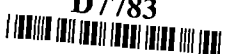


**INTEGRATED MANAGEMENT OF ROOT ROT DISEASE
IN ACID LIME (*Citrus aurantifolia* Swingle)**

BY
B. GOVINDA RAJULU
M.Sc.(Ag.)

**THESIS SUBMITTED TO THE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF**

**DOCTOR OF PHILOSOPHY
IN THE FACULTY OF AGRICULTURE**

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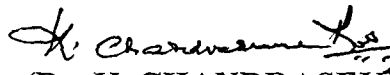
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Mr B. GOVINDA RAJULU, has satisfactorily prosecuted the course of research and that the thesis entitled "**INTEGRATED MANAGEMENT OF ROOT ROT DISEASE IN ACID LIME (*Citrus aurantifolia* Swingle)**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any University.

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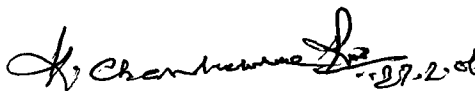
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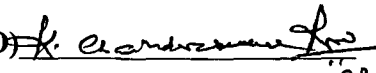
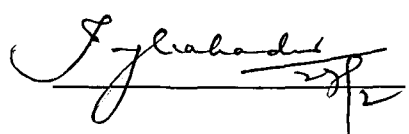
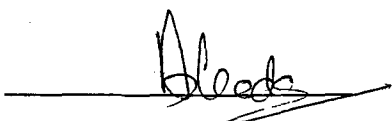
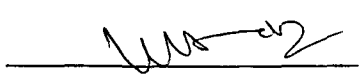
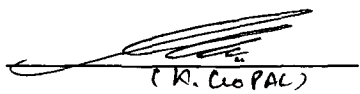
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This is to certify that the thesis entitled "**INTEGRATED MANAGEMENT OF ROOT ROT DISEASE IN ACID LIME (*Citrus aurantifolia* Swingle)**" submitted in partial fulfilment of the requirements for the degree of 'Doctor of Philosophy in Agriculture' of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Mr B. GOVINDARAJULU 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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LIST OF SYMBOLS AND ABBREVIATIONS

%	-	percentage
µg	-	micro gram
@	-	at the rate of
a.i.	-	active ingredient
AICRP	-	All India Co-ordinated Research Project
CBR	-	Cost benefit ratio
°C	-	Degree celsius
cfu	-	colony forming units
cm	-	centimeter
DAI	-	days after incubation
DAS	-	days after sowing
dSm ⁻¹	-	Deci siemen per meter
Fig.	-	Figure
g	-	gram
h	-	hour
ha	-	hectare
i.e.,	-	that is
kg	-	kilogram
l	-	liter
m ²	-	square meter
mg	-	milli gram
ml	-	milliliter
mm	-	millimeter
no.	-	number
ppm	-	parts per million
psi	-	pressure per square inch
viz.	-	namely

ACKNOWLEDGEMENTS

I earnestly revere the Lord Sri Venkateswara for his boundless blessings, and accompanied me in all endeavours.

I humbly express my profound sense of gratitude to my Major Advisor and Chairman of the Advisory Committee Dr. K. Chandrasekhara Rao, Director of Extension, ANGRA Hyderabad for his noble hearted help, valuable guidance, constructive criticism and constant encouragement throughout the course of investigation and presentation of this thesis. I deem it a privilege to have worked under him and gratefully acknowledge his keen insight into technological research.

It gives me greatest pleasure to express my sincere thanks to Dr. T.B. Gour, Principal Scientist, Entomology (Retd.), AICRP on Crop Residues, Rajendranagar, Hyderabad and member of Advisory Committee for his meticulous reasoning to refine this thesis.

I am indeed greatly indebted and thankful to Dr. B. Chenchu Reddy, Associate Director, S.V. Agricultural College, Tirupati and member of my Advisory Committee for his valuable constructive suggestions, encouragement and guidance during the execution of research work.

I am also thankful to Dr. K. Haribabu, Principal Scientist (Horticulture), Agricultural Research Station, Anantharajupet and member of my Advisory Committee for his valuable guidance and timely suggestions during the course of study.

I wish to proffer my genuine thanks to Dr. K. Gopal, Senior Scientist, Plant Pathology, AICRP on citrus, Tirupati and member of my Advisory Committee for his scholarly guidance, identification of organisms and for extending necessary lab facilities during the course of research work.

I humbly express my sincere thanks to Dr. T. Vithal Reddy, Professor and Head, Department of Plant Pathology, College of Agriculture, Rajendranagar for his valuable suggestions at each and every stage of my study.

I always remember the help of Dr. D. Raja Ram Reddy, Professor, Dr. C. Pramesh Chandra Kumar, Professor, Department of Plant Pathology for their kind cooperation in the preparation of manuscript.

I would like to express my heartfelt thanks to Dr. R. Ravindra Babu, Professor, Dr. P. Narayana Reddy, Professor, Dr. V. Krishna Rao, Professor, Department of Plant Pathology for their help during the course of this research work.

Thanks are also due to Dr. B.S. Kulkarni, Professor and Head, Department of Statistics, College of Agriculture, Rajendranagar and Dr. D. Munirathnam, Senior Scientist, RARS, Tirupati for their help in statistical analysis of the experimental data.

I am grateful to Dr. A. Padma Raju, Director of Research (Retd.) and Dr. P. Raghava Reddy, the present Director of Research, ANGRAU, Rajendranagar, Hyderabad for providing facilities and valuable suggestions during course of investigation. I also thank Dr. P. V. Reddy, Associate Director of Research, Regional Agricultural Research Station, Tirupati for providing facilities and guidance during the course of investigation.

I extend my deep sense of reverence and gratitude to Dr. K.V. Seshadri, Principal Scientist (Horticulture) and also to all the staff members of Citrus Research Station, Petlur for their help and co-operation during the course of my investigation.

From the inner core of my heart, I express my gratitude and special thanks to my seniors Dr. R. Jagadeeshwar, Dr. Krishna Prasadji, Dr. Bhagawan, Dr. Padmodaya, Dr. Sudhakar for their help and constant encouragement during course of my study.

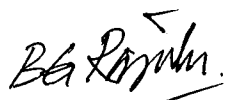
Diction is enough to express my affectionate gratitude and heartfelt regards to my beloved parents who constantly inspired, guided and moulded me to the present position.

I am in dearth of words to express my feelings towards my uncle Dr. K.V. Satyanarayana and aunt Dr. Smt. K. Radha Rani for their unparallel affection, moral support and persistent encouragement.

I affectionately acknowledge the cooperation and encouragement of my wife Smt. B. Vijaya and my beloved children Vinisha and Sai Vishwant during the course of my research work and presentation of thesis.

The whole hearted encouragement, cooperation and splendid company extended by my dear friends Dr. Khayum, Dr. Swaroop, Sri Subramanyam Reddy, Gopi, Seenu, Sudha, Madhu, Anand Ram, Hemanth Kumar and Krishnam Raju.

I would like to express my special thanks to Mr. Raju Graphics, Mr. Muni Yadav and Mr. Srikanth for neat laser typeset of the thesis.


(B. GOVINDA RAJULU)

DECLARATION

I, **B. GOVINDARAJULU**, hereby declare that the thesis entitled "**INTEGRATED MANAGEMENT OF ROOT ROT DISEASE IN ACID LIME (*Citrus aurantifolia* Swingle)**" submitted to Acharya N.G. Ranga Agricultural University, Hyderabad for the degree of **Doctor of Philosophy in Agriculture** is a result of original research work done by me. I also declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

Place : Hyderabad

Date :


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Faculty	:	AGRICULTURE
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Year of submission	:	2006

ABSTRACT

Studies on “Integrated management of root rot disease in acid lime (*Citrus aurantifolia* Swingle)” was carried out at Citrus Research Station, Petlur and at All India Co-ordinated Research Project (AICRP) on Tropical Fruits (Citrus), Tirupati, Andhra Pradesh (A.P) to ascertain the disease incidence in acid lime growing areas of the state and its management through integrated disease management strategies both in nursery and main field.

A systematic survey on the occurrence of root rot disease associated with acid lime was carried out in Chittoor, Nellore, Kadapa, Guntur and West Godavari districts of A.P. The disease incidence varied with area and it was in the range of 10.21 to 22.95 per cent. Among the five districts surveyed, maximum per cent of disease incidence was recorded in Nellore (20.25) followed by Kadapa (18.6), Chittoor (15.61) Guntur (13.7) and West Godavari (11.98). When the type of acid lime planting material was considered, seedlings showed low disease incidence (15.39%) than budlings (19.00%). Acid lime plantations raised on shallow soils were more prone to disease incidence (19.46%) than plantations raised on deep soils (12.8%). Limited irrigation contributed to higher disease incidence (20.66%) when compared to acid lime plantations with optimum irrigations (13.94%). Regarding water quality on incidence of root rot disease in the acid lime orchards, irrigated water with normal E.C showed less root rot disease incidence (14.51%) than the water with high E.C (35.08%). With respect to methods of irrigation, lowest disease incidence was recorded in basin irrigated acid lime plantations (15.84) than flood irrigation method (25.46%). With regard to effect of fertilizers,

inorganic fertilizer applied acid lime plantations showed high disease incidence (22.0%) than the organic fertilizer used ones (15.14%).

Pathogenicity tests revealed that maximum disease incidence (79.7%) was noticed when 8.0 per cent inoculum density was used. Among the 15 isolates of *Fusarium solani* isolated from different areas, PCF₂ isolate was categorized as extremely aggressive with maximum disease incidence (83.3%) and it was further confirmed as *Fusarium solani* (Mart.) Sacc. (ITCC 5742) at IARI, New Delhi.

With respect to isolation of native bioagents from the acid lime rhizosphere, eight non *Trichoderma* fungi on PDA, twelve *Trichoderma viride* isolates on *Trichoderma* selective medium (TSM) and three bacterial isolates on nutrient agar medium were isolated and found that *T. viride* isolates exhibited inhibitory activity against the pathogen *F. solani* (PCF₂). Among the twelve native and three commercial *T. viride* isolates, PCT₆ isolate grew 78.3 mm within 48 h and categorized as very fast growing. In morphological and phenotypic characters all the isolates have shown more or less similar characters. In dual culture technique *T. viride* (PCT₆) inhibited the test pathogen *F. solani* more effectively (73.6%) than the others. The PCT₆ isolate was further confirmed as *Trichoderma viride* Pers. (ITCC 5740) at IARI, New Delhi.

Among the six substrates tested for mass multiplication of the potential native *T. viride* isolate (PCT₆), neem cake supported maximum population (20.7×10^5 cfu/g) followed by pressmud (19.7×10^5 cfu/g) and farm yard manure (18.5×10^5 cfu/g).

Carbendazim and mancozeb at 100, 500 and 1000 ppm concentrations completely inhibited the growth of the test fungus. In the compatibility studies, mancozeb was selected for further studies in management of *F. solani* (PCF₂) as it was found very effective against test pathogen and safe to the native bioagent *T. viride* (PCT₆) up to 500 ppm.

The experiment taken up in the nursery showed that soil solarization increased soil temperature up to 12°C and retained 4 per cent moisture besides reducing the weeds (88.9%) and population density of *F. solani* (81.66%). Among the 12 treatments, the treatment involving *T. viride* (PCT₆) (100g) in combination with FYM (2.5 kg) and mancozeb (0.2%) recorded highest seedlings emergence (96.9 %) with least root rot incidence (4.0%) compared to control (70.1% seedling emergence with 68.9% disease incidence). With respect to Cost - Benefit ratio (CBR), the treatment combination of *T. viride* (PCT₆), FYM and mancozeb showed highest CBR 1 : 35 followed by the treatment combination of *T. viride* (PCT₆), FYM and neem cake (1 : 32).

In the field experiment, combined application of *T. viride* (PCT₆) @ 200 g + FYM @ 10 kg + neem cake @ 1 kg + mancozeb @ 0.3% per tree twice during the months of June and September reduced the rotting of roots by 78.3 per cent and recorded 100 per cent survival of acid lime trees.

INTRODUCTION

CHAPTER I

INTRODUCTION

Citrus is one of the important group of fruit crops grown throughout the world and is grown in more than 50 countries. It is one of the choicest fruits having high consumer preference both as fresh fruit as well as for its refreshing processed juice. Globally a gradual increase in production of citrus occurred since 1985.

Citrus occupies an important place in the horticultural wealth and economy of India as the third largest fruit industry after mango and banana, grown in an area of 5.66 lakh ha with a total production of 56.77 lakh tonnes and productivity of 10.1 tonnes/ha. (Singh, 2001). The area under citrus in A.P during 2003-04 has been estimated as one lakh hectares with production of 10.17 lakh tonnes, ranking second in area and production after Maharashtra (Directorate of Economics and Statistics, 2004).

In A.P the important commercial citrus fruits are acid lime (Kagazilime) and sweet orange (Sathgudi). Acid lime (*Citrus aurantifolia* Swingle) occupies first place both in area (0.37 lakh ha) and production (2.67 lakh tonnes) in state (Centre for monitoring Indian Economy, 2005).

The area under acid lime cultivation is increasing year after year at faster rate disproportionate to the production and productivity. The main factors of acid lime decline which are impeding the production are diseases, shallow calcareous soils, poor quality irrigated water and imbalanced nutrition. Among the diseases responsible for acid lime decline, the root rot is the major constraint affecting both

life span and production under field conditions resulting in substantial losses (Rs. 20 millions). Ramakrishnan (1954) stated that the root rot (*Fusarium* spp., *Macrophomina* spp. and *Diplodia* spp.) was common in citrus in North Arcot district of Tamil Nadu and the neighbouring Chittoor and Kadapa districts of A.P. Reddy and Paparao (1960) observed the root rot on sweet orange and acid lime caused by *Fusarium* spp., *Rhizoctonia* spp. and *Diplodia* spp. and documented their individual and cumulative effects on the disease incidence. Bender *et al.* (1982) reported dry root rot of citrus seedlings due to *Fusarium solani*. The disease occurred both in nursery and mainfield (Gopal *et al.*, 2000).

Management of root rot of acid lime with chemicals under field conditions is however much more difficult because the fungus occurs systemically throughout the root system of mature trees. Several disease management methods such as physical, biological and chemical proved to be effective against soil borne pathogens. Sharma and Mishra (1995) reported that *Trichoderma* spp. have gained importance as sole application or integrated with lower doses of fungicides for the management of soil borne plant pathogens. Management of this pathogen (*F. solani*) is difficult through application of fungicides as it has wide host range. Though drenching of carbendazim (0.1%) followed by another drenching of mancozeb (0.25%) or chlorothalonil (0.2%) was found effective (Satyanarayana and Reddy, 1994), it is uneconomical with short time gain besides its effect on soil microflora and fauna and pollute the soil and ecosystem in general. In view of the occurrence of the disease (15-20%) and economic loss caused (Rs. 20 millions) and high cost of fungicidal control with unquantifiable harmful effects on

ecosystem, most of the acid lime growers who are small and marginal farmers in semi arid tropics of the state cannot bear the huge cost of production. The present investigation on integrated disease management strategies which are most effective in the long run with environmentally sound sustainable horticultural systems were planned with the following objectives.

1. To survey the occurrence of root rot disease in acid lime growing areas of Andhra Pradesh.
2. Isolation and identification of organism associated with root rot of acid lime.
3. Isolation of fungi and bacteria from the *rhizosphere*.
4. *In vitro* screening of native fungal, bacterial isolates and commercial bio-control agents against root rot pathogen of acid lime to identify potential bio-control agents.
5. Screening of substrates for mass multiplication of potential bio-control agents.
6. Screening of fungicides and natural products against root rot pathogen *in vitro*.
7. To test the compatibility of fungicides and natural products with the potential bio-control agents.
8. To develop an integrated approach for the management of root rot disease in nursery and grown-up trees of acid lime.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Scanning of relevant literature revealed that very meagre information is available on root rot disease of citrus and other fruit crops from India as well as from other countries and hence, related literature on other crops is also presented so as to understand the work carried out on this subject.

2.1 SURVEY AND OCCURRENCE

Fawcett (1936) reported the association of *Rhizoctonia*, *Pythium* and *Phytophthora* as a cause of damping-off and dry root-rot of citrus from California.

Ramakrishnan (1954) for the first time observed the occurrence of dry root-rot from India on citrus in Tamil Nadu, Andhra Pradesh (A.P) and Karnataka and stated that it was due to rotting of roots caused by *Diplodia natalensis* and *Fusarium solani*.

Srivastava and Singh (1954) reported severe seedling root-rot of grape fruit (*C. paradisi*) caused by *Pythium debaryanum* and *Rhizoctonia solani* from India.

Rangacharulu *et al.* (1958) studied the effect of certain root stocks on Sathgudi sweet orange and found that budlings on sour orange, herale and lemon were highly susceptible to dry root-rot and declined suddenly due to unknown causes.

Reddy and Paparao (1960) observed the root rot on sweet orange and acidlime caused by *Fusarium*, *Rhizoctonia* and *Diplodia* spp. and studied their

individual and cumulative effects on the incidence of dry root-rot in major citrus growing areas of Andhra Pradesh.

Young citrus plants in the nurseries in Punjab were subjected to wilt and root-rot incited by *Fusarium*, *Rhizoctonia* and *Verticillium* (Bedi, 1961).

Lele *et al.* (1969) reported mortality of Citrus species due to *Rhizoctonia*, *Diplodia* and *Fusarium* spp. in a fairly large populations of six to eighteen months old plants of Trifoliate orange (*Poncirus trifoliata*) Rafinesque and its hybrids (*Carrizo citrange*), *Troyer citrange* and *Rubidouie* spp. grown as root stock.

Suhag (1976) observed the serious decline of guava trees due to root rot for the first time in Narnaund of Hissar district in Haryana state. Timmer *et al.* (1979) reported the serious wilt and die back of green house Mexican lime (*Citrus aurantifolia*) seedlings in Florida. Symptoms on *Citrus excelsa* were similar to those on Mexican lime, but symptoms on Rangapur lime were less pronounced when the pathogen (*Fusarium solani*) was inoculated.

Troutman *et al.* (1979) observed root-rot in 15 to 20 year old Citrus groves where rough lemon (*Citrus jambhiri*) was used as the rootstock. Omprakash and Singh (1980) observed wide spread root-rot and damping off of mango seedlings in cultivar of Dashehari at Central Mango Research Station, Rehmankhera, Lucknow.

Vanderweyen and Serrhini (1981) reported dry root-rot of sour orange seedlings caused by *Fusarium* spp. from Morocco. Bender *et al.* (1982) reported dry root rot as a destructive disease in California and it has also been reported from citrus growing areas in Arizona, Puerto Rico and South Africa. In California, dry

root rot caused by (*F. solani*) was most commonly seen in the Northern and Coastal citrus growing countries, although an out break of the disease has been reported from Coachella valley. The disease trends to be more prevalent in mature groves which are at the peak of fruit production.

Lele and Kapoor (1982) reported different *Phytophthora* spp. as the cause of citrus root rot from peninsular India. Lio *et al.* (1983) observed occurrence of various phytopathogenic fungi, viz., *Fusarium solani*, *F. equisetii*, *F. oxysporum*, *Phytophthora citrophthora* and *P. nicotianae* for the cause of root-rot in the nursery of sour oranges in Sicily.

Laville and Vogel (1984) investigated the reaction of different rootstocks to root-rot causing fungi and observed that *Poncirus trifoliata* was resistant to *Phytophthora*.

Kore and Patil (1985) reported dry root rot disease in mango seedlings at College of Horticulture, Parbhani and stated that the disease was 60 per cent in stone grafted seedlings and 20 per cent in seedlings.

Graham *et al* (1985) reported that *F. solani* caused root necrosis when starch reserves in the root were reduced and infection occurs after the tree had been subjected to stress. Blaker and McDonald (1986) observed that soil salinity enhanced the root-rot in citrus nurseries of California.

Wehner *et al* (1986) surveyed four registered citrus nurseries in the Transvaal and three in the Eastern cape in South Africa for the occurrence of

fungal root pathogens. *F. solani* and *F. oxysporum* were present in all the nurseries examined.

Malikoutsaki *et al* (1987) reported severe dry rot and collar rot of citrus plants as well as seedlings by *Fusarium* spp. from Greece. Kore and Mashalkar (1987) reported that dry root-rot of sapota grafts caused by *Fusarium solani* at Parbhani was to the tune of 40 and 50 per cent respectively.

Wutscher and Bistline (1988) reported from Southern Florida the performance of Hamilin orange on 30 Citrus root-stocks to *Phytophthora* root-rot *Poncirus trifoliata*, Trifoliolate hybrids and Tangelo (*C. paradisi* x *C. reticulata*) root stocks of Citrus survived better than on mandarin (*C. reticulata*) and sour orange (*C. aurantium*) root-stocks.

Labuschagne *et al* (1989) confirmed that interaction between *F. solani* and *Tylenchulus semipenetrans* on citrus roots influenced the development of root rot disease. They also demonstrated the interaction under simulated field conditions and found that combination of the two pathogens significantly reduced plant height in green house by 14.5 per cent and the increase in stem diameter in the field by 12.5 per cent in comparison with controls.

Jayachandra (1990) conducted a detailed survey on the incidence of dry root rot in Sathgudi sweet orange in Ananthapur, Kadapa and Kurnool districts of Rayalaseema in A.P and the results revealed that dry root rot was more prevalent in bearing orchards than to Sathgudi sweet orange trees budded on Jambheri (Rough lemon) root stock were more severely affected 1-30 per cent disease incidence recorded than Rangpur lime root stock.

Kore and Mane (1992) observed that dry root-rot of Kagzilime seedlings caused by *F. solani* from Parbhani region of Maharashtra and stated that 20 per cent of soil moisture and half dose of N.P.K. favoured the disease development. The pathogen was also found to infect sweet orange, urd bean, brinjal, sponge gourd, chilli, tomato, mango and ladys finger seedlings besides its own hosts.

Prakasam *et al.* (1992) reported the occurrence of dry root-rot disease in mandarin orange (*Citrus reticulata*) in Shevroy hills, caused by *Fusarium* sp. Rathore *et al.* (1993) recorded 55-60% disease incidence in seedlings of *Citrus aurantifolia* in nurseries growing under shade following irrigation in August and September.

Ansar *et al.* (1994) reported *F. solani* as the cause of citrus quick decline in Pakistan. Reddy *et al.* (1999) reported that dry root rot was major disease in both nursery and main field affecting life span and production of acid lime in Andhra Pradesh and the disease incidence ranged from 5 to 75 per cent.

Verma and Singh (1999) observed severe incidence of dry root rot resulting in mass mortality of young seedlings of commercial citrus root stocks Jettikhatti (*Citrus jambheri*), Pectiniferra (*C. depressa*) and Cleopatra (*C. reshini*) for the first time in Punjab, India and the causal organism was identified as *Fusarium solani*.

Gopal *et al.* (2000) reported that the dry root-rot was the major disease responsible for citrus decline in most of the citrus growing areas in A.P. especially

Anathapur, Prakasam, Kurnool, Mahaboobnagar, Cuddapah, Nalgonda and Karimnagar districts.

Studies conducted by Derick and Timmer (2000) under field conditions revealed that dry root rot caused sudden death of citrus plants in soils having high clay content, excess moisture and poor aeration.

2.1.1 Symptomatology

Lele *et al.* (1969) reported that the root system gradually withered and dried in trifoliate orange (*Poncirus trifoliata*) plants affected by dry root-rot and observed the moist decay of the bark in the process of peeling and slimy decay that affected main roots near the crown and below the soil level. The exposed cortical tissues showed a deep and dark brown stain bearing black irregular streaks on the surface. Severely affected plants showed shredding of bark with acute drying. Yellowing and shredding of leaves observed in later stages.

Timmer *et al.* (1979) observed wilt and dieback of green house grown Mexican lime (*Citrus aurantifolia*) seedlings for several years in Florida and expressed early symptoms as reticulate chlorosis and epinasty of the young leaves followed by wilting, leaf abscission, shoot dieback and gum exudation. They also observed that initial symptoms are sectorial then the dieback progress rapidly and plants seldom die with in 4-6 weeks.

Nemec *et al.* (1980) described symptoms due to *Fusarium solani* causing dry rot of citrus seedlings and observed dull green dehydrating leaves leading to severe leaf wilt followed by desiccation and leaf drop. Underground roots of infected seedlings showed vessel plugging and rotting of secondary fibrous roots.

Bender *et al.* (1982) observed that the citrus trees affected with dry root-rot showed moist, dark decay in the bark, which later became cracked in appearance. The wood below dead bark was hard and dry with a brownish grey stain. The disease persists in the wood for many years with slight wilting symptoms under dry conditions. However, in later stages the leaves turned yellow leading to the death of the trees.

Kore and Patil (1985) described initial and advanced symptoms of dry root rot of mango seedlings caused by *F. solani*. In initial stages of the disease, the colour of young leaves became light green and finally light yellow and successfully progress other leaves from tip downwards. In advanced stage leaves become shriveled, dried, dropped down and finally entire plant dries. The tap root and base of stem showed development of brown to blackish colour either uniformly or in streaks along the bark of the stem.

Rajput and Sriharibabu (1985) reported that the dry root-rot of citrus was characterized by moist decay of bark in early stages. At later stages, the bark became dry and shredding exposing the hard dead wood underneath. Foliage became yellow and later dropped down rendering the twigs bare. The affected twigs flowered profusely and bear a heavy crop of small sized fruits which died prematurely. The infected roots emitted foul odour.

Reddy and Murti (1985) described the symptoms as moist decay of the bark in the early stages, followed by drying and shredding of the bark leaving hard dead wood underneath in later stages. The affected roots emitted bad odour and the leaves became yellow followed by dropping of foliage. Increased flowering,

heavy crop of small sized fruits and death of the plant were the other characters associated with the disease.

Prakasam *et al.* (1992) noticed that mandarin orange trees showed yellowing of leaves followed by withering and drying of branches. The root portions also exhibited extensive rotting. The infected trees produced large crop of small sized fruits and extensive premature shedding of fruits was also noticed.

Kore and Mane (1992) observed symptoms of dry root rot disease of Kagzilime seedlings (*Citrus aurantifolia* Swingle) incited by *F. solani*, as light green to light yellow colour of younger leaves which dried and dropped down later. Secondary roots were completely rotted shredding of bark of main roots with blackish brown streaks.

Sathyanarayana and Reddy (1994) mentioned that sweet orange trees on Jambheri rootstock are more susceptible to dry root rot than those on the Rangpurlime rootstock. The symptoms are characterized by wilting, drooping and yellowing of foliage. Moist decay of root bark in early stages and a dry shredded condition in later stages. Affected roots emits bad odour. Excessive flowering, heavy crop of small-sized fruit and ultimate death of affected trees takes place in last stages.

Verma and Navtej Singh (1999) reported the typical symptoms of root rot of citrus seedlings in nurseries are characterized by yellowing, inward and upward rolling and drying of leaves followed by severe wilting and premature leaf drop. The infection on the underground roots of the infected seedlings exhibit discoloration, rotting and detachment of secondary fibrous roots.

2.2 ETIOLOGY

Roberts and Kraft (1971) observed symptoms after a 10-day incubation period in rapid pathogenicity test and the results were similar to those obtained in the green house after six weeks in pea.

Menge *et al.* (1980) showed that *F. solani* (Mart.) Sacc. a fungus that normally inhabits citrus soil was the most commonly isolated organism from diseased wood.

Bender *et al.* (1982) isolated *F. solani* in all the isolations made from stained wood of dry root rot of citrus trees. The fungal isolates appeared visually in various colours (pinkish-orange to violet) growth rate and spore production.

Chandra *et al.* (1983) studied three isolates of *F. oxysporum* and seven isolates of *F. solani* of tomato and reported that the isolates within species differed in their potentiality to cause pre or post emergence mortality of seedlings and induced wilt in matured plants.

Morgan and Timmer (1984) inoculated Mexican lime seedlings with 0, 500, 1000, 2000, 4000 and 8000 microconidia of *Fusarium oxysporum* f.sp. *citri* per gram of potting media. The percent infection and mean disease severity rating increased with increasing inoculum density of the pathogen.

Kore and Patel (1985) tried seven soil inoculation techniques for standardizing inoculation technique and the results indicated that the disease

development was best when soil (sand + soil + FYM) inoculations made eight days prior to transplanting.

Etiological studies conducted by Wehner *et al.* (1986) showed presence of *Fusarium* spp. from rotted and apparently healthy roots of citrus nurseries.

Malikoutsaki *et al.* (1987) isolated *Fusarium* spp. from roots of affected citrus trees and pathogenicity was proved by artificial inoculation to the potted plants.

Labuschagne *et al.* (1987) isolated *F. solani* and *F. oxysporum* from major and feeder roots of symptomless rough lemon seedlings, as well as from symptomless and declining sweet orange trees on rough lemon rootstock and reported that the average incidence of both fungi did not differ significantly between healthy and declining trees. The mean incidence of *F. oxysporum* was (19.9%) significantly lower than that of *F. solani* (39.8%)

Active lesions of natural and induced cankered bark of 'Hamlin' sweet oranges consistently yielded *F. solani* on isolation (Nemec, 1987).

Whiteside *et al.* (1989) reported that dry root rot was a destructive and localized disease caused by a common soil and root - inhibiting fungus *Fusarium solani* (Mart.) Sacc. Further, they found that citranges, citromella, roughlemon, and cleopatra mandarin were more susceptible than sour and sweet orange rootstocks.

Nemec *et al.* (1989) proved that *F. solani* can be pathogenic alone and the primary pathogen causing fibrous and scaffold root rot on citrus trees in Florida.

Highest populations of *F. solani* were recorded in the first 30 cm of the soil profile in citrus grooves.

Jagannathan (1989) isolated *Rhizoctonia solani* from diseased stems and roots of acid lime. Reddy and Raju (1991) found Gwalior isolate was most aggressive among 13 isolates of *Fusarium udum* (pigeonpea wilt) and indicated the existence of two distinct pathological strains. Prakasam *et al.* (1992) isolated *Fusarium* spp. from the infected root samples of mandarin orange trees.

Dandurand and Menge (1992) studied the influence of *F. solani* on citrus root rot caused by *Phytophthora parasitica* and *P. citrophthora* and found that sweet orange seedlings inoculated with *P. citrophthora* alone had significantly lower the top weights (56%), total weights (53%), feeder root weights (49%) and percentage of living roots (71%) than those plants inoculated with *P. parasitica*. They compared the plants inoculated with *P. parasitica* alone, *F. solani* alone or non-inoculated controls. Seedlings which were co-inoculated by root dipping in to zoospore suspensions of *P. parasitica* and planted into soil infested with *F. solani* had significantly reduced total root weights (60, 71 and 75 %), feeder rot weights (61, 72 and 76%) and feeder root length (62, 71 and 75%).

Kore and Mane (1992) found symptoms 21 days after inoculation on one month old acid lime seedlings raised in pots having sterile soil. Rathore *et al.* (1993) isolated *Fusarium acuminatum* (*Gibberella acuminata*) from affected seedlings of acid lime (*Citrus aurantifolia*) in sirohi nurseries of Rajasthan.

Ansar *et al.* (1994) stated that *F. solani* was predominantly associated with citrus quick decline and its pathogenicity was confirmed experimentally. Thomson

et al. (1995) isolated *Phytophthora nicotianae* and *Pythium* spp. from diseased feeder roots and rhizosphere soils of citrus orchards in the Transvaal Province of South Africa.

Liu-Jing Jing *et al.* (1995) isolated *F. solani* from the root lesions of *Citrus erythrosa* and *C. sinensis* in China and further studies revealed that the toxins of *F. solani* inhibited the growth of seedlings.

Ippolito *et al.* (1995) observed dry root rot of citrus exclusively on clementine mandarin grafted on sour orange and *F. solani* was isolated from the discolored wood.

Baker and Tatum (1998) isolated *F. oxysporum* from the diseased roots of blight-affected orange trees (*Citrus sinensis* osb.). Verma and Singh (1999) isolated *F. solani* from the roots of the infected citrus root stocks viz., *Jattikhatti*, *Cleopatra* and *Pectinefera* seedlings.

Okigbo and Emoghene (2000) isolated the *Ganoderma philippii* from the affected (dead) stems of orange plants.

Ramteke (2003) isolated *F. solani* from infected roots of sour orange (*Citrus jambhiri*) rootstock.

Reddy *et al.* (2003) isolated *F. solani* from the infected rhizomes of turmeric collected during the survey of turmeric fields of Kadapa and Guntur districts of Andhra Pradesh.

Dhanbir Singh and Akhliesh Singh (2004) conducted pathogenicity tests for Fusarial wilt of chilli and proved that *Fusarium* spp. developed infection both

in test tube and potted seedlings and pathogens were reisolated. *F. solani* was more virulent as compared to *F. oxysporum*. Recovery percentage of *F. solani* was 76 and 66 per cent where as *F. oxysporum* was 46 and 50 per cent from the test tube and potted seedlings respectively.

2.3 ISOLATION OF RHIZOSPHERE MICROFLORA

Trichoderma lignorum was isolated by Weindling and Emerson (1936) which was found to control damping off diseases in citrus caused by *Rhizoctonia solani*.

Rangaswami and Vasantharajan (1962) studied citrus rhizosphere microflora and found that bacteria were about 40 to 90 times, actinomycetes 2 to 6 times and fungi 3 to 6 times more abundant in rhizosphere than in the soils. They also compared the populations on the growing and non-growing roots of the trees and revealed that the growing roots harbored 2 to 3 times more bacteria and actinomycetes, then the non-growing roots. In fungi there was lower population in the rhizosphere of growing than non-growing roots. Further, they confirmed that no difference between the populations in the rhizospheres at the time of active growth and quiescent periods of the trees.

Gupta *et al.* (1974) isolated several fungi such as *Aspergillus* spp., *Cephalosporium* spp., *Cladosporium* spp., *Fusarium* spp., *Myrothecium* spp., *Pencillium* spp., *Rhizoctonia* spp., *Sclerotium* spp., and *Trichoderma viride* from the rhizosphere of pea plants.

Twenty five fungi were isolated from rhizosphere of lentil (*Lonus esculenta*) by soil plate method (Arora and Dwivedi, 1979).

Naqui and Chauhan (1980) isolated *T. viride*, *Aspergillus fumigatus*, *A. niger*, *A. sydowi*, *Penicillium jantho-citrinum*, *Cladosporium*, *Cladosporioides*, *F. moniliforme*, *F. oxysporum*, f.sp. *capsici*, *F. solani* from rhizosphere of chilli seedlings.

Hadwar and Khara (1980) isolated the *Trichoderma pseudokoningii* from the rhizosphere of tomato seedlings in Punjab. Heredia *et al.* (1988) isolated sixteen species of fungi from rhizosphere of spinach plants.

Bhattacharya and Bora (1995) studied rhizosphere microflora of tea in different seasons. Fungi were found to be dominant in autumn, while bacteria in rainy, summer and actinomycetes in spring. Among fungi *Alternaria*, *Aspergillus*, *Cladosporium*, *Curvularia* and mucor was present in abundance.

Li-qiqim *et al.* (2000) isolated *Bacillus* from the rhizosphere of watermelon and citrus which inhibited the growth and sclerotial germination of *Rhizoctonia solani*.

Sivakumar *et al.* (2000) reported that the *Pseudomonas fluorescens* Pf-1, isolated from the crop rhizosphere, exhibited inhibitory action against *Rhizoctonia solani* f.sp. *sasaki* Exur. causing banded leaf and sheath blight of maize.

Rakesh *et al.* (2004) conducted a pot culture experiment and determined that rhizosphere fungal population was significantly reduced by VAM inoculation while bacterial and actinomycetes populations were increased in chickpeas.

Tilak *et al.* (2004) studied that rhizosphere populations of bacteria, fungi and actinomycetes were higher in paddy straw – amended soils than in control soils of barley.

Plant pathologists and microbiologists about 75 years ago thought and were of the opinion that there were some antifungal substances in and around rhizosphere of the plant which controlled plant diseases (Srivastava, 2004).

2.4 ANTAGONISTIC ACTIVITY OF RHIZOSPHERE MICROFLORA

The potential use of fungal antagonists as bio-control agents 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*.

Rangaswami and Vasantharajan (1962) studied the fungal and actinomycetes floras of the rhizospheres of growing and non-growing roots of three species of citrus (Pummelo, Sathgudi and Mandarin) and compared with the soil. The results indicated that *Aspergilli* and *Penicillia* were more numerous in the rhizosphere and *Mucor* and *Rhizopus* were less abundant in the rhizosphere of both growing and non-growing roots than in soil. They estimated fungal populations in the rhizosphere and found that 4 to 8 times greater than in soil. They noted that streptomycetes occurred in greater numbers in the rhizosphere and showed antagonistic properties against bacteria.

Fluorescent *Pseudomonads* also have potential as biocontrol agents of *Pythium* and *Rhizoctonia* in cotton seedlings (Howell and Stipanovic, 1979 & 1980).

Bell *et al.* (1982) studied *in vitro* antagonism of *Trichoderma harzianum* against six fungal plant pathogens namely viz., *Sclerotium rolfsii*, *Ceratobasidium cornigerum*, *Phytophthora parasitica* f. sp. *nicotianae*, *Pythium aphanidermatum*, *P. myriotylum* and *R. solani*. They observed that a single isolate of *Trichoderma* can be highly effective against an isolate of a pathogen species but may have only minimal effects on other isolates of the same species.

Studies conducted by Raghavendra Rao (1984) found that antagonistic effect of mycelial and spore suspension was more effective than the culture filtrate of *Trichoderma viride* in reducing the mortality of citrus seedlings.

Apdel-Rahim and Abhu-Surriah (1985) in their *in vitro* studies proved that culture filtrates of *T. harzianum* and *T. koningii* were highly antagonistic to *Rhizoctonia solani* blight of okra.

Dubey and Dwivedi (1988) proved the antagonistic potential of *Trichoderma viride* isolated from soybean rhizosphere against *Macrophomina phaseolina* *in vitro* tests.

Nemec *et al.* (1989) compared the distribution of *Fusarium* spp. and other microflora in soil and rhizosphere of rough lemon roots and observed that *Fusarium* spp. cfu accounted for approximately eight per cent of the total fungal population in soil and 23 per cent of the total rhizosphere. The bacteria and

Pseudomonas spp. exhibited a greater rhizosphere enrichment than actinomycetes or fungal flora.

Dhedi *et al.* (1990) reported that five bacterial isolates, two actinomycetes and five fungi including *T. harzianum* and *T. viride* were antagonistic to *Fusarium oxysporum* f.sp. *ciceri*, the causal agent of vascular wilt of bengalgram.

Rathore *et al.* (1992) observed that the *Trichoderma viride* reduced the colony diameter of *Fusarium solani* (ginger rhizome rot) by production of volatile substances.

Dwivedi (1992) isolated microbes from the soils of Guava orchards and found that *Trichoderma* spp. were the most effective inhibitors of the growth of test pathogens. *T. lignorum* inhibited the growth of *F. oxysporum* f.sp. *psidii* and *F. moniliforme* (*Gibberella fujikoroii*) by 70% while *T. viride* inhibited the growth of *F. oxysporum* f.sp. *psidii* by 71.4 per cent. *T. harzianum* reduced the growth of *F. solani* and *F. longipes* by 60.0 and 60.4 per cent respectively.

Sitaramaiah and Parvathidevi (1993) tested the antagonistic nature of *T. harzianum* against *Fusarium solani* and *Sclerotium rolfsii* in seeded agar method and dual culture method.

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

Selvarajan and Jeyarajan (1996) showed the effect of eight fungal and bacterial antagonists against *Fusarium solani*, among which isolate-3 of *T. viride* and *T. hamatum* showed maximum inhibition zone.

Dubey (1998) investigated that the three fungal antagonists, *Trichoderma viride*, *T. harzianum* and *Gliocladium virens* significantly inhibited the mycelial growth and sclerotial production of *Thanetophorus cucumeris* (*Rhizoctonia solani*). Among the three, *G. virens* was more effective. Complete mycelial inhibition and 92% inhibition of sclerotial formation were recorded in 4:1 ratio of *G. virens* and *T. cucumeris*.

Hazarika and Das (1998) studied the potential of Assam and Tamil Nadu isolates of *Trichoderma harzianum*, *T. viride* and *T. virens* against *Rhizoctonia solani* the French bean root rot pathogen under *in vitro* conditions. Assam isolates of *T. harzianum* (78.4 per cent) and *T. viride* (75.8 per cent) were found to be more effective than isolates from Tamil nadu in inhibiting mycelial growth of *R. solani* in dual culture.

Dubey (2000) evaluated different biocontrol agents viz., *Trichoderma viride*, *T. harzianum* and *G. virens* evaluated against *Rhizoctonia solani* *in vitro* by dual culture technique. *Gliocladium virens* was most effective, which inhibited maximum mycelial growth (59.8%) and sclerotial production (70.0%) followed by *T. viride*.

Anitha *et al.* (2000) reported that the antagonistic activity of *Trichoderma* spp. was demonstrated on suppression of *S. rolfsii* in *in vitro* tests. The inhibitory

zone developed by *T. harzianum*, *T. viride*, *T. koningii* were 7.6 mm, 5.5 mm and 4.3 mm respectively. Inhibition test is performed *in vitro* only.

Patel and Anahosur (2001) reported antagonistic activity of *Trichoderma harzianum* against *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii* in chickpea.

Deepak Kumar and Dubey (2001) screened isolates of *T. viride*, *T. harzianum* and *Gliocladium virens* against *F. solani* f.sp. *pisi* causing collar rot of pea by dual culture and found that Ranchi isolate of *T. harzianum* and *G. virens* superiorly performed over all the others.

Srinivasulu *et al.* (2001) tested three species of *Trichoderma* and found that *T. harzianum* inhibit maximum mycelial growth of *Ganoderma applanatum* and *G. lucidum* (15 mm growth) as against 55 to 60 mm growth in control plates on PDA *in vitro* conditions.

T. harzianum was the most effective in causing significant suppression of growth and sclerotial formation of *R. solani* *in vitro* through production of volatile and non-volatile antibiotics, followed by *Gliocladium virens* and *T. aureoviride* (Bunker and Mathur, 2001). *Trichoderma harzianum* suppressed the growth of *M. phaseolina* by over growth which showed its mycoparasitism (Patel and Anahosur, 2001).

The principal mechanism observed was coiling of antagonistic hyphae around the hyphae of pathogen and lysing. The mechanism of inhibition may be

competition for food and space, production of antibiotics and mycoparasitism (Deepak Kumar and Dubey, 2001).

Bunker *et al.* (2001) isolated five isolates of biocontrol agent *Trichoderma* spp. from rhizosphere and evaluated *in vitro* against *R. solani*. *T. harzianum* was the most effective in causing significant suppression of both growth and sclerotial formation of *R. solani*. It caused above 60 per cent inhibition of growth and sclerotial formation after 7 days of incubation followed by *T. viride* (ITCC-1433) which caused about 50 per cent inhibition of growth after 3 days of incubation, but about 60 per cent inhibition of growth and above 50 per cent of sclerotial formation after 7 days. On further incubation upto 12 days it caused 6 per cent inhibition of sclerotial formation.

Desai and Srikant Kulkarni (2002a) reported that the effect of non volatiles produced by nine biocontrol agents on the hyphal growth and sclerotial formation of *Macrophomina phaseolina* (Tassi) Goid in M-35-1 sorghum variety. Cent per cent hyphal inhibition by sterilized (ST) non-volatile was recorded in (T2) *Aspergillus niger* and (T3) *A. niger* (K). Further *Gliocladium virens* (44.22) and (T8) *T. viride* II (43.32) which were on par with each other. Significantly higher hyphal inhibition was recorded in (T3) *A. niger* K (79.29) which was on par with (T1) and (T6). *T. harzianum* and (T6) *T. viride* II was recorded cent per cent sclerotial inhibition in *A. flavus* (T1), *A. niger* (T2), *A. niger* (K) (T3) and *G. virens*.

Xavier *et al.* (2002) described the role of arbuscular mycorrhizal fungi (AMF) as plant growth stimulators, bioprotectors against pathogenic bacteria,

fungi and viruses and providers of special benefits such as salt and drought tolerance.

Tripathi and Johri (2002) reported that the antagonistic potential of fluorescent pseudomonads (FLP) recovered from rhizoplane (Pen-4) and rhizosphere of pea (PRS-1) were studied for *in vitro* inhibition of *Colletotrichum dematium*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Pen-4 and PRS-1 showed strong antagonism against *C. dematium* and *R. solani* by 59 and 69% respectively in iron-rich medium but were infective in absence of Fe^{3+} .

The efficacy of exotic isolates of bioagents viz., *T. viride*, *T. harzianum*, *G. virens*, *P. fluorescens* (D) and *P. fluorescens* (T) was assessed against three test pathogens, *F.o. f.sp. ricini*, *M. phaseolina* and *S. rolfsii* by dual culture technique. The per cent growth reduction of the pathogen was maximum (45.33) with *T. viride* against *F.o. f.sp. ricini* and maximum per cent reduction (47.56, 46.89) with the *T. harzianum* and *T. viride* against *M. phaseolina* and *S. rolfsii* respectively (Humayun, 2002).

Gesheva (2002) isolated antagonistic actinomycete genus streptomycetes *Micromonospora* and *Nocardia* in rhizosphere soils under citrus trees (Lemon and orange) and reported that the antagonistic streptomycete strains produced antibacterial or antifungal antibiotics.

Sharma and Chandel (2003) Screened biocontrol agents against *F. oxysporum* f.sp. *gladioli* and obtained maximum inhibition of mycelial growth of *F. oxysporum* with *T. harzianum* (75.36%) followed by *T. viride* (70.95%) and *T. virens* (61.43%), which attributed to the antibiosis.

Gopalakrishnan *et al.* (2003) tested twelve *Trichoderma* isolates against damping-off of cotton caused by *Rhizoctonia solani*. The native cotton isolate *T. viride* exhibited the fastest growth rate and maximum effective against *R. solani* recording 56.2 per cent inhibition in dual culture.

Ramteke *et al.* (2003) evaluated the antifungal activity of *Withania somnifera* root extract against the dry root rot of citrus and found that methanolic extract of *W. somnifera* (1%) inhibited the growth of *F. solani* more than that of the clotrimazole (1%)

Thangavelu *et al.* (2004) isolated *Trichoderma* spp. from rhizosphere of banana (*Musa* spp.) from different areas of Tamilnadu and evaluated their antagonistic potential against *Fusarium oxysporum*, and found that *T. harzianum* isolate Th-10 was the most effective in inhibiting the mycelial growth *in vitro*.

Shah *et al.* (2005) studied antagonistic activities of rhizosphere fungi and *Trichoderma* spp. against *Fusarium udum* and found that *T. harzianum* achieved maximum inhibition (73.07%) followed by *T. viride* (70.76%) in pigeonpea.

2.5 MASS MULTIPLICATION OF BIOAGENTS

Henis *et al.* (1978) mixed 10 g of wheat bran with 20 ml tap water autoclaved it for 1 h for two consecutive days and used for mass multiplication of *T. harzianum*.

Elad *et al.* (1980) combined wheat bran, saw dust and water in the ratio of 3:1:4(v/v) autoclaved for 1 h for two successive days for multiplying *T. harzianum*.

Padmanabhan and Alexander (1984) utilized a medium containing 100 g of sand and 20 g sorghum grains for multiplication of *T. viride*.

Papavizas *et al.* (1984) standardized easily available inexpensive, molasses and brewer's yeast for multiplication of antagonists at industrial level. They attained maximum biomass 15 days after incubation.

Mukopadhyay *et al.* (1986) used wheat bran and saw dust medium for multiplying *T. harzianum* and *T. koeningii*. Upadhyay and Mukopadhyay (1986) multiplied *T. harzianum* on presoaked sorghum grains in two per cent sucrose solution for overnight.

One of the most critical obstacles to biological control by direct massive soil augmentation has been the lack of methods for culturing and delivering antagonist to soil. The production and commercialization of biological products is a dynamic process. The development of a stable, cost effective and easy to apply bio-control formulation is critical for advancement of biological control of plant pathogens with introduced antagonists. A suitable medium for mass multiplication should consists of inexpensive, readily available bio-products with appropriate nutrient balance (Lisanasky, 1985).

Maiti and Sen (1985) noted high level population of *Trichoderma* (73.30×10^5 /g soil) when it was added to soil on a wheat – bran formulation in combination with calcium ammonium nitrate.

Bharadwaj *et al.* (1987) reported that shelled cobs and wheat straw crushed to powder supported maximum growth of *T. viride*, *T. harzianum* and *T. hamatum* followed by farm yard manure.

Jacob (1989) observed farm yard manure and gobar gas effluent as the best substrates for the mass multiplication of *T. harzianum* and *T. viride*. Among these two, FYM supported higher population of antagonists.

Kousalya and Jeyarajan (1990) screened 18 agricultural bio-products for multiplication of *T. viride* and *T. harzianum* of which tapioca rind (27.2×10^6 , 26.1×10^6 cfu/g) and tapioca refuse (26.4×10^6 , 25.8×10^6 cfu/g) were found to be the best followed by well decomposed farm yard manure (FYM) (25.9×10^6 , 25.6×10^6 cfu/g) and pressmud (25.2×10^6 , 24.8×10^6 cfu/g) respectively.

Bhat *et al.* (1992) reported that gypsum had a significant influence on the fungal population of the groundnut rhizosphere. Sangeetha *et al.* (1993) evaluated different substrates for the growth of *T. viride* and *T. harzianum*. Farm Yard Manure supported more population of *T. viride* followed by wheat bran and rice bran. Jacob and Sivaprakasam (1995) reported maximum growth of *T. harzianum* and *T. viride* in FYM at 40 to 60 (W/W) moisture content for 14 days.

Jayaraj and Ramabadran (1996) screened seven organic substrates for mass multiplication of *T. harzianum*, among which pigeon pea husk (63.5×10^6 cfu/g) was found to be the best followed by tapioca waste (56.2×10^6 cfu/g) and press mud (50.8×10^6 cfu/g). Shamarao *et al.* (1998) screened various substrates for mass multiplication of *Trichoderma viride* and found that wheat bran was suitable for promoting sporulation and among oil cakes, pongamia cake was best.

Indu *et al.* (1996) isolated *T. harzianum* from coorg mandarin (*Citrus reticulata* Blanco) orchards in Kodagu was used for mass multiplication on organic wastes and achieved high number of cfu/g on coffee fruit skin (1.3×10^{10} to 40.4×10^{10}) in the 1.5 kg unsealed poly propylene bags where as low number of cfu i.e. 1.0×10^6 to 3.5×10^6 and 2.6×10^6 to 5.3×10^6 was recorded in the 5 kg unsealed bags and 1.5 kg sealed bags respectively. The other organic wastes poultry manure and coffee fruit skin – biogas slurry also gave high cfu i.e 4.3×10^9 and 9.2×10^{12} respectively, in the 1.5 kg unsealed bags.

Shamarao *et al.* (1998) tested ten different substrates and tested ten different substrates and found that the population of *T. viride* on wheat bran was 215.03×10^4 cfu/g followed by 193.33×10^4 and 115.0×10^4 cfu/g for farm yard manure and dung, respectively. Among the oil cakes pongamia cake was the best followed by neem cake.

Lilly and Santhanakrishnan (1998) conducted an experiment to determine the best substrate for rapid VAM fungi inoculum production and found that composted coir pith was superior in terms of infection percentage, extra metrical hyphae and spore count than compost, pressmud, vermiculate, perlite and vermiculate-perlite (1:1) substrates.

Hari and Somasekhara (1998) tested substrates for mass multiplication of the biocontrol agents *T. viride* and *Paecilomyces lilacinus* and found that *T. viride* (3.1×10^8 cfu/g) population was more on sugarcane bagasse and that of *P. lilacinus* (2.18×10^8 cfu/g) population occurred on pressmud which was much higher than sorghum grains (5×10^6 and 1×10^6 respectively).

Prakash *et al.* (1999) evaluated four substrates for mass production of the biological control agents *T. harzianum* and *T. virens* and found that tea waste was the best media. Cultures produced in tea waste can be stored 3 months without reduction of biocontrol agents populations.

Prasad and Rangeswaran (2000) reported molasses - soy medium (MSM) could serve as a better alternative to molasses yeast medium for large scale production of *Trichoderma* spp. and proved that molasses-soy medium with soy level 10gl^{-1} of molasses medium gave maximum yield of biomass, viable propagules and spores when compared to the standard molasses-yeast medium.

Ramarethinam *et al.* (2000) reported that mycoparasitism of *Trichoderma* against pathogens will be more virulent only at high population level i.e more than 2×10^6 cfu/g of formulated products.

Thangavelu *et al.* (2004) tested five different organics substrates (rice brawn, rice chaffy grain, farm yard manure, banana pseudostem and dried banana leaf) for mass production of potential *T. harzianum* and found that the strain th-10 produced high density of propagules (4.6×10^3 cfu/g of leaf) in dried banana leaf and addition of jaggery (10% w/v) increased the multiplication of *T. harzianum* which survived for more than six months on the stored substrates.

Nazam and Singh (2004) tested three substrates for multiplication of bioagents *T. harzianum* (PBAT-43) and *Pseudomonas fluorescens* (PBAP-27). The results revealed that *T. harzianum* population was more in cow dung (76.8×10^{10}) followed by pressmud (28.4×10^8) where as *P. fluorescens* was more in cow dung (42.6×10^{12}) and least in press mud (1.6×10^6) respectively.

2.6 *IN VITRO* SCREENING OF FUNGICIDES AND NATURAL PRODUCTS AGAINST ROOT ROT PATHOGENS

Dilip *et al.* (1991) tested sixteen fungi toxicants against *F. solani in-vitro* and found that captafol as most toxic besides thiram, mancozeb and MEMC also inhibited spore germination and mycelial growth and had EC₅₀ concentration of less than 10 µg ml⁻¹.

Hundoo *et al.* (1992) screened seven fungicides for their toxicity against *F. solani* root rot of eggplant (*Solanum melongena* L.) and found that JK stein (methyl benzimidazole carbamate) showed strongest activity inhibiting the growth at 255 g/ml.

Strauss and Labuschagne (1993) tested the efficacy of fungicides against mycelial growth of *F. solani* from citrus roots and found that prochloraz as the most effective and fosetyl the least effective in their *in vitro* studies.

Wahid *et al.* (1995) found that carbendazim at 10 ppm completely inhibited growth of *Fusarium moniliforme*, *Fusarium solani* and *Fusarium oxysporum* isolated from soybean seeds.

Bajwa *et al.* (1998) evaluated eight fungicides against *R. solani* root rot of bottle gourd and the results revealed that *in vitro* growth was inhibited by diniconazole and chlorothalonil followed by propineb, mancozeb and carboxin. Propineb, diniconazole and chlorothalonil were more effective inhibiting sclerotia

formation. Diniconazole, mancozeb and chlorothalonil were most effective in inhibiting the germination of sclerotia.

Complete inhibition of *Rhizoctonia solani* was observed with Rhizolex at a concentration of 1 ppm and thiophanate methyl at a concentration of 20 ppm (Sharma and Rai, 1999).

Singh *et al.* (2000) tested the efficacy of six fungicides against *F. solani* and reported that copper oxychloride and captan showed fungistatic action where as thiophanate-methyl, carbendazim, hexaconazole and mancozeb showed fungicidal action.

Effect of *Trichoderma* spp. viz., *T. harzianum*, *T. viride* and *T. koningii* were tested *in vitro* using dual culture technique to assess the antagonistic potential of the three *Trichoderma* spp. and poisoned food technique was used to study the efficacy of two chemicals viz., thiram 75 WP and Dithane M-45 75 WP @ 0.1% of its commercial product against the *S. rolfsii*. Out of three *Trichoderma* spp. *T. harzianum* was more inhibitory to *S. rolfsii* which showed 61.5% inhibition in mycelial growth of the pathogen. This was followed by *T. viride* and *T. koningii* respectively causing 59.1% and 57.2% growth inhibition. Sclerotial production was reduced and significantly ranged between 84.1% and 94.2%. *T. harzianum* inhibited more than 90% sclerotial production followed by *T. viride* 86.8% and *T. koningii* 84.1%. Dithane M-45 inhibiting mycelial growth (76.5%) and sclerotial production (98.6%) of *S. rolfsii* in comparison to thiram where mycelial growth and sclerotial production were 70.3% and 96.5% respectively (Pranab Datta and Das, 2002).

Desai and Srikant Kulkarni (2002) evaluated the effect of urea on the growth and sporulation of *Macrophomina phaseolina* (Tassi) Goid and native isolate of *Trichoderma viride* and *T. harzianum*. Mycelial and sclerotial inhibition of *M. phaseolina* ranged from 4.24 to 100 and 12.00 to 100 per cent. Cent per cent inhibition of growth and sporulation of all the three test fungi were recorded at 1000 ppm concentration.

Tripathi and Johri (2002) investigated the integrated use of fungicides and fluorescent pseudomonads recovered from rhizoplane (PEn-4) and rhizosphere of pea (PRS-1) and wheat (WRS-24) in *in vitro*. They observed the enhanced inhibitory potential of the fungicides and fluorescent pseudomonads against *Colletotrichum dematium*, *Rhizoctonia solani* and *Sclerotium rolfsii*. In association with vitavax, WRS-24 inhibited *C. dematium* by only 1%; this was in contrast to 39% inhibition by vitavax alone. However, average mycelial extension of *R. solani* and *S. rolfsii* against PEn-4 and WRS-24 was 78, 84 and 21, 41 (mm) dia respectively against 23, 46 on bavistin containing medium.

Narayanbhat and Srivastava (2003) tested 4 neem *Azadirachta indica* products viz., Nimbicidin, Neemazal, PJMC, and PJMD against *F. solani* and found that none of the formulations, even at 5000 ppm was completely inhibitory to the test pathogen. They also tested 14 fungicides by using poisoned food technique against *F. solani* dry root rot of ginger and found that indofil M-45 (mancozeb), bavistin 15 WP (carbendazim), benlate 15 WP (benomyl), saaf (carbendazim 12% and mancozeb 63%), tilt 25 EC (propiconazole) and topas (penconazole) could inhibit the growth of the pathogen.

Haritha (2003) screened five fungicides i.e mancozeb, copper oxychloride, metalaxyl, chlorothalonil and hexaconazole using poisoned food technique against *P. aphanidermatum* and reported that metalaxyl shows more per cent inhibition over control from 50 ppm onwards, whereas mancozeb and hexaconazole at 500 and 1000 ppm concentrations and COC complete inhibition from 250 ppm concentrations.

2.7 COMPATIBILITY OF ANTAGONISTS WITH FUNGICIDES IN VITRO CONDITIONS

Whipps and Lumsden (1991) reported that pathogenic activity was made to a tolerable or permissible level only when the biocontrol agents were compatible with agro-chemicals.

Krishnamoorthy and Bhaskaran (1993) compared the effect of soil drenching fungicides on the growth and sporulation of *T. viride* and *T.harzianum*. They observed copper oxychloride did not inhibit the mycelial growth, but *T. viride* did not sporulate in presence of copper oxychloride.

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

Goutam *et al.* (1995) tested compatibility of six *Trichoderma* spp. with fungicides and biocides and found that carbendazim at 200 ppm arrested the mycelial growth of all the six *Trichoderma* spp. In case of biocides, *T. viride*

(Tv-5) was compatible with both the formulation of neem product at 100 and 250 ppm.

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

Karpagavalli (1997) reported the effect of different fungicides (viz., carbendazim, thiram, copper oxychloride) on the growth of *T. harzianum* and *T. viride* at 500, 1250 and 2500 ppm concentrations *in vitro*. All the fungicides inhibited the growth of antagonistic fungi at all concentrations in liquid medium. When compared to control they recorded 430.00 mg and 410.00 mg dry weight for *T. harzianum* and *T. viride* respectively. Among the fungicides tried, COC recorded the lowest inhibition followed by thiram in *T. harzianum*. In case of *T. viride* the mycelial dry weight in 500 and 1250 ppm concentrations of COC was on par with the control. Inhibition was maximum in carbendazim at 2500 ppm concentration. The mycelial dry weight decreased with increased concentration of fungicides.

Shanmugum^o and Varma (1998) evaluated certain fungicides against *T. viride in vitro* and reported that mancozeb showed comparatively low rate of inhibition against *T. viride* and compatible.

Sharma *et al.* (1999) ascertained that *Trichoderma harzianum* and *T. pseudokoningii* which were compatible with Dithane M-45 in all concentrations of 100, 500, 1000 and 2000 ppm:

Goudar and Kulkarni (1999) studied the compatibility effect of antagonists and seed dressers against *F. udum* and proved that wilt incidence of pigeonpea was least in carbendazim + *T. viride* (16%) followed by carbendazim + *T. harzianum* (20%).

Results revealed that *T. viride* did not show sensitivity with the lower doses of Vitavax, Rovilan, Indofil M-45, Thiram, Captafol and Ridomil. Bayleton and bavistin were inhibitory to *T. viride*, therefore, these two fungicides cannot be used in integrated management strategies (Abha Agarwal and Tripathi, 1999).

Gupta *et al.* (1999) reported that the biocontrol agent *Trichoderma pseudokoningii*, chemical fungicide (Dithane M-45 as mancozeb) and phytohormones (Indole-3-acetic acid, IAA and 1-Naphthalene acetic acid, NAA) were evaluated individually and in combination for their effects on disease control and plant development. In plates, *T. pseudokoningii* was compatible with dithane M-45 at 0.1% and IAA and NAA at 20 ppm concentrations against *F. solani*.

Anandh *et al.* (1999) reported that at 25 ppm of carbendazim the mycelial growth of *Trichoderma harzianum*, *A. niger*, *A. flavus* was inhibited completely.

The compatibility of certain biocontrol agents *T. harzianum*, *Verticillium chlamidosporium* and *T. pseudokoningii* with pesticides and fertilizers was investigated. Compatibility with dithane M-45, carbofuran and sebufos, with urea, superphosphate, murate of potash and NPK 15:15:15 and with neem seed cake (*Azadirachta indica*) was determined by exposing the biocontrol agents to medium treated with 100, 500, 1000 and 2000 ppm. Carbofuran, sebufos, neem seed cake and the chemical fertilizers were compatible with all the biocontrol agents at all the

concentrations tested. Dithane M-45 was compatible with all the biocontrol agents at low concentrations (Sharma *et al.*, 1999).

Six fungicides (iprodione, mancozeb, metalaxyl + thiram, thiram, tolclofos-methyl, zineb), four insecticides (pirimofos methyl, cypermethrin, dimethoate, malathion) and one herbicide (chlorpropan + centrimide) were incorporated into agar at a range of concentrations up to twice the recommended dosage and the effects on radial growth and spore germination on *Coniothyrium minitans* was determined. Only the fungicides had significant effect on *C. minitans*. *C. minitans* was highly sensitive to iprodione EC50 7-125 g a.i. ml⁻¹), moderately sensitive to thiram EC50 52-1065 g a.i. ml⁻¹) but less sensitive to the other tested pesticides (Whipps *et al.*, 2001).

Ramarethinam *et al.* (2001) reported that the mancozeb (75% WP) and copper oxychloride (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 oxychloride completely inhibited the growth of *T. viride*. Fungicides like carbendazim (50% WP), hexaconazole (5% EC), propiconazole (25% WP) 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 *T. 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⁻¹ of carbendazim inhibited 90% (ED90) growth of the fungus in *in vitro* metalaxyl (0.1%) and carbendazim

(0.0065 %) seemed to have a safe tolerance limit (ED50) for the bio-agent. Among six non-systemic fungicides captafol and chlorothalonil even at 2317 $\mu\text{g ml}^{-1}$ and 2037 $\mu\text{g ml}^{-1}$ were tolerable (ED90) by the fungus. The concentrations i.e., 169, 375 and 625 $\mu\text{g ml}^{-1}$ of captan, captafol and chlorothalonil seemed to be a safe tolerance limit for the biocontrol agent. Mancozeb and copper oxychloride inhibited maximum growth (ED90) at 285 and 805 $\mu\text{g ml}^{-1}$ but ED50 value of these were 231 $\mu\text{g ml}^{-1}$ and 356 $\mu\text{g ml}^{-1}$ only. However, thiram inhibited the growth to the maximum (ED90) at 145 $\mu\text{g ml}^{-1}$ concentration only and 25 $\mu\text{g ml}^{-1}$ seemed to be safe tolerance limit.

Deepak Kumar and Dubey (2001) studied the compatibility between different biocontrol isolates and commonly used fungicides benlate (benomyl), bavistin (carbendazim), topsin-M (thiophanate methyl), thiram (tetramethyl thiuram disulphide), vitavax (carboxin), captaf (captan) at various concentrations and reported that benomyl, carbendazim and thiophanate methyl inhibited cent per cent mycelial growth of *T. harzianum* at 0.025 and 0.05 concentrations *in vivo*. Next effective fungicide was carboxin (48.0 and 52.0 per cent inhibition at 0.025 and 0.05 concentrations) followed by captan and thiram. Thiram, carboxin and captan were effective against the pathogen but they were less effective against *T. harzianum*.

Five different pesticides viz., MEMC, carbendazim, phorate, carbofuran and metribuzin were evaluated against *Trichoderma harzianum*, *T. viride* and *Chaetomium globosum* by poisoned-food technique *in vitro*. It was observed that both the fungicide (MEMC and carbendazim) and one insecticide (phorate) have

been found to inhibit the mycelial growth of all the three tested bio-inoculants strongly up to 5 days of incubation. Carbofuran showed slight inhibitory effect on *T. harzianum* (37.82%), *T. viride* (5.20%) and *C. globosum* (1.22%). Hence it proved safer insecticide than phorate. Herbicide metribuzin found to inhibit more mycelial growth of *T. harzianum* (87.32%) and *T. viride* (61.85%) and total inhibition of *C. globosum* (Bhat and Sabalpara, 2001).

Five different pesticides viz., phorate, carbofuran were evaluated against *Trichoderma harzianum*, *T. viride* and *Chaetomium globosum* by poisoned food technique *in vitro*. Carbofuran showed slight inhibitory effect on *T. harzianum* (37.82%), *T. viride* (5.20%) and *C. globosum* (1.22%) (Bhat *et al.*, 2001).

Bhat *et al.* (2001) tested the effect of metribuzin against *Trichoderma harzianum*, *T. viride* and *Chaetomium globosum* by poisoned food technique. Herbicide metribuzin found to inhibit more mycelial growth of *T. harzianum* (87.32%) and *T. viride* (61.85%) and total inhibition of *C. globosum*.

Malathi *et al.* (2002) evaluated the effect of carbendazim and thiophanate-methyl on the growth of *Pseudomonas fluorescens* (ARR 1G, ARR2, ARR10, CHAO, EP1, FP7, KKM2, Pfl, VPT2, VP4 and VPY10) isolates *in vitro* and reported that carbendazim and thiophanate methyl at 100 ppm enhanced the growth of *P. fluorescens* isolates. The antagonistic bacteria multiplied even when the fungicide rate was increased to 500 ppm.

Desai *et al.* (2002) tested the efficacy of six commonly used fungicides, 12% carbendazim+63% mancozeb (Campanion 75%), carbendazim (Bavistin 50 WP), carboxin (Vitavax 75 WP), 8% metalaxyl + 64% mancozeb (Matco 8-64

72 WP), mancozeb (indofil M-45 75 WP), mancozeb + thiophanate methyl and tricyclazole (Beam 75 WP). The per cent inhibition of hyphae and sporulation of *T. harzianum* in different test chemicals at 500 ppm ranged from 2.8 to 100 and 42.30 to 100% respectively. Similarly, the inhibition ranged at 1000 ppm was 4.49 to 100 and 4.17 to 100% respectively. The stimulatory effect of MATCO on hyphal growth (2.80) and sporulation (42.30) at 500 ppm was recorded. Mancozeb at 500 ppm recorded a lower inhibition of hyphae (5.70%) and sporulation (11.02%).

Desai and Srikant Kulkarni (2002) investigated that the urea, as a nitrogen source, was found to be lethal to native isolates of biocontrol agents *Trichoderma viride* and *T. harzianum*. Extent of inhibition over control of mycelium and sporulation of *T. viride* and *T. harzianum* ranged from 4.30 to 100; 9.68 to 100; and 4.52 to 100; 9.76 to 100 per cent, respectively. Cent per cent inhibition of growth and sporulation of all the two test fungi was recorded at 1000 ppm concentration. Regrowth study indicated the fungistatic nature of urea.

Haritha *et al.* (2003) studied that *in vitro* compatibility of fungicides with *Trichoderma* sp. and reported that metalaxyl has inhibited the radial growth by 18.52 per cent over control even at 1000 ppm. Whereas, mancozeb and copper oxychloride inhibited the radial growth of *Trichoderma* sp. by 76.66 and 44.44% at 1000 ppm and complete inhibition of growth at 100 ppm concentration of hexaconazole.

Narayanbhat and Srivastava (2003) tested 14 fungicides against the native *Trichoderma* spp. and found that carbendazim, benomyl, emisan (methoxy ethyl

mercury chloride), saaf (carbendazim 12% and mancozeb 62%), propiconazole and hexaconazole at all concentrations completely inhibit the growth of *T. viride*, where as mancozeb showed fungistatic property against *T. viride* with an inhibition of 31.5 per cent at 500 ppm.

Durai (2004) evaluated two systemic and non-systemic fungicides for their compatibility with *P. fluorescens*. Of the fungicides tested, thiram at 1000 ppm shown the highest inhibition (4.7%). Mancozeb was found to be compatible with *P. fluorescens* (B1) even at 50 ppm concentration.

Sendhilvel *et al* (2004) tested the compatibility of azoxystrobin 25 SC with biocontrol agents and found that no inhibition of mycelial growth of *G. virens* in all the concentrations viz., 125, 150, 200 and 250 ppm and inferred that *G. virens* is perfectly compatible with azoxystrobin.

2.8 DISEASE MANAGEMENT

Horticulture 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. In general, control of the soil borne diseases are very difficult. Integration of disease management methods like physical, biological and chemical proved to be effective against soil borne pathogens.

2.8.1 Physical method – Soil solarization

Solar heating of soil is a soil disinfestation method for disease control. 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. Soil solarization is 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.

In the studies carried out by several workers (Katan *et al* and 1976; 1980; Grinstein *et al.*, 1979) in various regions in Israel during July – August in different years, the recorded temperatures of mulched soil at depths of 5 and 20 cm were 46 to 55°C and 39 to 45°C respectively.

In California, U.S.A., temperatures of solarized soil were reached upto 60°C at 5cm depth (Pullman *et al.*, 1981). Al Raded (1979) reported from Jordan that maximal temperatures of solarized soils at depths of 5 and 20 cm were 44 to 52°C and 36 to 38°C respectively.

Chen *et al.* (1991) observed that soil solarization with polyethylene sheet increased soil temperatures, decreased microbial populations, changed the chemical composition and physical structure of soil, maintained high moisture levels and decreased the disease incidence.

Arun *et al.* (1993) reported that mulching with blue transparent sheet (150 gauge) increased average temperature from 42°C to 46°C at a depth of 5 cm at 12.30 h. The *Fusarium* propagules in non-rhizosphere soils of pigeonpea could not be recovered after 45 days of mulching.

Kalpanasastry and Chattopadhyay (1999) observed that the soil temperature at 5 cm depth varied widely among different treatments, especially in irrigated ploughed soil solarized plots. The maximum temperature of 52.5°C was recorded in solarized plots when compared to 44.4°C in untreated control. The soil population of *Fusarium oxysporum* f.sp.*carthami* was also reduced to non-detectable levels after six weeks of soil solarization. There was a significant rise in population of soil antagonists, actinomycetes and fluorescent *Pseudomonas*.

Thakare *et al.* (2002) reported that rhizosphere flora was found abundant in groundnut grown under mulched condition compared to non-mulch and due to the ability of polyethylene mulch increased soil temperature and soil moisture, enhanced the efficiency of soil microorganisms and reduced the *Rhizoctonia* populations.

Hemanth *et al.* (2004) conducted soil solarization with transparent polyethylene mulch (25µm) in naturally infested nursery beds against damping-off of papaya for 40 days and recorded average maximum temperature of 47.3°C at 10 cm soil depth in solarized beds which was 14.6°C higher than the non-solarized beds. Soil solarization during summer months under irrigated conditions was found effective to bring down the soil population of *Fusarium* spp. and *Pythium* spp. to zero level up to 30 cm depth.

2.8.1.2 Effect of soil solarization on fungal pathogens

Katan *et al.* (1976) studied the effect of solar heating on the inoculum of *Verticillium dahliae* and *Fusarium oxysporum* buried in mulched and non mulched soils at various depths. After 2 weeks under polyethylene sheets *V.dahliae* was eliminated completely at depths of 0 to 25 cm. The population of *Fusarium* was reduced by 94 to 100 per cent at 5 cm depth.

Grinstein *et al.* (1979) reported the effect of solar heating in the control of *Sclerotium rolfsii* in peanut. Soils mulched with clear polythene sheet for six weeks during July-August sown peanuts resulted in a significant decrease in diseased plants, rotten pods and there by an increase pod yield up to 52.8 per cent.

Katan *et al.* (1980) reported that the solar heating significantly reduced the incidence and severity of pink disease caused by *Pyrenochaeta terristris* by 73 to 100 per cent during 6 to 7 months of plant growth. The infection caused by *Rhizoctonia solani* and *Fusarium* spp. were also considerably reduced.

Katan *et al.* (1983) studied in detail the short and long term effects of soil solarization and cropping sequence on *Fusarium* wilt and yield of cotton. Soil solarization effectively reduced the *Fusarium* population in the soil and decreased wilt incidence in cotton plants. 90 per cent of the plants in untreated plots showed disease compared to 5 per cent in solarized plots hundred days after planting.

Chauhan *et al.* (1988) stated that chickpea and pigeonpea wilt caused by *Fusarium* propagules was greatly reduced in both dry and irrigated conditions immediately after soil solarization as compared to non-solarized treatments.

Lodha and Vidya (1990) studied the influence of soil solarization on the viability of *Fusarium solani* in an arid environment at Jodhpur and reported that mulching during June increased soil temperatures as much as 12 and 9°C at 5 cm soil depth in dry and wet plots resulting in a pronounced reduction of *F. solani* population from 250 propagules/g soil to 13 (dry) and 7 (wet) propagules/g during a period of 15 days.

Solarization by covering the soil with clear transparent polythene sheet for 6 weeks during hot summer months showed 80.8 per cent reduction of *Fusarium oxysporum* f.sp. *ciceri*, 80.6 per cent weed population and 90.5 per cent of their dry weight over control in chickpea (Krishna Rao and Krishnappa, 1995).

Harendarraaj *et al.* (1997) reported that soil solarization with transparent polythene mulch (25µm) was effective in controlling the pathogens causing damping – off incidence. Soil mulching with polythene resulted in an increase of 13.5°C temperature at 8 cm of soil depth with an average maximum temperature of 49.7°C. Soil solarization for 40 days killed *Pythium* spp. and *Fusarium* spp. upto 30 cm soil depth.

Gade *et al.* (1999) reported that the seedling mortality in *Citrus jambhiri* caused by *Fusarium* spp. was effectively controlled by soil solarization for 45 days using transparent polythene sheet in Maharashtra. Experiments on solar heating

using polyethylene mulching for the control of soil borne diseases were carried out in Israel during 1973 and 1974.

Kaushik *et al.* (2002) proved that soil solarization effectively brought down the population of *F. oxysporum* f.sp. *dalbergie*, *F. solani*, *P. aphanidermatum*, *R. solani* and *R. bataticola* to zero level in comparison to 37.5, 42.6 and 38.3 propagules per gram of soil respectively, in non-solarized/naturally infested soils.

Sharma *et al.* (2004) demonstrated soil solarization in mango nurseries with transparent polyethylene sheets (25, 50 and 100 μm) resulted increased soil temperatures by more than 12.7, 9.3 and 4.1°C at 10, 20 and 30 cm depths respectively as compared to unsolarized soils. The wilt pathogen *F. solani* killed upto 30 cm depth in solarized soils, whereas remained viable at all depths in unsolarized soils.

2.8.2 Biological methods

The potential use of fungal antagonists as bio-control agents of plant diseases was suggested more than 70 years ago by Weindling (1932) who was the first to demonstrate the parasitic activity of *Trichoderma* spp against *Rhizoctonia solani*.

Biological control of plant pathogens by the addition of antagonistic microorganism to the soil has become a powerful tool because of fast development

of resistance in fungal pathogens against site specific fungicides and advancement in fermentation and genetic manipulation technologies (Baker and Cook, 1974).

Davis (1980) noticed that mycorrhizal plants were more resistant to disease than plants without mycorrhizae and growth of citrus seedlings was increased by *Glomus fasciculatus* application. Dubey and Dwivedi (1988) reported the antagonistic potential of *Trichoderma viride* against *Macrophomina phaseolina*.

In recent years *Trichoderma* spp. have proved to be potential bio-agents in controlling several soil borne pathogens under both *in vitro* and *in vivo* conditions. (Sidovara, 1980; Chet, 1981; Elad *et al.*, 1981; Cook and Baker, 1983; Papavizas *et al.*, 1988 and Mukherjee *et al.*, 1989).

Gaur and Sharma (1991) noticed that *T. viride* present in the rhizosphere soil of resistant pigeon pea cultivar NP 15 as well as susceptible cultivar T 21, as the most effective bioagent in controlling the wilt disease caused by *F. oxysporum* f.sp. *udum*.

Weideman and Wehner (1993) reported that *T. harzianum* and *F. oxysporum* enhanced shoot growth rate and shoot mass of Roughlemon citrus seedlings in soils artificially infested with *Phytophthora nicotianae*.

Kumar (1993) found that the pre and post-emergence damping off of *Pinus roxburghii* caused by *Fusarium solani* was effectively controlled by incorporating the *Trichoderma viride* in the potting mixture before sowing.

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

Mukhopadhyay (1995) successfully used *Gliocladium virens* and *Trichoderma harzianum*, for treating various crops like chickpea, lentil, groundnut, tomato and cauliflower for protection against a wide range of soil borne pathogens viz., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium* spp. and *Fusarium oxysporum*.

Sheela *et al.* (1995) found that the *Trichoderma hamatum* reduced the wilt incidence by 33.34 per cent in egg plant caused by *Fusarium solani*. Rajendran and Ranganathan (1996) studied that combined seed treatment of *T. viride* and *Pseudomonas fluorescens* reduced onion basal rot incidence both in pot and in field conditions.

Reddy *et al.* (1996) observed maximum percentage root mycorrhizal colonization in plants inoculated with *G. macrocarpum* followed by *G. mosseae*. Shamim *et al.* (1997) noticed reduction in infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* by seed dressing with *Pseudomonas aeruginosa*, *Paecilomyces lilacinus* and *Trichoderma koningii*.

Raghuchander *et al.* (1997) demonstrated that dipping suckers of banana in the suspension of *Pseudomonas fluorescens* (10^6 cfu/ml) and *Trichoderma viride* (10^6 cfu/ml) along with an application of 500 g wheat bran and sawdust inoculum

(1:3) of the respective biological agent three months after planting, effectively reduced the *Fusarium* wilt incidence and produced the highest yield.

Widmer *et al.* (1998) isolated *Acremonium* from composted municipal waste (CMW) and found that suppressive to *Phytophthora nicotiana*. This fungus parasitized hyphae of *P. nicotiana* *in vitro* conditions. Application of fresh CMW (< 6 months) proved to be best for the management *Phytophthora* root rot in citrus nurseries and orchards.

Sharma *et al.* (1999) conducted pot culture experiments under glasshouse conditions. Coating of seeds with different biocontrol agents like *Trichoderma harzianum* and *Absidia cylindrospora* reduced the *Sclerotinia sclerotiorum* causing chickpea stem rot. Minimum incidence (4.52) of the disease was recorded in treatment where seeds were coated with *T. harzianum* as compared to control (43.44%).

Manoranjitham *et al.* (1999) reported that application of *T. viride* and *Pseudomonas fluorescens* either individually or in combination, highly reduced the pre and post emergence damping-off of chilli.

Ram *et al.* (1999) found that bio-control agent (BCA) *T. harzianum* isolated from rhizome rot suppressive soils, reduced the rhizome rot disease and increased plant stand and yield of ginger.

Prasad and Rangeswaran (2000) conducted a microplot test under field conditions to evaluate a modified wheat bran-kaolin formulation of *Trichoderma harzianum* in controlling seed rot and damping-off of chickpea incited by

Rhizoctonia solani. Significant ($P < 0.01$) control of the disease was achieved in the plots treated with two doses (100 and 200 g/m²) of the granular formulation when compared to bioagent treated seed plots or fungicide-treated plots. The disease incidence ranged between 10 and 15 per cent in granule treated plots where in other treatments, 51 to 57 per cent damping-off was observed.

Chandrasekhar *et al.* (2000) investigated the efficacy of native fungal antagonist, *Trichoderma viride* against root rot pathogen of brinjal as a talc based product under field conditions. The lowest root rot disease incidence of 9.4% was recorded in the plots treated with *T. viride* @ 4 g kg⁻¹ of seed and 3 g/20 m² followed by the application of *T. viride* @ 2 g kg⁻¹ of seed and 3 kg/20 m² (11.6%). The maximum per cent disease incidence (22.6%) was observed in the untreated control plots.

Jones and Badnur (2001) estimated total bacteria, fungi, actinomycetes, *Azotobacter*, P solubilizing microorganisms from chickpea rhizosphere and found to be more in mycorrhiza treated plants (146.61) compared to non-mycorrhizal plants (75.02).

Srivastava *et al.* (2001) recorded low percent wilting of Guava trees (mean 62%) in VA-Mycorrhiza treated trees (@ 5kg/tree) than the control (mean 90.3%) in the management of *F. oxysporum* f.sp. *psidii* in alphisols of Chota nagpur.

Jones (2001) described a new biological pesticide, messenger, which activates natural plant defence and growth systems. The active ingredient of messenger is harpin, a protein produce in nature by certain bacterial plant pathogens. Messenger has been tested on more than 40 crop groups and it is

effective on a number of crops, including citrus, cotton, wheat, cucumber, tobacco, straw berry, tomato and peppers. Messenger provides effective control of soil-borne pathogens, including *Fusarium* spp. and it can be used as a component of integrated pest management programmes.

Nagaraju *et al.* (2002) reported that the commercial formulation of *P. fluorescens*, *T. viride* and *T. harzianum* were used as seed treatment, root dip and foliar spray. The mean performance showed that application of *P. fluorescens* as root dip and foliar spray effectively reduced the severity of *R. solani* up to 62.13% and resulting in 47.68% higher grain yield compared with untreated check.

Ramteke (2003) tested the antifungal activity of *Withania somnifera* root extract against *F. solani* and found that the extract had higher inhibitory effect on the growth of *F. solani* the causal agent of root rot disease in *Citrus jambhiri*.

Bornali *et al* (2003) inoculated VAM to the tea seedlings showed increase in height, number of leaves, number of branches than the uninoculated seedlings.

Reddy *et al.* (2003) tested three biological agents *T. harzianum*, *Gliocladium* and VAM and found that *T. harzianum* as the most effective bioagent in the control of rhizome rot of turmeric caused by *F. solani*.

Sharma *et al.* (2004) observed that the biological antagonist *T. viride* controlled the root rot of *Prosopis cineraria* caused by *F. solani* to 50% under *in vivo* conditions.

Thangavelu *et al.* (2004) demonstrated that soil application of *T. harzianum* Th-10 as dried formulation effectively controlled *Fusarium* wilt with an efficacy comparable to that of the fungicide carbendazim.

2.8.3 Organic amendments in disease management

Zakaria *et al.* (1980) found that soybean, linseed and cotton seed meal amendments reduced *Fusarium* population in the soil.

Krishnamohan and Kandaswamy (1986) reported that the addition of farm yard manure at the rate of 10 t/ha along with inoculation of *Trichoderma viride* in soil increased the germination of cotton and reduced the post- emergence mortality of seedlings due to *Rhizoctonia* spp.

Maiti *et al.* (1987) found that addition of groundnut cake in combination with *Gliocladium virens* checked the spread of stem rot disease in groundnut by reducing sclerotial population of the pathogen, *Sclerotium rolfsii*.

Sivaprakasam (1990) studied that addition of organic matter as one of the effective method of bio-control and considerable evidence proved that the addition of amendments decreases the inoculum density of soil borne pathogens resulting in the decreasing of disease incidence.

Nitta (1990) reported that application of FYM (farm yard manure) to sugar beet increased the activating of root system and decreased root damage due to fungal diseases.

Pandey *et al.* (1991) deliberated that soil amended with neem cake had significant increase in rhizobium, azotobacter and fungi recorded the greatest increase in plant growth of green gram.

Naik *et al.* (1993) estimated total bacteria, fungi, actinomycetes and free-living nitrogen fixers in wheat rhizosphere after crop harvest and found a greater difference compared to initial counts in non-rhizosphere test soil viz., 3.2×10^6 , 5×10^3 , 2×10^4 and 1.9×10^4 per gram respectively. The plants inoculated with *Glomus fasciculatum*, FYM amendment stimulated bacteria, actinomycetes and free-living nitrogen fixers.

Harender *et al.* (1996) reported that reduction of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* with the use of oil cakes from groundnut, mustard, sesamum and cotton seed. They also stated that groundnut cake was superior to mustard as it not only reduced disease incidence but also improved plant growth.

Karthikeyan and Karunanithi (1996) observed the reduction of banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* with the use of neem cake as an organic amendment at the rate of 250 Kg/ha.

Rajendran and Ranganathan (1996) noticed combined seed treatment of *T. viride* and *Pseudomonas fluorescens* reduced onion basal rot incidence both in pot and in field conditions.

Selvarajan and Jeyarajan (1996) showed the effect of eight fungal and bacterial antagonists against *Fusarium solani*, among which isolate-3 of *T. viride* and *T. hamatum* showed maximum inhibition zone against *F. solani*.

Randhava and Parmar (1996) observed that wilt incidence caused by *Fusarium solani* was reduced by addition of neem cake to the soil and population of fungi, bacteria and actinomycetes was doubled.

Goudar *et al.* (1998) noticed that the organic amendments groundnut cake, neem cake and farm yard manure effectively reduced the *Fusarium* wilt of pigeonpea caused by *Fusarium udum*.

Hundekar *et al.* (1998) recorded the reduction of inoculum levels of *Macrophomina phaseolina* in the soil by the use of organic amendments.

Patel (1998) found that seed treatment, soil drench of carbendazim coupled with soil application of *T. harzianum* reduced the fungal count maximum, FYM reduced *Fusarium* spp. and *Macrophomina phaseolina*, but stimulated the other rhizosphere microflora in chickpea under irrigated conditions.

Padmodaya and Reddy (1999) studied that neem cake was superior than other organic amendments and reduced seedlings disease in tomato caused by *Fusarium solani* f.sp. *lycopersici*.

Manoranjitham *et al.* (1999) demonstrated that application of *T. viride* and *Pseudomonas fluorescens* either individually or in combination, highly reduced the pre and post emergence damping-off of chilli.

Biswas and Sen (2000) proved that *T. harzianum* reduced the stem rot of groundnut upto 83 per cent when it was applied as wheat bran sand mixture to the soil @ 30 g / kg of soil.

Sanjay and Kaushik (2001) evaluated the efficacy of six species of *Trichoderma*, *Gliocladium virens* and *Chaetomium globosum* against the forest tree nursery damping-off fungi, viz., *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* *in vivo*. The highest seed germination (86.6%) and seedling survival (83.4%) were recorded in *A. nilotica* on treatment with *T. viride*, followed by *T. longibrachiatum*. *Dulbergia sisso* seeds on treatment with *T. viride* and *T. longibrachiatum* showed significantly higher seed germination and seedling survival compared to control.

Neelamegam and Govindarajalu (2002) conducted experiments to control damping-off of tomato caused by *P. indicum* by using *Trichoderma viride* alone (or) in combination with farmyard manure (FYM). Addition of FYM in *P. indicum* inoculated unsterilized soil resulted in low seed germination (41%) in comparison to sterilized soil (53%). However, the integrated application of *T. viride* and FYM still increased the seed germination in unsterilized soil to 100 per cent as against 84 per cent in sterilized soil.

Mamata and Sharma (2002) studied the effect of oil cakes, viz., neem cake and cotton cake, mustard cake on white root rot of apple caused by *Dematophora necatrix* and found that neem cake was most effective in controlling the disease.

Burhanuddin and Kusum Mathur (2004) observed the highest suppression of *F. solani* root rot disease (2.5% mortality) and maximum dry mass weight

(19.4 g) with *Streptomyces* spp. (Cot-Coi-b-1), followed by *T. harzianum* (Th₂) and *Bacillus subtilis* (Ch-Mb-Kb-3) in soybean.

2.8.4 Chemical methods

Vazoquez and Ramallonilda (1969) reported that soil treatments with methyl bromide and vapam gave a high percentage of healthy rough lemon nurseries by controlling *Rhizoctonia*, *Pythium* and *Fusarium* spp. causing damping off disease.

Gegenava *et al.* (1976) found that seed treatment with thiram was more effective against the root rot and *Fusarium* wilt of trifoliate orange and citrus seedlings.

Suhag (1976) treated the diseased trees with 0.2% benlate or bavistin soil drenching during March, June, September, December months regenerated the guava trees to normal fruits.

David and Pieczarka (1979) reported the common root-rot of celery seedlings caused by *Rhizoctonia solani* and *Fusarium solani* was effectively controlled with weekly drenches by using dithane M-45 + difoltan 4f.

Laville and Chalandon (1982) studied the effect of fosetyl-Al on the *Phytophthora* root-rot when applied at the rate of 2000 ppm as a foliar spray and protected trees against any subsequent attack for 90 days after treatment.

Davis (1982) demonstrated soil drenching with systemic fungicide metalaxyl and foliar spray with fosetyl-aluminium effectively controlled *Phytophthora* root rot of sour orange seedlings.

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Padule *et al.* (1983) reported that Bordeaux mixture, captan and brassicol though given more or less similar control of *Rhizoctonia* root-rot of Kagzilime seedling, the seedling stand and vigour were more in the seed beds treated with Bordeaux mixture.

Raghavendra Rao (1984) found that seed treatment with carbendazim and soil drenching with captan and captafol was highly effective in controlling damping-off of citrus seedlings.

Rajput and Sriharibabu (1985) recommended to cut and destroy the diseased rotted-roots followed by application of Bordeaux paste to cut surfaces in early stages effectively reduced the root rot in citrus. They also suggested to avoid the cultural practices which were likely to cause injury to the roots and thereby making in roads for the fungal infection.

Sharma (1989) tested nine fungicides against *Rhizoctonia solani* the damping-off of *C. Jambhiri* (rough lemon) seedlings and found that TBZ (tebuconazole) as soil drench, resulted 94% seedling survival, followed by carbendazim, quintozone and thiram.

Jagannathan (1989) reported that among the seven fungicides tested against root rot of acid lime caused by *Rhizoctonia solani*, germination and plant survival was maximum with benomyl (96.5%) followed by carbendazim (91.2%) and minimum with PCNB (Quintozone).

Dilip *et al.* (1991) conducted an experiment on chemical management of root rot of cow pea caused by *F. solani* and found that mancozeb and captafol seed treatments gave maximum disease control.

Shukla (1992) reported that out of six fungicides ziram, shield (mancozeb), karathane (dinocap) and bavistin (carbendazim) were most effective against *F. solani* causing root rot in neem.

Hundoo *et al.* (1992) studied that kavach (chlorothalonil) and JK stein (methyl benzimidazole carbamate) showed best disease control of *F. solani* root rot of egg plant in glass house conditions giving 67% and 63% protection respectively.

Kore and Mane (1992) reported that dry root-rot of kagzilime was effectively reduced by seed treatment with carbendazim and copper fungicides.

Roux *et al.* (1992) observed that fumigation with methyl bromide to reduce numbers of *Phytophthora nicotianae*, *Fusarium solani*, *Fusarium oxysporum* and elimination of *T. semipenetrans* from the soils of citrus

Straus and Labuschagne (1993) in their *in vivo* studies found that drenching with prochloraz at 4000 5g a.i/cm³, benomil, thiabendazole and flusilazole + carbendazim mixture (4000 5g a.i/cm³ and 8000 5g a.i/cm³) also significantly reduced the incidence of *F. solani* in the roots of artificially infected rough lemon seedlings.

Sharma *et al.* (1993) observed that the soil drenching with carbendazim (0.1%) was highly effective against wilt of mango seedlings caused by *Fusarium solani* (Mart.) Sacc.

Satyanarayana and Ramasubba Reddy(1994) reported that dry root rot of sweet orange can be effectively checked by drenching the tree basins with 0.1 per cent carbendazim followed by another drenching of 0.25 per cent mancozeb or 0.2 per cent chlorothalonil, 12-24 h after irrigation at monthly intervals (one liter chemical fluid per sq.m area).

Verma and Navatej Singh (1999) evaluated the efficacy of different fungi toxicants against *Fusarium* root rot of citrus seedlings in two months old nurseries in Punjab. Soil drenching with 0.2 per cent carbendazim and 1.2 per cent Bordeaux mixture gave the maximum protection against the disease with 85.9 per cent and 82 per cent disease reduction over the control

Correction of stress factors predisposing the dry root rot disease by providing good drainage, soil aeration and treatment with benzimidazole fungicides provide effective control (Naqvi, 1999).

Thind *et al.* (2004) conducted a field experiment to evaluate the efficacy of the fungicides in the management of *Phytophthora* disease of kinnow mandarins for two seasons. Maximum reduction (88.4%) in lesion size was achieved in ridomil MZ followed by aliette (85%) and also observed the reduction of *Phytophthora* propogules density in citrus rhizosphere to the maximum (85.1%) with redomil MZ as compared to akomine, kavach and aliette.

2.8.5 Integrated management

Timmer and Leyder (1978) proved that pre-plant fumigation with methyl bromide, sodium-N methyl dithio carbamate and heat treatment temporarily

eliminated *Phytophthora parasitica* var. *nicotianae* and reduced root rot and damping-off of sour orange seedlings more effectively.

A combination of *T. harzianum* and PCNB at 1-2 mg per g gave better control than either component alone against damping-off of bean, tomato and egg plant seedlings caused by *Rhizoctonia solani* (Hadar *et al.*, 1979) and of stem rot of carnation in the glass house (Elad *et al.*, 1981).

Krishnamohan and Kandaswamy (1986) reported that the addition of farm yard manure at the rate of 10 t/ha along with inoculation of *Trichoderma viride* in soil increased the germination of cotton and reduced the post-emergence mortality of seedlings due to *Rhizoctonia* spp.

Effect of fungicides and *T. harzianum* on *Rhizoctonia* collar rot of passion fruit under inoculated conditions was studied. In disease control studies, collar rot symptoms were not observed in rizolex, bavistin and *T. harzianum* treatments, while in the other treatments, incidence of collar rot was 50-100 per cent after 60 days of inoculation with *R. solani*. Specific rizolex treatment (44.0%), while in other treatments, it was at par with control. Lesion length was significantly less in bavistin (3.13 cm), foltaf (3.38 cm), rizolex (3.75 cm) and *T. harzianum* (4.13 cm) treatments than control (6.63 cm) (Sawant *et al.*, 1994).

Krishna Rao and Krishnappa (1994) reported that soil solarization provided better seedling emergence (84.67%) than seed treatment with carbendazim (82.67%) or carbosulfan (79.67%) or soil application of *Glomus fasciculatum* (71.33%) in *Meloidogyne incognita* – *Fusarium* infected chickpea field.

Rajeswari (1997) reported that dry root-rot of mung bean caused by *Macrophomina phaseolina* was effectively controlled by integration of *Trichoderma harzianum* as soil application (5gm/kg) + seed treatment (10^8 cfu/ml) with sublethal doses of carbendazim (0.02%) significantly reduced the dry root-rot incidence (95.3%) over soil treatment (91.5 %) and seed treatment alone.

Ushamalini *et al.* (1997) reported that the efficacy of biocontrol agents, farmyard manure (FYM) and plant products against cowpea wilt caused by *Fusarium oxysporum* f.sp. *tracheiphilum*. Seed treatment with *Trichoderma harzianum* registered 98.8 per cent seed germination and was on par with the standard treatment of fungicide carbendazim compared to the lower germination per cent of 93.2 in control. The rhizosphere and non-rhizosphere population of fungi, bacteria and actinomycetes was more in neem cake applied plots whereas the population of *Trichoderma* was more in pots sown with seeds treated with talc based product of *T. viride* and *T. harzianum* with respect to *Trichoderma* the minimum root to shoot (R/S) ratio of 1-55 was recorded by neem cake at 20 days after sowing (DAS), while at 40 and 60 DAS, farmyard manure and *Delonix regia* recorded the minimum R/S ratio of 1/30 and 1-33 respectively.

Dhrub Singh and Andotra (1997) suggested that trash burning and FYM incorporation to the nursery beds followed by drenching with thiram @ 0.3% for effective management of seedling mortality in citrus.

Sreeramulu *et al.* (1998) conducted an experiment on the efficiency of VA-mycorrhiza, *Glomus fasciculatum* and *T. harzianum* in damping-off and black shank disease of tobacco seedlings under nursery conditions in comparison with

the recommended fungicides viz., copper oxychloride (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.

Hegde and Anahosur (1998) reported that the application of neem cake + *T. harzianum* + Ridomil-MZ + garlic mustard seed extract + mulching of the wet soil with transparent polythene sheet in hot summer was the best treatment and resulted in maximum survival of betel vines 88.89 per cent compared to 11.11 per cent in control. The soil temperature increased from 24.5°C to 29.6°C. The population of *Phytophthora capsici* was reduced to 25.76 per cent from 89.76 per cent in control. Also there was considerable increase of colonies per gram of soil of *T. harzianum* (155.55×10^3) other fungi (47.33×10^3) bacteria (85.0×10^4) and actinomycetes (35.00×10^3) which have suppressed *P. capsici* with their antagonistic effect.

Venkateswar Rao (1998) reported that an integration of soil solarization (six weeks), *T. viride* (native) and mancozeb (0.3%) effectively (82.2%) controlled the stem and root rot of sesame caused by *Macrophomina phaseolina* under field conditions.

Patel and Anahosur (1998) enumerated rhizosphere microflora of chickpea under irrigated conditions and found that carbendazim as seed treatment and soil drench coupled with soil application of *T. harzianum* reduced maximum fungal count of *Fusarium* spp. and *Macrophomina phaseolina*. FYM also reduced

pathogen population and stimulated all other microflora more than the other treatments there by probably operated multicorner antagonism against *Fusarium* spp. and *M. phaseolina*.

Gour *et al.* (1999) recommended application of *Trichoderma viride* 0.5 per cent formulation + 0.2 per cent copper oxychloride in early stages of infection for reducing the root-rot disease intensity in citrus.

Verma and Singh (1999) reported that wheat straw mulching in the nursery beds followed by soil drenching with thiram @ 0.3 per cent also reduced seedling mortality in citrus.

Ram *et al.* (1999) reported that integration of soil application of BCA (*T. harzianum*) with fungicidal rhizome treatment [bavistin (carbendazim) + ridomil MZ (metalaxyl + mancozeb)] increased the efficiency of rhizome rot disease control caused by *F. solani*.

Gupta *et al.* (1999) reported that under field conditions, integration of soil application of *T. pseudokoningii* (formulated product, NURSERY GUARD) with mancozeb (0.1%) provided 47.62 to 52.4 per cent root rot (*F. solani*) disease control which resulted in significant increase (over the control) in sprouting of mulberry stem-cuttings (53.1-63.3%) and plant survival (52.6-57.8%).

Anitha and Tripathi (2000) reported greenhouse studies and field experiments were conducted on integrated disease management of seedling diseases of okra caused by *Rhizoctonia solani* and *Pythium aphanidermatum* (Edson) Fitzp. By using a combination of varietal resistance, chemical and

biological means of control. Seed treatment with carbendazim followed by soil application of *Trichoderma viride* Pers was proved effective as disease incidences recorded were 5 and 18.33 per cent varsha upahar and pusa sawani varieties, as compared to 45 and 62 per cent incidences observed in control respectively.

Dubey (2000) reported that the seeds treated with *G. virens* in integration with thiram gave maximum germination (93.0%), minimum disease incidence (8.6%) and intensity (9.9%) with highest yield (2020 kg ha⁻¹) followed by *G. virens* treated groundnut seeds against *Rhizoctonia solani*.

Bunker and Mathur (2001) evaluated three biocontrol agents viz., *T. harzianum*, *T. aureoviride* and *T. viride* were evaluated individually and in combinations and in integration with Bavistin seed treatment in pathogen infested soil in pots for suppression of dry root rot pathogen *Rhizoctonia solani* in bell pepper (*Capsicum frutescens*) cv. California wonder. Mortality in these treatments was 9.1 and 12.9 per cent as compared to 20 and 13 per cent in their integration with bavistin seed treatment after 45 days; and 12.0 per cent as against 14.7 and 17.3 after 60 days of sowing. In treatments where *Trichoderma* spp. were applied on seeds, their cfu ranged from 0.52 – 0.69 x 10⁵ g⁻¹ soil, and where bavistin seed treatment, their cfu ranged from 0.77 – 0.81 x 10⁵ g⁻¹ soil. The highest inoculum density of *Trichoderma* spp. (1.21 x 10⁵ g⁻¹ soil) was in the seed plus soil treatment of *T. harzianum* + *T. aureoviride* followed by seed plus soil application of *T. harzianum* + *T. viride* (0.99 x 10⁵ g⁻¹ soil) and soil application of *T. harzianum* + *T. viride* in integration with bavistin seed treatment (90.92 x 10⁵ g⁻¹ soil).

Srinivasulu (2001) studied that *T. viride* 50g + neem cake 5kg/palm/year checked the linear spread of *Ganoderma* to maximum extent (22 cm) with in a period of four months from date of treatments imposed and untreated palm record linear spread of 77.6 cm in coconut.

Nagaraju *et al.* (2002) evaluated the efficacy of two antagonists *T. viride* and *T. harzianum* in reducing *R. solani* of paddy under transplanted conditions. The biological control agents were applied as basal treatments (or) as root dip + a spray (R+S). A chemical control treatment consisting of seed treatment with carbendazim (2 g kg⁻¹ seed) + one spray of hexaconazole (2 ml litre⁻¹) was also applied. Among the biocontrol agents R+S treatment with *P. fluorescens* was the most effective, reducing the disease incidence by upto 62.13% of that in untreated control followed by T+S treatment with *T. viride* (58.87%) and the R+S *P. fluorescens* treatment reduced in the highest grain yield of rice 5414 kg ha⁻¹ over-aged both years, 47.68% above the yield in the untreated control.

Vanitha and Suresh (2002) reported on effect of biocontrol agents and amendments (non chemicals) on the management of collar rot of brinjal. Lesser disease incidence of collar rot was recorded (9.44%) in *T. viride* seed treatment + soil application of adathoda leaf powder and FYM treated plots. The fungicide (carbendazim) seed treatment alone given recorded 10.83% of disease incidence in this experiment which used were effectively control the disease incidence compared to control, the disease incidence of 39.30 per cent, with regard to yield the highest of 12000 kg P lot⁻¹ was registered in both *T. viride* (ST) + Adathoda

leaf powdered application. FYM (SA) treated plots as well as the fungicide carbendazim treated plots.

Mathur *et al.* (2002) conducted an experiment by integration of soil solarization and pesticides for management of rhizome rot of ginger caused by *F. solani* and *Pythium myriotylum*. They observed that all the treatments in the solarized fields had significantly less disease compared to the untreated control. Least disease was recorded in captan seed dressing + phorate soil application (2.6%) followed by ridomil MZ seed treatment and phorate soil application + ridomil MZ drench (2.9%), respectively.

Kaushik *et al.* (2002) reported that the soil solarization with 25 μ m transparent polythene sheets during May-July resulted in significant reduction in soil population of the damping-off fungi viz., *Fusarium oxysporum* f.sp. *dalbergiae*, *F. solani*, *Pythium aphanidermatum*, *Rhizoctonia solani* and *R. bataticola* compared to non-solarised soil. Solarization coupled with biocontrol agents – *T. viride*, *T. harzianum*, *T. longibrachiatum* and *P. fluorescens* and sub-lethal doses of captan and carbendazim exhibited significantly higher seed germination and seedling survival of *A. nilotica*, *Albizia lebbeck*, *Azadirachta indica* and *Dalbergia sisso*.

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 *P. fluorescens* was distinctly superior.

The effect of carbendazim and thiophanate methyl on the growth of *Pseudomonas fluorescens* (ARR1G, ARR2, ARR10, CHAO, FP1, FP7, KKM1, KM2, Pfl, VPT2, VPT4 and VPY10) isolates was evaluated under glasshouse conditions. The combined application of antagonist *P. fluorescens* + thiophanate methyl resulted in the highest plant survival rate (81.67%) (Malathi *et al.*, 2002).

Ziedan *et al.* (2002) reported that treatment of mandarin rhizospheric soil with *B. megatherium* and fungicides [20 g captan (vitavax) + 20 g topsin M-70 (thiophanate-methyl)] per tree effectively controlled *Fusarium* root-rot and reduced the disease severity than the control and population density of *Fusarium* spp. was reduced around the feeder roots of mandarin trees.

Mohanbabu *et al.* (2002) reported that seed treatment *T. viride* I₃ and neem cake application recorded the minimum root rot (*Macrophomina phaseolina* (Tassi) Goid) incidence (6.47%) of black gram in acid soil as compared to *T. viride* I₃ alone (7.52%) and neem cake application alone (8.72%).

Neelamegam and Govindarajalu (2002) opined that integration of FYM + *T. viride* can serve as an excellent food base to improve the proliferation and antagonistic activity of *T. viride* which may lead to effective biological control of damping-off of tomato caused by *Pythium indicum*.

Koushik *et al.* (2002) recorded highest seedling survival (85.1 to 92.6%) in integrated treatment of soil solarization either of fungicides (carbendazim/captan) in the management of damping-off disease in forest nurseries

Gnanavel and Jayaraj (2003) reported the control of damping-off of tomato caused by *Pythium aphanidermatum* by using *Trichoderma viride* alone or in combination with soil solarization. *T. viride* along with soil solarization effectively reduced the pre and post-emergence damping-off by 75.23 per cent and 88.34 per cent, respectively over control with concomitant increase in seedling vigour index (1182.0) and biomass of tomato (656 mg/m²).

Durai (2004) conducted pot culture experiments on integrated disease management for charcoal rot of sesamum under glasshouse conditions. Out of 15 treatment combinations maximum disease reduction was achieved by integrated use of seed treatment with mancozeb (100 ppm) + soil application of native *T. viride* (10 g/kg soil) and *Pseudomonas fluorescens* (20 ml/kg of soil) as this treatment recorded PDI only 10.67. This treatment not only reduced the disease incidence to a maximum extent, but also recorded maximum plant height (22.29 cm), shoot length (5.41 cm), and root length (4.27 cm), maximum dry weight of shoot (76.28 mg) and root (52.31 mg) of sesame when compared to all other treatments.

Mohamedy (2004) reported that under field conditions, soil application of Topsin-M + Plant guard or *T. harzianum* twice caused a significant reduction on *Fusarium* root rot incidence in sour orange and Rangpur lime citrus rootstocks.

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MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The materials and methods used in the present investigation are broadly described under the following heads.

3.1 EXPERIMENTAL SITE

Experiments pertaining to the present research work were carried out at Citrus Research Station, Petlur and AICRP on Tropical Fruits (Citrus), Tirupati. Citrus Research Station, Petlur, site is geographically located at latitude $13^{\circ} 57'$ and longitude $79^{\circ} 35'$ at an altitude of 71.87m and the AICRP on Tropical Fruits (Citrus) site is at 13° North latitude at 79° East longitude and at an altitude of 182.9 m in tropical belt of South India.

3.2 CLIMATE

The experimental sites come under Semi-Arid Tropical Southern Zone of A.P based on Agro-climatic conditions. Southern Zone is characterized by fairly hot summer ($>40^{\circ}\text{C}$) and rainfall receiving in two spells viz., South-West (June – September) and North-East (October–January) monsoon period. Normally the rainfall is more in North-East monsoon than South–West monsoon period.

3.3 DISEASE SURVEY

Extensive survey for the occurrence of root rot incidence of acid lime (*Citrus aurantifolia* Swingle) was conducted during summer months of 2001-02 across the acid lime growing areas such as Chittoor, Nellore, Kadapa, Guntur and West Godavari districts of Andhra Pradesh. In each district, major acid lime

growing tracts of five mandals were chosen to know the prevalence and distribution of root rot disease. The disease was surveyed on the basis of characteristic expression of symptoms on leaves and branches of bearing trees. The disease incidence was expressed in percentage.

During the survey root samples of suspected plants infected with root rot disease were collected from the field trees. The roots were examined for the presence of brown, necrotic lesions, as well as shredding of lateral and fine roots. The diseased root samples were collected in polythene bags with proper labelling and brought to the laboratory for isolation studies.

Information regarding plant material used, depth of soils, water quality, irrigation conditions, methods of irrigation, manurial schedule etc., were recorded during survey with the help of a questionnaire which is given under Appendix A.

3.4 GLASSWARE

Borosil/Corning make flasks (250, 500 and 100 ml) Petri plates (90 mm diameter) test tubes (150 x 15 mm) microscope slides (2.5 x 7 x 2 mm) and pipettes (1, 5 and 10 ml) were used throughout the research work.

3.4.1 Cleaning of Glassware

The glassware was first washed with tap water before placing them into cleaning solution of the following composition:

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

The glassware was kept in the cleaning solution for 24 h, later thoroughly washed with tap water and finally rinsed with distilled water and dried.

3.5 CHEMICALS AND EQUIPMENTS

Chemicals used were of ‘Analytical Grade’ (B.O.D Anala; E. Merck). The pH of the medium was adjusted to the required level with N/10 NaOH and N/10 Hcl. Compound microscope (100X, 400X magnification) was used for observing the causal agents. Trinocular research microscope attached with camera and Adobe Photoshop software was used for micro-photography. Weighments were done on single pan electronic balance with a sensitivity of 0.0001g. The spore concentration of the test pathogen was determined by using a haemocytometer. Portable soil temperature thermometers with stainless steel probes were used to record soil temperature and Moisture Scan Diviner 2000 series was used for measurement of soil moisture during the soil solarization period.

3.5.1 Media used

The composition of the different media used during the course of investigation is given below:

i) Potato dextrose agar medium (PDA)

Peeled potato slices	:	200 g
Dextrose	:	20 g
Agar	:	20 g
Distilled water	:	1000 ml

ii) *Trichoderma* selective medium (Elad and Chet, 1983)

KH_2PO_4	:	0.2 g
MgSO_4	:	0.9 g
Kcl	:	0.15 g
NH_4NO_3	:	1.0 g
Glucose	:	3.0 g
Agar	:	15.0 g
Distilled water	:	1000 ml
Rose Bengal*	:	0.15 g
Chloramphenical	:	0.25 g
Metalaxyl	:	0.3 g
pH	:	6.5

* Added just before plating

iii) Nutrient agar medium

Beef extract	:	1 g
Yeast extract	:	2 g
Peptone	:	5 g
Nacl	:	5 g
Agar	:	15 g
Distilled water	:	1000 ml

3.5.2 Sterilization

Sterilization of glassware (wrapped in butter paper) and media was achieved by autoclaving at 15 lbs psi (121.6°C) for 20 minutes. The soil mixtures

were sterilized by autoclaving at 15 lbs psi 1 h for 3 consecutive days. The root bits were surface sterilized by 0.1% mercuric chloride followed by three changes in sterile water. All cultural work and *in vitro* experiments were carried out under aseptic conditions under laminar flow chamber using flame for sterilizing the tips of inoculation needle and forceps.

3.5.3 Laboratory techniques

General laboratory techniques followed were those described by Dingra and Sinclair (1995) with slight modification wherever necessary.

3.6 ISOLATION AND IDENTIFICATION OF THE PATHOGEN

Root samples of acid lime trees showing typical symptoms of root rot disease were collected from the Citrus Research Station (CRS), Petlur and also from the orchards of acid lime during the survey. For isolation of the pathogen, infected roots and root bits were washed thoroughly under tap water followed by rinsing in sterile water. Root bits (5-8 mm) were cut from the root portions showing brown discolouration of the bark. These bits were placed in a Petri plate containing 0.1% mercuric chloride and surface sterilized for 30 seconds, followed by three washings with sterile water. Then the bits were transferred to the Petri plate containing sterile discs of blotting paper. The dried bits were subsequently transferred to the Petri plates of potato dextrose agar medium (PDA) and incubated at $25 \pm 1^{\circ}\text{C}$. The isolates were purified by establishing single spore cultures by single hyphal tip method (Rangaswamy and Mahadevan, 1999). Pure cultures were maintained on freshly prepared PDA slants at 5°C and periodically transferring to fresh medium. The *Fusarium* isolate obtained from Citrus Research

Station (CRS), Petlur was used for pathogenicity test by soil inoculation method in pot culture.

3.6.1 Identification of cultures

The pathogenic isolates were mainly identified on the basis of cultural and morphological characters (Subramanian 1971, Barnett 1962; Sarbhoy and Agarwal, 1990).

3.6.2 Pathogenicity

Fusarium isolate of Citrus Research Station (CRS), Petlur was used to study the pathogenicity and effect of different inoculum levels for determining optimum damage threshold level. A pot culture experiment was conducted to study the pathogenicity of the CRS, Petlur *Fusarium* isolate by soil inoculation following the procedure given by Haware (1980).

The inoculum multiplied on sand-sorghum medium (SSM) was added to the sterilized soil at 2.0, 4.0, 6.0, 8.0 and 10.0 per cent (w/w) in the pots (10 x 8"). Soil mixture without inoculum served as control. Each pot was transplanted with 30 days old 20 healthy acid lime seedlings of local variety in each replication for each inoculum density level. Five replications were maintained for each inoculum density level of CRS, Petlur *Fusarium* isolate and the data on the root rot disease incidence was recorded after 45 days of planting.

Further, the roots of diseased plants were used for reisolation of pathogen and compared with the original *Fusarium* isolate obtained from CRS, Petlur. The isolate was sent to ITCC, IARI, New Delhi for identification. Optimum inoculum

density level obtained in the test was used for categorization of other *Fusarium* isolates which were obtained from different places during the survey.

3.7.1 Preliminary screening of different isolates by rapid pathogenicity vial test (Roberts and Kraft, 1971)

Two months old seedlings were uprooted from the pots by flooding with water to loosen the pot mixture. Roots were gently washed under running tap water and finally washed with sterile water. Acid lime seedlings were then placed with roots immersed in distilled water in the beakers. Spore suspensions of the different isolates were prepared by flooding seven day old PDA culture plates with sterile water. The resultant spore suspension (1×10^8 conidia/ml) was strained through muslin cloth and 40 ml of such spore suspension was transferred into test tubes (12" x 1.5"). The acid lime seedlings were then placed in each test tube such that the roots were immersed in spore suspension and cotton was placed around the plant at the mouth of the test tube. The test tubes were set in a pot containing soil and incubated at room temperature for observation of symptoms expression.

3.7.2 Categorization of different isolates obtained during the survey

Sand sorghum media (SSM) was used for developing mass inoculum of different isolates in the laboratory. The SSM was prepared by mixing 100 g sorghum grain and 50 g dry sand with 50 ml distilled water, autoclaved for 1 h at 15 lb pressure in 500 ml flasks twice and then inoculated with three mycelial agar discs (8 mm dia) cut from periphery of one week old colony and incubated at

27 $\pm$ 1°C for ten days. Inoculum of different isolates was mixed separately in sterilized soil with 8.0 per cent inoculum density.

The soil inoculated with pathogen filled in pots was kept for three days for multiplication. Twenty acid lime seedlings of 30 days old local variety were transplanted in pots. Each treatment was replicated thrice. Observations on number of seedlings infected were counted after 45 days of planting and expressed in percentage. Based on mean root rot incidence they were classified as aggressive (1-25%), moderately aggressive (26-50%), highly aggressive (51-75%) and extremely aggressive (76-100%) (Chohan and Jasmith Kaur, 1976). Further, the roots of the diseased plants infected with the highly aggressive isolate were used for reisolation of pathogen and the same isolate was sent to the ITCC, IARI, New Delhi for further, identification and confirmation.

3.7.3 Effect of different temperatures on growth and sporulation of test fungus

To determine minimum, optimum and maximum temperature and optimum pH for growth and sporulation of *Fusarium*, the isolate found aggressive in the categorization test was used. Twenty five ml solid medium was distributed in flasks, autoclaved and were inoculated and incubated for 14 days at different temperatures viz., 10, 15, 20, 25, 30, 35 and 40°C for observations. The data in terms of mycelial dry weight of the fungal vegetative growth and extent of sporulation were recorded at 14 days after incubation to determine minimum, optimum and maximum temperature for growth and sporulation of test fungus.

3.7.4 Effect of different pH levels on growth and sporulation of test fungus

In order to study the effect of different hydrogen ion concentrations on growth and sporulation at different pH levels viz., 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0, the pH of the basal medium was adjusted with the help of BDH narrow range pH paper using N/10 NaOH. The flasks containing 25 ml of the basal medium, adjusted to different pH levels, were autoclaved, inoculated and incubated at $25 \pm 1^\circ\text{C}$ for 14 days.

3.8 COLLECTION OF SOIL SAMPLES FOR ISOLATION OF RHIZOSPHERE FUNGI AND BACTERIA

Soil samples were collected from the rhizosphere during active growing period (March to May, 2001) of local acid lime variety. The soil samples were collected along with fibrous roots at a depth of 30 cm around the rhizosphere of acid lime diseased trees. The selected root samples were shaken to remove the bulk of the extraneous soil. The soil particles intimately adhering to the roots were collected and mixed to provide a composite rhizosphere soil. The collected rhizosphere samples were kept in sterile polythene bags and brought to the laboratory with proper labelling for isolation of bioagents.

3.8.1 Isolation of fungi and bacteria from rhizosphere soil

For isolation of rhizosphere microflora, the method proposed by Johnson and Curl (1972) was followed. From each pooled sample, one gram of soil was taken in a 250 ml conical flask with 99 ml of sterile distilled water. The sample was agitated for 5 min. Serial dilution of soil suspensions were prepared by

pipetting out one ml and mixing with 9 ml of sterile distilled water. One ml of required dilution 1:10000 (10^{-4}) for fungi and 1: 100000 (10^{-5}) for bacteria was poured in sterilized Petri dishes containing suitable media and the Petri dishes were rotated gently to distribute the spores throughout the medium and allowed for solidification. After solidification the plates were incubated at room temperature $28 \pm 2^\circ\text{C}$. PDA medium was used for isolation of general fungi, where as *Trichoderma* selective medium (TSM) was used for isolation of *Trichoderma* spp. and nutrient agar medium for isolation of bacteria. For each dilution, three replications were maintained. After evaporation of soil moisture, the flasks were placed in hot air oven at 105°C for 24 h. The flasks with the shade dried soil were weighed accurately. The dry weight of the soil was obtained by subtracting the weight of the empty conical flask. This dry weight was utilized to express the pattern of distribution of microflora quantitatively. The plates were examined daily and counts were made on 3rd day for bacteria and 7th day for fungi with the help of a 'Quebec' colony counter. Occurrence of bacteria and total number of fungi per gram of oven dry soil was calculated.

3.8.2 Maintenance of cultures

The fungi and bacteria isolated from the rhizosphere were transferred to culture tubes containing appropriate medium for further examination and identification.

3.9 ISOLATION AND MAINTENANCE OF *TRICHODERMA* FROM COMMERCIAL FORMULATIONS

3.9.1 Commercial formulations of *Trichoderma* spp.

To isolate *Trichoderma* spp. from the commercial formulations, 4 g of the commercial formulation of each of the isolate was added to 100 ml sterile distilled water and 0.5 ml of the preparation was aseptically transferred into TSM containing plates. The inoculated plates were incubated at $28 \pm 2^{\circ}\text{C}$ for one week and the resultant *Trichoderma* colonies were isolated and reidentified. Cultures of *Trichoderma* spp. were maintained on PDA by periodic transfers for further studies. The details of commercial biocontrol agents used in the study are given in Table 1.

Table 1: Details of native and commercial biocontrol agents used in the present study

Trade Name	Bio-agent	Formulation	Manufacturing company / Source of supply
Bio-cure-F	<i>T. viride</i>	Talc	T-Stanes & Company limited, 8/23-24, race course, Road, Coimbatore-641018
Bio-root	<i>T. viride</i>	Talc	The Pragathi Agro Bio-products No.6, Red lands, Dargamitta, Nellore-524003.
Sunderma	<i>T. viride</i>	Talc	Sun Agro Biosystem private limited, 3/340, Main Road, Madanandapuram, Porur, Chennai-600 116

3.10 CATEGORIZATION OF BIOAGENTS ON THE BASIS OF THEIR GROWTH RATE ON PDA MEDIUM

Depending on the growth rate on PDA medium, 15 isolates of *Trichoderma* sp. were categorized into three groups viz., very fast, fast and medium growing colonies. The colonies of isolates which grew 75 to 90 mm in diameter within 72h of incubation were categorized as medium growing and the isolates whose colonies grew 75 to 90 mm in diameter within 60 h of incubation were categorized as fast growing, whereas the colonies which grew 75 to 90 mm in diameter within 48 h of incubation were categorized as very fast growing.

3.10.1 Phenotypic characters

To know the variability among the different isolates in their phenotypic characters, observations were taken regarding their colony characters on PDA medium.

3.11 *IN VITRO* SCREENING OF NATIVE ISOLATES AND COMMERCIAL BIOAGENTS FOR ANTAGONISTIC ACTIVITY AGAINST TEST PATHOGEN BY DUAL CULTURE TECHNIQUE

Twelve native and three commercial isolates of *Trichoderma* were screened for their antagonistic activity by dual culture on PDA in Petri plates, both the fungi were inoculated at the same time. Discs (8 mm) cut from the margin of vigorously growing cultures were placed at the opposite periphery of the plates. The plates were incubated at room temperature. *Trichoderma* isolates were selected for their potential based on their growth after contact with the test

pathogen. Likewise three native bacterial isolates were also tested against the test pathogen.

The percentage of inhibition was calculated by the formula :

$$\text{Per cent inhibition of } F. \text{ solani} = \frac{\text{Growth of } F. \text{ solani in control} - \text{Growth of } F. \text{ solani in treatment}}{\text{Growth of } F. \text{ solani in control}} \times 100$$

3.12 EFFECT OF DIFFERENT SUBSTRATES ON MASS MULTIPLICATION OF *TRICHODERMA* SPP

The substrates used were farm yard manure, gypsum, neem cake, vermicompost, citrus rind and press mud. All the substrates were powdered, sieved through 60 mesh added with appropriate amount of water so as to obtain 50 per cent moisture level and autoclaved at 15 psi for one hour for two successive days. Then two discs of seven day old culture of the potential *Trichoderma* isolate (PCT₆) were added separately into conical flasks and incubated at room temperature. Observations regarding population level in different substrates were taken after 7, 14, 21 and 28 days of incubation by dilution plate technique.

3.12.1 Mass multiplication of *Trichoderma* isolate on different substrates

FYM : Dry decomposed FYM (50 g) was taken into 250 ml conical flasks. To this 24 ml tap water was added as per the moisture level so as to adjust to the 50 per cent (w/v) and mixed thoroughly. The flasks were autoclaved for 1h at 15 psi for two successive days. Each flask was inoculated with *Trichoderma* isolate (PCT₆).

Gypsum: Gypsum (50 g) was taken in each of the 250 ml conical flasks and 23 ml tap water was added so as to get 50 per cent (w/v) moisture level.

Neem cake : Neem cake (50 g) was taken in each of the 250 ml conical flasks and 23 ml tap water was added so as to get 50 per cent (w/v) of moisture level.

Vermicompost : Vermicompost comprising 50 g was taken in each of the 250 ml conical flasks and 21 ml of tap water was added so as to get 50 per cent (w/v) of moisture level.

Citrus rind : Citrus rind (50 g) was taken in each of the 250 ml conical flasks and 24 ml tap water was added for adjusting moisture level to 50 per cent.

Pressmud : Pressmud (50 g) was taken in each of the 250 ml conical flasks and 23 ml tap water was added for adjusting moisture to 50 per cent (w/v).

3.12.2 Determination of moisture per cent in substrates

Oven dry method was used for determination of moisture per cent in different substrates. Empty porcelain dishes with lids were dried in a hot air oven for 15 min and then transferred to a desiccator for cooling. The sterilized dishes with lids were weighed and five grams of substrate was taken in to the dishes and their weight was recorded. Then the dish was put in hot air oven at 105⁰ C for 6 h. After that the dish was removed from the hot air oven and placed into desiccator again for cooling and was finally weighed. The moisture present in the substrates was calculated by using the following formula.

$$\text{Moisture percentage} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.12.3 Population assessment of potential *Trichoderma* isolate in different substrates by dilution plate technique

One gram of substrate was taken and mixed in 9 ml of sterile distilled water in a test tube to achieve 10^{-1} dilution. From this suspension, one ml was transferred to 9 ml of sterile water in test tube (10^{-2}). Thus serial dilutions were done by transferring 1 ml of the suspension to the subsequent tubes to get 10^{-6} dilutions. One milliliter of the desired suspension was transferred to sterile Petri plates. Fifteen ml of melted and cooled TSM was added in the same Petri plate, rotated gently and allowed to solidify and incubated at room temperature. Colony forming units (cfu) present in 1 g of the substrate was calculated by using the following formula :

$$\text{cfu/g} = \frac{\text{cfu/Plate} \times \text{Dilution factor} \times 10 \text{ ml water}}{\text{Amount plated} \times \text{weight of inoculum taken}} \times 100$$

3.13 *IN VITRO* SCREENING OF FUNGICIDES AND NATURAL PRODUCTS AGAINST TEST PATHOGEN

The effect of six fungicides viz., copperoxychloride (50% W.P), mancozeb (75% WP), chlorothalonil (75% WP), carbendazim (50% WP), propiconazole (25% EC) and hexaconazole (5% EC) in different concentrations of 100, 500, 1000 ppm were evaluated for their efficacy on *F. solani*. Likewise two natural products nimbidine and ovis were tested for their efficacy. The details of the

fungicides and natural products employed in the present investigations are given in Table 2.

3.13.1 Poisoned food technique (Nene and Thapliyal, 1993)

Potato dextrose agar medium was prepared and sterilized in an autoclave. Ten ml of fungicidal solution was mixed thoroughly with 10 ml melted PDA medium and poured into the Petri plate under aseptic conditions and allowed to solidify. Controls were maintained using sterile water. From seven day old culture plates, 8 mm discs were cut from the outer margin of vigourously growing test fungus with sterilized cork borer and transferred to the centre of the plate containing fungicide poisoned medium. Controls were maintained by placing fungal discs in untreated plates. The entire work was carried out under aseptic conditions and plates were then kept at room temperature. The radial mycelial growth was measured when the growth of the test fungus completely occupied the control plates. The per cent inhibition was calculated by the formula :

$$\text{Per cent inhibition} = \frac{\text{Radial growth of colony in control plate} - \text{Radial growth of colony in treated plate}}{\text{Radial growth of colony in control plate}} \times 100$$

3.14 COMPATIBILITY OF FUNGICIDES AND NATURAL PRODUCTS WITH POTENTIAL BIOCONTROL AGENT

The compatibility of the potential native *Trichoderma* (PCT₆) isolate with the six fungicides and two natural products were tested by poisoned food technique. The procedure mentioned earlier in the screening of fungicides and natural products against *F. solani* was followed.

Table 2 : Details of fungicides and natural products employed in the investigation

S. No.	Trade name	Common name	Active ingredient	Chemical name	Source of supply
A. FUNGICIDES					
1.	Blitox	Copper oxychloride	50% WP	Dicopper chloride trihydroxide	Rallies India Ltd. Agrochemicals Division, Regd. Office, Rallis House, 21, D Sukhadavala Marg, Mumbai
2.	Indofil M-45	Mancozeb	75% WP	Manganese ethylene bis- ^u dithiocarbamate plus zinc	Indofil Chemicals Company – A Division of Modipon Ltd., Nirlon House, 3 rd Floor, Dr A Beasant Road, Worli, P.O.Box 9112, Mumbai 400 025.
3.	Kavach	Chlorothalonil	75% WP	Tetrachloroisophthalonitrile	Novartis India Limited, Tata Road, Mumbai
4.	Bavistin	Carbendazim	50% WP	Methyl benzimidazole – 2 yl carbamate	BASF India Ltd., P.O.Box No.19108, S K Ahire Marg, Mumbai – 400 025.
5	Tilt	Propiconazole	25% EC	1-{[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]mythyl}-1H-1,2,4,-triazole	Syngenta India Limited, Tata Road, Mumbai
6	Contaf	Hexaconazole	5% E	(RS)-2-(2-4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol	Rallis India Ltd. Agrochemicals Division, Regd. Office, 21 DS Sukhadavala Marg, Mumbai.
B NATURAL PRODUCTS					
1	Nimbidine			Azadiractin	T. Stanes and Company Limited, 23, Race Course Road, Coimbatore
2	Ovis			Citronella oil	Ocean Agro India Limited, GIDC, Nandesari, Baroda

3.15 INTEGRATED DISEASE MANAGEMENT IN ACID LIME NURSERY

Nursery experiment

In the present study, different approaches were attempted in order to identify the most effective and economical approach for the management of *Fusarium* root rot disease of acid lime seedlings with integration of physical, biological and chemical methods. Based on *in vitro* performance, the potential bioagent, *T. viride* (PCT₆) and safest fungicide mancozeb (75% WP) were selected to test their ability to control root rot of acid lime. The nursery experiment was conducted in a sick plot.

3.15.1 Preparation of *Fusarium solani* sick plot

A sick plot of *F. solani* was prepared by applying biomass of the disease affected roots collected from previous years. Biomass with acid lime plant debris (roots) was evenly spread and mixed with soil by ploughing. The same area was used for conducting the nursery experiment. The soil in the nursery was further made sick by applying biomass of *F. solani*, before conducting the experiment (Appendix B).

3.15.2 Preparation of *Trichoderma viride* (PCT₆) formulated product

Modified molasses yeast media was prepared as described by Chandrasekhar *et al.* (1998). Molasses yeast media was prepared by adding 5g of yeast and 100g of Sugar (instead of molasses) in 1 liter of distilled water. Thoroughly dissolved 300 ml medium was transferred to saline bottles and plugged with non-absorbent cotton. The saline bottles with media were

sterilized in an autoclave at 15kg/cm^2 (121.6°C) for 1 h and cooled to room temperature and 150 mg ciproflaxin was added and thoroughly mixed. Eight mm mycelial discs of 5 day old culture of *T. viride* was transferred to each saline bottle and incubated for 10 days. The medium was agitated by keeping the saline bottles on a rotary shaker for one day and draining the contents. This broth culture contains fungal mat, spores and antibiotics. One liter of broth culture was mixed with 2 kg talc powder and 10 g of carboxy methyl cellulose was added. Talc based *T. viride* formulation was packed in opaque polythene bags after air drying and sieving (12% moisture content) and kept in cool dry place. This formulated product was used in nursery and main field experiments.

3.15.3 Lay out of the experiment

The nursery beds of one m^2 each area were prepared. Totally 36 sub plots were prepared in 12 rows and each treatment was replicated three times (Fig.1). The treatments were allotted to the individual plots by following randomized block design (RBD). The details of each treatment are as follows.

$T_1 =$ *Trichoderma viride* (*T. viride*) (100g)

$T_2 =$ Farm Yard Manure (FYM) (2.5 kg)

$T_3 =$ Vesicular arbuscular mycorrhiza (VAM) (100g)

$T_4 =$ Neem cake (NC) (0.5 kg)

$T_5 =$ Mancozeb (M) (0.2%)

$T_6 =$ Soil solarization (SS)

$T_7 =$ *T. viride* (100g) + FYM (2.5 kg)

$T_8 =$ *T. viride* (100g) + FYM (2.5 kg) + VAM (100g)

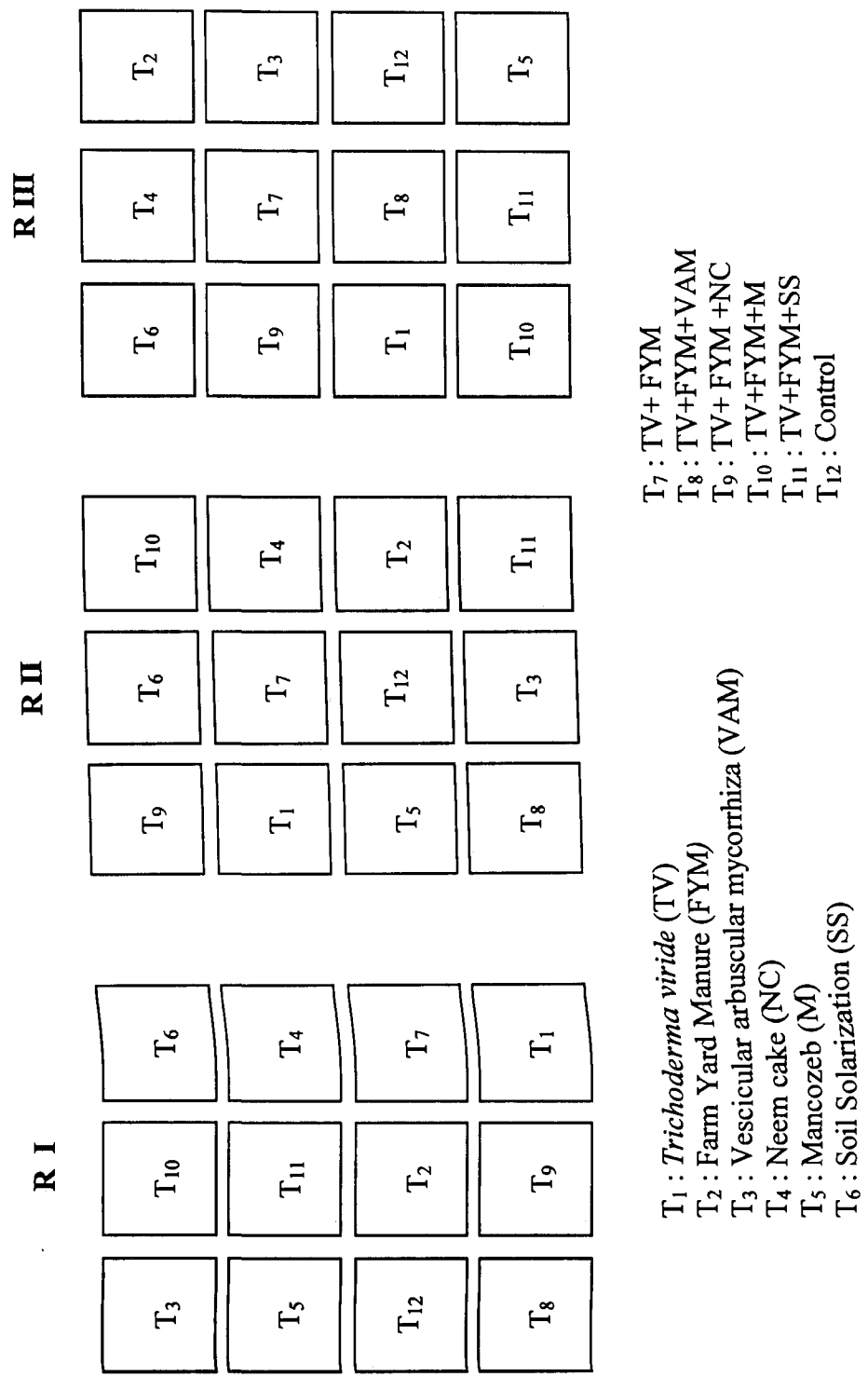


Fig 1: Layout of the nursery experiment showing randomised arrangement of different treatments

$T_9 = T. viride$ (100g) + FYM (2.5 kg) + NC (0.5 kg)

$T_{10} = T. viride$ (100g) + FYM (2.5 kg) + M (0.2%)

$T_{11} = T. viride$ (100g) + FYM (2.5 kg) + SS (100 μ m)

$T_{12} =$ Control

3.15.4 Soil solarization

Six weeks of peak summer with higher ambient temperature, sunshine hours and low rainfall was identified in the months of May and June every year according to the meteorological data of previous years. Polyethylene mulching was carried out from 2nd May to 12th June, 2003 (Appendix C).

Nursery beds were irrigated to field capacity one day prior to mulching in order to increase the thermal sensitivity of resting structures of pathogen and to improve heat conduction. Soil was mulched manually on prepared flattened beds with transparent polyethylene film (100 μ m thick). Special care was taken to keep the soil in good tilth before spreading the polyethylene sheet from all the sides, burying and sealing along the edges with the top soil allowing close contact between the polyethylene sheet and the soil.

The 1 x 1 meter nursery beds were remained covered for a period of 42 days maintaining appropriate controls. Soil temperatures at 5, 10 and 15 cm soil depth was recorded daily at 2.30 P.M during the period when temperatures reached peak. Portable soil temperature thermometers with stainless steel probes were used for the measurement of temperatures. Moisture Scan Diviner 2000 series was used for measurement of soil moisture.

3.15.5 Efficacy of different treatments in the management of root rot of acid lime

The nursery beds were exposed to sunlight (soil solarization) for 42 days. On 43rd day the polyethylene sheet was removed and earthened up. The talc based native potential bioagent *Trichoderma viride* @ 100 g, FYM 2.5 kg, Neem cake 0.5 kg and VAM 100 g per square meter were applied to the respective beds by way of uniform sprinkling and incorporation into soil. Mancozeb @ 2.0g in 1 liter of water was drenched per square meter area of the beds and neem cake was also incorporated 1 week before sowing. Acid lime (local variety) seeds @ 10 g/m² area were sown in the beds two days after imposing the other treatments. The seeds were sown in rows with an inter row spacing of 10 cm. The seed beds were covered with green leaf and regularly watered as per requirement. The seedlings were observed up to 180 days and recorded the shoot length, root length, germination (%) and disease incidence.

$$\text{Per cent disease incidence} = \frac{\text{No. of seedlings infected}}{\text{Total no. of seedlings assessed}} \times 100$$

3.16 INTEGRATED MANAGEMENT OF ROOT ROT DISEASE IN ACID LIME ORCHARD

A field experiment to manage *Fusarium* root rot in acid lime was conducted during 2004 on six years old trees with initial incidence of disease at Citrus Research Station, Petlur.

The best result obtained in the nursery i.e. integration of *T. viride* (PCT₆), FYM, mancozeb and neem cake was imposed two times during June and

September, 2004 to the plant basins of the identified diseased trees. For assessing the root rot incidence, roots from one square foot area up to a depth of a foot were collected randomly from four corners of each tree basin (All India Co-ordinated Research Project, 1990). The samples were air dried for two days and the proportion of healthy and rotted roots were calculated by weight. The per cent rotting was assessed before imposing treatments and 180 days after treatments.

3.17 STATISTICAL ANALYSIS

The data obtained from the different experiments (transformed using angular transformation wherever necessary) was statistically analysed (Panse and Sukhatme, 1978) using complete randomized design (CRD) and analysis of variance technique. The data pertaining to nursery experiment was statistically analysed using randomized block design (RBD). Data on shoot length, root length and germination per cent of acid lime seedlings were recorded at 180 DAS. Seedling vigour index was calculated by using the formula given by Abdul Baki and Anderson (1973).

Seedling vigour index = root length (cm) + shoot length (cm) x germination (%).

RESULTS

CHAPTER IV

RESULTS

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

4.1 SURVEY IN ACID LIME GROWING AREAS OF ANDHRA PRADESH

A systematic survey on the occurrence of root rot disease associated with acid lime (*Citrus aurantifolia* Swingle) was carried out in Chittoor, Nellore, Kadapa, Guntur and West Godavari districts of A.P during 2001-02 (Fig. 2).

Data presented in Table 3 revealed that disease incidence varied with the area and was in the range of 10.21 per cent (Pedavegi, West Godavari Dt.) to 22.95 per cent (Gudur, Nellore Dt.). Among the five districts surveyed root rot disease incidence was high in Nellore district (20.25%) followed by Kadapa (18.60%), Chittoor (15.61%), Guntur (13.71%) and West Godavari (11.98%). The data collected on type of soils during survey revealed that acid lime orchards grown in sandy clay loams are more prone to the root rot disease.

Results of survey with respect to influence of planting material on per cent root rot incidence was comparatively more in budlings (19.00%) than seedling trees (15.39%) (Table 4). Similarly, the disease incidence recorded was comparatively more in the acid lime trees grown in shallow soils (19.46%) than deep soils (12.80%) (Table 5). Also the orchards irrigated with normal E.C ($<0.5 \text{ dsm}^{-1}$) water showed least disease incidence (14.51%) as compared to orchards

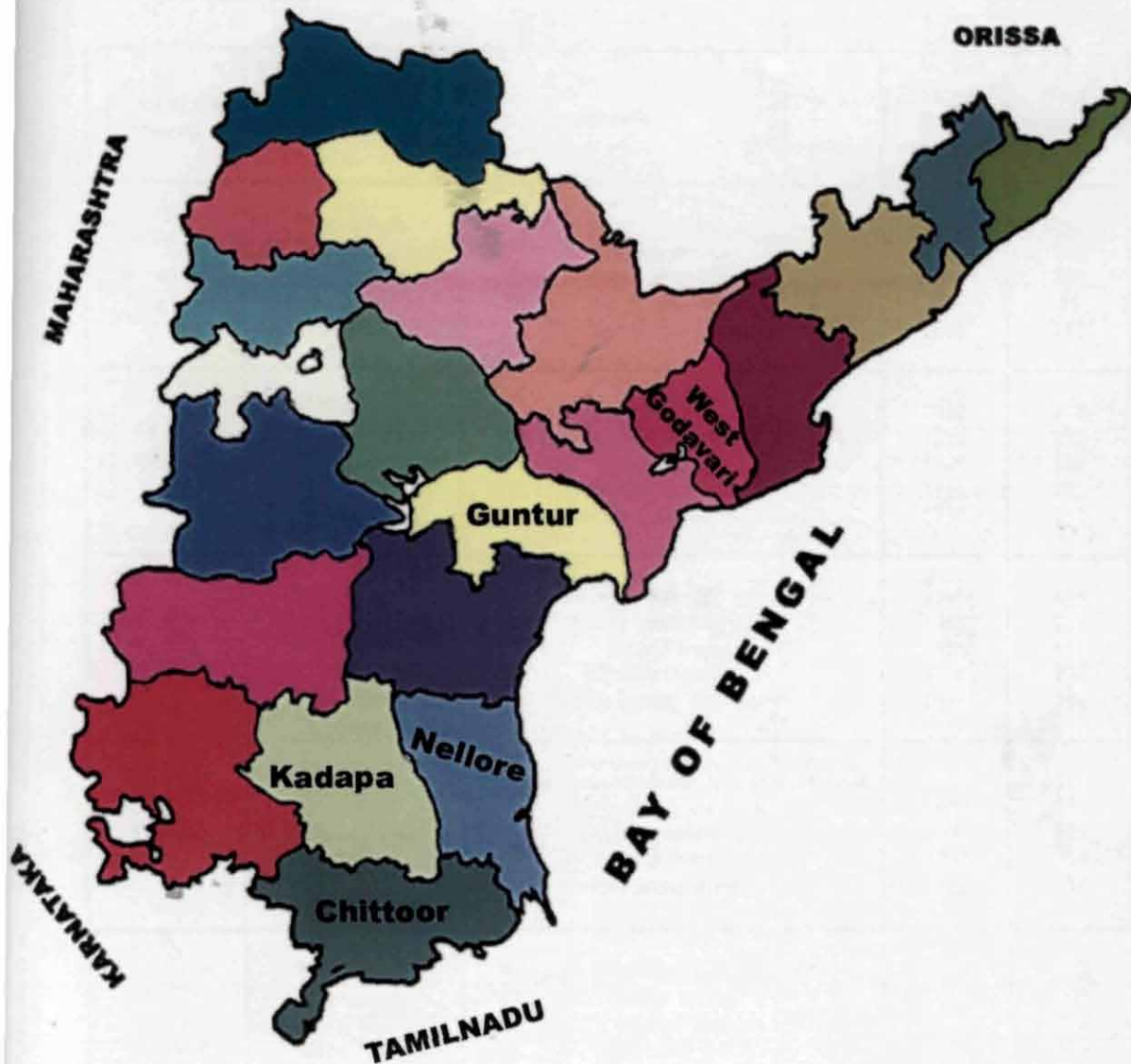


Fig.2: Districts surveyed for acid lime root rot disease in Andhra Pradesh during 2001- 02

Table 3 : Incidence of root rot disease in major acid lime growing districts of Andhra Pradesh

Name of the District	Name of the Mandal	Type of soils	No. of trees examined	No. of diseased trees	Mandal wise disease Incidence (Per cent)
Chittoor	Thottambedu	Sandy clay loams	1603	253	15.78
	Gudipala	Sandy loams	1283	140	10.91
	B.N. Kandriga	Sandy clay loams	1940	436	22.47
	Ramachandrapuram	Sandy clay loams	685	75	10.95
	Sri Kalahasthi	Sandy clay loams	1005	113	11.24
	Mean				15.61
Nellore	Rapur	Sandy clay loams	1850	418	22.59
	Balayapalli	Sandy loams with calcareous sub soil	1295	283	21.85
	Dakkili	Sandy loams	1705	333	19.53
	Gudur	Sandy clay loams	1320	303	22.95
	Venkatagiri	Sandy clay loams with calcareous sub soil	1700	257	15.12
	Mean				20.25
Kadapa	Obulavaripalli	Sandy loams Si. clay	2990	625	20.90
	Rly. Kodur	Sandy loams, Si. clay	1360	265	19.49
	Penagaluru	Sandy loams	3330	651	19.55
	Vemula	clay loams	3370	571	16.94
	Pulivendula	Sandy loams, clay loams	1630	246	15.09
	Mean				18.60
Guntur	Tenali	Sandy loams deltaic calcareous black soils	1550	229	14.77
	Pattipadu	Clay to Sandy clay loams	1800	257	14.27
	Guntur Rural	Clay loams	2000	279	13.95
	Duggirala	Clay loams	2200	304	13.81
	Chundur	Red sandy loams	2100	254	12.09
	Mean				13.71
West Godavari	Devarapalli	Sandy clay loams, clay loams	2830	378	13.36
	Gopalpuram	Sandy loams	2600	308	11.85
	Kamavarapu Kota	Sandy loams	2080	250	12.02
	Tadepalligudem	Sandy clay loams	2550	302	11.84
	Pedavegi	Sandy clay loams	1880	192	10.21
	Mean				11.98

Table 4 : Incidence of root rot disease in seedling and budling orchards of acid lime in AP

Plant material	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
Seedlings	42146	6485	15.39	2.7652*
Budlings	6510	1237	19.00	

* Significant at 0.05 level

Table 5 : Effect of soil depth on the incidence of acid lime root rot in AP

Soil depth	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
Shallow soils (30 cm)	22436	4365	19.46	4.7569*
Deep soils (90 cm)	26220	3357	12.80	

* Significant at 0.05 level

Table 6 : Effect of water quality on the incidence of acid lime root rot disease in AP

Water quality	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
Normal E.C (<0.5 dSm ⁻¹)	45446	6596	14.51	3.0061*
High E.C (>0.5 dSm ⁻¹)	3210	1126	35.08	

* Significant at 0.05 level



Plate 1: Field infected plant showing loss of turgidity in leaves during initial stages



Plate 2: Field infected plant showing yellowing of foliage



Plate 3: Field infected plant showing profused flowering



Plate 4: Field infected plant showing excessive fruit set



Plate 5: Diseased tree showing severe wilting followed by drying



Plate 6: Roots showing rotting, moist decay and shredding of bark

Table 7 : Effect of irrigation on the incidence of acid lime root rot in AP

Irrigation conditions	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
Optimum irrigation	34690	48364	13.94	2.6882*
Limited irrigation	13966	2886	20.66	

* Significant at 0.05 level

Table 8 : Effect of method of irrigation on the incidence of acid lime root rot disease in AP

Method of irrigation	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
Basin irrigation	37705	5974	15.84	2.8172*
Flood irrigation	1756	447	25.46	

* Significant at 0.05 level

Table 9 : Effect of fertilizers on the incidence of acid lime root rot disease in AP

Type of fertilizer	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
In-organic	30040	6608	22.00	2.6973*
Organic	7250	1105	15.14	

Table 7 : Effect of irrigation on the incidence of acid lime root rot in AP

Irrigation conditions	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
Optimum irrigation	34690	48364	13.94	2.6882*
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Table 9 : Effect of fertilizers on the incidence of acid lime root rot disease in AP

Type of fertilizer	Total no. of plants examined	Total no. of diseased plants	Disease incidence (%)	t- value
In-organic	30040	6608	22.00	2.6973*
Organic	7298	1105	15.14	

* Significant at 0.05 level

4.2 ISOLATION AND IDENTIFICATION OF ORGANISM ASSOCIATED WITH ROOT ROT

Isolation of the pathogen was made from the diseased acid lime roots showing typical root rot symptoms. From the Table 10 it was found that out of the 62 infected root samples collected during the survey from various locations, 38 samples yielded *Fusarium* spp. on isolation. Further, 13 samples yielded *Ganoderma* spp and *Rhizoctonia* spp. and 11 samples yielded none of the pathogens.

4.2.1 Pathogenicity

4.2.1.1 Pot culture experiment

Pathogenicity was proved on 30 days old seedlings raised in pots having sterile soil. The symptoms appeared 45 days after inoculation. All the seedlings in the inoculated pots were infected and showed typical root rot symptoms which includes yellowing, rolling and drooping of leaves and drying of seedlings (Plate 7).

4.2.1.2 Inoculum density

To determine the optimum inoculation level, five different levels of inoculum of *F. solani* were tested in pot culture experiment. Maximum per cent root rot incidence (81.00) was observed in 10.0 per cent inoculum density and it was on par with 8.0 per cent inoculum density (79.7%). Minimum per cent root rot incidence (12.00) was observed in 2.0 per cent inoculum density (Table 11). Thus,

Table 10 : Distribution of root rot disease in acid lime growing districts of Andhra Pradesh

District	Mandal	Isolation of Fungi from the roots		
		<i>Fusarium</i>	Others	None
Chittoor	Tottambedu	+	-	
	Tottambedu	-	-	*
	Gudipala	-	+	
	Gudipala	+	-	
	B.N. Kandriga	-	+	
	B.N. Kandriga	+	-	
	B.N. Kandriga	+	-	
	Kalahasthi	+	-	
	Kalahasthi	+	-	
	Ramachandra puram	+	-	
Nellore	Venkatagiri	+	-	
	Venkatagiri	+	-	
	Balayapalli	-	-	*
	Balayapalli	+	-	
	Balayapalli	-	+	
	Balayapalli	+	-	
	Rapur	+	-	
	Rapur	+	-	
	Rapur	-	-	*
	Rapur	+	-	
	Dakkili	-	+	
	Dakkili	+	-	
	Dakkili	+	-	
	Dakkili	-	-	*
	Gudur	+	-	
	Gudur	-	-	*
Kadapa	Vemula	+	-	
	Vemula	-	-	*
	Vemula	+	-	
	Pulivendula	+	-	
	Pulivendula	-	-	*
	Obulavaripalli	+	-	
	Obulavaripalli	+	-	
	Obulavaripalli	-	-	*
	Kodur	+	-	
	Kodur	+	-	
	Kodur	-	-	*
	Penagaluru	+	-	
	Penagaluru	-	+	



Un-inoculated pot showing
healthy acid lime seedlings

Inoculated pot showing
drying of acid lime seedlings

Plate 7: Pathogenicity test with *F. solani* in pot culture experiment

Table 11 : Effect of different inoculation levels of *Fusarium* isolate (CRS, Petlur) on root rot incidence of acid lime

S. No.	Inoculum density per cent (w/w)	Root rot incidence (%) ^a
1	2.0	12.0 (20.27) ^b
2	4.0	47.3 (43.45)
3	6.0	54.7 (47.70)
4	8.0	79.7 (63.22)
5	10.0	81.0 (64.16)
6	Control	0.0 (0.0)

SEm± 0.71
CD at 5% 2.40

^a = Mean of five replications

^b = Figures in parentheses are angular transformed values

the inoculum density of 8.0 per cent was found to be optimum for causing sufficient damage.

4.2.1.3 Vial test

Preliminary screening of 38 isolates of *Fusarium* spp. by rapid pathogenicity vial test revealed that only 15 isolates produced pathogenic reaction expressing symptoms of root rot (Plate 8) and the remaining isolates were discarded due to their non-pathogenic nature. To determine the identity of the species, the cultural and morphological characters of pathogenic isolates were studied and all the 15 isolates were identified as *Fusarium solani* (Plate 9).

4.2.1.4 Categorization of *F. solani* isolates

Fifteen *F. solani* isolates (PCF₁ to PCF₁₅) isolated from different locations (Table 12) were used for categorization based on disease incidence.

On perusal, the Table 13 showed that PCF₂ recorded the maximum root rot incidence (83.3%) followed by PCF₈ (73.3%) and the isolate PCF₁₃ recorded the least root rot incidence (23.3%). Based on disease incidence, the isolate PCF₁₃ was categorized as slightly aggressive (1-25% root rot incidence), isolates PCF₃, PCF₄ and PCF₁₅ as moderately aggressive (26-50%), isolates PCF₁, PCF₅, PCF₆, PCF₇, PCF₈, PCF₉, PCF₁₀, PCF₁₁, PCF₁₂, PCF₁₄ as highly aggressive (51-75%), and PCF₂ as extremely aggressive (76-100%) with 83.3 per cent disease incidence. The extremely aggressive isolate PCF₂ was reisolated and further confirmed as *F. solani* (ITCC 5742) at IARI, New Delhi.



Un-inoculated

Inoculated

Plate 8: Vial test for preliminary screening of *Fusarium* isolates

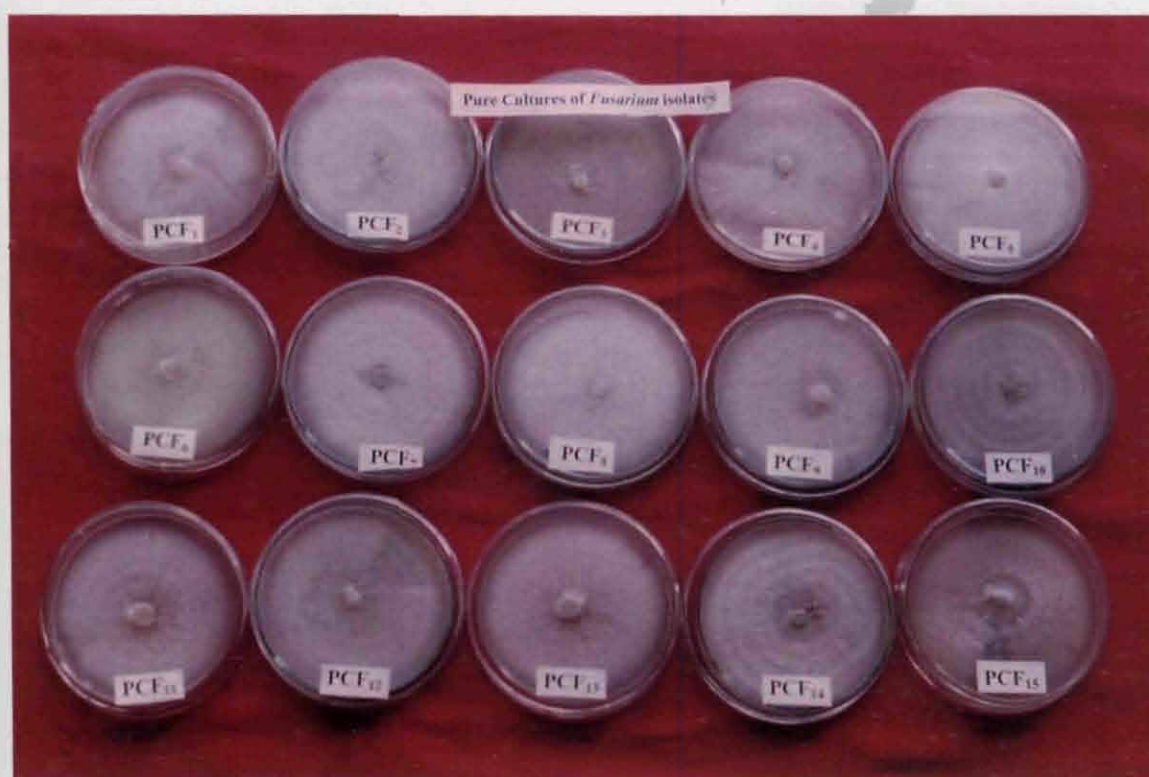


Plate 9: Pure cultures of *F. solani* isolates of different locations

Table 12 : Accession numbers of different *Fusarium isolates* along with place of collection of root rot samples from acid lime orchards

Accession number	Location
PCF ₁	CRS, Petlur, Venkatagiri
PCF ₂	Rapur
PCF ₃	Gudur
PCF ₄	Dakkili
PCF ₅	Balaya palli
PCF ₆	Vemula
PCF ₇	Rly. Kodur
PCF ₈	Obulavari palli
PCF ₉	Penagalur
PCF ₁₀	Tenali
PCF ₁₁	Gopalapuram
PCF ₁₂	Devarapalli
PCF ₁₃	Gudipala
PCF ₁₄	B.N. Kandriga
PCF ₁₅	Pulivendula

PCF = Petlur Citrus *Fusarium*

Table 13 : Categorization of different *F. solani* isolates obtained during the survey

Isolate	No. of seedlings/pot	No. of seedlings infected	Root rot incidence (%)	Mean root rot incidence (%)	Class of aggressiveness
PCF ₁	20	12	60.0	61.7	H.A
	20	13	65.0		
	20	12	60.0		
PCF ₂	20	16	80.0	83.3	E.A
	20	18	90.0		
	20	16	80.0		
PCF ₃	20	9	45.0	46.6	M.A
	20	8	40.0		
	20	11	55.0		
PCF ₄	20	6	30.0	33.3	M.A
	20	7	35.0		
	20	7	35.0		
PCF ₅	20	13	65.0	63.3	H.A
	20	12	60.0		
	20	13	65.0		
PCF ₆	20	11	55.0	60.0	H.A
	20	12	60.0		
	20	13	65.0		
PCF ₇	20	15	75.0	70.0	H.A
	20	15	75.0		
	20	12	60.0		
PCF ₈	20	14	70.0	73.3	H.A
	20	15	75.0		
	20	14	70.0		
PCF ₉	20	11	55.0	58.3	H.A
	20	13	65.0		
	20	11	55.0		
PCF ₁₀	20	15	75.0	66.6	H.A
	20	12	60.0		
	20	13	65.0		
PCF ₁₁	20	10	50.0	53.3	H.A
	20	10	50.0		
	20	12	60.0		
PCF ₁₂	20	11	55.0	58.3	H.A
	20	12	60.0		
	20	11	55.0		
PCF ₁₃	20	12	60.0	60.0	H.A
	20	11	55.0		
	20	12	60.0		

Table 13 : Categorization of different *F. solani* isolates obtained during the survey

Isolate	No. of seedlings/pot	No. of seedlings infected	Root rot incidence (%)	Mean root rot incidence (%)	Class of aggressiveness
PCF ₁	20	12	60.0	61.7	H.A
	20	13	65.0		
	20	12	60.0		
PCF ₂	20	16	80.0	83.3	E.A
	20	18	90.0		
	20	16	80.0		
PCF ₃	20	9	45.0	46.6	M.A
	20	8	40.0		
	20	11	55.0		
PCF ₄	20	6	30.0	33.3	M.A
	20	7	35.0		
	20	7	35.0		
PCF ₅	20	13	65.0	63.3	H.A
	20	12	60.0		
	20	13	65.0		
PCF ₆	20	11	55.0	60.0	H.A
	20	12	60.0		
	20	13	65.0		
PCF ₇	20	15	75.0	70.0	H.A
	20	15	75.0		
	20	12	60.0		
PCF ₈	20	14	70.0	73.3	H.A
	20	15	75.0		
	20	14	70.0		
PCF ₉	20	11	55.0	58.3	H.A
	20	13	65.0		
	20	11	55.0		
PCF ₁₀	20	15	75.0	66.6	H.A
	20	12	60.0		
	20	13	65.0		
PCF ₁₁	20	10	50.0	53.3	H.A
	20	10	50.0		
	20	12	60.0		
PCF ₁₂	20	11	55.0	56.6	H.A
	20	12	60.0		
	20	11	55.0		
PCF ₁₃	20	4	20.0	23.3	S.A
	20	5	25.0		
	20	5	25.0		
PCF ₁₄	20	14	70.0	68.3	H.A.
	20	15	75.0		
	20	12	60.0		
PCF ₁₅	20	12	60.0	50.0	M.A
	20	10	50.0		
	20	8	40.0		

S. A : Slightly aggressive 1-25% M.A: Moderately aggressive 26-50%
H.A : Highly aggressive 51-75% E.A : Extremely aggressive 76-100%

4.2.2 Cultural and morphological characters of *F. solani* (PCF₂)

Studies on cultural and morphological characters of *F. solani* (PCF₂) showed that it took five days to cover the petriplate completely. Colony colour of the PCF₂ isolate was brownish white on the agar surface with little aerial mycelium (Plate 10). Microconidia were abundant which were single septate, hyaline, cylindrical wedge shaped and measured about 9-16 x 2-4µm in size. Macroconidia were thick walled, subcylindrical, slightly curved, short and bent apical and indistinctly septate, predominantly three septate and 20-60 x 3.5-7µm in size (Plate 11). Chlamydospores terminal or intercalary, globose to subglobose in single, pairs or chain (Plate 12).

4.2.3 Effect of different temperatures on growth and sporulation of *Fusarium solani* (PCF₂)

Effect of different temperatures on vegetative growth and sporulation of the fungus *F. solani* grown at different temperatures viz., 10, 15, 20, 25, 30, 35 and 40°C and data in terms of average mycelial dry weight and sporulation obtained after 14 days of incubation are tabulated (Table 14).

The fungus yielded maximum mycelial dry weight (480 mg) at 30°C with maximum extent of sporulation i.e. more than 50 conidia / cm² followed by 455 mg mycelial dry weight at 25°C and minimum mycelial dry weight (176 mg) at 10°C with poor sporulation i.e. less than 20 conidia/cm².

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Plate 10: Pure culture of *F. solani* (PCF₂) on PDA medium used in different studies

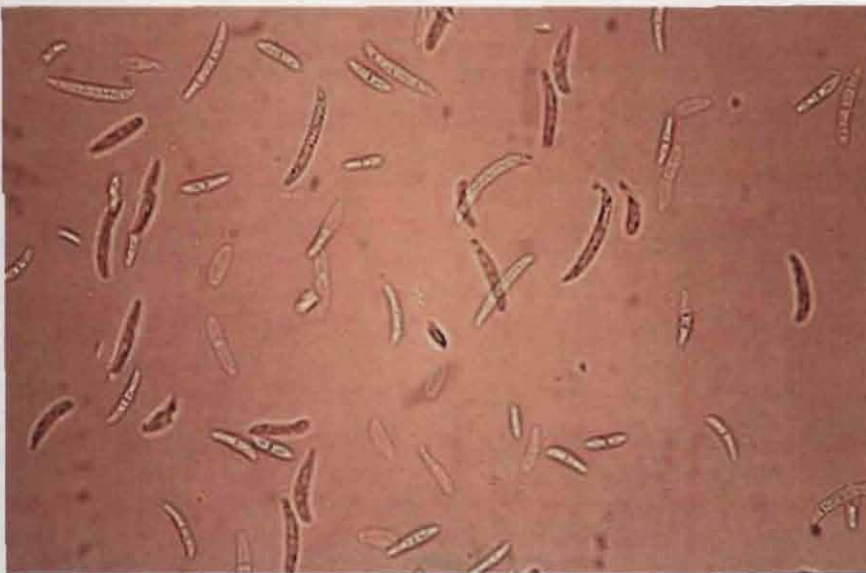


Plate 11: Micro and macro conidia of *F. solani* (PCF₂)

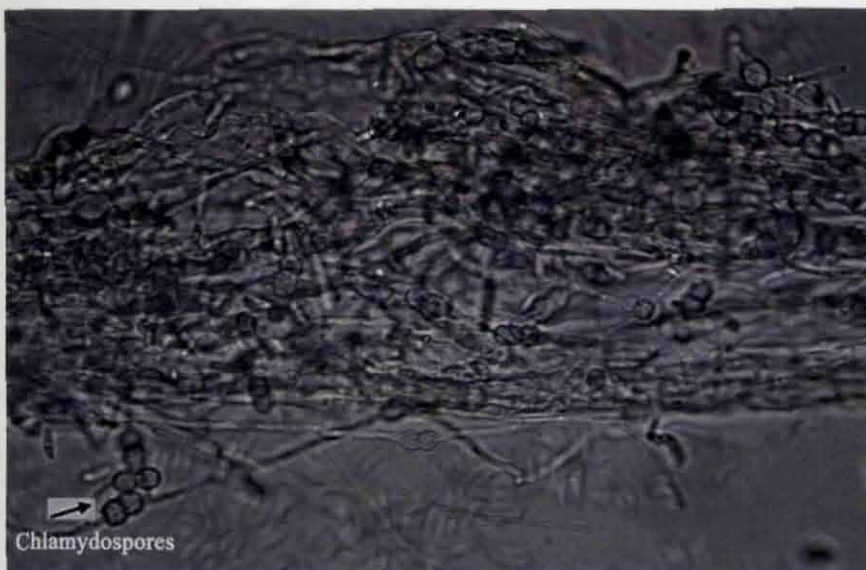


Plate 12: Spherical thick walled chlamydospores of *F. solani* (PCF₂)

Table 14 : Effect of different temperature on growth and sporulation of *F. solani* (PCF₂) after 14 days of incubation

Temperature (°C)	Mycelial dry weight (mg)	Sporulation
10	176	+
15	203	+
20	377	+++
25	455	++++
30	480	++++
35	310	++
40	190	+

SEm± 4.60

CD at 5% 18.72

Poor = + (< 20 conidia/cm²)
 Fair = ++ (< 40-20 conidia/cm²)
 Good = +++ (< 50-40 conidia/cm²)
 Excellent = ++++ (> 50 conidia/cm²)

4.2.4 Effect of different pH levels on growth and sporulation of *Fusarium solani* (PCF₂)

To determine the most suitable pH for vegetative growth and sporulation, seven different pH levels of media from 3.0 to 9.0 were tested. It is observed from the Table 15, that maximum mycelial dry weight of 490 mg with excellent sporulation (> 50 conidia /cm²) was at 6.0 pH followed by mycelial dry weight of 430 mg at pH 5.0 with an excellent sporulation (> 50 conidia / cm²). The fungus yielded least vegetative growth (140 mg) at 9.0 pH followed by (175 mg) at 3.0 pH.

4.3 ISOLATION OF FUNGI AND BACTERIA FROM THE RHIZOSPHERE

4.3.1 Fungal and Bacterial flora

The information on the number of fungal and bacterial colonies per gram of acid lime rhizosphere soil (cfu/g dry soil) are presented in the Table 16. Eight fungi viz., *Aspergillus niger*, *A. flavus*, *Rhizopus stolonifer*, *Penicillium oxalicum*, *P. nigricans*, *Fusarium solani*, *Helminthosporium* spp., *Mucor* spp and 12 isolates of *Trichoderma viride* were isolated by soil dilution plate method. Maximum number of colonies per gram of rhizosphere soil was recorded by *A. niger* (3.71×10^5) followed by *A. flavus* (2.72×10^5), *Fusarium* spp. (0.27×10^5), *Penicillium oxalicum* (0.6×10^5), *P. nigrican* (0.5×10^5) and *Helminthosporium* spp. (0.1×10^5). Among these, *A. niger* was most predominant in the rhizosphere of acid lime. The *Trichoderma* isolates showed optimum ($0.28-0.91 \times 10^5$) population. *Rhizopus* (0.18×10^5) and *Mucor* spp. (0.2×10^5) were less abundant in the

Table 15 : Effect of different pH levels on growth and sporulation of *F. solani* (PCF₂) after 14 days of incubation

pH levels	Mycelial dry weight (mg)	Sporulation
3.0	175	--
4.0	290	++
5.0	430	++++
6.0	490	++++
7.0	300	++
8.0	200	+
9.0	140	--

SEm± 3.01

CD at 5% 9.46

Poor = + (< 20 conidia/cm²)
Fair = ++ (< 40-20 conidia/cm²)
Good = +++ (< 50-40 conidia/cm²)
Excellent = ++++ (> 50 conidia/cm²)
-- = (No conidia)

Table 15 : Effect of different pH levels on growth and sporulation of *F. solani* (PCF₂) after 14 days of incubation

pH levels	Mycelial dry weight (mg)	Sporulation
3.0	175	--
4.0	290	++
5.0	430	++++
6.0	490	++++
7.0	300	++
8.0	200	+
9.0	140	--

SEm± 3.01

CD at 5% 9.46

Poor = + (< 20 conidia/cm²)
Fair = ++ (< 40-20 conidia/cm²)
Good = +++ (< 50-40 conidia/cm²)
Excellent = ++++ (> 50 conidia/cm²)
-- = (No conidia)

Table 16 : Qualitative and quantitative composition of rhizosphere microflora of acid lime local variety during 2001-02

Fungi	Number of colonies x 10 ⁵ /g dry sample
General fungi	
<i>Aspergillus niger</i>	3.71
<i>Aspergillus flavus</i>	2.72
<i>Rhizopus stolonifer</i>	0.18
<i>Penicillium oxalicum</i>	0.66
<i>Penicillium nigrican</i>	0.53
<i>Fusarium</i> spp.	0.27
<i>Helminthosporium</i>	0.36
<i>Mucor</i> spp	0.2
<i>Trichoderma viride</i>	
PCT ₁ ^a	0.28
PCT ₂	0.66
PCT ₃	0.80
PCT ₄	0.57
PCT ₅	0.91
PCT ₆	0.33
PCT ₇	0.16
PCT ₈	0.32
PCT ₉	0.25
PCT ₁₀	0.28
PCT ₁₁	0.25
PCT ₁₂	0.53
Bacteria	
PCB ₁ ^b	1698.8
PCB ₂	1489.7
PCB ₃	14.39.5

^a = PCT: Petlur citrus *T. viride*

^a = PCB: Petlur citrus Bacteria

|

|

rhizosphere. Among three isolates of bacteria, PCB₁ (1698.8×10^5) showed maximum population followed by PCB₃ (1489.7×10^5) and PCB₂ (1439.5×10^5).

44 **IN VITRO SCREENING OF NATIVE FUNGAL, BACTERIAL ISOLATES AND COMMERCIAL BIOCONTROL AGENTS**

4.4.1 **Interaction of soil mycoflora isolates (SMI) with *F. solani* (PCF₂)**

The interaction of soil mycoflora isolates (SMI) with *F. solani* were studied *in vitro* by dual culture technique. A total of 20 pure cultures isolated from the rhizosphere soil samples of acid lime local variety were used for screening against *F. solani* (PCF₂). The following four types of interactions were observed (Fig 3) on the 5th day when the check (*F. solani*) showed maximum growth in 9 cm diameter Petridishes on PDA.

Type I: Mutual intermingling: Both *F. solani* and SMI occupied equal area (4.5 cm each on 9 cm diameter petridish) at the same time without showing any apparent interaction. This type of interaction was observed in five fungal isolates of SMI.

Type II: Over growth of pathogen: *F. solani* overgrew (9 cm dia) the SMI and the latter was greatly inhibited and three fungal isolates exhibited this type of interaction.

Type III: Over growth of SMI: The SMI overgrew (9 cm dia) *F. solani* greatly inhibiting the later and no fungal isolates were found to suppress *F. solani* by such parasitic growth.

Type IV: No intermingling or slight inhibition: Both organisms mutually inhibited but approached each other until almost in contact (4.3 cm each) when

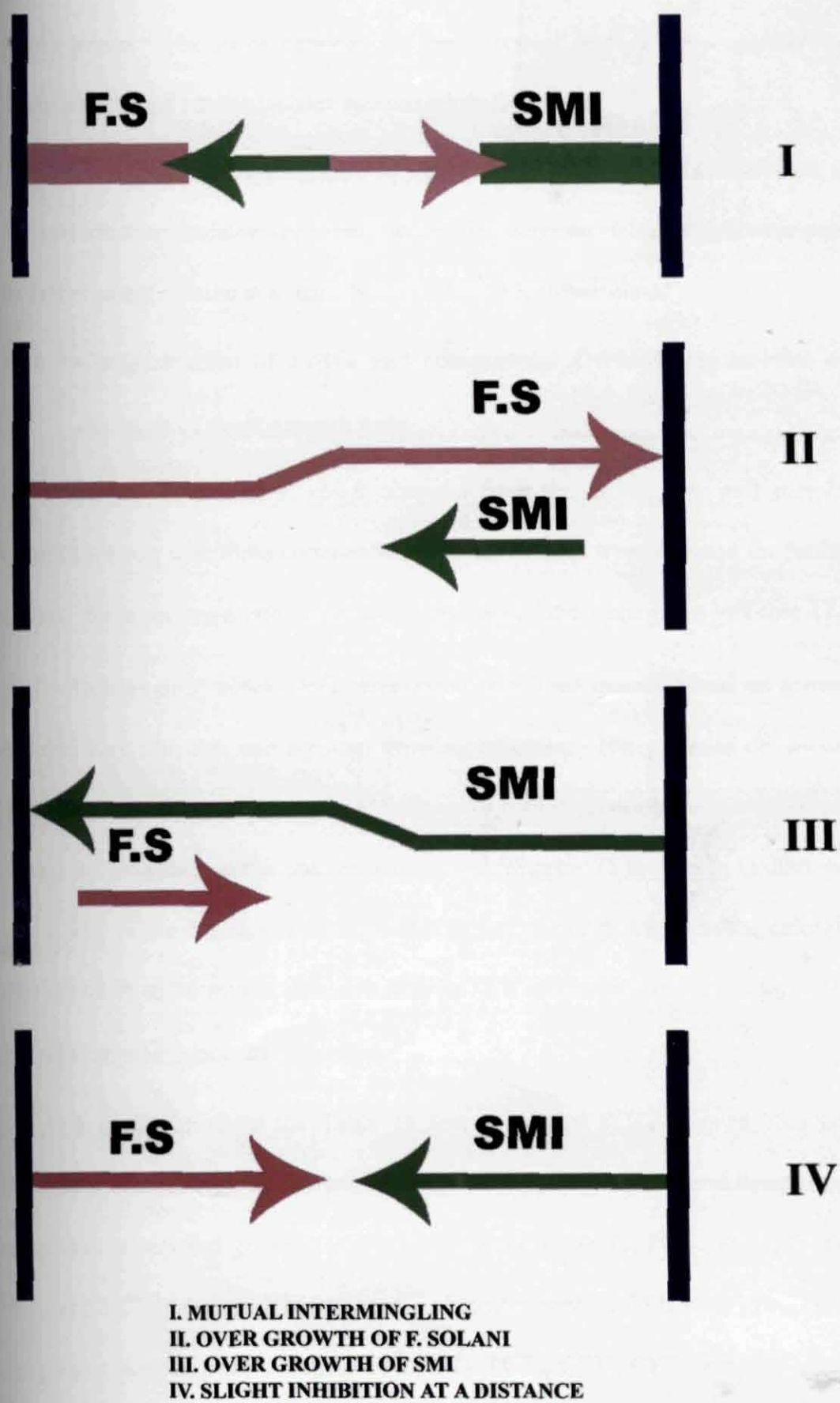


Fig.3: Types of interactions between soil mycoflora isolates (SMI) and *F.solani*

growth ceased. The space between the two colonies, though very narrow was clearly marked and twelve isolates showed slight inhibition.

The organisms showing type IV interaction were observed as inhibitors of *F. solani* and these isolates were identified as *Trichoderma viride*, which were used for further categorization and identification of an effective isolate.

4.4.2 Categorization of native and commercial *Trichoderma* isolates on the basis of their growth rate

Twelve isolates of *T. viride* obtained from the *rhizosphere* soil samples using TSM along with three commercial *T. viride* isolates were selected for further studies. The accession numbers of these *T. viride* isolates were given in Table 17.

Isolates of *T. viride* were categorized into three groups based on growth rate viz., very fast, fast and medium growing colonies. The colonies of isolates which grew 75 to 90 mm in diameter with in 48 h of incubation were categorized as very fast growing and the isolates whose colonies grew 75 to 90 mm in diameter with in 60 h of incubation were categorized as fast growing, where as the colonies which grew 75 to 90 mm in diameter with in 72 h of incubation were categorized as medium growing isolates.

It is evident from the Table 18 that PCT₁ and PCT₆ showed 76.3 and 78.3 mm in diameter of colony growth respectively, within 48 h and hence were categorized as very fast growing isolates. Isolates PCT₂, PCT₄, PCT₅, PCT₉, PCT₁₀, PCT₁₁ and PCT₁₂, showed 78 to 82 mm in diameter with in 60 h were grouped as fast growing where as the isolates PCT₃, PCT₇, PCT₈, CFT₁, CFT₂ and CFT₃ were

Table 17 : Accession numbers of different *Trichoderma* isolates along with place of collection of rhizosphere samples from acid lime orchards

Isolate	Location
PCT ₁ ^a	Gopalapuram
PCT ₂	Gudipala
PCT ₃	Vemula
PCT ₄	Pattipadu
PCT ₅	Tenali
PCT ₆	Balayapalli
PCT ₇	B.N. Kandriga
PCT ₈	Guntur rural
PCT ₉	Devarapalli
PCT ₁₀	Gudur
PCT ₁₁	Venkatagiri
PCT ₁₂	Dakkili
CFT ₁ ^b	Coimbatore
CFT ₂	Chennai
CFT ₃	Nellore

^a = PCT: Petlur Citrus *Trichoderma*

^b = CFT: Commercial formulation of *Trichoderma*

Table 18 : Categorization of native and commercial *Trichoderma* isolates on the basis of their growth rate

Isolate	Colony diameter (mm) after hours						Growth rate ^c
	12	24	36	48	60	72	
PCT ₁ ^a	11.3	25.0	50.6	76.3	90.0	--	Very fast
PCT ₂	7.6	19.0	41.6	59.0	78.0	81.3	Fast
PCT ₃	4.6	13.0	32.0	42.6	67.0	75.3	Medium
PCT ₄	10.6	28.6	56.0	71.3	81.3	89.3	Fast
PCT ₅	7.0	20.6	42.0	59.3	82.0	90.0	Fast
PCT ₆	11.6	25.6	51.0	78.3	90.0	--	Very fast
PCT ₇	8.0	18.0	35.3	51.3	71.3	79.3	Medium
PCT ₈	2.3	14.3	38.0	48.0	69.3	80.0	Medium
PCT ₉	8.0	20.6	48.6	71.3	80.6	88.6	Fast
PCT ₁₀	7.0	19.6	43.3	62.0	80.6	83.3	Fast
PCT ₁₁	10.3	24.6	57.3	72.0	81.3	90.0	Fast
PCT ₁₂	10.6	21.3	46.0	62.3	81.3	85.0	Fast
CFT ₁ ^b	8.0	21.0	42.0	48.3	68.0	77.0	Medium
CFT ₂	8.0	19.3	41.0	53.6	72.0	80.6	Medium
CFT ₃	3.3	17.0	25.3	52.6	61.3	75.0	Medium

^a = PCT: Petlur Citrus *Trichoderma*

^b = CFT: Commercial formulation of *Trichoderma*

^c = 75 to 90 mm within 48 h -- Very fast

75 to 90 mm within 60 h -- Fast

75 to 90 mm within 72 h -- Medium

. Lea

categorized as medium growing isolates which exhibited 75 to 80.6 mm in diameter within 72 h of incubation.

4.4.3 Phenotypic characters of native and commercial *Trichoderma* isolates

To know the variability among the test isolates in their phenotypic characters, observations were taken regarding their colony characters on PDA medium and the results are summarized in Table 19. In all the 15 isolates the aerial mycelium growth was fluffy at early stages. Among the isolates, PCT₁, PCT₂, PCT₃, PCT₄, PCT₅, PCT₆, PCT₈ and PCT₁₂ isolates exhibited abundant sporulation after 5 days of incubation. PCT₇, PCT₉, PCT₁₁, CFT₁, CFT₂, and CFT₃ exhibited moderate sporulation. More than one ring was formed in all the isolates except PCT₈ and PCT₁₁. All the isolates produced bright yellow to yellow pigmentation (Plate 13).

4.4.4 *In vitro* screening of native and commercial *T. viride* isolates against *F. solani* (PCF₂)

In order to find out the effective isolate of *Trichoderma* against *F. solani*, 15 isolates were screened against the test pathogen. The results of the dual culture are presented in Table 20. All the 15 isolates in dual culture inhibited the test pathogen *F. solani* (Plate 14). The inhibition per cent of commercial isolates ranged from 65.2 to 70.8. The isolate PCT₆ exhibited more per cent of inhibition (73.6) followed by PCT₄ which showed 72.2 per cent inhibition over control (Plate 15). Least per cent of inhibition of 65.2 was recorded in the commercial

Table 19 : Phenotypic characters of native and commercial *Trichoderma* isolates

Isolate	Aerial mycelium in colony growth ^c	Sporulation ^d	Ring formation ^e	Pigmentation ^f
PCT ₁ ^a	++	+++	++	BY
PCT ₂	++	+++	++	BY
PCT ₃	++	+++	++	BY
PCT ₄	++	+++	++	BY
PCT ₅	++	+++	++	BY
PCT ₆	++	+++	++	BY
PCT ₇	++	++	++	BY
PCT ₈	++	+++	+	BY
PCT ₉	++	++	++	BY
PCT ₁₀	++	++	++	By
PCT ₁₁	++	++	+	BY
PCT ₁₂	++	+++	++	Y
CFT ₁ ^b	++	++	++	BY
CFT ₂	++	++	++	BY
CFT ₃	++	++	++	Y

^a = PCT : Petlur Citrus *Trichoderma*
^b = CFT : Commercial formulation *Trichoderma*
^c = Aerial mycelium : (++) Fluffy growth
^d = Sporulation : (+++) Abundant
 (++) Moderate
 (+) Less
^e = Ring formation : (++) More than one ring
 (+) One ring
^f = Pigmentation : BY - Bright yellow
 Y - Yellow

Table 20 : *In vitro* screening of native and commercial *Trichoderma* isolates against *F. solani*

S. No,	Isolates	Radial growth (mm) ^c	Per cent Inhibition over control
1	PCT ₁ ^a	11.2	68.8
2	PCT ₂	10.7	70.2
3	PCT ₃	12.5	65.2
4	PCT ₄	10.0	72.2
5	PCT ₅	10.8	70.0
6	PCT ₆	9.5	73.6
7	PCT ₇	11.5	68.0
8	PCT ₈	10.7	70.2
9	PCT ₉	10.2	71.6
10	PCT ₁₀	11.0	69.4
11	PCT ₁₁	10.5	70.0
12	PCT ₁₂	11.8	67.2
13	CFT ₁ ^b	12.5	65.2
14	CFT ₂	11.0	69.4
15	CFT ₃	10.5	70.8
	Control	36.0	--

SEm (±) 0.376

CD (P=0.05) 1.085

^a = PCT : Petlur Citrus *Trichoderma*
^b = CFT : Commercial formulation *Trichoderma*
^c = Mean of three replications



Plate 13: Pure Cultures of native and commercial *Trichoderma* isolates used against *F. solani* (PCF₂)



Plate 14: Antagonistic activity of native and commercial *Trichoderma* isolates against *F. solani* (PCF₂)



isolate CFT₁. Further, isolate PCT₆ was confirmed as *Trichoderma viride* (ITCC 5740) at IARI, New Delhi (Plate 16 and 17).

4.4.5 *In vitro* screening of bacterial isolates against *Fusarium solani*

Isolates of bacterium (PCB₁, PCB₂ and PCB₃) were tested for their efficacy against test pathogen *F. solani* under dual culture and radial growth of the test pathogen was measured along with the control. Results presented in the Table 21 revealed no significant difference among all the three isolates with respect to per cent inhibition over control (Plate 18 and 19).

4.5 SCREENING OF SUBSTRATES FOR MASS MULTIPLICATION OF POTENTIAL BIOAGENT

Six substrates were tested for the mass multiplication of PCT₆ isolate and the results are furnished in Table 22. It is evident that among the six substrates tested, neem cake was best in respect of colony forming units compared to the other substrates. The mean population was highest (20.7×10^5 cfu/g) in neem cake followed by pressmud (19.7×10^5 cfu/g), FYM (18.5×10^5 cfu/g) and lowest mean population (7.2×10^5 cfu/g) was recorded in citrus fruit rind. When the mean population after different periods of incubation was considered, the population gradually increased upto 21 days and reached a peak of 20.1×10^5 cfu/g and there after declined to 18.6×10^5 cfu/g (Plate 20).

4.6 *IN VITRO* SCREENING OF FUNGICIDES AND NATURAL PRODUCTS AGAINST *FUSARIUM SOLANI* (PCF₂)

To know the efficacy of fungicides and natural products against *Fusarium solani* six fungicides viz., copper oxychloride (50% WP), mancozeb (75% WP),



Plate 16: Plate showing colony growth of *T. viride* (PCT₆)

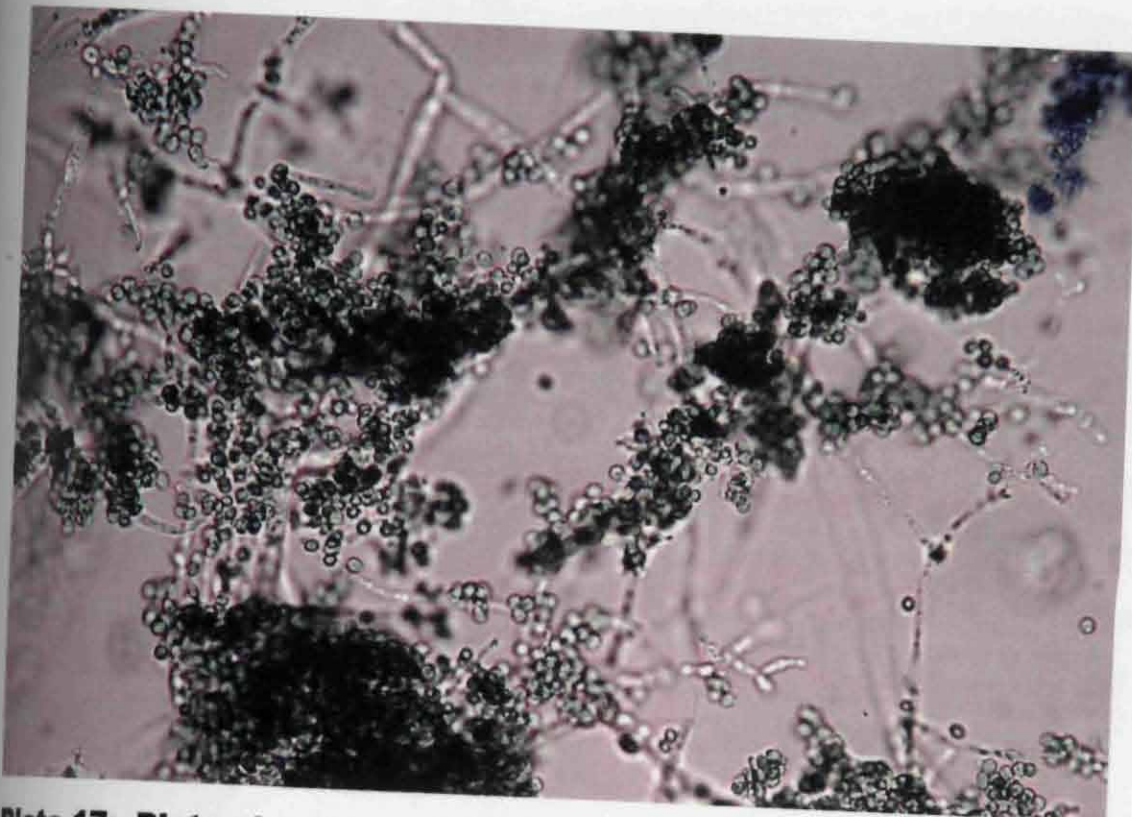


Plate 17: Plate showing phialides and phialospores of *T. viride* (PCT₆)



Plate 18: Pure cultures of native bacterial isolates used *in vitro* screening against *F. solani* (PCF₂)



Plate 19: *In vitro* screening of bacterial isolates against *F. solani* (PCF₂)



Plate 20: Mass multiplication of *T. viride* (PCT₆) in different substrates

chlorothalonil (75% WP), carbendazim (50% WP), propiconazole (25% EC), hexaconazole (5% EC) and two natural products, nimbidine and ovis were screened against *F. solani* at different concentrations viz., 100, 500, 1000 ppm in *in vitro* by adopting poisoned food technique.

Results presented in Table 23 clearly showed that mancozeb (75% WP) and carbendazim (50% WP) were very effective against the test pathogen followed by propiconazole (25% EC) and hexaconazole (5% EC), while least inhibition of growth was observed in the natural products, nimbidine and ovis.

Mancozeb (75% WP) and carbendazim (50% WP) recorded 100 per cent inhibition of growth even at 100 ppm followed by propiconazole (25% EC), (77.7, 84.0 and 89.3 per cent inhibition of growth at 100, 500 and 1000 ppm respectively).

Hexaconazole at 100, 500, 1000 ppm recorded 71.5, 77.1 and 80.0 per cent inhibition of growth, respectively. Chlorothalonil (75% WP) at 100, 500 and 1000 ppm recorded 55.5, 67.7 and 70.6 per cent inhibition of growth, respectively. Copper oxychloride (50% WP) recorded least per cent inhibition of growth when compared to the other fungicides.

Natural products, nimbidine and ovis were found to be least effective when compared to the fungicides. Nimbidine at 100, 500, 1000 ppm recorded 16.2, 16.6 and 21.5 per cent inhibition of growth, respectively. Ovis at 100 and 500 ppm recorded 22.2 and 28.8 per cent inhibition of growth, respectively. Ovis at 1000 ppm recorded 68.4 per cent inhibition of growth which is slightly more than copper oxychloride and nimbidine (Plate 21).

Table 23 : *In vitro* screening of fungicides and natural products against *F. solani* (PCF₂)

S. No.	Treatments	Concentration (ppm)	Mean radial mycelial growth (mm) ^a	Per cent inhibition
1	Copper oxychloride	100	38.5	14.4
		500	30.5	32.2
		1000	26.7	40.6
2	Mancozeb	100	0.0	100
		500	0.0	100
		1000	0.0	100
3	Chlorothalonil	100	20.0	55.5
		500	14.5	67.7
		1000	13.2	70.6
4	Carbendazim	100	0.0	100
		500	0.0	100
		1000	0.0	100
5	Propiconazole	100	10.0	77.7
		500	7.2	84.0
		1000	4.8	89.3
6	Hexaconazole	100	12.8	71.5
		500	10.3	77.1
		1000	9.0	80.0
7	Nimbidine	100	37.7	16.2
		500	37.5	16.6
		1000	35.3	21.5
8	Ovis	100	35.0	22.2
		500	32.0	28.8
		1000	14.2	68.4
9	Control		45.0	

SEm ±
CD at 5%

0.962
1.955

^a = Mean of three replications

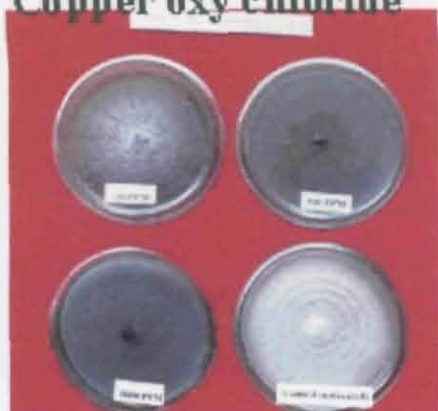
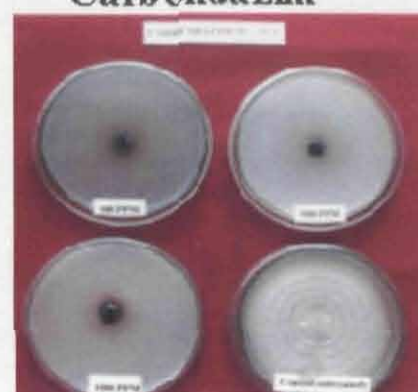
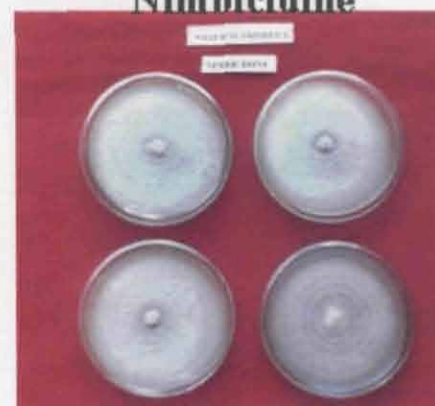
Copper oxy chloride**Mancozeb****Chlorothalonil****Carbendazim****Propiconazole****Hexaconazole****Nimbidine****Ovis**

Plate 21: In vitro Screening of fungicides and natural products against *F. solani* (PCF₂)

4.7 COMPATIBILITY OF FUNGICIDES AND NATURAL PRODUCTS WITH POTENTIAL BIOCONTROL AGENTS

Six fungicides viz., copper oxychloride (50% WP), mancozeb (75% WP), chlorothalonil (75% WP), carbendazim (50% WP), propiconazole (25% EC), hexaconazole (5% EC) and two natural products nimbidine and ovis were used to test their compatibility with *T. viride* (PCT₆) at different concentration viz., 100, 500 and 1000 ppm by poisoned food technique (Table 24). Among the fungicides, mancozeb (75% WP) was found to be highly compatible with *T. viride* (PCT₆) at 100 and 500 ppm with least per cent of inhibition of 12.8 and 41.1, respectively.

The next best compatible fungicide was copper oxychloride and it was found to be safe at 100 ppm with 0.0 per cent of inhibition. However, at 500 and 1000 ppm per cent inhibition of growth was 52.2 and 54.2, respectively.

Chlorothalonil (75% WP) showed inhibition on growth of PCT₆ isolate and it was in the range of 88.4 to 100 per cent from 100 ppm to 1000 ppm. Carbendazim (50% WP), propiconazole (25% EC) and hexaconazole (5% EC) recorded 100 per cent inhibition of growth even at 100 ppm.

Natural products, nimbidine and ovis showed inhibition on growth of *Trichoderma viride* (PCT₆) and it was in the range of 11.7 to 53.3 per cent (Plate 22).

Table 24 : Compatibility of *T. viride* (PCT₆) with fungicides and natural products

S. No.	Treatments	Concentration (ppm)	Mean radial mycelial growth (mm) ^a	Per cent inhibition of growth over control
1	Copper oxychloride	100	45.0	0.0
		500	21.5	52.2
		1000	20.5	54.2
2	Mancozeb	100	39.2	12.8
		500	26.5	41.1
		1000	20.3	54.8
3	Chlorothalonil	100	6.5	85.5
		500	5.8	87.1
		1000	5.2	88.4
4	Carbendazim	100	0.0	100.0
		500	0.0	100.0
		1000	0.0	100.0
5	Propiconazole	100	0.0	100.0
		500	0.0	100.0
		1000	0.0	100.0
6	Hexaconazole	100	0.0	100.0
		500	0.0	100.0
		1000	0.0	100.0
7	Nimbidine	100	35.7	20.6
		500	34.0	24.4
		1000	31.8	29.3
8	Ovis	100	33.3	11.7
		500	28.8	36.0
		1000	21.0	53.3
9	Control		45.0	
SEm ±			0.722	
CD at 5%			1.479	

Mean of three replications

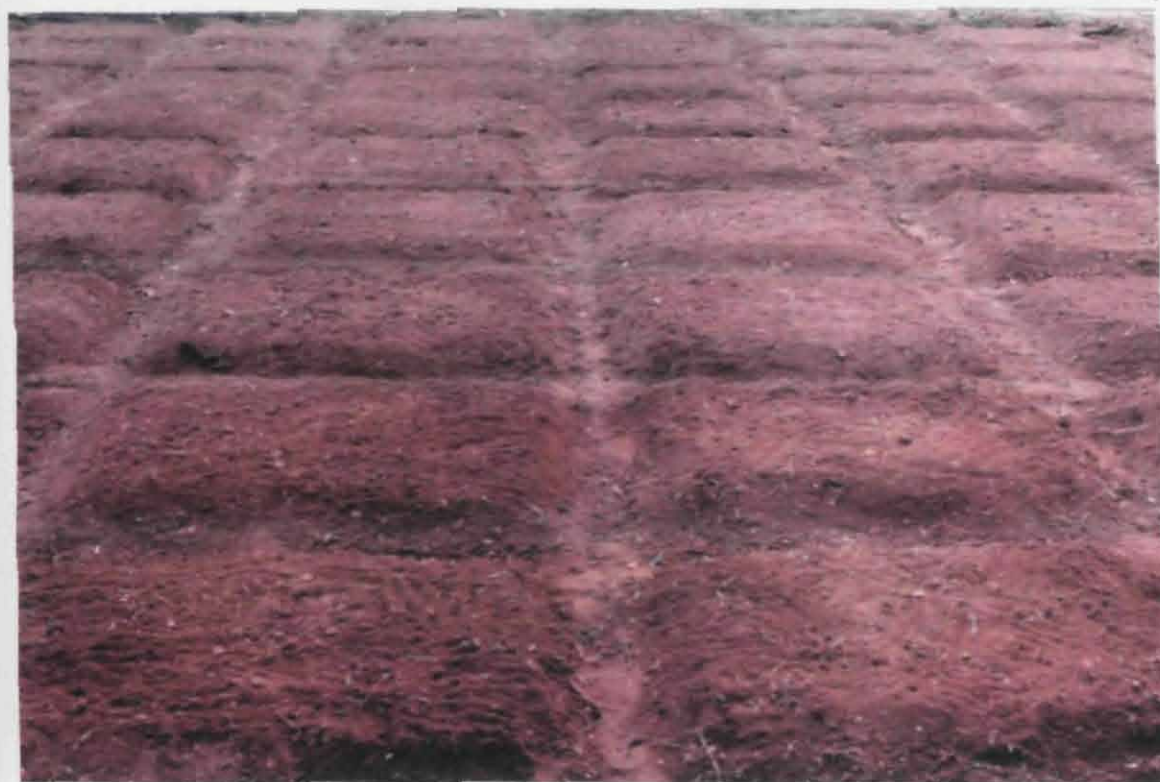


Plate 24: Layout of nursery (1x1m) experiment



Plate 25: Soil solarization of nursery beds with polyethylene sheet

4.8 INTEGRATED APPROACH FOR THE MANAGEMENT OF ROOT ROT DISEASE IN NURSERY AND GROWN UP TREES OF ACID LIME

Nursery experiment : In order to find out the effect of *T. viride* (PCT₆), farm yard manure, VAM, neem cake, mancozeb, soil solarization individually and in combination on the root rot incidence an experiment was conducted during the year 2003 in a sick plot (Plate 23).

4.8.1 Effect of soil solarization at different depths of soil in solarized and non-solarized treatments

Soil temperature, recorded at different depths daily at 2.30 P.M during the solarization period (2nd May to 12th June, 2003) are presented in Table 25. Maximum temperature in soil solarized plots was 58, 55 and 51°C at 5, 10 and 15 cm depths, respectively. In non-solarized plots the temperature was 45, 40 and 38°C at 5, 10 and 15 cm depths, respectively (Plate 24 and 25).

4.8.1.1 Effect of soil solarization on soil moisture at different soil depth

Soil moisture estimated in solarized and non-solarized plots at different depths are presented in Table 26. There was significant difference in soil moisture between solarized (11.75%) and non-solarized (7.75%) plots (Plate 26).

4.8.1.2 Effect of soil solarization on population density of *F. solani* at different soil depths

Population density of *F. solani* was recorded immediately after termination of polyethylene sheet (solarization). Data regarding population density of *F. solani* at different depths in solarized and non-solarized plots are presented in the Table 27. From the data it is evident that solarization had a great effect which



Plate 23: Photograph showing field view of nursery experiment

Table 25 : Soil temperature recorded at different depths at 2.30 PM in non-solarized and solarized plots from 2nd May to 12th June, 2003

Soil depth (cm)	Soil temp °C (range)		Soil temp °C (mean)		t-value
	Non-solarized	Solarized	Non-solarized	Solarized	
5	36-45	47-58	41.10	54.36	23.766 ^a
10	34-40	43-55	38.55	50.90	22.211 ^a
15	33-38	42-51	36.29	47.29	26.447 ^a

^a = Significant at 0.01 level

Table 26 : Effect of soil solarization on soil moisture at different depths in solarized and non-solarized plots

Soil depth (cm)	Moisture (%)		
	Solarized	Non-solarized	Mean
10	10.78 (19.04)	6.24 (14.12)	8.51 (16.58)
20	11.31 (19.54)	7.17 (15.49)	9.24 (17.51)
30	13.17 (21.23)	9.85 (18.20)	11.51 (19.72)
Means	11.75 (19.93)	7.75 (15.93)	--

CD at 5%

solarization - 0.35
Soil depths - 0.21
Interaction - 1.43

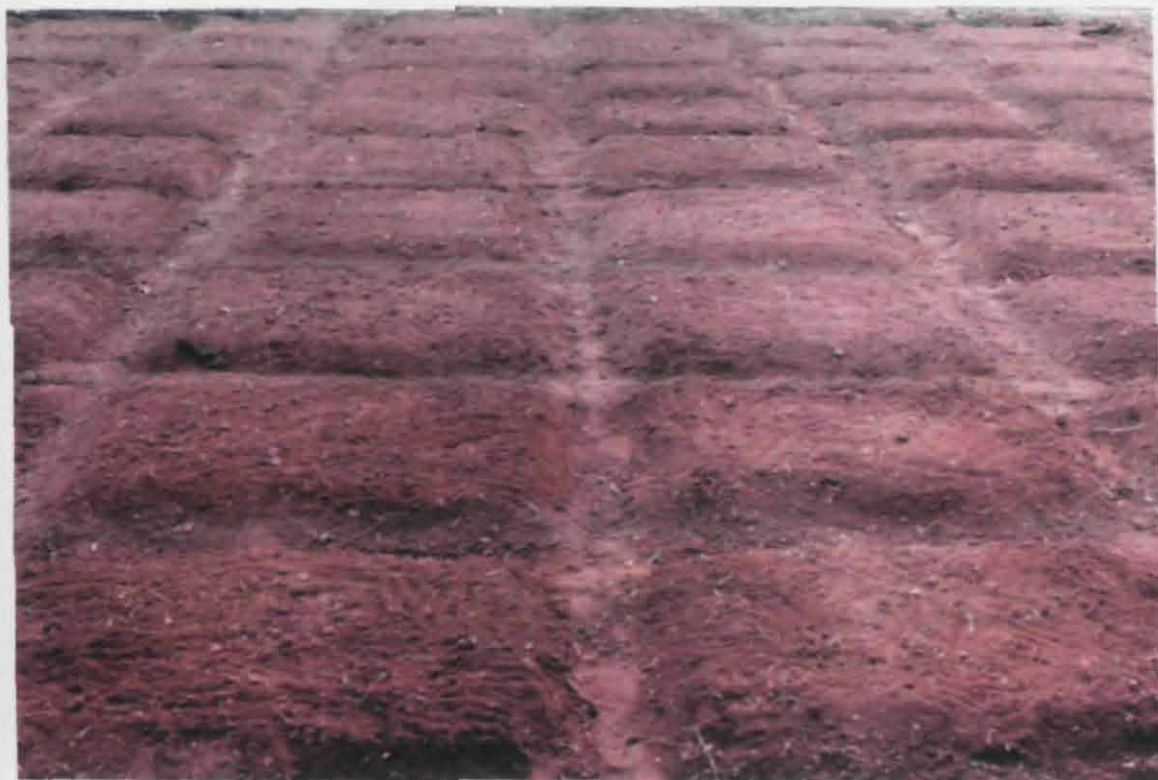


Plate 24: Layout of nursery (1x1m) experiment



Plate 25: Soil solarization of nursery beds with polyethylene sheet



Plate.26: Photograph showing soil thermometers for measurement of temperature.



Plate.27: Photograph showing more weed growth in unsolarized than solarized.

Table 27 : Effect of soil solarization on population density of *F. solani* (cfu x 10³/g) in solarized and non-solarized plots

Treatment	Soil depth (5 cm)	Per cent reduction over control	Soil depth (10 cm)	Per cent reduction over control	Soil depth (15 cm)	Per cent reduction over control
Solarized	0.00	100.0	0.00	100.0	0.66 ^a	81.66
Un-solarized (Control)	3.00	--	3.46	--	3.60	--

^a = Significant at 1% according to 't' test

Table 28 : Effect of soil solarization on weed growth in solarized and non-solarized plots

Treatment	Weed count	Percent reduction over control	Fresh weight (g)	Percent reduction over control	Dry weight (g)	Percent reduction over control
Solarized	14.5 ^a	88.9	8.96 ^a	94.9	7.2 ^a	91.8
Un-solarized (Control)	131.21	0.0	177.60	0.0	88.36	0.0

^a = Significant at 1% according to 't' test

Table 29 : Effect of different treatments on root rot disease (*F. solani*) in acid lime nursery (Local variety)

S. No.	Treatments	Root rot incidence (%)	Per cent decrease over control
T ₁	<i>Trichoderma viride</i> (PCT ₆)	21.3 ^a	69.0
T ₂	Farm yard manure	40.8	40.7
T ₃	VAM	25.3	63.2
T ₄	Neem cake	27.0	60.8
T ₅	Mancozeb	10.8	80.4
T ₆	Soil solarization	19.2	72.1
T ₇	T ₁ + T ₂	20.8	69.8
T ₈	T ₁ + T ₂ + T ₃	19.7	71.4
T ₉	T ₁ + T ₂ + T ₄	8.6	87.5
T ₁₀	T ₁ + T ₂ + T ₅	4.0	94.1
T ₁₁	T ₁ + T ₂ + T ₆	8.2	88.0
T ₁₂	Control	68.9	0.0
SEm		3.038	
CD at 5%		11.929	

^a = Mean of three replications



T₉: *T. viride* (PCT₉) + FYM + Neem cake

**T₁₁: *T. viride* (PCT₁₁) + FYM +
Soil solarization**



T₁₀: *T. viride* (PCT₁₀) + FYM + Mancozeb

T₁₂: Control



4.8.2.1 Effect of different treatments on plant growth parameters of acid lime nursery (local variety)

Results presented in Table 30 revealed that *T. viride* (PCT₆) in combination with farm yard manure and mancozeb increased the shoot length (17.7 cm), root length (19.5 cm), germination (96.9%) and seedling vigour index (3604.6) of acid lime seedlings followed by *T. viride* (PCT₆) in combination with FYM and soil solarization with shoot length (15.7 cm) root length (17.6 cm), germination (92.2%) and seedling vigour index (3010.2).

4.8.2.2 Cost - Benefit ratio in management of root rot incidence in acid lime nursery (local variety)

Cost – Benefit ratio (CBR) of the different treatments was calculated and presented in Table 31. Among the individual treatments, mancozeb gave maximum CBR of 1 : 33.28 followed by soil solarization (1:31.35). Among the integrated treatments, the treatment involving *T. viride* (PCT₆), FYM and mancozeb was the most economical with CBR of 1:35.18 followed by treatment *T. viride* (PCT₆), FYM and neem cake (1:32.08).

4.8.3 Integrated management of root rot disease in grown-up acid lime trees

The best result obtained in the nursery experiment i.e. integration of *T. viride* (PCT₆), FYM, neem cake and mancozeb was tested with different doses on six year old acid lime trees for effective management of the disease. The effect of the different treatments were recorded and the data are presented in the Table 32.

Table 30 : Effect of different treatments on germination and growth of acid lime nursery (Local variety)

S. No.	Treatments	Shoot length (cm) ^a	Root length (cm) ^a	Germination (%)	Seedling vigour index ^b
T ₁	<i>Trichoderma viride</i>	13.7	15.4	81.7	2377.4
T ₂	Farm yard manure	11.3	12.4	75.6	1791.7
T ₃	VAM	12.6	16.5	80.6	2345.4
T ₄	Neem Cake	12.5	15.1	77.3	2133.4
T ₅	Mancozeb	14.6	15.8	88.1	2678.2
T ₆	Soil solarization	14.5	15.6	86.1	2591.6
T ₇	T ₁ + T ₂	13.8	15.4	82.1	2397.3
T ₈	T ₁ + T ₂ + T ₃	12.7	16.4	84.8	2467.6
T ₉	T ₁ + T ₂ + T ₄	15.6	17.1	90.0	2943.0
T ₁₀	T ₁ + T ₂ + T ₅	17.7	19.5	96.9	3604.6
T ₁₁	T ₁ + T ₂ + T ₆	15.7	17.6	92.2	3010.2
T ₁₂	Control	7.3	8.6	70.1	1114.5
SEm		0.35	0.40	4.015	
CD at 5%		1.0	1.25	15.761	

^a = Mean of three replications

^b = Seedling vigour index = root length (cm) + shoot length (cm) x germination (%)

Table 32 : Effect of different doses of integrated treatment in per cent reduction of rotted roots in acid lime trees at 180 days after treatment

SL No	Treatments	Percentage reduction in rotted roots
T ₁	Trichoderma (100g) + FYM (2.5kg)+neem cake (0.5 kg) + mancozeb (0.2%)	37.3 ^a
T ₂	Trichoderma (150g) + FYM (5kg)+neem cake (0.75kg) + mancozeb (0.25%)	51.9
T ₃	Trichoderma (200g) + FYM (10kg)+ neem cake (1kg) + mancozeb (0.3%)	78.3
T ₄	Control	00.0

SEm $\pm$

0.254

CD at 5%

3.501

^a = Mean of six replications

Table 32 : Effect of different doses of integrated treatment in per cent reduction of rotted roots in acid lime trees at 180 days after treatment

Sl. No	Treatments	Percentage reduction in rotted roots
T ₁	Trichoderma (100g) + FYM (2.5kg)+neem cake (0.5 kg) + mancozeb (0.2%)	37.3 ^a
T ₂	Trichoderma (150g) + FYM (5kg)+neem cake (0.75kg) + mancozeb (0.25%)	51.9
T ₃	Trichoderma (200g) + FYM (10kg)+ neem cake (1kg) + mancozeb (0.3%)	78.3
T ₄	Control	00.0

SEm $\pm$

0.254

CD at 5%

3.501

^a = Mean of six replications



Plate 29: Photograph showing trees which are imposed with treatments in field experiment

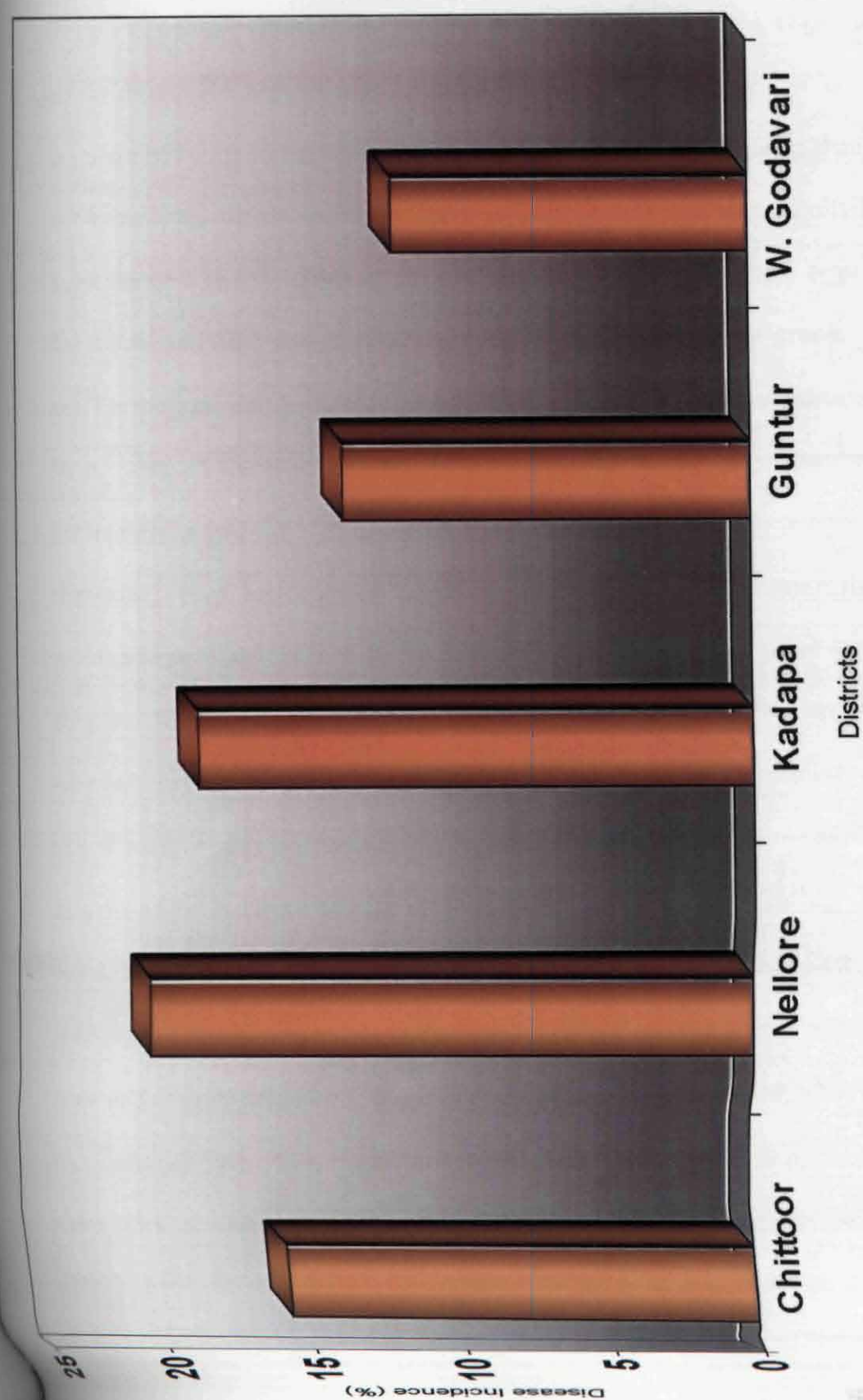


Fig.4: Incidence of root rot in acid lime growing districts of Andhra Pradesh

Chittoor (15.61%), Guntur (13.71%) and West Godavari (11.98%). High incidence of the disease in Nellore district could be due to the presence of continuous inoculum of *F. solani* because of monocropping of acid lime and also shallow and calcareous soils which predispose the acid lime trees to root rot. Similarly, high incidence of 5 to 75 (Reddy *et al.*, 1999) and 29.1 per cent (Gopal *et al.*, 2005) disease on acid lime was reported from A.P. where acid lime was grown in larger area for the past four decades. Few workers also reported high incidence of 1 to 30 per cent of the disease from different districts of A.P (Jayachandra, 1990; Satyanarayana and Reddy, 1994) on sweet orange where rough lemon was the root-stock. High incidence of the disease is expected in roughlemon root-stock sweet orange orchards due to its susceptibility to root rot disease caused by *F. solani* (Gopal *et al.*, 2005). Further, it was also noted that budling orchards recorded high incidence of the disease (19.0%) compared to seedling orchards (15.4%). The higher incidence could be due to the use of rough lemon as root-stock as most of the nursery-men use it as root-stock material due to its high bud take and fast growth nature (Gopal *et al.*, 2005). Thus, it clearly indicates that budling orchards are highly susceptible to the disease. The disease incidence recorded was more in the orchards grown in shallow sandy clay loam soils (19.46%) than the deep soils (12.8%). This might be attributed due to the presence of hard pan of either stone or calcareous or gravel in the sub-soil, which are impervious to root system to develop and disturb the root physiology. This is in accordance with the findings of Naidu and Rao (1958) who found that hard clay pan formation and unfavourable soil moisture retention in the sub-soils led to inadequate oxygen at

the root zone which is predominantly responsible for dry root rot. Reddy and Paparao (1960) also opined that shallow soils favour early decline of sweet orange and acid lime due to root rot. Similar observations were made by Nemec *et al.* (1989) that *Fusarium* spp. are prevalent in sandy and clay grove soils of Florida. Blaker and Mc Donald (1986) also observed that soil salinity enhanced the root rot in citrus nurseries.

Further, the data from the survey also revealed that orchards irrigated with water having high EC (35.08%), limited irrigation (20.66%), flood irrigation (25.56%) and continuous use of inorganic fertilizers (22.0%) were more prone to the root rot incidence compared to normal EC (14.51%), optimum irrigation (13.94%) and organic fertilizers (15.14%). On mandarin plantations, the occurrence of dry root rot disease was associated with excessive moisture or lack of sufficient moisture, direct contact with trunk, poor aeration, heavy soils, frequent injuries to roots, excessive manuring at long intervals, close planting (Ramakrishnan, 1954; Prakasam and Jayarajan, 1992) and shallow soils (Mariakulandia and Durairaj, 1958). Kore and Mane (1992) stated that limited irrigation with 20 per cent soil moisture and half dose of NPK favoured the disease development. Because of soil borne nature of the pathogen these factors, mostly in combination influence the occurrence or favour the initiation and spread of the disease. The combination of the factors which are directly or indirectly responsible for initiation and spread of the disease may vary from one area to another because of environmental, soil and management practices.

5.2 ISOLATION AND IDENTIFICATION OF ORGANISM ASSOCIATED WITH ROOT ROT OF ACID LIME

Distribution of root rot disease of acid lime in different districts of A.P revealed that 61.2 per cent of the infected samples yielded *Fusarium* spp. and 20 per cent of *Ganoderma* and *Rhizoctonia* spp. These results are in agreement with the results of Gopal *et al* (2001) who reported that 80 per cent of acid lime root rot samples yielded *F. solani*. Nemec *et al.* (1989) also studied distribution of *Fusarium* spp. and indicated that *Fusarium* spp. was widely distributed in citrus groves throughout Florida. They also reported *F. solani* as the predominant and principal fungus associated with citrus roots and soil in Florida. Similarly, Vanderweyen and Serrhini (1991), Bender *et al.* (1982), Malikoutsaki *et al.* (1987), Prakasam *et al.* (1992), Graham *et al.* (1985), Ansar *et al.* (1994), Verma and Navtej Singh (1999) and Gopal *et al.* (2000) reported *F. solani* as the major cause for root rot in citrus.

Bender *et al.* (1982) isolated *F. solani* in all the isolations (100%) made from citrus trees with stained wood. Further, Wehner *et al* (1986), Menge *et al.* (1980), Prakasam *et al.* (1992), Timmer *et al.* (1979), Jagannathan (1989), Malikoutsaki (1987), Whiteside *et al.* (1989), Labuschagne *et al.* (1987), Verma and Navtej Singh (1999), Rathore *et al.* (1993), Liu-Jing Jing *et al.* (1993), Ramteke (2003) isolated *F. solani* from rotted and apparently healthy roots of different citrus species.

5.2.1 Pathogenicity

The studies on pathogenicity test in pot culture revealed that disease incidence increased as the inoculum density level increased. The optimum damaging threshold level was 8.0 per cent inoculum causing 79.7 per cent disease incidence (Fig. 5). The symptoms appeared 45 days after inoculation. Acid lime seedlings showed typical root rot symptoms which includes yellowing, rolling of leaves and wilting of seedlings. In a similar study Graham *et al.* (1985) and Nemec *et al.* (1988) observed typical symptoms of the disease one month after inoculation of young potted citrus plants of rough lemon with *Fusarium solani*. Kore and Mane (1992) also found similar symptoms after 21 days of inoculation on one month old acid lime seedlings raised in pots having sterile soil. These results more or less coincide with the report of Dhanbir Singh and Akhliesh Singh (2004) who found that *F. solani* developed infection on one month old potted seedlings causing 65.50 per cent of disease incidence. Gopal *et al.* (2005) reported that 7.5 per cent inoculum density caused 85.3 per cent in pot culture tests. Morgan and Timmer (1984) opined that *Fusarium* wilt incidence of Mexican lime increased (0.5 to 2.5%) with increase in inoculum density (500 to 1000 propagules per gram of potting media). Thus, the present study is in confirmity with the results reported by Chandra *et al.* (1983), Malikoutsaki *et al.* (1987) and Srivastava (2004).

5.2.2 Categorization of *Fusarium* isolates

In the present investigation, fifteen isolates of *Fusarium solani* isolated from different locations were used for categorization based on disease incidence.

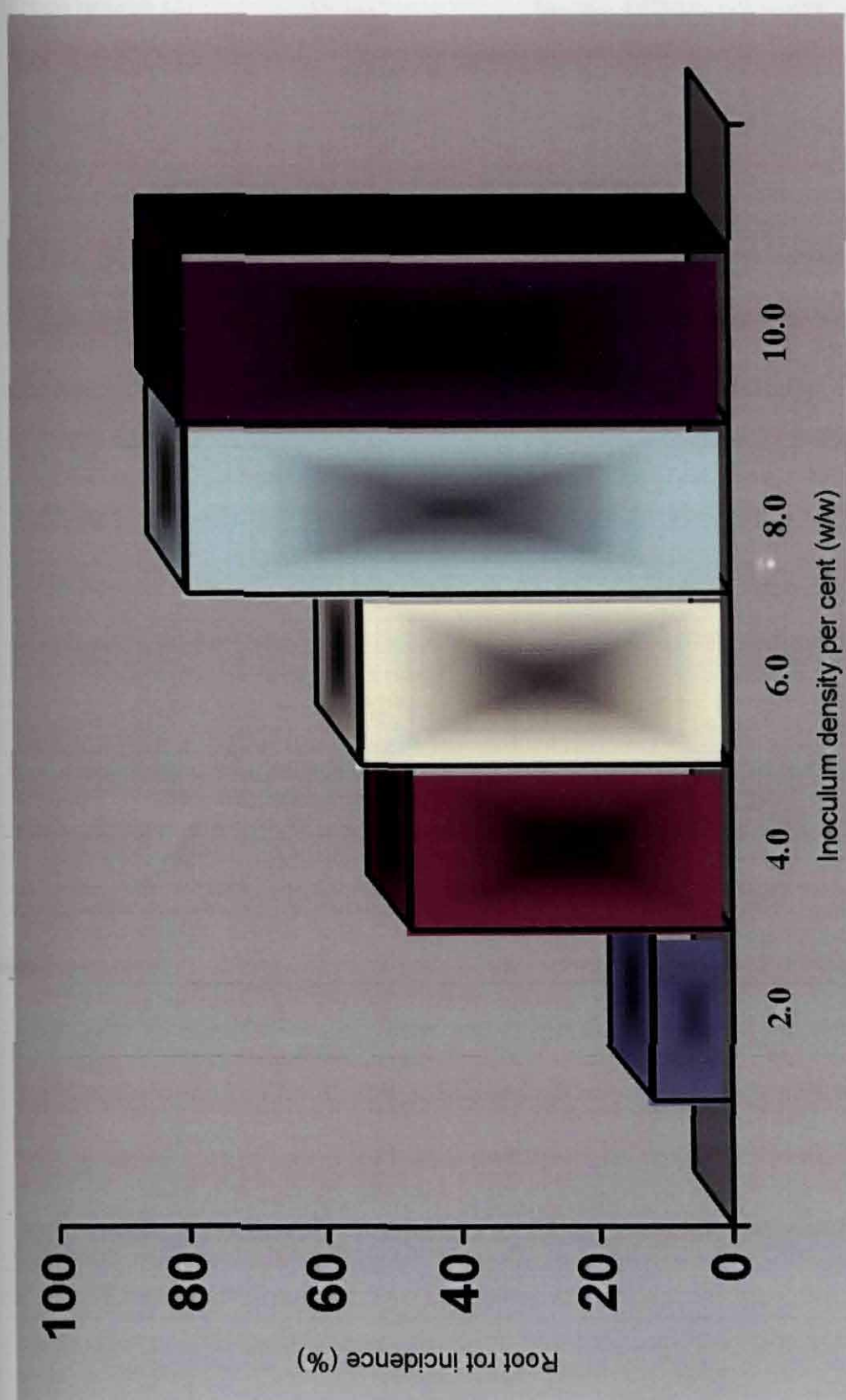


Fig.5: Effect of different inoculum levels of *Fusarium* isolate (CRS, Petiur) on root rot incidence of acid lime

These isolates differed in their ability to cause disease. Among the fifteen isolates, PCF₂ (Rapur) recorded the maximum percentage (83.3) of seedling root rot incidence and categorized as extremely aggressive isolate. Whereas, the rest of isolates recorded disease incidence in the range of 23.3 to 73.3 per cent. These isolates were classified into 4 classes according to Chohan and Jasmith Kaur (1976) who classified the isolates of *Rhizoctonia bataticola* into five classes i.e non-aggressive (no apparent root rot symptoms), slightly aggressive (1 to 25% mortality of seedlings) moderately aggressive (26 to 50% mortality of seedlings), highly aggressive (51 to 75% mortality of seedlings), extremely aggressive (76 to 100% mortality of seedlings). Chandra *et al.* (1983) studied three isolates of *Fusarium oxysporum* and seven isolates of *F. solani* of tomato and reported that the isolates within a species differed in their potential to cause pre or post-emergence killing of seedlings and induce wilt in mature plants. Reddy and Raju (1991) found that Gwalior isolate was most aggressive among thirteen isolates of *Fusarium udum*. Champawat and Pathak (1989) reported that isolate T₄ (49.30%) was most virulent among nine isolates of *Fusarium oxysporum f.sp. cumine* which causes wilt of cumin. Prameela Devi and Singh (1998) studied the pathogenic variation in six groups of *Macrophomina phaseolina* isolates and found that MP-2, MP-3 groups cause high per cent incidence of 84 and 80, respectively.

5.2.3 Effect of different temperatures on growth and sporulation of *F. solani* (PCF₂)

The test fungus incubated at different temperatures has shown maximum vegetative growth and abundant sporulation at 30°C, which was close to the

average atmospheric temperature prevailing during February and March, synchronizing with a period of maximum development of disease on acidlime under natural field conditions. The fungus showed minimum vegetative growth and low level of sporulation at low (10°C) and high (40°C) temperatures. Agarwal and Sarbhoy (1978) used a wide range of temperatures (15 to 40°C) for the growth of *F. solani* and found that the maximum growth and sporulation was obtained between 25 and 30°C. Further, they also observed steep fall in growth rate of pathogen above 35°C (Fig. 6).

5.2.4 Effect of different pH levels on growth and sporulation of *F. solani* (PCF₂)

Hydrogen ion concentration (pH) had marked influence on the growth and sporulation of *F. solani* in the present investigation. The fungus exhibited a distinct increasing trend in the vegetative growth from pH 3.0 to 6.0 (Fig. 7). It grew best at pH 6.0 followed by pH 5.0 and maximum sporulation was observed at pH 6.0. The results are more or less similar to those reported by Sharma and Agnihotri (1971) who observed maximum growth and sporulation of *F. solani* at pH 5.8. Bhargava (1962) opined that acidic pH 4.5 to 6.0 favoured best growth of *F. solani*. Similar findings were observed by Agarwal and Sarbhoy (1977) that pH 4.5 and 6.0 supported the growth of both *F. oxysporum* and *F. solani*.

5.3 ISOLATION OF FUNGI AND BACTERIA FROM THE RHIZOSPHERE

Eight general fungi, 12 *Trichoderma* spp. and three bacterial spp. are associated with the rhizosphere of acid lime. *Aspergillus niger* showed maximum

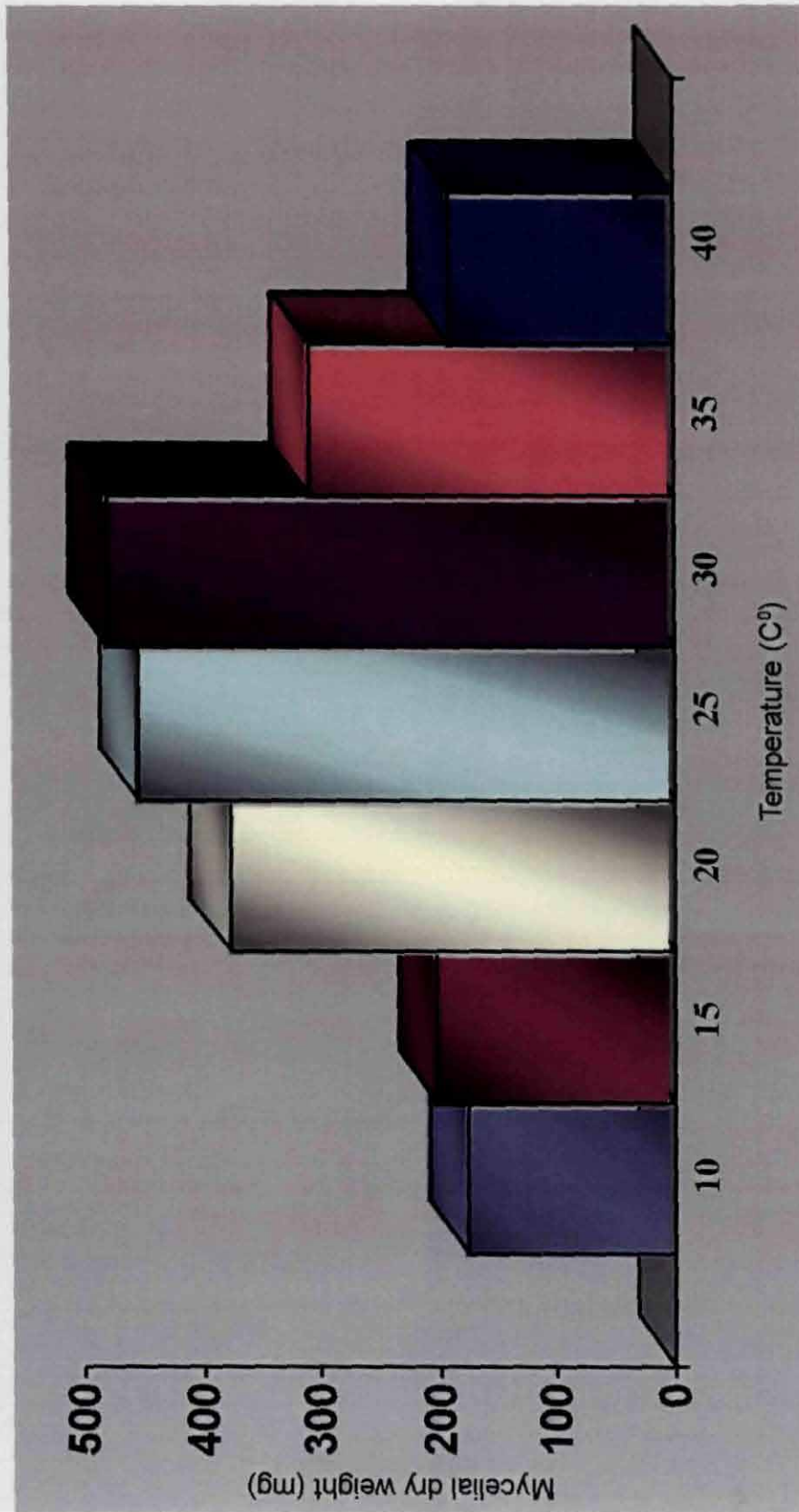


Fig.6: Effect of different temperatures on growth and sporulation of *Fusarium solani* (PCF₂) after 14 days of incubation

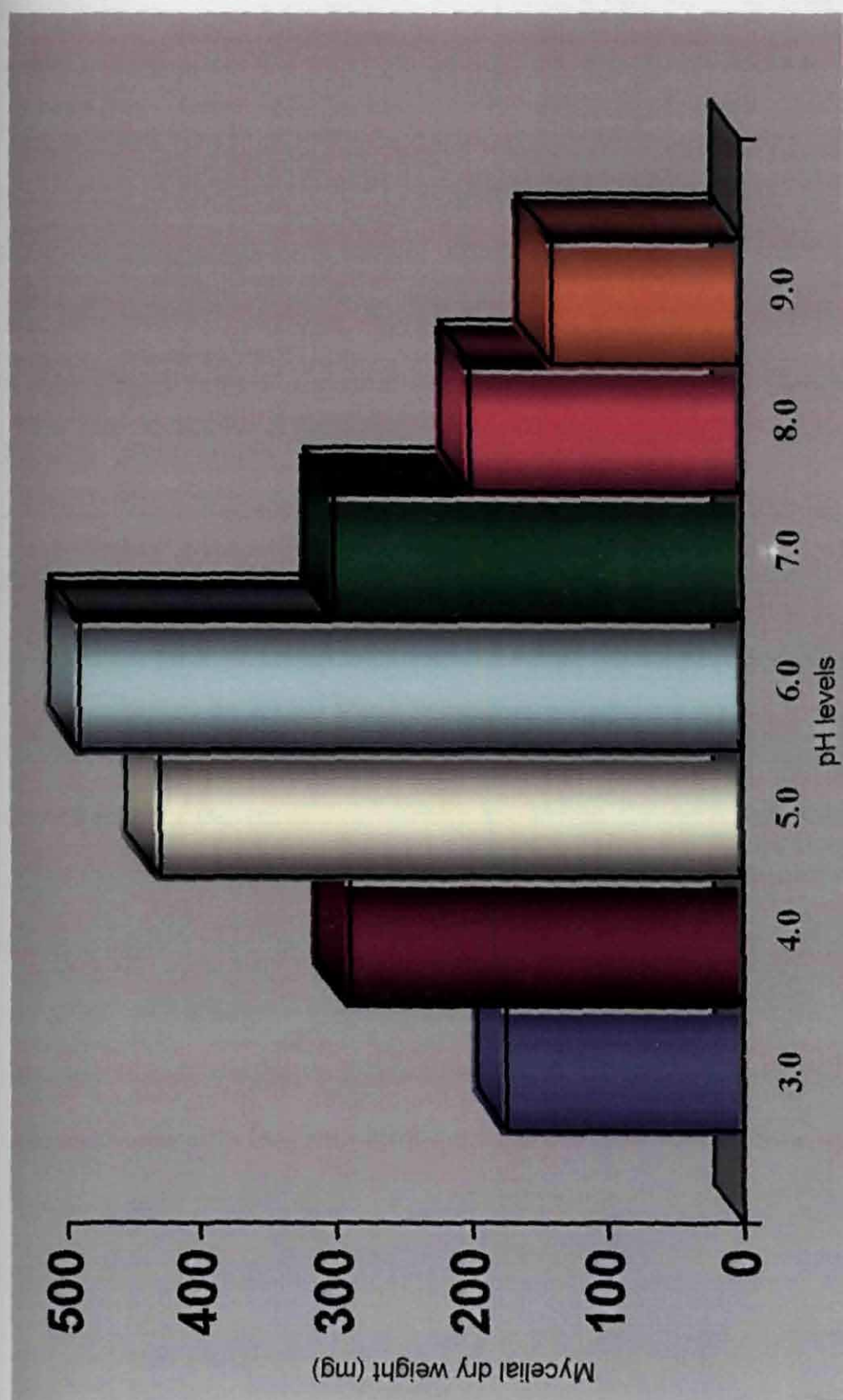


Fig.7: Effect of different pH levels on growth and sporulation of *Fusarium solani* (PCF₂) after 14 days of incubation

population (3.71×10^5) followed by *A. flavus* (2.72×10^5) whereas *Mucor* spp. recorded less population (0.2×10^5) per gram of soil. Among the bacterial isolates PCB₁ showed maximum population than others (1698.8×10^5). Similar results were obtained by Rangaswami and Vasantharajan (1962) indicating *Aspergilli* and *Penicillia* were more numerous whereas *Mucor* and *Rhizopus* were less abundant in the rhizosphere soils of citrus. They also reported that bacteria were about 40 to 90 times, fungi 3 to 6 times more abundant in the rhizosphere soils than in non-rhizosphere soils. Nemec *et al.* (1989) also reported that total bacteria and *Pseudomonas* spp. exhibited a greater rhizosphere enrichment than actinomycetes and fungal flora.

5.4 IN VITRO SCREENING OF NATIVE FUNGAL, BACTERIAL AND COMMERCIAL ISOLATES OF BIO-AGENTS

Fungi, in the genus *Trichoderma* are among the most promising bio-control agents controlling a variety of pathogens. Among all the biological agents, more research has been done on *Trichoderma* spp. Based on easy availability, rapid growth on various substrates and most effective antagonistic activity against wide range of plant pathogens. *Trichoderma* spp. has been found to be better bio-agent when compared to other organisms. For the past three decades, a lot of research has been carried out on the antagonistic nature of several species of the genus *Trichoderma* (Papavizas, 1985; Chet and Baker, 1981).

In the present investigation too higher antagonistic activity was observed with only *Trichoderma* isolates among the rhizosphere mycoflora isolated for the

study. Based on growth rate on PDA potential isolates were selected. In this study among 12 native isolates of *Trichoderma* and three commercial *Trichoderma* isolates, PCT₁ and PCT₆ having a growth of more than 75 mm in diameter with in 48 h were categorized as very fast growing isolates. Isolates PCT₂, PCT₄, PCT₅, PCT₉, PCT₁₀, PCT₁₁ and PCT₁₂ growth was more than 75 mm within 60 h were categorized as fast growing isolates and PCT₃, PCT₇, PCT₈, CFT₁, CFT₂ and CFT₃ showed more than 75 mm growth after 72 h were categorized as medium growing isolates. Similar studies on identification of *Trichoderma* isolates based on growth were reported by Singh *et al.* (1994) who isolated 27 isolates of *Trichoderma harzianum* from soil samples collected randomly from fallow agricultural fields throughout the Punjab and studied their growth rate on PDA medium and categorized into medium, fast and very fast growing. Domsch *et al.* (1980), Cook and Baker (1983) also reported similar observation regarding growth rate. Similarly Kavitha *et al.* (2004) isolated 12 number of *Trichoderma* isolates from rhizoplane of sweet orange from different areas and found T₂ and T₄ as best isolates based on growth rate (76-78 mm diameter within 48 h).

Further, in the present studies certain phenotypic characters viz., aerial mycelium, colony growth, pigment production, sporulation and ring formation were taken into account to study the variability among different isolates. Fifteen isolates showed more or less similar characters. In early stages these isolates showed fluffy aerial mycelium on PDA medium. All the isolates showed abundant sporulation and more than one ring formation except PCT₈ and PCT₁₁ which showed one ring formation. These observations on phenotypic characters (colony

characters) showed no difference from those made earlier by Rifai (1969) and Souza *et al.* (2001). Similarly, Cook and Baker (1983), Singh *et al.* (1994) and Viswanath (2003) have recorded variation in the growth rate of different *Trichoderma* isolates.

The antagonistic nature of 12 native isolates and three commercial isolates of *T. viride* were studied in the present investigation using dual culture technique showed clear variation in the degree of parasitism from one isolate to another. Among these, PCT₆ which recorded highest per cent inhibition (73.6%) was selected for further studies (Fig. 8). Similar observations regarding the antagonistic activity of *Trichoderma* isolates have been recorded by several workers. Maiti *et al.* (1987) obtained a clear variation in antagonistic potential under *in vitro* screening against *Sclerotium rolfsii*. Bell *et al.* (1982) tested antagonistic activities of 77 isolates primarily of *T. harzianum* against six plant pathogens and recorded highly significant difference between pathogen-antagonistic interactions. Selvarajan and Jeyarajan (1996) from their studies using dual culture technique reported that *T. viride*, *T. hamatum* showed maximum inhibition zone (9.3 mm) against the test pathogen *F. solani*. Postucha (1999) stated that among several antagonistic organisms, *T. koningii* and *T. viride* isolated from soybean roots inhibited *F. solani*. Souza *et al.* (2001) tested the antagonistic potential of eight isolates of *T. harzianum* against four fungal plant pathogens (*Rhizoctonia solani*, *Phytophthora parasitica*, *Colletotrichum capsici* and *Sclerotium rolfsii*). Similarly, Kavitha *et al.* (2004) found T₂ and T₄ as best isolates under dual culture against *F. solani* causing dry root rot in acid lime. In the present studies inhibition of

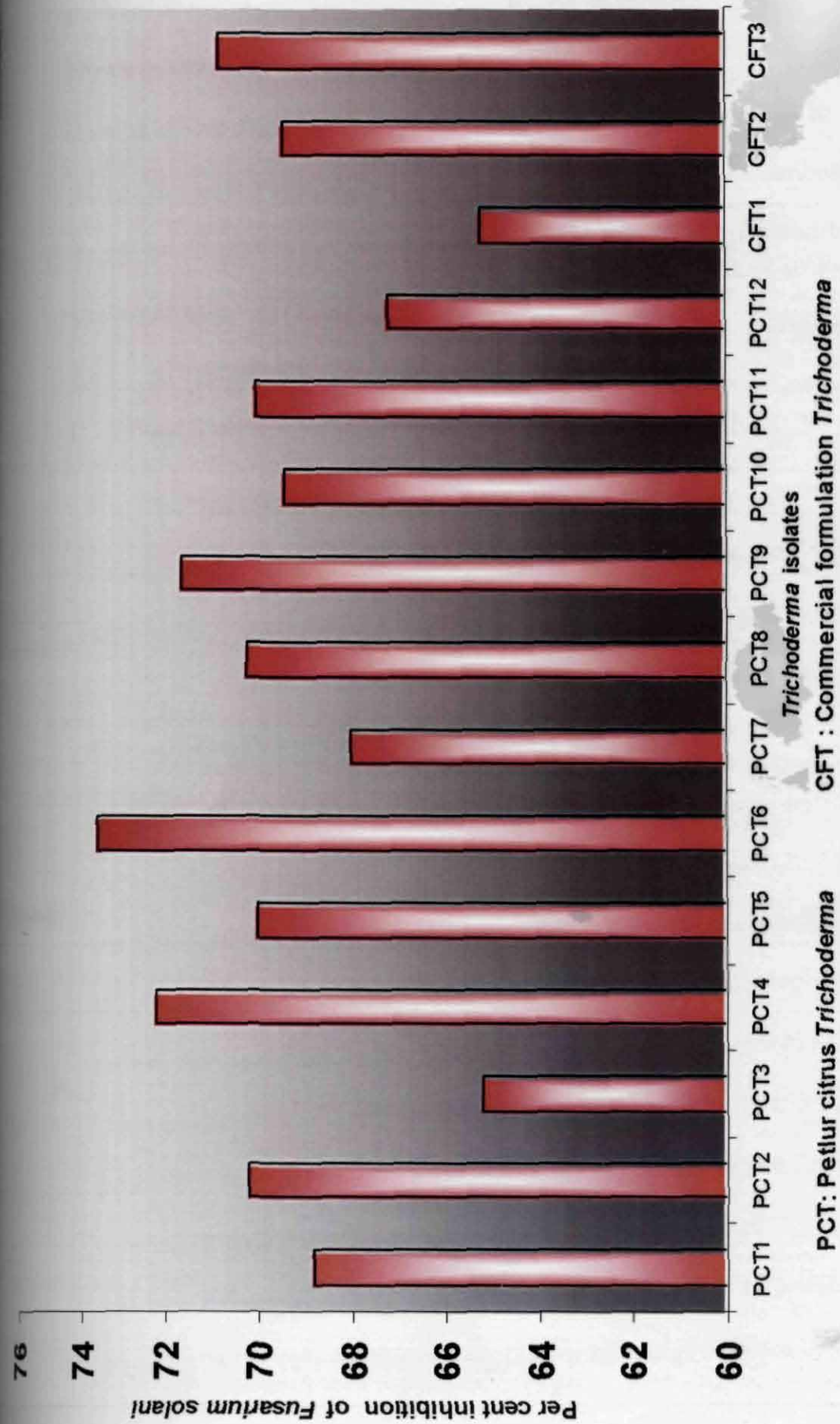


Fig 8: In vitro screening of native and commercial *Trichoderma* isolates against *Fusarium solani*(PCF₂)

growth of *F. solani* could be attributed mainly due to antibiosis or hyperparasitism. Likewise, three bacterial isolates (PCB₁, PCB₂ and PCB₃) which were also screened against *Fusarium solani* found ineffective when compared to *T. viride* (PCT₆). The low biocontrol activity of these three isolates may be attributed due to the non-production of antibiotics siderophores, ammonia cyanide and hydrolytic enzymes (Baker, 1987).

5.5 SCREENING OF SUBSTRATES FOR MASS MULTIPLICATION OF POTENTIAL BIOAGENT

For success of biological control of soil borne plant pathogens using *Trichoderma* spp, suitable substrates which are easily available, cost effective and supports for production of maximum colony forming units is very much essential and it is reported to be $2-5 \times 10^6$ (Ramarethinam *et al.* 2000; Adams, 1990) for higher efficiency under field conditions.

In the present investigation, of the various substrates tested for mass multiplication of potential bio-agent *T. viride* (PCT₆), neem cake (20.7×10^5 cfu/g) was found to be the best followed by pressmud (19.7×10^5 cfu/g) and FYM (18.5×10^5 cfu/g) where population of *T. viride* (PCT₆) was observed upto 28 days. Further, the population steadily increased upto 21 days then showed a slow decline. These results indicate that neem cake recorded maximum population at 21 days of incubation compared to other substrates. Similarly Kousalya and Jeyarajan (1990) screened 18 agricultural bio-products for mass multiplication of *T. viride* and found that well decomposed farm yard manure (FYM) (25.9×10^6) was at par with pressmud 25.2×10^6). Pigeonpea husk (63.5×10^6 cfu/g) followed by tapioca waste

(56.2×10^6 cfu/g) and pressmud (50.8×10^6 cfu/g) were reported as best organic substrates for the mass multiplication of *T. harzianum* by Jayaraj and Ramabadran (1996). Gangadharan and Jayarajan (1990) tested 18 agricultural by-products and wastes as substrates for mass multiplication of *T. viride* and reported that pressmud (25.2×10^6 cfu/g) was at par with decomposed FYM (25.9×10^6 cfu/g). Shamarao *et al.*, (1998) also recorded maximum population of *T. viride* on wheat bran (215.03×10^4 cfu/g) followed by FYM (193.33×10^4 cfu/g). Kavitha (2002) identified wheat bran (28.5×10^5 cfu/g) as good substrate for mass multiplication of *Trichoderma* isolates followed by Jowar grain flour (26.4×10^5 cfu/g).

5.6 *IN VITRO* SCREENING OF FUNGICIDES AND NATURAL PRODUCTS AGAINST ROOT ROT PATHOGEN

The efficacy of six fungicides and two natural products against *F. solani* (PCF₂) was studied at different concentrations viz., 100, 500, 1000 ppm by poisoned food technique. The results revealed that the fungicides mancozeb, chlorothalonil, carbendazim, propiconazole and hexaconazole were found effective compared to natural products, however, their efficiency differed at different concentrations. Mancozeb and carbendazim at 100, 500 and 1000 ppm concentrations were very effective compared to others and recorded complete inhibition of growth of *F. solani* followed by propiconazole and hexaconazole (Fig. 9). Several workers also reported the efficacy of different fungicides in inhibiting the growth of *Fusarium* sp. Similar findings are reported on complete inhibition of *F. solani* a causal agent of dry root rot of acid lime by carbendazim even at 50 ppm followed by captafol (Vijaya Kumar, 2001) and on *F. oxysporum* f.sp. *ciceri* a causal agent of chickpea wilt by carbendazim (Singh *et al.*, 1993;

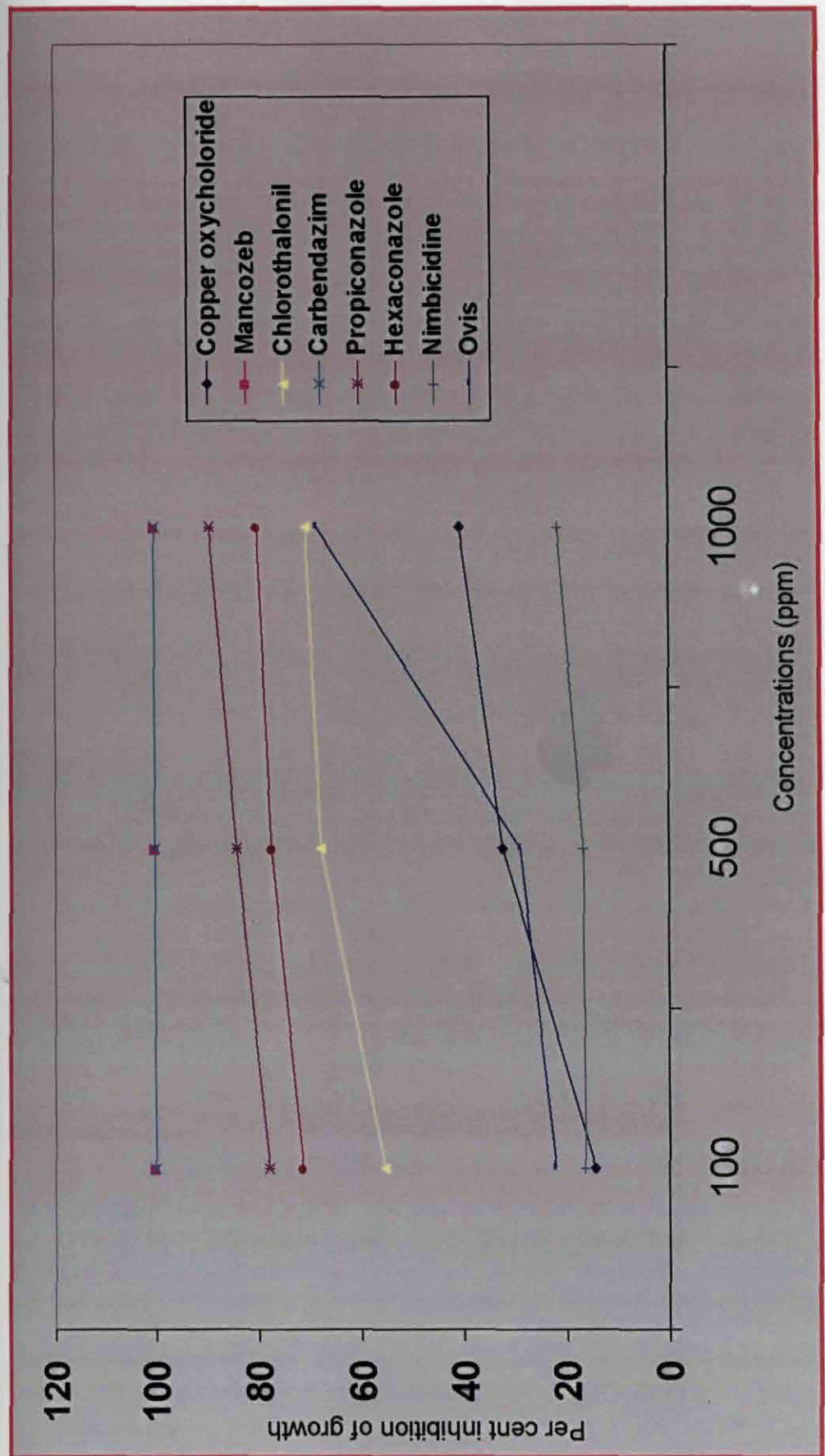


Fig.9: *In vitro* screening of fungicides and natural products against *Fusarium solani* (PCF₂)

Gupta *et al.* 1997). Similarly the results are in agreement with Iboton Singh *et al.* (2000) who proved that carbendazim (0.1%) and mancozeb (0.2%) completely inhibited the linear fungal growth (0.0 cm) and sporulation (0.0 mg) of *F. solani* in *in vitro* conditions. They also stated that copper oxychloride 50 WP at 0.1 per cent concentration was not as effective as other fungicides tested for the control of *F. solani*. Dilip *et al.* (1991) also proved that mancozeb EC 50 (concentration $< 10 \mu\text{g}^{-1}$) completely inhibited spore germination and mycelial growth of *F. solani*.

The results of present study is quite similar with the findings of Narayanabhat and Srivastava (2003) who have reported complete inhibition of *F. solani* at 250, 500 and 1000 ppm of carbendazim and mancozeb. Further, with respect to natural products viz., nimbidine and ovis, inhibitory activity on *F. solani* was least i.e. 68.4 per cent with ovis and 21.5 per cent with nimbidine at 1000 ppm, however, the natural product ovis was significantly superior to copper oxychloride (40.6%). Narayanabhat and Srivastava (2003) also opined that among nimbidine, neemagel, PJMD and PJMO tested against *F. solani* none were completely inhibited the pathogen in poisoned food test even at 5000 ppm.

5.7 COMPATIBILITY OF FUNGICIDES AND NATURAL PRODUCTS WITH POTENTIAL BIOCONTROL AGENTS

Bio-agents have gained considerable importance as sole application or integrated with lower doses of fungicide for the management of soil borne plant pathogens. Since, the biocontrol agent has to be applied in to soil, it becomes imperative to ascertain its tolerance to fungicides used in crop protection

technology. Further, it was also opined that biological approach can be successful only if antagonists are compatible with fungicides and biopesticides (Papavizas, 1985).

In the present investigation an attempt was made to study the compatibility of six fungicides and two natural products with the potential *T. viride* (PCT₆) under *in vitro* conditions to identify the best for use in integrated management of the disease. The results revealed that out of the six fungicides, mancozeb was highly compatible with *T. viride* (PCT₆) followed by copper oxychloride. Carbendazim, propiconazole and hexaconazole showed 100 per cent inhibition of mycelial growth of *T. viride* (PCT₆) even at 100 ppm concentration (Fig. 10). Several workers have also reported the compatibility of different fungicides with biocontrol agents. The present results are more or less similar to Ramarethinam *et al.* (2001) who reported that the *Trichoderma viride* (Srm 62) isolate grew well in culture medium containing mancozeb and copper oxychloride even at the concentration as high as 500 ppm. According to the data on per cent inhibition of *T. viride* (Srm 62) by different chemicals, they have stated that mancozeb and copper oxychloride can be best used in an integrated manner with *T. viride* in the control of plant diseases. They also reported that the fungicides like carbendazim (50% WP), hexaconazole (5% EC) and propiconazole (25% EC) completely inhibited the growth of *T. viride* even at 100 ppm concentration *in vitro* and it was also true in case of *T. harzianum* with carbendazim (Anandh, 1999). Pratibha Sharma and Prem Dureja (2004) tested the sensitivity of 12 isolates of *Trichoderma* spp. against some commonly used fungicides and categorised mancozeb and captan as the low risk one and

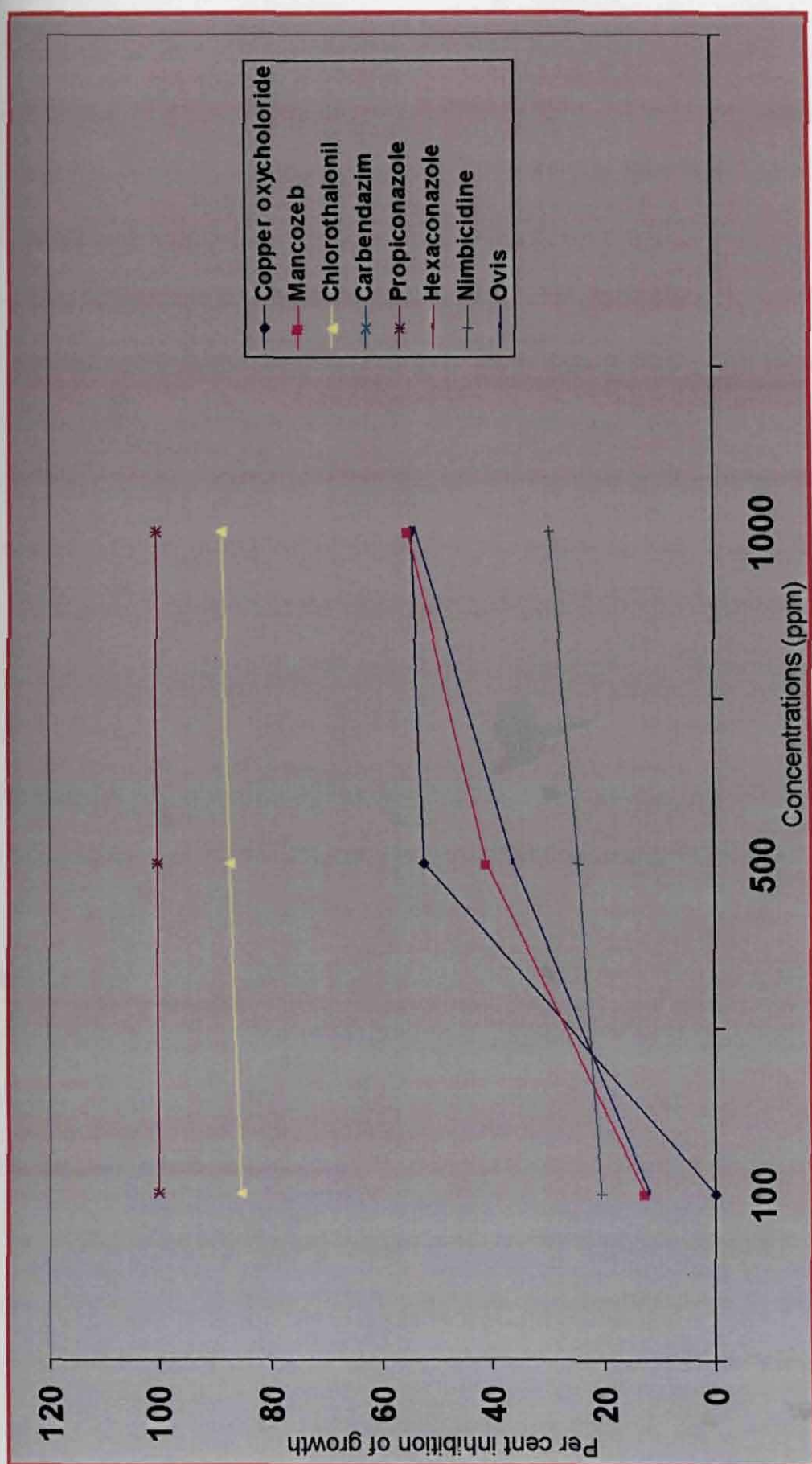


Fig.10: In vitro compatibility of *Trichoderma viride* (PCT_e) with fungicides and natural products

carbendazim as high risk fungicide based on their growth and sporulation. Sharma *et al.* (1999) opined that *T. harzianum* and *T. pseudokoningii* are highly compatible with mancozeb at all concentrations of 100, 500, 1000 and 2000 ppm. Singh *et al.* (1995) also reported that the growth of the isolates *T. viride* and *T. kongii* were not inhibited at 500 ppm of captafol and mancozeb. Mancozeb was compatible with bio-control agents at low concentrations and can be used in integrated management strategies (Abha Agarwal and Tripathi, 1999; Shanmugam and Verma, 1998 and Gupta *et al.*, 1999). Desai *et al.* (2002a) also reported that mancozeb at 500 ppm recorded a lower inhibition of hyphae (5.70%) and sporulation (11.02%) of *T. harzianum*. Whereas, Haritha (2003) and Narayana Bhat and Srivastava (2003) reported that mancozeb and COC inhibited the radial growth of *Trichoderma* spp. by 76.66 and 44.44% at 1000 ppm and complete inhibition of growth at 100 ppm concentration of hexaconazole. The results are also in agreement with the works of Mukhopadyay *et al.* (1986); Sawant and Mukhopadyay (1990); Sharma and Mishra (1995); Rajan and Sharma (1996); Sharma *et al.* (2001); Karpagavalli and Ramabhadran (2001); Deepak Kumar and Dubey (2001); Bhat and Sabalpara (2001); Srinivasulu *et al.* (2001); Durai (2004); Desai and Srikant Kulkarni (2004); Prathibha Sharma and Prem Dureja (2004) who also found good growth of *Trichoderma* isolates at low and medium concentrations of various fungicides.

With regard to the natural products, nimbidine and ovis showed high compatibility with *T. viride* (PCT₆) upto 1000 ppm however, due to their less effectiveness against *F. solani*, natural products were not utilized for further studies. Goutam Mandal *et al.* (1995) also reported the high compatibility

of *T. viride* (TV-5) with neem products, antifeedant neem (hexane insoluble) and neem oil (hexane soluble) at 100 and 250 ppm

5.8 INTEGRATED MANAGEMENT OF ROOT ROT DISEASE IN ACID LIME NURSERY AND GROWNUP TREES OF ACID LIME

In the present investigation, individual as well as combined effect of certain physical, biological and chemical methods were tried with an integrated approach to study the management of root rot of acid lime seedlings in nursery.

Temperature under polythene sheet exceeds 50°C at 10 cm soil depth found lethal to many microorganisms which is effectively being used for many soil borne plant pathogens (Pullman *et al.*, 1981). Soil solarization with transparent polyethylene sheet (100 µm) in nursery experiment, soil temperature exceeded 50°C and increased by 12°C in solarized plots over the unsolarized plots both at 5 and 10 cm depth. Kaushik (2002) reported soil solarization by mulching with polythene sheet (25µm) led to rise in temperature, being the average highest temperature of 56.3°C at 8 cm soil depth which is 16.6°C higher compared to non-solarized beds. The increase in soil temperature by soil solarization reduced the disease severity in the solarized ones than the unsolarized plots (Katan *et al.*, 1983; Vijaya Kumar, 2001) (Fig. 11, 12 and 13).

There was more conservation of moisture (4.0 %) in solarized plots and the results are in agreement with Stapleton and Devay (1987) and Vijaya Kumar (2001). The conservation of moisture in solarized plots might be attributed to condensation of moisture beneath polythene sheet (Chauhan *et al.* 1988).

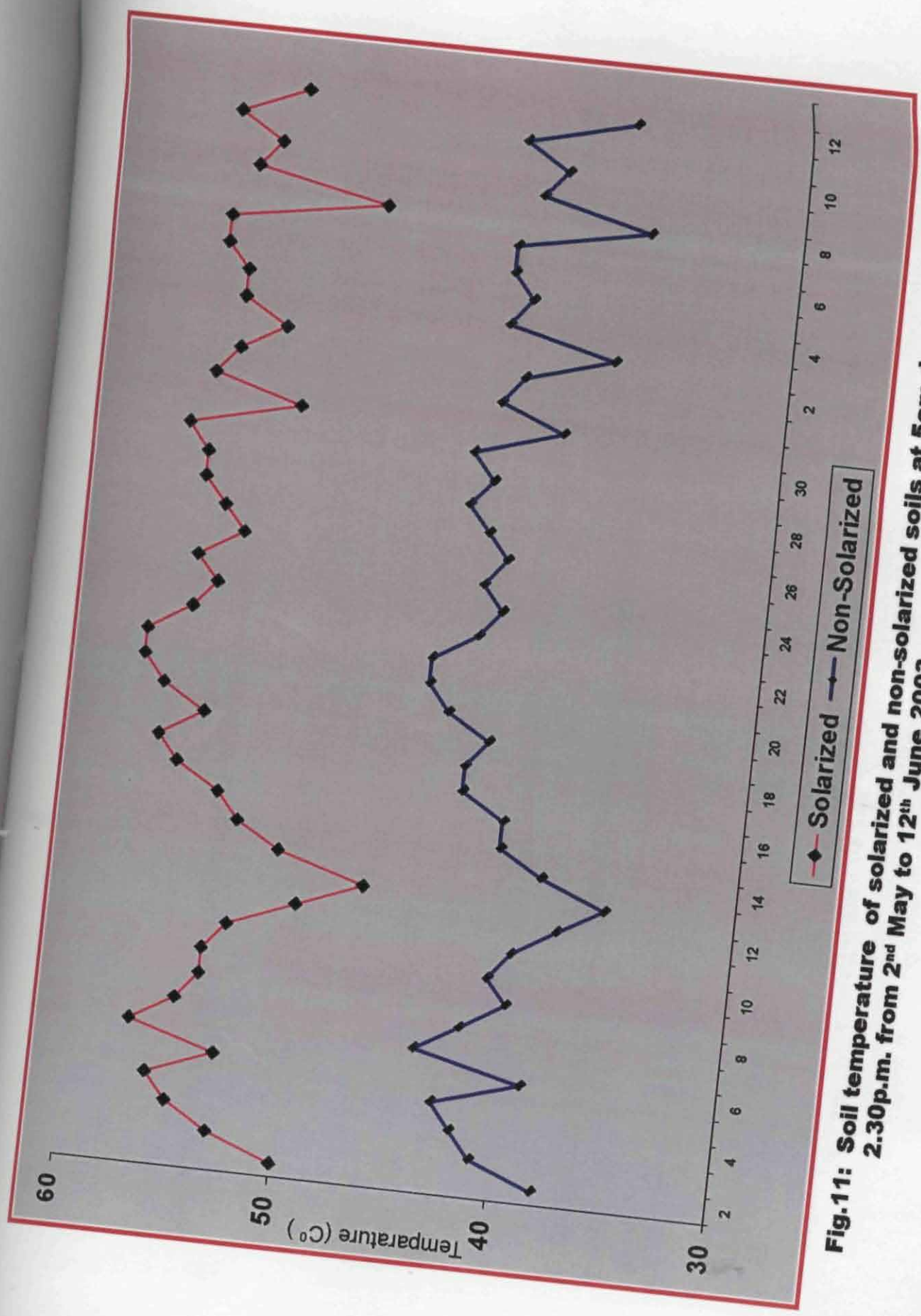


Fig.11: Soil temperature of solarized and non-solarized soils at 5cm depth recorded at 2.30p.m. from 2nd May to 12th June 2003

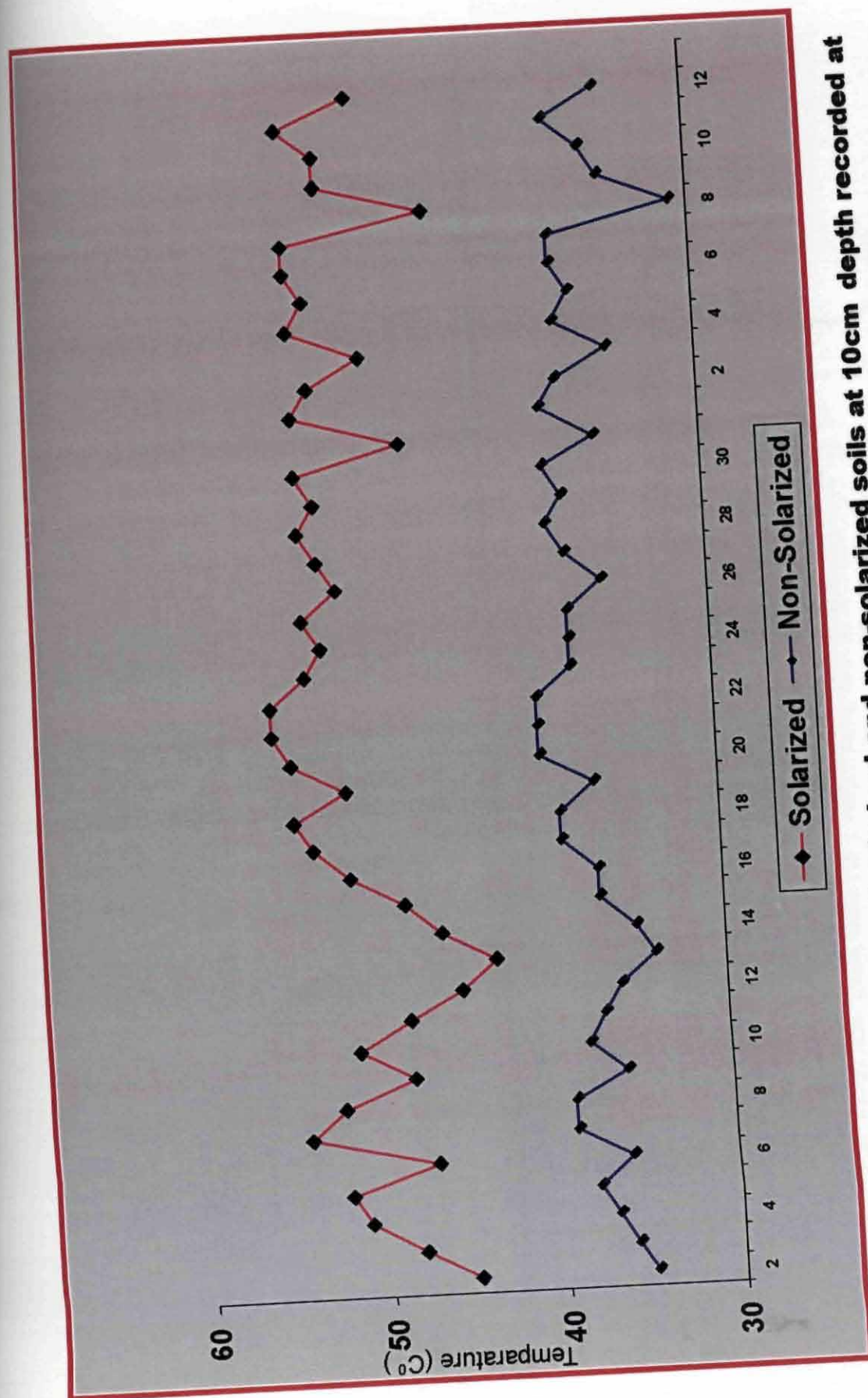
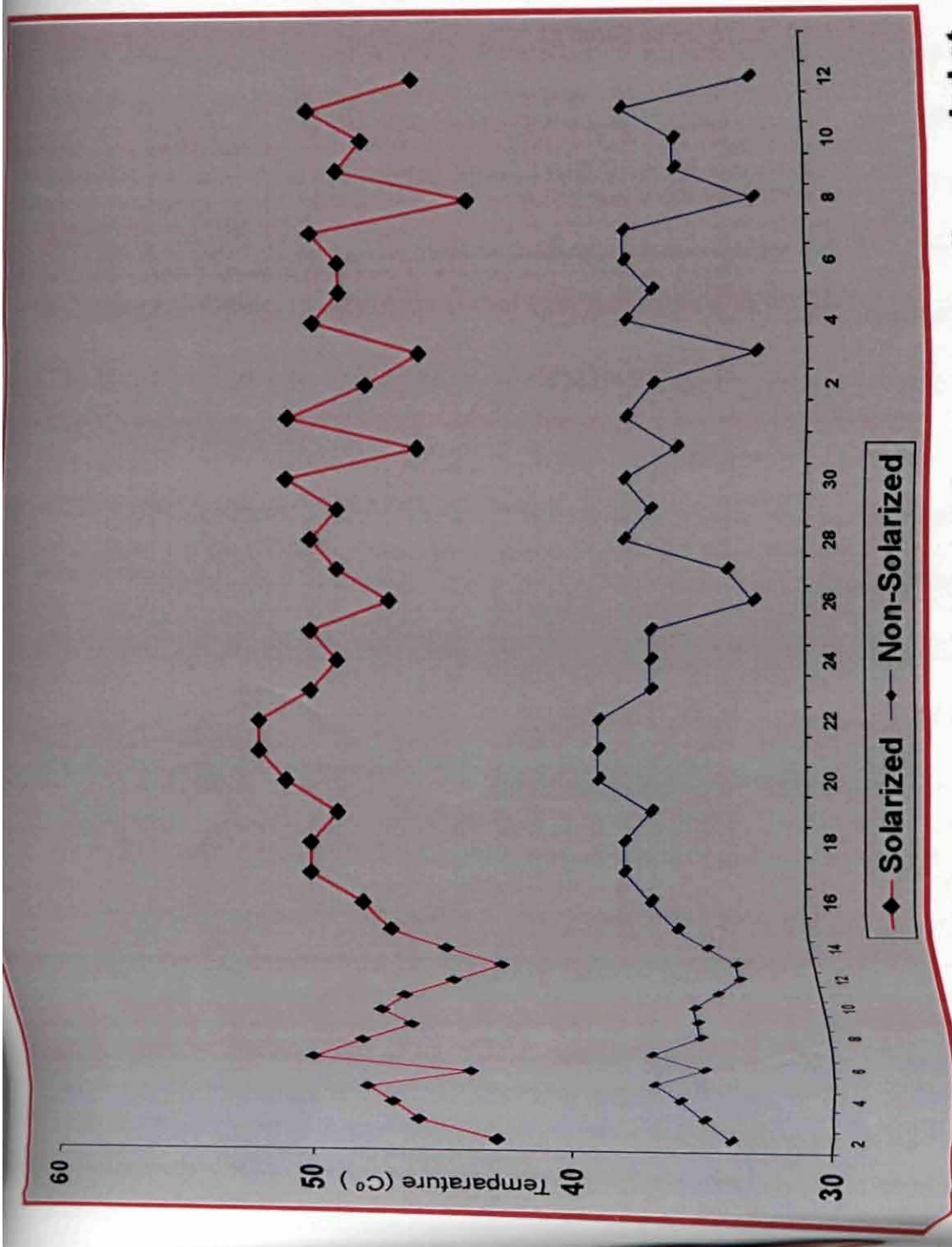
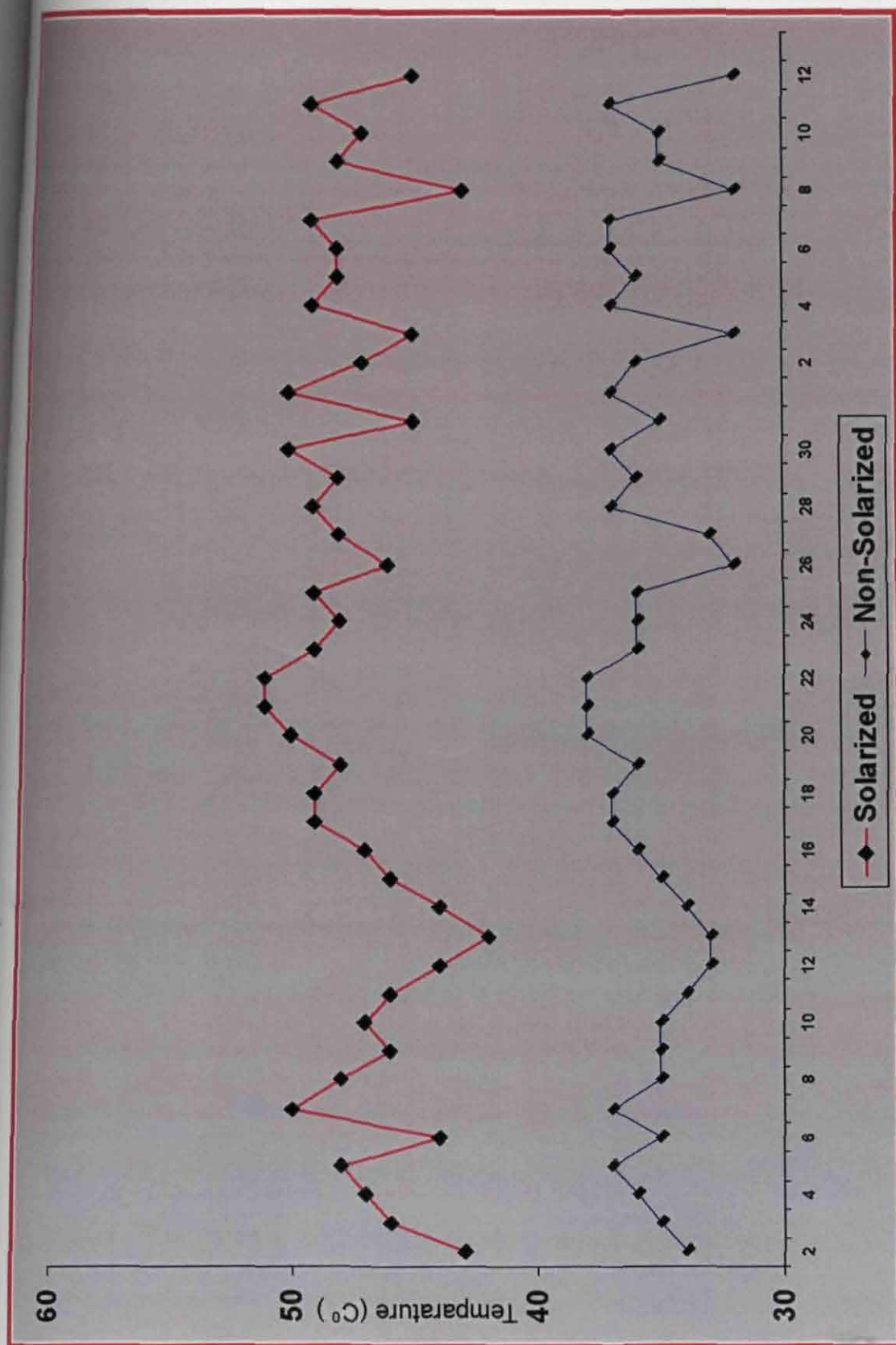


Fig.12: Soil temperature of solarized and non-solarized soils at 10cm depth recorded at 2.30p.m. from 2nd May to 12th June 2003





Soil solarization resulted in the maximum per cent reduction of weed counts (88.9%), fresh weight (94.9%) and dry weight (91.8%) compared to unsolarized plots. In general, most of the annual and perennial weeds (*Amaranthus*, *Anagallis*, *Avena*, *Capsella*, *Chenopodium*, *Digitaria*, *Eleusine* and *Solanum*) are effectively controlled by soil solarization (Katan *et al.*, 1983). In the present investigation too, the population of weeds were less than that of unsolarized plots. This reduction could be due to rise in temperature to higher levels by transparent polyethylene sheet which caused death or damage to the weed seeds present in the soil to a greater extent. Krishna Rao and Krishnappa (1995) and Patel *et al.* (1995) also reported reduction of weed growth by soil solarization. Vijaya Kumar (2001) and Nanjappa *et al.* (2005) reported that soil solarization is effective in controlling most of the annual weeds however, the perennials such as *Cynodon dactylon*, *Cyperus rotundus* and *Convolvulus arvensis* are gradually recovered.

Soil solarization reduced the population levels of *F. solani* to an extent of 81.7 per cent compared to unsolarized plots. This could be due to the high thermal temperatures affecting the survival of *F. solani* in the soil. Use of soil solarization in different host pathogen systems are reported by different workers. Seedling mortality in *Citrus jambhiri* caused by *Fusarium* sp. was effectively controlled by soil solarization for 45 days using transparent polythene sheet in Maharashtra (Gade *et al.*, 1999). Under cotton-*Fusarium* pathosystem, Katan *et al.* (1983) reported high reduction of *Fusarium* population in the soil due to solarization with decreased wilt incidence. Kaushik *et al.* (2002) also reported that soil solarization brought down the population of *F. solani* to

zero level up to 15 cm depth in comparison to 42.6 propagules per gram of soil in non-solarized naturally infested soils. Soil solarization for 40 days was found effective to kill the viable propagules of *Fusarium* spp. in the papaya-*Fusarium* pathosystem under nursery conditions (Singh *et al.*, 2004).

From the results obtained in the present study on soil solarization, it is evident that solarization of soil can be very well adopted for solar heating of nursery beds to control soil borne diseases more efficiently as it involves covering small area only. This method involves no phytotoxicity or hazards of pesticide residues and does not require sophisticated machines and can be done manually.

The present studies on integrated management of root rot of acid lime in nursery, among the individual treatments drenching the nursery beds with mancozeb was more effective with least root rot incidence (10.8%) followed by soil solarization (19.2%) and *Trichoderma* (21.3%). In turmeric - *F. solani* pathosystem soil application of *T. harzianum* alone resulted in reduced rhizome rot disease incited by *F. solani* and increased the plant biomass (Reddy *et al.*, 2003). In the present investigation too mancozeb combined with *T. viride* (PCT₆) and farm yard manure recorded least root rot incidence (4.0%) (Fig. 14) with high seedling vigour index (3604.6) followed by *T. viride* (PCT₆) combined with farm yard manure and soil solarization (8.2% disease incidence and 3010.2 vigour index). Further, it was at par with *T. viride* (PCT₆) combined with farm yard manure and neem cake (8.6% disease incidence and 2943.0 vigour index). Sateesh and Venu (2004) reported that neem cake 7.5 per cent + *T. viride* 1 per cent recorded no incidence of *Fusarium* wilt in banana. Pandey *et al.* (1991) opined that soil

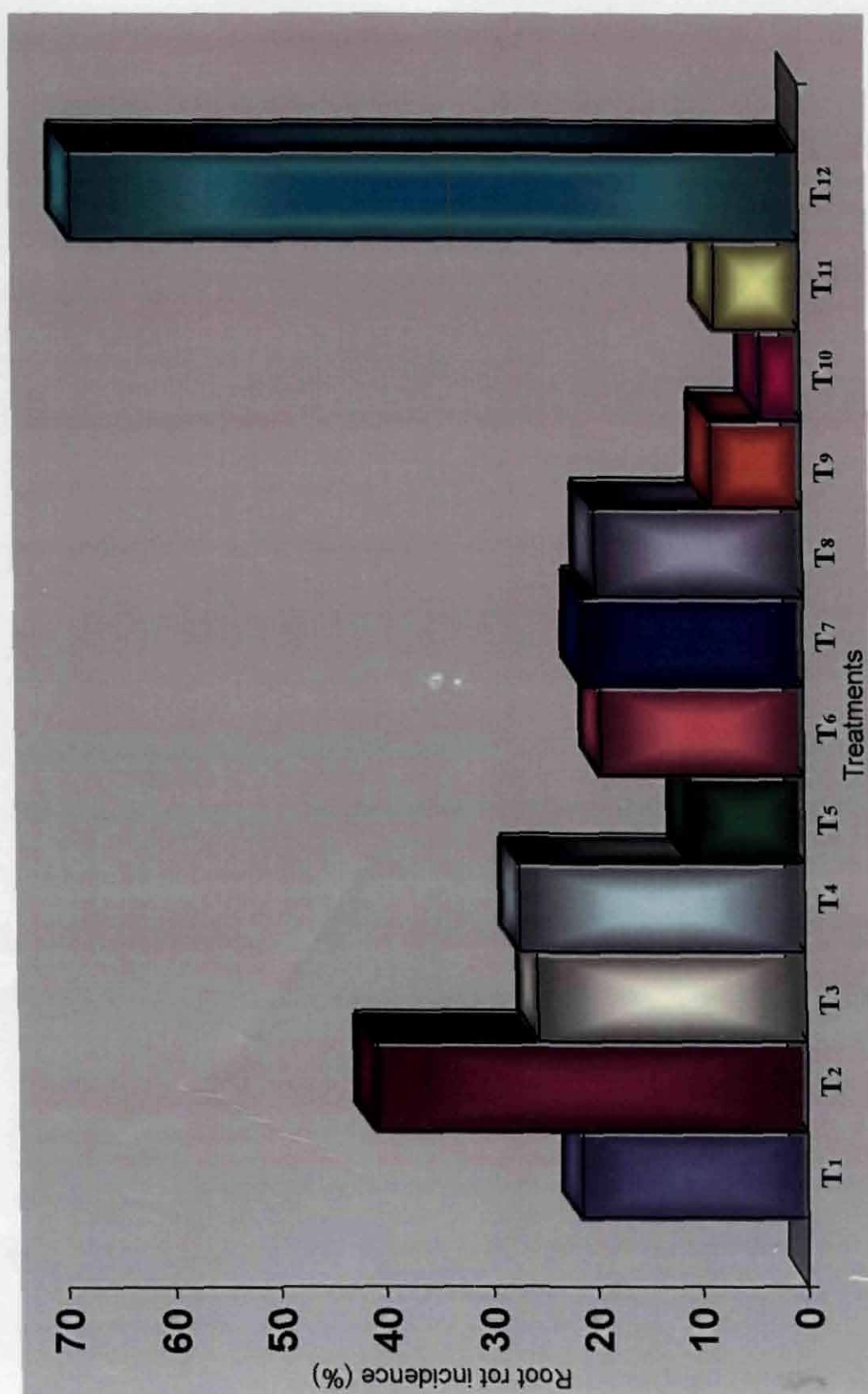


Fig.14: Effect of different treatments on root rot disease (*Fusarium solani*) in acid lime nursery (Local variety)

VAM + vermi compost for the management of root rot disease. The results revealed that prophylactic application of integrated treatments twice a year during September and December months for five years showed no incidence of root rot in integrated treatments where as 33.2 per cent root rot disease was recorded in control. The integration of farm yard manure and neem cake can serve as an excellent food base to improve the proliferation and antagonistic activity of *T. viride* besides mancozeb acts as a root dresser to combat the disease progress. This may lead to effective integrated control of root rot disease in acid lime caused by *F. solani*.

FUTURE LINES OF RESEARCH

The results of the present study indicated the need for future research in the following areas.

- Identification of native bio-agents for each agro-ecosystem in the state, preferably having broad spectrum antifungal activity against citrus soil borne pathogens.
- Working out specific soil amendments for different soil types with different pH levels required for better survival of bio-agents used in IDM of dry root rot disease.
- Studies on sensitivity of potential fungal bio-control agents against fertilizers, insecticides and herbicides.
- Molecular characterization of bio-agents to study the diversity and effective use.

- Development of mutants in bio-agents for wide adoptability and effective use.
- Molecular characterization of the isolates of *F. solani* through random amplified polymorphic DNA (RAPD) analysis to study the diversity among them.
- Identification of need based package as prophylactic measure to control the soil borne diseases for each agro-ecological system in the state.

SUMMARY

CHAPTER VI

SUMMARY

Root rot of acid lime is a very destructive disease particularly in young bearing orchards. A systematic survey conducted during the year 2001-02 on occurrence of root rot disease of acid lime in five acid lime growing districts viz., Chittoor, Nellore, Kadapa, Guntur and West Godavari districts revealed that maximum per cent of disease incidence of 20.25 in Nellore followed by Kadapa (18.6%) district.

Observations on acid lime trees infected with root rot revealed that the symptoms start as yellowing and drying of foliage followed by severe wilting and premature leaf and fruit drop on ground surface. The infection on roots showed discoloration and rotting of secondary fibrous roots.

Budlings are more prone to the root rot disease compared to seedling trees. Acid lime orchards grown in shallow soils recorded more incidence of disease (19.46%) than deep soils (12.8%). The trees irrigated with normal E.C water showed less disease (14.51%) incidence when compared to limited irrigation (20.66%). Flood irrigation and inorganic fertilizer application to the acid lime orchards recorded high disease incidence (25.46 and 22.0%) respectively.

Pathogen associated with acid lime root rot samples yielded *Fusarium* sp, and after proving Koch's postulates it was identified as *F. solani* (ITCC 4657) at IARI, New Delhi. The pathogen could readily infect the young acid lime seedlings in soil inoculation and root rot symptoms were produced 45 days after inoculation.

The effect of different inoculum levels of *Fusarium solani* on root rot incidence of acid lime seedlings in pot culture studies revealed maximum increase of root rot incidence (79.7%) when 8.0 per cent (w/w) density of inoculum was used.

Majority of the infected root samples collected from different acid lime growing areas yielded *Fusarium* sp. on isolation. Among the 15 *F. solani* isolates, PCF₂ recorded the maximum per cent of root rot incidence (83.3%) and was found to be extremely aggressive. It was further confirmed as *F. solani* (Mart.) Sacc. (ITCC 5742) at IARI, New Delhi.

Fusarium solani (PCF₂) was found to grow over a wide range of temperatures varying from 10°C to 40°C with maximum biomass obtained at 30°C. The fungus yielded the maximum vegetative growth (490 mg) and excellent sporulation at pH level 6.0.

A total of 12 native *T. viride* isolates and three native bacterial isolates obtained from the rhizosphere soil samples from disease prone orchards and three commercial *T. viride* isolates were studied for their growth rate. Among the isolates of *Trichoderma* sp. PCT₁ and PCT₆ recorded 76.3 and 78.3 mm growth of colony diameter, respectively, within 48 h and were categorized as very fast growing. In the remaining isolates, seven isolates PCT₂, PCT₄, PCT₅, PCT₉, PCT₁₀, PCT₁₁ and PCT₁₂ were found to be fast growing and six isolates PCT₃, PCT₇, PCT₈, CFT₁, CFT₂ and CFT₃ were categorized as medium growing isolates.

All the 15 *Trichoderma* isolates inhibited the test pathogen *F. solani* in dual culture. However, the isolate PCT₆ recorded more per cent of inhibition (73.6) than the others and the same was further confirmed as *Trichoderma viride*

Pers. (ITCC 5740) at IARI, New Delhi. The three native bacterial isolates were ineffective against test pathogen. No significant difference was found among them.

Among the six substrates tested for the mass multiplication of *T. viride* (PTC₆), neem cake was found to be the best substrate and recorded maximum population (20.7×10^5 cfu/g) 21 days after incubation followed by pressmud (19.7×10^5 cfu/g) and FYM (18.5×10^5 cfu/g). Lowest population was observed in citrus fruit rind (7.2×10^5 cfu/g).

Mancozeb (75% WP) and carbendazim (50% WP) were found to be highly effective and resulted in 100 per cent inhibition of growth of *F. solani* even at 100 ppm. Natural products nimbiacidine and ovis proved ineffective as they did not inhibit 50 per cent growth even at 500 ppm.

Among the fungicides tested with the potential *Trichoderma* isolate (PCT₆) for their compatibility, mancozeb was found to be highly compatible and safe upto 500 ppm as it inhibited less than 50 per cent of growth. Mancozeb was found effective against *F. solani* and compatible with *Trichoderma*, hence it was selected for further studies on the integrated management of root rot disease.

Soil solarization with polyethylene sheet for 42 days increased soil temperature by 12°C and retained soil moisture upto 4.0 per cent. Soil solarization reduced the weed count (88.9%), fresh weight (94.9%), dry weight (91.8%) of weeds and population density of *F. solani* (81.66% at 15 cm depth) compared to unsolarized plots.

In the nursery experiment, mancozeb in combination with other treatments recorded more seedling vigour index. Mancozeb in combination with farm yard manure and *T. viride* was found to be the most effective in decreasing root rot incidence (94.1%) and was adjudged as the best integrated treatment in respect of Cost – Benefit ratio (1 : 35) followed by *T. viride* in combination with farm yard manure and neem cake (1 : 32).

In the field experiment, among the different treatments tested, the integration of *T. viride* (PCT₆) @ 200g + FYM @ 10 kg + neem cake @ 1 kg + mancozeb @ 0.3% per tree basin for twice during the months of June and September was found to be the best in reducing the rotting of roots (78.3%) and recorded 100 per cent survival of trees followed by the treatment, *T. viride* (PCT₆) @ 150 g + FYM @ 5 kg + neem cake @ 0.75 kg + mancozeb @ 0.25% which reduced the rotting of roots by 51.9 per cent. The treatment, *T. viride* (PCT₆) @ 100 g + FYM @ 2.5 kg + neem cake @ 0.5 kg + mancozeb @ 0.2% was found to be the least effective compared to other treatments which reduced the rotting of roots only upto 37.3 per cent.

LITERATURE CITED

LITERATURE CITED

- * Abdel-Rahim A M and Abu-Surrieh A A 1989 Biological control of *Rhizoctonia solani* the causal agent of seedling blight in okra. Arab-Journal of Plant Protection 7(2) : 167-171.
- Abdul Baki A A and Anderson J D 1973 Vigour determination in soyabean seed by multiple criteria. Crop Science 13 : 630-633.
- Abha Agarwal and Tripathi H S 1999 Biological and chemical control of Botrytis grey mold of chickpea. Journal of Mycology and Plant Pathology 29(1): 52-56.
- Adams P B 1990 The potential of mycoparasites for biological control of plant disease. Annual Review of Phytopathology 28: 59-72.
- Agarwal D K and Sarbhoy A K 1978 Physiological studies on four species of *Fusarium* pathogenic to soybean. Indian Phytopathology 31: 24-31.
- * Al-Hamdony M A 1988 Efficacy of isolates of *Trichoderma* spp to suppress *Rhizoctonia solani* in sesame. Journal of Agriculture and Water Resource Resaearch Plant Production 7: 107-114.
- All India Co-ordinated Research Project 1990 Control of dry root rot in Sathgudi sweet orange, Seasonal report, Tirupati pp.15.
- * Al-Raded A M M 1979 Soil disinfestations by plastic tarping. M.Sc (Ag) Thesis, University of Jordan pp. 95.
- Anandh K, Ramanujam K and Prakasam V 1999 Effect of fungicides on the incidence of contaminants and yield of *Pleurotuseous*. Journal of Mycology and Plant Pathology 29(3): 289-293
- Anita Chowdary 1997 Studies on sclerotial wilt of bell pepper M.Sc (Ag) thesis submitted to ANGRAU, Hyderabad pp. 169.
- Anita K and Tripathi N N 2000 Integrated management of seedling diseases of okra caused by *Rhizoctonia solani* and *Pythium aphanidermatum* (Edson) Fitzp. Indian Journal of Plant Protection 28(2) : 127-131.
- Anita Mazinder Buragohain and Islam M 2000 Antagonistic activity of *Trichoderma* spp. against *Sclerotium rolfsii* (Sacc.) Curzi. Pestology, Volume XXIV, no.12.

- * Ansar M, Saleem A and Harmeed K 1994 Studies on cause of citrus quick decline in the Punjab (Pakistan). Pakistan Journal of Phytopathology 6: 38-40.
- Arora D K and Dwivedi R S 1979 rhizosphere fungi of *Lens esculenta* Moench antagonistic to *Sclerotium rolfsii* sacc. Soil Biology and Biochemistry 11: 563-566
- Arun Arya Mathew D S and Arya A 1993 Studies on rhizosphere microflora of pigeonpea: qualitative and quantative incidence of microorganisms after solarization. Indian Phytopathology 46(2): 151-154.
- * Bajwa K M, Tasleem U Z, Zaman Khan and Bajwa M N 1998 Effect of fungicides on *Rhizoctonia solani* Kahn causing root rot of bottlegourd. Pakistan Journal of Phytopathology 10(1) : 23-25.
- Baker K F 1987 Evolving concepts of biological control of plant pathogens. Annual Review of Phytopathology 25 : 67-85.
- Baker K F and Cook R J 1974 Biological control of Plant Pathogens. WH Freeman and Company San Francisco pp. 433.
- Baker K P and Cook J R 1982 Biological control of plant pathogens. American Phytopathological Society 433 pp.
- * Baker R A and Tatum J H 1998 Novel anthraquinones from stationary cultures of *Fusarium oxysporum*. J. Ferment. Bioeng. 85(4):359-361.
- Barnett 1962 Illustrated genera of imperfect fungi. Bergess Publishing Company, Minneapolis, USA pp. 225.
- * Bashar M A and Khatun M T 1999 Rhizosphere and rhizoplane mycoflora of jute (*Corchorus capsularis* L.) and their antagonistic potential against *Macrophomina phaseolina* (Tassi) Goid. Bangladesh Journal Botany 28: 69-77.
- Bedi K S 1961 The wilt disease of citrus nurseries. Punjab Horticulture Journal 1: 141.
- Bell D K, Wells H D and Markham C R 1982 *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72: 379-82.
- Bender G S, Menge J A, Ohr W D and Burns R M 1982 Dry root-rot of citrus: it's meaning for the grower. Citrograph 67: 249-254.
- Bhardwaj S S, Gupta P K and Shyam K R 1987 Evaluation of substrates for enhanced growth of *Trichoderma* spp. Journal of Biological Control 1: 151-152.

- Bhargava S N 1962 Physiological and pathological studies of some fungi causing storage rots. Ph.D Thesis, Allahabad University, Allahabad, India.
- Bhat D, Shetty H S, Prakash H S and Karanth N G K 1992 Influence of gypsum amendment to soil on *Trichoderma harzianum* and *Aspergillus flavus* in the rhizosphere of groundnut. *Advances in Plant Sciences* 5(2): 506-510.
- Bhat T K and Sabhalpara A N 2001 Sensitivity of some bio-inoculants to pesticides. *Journal of Mycology and Plant Pathology* 31(2): 267.
- Bhatnagar G C and Prasad N 1966 Studies on *Fusarium* twig disease of lime (*Citrus aurantifolia* Swingle). *Indian Phytopathology* 19: 257-261.
- Bhattacharya M K and Bora K N 1995 Rhizosphere microflora of Tea in relation to age of the plants. *Indian Journal of Mycology and Plant Pathology* 25 (3): 263-265.
- Biswas K K and Sen C 2000 Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum*. *Indian Phytopathology* 53: 290-295.
- Blaker N S and Mc Donald J D 1986 The role of salicity in the development of *phytophthora* root rot of citrus. *Phytopathology* 76 : 970-975.
- Bohra Burhanuddin and Kusum Mathur 2004 Biocontrol agents and neem formulation for suppression of *Fusarium solani* root rot in soybean. *Journal of Mycology and Plant Pathology* 34(2): 408-409.
- Bornali Bora Barua S C and Barua R 2003 Growth of the seedlings inoculated with vesicular arbuscular mycorrhiza and phosphate solubilising fungi. *Journal of Plantation Crops*, 31 (3): 23-26.
- Bunker R N and Mathur K 2001 Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root rot of chilli. *Journal of Mycology and Plant Pathology* 31(1): 50-53.
- Bunker R N and Mathur Kusum 2001 Integration of biocontrol agents and fungicide for suppression of dry root rot of *Capsicum frutescens*. *Journal of Mycology and Plant Pathology* 31(3): 330-334.
- Champawat R S and Pathak V N 1989 Cultural morphological and pathogenic variations in *Fusarium oxysporum* f.sp. *cumini*. *Indian journal of Mycology and Plant Pathology* 19: 178-183.
- Chandra S, Raizada M and Gaur A K S 1983 Pathological variability in *Fusarium oxysporum* and *Fusarium solani*. *Indian Phytopathology* 36: 36-40.

- Chandrasekhar G, Ayyappan S and Balakrishna Murthy P 2000 Biocontrol of root rot diseases of brinjal (eggplant). *Pestology* 24(6): 45-47.
- Chandrasekhar G, Nakkeeran S, Ramiah M and Sabita Doraiswamy 1998 Characters and mass multiplication of *Trichoderma*. Manual on *Trichoderma* Mass Production Technology 4: 16-24.
- Chauhan Y S, Nene Y L, Johnsen C and Haware M P 1988 Effect of soil solarization on pigeonpea and chickpea. *ICRISAT Res. Bull. No. 11*: 1-16.
- Chen Y, Ganliel A, Stapleton J J and Avid T 1991 Chemical Physical and microbial changes related to plant growth in disinfected soils In: J. Katan and J.E. Devay (Eds.) *Soil solarization* CRS Press. Boca Raton, FL, USA pp 103-129.
- Chet I, and Baker R 1981 Isolation and bio-control potential of *Trichoderma harzianum* from soil naturally suppressive to *Rhizoctonia solani*. *Phytopathology* 71: 286-290.
- Chohan J S and Jasmit Kaur 1976 Cultural studies on *Rhizoctonia bataticola* causal agent of root rot of sunflower. *Indian Journal of Mycology and Plant Pathology*. 6: 140-144.
- Citrus research station, Petlur 2005 Integrated management of root rot disease in acid lime, Annual report, Petlur pp.65.
- CMIE 2005 Centre for Monitoring Indian Economy Private limited, Bombay.
- Cook R J and Baker 1983 The nature and practices of biological control of plant pathogens. St. Pant Minnesota, American Phytopathological Society pp. 539.
- Dandurand L M and Menge J A 1992 Influence of *Fusarium solani* on citrus root rot caused by *Phytophthora parasitica* and *Phytophthora citrophthora*. *Plant and Soil* 144: 13-21.
- David J and Pieczarka 1979 Root rot of celery seedlings caused by *Rhizoctonia solani* and *Fusarium solani*. *Phytopathology* 69 : 1042.
- Davis R M 1980 Influence of *Glomus fasciculatus* on *Thielaviopsis basicola* root rot of citrus. American Phytopathological Society. *Plant Disease* 64: 839-840.
- Davis R M 1980 Control of *Phytophthora* root and root-rot of Citrus with systemic fungicides. *Plant Disease* 66: 218-220.

- * Deb P R and Dutta B K 1991 Studies on biological control of foot-rot disease of soybean caused by *Sclerotium rolfsii* Sacc. Zeitschrift für Pflanzenkrankheiten and Pflanzenschutz. 98(5): 539-554.
- Deepa Khulbe and Choube H S 2002 Effect of soil solarization and its integration with bioagents and fungicides on incidence of damping-off and plant growth response of vegetable crops in nursery. Journal of Mycology and Plant Pathology 32(3): 413.
- Deepak Kumar and Dubey S C 2001 Management of collar rot of pea by the integration of biological and chemical methods. Indian Phytopathology 54(1): 62-66.
- Derrik K S and Timmer L W 2000 Citrus blight and other diseases of recalcitrant etiology. Annual Review of Phytopathology 38: 198-199.
- Desai S A and Srikant Kulkarni 2002a Effect of urea on the growth and sporulation of *Macrophomina phaseolina* (Tassi) Goid and native isolates of *Trichoderma viride* Pers. and *Trichoderma harzianum* Rifai. Karnataka Journal of Agricultural Sciences 15(1): 77-79.
- Desai S A and Srikant Kulkarni 2002b Fungistatic effect of chlorpyrifos on the growth of *Trichoderma viride* Pers. Karnataka Journal of Agricultural Sciences 15(2): 384-385.
- Desai S A and Srikant Kulkarni 2004 Effect of fungicides, insecticides and weedicides on the growth and sporulation of native *Trichoderma harzianum* Rifai. Karnataka Journal of Agricultural Sciences 17(1): 57-62.
- Dhanbir Singh and Akhliesh Singh 2004 Fusarial wilt – A new disease of chilli in Himachal Pradesh. Journal of Mycology and Plant Pathology 34(3) : 885-886.
- Dhedi B M, Om Gupta, Patel V A and Gupta O 1990 Antagonistic effects of microorganisms to *Fusarium oxysporum* f.sp. *ciceri*. Indian Journal of Mycology and Plant Pathology 20(1): 70-71.
- Dhrubsingh and Andotra P S 1997 Effect of soil amendments for the management of citrus seed mortality in Nursery beds. Journal of Mycology and Plant Pathology 27: 71-72.
- Dilip M and Rajendra K Grover 1991 Chemical control of root rot of cow pea in relation to altered pathogenicity of *Fusarium solani*. Indian Phytopathology 44(4): 462-469.

- Dingra D and Sinclair B 1995 Basic plant pathology methods. Lewis Publishers, London pp. 434.
- Directorate of Economics and Statistics 2004 An out line of Agriculture situation in Andhra Pradesh 2003 – 2004. Government of Andhra Pradesh.
- Domsch K H, Gams W and Anderson T H 1980 Compendium of soil fungi. Volume I. Academic Press, New York, 794-809.
- Dubey R C and Dwivedi R S 1988 Antagonism between *Macrophomina phaseolina* and some rhizosphere microfungi of soybean. Indian Journal of Tropical Plant Diseases 6: 89-94.
- Dubey S C 1998 Evaluation of fungal antagonists of *Thanetophorus cucumeris* causing web blight of horsegram. Journal of Mycology and Plant Pathology 28(1): 15-18.
- Dubey S C 2000 Biological management of web blight of groundnut (*Rhizoctonia solani*). Journal of Mycology and Plant Pathology 30(1): 89-90.
- Dubey S C and Patel B 2001 Evaluation of fungal antagonists against *Thanetophorus cucumeris* causing web blight of urd and mung bean. Indian Phytopathology 54(2): 206-209.
- Durai M 2004 Management of charcoal rot of sesame (*Sesamum indicum* L.) incited by *Macrophomina phaseolina* (Tassi.) Goid. M Sc (Ag.) Thesis submitted to Acharya N G Ranga Agricultural University, Hyderabad.
- * Dwivedi S K 1992 Effect of culture filtrates of soil microbes on pathogens inciting wilt diseases of guava (*Psidium guajava* L.) under *in vitro* conditions. National Academy Science Letters 15(2) : 33-35.
- Elad Y and Chet I 1983 Improved selective media for isolation of *Trichoderma* and *Fusarium* spp. Phytoparasitica 11 : 55-58.
- Elad Y, Chet I and Katan J 1980 *Trichoderma harzianum* A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70: 119-121.
- Elad Y, Chet I and Katan J 1980a *Trichoderma harzianum* : A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70: 119-121.
- Elad Y, Chet I, Boyle P and Henis Y 1983a Parasitism of *Trichoderma* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy-flouorescent microscopy. Phytopathology 73: 85-88.

Elad Y, Hadar Y, Hadar E Chet I and Henis Y 1981 Biological control of *Rhizoctonia solani* by *Trichoderma harzianum* in carnation. Plant Disease 65: 675-677.

Fawcett H S 1936 Citrus diseases and their control. Mc Graw Hill Book Co., Inc New York 97-100

Gade R M, Palows A N, Giri G K and Patil S R 1999 Soil solarization for control of mortality in *Citrus jambhiri* Lush. Paper presented in International symposium on citriculture held at Nagpur. November 23-27, 1999 at NRCC, Nagpur, India. pp. 163.

Gaikwad S J and Kapgate D J 1990 Biological control of *Sclerotium* root rot of sesamum PKV Research Journal 14: 87-89.

Gangadharan G and Jayarajan R 1990 Mass multiplication of *Trichoderma* spp. Journal of Biological Control 4(1) : 70-71.

Gaur V K and Sharma L C 1991 Microorganisms antagonistic to *Fusarium udum* Butler. Proceedings of the Indian National Sciences Academy Part-B, Biological Sciences 57: 85-88

* Gegenava G V, Nishnianidze M O, Gogiberdize G S and Khitrov D P 1976 The effectiveness of some fungicides against disease of trifoliate orange seeds and citrus seedlings. Trudy – Nauchno – Issle dovate skogo – Instituta – Zashihitty – Rastenii – Groz USSR 28: 102-107.

* Gesheva V 2002 Rhizosphere microflora of some citrus as a source of antagonistic actinomycetes. European Journal of Soil Biology 38(1): 85-88.

Gnanavel I and Jayaraj J 2003 Integration of soil solarization and *Trichoderma viride* Pers. Fr to control damping off of tomato (*Lycopersicon esculentum* Mill.). Journal of Biological Control 17(1): 99-102.

Gopal K, Ahammed S K, Sreenivasulu Y, Gopi V, Malathi V G, and Madhusudhan P 2005 Eco-friendly management of *F. solani*, an incitant of dry root rot in acid lime (*Citrus aurantifolia* Swingle) poster presentation Global Conference-II, Plant Health-Global wealth, MPUAT, Udaipur 25th to 29th November, 2005 pp.180.

Gopal K, Subbi Reddy G, Reddy M R S, Chenchu Reddy B, Ramakrishna Rao A, Madhavi M and Venkataramana K T 2000 Citrus decline in Andhra Pradesh - causes and their management. In: Hi-tech citriculture (Ed. Shyam singh and Ghosh) published by ICAR, NRCC, Nagpur, India pp 702-711.

- Gopal K, Vijayakumar B and Rao A R K 2001 A major fungal threat to citrus plantations in A.P. National symposium on plant protection strategies for sustainable Agri-Horticulture. Society of Plant Protection Sciences, IARI, New Delhi, pp. 123.
- Gopalakrishnan C, Ramanujam B, Prasad R D, Rao N S and Rabindra R J 2003 Screening and selection of potential *Trichoderma* isolates for the control of cotton seed rot and damping-off. *Journal of Biological Control* 17(2): 161-165.
- Goudar and Srikant Kulkarni 1999 Compatibility effect of antagonistic and seed dressers against *Fusarium udum*-The causal agent of pigeonpea wilt. *Karnataka Journal of Agricultural Sciences* 12: 197-199.
- Goudar S B, Srikant -Kulkarni and Kulkarni S 1998 Effect of organic amendment on *Fusarium udum* Butler the causal agent of wilt of pigeon pea. *Karnataka Journal of Agricultural Sciences* 11: 690-692.
- Gour T B, Chandrasekhara Rao K and Ramasubba Reedy M 1999 Citrus insect pests diseases nutritional disorders. Acharya N.G. Ranga Agricultural University Rajendra Nagar Hyderabad pp.85.
- Goutam Mandal, Srivastava K D and Aggarwal R 1995 Antagonistic effect of *Trichoderma* spp on *Ustilago segetum* var. *tritici* and their compatibility with fungicides and biocides. *Indian Phytopathology* 48(4): 466-470.
- Graham J H, Brlansky R H, Timmer L W, Lee R F and Marais L J 1985 Comparison of Citrus tree declines with necrosis of major roots and their association with *Fusarium solani*. *Plant Disease* 69: 1055-58.
- Grinstein A, Elad Y, Katan J and Chet 1979 Control of *Sclerotium rolfsii* by means of a herbicide and *Trichoderma harzianum* *Plant Disease Reporter* 63: 823-826.
- Grinstein A, Katan J, Abdul Razik A, Zeydam O and Elady 1976 Control of *Sclerotium rolfsii* and weeds in peanuts by solar heating of the soil. *Plant Disease Reporter* 63: 1056-1059.
- Gupta B 1974 Effect of rhizosphere mycoflora *Rhizobium legumimosarum* MSc (Ag.) thesis, APAU, Hyderabad.
- Gupta S K, Upadhyay J P and Ojha K L 1997 Effect of fungicidal seed treatment on the incidence of chickpea wilt complex. *Annals of Plant Protection Science* 5: 184-187.

- Gupta V P, Sharma D D, Rekha M and Chandrasekhar D S 1999 Integration of *Trichoderma pseudokoningii* with agrochemicals for disease management and plant development in mulberry. Archives of Phytopathology and Plant Protection 32(6): 521-529.
- Hader Y, Chet I and Henis Y 1979 Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology 69: 64-68.
- Hadwar H A and Khara H S 1980 A new record of *Trichoderma* from India. Indian Journal of Ecology 17: 121-124.
- Harender Raj M L, Bharadwaj and Sharma N K 1997 Soil solarization for the control of damping-off of different vegetable crops in the nursery. Indian Phytopathology 50(4): 524-28.
- Hari K and Somasekhar N 1998 Utilisation of sugarcane wastes for the mass multiplication of fungal biocontrol agents. Cooperative Sugar 29(9) : 637-638.
- Haritha V 2003 Studies on the management of damping-off disease incited by *Pythium aphanidermatum* (Edson) Fitzp. in tobacco nurseries. M Sc (Ag.) Thesis submitted to Acharya N G Ranga Agricultural University, Hyderabad.
- Haware M P 1980 Methods of artificial inoculation and disease rating of root pathogens in Phytopathological techniques (ed. JN Chand and G S Sharma), pp. 32-35.
- Hazarika D K and Das K K 1998 Biological management of root rot of French bean (*Phaseolus vulgaris* L.) caused by *Rhizoctonia solani*. Plant Disease Research 13(2): 101-105.
- Hegde S G and Anahosur K H 1998 Integrated management of foot rot of black pepper. Karnataka Journal of Agricultural Sciences 11(1): 78-82.
- Hemanth Kumar Singh, Sunil Kumar and Singh H K 2004 Soil solarization for the control of damping-off of Papaya (*Carica Papaya* L.) in the nursery. Progressive Horticulture 36(1): 177-179.
- Henis Y, Gaffar A and Baker R 1978 Integrated control of *Rhizoctonia solani* damping off of radish: Effect of successive plantings, PCNB and *Trichoderma harzianum* on pathogen and disease. Phytopathology, 68: 900-907.

- Howell C R and Stipanovic R D 1979 Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with antibiotic produced by the bacterium. *Phytopathology* 70: 712-715.
- Humayun P 2002 Studies on biological control of soil borne plant pathogens. M.Sc.(Ag.) Thesis, Acharya N.G. Ranga Agricultural University, Hyderabad.
- Hundekar A R, Anhosur K H, Patil M S, Kalappanavar I K and Chattannavar S N 1998 *In vitro* evaluation of organic amendments against stalk rot of sorghum. *Journal of Mycology and Plant Pathology* 28:26-30.
- Hundoo S and Dwivedi R S 1997 Chemical control of *Rhizoctonia solani* Kuhn causing damping off of egg plant (*Solanum melongena* L.). *Crop Research Hissar* 13(2) : 445-454.
- Hundoo S, Dwivedi R S and Sucheta Hundoo 1992 Chemical control of *Fusarium solani*, the incitant of root rot of egg plant (*Solanum melongena* L.). *Journal of Phytopathological Research* 5(1-2) : 89-92.
- Hykamachi M 1994 Plant growth promoting fungi from turf grass rhizosphere with potential for disease suppression. *Soil micro-organisms* 44: 53-68.
- Ibton Singh N, Tombisana Devi R K and Pramo Devi P 2000 Effect of fungicides on growth and sporulation of *Fusarium solani*. *Indian Phytopathology* 53: 327-328.
- Indu S, Sawant and Sawant S D 1996 A simple method for achieving high CFU of *Trichoderma harzianum* on organic wastes for field applications. *Indian Phytopathology* 49(2): 185-187
- * Ippolito A, Cico-V-de and De-Cicco V 1995 Dry root rot of citrus in Apulia and Basilicata. *Informatore Fitopatologico* 45 (12): 51-53.
- Jacob K C 1989 Biological control of root rot of blackgram *Vigna mungo* (L.) Hepper by introduction of antagonists Ph.D thesis. Tamil Nadu Agricultural University, Coimbatore, India pp. 214.
- Jacob K C and Sivaprakasam K 1995 Development of a cheap system for mass multiplication and delivery of *Trichoderma harzianum* and *T. viride*. In *crop diseases: Innovative techniques and management* (ed) Sivaprakasam K and Seetharaman K, Kalyani Publishers, New Delhi pp 143-147.
- Jagannathan 1992 Chemical control of damping off of acid lime. *Madras Agricultural Journal Research Notes*, pp. 418-420.

- Jagannathan R 1989 Chemical control of damping-off of acid lime. South Indian Horticulture 37 (6):343.
- Jayachandra 1990 Studies of survey and chemical management of root-rot of sweet orange var. Sathgudi *Citrus sinensis* (L.) Osb. MSc(Ag.) thesis, APAU, Hyderabad pp 23-24.
- Jayaraj J and Ramabhadran R 1996 Evaluation of certain organic substrates and adjuvants for the mass multiplication of *Trichoderma harzianum* Rifai. Journal of Biological Control 10: 129-131.
- Jayarajan and Ramakrishnan 1995 Mass production of *Trichoderma viride* for biological control. Indian Journal of Mycology and Plant Pathology 25 (1&2):125.
- Jeyarajan R, Ramakrishnan G, Dinakaran D and Sridhar R 1994 Development of product of *Trichoderma viride* and *Bacillus subtilis* for biocontrol of root rot disease. In: Dwivedi B K (ed.). Biotechnology in India. Bioved Research Society, Allahabad, India.
- Johnson L F and Curl E A 1972 Methods for research on the ecology of soil borne pathogens. Burgees Publications pp. 274.
- Jones J 2001 Harpin. Pesticide Outlook 12(4): 134-135.
- Jones Nirmalnath P and Badnur V P 2001 Effect of inoculation of VA Mycorrhizal fungi on the rhizosphere microflora, growth and yield of chickpea. Karnataka Journal of Agricultural Science 14(2): 523-527.
- Kallurmuth R S and Rajasab A H 2000 Rhizosphere mycoflora of onion (*Allium cepa* L.) crop with special reference to *Aspergillus niger*. Journal of Mycology and Plant Pathology 30(3): 339-342.
- Kalpanasastry R and Chattopadhyay C 1999 Effect of soil solarization on *Fusarium oxysporum* f.sp. *carthami* populations in endemic soils. Indian phytopathology 52: 51-55
- Karpagavalli S 1997 Effect of different fungicides on the growth of *Trichoderma*. Indian Journal of Plant Protection 25(1): 82-83.
- Karpagavalli S and Ramabhadran R 2001 Effect of fungicides and *Trichoderma* species on cellulolytic enzyme production, damping-off incidence and seedling vigour of tomato. Plant Disease Research 16(2): 179-185.
- Karthikeyan A and Karunanithi K A 1996 Influence of organic amendment on the intensity of *Fusarium* wilt of banana. Plant Disease Research 11: 180-181.

- Katan J 1981 Solar heating of soil for control of soil borne pests. *Annal Review of Phytopathology* 19: 211-236.
- Katan J 1995 Soil solarization, a non chemical tool in plant protection. In a special issue of the global conference on advances in research on plant diseases and their management 12-17 Feb. 1995 Udaipur, India. *Indian Journal of Mycology and Plant Pathology* 25 : 46-47.
- Katan J, Fishler G and Grinstein A 1983 Short and long term effects of soil solarization and crop sequence on *Fusarium* wilt and yield of cotton in Israel. *Phytopathology* 73: 1215-1219.
- Katan J, Green Berger A, Alon H and Grinstein A 1976 Solar heating by polythene mulching for the control of diseases caused by soil borne pathogens. *Phytopathology* 66: 683-688
- Katan J, Totem I, Finkel Y and Daniel J 1980 Solar heating of the soil for the control of pink root and other soil diseases in onions. *Phytoparasitica* 8 : 39-50.
- Kaushik J C, Sanjay Arya and Gupta P P 2002 Management of damping-off disease in forest nurseries by soil solarization and biocontrol agents. *Forage Research* 28(1): 29-31.
- Kavitha M 2002 Studies on biological control of dry root rot of acid lime (*Citrus aurantifolia* swingle) caused by *Fusarium solani*. M.Sc.(Ag.) Thesis, Acharya N.G. Ranga Agricultural University, Hyderabad.
- Kavitha M, Gopal K, Anandam R J, Prasad Babu G 2004 Evaluation of native isolates of *Trichoderma* in the control of dry root rot in acid lime. *Journal of Mycology and Plant Pathology* 34(2): 384-386.
- Kore S S and Mane A V 1992 Dry root-rot of Kagzilime seedling caused by *Fusarium solani*. *Journal of Maharashtra Agricultural Universities* 17: 276-278.
- Kore S S and Mashalkar S I 1987 Dry rot disease of sapota caused by *Fusarium solani* (Mart.) Sacc. *Journal of Maharashtra Agricultural Universities* 12 : 279-282.
- Kore S S and Patil D S 1985 Dry rot disease of mango seedlings caused by *Fusarium solani*. *Indian Journal of Mycology and Plant Pathology* 15: 287-288.
- Kouslya and Jayarajan 1990 Mass multiplication of *Trichoderma* spp. *Journal of Biological Control* 4(1): 70-71.

- Krishna Rao V and Krishnappa K 1994 Effect of soil solarization, V A mycorrhizal fungus and seed dressing chemicals on seedlings emergence of chickpea in *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *ciceri* infected field. Indian Journal of Nematology 24: 250-252.
- Krishna Rao V and Krishnappa K 1996 Rhizosphere mycoflora of chickpea infected with *Meloidogyne incognita*. Indian Journal of Mycology and Plant Pathology 26: 103-104.
- Krishnamohan G and Kandaswamy T K 1986 Effect of organic amendments and seed dressing on *Rhizoctonia solani* in cotton. In : biological control of plant diseases. A workshop, Department of Plant Pathology CPSS, TNAU, Coimbatore, pp. 34 (Abstract).
- Krishnamoorti A S and Bhaskaran R 1993 Biological control of damping-off disease of tomato caused by *Pythium indicum* Balakrishnan. Journal of Biological Control 4: 52-54.
- Krishnarao V and Krishnappa K 1995 Effect of soil solarization, VA Mycorrhizal fungus and seed dressing chemicals on seedling emergence of chickpea in *Meloidogyne incognita*-*Fusarium oxysporum* f. sp *cicer* infected field. Ph.D. Thesis University of Agricultural Sciences, GKVK, Bangalore.
- Kumar R N 1993 Biological control of damping-off disease in chirpine. Indian Journal of Mycology and Plant Pathology. 23: 237-240.
- Labuschagne N, Kotze J M and Putterill J F 1987 Incidence of *Fusarium solani* and *F. oxysporum* in citrus roots and infection by *F. solani*. Phytophylactica 19 : 315-318.
- Labuschagne N, Vandervegte F A and Kotze J M 1989 Interaction between *Fusarium solani* and *Tylenchulus semipenetrans* on citrus roots. Phytophylactica 21: 29-33.
- Laha G S, Singh R P and Verma J P 1992 Biocontrol of *Rhizoctonia solani* in cotton by fluorescent Pseudomonads. Indian Phytopathology 45(4): 412-415.
- Laville E Y and Chalandon A J 1982 Control of *phytophthora gummosis* in citrus with foliar sprays of Fosetyl A l, a new systemic fungicide. Proceedings of International Society of Citriculture 1 : 346-349.
- * Laville E Y and Vogel R 1984 Root rot attacks in citrus orchards in Corsica. Arboriculture Fuitiere 369 : 34-36.
- Lele V C and Kapoor J N 1982 *Phytophthora* on citrus in central and peninsular India. Indian Phytopathology 35: 407-410.

- Lele V C and Kapoor J N 1982 *Phytophthora* on citrus in central and peninsular India. Indian Phytopathology 35: 407-410.
- Lele V C, Rayachowdhari S P and Ashram 1969. Dry root-rot of Trifoliate orange. A new nursery disease. Indian phytopathology 21: 497-499.
- Lilly S S and Santhakrishnan P 1998 Coirpith compost a suitable medium for mass multiplication of *Glomus fasciculatum*. Madras Agricultural Journal 85 (5-6) : 240-243.
- * Lio G M S, Perrota G and Tuttobene R 1983 Research on pathogenic fungi present in the soil and sour orange seedbeds in Sicily. Informaont are- Fitipathologica 33: 49-55.
- * Li-Qiqim, Lin-Wei Chen, YongNing Peng, HaoWen Meng and JiaoRong 2000 Biological control sheath blight of rice by *Bacillus* sp. Chinese Journal of Biological Control 16 (4):160-162.
- * Lisanasky S G 1985 Production and commercialization of pathogens pp 210-218 in: Biological pest Control (Eds) Hussey N W and Scopes N. Blandford Press, Poole, U.K.
- * Liu-JingJing, Yin-GongYi, Liu JJ and Yin G Y 1995 Studies on the root rot and yellow leaf disease of citrus in Jiangsu and Hubei provinces. Journal of Nanjing Agricultural University 16(1): 38-44.
- Lodha S and Anjali Vaidya 1990 Influence of soil solarization on the viability of *Fusarium solani* in an arid environment. Arid Soil Research 4: 199-202.
- Maiti D, Khatua D C and Sen C 1987 Effect of oil cakes and a potential antagonist on survival of Sclerotia of *Sclerotium rolfsii* stem rot of groundnut. In : Biological control of plant diseases. CPPS, TNAU, Coimbatore.
- Malathi P, Viswanathan R, Padmanabhan P, Mohanraj D and Sunder A R 2002 Compatibility of biocontrol agents with fungicides against red rot disease of sugarcane. Sugar Technology 4(3-4): 131-136.
- Malikoutsaki M M, Bourbos V A and Skoudridakis M T 1987 Dry root-rot a very citrus disease in Greece. Bulletin -OEPP 17: 335-340.
- Mamata Sharma and Sharma S K 2002 Effect of organic amendments on soil microbial population in relation to *Dematophora necatrix* causing white root rot of apple. Plant Disease Research 17(1): 9-15.
- Manoranjitham S K, Prakasam V and Rajappan K 1999 Effect of antagonists on *Pythium aphenidermatum* (Edison) Piz and the growth of chilli seedling. Journal of Biological Control 13: 101-106.
- Mariakulandai A and Durairaj D J 1958 Some chemical aspects of mandarin orange decline in wynaad. South Indian Horticulture 6: 23-35.

- Mathur K and Bhatnagar K 1994 Effect of volatile and non-volatile substances produced by *Trichoderma viride* on stem blight pathogen of cowpea. *Journal of Biological Control* 8: 64.
- Mathur K, Ram D, Poonia J and Lodha B C 2002 Integration of soil solarization and pesticides for management of rhizome rot of ginger. *Indian Phytopathology* 55(3): 345-347.
- Meera M S, Shivanna M V, Kageyamma K and Hyakumanchi M 1994 Plant growth promoting fungi from zoysia grass rhizosphere as potential inducers of systemic resistance in cucumbers. *Phytopathology* 84: 1399-1406.
- Mehta R D, patel K , Roy K K and Mehta M H 1995 Biological control of soil borne pathogens with *Trichoderma harzianum*, Department of Microbiology, M.S. University of Baroda, Gujarat, India). *Indian Journal of Mycology and Plant Pathology* 25(1&2): 128.
- Menge J A, Johnson E L V, Sibert D and Burns R M 1980 Dry root rot of citrus-A disease complex, 72nd Annual Meet, American Phytopathological Society pp. 474.
- * Mohamedy R S R 2004 Integration between biological and chemical treatments to control *Fusarium* root rot of some citrus rootstocks under saline soil conditions. *Annals of Agricultural Science Cairo* 49 (1): 357-375.
- Mohanbabu R, Seetharaman K, Ebenegar E G and Sageena A 2002. Efficacy of *Trichoderma viride* Pers. Gray and neem cake amendment in different soils against blackgram root-rot caused by *Macrophomina phaseolina* (Tassi.) Goid. *Journal of Biological Control* . 16: 49-55.
- Morgan K T and Timmer L W 1984 Effect of inoculum density, nitrogen source and saprophytic fungi on *Fusarium* wilt of Mexican lime. *Plant and Soil* 79 : 203-210.
- Mukherjee P K, Upadhyay J P and Mukhopadhyay A N 1989 Biological control of *Pythium* damping off of cauliflower by *Trichoderma harzianum*. *Journal of Biological Control* 3: 119-124.
- Mukhopadhyay A N, Birambhatt A and Patel G J 1986 *Trichoderma harzianum* – a potential bio-control agent for tobacco damping-off. *Tobacco Research* 12(1): 26-35.
- Mukopadhyay A N 1995 Exploitation of *Gliocladium virens* and *Trichoderma harzianum* for biological seed treatment against soilborne diseases. *Indian Journal of Mycology and Plant Pathology* 25: 124.

- Mukopadhyay A N, Patel G J and Brahmabhatt A 1986 *Trichoderma harzianum* - a potential bio-control agent for tobacco damping-off. Tobacco Research 12: 26-35.
- Nagaraju P, Naresh D and Biradar D 2002 Biological control of sheath blight (*Rhizoctonia solani*) in transplanted rice (*Oryza sativa*). Indian Journal of Agricultural Sciences 72(5): 306-307.
- Naidu Venkata C and Rao Krishna D 1958 Note on investigation of the soil of the fruit research station, Anantharajapet, Kodur, with reference to citrus decay or dry root rot. The Andhra Agricultural Journal 5: 55-59.
- Naik Gaonkar, Sreenivasa S B and Alagawadi A R 1993 Rhizosphere microflora, growth and yield of wheat as influenced by inoculation of *Glomus fasciculatum* in conjunction with organic amendments. Karnataka Journal of Agricultural Science 6(4): 371-377.
- Nanjappa H V, Rachandrappa B K, Soumya T M and Thimmegowda M N 2005 Soil solarization – An eco-friendly technology for weed and pest control Indian Farming 14-16.
- Naqui S M A and Chauhan S K 1980 Effect of root exudates on the spore germination of rhizosphere and rhizoplane mycoflora of chilli cultivars. Plant and Soil 55: 397-402.
- Naqvi S A M A 1999 Major fungal diseases of citrus and their management. Citriculture. In: Manual of Winter school, NRCC, Nagpur pp 273-282.
- Narayana Bhat M and Srivastava L S 2003 Evaluation of some fungicides and neem formulations against six soil borne pathogens and three *Trichoderma* spp. *in vitro*. Plant Disease Research 18(1): 56-59.
- Nash S M and Synder W C 1962 Quantitative estimation of plate counts of propagules of the bean root rot *Fusarium* in the field soils. Phytopathology 52: 567-572.
- Nazam W Z and Singh 2004 Development of improved technology for mass multiplication and delivery of fungal (*Trichoderma*) and bacterial (*Pseudomonas*) bio-control agents. Journal of Mycology and Plant Pathology 34(3) : 732-741.
- Neelamegam R and Govindarajalu T 2002 Integrated application of *Trichoderma viride* Pers. Fr. And farmyard manure to control damping off of tomato (*Lycopersicon esculentum* mill.). Journal of Biological Control 16(1): 65-69.

- Nemec S 1982 Water relations of citrus seedlings inoculated with *Fusarium solani* Phytopathology 72(7): 969
- Nemec S 1987 *Fusarium solani* association with branch and trunk cankers on citrus by cold weather in Florida. Mycopathologia.
- Nemec S Barker R and Burnett H 1980 Pathogenicity of *Fusarium solani* to citrus roots and its possible role in blight etiology. Proc. Florida State Hort. Soc. 93: 36-41.
- * Nemec S, Robert A, Baker R and James H Tattum 1988 Toxicity of dihydrofusarubin and Isomarticin from *Fusarium solani* to citrus seedlings Soil Biology Biochemistry 20: 493-499.
- Nemec S, Zaldotowicz R M and Chandler J L 1989 Distribution of *Fusarium* spp and selected mycroflora in citrus soils and rhizosphere associated with healthy and blight diseased citrus in Florida. Phytophylactica 21: 141-146.
- Nene Y L and Thapliyal P N 1993 Fungicides in plant disease control. 3rd edition, Oxford and IBH Publishers, New Delhi, pp. 691.
- Nitta T 1990 The stimulation of root activity and a reduction in root diseases of upland crops through improvements in the rhizosphere microflora. Research Journal of Food and Agriculture 13(6): 15-20.
- Okigbo R and Emoghene A 2000 Root and butt rot of orange trees in South-Western Nigeria. Fruits-Paris 55 (5): 309-314.
- Om Prakash and Singh U N 1980 Root-rot and damping-off of mango seedlings caused by *Rhizoctonia solani*. Journal of Mycology and Plant Pathology 10: 69.
- Padmanabhan P and Alexander K C 1984 Biological control of cane seedling root rot. Sugarcane 4: 11-12.
- Padmodaya B, Reddy H R 1999 Screening of antagonists against *Fusarium oxysporum* f.sp *lycopersici* causing seedling disease and wilt in tomato. Journal of Mycology and Plant Pathology 28 (3): 339-341.
- Padule D N, Nevase A G and Patil B P 1983 Control of Rhizoctonia damping off in the nursery of Kagzilime (*Citrus aurantifolia*). Pesticides 17: 6-7.
- Pandey G, Dwivedi B K and Singh R B 1991 Effect of organic amendments on the root knot disease of green gram and its rhizosphere micrflora. New Agriculturalist 2(2): 203-206.

- Pandey K K and Upadhyay J P 2000 Microbial population from rhizosphere and non-rhizosphere soil of pigeonpea: Screening for resident antagonist and mode of mycoparasitism. *Journal of Mycology and Plant Pathology* 30 : 7-10.
- Panse V G and Sukhatme P V 1978 Statistical methods for Agricultural workers. Indian Council of Agricultural Research, New Delhi pp. 136.
- Papavizas 1985 *Trichoderma* and *Gliocladium*, biology, ecology and potential for biocontrol. *Annual Review of Phytopathology* 23: 23-54.
- Papavizas C E and Lewis J A 1988 The use of fungi in integrated control of plant disease. In: *Fungi in biological control systems* pp. 235-253 (eds. By M.N. Burge Manchester): Manchester University Press, U.K.
- Papavizas G C, Dann M T and Ristaino B J 1984 Liquid fermentation technology for experimental production of biocontrol fungi. *Phytopathology* 74 : 1171-1175.
- Patel H R, Makwana M G and Patel B N 1995 The control of nematodes and weeds by soil solarization in tobacco nurseries, effect of the film thickness and of the covering duration. *Plasticulture* 107: 23-28.
- Patel S B and Patel H R 1998 Integration of *Tylenchorynchus vulgaris* and *Pythium aphanidermatum* on beedi tobacco. *Journal of Mycology and Plant Pathology* 28 : 177-180.
- Patel S T and Anahosur K H 2001 Potential antagonism of *T.harzianum* against *Fusarium* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii*. *Indian Journal of Mycology and Plant Pathology* 31 : 365.
- * Postucha A 1999 Protective effect of antagonistic microorganisms in limiting soybean (*Glycine max* (L.) Merrill) root diseases. *Annales Universitatis Mariae Curie Sklodowska Section EEE, Horticultura* 7: 119-135.
- Prakasam V, Jeyarajan R and Thamburaj S 1992 Occurrence of a dry root rot disease in mandarin plantations in shevroy hills. *South Indian Horticulture* 40: 234.
- Prakash M G, Gopal K V, Anandaraj M and Sarma Y R 1999 Evaluation of substrates for mass multiplication of fungal biocontrol agents. *Trichoderma harzianum* and *T. virens*. *Journal of Spices and Aromatic Crops* 8(2) : 207-210.
- Prameela Devi T and Singh R H 1998 Studies on virulence of *Macrophomina phaseolina* isolates from blackgram and greengram. *Journal of Mycology and Plant Pathology* 28(1) : 196-201.

- Pranab Datta and Das B C 2002 Management of collar rot of tomato by *Trichoderma* spp. and chemicals. Indian Phytopathology 55 : 235-237.
- Prasad R D and Rangeswaran R 2000 Effect of soil application of a granular formulation of *Trichoderma harzianum* on *Rhizoctonia solani* incited by seed rot and damping off of chickpea. Journal of Mycology and Plant Pathology 30(2): 216-220.
- Pratiba Sharma and Prem Dureja 2004 Evaluation of *Trichoderma harzianum* and *T. viride* isolates at BCA pathogen crop interface. Journal of Mycology and Plant Pathology 34(1): 47-55.
- Pullman G S, De Vay J E And Garber R H 1981 Soil solarization and thermal death: A logarithmic relationship between time and temperature for soil borne plant pathogens. Phytopathology 71 (9): 959-964.
- Pushpavathi B 1995 Role of *Fusarium oxysporum* spp. *ricini* (Wr) Gordon in castor wilt complex. MSc (Ag) thesis, ANGRAU, Hyderabad pp. 109.
- Raghavendra Rao N N 1984 Control of pre and post emergence damping-off of citrus seedlings. Pesticides 31-32.
- Raghuchander T, Jayashree K and Samiyappan R 1997 Management of *Fusarium* wilt of banana using antagonistic microorganisms. Journal of Biological control 11 : 101-105.
- Rajan P P and Sharma Y R 1996 Compatibility of potassium phosphate (Akomin 40) with different species of *Trichoderma* and *Gliocladium virens*. Biotechnology of spices, medicinal and aromatic plants. Proceedings of the National Seminar on Biotechnology of Spices and Aromatic Plants, Calicut, India, 24-25 April, 1996, pp.150-155.
- Rajendran K and Ranganathan K 1996 Biological control of onion basal rot (*Fusarium oxysporum* f.sp.*cepae*) by combined application of fungal and bacterial antagonists. Journal of Biological Control 10 : 97-102.
- Rajeswari B 1997 Studies on biological control of *Macrophomina phaseolina* (Tassi.) Goid incitant of dry root rot of Mungbean. Ph.D. thesis, Acharya N.G. Ranga Agricultural University, Hyderabad pp 142-188.
- Rajput C B S and Sri Haribabu R 1985 Citriculture. Kalyani Publishers, New Delhi pp. 282.
- Rakesh Kumar Jalali B L and Hari Chand 2004 Effect of different VAM fungi on nodulation, nitrogenase activity and rhizosphere microflora of chickpea. Legume Research 27(1): 50-53.
- Ram P, Mathur K and Lodha B C 1999 Integrated management of rhizome rot of ginger involving bio-control agents and fungicides. Journal of Mycology and Plant Pathology 29 (3): 416-420.

- Ramakrishnan T S 1954 The deterioration of Mandarin orange in Madras state. South Indian Horticulture 2: 52-56.
- Ramarethinam S, Murugesan M V and Marimuthu S 2001 Compatibility studies of fungicides with *Trichoderma viride* used in the commercial formulation Bio-Cure-F. Pestology 25(5): 2-6.
- Ramarethinam S, Murugesan N V Viswanathan S R and Marimuthu S 2000 Effect of climatological factors on the viability of conidial spores of *Trichoderma* sp. talc-based commercial preparation – Bio-cure-F. Pestology vol. XXIV No.8 P2-8.
- Ramteke A P, Kalore B R and Patil S R 2003 Evaluation of antifungal activity of *Withania somnifera* root extract against the fungal root infections in citrus. JNKVV Research Journal 37(1): 109-110.
- Ranagacharuyulu V S, Anjaneyulu Naidu N and Narasimha Rao T L 1958 Performance of Sathgudi orange (*Citrus sinensis* Osbeck) on different rootstocks at Kodur (South India). The Indian Journal of Horticulture 15 : 35-58.
- Randhava N S and Parmar B S 1996 Neem society of pesticide science, India, New age International (P) Lto Publishers, New Delhi.
- Rangaswami G and Mahadevan A 1999 Disease of crop plants in India, Fourth Edition, Prentice hall of India Private Limited, New Delhi pp 291-293.
- Rangaswami G and Vasantharajan V N 1962 Studies on the rhizosphere microflora of citrus trees. Canadian Journal of Microbiology 8: 473-489.
- Rangeshwaran R and Prasad R D 2000 Isolation and evaluation of rhizospheric bacteria for biological control of chickpea wilt pathogens. Journal of Biological control 14(1):9-15.
- Rathore R S, Solanki J S and Bisnoi H R 1993 A new seedling wilt of acid lime. Madras Agricultural Journal 80 (12): 704.
- Rathore V R S, Kusum – Mathur, Lodha B C and Mathur K 1992 Activity of volatile and non-volatile substances produced by *Trichoderma viride* on ginger rhizome rot pathogens. Indian Phytopathology 45: 253-254.
- Reddy B C, Govindarajulu B, Aariff Khan M A and Hameedunissa Begum 1999 Root-rot diseases the major cause of acid lime decline in Andhra Pradesh. Paper presented in international symposium on citriculture (November 23-27) held at NRCC, Nagpur, India pp. 157.

- Reddy B, Bagyaraj D J and Mallesha B C 1996 Selection of efficient VA Mycorrhizal fungi for acid lime. Indian Journal of Mycobiology 36: 13-16.
- Reddy G S and Murti V D 1985 citrus diseases and their control. Indian council of Agricultural Research New Delhi pp. 79.
- Reddy G S and Papa Rao A 1960 Problems of sweet orange decline in Andhra Pradesh. Andhra Agricultural Journal 7: 175-188.
- Reddy M N, Devi M C and Sreedevi N V 2003 Biological control of rhizome rot of turmeric (*Curcuma longa* L.) caused by *Fusarium solani*. Journal of Biological Control 17 (2): 193-195.
- Reddy M V and Raju T N 1991 Pathogenic variability in pigeonpea wilt pathogen *Fusarium udum*. In: Plant disease problems in central India (Ed: K. Muralidharan and C S Reddy). Indian Phytopathological Society pp 32-34.
- Rifai M A 1969 A revision of the genus *Trichoderma* Common Wealth Mycological Institute Mycol. 116: 56
- Roberts D D and Kraft J M 1971 A rapid technique for studying *Fusarium* wilt of peas. Phytopathological Notes 342-343.
- Roux H F, Wehner F C, Kotze J M and Pretorius M C 1992 Comparison of soil solarisation with methyl bromide fumigation in a citrus replant situation. Citrus Journal 2 : 41-44.
- Rupe J C, Correll J C, Gherbu J C and Becton C M 2001 Differentiation of the sudden death syndrome pathogens from other isolates of *Fusarium solani* based on cultural morphology pathogenicity and mitochondrial DNA restriction fragment length polymorphisms. Canadian Journal of Botany 79: 829-835.
- Sangeetha Panicker and Jeyarajan R 1993 Mass multiplication of biocontrol agent *Trichoderma* spp. Indian Journal of Mycology and Plant Pathology 23: 328-330.
- Sanjay Arya and Kauhik J C 2001 Efficacy of *Trichoderma* species and *Gliocladium virens* as biocontrol agents against damping off of forest nurseries. Plant Disease Research 16(1): 46-51.
- Sarbhoj A K and Agarwal D K 1990 Description of tropical plant pathogenic fungi. Mehrotra Publishing House, New Delhi, pp. 38.
- Satish S and Venu D V 2004 Effect of various amendments and *Trichoderma viride* on the *Fusarium* wilt of banana. Mysore Journal of Agricultural Science 38(4): 546-550.

- Satyanarayana G and Ramasubba Reddy 1994 Citrus cultivation and protection Wiley Eastren Limited New Delhi pp. 30.
- Savanth Indu S, Savant S D and Ganapathy M M 1994 Collar rot of passion fruit caused by *Rhizoctonia solani* and its control. Indian Phytopathology 202-205.
- Sawant I S and Mukhopadyay A N 1990 Integration of metalaxyl with *Trichoderma harzianum* for the control of Pythium damping-off in sugarbeet. Indian Phytopathology 43: 535-541.
- Selvarajan R and Jeyarajan R 1996 Inhibition of chickpea root-rot pathogens, *Fusarium solani* and *Macrophomina phaseolina*, by antagonists. Indian Journal of Mycology and Plant Pathology 26: 248-251.
- Sendhivel V, Marimuthu T and Raguchander T 2004 Compatibility of Azoxystrobin 25Sc with biocontrol agents. Pestology XXVIII (10): 61-63.
- Shah U R, Ingole M N and Patil M J 2005 Antagonistic efficiency of rhizosphere fungi and *Trichoderma* against *Fusarium udum* causing wilt in pigeonpea. Journal of Plant Disease Sciences. 1(1):88-91.
- Shamaro Jahagirdar, Siddaramaiah A L, Narayanaswamy H and Jahagirdar S 1998 Screening substrates for mass multiplication of *Trichoderma viride*. Karnataka Journal of Agricultural Sciences 11: 233-236.
- * Shamim S, Ahmad N, Atta-Ur-Rahman, Enteshamul-Haque S, Ghaffar A 1997 Efficacy of *Pseudomonas aeruginosa* and other bio-control agents in the control of root rot infection in cotton. Acta-Agrobotanica 50: 5-10.
- Shanmugam V and Varma A S 1998 *In vitro* evaluation of certain fungicides against selected antagonists of *Pythium aphanidermatum* the incitant of rhizome rot of ginger (Abstract). Journal of Mycology and Plant Pathology 28: 70.
- Sharma D D, Gupta V P and Chandrashekar D S 1999 Compatibility of certain biocontrol agents with chemical pesticides and fertilizers. Indian Journal of Sericulture 38(1): 79-82.
- Sharma I M 1989 Damping-off of citrus Jambhiri (Jatti Khatti) seedlings, its cause and control in Himachal Pradesh. Progressive Horticulture 21(4): 254-256.
- Sharma K B and Agnihotri J P 1971 Influence of nutrition and pH on growth and sporulation of *Fusarium orthoceras* var. *kentis*. Indian Phytopathology 24 : 553-558.

- Sharma M and Badiyala S D and Sharma N K 1993 Effect of fungicidal drenching against wilt of mango seedlings caused by *Fusarium solani* (Mart.) Sacc. Indian Journal of Mycology and Plant Pathology 28(3): 326-327.
- Sharma R C and Rai S N 1999 Chemical control of banded leaf and sheath blight of maize. Indian Phytopathology 52 : 94-95.
- Sharma S D and Mishra A 1995 Tolerance of *Trichoderma harzianum* to agrochemicals. In: Abst. Global conference on Advances in Research on Plant Diseases and Their Management. February 12-17, 1995. Rajasthan College of Agriculture, India, p.162.
- Sharma S D Ashok Mishra 1995 Tolerance of *Trichoderma harzianum* to agrochemicals. Indian Journal of Mycology and Plant Pathology 25 (1&2): 129.
- Sharma S D, Mishra A, Pandey R N and Patel S J 2001 Sensitivity of *Trichoderma harzianum* to fungicides. Journal of Mycology and Plant Pathology 31(2): 251-253.
- Sharma S K, Verma V S, Anita Sharma and Meenu Gupta 2004 Effect of soil solarization on microflora with special reference to *F. solani* in mango nurseries. Plant Disease Research 19(2):144-150.
- Sharma S N and Chandel S S 2003 Screening of biocontrol agents *in vitro* against *Fusarium oxysporum* f.sp. *gladioli* and their mass multiplication on different organic substrates. Plant Disease Research, Ludhiana 18(2) : 135-138.
- Sharma S, Pal R, Gupta P P and Kaushik J C 2004 Management studies on root of *Prosopis cineraria* caused by *Fusarium solani*. Journal of Tropical Forest Science 16(1): 71-77.
- Sheela J, Sivaprakasam K and Seetharaman K 1995 Biological control of *Fusarium* wilt of egg plant. Madras Agricultural Journal 82: 199-201.
- * Sidovora E E 1980 Biologicheskie metodi barbis Fitopatogennium V Khuge. Itologinaku U Teknuku Zashita Rastenui. T.G.MIZD. VO. VINITOAN SSSR, 116-154 (In Russian).
- Singh N I, Devi R K T and Devi P P 2000 Effect of fungicides on growth and sporulation of *Fusarium solani*. Indian Phytopathology 53(3) : 327-328.
- Singh R N, Upadhyaya J P and Ojha K L 1993 Management of chickpea wilt by fungicides and *Gliocladium*. Journal of Applied Biology 3: 46-51.

- Singh R S, Jindal A, Singh D and Singh T 1995 Selection of *Trichoderma* isolates against common fungicides for their use in integrated plant disease management. Indian journal of Mycology and Plant Pathology 25(1&2) : 127.
- Singh R S, Mann S S, Jindal A and Kaur 1994 Variation of isolates of *Trichoderma harzianum* from Punjab soils. Plant Disease Research 10: 10-15.
- Singh R S, Sing S and Kang M S 1992 Effect of culture filtrate of *Trichoderma* species on mycelial growth and Sclerotia formation of *Rhizoctonia solani* causing black scurf of potato. Indian Journal of Mycology and Plant Pathology. 22: 90.
- Singh S 2001 Citrus. International book distributing company. Lucknow pp. 3.
- Sinha and Prasad 1989 Chemical management of *Alternaria* blight of Cauliflower seed crop. Indian Journal of Mycology and Plant Pathology 19(2) : 204-205.
- Sitaramaiah K and Parvathi Devi G 1993 Antagonistic effect of *Trichoderma harzianum* on betelvine isolates of *Fusarium solani* and *Sclerotium rolfsii* in vitro. In Advance in betelvine production technology (ed: RK Chourasia M L Nayak and M N Khare) pp 68-69.
- Sivakumar G, Sharma R C and Rai S N 2000 Biocontrol of banded leaf and sheath blight of maize by peat based *Pseudomonas fluorescens* formulation. Indian Phytopathology 53(2): 190-192.
- Sivaprakasam K 1990 Soil amendment for crop disease management in P Vidhyasekaran (ed.) Basic Research for Crop Disease Management Daya publishing house, New Delhi.
- Souza Alvira D, Jayanta K, Roy Bibekananda mahanty and Dasgupta B 2001 Screening of *Trichoderma harzianum* against major fungal pathogens of betelvine. Indian Phytopathology 54 (3): 340-345.
- Sreeramulu K R, Onkarappa T and Narayanaswamy H 1998 Biocontrol of damping-off and black shank disease in tobacco nursery. Tobacco Research 24(1): 1-4.
- Srinivasulu B, Aruna K and Rao D V R 2001 Biocontrol of *Ganoderma* wilt of coconut palm. South Indian Horticulture 49 : 240-242.
- Srivastava V K 2004 *Trichoderma* spp - A boon for better crop health. Pestology Vol. XXVIII (8): 40-45.

- Srivastava A K Ahmed R Kumar S and Sukhadamohandas 2001 Role of VA-Mycorrhiza in the management of wilt disease of guava in the alfisols of Chotanagapur. *Indian Phytopathology* 54(1): 78-81.
- Srivastava H C and Singh B 1954 Seedling trouble in citrus beds and their control. *Science Culture* 20: 93-95.
- Stapleton J J and Devay J E 1987 Effect of combining soil solarization with certain nematicides on target and non-target organism and plant growth. *Annual Applied Nematology* 1: 107-112
- Strauss J and Labuschagne N 1993 In vitro and in vivo effect of fungicides on *Fusarium solani* associated with citrus roots. *Applied Plant Science* 7(2) : 56-59.
- Subramanian C V 1971 Hypomycetes. Indian Council of Agricultural Research, New Delhi pp. 930.
- Suhag L S 1976 Observations on guava decline in Haryana and its control. *Pesticides* 10: 42-44.
- Thakare A R, Raut B T, Chavan P N, Tini Pillai and Pillai T 2002 Rhizosphere microflora of groundnut in mulched and non-mulched conditions. *Karnataka Journal of Agricultural Sciences* 15(1): 163-166.
- Thangavelu R, Palaniswami A and Velazhahan R 2004 Mass production of *Trichoderma harzianum* for managing *Fusarium* wilt of banana. *Agricultural Ecosystems and Environment* 103(1) : 259-263.
- Thind S K, Thind T S and Arora J K 2004 Prevalence of citrus *Phytophthora* in Kinnow orchards and its fungicidal management. *Plant Disease Research* 19(1): 31-35.
- Thompson A H, Phillips A J L and Nel E 1995 *Phytophthora* and *Pythium* associated with feeder root rot of citrus in the Transvaal province of South Africa. *Journal of Phytopathology* 143: 37-41.
- * Tilak K V B R, Saxena A K and Dwivedi A K 2004 Population dynamics of rhizosphere microflora of mycorrhizal and nonmycorrhizal barley (*Hordeum vulgare*) in organic amended soils. *Acta Botanica Hungarica* 46(3) : 417-421.
- * Timmer L K L and Leyder R F 1978 Relationship of seedbed fertilization and formalin to infection of sour orange seedlings by mycorrhizal fungi and *Phytophthora parasitica*. *Journal of American Society of Horticultural Science* 4 : 537-541.

Timmer L W, Garnsey S M, Grimm G R, Gholi N E E L and Schoulties C L 1979
Wilt and dieback of Mexican lime caused by *Fusarium oxysporum*.
Phytopathology 69(7): 730-733.

Tripathi M and Johri B N 2002 In vitro antagonistic potential of fluorescent
pseudomonas and control of sheath blight of maize caused by *Rhizoctonia*
solani. *Indian Journal of Microbiology* 42: 207-214.

* Troutman J L, Mc Donald H and Matejka J C 1979 Decline of citrus on rough
lemon rootstocks in Arizona. University of Arizona USA, pp. 896-897.

Upadhyay J P and Mukhopadhyay A M 1986 Biological control of *Sclerotium*
rolfsii by *Trichoderma harzianum* in sugarbeet. *Tropical Pest Management*
32: 215-220.

Ushamalini C, Rajappan K and Kousalya Gandadharan 1997 Control of cowpea
wilt by non-chemical means. *Plant Disease Research* 12(2): 122-129.

* Vanderweyen and Serrhini 1981 Citrus dry root-rot in Morocco. *Comptes*
Rendus-desseances-de-l Academic-d Agriculture-de France 67: 1492-95.

Vanitha S and Suresh M 2002 Management of collar rot of brinjal (*Sclerotium*
rolfsii) by non-chemical methods. *South Indian Horticulture* 50(4-6) :
602-606.

* Vazquez D E and Ramallonilda E 1969 El control of de damping off en
almacigos de citrus (Control of damping off in citrus seed beds). *Revta ind.*
agric. Tucuman 46 : 87-96.

Venkateswar Rao K 1998 An integrated approach for management of sesamum
stem and root rot caused by *Macrophomina phaseolina* (Tassi) Goid. M Sc
(Ag.) Thesis submitted to Acharya N G Ranga Agricultural University,
Hyderabad.

Verma K S and Navtej Singh 1999 Occurrence and control of dry root-rot of citrus
seedlings. *Plant Disease Research* 14: 31-34.

Vijaya Krishna Kumar K 1997 Integrated approach for management of damping-
off disease in tomato caused by *Pythium aphanidermatum* (Edson) MSc
(Ag.), ANGRAU, Hyderabad.

Vijaya Kumar B 2001 Studies on Dry root rot disease acidlime (*Citrus aurantifolia*
Swingle) Nursery M.Sc (Ag.) thesis submitted to the Acharya N.G. Ranga
Agricultural University, Hyderabad.

- Viswanath 2003 Studies on dry root rot disease of citrus : Molecular characterisation of potential native fungal antagonist and biological control. M.Sc.(Ag.) Thesis, ANGRAU, Hyderabad.
- Wahid A, Javed M S and Idress M 1995 chemical control of *Fusarium* root-rot wilt and collar rot of soyabean (*Glycine max* L). Pakistan Journal of Phytopathology 7: 21-24.
- Wehner F C, Hantie Combrink and Kotze J M 1986 Root pathogens in South African citrus nurseries 19: 107-108.
- Weideman H and Wehner F C 1993 Greenhouse evaluation of *Trichoderma harzianum* and *Fusarium oxysporum* for biological control of citrus root rot in soils naturally and artificially infested with *Phytophthora nicotianae*. Phytophylactica 25 : 101-105.
- Weindling R 1932 *Trichoderma lignorum* as a parasite of other fungi. Phytopathology 22 : 837-845.
- * Whipps J M, Budge S P, Elad Y (ed.), Freeman S (ed.) and Monte E 2001 Integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using the mycoparasite *Coniothyrium minitans*. Bulletin OILB SROP 24(3): 217-220.
- Whipps M J and Lumsden R D 1991 Biological control of *Pythium* species. Biocontrol Science and Technology 1: 80-90.
- Whiteside J O, Gadney S M and Timmer W 1989 Compendium of citrus diseases APS press, APS pp 14-15.
- Widmer T L, Graham J H and Mitchell D J 1998 Composted municipal waste reduces infection of citrus seedlings by *Phytophthora nicotianae*. Plant Disease 82(6):683-688.
- Wutscher H K and Bistline F W 1988 Performance of Hamlin orange of 30 citrus rootstocks in Southern Florida. Journal of American Society of Horticultural Science 113 : 493-497.
- * Xavier L J C, Boyetchko S M, Khachatourians G G and Arora D K 2002 Arbuscular mycorrhizal fungi as biostimulants and bioprotectants of crops. Applied Mycology and Biotechnology 2: 311-340.
- Zakaria M A, Lockwood J L and Filonow A B 1980 Reduction in *Fusarium* population density in soil by Volatile degradation products of oilseed meal amendments. Phytopathology 70 : 495-499.

-
- * Ziedan E H A and Farrag E S H 2002 Biological control of root rot disease on mandarin by antagonistic strain of *Bacillus megatherium*. Annals of Agricultural Science Cairo 47 (3): 1021-1031.

The pattern of 'Literature cited' presented above is in accordance with the 'Guidelines for thesis presentation for Acharya N.G Ranga Agricultural University, Hyderabad.

- * Original not seen

APPENDICES

APPENDIX A

PROFORMA FOR ROOT ROT DISEASE SURVEY IN ACID LIME

Date of Survey :

1. Name of the grower :

Location : Mandal : District:

2. Details of the orchard :

a) Extent (Acres/No. of Plants):

b) Source of plant material :

c) Age of the crop :

d) Variety :

e) Seedling/budling :

f) Rootstock :

g) Soil type :

h) Soil depth :

i) Water source (well/bore/
canal) :

j) Water quality :

k) Method of irrigation :

l) Optimum/limited irrigation :

m) Fertilisers used :

n) Pesticides used :

3. No. of trees examined :

4. No. of root rot affected trees :

5. Percentage affected trees :

APPENDIX B

CALCULATION OF POISSON DISTRIBUTION

No.of colonies	No. of Petriplates
36	4
39	3
42	3
43	3
45	3
47	3
48	4
50	3
350	26

$$36 \times 4 + 39 \times 3 + 42 \times 3 + 43 \times 3 + 45 \times 3 + 47 \times 3 + 48 \times 4 + 50 \times 3$$

$$= 144 + 117 + 126 + 129 + 135 + 188 + 144 + 150$$

$$= \frac{1133}{26} = 43.57$$

Standard deviation (S.D) was calculated for all the observations and it was 7.51, 2.82, 0.42, 0.09, 0.16, 0.59, 2.07, 6.13 = 20.79

$$\text{Standard deviation (S.D)} = \frac{20.79}{25} = 0.8316$$

$$\text{Variance : } \sqrt{0.8316} = 0.91192106$$

$$\begin{aligned} \text{Critical difference (C.D)} &= \frac{(0.91192106)^2}{43.57} = \frac{0.8316}{43.57} \\ &= 0.019087 \end{aligned}$$

So critical difference < 1 = So it follows poisson distribution.

Variance < Mean population

$$0.912 < 43.57$$

APPENDIX C

**METEOROLOGICAL DATA AT AICRP ON TROPICAL FRUITS (CITRUS),
TIRUPATI FOR THE MONTH OF MAY, 2003**

Date	Maximum temperature	Minimum temperature	RH ₁	RH ₂	Rainfall	Sunshine hours
1-5-2003	38.0	25.0	61.0	43.0	--	9.0
2-5-2003	37.5	26.0	71.0	32.0	--	10.2
3-5-2003	40.0	26.0	64.0	28.0	--	10.3
4-5-2003	41.5	26.6	49.0	27.0	--	10.5
5-5-2003	42.0	27.2	53.0	28.0	--	10.0
6-5-2003	38.5	27.0	81.0	29.0	--	1.5
7-5-2003	43.0	26.8	56.0	25.0	--	8.2
8-5-2003	41.0	28.5	72.0	22.0	--	5.8
9-5-2003	39.5	27.2	60.0	19.0	--	7.4
10-5-2003	40.0	26.0	51.0	17.0	--	8.0
11-5-2003	39.0	25.8	64.0	23.0	--	7.8
12-5-2003	37.0	27.0	67.0	28.0	--	8.0
13-5-2003	35.0	26.2	56.0	25.0	--	0.0
14-5-2003	37.5	26.1	84.0	28.0	--	0.0
15-5-2003	39.5	27.5	47.0	23.0	--	0.6
16-5-2003	40.0	28.0	46.0	24.0	--	0.0
17-5-2003	42.0	27.5	50.0	30.0	--	7.0
18-5-2003	42.5	29.0	40.0	34.0	--	9.0
19-5-2003	41.0	29.5	48.0	40.0	--	8.4
20-5-2003	43.5	30.0	43.0	22.0	13.2	8.4
21-5-2003	44.0	29.5	45.0	21.0	22.0	8.0
22-5-2003	44.0	27.5	48.0	30.0	--	9.2
23-5-2003	43.0	27.5	45.0	32.0	--	7.2
24-5-2003	41.5	27.0	47.0	44.0	--	6.4
25-5-2003	42.0	28.5	43.0	44.0	--	7.0
26-5-2003	40.5	24.0	42.0	25.0	--	4.6
27-5-2003	42.0	21.5	55.0	29.0	--	9.8
28-5-2003	42.5	22.4	46.0	31.0	--	9.4
29-5-2003	42.2	23.4	49.0	26.0	-	10.0
30-5-2003	43.0	25.5	48.0	27.0	--	8.0
31-5-2003	38.5	26.4	44.0	29.0	--	0.0
Total	1221.7	799.7	1583	802	35.2	201.7
Mean	39.4	26.64	54.0	25.87	--	6.5

**METEOROLOGICAL DATA AT AICRP ON TROPICAL FRUITS (CITRUS),
TIRUPATI FOR THE MONTH OF JUNE, 2003**

Date	Maximum temperature	Minimum temperature	RH ₁	RH ₂	Rainfall	Sunshine hours
1-6-2003	42.5	27.2	81.0	36.0	---	6.0
2-6-2003	41.0	27.5	61.0	34.0	--	5.0
3-6-2003	38.5	27.0	53.0	33.0	--	1.6
4-6-2003	42.0	26.2	55.0	26.0	--	8.8
5-6-2003	41.5	29.0	46.0	25.0	--	9.0
6-6-2003	42.0	27.4	46.0	28.0	--	10.0
7-6-2003	42.0	28.0	47.0	28.0	--	8.2
8-6-2003	36.0	28.5	63.0	32.0	45.2	2.4
9-6-2003	41.0	29.0	38.0	54.0	--	8.0
10-6-2003	40.0	26.0	72.0	48.0	--	8.8
11-6-2003	42.0	26.5	57.0	41.0	6.0	8.2
12-6-2003	38.5	27.0	57.0	34.0	--	5.2
13-6-2003	35.0	24.0	78.0	56.0	--	0.0
14-6-2003	33.0	22.0	83.0	60.0	--	0.0
15-6-2003	35.5	22.0	77.0	48.0	33.0	4.0
16-6-2003	36.5	26.0	74.0	46.0	1.2	8.6
17-6-2003	38.5	25.5	62.0	38.0	--	6.0
18-6-2003	36.5	27.5	68.0	41.0	--	0.0
19-6-2003	36.5	26.5	72.0	41.0	--	2.0
20-6-2003	37.5	27.0	56.0	35.0	--	6.0
21-6-2003	37.5	27.8	74.0	37.0	--	3.0
22-6-2003	37.0	27.0	66.0	39.0	--	2.2
23-6-2003	38.0	28.0	68.0	42.0	--	8.0
24-6-2003	36.5	28.5	66.0	38.0	--	1.0
25-6-2003	36.0	25.0	68.0	28.0	4.5	1.0
26-6-2003	36.0	26.0	56.0	34.0	--	5.0
27-6-2003	38.5	25.0	47.0	40.0	--	7.2
28-6-2003	32.5	27.0	56.0	37.0	--	6.0
29-6-2003	33.5	23.0	84.0	54.0	47.0	1.0
30-6-2003	35.0	23.5	71.0	47.0	--	2.0
Total	1173.9	790.6	1902	1180	136.9	173.0
Mean	39.13	26.3	63.4	39.53	5	5.7

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