

**“STUDIES ON COLLAR ROT OF GROUNDNUT  
CAUSED BY *Aspergillus niger* van Tieghem ”**

By

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

MASTER OF SCIENCE IN AGRICULTURE



**DEPARTMENT OF PLANT PATHOLOGY**  
AGRICULTURAL COLLEGE, BAPATLA – 522 101  
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2004

## CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON COLLAR ROT OF GROUNDNUT CAUSED BY *Aspergillus niger* van Tieghem**” submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** in the major field of **PLANT PATHOLOGY** of the Acharya N.G. Ranga Agricultural University, Hyderabad is a record of the bonafide research work carried out by **Mr. T. RAJASEKHARAM** 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 degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

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## DECLARATION

I Mr. **T. RAJASEKHARAM** here by declare that the thesis entitled “**STUDIES ON COLLAR ROT OF GROUNDNUT CAUSED BY *Aspergillus niger* van Tieghem**” submitted to the Acharya N.G. Ranga Agricultural University for the degree of **Master of Science in Agriculture** in the major field of **PLANT PATHOLOGY** is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier.

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Mr. **T. RAJASEKHARAM** has satisfactorily prosecuted the course of research and that the thesis entitled “**STUDIES ON COLLAR ROT OF GROUNDNUT CAUSED BY *Aspergillus niger* van Tieghem**” submitted is the result of original research work and of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any university.

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## LIST OF SYMBOLS AND ABBREVIATIONS

(Source : Plant Pathologist pocket book)

%	:	per cent
°C	:	degrees centigrade
cm	:	centimeter
<i>et al.</i>	:	and others
fig	:	figure
g	:	gram
h	:	hour
i.e	:	that is
kg	:	kilogram
lb	:	pound
m ha	:	million hectare
m t	:	million tonnes
min	:	minutes
ml	:	milli litre
mm	:	milli meter
mg	:	micro gram
µm	:	micro meter
no.	:	number
sp. & spp.	:	species (singular & plural)
cv	:	cultivar
<i>viz.</i> ,	:	namely
w/w	:	weight/weight
M	:	molar
CD	:	Critical difference
SEm	:	Standard error of mean

## ACKNOWLEDGEMENTS

*Accomplishment of this thesis is the result of benevolence of **Almighty**, blessings of my **teachers**, love of my **parents** and motivation of my **friends**.*

*I humbly place on record my respect and profound sense of gratitude to the esteemed Chairman of my Advisory Committee, **Dr. C. Subba Reddy**, Professor, Department of Plant Pathology, Agricultural College, Bapatla for his indefatigable guidance, unstinting attention and constructive criticism which provided commendable encouragement to carry out this work.*

*I am deeply indebted to **Dr. K.V.M. Krishna Murthy**, Professor, Department of Plant Pathology, Agricultural College, Bapatla for his encouragement in conducting research and meticulous reasoning to refine the dissertation to recon with set standards.*

*I extend my sincere thanks to **Dr. V. Ramasubba Rao**, Professor, Department of Entomology, Agricultural College, Bapatla for his valuable help rendered during the course of investigation.*

*It is pride and honour to express my heartfelt thanks and gratitude to the celebrated personality **Dr. M. Subba Rao**, Professor and Head, Department of Plant Pathology, for his sustained help, unfailing cooperation and meticulous guidance to accomplish my study.*

*I sincerely extend my respect and profound gratitude to **Dr. R. Ravindra Babu**, Associate Professor, Department of Plant Pathology, Agricultural College, Bapatla for his convivial exhortation and constant encouragement.*

*I owe respectable regards to the staff members of Department of Statistics and Mathematics, **Dr. V. Srinivasa Rao**, Asst. Professor and **Sri. K. Jayaramaiah**, Asst. Professor for giving worthy suggestions and guidance during the course of the study.*

*I avail the opportunity to express my deep sense of gratitude to **Sri. Krishnam Raju**, Scientist, ARS, Vizianagaram for his unforgettable help during my research work.*

*I am in dearth of words to express my heartfelt feelings to my beloved parents **Sri. T. Kusa Raju** and **Smt. T. Vanajakshi**, brother **Rajesh**, Sister **Indira**, brother-in-law **Ramesh**, wonder kids **Akash naidu**, **Sivaprasad naidu** without whose affection, moral support and persistent encouragement this thesis had not seen the ray of light.*

*Mere words cannot express my feelings to my childhood friends, **Ramesh, Mahesh, Ganesh, Ravi, Radhakrishna, Madhu and Trinath** for their constant help during the course of study.*

*Words fail me in expressing my profound sense of gratitude to my beloved and ever lasting friends **Sharma, Satish<sup>2</sup>, Sridhar, Kumar Raja, Gokhale, Mouli, Raja, Anjaneyulu, Murali, Appari, Sasi, Rajsekhar, Sitaram, Sekhar, Ravikiran, Ratna Babu, Tejeswara Rao, Sankar, Sunil, Krishna, Janardhan, Ranga Rao, Srinivasa Reddy, Sampath, Chandrasekhar** for their untiring help and constant encouragement rendered to me during the entire educational career.*

*I extend my deep felt regards to my genius senior friends **Rajkumar, Kavitha, Bhuvanewari, Prasad, Mohan, Siddaramappa, Mangala** and **Anjali** for their meticulous guidance during the entire period of study.*

*I extend my heartfelt thanks to my beloved senior **Dr. M.S.V. Chalam** , for his help and constant encouragement during my study.*

*I express my immense and whole hearted thanks to my colleague friends, **Suraj, Madhumohan** and my juniors **Srinivasa Rao** and **Srikanth** for their valuable help during my post graduate study.*

*I extend my sincere thanks to **Sri. Abdul Hakeem**, Librarian and other staff of library for their help during my study.*

*I acknowledge **Acharya N. G. Ranga Agricultural University** for providing financial assistance during the course of study.*

*A word of appreciation goes to **Mr. Rohit** for meticulous typing and printing of this thesis in a neat form.*

**Date :**

**(T. RAJASEKHARAM)**

## ABSTRACT

Author : T. RAJASEKHARAM  
Title of the thesis : **STUDIES ON COLLAR ROT OF  
GROUNDNUT CAUSED BY *Aspergillus  
niger* van Tieghem**  
Submitted for the award of : Master of Science in Agriculture  
Department : **PLANT PATHOLOGY**  
Chairman : **Dr. C. SUBBA REDDY**  
University : Acharya N.G. Ranga Agricultural  
University  
Year of Submission : 2004

Groundnut is one of the principal oil seed crops grown in India and is being infected by a number of fungal diseases. Among the various diseases, pre and post-emergence damping off and collar rot are important diseases caused by *Aspergillus niger* van Tieghem. The seeds affected by *A. niger* which were covered by black fungal spore mass rot in soil and fail to germinate. The symptoms of collar rot include covering of collar region of the plant at soil surface with the sporulating fungal mass and seedling infection in the cotyledonary hypocotyls region shortly after emergence.

The field survey conducted in North Coastal Zone of Andhra Pradesh revealed that the disease was more predominant in areas having light textured soils grown with spanish bunch cultivars.

An inoculum level 50 per cent (w/w) of test pathogen (*A. niger*) was found to be the optimum infection threshold level with 66.67 and 53.33 per

cent of pre and post-emergence damping off and collar rot incidence, respectively.

The antagonistic effect of ten isolates of *Trichoderma* spp. were more pronounced in seeded agar method. Of the ten isolates, T<sub>1</sub>, T<sub>2</sub>, T<sub>18</sub>, T<sub>21</sub> and T<sub>40</sub> were found to show more antagonistic effect. When commercial formulations of *Trichoderma* spp. and bacterial antagonists (*Pseudomonas fluorescens* and *Bacillus subtilis*) were compared with the native isolates, *B. subtilis* was found to be more antagonistic against growth of *A. niger* than all other biocontrol agents, but native isolates T<sub>1</sub>, T<sub>2</sub>, T<sub>18</sub>, T<sub>21</sub> and T<sub>40</sub> were more effective than commercial formulation of *T. viride* and *T. harzianum*.

The plant extracts of *Allium sativum* was most effective followed by *Polyalthia longifolia*, *Annona squamosa* and *Pongamia glabra* where as *Ocimum sanctum*, *Piper betle* and *Azadirachta indica* were found least effective. Comparison of the native isolates of *Trichoderma* spp., *P. fluorescens* and *Bacillus subtilis* with the best plant extracts revealed that extracts of *A. sativum* was most effective in checking the growth of *A. niger* followed by *B. subtilis* and T<sub>1</sub> isolate.

Seed treatment with non conventional chemicals significantly reduced the pre and post-emergence damping off and collar rot incidence. Among the non conventional chemicals, Lithium sulphate (10<sup>-3</sup>M), Barium nitrate (10<sup>-4</sup>M), Salicylic acid (10<sup>-5</sup>M) and Gibberillin A<sub>3</sub> (10<sup>-6</sup>M) were found to be more effective.

## CHAPTER – I

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.), one of the important oil seed crops of the world is believed to be a native of South America. It was introduced to East Asia from South America and from there to India by Spaniards during the 19<sup>th</sup> Century. India gradually became a major groundnut producing country of the world with in a span of 5-6 decades. This crop assumed great significance in the present day economy.

India ranks second in the world production of groundnut with an area of 7.28 m ha producing 7.85 m t annually (Agri India green pages, 1999-2001). In Andhra Pradesh, it is grown over 16.98 lakh ha with an annual production of 12.50 lakh tonnes (Directorate of Economics and Statistics, Andhra Pradesh, 2003).

Groundnut is attacked by several soilborne pathogens causing diseases like collar rot, charcoal rot, *Sclerotium* wilt, damping off, seed rot and yellow mould which limit the yield considerably. These diseases largely account for the death of the seedlings. Of these, pre and post-emergence damping off and collar rot caused by *Aspergillus niger* van Teighem was the most prevalent disease causing seedling losses upto 50 per cent (Chohan, 1969; Mc Donald, 1978).

In North-Coastal Zone of Andhra Pradesh, collar rot disease is one of the major problems in traditional groundnut growing areas in recent years and no precise information is available on the extent of disease incidence in this region. Though wide group of fungicides are being suggested for use, these fungicides cause hazardous effect on environment, crop ecology, soil biology and human health (Papavizas, 1985). Hence, an attempt was made in the present study to evolve ecologically safe and environmentally friendly methods of disease management with the following objectives.

1. To study the collar rot of groundnut disease incidence in North-Coastal Zone of Andhra Pradesh.
2. To study the antagonistic effects of biocontrol agents.
3. To study the effect of plant extracts on growth of *Aspergillus niger*.
4. To study the effect of non conventional chemicals against the groundnut collar rot.

## CHAPTER - II

### REVIEW OF LITERATURE

Screening of literature revealed that very meager information is available on collar rot of groundnut from India as well as abroad and the same is presented along with the information on other crops in this chapter so as to understand the work carried out on this subject . The available literature on groundnut collar rot has been reviewed and presented under the following sub-heads.

#### **2.1 DISTRIBUTION OF GROUNDNUT COLLAR ROT DISEASE**

Collar rot or seedling blight is present in all most all the groundnut growing areas of the World. The disease was first reported from Java (Jochems, 1926) and subsequently from Australia (Morwood 1945, 1946,1953; Purss, 1960), India (Jain and Nema 1952; Kulashreshtra *et al.*, 1964; Ravindranath and Thobbi, 1967), East Africa (Gibson, 1953), USA (Jackson, 1962, His, 1966), Israel (Joffee, 1972) and Nigeria (Mc Donald, 1978). In India the post-emergence seedling disease in groundnut caused by *Aspergillus niger* van (Teighem) was found to be prevalent in the states of Andhra Pradesh (Kulashreshta *et al.*,1964; Ravindranath and Thobbi , 1967; Rao *et al.*, 1997; Raju, 2001), Punjab (Chohan, 1965; Gupta and Chohan, 1970) and Rajastan (Sharma *et al.*,1973).

## **2.2 SYMPTOMATOLOGY**

### **2.2.1 Symptoms of Pre-emergence Seed rot Caused by *Aspergillus niger* van Tieghem**

Morwood (1945) reported that the rot symptoms first appeared on the seed and as a result seed failed to germinate. Nema *et al.* (1955) reported that collar rot disease of groundnut appeared in two phases as pre-emergence and post-emergence. Mercer (1978) found that seeds failed to germinate due to pre-emergence death of groundnut seeds. Subrahmanayam *et al.* (1992) observed that pre-emergence seed rot infected seeds failed to germinate and were covered with masses of black conidia which imparted them a sooty appearance.

### **2.2.2 Symptoms of Post-emergence Seedling Disease and Collar Rot Caused by *Aspergillus niger***

According to Morwood (1945) crown rot and seedling blight appeared as a rot in the initial stage. The symptoms appeared first on the seed and then on the leaves and finally on the stem. The disease resulted in a dry shredded effect of wet and slimy rotting nature on seedling, besides breakdown of stem at different stages starting from germination to maturity. Collar rot affected plants showed a general wilting besides rotting of tissues just below the ground level. The affected portion turned dark, shunken and

shredded and later covered by black masses of spores of the pathogen.

Jain and Nema (1952) reported that the first indication of the disease was the appearance of a circular, brownish spot on the cotyledons. The discolored area rapidly became soft and rotten and spread on to the stems and hypocotyls which also become yellow, soft, rotten and collapse. The affected stems become shredded. Greyish white mycelium and black fruitifications of the pathogen appeared on the surface of the affected parts. Gibson (1953) found that the peak death rate occurred about the 17<sup>th</sup> day after planting and that the period of dying of emerged plants extended from about 10 to 30 days due to collar rot incidence.

Wardsworth and Melouk (1978) reported that the most obvious symptom was the sudden wilting of young plants. Infection of seedlings commonly occurred in the cotyledon and hypocotyl regions shortly after germination and as the disease progressed rapidly. The plants may die within 30 days after sowing. If plants survive, lesions are characterized by shredded tissues and usually the diseased areas were profusely covered with dark masses of mycelium, conidiophores and conidia. They also observed a major symptom of crown rot infection in older plants, with dead central stem, often broken at the soil line in Spanish peanuts.

Sharma *et al.* (1973) found that crown rot was the most serious disease of groundnut in early stage of growth and they further indicated that the emerged seedlings were affected at collar region causing yellowing of lower leaves, slow death due to blighting effect on the shoot, finally leading to death of crown portion.

Suzui and Makino (1980) reported that collar rot was characterized by wilting symptoms, affecting one or more branches and led to death of seedlings followed by rotting of hypocotyl. The affected hypocotyl showed a yellow brown lesion, which progressed both towards upper branches and root system and turned black latter. In some cases profuse sporulation of *A. niger* was observed on the infected stem portion .

The first symptom observed on seedlings was rapid desiccation of the entire plant, as the infection progress, affected tissue was covered by sporulating fungus at the soil surface. The entire collar region become dark brown and shredded. Seedling infection commonly occurred in the cotyledon and hypocotyl region shortly after emergence. The infected areas were water soaked, light brown and covered with black fungal spores (Subrahmanayam *et al.*, 1992).

### **2.3 THE PATHOGEN**

The causal organism for the post-emergence seedling blight was originally reported as *Aspergillus niger* van Tieghem (Jochems, 1926). But, Boedzin (1938) identified it as *A. pulverulentus* (Mc Alpine) Thom which was taxonomically very close to *A. niger* (Raper *et al.*, 1965). The morphology of fungus was described by Raper *et al.* (1965). The conidiophores were described as upright and simple, terminally with a globose swelling, bearing phialides radiating from the entire surface. Conidia were found one celled, globose, light to dark brown, produced basipetally. Mycelium was observed to be hyaline, branched and septate. In India the collar rot disease was caused by both *A. niger* and *A. pulverulentus* (Chohan, 1965, 1969) but the former was predominant (Ravindranath, 1975).

### **2.4 SURVEY ON COLLAR ROT DISEASE INCIDENCE**

Mishra and Ghewande (1983) observed that collar rot of groundnut was prevalent in almost all parts of the country being more during *kharif* than *rabi*.

Mayee (1986) reported that crown rot caused by *A. niger* was an important disease in groundnut which usually occurred in its early period of growth. Savary (1987) conducted a survey on groundnut diseases on the various traditional cropping regions of Ivory coast and observed 2.9 to 19.3 per cent seedling diseases caused by

*A. niger*. Wongkaew *et al.* (1989) conducted a survey on occurrence of groundnut diseases and observed that the seedling blight caused by *A. niger* appeared to be one of the most important diseases found in the irrigated areas. An extent of the 3 to 10 per cent incidence was normally observed in most of the fields.

Gray *et al.* (1990) conducted a survey in the Bay region of Somalia in East Africa and identified that the collar rot disease of groundnut caused by *A. niger* was most prevalent. Shim *et al.* (1996) collected ungerminated groundnut seeds and seedlings from the different locations and *A. niger* was found to be the most frequently associated with seeds and seedlings of groundnut.

Rao *et al.* (1997) conducted a survey on occurrence of seedling diseases of groundnut in Andhra Pradesh. Among the seedling diseases recorded collar rot caused by *A. niger* was common one and mean disease incidence of collar rot ranged from 1.0 to 10.0 per cent.

## **2.5 BIOLOGICAL CONTROL**

The potential use of fungal antagonists as biocontrol agents of plant diseases was suggested more than 75 years ago by Weindling (1932) who was the first to demonstrate the antagonistic activity of *Trichoderma* spp. against *Rhizoctonia solani*.

In recent years *Trichoderma* spp. have proved to be potential biocontrol agents in controlling several soil borne diseases under

both green house and field conditions (Hardar *et al.*, 1979; Sidorova, 1980; Chet and Baker, 1980, 1981; Elad *et al.*, 1981; Seikatov, 1982; Cook and Baker, 1983). *Trichoderma*, an antagonistic fungi inhibits a range of test pathogens by a different mechanisms of antagonism such as by producing non-volatile antibiotics (Dennis and Webster, 1971a), volatile antibiotics (Dennis and Webster, 1971b) and by coiling around hyphae of different plant pathogens (Dennis and Webster, 1971c).

#### **2.5.1 Antagonistic Activity of *Trichoderma* spp. Against *Aspergillus* spp. *in vitro* and *in vivo***

Chohan and Singh (1973) observed *Trichoderma polysporum* was parasitic over the colonies of *A. niger*. Rai *et al.* (1980) showed parasitization of *Aspergillus niger*, *A. candidum* and *A. luchuesis* by *T. viride* which showed coiling, penetration and hyphal growth inside the conidiophores of *Aspergillus* spp. and reported volatile and non volatile metabolites of *T. viride* were inhibitory to radial growth of all *Aspergillus* spp. tested.

Lashin *et al.* (1989) found that linear growth of three isolates of *A. niger* (A1, A2 and A3) were greatly reduced in the presence of *T. harzianum in vitro* and disease incidence was low in soils treated with *T. harzianum* at seedling and vegetative growth stages.

Hussain and Lane (1992) reported broad antifungal activity of active preparations of *T. viride*, *Ampelomyces quisqualis*, *Gliocladium roseum* towards *Penicillium*, *Aspergillus*, *Botrytis* and Yeasts.

Chowdhury (1992) reported that *T. viride* was found to be effective in inhibiting the growth of *A. flavus* isolated from groundnut seeds to an extent of 72.5 per cent *in vitro*. Pandey *et al.* (1993) found that *A. pullulans*, *T. harzianum* were found to be strongly antagonistic to *A. niger*.

Calistru *et al.* (1997) reported the aggressive nature of *Trichoderma* spp. against *A. flavus* was due to liberation of cellular amylolytic, pectinolytic, proteolytic and cellulolytic enzymes. Dasgupta and Raj (1998) reported that *T. harzianum* inhibited the growth of two virulent isolates of *A. niger* by 63 and 58.1 per cent, respectively, 72 h after inoculation in dual culture.

Prabhu and Urs (1998) studied the antagonistic activity of six isolates of *Trichoderma* against *A. niger* *in vitro* and in pot culture experiments. The results indicated that there was a significant reduction in seed rot and collar rot of groundnut to an extent of 13.5 and 26 per cent, respectively.

Parakhia *et al.* (1998) found that biocontrol agents *Bacillus* spp., *Pseudomonas fluorescens*, *T. harzianum* were moderately

effective as compared to fungicides against the collar rot infection in groundnut.

Desai *et al.* (2000) characterized 26 isolates of *Trichoderma* spp. for biocontrol potential against *A. flavus* in dual culture technique and observed growth inhibition of *A. flavus* by production of non volatile antibiotics by *Trichoderma* isolates. A total of 16 *Trichoderma* isolates were isolated from rhizosphere of groundnut plants and screened for their antagonistic activity against *A. niger* by dual culture technique and found that *Trichoderma* isolates were effective in reducing the pre-emergence rotting both when applied as seed treatment and as soil amendment compared to control (Kishore *et al.*, 2001).

Srilakshmi *et al.* (2001) studied the antagonistic activity of different *Trichoderma* spp. isolated from the rhizosphere of groundnut. Out of the 212 isolates tested, 145 were antagonistic to *A. flavus* (Af 11-4). Among these, 39 isolates showed clear inhibition zone against Af 11-4 strain.

Raju and Krishnamurthy (2002) reported that bio-control agents *T. viride* and *T. harzianum* reduced the growth of *A. niger* to the extent of 51.07 and 46.41 per cent, respectively when compared to the control in *in vitro*.

Subbiah and Indra (2003) reported that *T. viride* caused maximum percentage growth inhibition of *A. niger* (52.06%) followed by *T. harzianum* (49.48%) *invitro*. Thirumala Rao and Sitaramaiah (2000) reported that *T. koningii* was the most effective antagonist to *A. niger* which inhibited growth by 46.4 per cent followed by *T. viride* (42.9 %) where as *T. hamatum* recorded the least growth inhibition (27.3%).

### **2.5.2 Bacterial Antagonists Against the *Aspergillus* spp.**

Narain and Mohanty (1983) reported three bacterial antagonists, *Xanthomonas campestris* pv *oryzae* (YB), *Bacillus subtilis* (WB and An 771) inhibited the growth of *Aspergillus niger*, *A. flavus*, *Fusarium* spp., *Fusarium oxysporum* and *Colletotrichum* spp. Among the bacterial antagonists, AN 771 and YB most effectively inhibited the growth of *A. niger*.

Podile and Prakash (1996) found that *Bacillus subtilis* AF1 suppressed *A. niger* growth to an extent of 90 per cent and bacterial cells multiplied *in situ* and colonized the mycelial surface. Growth of AF1 resulted in damage to the cell walls followed by lysis.

Sheela *et al.* (1998) reported that soil application of *Pseudomonas fluorescens* recorded the lowest collar rot incidence (23.33%) in groundnut crop.

Dileep *et al.* (1999) studied the effect of fluorescent pseudomonad strains FPC 32 and FPO 4 against collar rot fungi, *A. niger*. The two strains FPC 32 and FPO 4 showed *in vitro* antibiosis and protected against collar rot of peanut *in vivo*.

Sheela and Packiaraj (2000) showed that seed treatment with *Pseudomonas fluorescens* along with soil application of neem cake was found to be the best in reducing the collar rot incidence in groundnut crop to the extent of 6.63% as against 18.77% in control.

## **2.6 EFFECT OF PLANT EXTRACTS ON GROWTH OF *Aspergillus* spp. AND OTHER FUNGI *in vitro*.**

Chakravarthy and Pariya (1977) reported total inhibition of germination of *Sclerotium rolfsii*, *Alternaria tenuis* and *Aspergillus niger* by root and leaf extracts of *Achyranthes aspera*. Narian and Satapathy (1978) reported antifungal activity of leaf, flower, stem and root extracts of *Vinca rosea* against *Helminthosporium nodulosum*, *Sclerotium rolfsii*, *Pestalotia* spp, *Fusarium oxysporum* and *Aspergillus niger* from various crops.

Guerin and Raveillera (1984) showed that the extracts of *Ruscus aculeatus*, *Illium verum*, *Hibiscus sabdariffa*, *Rhamnus frangula* and *Tamarindus indica* inhibited the growth and spore germination in many fungi tested (*Candida albicans*, *Aspergillus niger*, *A. fumigatus*, *Trichophyton* spp. and *Fusarium oxysporum*).

Saxena and Tripathi (1985) reported that leaves of *Lantana camara* were effective in inhibiting spore germination of *Aspergillus fumigatus*, *A. niger*, *Mucor mucedo* and *Alternaria alternata*.

Lokesh *et al.* (1986) reported that extracts of *Parthenium hysterophorus*, *Thuja orientalis*, *Eucalyptus citridora* and *Melia azadirachta* inhibited the growth of *A. flavus*. Navrekhar and Patil (1986) reported inhibition of spore germination of *Alternaria* spp., *Aspergillus* spp. and *Fusarium* spp. and sclerotial germination of *Sclerotium* spp. by *Allium sativum* at low concentration (5%) followed by *A. cepa*, *Ocimum sanctum*, *Rosa* spp. and *Nerium* spp.

Tiwari *et al.* (1987) reported that leaf extracts of *Aegle marmelos* and *Eupatorium capillofolium* completely inhibited the mycelial growth of *Penicillium oxalicum* and *A. flavus*.

The essential oil of *Ocimum basilicum* at 1.5 ml/L completely inhibited the mycelial growth of 22 species including *A. flavus* and *A. parasiticum* (Dube *et al.*, 1989).

Lakshmanan and Mohan (1989) reported that the water extracts of garlic, *Bougainvillea* and *Azadirachta* significantly inhibited the mycelial growth of *Thanatephorus cucumeris*. Rizki *et al.* (1989) tested 32 plant extracts against *A. niger*, *A. flavus* and

*Penicillium citrivum*. Of the plant extracts tested, only 11 species showed antifungal activity.

Sinha and Saxena (1990) reported that latex of *Euphorbia hirta* effectively inhibited the growth of *Aspergillus niger*. Yasmeen and Saxena (1990) reported that different extracts of fern, *Adiantum caudatum*, *Diplazium esculentum* and *Pteris vittata* reduced the growth of *Alternaria brassicola* and *Aspergillus niger*. Rhizome extracts were found more toxic than leaf extracts.

Bansal and Sobti (1990) reported that seed treatment with neem extract and turmeric powder at 2.0 per cent concentration effectively checked the collar rot and crown rot of groundnut and these treatments were on par with thiram (0.3%) and dithane M-45 (0.2%) in reducing the incidence of both species of *Aspergillus*.

Mishra *et al.* (1992) studied effect of plant extracts of *Cinnamomum zylanicum*, *Crysentia curieta*, *Psidium guava*, *Alangium salvinofolicum*, *Strychnos potatorum*, *Strychnos nuxvomica*, *Thuja orientalis* and *Laserstroemia parviflora* on six fungal pathogens of rice *viz.*, *Pyricularia oryzae*, *Rhizoctonia* spp, *Fusarium*, *Curvularia*, *A. niger*, *A. flavus* and the results indicated that the aqueous extracts of *P. guava*, *S. potatorum*, *T. orientalis* and *L. parviflora* exhibited greater fungitoxicity than ethanolic extracts.

Mishra and Tewari (1992) found that ethanolic extract and essential oil from the leaf of *Polyalthia longifolia* effectively reduced the growth of test fungi viz., *Pyricularia oryzae*, *Rhizoctonia solani*, *Fusarium moniliforme*, *Aspergillus niger* and *Curvularia lunata*. Ethanolic extract besides being more effective, possessed broad spectrum fungitoxicity than essential oil.

Singh *et al.* (1993) reported that the aqueous leaf extracts of some medicinal plants were effective against *A. flavus*. Among them, the leaf extracts *Azadirachta indica* and *Ocimum sanctum* were found to be most effective.

Mahapatra and Tewari (1994) tested ethanol and essential oil extracts of *Ocimum sanctum* against *Aspergillus niger* and *A. flavus*. Ethanolic and essential oil extract treated seed of groundnut in either 0.05 or 0.1% concentrations and carbendazim 0.1% restricted the infection of both test pathogens to 1-7% over the control (65.7 – 63.4%).

Arun *et al.* (1995) reported that the extracts of *Bignonia* leaves and garlic bulbs suppressed radial growth of *A. niger*. Pai and Platt (1995) reported that garlic extracts worked effectively against *A. niger*. Sobti *et al.* (1995) tested inhibitory effect of *Azadirachta indica*, *Polyalthia longifolia* and *Ocimum gratissimum* on *Aspergillus niger*, *Macrophomina phaseolina* and *Aspergillus*

*flavus* and concluded that *Ployalthia longifolia* extract was almost as effective as thiram (0.3%) in controlling the test fungi.

Jatisatiener *et al.* (1996) reported that dried powder of *Fugeria caryophylla*, *Acorus calanus*, *Illium verum*, *Gloriosa superba*, *Mammea siaensis*, *Stemona tuberosa* and *Piper retrofractum* in potato dextrose agar medium were found effective on *Fusarium* spp., *Colletotrichum* spp., *Alternaria* spp. and *Aspergillus* spp.

Pan and Deb (1997) studied the effect of different plant extracts *in vitro* and reported that *Acacia nilotica* bark extract effectively inhibited the growth of *A. niger*

Sharma (1998) found that *Trichoderma harzianum*, *T. viride*, *Azadirachta indica* and *Agave americana* were most effective in reducing mycelial growth of *Fusarium oxysporum*, *Fusarium oxysporum zingiberi* and *Pythium aphanidermatum* causing yellow and rhizome rot of ginger, respectively.

Radha *et al.* (1998) tested the antifungal properties of garlic (*Allium sativum* L.) extracts, extracted with different solvents against *Colletotrichum falcatum*. Acetone and methanolic extracts were found to be significantly effective in inhibiting the growth of test fungi.

Yin *et al.* (1998) reported inhibitory effect of extracts of garlic bulbs, green garlic and green onion on the growth of *A. niger* and *A. flavus*. Concha *et al.* (1998) concluded that Tea tree oil was inhibitory to all nine isolates of *A. niger* tested.

Onuegbu (1999) showed that there was no significant difference between treatments of 100 ppm dithane M-45, 1% Clorox and 20% leaf extracts of *Vernonia amygdalina* in control of the mould growth of mung bean (*Vigna radiata*) caused by *Aspergillus niger* and *A. flavus*.

## **2.7 COMPARISION OF PLANT EXTRACTS WITH BIOCONTROL AGENTS**

Humanthegowda and Adiver (2001) reported that extracts of *Parthenium hystriophyrus* and *Polyalthia longifolia* were effectively inhibited the growth of *Sclerotium rolfsii* causing stem rot of groundnut when compared to *T. harzianum*.

Jacob *et al.* (1988) evaluated fungal antagonists and leaf extracts *Invitro* against *Pythium aphanidermatum*. The results revealed that *Eucalyptus teratocornis* and *Emelia conchifolia* effectively inhibited the growth of *P. aphanidermatum* which were on par with *T. harzianum* and *T. viride*.

Sharma *et al.* (1998) screened various parts of the plant of *Cassia italica* and *C. siamea* extracted in petroleum ether, benzene,

chloroform, alcohol and water against *A. niger*, *A. flavus*, *Fusarium moniliforme* and *Curvularia lunata*. The alcohol and sequential extracts of plant parts inhibited growth of almost all test fungi.

## **2.8 EFFECT OF NON CONVENTIONAL CHEMICALS ON VARIOUS DISEASES CAUSED BY PLANT PATHOGENS.**

Giri and Sinha (1983) studied the effects of different heavy metal salts at different concentrations on rice seedling against brown spot infection and observed that great and durable protection was obtained with wet seed treatment by Ferric chloride and Nickel nitrate and were most effective in reducing the disease incidence. Where as, Mercuric chloride was proved to be the least effective.

A group of non conventional chemicals were tested by Chowdhury (1995) at different concentrations for their possible effect against *Puccinia arachidis* causing rust infection of groundnut. The results revealed that all the chemicals tested provided significant protection and best results were obtained with Indole Acetic Acid and they also observed that treated plants recorded higher levels of phenolics, protein and oxidase activities as compared to untreated control.

Das Gupta *et al.* (1998) studied the effect of seed soaking with different growth regulators on collar rot disease incidence of

groundnut. The results revealed that all the growth regulators caused a significant reduction in collar rot incidence. Chitosan (0.1%) appear to be more effective in controlling collar rot disease. Non conventional chemicals having potential to accumulate phenolic substances were tested by Dasgupta *et al.* (1999) at different concentrations for their possible effect on defense mechanism in groundnut against collar rot. The results revealed that seeds treated with Mercuric chloride ( $10^{-4}$ M) showed highest reduction (69.01%) of disease incidence followed by Barium sulphate ( $10^{-2}$ M) and Barium nitrate ( $10^{-4}$ M) with 59.15 and 56.34 per cent, respectively.

Chowdhury (2000) tested the effectiveness of four different non conventional chemicals at concentrations of  $10^{-3}$  to  $10^{-6}$  M against *Fusarium* wilt of chickpea. The results revealed that of the chemicals tested, Indole acetic acid, Cycloheximide and Cycocel showed very strong protective effect at relatively low concentrations by reducing wilt symptoms to an extent of 45 to 57 per cent.

Das Gupta *et al.* (2000) tested the effectiveness of some non conventional chemicals at different concentrations in reducing collar rot disease. Under glass house conditions. They observed that all the test chemicals at their respective concentrations significantly reduced the disease incidence as compared with untreated control. Barium sulphate ( $10^{-2}$  M) showed the highest reduction of collar rot incidence (66.5%) followed by Ferric chloride ( $10^{-2}$ M) 58.25 per cent.

## **CHAPTER - III**

### **MATERIALS AND METHODS**

The investigation on “Studies on collar rot of groundnut caused by *Aspergillus niger* van Tieghem were carried out at Department of Plant Pathology, Agricultural College, Bapatla. The general techniques such as preparation of media, isolation of fungi, maintenance of cultures as advocated by Riker and Riker, 1936; Tuite, 1969; Dhingra and Sinclair, 1995 were followed in the present investigation.

#### **3.1 LOCATION AND CLIMATE**

The experimental site located at the Agricultural College, Bapatla which is located at 15° 54<sup>1</sup> N latitude and 80° 30<sup>1</sup> longitude with an altitude of 5.44 above MSL and about 7 km away from the Bay of Bengal.

The weather data recorded during the crop period and the weekly maximum and minimum temperatures ranged from 32.2 to 35.9<sup>0</sup>C and 24.5 to 26.9<sup>0</sup>C, respectively. While the average maximum and minimum temperature during the crop period were 32.5 and 25.4<sup>0</sup>C, respectively. The weekly mean relative humidity ranged from 65.2 to 85.0 per cent with an average of 78.7 per cent.

## **3.2 GLASSWARE AND CHEMICALS**

Borosil, Anumbra and Corning brand glassware were used. Chemicals used in this study were of 'Analytical Grade' (Loba; E Merck, B.D.H. Analar).

### **3.2.1 Cleaning of Glassware**

Glassware was first washed with detergent solution followed by thorough cleaning in tap water. The glassware was kept in chromic acid mixture (potassium dichromate 75g, concentrated sulphuric acid 500 ml and distilled water 500 ml) for a period of 24 hours and later rinsed with distilled water for three times before use.

## **3.3 POTS**

Earthen pots of 30 X 30 cm were used to raise groundnut seedlings in the present study. Sand and clay in the ratio of 3 : 1 was used as potting medium. Fertilizers were applied as per recommendation to the groundnut crop.

## **3.4 SEED MATERIAL**

The seed samples of groundnut variety, JL- 24 was collected from Agricultural Research Station, Vizianagaram. The variety was employed to study the effect of different inoculum levels on groundnut collar rot incidence and the effect of seed treatment with non conventional chemicals in the management of collar rot.

### 3.5 NUTRIENT MEDIA

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

#### 3.5.1 Potato Dextrose Agar Medium (PDA)

Peeled potato	-	200g
Dextrose	-	20g
Agar	-	20g
Distilled water	-	1000ml

Potato extract was prepared by boiling peeled potato tubers in 500 ml of distilled water and to another 500 ml of distilled water, agar powder was added and melted by heating. Both the solutions were filtered through a muslin cloth into a third container. The volume of the filtrate was made upto one litre by addition of distilled water. The pH of the medium was adjusted to 6.0 to 6.5 by adding either 1.0 N sodium hydroxide or 1.0 N hydrochloric acid, before sterilization in an autoclave at 15 lb/inch<sup>2</sup> for 15 min at 121<sup>0</sup>C.

#### 3.5.2 *Trichoderma* Selective Medium (TSM) (Elad and Chet, 1983).

Mg SO <sub>4</sub> . 7 H <sub>2</sub> O	-	0.2g
K <sub>2</sub> HPO <sub>4</sub>	-	0.9g
KCl	-	0.15g
NH <sub>4</sub> NO <sub>3</sub>	-	1.0g

Glucose	-	3.0g
Chloramphenicol	-	0.25g
Fenaminosulf	-	0.30g
Quintozone	-	0.20g
Rosebengal	-	0.15g
Agar	-	20.0g
Captan	-	0.02g
Distilled water	-	1000 ml

### **3.5.3 Kings B medium (King *et al.* , 1954).**

Peptone	-	20g
K <sub>2</sub> HPO <sub>4</sub>	-	0.9g
Glycerol	-	15ml
MgSO <sub>4</sub> 7H <sub>2</sub> O	-	6g
Agar	-	15g
Cycloheximide	-	75mg
Ampicillin	-	50mg
Chloromphenicol	-	12.5mg
Distilled water	-	1000ml

### **3.5.4 Czapek – Dox agar (Dox, 1910)**

NaNO <sub>3</sub>	-	2.0g
K <sub>2</sub> HPO <sub>4</sub>	-	1.0g
Mg SO <sub>4</sub> 7 H <sub>2</sub> O	-	0.5g
FeSO <sub>4</sub> 7H <sub>2</sub> O	-	0.01g
Sucrose	-	30.0g

Agar	-	15.0g
Distilled water	-	1000ml

### **3.5.5 Sand Maize meal Medium (SMM) (Dasgupta *et al.*, 1998)**

Sand Maize meal Medium (SMM) was used for developing mass inoculum in laboratory (Plate 1). The SMM was prepared by mixing sand and maize meal in the ratio of 3:1 with 50 ml distilled water in 250 ml conical flasks and autoclaved at 15 lb/inch<sup>2</sup> for 30 min. at 121<sup>0</sup>C. Flasks were subsequently inoculated with test fungus and incubated at room temperature ( 30 ± 2<sup>0</sup> C).

### **3.6 STERILIZATION**

All the glassware used in the present investigation were sterilized in a hot air oven at 160<sup>0</sup>C for 120 min. All the nutrient media was sterilized in an autoclave at 15 lb/inch<sup>2</sup> for 15 min at 121<sup>0</sup>C.

Seed material wherever necessary was surface sterilized with one per cent sodium hypochlorite and washed three times in sterile distilled water.

The earthen pots used in pot culture experiments were disinfected with one per cent sodium hypochloride and potting medium used in pot culture experiments was sterilized in an autoclave at 15 lb/ inch<sup>2</sup> for 30 min at 121<sup>0</sup>C.

### **3.7 SURVEY**

### **3.7.1 Field Survey for Assessment of groundnut Collar rot Incidence Caused by *Aspergillus niger* van Teighem in North Coastal Zone of Andhra Pradesh.**

Survey for the occurrence of collar rot incidence of groundnut (Plate 2) and collection of soil samples for isolation of native *Trichoderma* spp. was conducted in North Coastal Zone of Andhra Pradesh (Srikakulam, Vizianagaram, Visakhapatnam and up lands of East Godavari) during *rabi*, 2003-2004. In each district two to three major groundnut growing mandals, from each mandal two to four villages and from each village, five fields were selected. From each field 4-6 places (depending upon the area of the field) were selected at random on diagonal lines of the field (Singh, 1984). Totally about 10 to 15 spots were selected at random for taking soil and plant samples representing whole field. A sample of 250 g of soil along with diseased plants were collected in polythene bags at each field and finally composite samples of 500 g was prepared for each mandal. The relevant information on type of soil, crop variety, date of sowing and disease incidence were recorded.

The collar rot disease incidence was calculated using the following formula (Mayee and Datar, 1985).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

### **3.7.2 Effect of Agroecological Factors on Distribution and Occurrence of *Aspergillus niger* Associated with Groundnut in North Coastal Zone of Andhra Pradesh.**

The information obtained from the survey have been further utilized to know the interaction of various agro-ecological factors *viz.*, soil type, cultivars of groundnut, age of the crop on the distribution and occurrence of groundnut collar rot disease incidence caused by *A. niger*.

### **3.8 ISOLATION OF *Trichoderma* spp. FROM SOIL AND MAINTENANCE OF THE CULTURES**

Soil samples collected during the survey were used for isolation of native isolates of *Trichoderma* spp. by serial dilution pour plate technique (Johnson and Curl, 1972) using *Trichoderma* selective medium. Ten grams of soil was taken and mixed with 90 ml of sterile distilled water in a conical flask to achieve  $10^{-1}$  dilution. From this one ml was transferred to nine ml of sterile distilled water taken in another test tube so as to get  $10^{-2}$  dilution. A series of successive dilutions were made upto  $10^{-4}$  and from which one ml of soil suspension was transferred to a sterile Petridish into which 15 ml of sterile *Trichoderma* selective medium was poured and the petridish was rotated gently for even distribution of sample in the medium. After solidification of medium the plates were incubated at room temperature of  $30 \pm 2^{\circ}\text{C}$  for 4-5 days for development of colonies.

The isolates of *Trichoderma* spp. were identified based on their colony and morphological characters. The measurements of conidiophore and conidia were recorded and compared with the standard measurements of *Trichoderma* spp. given by Rifai (1969). Different isolates were maintained separately on PDA slants for testing their growth rates and antagonistic activity against *A. niger*.

### **3.9 ISOLATION AND IDENTIFICATION OF THE PATHOGEN**

#### **3.9.1 Isolation of Pathogen**

The causal organism of collar rot disease, *Aspergillus niger* was isolated from the groundnut plants showing collar rot symptoms. The infected plants were collected from farmers' fields during survey. The collar region from the infected plants was cut into 5 mm size bits along with a little of healthy portion. These bits, after surface sterilization with one per cent sodium hypochlorite for one minute followed by rinsing in three changes of sterile distilled water were transferred on to potato dextrose agar medium in Petridishes aseptically and incubated at room temperature ( $30 \pm 2^{\circ}\text{C}$ ) in the laboratory. Seven days after incubation, when the growth of the fungus was seen on the medium, it was transferred aseptically to potato dextrose agar slants.

#### **3.9.2 Purification of Culture**

The fungal culture was purified by single spore isolation. The spore suspension was prepared from 7 day old culture. The

concentration of the original spore suspension was determined by counting the number of spores carried in 2 mm loop. The loop full of suspension containing 75-100 spores was mixed in 10 ml of two per cent sterilized plain water agar and poured aseptically into Petridishes. After incubation of the Petridishes for 12 h the spores well spaced out were located by microscope and were marked with a glass marking pencil. Each marked single spore along with a bit of agar medium was aseptically cut and scooped with the help of a sterilized scalpel and transferred to potato dextrose agar slants. The culture was maintained by sub-culturing at 15-20 days interval.

### **3.9.3 Identification of Fungus as *Aspergillus niger***

The identity of *A. niger* isolated during the study was confirmed based on conidiphore and conidial characters. The measurements of the conidiophores and conidia were recorded and compared with the standard measurements of the species given by Onions (1966), "Illustrated Genera of Imperfect Fungi" (Barnett and Hunter, 1972) and "The Genus *Aspergillus*" by Raper *et al.* (1965).

The following formula was employed for comparison.

$$\mu = \bar{X} \pm t (0.05) \times \text{SEm}$$

where,

$$\mu = \text{Size of conidia or conidiophores}$$

$X$  = mean of 100 observations (conidia or conidiophores)

$t_{100} (0.05) = 1.96.$

SEm = Standard error of the mean.

The fungal species was identified based on the “ $\mu$ ’ value falling between confidence limits.

### **3.10 PATHOGENICITY STUDIES**

The test pathogen, *A. niger* maintained on sand maize meal medium, was used to study the effect of different inoculum levels so as to find out the optimum infection threshold level with reference to pre and post-emergence damping off and collar rot incidence associated with groundnut.

A pot culture experiment was conducted in the polyhouse to study the pathogenicity of *A. niger* by soil infection as per the procedure given by Haware (1980) with slight modification. The potting mixture as earlier mentioned was sterilized in an autoclave. The inoculum multiplied on sand maize meal medium as earlier mentioned, was prepared in the following concentrations 10.0, 20.0, 30.0, 40.0 and 50.0 per cent (w/w). Top one inch of soil in the pots was replaced with different levels of inoculum mixture. Then a thin layer of soil was applied on the surface. Pots were watered as and when required. Soil mixture without inoculum served as control. Each pot was sown with the surface sterilized seed (JL 24). Six treatments

were maintained with three replications. The data on collar rot disease incidence was recorded and expressed in percentage.

### **3.11 EFFECT OF DIFFERENT *Trichoderma* NATIVE ISOLATES ON GROWTH OF *Aspergillus niger* IN VITRO**

The antagonistic effect of the different *Trichoderma* spp. isolated from soil samples tested against the growth of *A. niger* by dual culture technique (Dennis and Webster, 1971c) and seeded agar method. In dual culture technique, sterilized potato dextrose agar medium melted and cooled at 45<sup>0</sup>C was poured aseptically into sterilized petridishes. Mycelial discs of 5mm diameter were cut from the edge of the four day old culture of each *Trichoderma* spp. and *A. niger* with the help of sterilized cork borer, were placed simultaneously on the periphery of the medium exactly opposite to the *Trichoderma* disc where as in seed agar method, the spore suspension of isolates of *Trichoderma* spp. were separately prepared and the spore suspension was diluted further with sterile water so as to have a suspension with a concentration of 2 X 10<sup>5</sup> spores/ml. Five ml of spore suspension of each isolate of *Trichoderma* spp. was mixed with 95 ml molten but slightly warm potato dextrose agar. The seeded agar was poured at the rate of 15 ml per Petridishe and was allowed to solidify. The seeded Petridishes were centrally inoculated with 5 mm mycelial disc of *A. niger*. For each isolate three replications were maintained. Then the plates were incubated at room temperature (28 ± 2<sup>0</sup>C). Petridishes inoculated with only *A.*

*niger* served as control. Five days after incubation, the colony diameter of the pathogen and *Trichoderma* spp. were measured and per cent inhibition of the pathogen over control was calculated by the following formula.

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

### **3.11.1 Comparison of Native Isolates of *Trichoderma* spp. with Commercial Formulations of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*.**

The native isolates of *Trichoderma* spp. were compared with commercially formulated products of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* (Table 1) to find out their relative antagonistic potential against mycelial growth of *A. niger* by dual culture technique. Three replications were maintained and PDA inoculated with *A. niger* alone served as control. The plates were incubated at room temperature. At the end of incubation period, the colony diameter was measured and expressed as per cent inhibition over control.

### **3.12 EVALUATION OF PLANT EXTRACTS AGAINST *Aspergillus niger***

To study the effect of plant extracts on growth of *A. niger*. Plant extracts of the following species were used.

<b>Name of the plant species</b>	<b>plant part used</b>
1. <i>Annona squamosa</i>	kernel
2. <i>Allium sativum</i>	bulb
3. <i>Polyalthia longifolia</i>	seed
4. <i>Ocimum sanctum</i>	leaf
5. <i>Curcuma longa</i>	rhizome
6. <i>Piper betle</i>	leaf
7. <i>Azadirachta indica</i>	leaf
8. <i>Azadirachta indica</i>	seed
9. <i>Pongamia glabra</i>	seed
10. <i>Acacia arabica</i>	bark

### **3.12.1 Preparation of plant extract**

Fresh plant materials of different plant species was thoroughly cleaned, surface sterilized with 2% sodium hypochlorite and washed well with sterile water. The predetermined plant parts were grounded along with sterile water at the rate of 1:1 w/v using pestle and mortar and the macerate was filtered through a Whatman No.1 filter paper in sterilized condition to get the clear plant extract (100%).

### **3.12.2 Effect of Plant extracts on growth of *Aspergillus niger***

The extract of each plant species at each of the three concentrations *viz.*, 5, 10 and 15 per cent tested against the *A. niger* by poisoned food technique (Nene and Thapliyal, 1971). Different

concentrations of plant extracts 5, 10 and 15 per cent was achieved by mixing 5, 10 and 15 ml of plant extracts in 95, 90 and 85 ml potato dextrose agar medium, respectively. The medium was then poured into Petridishes and allowed to solidify. Discs of 5 mm size cut from the edge of colony of *A. niger* were transferred on to the preserved medium and PDA medium without the plant extract served as control. Three replications were maintained for each plant extract. The colony diameter was recorded and per cent inhibition of growth of *A. niger* over control was estimated by using the following formula.

$$\text{Per cent inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

### **3.13 COMPARISON OF THE PLANT EXTRACTS WITH THE *Trichoderma* NATIVE ISOLATES, *Pseudomonas fluorescens* and *Bacillus subtilis* AGAINST *Aspergillus niger* IN VITRO**

In the above experiment, the plant extracts which were found to be the best were compared with biocontrol agents against *A. niger*. Among the ten plant extracts, *Annona squamosa* (15%), *Pongamia glabra* (15%), *Allium sativum*(10%) and *Polyalthia longifolia* (15%) were used. These were compared with T<sub>1</sub>, T<sub>2</sub>, T<sub>18</sub>, T<sub>21</sub>, T<sub>40</sub> isolates of *Trichoderma* spp., *Pseudomonas fluorescence* and *B. subtilis*. Poisoned food technique was employed in respect of the plant extracts and seeded agar method was used for antagonistic potential of biocontrol agents as explained earlier. The petridishes that did not

contain any plant extract or biocontrol agents served as control. Each treatment was replicated three times and observations were recorded on the colony diameter of the *A. niger* as expressed in percentage inhibition over control.

### **3.14 EFFECT OF NON CONVENTIONAL CHEMICALS AGAINST *Aspergillus niger* IN VIVO**

The collar rot of groundnut caused by *Aspergillus niger* van Tieghem is a very common and destructive diseases in India and causes considerable loss up to 50 per cent (Chohan, 1969 ; Mc Donald, 1978). Though several fungicides have been recommended for managing this pathogen, their threat to environment, crop ecology, soil biology and human health (Papavizas, 1985). A lot of information was available on use of different groups of non conventional chemicals with little or no direct toxicity that could reduce disease incidence significantly in many crops and restricted damage to tolerable limits (Lazarovits,1988; Sinha,1989). In this context, an experiment was conducted to reduce the collar rot incidence through developing systemic defense mechanism in host by using non conventional chemicals.

#### **3.14.1 Effect of seed treatment with heavy metal salts and weak acids on *Aspergillus niger*.**

To study the effect of non conventional chemicals on the pre and post-emergence damping off and collar rot incidence caused by *A. niger*. Ten non conventional chemicals viz., Lithium sulphate,

Cupric sulphate, Cupric chloride, Boric acid, Zinc sulphate, Ferrous sulphate, Manganese sulphate, Salicylic acid, Barium nitrate and Barium chloride were tested at different concentrations *viz.*,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  M in pot culture experiments. From the earlier experiment best inoculum concentration was taken (50% w/w) and added to the pots as mentioned earlier. The above mentioned chemicals were prepared in  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  M concentration solutions. Surface sterilized seeds were soaked for 24 hours in the chemical solutions. Each pot was sown with ten groundnut seeds (JL 24). Seeds soaked in sterile distilled water served as control. The observations on pre and post-emergence damping off upto 30 days and collar rot incidence upto 50 days were recorded and expressed in the disease percentage and disease control over control.

#### **3.14.2 Effect of Seed Treatments with Growth Hormones on Groundnut Collar Rot Incidence**

To study the effect of growth hormones on pre and post-emergence damping off and collar rot caused by *A. niger*. Five growth hormones *viz.*, Salicylic acid, Indole acetic acid, Maleic hydrazide, Gibberillin<sub>3</sub> and N<sup>6</sup>-Benzyladenine were tested at different concentrations *viz.*,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M in pot culture experiments. Pots were inoculated with inoculum as mentioned earlier. Seeds were soaked in chemical solutions at  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M concentrations for 24 h. Ten seeds per pot were sown

and seeds soaked in sterile distilled water served as control. Observation on pre and post-emergence damping off and collar rot incidence were recorded.

### **3.15 STATICAL ANALYSIS**

The data on screening of antagonistic effect of native isolates *Trichoderma* spp. against *A. niger*, comparison of native isolates with commercial formulation and comparison of native isolates with plant extracts were statistically analysed using completely randomized block design. Factorial completely randomized block design was adopted for the experiments conducted to study the effect of plant extracts against *A. niger* and effect of non conventional chemicals on groundnut collar rot incidence. The data showing percentage were transformed by angular transformation and were statistically analysed (Gomez and Gomez, 1984).

## CHAPTER- IV

### RESULTS

#### 4.1 SURVEY

##### 4.1.1 Prevalence of groundnut collar rot caused by *Aspergillus niger* in North Coastal Zone of Andhra Pradesh

A preliminary field survey was conducted to assess the incidence of groundnut collar rot caused by *A. niger* in major groundnut growing mandals of North Coastal zone of Andhra Pradesh and the results were presented in Table 2. A total of 29 villages were surveyed from four districts.

Incidence of collar rot was recorded in all the fields surveyed with minimum incidence (0.3%) in Mahadevipuram village, Vajrapukotturu mandal of Srikakulam district and a maximum of 10.52 per cent in Vadisaluru village, Rangampeta mandal of East Godavari district with an average of 4.07 per cent. Among the four districts surveyed, highest mean incidence of 6.04 per cent was recorded in uplands of East Godavari followed by Vizianagaram (4.53%). The lowest mean incidence of 2.32 per cent was recorded in Srikakulam district.

#### **4.1.2 Effect of agro - ecological factors on groundnut collar rot incidence**

The data recorded during the field survey was further analysed based on agro - ecological factors *viz.*, soil type, cultivars and age of the crop on the distribution and occurrence of groundnut collar rot incidence.

##### **4.1.2.1 Soil type**

A total of four types of soils were observed during the field survey in four districts. Among the four soil types (Table 3 and Fig. 1), highest collar rot incidence (5.49%) was recorded in sandy loam soils followed by loams (3.84%) and sandy clay loams (2.64%) where as clay loam soils recorded lowest collar rot incidence (0.97%).

##### **4.1.2.2 Cultivars**

The collar rot incidence was recorded in Spanish bunch (JL 24, TAG 24 and TMV 2) and Virginia bunch (K 3) cultivars. The highest mean collar rot incidence was observed in Spanish bunch cultivars like JL 24 (4.75%) followed by TAG 24 (4.39%) and TMV 2 (3.26%) where as Virginia bunch type cultivar (K 3) showed less incidence (2.3%) (Table 4 & Fig. 2).

##### **4.1.2.3 Age of crop**

The collar rot disease recorded in groundnut crop during field survey was analysed with different age groups of crop growth. The highest mean disease incidence (5.93%) was recorded during the early period of the crop growth (15-20 days), followed by 3.64 per cent incidence during 20-25 days and 3.49 per cent during 25-30 days of crop growth while, least disease incidence (2.75 %) was recorded during 35-40 days period of crop growth (Table 5, Fig. 3).

#### **4.1.3 Native isolates of *Trichoderma* spp. from North Coastal Zone of Andhra Pradesh**

Out of the 29 soil samples drawn from non rhizosphere region during the field survey, 45 *Trichoderma* isolates were obtained (Table 6); 17 from Srikakulam district, 12 from Visakhapatnam and 9 from Vizianagaram. Less population (7 isolates) of native isolates of *Trichoderma* spp. were obtained from uplands of East Godavari.

#### **4.2 ISOLATION AND IDENTIFICATION OF PATHOGEN**

The infected groundnut plants showing typical symptoms of collar rot disease were collected during the field survey from the farmer's fields. The pathogen was isolated and purified by single spore isolation as explained under materials and methods.

The colony growth on Czapek agar was rather restricted and on potato dextrose agar was more rapid attaining 5-6 cm diameter of radial growth in 4 days. In culture, the colony was white or light

yellow initially, later turning black to dark black in colour (Plate 3), some times showing concentric zonation, reverse of colony was white to yellow in colour. The conidiophores were found to be hyaline or fairly brownish near apex, unbranched, erect and to terminate in an inflated apex upon which radiating phialides were formed (Plate 4). Conidia produced in chains, single celled, pale to dark brown and less globose with prominent ridges being usually discontinuous with very rough or echinulate surface (Table 7).

The measurements of the conidiophores and conidia were found comparable with the standard measurements of the species as given by Onions (1966) and in "The genus *Aspergillus*" (Raper *et al.*, 1965). Hence, the identity of the fungus was confirmed as *Aspergillus niger* van Tieghem. The pathogenicity of *A. niger* was established by inoculating the seeds with conidial suspension ( $2 \times 10^5$  spores/ml) of the fungus thus proving the Koch's postulates (Plate 5).

#### **4.3 SYMPTOMS OF THE DISEASE**

The ungerminated seeds were found to be covered with mass of black conidia and the seeds were found rotten. Seedlings having typical collar rot symptoms wilted. The rot was characterized by yellow brown lesion with profusely sporulating growth of *Aspergillus niger*. The hypocotyls become completely necrotic. In late infection, *i.e.* beyond the seedling stage, collar region showed water soaked and light brown lesions covered with the sporulating fungal

mycelium leading to rotting and death of the whole plant (Plate 5 and 6).

#### **4.4 EFFECT OF DIFFERENT INOCULUM LEVELS OF *Aspergillus niger* ON PRE AND POST- EMERGENCE DAMPING OFF AND COLLAR ROT INCIDENCE OF GROUNDNUT *IN VIVO***

In order to study the effect of different inoculum levels of *A. niger* on pre and post-emergence damping off and collar rot infection of groundnut *in vivo* to determine the optimum infection threshold level, an experiment was conducted with the different levels of inoculum comprising of 10, 20, 30, 40 and 50 per cent (w/w) was applied by replacing top one inch soil as earlier explained under materials and methods.

The results presented in table 8 and fig. 4 indicated that the inoculum at all levels resulted in significantly higher percentage of infection than in control. The pre and post-emergence and collar rot incidence increased with increase in inoculum levels. The pre and post-emergence damping off increased from 10.0 to 66.67 per cent with an increase in level of inoculum from 10 to 50 per cent. Inoculum level at 50 per cent caused the maximum infection of 66.67 per cent which was significantly superior to all other inoculum levels followed by 40 per cent and 30 per cent inoculum levels which recorded 53.33% and 46.67% diseases incidence, respectively.

The higher collar rot incidence was recorded with increase in the inoculum level from 10 to 50 per cent, ranging from 7.87 to 53.33 per cent. The highest collar rot incidence (53.33%) was recorded at 50 per cent inoculum level which was significantly superior over all other treatments followed by 40 per cent and 30 per cent inoculum levels which recorded 43.33 per cent and 24.44 per cent disease incidence.

#### **4.5 BIOLOGICAL CONTROL**

##### **4.5.1 Effect of Native *Trichoderma* isolates on *Aspergillus niger* *in vitro***

An experiment was conducted to study the effect of different native isolates of *Trichoderma* spp. on the growth of *Aspergillus niger in vitro*.

From the data presented in table 9 and fig.5, it could be inferred that *Trichoderma* isolate T<sub>21</sub> had maximum growth inhibition of *A. niger* (75.44 %) which was significantly superior over all other isolates. The next best isolates were T<sub>1</sub> (70.34%), T<sub>40</sub> (69.99%) and T<sub>18</sub> (67.62%) which were significantly superior to the remaining isolates and were on par with each other. T<sub>38</sub> isolate (58.48%) was least effective in dual culture method (Plate 7), T<sub>1</sub> isolate in seed agar method (Plate 8) inhibited 85.00 per cent growth of *A. niger* which was significantly superior to all other isolates followed by T<sub>2</sub> (81.93%) and T<sub>21</sub> (81.11%). T<sub>27</sub> isolate (72.84%) was least effective.

#### **4.5.2 Comparison of native *Trichoderma* isolates with commercial formulations of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis***

Native isolates of *Trichoderma* spp were significantly superior to the commercial formulations of *T. viride*, *T. harzianum* and *P. fluorescens* in inhibiting the growth of *A. niger* ((Table 10, Fig.6 & Plate 9) except *B. subtilis* (Plate 10) which recorded maximum growth inhibition (77.21%) and was on par with T<sub>21</sub> isolate (75.21%). *P. fluorescens* was least effective which recorded 31.88 per cent inhibition over control.

#### **4.6 EFFECT OF PLANT EXTRACTS ON GROWTH OF *Aspergillus niger* IN VITRO**

In the present study, all the plant extracts (Plate 11 to 19) tested at all concentrations were significantly effective in reducing the growth of *A. niger* when compared to control (Table 11, Fig. 7). The inhibition of growth of *A. niger* ranged from 1.57 to 7.73 cm as against 8.51 cm in control. Irrespective of the concentrations, *Allium sativum* was proved to be the most effective botanical and recorded the highest reduction of growth (81.51%) which was significantly superior to all other plant extracts. The next best treatment was *Polyalthia longifolia* (60.18%) followed by *Annona squamosa* (55.87%) and *Pongamia glabra* (54.44%). *Ocimum sanctum* was least effective in reducing the fungal growth (9.13%).

The plant extracts irrespective of the species were found to be most effective at 15 per cent concentration. Maximum reduction of mycelial growth (41.61%) was observed at 15 per cent concentration which was significantly superior over rest of the concentrations.

The interaction between plant extracts and concentrations was significant. All the plant extracts were increasingly effective in reducing the mycelial growth with increase in concentrations. Maximum reduction of mycelial growth (100%) was observed with *Allium sativum* at 10 per cent concentration which was significantly superior over all other combinations followed by *Polyalthia longifolia* (77.73%), *Annona squamosa* (74.61%) and *Pongamia glabra* (70.33%) at 15 per cent concentration where as no mycelial growth reduction (0.0%) was observed with *Ocimum sanctum* (10%), *Curcuma longa* (5%) and *Azadirachta indica* seed extract (5%).

#### **4.7 COMPARISION OF PLANT EXTRACTS WITH BIOLOGICAL CONTROL AGENTS**

An experiment was conducted to study the efficacy of plant extracts in comparison to *Trichoderma* isolates (Plate 20) *Psuedomonas fluorescens* and *Bacillus subtilis* against the *Aspergillus niger in vitro*.

*Allium sativum* (10%) had maximum inhibiting effect (100.00%) on *A. niger* which was significantly superior to all other plant

extracts and bio-control agents and it was followed by *Bacillus subtilis* (Plate 21) and T<sub>1</sub> isolate which recorded 84.52 and 84.49 per cent growth inhibition over control respectively, which were on par with each other. *Pseudomonas fluorescens* (Plate 22) was least effective and recorded 62.95 per cent inhibition over control (Table 12 & Fig.8).

#### **4.8 NON CONVENTIONAL CHEMICALS**

##### **4.8.1 Effect of Heavy Metal Salts and Weak Acids on Pre and Post-emergence Damping off and Collar Rot Incidence Caused by *Aspergillus niger*.**

###### **4.8.1.1 Pre and post-emergence damping off**

Data pertaining to the pre and post-emergence damping off caused by *A. niger* were presented in table13 and fig.9. The disease incidence was significantly influenced by chemicals and interaction of chemicals and concentrations but concentrations were found to be non significant. Irrespective of the concentrations tested, Lithium sulphate followed by Barium nitrate were most effective and significantly superior to all other treatments by recording 32.91 and 35.51 per cent disease incidence, respectively. The next best treatments were Cupric sulphate (39.41%), Cupric chloride (40.58%) and Barium chloride (41.73%) and were on par with each other where as Boric acid was found to be least effective(51.01%).

The interaction between chemicals and their concentrations was found significant. Differences among the chemicals in respect

of their ability to reduce disease incidence was influenced by the concentrations. Similarly, the differences observed among the concentrations varied with the type of chemical used. But the test compounds showed a fairly strong effect at low concentrations than at higher concentrations. Maximum disease control was observed with Lithium sulphate ( $10^{-3}$  M) 63.88 per cent followed by Barium nitrate ( $10^{-4}$ M) 55.88 per cent and Cupric sulphate ( $10^{-4}$ M) 50.00 per cent which were most effective and significantly superior over all other treatments.

#### **4.8.1.2 Collar rot**

The relevant data on collar rot incidence were presented in table 14 and fig.10. The collar rot incidence was significantly reduced by all the chemicals tested compared to control. Barium nitrate followed by Lithium sulphate, Cupric chloride and Ferrous sulphate were most effective and significantly superior to all other treatments by recording 17.45, 18.02, 18.26 and 18.99 per cent collar rot incidence respectively, which were on par with each other. Boric acid was least effective by recording 27.59 per cent disease incidence. The effect of concentrations on collar rot incidence irrespective of chemicals was found to be non significant.

The interaction between chemicals and concentrations was found to be significant. Differences among the chemicals was influenced by the different concentrations. Similarly, the effect of different concentrations varied with the different chemicals.

Maximum disease control was observed with Lithium sulphate ( $10^{-3}$ M) 71.57 per cent over control followed by Barium nitrate ( $10^{-4}$ M) 68.36 per cent, Cupric sulphate ( $10^{-4}$ M) 66.69 per cent, Manganese sulphate ( $10^{-2}$ M) 62.22 per cent, Cupric chloride ( $10^{-4}$ M) 56.58 per cent and Zinc sulphate ( $10^{-4}$ M) 54.05 per cent which were most effective and significantly superior to all the other treatments.

#### **4.8.2 Effect of seed soaking with growth hormones on pre and post-emergence damping off and collar rot caused by *Aspergillus niger*.**

##### **4.8.2.1 Pre and post-emergence damping off**

Perusal of data in table 15 and fig. 11 revealed that there was a significant influence of chemicals and interaction between chemicals and concentrations on pre and post-emergence damping off where as influence of concentrations was found to be non significant. The disease incidence was recorded as 32.50, 36.33 and 37.50 per cent, respectively with Salicylic acid, GibberilinA<sub>3</sub> and Indole Acetic Acid and were significantly superior to all other treatments. N<sup>6</sup>-benzyladenine was least effective by recording 47.50% disease incidence.

With most of the chemicals tested, no significant differences existed between the effect of concentrations and in some cases even stronger protection was achieved with the lower concentrations. Among the combination of chemicals and concentrations, Salicylic acid ( $10^{-5}$ M) was the most effective by

reducing the 75.00 per cent of pre and post-emergence damping off incidence over control followed by GibberillinA<sub>3</sub> (10<sup>-6</sup>M) and Maleic hydrazide (10<sup>-6</sup>M) which recorded 63.64 and 59.09 per cent inhibition over untreated control, respectively and were on par with each other where as N<sup>6</sup>-benzyladenine (10<sup>-5</sup>M) was least effective by recording 8.33 per cent inhibition over control.

#### **4.8.2.2 Collar rot**

Perusal of the data in table 16 and fig. 12 revealed that the collar rot incidence significantly differed with all chemicals tested compared to control. Minimum disease incidence was observed with salicylic acid (19.83%) followed by Indole Acetic Acid (21.37%) and GibberillinA<sub>3</sub> (24.61%) which were significantly superior to all other treatments and were on par with each other. Maximum disease incidence was observed with N<sup>6</sup>-benzyladenine (32.55%). The effect of concentration on collar rot disease incidence irrespective of chemicals was found non significant.

The interaction between chemicals and concentrations was found to be significant. Differences among the chemicals was influenced by the different concentrations. Similarly the effect of different concentrations varied with the different chemicals. Maximum disease reduction (79.36%) was observed with Salicylic acid (10<sup>-5</sup> M) over untreated control followed by GibberilinA<sub>3</sub> (10<sup>-6</sup>M), Indole Acetic Acid (10<sup>-6</sup>M) and Maleic hydrazide (10<sup>-6</sup>M) which recorded 72.02, 70.24 and 64.29 per cent, respectively were most

effective and sufficiently superior to all other treatments and were on par with each other where as Maleic hydrazide ( $10^{-4}\text{M}$ ) was least effective by recording 6.88 per cent inhibition over control.

**Table. 1 List of commercial formulations of *Trichoderma viride*,  
*T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis***

<b>S.No</b>	<b>Name of the product</b>	<b>Organism</b>	<b>Manufacturer</b>
1	Biocure – F	<i>Trichoderma viride</i>	T. stains company Ltd., Coimbatore
2	Biocure – B	<i>Pseudomonas fluorescens</i>	T. stains company Ltd., Coimbatore
3	Ecoderma	<i>T. harzianum</i>	Margo biocontrol Pvt. Ltd., Bangalore
4	Biotok	<i>Bacillus subtilis</i>	Tocklai experimental station, Tea Research Association, Jorhat, Assam

**Table 3. Influence of soil type on groundnut collar rot incidence**

<b>S.No</b>	<b>Soil type</b>	<b>Number of villages</b>	<b>Mean disease incidence (%)</b>
1	Sandy loam	15	5.49
2	Loam	3	3.84
3	Sandy clay loam	8	2.64
4	Clay loam	3	0.97

**Table 4. Influence of cultivars on groundnut collar rot incidence**

<b>S.No</b>	<b>Cultivar</b>	<b>Number of villages</b>	<b>Mean disease incidence (%)</b>
1	JL-24	14	4.75
2	TAG -24	4	4.39
3	TMV-2	9	3.26
4	K -3	2	2.30

**Table 5. Influence of age of crop on groundnut collar rot incidence**

<b>S.No</b>	<b>Age of crop</b>	<b>Number of villages</b>	<b>Mean disease incidence (%)</b>
1	15-20	8	5.93
2	20-25	4	3.74
3	25-30	9	3.49
4	30-35	5	3.18
5	35-40	3	2.75

**Table 6. Number of soil samples collected and *Trichoderma* spp. isolated from the major groundnut growing mandals of North Coastal zone of Andhra Pradesh**

<b>Name of the district</b>	<b>Name of the mandal</b>	<b>No. of soil samples collected</b>	<b>No. of <i>Trichoderma</i> isolates isolated</b>
<b>Srikakulam</b>	Vajrapukotturu	3	7
	Laveru	3	5
	Ranastalam	3	5
<b>Vizianagaram</b>	Bhogapuram	4	5
	Denkada	3	4
<b>Visakhapatnam</b>	Bheemunipatnam	3	5
	Anandapuram	3	7
<b>East Godavari</b>	Peddapuram	4	3
	Rangampeta	3	4

**Table 7. Characters of mycelium, conidiophores and conidia of *Aspergillus niger***

<b>Character</b>	<b>Mycelium</b>	<b>Conidiophore</b>	<b>Conidia</b>
<b>Length</b>	-	1.5 – 3.0 mm	4 – 5 $\mu$ m
<b>Width</b>	-	15 – 20 $\mu$ m	4 – 5 $\mu$ m
<b>Colour</b>	White to light yellow	Hyaline to light brown	Pale to dark brown
<b>Shape</b>	-	Simple, elongate	Globose
<b>Septation</b>	Septate	No septation	No septation Produced chains of conidia basipetally borne on the sterigmata on the swollen apex of conidiophore

**Table 8. Effect of different inoculum levels of *Aspergillus niger* on pre and post – emergence damping off and collar rot incidence of groundnut cv. JL 24**

S.No	Inoculum density (w/w)	*Pre and post-emergence damping off (%)	*Collar rot (%)
1	10	10.0 (18.45)	7.87 (13.39)
2	20	23.33 (27.78)	13.09 (21.20)
3	30	46.67 (43.08)	24.44 (29.46)
4	40	53.33 (46.92)	43.33 (41.15)
5	50	66.67 (54.78)	53.33 (46.92)
6	Control	0.00 (0.0)	0.0 (0.0)

SEm  $\pm$

1.986

1.431

C.D (0.05)

6.260

4.510

\* Mean of three replications

Figures in parentheses are angular transformed values.

**Table 9. *In vitro* screening of *Trichoderma* native isolates against *Aspergillus niger***

S.No	<i>Trichoderma</i> isolate	Dual culture method		Seeded agar method	
		*Mycelial growth of <i>Aspergillus niger</i> (cm)	Inhibition over control (%)	*Mycelial growth of <i>Aspergillus niger</i> (cm)	Inhibition over control (%)
1	T <sub>1</sub>	2.50	70.34	1.27	85.00
2	T <sub>2</sub>	2.80	66.79	1.53	81.93
3	T <sub>18</sub>	2.73	67.62	1.80	78.44
4	T <sub>20</sub>	3.27	61.21	2.10	75.20
5	T <sub>21</sub>	2.07	75.44	1.60	81.11
6	T <sub>22</sub>	2.90	66.60	1.93	77.33
7	T <sub>27</sub>	3.17	62.40	2.30	72.84
8	T <sub>29</sub>	3.50	58.48	2.20	74.02
9	T <sub>38</sub>	3.90	53.74	2.00	76.38
10	T <sub>40</sub>	2.53	69.99	1.80	78.44
11	Control	8.43	-	8.47	-

SEm<sub>±</sub> 0.089 0.620

C.D (0.05) 0.254 0.184

\* Mean of three replications

**Table 10. Comparison of native *Trichoderma* isolates with commercial formulations of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis***

S.No	Biocontrol agent	*Mycelial growth of <i>Aspergillus niger</i> (cm)	Inhibition over control (%)
<b>Native isolates</b>			
1	<i>Trichoderma</i> isolate (T <sub>1</sub> )	2.52	70.25
2	<i>Trichoderma</i> isolate (T <sub>2</sub> )	2.79	67.06
3	<i>Trichoderma</i> isolate (T <sub>18</sub> )	2.71	68.00
4	<i>Trichoderma</i> isolate (T <sub>21</sub> )	2.10	75.21
5	<i>Trichoderma</i> isolate (T <sub>40</sub> )	2.56	69.78
<b>Commercial formulations</b>			
6	<i>Trichoderma viride</i>	3.50	58.68
7	<i>Trichoderma harzianum</i>	3.77	55.49
8	<i>Pseudomonas fluorescens</i>	5.77	31.88
9	<i>Bacillus subtilis</i>	1.93	77.21
10	Control	8.47	-

SEm± 0.071

C.D (0.05) 0.150

\* Mean of three replications

**Table 12. Comparison of the plant extracts with native isolates of *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis***

<b>S.No</b>	<b>Plant extract/ biocontrol agent</b>	<b>*Mycelial growth (cm)</b>	<b>Inhibition over control (%)</b>
1	<i>Annona squamosa</i> (15%)	2.23	73.87
2	<i>Allium sativum</i> (10%)	0.00	100.00
3	<i>Polyalthia longifolia</i> (15%)	1.93	77.37
4	<i>Pongamia glabra</i> (15%)	2.57	69.87
5	<i>Pseudomonas fluorescens</i>	3.16	62.95
6	<i>Bacillus subtilis</i>	1.29	84.52
7	<i>Trichoderma</i> isolate (T <sub>1</sub> )	1.32	84.49
8	<i>Trichoderma</i> isolate (T <sub>2</sub> )	1.54	81.19
9	<i>Trichoderma</i> isolate (T <sub>18</sub> )	1.85	78.31
10	<i>Trichoderma</i> isolate (T <sub>21</sub> )	1.69	80.76
11	<i>Trichoderma</i> isolate (T <sub>40</sub> )	1.88	77.96
12	Control	8.53	-

SEm $\pm$  0.081

C.D (0.05) 0.230

\* Mean of three replications

**Table 11. Effect of plant extracts on growth of *Aspergillus niger* in vitro**

S.No	Plant extract	Concentration of plant extract							
		5%		10%		15%		mean	
		*Colony diameter (cm)	*Inhibition of mycelial growth over control (%)	Colony diameter (cm)	Inhibition of mycelial growth over control (%)	Colony diameter (cm)	Inhibition of mycelial growth over control (%)	Colony diameter (cm)	Inhibition of mycelial growth over control (%)
1	<i>Annona squamosa</i>	5.10	40.23 (39.36)	4.00	52.76 (46.58)	2.17	74.61 (59.75)	3.75	55.87 (48.56)
2	<i>Allium sativum</i>	4.73	44.54 (41.86)	0.00	100.00 (90.00)	0.0	100.00 (90.00)	1.57	81.51 (73.95)
3	<i>Polyalthia longifolia</i>	4.90	42.58 (40.73)	3.37	60.24 (50.91)	1.90	77.73 (61.84)	3.38	60.18 (51.16)
4	<i>Ocimum sanctum</i>	8.53	00.00 (00.00)	7.87	7.09 (15.40)	6.80	20.32 (26.77)	7.73	9.13 (14.06)
5	<i>Curcuma longa</i>	8.53	00.00 (00.00)	8.41	0.00 (0.00)	5.63	33.99 (35.65)	7.54	11.33 (11.88)
6	<i>Piper betle</i>	7.67	10.15 (18.53)	7.13	15.74 (23.36)	6.70	21.15 (27.37)	7.16	15.68 (23.09)
7	<i>Azadirachta indica</i> (Leaf extract)	8.20	3.90 (11.20)	7.67	9.44 (17.82)	6.30	24.84 (29.88)	7.38	12.73 (19.64)
8	<i>A. indica</i> (seed extract)	8.53	0.00 (0.0)	8.03	5.11 (12.98)	5.90	30.85 (33.74)	7.48	11.99 (15.57)
9	<i>Pongamia glabra</i>	5.03	41.01 (39.82)	4.07	51.97 (46.13)	2.53	70.33 (57.00)	3.87	54.44 (47.65)
10	<i>Acacia arabica</i>	7.43	12.89 (21.02)	6.50	23.22 (28.80)	5.63	33.98 (35.65)	6.52	23.36 (28.49)
11	Control	8.53	0.00 (0.0)	8.47	0.00 (0.00)	8.53	0.00 (0.00)	8.51	0.00 (0.00)
	Mean	7.01	17.75 (19.32)	5.96	29.59 (30.18)	4.73	44.34 (41.61)		

	Colony diameter		Per cent inhibition	
	SEm±	C.D (0.05)	SEm±	C.D (0.05)
Plant extract	0.0427	0.0086	0.3572	0.9902
Concentration	0.0223	0.0619	0.1865	0.5271
Plant extract X concentration	0.0741	0.2053	0.6186	1.7151

\* Mean of three replications

Figures in parentheses are angular transformed values

**Table 14. Effect of seed soaking in heavy metal salts and weak acids on groundnut collar rot incidence caused by *Aspergillus niger* in vivo**

S.No	Chemicals	Concentration of chemicals used						Mean
		10 <sup>-2</sup> M		10 <sup>-3</sup> M		10 <sup>-4</sup> M		
		*Collar rot (%)	Disease control (%)	Collar rot (%)	Disease control (%)	Collar rot (%)	Disease control (%)	
1	Lithium sulphate	16.98 (24.29)	55.70	12.63 (20.79)	71.57	24.44 (29.46)	40.55	18.02 (24.84)
2	Cupric sulphate	23.33 (28.86)	39.13	17.77 (24.91)	60.01	13.69 (21.70)	66.69	18.26 (25.16)
3	Cupric chloride	31.67 (33.86)	17.37	23.33 (28.64)	47.50	17.85 (24.80)	56.58	24.28 (29.09)
4	Boric acid	33.33 (35.00)	13.04	26.11 (30.61)	41.25	23.33 (28.86)	43.25	27.59 (31.49)
5	Zinc sulphate	30.00 (30.08)	21.73	28.87 (32.36)	35.03	18.89 (25.74)	54.05	25.92 (30.39)
6	Ferrous sulphate	20.55 (26.88)	46.39	16.98 (24.29)	61.79	19.44 (26.06)	52.71	18.99 (25.74)
7	Manganese sulphate	14.48 (22.33)	62.22	24.44 (29.46)	45.00	30.00 (33.08)	27.02	22.97 (28.29)
8	Salicylic acid	30.55 (33.51)	20.29	27.78 (31.74)	37.49	24.44 (29.14)	40.55	27.59 (31.46)
9	Barium nitrate	19.44 (26.06)	49.28	19.84 (26.20)	55.35	13.09 (21.20)	68.16	17.46 (24.49)
10	Barium chloride	23.33 (28.64)	39.13	21.74 (27.66)	51.10	26.11 (30.61)	36.49	23.73 (28.98)
11	Control	38.33 (38.08)		44.44 (41.75)		41.11 (39.83)		41.29 (39.89)
	Mean	25.64 (30.05)		23.99 (28.95)		22.94 (28.22)		

	SEm ±	CD (0.05)
Chemicals	1.47	4.07
Concentration	0.77	NS
Chemicals X Concentration	2.41	6.69

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

**Table 15. Effect of seed soaking with growth hormones on pre and post- emergence damping off incidence caused by *Aspergillus niger in vivo***

S.No	Chemicals	Concentration of chemicals used						Mean
		10 <sup>-4</sup> M		10 <sup>-5</sup> M		10 <sup>-6</sup> M		
		*Pre and post-emergence damping off (%)	Disease control (%)	Pre and post-emergence damping off (%)	Disease control (%)	Pre and post-emergence damping off (%)	Disease control (%)	
1	Salicylic acid	37.50 (37.73)	34.78	15.00 (22.50)	75.00	45.00 (42.12)	18.18	32.50 (34.11)
2	Indole Acetic Acid	52.50 (45.00)	8.69	35.00 (36.22)	41.66	25.00 (29.89)	54.55	37.50 (37.57)
3	Maleic hydrazide	50.00 (45.00)	13.04	47.50 (43.56)	12.50	22.50 (21.23)	59.09	40.00 (38.93)
4	Gibberillin A <sub>3</sub>	45.00 (42.12)	21.74	50.00 (45.00)	16.66	20.00 (26.19)	63.64	36.33 (37.78)
5	N <sup>6</sup> – Benzyladenine	47.50 (45.56)	13.91	55.00 (47.88)	8.33	40.00 (37.17)	27.27	47.50 (43.54)
6	Control	57.50 (49.34)	-	60.00 (50.77)	-	55.00 (49.88)	-	57.50 (49.33)
	Mean	48.33 (44.03)		43.75 (40.99)		34.58 (35.88)		

SEm ±

CD (0.05)

\* Mean of three replications

Chemicals

2.27

6.42

Figures in parenthesis are angular transformed

Concentration

0.97

NS

values

Chemicals X Concentration

3.21

9.07

**Table. 16 Effect of seed soaking with growth hormones on collar rot incidence caused by *Aspergillus niger* in vivo**

S.No	Chemicals	Concentration of chemicals used						Mean
		10 <sup>-4</sup> M		10 <sup>-5</sup> M		10 <sup>-6</sup> M		
		*Collar rot disease incidence (%)	Disease control (%)	Collar rot disease incidence (%)	Disease control (%)	Collar rot disease incidence (%)	Disease control (%)	
1	Salicylic acid	27.97 (31.73)	32.19	9.03 (15.22)	79.36	22.50 (28.28)	50.00	19.83 (25.07)
2	Indole acetic acid	27.50 (31.17)	33.33	23.21 (28.47)	46.95	13.39 (21.45)	70.24	21.37 (27.03)
3	Maleic hydrazide	39.41 (38.79)	6.88	31.67 (33.86)	27.61	16.07 (23.40)	64.29	29.05 (32.23)
4	Gibberillin A <sub>3</sub>	31.25 (33.95)	24.24	30.00 (32.89)	31.43	12.59 (20.77)	72.02	24.61 (29.21)
5	N <sup>6</sup> – Benzyladenine	27.92 (31.77)	32.32	40.00 (38.95)	8.57	29.74 (32.57)	33.91	32.55 (34.43)
6	Control	41.25 (39.80)	-	43.75 (41.25)	-	45.00 (42.12)	-	43.33 (41.06)
	Mean	32.55 (34.43)		29.61 (32.49)		23.21 (28.09)		

	S.E.m ±	CD (0.05)
Chemicals	1.81	5.12
Concentration	1.28	NS
Chemicals X Concentration	3.14	8.87

\* Mean of three replications  
 Figures in parenthesis are angular transformed values

**Table 13. Effect of seed soaking in heavy metal salts and weak acids on groundnut pre and post-emergence damping off caused by *Aspergillus niger in vivo***

S.No	Chemicals	Concentration of chemicals						Mean
		10 <sup>-2</sup> M		10 <sup>-3</sup> M		10 <sup>-4</sup> M		
		*Pre and post-emergence damping off (%)	Disease control (%)	Pre and post-emergence damping off (%)	Disease control (%)	Pre and post-emergence damping off (%)	Disease control (%)	
1	Lithium sulphate	32.06 (34.36)	43.43	21.67 (27.52)	63.88	45.00 (42.12)	20.59	32.91 (34.67)
2	Cupric sulphate	43.22 (41.07)	23.73	46.67 (43.09)	22.22	28.33 (32.14)	50.00	39.41 (38.77)
3	Cupric chloride	44.40 (41.76)	21.65	42.34 (40.58)	29.43	35.00 (36.18)	38.24	40.58 (39.51)
4	Boric acid	48.05 (43.88)	15.21	51.67 (45.96)	13.88	53.33 (46.92)	5.89	51.01 (45.59)
5	Zinc sulphate	49.99 (44.99)	11.79	43.33 (41.16)	27.78	51.67 (45.96)	8.82	48.33 (44.04)
6	Ferrous sulphate	47.21 (43.38)	16.69	38.33 (38.16)	36.11	48.33 (44.04)	14.72	44.63 (41.86)
7	Manganese sulphate	31.03 (33.66)	45.24	46.67 (43.08)	22.21	55.00 (47.88)	2.94	44.23 (41.54)
8	Salicylic acid	49.45 (44.86)	12.21	43.64 (41.32)	27.27	41.67 (40.17)	26.47	45.02 (42.12)
9	Barium nitrate	46.53 (42.99)	17.89	35.00 (36.23)	41.67	25.00 (29.92)	55.88	35.51 (36.39)
10	Barium chloride	35.18 (36.37)	37.92	41.67 (40.10)	30.55	48.33 (44.04)	14.72	41.73 (40.17)
11	Control	56.67 (48.84)	-	60.00 (50.79)	-	56.67 (48.85)	-	57.78 (49.49)
	Mean	44.01 (41.47)		42.82 (40.73)		44.39 (41.66)		

	SEm ±	CD (0.05)
Chemicals	1.42	3.95
Concentration	0.74	NS
Chemicals X concentration	2.47	6.83

\* Mean of three replications

Figures in parentheses are angular transformed values

**Table 2. Collar rot incidence of groundnut caused by *Aspergillus niger* in North Coastal Zone of Andhra Pradesh**

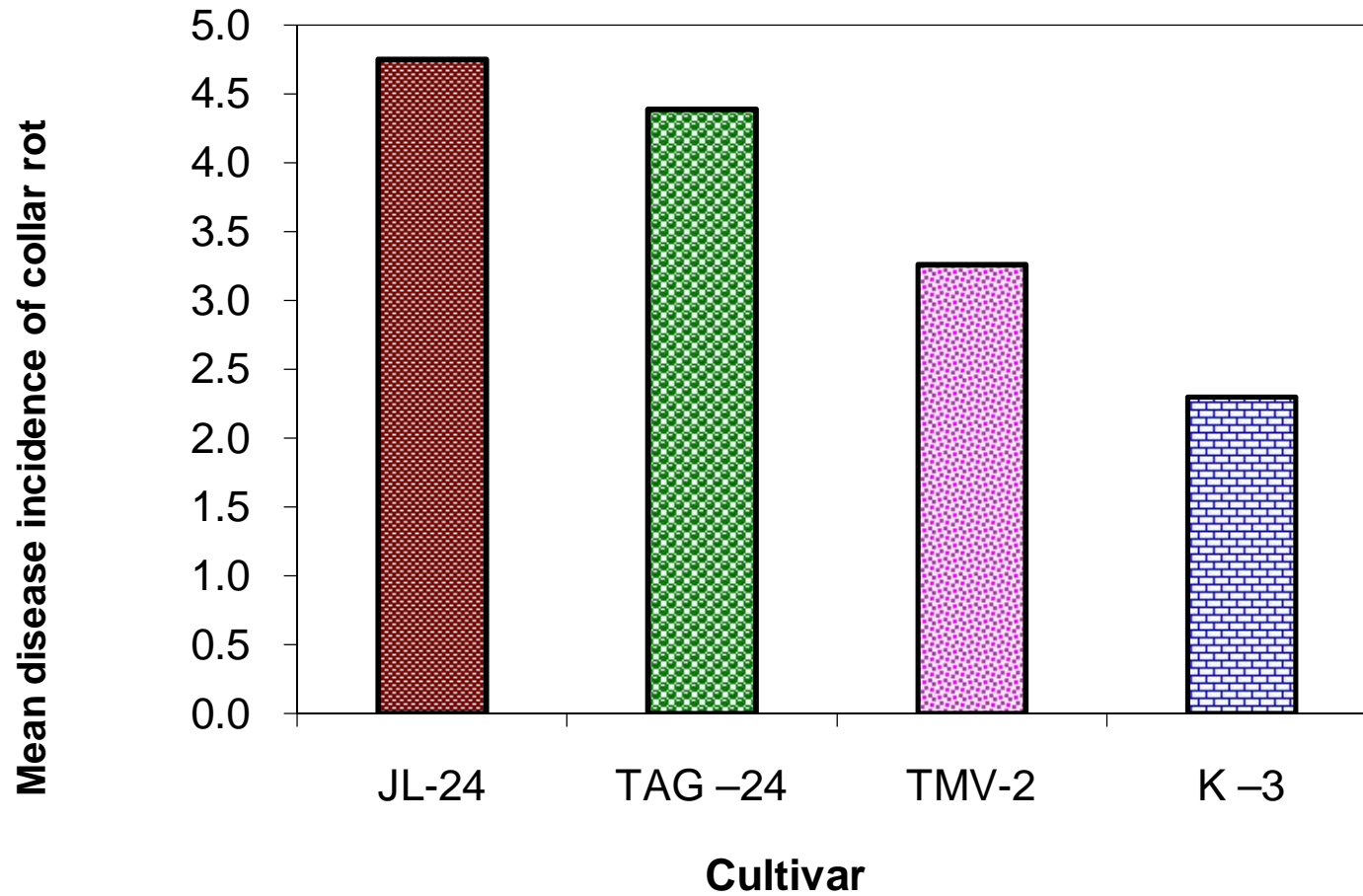
Name of the District	Name of the mandal/village	Soil Type	Variety	Age of crop	Mean population	Per cent collar rot incidence	Average		
							Mandal	District	
SRIKAKULAM	<b>Vajrapukotturu</b>								
	▪ Mahadevipuram	Clay loam	TMV 2	30	33.3	0.30	0.87	2.32	
	▪ Peddabanapuram	Sandy clay loam	TMV 2	17	34.2	1.50			
	▪ Bhendi	Clay loam	TMV 2	25	32.7	0.80			
	<b>Laveru</b>								
	▪ Laveru	Sandy clay loam	JL 24	18	30.2	2.62	2.43		
	▪ Bejjpuram	loam	JL 24	23	31.8	2.84			
	▪ Tallavalasa	Sandy clay loam Clay loam	JL 24	22	28.9	1.83			
	<b>Ranastalam</b>								
▪ Ranastalam	Sandy clay loam	JL 24	27	30.5	3.52	3.68			
▪ Bavarajupalem	loam	JL 24	19	25.2	4.12				
▪ Kosta	Sandy clay loam Sandy clay loam	JL 24	25	28.9	3.41				

<b>VIZIANAGARAM</b>	<b>Bhogapuram</b>							
	▪ Ravada	Sandy loam	JL 24	33	26.7	4.21		
	▪ Chakivalasa	Sandy loam	JL 24	32	24.8	5.32	3.90	4.64
	▪ Polipalli	Sandy loam	TAG 24	28	23.4	3.84		
	▪ A. Rayavalasa	Sandy clay loam	TAG 24	36	23.5	2.24		

Cont..

Name of the District	Name of the mandal/village	Soil Type	Variety	Age of crop	Mean population	Per cent collar rot incidence	Average	
							Mandal	District
VISAKHAPATNAM	<b>Bheemunipatnam</b>							
	▪ Yathakummripalem	Sandy loam	JL 24	28	28.2	5.30	3.69	3.13
	▪ Sangivalasa	Sandy loam	JL 24	25	24.4	3.52		
	▪ Tallavalasa	Sandy loam	TAG 24	18	26.2	4.24		
	<b>Anandapuram</b>							
	▪ Anandapuram	Sandy loam	TMV 2	36	27.50	2.62	2.58	
▪ Peddivalasa	Sandy loam	K 3	28	30.24	2.00			
▪ Dakamari	Sandy loam	JL 24	22	29.80	3.12			
EAST GODAVARI	<b>Peddapuram</b>							
	▪ Nagaram	Loam	TMV 2	23	28.6	4.61	6.54	6.04
	▪ Naikampalli	Sandy loam	TMV 2	17	24.5	9.31		
	▪ Katravulapalli	Sandy loam	JL 24	19	26.5	8.82		
	▪ Yellamilli	Loam	TMV 2	35	29.8	3.42		
	<b>Rangampeta</b>							
	▪ Rangampeta	Loam	TMV 2	34	23.4	3.50	5.53	
▪ Vadisaluru	Sandy loam	JL 24	17	20.8	10.50			
▪ Subadrampeta	Sandy clay loam	K 3	30	30.3	2.60			

**Fig. 2 Influence of cultivars on groundnut collar rot incidence caused by *Aspergillus niger* in North-Coastal Zone of Andhra Pradesh**



**Fig. 3 Influence of age of crop on groundnut collar rot caused by *Aspergillus niger* in North-Coastal Zone of Andhra Pradesh**

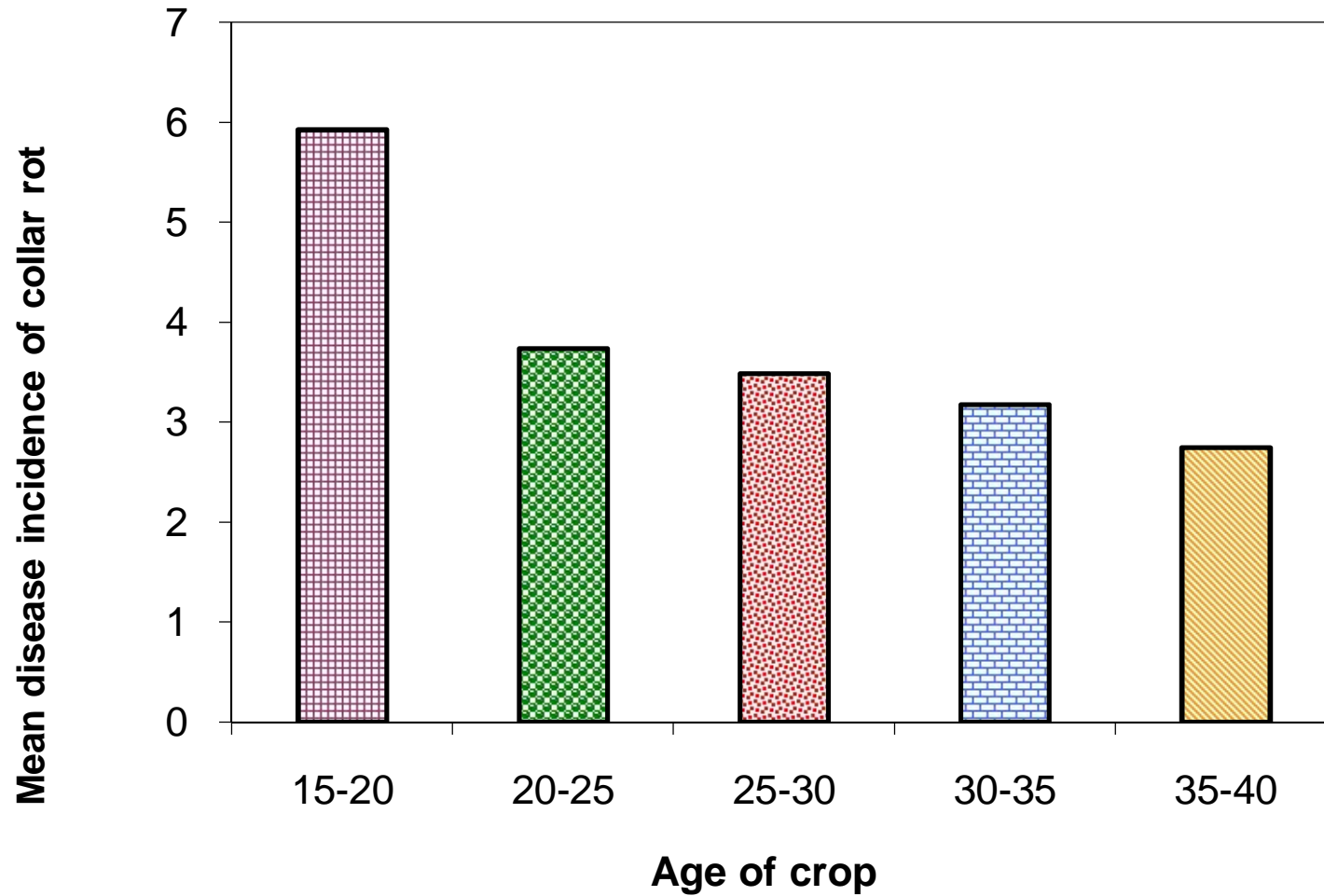


Fig.4 Pre and post emergence damping off and collar rot

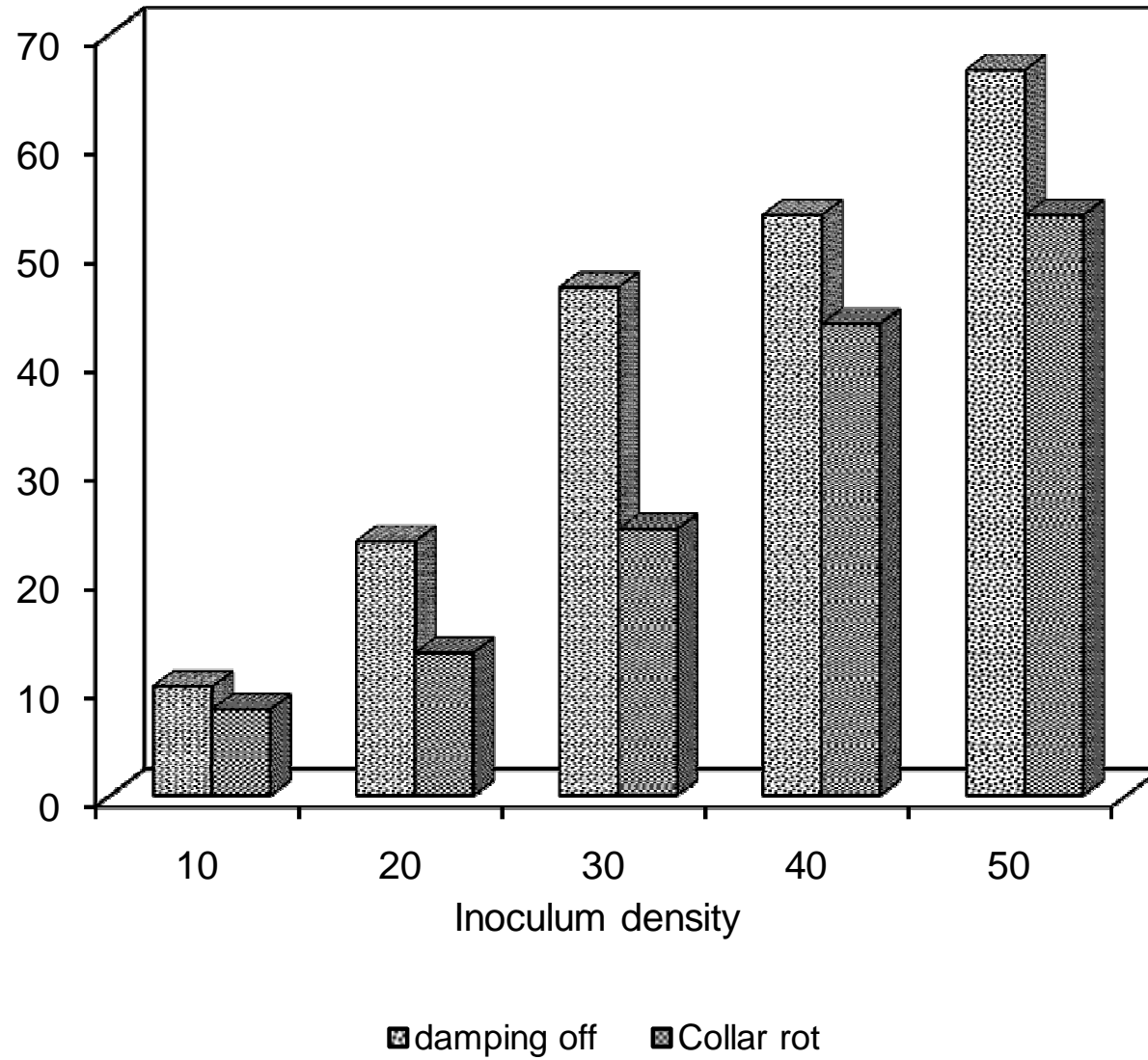
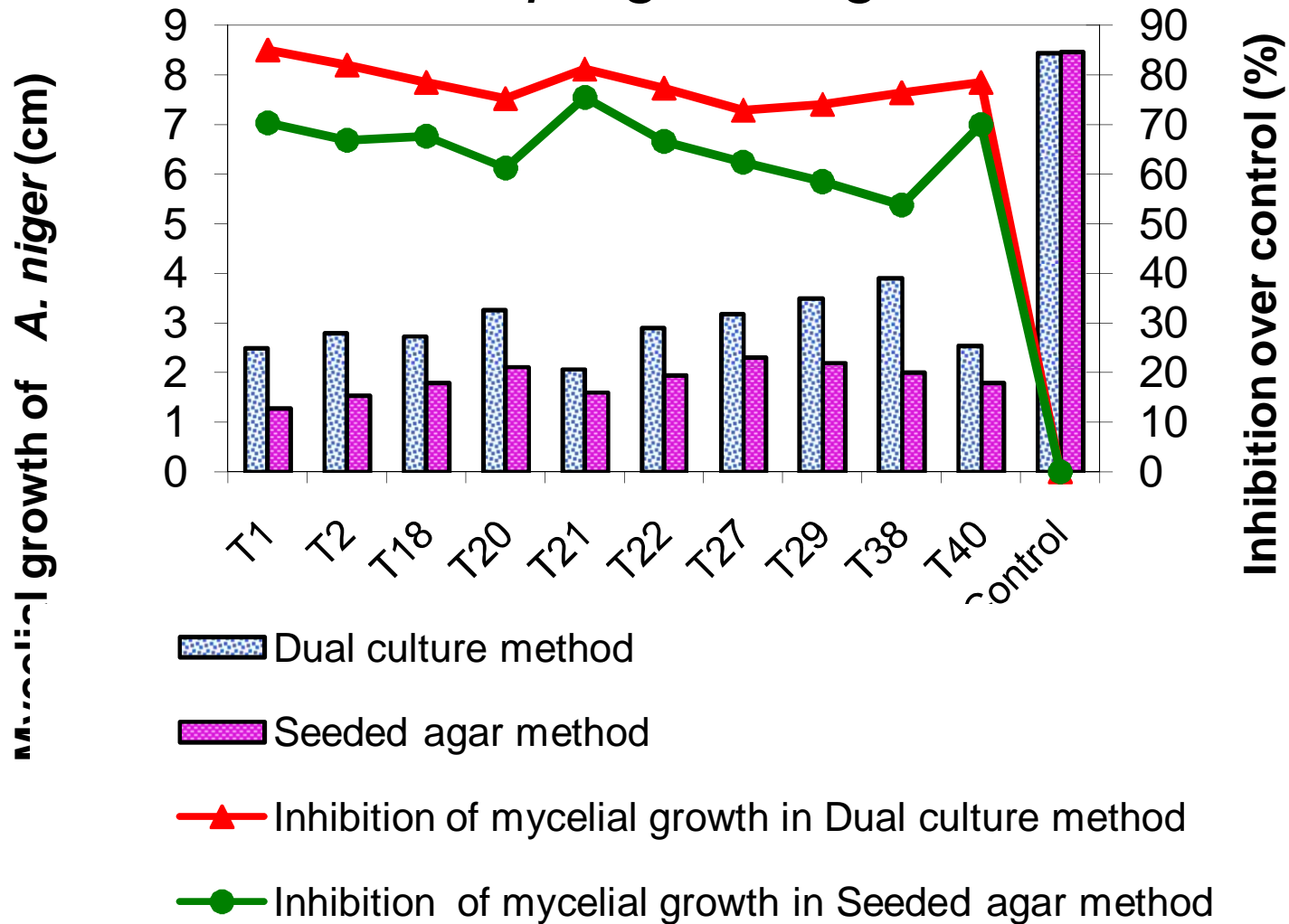
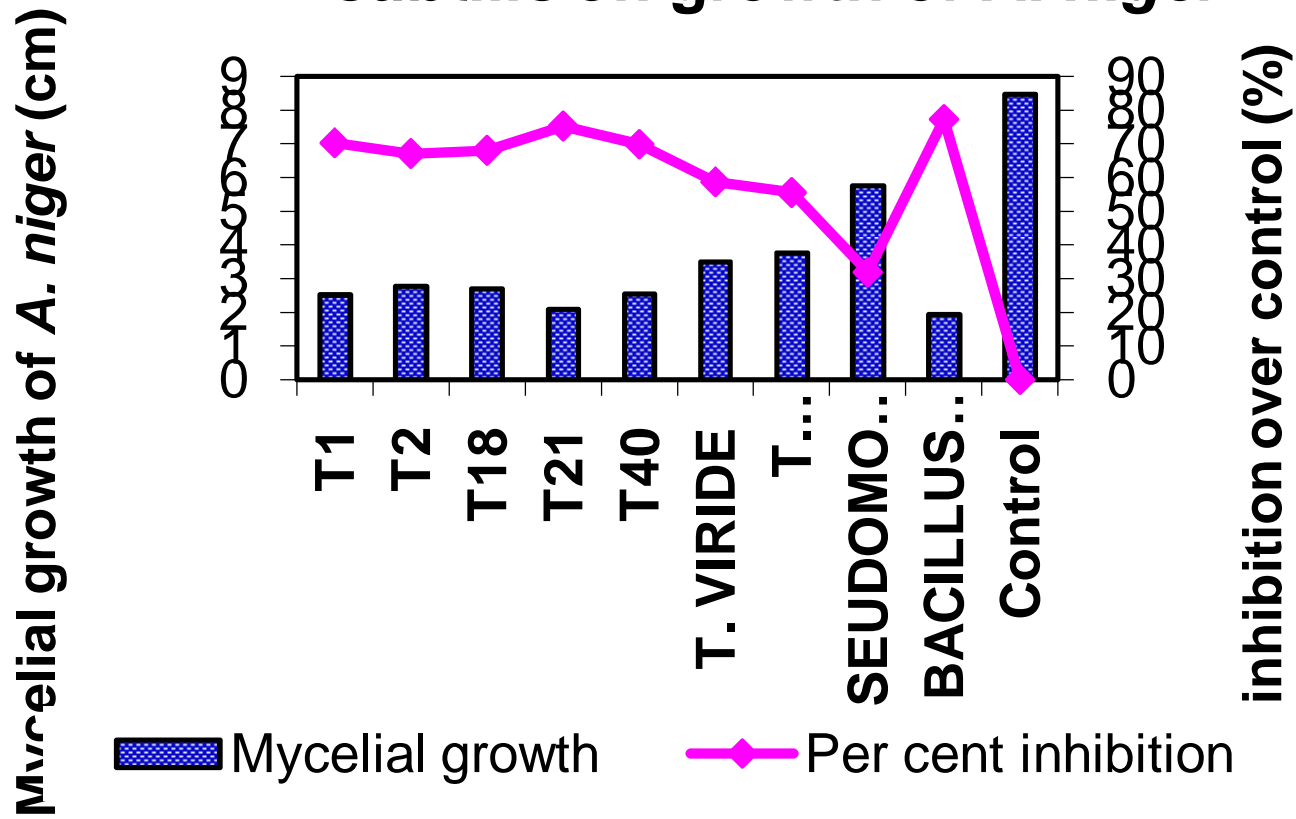


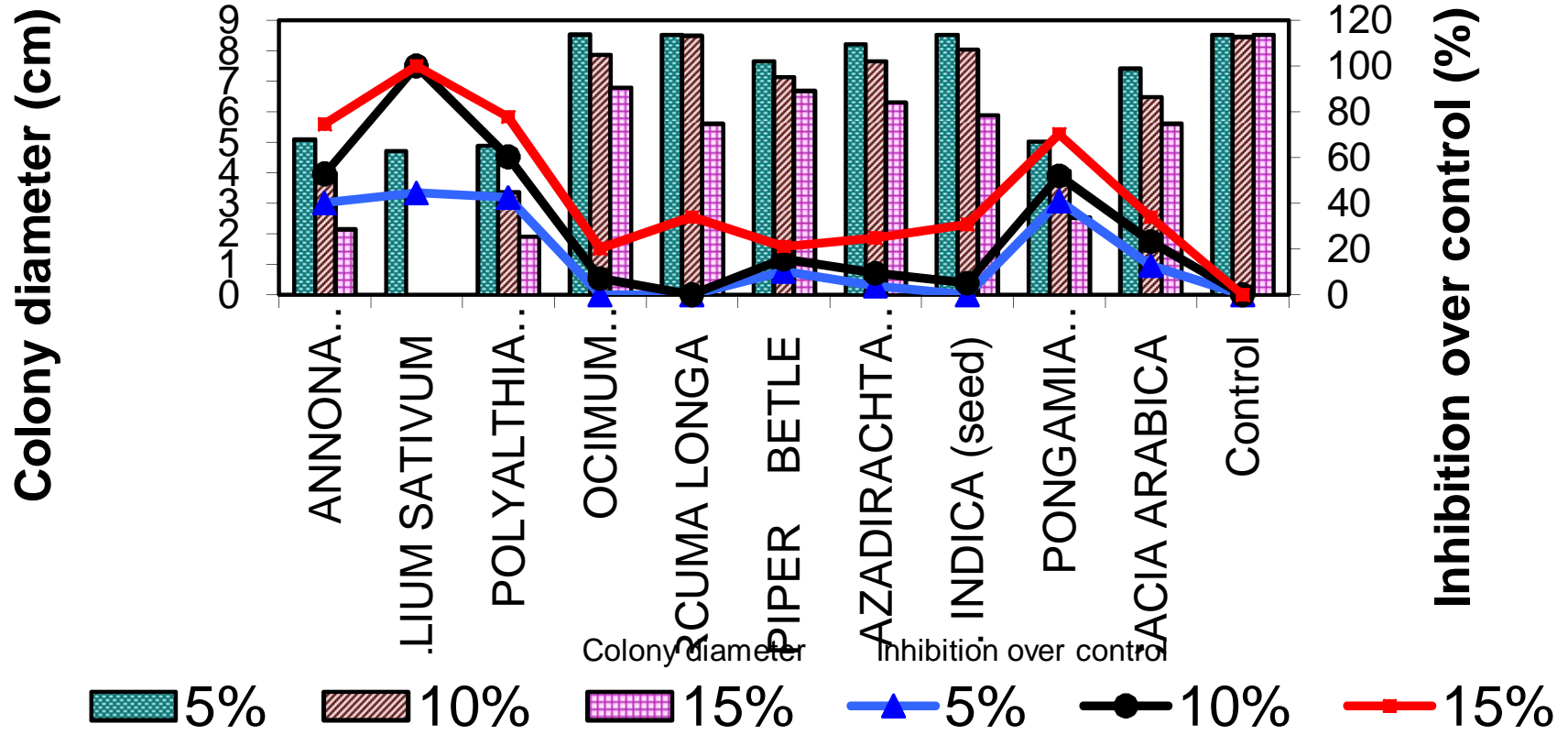
Fig. 5 Effect of *Trichoderma* native isolates on *Aspergillus niger* in vitro



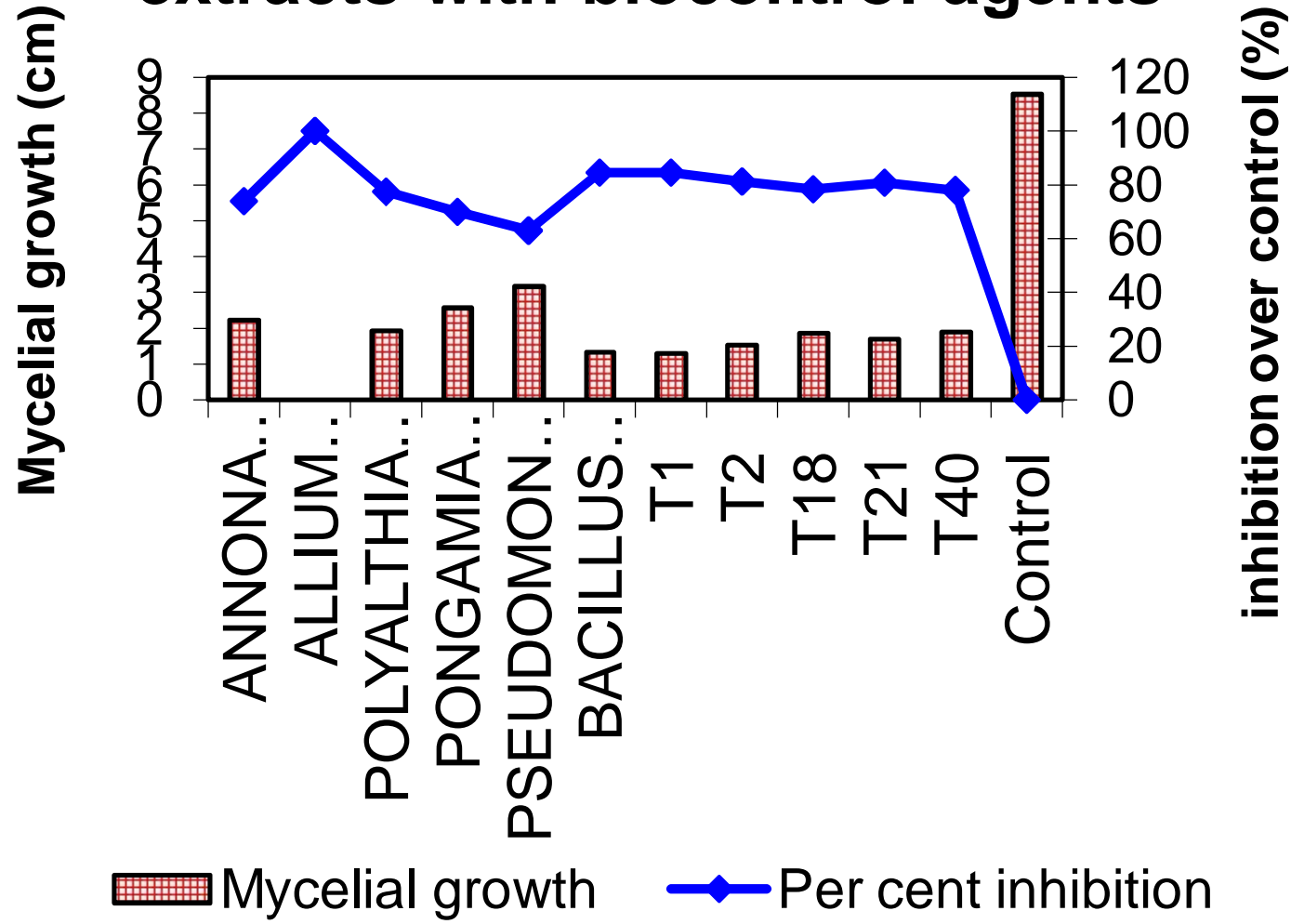
**Fig. 6 Effect of native *Trichoderma* isolates and commercial formulations of *T. viride*, *T. harzianum*, *P. fluorescens* and *B. subtilis* on growth of *A. niger***



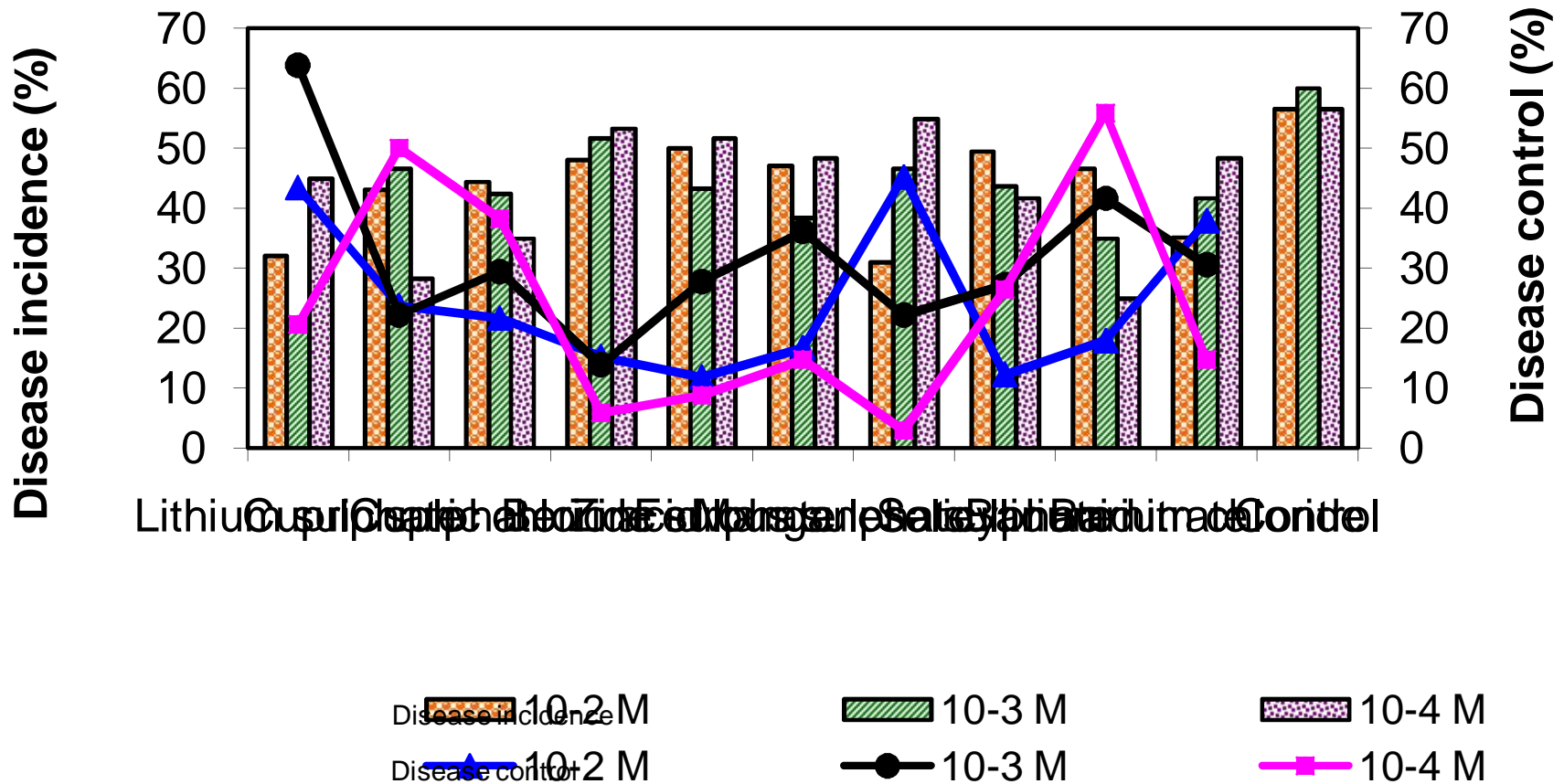
**Fig. 7 Effect of plant extracts on growth of *Aspergillus niger* in vitro**



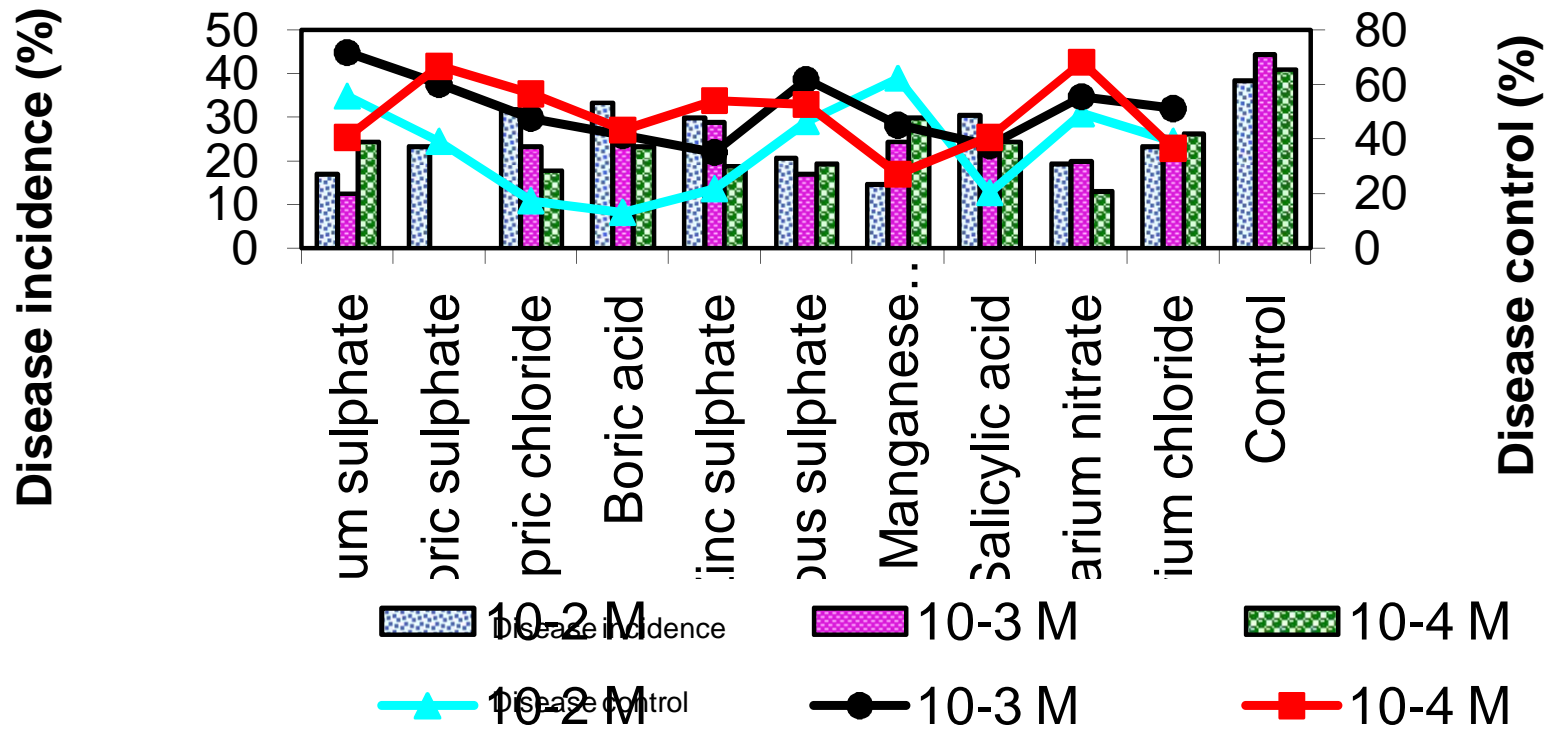
**Fig. 8 Comparison of plant extracts with biocontrol agents**



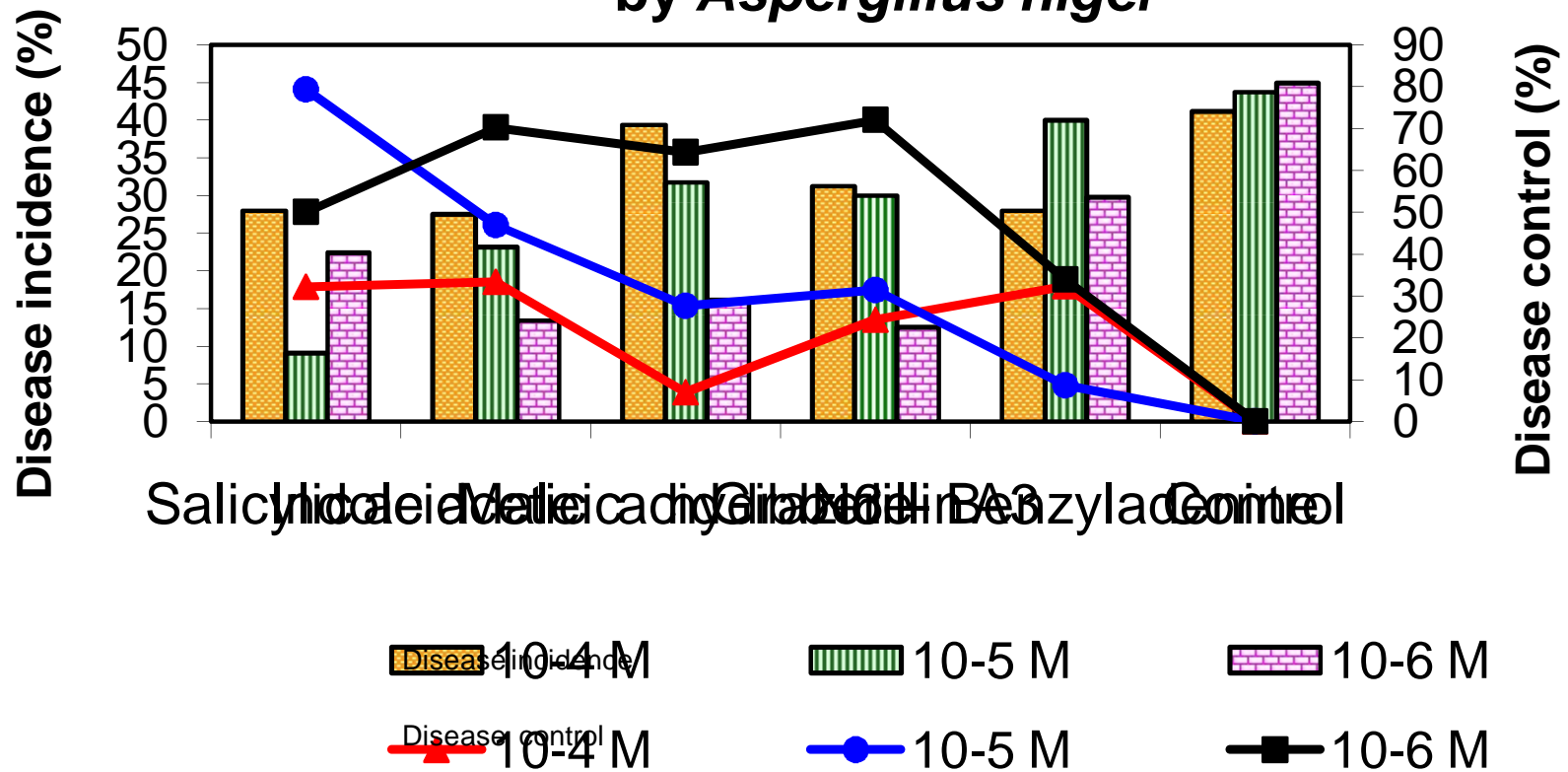
**Fig. 9 Effect seed soaking with heavy metals and weak acids on pre and post-emergence damping off of groundnut caused by *Aspergillus niger***



**Fig. 10 Effect of seed soaking in heavy metals salts and weak acids on groundnut collar rot incidence caused by *Aspergillus niger***



**Fig. 12 Effect of seed soaking with growth hormones on groundnut collar rot incidence caused by *Aspergillus niger***



## CHAPTER – V

### DISCUSSION

#### 5.1 SURVEY

##### 5.1.1 Prevalence of groundnut collar rot caused by *Aspergillus niger* in North Coastal Zone of Andhra Pradesh

The data revealed that there was variation in collar rot incidence in different mandals with a high incidence of 10.3 per cent in Vadisaluru village, Rangampeta mandal of East Godawari district, while it was low (0.3%) in Mahadevipuram village, Vajrapukotturu mandal of Srikakulam district. Among the four districts surveyed, highest mean incidence of 6.1 per cent were recorded in East Godawari and lowest incidence of 2.3 per cent was recorded in Srikakulam district. Collar rot incidence in Visakhapatnam district (0.9%) and Srikakulam district (1.0%) was reported by Rao (1997). Savary (1987) reported to the extent of 2.9 to 19.3 per cent mean disease incidence of *A. niger* in traditional cropping regions of Ivory Coast.

##### 5.1.2 Effect of agroecological factors on groundnut collar rot incidence

###### 5.1.2.1 Soil type

In the present study differences in the collar rot incidence were observed in respect of different soil types. The results (table 3) showed that sandy loam soils recorded maximum collar rot

incidence (5.41%) followed by loams (3.84%) and sandy clay loam (2.64%) whereas clay loam soils recorded very low collar rot incidence (0.97%). Incidence of collar rot due to *A. niger* was reported to be generally high in light sandy soils and similar results were also reported by Gibson (1953) and Chohan (1969). Heavy soils were reported to be least favourable for seed rot and collar rot diseases of groundnut (Ashworth *et al.*, 1964). The aeration of the soil will be influenced by soil texture and the amount of free oxygen available obviously decides the activity of soil borne fungus. A critical stage of oxygen competition between plants and microorganisms arises during seed germination. Sandy soils with more number of macropores compared to clay soils, can hold adequate air though they are poor in water holding capacity (Baver *et al.*, 1962). This could be the probable reason for high percentage of collar rot incidence in sandy loams when compared to clay loams. Reduction in population of groundnut due to collar rot incidence in sandy loam soils of Nellore and Chittoor districts of Andhra Pradesh was also earlier reported (Annual Report of RARS, Tirupati, 1982).

#### **5.1.2.2 Cultivars**

The results presented in Table 4 revealed that the maximum incidence of collar rot was recorded in Spanish bunch cultivars like JL-24 (4.75%) followed by TAG 24 (4.31%) and TMV-2 (3.26%). The

minimum disease incidence (2.3%) was recorded in Virginia bunch type cultivar (K-3).

Mixon (1980) observed less seed colonization by *A. niger* in varieties resistance to collar rot disease than susceptible varieties. Differences in the incidence of aflatoxigenic fungi in different varieties of groundnut were reported earlier (Verma *et al.*, 1996).

Differences among the varieties in respect of the collar rot incidence caused by *A. niger* observed in present study may be attributed to differences in the varietal characters. Pettit *et al.* (1989) expressed that differences in structural and biochemical features of different plant parts could be the reason for differential resistance of different groundnut varieties.

#### **5.1.2.3 Age of crop**

The reaction of the host plant to infection by a pathogen varies with plant age. Data presented in Table 5 revealed that highest mean disease incidence (5.93%) was recorded during the early period of the crop growth (15-20 days). Whereas during the 35-40 days period of crop growth less disease incidence (2.75%) was recorded. It is obvious that the young plants were more prone to disease than aged plants. The findings of the present study were corroborated by Dange and Saradava (1987) who reported that

groundnut seedlings at 2<sup>nd</sup> week were more prone to the attack by *A. niger* than old seedlings.

The earlier reports in several crops indicate that the influence of plant age on disease reaction of host is variable. Ratnoo and Bhatnagar (1992) reported that cow pea plants were more susceptible to anthracnose disease at early stage and susceptibility decreased gradually with increase in age. Chohan and Kaur (1976) observed that certain isolates of *Macrophomina phaseolina* were more aggressive against younger seedlings while some isolates on older seedlings. The relative resistance of the older seedlings to *A. niger* might be due to biochemical changes that occurred in the mature tissue normally (Tarr, 1972). As seedlings at different ages were inoculated and the development of the disease was observed under similar environmental conditions the differences in the degree of susceptibility at different ages may not be attributable to the influence of the environmental conditions.

## **5.2 THE PATHOGEN**

The causal organism was isolated from groundnut plants showing symptoms of collar rot disease and identified as *A. niger* based on the characters of hyaline, septate mycelium and reproductive structures. The characters and measurements of fungal structures were found comparable to those of the species reported earlier (Thom and Raper, 1945; Raper *et al.*, 1965). Many

earlier workers reported *A. niger* as the incitant of collar rot disease in groundnut (Jochems, 1926; Jain and Nema, 1952; Chohan, 1965, 1969). In the present study, the pathogenicity of the fungus was established by artificial inoculation of groundnut seeds with conidial suspension of the fungus (Bhatia and Gangopadhyay, 1999) and also by inoculating the collar region of the seedlings with a mixture of spores and mycelium of the fungus (Gibson, 1953).

### **5.3 SYMPTOMS OF THE DISEASE**

The symptoms of collar rot disease of groundnut seedlings studied were ranged from pre-emergence rotting, death of emerged seedlings and the collar region became dark brown and shredded soon after wilting and sudden death of seedlings. The infected area become water soaked and light brown and were covered with black fungal spores. Similar observations were reported earlier (Boyle, 1951; Jain and Nema, 1952; Gibson, 1953). It was reported that *A. niger* would attack cotyledon, stem, hypocotyls and kernels of groundnut. Pre-emergence rotting of the seeds under favourable conditions was found to be common (Jain and Nema, 1952). The disease was observed to occur only at early stage of growth characterized by wilting and sudden death of seedling (Verma, 1971) and peak death rate occurred about the 17<sup>th</sup> day after planting and that the period of drying of emerged plants extended from about 10 to 30 days (Gibson, 1953).

#### **5.4 EFFECT OF DIFFERENT INOCULUM LEVELS OF *Aspergillus niger* ON PRE AND POST-EMERGENCE DAMPING OFF AND COLLAR ROT INCIDENCE OF GROUNDNUT**

Quantitative evaluation of different inoculum levels of *Aspergillus niger* was studied to find out optimum infection threshold level under prevailing environmental conditions. It revealed that pre and post-emergence damping off and collar rot disease incidence increased from 10.00 to 66.67 and 7.87 to 53.33 per cent, respectively as the inoculum level increased from 10 to 50 per cent. The inoculum level at 50 per cent (50 g inoculum /100 g soil) was found to be optimum infection threshold level causing 64.8 per cent disease incidence.

Narain and Kar (1990) reported 26 per cent infection of *Aspergillus niger* in groundnut pot culture. Malakondaiah (2000) reported that 7.5 per cent (75 g/kg soil) inoculum level was found to be the optimum threshold level for causing collar rot incidence to the extent of 65.00 per cent. The present results clearly demonstrated that the intensity of collar rot incidence is proportionate to the inoculum level, which was in agreement with Baker (1968).

## 5.5 ISOLATION OF *Trichoderma* spp. FROM SOIL SAMPLES COLLECTED DURING FIELD SURVEY

In the present study isolates of *Trichoderma* spp. obtained from soil samples which were collected during the field survey for collar rot disease incidence, showed quantitative differences. The isolates exhibited differences in their growth rate on PDA and Czapek dox medium and showed differences in their morphology based on which, they were categorized into 10 groups. Of the 45 isolates, 10 morphologically different groups were obtained viz., *Trichoderma* isolate T<sub>1</sub>, T<sub>2</sub>, T<sub>18</sub>, T<sub>20</sub>, T<sub>21</sub>, T<sub>22</sub>, T<sub>27</sub>, T<sub>29</sub> T<sub>38</sub> and T<sub>40</sub>.

Srilakshmi *et al.* (2001) obtained 212 isolates from soil samples. Out of 212 isolates, 39 isolates showed antagonism against *Aspergillus flavus* which were identified based on morphology and colony characters.

Kishore *et al.* (2001) obtained sixteen *Trichoderma* isolates from the rhizosphere soil of groundnut plants and were identified into four species aggregates viz., *hametum*, *harzianum*, *longibrachiatum* and *viride* based on morphology and colony characters.

## **5.6 ANTAGONISTIC EFFECT OF ISOLATES OF *Trichoderma* spp. ON GROWTH OF *A. niger* IN VITRO**

In the present investigation, the antagonistic effect of all the 10 isolates of *Trichoderma* against *A. niger* was studied by dual culture and seeded agar methods. Even though all the 10 isolates significantly inhibited growth of *A. niger* over control, differences were found among the isolates in respect of their relative antagonistic potential. The isolates T<sub>21</sub>, T<sub>1</sub>, T<sub>40</sub>, T<sub>18</sub> and T<sub>2</sub> showed more per cent inhibition of growth 75.44, 70.34, 69.99, 67.62 and 66.79 per cent, respectively than others in dual culture method. Where as, in seeded agar method the isolates T<sub>1</sub>, T<sub>2</sub>, T<sub>21</sub>, T<sub>18</sub> and T<sub>40</sub> showed more per cent inhibition of growth with 85.00, 81.93, 81.11, 78.44 and 76.44 per cent, respectively.

There are many well documented studies on the antagonistic effect of *Trichoderma* spp. against the growth of *A. niger* (Chohan and Singh, 1973; Rai *et al.*, 1980; Lashin *et al.* , 1989; Hussian and Lane, 1992; Raju and Krishnamurthy, 2002; Subbiah and Indra, 2003). Chohan and Singh (1973) observed that *Trichoderma polysporum* was parasitic over the colonies of *A. niger*. *T. viride* parasitised *A. niger* by coiling, penetration and hyphal growth inside the conidiophores of *A. niger* (Rai *et al.*, 1980).

Dennis and Webster (1971a, 1971b, 1971c) showed that many isolates of *Trichoderma* spp. produced non volatile antibiotics and

parasitised by coiling around the hyphae of different fungal pathogens. The role of competition, lysis, antibiotics and mycoparasitism in the antagonism exhibited by *Trichoderma* isolates was described earlier by Papavizas and Lumsden, 1980; Elad *et al.*, 1982 and Cook and Baker, 1983.

#### **5.7 COMPARISON OF NATIVE ISOLATES OF *Trichoderma* spp. WITH COMMERCIAL FORMULATIONS OF *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* AND *Bacillus subtilis* IN THEIR EFFECT ON GROWTH OF *Aspergillus niger***

Significant differences were found between the native isolates of *Trichoderma* and commercial formulations of *T. viride*, *T. harzianum*, *P. fluorescens* and *B. subtilis* in respect of antagonistic effect against *A. niger*.

However, the results showed a clear cut superiority of native isolates of *Trichoderma* over the commercial formulations of *T. viride*, *T. harzianum* and *P. fluorescens*. The formulated product of *B. subtilis* and T<sub>21</sub> isolate showed significantly more antagonistic effect against the growth of *A. niger* than the remaining biocontrol agents. Commercial formulations of *P. fluorescens* was observed to be least effective than all other biocontrol agents.

Based on these findings it can be assumed that marked differences were present between native isolates and commercial formulations of biocontrol agents which were relatively antagonistic against *A. niger*. Ramarathinam *et al.* (2000) reported antagonistic effect of commercial formulation of *Trichoderma* was directly correlated with agroclimatic conditions of particular site and season.

#### **5.8 EFFECT OF PLANT EXTRACTS ON THE GROWTH OF *Aspergillus niger* IN VITRO**

The efficacy of natural plant extracts against various plant pathogens has been catching the imagination of several investigators. Although the work is neither expected to revolutionize plant protection techniques nor produce effective substitute for chemicals over night, but it is expected to stimulate the scientists and industrialists to realize that potential alternative, which is less hazardous. In this context the information obtained in the present investigation can be useful to study the anti-fungal characters of the plant extracts.

In the present investigation effect of 10 plant extracts were studied on growth of *A. niger*. It was observed that out of 10 plant extracts tested, *A. sativum* was most effective in reducing the growth of *A. niger* (100%) followed by *Polyalthia longifolia*

(97.73%), *Annona squamosa* (74.61%) and *Pongamia glabra* (70.33%).

The findings of the present study were reinforced by the observations of Arun *et al.* (1995) who reported the fungitoxic activity of *Allium sativum* against the *A. niger*, while Radha *et al.* (1998) found marked inhibition of mycelial growth of *Colletrichum falcatum* by *Allium sativum*. Sobti *et al.*, 1995; Mishra and Tewari, 1992; Annapurna *et al.*, 1983 reported the antifungal activity of seed extract of *Polyalthia longifolia* on *A. niger*.

The fungitoxicity of the *A. sativum* bulb extract in the present study might be due to antifungal substances present in garlic namely sulphur containing compounds *viz.*, allicin, allylpropyl disulphide, diallyl disulphide or due to an enzyme, allinase (Radha *et al.*, 1998) and the organic fraction of *A. sativum*, allicin was more effective against *A. niger* (Arun *et al.*, 1995).

#### **5.9 COMPARISON OF NATIVE ISOLATES OF *Trichoderma* spp. AND BACTERIAL ANTAGONISTS (*Pseudomonas fluorescens* AND *Bacillus subtilis*) WITH PLANT EXTRACTS ON THEIR EFFECT ON GROWTH OF *A. niger* IN VITRO**

In the present study, native isolates of *Trichoderma* spp., and plant extracts of *Annona squamosa* (15%), *Allium sativum* (10%), *Polyalthia longifolia* (15%) and *Pongamia glabra* (15%) were

tested for their efficacy against *A. niger* which were proved effective from the previous experiments along with other bacterial antagonists like *Pseudomonas fluorescens* and *Bacillus subtilis*.

The isolates of *Trichoderma* spp., *P. fluorescens*, *B. subtilis* and plant extracts showed inhibition of growth of the *A. niger* which ranged from 62.95 to 100.00 per cent. *A. sativum* showed the highest inhibition of growth of *A. niger* (100.0%) followed by *B. subtilis* and T<sub>1</sub> isolate, which caused 84.52 and 84.49 per cent growth reduction of *A. niger*, respectively. The lowest record of inhibition (62.95%) was by *P. fluorescens*. According to Jacob *et al.* (1988) plant extracts were more effective than the biocontrol agents against *Pythium aphanidermatum*. Humanthegowda and Adiver (2001) found that the plant extracts of *Parthenium hysterophorus* and *Polyalthia longifolia* were more effective than *T. harzianum* against the *Sclerotium rolfsi*.

## **5.10 NON CONVENTIONAL CHEMICALS**

Among the several adverse effects caused by *Aspergillus niger* on groundnut, pre and post-emergence damping off and collar rot were wide spread. Several workers have tried to manage this damage by seed dressing with different fungicides (Wadibhasme *et al.*, 1991; Rao *et al.*, 1998; Raju *et al.*, 2002). However, it was observed that seed dressing provided protection only for a limited period. Although, application of fungicides to soil and plants can

cause disease reduction. They are a threat to the environment, crop ecology, soil biology and human health. Hence, an attempt was made in the present study an experiment was conducted to reduce the seed and seedling losses caused by *A. niger* through inducing the systemic defence mechanism in host by non conventional chemicals.

#### **5.10.1 Effect of heavy metal salt and weak acids on pre and post-emergence damping off and collar rot incidence caused by *Aspergillus niger***

In the present study, 10 heavy metal salts and weak acids *viz.*, Lithium sulphate, Cupric sulphate, Cupric chloride, Boric acid, Zinc sulphate, Ferrous sulphate, Manganese sulphate, Salicylic acid, Barium nitrate and Barium chloride were tested each at different concentrations *viz.*,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  M for their effect on pre and post-emergence damping off and collar rot caused by *A. niger*. All the chemicals caused a significant reduction in pre and post-emergence damping off and collar rot incidence (Table 13 and 14). Among the chemicals at different concentrations disease incidence was varied. There is a little direct relationship between concentration of their chemicals and their protective effect. A maximum of 63.88 per cent pre and post-emergence damping off disease control was recorded with Lithium sulphate ( $10^{-3}$ M) followed by Barium nitrate ( $10^{-4}$ M) and Cupric sulphate ( $10^{-4}$ M) with 55.88 and 50.00 per cent, respectively. Where as highest reduction of

collar rot disease was observed when the seeds were treated with Lithium sulphate ( $10^{-3}$ M) 71.57 per cent over control followed by Barium nitrate ( $10^{-4}$ M), Cupric sulphate ( $10^{-4}$ M), Manganese sulphate ( $10^{-2}$ M), Cupric chloride ( $10^{-4}$ M) and Zinc sulphate ( $10^{-4}$ M) with 66.69, 62.22, 56.58 and 54.05 per cent, respectively. The results were in agreement with the findings of Dasgupta *et al.* (1999; 2000) who observed that pre and post-emergence damping off and collar rot of groundnut were effectively reduced disease incidence when seeds were pre soaked in heavy metal salts. The disease controlling activity of the non conventional chemicals might be due to the rapid development of fungitoxic substances in treated plants. Production of such substances in susceptible tissue might be due to a conditioning effect of treatment with heavy metal salts which made susceptible plants respond to infection like resistant plants by producing large amounts of fungitoxic substances (Giri and Sinha, 1983) and the protection might be due to the certain biochemical changes in treated plants like increased levels of phenolics, peroxides and reduced activity of polygalactronase enzyme (Dasgupta *et al.*,1999). It appeared that the wet seed treatment with these 10 compounds not normally used for plant protection and also having no or little fungitoxicity at the concentrations employed could considerably limit the disease development in groundnut. These observations coupled with the fact that test compounds showed a fairly strong effect at diluted concentrations imply that in controlling collar rot these chemicals

might have acted by conditioning susceptible groundnut plants for a more vigorous and dynamic defense response to the pathogen. Such conditioning of the host might be based on the activation of the latent defense potential that normally remain suppressed in a compatible host-pathogen interaction and this would mean induction of resistance. The acquired dynamic defence appeared to be the most significant aspect of activated resistance by biological induction of resistance (Kuc, 1981) and chemical activation of resistance (Lazarovits, 1988). These chemicals are non toxic, non hazardous and targetted at the host parasite complex and act only indirectly on the pathogen through the host, the chances of pathogen developing any tolerance to them are remote and they also not expected to have any ill effects on the non target bio ecosystem as quite often happens with conventional plant protection chemicals.

#### **5.10.2 Effect of plant hormones on pre and post emergence damping off and collar rot disease incidence caused by *Aspergillus niger*.**

In the present study five growth hormones *viz.*, Salicylic acid, Indole Acetic Acid, Maleic hydrazide, Gibberilin A<sub>3</sub> and N<sup>6</sup>-benzyladenine were treated at three concentrations *viz.*, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup>M separately for their effect on pre and post-emergence damping off and collar rot disease incidence caused by *A. niger*.

Perusal of data in Table 15 and 16 revealed that there was significant influence of chemicals on pre and post-emergence damping off and collar rot disease incidence. Salicylic acid ( $10^{-5}$ ) was most effective by reducing 75.00 per cent of pre and post-emergence disease incidence followed by Gibberellin A<sub>3</sub> ( $10^{-6}$ M) and Malic hydrazide ( $10^{-6}$ M) were recorded 63.64 and 59.09 per cent disease reduction over untreated control, respectively where as maximum reduction of collar rot disease incidence was observed when seeds were pre-soaked with Salicylic acid ( $10^{-5}$ M) 79.36 per cent over control followed by Gibberellin A<sub>3</sub> ( $10^{-6}$ M), Indole acetic acid ( $10^{-6}$ M) and Maleic hydrazide ( $10^{-6}$ M) which recorded 72.02, 70.24 and 64.29 per cent, respectively.

The findings of the present study were corroborated by Dasgupta *et al.* (1998) who reported that Chitosan (0.3%), 2,4,5-trichlorophenoxy acetic acid ( $10^{-6}$ M) and Indole Acetic Acid ( $10^{-5}$ M) more effective against the pre-emergence seed rot and collar rot infection. Chowdhury (1995) reported that Indole acetic acid was more effective and reduced the rust symptoms in groundnut. Indole acetic acid ( $10^{-5}$ M) and cycloheximide ( $10^{-6}$ M) were most effective in reduction of *fusarium* wilt infection in chickpea (Chowdhury, 2000). In case of sheath rot of rice Ghoshal and Purakayastha (1984) reported that Gibberellin reduced the disease incidence by conditioning the susceptible host cells.

From the above discussion, the following conclusions would be drawn

1. In North Coastal zone of Andhra Pradesh, groundnut collar rot incidence varied from 0.3 per cent to 10.3 per cent and light textured soils, Spanish bunch cultivars and in early period of crop growth recorded highest disease incidence.
2. Native isolates of *Trichoderma* spp. were superior than commercial formulations of *T. viride*, *T. harzianum* and *Pseudomonas fluorescense* in reduction of growth of *Aspergillus niger*. But, the commercial formulations of *Bacillus subtilis* was most effective *in vitro*.
3. Growth of *Aspergillus niger* was completely checked by bulb extract of *Allium sativum* at 10 per cent concentration *in vitro*.
4. Groundnut pre and post emergence damping off and collar rot incidence was significantly reduced by wet seed treatment with Lithium sulphate ( $10^{-3}$ M), Barium nitrate ( $10^{-4}$ M), Salicylic acid ( $10^{-5}$ M) and Gibberlin A<sub>3</sub> ( $10^{-6}$ M).

## CHAPTER – VI

### SUMMARY

In the present investigation, collar rot incidence was recorded during field survey in North Coast Zone of Andhra Pradesh. In general, the disease incidence ranged from 0.3 to 10.52 per cent. Highest mean incidence of 6.04 per cent was recorded in uplands of East Godavari followed by Vizianagaram (4.53%), Visakhapatnam (3.13%). The lowest incidence of 2.32 per cent was recorded in Srikakulam district.

Among the four soil types surveyed, sandy loams recorded the highest disease incidence (5.49%). Groundnut cv. JL 24 was the most susceptible variety and recorded 4.75 per cent disease incidence. In general, highest collar rot disease incidence was observed during the early period of crop growth (15-20 days).

The causal organism of groundnut collar rot was isolated from diseased groundnut plants and identified as *A. niger* based on morphology and colony characters of the fungus.

Among the five levels of inoculum tested to know the infection threshold of *A. niger*, 50 per cent inoculum level was found to be optimum with 66.67 and 53.33 per cent pre and post-emergence damping off and collar rot incidence, respectively in pot culture experiments.

Forty five isolates of *Trichoderma* spp. were isolated from soil samples collected from groundnut growing fields of North-Coastal Zone of Andhra Pradesh and of them 10 morphologically different isolates were identified.

The native isolates of *Trichoderma* spp. significantly inhibited the growth of *A. niger* over control. The isolates T<sub>1</sub>, T<sub>2</sub>, T<sub>18</sub>, T<sub>21</sub> and T<sub>40</sub> recorded more inhibition than others in dual culture and seeded agar methods. The extent of inhibition was more pronounced in seeded agar method than in dual culture method.

The native isolates and commercial formulations of *T. viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were compared *in vitro* for their antagonistic effect against growth of *A. niger*. The native isolates of *Trichoderma* spp. showed a clear cut superiority over commercial formulations. But the commercial formulation of bacterial antagonist *Bacillus subtilis* showed significantly more antagonistic effect on the growth of *A. niger* than native isolates. *P. fluorescens* was least effective.

In the present study, increase in the inhibition of growth of *A. niger* was observed with increase in the concentration of plant extracts used. Extract of *Allium sativum* was most effective in inhibiting the growth of *A. niger* followed by *Polyalthia longifolia*, *Annona squamosa* where as extracts of *Ocimum sanctum*, *Curcuma*

*longa* and seed extract of *Azadirachta indica* were found to be least effective on growth of *A. niger*.

The efficacy of plant extracts *viz.*, *Annona squamosa*, *Allium sativum*, *Polyalthia longifolia* and *Pongamia glabra* were compared with the native isolates of *Trichoderma* spp. and bacterial antagonists *P. fluorescens* and *B. subtilis* in inhibiting the mycelial growth of *A. niger*. *A. sativum* was the most effective than the biocontrol agents followed by *B. subtilis* and T<sub>1</sub> isolate. No significant difference was observed among *B. subtilis* and T<sub>1</sub> isolate.

Seed soaking with heavy metal salts and weak acids significantly reduced the pre and post-emergence damping off and collar rot disease incidence over control. Lithium sulphate (10<sup>-3</sup> M), Barium nitrate (10<sup>-4</sup>M), Cupric sulphate (10<sup>-4</sup>M) reduced the pre and post-emergence damping off to the extent of 63.88, 55.88 and 50.00 per cent, respectively and were most effective than all other tested chemicals. Collar rot incidence was reduced by Lithium sulphate (10<sup>-3</sup>M), Barium nitrate (10<sup>-4</sup>M), Cupric sulphate (10<sup>-4</sup>M) and Zinc sulphate (10<sup>-4</sup>M) to the extent of 71.57, 68.36, 66.93, 62.22, 56.58 and 54.04 per cent, respectively.

Seed treatment with growth hormones significantly reduced the pre and post-emergence damping off and collar rot incidence over control. Salicylic acid ((10<sup>-5</sup>M), GibberillinA<sub>3</sub> ((10<sup>-6</sup>M) and

Maleic hydrazide ( $10^{-6}\text{M}$ ) were found effective in reducing pre and post-emergence damping off with 75.00, 63.64 and 59.09 per cent reduction, respectively. Collar rot incidence was effectively reduced by Salicylic acid ( $10^{-5}\text{M}$ ), Gibberillin  $A_3$  ( $10^{-6}\text{M}$ ), Indole acetic acid ( $10^{-6}\text{M}$ ) and Maleic hydrazide ( $10^{-6}\text{M}$ ) to the extent of 79.36, 72.02, 70.24 and 64.29 per cent, respectively.

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