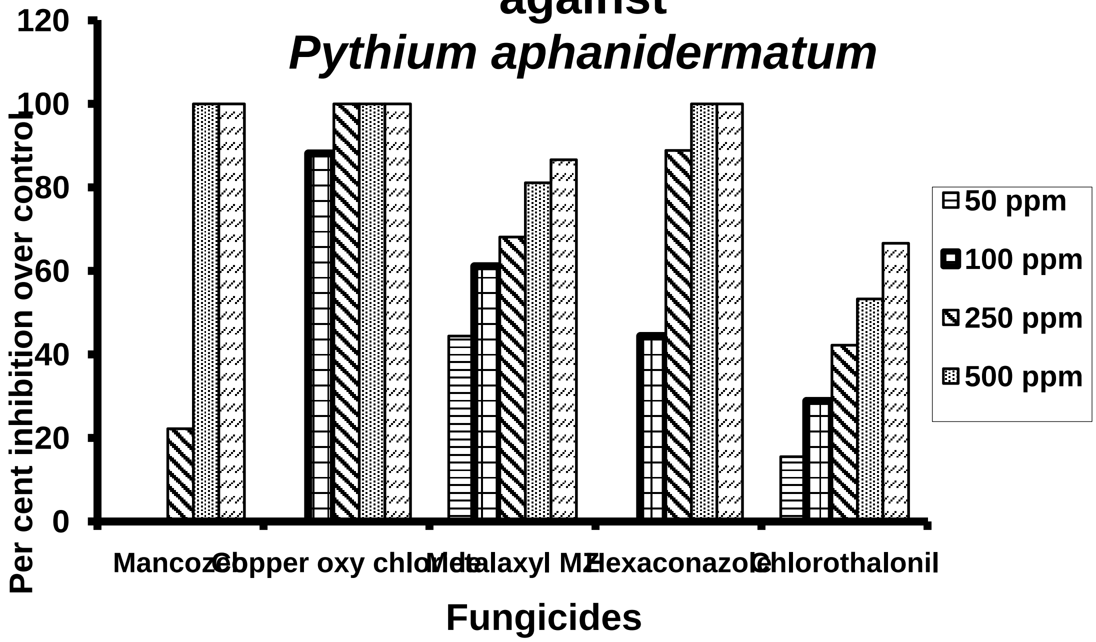


Fig-1: ***In vitro*** **efficacy of soil microflora against *Pythium aphanidermatum***

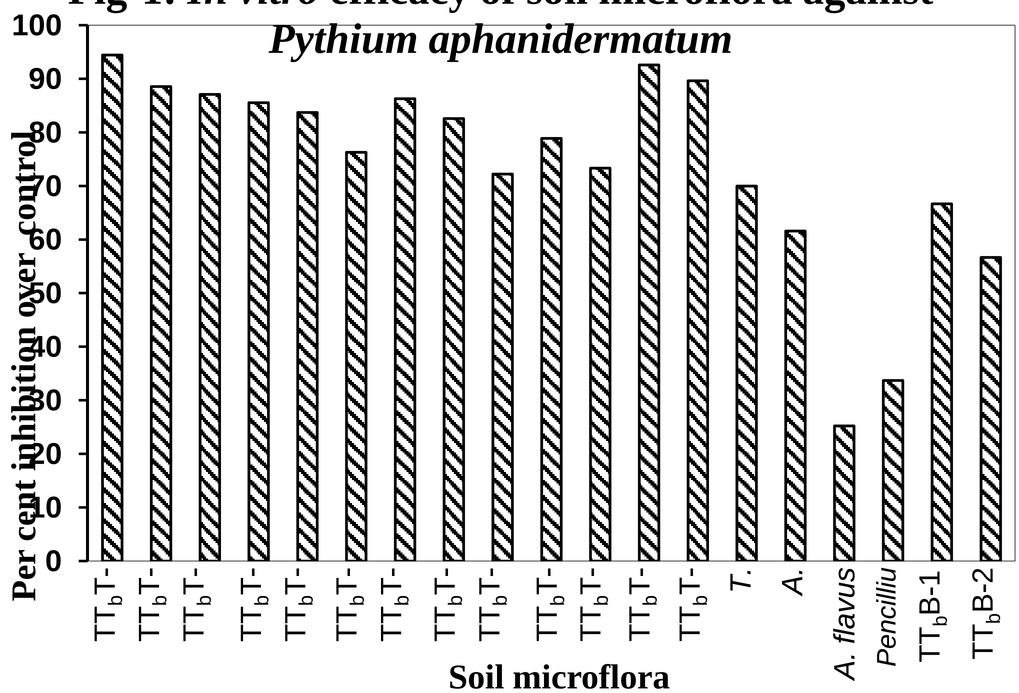


Fig-3: Compatibility of *Trichoderma* spp. (TT_bT-1) with different fungicides

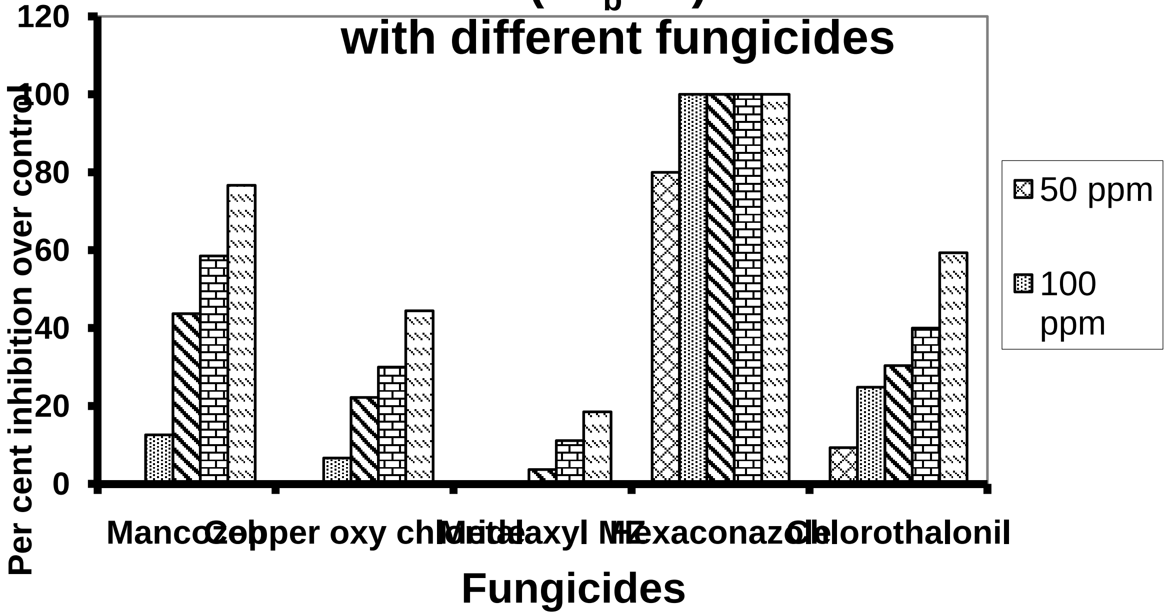


Fig-4: Effect of soil solarization on soil temperature

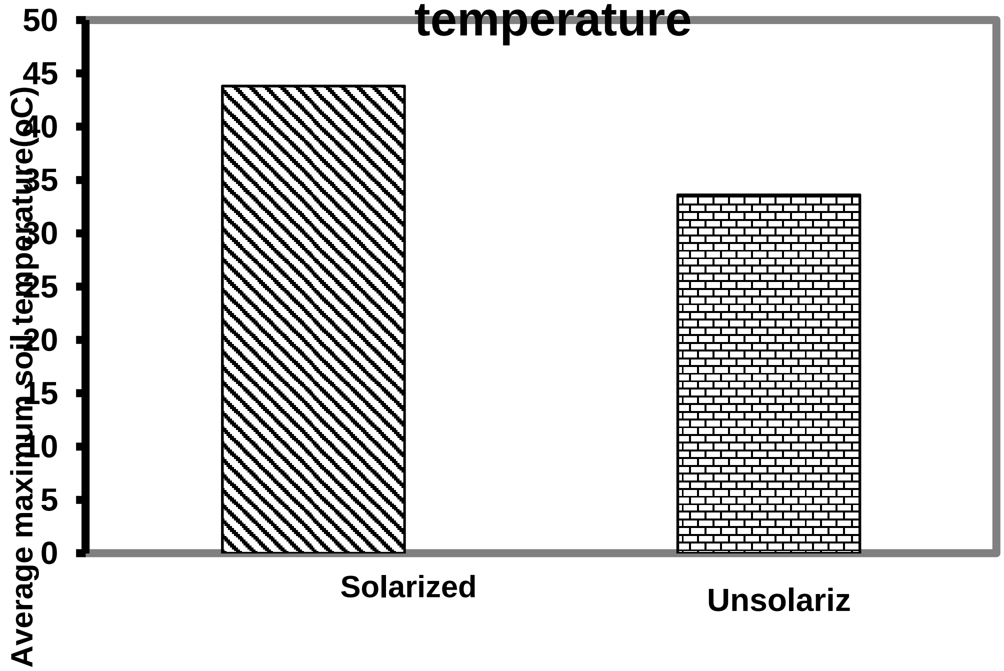


Fig-5: Effect of soil solarization on soil moisture content at 10 cm depth

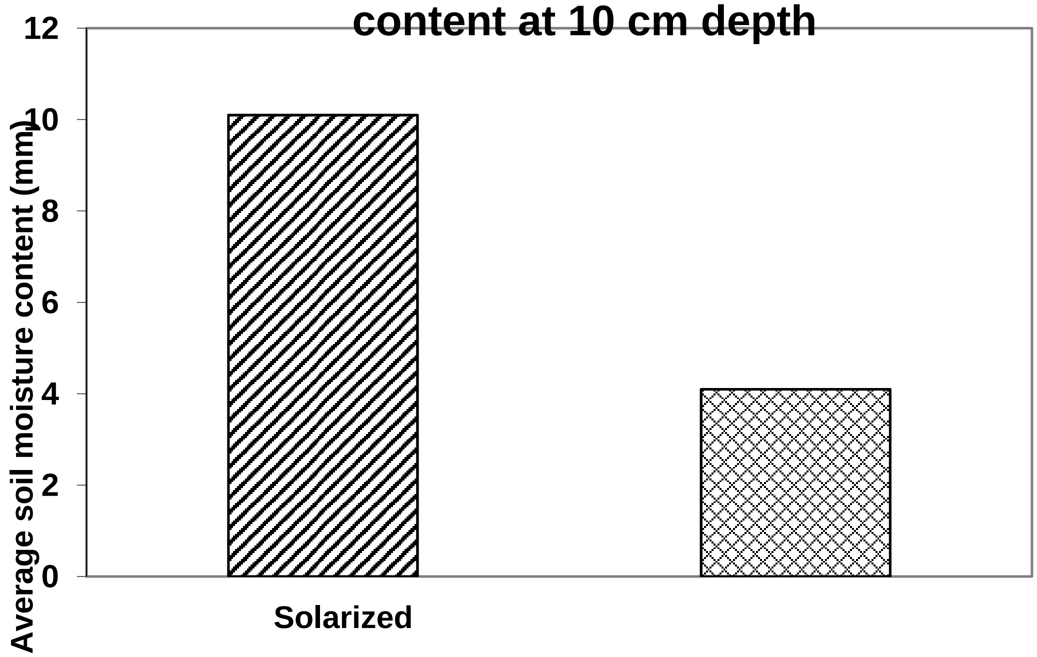


Fig-6: Effect of soil solarization on weed count and their fresh weight and dry weight

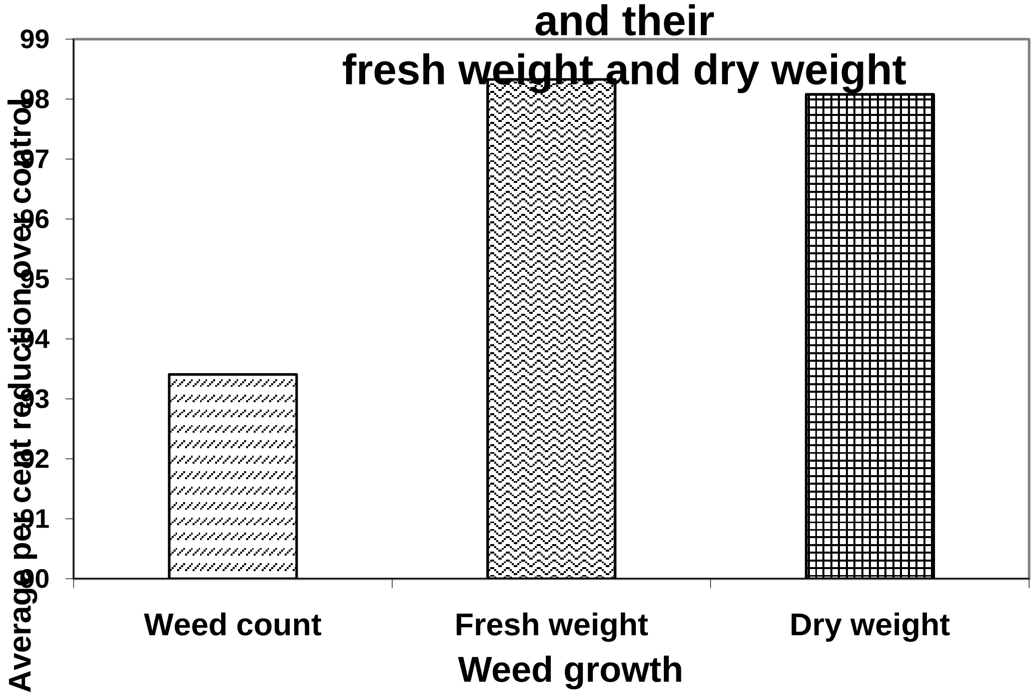


Fig-7: Effect of different treatments on germination percentage of Tobacco seeds

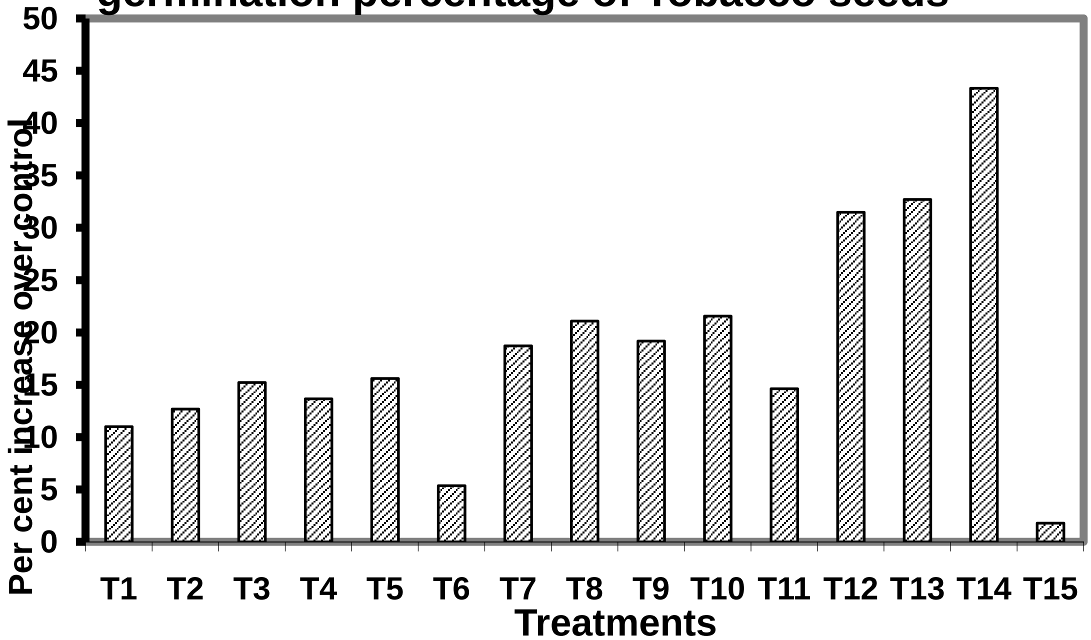


Fig: 8 Effect of different treatments on per cent disease incidence (damping-off)of tobacco seedlings at 40 DAS

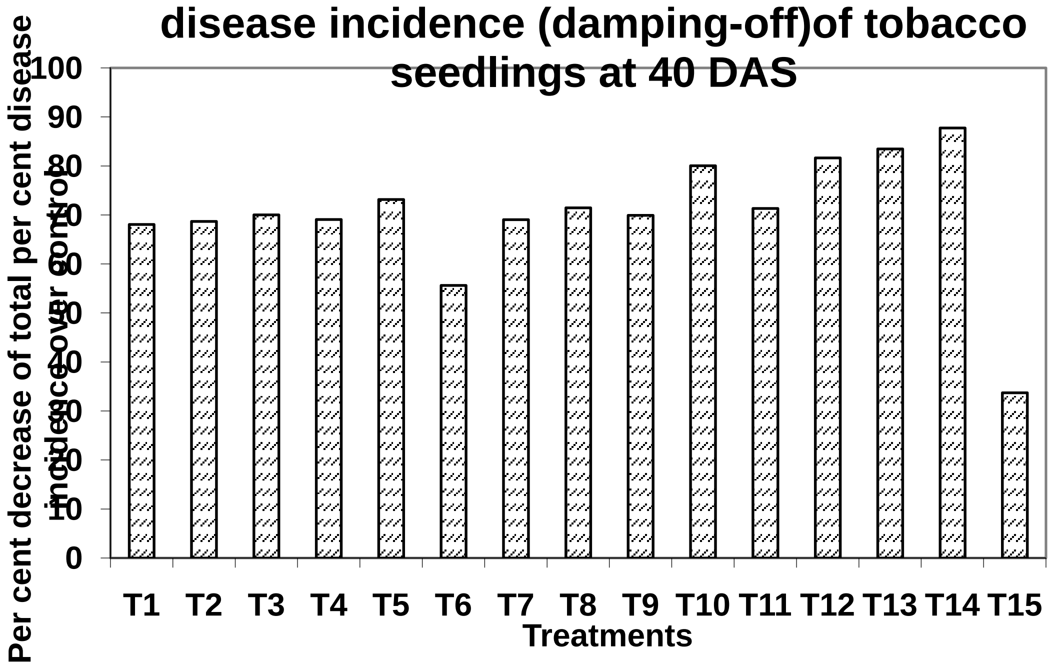


Fig-10: Effect of different treatments on plant height of tobacco seedlings at 10, 20, 30 and 40 DAS

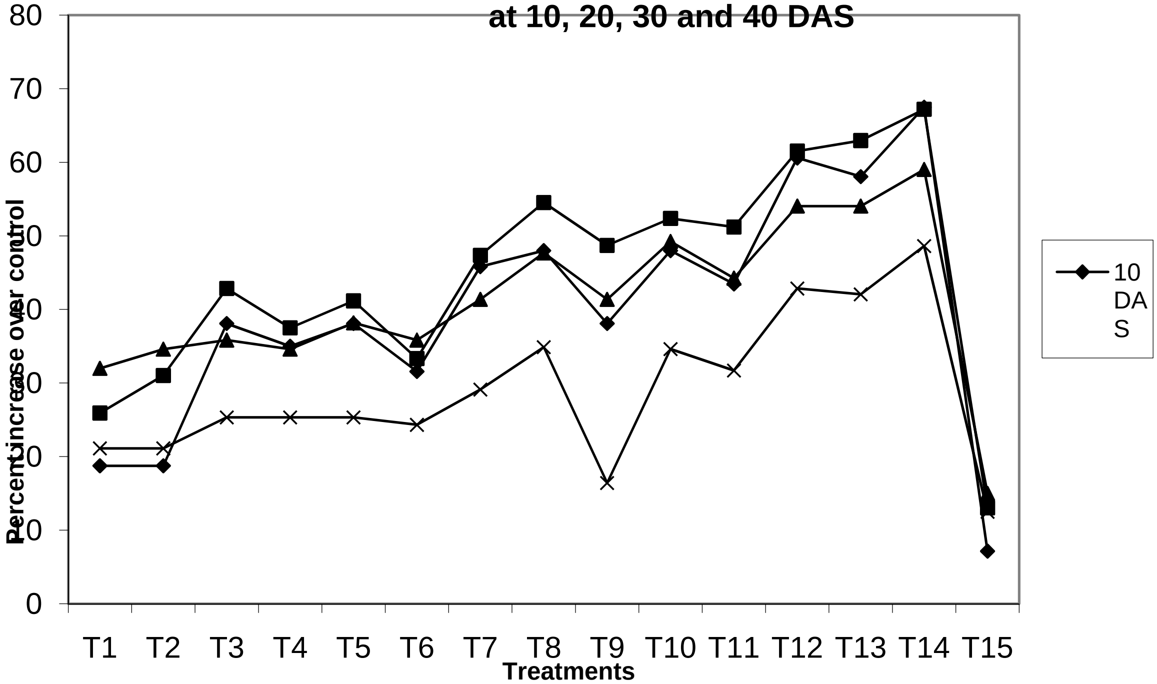


Fig-9: Effect of different treatments on per cent disease incidence (damping-off) of tobacco seedlings at different days after sowing

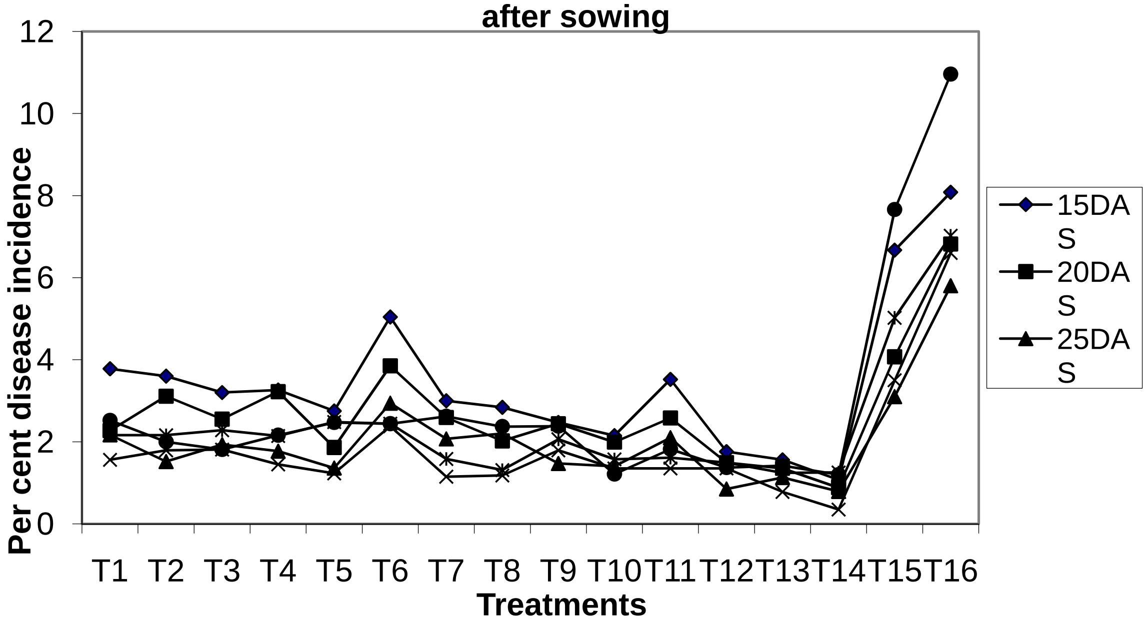
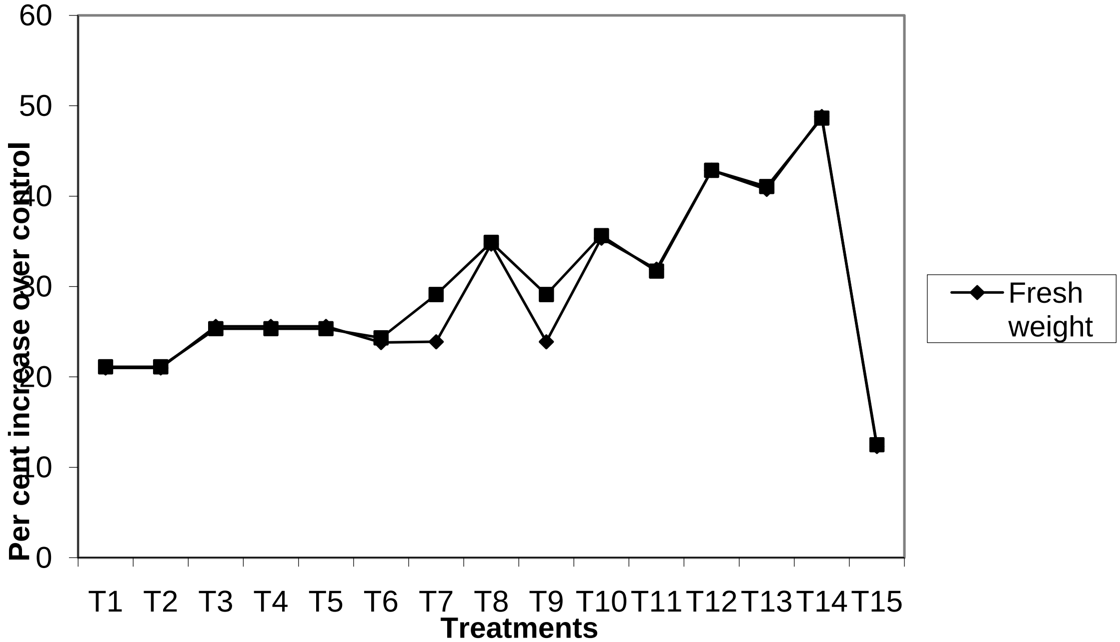


Fig-11: Influence of different treatments on fresh weight and dry weight of tobacco seedlings at 40 DAS



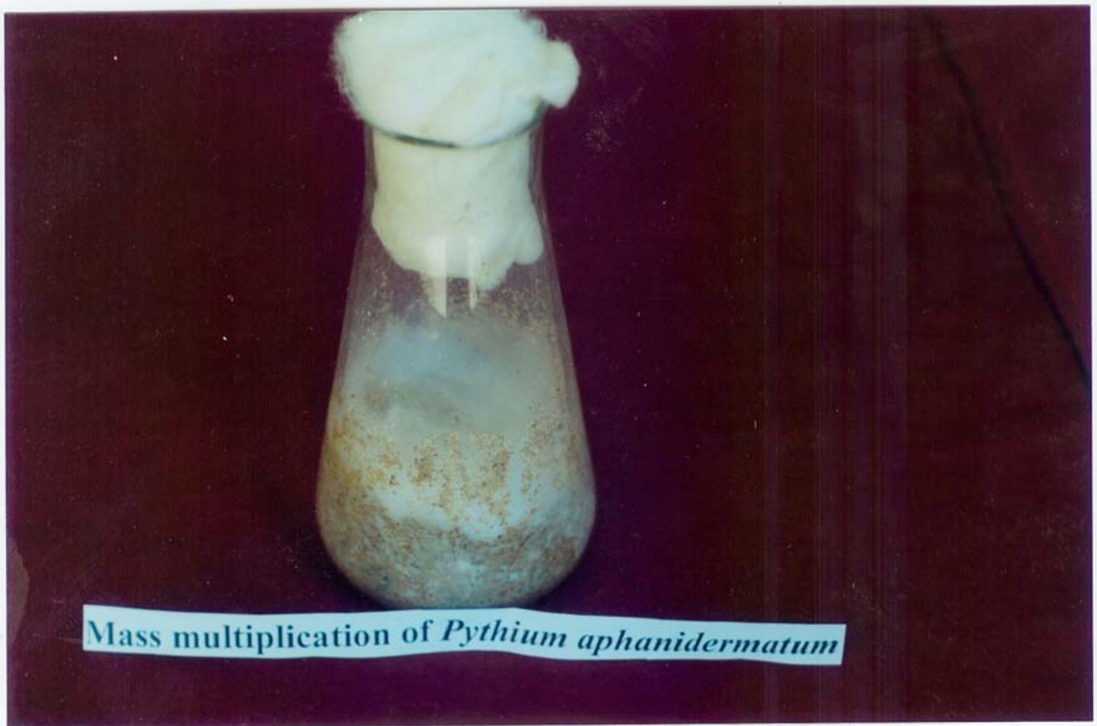


Plate 24 : Mass multiplication of *Pythium aphanidermatum*

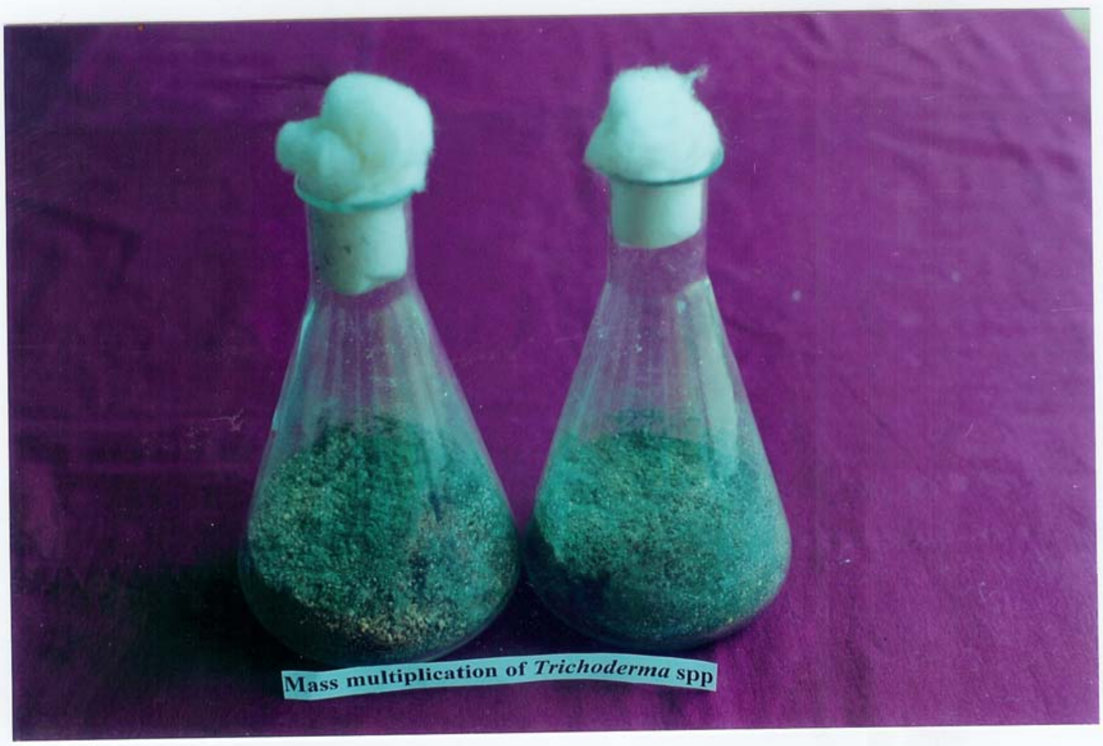


Plate 25 : Mass multiplication of *Trichoderma* spp. (TT_bT-1)

Plate 1 : Photograph shows the bed covered with polyethylene sheet and it is tightly bounded with soil at all the sides

Plate 2 : The field showing the solarized and un-solarized beds with soil thermometers

Plate 3 : **Pure culture of test pathogen *Pythium aphanidermatum***

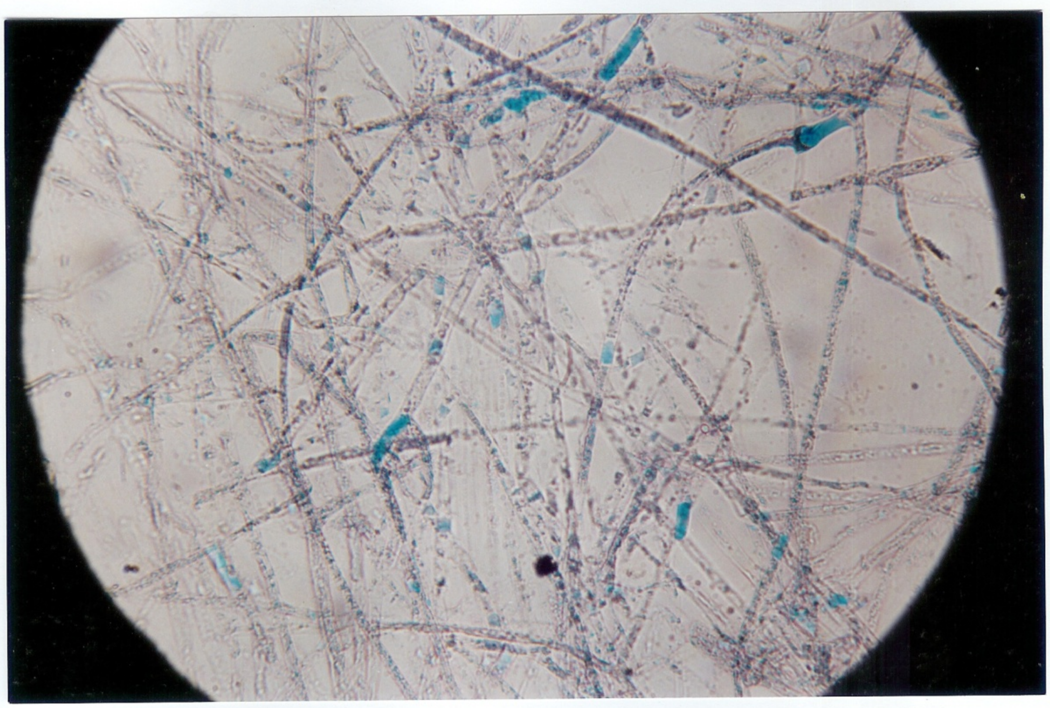


Plate 4 : **Photomicrograph of *Pythium aphanidermatum***

Plate 5 : The rhizosphere microflora of tobacco. A) Soil bacteria B) Soil mycoflora

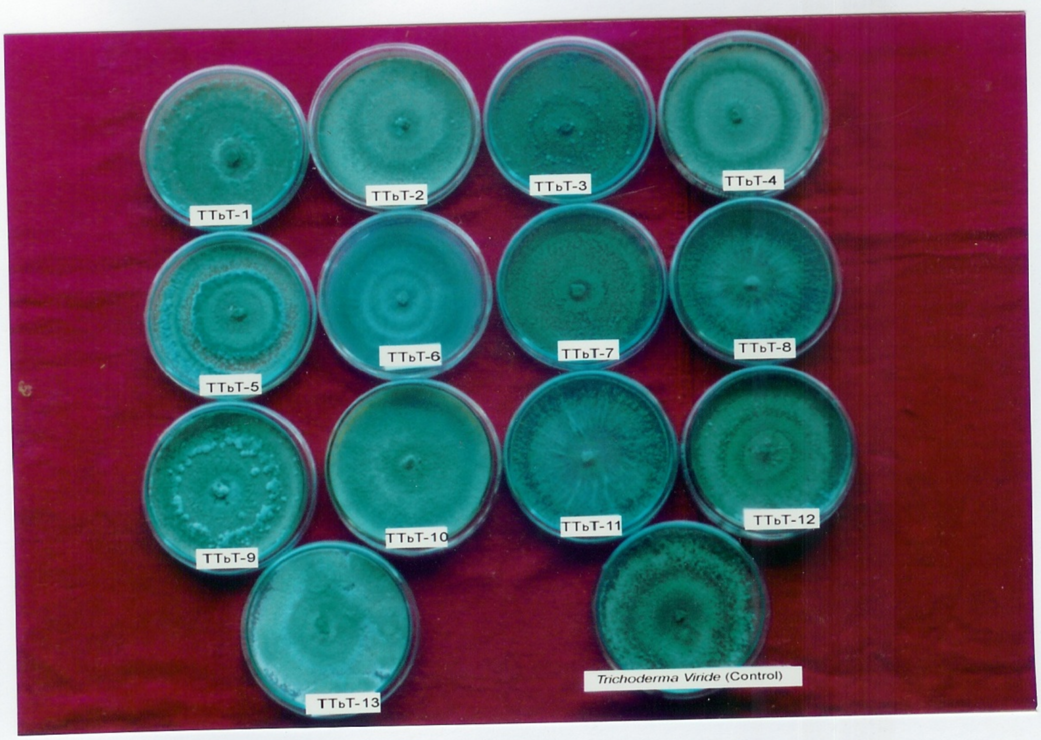


Plate 6 : Pure cultures of *Trichoderma* isolates

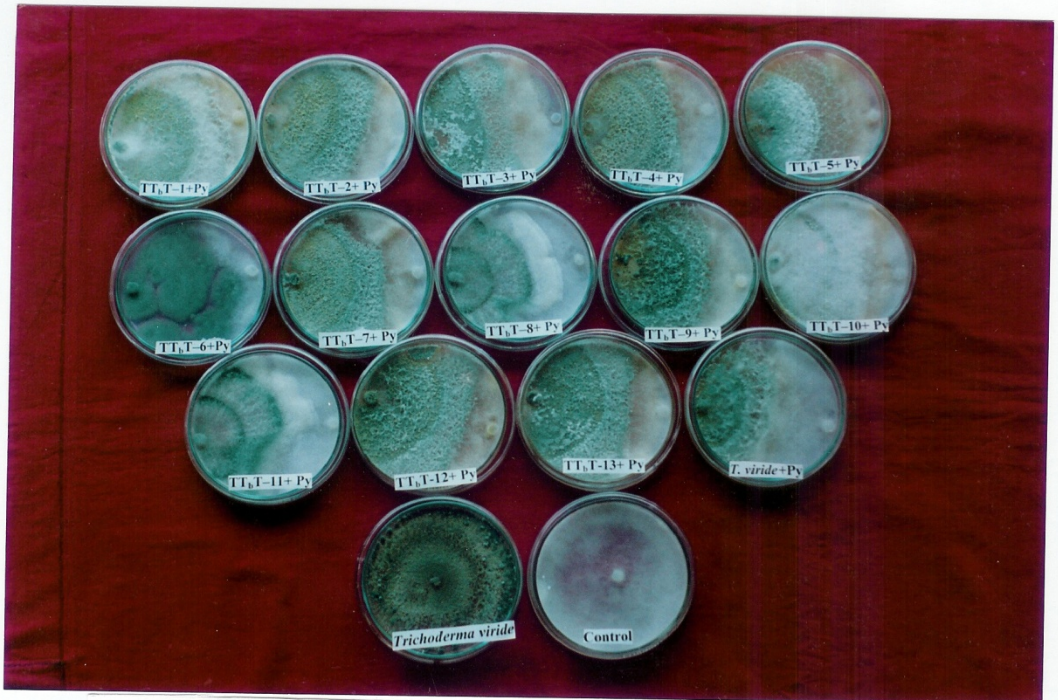


Plate 7 : Photograph showing antagonistic activity of *Trichoderma* isolates against *Pythium aphanidermatum* in dual culture technique

Plate 8 : Photograph showing antagonistic activity of two effective *Trichoderma* isolates (TT_bT-1 and TT_bT-12) against *Pyhium aphanidermatum* in dual culture technique

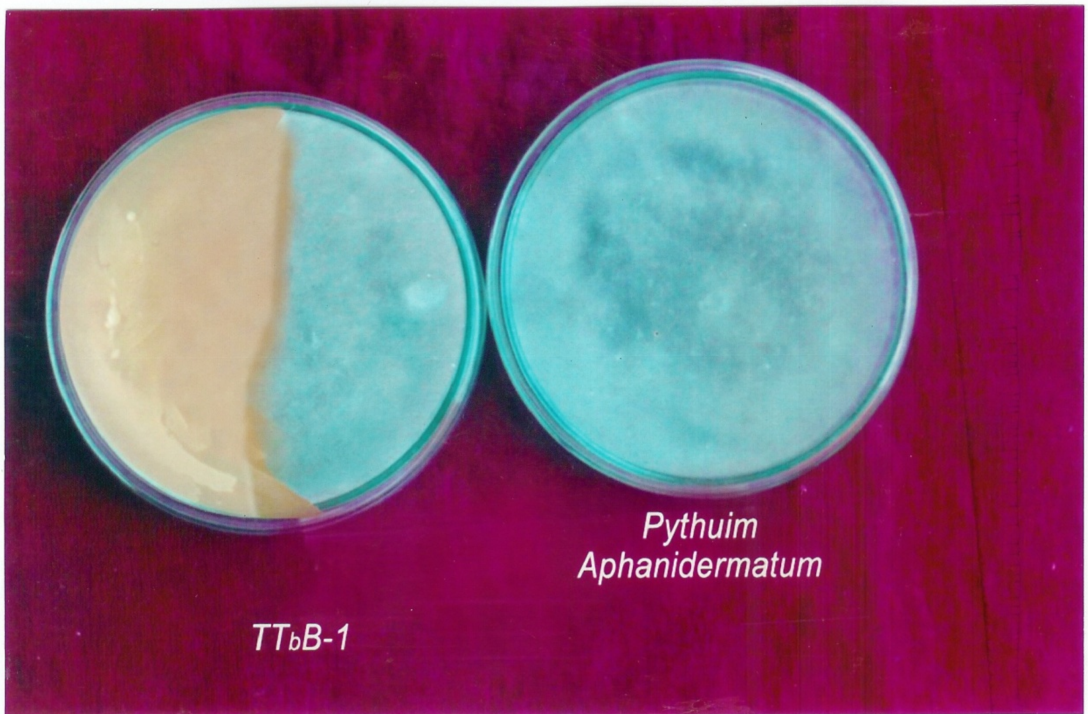


Plate 11 : Antagonistic activity of TT_bB-1 against *Pythium aphanidermatum*

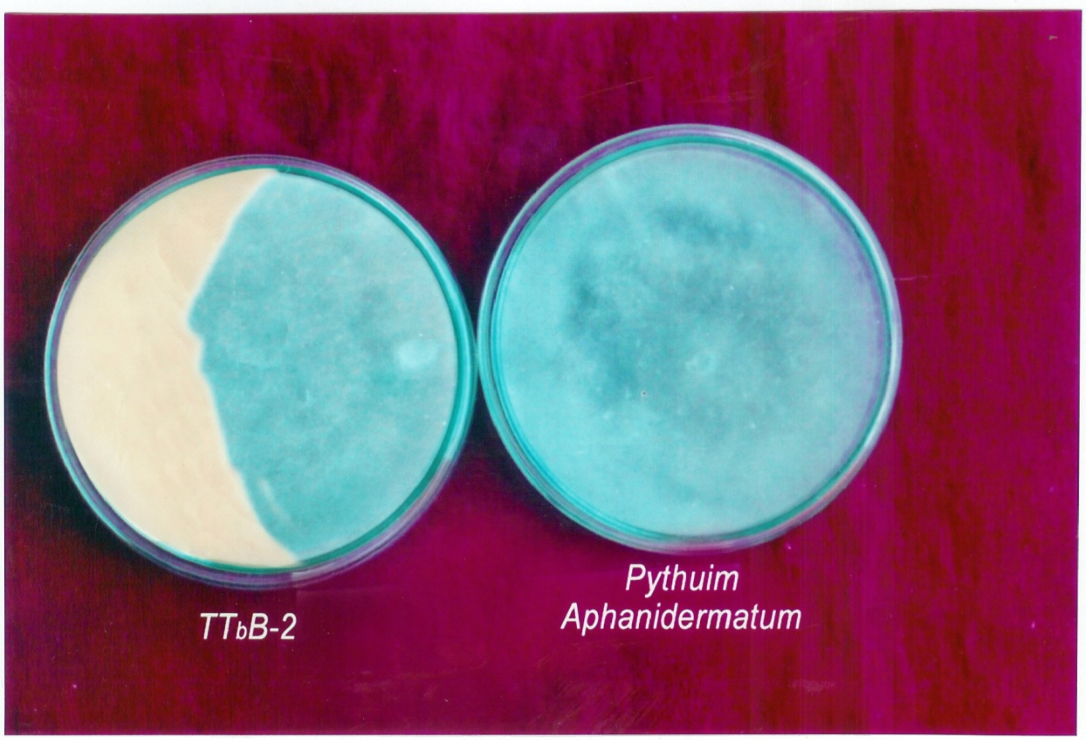


Plate 12 : Antagonistic activity of TT_bB-2 against *Pythium aphanidermatum*

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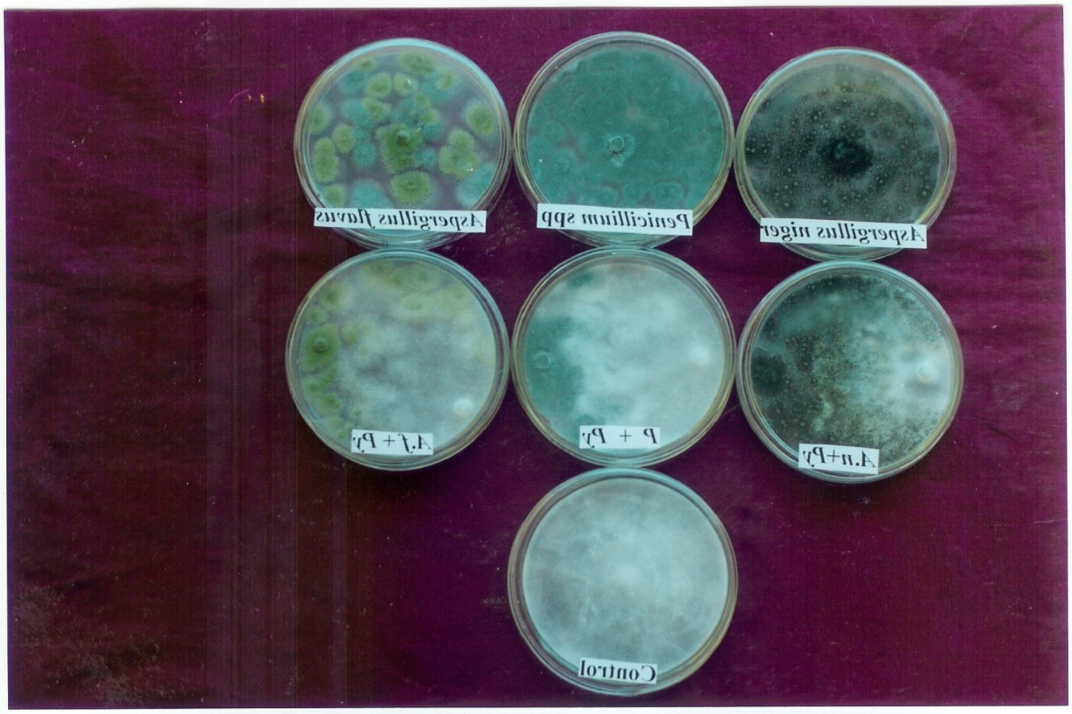


Plate 9 : Pure cultures of *Aspergillus niger*, *A. flavus*, *Pencillium* spp. and their antagonistic activity against *Pythium aphanidermatum*

Plate 10 : Pure cultures of two bacterial isolates

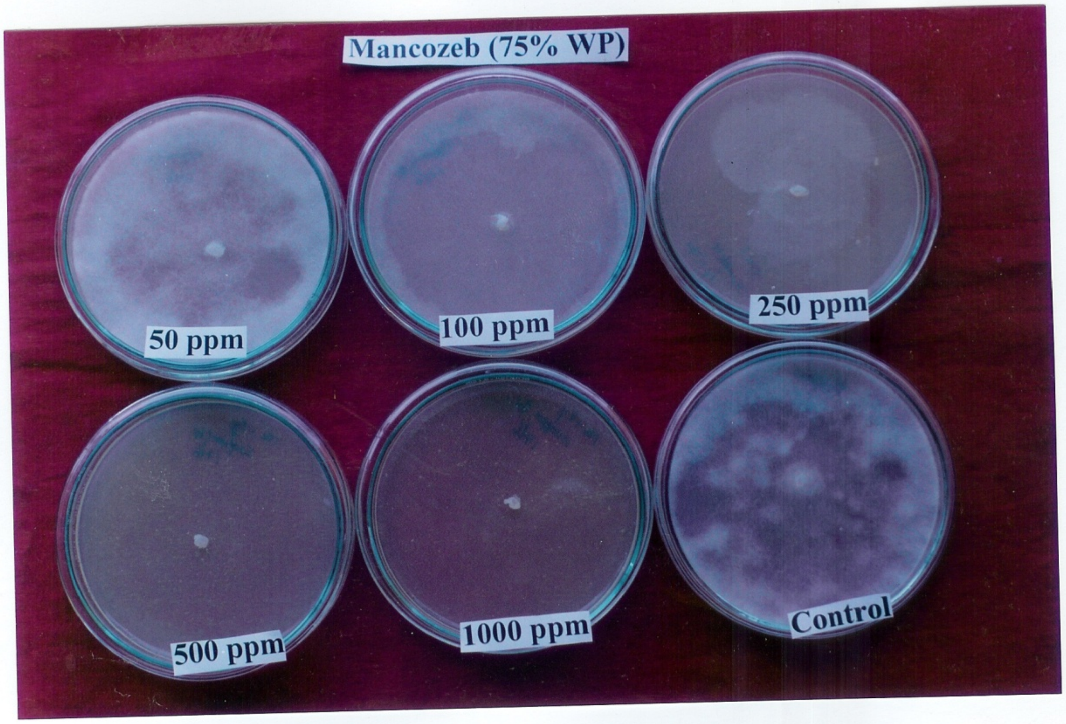


Plate 13 : *In vitro* efficacy of mancozeb against *Pythium aphanidermatum*

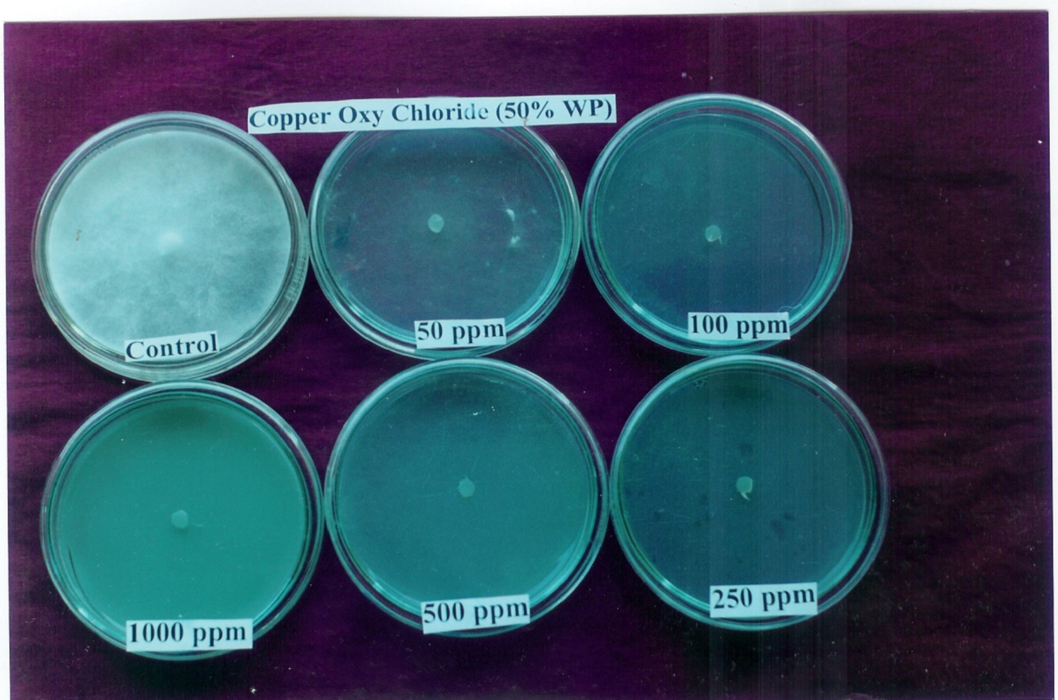


Plate 14 : *In vitro* efficacy of copper oxy chloride against *Pythium aphanidermatum*

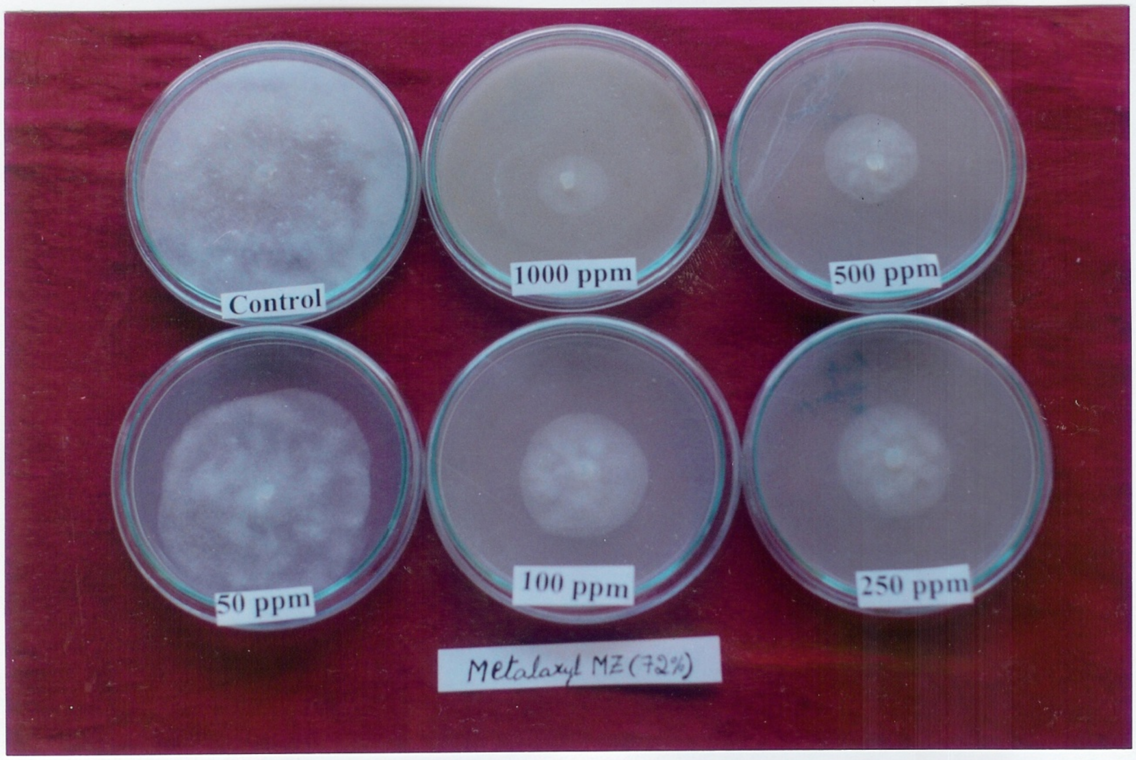
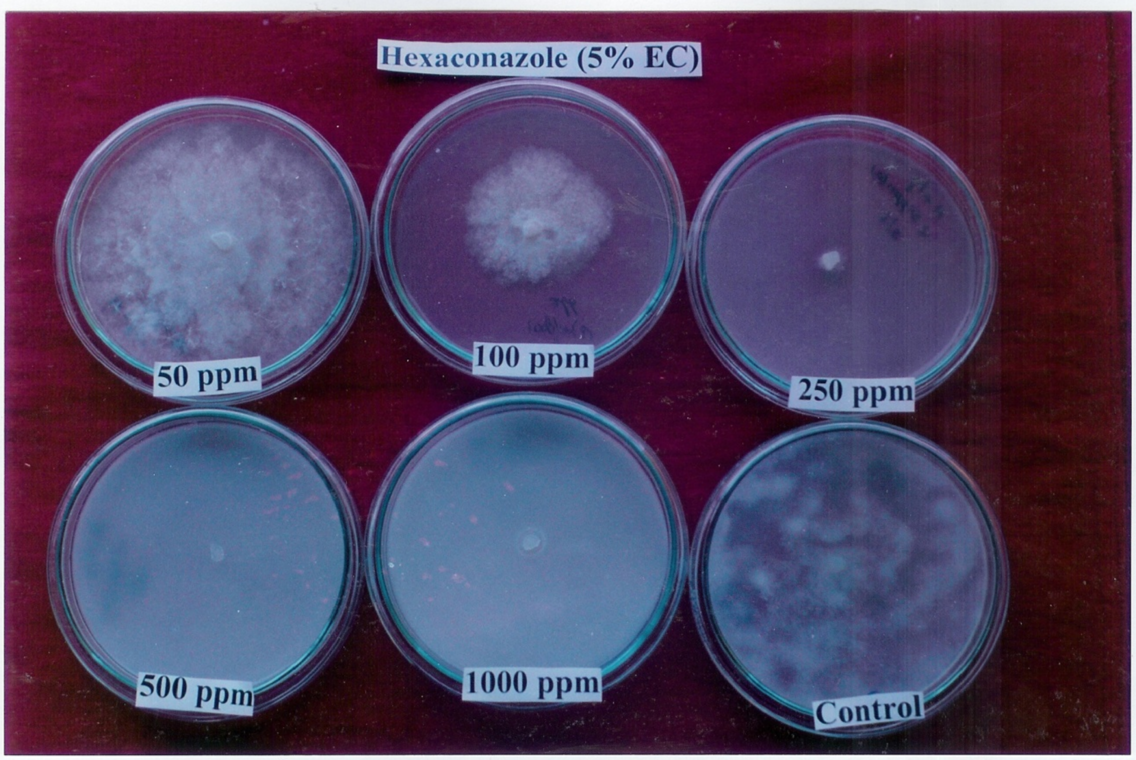


Plate 15 : *In vitro* efficacy of metalaxyl MZ against *Pythium aphanidermatum*



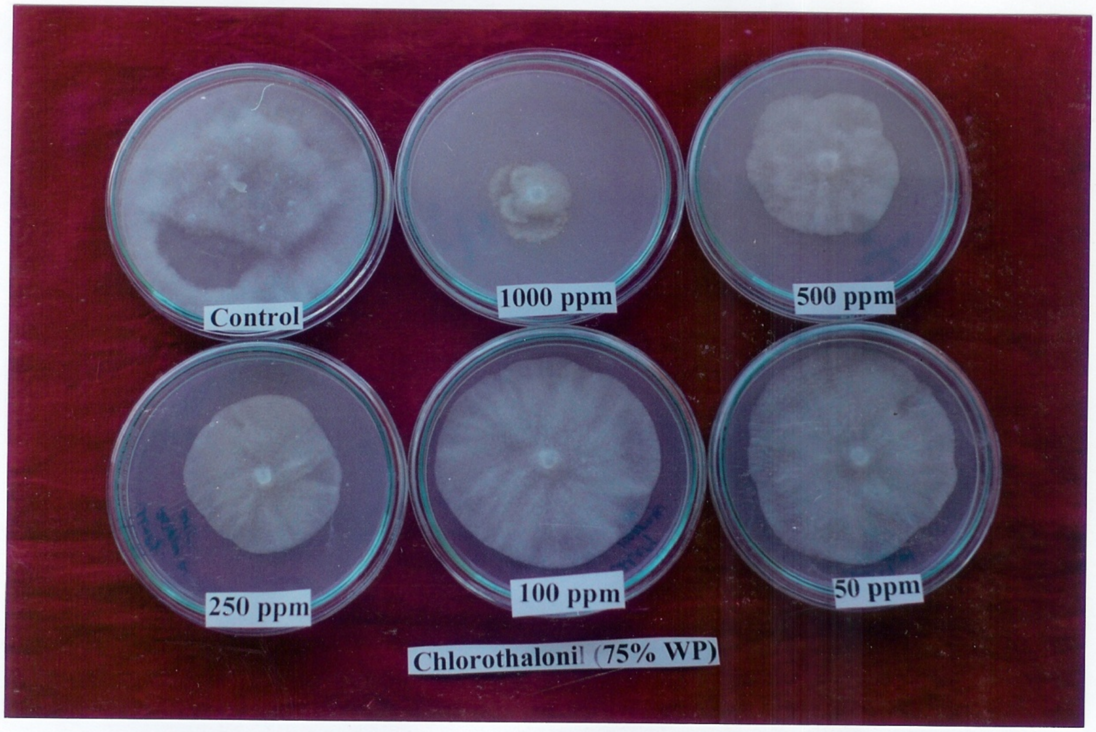


Plate 17 : *In vitro* efficacy of chlorothalonil against *Pythium aphanidermatum*

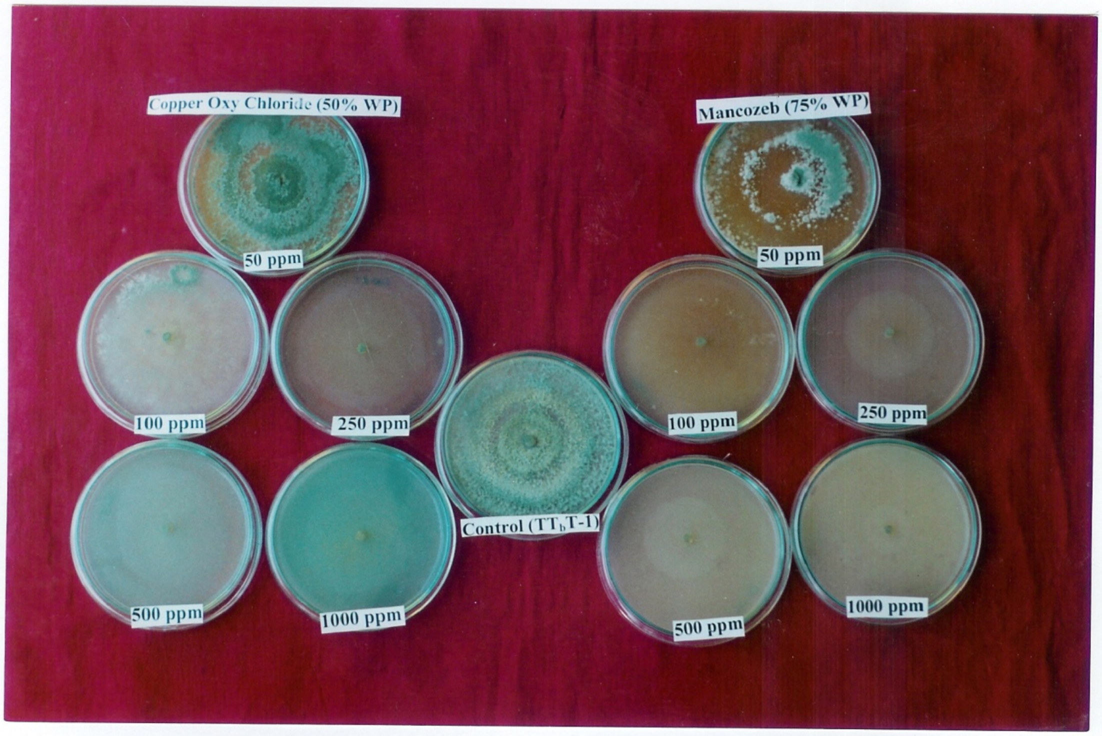


Plate 18 : Compatibility of *Trichoderma* isolate (TT_bT-1) with copper oxy chloride and mancozeb

Plate 19 : Compatibility of *Trichoderma* isolate (TT_bT-1) with metalaxyl MZ

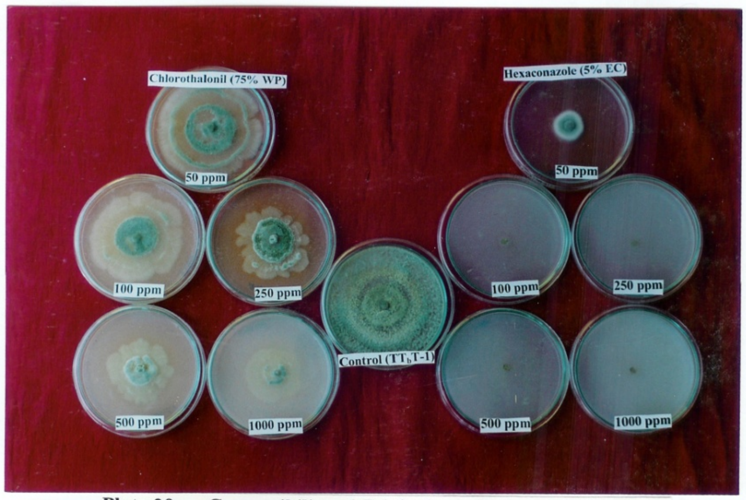


Plate 20 : **Compatibility of *Trichoderma* isolate (TT_bT-1) with chlorothalonil and hexaconazole**

Plate 21 : **Photograph showing the condensing water inside the polyethylene sheet in solarized plots**

Plate 26 : **Inoculated control (T_{16}) showing less number of plants, less plant height and more number of weeds**

Plate 27 : **Integrated treatment (T_{14}) showing more number of plants, increased plant height and no weeds**

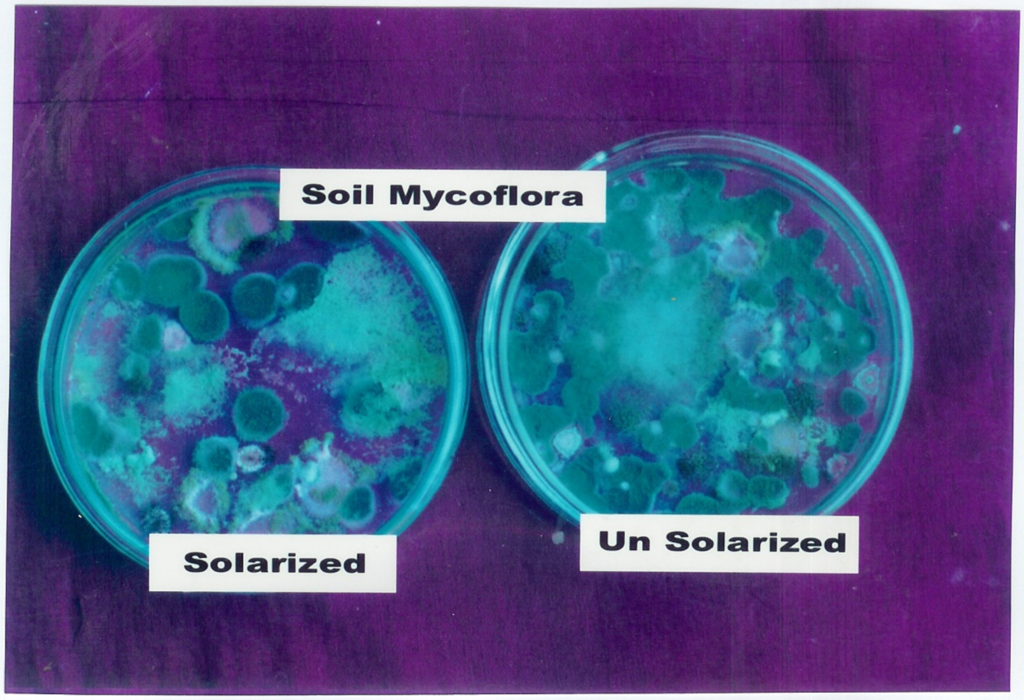


Plate 22 : Photograph showing the soil mycoflora in solarized plots

Plate 23 : The solarized plot showing no weeds after removal of polyethylene sheet and un-solarized bed with full of weeds

CHAPTER - IV

RESULTS

The results pertaining to the present investigation are presented under the following sections.

4.1 ISOLATION AND IDENTIFICATION OF THE PATHOGEN

4.1.1 Isolation

Isolation of the pathogen was made from the diseased seedlings showing typical post emergence damping-off symptoms. The fungus associated with the disease was isolated, purified and maintained on potato dextrose agar (PDA) medium. The pathogen growth on PDA medium was better than on CMA medium.

4.1.2 Identification

To determine the identity of the species, its cultural and morphological characters were studied. A fluffy, aerial growth was formed on PDA, but on this medium oospores and sporangia were scanty (Plate 3). The mycelium was irregularly branched, coenocytic when young, the filaments being full of granular protoplasm (Plate 4). In older cultures, the filaments were hyaline and septate. The fungus was found to produce sporangia. The fungus grew well from 26 to 37°C. On the basis of morphological and cultural characters, growth habit and temperature relations, the fungus was identified as *Pythium aphanidermatum.*

The microflora were isolated from tobacco rhizosphere by following the procedure of serial dilution pour plate technique. The various fungi and bacteria isolated were listed in table 1 (Plate 5).

Thirteen isolates of *Trichoderma* spp. from various soil samples collected from Kalikiri area were used and the numbers were given as TT_bT - 1 to TT_bT - 13 (Plate 6).

4.3 IDENTIFICATION OF RHIZOSPHERE MICROFLORA

The isolated microflora were identified up to the genus or species level on the basis of their growth, colour and other characters produced by them on PDA.

Aspergillus niger

The growth of the fungus was rapid on PDA. Spherical heads were found consisting of chains of conidia radiating from swollen knob of conidiophore. Spore bearing hypha showed sterigmata on which chains of spores were borne. The heads on sporangiophores were black in colour.

Aspergillus flavus:

The fungus exhibited rapid growth with greenish colour on PDA. The conidiophore heads are lemon green and conidia were pyriform to globose.

***Penicillium* spp:**

The fungal colonies were green to olive green with green mycelium. The conidiophores were short. The conidia were on the tips of conidiophores which were cylindrical to globose.

***Trichoderma* spp:**

The fungal colonies were yellowish green to dark green in colour. The conidia were round to oblong in shape which were formed in chains on tips of phialides.

Bacterial isolates:

TT_bB-1 isolate was isolated on *Pseudomonas fluorescencs* (King's B) medium and TT_bB-2 was isolated on *Bacillus subtilis* medium (Plate 10).

4.4 *In vitro* SCREENING OF THE ANTAGONISTIC MICROFLORA AGAINST *P. aphanidermatum*.

The microflora namely *A. niger, A. flavus, Pencillium* spp. *Trichoderma* spp. and two bacterial spp. which were isolated from rhizosphere of tobacco seedlings and screened for their antagonistic activity against *Pythium aphanidermatum* by dual culture technique (Plate 7 & 9) and the results are presented in table 2.

Maximum antagonistic activity was recorded in case of TT_bT-1 isolate which has given 94.44 per cent inhibition of the pathogen over the control.

TT_bT-12 isolate also showed more inhibition percentage (92.60) which was statistically on par with TT_bT-1 isolate (Plate 8).

There was significant difference between isolate TT_bT-12 and TT_bT-13 which showed 92.60 and 89.63 per cent inhibition, respectively. TT_bT-13, TT_bT-2, and TT_bT-3 isolates were on par with each other. Similarly, TT_bT-3, TT_bT-7 and TT_bT-4 isolates; TT_bT-4, TT_bT-5 and TT_bT-8 isolates; TT_bT-6 and TT_bT-10 isolates; TT_bT-9 and TT_bT-11 isolates were on par with each other in inhibiting the growth of the pathogen (Table 2, Fig-1).

With regard to the inhibition of the growth of the pathogen there was highly significant difference between *Aspergillus niger* (61.11%), *Aspergillus flavus* (25.22%) and *Pencillium* spp. (42.60%) (Table 2).

The bacterial isolates were also shown more significant difference in inhibiting the pathogen growth *in vitro* i.e. TT_bB-1 (66.66%) and TT_bB-2 (56.66%) (Plate 11 & 12).

Trichoderma isolates showed higher percentage of inhibition when compared to other soil microflora i.e. *A. niger, A. flavus, Pencillium* spp. and bacterial isolates. TT_bT-1 and TT_bT-12 isolates took minimum time (72 hrs) to completely over grow the *P. aphanidermatum* colony (Table 2).

4.5 *In vitro* SCREENING OF FUNGICIDES AGAINST *Pythium aphanidermatum*

The radial growth of pathogen in *in vitro* was reduced by 44.44 per cent by metalaxyl (Ridomil) MZ (72% WP) at 50 ppm concentration. This was followed by chlorothalonil (75% WP) which has shown 15.55 per cent inhibition. Whereas there is no inhibition by other fungicides i.e. mancozeb (75%WP), copper oxy chloride (50% WP) and hexaconazole (5% EC) at this concentration (Table 3, Fig-2).

The radial growth of pathogen was highly reduced by copper oxy chloride (88.15%), metalaxyl MZ (61.11%) and hexaconazole (44.44%) at 100 ppm. There is no inhibition by mancozeb at this concentration.

Copper oxy chloride showed complete inhibition of radial growth from 250 ppm to 1000 ppm in *in vitro* where as hexaconazole showed 88.88 per cent inhibition at 250 ppm and at 500 and 1000 ppm there was complete inhibition. Mancozeb showed 22.22 per cent inhibition at 250 ppm and there was complete inhibition of growth at 500 and 1000 ppm. Metalaxyl MZ has given 68.15, 81.11 and 86.66 per cent inhibition at 250, 500 and 1000 ppm respectively (Plate 13 - 17).

This study indicate that the fungicide metalaxyl MZ has shown superiority in inhibition of growth in *in vitro* even from 50 ppm onwards when compared to rest of the fungicides. So this is an effective fungicide among all the fungicides used.

4.6 COMPATIBILITY OF *Trichoderma* spp. (TT_bT-1) WITH FUNGICIDES

Metalaxyl MZ (72%WP) was highly compatible with *Trichoderma* spp. at all the concentrations. The data in table 4 shows that there was no inhibition of radial growth in *in vitro* at 50 and 100 ppm where as only 3.71, 11.11 and 18.52 per cent inhibition was recorded at 250, 500 and 1000 ppm, respectively (Plate 19). This was followed by copper oxy chloride which also showed no inhibition at 50 ppm and 44.44 per cent inhibition was recorded even at 1000 ppm. Chlorothalonil and mancozeb inhibited the growth by 59.33 and 58.52 per cent respectively at 1000 ppm. Hexaconazole inhibited the radial growth even from 50 ppm (80 per cent) and there was complete inhibition from 100 ppm to 1000 ppm (Table 4, Fig. 3, Plate 18 & 20).

Thus metalaxyl MZ (Ridomil) was highly compatible with bio-agent and also it is effective against pathogen. Hence, this fungicide was selected for experimentation in field.

4.7 EFFECT OF SOIL SOLARIZATION ON SOIL TEMPERATURE

The average temperature recorded in solarized plots is 43.83°C which is 10.20°C more than un-solarized plots in which average soil temperature was

33.63°C (Table 5, Fig. 4). There was statistically significant difference between the temperatures recorded in solarized and un-solarized plots.

4.8 EFFECT OF SOIL SOLARIZATION ON SOIL MOISTURE

The average soil moisture content in solarized plots was 10.10 mm which was 6.80 mm more than the average soil moisture content of un-solarized plots (4.10 mm) at 10 cm depth (Table 6, Fig. 5; Plate 21).

4.9 EFFECT OF SOIL SOLARIZATION ON SOIL MYCOFLORA

The effect of soil solarization was high on soil mycoflora. There was much reduction in fungal colonies in solarized soil samples than un-solarized soil samples. There was much reduction in *A. niger* population in solarized soils. The number of colonies of mycoflora in solarized soils at 10^{-4} dilution recorded as 29 when compared to unsolarized, where 68 colonies were recorded (Table 7, Plate 22).

4.10 EFFECT OF SOIL SOLARIZATION ON WEED GROWTH

Due to soil solarization, average number of weeds were reduced by 93.41 per cent in solarized plots (Plate 23) when compared to un-solarized plots and the fresh weight and dry weight of weeds were also significantly reduced in solarized plots (98%) when compared to unsolarized plots (Table 8, Fig. 6).

4.11 MASS MULTIPLICATION OF *P. aphanidermatum*

The pathogen was mass multiplied on autoclaved sand maize meal medium. Milky white mycelium initiated within one day after inoculation. The fungus covered the entire substrate in the flasks in 10 days when incubated at room temperature (Plate 24).

4.12 MASS MULTIPLICATION OF *Trichoderma* spp. (TT_bT-1)

The potential antagonist TT_bT-1 isolate of *Trichoderma* spp. was mass multiplied on wheat bran. This medium was most suitable for growth and sporulation. Green coloured growth was observed in the flasks 36 hrs after inoculation and the substrate was completely covered by antagonist in 12 to15 days after inoculation (Plate 25).

4.13 EFFECT OF DIFFERENT TREATMENTS ON GERMINATION PERCENTAGE OF TOBACCO SEEDS

Germination percentage was increased by 43.33 per cent over inoculated control in T_{14} treatment with a germination percentage of 85.50 (Table 9) and in case of control it was recorded as 48.45. After T_{14}, next best treatments were T_{13} and T_{12} in which the germination percentages recorded as 72.01 and 70.72, respectively and these two were on par with each other. Lowest germination percentage was recorded in T_{15} and T_6 treatments. Where as in T_1, T_2, T_3, T_4, T_5 and T_{11} has given almost equal germination percentages i.e. there was no significant difference statistically. The treatments T_7, T_8, T_9, and T_{10}, have given equal germination percentages, statistically (Table 9, Fig.7).

4.14 EFFECT OF DIFFERENT TREATMENTS ON PER CENT DISEASE INCIDENCE OF TOBACCO SEEDLINGS AT 15, 20, 25, 30, 35 AND 40 DAS

The per cent disease incidence was decreased in general from 15 days to 30 days and again increased up to 40 days after sowing (Table 10, Fig. 9).

Soil solarization (T_1) individually controlled the disease by 68.07 per cent over control. When it was combined with T_2, T_3, T_4 and T_6 there was no much difference in disease control, however, it has given significant disease control when combined with T_5 where seed treatment and soil drenching with effective fungicide was performed. And when it was combined with both T_3 and T_6 (T_{12}), T_5 and T_6 (T_{13}) it has given significant disease control i.e. the disease was controlled up to 81.65 and 83.48 per cent, respectively, over control. The combination of all the treatments i.e. T_1, T_3, T_5 and T_6 (T_{14}) has given good control of disease incidence (87.77%) over control.

Among all the treatments T_{15} has given lowest per cent disease control i.e. 33.73 per cent, next was T_6 in which per cent disease control recorded was 55.65 per cent over control. Treatments T_2, T_3, T_4 and T_5 have given no significant difference statistically regarding per cent decrease of total disease incidence i.e. they were on par with each other. In the same way T_7, T_8 and T_9 were also on par with each other (Fig. 8).

The cumulative disease incidence at 40 days after sowing recorded was only 5.54 in T_{14} against 45.3 in control. And next best treatments were T_{13} and T_{12} in which only 7.48 and 8.31 was recorded. In all the treatments the disease incidence was reduced significantly when compared with control.

Number of healthy seedlings were also increased in T_{14} treatment in which 650 plants were recorded where as only 246.33 plants were recorded in control. In T_{13} and T_{12} treatments the number of healthy seedlings recorded were 535 and 520.66 respectively (Plate 26 & 27).

Disease growth rate was also much less in case of T_{14} treatment (3.20) where as in control it was recorded as 59.95. Next best treatments were T_{13} and T_{12} where the disease growth rate was recorded as 4.01 and 6.75, respectively. In case of T_{10} also less disease growth rate (8.86) was recorded. The data in Table 9 indicate that disease growth rate was reduced in integrated treatments when compared to individual treatments.

4.15 EFFECT OF DIFFERENT TREATMENTS ON SHOOT LENGTH, ROOT LENGTH AND PLANT HEIGHT AT 10, 20, 30 AND 40 DAS.

The shoot length, root length and plant height were increased significantly over control (Table 11). In treatment T_{14} plant height was increased by 67.5, 67.21, 59.03 and 48.62 per cent over control at 10, 20, 30 and 40 DAS, respectively. The next best treatments were T_{12} and T_{13} in which plant height was increased 42.85 and 41.05 per cent over control at 40 DAS.

Next better treatments were T_{10} and T_8 where in 35.63 and 34.88 per cent increase over control were recorded at 40 DAS. In general, in all the treatments there was significant increase in plant height over control (Table 12, Fig. 10).

4.16 EFFECT OF DIFFERENT TREATMENTS ON FRESH WEIGHT AND DRY WEIGHT OF TOBACCO SEEDLINGS AT 40 DAS

The fresh weight and dry weight of seedlings was also increased significantly over control. Here also, in integrated treatment (T_{14}) 48.80 and 48.62 per cent increase was recorded over control regarding fresh weight and dry weight, respectively. Next best treatments were T_{12} and T_{13} where 42.85 and 40.74; 42.85 and 41.05 per cent increase over control was recorded with respect to fresh weight and dry weight. In other treatments also the increased fresh weight and dry weight over control were recorded. In T_1 and T_2, T_3, T_4 and T_5 treatments the fresh and dry weights recorded were on par with each other (Table 13, Fig. 11).

Table 1: Different soil microflora isolated from the tobacco rhizosphere

Soil Microflora	Colour of the colony
Soil Fungi 1) *Aspergillus niger*	Black
2) *A.flavus*	Green
3) *Penicillium* sp.	Olive green
4) *Trichoderma* sp. (TT_bT-1 to TT_bT-13)	Light green to Dark green
Soil Bacteria 1) TT_bB-1	Light yellow
2) TT_bB-2	Dusty White

Table 2: *In vitro* efficacy of soil microflora against *P. aphanidermatum*

S. No.	Soil microflora	*Growth of *P. aphanidermatum* (mm)	Per cent inhibition over control
1	TT_bT-1	5.00 (12.92)**	94.44 (76.31)
2	TT_bT-2	10.30 (18.72)	88.55 (70.27)
3	TT_bT-3	11.60 (19.91)	87.11 (68.95)
4	TT_bT-4	13.00 (21.13)	85.55 (67.70)
5	TT_bT-5	14.66 (22.46)	83.71 (66.19)
6	TT_bT-6	21.33 (27.49)	76.30 (60.37)
7	TT_bT-7	12.33 (20.53)	86.30 (68.28)
8	TT_bT-8	15.66 (23.26)	82.60 (65.35)
9	TT_bT-9	25.00 (30.00)	72.22 (58.18)
10	TT_bT-10	19.00 (25.84)	78.88 (62.65)
11	TT_bT-11	24.00 (29.33)	73.33 (58.89)
12	TT_bT-12	6.66 (14.89)	92.60 (74.21)
13	TT_bT-13	9.33 (17.76)	89.63 (71.19)
14	*T.viride*	27.00 (31.31)	70.00 (56.79)
15	*A. niger*	35.00 (36.27)	61.60 (51.71)
16	*A. flaves*	67.30 (55.22)	25.22 (30.13)
17	*Pencillium* spp.	51.66 (45.92)	33.71 (35.49)
18	TT_bB-1	35.00 (33.21)	66.66 (54.76)
19	TT_bB-2	39.00 (38.65)	56.66 (48.85)
20	Control	90.00 (71.57)	--

SEm (±) (0.89)
CD (P=0.05) (2.54)

* Mean of three replications

** Figures in parentheses are angular transformed values

Table 3: *In vitro* efficacy of fungicides against *Pythium aphanidermatum*

Name of Fungicide	Concentration (ppm)	*Diameter of growth (mm)	Percent inhibition over control
Mancozeb (75% WP)	50	90.00 (71.57)**	0.00 (0.00)
	100	90.00 (71.57)	0.00 (0.00)
	250	70.00 (56.79)	22.22 (28.11)
	500	0.00 (0.00)	100.00 (90.00)
	1000	0.00 (0.00)	100.00 (90.00)
Copper oxy Chloride (50% WP)	50	90.0 (71.57)	0.00 (0.00)
	100	10.66 (19.00)	88.15 (69.82)
	250	0.00 (0.00)	100.00 (90.00)
	500	0.00 (0.00)	100.00 (90.00)
	1000	0.00 (0.00)	100.00 (90.00)
Metalaxyl MZ (72% WP)	50	50.00 (45.00)	44.00 (41.55)
	100	35.00 (36.27)	61.11 (51.41)
	250	28.66 (32.33)	68.15 (55.61)
	500	17.00 (24.35)	81.11 (64.23)
	1000	12.00 (20.27)	86.66 (68.65)
Hexaconazole (5% EC)	50	90.00 (71.57)	0.00 (0.00)
	100	50.00 (45.00)	44.44 (41.78)
	250	10.00 (18.43)	88.88 (0.54)7
	500	0.00 (0.00)	100.00 (90.00)
	1000	0.00 (0.00)	100.00 (90.00)
Chlorothalonil (75% WP)	50	76.00 (60.67)	15.55 (23.18)
	100	64.00 (53.13)	28.88 (32.52)
	250	52.00 (46.15)	42.22 (40.51)
	500	42.00 (40.40)	53.33 (46.89)
	1000	30.00 (33.21)	66.66 (54.76)
Control		90.00 (71.57)	
SEm (±)		(0.44)	
CD (P=0.05)		(1.26)	

* Mean of three replication

** Figures in parentheses are angular transformed values

Table 4 : Compatibility of *Trichoderma* spp. (TT_bT-1) with different fungicides

Name of Fungicide	Concentration (ppm)	*Diameter of growth (mm)	Percent inhibition over control
Mancozeb (75% WP)	50	90.00 (71.57)**	0.00 (0.00)
	100	78.66 (62.44)	12.60 (20.79)
	250	50.66 (45.34)	43.71 (41.38)
	500	37.33 (37.64)	58.52 (49.89)
	1000	21.00 (27.27)	76.66 (61.14)
Copper oxy Chloride (50% WP)	50	90.00 (71.57)	0.00 (0.00)
	100	84.00 (66.42)	6.66 (15.00)
	250	70.00 (56.79)	22.22 (28.11)
	500	63.00 (52.54)	30.00 (33.21)
	1000	50.00 (45.00)	44.44 (41.78)
Metalaxyl MZ (72% WP)	50	90.00 (71.57)	0.00 (0.00)
	100	90.00 (71.57)	0.00 (0.00)
	250	86.66 (68.53)	3.71 (11.09)
	500	80.00 (63.43)	11.11 (19.46)
	1000	73.33 (58.89)	18.52 (25.47)
Hexaconazole (5% EC)	50	18.00 (25.10)	80.00 (63.43)
	100	0.00 (0.00)	100.00 (90.00)
	250	0.00 (0.00)	100.00 (90.00)
	500	0.00 (0.00)	100.00 (90.00)
	1000	0.00 (0.00)	100.00 (90.00)
Chlorothalonil (75% WP)	50	81.60 (64.60)	9.33 (17.76)
	100	67.66 (55.37)	24.82 (29.87)
	250	62.66 (51.77)	30.37 (33.40)
	500	54.00 (47.29)	40.00 (39.23)
	1000	36.60 (37.23)	59.33 (50.36)
Control		90.00 (71.57)	--
SEm (±)		(0.43)	
CD (P=0.05)		(1.21)	

* Mean of three replications

** Figures in parentheses are angular transformed values

Table 5: Effect of soil solarization on soil temperature

No. of weeks	*Temperature in solarized plots (°C)	*Temperature in un-solarized plots (°C)	Difference (°C)
1 Week (27/4/2003 to 3/5/2003)	42.50	33.50	9.0
2 Week (4/5/2003 to 10/5/2003)	44.66	32.83	11.83
3 Week (11/5/2003 to 17/5/2003)	38.85	29.58	9.0
4 Week (18/5/2003 to 24/5/2003)	46.00	36.00	10.00
5 Week (25/5/2003 to 31/5/2003)	42.00	33.00	9.00
6 Week (1/6/2003 to 7/6/2003)	49.00	36.60	12.4
Average	43.83	33.63	10.20

t-test value(P=0.05) 6.31

*Mean of three replications

Table 6: Effect of soil Solarization on soil moisture content at 10 cm depth.

No. of weeks	*Soil moisture content in solarized plots (mm)	*Soil moisture content in un-solarized plots (mm)	Difference
1 Week (27/4/2003 to 3/5/2003)	9.57	3.38	6.19
2 Weeks (4/5/2003 to 10/5/2003)	9.45	3.67	5.78
3 Weeks (11/5/2003 to 17/5/2003)	9.33	6.18	3.15
4 Weeks (18/5/2003 to 24/5/2003)	9.68	2.90	6.78
5 Weeks (25/5/2003 to 31/5/2003)	11.09	3.89	7.20
6 Weeks (1/6/2003 to 7/6/2003)	11.48	4.60	6.88
Average	10.10	4.10	6.00

t-test value(P=0.05) 10.75

*Mean of three replications

Table 7 : Effect of soil solarization on soil mycoflora

Treatment	**Soil mycoflora (x 10^4 CFU /g of soil)**
Solarized	29
Unsolarized	68

Table 8 : Effect of soil solarization on weed count and their fresh weight and dry weight.

	Solarized	Un-solarized	Percent reduction	t-value (P = 0.05)
Weed count / plot*	7.25	109.44	93.41	8.26**
Fresh weight (g)*	4.48	269.45	98.33	80.29**
Dry weight (g)*	3.60	189.59	90.08	27.82**

* Mean of nine replications
** Significant

Table 9 : Effect of different treatments on germination percentage of tobacco seeds.

Treatments	*No. of seeds germinated	*Germination percentage	Per cent increase over control
T_1	441.00	54.45 (47.52)**	11.01 (19.87)
T_2	444.00	55.49 (48.10)	12.68 (20.79)
T_3	453.33	57.16 (49.08)	15.23 (22.95)
T_4	449.00	56.12 (48.50)	13.66 (21.64)
T_5	459.33	57.41 (49.26)	15.60 (23.26)
T_6	409.66	51.20 (45.69)	5.37 (13.31)
T_7	477.0	59.62 (50.53)	18.73 (25.62)
T_8	491.33	61.41 (51.59)	21.10 (27.35)
T_9	479.66	59.95 (50.71)	19.18 (25.91)
T_{10}	494.32	61.78 (51.77)	21.57 (27.62)
T_{11}	454.00	56.75 (48.85)	14.62 (22.46)
T_{12}	565.66	70.72 (57.23)	31.49 (34.08)
T_{13}	576.00	72.01 (58.05)	32.71 (34.88)
T_{14}	684.00	85.50 (67.62)	43.33 (41.15)
T_{15}	394.66	49.33 (44.60)	1.78 (7.49)
T_{16}	387.66	48.45 (44.08)	

SEm ($\pm$) (0.53)
CD(P=0.05) (1.08)
*Mean of three replications
**Figures in parentheses are angular transformed values

Table 10 : Effect of different treatments on per cent disease incidence of tobacco seedlings at 15, 20, 25, 30, 35 and 40 DAS.

Treatments	*Disease Incidence						Total disease incidence	Percent decrease over control	Disease growth rate**	*No. of healthy seedlings
	15 DAS	20 DAS	25 DAS	30 DAS	35 DAS	40 DAS				
T_1	3.78	2.27	2.17	1.56	2.16	2.52	14.46 (22.38)***	68.07 (55.61)	19.81	378.66
T_2	3.60	3.11	1.52	1.79	2.16	2.00	14.18 (22.14)	68.69 (55.98)	18.03	382.33
T_3	3.20	2.55	1.93	1.81	2.28	1.81	13.58 (21.64)	70.02 (56.79)	16.41	306.66
T_4	3.26	3.22	1.77	1.45	2.14	2.16	14.00 (21.97)	66.88 (54.88)	20.42	380.33
T_5	2.75	1.86	1.36	1.23	2.48	2.47	12.15 (20.36)	73.17 (58.82)	16.57	408.66
T_6	5.64	3.85	2.94	2.38	2.44	2.44	20.09 (26.64)	55.65 (48.27)	25.83	330.66
T_7	3.00	2.59	2.07	1.15	1.58	2.62	13.01 (21.13)	71.28 (57.61)	16.35	416.66
T_8	2.84	2.02	2.20	1.18	1.31	2.37	11.92 (20.18)	73.68 (59.15)	14.07	434.33
T_9	2.47	2.44	1.47	1.79	2.06	2.38	12.61 (20.79)	72.16 (57.54)	17.86	420.66
T_{10}	2.15	1.99	1.40	1.35	1.57	1.21	9.67 (18.15)	78.65 (67.51)	8.86	452.00
T_{11}	3.52	2.58	2.10	1.35	1.61	1.82	12.98 (12.13)	71.34 (57.61)	14.76	428.33
T_{12}	1.76	1.49	0.85	1.35	1.49	1.37	8.31 (16.74)	81.65 (64.60)	6.75	520.66
T_{13}	1.56	1.35	1.13	0.78	1.24	1.42	7.48 (15.89)	83.48 (66.03)	4.01	535.00
T_{14}	1.07	0.88	0.79	0.35	1.25	1.20	5.54 (13.56)	87.77 (69.56)	3.20	650.00
T_{15}	6.67	4.07	3.10	3.50	5.02	7.66	30.02 (33.21)	33.73 (35.49)	44.31	305.00
T_{16}	8.08	6.82	5.82	6.60	7.02	10.96	45.30 (42.30)		59.95	246.33

SEm (±) (0.35)
CD (P=0.05) (0.71)

* Mean of three replications

** Formula y = a + bx

*** Figures in parentheses are angular transformed values

Table 11 : Effect of different treatments on root length and shoot length of tobacco seedlings at 10, 20, 30 and 40 DAS

Treatments	*Root length / plant (cm)				*Shoot length / plant (cm)			
	10 DAS	20 DAS	30 DAS	40 DAS	10 DAS	20 DAS	30 DAS	40 DAS
T_1	0.5	0.9	2.0	3.3	1.1	1.8	3.0	3.8
T_2	0.5	1.0	2.1	3.3	1.1	1.9	3.1	3.8
T_3	0.7	1.2	2.1	3.5	1.4	2.3	3.2	4.0
T_4	0.7	1.2	2.1	3.5	1.4	2.2	3.1	4.0
T_5	0.7	1.1	2.3	3.5	1.3	2.1	3.2	4.0
T_6	0.6	1.0	2.2	3.4	1.3	2.0	3.1	4.0
T_7	1.0	1.8	2.7	3.7	1.4	2.0	3.1	4.2
T_8	1.0	1.9	3.1	4.0	1.5	2.5	3.4	4.6
T_9	0.8	2.0	2.8	3.7	1.3	1.9	3.0	4.2
T_{10}	0.9	1.8	3.0	4.1	1.6	2.4	3.7	4.6
T_{11}	0.9	1.8	3.0	3.9	1.4	2.3	3.1	4.3
T_{12}	1.3	2.3	3.4	4.6	2.0	2.9	4.0	5.2
T_{13}	1.2	2.6	3.5	4.5	1.9	2.8	3.9	5.0
T_{14}	1.6	2.8	3.8	5.1	2.4	3.3	4.5	5.8
T_{15}	0.5	0.8	1.7	2.9	0.9	1.5	2.3	3.5
T_{16}	0.4	0.6	1.3	2.6	0.9	1.4	2.1	3.0

*Mean of three replications

Table 12 : Effect of different treatment on plant height at 10, 20, 30 and 40 DAS

Treatments	*Plant height							
	10 DAS	**Per cent increase over control**	**20 DAS**	**Percent increase over control**	**30 DAS**	**Percent increase over control**	**40 DAS**	**Percent increase over control**
T_1	1.6 (7.27)**	18.75 (25.70)	2.7 (9.46)	25.92 (30.59)	5.0 (12.92)	32.00 (34.45)	7.1 (15.45)	21.12 (27.35)
T_2	1.6 (7.27)	18.75 (25.70)	2.9 (9.80)	31.03 (33.83)	5.2 (13.18)	34.61 (36.03)	7.1 (15.45)	21.12 (27.35)
T_3	2.1 (8.33)	38.09 (38.12)	3.5 (10.78)	42.85 (40.92)	5.3 (13.31)	35.84 (36.75)	7.5 (15.89)	25.33 (30.20)
T_4	2.0 (8.13)	35.00 (36.27)	3.2 (10.30)	37.50 (37.76)	5.2 (13.18)	34.61 (36.03)	7.5 (15.89)	25.33 (30.20)
T_5	2.1 (8.33)	38.09 (38.12)	3.4 (10.63)	41.17 (39.93)	5.5 (13.56)	38.18 (38.17)	7.5 (15.89)	25.33 (30.20)
T_6	1.9 (7.92)	31.57 (34.20)	3.0 (9.97)	33.33 (35.24)	5.3 (13.31)	35.84 (36.75)	7.4 (15.79)	24.32 (29.53)
T_7	2.4 (8.91)	45.83 (42.65)	3.8 (11.24)	47.26 (43.51)	5.8 (13.94)	41.37 (40.05)	7.9 (16.32)	29.11 (32.65)
T_8	2.5 (9.10)	48.00 (43.85)	4.4 (12.11)	54.54 (47.58)	6.5 (14.77)	47.69 (43.68)	8.6 (17.05)	34.88 (36.21)
T_9	2.1 (8.33)	38.09 (38.12)	3.9 (11.39)	48.71 (44.26)	5.8 (13.94)	41.37 (40.05)	7.9 (15.00)	29.11 (32.65)
T_{10}	2.5 (9.10)	48.00 (43.85)	4.2 (11.83)	52.38 (46.38)	6.7 (15.00)	49.25 (44.60)	8.7 (17.15)	35.63 (36.63)
T_{11}	2.3 (8.72)	43.47 (41.27)	4.1 (11.68)	51.21 (45.69)	6.1 (14.30)	42.26 (41.73)	8.2 (16.64)	31.70 (34.27)
T_{12}	3.3 (10.47)	60.60 (51.12)	5.2 (13.18)	61.53 (51.65)	7.4 (15.79)	54.05 (45.06)	9.8 (18.24)	42.85 (40.92)
T_{13}	3.1 (10.14)	58.06 (49.95)	5.4 (13.44)	62.96 (52.54)	7.4 (15.79)	54.05 (45.06)	9.5 (17.95)	41.05 (39.87)
T_{14}	4.0 (11.54)	67.50 (55.24)	6.1 (14.30)	67.21 (55.06)	8.3 (16.74)	59.03 (50.18)	10.9 (19.28)	48.62 (44.20)
T_{15}	1.4 (6.80)	7.14 (15.45)	2.3 (8.72)	13.04 (21.13)	4.0 (11.54)	15.00 (22.76)	6.4 (14.65)	12.5 (20.70)
T_{16}	1.3 (6.55)		2.0 (8.13)		3.4 (10.63)		5.6 (13.69)	
SEm (±)	(0.31)		(0.19)		(0.15)		(0.12)	
CD (P=0.05)	(0.64)		(0.40)		(0.31)		(0.26)	

*Mean of three replications

** Figures in parentheses are angular transformed values

Table 13 : Effect of different treatments on fresh weight and dry weight of tobacco seedlings at 40 DAS.

Treatments	*Fresh weight / plant (g)	Per cent increase over control	* Dry weight / plant (g)	Per cent increase over control
T_1	0.81 (5.13)**	20.98 (27.27)	0.71 (4.80)	21.12 (27.35)
T_2	0.81 (5.13)	20.98 (27.27)	0.71 (4.80)	21.12 (27.35)
T_3	0.86 (5.44)	25.58 (30.48)	0.75 (5.13)	25.33 (30.20)
T_4	0.86 (5.44)	25.58 (30.40)	0.75 (5.13)	25.33 (30.20)
T_5	0.86 (5.44)	25.58 (30.40)	0.75 (5.13)	25.33 (30.20)
T_6	0.84 (5.13)	23.80 (29.20)	0.74 (4.80)	24.32 (29.53)
T_7	0.90 (5.44)	28.88 (32.52)	0.79 (5.13)	29.11 (32.65)
T_8	0.98 (5.74)	34.69 (36.09)	0.86 (5.44)	34.88 (36.21)
T_9	0.90 (5.44)	28.88 (32.52)	0.79 (5.13)	29.11 (32.65)
T_{10}	0.99 (5.74)	35.35 (36.45)	0.87 (5.44)	35.63 (36..63)
T_{11}	0.94 (5.44)	31.91 (34.39)	0.82 (5.13)	31.70 (34.27)
T_{12}	1.12 (6.02)	42.85 (40.86)	0.98 (5.74)	42.85 (40.86)
T_{13}	1.08 (6.02)	40.74 (39.64)	0.95 (5.74)	41.05 (39.82)
T_{14}	1.25 (6.55)	48.80 (44.31)	1.09 (6.02)	48.62 (44.20)
T_{15}	0.73 (4.80)	12.32 (20.53)	0.64 (4.44)	12.5 (20.70)
T_{16}	0.64 (4.44)		0.56 (4.05)	

SEm (±) (0.06) (0.39)
CD (P=0.05) (0.13) (0.79)

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

CHAPTER-V

DISCUSSION

Although tobacco is an important crop which gives high revenue through its commercial cultivation, it is susceptible to many diseases at different stages of its growth mainly starting from nursery stage i.e. at pre-emergence and post emergence stages resulting in the loss of whole crop.

Ramakrishna Iyer (1929) reported for the first time from India, the occurrence of *Pythium aphanidermatum* causing damping-off of seedlings in nurseries. In general, control of soil borne pathogens is very difficult. Hence, the combination of all the management methods, i.e. biological, cultural and chemical were used for significant control.

The results obtained in the present investigation were discussed in detail in this chapter under the following sections.

5.1 RHIZOSPHERE MICROFLORA

The rhizosphere refers to the thin layer adhering to the root after the loose soil and clumps have been removed by shaking. The microbial population and activity in this region is always higher and more complex than in soil away from roots. The rhizosphere microbes have great significance in maintaining the soil fertility and biological control of soil borne plant pathogens. It is well known that, root exudates have a direct influence on the microbial population in the rhizosphere. The fungistatic effect in the rhizosphere against soil borne plant pathogens and root pathogens is believed to be due to these microbes which release certain antibiotics. The metabolic activities of microbes in root region are of vital importance for plant growth and management of soil borne plant pathogens (Balandrean and Knowles, 1978).

In the present study, four fungi and two bacterial isolates were isolated from rhizospere soil of tobacco plants, viz., *A. niger, A. flavus, Penicillum sp.* and *Trichoderma* spp (13 isolates from different soil samples TT_bT-1 to TT_bT-13) and two bacterial isolates (TT_bB-1 and TT_bB-2) (Table 1). Among them, two *Trichoderma* isolates TT_bT-1 and TT_bT-12 were most effective against the pathogen. Krishna Rao and Krishnappa (1996) isolated seventeen species of fungi from rhizosphere of chick pea by employing soil dilution plate technique. These were *Alternaria* sp, *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, Chactorimium* sp., *Cladosporium* sp., *Cunninghamella* sp., *Curvularia* sp., *Fusarium oxysporum* f.sp.,*ciceri*, *P. solani, Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Phoma* sp., and *S. rolfsii*.

Vijay Krishna Kumar (1997) isolated rhizosphere mycoflora from tomato plants viz., *Aspergillus niger, A. flavus, Trichoderma harzianum, Cladosporium fulvum, Penicillium* sp., *Rhizopus sp.,* and *Fusarium* sp. and tested them against *P. aphanidermatum* causal agent of damping-off incidence in tomato. Among them, *T. harzianum* was more effective.

The fungi isolated from rhizosphere of tobacco were found to be the most common fungi occurring in the rhizosphere. The reason for occurrence of microbes in the rhizosphere may be attributed to simple sugars, amino acids and many other compounds which are exuded by the plants and are made available to these microbes.

It is in the rhizosphere that many of the interactions between soil borne pathogens and potential bio-control agents will occur, and hence it is important to understand the behaviour of microbes in this region if one has to manipulate them to reduce the disease incidence.

5.2 *In vitro* SCREENING OF THE RHIZOSPHERE MICROFLORA AGAINST PATHOGEN

The *in vitro* screening of the microflora isolated from tobacco rhizosphere against *Pythium aphanidermatum* revealed that all the antagonists reduced the growth of the test fungus significantly. In terms of per cent growth reduction of test pathogen there were significant differences. There was significant difference between the per cent reduction by *Trichoderma* isolates and other isolates including bacteria (Table 2).

Pathogen was controlled effectively *in vitro* by *Trichoderma* isolates and the antagonist completely over grown on *Pythium* and this could be attributed to the fact that the antagonist had released antibiotics which were inhibitory to the growth of *P. aphanidermatum.* A similar observation was made by Devaki *et al,* (1992) who reported that the volatile and non volatile metabolites produced by *T. harzianum* were both fungistatic and fungicidal to the *P. aphanidermatum.*

The results indicate that *Trichoderma* isolates are destructive mycoparasites on *P. aphanidermatum.* There are reports regarding coiling of *Trichoderma* around *Pythium* hypha. Formation of septa in *Pythium* hypha is attributed to an attempt to prevent the draining out of the cell contents due to lysis (Mukhopadhyay *et al.,* 1986).

Shanmugam and Varma (1998) also reported that among the diversified group of microflora i.e. *Rhizopus, Aspergillus, Trichoderma* and *Eupenicillium,* one actinomycete, *Streptomyces* sp. and four species of bacteria B_1, B_2, B_3 and B_4, *T. viride* was an efficient antagonist against *P. aphanidermatum* which is causal agent of ginger rhizome rot in dual culture.

These results were also similar with the results of Sawant and Mukhopadhyay (1990) who reported that in dual culture experiments, *P. aphanidermatum* colony was completely over grown by *T. harzianum* with in four days of contact. The mechanism of mycoparasitism was by close contact, coiling, penetration and disintegration of the protoplasm.

Thus, *Trichoderma* isolates were proved superior to all other antagonists and therefore selected for further field studies.

5.3 *In vitro* SCREENING OF FUNGICIDES AGAINST *Pythium aphanidermatum*

Five fungicides i.e. mancozeb (Indofil M-45), copper oxy chloride (Blitox 50%WP), metalaxyl MZ (Ridomil MZ 72%WP), chlorothalonil (Kavach 75%WP) and hexaconazole (Contaf 5%EC) at five concentrations i.e. 50, 100, 250, 500 and 1000ppm were screened *in vitro* for their efficacy on *P. aphanidermatum* which is a causal agent of damping-off of tobacco (Table 3). Among these metalaxyl MZ was found effective even from 50ppm onwards and from 100ppm the radial growth of pathogen was inhibited more than 50 per cent and from 500 ppm onwards the radial growth was inhibited more than 80 per cent. These results are in agreement with Jain *et al.* (1984) who found that metalaxyl completely inhibited the growth of *P. aphanidermatum* at 100ppm *in vitro.* Nagarajan and Reddy (1980) also reported that metalaxyl (Ridomil 25WP) is a promising fungicide for the control of both damping-off and leaf blight diseases of tobacco @ 0.1, 0.2 and 0.3 per cent concentrations.

Chlorothalonil has given 15 to 55 per cent reduction in growth of the pathogen over control at 50ppm and at 1000ppm the inhibition increased to 66.66 per cent. Whereas copper oxy chloride has given 88.15 per cent inhibition at 100ppm and complete inhibition at 500 and 1000ppm. Mancozeb has also controlled the pathogen completely at 500 and 1000ppm. Where as hexaconazole reduced the growth of the pathogen completely at 500 and 1000ppm. These results coincide with the reports of Rajib Kumar De and Mukhopadhyay (1994) who reported the fungicides apron (5-25 μg ml^-), captaf (5-25 μg ml^-) and thiram (25-100 μg ml^-) were found to be inhibitory to *P. aphanidermatum.* Apron was found to be the most effective and has completely inhibited the growth of the test fungus.

The results are also similar with Narayana Bhat and Srivastava (2003) who have reported that copper oxy chloride, mancozeb and hexaconazole have completely inhibited the growth of *P. aphanidermatum in vitro* at 500 and 1000ppm concentrations whereas mancozeb inhibited the growth of *Pythium* even at 250ppm concentration.

5.4 COMPATIBILITY OF *Trichoderma* spp. WITH DIFFERENT FUNGICIDES

The effective antagonist *Trichoderma* isolate TT_bT-1 was tested for its compatibility with five fungicides *in vitro* i.e. mancozeb, copper oxy chloride, metalaxyl MZ, chlorothalonil and hexaconazole at 50, 100, 250, 500 and 1000ppm concentrations (Table 4). Among these, metalaxyl MZ has shown less effect on the radial growth and sporulation of *Trichoderma* isolate. Only 18.52 per cent inhibition was recorded even at 1000ppm of metalaxyl.

The copper oxy chloride, mancozeb and chlorothalonil showed 44.44, 76.66 and 59.33 per cent inhibition respectively, over control at 1000ppm. Hexaconazole inhibited the growth even from 50ppm (80 per cent) and from 100ppm it has completely inhibited the growth of *Trichoderma*. These results were supported by findings of Mukhopadhyay *et al.* (1986) who reported that the radial growth of *Trichoderma harzianum* was not effected by metalaxyl at 0.1, 1, 10 and 100ppm concentrations. Similarly, Sawant and Mukhopadhyay (1990) also reported that the radial growth of *T. harzianum* was not inhibited by metalaxyl at 50ppm while at 500 and 1000ppm 19 and 30 per cent inhibition was recorded. Like wise Sharma and Mishra (1995) have found that metalaxyl, chlorothalonil and captafol showed little inhibition of growth and spore germination of *T. harzianum.*

Ramarethinam *et al.* (2001) also reported that mancozeb(75%WP) and copper oxy chloride (88%W/W) did not inhibit the growth of *T. viride* at 100 and 500ppm: however copper oxy chloride completely inhibited at 1000ppm. Fungicides like carbendazim (50%WP), hexaconazole (5%EC) and propiconazole (25%) completely inhibited the growth of *T. viride* even from 100ppm *in vitro*. Narayana Bhat and Srivastava (2003) and Sharma *et al.* (2001) also reported similar observations.

5.5 EFFECT OF SOIL SOLARIZATION ON SOIL TEMPERATURE

Use of clear plastic polyethylene sheet for solarization increased the average maximum soil temperature to 43.83^{0}C where as in unsolarized plots the soil temperature recorded was only 33.63°C. The difference was +10.20^{0}C (Table 5). This data is similar with the results of Abdul Wajid *et al.* (1995) who reported the temperature of solarized beds as 54°C at 5cm depth. Harender Raj *et al.* (1997) reported that soil mulching with polythene resulted in an increase of temperature by 13.5°C at 8 cm depth with an average maximum temperature of 49.7°C. Coelho *et al.* (1999) found that the soil temperature in soil solarized plots reached to a maximum of 47^{0}C at 10 cm depth compared to 41^{0}C at 25 cm. Reddy *et al.* (2001) also reported that soil solarization with clear polythene sheet for 6 weeks during hot summer months showed an increase in soil temperature to 48°C. Kusum Mathur *et al.* (2002) reported that a temperature of 59°C was recorded on the surface of mulched soil, which was 15°C higher than that of non- mulched surface.

5.6 EFFECT OF SOIL SOLARIZATION ON SOIL MOISTURE CONTENT

The average soil moisture content in solarized plots recorded was 10.10 mm in comparision with 4.10 mm in unsolarized plots. The increase in soil moisture content is +6.00 in solarized plots when compared to unsolarized plots at 10 cm depth (Table 6). Reddy *et al*. (2001) reported that soil moisture was conserved 4.5 per cent in solarized plots when compared to unsolarized control.

5.7 EFFECT OF SOIL SOLARIZATION ON SOIL MYCOFLORA

After soil solarization the fungal population has significantly reduced when compared to soil samples of unsolarized beds (Table 6). Stapleton and Devay (1982) reported that population densities of *Agrobacterium* spp., fluorescent pseudomonads, gram positive bacteria and fungi were greatly reduced in solarized soils. Actinomycetes and thermophilic fungi attained higher population densities following soil solarization.

5.8 EFFECT OF SOIL SOLARIZATION ON WEED GROWTH

Due to soil solarization the weed count, and it's fresh weight and dry weight were reduced significantly i.e. 93.41, 98.33 and 98.08 per cent reduction was recorded over unsolarized control plots. The main dominating weed in the plots was *Cyperus* spp (Table 8). Sudha *et al* (1998) reported that soil solarization with transparent polythene sheet for 30 days controlled the weeds effectively. Many weeds belong to the family Graminae were found to be sensitive to soil solarization (Katan, 1981). Nut grass (*Cyperus rotundus*) was partially controlled by soil solarization (Rubin and Benjamin, 1981). Pandey and Pandey (2002) reported that all the dicots were eliminated up to 15 days after the removal of polythene sheet while maximum reduction was observed in *Echinocloa* spp, followed by *Cyperus rotundus.*

5.9 EFFECT OF DIFFERENT TREATMENTS ON GERMINATION PERCENTAGE OF TOBACCO SEEDS

In solarized plots the germination percentage was increased generally. In treatment T_1 (only soil solarization) has given 54.45 per cent germination which was 11.01 per cent more than control. When it was combined with bio-agents (T_7 and T_8), with chemical treatment (T_9 and T_{10}), with organic amendments (T_{11}), with both bio-agent and neem cake (T_{12}), chemical and neem cake (T_{13}) and with all the treatments (T_{14}) the germination percentage increased significantly. The seed treatment with bio-agent (T_2) and both seed treatment and soil application of *Trichoderma* (T_3) increased the germination percentage by 12.68 and 15.23, respectively, over control. However, when these are combined with soil solarization, neem cake, fungicidal seed treatment and drenching it has given more per cent increase of germination percentage over control i.e. 43.33 per cent. Application of neem cake individually (T_6) did not increase the germination percentage significantly but when it was combined with other treatments it has shown its synergistic effect (Table 9). These findings were in agreement with the reports of Abdul Wajid *et al*. (1995) who proposed the best schedule for integrated disease management in FCV tobacco nurseries. Solarization for six weeks from February to March + soil amended with neem cake @ 400 g/m + foliar spray of metalaxyl @ 0.2 per cent at 30DAS yielded maximum healthy transplants.

Patel *et al.* (1996) also reported integration of solarization +metalaxyl MZ @ 2.16 Kg/ha yielded maximum number of transplantable seedlings in bidi tobacco. Similarly, Narayana Swamy *et al.* (2002) also reported that integration of soil solarization four weeks along with incorporation of neem cake @ 400 g/m before sowing in addition to the need based spray of metalaxyl MZ 72 WP @ 0.2% at 30DAS has significantly increased the seedling emergence.

Similarly, Krishnamoorthy (1987) reported the higher germination percentage of tomato seeds in the treatment comprising of soil amendment with neem cake (25t/ha) + seed treatment with *T. viride.*

5.10 EFFECT OF DIFFERENT TREATMENTS ON PER CENT DISEASE INCIDENCE IN TOBACCO NURSERIES

According to the data in table-9 the cumulative per cent disease incidence at 40 DAS was decreased significantly in integrated treatment (T_{14}) in which soil solarization , seed treatment with bio-agent (4g/Kg seed), soil application of bio-agent (50g/bed), seed treatment with metalaxyl MZ (3g/lit/m^2 and neem cake 9500g/ bed) were used in combination. The per cent decrease of incidence in T_{14} was 87.77 over control. Treatments T_{13} and T_{12} in which bio-agent and fungicide application were excluded, respectively, have also reduced the disease incidence by 83.48 and 81.65 per cent respectively, over control. Soil solarization (T_1) alone reduced the disease incidence by 68.07 per cent. Individual applications of bio-agents (T_2,T_3) and fungicide (T_4,T_5) and also integration of T_1 with bio-agent (T_7,T_8); with fungicide (T_9,T_{10}) and with T_6 have given almost similar reduction in disease incidence over control. Lowest reduction of disease was recorded in T_6 treatment i.e. only 55.65 per cent over control. Mukhopadhyay *et al*. (1986) reported that integration of seed treatment with metalaxyl (3% slurry) and application *T. harzianum* (50g/Kg soil) gave an excellent control of the damping-off disease of tobacco. Abdul Wajid *et al.* (1995) reported that soil solarization for six weeks + neem cake @ 400g/m^2 + foliar spray of metalaxyl (Ridomil MZ) @ 0.2% at 30DAS gave 81.3 to 95.5 per cent control of disease in FCV tobacco nurseries. Lakra (2003) reported that incidence of post-emergence damping-off was minimum (5.5%) when seed and soil were treated with captan (4g/Kg seed and 5g/m^2, respectively) followed by seed + soil treatment with *T.viride* @ 4g/Kg seed and 5g/m^2 (13.5%) when compared to control (42.6%).

Data represented in table-9 indicated that the disease was reduced up to 30DAS and again it increased from 35DAS. This is mostly due to the no application of bio-agent or fungicide after sowing. Shenoi *et al*. (1993) reported that there was sudden build up in disease pressure after 30 DAS which indicated the limited efficacy of soil solarization and neem cake, and the necessity of using chemical fungicides like metalaxyl MZ (Ridomil MZ) subsequently for management of damping-off. They also reported that application of neem cake @ 400g/m^2 + solarization for six weeks + metalaxyl MZ (Ridomil MZ) foliar sprays of 0.2% at 25 and 40DAS has significantly reduced the disease even at 40DAS (99 per cent) where as at 25 and 30DAS hundred per cent reduction of disease was recorded.

5.10 EFFECT OF DIFFERENT TREATMENTS ON PLANT HEIGHT

In all the treatments the root length, shoot length and plant height significantly increased over control. In integrated treatment (T_{14}) the plant height increased by 67.5, 67.21, 59.03 and 48.62 at 10, 20, 30 and 40DAS, respectively, over control. Next best treatments were T_{12} and T_{13} in which the plant height was increased by 42.85 and 41.05 per cent over control at 40DAS (Table 12). Sreeramulu *et al.* (1998) reported similar results that plant height was 17.9cm in the treatment *Glomus fasciculatum* + *T. harzianum* where as in control it was only 11.3cm. Further, they also noted that root length was 6.5cm in integrated treatment where as in control it was 3.2cm.

5.11 EFFECT OF DIFFERENT TREATMENTS ON FRESH WEIGHT AND DRY WEIGHT OF TOBACCO SEEDLINGS

The fresh weight and dry weight of seedlings also increased significantly over control in all the treatments. In integrated treatment (T_{14}) 48.8 and 48.62 per cent increase was recorded over control regarding fresh weight and dry weight, respectively (Table 13). Next best treatments were T_{12}

and T_{13} where 42.85 and 40.74; 42.85 and 41.05 per cent increase over control were recorded with respect to fresh weight and dry weight. These results are similar to that of Windham *et al.* (1986) who reported that addition of *Trichoderma* sp. to autoclaved soil increased the root and shoot dry weights of tomato (213 to 275%) and tobacco (259 to 318%) over control, eight weeks after planting.

CHAPTER – VI

SUMMARY

The current investigations on tobacco nurseries were carried out to find out the best method to control damping-off disease caused by *Pythium aphanidermatum* by using effective bio-agent (*Trichoderma* sp.) (4g/kg seed and 100g/m^2) effective chemical, metalaxyl (Ridomil MZ 72% WP) (3g/kg seed and 3g/lit/m^2), soil solarization (6 weeks) and neem cake (1kg/m^2) individually and in combination. The effective bio-agent and effective chemical were identified *in vitro* conditions by using dual culture technique and poisoned food technique, respectively.

In *in vitro* conditions, all the soil microflora which were isolated from rhizosphere were tested against *Pythium aphanidermatum.* Among all the soil microflora *Trichoderma* isolates (TT_bT-1 to TT_bT-13) have given more per cent inhibition of the pathogen (> 70 per cent).The isolate TT_bT-1 has shown 94.44 percent inhibition of the pathogen over control. Next best isolate was TT_bT-12 which has shown 92.60 per cent inhibition. *Aspergillus niger* has also shown 61.6 per cent inhibition over control. Among the two bacterial isolates, TT_bB-1 has shown 66.66 percent inhibition over control.

The effective fungicide was identified *in vitro* using poisoned food technique. Out of five fungicides tested i.e. mancozeb (75% WP), copper oxy

chloride (50% WP), metalaxyl MZ (72% WP), chlorothalonil (75% WP) and hexaconazole (5% EC), metalaxyl MZ shown more per cent inhibition over control from 50 ppm onwards. Where as mancozeb and hexaconazole inhibited the radial growth of the pathogen completely at 500 and 1000 ppm concentrations and copper oxy chloride shown complete inhibition even from 250 ppm. Chlorothalonil inhibited the radial growth of the pathogen 66.66 per cent over control at 1000 ppm. Regarding compatibility of fungicides with *Trichoderma* sp. metalaxyl MZ has inhibited the radial growth by 18.52 per cent over control even at 1000 ppm. Where as mancozeb and copper oxy chloride inhibited the radial growth of *Trichoderma* sp. by 76.66 and 44.44 per cent respectively, over control at 1000 ppm. Hexaconazole has completely inhibited the growth even at100 ppm concentration.

In field conditions, soil solarization was done with a clear plastic polyethylene sheet for six weeks in the hot summer months. The temperature and moisture content of solarized soils at 10 cm depth increased to 43.83^{o}C with a soil moisture of 10.10 mm, respectively where as in un-solarized soils a temperature of 33.63^{o}C and 4.10 mm soil moisture were recorded. Due to soil solarization weed population, fresh weight and dry weight of weeds were reduced by more than 90 per cent over un-solarized soils. The soil mycoflora also reduced by more than 50 per cent due to soil solarization.

In nursery beds different treatments viz., bio-agent, fungicide, neem cake and soil solarization either alone or in combination were imposed to see the effect on germination percentage of tobacco seeds, disease incidence, plant height, fresh weight and dry weight of seedlings. Among all the treatments, the integrated treatment (T_{14}) consisting of soil solarization for six weeks, neem cake (1 kg/m^2) seed and soil application of bio-agent TT_bT-1 isolate of *Trichoderma* spp. (4g/kg seed+3g/lit/m^2), seed and soil drenching of metalaxyl MZ (3g/Kg seed + 3g/l/m^2) was the best one in which 85.5 per cent germination, 5.54 per cent disease incidence, 3.20 per cent disease growth rate, 1.25g of fresh weight and 1.09 g of dry weight were recorded. Where as the bio-agent, fungicide, neem cake and soil solarization applied individually also given increased germination percentage, decrease in disease incidence and disease growth rate but it was much less when compared to combined use. When the soil solarization was integrated with bio-agent (T_{12}) and fungicide (T_{13}) individually, the results were almost similar in both cases.

Hence, the conclusion for this study is the integrated use of biological, physical, chemical and cultural practices is the best method for controlling a soil borne disease i.e. damping-off tobacco rather than the individual application.

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