INVESTIGATION ON THE COLLAR ROT DISEASE OF GROUNDNUT CAUSED BY *Aspergillus niger* van Teighem AND ITS MANAGEMENT IN WEST BENGAL

A THESIS
SUBMITTED TO THE
BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
PLANT PATHOLOGY

BY

SUKANTA DAS GUPTA

DEPARTMENT OF PLANT PATHOLOGY
FACULTY OF AGRICULTURE
BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
MOHANPUR, NADIA,
WEST BENGAL
1995

Dedicated

to my

Parents

BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
FACULTY OF AGRICULTURE

Satya Kinkar Raj, M.Sc. (Ag.), Ph.D.
Reader in Plant Pathology

Dept. Of Plant Pathology
Mohanpur, Nadia, W.B.

Date :

CERTIFICATE

This is to certify that the work recorded in the thesis entitled Investigation on the collar rot disease of groundnut caused by Aspergillus niger Van. Teighem and its management in West Bengal submitted by Sri Sukanta Das Gupta for the award of the Degree of Doctor of Philosophy in Plant Pathology of the Bidhan Chandra Krishi Viswavidyalaya, is record of faithful and bonafide research work carried out by Sri Das Gupta under my direct supervision and guidance. The result of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation have been duly acknowledged.

25/12/95

(**Satya Kinkar Raj**)
Supervisor

Acknowledgment

I avail myself of the opportunity to express my deepest sense of gratitude to Dr. S.K.Raj, Reader in Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya for his noble supervision, constant encouragement, ceaseless and liberal guidance and sustained interest throughout the entire course of this investigation.

I greatfully acknowledge the valuable advice and helpful suggestions of Prof. A.K.Sinha, Prof. C. Sen, Prof. S. Chowdhuri, Prof. S. Mukhopadhyay, Prof. N. Mukherjee, Prof. P.K.Sengupta, Dr. D.K.Chakraborty, Dr. D.C.Khatua and Dr. A.K.Chowdhuri,

I am greatful to those Plant Pathologists whose research papers greatfully influenced my concepts, philosophies and interpretations in this regard.

I am greatful to Sri S. Chatterjee and all other research scholars of the department for their continuous encouragement and help during the course of study.

Words are quite inadequate to express my indebteness to my beloved parents, brothers and sister for their blessings and inspiration.

I do express my deepest sense of gratitude to Smt. J. Dasgupta for her constant help and inspiration.

Finally the financial assistance in the form of Research Scholarship of B.C.K.V is duly acknowledged.

Sukanta Das Gupta

Date : 28th Dec '95

(SUKANTA DAS GUPTA)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

10.	Plate-10	Growth and Sporulation of 5 different isolates of A. niger on CDA medium at different pH.
11.	Plate-11	Growth and Sporulation of 5 different isolates of A. niger on CDA medium at different pH.
12.	Plate-12	Sclerotia produced by A. niger on surface of inoculated soil.
13.	Plate-13	Overgrowth of T. harzianum on colony of A. niger
14.	Plate-14	Overgrowth of T. harzianum on colony of A. niger in dual culture
15.	Plate-15	Groth of T. harzianum on Sclerotium of A. niger
16.	Plate-16	Groth of T. harzianum on Sclerotium of A. niger
17.	Plate-17	Groth of T. harzianum on Sclerotium of A. niger
18.	Plate-18	Showing normal germination of sclerotium of A. niger (Left) and inhibited germination of sclerotia of A. niger by T. harzianum (Right)
19.	Plate-19	Inhibited germination of sclerotium of A. niger by T. harzianum
20.	Plate-20	Inhibited germination of sclerotium of A. niger by T. harzianum

INTRODUCTION

Groundnut or peanut (Arachis hypogaea L.) is one of the most important crops in dry land agriculture of Asia, central and South America and Africa. This crop is grown on nearly 20 million hectares under a very wide range of environmental conditions in all the continents between 40 degree N and 40 degree S of equator. Annual world production of fruits (kernels in shell) is about 18.5 million tonnes, of which about 70% consists of seeds. More than 70% of the total production comes from developing countries. Although yield in experimental farm may reach 4-5 tonnes/ha, that in farmers field in the tropics varies between 0.5 and 1.5 tonnes/ha. India produced 70.66 lakh tonnes in 1991-92(INDIA 1993, Published by Ministry of Information and broadcasting, Govt. Of India).

Groundnut provides about 20% of the total world vegetable oil production, used primarily for cooking as such or after hydrogenation. It is used both as refined oil and in vanaspati industry. It is also used in soap, cosmetics, lubricant industries. Kernels are also eaten as such, roasted or sweetened. They are rich in protein and vitamin A,B and some members of Vitamin B-12 group.

Their calorific value is 349 per 100 grams. After oil extraction the residual cake is an excellent animal feed, with high nitrogen content.

Different seed borne mycoflora cause various types of damage to seeds and seedlings under congenial condition in storage and in field. In storage, infected seeds loose their viability. In the field Aspergillus niger causes collar rot or crown rot. A. flavus causes aflaroot disease of seedlings. Further more, a toxin, afla-toxin produced by A. flavus is also toxic to animals and human beings. Rhizopus spp. always cause seed rotting. Many other micro organisms are also associated with the seeds which although may not be pathogenic to the host, play an important role in seed germination, plant stand and initial health of the plant.

Crown rot disease of groundnut, also known as collar rot or Aspergillus blight is caused by Aspergillus niger van.Teighem. It is most prevalent pathogen wherever groundnut is cultivated. Being both seed and soil borne it causes considerable seedling mortality in the early stage of crop growth, thus reducing the stand, there by affecting the pod yield and causing economic loss to the farmers. Crown rot of groundnut causes in some areas upto 50% yield loss.

Groundnut cultivation is gaining importance as oilseed crop in this state because of enhanced irrigation facilities. For repeated cultivation of groundnut for years in same field, inoculum density of A niger increases at a constant rate and currently threatening groundnut production. Although some fungicides are capable of checking seed rot and pre-emergence damping off, but considerable losses are still caused in the

Plate-1 Collar rotted groundnut seedlings

Plate-2 Collar rotted groungnut seedlings

post-emergence stage. The only effective way to manage the disease is to grow resistant varieties. In the present study screening of resistant varieties was undertaken with the following objectives :

i) to confirm, under artificial conditions of epiphytotics, the extent of resistance of the varieties that exhibited resistance in earlier studies.

ii) to locate fresh sources of resistance in the groundnut germplasms collected from National Research Centre for Groundnut, Timba wandi, Junagadh, Gujarat, India and

iii) to use the resistant germplasm in further breeding for crown rot resistance.

Biological control of soil borne diseases is a popular and challenging goal and has been a focus for research over many years. Biological control is attractive in an environmental and economic sense because it offers durable, safe and cost-effective alternative to soil applied chemicals.

Trichoderma harzianum has been used against various plant pathogens in different crops (Harder et al., 1979; Henis et al., 1979; Lewis and Papavizas, 1980; Well et al., 1972; Backman and Rodriguez Kabana, 1975; Gupta et al., 1979). There are several positive examples of successful application of biocontrol agents (Cook and Baker, 1983).

During the course of isolation of mycoflora from rhizosphere soil of groundnut, it was observed in Petri-dishes that Trichoderma grew frequently over the colonies of A. niger. When hyphae were mounted from this zone and seen under light microscope, frequent coiling around the conidiophores of A. niger was observed by hyphae of T. harzianum. Therefore, under present investigation the mycoparasitism of T. harzianum against A. niger and its efficacy as biocontrol agent against crown rot disease of groundnut caused by A. niger was studied.

At present, the use of toxic chemicals probably constitutes the most common approach to plant disease control. The ever increasing and sometimes the injudicious application of such toxic chemicals as a part of the normal crop husbandry practice, pose a potential threat to nature and life because of resultant pollution of our environment. Most of them are severely hazardous for ecological balance in nature. Further, complexity develops from the fact that some pathogens are developing resistance or tolerence to some of the fungicides, particularly the systemic ones, because of too frequent exposure to these compounds. As a result of this, newer and newer fungcides are being developed and used and this aggravates the situation. A conventional and at the same time safer and more dependable approach involves the use of disease resistant varieties, but suitable resistant varieties are neither always available nor easy to develop. Not only that, long period is needed to produce such varieties, but a resistant

variety rarely survives the attack from different races of the pathogen for long and suffer great damage. So, in most cases of disease outbreak in severe form there is little alternative left but to resort to chemical control by the use of such toxic substances inspite of their hazardous after effects. In this situation there is a growing feeling that fresh avenues must be explored and some alternative methods developed that will be safer, less hazardous, economical and also easy to apply. Use of non-toxic or mildly toxic chemicals that may be systemic in action and alter the host metabolism in a direction unfavorable to the pathogen or strengthen its defense potential and thus limit the pathogen's activity before much damage occurs in the plant, may be one of the possible approaches in this direction. Attempt to induce resistance in plants or to stimulate their natural defence mechanism by different treatments may be worthwhile proposition and may provide a safer measure for plant disease control in the long run. The term induced resistance means that resistance is induced in the susceptible varieties of plants against their pathogen or prior interaction with certain parasitic organisms, related or unrelated, their metabolites, viruses and various chemicals.

The above alternative approach to chemical control has been extensively investigated at the plant pathology laboratory, Bidhan Chandra Krishi Viswavidyalaya using phytoalexin induce

chemicals as the `inducer' for the development of resistance in plant hosts against a diverse group of pathogens.

Observations indicated that such treatments conditioned susceptible host towards more vigorous responses to infection including greater accumulation of phytoalexin type fungi toxic substance and/or some other metabolic changes that characterised the defence responses of resistant plants.

This appears to be a case of activation of host's innate defence potential that normally remains suppressed in compatible host pathogen interactions.

In the background of such information, present investigation programme was taken with the following specific objectives.

i) to make comparative study of the effect of seed soaking with some heavy metal salts, on induction of resistance in groundnut as a disease control measure against crown rot disease caused by A. niger.

ii) to study certain biochemical responses of plants in different treatments to infections with a view to determine if any correlation exists between the changes in such responses and the induction of resistance, and

iii) to study their effect on various components of yield of crop.

CHAPTER - II

REVIEW OF LITERATURE

The pathogen :

Aspergillus niger is a soil and seed borne pathogen, causing seed rot, collor rot disease of groundnut (Arachis hypogaea L.) in tropical climate. Collar rot is also known as crown rot. Many workers have worked on various aspects with the diseases on groundnut in our country and abroad. Those works may be summarised as follows :

1. Varietal response to A. niger infection :

Jochems (1926) first described the disease crown rot caused by A.niger in groundnut and used inoculation technique with A . niger to develop disease symptoms on groundnut plant artificially, later on Chohan et al. (1969,1970) inoculated a large number of groundnut varieties using two techniques : seed inoculation and soil inoculation developed in Punjab Agril. University, Ludhiana. To test the world collection of groundnut germplasms they took 734 varieties to screen against the crown rot disease. Of which 20 germplasms showed complete freedom from the attack of crown rot pathogen A . niger. The others

varieties showed the incidence of crown rot varying from 3.7 to 100 per cent. In 34 germplasms no plant escaped the attack. While conducting further test with 20 groundnut germplasms they found similar reaction as compared to earlier results. Kirpal et al. (1970) found only one exotic variety from Sudan to be significantly resistant to the disease. 40 groundnut varieties, previously found resistant to or tolerant to A. niger, were tested again for their reactions to this fungus under artificial epiphytotic conditions by Chahal et al. (1974).

They found only two varieties showed resistant, four tolerant and thirty four susceptible reactions with the pathogen. From a four year trial with 44 groundnut varieties Mathur et al. (1970) screened out only 5 varieties that showed least susceptible reaction, 36 varieties moderately susceptible and 3 varieties were most susceptible to A. niger . They also recorded that spreading types were more susceptible than bunching ones. Mixon (1950) made a comparative study using 3 screening methods for evaluating pod and seed infections of different groundnut genotypes by Aspergillus sp. which revealed that different genotypes had different levels of resistance in seed colonisation. With all 3 methods seed colonisation was considerably less for resistant genotypes. Seed colonisation of the more susceptible genotypes was noticeably less with pod inoculation than with seed inoculation. Dange and Saradava (1985) screened out in sandy soil, 5 cultivars resistant to

seed rot as well as collar rot from 24 groundnut cultivars. Pettit et al. (1989) studied the extent of damage caused by A. niger to pegs, shells and kernels of different groundnut cultivars. They also observed structural and biochemical changes of infected groundnut plant parts that may be employed as a tool for assessing varietal reaction to the pathogen.

ii. **Survival of A. niger in soil** :

Chohan (1967) studied the survival of A. niger, var. Teighem and the soil factors influencing it. The fungus survived in soil wherein it served as the source of primary inoculum. It remains viable even after 15 months storage at the various levels of relative humidity, but it looses its power of growth at 100 per cent R.H. after 2 months of storage. In sterilised soil, it remained viable upto 9 months storage at various levels of soil moisture.

iii. **Physical injury of seeds as a predisposing factor for crown rot disease in grountnut** :

Several workers reported that seed or seedling injury predisposed the groundnut seedlings to A. niger infection. Ashworth et al. (1964) stated non-injured and mechanically injured plants were only slightly prone to infection. While needle injury to kernels by shelling machine had a considerable

bearing on the disease, although this was not always true when deliberate injuries were induced in the laboratory. Gibson (1953) observed that needle scratch into hypocotyl tissue increased crownrot symptoms on groundnut. The presence of injury on the surface of the seed seems to be essential for penetration and infection by the collor-rot fungus A. niger (Chohan, 1969).

IV. Effect of soil moisture on crown rot development :

Soil moisture, along with other soil and environmental factors plays an important role in development of crown rot disease incidence of groundnut caused by A. niger. At eleven percent soil moisture, the germination of the seed was very slow, thereby allowing A, niger to attack and cause the slow rotting of seed (Chohan 1965; Kang & Chohan 1966,1967). Again seed rot was high at the highest soil moisture level due to excessive moisture, which is conducive to A. niger infection and development (Chohan,1965). At the soil moisture level of 13 and 16 percent, groundnut seedlings infected about 80 percent by collar rot - the highest - and it was the lowest at the highest soil-moisture level. Gibson (1953) observed that moisture level at and above 30 per cent, there was a little variation in the susceptibility of the goundnut plant to the disease, but as the root environment became wetter, the seedling became less liable to death from such infection. At moisture levels below

30 per cent where gross effects of water stress were observed on the seedling, both disease and total mortality markedly decreased. Yi-Cheng-Lin (1982), stated that in field inoculation experiments peanut plants might be killed by using mycelium mat, wheat inoculum or corn stalk as inoculum under water stress condition with wounded crown. However, irrigation of peanut field to maintain moisture at field capacity (-1/3 bars) within 21 days after planting could protect the plants from severe damage caused by A. niger.

V. Role of hydrolytic enzymes in pathogenesis of crown rot disease of groundnut caused by A. niger.

Many of the phytopathogenic micro organisms use cell wall degrading enzymes as one of the tools for infection and colonization in the host tissues was first identified by Debary (1853). The role of cell wall degrading enzymes in the pathogenicity of fungi and bacteria towards higher plants has been reviewed by Wood (1960), Bateman and Miller (1966). Pectolytic enzymes are supposed to be involved in the soft rot of plants caused by A. niger. The pectolytic enzyme produced by this fungus during pathogenesis can account for the maceration of cell wall and middle lamella of host tissue (Bisen 1972,1978). Several studies have shown that the enzyme

is responsible for the maceration of plant tissue is also responsible for the death of plant tissues (Basham and Bateman 1975, Bisen 1978, Byrde et al. 1973, Garibaldi & Beteman 1971, Hall and Wood 1974, Mount et al. 1970). Bhatia and Chohan (1970) also observed that the PG enzyme plays on important role in tissue maceration caused by A. niger. Agarwal and Gupta (1986) concluded that both the pectolytic and cellulolytic enzymes were involved in pathogenesis and also that the C_x produced by A niger was much higher as compared to others.

Leakage of electrolytes is known in many plant diseases (Burkowiez and Goodman 1967, Byrde et al. 1973, Bisen 1978, Hall and Wood 1974, Lai et al. 1968, Mount et al. 1970). Sometimes non-enzymatic toxins and mostly pectic enzymes are suggested to be involved with this phenomenon.

VI. Biocontrol :

The control of groundnut soil borne diseases by infesting the soil with Trichoderma lingorum had been found to be effective (Weindling, 1932). Moreover, it was recorded that addition of T. ligorum spores to the soil caused a significant decrease in peanut pod rot incidence (Abo-Arkoub 1973). Trichoderma harzianum and T. viridie were found to compete with organisms which cause crown or pod rot disease in peanut and damping off on other hosts (Garren, 1967 and Hadar et al, 1979). Rao

(1962), Joffee (1969) observed marked accumulation of Trichoderma sp. in groundnut rhizosphere. Even in stored grains of groundnut and other crops Trichoderma sp. were found to be predominant fungi (Joffee, 1968). The importance of soil organisms in controlling some soil-borne pathogens through antagonistic mycoflora and/or competitive activities was extensively discussed by Garren, 1967 and Hadar et al, 1979. Biological control of F. oxysporum, R. solani and S. rolfsii which attack peanut plants was effective when T. harzianum was added to the soil four days prior to sowing or one day before soil infestation with each of the three pathogens (El-Sherif, 1988). Chohan (1970 & 1971) observed that T. viride and a Streptomyces sp. had a highly antagonistic effect on A. niger in vitro and T. viride had a parasitic effect also on A. niger. He also found that in sterilized soil, the addition of T. viride one month before sowing, had a remarkable effect in decreasing seed-rot and collar rot caused by A. niger, both in sterilized and unsterilized soil. Rai et al. (1980) found that T. viride was parasitic on three species of Aspergillus. The mycoparasitism was characterised by frequent coiling, penetration and hyphal growth of the parasite inside the conidiophores of Aspergillus. The volatile and non volatile metabolites of T. viride, more or less inhibited radial growth of all the test Aspergillus spp. Co_2 and ethanol production by T. harzianum were considered responsible for the growth & sporulation inhibition of A. niger (Hutchinson & Cowan, 1972).

Soil-borne fungi, inducing damping-off, attack seeds and seedlings. Host can be protected by seed treatment with such antagonists as Gliocladium virens (Papavizas, 1985)or Trichoderma spp. (Harman et al, 1980, 1981). The potential of Trichoderma spp. to increase growth was reviewed by Baker (1988 a). The mechanisms associated with the phenomenon appear to be inhibition of minor pathogens (Baker and Cook, 1988; Loper, 1988, Salt 1978) and the production by Trichoderma spp. of a growth stimulating factor (Windham et al, 1986). The rhizosphere competent mutants of Trichoderma especially induced spectacular increased growth responses (Ahmed and Baker, 1987 a, 1988 a) and these were observed consistently in commercial bedding plant operations (Chang et al, 1986). T. harzianum isolated from diseased Sclerotia of S. rolfsii, effectively controlled S.rolfsii on blue lupins, tomatoes groundnut (Hamer et al, 1972). Sanforn (1956) found that Trichoderma sp. inhibited sclerotia formation in R. solani and penetrated cells. Chi (1960) found that Trichoderma sp. become coiled about hyphae of F.oxysporum, F. solani and F. roseum causing changes in the protoplasm of these fungi. Brain and McGowan (1945) isolated an antibiotic substance 'viridin' from number of strains of Trichoderma viride . Dennis & Webster (1971) observed that the hyphae of Trichoderma coiled around or invade the hyphae of several fungi. Durrell (1968) observed that T. viride destroyed several fungi expecially Phycomycetes by penetration and digestion. In extreme cases the invading hyphae

fill the lumen of the cell and its contents are destroyed. The coenocytic nature of the phycomycete hyphae offers little or no resistance once parasite has entered. In R. solani the invading hyphae may proceed from cell to cell through pore in the cross septum. Harman et al (1980) observed that seed treatment with Trichoderma sp. are nearly as effective as chemical seed treatments. Moreover this agent become established in soil and protected subsequent generations of seedlings from attack. This indicates that seed treatment with this antagonist may be effective against soil borne diseases of older plants (e.g. root rot) Trichoderma harzianum has been used as a successful biocontrol agent for several soil borne plant pathogen (Jordon & Erb, 1976). Mycoparasitism has a great impact on survival of plant pathogens. Park (1965) stated that parasitism and predation contribute to survival and disappearance of micro organisms from soil. Both the active and inactive phases of the pathogens are affected by the mycoparasites. Mycoparasitis and predation both inevitably reduce the activity and survival of plant pathogens which may ultimately diminish their active colonization on hosts (Barnet et al 1973; Boosalis & Mankau, 1965; Old and Wong, 1976) Park 1965; Snesh et al. , 1977). Mycoparasites soften and disintegrate the sclerotia of the host (Ayers & Adams 1981).

Biocontrol by adding large amount of T. harzianum with its food base to soil is exemplified by the work of Wells et, al,

(1972). The efficacy of biocontrol with solid inoculants depended on temperature, type of inoculum used, time of introduction of the antagonists to soil in relation to the time of sowing (Elad et al, 1980), the rate of application (inoculum density) of the antagonist (Elad et al, 1981; Hadar et al, 1979) and the inoculum density of the pathogen (Wu, W.S. 1982). Application of Trichoderma or Gliocladium to seed were suggested (Harman et al, 1981)as an alternative approach to introducing them into soil. This method requires smaller amount of biological material than in furrow or broadcast application. The advances in seed treatment with Trichoderma, which have taken place during last few years (Chet & Baker, 1981; Harman et al, 1980; Harman et al, 1981; Papavizas et al, 1982; Papavizas & Lewis, 1983; Wu, W.S, 1982; Kommedahl et al, 1981; Elad et al, 1982), provide a clear picture on the promises and difficulties inherent in this approach.

It is difficult at this time to predict the full extent to which seed treatment will develop and expand as aviable biocontrol technology over the next years. Development and expansion will depend on understanding and solving several problems, none of which is as important as the economic feasibility and acceptibility of such technology by industry and agriculture. From basic stand point, other considerations are also important. Success will depend on isolates used (Hadar et al. 1984; Papavizas & Lewis 1983; Papavizas 1982), the age

of the seed inoculant (Kommedahl at el. 1981), the soil temperature & soil reaction (Harman et al. 1980,1981), the kind of soil and its microbiota (Harman et al. 1984), the nutritional status of the inoculant (Harman et at. 1981), the inoculum density of the seed (Harman et al. 1981, Papavizas et al. 1982), the inoculum potential of the pathogen in the soil (Wu, W-S, 1982) and even the timing of planting (Kommedahl et al. 1981). Nelson et al. 1983) found that the proliferation of Trichoderma in soil is influenced by the degree of decomposition of hardwood bark compost. Trichoderma populations do not change in soil in response to nature of compost. Indigenous or introduced Trichoderma is know to have greater tolerance for broad-spectrum biocides than many other soil micro-organism and to colonize the treated soil more rapidly than other soil competitors (Munnecke, D.E. 1972). Biocides can stress, weaken and render pathogen propagules more susceptible to attack by the antagonists. Sclerotia of S. rolfsii 'weakened' by sublethal concentration of methamsodium, become susceptible to invasion and degradation by T. harzianum (Henis & Papavizas, 1983).

Richardson (1954) & Davet (1981) showed that the fungicide thiram, used extensively for seed treatments, had selective & beneficial effect on Trichoderma. Papavizas (1981) observed that infusion of pea seed with metalaxil before coating it with conidia of T. harzianum improved the survival of conidia and

even increased the number of colony-forming units in the rhizosphere compared with the number in the rhizosphere from seed that received conidia only. Indirect evidence on the non-target effects of benomyl in soil was provided by Backman & Rodriguezkabana (1975,1977), who showed that benomyl applied to control foliar diseases of peanuts resulted in a lower yield because of more severe Southern stem blight (S. rolfsii). Benomyl had no effect on the pathogen but was very toxic to Trichoderma.

The dominance of Trichoderma following application of sublethal rates of pesticides is of special interest in biological control because of the ability of the genus to proliferate in the treated environment as well as its demonstrated ability to produce antibiotics, compete for nutrients and acts as a mycoparasite.

Treatment of soil with steam (Baker & Cook, 1974), solarization (Katan, 1981) helps the proliferation of Trichoderma in soil. Relatively repid spread of Trichoderma sp. was observed in sterile soil (Norton, 1954).

The effectiveness of Trichoderma as seed treatments is probably determined not only by their biocontrol qualities but also by their abilities to multiply in the rhizosphere. When applied to seed Trichoderma inoculum multiplies around the sites of

application, but not along the root surfaces away from the cotyledon attachment, it may suppress pathogens causing seed rot and seedling diseases, but not those that cause root diseases. Lack of establishment or movement of Trichoderma in plant rhizosphere was observed by Papavizas (1981) and Chao, Harman & Nelson (Unplublshed data). Recently Baker (1989) reported that mutants or Trichoderma are able to colonise root rhizosphere after seed inoculation. They are able to protect developing roots from microbial attack & can produce a plant growth stimulating factor.

There are several possible explanations for the decline of added Trichoderma inocula or the inability of the fungus to proliferate in the plant rhizosphere, including lack of proper nutrients, the presence of toxic substances in the root exudations or the presence of antagonistic or competing microorganism (Chao, Harman & Nelson, unpublished data) at the rhizosphere or rhizoplane level. Pseudomonus spp. may compete with the biocontrol agent for iron in the rhizosphere (Hubbard et al. 1983) and produce matabolites toxic to Trichoderma.

One of the most critical obstacles of biocontrol by direct massive soil augmentation or seed treatment with biocontrol fungi such as Trichoderma, has been the lack of knowledge of methods for mass culturing and delivering the fungi. Solid media for the experimental production of Trichoderma have

frequently been used in laboratory and green house studies. Such media also have been tested in the field (Devet et al. 1981; Elad et al. 1980; Elad et al. (2) 1980) and several were tabulated in review of Papavizas & Lewis (1981). Though further refinement is needed, some of the promising methods for mass production of biomass of biocontrol micro-organisms are deep tank fermentation for liquid formulations (kenney & Couch, 1981; Papavizas et al. 1984). Dry formulation and encapsulated propagales are other important recent development (Walker & Connick, 1983). Lewis & Papavizas (1987) applied alginate pellets containing Trichoderma hamatum to soil and succeded in reducing the incidence of disease caused by R. solani . Knudsen & Bin (1990) observed that temperature had a significant positive effects on radical growth rate from alginate pellets, applied in soil. And hyphal density declined exponentially with distance from pellets and density was significantly larger with bran in drier soil. Broadcasting, furrow application, root zone application and seed coating are four different techniques for the application of Trichoderma sp. as biocontrol agent and each has been found to be effective in the field Chet and Baker (1980) found that the minimal effective amount of Trichoderma is about 1×10^6 colony-forming unit (CFU)/ gm of soil. But still further advancement is needed for development of a stable, cost-effective and easy-to-apply biocontrol formulation for control of plant pathogens with introduced antagonists (Lisansky, 1985).

T. harzianum has been found to stimulate the growth of plants, including various floricultural & horticultural plants (Baker et al. 1984; Chang et al. 1986, Baker, 1989). The growth stimulation by T. harzianum could be the result of production by the fungus of plant hormones, increased uptake of nutrients by plants on the control of one or more subclinical pathogens. But Kohl & Schlosser (1989) observed that in non-infested soil, seed treatment with conidia or fresh mycelium of 9 strains of Trichoderma sp. slightly reduced the no. of emerged seedlings and retarded root growth of sugarbeet. Under aggravated condition with an increased amount of inoculum, the seedlings were severely damaged, especially by strain of T. viride. Roots were short, brownish and sometimes twisted.

Danielson & Davey (1973) stated that T. harzianum was most frequently found in relatively warm regions. Wells et al. (1972) observed that T. harzianum caused severe injury to the sclerotia of S. rolfsii. While working with 5 spp. of Trichoderma Widden & Scattolin (1988) observed that all spp. were antagonistic towards each other. In biocontrol of S. rolfsii under glass house condition T. harzianum was found to produce extracellularp - (1,3) gluconase & Chitinase (Chet & Elad, 1982). Rodges (1989) reported a nobel Pyridine with fungicidal activity against a range of plant and human diseases produced by T. harzianum and a nobel metabolite of Bacillus

subtitis which has a high level of broad spectrum fungicidal activity. Espuny et al. (1980) reported that a Streptomyces spp. are antagonistic against Aspergillus sp. Narain & Mohanty (1983) reported that detached groundnut Kernels and stems pretreated with living cells of the bacterial antagonists, showed much reduced or no colonization when inoculated with A. niger. Espuny et al. (1982) reported number of bacterial antagonists which showed wide spectrum of activity against 11 plant pathogenic fungi including Aspergillus sp. Eight out of 127 actinomycetes isolated from 47 soil samples showed strong antifungal activity against 4 test fungi including A. niger (Paul and Banerjee, 1984). While working with 644 bacterial isolates obtained from soil and the rhizosphere of some leguminous plants against one hyperparasite and 6 pathogenic fungi frequently associated with leguminous and graminaceous plants Gagne et al. (1985) observed that more than half of the bacterial isolates inibited at least one fungus and had an inhibitory eftect on all fungi tested.

In general higher percentage of inhibitor bacteria were obtained from rhizosphere than non rhizosphere soil. Sclerotia of pathogenic fungi sometimes are found to loose their germinability due to antagonistic effects of some bacterial isolates from soil (Gakulapalan & Nair, 1984). Sclerotia can be invaded by other fungi in the soil which may or may not suppress germination (Wicklow & Wilson, 1986). Sclerotia of A. flavus may be colonised by several antagonistic fungi including

Trichoderma spp. any of which can render the sclerotia inviable or suppress germination (Stack & Pettit, 1984).

VII. Effect of cell free culture filtrate of various isolates of A. niger on groundnut.

The culture filtrate of A. niger play an important role in pathogenesis, growth of plant and crown rot disease incidence of groundnut caused by A. niger. R.W. curtis 1988 a & b reported that when culture filtrates of the fungus, A. niger are used to treat the growing points of bean seedlings, severe curvatures and malformations are produced on the subsequent growth of the plant. Malformations consisted of greatly thickened stems and petioles, tumour like stem projections severely twisted stems, and stems enlarged in only one plane to produce a stem that was wide and relatively flat. Most frequently, curvatures consisted of strong downward bendings of the elongating stem and the compound leaves. In addition, elongation of the first and second internodes above the primary leaves was inhibited. Little or no effect was noted when corn seedlings were treated with the culture filtrate. In a number of cases the roots on the A. niger extract formed several complete circles in a light concentration to give the appearance of a corkscrew. It has appeared that best concentration for producing root curvatures are between 1/20

and 1/50 or the normal concentration of the culture filtrate. He also reported (1958) that corn root curvatures caused by culture filtrates of A. niger are caused by the same compound which causes curvatures and `malformation' on the stems and petioles of the bean plants. The name malformin was proposed for the active compound (Curtis 1958 c) which was found to be a neutral peptide containing four amino acids, valine, leucine, isoleucine and 1/2 cystine. It is a cyclic peptide and its molecular formula was proposed to be $C_{23}H_{39}N_5O_5S_2$.

Postlethwait and Curtis (1959) reported the histology of malformation produced on bean plants by culture filtrate of A. niger. Reiss (1970) stated that pea plants when grown in culture filtrate of A. niger showed signs of wilting after 10 days and inhibition of growth. Omprakash and Siradhana (1978) reported that at higher concentrations the antimetabolites produced by A. flavus, A. niger, A. tamarii and A. terreus, in a liquid culture caused complete wilting of the seedlings but at lower concentration only tip and margin burning. Kulfinski and Pappelis (1980) reported that a virulent onion isolate of A. niger produced a heat stable compound both in vivo and in vitro which induced reductions in nuclear area and nuclear dry mass in inner epidermal cells of onion bulb scales. Desai et al. (1981) reported that culture filtrate or A. niger was toxic to Guizotia abyssinia seeds and inhibited germination by 11-100% and root and shoot elongation by upto 100%. Vishnuvat &

Shukla (1981) reported that out of 25 fungi tested maximum reduction in germination of lentil seed was obtained with culture filtrate from A. niger, A. flavus and A. terreus. Ghewande et al. (1984) reported that among 15 cultivars of groundnut and 7 isolates of A. flavus, the inhibition of seed germination, root and shoot growth was observed in the range 5-80%, 3.61-100% and 5-100% respectively. Haider et al. (1986) reported that culture filtrates of sed borne A. flavus, A. niger, Penicillum expansum, Alternaria alternate and Fusarium equiseti separately and in mixture were toxic to safflower seeds, inhibitine germination by upto 100% and root and shoot elongation by 80.5-100%.

VIII. **Effect of seed treatment with heavy metal salts on disease incidence**

Much information has now accumulated on the effectiveness of treatments with various kinds of chemicals in inducing resistance in crop hosts against their pathogens as a possible disease control measure. Resistance has thus been induced in a wide variety of crops active against a multitude of pathogens, both soil borne and air borne pathogens in nature. More interesting of these reports are being reviewed here.

i) **Inorganic salts as inducers of phytoalexin**

Salts of copper, which are fungitoxic in nature, have been used most, but in such cases the nature of treatment and the concentration used precluded any direct toxic action as the possible cause behind the reduction of symptoms. Copper salts induced anti-mildew activity in cereals (Olesen, 1938; Byrde et al 1953). Seed treatment with copper sulphate and boric acid reduced brown spot symptoms in rice seedlings (Bouchereau and Atkin, 1950). Lithium salts effectively controlled both rust (Kent, 1941) and powdery mildew (Beckman, 1958; Byrde et al., 1953). Sodium borate application in root of wheat reduced both brown and yellow rust under field conditions (Gigante 1953) and linseed rust (Heggeness H.C., 1942). Rodigin and krasnova (1959) reported that seed treatment with Boric acid, copper sulphate and zinc sulphate were effective against leaf rusts of wheat. Zinc chloride reduced fireblight of pears (Beckman, 1958). Root application or seed treatment with manganese sulphate gave effective control of Fusarium wilt of pigeon pea. Complete control being achieved with 100-200 ppm (Beckman, 1958, Carorasco et al., 1978). Kiss and Pozsar (1977) could reduce infection by Uromyces appendiculatus in French bean by foliar spray with magnesium salts. Covey (1971) and Balazas et al.(1978) observed many wheat varieties suffered less damage when potassium or sodium

chloride was added to the soil. Powdery mildew infection of turnip and cabbage incited by Erysiphe polygoni was found to be significantly less on the plants grown in soil with 0.1 to 10% w/w zinc frit (Tomlinson and Webb, 1958). Terpenoids compounds, which were important in the defence reaction of the sweet potato, were found to accumulate in the roots when treated with mercuric chloride (Oguni et al., 1976). Several nickel salts were found to have eradicant action against leaf rust of rye (Keil et al, 1958). Carter and Wain (1964) could induce resistance in bean against Botrytis cinerea by the use of zinc sulphate and manganese chloride. Wet seed treatment with dilute concentrations of some phytoalexin inducing compounds and related chemicals can provide rice plants substantial protection from brown spot and blast (Giri and Sinha 1983a, b, Sinha and Hait 1982).

IX. Effect of different fungicides in control of crown not of groundnut

Frank (1969) conducted a laboratory and field trial with several fungicides, of which 3 parts of 75%. Captan + 1 part of 75% PCNB (quintozene) in 3 g/kg of seed gave satisfactory control of both crown rot disease caused by A. niger and seed rot caused by Rhizopus spp. Aulakh and Sunar (1970) reported that Benlate, Brassicol, Ceresan, demosan 100, Fertix 300 and

Ziram were most effective upto 6 weeks with varing percentage of germination. Kumar and Khare (1970) reported that out of 8 fungicides tested Fertix (3.% organic Hg) was best. Mathur & Sharma (1971) saw that of 11 fungicides tested for 5 yrs. the ceresan @ 3 g/kg and thiram @ 4 g/kg improved germination most, while agrosan @ 3 g/kg was the cheapest and as effective as ceresan. Whitehead and Thirumalachar (1971) reported that Aureofungin (2/ U g/ml) in vitro inhibited A. niger strain. Sidhu and Chohan (1971) reported that Thiram and Captan applied as dry seed dressing at 1:200 and 1:300, respectively gave the best control of A. niger and increased germination, followed by Thiram or Captan + Agrosan GN. Venkataraman and Rajyalakshmi (1971) saw that Ceresan, Dithan-M-45, 2-4-D, Dalapon, propazine, Cotoron, linuran and Diuron suppressed the bacteria and actinomycetes but not soil fungi. Aspergillus spp. the predominant fungi, were tolerant of most of the pesticides. Lalithakumari et al. (1972) reported that Thiram gave the best protection against seed spoilage of groundnut caused by various seed borne fungi. There are conflicting reports regarding the efficacy of organo-mercurials in controlling A. niger, collar rot of groundnut. Morwood (1945) reported that Agrosan G N and Ceresan are effective in controlling A.niger . Later, Morwood (1953) and Purss (1960) observed that the post-emergence loss of plants was higher in seeds treated with organomercurials than in seeds treated with other fungicides. Neme et al. (1955) tested various organomercurials and observed that all the

materials reduce the pre and post emergence damping off of groundnut plants as compared to the control.

This is due to the reason that certain isolates of A. niger were mercury tolerant and concluded that this effect of organomercurials was due to their selective action upon the fungal flora of the soil region of the treated seeds operating to the advantage of A. niger

Agnihotri and Sharma (1972) showed that thiram was found to be better than Agrosan GN, Ceresan wet but inferior to seed Dresser 6335, Seed dresser 6334 and Fertix 85. Sharma & Agnihotri (1969), observed that mycoflora associated with the groundnut seeds could effectively be controlled by seed dresser 6335 and Seed Dresser 6334 and that A. niger and species of Fusarium and Sclerotium could not completely be inhibited by Agrasan GN, Brassicol, Harvason, Ceresan wet and Thiram. Sharma et al. (1973) reported seed treatment with fungicidal mixture S.D. 6335 (3% organic Hg) and Captan in 1:1 ratio gave effective control of A. niger and increased germination, stand density and yield. Aulakh and Chohan (1974) reported that out of 8 fungicides tested PCNB gave highest control and it was most effective when the kernels were treated 3 months before sowing. Thiram treatment also gave a very prominent disease control, but it should be used just at the time of sowing of goundnut in the field. Kodmelwar et al. (1977) reported that

Cuman at all concentrations and Ziride at 3000 and 4000 ppm were found effective as compared to other fungicides viz. Dithane Z-78, Zineb-75 and Lonacol in controlling A. niger, Wangikar and Kodmelwar (1977) stated that Bavistin, Derosal, Benlate, Thiram, Cersan, Cuman, Captan and Vitavax showed effectiveness in descending order in controlling the growth of A. niger at 2000 ppm concentration. Derosal at 3000 ppm conc. completely inhibited the growth of fungus. Dithane M-45 and Brassicol showed less effectiveness against A. niger at both the concentrations. Mathur & Sharma (1977) reported that Bavistin & Benlate were found most promising fungicide among 9 other fungicides tested against A. niger. There was 100% inhibition of growth even at 10 ppm with both these fungicides. Campogram M was found to be the poorest for inhibition of growth amongst all the fungicides tried. Gaur and Ahmed (1983) obtained highest control with carbendazim @ 1.5 g/kg of seed against collar rot disease. Murphy and Levy (1983) demostrated the production of copper oxalate by A . niger in relation to its tolerance to copper fungicides. Siddaramaiah et al. (1979) reported as seed treatment at 1 g/kg of seeds Bavistin followed by Thiram, Difolatan, Brestan and bayleton were better than Captan in reducing the pre-emergence and post-emergence mortality due to A. niger. Kocaturk and Maden (1977) observed that in glasshouse and field trials Pomarsol Forte, Dexonal, Ceresan UT 687 and Busan 30 were effective as seed treatment against seed borne fungi mainly spp. of Fusarium,

Pencillium, Aspergillus, Macrophomina, Botryodiplodia & Mucor. Babich and Stotzky (1973) reported that a 10 mM conc. of zinc reduced mycelial growth of A, niger. The effect of zinc in the presence of high NaCl conc. was variable. A . niger tolerated higher conc. of zinc in the presence of NaCl. Lal and Jayamma (1978) reported that mixtures of Captan and Thiram, both at the higher and lower conc. were superior to Captan + Ceresan or thiram + ceresan. All 3 fungicides were biologically compatible. Siddaramaiah et al. (1979) reported that seed treatment with carbendazim gave the best results, reducing pre- and post emerence death caused by A. niger from 3% to 3.5% followed by Thiram (7%) and captafol (10%). Roy (1980) found that A. niger was able to solubilize copper carbonate and cupramar (Copper oxychloride) in vitro, indicating a potential mechanism of resistance to copper fungicides. Sidaramaiah et al. (1981) reported that in vitro A. niger A. flavus were completely inhibited by 2000 ppm of Basalin (aniline Lano (methoxy methyl acetanilide) or Tok-E-25 (1-4-dihydrophenol 1-4-nitrophen ether). In pot culture, pre-emergence death was more frequent in seeds inoculated with A. niger in in-vitro studies. Gupta & Sengupta (1983) obtained fungicidal activity almost with all the rhodanines tested in in-vitro studies. Dimov (1983) on the basis of laboratory and field trials, recommended semi moist treatment with Vitavax (Carboxin) 200 or 75 at 200 g/ 100 kg. seed a TMTD (Thiram) at 300 g/ 100 kg for reducing seed & seedling rot & increasing germination. 5-

chloro-1-methyl 1-4-nitroimidazole completely inhibited A. niger growth at 600-1000 ppm (Gaj dzinski - Mrockiewicz 1983). Soil drenching with thiram & Dithane-M-45 (Mancozeb) gave highest yield of groundnut (Natarajan et al. 1983). Agrosan GN @ 3 g/kg of seed and Captan, Ceresan dry, Bavistin and Vitavax all at 2 g/kg of seed effectively controlled the storage fungi including A. niger in table pea (Kumar et al. 1983).

Devi and Polasa (1984) reported that at low concentration (25-100 ppm) Bavistin (Carbendazim) stimulated the growth of several Aspergillus but at higher concentration growth and toxin production were completely inhibited. Ravindar and Lingaiah (1984) stated that the toxicity of the metal complexes against A. niger followed the order Pd> Cu> Ni> Fe> Co, which parallels the stability order of the complexes. Srivastava et al. (1984) observed that 5,3/5 and 2-substituted (1,3,4) - oxadiazoles and their related products have strong activity against A. niger at 1:1000. Singh D.B. (1984) reported that A. niger on phylloplane of mustard is fairly tolerent to mycostatin treatment. Singh et al. (1985) observed on grapes. A. niger was best controlled by diphenyl used protectant and eradicant, stable bleaching powder was second of the 4-chemical tested while sodium metabisulphite was good protectant but not eradicant. Reddy et al (1985) reported that 2-4 dihydroxy acetophenone phenylhydrazone was effective gainst A. niger in vitro among 7 compounds tested. Krishnaiah (1985) observed that

8 commercial Decco food grade fruit coatings, namely WT-12, WT-22 and WT-23 each containing the fungicide orthophenyl phenol @ 1.8, 2.5 and 2.5%respectively, were effective in controlling A. niger on banana fruits. Dange and Saradava (1985) suggested that carbendazim and benomyl (1 g/kg seed) can effectively control seed rot and collar rot of groundnut caused by A. niger in sandy soil. Fungicidal seed dressing increased germination by 2 to 61%, reduced incidence of abnormalities and lessened death of seedling of groundnut (Harrison, 1985). Shekhawat et al. (1986) reported that carbenddazim, Benomyl, Carboxin and ethyl mercury chloride were most effective of 11 fungicides against A. niger in vitro at 1500 ppm. They also effectively controlled pre-and post-emergence collar rot in the blotter and pot tests when used as seed dressing at 2-2.5 g/kg of seed. Sumbali and Mehrotra (1986) reported that Idodine-potassium-iodide effectively reduced peach rot caused by A. niger. Lande et al (1986) reported that thiram 0.4%, Captan 0.25% and a combination of both were promising in control of pathogenic seed contaminants which were mainly Aspergillus ssp. Post-harvest Aspergillus rot of Banarasi lemon was best reduced in fruit treatment with Waxol 0-12+ Capan at 0.1% followed by Captan 0.1% alone (Sharma et al. 1986). Tyagi (1986) suggested that as ammonia fumigation at low concentration (5 & 10 ppm) are fungistatic for A. niger and several other fungi, periodical fumigation with this can be done in store houses at low concentration. Indolinone derivatives were found to have

antifungal activity against A. niger in vivo and in Vitro (Singha & Jha, 1989). Saha and Raj (1989) reported that among 7 fungicides, Dithane-M-45 and Emisan-6 showed best results to check the seed mycoflora of groundnut including A. niger Chattopadhya and Raj (1981) stated that seed treatment with Bavistin (Carbendaxim) reduced population of A. niger in wet soil, but Emisan seed treatment reduce more number of rhizosphere mycoflora than Bavistin or Dithan-M-45. Reddy et. el. (1991) reported that of 5 fungicides tested in vitro, carbendazim was most effective in controlling the growth of A. niger followed by carbendazim + Thiram, and Captan. In vivo studies of seedling vigour showed that carbendazim + Thiram was the most effective treatment for enhancing seed germination. shoot, root growth & total dry weight/plant. Wadibhasme et al. (1991) reported that 6 foliar sprays of carbendazim (0.1%) + phosphamidan (0.02%) or Mancozeb (0.2%) + Phosphamidan (0.2%) at 10-day interval gave good control of seedling blight of groundnut caused by A. niger.

X. ROLE OF GROWTH REGULATORS ON INDUCED RESISTANCE

Induced resistance against plant disease means development of resistance in susceptible plants after treatment with biotic or abiotic agents. It is very effective against some of the most serious human and animal diseases. But against plant disease, only recently some serious attention is being paid to

induced resistance, though evidence for it against plant diseases had been known for many years.

Resistance induced in plants is usually non-specific both in respect of inducer agents and the pathogens against which it is effective. Both biotic and abiotic agents are used as inducer. Among biotic components, inducers may be the same pathogen that are used in challenge inoculation, it's avirulent or mildly virulent forms, other pathogens or even saprophytes (Ryan 1975). Modified biotic agents like heat killed or sonicated cells, culture filtrate of pothogen or non-pathogen, spore germination fluid or even various cell components like lipo-poly sacharide, protein, nucleic acid etc. There are excellent reviews on different aspects of biologically induced resistance.

Though induced resistance is usually non-specitic in it's action, sometime specific induce-host-challenger combination may provide the best protection. Localised (Rose, 1961 a) or systemic (Ross 1961 b; Kuc 1983) nature of resistance can be induced in plants to an extent greatly depend on inducer-plant-challenger combination. Induced resistance is usually locally effective but there are cases where a systemic effect has been recorded. It may be non-persistant or peristant. Such variation in effect depend upon the nature of inducer-host-challenger combination.

Besides such biotic agents or their components and metabolites, various chemicals have been shown to induce resistance in plant hosts against their pathogens. Resistance thus induced is rarely specific, usually more general in nature, may be effective against more than one pathogens, systemic in certain cases and also persistent over most or a major part of crop life.

Compounds of diverse chemical nature and biological actions have tested for this purpose. More effective ones include metal salts, plant disease management have been reviewed by Yarwood (1959), Van Andel (1966), Marsh (1977) Sinha (1984).

Growth regulators of diverse origin play an important role in induction of resistance in various plant. Sinha and Giri (1979) found that of different method of application, wet seed treatment provided the best and most persistent protection. Sinha and Hait (1982) achieved strong protective effects with sodium selenite, thiogly collic acid, cysteine, P-chloromur curibenzoate and cycloheximide used in seed treatment against brown spot disease of rice at very low concentration (10^{-4} - 10^{-6}M)

Many of the effective chemicals showed better effect at lower concentration suggesting that they acted through their effect on host metabolism.

Akai (1955) reported that 1,4- dihydronapthoic acid, Vitamin K4 pentachlorophenol and it's derivative provided excellent control of brown spot disease in rice seedling. He also reported that root dipping in B napthoxyacetic acid or B Indole acetic acid solution at 10^{-5} concentration was effective in controlling brown spot disease of rice. Davis (1952) reported significant increase in resistance of Nicotiana glutinosa against TMV when treated with 4-chloro-3,5-dimethyl - phenoxy ethanol. Intensity of chocolate spot of broad bean could be reduced by application of -Napthyloxy acetic acid or- Napthanol (Bryd et al. 1953) or 2,4,6-trichlorophenoxy acetic acid (2,4,6-T) (Fawcett et al., 1955,1957). Attempt to induce resistance against wilt diseases with plant growth regulating substances yielded many interesting results. Significant reductions in Fusarium by host injection with 2,3,6-trichlorophenoxyacetic acid (2,3,6-T) (Smalley,1962). and some other growth regulating substances (Beckman,1958). Sinha and Sengupta (1986) reported that treatment with cycloheximide, IAA, ferric chloride effectively inhibit bunt symptom in their susceptible cultivars Pusa -2.21 and CRM-13-3241. Manibhusan Rao et al. (1987) reported that phenyl Acetic Acid and it's meta, para and ortho hydroxy derivatives are the effective inducer of resistance against Corticium sasakii infection in CV. TKM-9.

Almost in every case of effective treatments the protected plats exhibited growth inhibition suggesting that a change in pectic constituents of the plant cell wall may have been causally associated in some way with limilation of pathogen activity.

Sumbali and Mehrotra (1981) noticed reduction of post harvest decay of apple, pear and peach fruit by different growth regulators at 1000 ppm. Hale et al. (1981) observed that reduction in disease susceptibility of groundnut plants towards Pythium myriotylum by foliar application of growth regulators like Gibberellin A_3, 2-4-D. Soleman et al. (1988) reported that amount of disease in faba beans caused by Rhizoctonia solani was decreased to 29.17% and 40.28% by application of 500 ppm cycocel + $CaHPO_4$, $CaSO_4$ and 100 ppm IAA + $CaCl_2$ respectively, compared with the controls (68.07 and 72.92% respectively). Length of radicle epicotyl and plant dry weight were not significaltly affected by treatments. The number of lateral roots, hypocotyl length ad stem length of treated plants were significantly increased when compared with the controls. The numbers of xylem vessels was decreased and the thickness of the phloem zone was increased by infection. Application of IAA and cycocel combined with Ca treatments reduced the effects of infection on the cortex, phloem and xylem vessels of faba bean. Dahne reported that when growth regulators were applied to different plants before inoculation those containing ethylene

had a strong effect on disease development. Ethrel reduced host susceptibility to obligate biotrophic fungi and increased it to perthotrophic fungi and bacterial diseases.

Lee (1990) observed soaking of groundnut seeds in solutions of 0,50 and 100 ppm gibberellic acid and 50,10 and 200 ppm IAA prior to sowing produced plants with longer main stems, more branches, higher chlorophyll content, greater no. of flowers, internodes and pods and greater grain weight/plant than in the control. Seedling emergence and time to flowering were also improved by growth regulator seed treatment.

XI. Synergistic action of oxalic acid and pectic enzyme on the collar rot of groundnut caused by Aspergillus niger

Causal involvement of oxalic acid in pathogenesis of A. niger of different host have been reported by different workers at different time. Tanaka and Nonaka (1977) demostrated that a crude enzyme preparation and oxallic acid from culture filtrate of A. niger exhibited as exceedingly great macerating activity in onion tissue. Bateman and Beer (1965) and Maxwell and lumsden (1970) have suggested a synergistic effect of oxalic acid and several extracellular enzymes in maceration of host tissue.

Gibson (1953), Higgins (1927) and Kritzman (1977) reported that oxalic acid production by pathogens was a key factor in enabling this organism to attack living plants.

Bateman and Beer (1965) reported endo- PG would not hydrolyse a calcium pectate complex except in presence of Oxalic acid. They suggested that oxalic acid enhanced the activity of polygalacturonase by letting the susceptible tissues down to the optimum pH values for the enzyme. Tanaka and Nonaka (1981) cosidered that synergistic action of the enzyme and Oxalic acid to be a significant factor in the rapid destruction of onion bulbs by A niger.

CHAPTER-III

Materials and Methods

The materials used and methods followed in the present work are described below :

3.1 **The pathogen**

Aspergillus niger van. Tieghem was isolated from soils of 4 agroclimatic zones of West Bengal and from infected groundnut kernels. The fungal isolates were identified from Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.

3.2 **Isolation of pathogenic isolates**

To isolate the pathogenic isolotes of A. niger, soil samples from cultivated fields from various agroclimatic zones of West Bengal were collected. The fungus was selectively isolated from soil samples using modified M3SIB medium. Among 25 nos. of isolates only 9 isolates showed distinct virulence against the test plant CV. JL-24 of groundnut in glass house condition. One isolate was collected from infested seed of groundnut. Routine maintenance of the fungal isolates was done in PDA medium.

Plate-7 Root curvature induced by A. niger

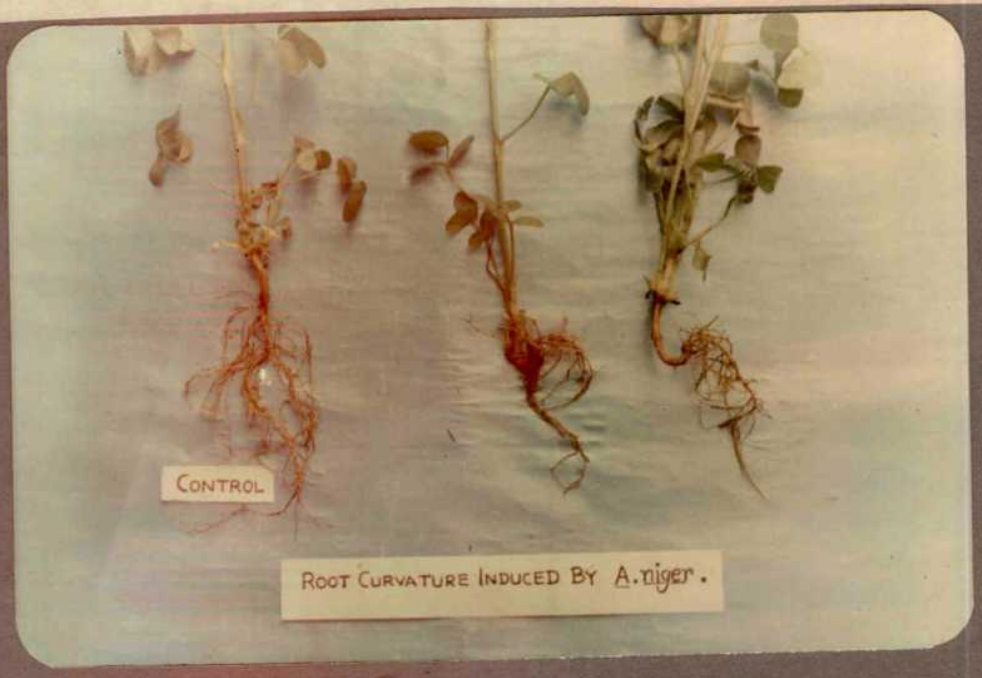


Plate-8 Growth of five different isolates of A. niger in Martin's Dextrose Peptone-Rose Bengal Agar medium. 1→Isolate 2; 2→Iso.3; 3→Iso.5 4→Iso.16; 5→Iso.18

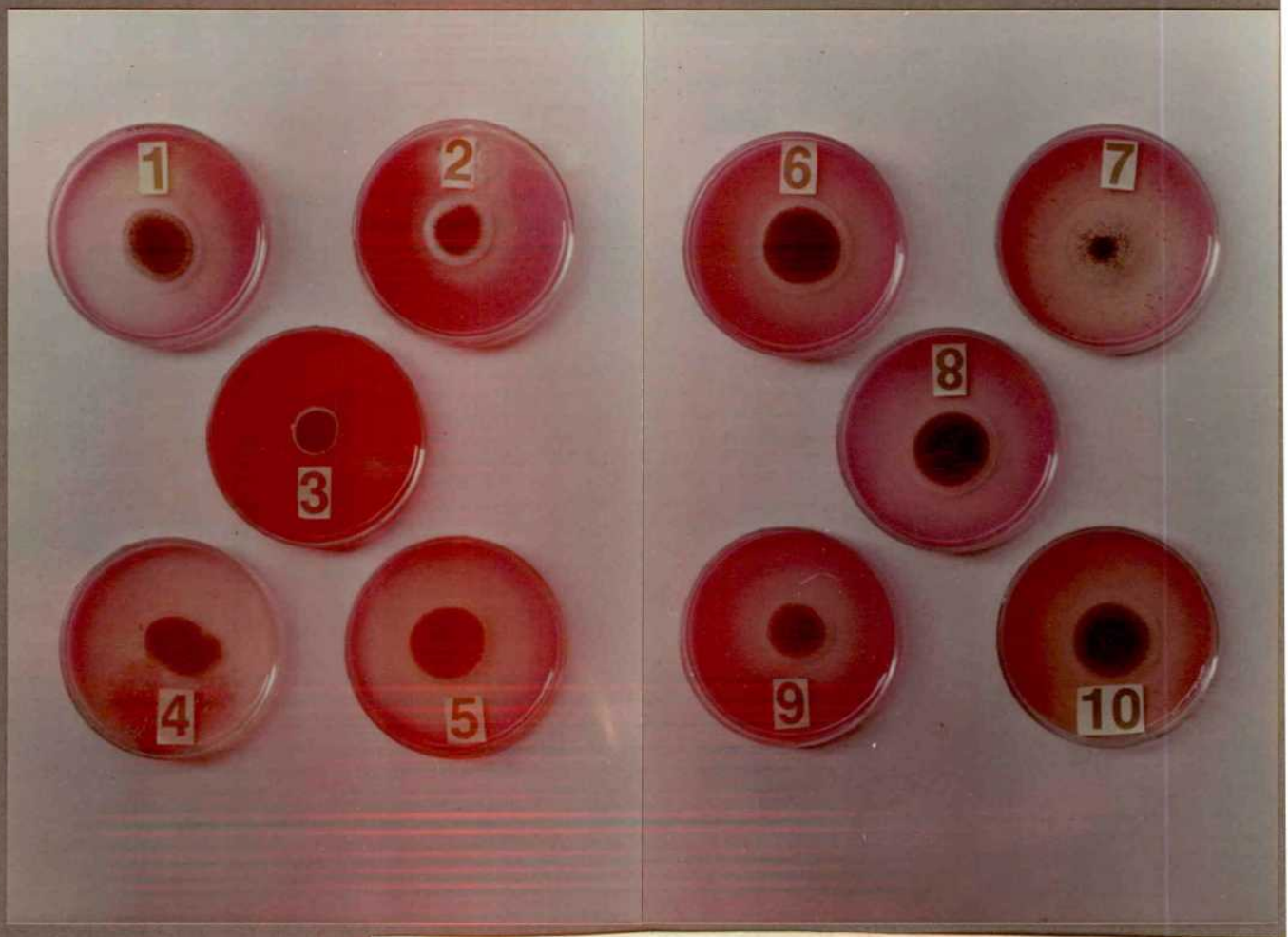


Plate-9 Growth of five different isolates of A. niger i Martin's Dextrose Peptone-Rose Bengal Agar medium 6→Iso.20; 7→Iso.21; 8→Iso.22 9→Iso.23; 10→Iso.24.

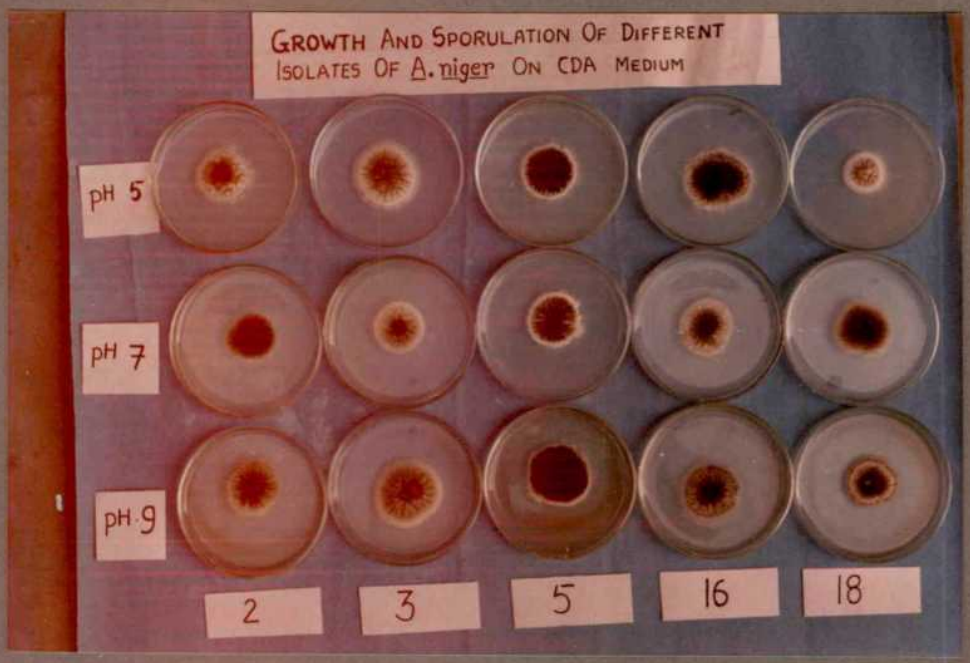


Plate-10 Growth and Sporulation of 5 different isolates of A. niger on CDA medium at different pH.

Plate-11 Growth and Sporulation of 5 different isolates of A. niger on CDA medium at different pH.

3.3 **The host**

Groundnut (Arachis hypogaea L.) collected from Main oilseed Research Station, G.A.U. Junagadh, Gujarat for varietal screening. In general, JL-24 variety was used throughout the whole period of investigation.

3.4 **Inoculation of Soil** :

The inoculum was mixed thoroughly with upper 5 cm doubly sterilized (at 20 psi pressure in autoclave for 2 hours for two consecutive days) soil of each pot @ 1:1 W/W. Inoculum was mixed with the soil before 10 days of sowing of seeds. The inoculum mixed soil was kept moist with moistened cotton. The pot was regularly irrigated with measured quantity of water.

3.5 **Sowing of seeds**

In general case, surface sterilized seeds (0.1 percent mercuric chloride for 1 minute and washed in sterile water) were sown in earthen pots (25 cm in diameter) for glass house experiments.

3.6 **Medium used**

1) Potato dextrose agar medium :

Peeled potato		200 g	
Dextrose		20 g	Auto claved
Agar Agar		20 g	at 15 lbs
Dist. water	to	1000 ml	psi for 15 m

2) Maize meal sand for 250 ml. flask.

maize meal	12.5 g	Autoclaved at
Sand	37.5 g	20 lbs.psi for
Water	50 ml	20 minutes.

3.7.1 **Varietal reaction to A. niger**

138 groundnut cultivars (@ 20 cultivars in a lot) were screened against seed rot and collar rot. Two virulent isolates of A. niger were selected for the experiment, one of which is soil isolate (Isolate 16) and another is seed isolate (Isolate 24). The screening experiment was conducted according to the method described by Dange et al. (1985) with some modifications. Isolates were multiplied on 100 g of sand maize meal (3:1) medium in polypropelene packets (10" X 6") 10 days at 28 ± 1 degree C and then mixed thoroughly with upper 8 cm of doubly sterilized sandy soil (1:3) kept in plastic bag (20 cm in diameter). These plastic bags were irrigated and kept in glasshouse for 3 days. After that 8 surface sterilized seeds of each groundnut cultivar were sown in each bag and three replications were kept of each cultivar. The bags were kept randomly in glass house to avoid positional effect of the surroundings and were irrigated as and when required. As the inoculum was mixed in soil many seeds rotted which were taken out carefully for count to find out the percent seed rot after 7 days of sowing. Ten day old seedlings were inoculated at

collar region with a mixture of mycelium and spores of A. niger multiplied on potato dextrose agar medium for 5 days at 28 ± 1 degree C. Inoculated collar region was covered with wet soil to keep the inoculum moist. The plants in each variety affected by collar rot were counted at 7 days intervals within a period of 45 days after sowing as there was no increase in disease later on and the percentage of plants attacked in each variety was calculated. The varieties were grouped as resistant (1-8.3%), tolerent (8.3-20%) and susceptible (>20%) on the basis of percent collar rot infection as described by Chahal et al. (1974), with slight modification for convenience and same criteria were adopted for seed rot also. Experiment was repeated twice by following the same procedure.

3.7.2 **Relative incidence of crown rot disease of groundnut caused by various isolates of A. niger in different seasons.**

A. Under glass house condition

10 Isolates of A. niger (2, 3, 5, 16, 18, 20, 21, 22, 23, 24) were selected for the experiment under glass house condition. Soils of each earthen pot were inoculated with inoculum of respective isolate of A. niger @ 1:1. The inoculum was mixed with soil 5 days before sowing and covered with cotton wool and kept in glass house with the supply of water as and when

required for the initial establishment of the pathogen in the soil. 8 nos. of groundnut seed (var. JL-24) were sown in each pot. For each isolates 5-pot replications were maintained. The experiments were conducted during 15 Jan-28 Feb. 1990, 15 May-30th June 1990, and 15th Oct.-30th Nov. 1990. Disease incidence was recorded upto 45 days of age after which no increase in disease incidence was recorded.

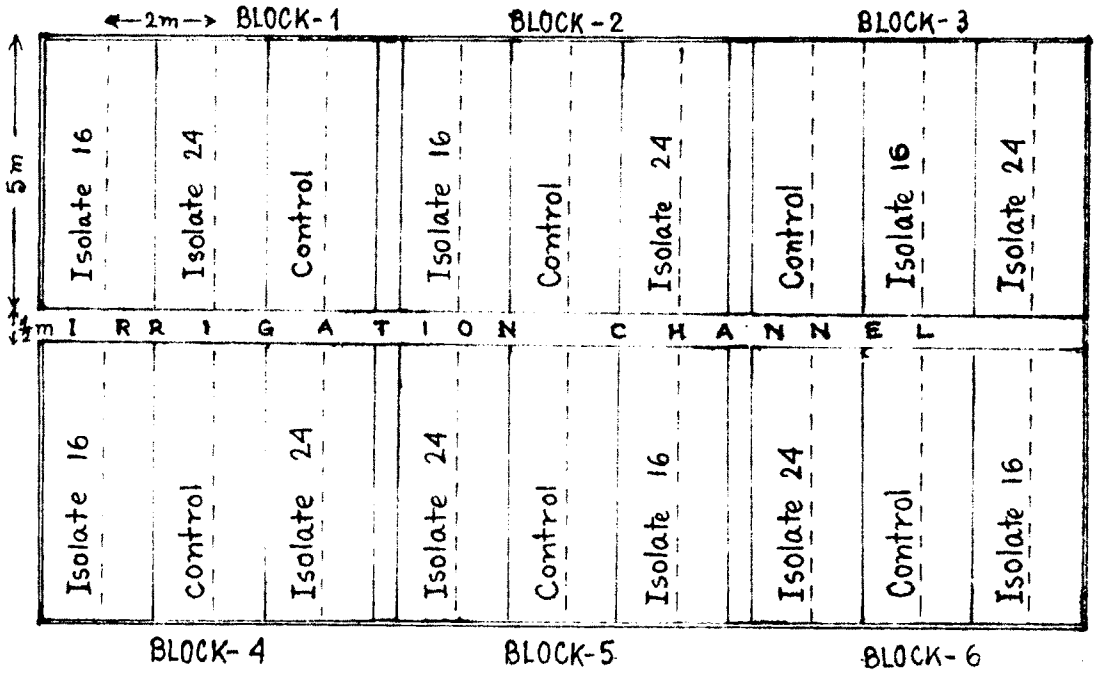
B. **Under field condition**

Similar experiments were conducted under field condition for two years. For the 1st year the experiment was conducted during Oct.-Nov. 1989, Jan.-Feb. 1990, and May-June 1990. And for the 2nd year the experiment were conducted during Oct-Nov, 1990, Jan-Feb 1991 and May-June 1991.

The experiment was conducted in sandy loam soil under upland condition with irrigation facilities. Well decomposed compost was added @ 12 tonnes/ha 15 days before sowing. Basal dosage of fertilizers i.e. N,P and K were added @ 30:60:30 just before final tillage.

The total field was divided into six blocks. Only two isolates of A. niger - isolate 16 and isolate 24 were used in the experiment. In each block each treatment was present. Each line represented each treatment. Each line was 5m in length and were placed 2 m apart. Inoculum was grown in sand-maize meal medium for 10 days at 28 ± 1 degree C. Each line were treated with 4 packets (100g each) of respective inoculum in upper 5 cm of depth. 50 nos. of surface sterilized seeds (var. JL-24) were inoculated with respective inoculum by simply rolling over the inoculum grown in PDA medium in Petridish for 7 days. Inoculated seeds were sown in each line at 5 cm depth and 10 cm appart and covered with inoculum soil mixture. Soil ridges were built on each side of the line to prevent flow of irrigation water from one line to another. Irrigation was given in a very controlled way. The disease incidence was recorded upto 45 days after sowing after which no increase in disease incidence was recorded. Uninoculated seeds were sown as control.

LAYOUT OF THE EXPERIMENT



3.7.3 To study the relative pathogenicity of various isolates of A. niger and persistence of infectivity of sick soil.

Kernels of groundnut (variety JL-24) were used in all the tests and they were surface-sterilized before sowing in the earthen pots. Soil was sterilized in an autoclave at 20 lb. pressure for 2 hours for two consecutive days. For artificial infestation of soil, inoculum of A. niger (isolate nos, 2,3,5,16,18,20,21,22,23,24) was prepared on sand-maize meal medium. Inoculum was mixed with sterilized soil kept in earthen pot @ 1:1. Survival of inoculum in soil was assessed by sowing

of surface sterilized kernels in sick soil during May-June 1990, Oct.-Nov. 1990, Feb-March 1991, and May-June 1991. Sterilized soil, without any inoculum added to it, served as the control. The pots were kept under glass house condition and were irrigated as and when required.

3.8. **Effect of physical injury on disease incidence**

Surface sterilised groundnut seeds (var. JL-24) were injured by needle scratching on the cotyledon. Inoculum was grown in sand-maize meal medium for 10 days. Autoclaved soil was inoculated 5 days before sowing of seeds @ 1:1 V/V and covered with cotton wool and kept in glasshouse. Watering was done as and when required. 8 nos. of seeds were sown in each earthen pot (10 cm in diameter). Soil inoculation was done with isolate - 16 and isolate-24 of A. niger separately. 7 day old seedlings developed from uninjured seeds in autoclaved soil were injured at the hypocotyl region by needle scratching and inoculated by placing 0.5 cm square of conidial and mycelial mat on the injured hypocotyl. The disease incidence was recorded.

3.9 **Effect of soil moisture on disease incidence**

Autoclaved sandy loam soil, filled in earthen pot (20 cm in diameter), inner side of which was covered with plastic sheet to regulate the soil moisture, was inoculated with A. niger, grown in sand-maize-meal medium for 10 days, @ 1:1 V/V. After

initial establishment of the pathogen in soil i.e. after 4 days, 10 surface sterilized seeds of groundnut (var. JL-24) were sown in each pot. The moisture level of the soil was maintained at 5%, 10%, 15% and 25% by weight by daily watering. The experiment was conducted with two isolates of A. niger i.e. isolate 16 and isolate 24 under glass house condition during May-June months 1990. The disease incidence was recorded upto 45 days after seeding after which no increase in disease incidence was recorded. In control, surface sterilized seeds were sown in autoclaved soil.

3.10 **In vivo, in-vitro enzyme production**

3.10.1 **Organism**

Various isolates of A. niger 2, 3, 5, 16, 18, 20, 21, 22, 23 and 24, maintained on PDA slants in the laboratory were used for the experiment.

3.10.2 **Estimation of in-vitro production of enzymes**

a) **Culture medium** : Ammonium oxalate medium tested by Gupta and Gupta (1967) was employed for studying the enzyme secretion. It contains 0.5% Glucose, 0.389% ammonium oxalate, 0.1% Potassium dihydrogen phosphate and 0.05% magnesium sulphate supplemented with 1% pectin. pH of the medium was adjusted to 5.5 before autoclaving by addition of 0.1 N NaOH. Aliquots of 25 ml of medium was taken in 150 ml Erlenmeyer flasks and autoclaved at 15 psi for 15 minutes.

b) **Cultural methods** : The inoculum in each case consisted of a disk of 8mm diameter cut from the margin of a freshly grown colony (5 days old) of the fungus on PDA medium. The cultures were incubated for six days at 28 $\pm$ 1 degree C in a B.O.D. incubator.

c) **Yield of organism** : Mycelium along with sporulation was harvested on a preweighed Whatman No. 42 filter paper, washed extensively with warm distilled water, heated overnight in an electric oven at about 90 degree C, cooled in a desicator and weighed. No further loss in weight was obtained for longer periods of drying. The pH of the crude culture filtrate was immediately recorded.

d) **Enzyme Preparation** : Crude culture filtrates obtained after harvesting mycelium were used for enzyme assay after dialysis at 4 degree C against distilled water for 16-18 hrs. and stored at 0 degree C until used.

e) **Enzyme substrate** :

a) 1% sodium polypectate solution was used as substrate for PG enzyme assay.

b) 1% CMC solution was used as substrate for Cx enzyme assay.

f) **Enzyme assay** : Boiled enzyme preparations were used as control. Reduction of viscosity of substate solution - viscosity loss was determined using a type 300 Fenske-Ostwald viscometer at 30 degree C (Bell et al., 1955). For PG enzyme assay the reaction mixture contained 4 ml of 1% Sodium ploypectate solution, 1 ml citric acid sodium citrate buffer (0.1 M), 1 ml enzyme preparation and water to 10 ml. For cellulase enzyme assay the reaction mixture contained 4 ml 1% CMC solution, 1 ml citric acid-sodium citrate buffer (0.1 M) and 1 ml enzyme preparation. The flow time as converted to percent loss in using the formula

$$V = \frac{E_o - E_t}{E_o - W_w} \times 100$$

V = % loss in viscosity

E_t = Flow time of the reaction mixture at time t

E_o = Flow time of the reaction mixture at zero time

E_w = Flow time of the distilled water.

RA/ml (Relative activity unit per ml) has been determined from the reciprocal of the time in minutes required for 50% loss in viscosity multiplied by 1000.

g) **Toxicity measurement** : Toxicity of the enzyme preparation was determined by the plasmolytic method of Tribe (1955), based on the principle that only live protoplasts retain neutral red in plasmolysing solutions. Twenty four disks of potato tuber (7 cm diameter, 0.5 mm thick) were placed in a reaction mixture containing 5 ml enzyme sample, 2 ml buffer and made upto volume 10 ml with water, at intervals 3 disks were removed from the reaction mixture, washed rapidly and then placed in 2 ml of the following solution for staining : 85 ml of 0.9 M KNO_3, 10 ml 0.1% Neutral Red solution and 5 ml 0.1 M phosphate buffer, pH 7.5 Disks quickly plasmolysed and accumulated the stain in this solution which after 20 minutes were replaced by 0.9 M KNO_3 containing 0.01 M phosphate buffer at pH 7.5. Stain rapidly leached from dead protoplasts whereas live cells retained red. The neutral red index of Tribe (1955) was used to record results on a continuous scale 0-5 in which 0 indicates that all protoplasts are dead and 5 indicates that all are living. The data were recorded accordingly.

h) **Maceration** : Maceration was accessed by the needle test. Reaction mixtures were the same as toxicity measurement and at similar intervals three disks were removed and tested by attempting to pull them apart using needles.

They were regarded as macerated when they showed no resistance.

i) **Permeability changes** : Permeability changes were measured by assessing increase in conductivity of solutions as electrolytes leaked out into them from tuber disks. Reaction mixtures were similar to toxicity measurement and at intervals three disks were removed from the reaction mixture, washed gently and placed in 15 ml glass distilled water. It was shaken for 10 seconds and the conductivity was then measured by using Systronics Direct Reading Conductivity meter 303. Results were expressed as the increase in conductivity during 15 minutes over control.

3.10.3 Estimation of in-vivo production of enzymes

3.10.3.1 Enzyme preparation

The groundnut plants were grown in sterilized soil in 4" earthen pots from surface sterilized seed (CV.JL-24) and 7 day old seedlings were inoculated with respective isolate of A. niger at the collar region. 5 g of infected tissues were collected from diseased plants of early stage (4 days after inoculation) of plant infection and was thoroughly ground along with 10 ml of distilled water and 10 ml of 5 N NaCl solution. The mixture was filtered by Whatman No. 42 filter paper and

were centrifuged at 2000 Rpm for 5 minutes and was dialysed at 4 degree C for 18-20 hours and then used as crude enzyme sample and stored at 0 degree C until used.

3.10.3.2 **Enzyme assay** :

Boiled enzyme preparations were used as control. Enzyme activity was assayed viscosimetrically using a type 300 Fenske-Ostwald viscometer. For PG enzyme assay, the reaction mixture contained 4 ml 1% sodium polypectate solution, 4 ml enzyme preparation and 2 ml citric acid-sodium citrate buffer.

3.10.4 **Synergistic action of oxalic acid and pectic enzyme on the collar rot of groundnut caused by Aspergillus niger.**

The groundnut plants were grown in sterilized soil in 4" earthen pots from surface sterilized seeds (CV. JL-24) and 7 day old seedlings were inoculated with respective isolate of A. niger at the collar region. 5 g of infected tissue were collected from diseased plants at early stage of plant infection and was thoroughly ground along with 10 ml of 5N NaCl solution. The mixture was filtered by Whatman No. 42 filter paper and were centrifused at 2000 rpm for 5 minutes and was dialysed at 4 degree C for 18-20 hours and then used as crude enzyme sample and stored at 0 degree C until used.

To 5 ml aliquot of the cell free crude enzyme sample in centrifuge tube 4 ml of calcium chloride-acetate buffer (pH 4.5) was mixed. The mixture was allowed to stand overnight and centrifuged at 3000 rpm for 10 minutes. The supernatent was discarded and the sediment was washed with 5 ml of 5% acetic acid saturated with calcium oxalate and was centrifuged. The residue was disolved in 5 ml of 4N H_2SO_4 and the solution was transfered to 100 ml flask and was heated to 80-90 degree C on a water bath. The solution was titrated with 0.02N potassium permanganate until a faint pink colour persisted. The amount of oxalic acid present in the crude enzyme sample was calculated. One ml of 0.02N potassium permanganate will react with 1.2653 mg of oxalic acid.

3.11 **Biocontrol**

Ten isolates of Aspergillus niger were selectively isolated from groundnut cultivated soils from different parts of West Bengal using M3 S1B medium. Their relative pathogenicity was tested against groundnut and was identified by Division of Mycology and Plant Pathology, I.A.R.I., New Delhi. The culture of Trichoderma harzianum was collected from Departmental laboratory, BCKV and both fungi were maintained in PDA.

3.11.1 **In Vitro**

A 5 mm discs of A niger (isolate 16, Isolate 24) were taken and transferred to P.D.A. media plates 2-3 cm apart from the edge.

The other side of the medium was inoculated with 5 mm disc of T. harzianum. Three replicates were used for each treatment and control. The plates were incubated at 28 ± 1 degree C and percentage of reduction was calculated after 24 and 72 hrs. To see the mycoparasitism, intermingled hyphae were mounted under microscope on slides after stain with cotton blue.

3.11.2 **In vivo**

3.11.2.1 **In glass house**

I. **Seed dressing with T. harzianum spore**

Four levels of spore suspensions i.e., 0,25,100 and 250 x 10^6 spores/ml were prepared from 7 days old cultures of T. harzianum grown on PDA medium.

Surface sterilized groundnut seeds (var. JL-24) were treated with each concentration of spore suspension @ 20 ml/20 g seeds. In control treatment seeds were treated with water only before sowing. Soil of earthen pots were infested with the highly virulent strain of A.niger (isolate 16) grown in autoclaved sand-maize meal medium for 10 days at 28 ± 1 degree C @ 0,30 and 50% of the soil weight. Four replication for each treatment were maintained

and the pots were watered as and when required. Percentage of seedrot and collar rot incidence were recorded.

II. The inoculum of both T. harzianum and A. niger (isolate 16) were grown in autoclaved sand-maize meal medium for 10 days at 28 ± 1 degree C. Sandy loam soil from upland cultivated field was collected for the experiment. Surface sterilized (with 4% formaldehyde solution) earthen pots (4" diameter) were used. Inoculum was mixed with soil (sterilized / unsterilized) @ 1:1 and upper 5 cm depth of the pots were filled with this mixture. Seeds of groundnut (var. JL-24) were surface sterilized with 0.25% sodium hypochlorite for 2 minutes and then rinsed thoroughly with sterile distilled water.

The experiment was conducted according to the following treatments.

1. Treatment 1 (T_1) - Inoculum of T. harzianum was mixed with sterilized soil @ 1:1 and kept for 7 days with slight irrigation under cotton wool plug for initial establishment of the organism in the soil and then 10 surface sterilized seeds were sown in each pot.

2. Treatment 2 (T_2)- Treatment was similar to T_1, but here normal soil was used instead of sterilized soil.

3. Treatment 3 (T_3) - Inoculum of A.niger (isolate 16) was mixed with sterilized soil @ 1:1 and kept for 7 days with slight irrigation under cotton wool cover and then 10 surface sterilized seeds were sown in each pot.

4. Treatment 4 (T_4) - Treatment was similar to T_3, but here normal soil was used.

5. Treatment 5 (T_5) - Inoculum of A. niger was mixed with sterilized soil @ 1:1 and kept for 7 days under cotton wool cover with slight irrigation. Seeds were surface sterilized and then inoculated with irrigation. Seeds were surface sterilized and then inoculated with **250 X 10^6** spore/ml spore suspension of T. harzianum culture, grown on PDA in Petri plates for 7 days @ 20 ml/ 20 g seeds and 10 such seeds were sown in each pot.

6. Treatment 6 (T_6) - Treatment was similar to T_5, but here normal soil was used instead of sterilized soil.

7. Treatment 7 (T_7) - Inoculum of T. harzianum & A. niger were mixed with sterilized soil both @ 1:1 and kept for 7 days with slight irrigation under

cotton wool cover and then 10 surface sterilized seeds were sown in sterilised soil @ 10 seeds/pot.

8. Treatment 8 (T_8) - treatment was similar to T_7 but here normal soil was used in the experiment.
9. Treatment 9 (T_9) - Surface sterilized seeds were sown in sterilised soil @ 10 seeds/pot.
10. Treatment 10(T_{10}) - Treatment was sililar to T_9 but here seeds were sown in normal soil instead of sterilised soil.

Four replications for each treatment were maintained. Pots were kept in glass house in randomised manner and were supplied with water as and when required. Disease incidence was recorded upto 45 days after sowing as there was no increase in disease later on.

3.11.2.2 **In field**

During dilution plating with rhizosphere soil of groundnut, Colony of Streptomyces spp. on PDA was found to inhibit further growth of A. niger colony in Petriplates. The bacterium was isolated into pure culture and tested for its antagonistic property against virulent isolates of A. niger in vitro condition. Getting promising response, a field experiment was carried out at Mandouri farm of B.C.K.V. during May-June, 1990 on upland sandy loam soil with irrigation facilities.

Biocontrol of crown rot disease with this bacterial isolate (Streptomyces sp) and T. harzianum was compared with seed treatment with different established fungicides e.g. Carbendazim (Bavistin 50 WP @ 0.1%) Copper oxychloride, (Blitox 50 WP @ 0.4%) and MEMC (Emisan-6 @ 0.25%). The layout of the experiment was designed according to factorial randomised block design. One month before sowing soil was ammended with well decomposed F.Y.M. @ 12.5 tonnes/ha and before final tillage basal dose of N.P. and K. fertilizers @ 30:60:30 kg/ha were added. The whole experimental plot was divided into 4 different blocks each of 10 m X 5 m in size. Irrigation channels were prepared suitably. Each line (one line = one treatment) was 5 metres and line to line i.e. treatment to treatment distance was 1 m. Ridges were prepared between each line to prevent flow of water from one line to another. Irrigation was given in a very controlled way so that water could not move from one treatment to another. Inocula of A. niger and T. harzianum were grown in packets (10 X 6"). Inocula of Streptomyces sp. and T. harzianum were prepared on PDA medium in Petri plates. Surface sterilized seeds (var. JL-24) were treated with respective fungicides. Some surface sterilized seeds were inoculated with spore suspension of the bacterium and T. harzianum culture separately so that the inoculum of respective organism could cover the outer surface of the seeds thoroughly. Inoculum of A. niger was mixed with sterilized soil @ 1:1 7 days before

sowing. 50 seeds were sown in each line at 10 cm distance & 5 cm depth. The experiment was conducted according to the following treatments.

1. Treatment-1 (T_1) : Surface sterilized seeds were inoculated with spore suspension (250 X 10^6 spores/ml) of T. harzianum and seeds were sown and then covered with A. niger inoculated soil.
2. Treatment 2 (T_2) : Surface sterilized seeds were inoculated with spore suspension (250 X 10^6/spores/ml) of T. harzianum @ 20 ml/20 g and seeds were sown and then covered with a soil mixture of A. niger and T. harzianum, prepared 7 days before sowing.
3. Treatment 3 (T_3): Surface sterilized seeds were treated with Copper oxychoride (Blitox 50 WP @ 0.4%) and then seeds were sown and then were covered with A. niger inoculated soil.
4. Treatment 4 (T_4) : Surface sterilized seeds were treated with Carbendazim (Bavistin 50 WP @ 0.1%) and sown and then covered with A. niger inoculated soil.
5. Treatment 5 (T_5) : Surface sterilized seeds were treated with MEMC (Emisan-6 @ 0.25%) and then seeds were sown and then covered with A. niger inoculated soil.

6. Treatment 6 (T_6) : Surface sterilized seeds were inoculated with spore suspension of Streptomyces sp. grown in Petriplates and then sown and covered with A. niger inoculated soil.

7. Treatment 7 (T_7) : Surface sterilized seeds were sown and covered with A. niger inoculated soil. It served as a control for seed treatment.

8. Treatment 8 (T_8) : Surface sterilized seeds were inoculated with spore suspension (250 X 10^6 spores/ml) of T. harzianum culture, grown in petriplates, @ 20 ml/20 g seed and then were sown and covered with normal soil.

9. Treatment 9 (T_9) : Surface sterilized seeds were treated with spore suspension of the Streptomyces spp. grown in Petriplates, @ 20 ml/20 g of seed and then sown & covered with normal soil.

10. Treatment 10 (T_{10}) :Surface sterilized seeds were sown and covered with normal soil. It served as a control for soil inoculation.Four replications for each treatment were maintained. Disease incidence were recorded upto 45 days after sowing as there was no increses in disease later on.

3.11.2.3 **In storage** :

To find out the effect of surface treatment of groundnut seeds by Trichoderma harzianum on storage, groundnut cultivar JL-24 and A. niger (Isolate 16) were taken for experiment.

A Spore suspension (i.e., 250 X 10^6) spores/ml for each T.harzianum & A. niger (isolate 16) from 7 days old cultures, grown on PDA medium at 28 ± 1^0 C, were prepared.

The experiment was conducted according to the following treatments :

1. Treatment 1 (T_1) (Treatment 1) : 200 nos. of surface sterilized groundnut seeds were treated with spore suspension of T. harzianum @ 20 ml/100 g seeds and dried in shadow.
2. Treatment 2 (T_2) : 200 nos. of groundnut seeds were treated with spore suspension of T. harzianum @ 20 ml/100 g seeds and dried in shadow and after 2 days those seeds were again treated with A.niger spore suspension. and then dried in shadow.
3. Treatment 3 (T_3) ; 200 nos. of groundnut seeds were treated with spore suspension of A.niger @ 20 ml/100 g of seeds and then dried in shadow.
4. Treatment 4 (T_4) : 200 nos of groundnut seeds were treated with distilled water only and dried in shadow.

All seeds were stored in paper packets separately. Incidence of seed & collor rotting was recorded at one month interval started from Feb. 1991 upto six months after treatment . Three

pot replications for each treatment were maintained. Disease incidence was recorded upto 45 days after sowing after which there was no increase in disease incidence.

3.11.2.4. **On sclerotia**

During field experiment with A. niger & T. harzianum selarotia produced by A. niger were found to be colonised by T. harzianum. T_o find out the effect of colonization of selerotia of A. niger by T. harzianum and an antagonistic bacteria, isolate 3 of A. niger a pathogenic isolate, which was found to produce abundant sclerotia on sand-maize meal medium, was selected.

Sclerotia of A. niger were allowed to grow on sand-maize medium for two months and then they were separated from medium washing in tap water and then air dried.

For experiment, different types of soil were collected from different parts of West bengal. 200 nos. surface sterilized (with 0.1% mercuric chloride solution for 1 min.) selerotia were placed in each type of soil (sterilized and normal soil), kept in small earthen pots in glasshouse condition. Some of the pots were poured with 100 ml of spore suspension of T. harzianum (300 X 10^6/ml) and Streptomyces sp. grown in PDA medium for 7 days. The pots were supplied wit water to keep the soil slightly moist.

At certain days of interval sclerotia from different treatments were separately collected and their germinability were tested in distilled water soaked filter paper in Petri plates on which some surface sterilized groundnut seeds (var.JL-24) were placed to stimulate germination of sclerotia and germination % was recorded.

The sclerotia from different treatments were physically examined under binocular to see the colonization by T. harzianum.

3.12. Effect of seed treatment with heavy metal salts in disease incidence

The present experiment was conducted to make a comparative study of the effect of seed soaking with some heavy metal salts, on induction of resistance in groundnut as a disease control measure against crown rot (c.o. Aspergillus niger).

3.12.1. The test chemicals

The following chemicals were used for seed soaking- Nitrate of i) Barium Sulphate of i) Zinc, (ii) Copper, (iii) Barium , (iv) Manganese Chloride of i) Iron ii) Zinc iii) Mercury iv) Copper. All these compounds have been reported as inducers of phytoalexin production in different plant species (Perrin and Cruickshank, 1965).

From the results of effect of these salts on germination of groundnut seeds, different concentrations were used for different salts e.g. Ba $(No)_3$ [10^{-2} M to 10^{-4} M)] $ZnSO_4$ [10^{-2} M to 10^{-4} M),

$CuSO_4$ [10^{-2} M to 10^{-3} M], $MnSO_4$ [10^{-2} M to 10^{-3} M], $Fe\,CI_3$ (10^{-3} M to 10^{-5} M) $ZnCl_2$ (10^{-2} M to 10^{-3} M), $HgCl_2$ (10^{-4} M to 10^{-6} M). and $CuCl_2$ (10^{-3} M to 10^{-5} M)

3.12.2. **Fungitoxicity assay of metal salts**

All the salts of different heavy metals to be tested against crown rot disease, were initially screened at different concentrations for their fungitoxic effect if any, on selerotial germinations of the pathogen. These concentration were near optimum for the production of phytoalexin in pea plants (Perrin and Cruickshanke, 1965). The fungi toxicity of test chemicals was assayed in Petridish. The filter papers were soaked in test chemical solution and 25 sclerotia of A. niger were placed on the filter paper in a Petridish. There were 3 Petridishes for each treatment. To stimulate germination of sclerotia 5 surface sterilized groundnut seeds (Var. JL-24) were placed in Petriplate. The whole Petridishes were incubated at 28 ± 1 degree Centrigate for 48 hrs. Then growth of fungus from selerotia was studied under a binocular. The observations were taken as follows :

1. No germination of sclerotia
2. Eruptive germination (where growth of fungus from selerotia was one or both sided)
3. Normal germination (where growth of fungus from selerotia was radially).

3.12.3. **Soaking of seeds**

Seeds were soaked in various salt solutions by keeping them submerged in them for 24 hours at room temperature. For the control, the seeds were soaked in equal volume of distilled water.

3.12.4. **Preparation of inoculum**

The inoculum of the pathogen was grown in sand-maize meal (3:1) medium in polypropetene packets (10" X 6") for 10 days at 28 ± 1 degree Centrigate temperature.

3.12.5. **Experiment in field**

Only those metallic salts which showed promising response at certain concentration in induction of resistance in groundnut against A. niger under glass house condition, were taken to see their efficiency in induction of resistance under field condition.

i) **Field preparation** : The experiment was conducted during May-June months 1990 in high land sandy loam soil having irrigation facilities at Mandouri Farm of BCKV at Mohanpur. Well decomposed compost was added @ 12 tonnes/ha 15 days before sowing, Basal dosage of fertilizers i.e. N,P & K were added @ 30:60:30, just before final tillage.

ii) **Layout** : The layout of the experiment was designed according to factorial randomised block design. Size of each Plot was 10 M X 5 M and length of each line was 5 m and were spaced at 1 m distance. Each line represented each treatment. Ridges were formed between the lines to prevent movement of irrigation water from the one treatment to other. Four replications for each treatment were maintained.

iii) **Inoculation of plants** : 50 seeds for each treatment were sown in each line at 10 cm distance. The 10 day old plants were inoculated at the collar region with inoculum of A. niger after mixing with double sterilized soil @ 1:1.

The disease incidence was recorded upto 45 DAS after which no increase in disease incidence was recorded.

3.13. Biochemical analysis

3.13.1. Assay of polygalacturonase (PG) enzyme activity

Viscosimetric method as described by Bell et al. (1955) with modification as suggested by Hancock et al (1964) was adopted. To 8 ml of appropriately bufferised substrate 4 ml of enzyme was added in an Ostwald's viscosimeter and flow time was recorded at 0,20,40,60,80 and 100 min. During this period the enzyme subtrate mixture was incubated at 30 degree Centrigate in a thermostatic water bath. The flow time of water and the flow time of a mixture of 8 ml bufferised substrate and 4 ml autoclaved enzyme (autoclaved for 10 min. at 15 Psi) was determined.

$$PDFt = \frac{E - E_t}{E - F_w} \times 100$$

where, E = Flow time of substrate + Inactivateed enzyme

E_t = Flow time of substrate + active enzyme preparation after time t.

E_w = Flow time of distilled water.

This % reduction in viscosity was plotted against time and time required for 50% reduction in flow time ($PDF_{T\ 50}$) was calculated. The specific enzyme activity was determined from the formula, 1/t X 100∅, where 't' is the time required in min for 50% reduction in viscosity.

In this way PG enzyme activity in different treatments were determined and tabulated.

3.13.2. **Estimation of total phenol**

Extraction : Tissue segments (1-2cm) were put in boiling ethyle alcohol (5-10ml/g tissue) in a water bath and was kept there for 5-10 min. The extract was cooled in a pan of cold water, and then the tissue was crushed thoroughly in a morter with pestle for 5-10 min. and the extract was passed through layers of cheese cloth. The ground tissue was extracted for 3 min. in boiling 80% alcohol (using 2-3 ml of alcohol for every gram of tissue). The second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and then was passed through cheese cloth and its volume was raised with 80% ethanol or reduced by evaporating it to a volume to represent 5 ml/g of tissue used.

Folin-ciocalteu method : Estimation of phenols with Folinciocalteu reagent is based on the reaction between phenols and as oxidising agent phospomolybdate which results in the formation of a blue complex (Brey and Thorpe, 1954).

Method : One ml of extract was pipetted into a graduated test tube, to which added 1 ml or Folin-cioalteu reagent followed by 2 ml of Na_2CO_3 (20%) solution. The tube was shaken and heated

in a boiling water bath for exactly 1 min and then cooled under a running tap. The resulting blue solution was diluted to 25 ml with water and its absorbance was measured at 650 mm in a colorimeter (Readings of unknown were taken from a standard curve made from different concentration of catechol). Results were expressed as catechol equivalent measured in terms of a standard curve prepared with catechol.

In this way, total phenols from different treatments were determined and results were tabulated.

3.12.3. **Estimation of peroxidase activity**

This tissue was crushed with cold phosphate buffer pH 6.0; 0.05 M 5 ml/g of tissue. The slurry was centrifused and the supernatant was taken and dialysed for 24 hours against the same buffer.

Reaction mixture :

a) 5 ml pyrogallol solution (0.05 M in 0.1 M phosphate buffer pH 6.0 i.e. 0.31 g pyrogallol in 5.0 ml buffer)

b) 0.1 ml dialysed extract.

c) 0.5 ml of 1.0% hydrogen peroxide.

All ingredients except H_2O_2 were taken in colorimetric tube and the null point was set at '0' at 420 mμ, then H_2O_2 was added and mixed immediately and O.D. taken at 420 mμ within 40-160 seconds.

The blanks were prepared without H_2O_2. In this way peroxidase activity was determined in different treatments and the results were tabulated,

3.13.4. Effect of seed treatment with the salts of heavy metal on growth characteristic and yield of groundnut

Since some of the salts in seed substantially reduced disease development in groundnut plants, it was of interest to determine how these affected various growth characters and yield attributes. Various observations were recorded in this connection from randomly selected six plants for each nine highly effective treatments. The results are recorded.

3.14. Effect of different fungicides on various isolates of A. niger

The fungicide Tested were :

Trade name	Active ingrediant	Dose	Source
Emisan - 6	Methoxy ethyle mercury chloride	0.25%	Excel Industries Ltd.
Blitox - 50	Copper oxychloride	0.4%	Rallis India Ltd.
Bavistin 50 WP	Methyl-2-benzimidazole carbamate	0.2%	BASF India Ltd.
Indofil M-45	Zinc + manganous ethylene - bisdithiocarbamate	0.2%	Indofil chem. Ltd.
Kabach 75 WP	Chlorothalonil	0.2%	Dimond Shymrock Corporation India

3.14.1. **Effect on growth and sporulation**

Measured quantity of fungicides was incorporated and mixed thoroughly into sterilized warm potato dextrose agar (PDA) medium in conical flasks to get 250, 500 and 1000 ppm concentration of each fungicides. 25 ml of poisoned medium was then poured into sterilized Petridishes, keeping four replicates of each treatment for each isolate of A. niger. PDA medium without fungicide served as control. After solidification of the medium, pure culture discs of 4 mm diameter taken from peripheral region from 5 days old culture, were inoculated in the centre of the plates. Ten isolates of A. niger (2, 3, 5, 16, 18, 20, 21, 22, 23 and 24) were used in the screening test. The plates were then incubated at 28 ± 1 degree C. The radial growth of the fungus was measured upto 120 hours after inoculation when the fungus covered the entire medium in Petridish in the control.

3.14.2 **Effect of fungicide on germination of sclerotia of sclerotia producing isolates of A. niger.**

Four sclerotia producing isolates of A. niger viz. isolate 3,16,18 and 23 were selected. They were allowed to produce sclerotia in sand maize meal medium, incubating at 28 ± 1

degree C for more than 21 days. After that the sclerotia were harvested by washing in tap water and then air dried.

Measured quantity of fungicides was incorporated and mixed thoroughly into sterilized warm PDA medium in conical flasks to get 250,500,1000 and 2000 ppm concentration of each fungicides. 25 ml of poisoned medium was then poured into sterilized Petridishes, keeping three replications for each treatment for each isolate of A. niger PDA medium, ten numbers of surface sterilized sclerotia were placed in each plate. And then incubated in 28 ± 1 degree C temperature.

To study the effect of these fungicides at 250,500,1000 & 2000 ppm concentration on growth of isolates, 3,16,18 and 23 of A. niger after germination from their sclerotia, single surface sterilized sclerotium was placed at the centre of Petridish poured with fungicide treated PDA medium. Three replications were maintained for each treatment and PDA medium without fungicide served as control. The plates were incubated at 28 ± 1 degree C temperature and radial growth of the fungus from sclerotia was recorded upto 120 hours after inoculation when the fungus covered the entire medium in Petridish in the control.

3.15. Effect of culture filtrate of various isolates of A. niger on groundnut.

3.15.1 Role in pathogenesis

To study the effect of culture filtrate on seedlings, 10 - day old seedlings (Var.JL-24) developed in autoclaved sand, were uprooted carefully to reduce root injury and placed in culture filtrate ammended Hoogland solution (1:9 v/v) in culture tubes. In control seedlings were placed in distilled water ammended Hoogland solution. Ten replicates were maintained for each treatment and they were kept in diffused sunlight. Observations upto 10 days were recorded.

10 different isolates, numbered as 2,3,5,16,18,20,21,22,23, and 24 of A. niger were grown in Potato Dextrose Broth (PDB) for 7 days. Pure culture filtrates were then collected from them. 100 nos. of groundnut seeds (var. JL-24) were soaked in steriled distilled water @ 10 seeds/pot were then sown in autoclaved soil and kept with occasional supply of water. Effect on seed germination and subsequent plant growth was recorded. Four pot replications were maintained for each treatment.

3.15.2. **Role in induced resistance**

Culture filtrates from PDB of each 10 isolate of A. niger were diluted 10 times with addition of sterile distrilled water. Groundnut seeds (Var. JL-24), were soaked obvernight in diluted culture filtrate and then sown in autoclaved soil kept in earthen pots (4" diam.) @ 10 seeds/ pot. For control groundnut seeds were soaked overnight in sterile distilled water. After 7 days of emergence seedlings were inoculated with inoculum of respective isolate, grown in sand-maize meal for 7 days at 28 ± 1 degree C. 3 replicates of each treatments were maintained in glass house with occasional supply of water and disease incidence were recorded upto 45 days of seedling age after which no increase in disease incidence was observed.

To see whether seed soaking with diluted culture filtrate of one isolate of A. niger is equally efficient in induction of resistance in groundnut against other isolate of A. niger, the experiment was conducted following the above method only here seed soaking was done with diluted culture filtrate of most virulent isolate (no. 16) of A. niger.

3.16 Effect of growth regulators on induced resistance in groundnut plant against A. niger

3.16.1. **Plant** : For experiment of crown rot disease CV.JL-24, a popular high yielding variety susceptible to the disease was used as the plant material.

3.16.2 **Fungus** :A virulent isolate of A. niger (isolate-16) was used to conduct the experiment concerned with crown rot of groundnut. The virulent isolate was intially obtained from the stock cultures maintained in the Department of Plant Pathology, B.C.K.V. , Mohanpur. The isolate was passed through its host plant to ensure its pathogenicity before use. Routine maintenance of the fungal culture was done on Potato-Dextrose-Agar medium.

3.16.3 **Test chemical** : The following growth regulators were used at specific concentration for seed soaking in solutions for 12 hours before sowing in field.

a) Chitosan : 1%, 0.3%, 0.1%

b) Cycocel : 10^{-3}M and 10^{-4} M

c) I.A.A. : 10^{-5}M

d) 2-4-D : 10^{-6}M

e) 2-4-5-T : 10^{-6}M

For control, the seeds were soaked in equal volume of distilled water.

3.16.4 **Preparation of inoculum**

The test fungus was grown in sand maize medium in poly propelene pack for 7 days. Then it was thoroughly mixed with doubly sterilized soil in 1:1 proportion to obtain the inoculum for the inoculation of groundnut plants.

3.16.5 **Field lay out**

The field was diverted into four blocks of 8m x 2 m size. For each treatment one line (2m in length) was selected in each block in a randomised manner. Seeds, soaked in respective growth regulator in specific concentration, were sown at 5 cm apart in each line and covered with inoculum

soil mixture. Line to Line i.e. treatment to treatment distance was 1 m. The field was irrigated as and when required in a very controlled way to prevent flow of water from one line to another.

The effect of growth regulators on disease susceptibility and growth characteristics and yield was recorded.

CHAPTER IV

EXPERIMENTAL RESULTS

4.1. Varietal reaction to A. niger

One hundred thirtyeight varieties or germplasms of groundnut (Arachis hypogaea L.) were screened against two isolates of A. niger (16 and 24) in glass house condition. This is the first time in West Bengal where a large number of germ plasms (Groundnuts) were screened against A. niger. Ten surface sterilised seeds of groundnut were sown in 25cm in diameter earthen pots. The inoculum was grown in Sand-maize meal medium in a 250ml conical flask. 10-day old culture was mixed up with well pulvarised soil and spread in the pot in 1:3 ratio (w/w). After 3 days 8 surface sterlised seeds were sown in each pot. Watering was done regularly. Rotted seeds were taken out carefully and regularly and ten-day old seedling were inoculated at collar region with a mixture of 5-day old mycelium and spores of A. niger grown in PDA medium and covered with wet soil. The detail procedure adopted has been given in chapter-III of this manuscript. Disease incidence (seed rot and collar rot) was recorded at percentage basis and disease reaction was counted with the score : Immune (I) value 1 to 8.3 tolerant (T), 8.3 to 20.0 moderately susceptible (MS) and above 20.0 is susceptible (s) (Chahal et. al. (1974). The data are presented in Table-4.1.

Table - 4.1 Varietal reaction to A. niger

Sl No.	Variety		A. niger Isolate-16		A. niger Isolate-24	Reaction
	Germplasm	Habit Group	Seed rot (%)	Collar rot (%)	Seed rot (%)	Collar rot (%)
1.	C-176	VR	16.6 (24.04)	12.5 (20.70) MS	16.6 (24.04)	8.30 (16.74) T
2.	C-175	VR	16.6 (24.04)	16.60 (24.04)MS	16.6 (24.04)	8.30 (16.74) T
3.	Rajkot Local	VR	20.8 (27.13)	4.10 (11.68) T	16.6 (24.04)	12.50 (20.70)MS
4.	2340	VB	33.3 (35.24)	20.80 (27.13) S	62.50 (52.24)	20.80 (27.13) S
5.	C - 15	VR	33.3 (35.24)	29.16 (32.65) S	25.00 (30.00)	16.60 (24.04)MS
6.	Chungja	VR	37.5 (37.76)	33.30 (35.24) S	29.10 (32.65)	12.50 (20.70)MS
7.	2956	VB	45.83 (42.59)	16.60 (24.04)MS	33.3 (35.24)	16.60 (24.04)MS
8.	Ah 7186	VR	50.00 (45.00)	20.80 (27.13) S	45.83 (42.59)	25.00 (30.00) S
9.	7300	VR	50.00 (45.00)	25.00 (30.00) S	41.60 (40.10)	25.00 (30.00) S
10.	3024	VR	54.00 (47.29)	16.60 (24.04)MS	45.83 (42.59)	24.10 (29.33) S
11	29.09	VB	54.10 (47.35)	16.60 (24.04)MS	37.5 (37.76)	16.60 (24.04)MS
12.	648	VR	54.10 (47.35)	20.80 (27.13) S	62.50 (52.24)	20.80 (27.13) S
13.	2523	VB	54.10 (47.35)	25.30 (30.20) S	62.50 (52.24)	12.50 (20.70)MS
14.	2592	VB	58.30 (49.78)	16.60 (24.04)MS	62.50 (52.24)	12.50 (20.70)MS
15.	Dipadiva Local	VR	62.50 (52.24)	12.5 (20.70) MS	58.30 (49.78)	12.50 (20.70)MS
16.	638	VR	62.50 (52.24)	16.60 (24.04)MS	66.60 (54.70)	16.60 (24.04)MS
17.	913-2	VR	66.60 (54.70)	16.60 (24.04)MS	50.00 (45.00)	20.80 (27.13) S
18.	648	VB	66.60 (54.70)	20.80 (27.13) S	50.00 (45.00)	16.60 (24.04)MS
19.	C-176	VR	75.00 (60.00)	8.30 (16.74) T	41.60 (40.10)	12.50 (20.70)MS
20.	C-176	VR	75.00 (60.00)	12.5 (20.70) MS	29.10 (32.65)	12.50 (20.70)MS
21.	583-13	VR	8.3 (16.74)	12.5 (20.70) MS	12.5 (20.70)	8.30 (16.74) T
22.	2710	VB	12.5 (20.70)	4.10 (11.68) T	12.5 (20.70)	4.10 (11.68) T
23.	Kaulikoro	VR	12.5 (20.70)	4.10 (11.68) T	20.80 (27.13)	16.60 (24.04)MS
24.	302/19	VR	12.5 (20.70)	8.30 (16.74) T	20.80 (27.13)	20.80 (27.13) S
25.	2496	VR	12.5 (20.70)	8.30 (16.74) T	20.80 (27.13)	20.80 (27.13) S
26.	EC 133155	VR	12.5 (20.70)	16.60 (24.04)MS	8.30 (16.74)	8.30 (16.74) T
27.	F-12	VR	16.60 (24.04)	4.10 (11.68) T	12.5 (20.70)	8.30 (16.74) T
28.	EC-21009	VR	16.60 (24.04)	12.5 (20.70) MS	45.80 (42.59)	37.5 (37.76) S
29.	S-7-1-6	VR	20.80 (27.13)	8.30 (16.74) T	16.60 (24.04)	8.30 (16.74) T
30.	2694	VB	20.80 (27.13)	12.5 (20.70) MS	12.5 (20.70)	12.50 (20.70)MS
31.	Lota Mangfali	VR	29.10 (32.65)	8.30 (16.74) T	25.00 (30.00)	8.30 (16.74) T
32.	G-211-31	VR	29.10 (32.65)	16.60 (24.04)MS	29.10 (32.65)	16.60 (24.04)MS
33.	578-6	VR	29.10 (32.65)	20.80 (27.13) S	41.60 (40.16)	20.80 (27.13) S
34.	4383-3	VR	33.30 (35.24)	8.30 (16.74) T	37.50 (37.76)	25.00 (30.00) S
35.	545-2	VR	45.80 (42.50)	16.60 (24.04)MS	45.80 (42.50)	37.5 (37.76) S
36.	593-11	VR	45.80 (42.50)	25.30 (30.20) S	62.50 (52.24)	20.80 (27.13) S
37.	578-8	VR	45.80 (42.50)	25.30 (30.20) S	37.50 (37.76)	25.00 (30.00) S
38.	575-5	VR	45.80 (42.50)	25.30 (30.20) S	37.50 (37.76)	20.80 (27.13) S
39.	371-1	VR	50.00 (45.00)	20.80 (27.13) S	54.10 (47.41)	20.80 (27.13) S
40.	371-1 (NRCG No.3380)	VR	58.30 (49.78)	20.80 (27.13) S	37.50 (37.76)	16.60 (24.04)MS

Sl No.	Variety: Germplasm	Habit Group	*A. niger* Isolate-16: Seed rot (%)	*A. niger* Isolate-16: Collar rot (%)	*A. niger* Isolate-24: Seed rot (%)	*A. niger* Isolate-24: Collar rot (%)
41.	2848	VB	4.10 (11.68)	4.10 (11.68) T	12.5 (20.70)	8.30 (16.74) T
42.	EC-20998	VB	8.3 (16.74)	4.10 (11.68) T	8.30 (16.74)	4.10 (11.68) T
43.	2976	VB	12.5 (20.70)	4.10 (11.68) T	8.30 (16.74)	12.50 (20.70)MS
44.	EC-20966	VB	20.80 (27.13)	25.30 (30.20) S	45.80 (42.50)	16.60 (24.04)MS
45.	RS-81	VR	25.30 (30.20)	20.80 (27.13) S	25.30 (30.20)	16.60 (24.04)MS
46.	30-33	VB	29.10 (32.65)	20.80 (27.13) S	12.5 (20.70)	13.30 (21.39)MS
47.	2825	VR	29.10 (32.65)	20.80 (27.13) S	25.30 (30.20)	25.00 (30.00) S
48.	EC-20978	VB	33.30 (35.24)	8.50 (16.95) T	25.30 (30.20)	12.50 (20.70)MS
49.	2971	VB	33.30 (35.24)	12.50 (20.70)MS	33.30 (35.24)	12.50 (20.70)MS
50.	2809	VB	33.30 (35.24)	20.80 (27.13) S	8.30 (16.74)	8.30 (16.74) T
51.	2957	VB	33.30 (35.24)	20.80 (27.13) S	37.50 (37.76)	25.00 (30.00) S
52.	2599	VR	33.30 (35.24)	25.30 (30.20) S	25.30 (30.20)	16.60 (24.04)MS
53.	EC-21001	VB	33.30 (35.24)	25.30 (30.20) S	37.50 (37.76)	12.50 (20.70)MS
54.	RS-87	VR	37.50 (37.76)	12.50 (20.70)MS	16.60 (24.04)	13.30 (21.39)MS
55.	2808	VR	37.50 (37.76)	16.60 (24.04)MS	25.30 (30.20)	25.00 (30.00) S
56.	EC-20979	VB	37.50 (37.76)	16.60 (24.04)MS	41.60 (40.16)	20.80 (27.13) S
57.	2799	VB	37.50 (37.76)	20.80 (27.13) S	29.10 (32.65)	20.80 (27.13) S
58.	S-7-1-5	VB	37.50 (37.76)	20.80 (27.13) S	20.80 (27.13)	12.50 (20.70)MS
59.	2971	VB	41.60 (40.16)	8.3 (16.74) T	33.30 (35.24)	12.50 (20.70)MS
60.	2446	VB	41.60 (40.16)	16.60 (24.04)MS	41.60 (40.16)	12.50 (20.70)MS
61.	EC-21004	VB	12.5 (20.70)	4.10 (11.68) T	12.50 (20.70)	20.80 (27.13) S
62.	PI 262101	VL	12.5 (20.70)	8.3 (16.74) T	16.60 (24.04)	12.50 (20.70)MS
63.	PI 268494	VL	16.60 (24.04)	12.50 (20.70)MS	25.30 (30.20)	37.50 (37.76) S
64.	PI 262110	VL	20.80 (27.13)	25.30 (30.20) S	33.30 (35.24)	25.30 (30.20) S
65.	PSR 104	VR	29.10 (32.65)	12.50 (20.70)MS	50.00 (45.00)	16.60 (24.04)MS
66.	P1 262097	VL	29.10 (32.65)	12.50 (20.70)MS	33.30 (35.24)	16.60 (24.04)MS
67.	PC 24400	VR	29.10 (32.65)	16.60 (24.04)MS	29.10 (32.65)	25.30 (30.20) S
68.	PI 270767	VL	29.10 (32.65)	25.30 (30.20) S	12.50 (20.70)	16.60 (24.04)MS
69.	PI 268522	VL	29.10 (32.65)	33.30 (35.24) S	54.10 (47.43)	25.30 (30.20) S
70.	DSA 160	VB	33.30 (35.24)	20.80 (27.13) S	33.30 (35.24)	25.30 (30.20) S
71.	PC 20981	VB	33.30 (35.24)	29.10 (32.65) S	33.30 (35.24)	29.10 (32.65) S
72.	PI 268514	VL	37.50 (37.76)	16.60 (24.04)MS	41.60 (40.16)	20.80 (27.13) S
73.	PI 268514	VL	37.50 (37.76)	16.60 (24.04)MS	33.30 (35.24)	33.30 (35.24) S
74.	Chandan Red	VL	37.50 (37.76)	16.60 (24.04)MS	45.80 (42.59)	8.3 (16.74) T
75.	DSA 181	VB	37.50 (37.76)	20.80 (27.13) S	20.80 (27.13)	29.10 (32.65) S
76.	PI 262107	VL	37.50 (37.76)	33.30 (35.24) S	29.10 (32.65)	12.50 (20.70)MS
77.	PI 262087	VL	41.60 (40.16)	25.30 (30.20) S	50.00 (45.00)	20.80 (27.13) S
78.	PI 262112	VL	45.80 (42.59)	29.10 (32.65) S	54.10 (47.43)	25.30 (30.20) S
79.	PI 1262085	VL	45.80 (42.59)	33.30 (35.24) S	70.80 (57.29)	20.80 (27.13) S
80.	PI 268589	VL	58.30 (49.78)	8.3 (16.74) T	50.00 (45.00)	16.60 (24.04)MS

Sl No.	Variety: Germplasm	Habit Group	A. niger Isolate-16: Seed rot (%)	A. niger Isolate-16: Collar rot (%)	A. niger Isolate-24: Seed rot (%)	A. niger Isolate-24: Collar rot (%)
81.	PI 268761	SB	16.60 (24.04)	8.3 (16.74) T	16.60 (24.04)	4.10 (11.68) T
82.	Savner Local	VL	25.30 (30.20)	12.50 (20.70)MS	45.80 (42.59)	8.3 (16.74) T
83.	MT 9512	VL	29.10 (32.65)	12.50 (20.70)MS	41.60 (40.16)	16.60 (24.04)MS
84.	Strain No. 11	VL	29.10 (32.65)	20.80 (27.13) S	50.00 (45.00)	8.3 (16.74) T
85.	PI 268989	VL	29.10 (32.65)	33.30 (35.24) S	54.10 (47.51)	8.3 (16.74) T
86.	S. Am. Coll 10	VL	33.30 (35.24)	16.10 (23.66)MS	41.60 (40.16)	12.60 (20.79)MS
87.	PI 270920	VL	33.30 (35.24)	20.80 (27.13) S	45.80 (42.59)	33.30 (35.24) S
88.	PI 261717	VL	33.30 (35.24)	37.50 (37.76) S	37.50 (37.76)	16.60 (24.04)MS
89.	PI 268527	VL	41.60 (40.16)	16.60 (24.04)MS	37.50 (37.76)	8.3 (16.74) T
90.	PI 268492	VL	41.60 (40.16)	20.80 (27.13) S	33.30 (35.24)	12.60 (20.79)MS
91.	S 720	VL	41.60 (40.16)	20.80 (27.13) S	41.60 (40.16)	12.60 (20.79)MS
92.	PI 260864	VL	41.60 (40.16)	29.10 (32.65) S	37.50 (37.76)	16.60 (24.04)MS
93.	PI 268753	SB	45.80 (42.59)	20.80 (27.13) S	45.80 (42.59)	16.60 (24.04)MS
94.	S. Am. Coll 89	VL	50.00 (45.00)	16.60 (24.04)MS	33.30 (35.24)	12.60 (20.79)MS
95.	Tenn. Red	VL	50.00 (45.00)	20.80 (27.13) S	75.00 (60.00)	12.60 (20.79)MS
96.	PI 2707 95	VL	58.30 (49.78)	12.60 (20.79)MS	58.30 (49.78)	20.80 (27.13) S
97.	PI 2620 45	VL	58.30 (49.78)	16.60 (24.04)MS	48.80 (42.59)	25.30 (30.20) S
98.	PI 270855	VL	62.50 (52.24)	25.30 (30.20) S	62.50 (52.24)	12.60 (20.79)MS
99.	PI 268772	SB	70.83 (57.20)	21.00 (27.27) S	70.83 (57.20)	8.3 (16.74) T
100.	PI 268781	VL	75.00 (60.00)	20.80 (27.13) S	58.30 (49.78)	20.80 (27.13) S
101.	PI 268777	SB	16.60 (24.04)	4.10 (11.68) T	16.60 (24.04)	4.10 (11.68) T
102.	PI 268705	SB	16.60 (24.04)	4.10 (11.68) T	16.60 (24.04)	4.10 (11.68) T
103.	PC 1717	SB	16.60 (24.04)	12.50 (20.70)MS	16.60 (24.04)	16.60 (24.04)MS
104.	0-103-10	SB	37.50 (37.76)	12.50 (20.70)MS	37.50 (37.76)	4.16 (11.77) T
105.	PI 268612	SB	37.50 (37.76)	12.50 (20.70)MS	25.30 (30.20)	8.3 (16.74) T
106.	PI 268601	SB	37.50 (37.76)	12.50 (20.70)MS	62.50 (52.24)	20.80 (27.13) S
107.	PI 268637	SB	37.50 (37.76)	16.60 (24.04)MS	45.80 (42.59)	16.60 (24.04)MS
108.	43 G- 68	SB	37.50 (37.76)	16.60 (24.04)MS	45.80 (42.59)	16.60 (24.04)MS
109.	EC- 1717	SB	37.50 (37.76)	29.10 (32.65) S	29.10 (32.65)	8.3 (16.74) T
110.	DH-3-29	SB	45.80 (42.59)	8.3 (16.74) T	20.80 (27.13)	8.3 (16.74) T
111.	PI 268772	SB	45.80 (42.59)	12.50 (20.70)MS	37.50 (37.76)	8.3 (16.74) T
112.	Dukut Boub	SB	45.80 (42.59)	16.60 (24.04)MS	20.80 (27.13)	8.3 (16.74) T
113.	PI 268614	SB	45.80 (42.59)	16.60 (24.04)MS	33.30 (35.24)	16.60 (24.04)MS
114.	PI 268677	SB	45.80 (42.59)	20.80 (27.13) S	54.10 (47.51)	12.50 (20.70)MS
115.	PC 6618	SB	45.80 (42.59)	20.80 (27.13) S	54.10 (47.51)	8.3 (16.74) T
116.	D 103	SB	50.00 (45.00)	8.3 (16.74) T	25.30 (30.20)	4.16 (11.77) T
117.	EC 9	SB	50.00 (45.00)	20.80 (27.13) S	58.30 (49.78)	12.00 (20.27)MS
118.	D No. 686	SB	58.30 (49.78)	8.3 (16.74) T	62.50 (52.24)	12.50 (20.70)MS
119.	DH -3-28	SB	58.30 (49.78)	16.60 (24.04)MS	58.30 (49.78)	20.80 (27.13) S
120.	PH-3-30	SB	75.00 (60.00)	12.50 (20.70)MS	41.60 (40.16)	20.80 (27.13) S

Sl No.	Variety: Germplasm	Habit Group	A. niger Isolate-16: Seed rot (%)	A. niger Isolate-16: Collar rot (%)	A. niger Isolate-24: Seed rot (%)	A. niger Isolate-24: Collar rot (%)
121.	C-421	SB	4.10 (11.68)	8.3 (16.74) T	8.3 (16.74)	8.3 (16.74) T
122.	JH 225	SB	4.10 (11.68)	12.50 (20.70)MS	20.80 (27.13)	4.10 (11.68) T
123.	Faizpur -1-5	SB	4.10 (11.68)	0.00 (0.00) I	16.60 (24.04)	8.3 (16.74) T
124.	C- No. 1780	SB	4.10 (11.68)	0.00 (0.00) I	8.3 (16.74)	8.3 (16.74) T
125.	GA- 188	SB	29.10 (32.63)	4.10 (11.68) T	58.30 (49.78)	16.60 (24.04)MS
126.	C-No. 1841	SB	33.30 (35.24)	20.80 (27.13) S	37.50 (37.76)	25.00 (30.00) S
127.	C- No. 2184	SB	33.30 (35.24)	29.16 (32.68) S	33.30 (35.24)	25.00 (30.00) S
128.	G-64-403	SB	41.60 (40.16)	20.83 (27.16) S	41.60 (40.60)	29.10 (32.65) S
129.	Issue-de-cuba	SB	45.80 (42.59)	25.00 (30.00) S	66.60 (54.70)	12.50 (20.7) MS
130.	C-817	SB	45.80 (42.59)	33.30 (35.24) S	33.30 (35.24)	20.80 (27.13) S
131.	G-969	SB	50.00 (45.00)	33.30 (35.24) S	71.50 (57.73)	41.60 (39.82) S
132.	JH 335	SB	54.16 (47.38)	20.80 (27.13) S	66.60 (54.70)	12.50 (20.7) MS
133.	JH 113	SB	62.50 (52.24)	8.3 (16.74) T	33.30 (35.24)	16.60 (24.04)MS
134.	IARI 6807	SB	62.50 (52.24)	16.60 (24.04)MS	54.10 (47.41)	20.80 (27.13) S
135.	Hou. No. 113	SB	62.50 (52.24)	20.80 (27.13) S	54.10 (47.41)	16.60 (24.04)MS
136.	EC- 4110	SB	66.60 (54.70)	4.10 (11.68) T	66.60 (54.70)	12.50 (20.7) MS
137.	Faizpur	SB	70.80 (57.29)	8.33 (16.77) T	62.50 (52.24)	16.60 (24.04)MS
138.	GO-053	SB	75.00 (60.00)	0.00 (0.00) I	70.80 (57.29)	12.50 (20.7) MS

Figures within Parenthesis are angularly transformed values

CD 5% values :

		Sed rot	Cllar rot
1. For cultivar	...	14.80	12.91
2. For isolate	...	4.71	4.10
3. For interaction (Cultivar x Isolate)	...	20.95	18.26

Among one hundred thirty eight germplasms or cultivars of groundnut, 3 showed immune reaction to A. niger (isloate-16) 29 cultivars or germplasms were tolerant, moderately susceptible reaction was noted in case of 52 germplasms or cultivars and 54 showed highly susceptible reaction to isolate 16 of A. niger while among the germplasms/cultivars 32 showed tolerant reaction, 61 cultivars/germplasms were moderately susceptible to solate 24 of Aspergillus niger. None of the cultivars/germplasms of groundnut showed immune reaction to isolate 24 of A. niger.

From the table it is clear that cultivars/germplasms are immune to isolate 16 are Faizpur 1-5, C-No. 1780 and GO-053.

Tolerant to collar rot (Aspergillus niger isolate 16) are Rajkot local, 2710, Kaulikoro, 302/19, 2496, F-12, 5-7-1-6, Lata Mongfali, 4383-3, 2848, EC-20998, 2976, EC 20978, 2971, EC21004, PI-262101, P 1268589, PI 268761, PI 268777, PI 268705, DH-3-29, D 103, D No. 686, C-421, GA 188, JH-113, EC 4110 and Faizpur, Moderately susceptible cultivars/germplasms are C-175, 2956, 3024, 2909, 2592, Dipadira Local VR, 638, 913.2 Cyprus-groundnut, 583-13, EC 1333155, EC 21009, 2694, G-211-31, 545-2, 2971, RS-87, 8808, EC 20979, 2799, S-7-1-5, 2446, PI 268494, PSR 104, PI 862097, EC 24400, PI 268514, PI 268514, Chandan Red, Savner local MT-9512, Strain No. 11, S.Am. Coll. 105, PI 268527, S.Am. Coll.89, PI 270795, PI 262045, EC 1717, O-103-10,

PI 268612, PI 268601, PI 288637, 4361-68, PI 268772, Dukut Boub, PI-2686-16, DH-3-28, DH 3.30, JH-225, IARI 6807 to isolate 16 of A. niger. Cultivers/germplasms showed highly susceptible reaction to isolate 16 of A. niger are 2340, C-15, Chungjia, AH 7186, 7300, 648, 2523, 648, 578-6, 573-11, 578-8, 575-5, 371-1, 371-1 (NRCO NO 3380), EC 20966, Rs-81, 30-33, 2825, 2809, 2957, 2599, EC 21001, PI 262000, PI-2707, PI 268522, DSA 166, EC 20981, DSA 181, PI 262107, PI 262087, PO 262112, PI 262085, PL 268989, PI 270920, PI 261717, PI 268492, S-720, PI 260864, PI 268753, Tenn Red, PI-270855, PI 268772, PI 268781, EC 1716, PI 268677, EC 6618, EC-9, C. NO. 1841, C. No. 2184, G-64-403, Issue-de-caba, C-817, G-969, JH 335, HOU. No. 113.

Isolate 24 of Aspergillus niger

Immune to : Nil

Tolerant to cultivar/germplasms of groundnut

C-175, C-176, 2710, 302/19, EC 133155, P-12, 5-7-1-6, Lota Mangfali, 8848, EC 20998, 2809, Chandan Red, PI-268761, Savner Local, Strain No. 11, PI 268989, PI 268527, pi 268772, PL-268777, PI-268705, O-103-10, PL-268612, eC 1716, DH-3-29, PI-268772, Dukut Boub, EC-6618, D-103, C-421, JH 225, Faizpur 1-5, C-No. 1780, Moderately susceptible cultivars/germplasms are :

Rajkot Local, C-15, Chungjia, 2956, 2523, 2592, Dipadiva Local, Ve-638, 648, C-176, Cyprus groundnut, Kaulikoro, 2694, G-211-31, 371-1 (NRCG No. 3380), 2976, EC 20968, R-S-81, 30-33, EC 20978, 2971, 2599,EC 21001, RS-87, 5-7-1-5, 2971, 2446, PI-262101, PSR 104, PI-262097, PI-270767, PI-262107, PI 268589, MT-9512, S. Am. Coll. 105, PI, 261717, PI 268492, S-720, PI 260864, PI 268753, S. Am. CLL. 89, Tenn Red. PI 270855, EC 1717, PI 268601, PI-268637, 43G-68, PJ,288614, PI 268614,PI-268677, EC 9, OnO 686, GA-188, Issue-de-caba, JH 335, JH 113, Hou-No. 113, EC 4110, Faizpur and GO-053. Susceptible reaction was observed in the cultivars/germplasms to isolate 24 of A. niger.

Ah 7186, 7300, 3024, 648, 913-2, 578-6, 4383-3, 545-2, 593-11, 578-8, 575-5, 371-1, 8825, 2957, 2808, EC 20979, 2799, EC 21004, PI.268494, PJ 262110, EC 24400, PI.298522, DSA 160, EC 20981, PJ 268514, PJ 268514, DSA 181, PJ 262087, PJ 262112, PI 262085, I 270920,PJ 270795, PJ 262045, PI 268781, DH 3-28, PH-3-30, C. No. 1841, C. No. 2184, G-64-403, C-817, G-969, IARI 6809.

From Table-4.1 it was noted that the groundnut varieties which showed resistant reaction to single or both isolates of A. niger in respect of seed rotting or collar rotting or both can be grouped in the following way.

[A] **Seed rot** :

1. To isolate 16 : i) Virginia bunch group -2848 (VB)
   ii) Virginia runner group-583-13,
   iii) Valencia -X (VL)
   iv) Spanish - JH-225,

2. To isolate 24 : i) Virginia bunch group-2809, 2976
   ii) Virginia runner group -EC 133155,
   iii) Valencia group - x
   iv) Spanish group - x

3. To both isolates :
   i) VB group -EC 20998
   ii) VR group -x
   iii) VL group - x
   iv) Spanish group-C-421, C-No. 1780.

[B] **Collar rot** :

1. To isolate - 16
   I) VB group- 2976, EC 20978, 2971, EC 21004
   ii) VR group -Rajkot local, C-176, Kaulikoro, 2496, 4383-3,
   iii) VL group -PI 262101, PI 268589,
   iv) SB group-D No.686, GA-188, jh-113, EC-4110, GO-053.

2. To isolate - 24
   I) VB group - 2809
   ii) VR group-C-175, C-178, Cyprus groundnut, EC-133155, 583-13.

iii) VL group- Chandan Red, PI 268527, Savner local strain No. 11, PI 268989, PI-268772.

iv) SB group-Dukut Boub, Pl. 268612, EC 1716, 0-1-3-10, PI 268772, EC 6618, JH-225.

3. To both isolates

I) VB group - 2710, 2848, EC 20998

ii) VR group- 302/19, F-12, S-7-1-6, Lota mungfali

iii) VL group- x

iv) SB group- PI 268761, PI 268677, PI 268705, DH-3-29, D-103, C-421, Faizpur 1-5, C. No. 1780.

[C] **Both seed rot and collar rot :**

1. To isolate 16 : I) VB group-2848

ii) SB group -Faizpur 1-5

2. To isolate 24 : I) VB group-2809

ii) VR group-EC 133155

3. To both isolates :

I) VB group - EC 20998

ii) SB group-C-421, C-No. 1780.

4.2 Relative Incidence of crown rot disease of groundnut in different seasons

Ten isolates of A. niger isolated from soils of four agro-climatic zones of West Bengal (isolates 2,3,5,16,18,20,21,22,23) and from infected seeds of groundnut (isolate-24) were inoculated with soil kept in 20 cm earthen pots separately. Cultures of A. niger were grown in sand maize meal medium and soil incoculation was done in different season. Details of soil inoculation are given in chapter-III of the manuscript. Seeds (CV. JL-24) of groundnut were sown in these pots inoculated with respective isolates of A. niger. Relative disease incidence was recorded in percent basis. Data are statistically analysed after transforming them into angular transformed values. The experiment was conducted both in glass house and in field conditions.

Table-4.2.1

Relative incidence of crown rot disease of groundnut in different seasons

Isolate	SEASON	Disease incidence (%) Seed rot	Collar rot	Plant infected but not dead
2	Jan.-Fed.'90	32.5(34.76)	22.5(28.32)	32.5
	May-June'90	25 (30)	40 (39.23)	30
	Oct.-Nov.'90	27.5(31.63)	17.5(24.73)	30
3.	Jan.-Fed.'90	35(36.27)	17.5(24.73)	30
	May-June'90	27.5(31.63)	40(39.23)	22.5
	Oct.-Nov.'90	30(33.21)	17.5(24.73)	25.0
5.	Jan.-Fed.'90	35(36.27)	12.5(20.7)	17.5
	May-June'90	20(26.57)	37.5(37.76)	10
	Oct.-Nov.'90	30(33.21)	20(26.57)	20
16.	Jan.-Feb.'90	42.5(40.69)	12.5(20.7)	15
	May-June'90	30.0(33.21)	37.5(37.76)	15
	Oct.- Nov'90	37.5(37.76)	22.5(28.32)	12.5
18.	Jan.-Feb.'90	30 (33.21)	12.5(20.7)	15
	May-June'90	15 (22.79)	37.5(37.76)	12.5
	Oct.-Nov.'90	30 (33.21)	20 (26.57)	15
20.	Jan.-Feb.'90	27.5(31.63)	12.5(20.7)	17.5
	May-June.'90	17.5(24.73)	32.5(34.76)	10
	Oct.-Nov.'90	30 (33.21)	22.5(26.32)	2.5

Isolate	SEASON	Disease incidence (%) Seed rot	Collar rot	Plant infected but not dead
21.	Jan.-Feb.'90	37.5(37.76)	17.5(24.73)	5
	May-June.'90	20(26.57)	37.5(37.76)	5
	Oct.-Nov.'90	27.5(31.63)	25 (30)	10
22.	Jan.-Feb.'90	32.5(34.76)	17.5(24.73)	2.5
	May-June.'90	17.5(24.73)	32.5(34.76)	5
	Oct.-Nov.'90	27.5(31.63)	20(26.57)	10
23.	Jan.-Fab.'90	35(36.27)	10 (18.43)	2.5
	May-June '90	12.5(20.7)	35 (36.27)	10
	Oct.-Nov.'90	22.5(26.32)	20 (20.57)	7.5
24.	Jan.-Feb.'90	35(36.27)	25 (30)	5
	May-June'90	25 (30)	42.5(40.69)	5
	Oct.-Nov.'90	32.5(34.76)	30(33.21)	12.5
Cont-	Jan-Feb.'90	4.1(11.68)	0(0)	0
rol	May-June'90	2.08(8.29)	2.08(8.29)	0
	Oct.-Nov'90	4.1(11.68)	0(0)	0

Figures in paranthesis are angular transformed values.

CD at 5%	**Seed rotting**	**Collar rotting**
For isolate	6.32	5.44
For time	3.28	2.84
For interaction	10.97	9.45

Plate-3 Collar rotted ground seedlings at different stage of attack

Plate-4 Collar rotted ground seedlings at different stage of attack

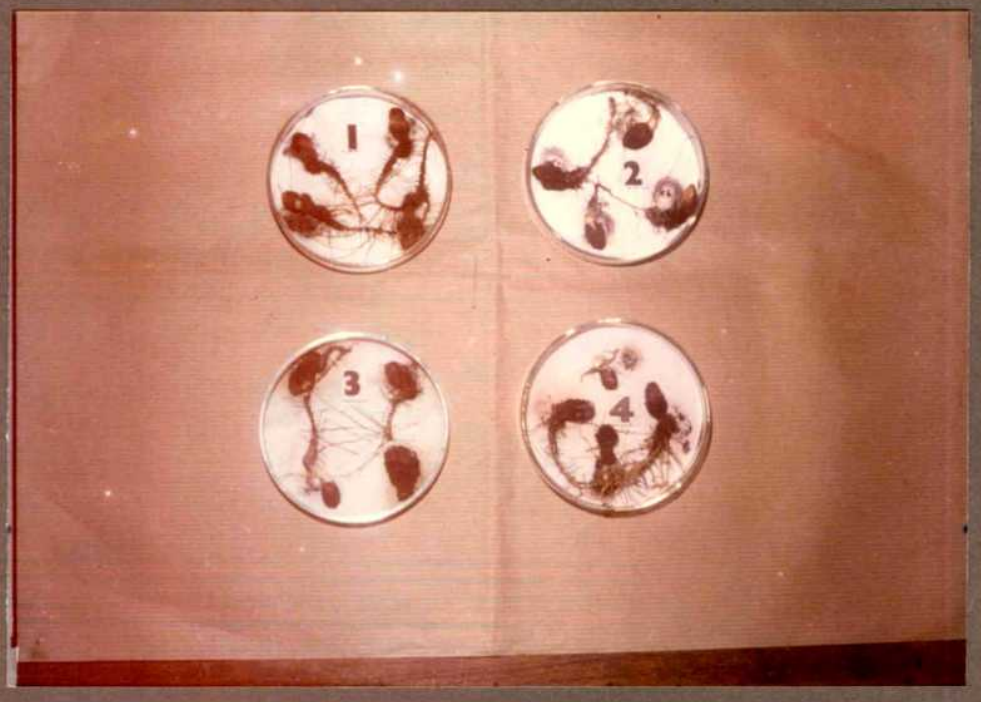


Plate-5 Groundnut seed rotting by four different isolates of A. niger 1→ Isolate-2; 2→ Iso.3; 3→ Iso.5; 4→ Iso.16.

Plate-6 Groundnut seed rotting by four different isolates of A. niger 5→ Iso-18; 6→ Iso.22; 7→ Iso.23; 8→ 24.

Fig.-1. Relative incidence of seed rot caused by various isolates of A.niger in glass house during different seasons

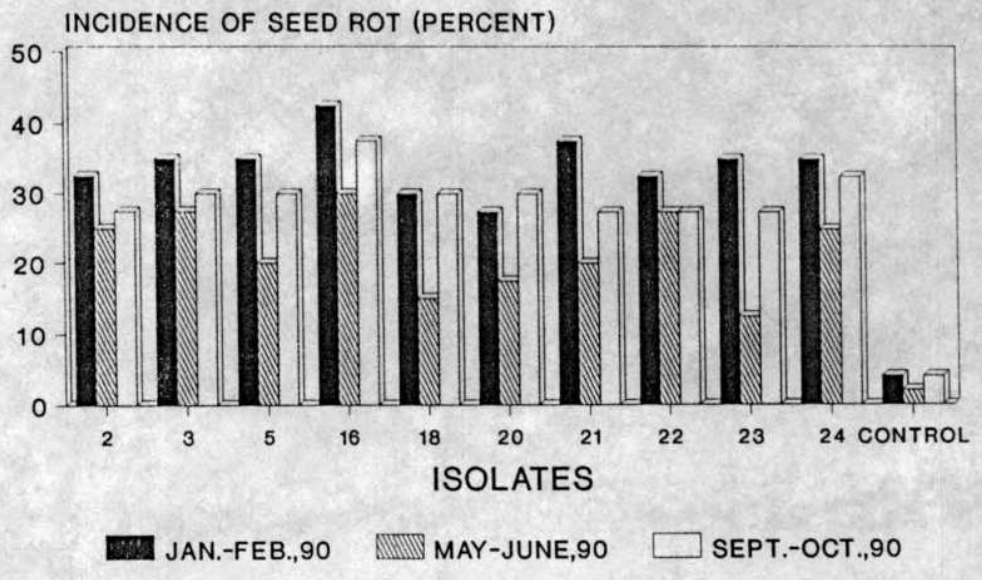
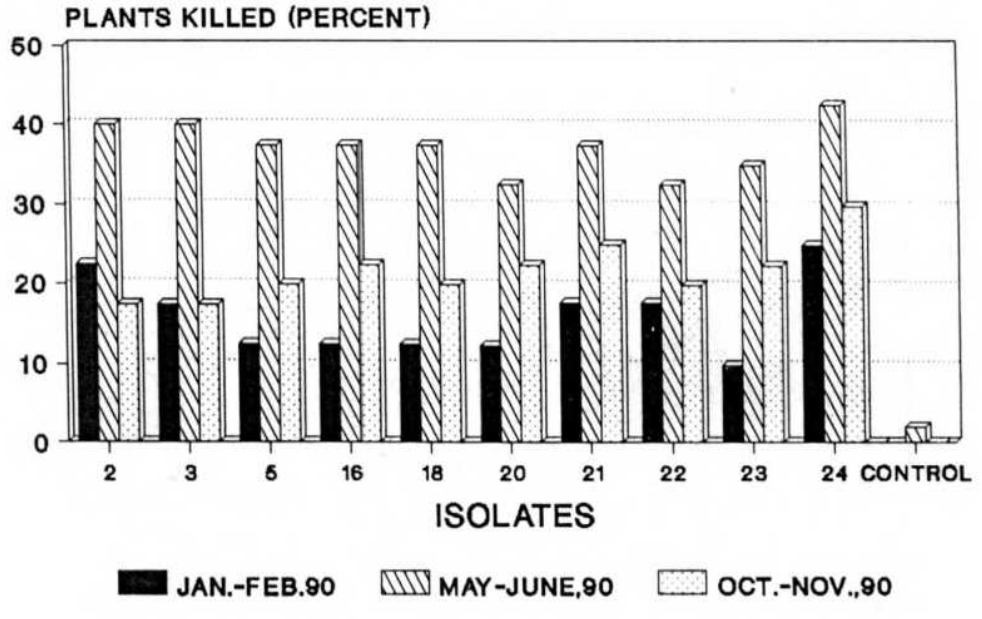


Fig.-2 Relative incidence of collar rot caused by various isolates of A.niger in glass house during different seasons



From Table 4.2.1. and Fig.1 - it is clear that seed rotting is maximum during Jan.-Feb., intermediate during Oct.-Nov. and minimum during May-June. There was a significnat difference in the extent of seed rotting in different seasons in case of all isolates except in control. The main effect of isolate was significant. The results show a non-significant interaction between isolate and time in case of seed rotting indicating that difference in isolate was not significantly affected by the time and that the time effects did not differ significantly with the isolates tested.

Collar rotting maximum during May-June, intermediate during Oct.-Nov. and minimum during Jan.-Feb. There was a significant difference in extent of collar rotting in different season in case of all isolates except in control. The main effect of isolate was significant. The result show a significant interaction between isolate and time, indicating that the difference in isolate was significnatly affected by the time and that the time effect differed significantly with the isolates tested.

Table 4.2.2 : Showing the relative incidence of crown rot disease of groundnut caused by two isolates of **A. niger** in different growing seasons under field condition.

Time	Treatment	Disease incidence (%)					
		Seed rot		Collar rot		Plant infected but not dead	
		'89-90	'90-91	'89-90	'90-91	'89-90	'90-91
Oct.-Nov.	16	33.33(35.24)*	26.66(31.08)	20(26.67)	17.33(24.60)	16	12.66
	24	27(31.31)	21.66(27.74)	19.33(26.08)	19.33(26.08)	13	11
	Control	7(15.34	7.66(16.5)	2(8.13)	4(11.54)	0	0
Jan.-Feb.	16	36.66(37.26)	32.66(34.85)	21(27.27)	18 (25.1)	17	14
	24	40.66(39.61)	37.(27.46)	22.66(28.24)	22.66(28.42)	11.33	11.66
	Control	10(18.43)	11.66(19.95)	3(9.97)	4(11.54)	0	0
May.-June	16	25.33(30.22)*	21.66(27.74)	30.66(33.62)	27.66(31.73)	9	7.33
	24	29.33(31.31)	26(30.66)	30.66(33.62)	32.33(34.65)	11.66	11
	Control	3(9.97)	5.33(13.33)	35.33(36.45)	7.66(16.7)	0	0

Cd5%	For Isolate	2.47	2.39	0.63	3.07
	For time	2.47	2.39	0.63	3.07
	For interaction	3.50	3.37	0.85	1.25

Both isolates of A. niger caused significantly higher amount of collar rotting with respect to control. Significant difference in respect of collar rotting was observed in different seasons. The result show a non-significant interaction between isolate and time, indicating that difference in isolate was not significantly affected by the time and that the effect of time did not differ significantly with the isolate tested.

In case of seed rotting also both isolates of A. niger caused significantly higher amount of seed rotting with respect to control. Significant difference in respect to seed rotting was observed in different seasons. The result shows a nonsignificant interaction between isolate and time, indicating

that difference in isolate was not significantly affected by the time and the effect of time did not differ significantly with the isolate tested.

Almost similar results were obtained during both year of observation.

4.3. **Relative pathogenicity of different isolates of A. niger and persistance of infectivity of sick soil**

Kernels of groundnut cv. JL-24 were used in all the tests and they were surface-sterilized before sowing in earthen pots. The soil of the pots were autoclaved at 20 lbs psi for 2 hours for two consecutive days. The inoculum was build up in sand-maize meal medium (isolate nos. 2,3,5,16,18,20,21,22,23 and 24). Inoculum was mixed up with soils of 20 cm in diameter earthen pots @1:1 in V/V. Infested soil was left for 2 weeks covered with polythene paper. 20 Seeds were sown per pot. Pots were irrigated as and when required. Persistance of infectivity of sick soil was assessed by using surface sterilised seeds in sick soil during May-June, 1990, October-November, 1991 and May-June, 1991.

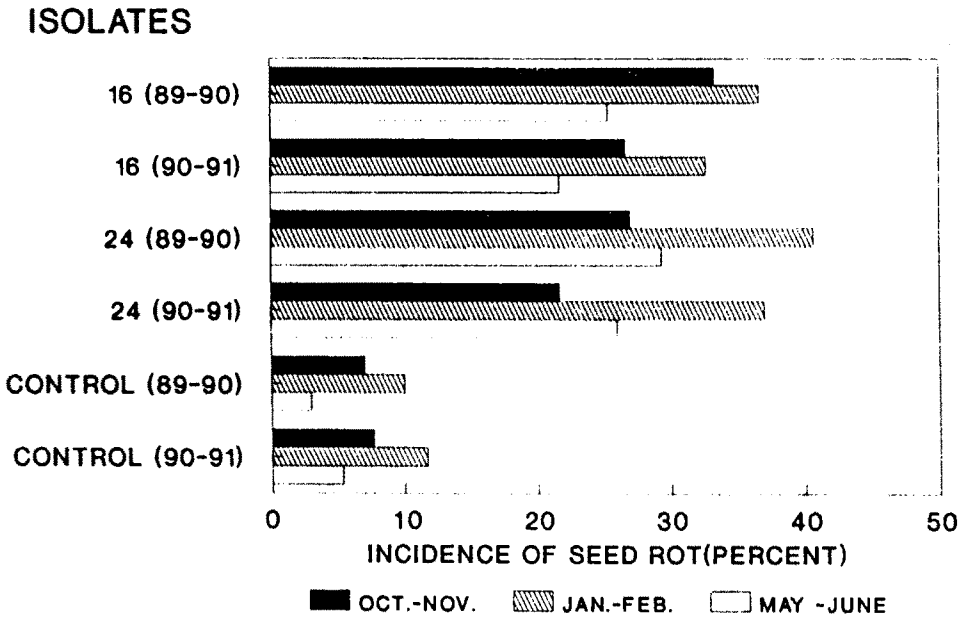
In one uninoculated pot surface sterilized seeds were sown which was treated as control. Three replications were maintained through out the experiement. Per plant disease index was recorded to assess the pathogenicity of different isolates. Data were statistically analysed using randomised block design. Detail of the procedure adopted has been given in Chapter III of the manuscript. Data are tabulated in Table 4.3.1.

Table 4.3.1 : Relative pahtogenicity of various isolates of A. niger the causal agent of crown rot disease of ground and persistance of infectivity of the sick soil under green house condition.

	Treat-ments	Per plant disease index (PDI)				CD 5%
		May-June '90	Oct.- Nov. '90	Feb.- March '91	May - June '91	
	2	20.8 (27.13) *	16.7 (24.12)	10.4 (18.81)	2.5 (9.1)	For isolates =
Seedrotting	3	31.3 (34.02)	22.9 (28.59)	6.2 (14.42)	2.5 (9.1)	5.13
	5	20.8 (27.13)	25.0 (30)	6.2 (14.42)	0 (0)	For time =
	16	35.4 (36.51)	27.1 (31.37)	14.9 (22.71)	14.6 (22.46)	3.23
	18	18.8 (25.7)	14.58 (22.44)	14.9 (22.71)	10 (18.43)	For interaction =
	20	25 (30)	10.4 (17.85)	6.3 (14.54)	2.5 (9.1)	10.24
	21	22.9 (29-27)	16.7 (24.12)	4.2 (11.83)	0 (0)	
	22	20.83 (27.15)	18.8 (25.7)	8.4 (16.55)	0 (0)	
	23	20.8 (27.13)	12.5 (20.7)	10.4 (18.81)	7.5 (15.89)	
	24	37.5 (37.76)	31.3 (34.02)	16.7 (24.12)	12.5 (20.7)	
	Control	0(0)	4.1 (11.54)	2 (8.13)	0.1 (1.81)	
Collar rotting	2	29.7 (33.02)	8.3 (16.74)	4.1 (11.68)	0 (0)	
	3	25.4 (30.26)	14.6 (22.46)	6.2 (14.42)	25 (9.1)	For isolate =
	5	33.3 (35.24)	18.8 (25.7)	6.2 (14.42)	0 (0)	5.44
	16	33.3 (35.24)	20.8 (27.13)	16.7 (24.12)	10.41	For time =
	18	35.4 (36.51)	12.5 (20.7)	8.4 (16.85)	0 (0)	3.45
	20	25 (30)	10.4 (18.81)	8.4 (16.85)	2.5 (9.1)	For interaction=
	21	27.1 (31.37)	12.6 (20.79)	8.4 (16.85)	2.5 (9.1)	10.88
	22	31.3 (34.02)	16.7 (24.12)	6.3 (14.54)	5 (12.92)	
	23	29.2 (32.71)	14.6 (22.46)	10.4 (18.81)	2.5 (9.1)	
	24	37.5 (37.76)	16.7 (24.12)	18.8 (25.7)	14.6 (22.46)	
	Control	0 (0)	2 (8.13)	2 (8.13)	0 (0)	

*** Values in paranthesis are angularly transformed values.**

Fig.-2. Relative incidence of seed rot caused by two isolates of A.niger in different seasons under field conditions



Horizontal chart

From the table-4.3.1, and Fig . 2 it is clear that there was significant difference in extent of seed rotting among isolates of A. niger in all seasons i.e. the main effect of isolate was significant. Seed rotting was maximum during May-June'90, lowest during May-June '91 intermediate during Oct.-Nov.'90 and Feb-March'91. The main effect of time was significant i.e. a gradual and significant reduction in respect of seed rotting was from May-June'90 to May-June'91. The result showed a nonsignificant interaction between isolate and time, indicating that the difference in isolate was not significantly affected by the time and that the time effect did not differ significantly with the isolates tested.

Similarly, significant difference in the extent of collar rotting was found among some isolates of A. niger in all seasons, i.e. the main effect of isolate was significant. Collar rotting is maximum during May-June'90, lowest during May-June'91, intermediate during Oct-Nov'90, Feb-March'91. The main effect of time is significant i.e. a gradual and significant reduction in respect of collar rotting was found from May-June'90 to May-June'91. The result show a non-significant interaction between isolate and time, indicated that the difference in isolate was not significantly affected by the time and that the time effect did not differ significantly with the isolates tested.

It was evident that isolate 16 and isolate 24 were more virulent than the other isolates of A. niger. In case of all isolates, it is clear that there is a gradual loss of infectivity of sick soil during one year period of time. According to virulence they can be arranged 24>16>3>18>5>22>2>23>21>20.

4.4 : **Effect of physical injury on disease incidence** :

Surface sterilized groundnut seeds (cv.JL-24) were sown in 10 cm in diameter earthen pots containing autoclaved soil. The inoculum was developed in sand maize meal medium for 10 days. Inoculum was mixed up with the potted soil @ 1:1 5 days' before sowing of seeds. The experiment was conducted with two isolates of A. niger (16 and 24). Seven days' old seedlings were injured at the hypocotyl with the help of a sterile needle and cotyledons of seeds were also injured to note the incidence of seed rotting and collar rotting symptoms. 8 seeds were sown in each pot. Seedlings of one pot were not injured.

Total one hundred seedlings were used in this experiment. Collar rot disease was recorded under glass house condition. No injury at hypocotyl of the seedlings and cotyledons of seeds and not inoculated pot was treated as control. Details of the procedure adopted has been given in Chapter-III of this manuscript. Data are analysed using RBD design and are tabulated in Table 4.4.1.

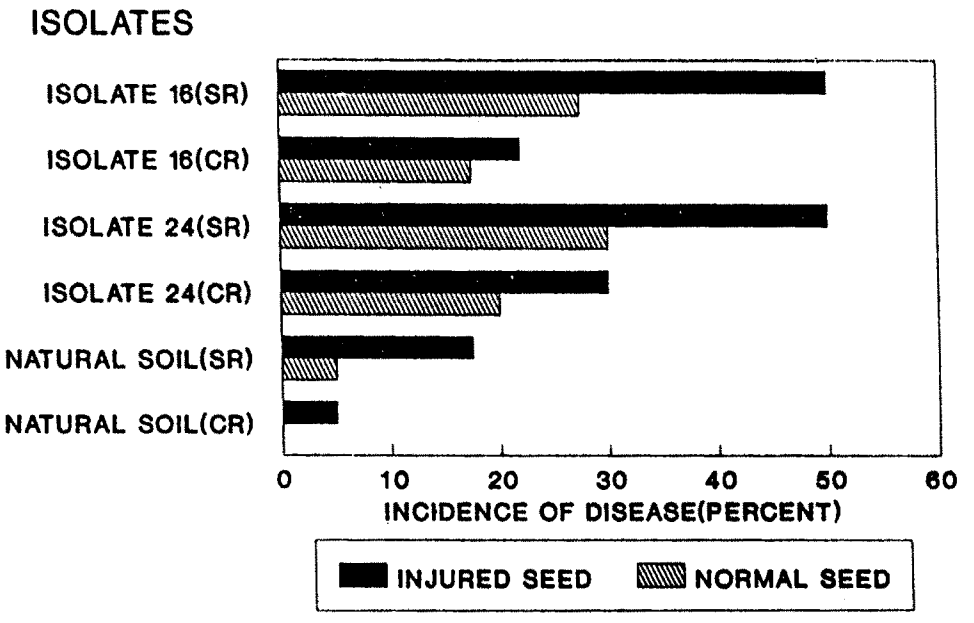
Table :- 4.4.1 Effect of physical injury of seed and seedlings on crown rot disease incidence of groundnut caused by *A. niger*

Treat-ment	On cotyledon						On hypocotyle			
							Collar rotting		Intact	
	seed-rotting	collar rotting	Healthy	seed-rotting	collar rotting	Healthy	Healthy seed-sedling	Injured seed-ling		
	%	%	%	%	%	%	%	%	%	
A. niger-16	50 (45)	22 (27.97)	28 (31.95)	27.5	(81.63)	17.5 (24.73)	55 (47.57)	42.5 (40.4)	22.5 (28.32)	71 (57.42)
A. niger-16	50 (45)	30 (33.21)	20 (26.57)	30	(33.21)	20 (26.57)	50 (45)	45 (42.13)	25 (30)	30 (31.21)
Control	17.5 (24.73)	5 (12.92)	77.5 (61.68)	5	(12.92)	0 (27.97)	95 (77.08)	5 (12.92)	0 (0)	95 (77.08)

CD 5% for	Seed rotting	Collar rotting	Collar rotting
Injured-Uninjured	5.180	4.530	4.820
Treatment (Isolate)	6.340	5.530	5.920
Interaction	9.005	7.850	8.380

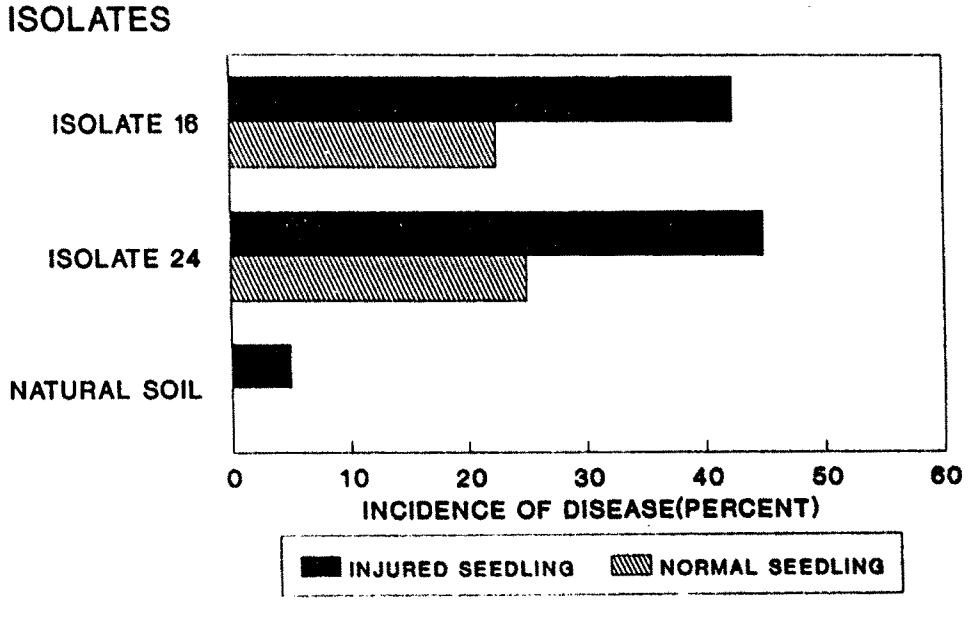
*** Values in paranthesis are angular transformed values.**

Fig.-3. Effect of seed injury on crown rot disease caused by two isolates of A.niger



SR-SEED ROT, CR-COLLAR ROT
Horizontal chart

Fig.-4. Effect of seedling injury on crown rot disease caused by two isolates of A.niger



Horizontal chart

Injury of the the seed in cotyledons Table 4.4.1 and Fig. 3 significantly increased the seed rotting caused by A. niger in case of both isolates i.e. Isolate-16 and Isolate-24 over control i.e. where intact seeds were sown. But no significant difference in seed rotting was observed between two isolates of A. niger. In natural soil where no inoculation was done in soil, there was significant increase in seed rotting.

Seed injury also increased collar rotting incidence significantly in case of isolate-24 and control but in isolate-16 this increase was marginal.

Seedling injury of the hypocotyle region significantly increased collar rotting in case of both isolates. Marginal increase was also recorded in control where no inoculation was done. [Fig. 4]

4.5 : **Effect of soil moisture on crown rot incidence of groundnut** :

Autoclaved sandy loam soil was filled up in 20 cm in diameter earthen pot. The inner side of the pot was covered with plastic sheet to avoid seepage of water. The soil was inoculated with 10 days' old cultures of A. niger, grown in sand maize meal medium @1:1 in ratio v/v, 4 days before sowing

of seeds. 10 surface sterilized seeds/pot of groundnut (cv.JL-24) were sown in each pot. Measured quantity of water was given to maintain 5%, 10%, 15% and 25% moisture level of the soil by weight in each day. In each case three replications were taken. Disease incidence was recorded with two isolates of **Aspergillus niger** (S16 and S24). Data were statistically analysed using RBD design. The details of the procedure adopted has been given in Chapter-III of the manuscript. Data are tabulated in Table 4.5.1.

Table 4.5.1 : Effect of soil moisture on crown rot disease incidence of groundnut caused by A. niger (Figures in paranthesis are angular transformed values).

Treatment	Moisture percentage of soil											
	5%			10%			15%			25%		
	A	B	C	A	B	C	A	B	C	A	B	C
Isolate-16	100	56.6 (48.79)	23.33 (28.86)	100	60 (50.77)	20 (26.57)	100	46.6 (43.05)	13.3 (21.39)	100	40 (39.23)	10 (18.43)
Isolate-24	100	60 (50.77)	20 (26.57)	100	63.3 (52.71)	16.6 (24.04)	100	46.6 (43.05)	10 (18.43)	100	43 (40.98)	3.3 (10.47)

· Vaule in paranthesis are angular transformed values

** Data is average of 3 replications

A = Percentage of seed germination and seedling emergence

B = Percentage of seed rotting

C = Percentage of collar rotting.

CD 5%	Seed rotting	Collar rotting
1. For isolate	5.93	3.83
2. For moisture %	6.87	4.44
3. For Interaction	11.89	4.76

Fig.-5. Effect of soil moisture on the incidence of crown rot disease caused by two isolates of A.niger

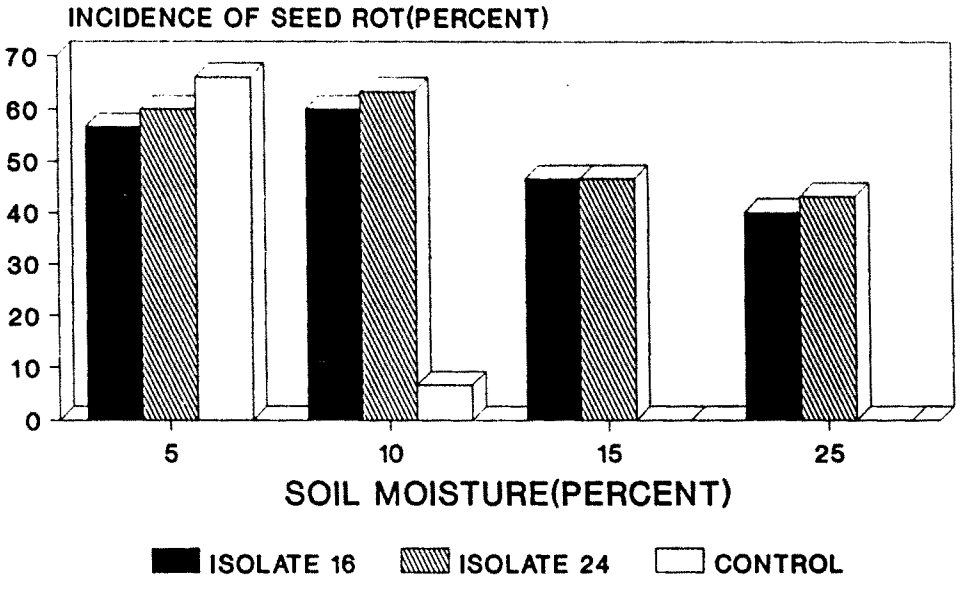
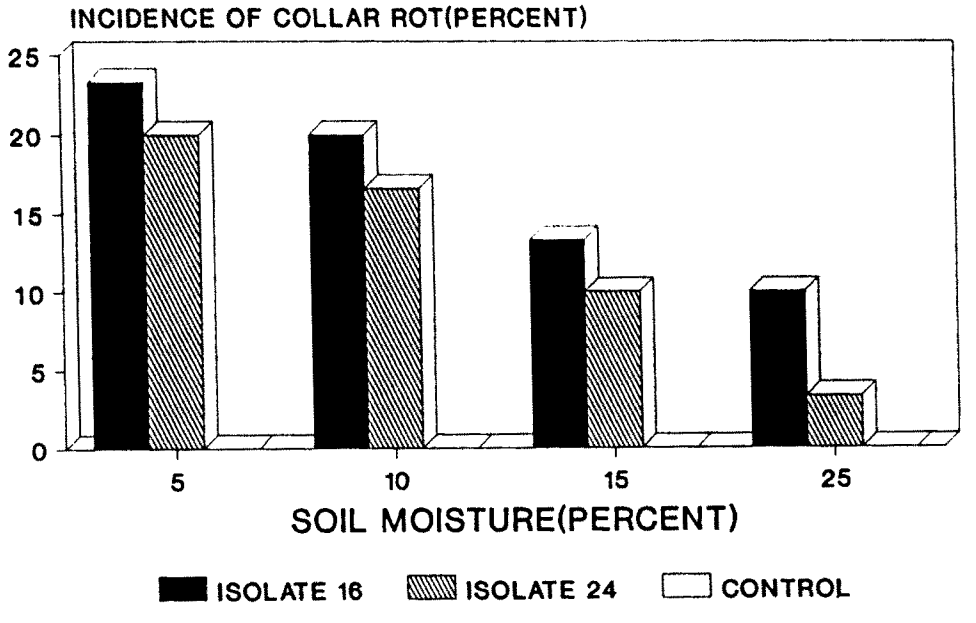


Fig.-6. Effect of soil moisture on the incidence of crown rot disease caused by two isolates of A.niger



From Table 4.5.1 and Fig.5 and Fig.6 it is observed that moisture level in soil played a significant role in seed rotting caused by A. niger in case of both isolates. 25% soil moisture level reduced the seed rotting significantly with respect to 5% & 10% level. Maximum incidence of seed rotting was observed at 10% soil moisture level in case of both isolates. The result show a nonsignificant interaction between isolate and soil moisture level, indicating that effect of isolate was not significantly affected by soil moisture level and that the soil moisture effect did not differ significantly with the isolate tested.

Similarly, it is also observed that moisture level in soil played a significant role in seedling collar rot caused by A. niger in case of both isolates. 15% and 25% soil moisture level reduced the collar rotting significantly with respect to 5% and 10% moisture level. Maximum incidence of collar rotting was observed at 5% moisture level in case of both isolates. The result show a nonsignificant interaction between isolate and soil mositure level, indicating that the effect of isolate was not significantly affected by the soil moisture and that the soil moisture effect did not differ significantly with the isolates tested. [Fig. 7]

4.6. Production of hydrolytic enzymes by various isolates of A. niger. (Both in vivo and in vitro.)

4.6.1. Production of PG & Cellulase enzymes both in vivo & invitro condition

Various isolates of A. niger (Isolates 2,3,5,16,18,20,21,22,23 and 24) were maintained in PDA slants and were used for in vitro production of PG and Cx enzymes. For in vitro production of enzymes Ammonium oxalate medium was used with Pectin (Gupta and Gupta, 1967)

For estimation of in-vivo enzyme production 7-day old groundnut plant (cv.JL-24) grown in autoclaved sandy soil in earthen pots, were inoculated with isolate 16 and isolate 24 of A. niger separately at the collar region. Crude enzyme sample was prepared from infected tissue.

Assay of PG enzyme was done with 1% Sodium Polypectate soultuion and for Cellulase 1% CMC was used. The enzymes activities were calculated Viscosimetrically. The flow time was converted into percent loss in viscosity using the formula

$$V = \frac{Eo - Et}{Eo - Ww} \times 100$$

where V = % loss in viscosity,

E_t = Flow time of the reaction mixture at time t,

E_o = Flow time at zero time,

E_w = Flow time of distilled water

RA/ml (Relative activity per mililitre) = Relative activity unit per ml. has been determined from the reciprocal of the time in minutes required for 50% loss in viscosity multiplied by 100 .

Detials of the procedure adopted has been given in Chapter III of this manuscript.

Table : 4.6.1. In vitro and in vivo enzyme production of various isolates of A. niger.

Isolates	In vitro		In vivo	
	Relative activity unit		Relative activity unit	
	PG	Cx	PG	Cx
2	18.34	10.98	13.33	6.84
3	45.45	11.11	14.08	7.14
5	42.55	8.8	13.15	6.49
16	50.50	20.83	16.94	8.5
18	80	10.81	13.60	6.84
20	57.14	9.8	10.75	6.84
21	28.98	9.009	9.9	6.82
22	25.64	9.8	8.47	6.73
23	25.31	11.62	8.09	7.40
24	42.55	14.28	15.15	8.36

Fig.-7. In vitro enzyme production
of various isolates of A.niger

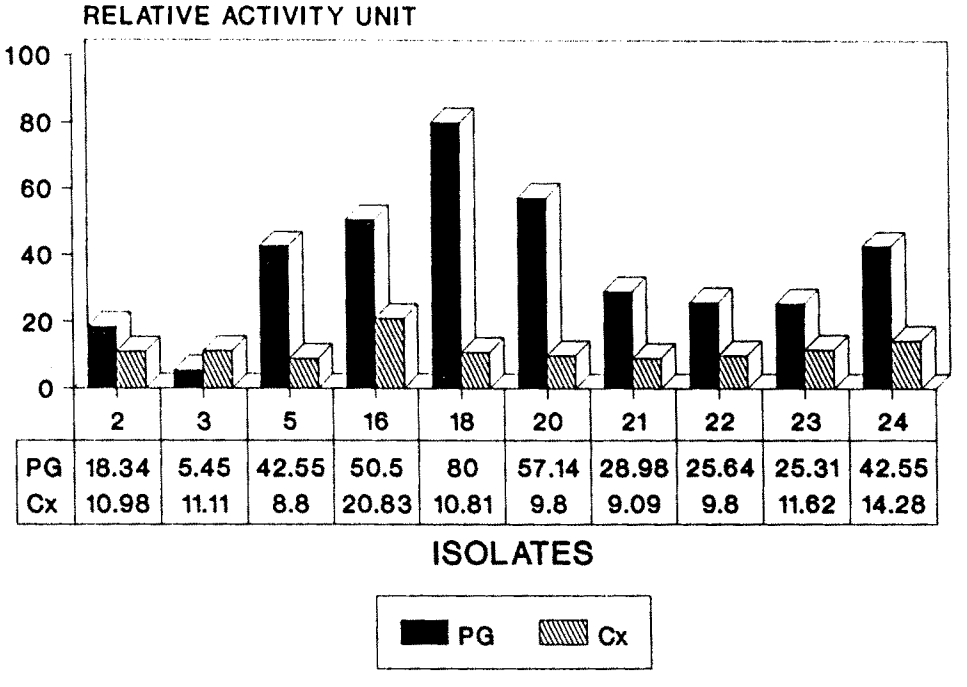
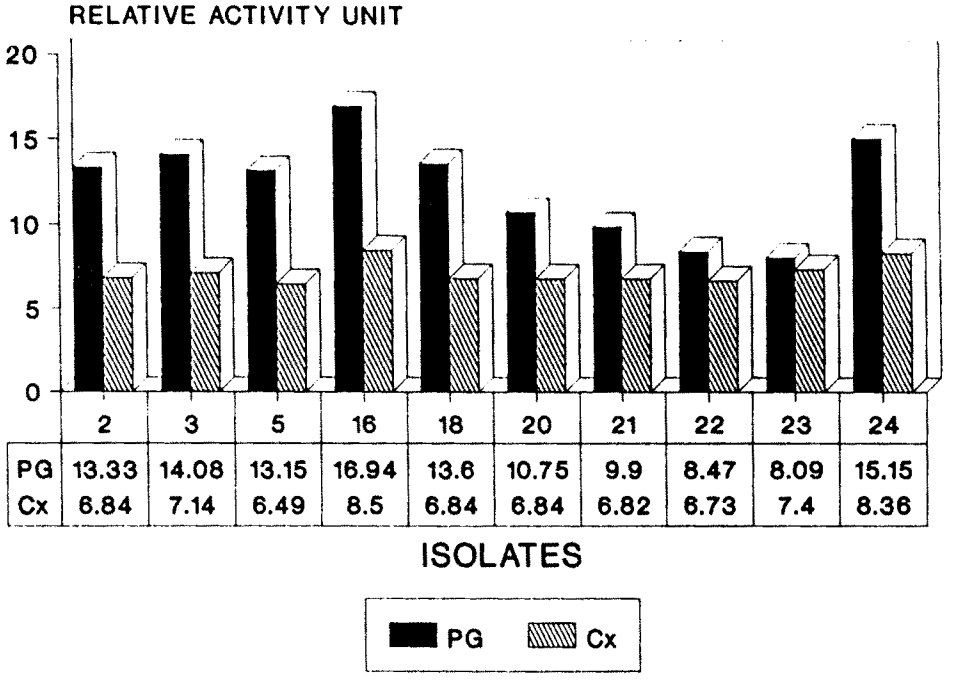


Fig. 8. In vivo enzyme production of various isolates of A.niger



	2	3	5	16	18	20	21	22	23	24
PG	13.33	14.08	13.15	16.94	13.6	10.75	9.9	8.47	8.09	15.15
Cx	6.84	7.14	6.49	8.5	6.84	6.84	6.82	6.73	7.4	8.36

From the Table 4.6.1 and figures 7 and 8 it is observed that all isolates of A. niger produced both PG and Cx enzymes both in in-vitro and in-vivo condition to various extents. Their activity in in-vivo is lower than those displayed in in-vitro condition which indicated the complexity and diversity of the factors operating in-vivo conditions. From the above study it can be concluded that both the pectolytic and cellulolytic enzymes are involved in pathogenesis and also that the Cx enzyme took active part in the breakdown of cellulose of the infected tissues and enhanced the effect of the pectolytic enzymes resulting in rapid decay. Relatively higher PG and Cx enzyme activity was recorded in case of isolate 16 and isolate 24 - the relative pathogenic abilities of which are significantly higher than other isolates.

4.6.2 Cell death and cell disintegration by hydrolytic enzymes produced by various isolates of A. niger.

Toxicity of the enzyme preparation was determined by plasmolytic method following Tribe (1955). Twenty four disks of potato-tuber (7cm in diameter, 0.5mm in thickness) were placed in a reaction mixture containing 5 ml of enzyme preparation, 2 ml of phosphate buffer (0.1N, pH 7.5) and made up the volume upto 10ml with distilled water. At intervals,

3 disks were removed from the reaction mixture, washed rapidly and then placed in 2ml of the following solution for staining (85 ml of 0.9N of KNO_3, 10 ml of 0.1% Neutral red and 5 ml of 0.1 N phosphate buffer, pH adjusted at 7.5).

Maceration was measured using 0.--5 scale, of which 0 indicates the total death of protoplast and 5 for all are living protoplast.

Details of the procedure adopted has been given in Chapter III of the manuscript.

Results are tabulated in table 4.6.2.

Table 4.6.2 Cell death and cell disintegration caused by hydrolytic enzymes produced by various isolates of A.niger on Ammonium oxalate medium.

Isolate	Reaction time						
	0 min.	30 min.	1 hr.	1 hr. 30 min	2 hr.	2 hr. 30 min	3 hr.
2	5	4	3m	2m	1m	0m	0m
Control 2	5	5	4	4	4	4	4
3	5	4m	2m	1m	1m	0m	0m
Control 3	5	5	5	4	4	4	4
5	5	4	3m	2.5m	2m	1m	0m
Control 5	5	5	5	5	4	4	4
16	5	3m	3m	2.5m	2m	1m	0m
Contorl 16	5	5	4	4	4	4	4
18	5	4	3m	2.5m	2m	1m	0m
Control 18	5	5	5	4	4	4	4
20	5	4.5	4m	3m	2m	1m	0.5m
Control 20	5	5	4	4	4	4	4
21	5	4	3m	2m	1m	0m	0m
Control 21	5	5	5	4	4	4	4
22	5	4	3m	3m	2m	1m	0m
Control 22	5	5	5	4	4	4	4
23	5	4	3m	3m	2.5m	1.5m	0m
Control 23	5	5	4	4	4	4	4
24	5	3m	2.5m	2m	1m	0m	0m
Control 24	5	4	4	4	4	4	4
Blank	5	5	5	5	5	5	5

m_1 - m_5 degree of maceration, 0-5 degree of living cells, 0 indicates all cells are dead, control means dead enzyme preparation of the isolate, blank means in sterile water.

From table 4.6.2 it is observed that cell death began before maceration was completed. There was no maceration in boiled enzyme preparation [Control and in blank sterile water check]. Slight cell death was recorded in all test controls. The speed of cell death and maceration was somewhat higher in isolate 16 and isolate 24.

4.6.3. **Change in permeability by enzyme preparation of various isolates of A. niger.**

Permeability changes were measured by assessing increase in conductivity of solution as electrolytes leaked out into them from tuber disks. Reaction mixtures were similar to toxicity measurement as mentioned in 4.6.2 and at intervals three disks were removed from the reaction mixture, washed gently and placed in 15 ml glass distilled water. It was shaken for 10 seconds and the conductivity was then measured by using Systonics Direct Reading conductivity meter 303. Results were expressed as the increase in conductivity (umhos_ during 15 minutes over control. Details of the procedure adopted has been given in chapter III of the manuscript.

Results are tabulated in Table 4.6.3.

Table 4.6.3. Effect of enzyme samples of different isolates of A. niger on change of permeability of potato tuber disks at pH 4.5.

Isolate No.	Change in conductivity (micromhos) at time interval. Time interval (in minutes)								
	0	15	30	45	60	75	90	105	120
2	210	325	340	425	525	390	375	380	350
Control 2	170	175	190	180	185	175	175	185	190
3	225	300	375	500	450	425	375	375	350
Control 3	140	130	175	175	180	175	160	125	125
5	240	350	375	550	450	450	400	375	375
Control 5	120	125	125	150	130	170	150	150	125
16	325	375	525	450	420	420	390	375	350
Control 16	130	150	125	125	150	160	160	140	140
18	250	390	525	410	430	415	375	360	315
Control 18	120	130	175	155	160	180	180	170	150
20	290	360	540	450	430	390	350	325	325
Control 20	120	160	175	185	140	145	150	125	110
21	260	350	425	550	475	460	435	410	390
Control 21	120	145	140	160	150	150	125	120	125
22	290	300	350	375	400	350	325	325	325
Control 22	125	150	175	175	180	160	140	140	140
23	290	340	390	430	400	375	340	325	300
Control 23	140	150	160	175	155	140	140	120	120
24	325	410	540	490	460	425	310	310	300
Control 24	140	140	160	140	125	125	120	110	110

The results (Table 4.6.3) and on permeability changed by enzyme preparation in defined media at pH 4.5 showed that there was gradual increase in the amount of loss of electrolytes from potato disks with the incubation period for 30 minutes to 45 min. and gradually fell off upto 120 minutes. Control (boiled enzyme preparation) had much less effect on permeability. Quick loss of electrolytes were recorded in isolates 16,18 and 20.

4.6.4. **Effect of various plasmolysing concentrations of glucose on cell separation, cell death and electrolyte loss.**

Maserations was assessed by needle test. Cell death due to toxicity of the enzyme preparation was determined by the plamolytic method of Tribe (1955), based on the principle that only live protoplasts retain neutral red in plasmolysising solution. 24 disks of potato tuber (7 cm diameter, 0.5mm thick) were placed in reaction mixture containing 5ml enzyme sample, 2 ml buffer and make upto volume 10 ml with water. At interval, 3 disks were removed from the reaction mixture washed rapidly and then placed in 2ml of the staining solution.

Disks quickly plasmolysed and accumulated the stain in this solution which after 20 min. were replaced by 0.9 M $KMNO_3$ containing 0.01 M phosphate buffer at pH 7.5 Stain rapidly leached from dead protoplasts where as live cell retained red.

Permeability changes were measured by assessing increase in conductivity of solution as electrolytes leaked out into them from tuber disks. Reaction mixtures were similar to toxicity measurement and at intervals three disks were removed from the reaction mixture, washed gently and placed in 15 ml glass distilled water. It was shaken for 10 seconds and the conductivity was then measured by using Systonics Direct Reading Conductivity meter 303. Results were expressed as the increase in conductivity (umhos) during 15 minutes over control.

The results are tabulated in Table 4.6.4 (i) and Table 4.6.4 (ii).

Table 4.6.4 (i) and Table 4.6.4 (ii) effect of various hypertonic plasmolysing solutions of glucose (0.1 M to 0.5M) on maceration, cell death and electrolyte loss, suggested that killing of protoplasts by an enzyme sample was delayed by higher plasmolysing concentration (0.3M to 0.5M). Glucose concentration of 0.5M completely prevented cell death. Similarly there was a gradual inhibition of electrolyte loss from 0.1M to 0.5M solution. Maximum inhibition of electrolyte loss took place at 0.5M glucose concentration.

Table 4.6.4 (ii) **Effect of various plasmolysing solutions of glucose on cell death and cell separation by enzyme preparation of 16 and 24 isolates of A. niger grown in ammonium oxalate medium (pH 4.5).**

Isolate	Time interval In Min.	0.0	0.1	0.2	0.3	0.4	0.5
16	0	5	5	5	5	5	5
	30	3	4	5	5	5	5
	60	2m *	3m	4	5	5	5
	90	1m **	2m	4m	5	5	5
	120	0m	1m	3m	4m	4	5
	150	0m	0m ***	2m	4m	4m	4
	180	0m	0m	2m	3m	4m	4
24	0	5	5	5	5	5	5
	30	3m	4	4	5	5	5
	60	2.5m	3m	4	5	5	5
	90	2m	2m	3m	4	5	5
	120	1m	2m	3m	4m	4m	5
	150	0m	1m	2m	3m	3m	4
	180	0m	0m	1m	2m	3m	4

*** m - m degree of maceration, ** 1-5 degree of living cell, *** 0 indicates all cells are dead.**

Table 4.6.4 (i) Effect of various plasmolysing concentration of glucose at pH 4.5 on permeability changes in potato tuber disks caused by enzyme preparation of isolate 16 and isolate 24 of **A. niger grown in ammonium oxalate.**

I Measured on Systonics Direct Reading Conductivity meter 303 (Micromhos) I

Isolates	Time interval in minutes	Glucose concentration (M)					
		0.0	0.1	0.2	0.3	0.4	0.5
	20	275	230	210	180	125	-
	40	325	290	240	205	140	-
	60	590	330	290	250	170	-
	80	470	310	260	180	120	-
16	100	525	270	180	150	105	-
	120	420	250	165	125	-	-
	140	310	235	140	115	-	-
	160	260	190	135	115	-	-
	180	225	180	125	105	-	-
	20	375	340	290	210	125	-
	40	500	460	320	250	140	-
	60	410	410	290	225	105	-
	80	330	310	250	190	105	-
	100	275	260	190	130	-	-
24	120	275	190	165	125	-	-
	140	210	180	140	110	-	-
	160	175	160	125	110	-	-
	180	175	130	125	105	-	-

4.6.5: Synergistic action of oxalic acid and pectic enzyme on the collar rot disease incidence of groundnut caused by A. niger.

To find out the role of oxalic acid in disease development, the in vivo production of oxalic acid by various virulent isolates of A. niger was estimated. The groundnut plants (CV. JL-24), grown in sterilized soil in earthen pots, were inoculated with respective isolate of A. niger at 7 DAS at collar region. Crude enzyme samples were prepared from each treatment. To 5 ml aliquots of the cell-free crude enzyme sample in centrifuge tube 4 ml of calcium chloride-acetate buffer (pH 4.5) was mixed. The mixture was allowed to stand overnight and centrifuged at 3000 g for 10 minutes. The supernatant was discarded and the sediment was washed with 5 ml of 5% acetic acid saturated with calcium oxalate and was centrifuged. The residue was disolved in 5 ml of 4 N Sulphuric Acid and the solution was tranferred to 100 ml flask and was heated to 80-90 degree centregate on a water bath. The solution was treated while hot with 0.02N Potassium permanganate until a faint pink colour persisted. The amount of oxalic acid present in the crude enzyme sample was calculated. One ml of 0.02N $KMNO_4$ will react with 1.2653 mg of oxalic acid.

The detail method followed has been mentioned in chapter III of this manuscript.

The data are recorded in Table 4.6.5.

Table - 4.6.5

Estimation of oxalic acid present in culture filtrate of various isolates of A. niger grown in Potato Dexhose broth for 10 days.

Isolate	pH of culture filtrate	Yield of o ganim (g)	Amount of oxalic acid produced (g)	In vibo Pr enzyme activity (RA/ml)
2	2.5	0.175	0.073	13.33
3	2.6	0.324	0.1	14.08
5	3	0.16	0.0696	13.15
16	2.5	0.17	0.121	16.94
18	2.2	0.116	0.088	13.6
20	2.3	0.195	0.068	10.75
21	2.2	0.195	0.0652	9.9
22	2.8	0.21	0.0639	8.47
24	2.5	0.22	0.111	15.15
			CD 5%	=14.19

* Values in parenthesis are angular transformed values

It is observed from the Table 4.6.5. that significantly higher degree of disease incidence was recorded with isolate 24 and isolate 16 of A. niger with respect to other isolates. The invivo PG enzyme activity as well as amount of oxalic acid produced by those two isolates were somewhat higher than that of other isolates. A synergistic effect of oxalic acid and PG enzyme was observed in maceration of host tissue.

4.7. **Biocontrol of A. niger and disease caused by it in groundnut.**

4.7.1. **In vitro**

To find out the antagonistic effect of T. harzianum against A. niger in invitro condition, P.D.A. plates with dual cultures were incubated at 28 ± 1 degree Centrigate and percentage of reduction was recorded after 24 hrs. and 72 hrs. and results were tabulated in Table 4.7.1. Detail of the method followed has been given in chapter III of this manuscript.

Table 4.7.1. Effect of T. harzianum on growth of pathogenic isolates of A. niger, expressed as reduction % of linear growth after 24 hrs. and 72 hrs of inoculation.

A. niger	**Liner growth (m.m)**					
Isolates	**24 hrs.**			**72 hrs**		
	R1	R2	Reduction(%)	R1	R2	Reduction (%)
Isolate 16	19**	9	52.63	43	17	60.46
Isolate 24	17	8	52	40	16	60

* Reduction % of Linear growth = $\frac{R_1 - R_2}{R_1} \times 100$

Where, R_1 = Radius of normal growth

R_2 = Radius of inhibited growth

** Data are average of 3 replications.

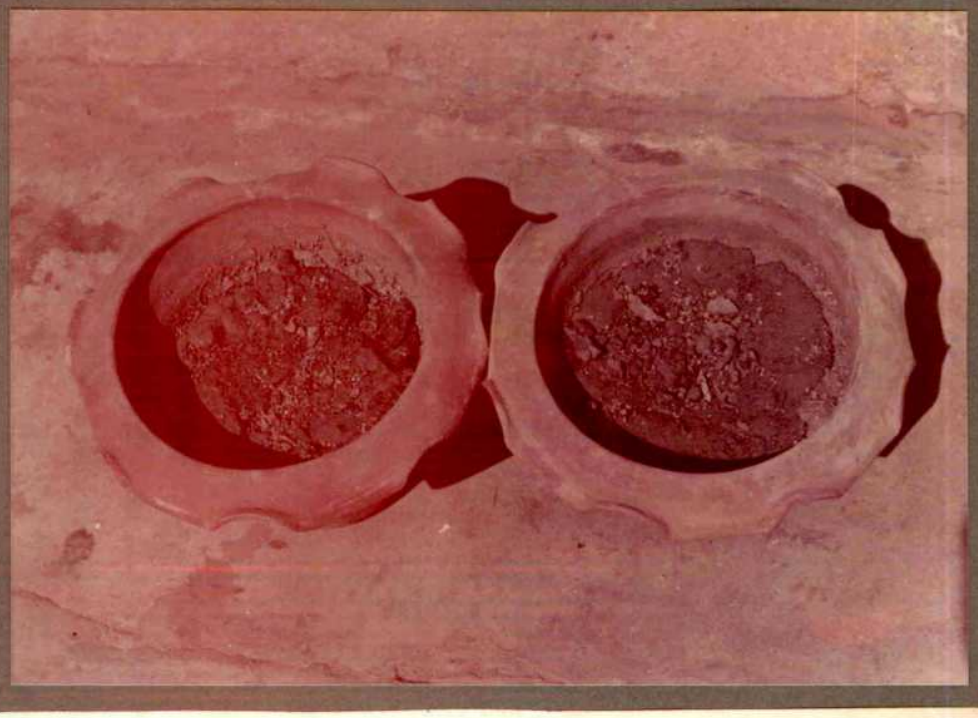


Plate-12 Sclerotia produced by A. niger on surface of inoculated soil.

Plate-13 Overgrowth of T. harzianum on colony of A. niger

of Trichoderma was recorded to be of a necrotroptic or destructive type. (Barnett and Binder 1973; Dennis 1971; Gupta 1979; Transmo and Dennis 1978; Upadhyay 1979). The hyphae of T. harzianum was found to produce branches inside the conidiophores after penetration. Pressure created by this caused rupturing of conidiophores.

4.7.2 **In vivo**

A. In glass house condition

4.7.2A (i) **Seed dressing with T. harzianum. spores.**

To find out the effect of seed dressing with T. harzianum against A. niger, the surface sterilized seeds of groundnut (Var. JL-24) were treated separately with four levels of spore suspensions i.e. 0, 25, 100 and 250 x 10^6 spores/ml of T. harzianum, @ 20 ml/20 g seeds and sown in soil, inoculated with a virulent isolate of A. niger (Isolate 16) @ 30 and 50% of the soil weight. Disease incidence was recorded upto 45 days after sowing after which no increase in disease incidence was observed. The detail method followed has been given in chapter III of this manuscript.

The data are recorded in Table 4.7.2A (i)

Table 4.7.2A (i) Effect of groundnut seed dressing with different level of T. harzianum spores on crown rot disease incidence under different inoculum pressure of A. niger (Isolate 16).

Percentage of A. niger inoculum in soil	T.harzianum spore inoculum (million/ml)							
	0	25	100	250	0	25	100	250
	2	0	0	0	2.5	0	0	0
30	48.5	34	21	12.5	20.5	14.5	11	8.5
50	67	43.5	28.5	22.5	28	17.5	8.5	3.5

Crown rot disease percentage increased with increasing the percentage of A. niger (Isolate - 16) inoculum in the soil as shown in table 4.7.2 A (i). Both seed rotting and collar rot incidence were found to decrease when the concentration of T. harzianum was increased under each inoculum percentage of A. niger.

The seed rot and collar rot incidence were decreased much below than half of the untreated seeds by 100 and 250 x 10^{-6} spores/ml of T. harzianum at both 30% and 50% of inoculum pressure of A. niger (isolate 16).

4.7.2A (ii) **Effect of T. harzianum on crown rot disease incidence of groundnut under glass house condition.**

The inocula of both T. harzianum and A. niger (isolate 16) were grown in autoclaved sand maize meal media for 10 days at 28 ± 1 degree centrigate. Surface sterilized earthen pots were filled with sandy loam cultivated soil (sterilized/unsterilized) and upper 5 cm depth of the soil in the pot were inoculated with T. harzianum and A. niger separately @ 1:1 (V/V). 10 surface sterilized seeds were sown in each pot.

The experiment was conducted with 10 different treatments (T_1, T_2.. T_{10})

The detail method followed has been given in chapter III of this manuscript.

The data are recorded in Table 4.7.2A (ii).

Table 4.7.2A (ii) **Effect of T. harzianum on Crown rot disease incidence of groundnut caused by A. niger under glass house condition.**

Treatments	Disease incidence		
	A	B	C
T_1	0 (0)*	0 (0)	0 (0)
T_2	0 (0)	0 (0)	0 (0)
T_3	30 (33.21)	27.5 (31.63)	12.5 (20.7)
T_4	22.5 (28.32)	27.5 (31.63)	15 (22.79)
T_5	7.5 (15.89)	10 (18.43)	10 (18.43)
T_6	5.0 (12.92)	7.5 (15.89)	10 (18.43)
T_7	10 (18.43)	7.5 (15.89)	5 (12.92)
T_8	7.5 (15.89)	5 (12.92)	5 (12.92)
T_9	0 (0)	0 (0)	0 (0)
T_{10}	2.5 (9.1)	0 (0)	0 (0)

* Figures in parenthesis are angular transformed values.

CD5% 12.55 8.26

A = Percentage of seed rotting and pre-emergence damping off

B = Percentage of collar rotting

C = Percentage of plant infected but rot dead

From the results of T_1 and T_2 of Table 4.7.2A (ii) it is evident that isolate of T. harzianum used in the experiment had no adverse effect on the plant. From T_3 and T_4 it is observed that A. niger incited more disease in sterilized soil than in normal soil. This may be due to better colonization of sterilized soil by A. niger because other competitive organism were absent there, T_5 and T_6 showed that surface inoculation with T. harzianum culture significantly reduced the seed and collar rot incidence both in sterilized and normal soil. But T_7 and T_8 showed that T. harzianum when inoculated into soil along with A. niger significantly reduced seed rotting and collar rotting of the plant in both types (sterilized/normal) of soil though slight better protection were observed in normal soil. But here incidence of seed rotting was somewhat higher than T_5 and T_6.

4.7.2.B In field

To find out the efficacy of biocontrol agent in comparison to other seed treating chemicals a field experiment was conducted to control crown rot disease of groundnut. The inocula of T. harzianum and A. niger were grown in sand maize meal medium at 28 ± 1 degree centrigate for 10 days. The inocula of Streptomyces sp. and T. harzianum were grown in P D A medium in Petri plates.

After proper manuring the whole experimental plot was divided into 4 different blocks each of 10 m x 5 m in size. Each line (5 m)

metre and were separated by ridges of soil to check flow of water from one treatment to another.

There seed treating chemicals viz. carbendazim (Bavistin 50WP @ 0.1%), Copper oxychloride (Blitox 50 WP @ 0.4%) and MEMC (Emisan - 6 @ 0.25%) were also used as seed treating chemicals for comparison.

Surface sterilized seeds (Cultivar JL - 24) were treated with seed treating chemicals and biocontrol agents separately before sowing as per requirement of the different treatments. 50 seeds were sown in each line (10 cm distance and 5 cm depth). Four replications were maintained for each treatment.

The experiment was conducted in 10 different treatments (Viz. T_{-1}, T_{-2}... T_{-10}). Disease incidence was recorded upto 45 days after sowing as there was no increase of disease later on.

The detail of the method followed has been mentioned in chapter III of this manuscript.

The data are recorded in Table 4.7.2.B. From the results of T_{-1} and T_{-7}, it is evident that seed inoculation with *T. harzianum* was very efficient in reducing the seed rotting and pre emergence damping off plants caused by *A. niger*, T_{-2} showed that soil inoculation with *T. harzianum* significantly reduced collar rotting. T_{-3} showed that Blitox 50 WP (Copper oxychloride 50%) was not much effective in reducing disease incidence both in respect of seed rotting and collar rotting. T-4 and T-5 showed that both Bavistin 50 WP (Carbendazim 50%) and Emisan 6 (MEMC -0.25%) at their recommended dosage reduced disease incidence significantly. From T-6 it was evident that seed inoculation with *Streptomyces* sp also reduced disease incidence to a significant extent. T-8, T-9 and T-10 showed that these two antagonistic organims i.e. *T. harzianum* and *Streptomyces sp*. were also effective in reducing the natural incidence of the disease.

Again all these treatments, except seed treatment with Blitox 50 WP, increased the number and weight of Pod and seed per pod and reduced the percentage of Pod infection significantly.

It is also noted that natural incidence of collar rotting caused by Sclerotium rolfsii was found to be significantly lower where seed and soil were inoculated with T. harzianum.

It is also clear that pod infection percentage has significantly decreased in the treatments of A. niger at the antagonistic effect with T. harzianum.

Table 4.7.2.B : Disease incidence, yield component and pod infection of groun Streptomyces sp. and different fungicides angaint A. niger

Treat-ment	Disease caused by *A. niger*		Disease caused by *S. rolfsii*	Pod/ plant	Seed / plant			Infected pot (%)
	Seed rot (%)	Collar rot (%)	Collar rot (%)	Number	Weight (gm)	Number	Weight (gm)	
T_1	6(14.18)	8 (16.43)	3 (9.97)	33	81.5	100.5	64.25	11.5 (19.82)
T_2	9.5 (17.95)	4 (11.54)	1 (5.77)	34.8	83.25	100	60.75	8.25 (16.69)
T_3	41 (39.82)	8.5 916.94)	4.5 (12.25)	17.0	42.25	43.5	27.25	50.5 (45.29)
T_4	8.5 (16.95)	4.5 (12.25)	3.5 (10.78)	40	94.75	113	76	14 (21.97)
T_5	9. (17.46)	5 (12.92)	5 (12.92)	30.75	74.75	85	55.25	18.25 (25.43)
T_6	13 (21.13)	6 (14.18)	3.5 (10.78)	28.25	70.25	81.25	51.75	14.75 (22.59)
T_7	44.5 (41.89)	15.5 (23.18)	3 (9.97)	16.25	36.25	48.75	31.75	73.25 (58.86)
T_8	4 (11.54)	0.(0)	1 (5.74)	35.5	87.5	88.5	57.75	3 (9.97)
T_9	6.5 (14.77)	0.(0)	5 (12.92)	31.00	74.5	95	62	5.75 (13.87)
T10	12 (20.27)	6 (14.18)	8 (16.430	23.9	57	69.75	44.2	10.7 (19.14)
CD 5%	3.67	3.52	3.26	3.44	7.19	4.17	4.05	0.46

* Figures in parentheses are angular tramformed value

4.7.3. Effect of surface treatment of groundnut seeds with T. **harzianum on storage**.

To find out the effect of surface treatment of groundnut seeds (Var. JL-24) with T. harzianum on storage, 200 nos. of seeds were sprayed with T. harzianum spore suspension, and after drying they were sprayed with A. niger spore suspension. 200 nos. of seeds were inoculated with A. niger only. And 200 nos. of seeds were kept untreated as control. To study the effect of treatment 10 seeds from each treatment were sown in sterilized soil kept in earthen pot. 3 pot replications were maintained for each treatment. To study the effect of storage testing was done at one month interval and continued upto 5th month. The detail of method followed has been mentioned in Chapter III of this manuscript. The results are tabulated in Table 4.7.3.

From Table 4.7.3, comparing the results of various treatments, it is evident that seed coating, with T. harzianum spores significantly reduced the seed rotting as well as collar rotting incidence at all time interval tested. The main effect of time was significant in both seed rotting and collar rotting incidence. The interaction between time and treatment was insignificant in case of seed rotting but in significant in case of collar rotting incidence.

Table 4.7.3 : Effect of seed treatment with T. harziamum on storage of goundnut

Treatment	1 Month		2 Month		3 Month		4 Month		5 Month	
	A	B	A	B	A	B	A	B	A	B
T. harziamum	3.3 (10.47)	0 (0)	0 (0)	0 (0)	3.3 (10.47)	0 (0)	13.3 (21.39)	0 (0)	16.6 (24.04)	0 (0)
T. harziamum + A.niger	13.3 (21.39)	0 (0)	3.3 (10.47)	0 (0)	13.3 (21.39)	6.6 (14.89)	30.0 (33.21)	13.3 (21.39)	33.3 (35.24)	10 (18.43)
A.niger	20 (26.57)	3.3 (10.47)	26.6 (31.05)	0 (0)	16.6 (24.04)	10 (18.43)	40 (39.23)	30.0 (33.21)	87.5 (69.3)	3.3 (10.47)
Control	6.6 (14.89)	6.6 (14.89)	13.3 (21.39)	0 (0)	13.3 (21.39)	0 (0)	40 (39.23)	6.6 (14.89)	44.66 (40.16)	16.6 (24.04)

A + Percentage of seed rotting

B = " " collar "

* Figures in parentheses are angular transformed values.

CD 5% 1. Seed rotting

a) For treatment = 6.61
b) For time = 7.37
c) For interaction = 14.78

a) For treatment = 0.42
b) For time = 0.65
c) For interaction = 10.88

4.7.4. Effect of soil inoculation with antagonistic agents on germination of sclerotia on A. niger.

To find out the antagonistic effect of T. harzianum and Streptomyces sp on germination of sclerotia of A. niger. grown in sand-maize meal, was allowed to grow sclerotia. The sclerotia were harvested and thoroughly cleaned in tap water and air dried. Soil from different parts of W.B. were collected. Soil, collected from Mohanpur, Shantinikentam, Canning, Cooch Behar and sand, were sterilized. With sterilized and normal soil, kept in earthen pots, sclerotia were mixed thoroughly and then spore suspension of T. harzianum and Streptomyces sp. were mixed with soil separately. 10

Sclerotia from each type of treatment were harvested at 15 days interval upto 90 days after inoculation and their germination percentage were tested in wet blotting paper in Petri plates. To stimulate gernination of sclerotia in petri plates 4 surface sterilized groundnut seeds (CV. JL - 24) were kept in the plates. The detail of mentod followed has been mentioned in Chapter III of this manuscript.

The effect of soil contaminated with T. harzianum and Streptonyces Sp. were tabulated in table 4.7.4 (a) and 4.7.4(b).

The chemical and physical characteristics of those soils are tabulated in table 4.7.4 (c).

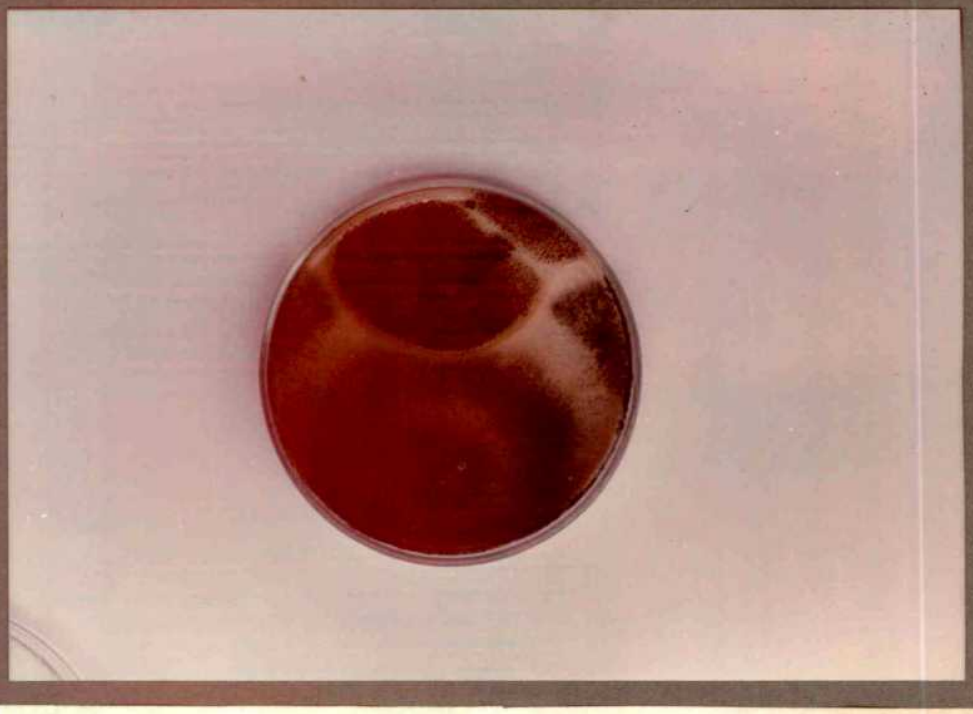


Plate-14 Overgrowth of T. harzianum on colony of A. niger in dual culture

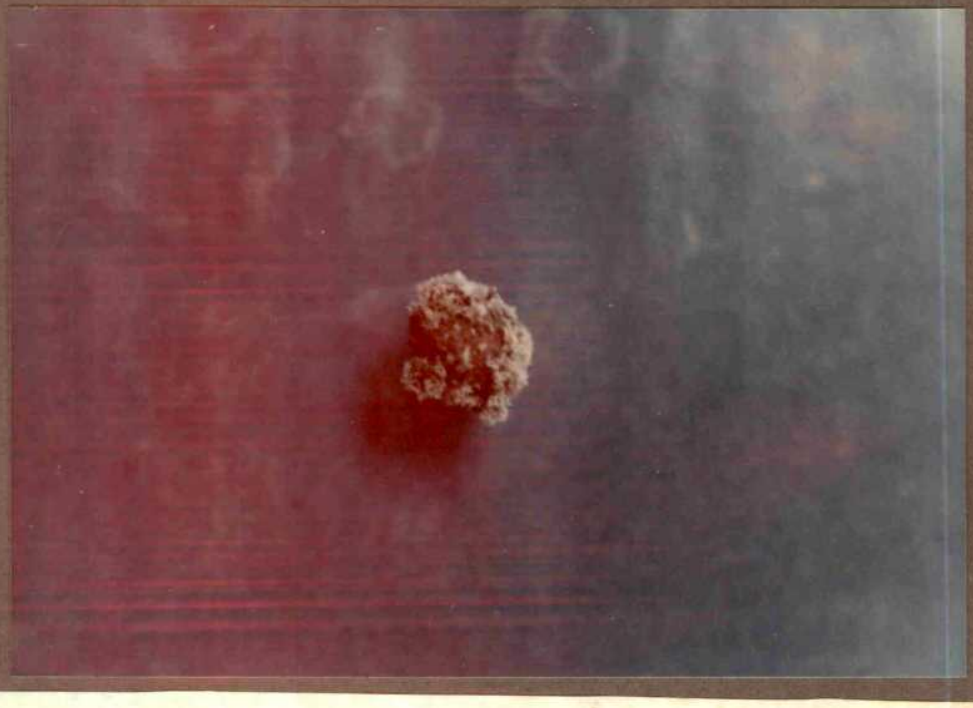


Plate-15 Growth of T. harzianum on Sclerotium of A. niger

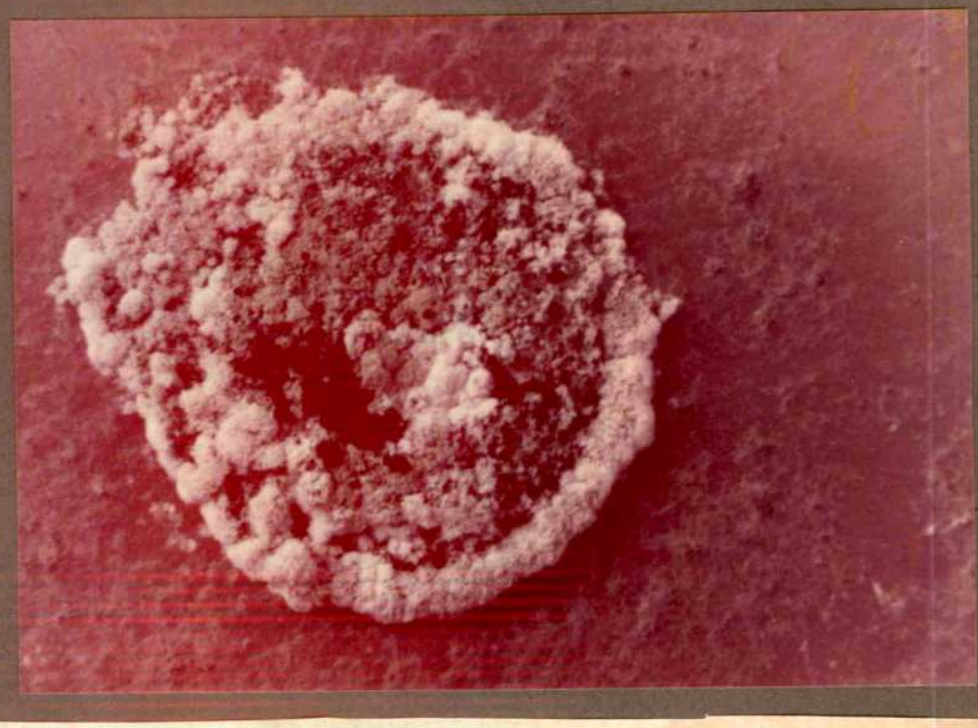


Plate-16 Groth of T. harzianum on Sclerotium of A. niger

Plate-17 Groth of T. harzianum on Sclerotium of A. niger

Plate-18 Showing normal germination of sclerotium of A. niger (Left) and inhibited germination of sclerotia of A. niger by T. harzianum (Right)

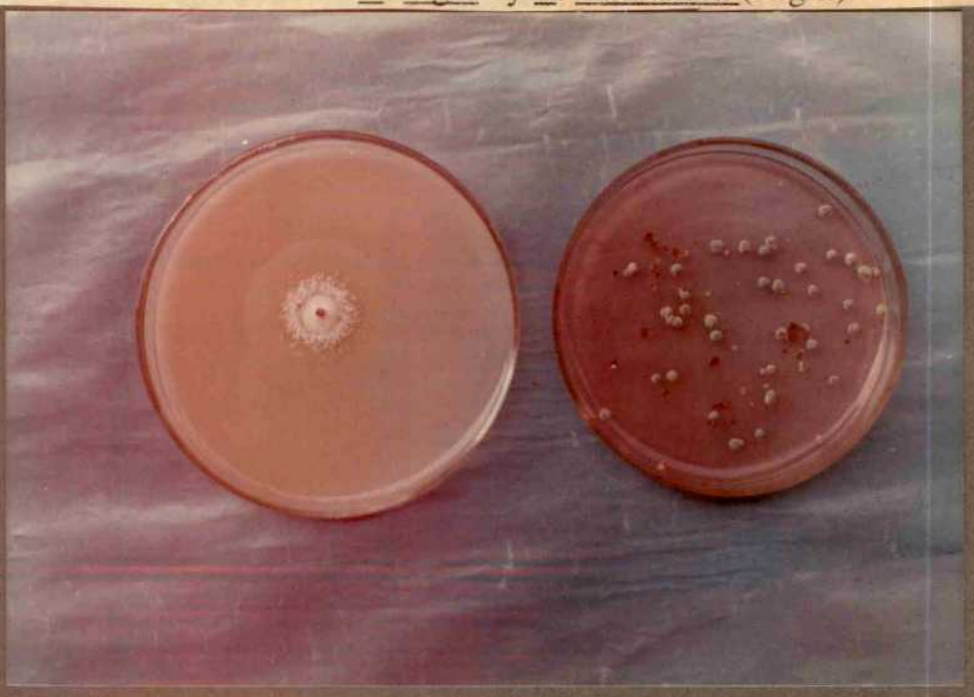


Plate-19 Inhibited germination of sclerotium of A. niger by T. harzianum

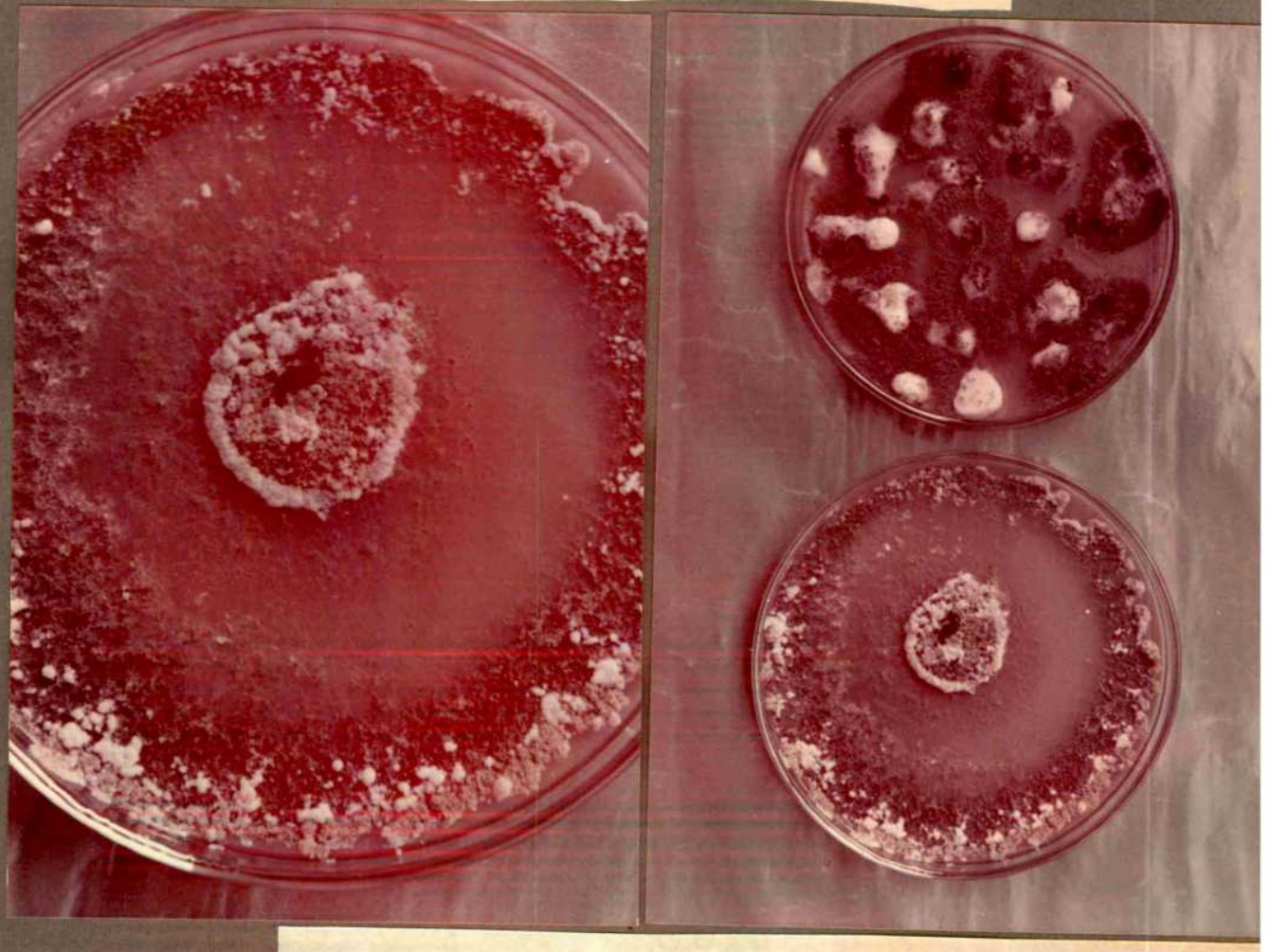


Plate-20 Inhibited germination of sclerotium of A. niger by T. harzianum

Table 4.7.4 (a). Effect of T. harzianum on germination of selerotia of A. niger as affected by different type of soil

Soil	Type of soil	% Germination of selerotic of A. niger							
		15 days		30 days		60 days		90 days	
		Unoculated	Inoculated	Unoculated	Inoculated	Unoculated	Inoculated	Unoculated	Inoculated
MOHANPUR	Sterilized	100	90.9	98.33	48.2	95.71	36.84	90.9	20.2
	Normal	95.23	81.81	91.23	64.28	91.66	60	85.29	45.33
SHANTINIKETAN	Sterilized	100	90.47	100	73.84	93.42	68.18	90.9	53.33
	Normal	86.66	82.85	85.33	80	84.3	77.14	85	57.66
CANNING	Sterilized	100	68.26	98	55.55	95	45.23	87.14	33.33
	Normal	95.65	86.63	82.35	73.9	81.11	60	73.9	52.33
COOCH-BEHAR	Sterilized	96.15	73.33	93.33	53.33	93.73	46.47	87.82	42.85
	Normal	93.54	86.66	92	77.85	85.71	73.84	80.23	86.66
SAND	Sterilized	100	84.46	100	73.68	100	60	91	58.82
	Normal	93.33	90.22	90.66	80.9	90.92	76	91	58.82

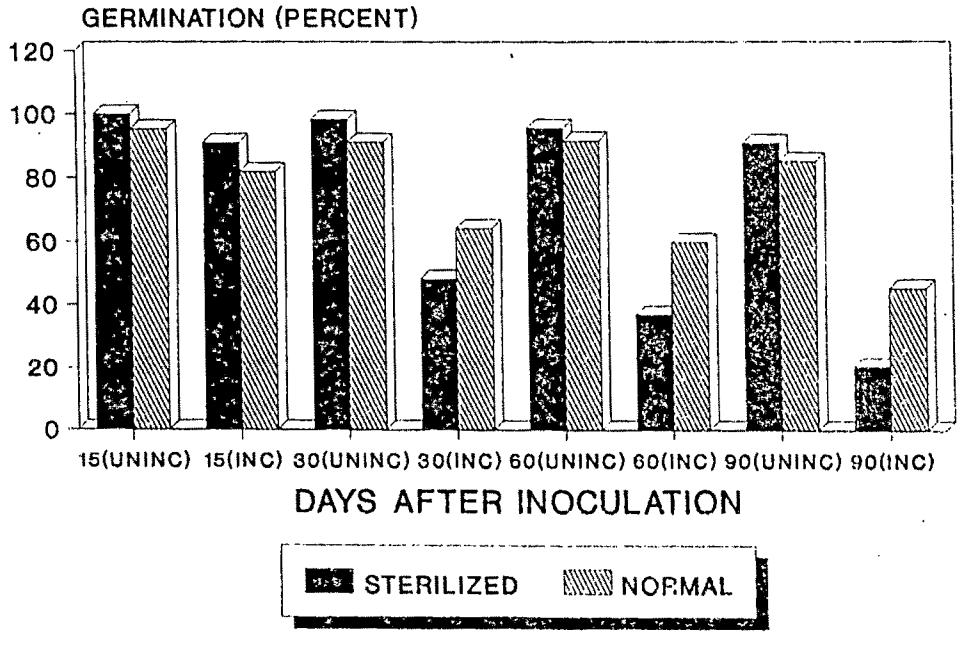
Table 4.7.4 (b). Experiment to findout the colonization of sclerotia by antagonistic bacteria (Streptomyces sp)

Soil	Type of soil	% Germination of selerotic of A. niger							
		15 days		30 days		60 days		90 days	
		Unoculated	Inoculated	Unoculated	Inoculated	Unoculated	Inoculated	Unoculated	Inoculated
MOHANPUR	Sterilized	100	73.25	98.33	32.00	95.71	7.8	90.9	0
	Normal	95.23	68.66	91.23	28.33	91.66	6.2	85.29	0
SHANTINIKET.	Sterilized	100	55.66	100	21.00	93.42	3.3	90.9	0
	Normal	86.66	48.54	85.33	18.33	84.3	3.0	85.00	0
CANNING	Sterilized	100	58.63	98	35.32	95.00	13.4	87.14	6.0
	Normal	95.65	52.00	82.35	31.33	81.11	11.32	73.9	3.55
COOCH-BEHAR	Sterilized	100	75.66	93.33	39.43	93.75	16.7	87.82	4.5
	Normal	93.54	71.33	92.00	37.33	85.71	15.32	80.23	3.3
SAND	Sterilized	100	59.00	100	30.13	100	12.0	91.00	6.3
	Normal	93.33	56.32	90.66	28.33	90.92	11.06	86.23	4.3

Table 4.7.4 (c) Soil Characteristic.

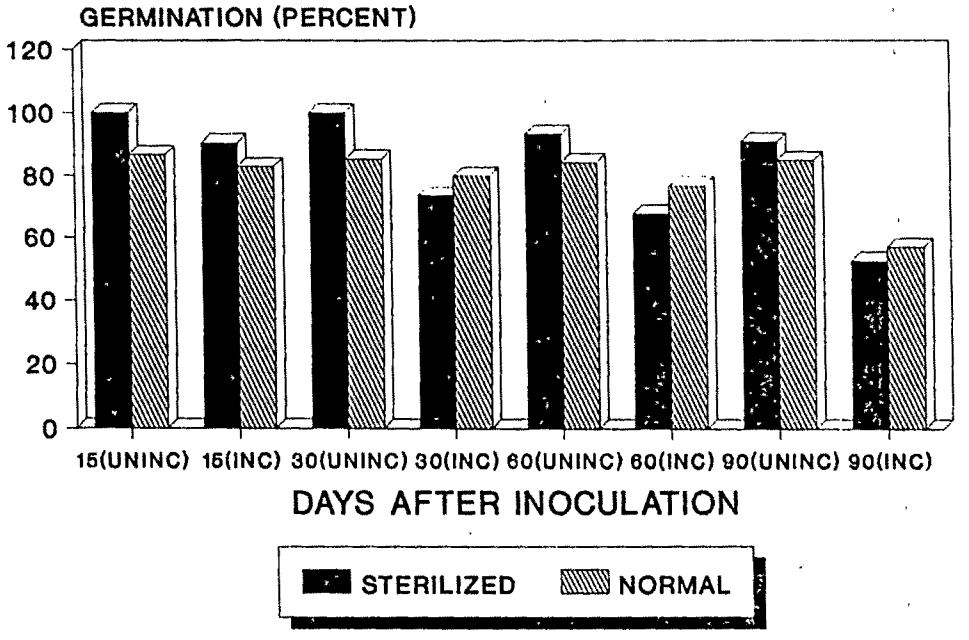
Name of place from where soil was collected	pH	EC mmhos/ cm	Organic C	Available $P_2 O_5$ (Kg/ha)	Available K (Kg/ha)
Canning (24-Parg.(S)	6.5	3.0	0.64	33.75	330
Cooch-Behar	6.7	0.3	0.60	30.0	182
Mohanpur (Nadia)	7.0	0.4	0.52	24.5	182
Santiniketan (Birbhum)	6.1	0.3	0.42	32.50	269

Fig. 9.Effect of T.harzianum on germination of sclerotia of A.niger under Mohanpur soil condition



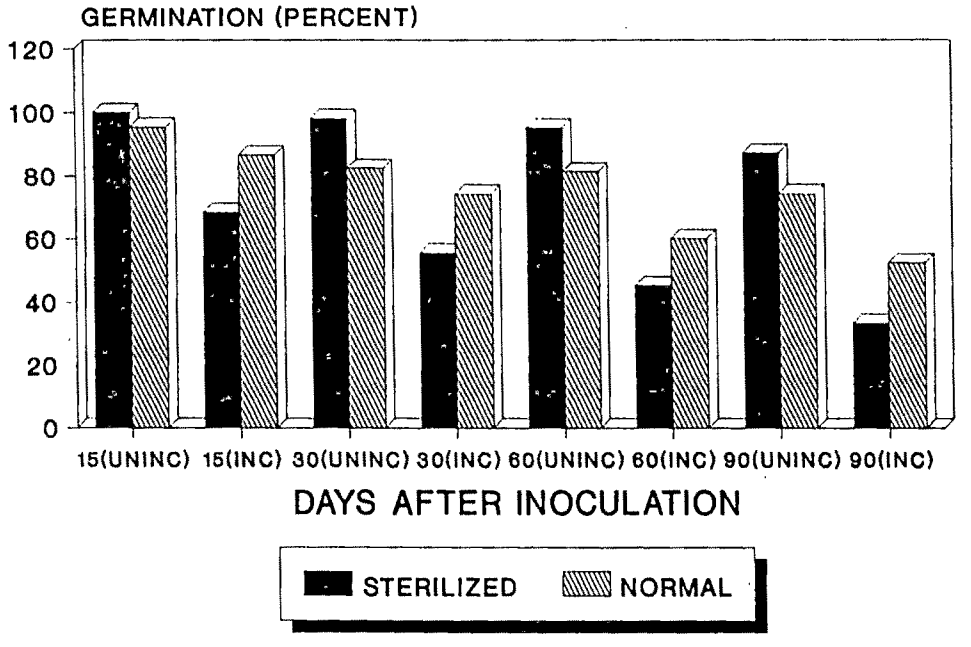
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 10. Effect of T.harzianum on germination of sclerotia of A.niger under Santiniketan soil condition



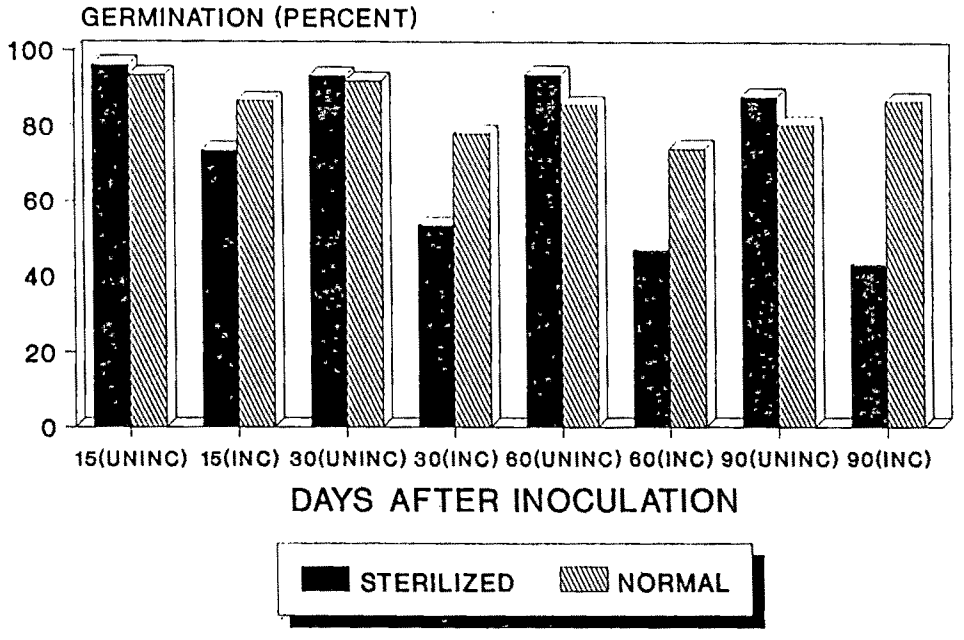
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 11. Effect of T.harzianum on germination of sclerotia of A.niger under Canning soil condition



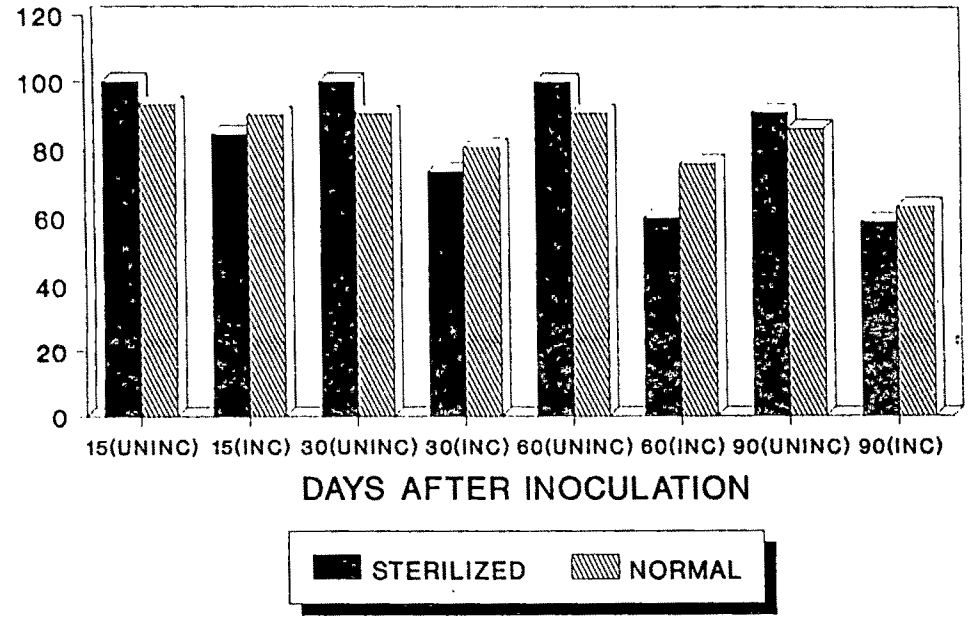
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 12. Effect of T.harzianum on germination of sclerotia of A.niger under Coochbehar soil condition



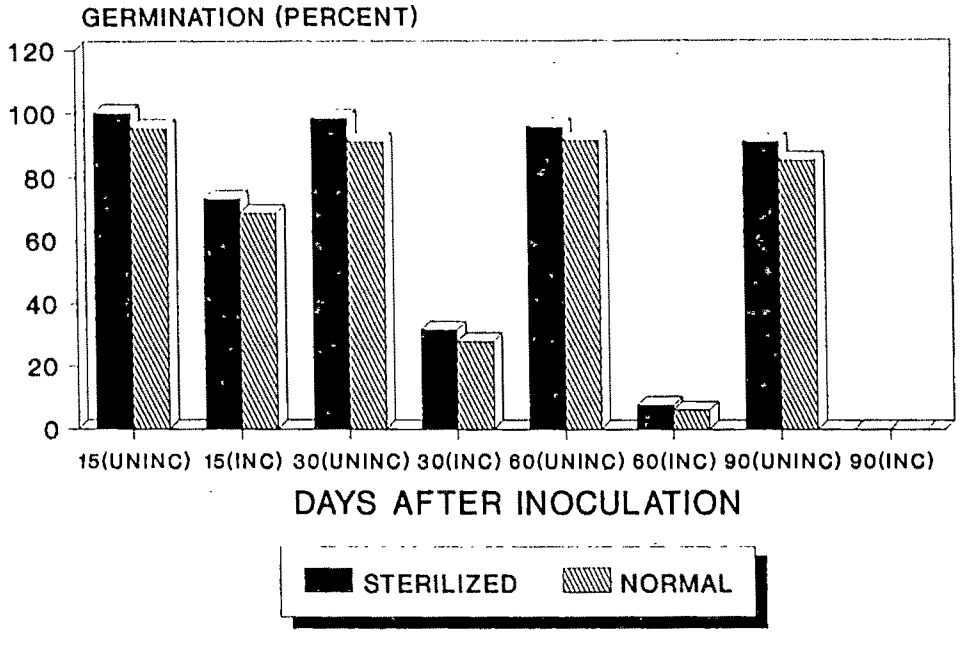
UNINC-UNINOCULATED, INC-INOCULATED

Fig 12.a Effect of T.harzianum on germination of sclerotia of A.niger under pure sand condition



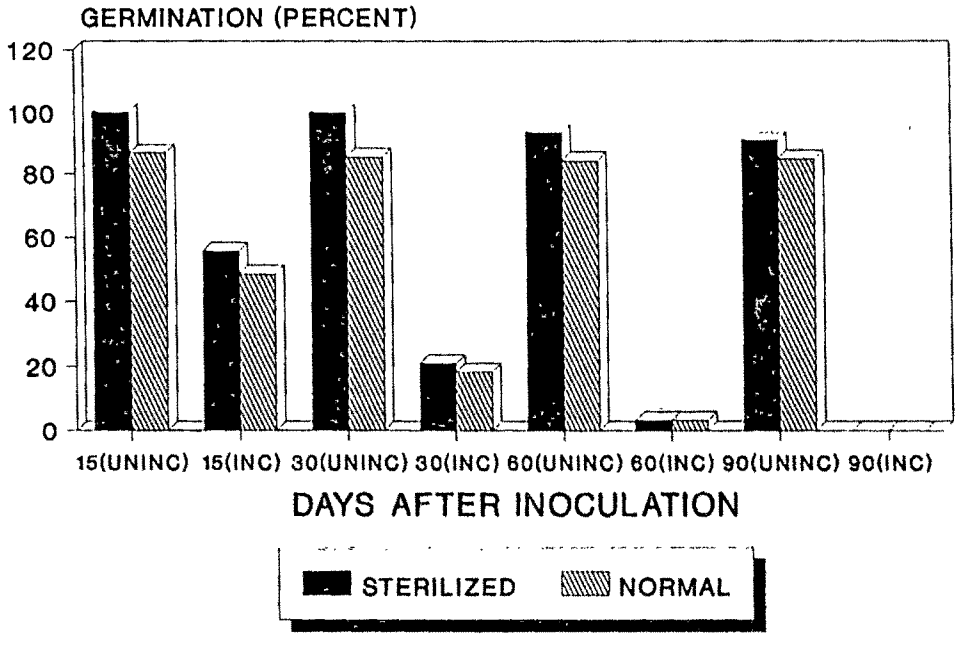
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 13. Effect of Streptomyces sp. on germination of sclerotia of A.niger under Mohanpur soil condition



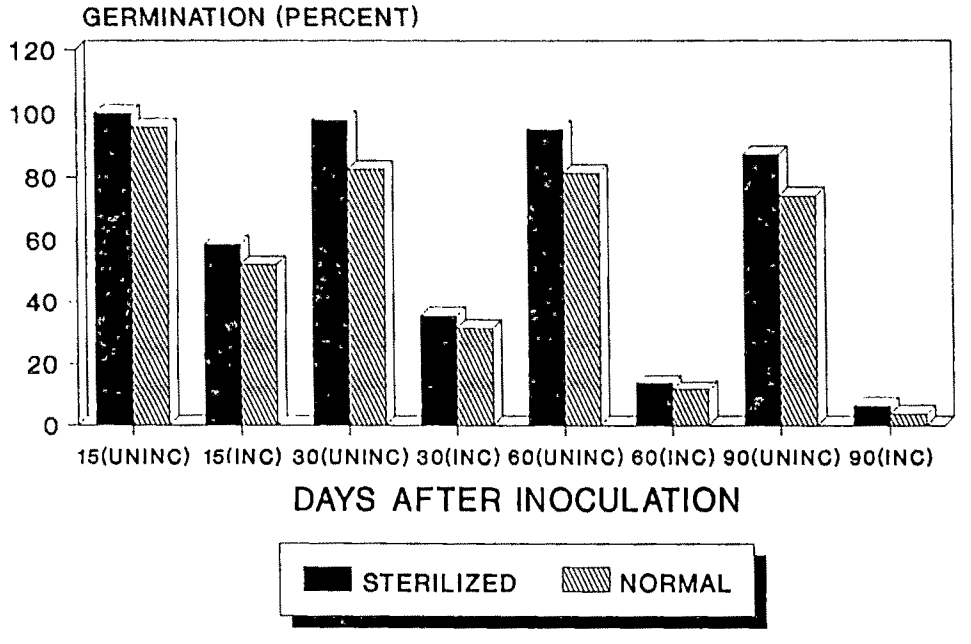
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 14. Effect of Streptomyces sp. on germination of sclerotia of A.niger under Santiniketan soil condition



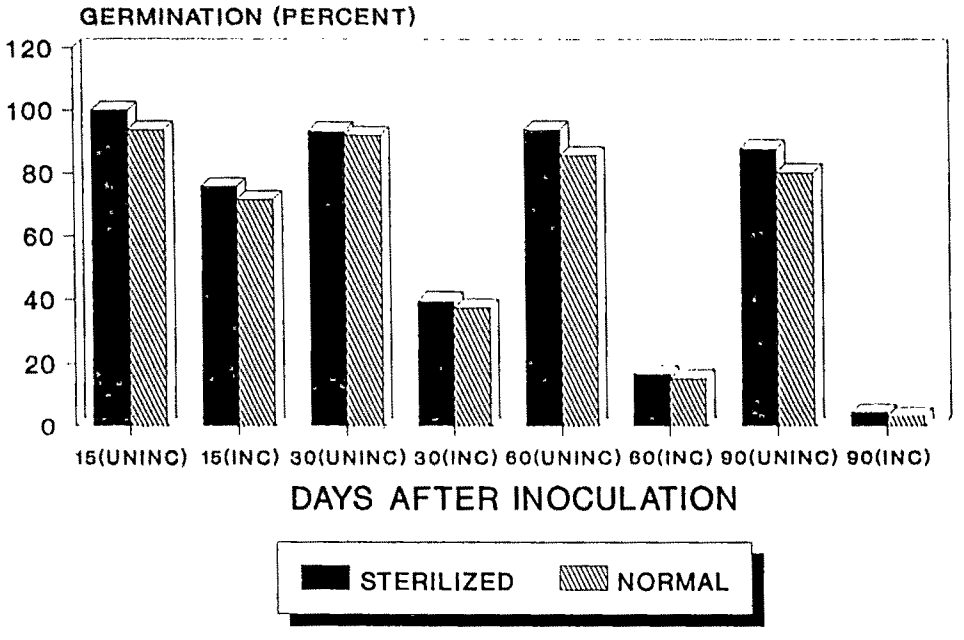
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 15. Effect of Streptomyces sp. on germination of sclerotia of A.niger under Canning soil condition



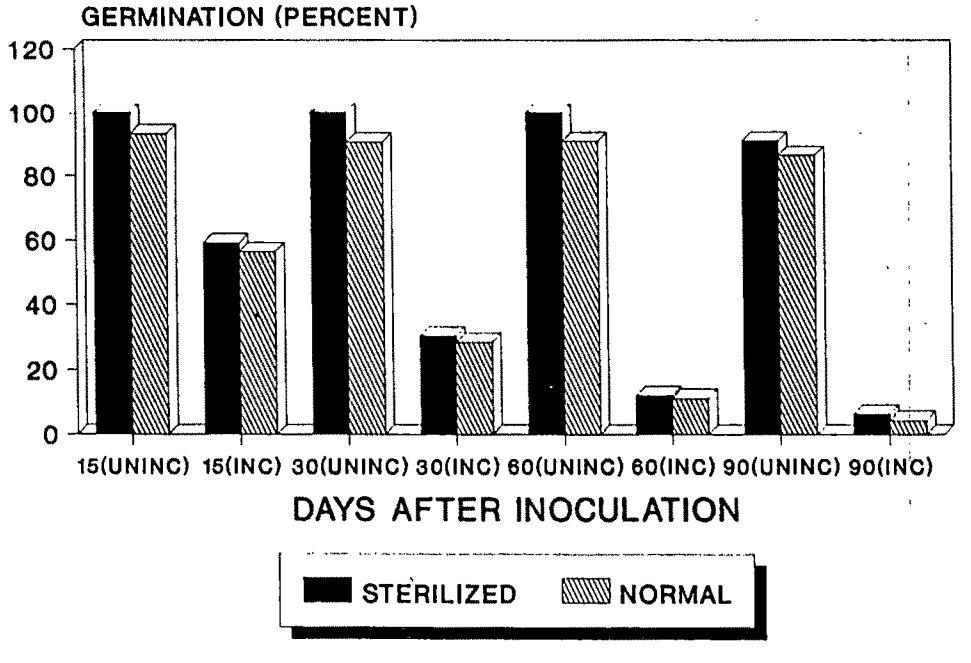
UNINC-UNINOCULATED, INC-INOCULATED

Fig. 16. Effect of Streptomyces sp. on germination of sclerotia of A.niger under Coochbehar soil condition



UNINC-UNINOCULATED, INC-INOCULATED

Fig. 17. Effect of Streptomyces sp. ong germination of sclerotia of A.niger under Pure sand condition



UNINC-UNINOCULATED, INC-INOCULATED

Comparing the results tabulated in table 4.7.4. (a), and table 4.7.4.(b) & Fig (9,10,11,12,13,14,15,16,17) it is clear that soil incoulation with T. harzianum / Streptomyces sp. appreciably reduced the germination percentage in various soil. With increasing time sclerotial germination percentage was found to reduce to a variable extent in different types of soils. The reduction of sclerotial germination was more in sterilized soil than in normal soil in both the cases when inoculated with T.harzianum, or Streptomyces Sp. In case of treatment with T. harzianum, the reduction in sclerotial germination was most prominent in soil of Mohanpur with neutral reaction but in case of treatment with Streptomyces Sp., this reduction was most prominent in soil of Mohanpur and Santiniketan with neutral and acidic reaction respectively.

4.8 Effect of seed treatment with various heavymetal salts on disease incidence.

4.8.1. Fungitoxicity assay of heavy metal salts.

All the salts of different heavy metals to be tested against crown rot disease of groundnut, were initially screened at a range of three concentrations 10^{-5}m to 10^{-2}m for their fungi toxic effect if any, on sclerotial germination of the pathogen.

Table 4.8.1 Effect of salts of different heavy metals at different concentrations on sclerotial germination of A. niger (Isolate 16).

Salts	Conc. (M).	Germination of 100 Sclerotia			
		Eruptive	Normal	No	Germination (%)
	10^{-2}	16	72	12	88
	10^{-3}	14	72	14	86
$Ba(No_3)_2$	10^{-4}	14	75	11	89
	10^{-2}	9	90	1	99
$ZnSO_4$	10^{-3}	13	85	2	98
	10^{-4}	7	92	1	99
	10^{-2}	10	88	2	98
$CuSO_4$	10^{-3}	12	86	2	98
	10^{-4}	08	80	12	88
	10^{-2}	14	74	12	88
$MnSO_4$	10^{-3}	12	74	14	86
	10^{-4}	13	71	16	84
	10^{-2}	20	76	4	96
$FeCl_3$	10^{-3}	16	72	12	88
	10^{-4}	15	73	12	88
	10^{-2}	22	70	8	88
$ZnCl_2$	10^{-3}	18	72	10	90
	10^{-4}	18	74	8	88
	10^{-4}	32	52	16	84
$HgCl_2$	10^{-5}	24	68	8	92
	10^{-6}	22	62	16	84
	10^{-3}	7	88	5	95
$CuCl_2$	10^{-4}	9	89	2	98
	10^{-5}	13	82	5	95
Water (Control)		22	62	16	84

Both the percentage, and pattern of sclerotial germination (Table 4.8.1) make it clear that none of the salt exhibited any discernible fungitoxic effect on the sclerotial germination at the concentration screened. Though proportion of eruptive germination were slightly higher in case at $HgCl_2$ (at 10^{-4}m) as compared to the control. But most of the test chemicals stimulated germination of sclerotia at their test concentration.

4.8.2. **Experiment in glass house** :

Surface sterilized groundnut seed, (Var. JL -24) were soaked in respective solution of heavy metal salts and sown in inoculated soil, kept in pot under glass house condition. Disease incidence was recorded (Table 4.8.2) upto 45 DAS as there was no increase in disease incidence after that period.

The detail of method followed has been mentioned in Chapter III, of this manuscript.

Table 4.8.2 Effect of seed socking with salts of different heavy metals on symptom expression under glass house condition.

Salt	Conc (m)	Disease Incidence A	B	C
Ba (No3)2	10-2	** (33.21) 30 (-35.7)*	(26.57) 20 (-39.9)	(10.47) 3.4 (-79.6)
	10-3	(29.93) 23.4 (-49-9)	(21.13) 13.4 (-59.8)	(18.43) 10 (-40.1)
	10-4	(31.05) 26.7 (-42.8)	(18.43) 10 (-69.9)	(18.43) 10 (-40.1)
ZnSO4	10-2	(36.29) 36.7 (-21.41)	(26.57) 20 (-39.9)	(21.47) 13.4 (-19.8)
	10-3	(41.21) 43.4 (-7.1)	(21.13) 13.4 (-59.8)	(15) 6.7 (-59.9)
	10-4	(31.29) 36.7 (-2.4)	(26.57) 20 (-39.9)	(18.43) 10 (-40.1)
CuSO4	10-2	(41.21) 43.4 (-7.1)	(28.93) 23.4 (-29.7)	(10.47) 3.4 (-79.6)
	10-3	(41.21) 43.4 (-7.1)	(21.13) 13.4 (-59.8)	(15) 6.7 (-59.9)
	10-4	(37.29) 36.7 (-21.4)	(24.12) 16.7 (-49.8)	(21.47) 13.4 (-19.8)
BaSO4	10-2	(45) 50 (+7.1)	(15) 6.7 (-79.9)	(15) 6.7 (-59.9)
	10-3	(31.05) 26.7 (-42.8)	(24.12) 16.7 (-49.8)	(10.47) 3.4 (-79.6)
	10-4	(41.15) 43.3 (-7.3)	(26.57) 20 (-39.9)	(21.47) 13.4 (-19.8)
MnSO4	10-2	(33.21) 30 (-35.7)	(31.05) 26.5 (-20.12)	(18.43) 10 (-40.1)
	10-3	(35.3) 33.4 (-28.5)	(21.13) 13.4 (-59.8)	(18.43) 10 (-40.1)
	10-4	(41.21) 43.4 (-7.1)	(26.57) 20 (-39.9)	(15) 6.7 (-59.9)
FeCl3	10-2	(41.21) 43.4 (-7.1)	(15) 6.7 (-79.9)	(18.43) 10 (-40.1)
	10-3	(29.23) 40 (-14.3)	(21.13) 13.4 (-59.8)	(18.43) 10 (-40.1)
	10-4	(37.29) 36.7 (-21.4)	(28.93) 23.4 (-29.7)	(21.47) 13.4 (-19.8)
ZnCl2	10-2	(33.21) 40 (-14.3)	(24.12) 16.7 (-49.8)	(24.88) 16.7 (0)
	10-3	(33.21) 40 (-14.3)	(26.57) 20 (-39.9)	(10.47) 3.4 (-79.6)
	10-4	(33.21) 40 (-14.3)	(18.43) 10 (-69.9)	(18.43) 10 (-40.1)
HgCl2	10-2	(37.29) 36.7 (-21.4)	(10.47) 3.3 (89.4)	(18.43) 10 (-40.1)
	10-3	(33.21) 40 (-14.3)	(18.43) 10 (-69.9)	(15) 6.7 (-59.9)
	10-4	(41.21) 43.4 (-7.1)	(18.43) 10 (-69.9)	(18.43) 10 (-40.1)
CuCl2	10-2	(41.21) 43.4 (-7.1)	(28.93) 23.4 (-29.7)	(18.43) 10 (-40.1)
	10-3	(35.3) 33.4 (-28.5)	(21.13) 13.4 (-59.8)	(21.47) 13.4 (-19.8)
	10-4	(41.21) 43.4 (-7.1)	(21.13) 13.4 (-59.8)	(21.47) 13.4 (-19.8)
Control		(43.11) 46.7	(35.24) 33.3	(24.32) 16.7
CD 5%		Non significant r	12.87	Non significant

A = % of seed rotting and preemergence damping, off

B = % of collar rotted plant

C = % of plant infected but not dead.

* Values in right parenthesis indicate the percentage reduation with reference to control;

** Values in left parenthesis are angular transformed values.

It appears from table 4.8.2 that though there was a considerable reduction in percentage of seed rotting or emergence damping off in the treatments, except $BaSO_4$ (10^{-2} M), over control, none of the test chemical at any concentration significantly reduced the seed rotting or emergence damping off over control. But a significant reduction in collar rotting varing from 29.7% to 79.9% over control was observed in case of the following chemicals at specific concentration - $Ba(NO_3)_2$ (10^{-4} M, 10^{-4} M), ZnSO4 (10^{-3} M) $ZnCl_2$ (10^{-4} M) $HgCl_2$ (in all the three tested concentration) and CuSO4 (10^{-3}M) $MnSO_4$ (10^{-3}M) CuCl2 (10^{-3} M, 10^{-4} M) and $FeCl_3$ (10^{-3} M, 10^{-4} M) concentrations. Again though there was a considerable reduction (0-79.6%) in partially infected plants (infected but not dead), by most of the chemical over control, but this reduction was not significant. Spectacular reduction in collar rotting incidence was recorded in treatment with $Ba(NO_3)$-10^{-4}M (-69.9%), BaSO4 - 10^{-2}M (-79.9%), $FeCl_3$ 10^{-3}M (-79.9%), $ZnCl_2$ - 10^{-4}M (-69.9%), $HgCl_2$ - 10^{-4}M (89.8%). over control.

4.8.3 **Experiment in field.**

These salts of heavy metals, which were found to induce resistance in groundnut plants against A. niger at a particular concentration significantly, were only selected for field experiment. The results of field experiment is recorded in the Table 4.8.3.

Table 4.8.3 **Effect of seed soaking of groundnut with different salts of heavy metals in induction of resistance against collar rot disease caused by A. niger (Isolate - 16) under field condition.**

Salts	Concentration (M)	Disease incidence A				B			
$Ba(NO_3)_2$	10^{-4} M	(28.66)	23	(-53.5)	*	(16.43)	8	(-62.8)	
$ZnSO_4$	10^{-3} M	(28.66)	23	(-53.5)	+	(14.77)	6.5	(-69.8)	
$CuSO_4$	10^{-4} M	(31.31)	22	(-45.5)	**	(20.27)	12	(-44.2)	
$BaSO_4$	10^{-2} M	(27.97)	22	(-55.6)		(15.34)	7	(-67.4)	
$MnSo_4$	10^{-2} M	(34.45)	32	(-35.4)		(18.91)	10.5	(-51.2)	
$FeCl_3$	10^{-4} M	(35.97)	34.5	(-30.3)		(22.38)	14.5	(-32.6)	
$ZnCl_2$	10^{-4} M	(31.31)	27	(-45.5)		(16.43)	8	(-62.8)	
$HgCl_2$	10^{-4} M	(25.10)	18	(-63.6)		(11.54)	4	(-81.4)	
$CuCl_2$	10^{-4} M	(30.66)	26	(-47.5)		(18.91)	10.5	(-51.2)	
Water (Control)	-	(44.43)	49.5			(27.27)	21.5		
C.D. (P=0.05)		5.23				3.50			

A = % of collar rotted plants.

B = % of infected but not dead plants.

* Results represent the average at 4 replications.

\+ Values in right parenthesis indicate the percentage reduction with reference to control.

** Values in left parenthesis indicate the angular transformed values.

The results in Table 4.8.3 basically confirms the results of the glass house experiment. In all the treatment a significant reduction by 30.3 to 63.6% in collar rotting was observed over the control. Stronger effects were recorded with mercuric chloride at 10^{-4} M, Barium nitrate at 10^{-4} M, Zinc suplhate at 10^{-4} M. These treatment also reduced the percentage of partially infected plants (Plants infected but not dead) drastically.

4.8.4. Biochemical changes in response to inoculation.

a) PG enzyme activity

Pectolytic enzyme is supposed to be involved in the soft rot of the plant tissue caused by A. niger. The pectolytic enzyme produced by this fungus during pathogenesis can account for the maceration of infected host tissue as well as for extensive cell wall breakdown (Bisen 1972, 1978). Studies have shown that the enzyme, responsible for the maceration of plant tissue, is also responsible for the death of plant cells (Basham and Bateman 1975, Bisen 1978, Byrde et

al. 1973, Garibaldi and Bateman 1971, Hall and Wood 1974, Mount et al. 1970). So, in effectivetreatment i.e. where a significant reduction in disease incidence was observed, such enzyme activity might have been effectively suppressed. So, a comparative study on the relative activity of PG enzyme by A. niger for disease development seemed to be quite justified. For this purpose, stem tissue were collected from the control plants and those of treatments.

The observations are presented in table 4.8.4(a). It was observed that plants in all treatments responded to inoculation by significantly lower PG enzyme activity as compared to that in the control. There seems to be good correlation between the suppession of PG activity and reduction in mortality percentage found in different treatments.

Table 4.8.4(a) **Effect of seed treatment with selected heavy metal salts on polygalacturonase enzyme activity in groundnut plants, exposed to inoculation with A. niger recorded at 10 days after inoculation.**

Treatment	Disease incidence Collar rotting	Unit of enzyme activity (RA/ml) Inoculated	%of reduction in enzyme activity due to noculation
1. $Ba(No_3)_2$, 10^{-4} M	(28.66)23(-53.5)*	15.1	63.8
2. $ZnSo_4$, 10^{-3} M	(28.66)23(-53.5)**	16.5	60.43
3. $CuSo_4$, 10^{-4} M	(31.31) 27 (-45.5)	19.7	52.75
4. $BaSo_4$, 10^{-2} M	(27.97) 22 (-55.5)	15.03	63.9
5. $MnSo_4$, 10^{-3} M	(34.65) 32 (-35.5)	13.03	68.8
6. FeC_{13}, 10^{-4} M	(35.97)34.5(-30.3)	14.9	64.27
7. ZnC_{12}, 10^{-4} M	(31.31) 27 (-45.5)	22.8	45.32
8. HgC_{12}, 10^{-4} M	(25.10) 18 (-63.5)	11.4	72.7
9. CuC_{12}, 10^{-4} M	(30.66) 26 (-47.5)	18.6	55.4
10. Control (Water)	(44.43) 49.5	41.7	

CD 5% for seed treatment = 5.23

* Value in left parenthasis is angular transformed value.

** Value in right parenthesis is percentage reduction over control.

b) Estimation of total phenol.

Since increased phenol biosynthesis and greater accumulation of phenol have often been associated with host defence reactions, particularly against rotting type pathogens, changes in phenol content in both treated and untreated plants in response to inoculation with A. niger were investigated.

Tissues were collected both from healthy and inoculated plants. Results are shown in Table 4.8.4 (b).

Table 4.8.4 (b) Effect of seed treatment with various salts of heavy metal on total phenol content in healthy and collar rot effected groundnut plants (C.V. - JL -24), recorded at 10 days after inoculation.

Seed Treatment	Conc. (M)	Phenol content (mg/g fresh weight of tissue) Healthy	Inoculated	% Increase due to inoculation
1. $Ba(No_3)_2$	10^{-4}	0.84	1.49	77.38
2. $ZnSo_4$	10^{-3}	1.05	1.48	40.95
3. $CuSo_4$	10^{-4}	1.27	1.66	30.70
4. $BaSo_4$	10^{-2}	0.8	1.54	92.5
5. $MnSo_4$	10^{-3}	0.69	1.54	123.18
6. $FeCl_3$	10^{-4}	0.67	1.35	101.44
7. $ZnCl_2$	10^{-4}	1.26	1.58	25.34
8. $HGCl_2$	10^{-4}	1.13	1.74	53.98
9. $CuCl_2$	10^{-4}	1.15	1.62	40.86
10. Control (Water)	-	0.85	0.873	2.70

C D at 5% For 1. Seed Treatment (ST) = 0.026

2. Helathy/Inoculated (H/T) = 0.012

3. Interaction (ST x H/T) = 0.038.

From Table 4.8.4(b) it appears that most of the chemicals except $Ba(NO_3)_2$, $MnSO_4$, $FeCl_3$, $BaSO_4$, increased phenol content of the healthy groundnut plant significantly. Plant in all treatments along with the control (water) responded to inoculation by producing significantly greater amount of total phenol. In plants in control this increase was small or marginal. The main effect of both chemicals and inoculation were significant. The result showed a significant interaction between chemical and inoculation, indicating that the difference in chemicals was significantly affected by the inoculation treatment and that the inoculation effect differ significantly with the chemicals tested.

C) **Estimation of peroxidase activity**

Since many of the treatments with heavy metal salts substantially reduced collar rot incidence in groundnut plants, one contributing factor may have been the limitation of lession size for which evidence is available. This may have occurred due to increased biosynthesis of lignin and its enhanced accumulation at the site of pathogen attack. Since peroxidase is the most important enzyme at the terminal phase of lignin biosynthesis, it was decided to assay activity of this enzyme in inoculated plants at 10 days after inoculation. Results are given in Table - 4.8.4 (c).

Table 4.8.4(c) : Effect of seed treatment with salts of selected heavy metal on peroxidase activity in groundnut plants (CV. JL-24)exposed to inoculation with A.niger, recorded at 10 days after inoculation.

Treatment	Conc.	Unit of enzyme activity/g of tissue/min.	% of increase in peroxidase due to treatment over control	Disease incidence	
				A	B
1. $Ba(NO_3)_2$	10^{-4}M	37.5	184.95	*(28.66)23(-53.5)	**(16.43)8(-62.8)
2. $ZnSO_4$	10^{-3}M	33	150.75	(28.66)23(-53.5)	**(14.77)6.5(-64.8)
3. $CuSO_4$	10^{-4}M	29.83	126.67	(31.31)27(-45.5)	(20.27)12(-44.2)
4. $BaSO_4$	10^{-2}M	41.16	212.76	(27.97)22(-55.6)	(15.34)7(-67.4)
5. $MnSO_4$	10^{-3}M	27.83	111.47	(35.97)32(-33.4)	(18.91)10.5(-51.2)
6. $FeCl_3$	10^{-4}M	26.83	103.87	(35.97)34.5(33.3)	(22.38)14.5(-32.6)
7. $ZnCl_2$	10^{-4}M	29	120.36	(31.31)27(-45.5)	(16.43)8(-62.8)
8. $HgCl_2$	10^{-4}M	42.5	222.94	(25.10)18(-63.6)	(11.54)4(-81.4)
9. $CuCl_2$	10^{-4}M	31.66	148.54	(30.66)26(-47.5)	(18.91)10.5(-51.2)
10. Control (water)	-	13.6	-	(44.43)49.5	(27.27)21.5
$CD_{5\%}$		1.11		5.23	3.5

.. % of seed germinated plants & % of infected but not dead plants.

* A change in absorption by 0.01 per minute at 420 mm was accepted as a unit of enzyme activity.

** Values in right parenthesis indicate percentage reduction with reference to control and in left parenthesis the angularly transformed value.

From Table 4.8.4 (c). it appears that plants in all treatments responded to inoculation by significantly greater pexoxidase enzyme activity as compared to control (water) by 103.87 to 222.94%. Mercuric chloride, the compound with most protective action caused the maximum and ferric chloride with least protective action caused minimum incrase in enzyme activity. Correlation in this respect was good.

4.8.5. **Effect of seed treatment with the salts of heavy metals on growth characteristic and yield response of groundnut.**

To study the effect of seed treatment with salts of heavy metal on groundnut plants, the analysis of effect of various growth and yield attributes was done. The results are tabulated in table 4.8.5. Growth analysis was done at 75 DAS.

(a) Plant height

Seed treatment with $Ba(NO_3)_2$-10^{-4}M, $ZnSO_4$, 10^{-4} M, $BaSO_4$, 10^{-2}M, $MnSO_4$, 10^{-3}M, and $ZnCl_2$, 10^{-4}M significantly increased the Plant height over control. Treatment with $CuSO_4$, 10^{-4}M, $FeCl_3$ 10^{-4}M, $CuCl_2$ 10^{-4}M and $HgCl_2$ 10^{-4}M had no significant effect.

(b) Root length

Seed treatment with $CuSO_4$, 10^{-4}M, $MnSO_4$ 10^{-3} M, $BaSO_4$, 10^{-2}M, significantly reduced the root lenght of groundnut over control. Where as significant reduction in root length was observed in treatment with $ZnSO_4$, 10^{-3}M, $FeCl_3$ 10^{-4}M, and $CuCl_2$ 10^{-4}M. Where as $Ba(NO_3)$ 10^{-4}M, $ZnCl_2$ 10^{-4}M, $HgCl_2$ 10^{-4}M had no significant effect.

Table 4.8.5. Effect of treatment with various salts of heavy metal on growth and yield atributers of groundnut (CV.JL - 24).

Treat-ment	Conc.	AT 75 DAYS AFTER SOWING											Yield (g/Plant) After harvest
		Plant height (cm)	Root length (cm)	No. of leaft-ets. (cm)	Dry wt. of lea-fted (g).	No. of root Nodules	Dry wt. of root nodules (g).	No. of Peg.	No. of Pod	No. of flower	Dry wt. of stem (g).	Dry wt. of root (g).	
1. Ba (No3)2	10-4	40.33	26.33	178.5	6.2	338.66	0.279	6.66	6.83	4.83	11	0.512	27.52
2. ZnSO4	10-3	41.33	18	211.33	9.26	629.83	0.431	25.83	10.66	7.5	12.83	0.659	29.92
3. CuSO4	10-4	36.16	30.16	303.83	10.75	306.50	0.331	18.66	11.83	7.66	13.33	0.805	26.50
4. BaSO4	10-2	42.33	31.16	306.83	13.66	467.16	0.367	10	16.66	6	16.66	0.988	30.17
5. MnSO4	10-3	41	30.5	432.83	14.41	751.00	1.02	15.33	19.16	23.5	23.5	1.156	24.92
6. FeCl4	10-4	27.5	19	197.33	3.54	165.83	0.153	9.83	6	6	5.25	0.362	16.17
7. CuCl2	10-4	32	24.5	217.16	6.25	362.50	0.729	14	11	12.83	9.66	0.912	26.33
8. ZnCl2	10-4	39.66	29.16	324.33	14.03	560.83	0.657	22.66	17.83	19.33	19.16	1.012	31.50
9. HgCl2	10-4	30.66	25	319.16	10.53	310.66	0.250	14.5	21.5	9.33	14.75	1.00	24.42
10. Control (Water)	–	34	27.83	324.83	9.58	237.00	0.271	10.33	8.33	10.66	10.41	0.721	23.00
C D 5%		5.45	2.86	55.63	1.73	114.92	0.093	4.004	5.10	4.05	3.63	0.179	1.63

(c) No. of leaflets

Significant increase in no. of leaflets was observed in treatment with $MnSO_4$ 10^{-3}M. over control. No significant effect was observed in treatment with $CuSO_4$, 10^{-4}M, $BaSO_4$, 10^{-2}M, $ZnCl_2$, 10^{-4} M, and $HgCl_2$ 10^{-4} M. Where as significant reduction in no. of leaflets was observed in the treatment with $Ba(NO_3)_2$ 10^{-4}M, $ZnSO_4$. 10^{-3}M, $FeCl_3$ 10^{-4}M and $CuCl_2$ 10^{-4}M.

(d) Dry weight of leaflets

Significant increase in dry weight of total leaflets was observed in the treatment with $BaSO_4$ 10^{-2}M $MnSO_4$ 10^{-3}M and $ZnCl_2$ 10^{-4}M over control. No significant effect was observed in the treatment with $ZnSO_4$, 10^{-3} M, $CuSO_4$ 10^{-4}M and $HgCl_2$ 10^{-4} M. Treatment with $Ba(NO_3)_2$ 10^{-4}M, $CuCl_2$ 10^{-4}M significantly reduced the dry wt. of leaflets of groundnut.

(e) Number of nodules

Seed treatment with $ZnSo_4$, 10^{-3}M, $BaSo_4$ 10^{-2}M, $MnSo_4$ 10^{-3}M, $CuCl_2$ 10^{-4}M and $ZnCl_2$ 10^{-4}M significantly incrased no. of root nodules over control i.e. treatment with water. Though increase in number of root nodules were also observed in those treatments with $Ba(No_3)_2$ 10^{-4}M, $CuSo_4$ 10^{-4} M, $HgCl_2$ 10^{-4} M but such increase was not significantly. A significant reduction in number of root nodules was observed in treatment with $FeCl_3$ 10^{-4}M.

(f) Dry weight of root nodules

Seed treatment with $ZnSo_4$ 10^{-3}M, $BaSo_4$, 10^{-2}M, $MnSo_4$ 10^{-3}M, $Cucl_2$ 10^{-4}M and $ZnCl_2$ 10^{-4} M significantly increased dry weight of root nodules over control. Slight increase was also observed in the treatments with $Ba(No_3)_2$ 10^{-4}M, $CuSo_4$ 10^{-4}M. Slight reduction was observed in the treatment with $HgCl_2$, 10^{-4} M and significant reduction was observed in treatment with $FeCl_3$, 10^{-4} M.

(g) Number of pegs

Significant incrase in number of peg was observed in seed treatment with $ZnSo_4$, 10^{-3}M, $CuSo_4$, 10^{-4}M, $MnSo_4$, 10^{3} M, $ZnCl_2$ 10^{4} M and $HgCl_2$ 10^{-4}M over control. Incrase in number of peg was also observed in treatment with $CuCl_2$ 10^{-4}M. Slight reduction was observed in treatment with $Ba(No_3)_2$ 10^{-4}M and $FeCl_3$ 10^{-4}M.

(h) Number of peg

Seed treatment with BaSo4 10^{-2}M, MnSo4 10^{-3}M, $ZnCl_2$ 10^{-4}M and $HgCl_2$ 10^{-4}M significantly incrased number of pegs of groundnut plants over control. Slight incrase was also recorded in treatments with $ZnSo_4$ 10^{-3}M, CuSo4 10^{-4} M and $CuCl_2$ 10^{-4} M. Slight reduction was recorded in treatment with $Ba(No_3)_2$ 10^{-4}M and $FeCl_3$ 10^{-4}M.

(I) Number of flower

Number of flowers were found significantly high in treatment with $MnSo_4$ 10^{-3}M, $ZnCl_2$ 10^{-4}M. Increase was also recorded in treatment with $CuCl_2$ 10^{-4}M. Slight reduction in flowering was recorded in

$ZnSo_4$ 10^{-3}M, CuSo4 10^{-4}M and $HgCl_2$ 10^{-4}M. Significant reduction in flowering was observed in treatments with $Ba(No_3)_2$ 10^{-4}M and $Ba(So_4)$ 10^{-2}M.

(j) Dry weight of stem

Seed treatment with BaSo4 10^{-2}M, $MnSo_4$ 10^{-3}M, $ZnCl_2$ 10^{-4}M and $HgCl_2$ 10^{-4}M significantly increased weight of stem. Slight increase was also recorded in treatment with $Ba(No_3)_2$ 10^{-4}M, $ZnSo_4$ 10^{-3}M, $CuSo_4$ 10^{-4}M, Slight reduction was recorded at $FeCl_3$ 10^{-4} M.

(k) Dry weight of root

It was observed that seed treatment with $BsSo_4$ 10^{-2}M, $MnSo_4$ 10^{-3}M, $CuCl_2$ 10^{-4}M, $Zncl_2$ 10^{-4}M and $HgCl_2$ 10^{-4}M significantly increased dry weight of root. Slight increase was also recorded with $CuSo_4$ 10^{-4}M. And slight reduction was recorded in ZnSo4 10^{-3}M treatment. But significant reduction was recorded in treatment with $Ba(No_3)_2$ 10^{-4}M and $FeCl_3$ 10^{-4}M.

(l) Yield per plant

Seed treatment with all heavy metal salts sigtnificantly incrased the yield/plant over control except in $FeCl_3$ 10^{-4}M treatment where significant reduction in yield/plant was observed.

4.9 Assay of various fungicides on growth of various isolates of A. niger.

To findout the fungitoxic activity of various fungicides against different isolates of A. niger poisoned Food Technique was followed. Five fungicides e.g. Emisan 6 (MEMC), Blitox 50WP (Copper oxychloride). Bavistin 50WP (Carbendazim), indofil M-45 (Mancozeb) and Kabach 75 WP (Chlorothalonil), were tested at three concentration e.g. 250,500 and 1000 ppm against ten isolates of A. niger. Sterilized Petriplates were poured with fungicide ammended PDA and were inoculated with A. niger at the centre. The plates were incubated at 28 ± 1 degree C temperature for 72 hours in a B.O.D. incubator. Four replications were maintained. Radial growth rate (mm/hrs.) was measured. Detail method followed has been mentioned in chapter III of this manuscript. Effect of fungicides on germination of sclerotia and growth of four sclerotial isolates of A. niger was recorded in Table 4.9 (b) and 4.9 (c) respectively.

Table 4.9 (a) Effect of different fungicides on growth of various isolates of *A. niger*

Fungicide	Concentration (ppm)	Radial growth in mm/hour									
		2	3	5	16	18	20	21	22	23	24
Emisan6	250	0.01	0.01	0	0.027	0.016	0	0	0	0	0
	500	0.01	0	0	0.019	0	0	0	0	0	0
	1000	0	0	0	0.016	0	0	0	0	0	0
Blitox 50 WP	250	0.32	0.35	0.35	0.36	0.36	0.38	0.37	0.33	0.31	0.36
	500	0.32	0.33	0.35	0.34	0.34	0.34	0.31	0.28	0.26	0.3
	1000	0.22	0.21	0.22	0.26	0.25	0.25	0.23	0.21	0.23	0.25
Bavistin 50WP	250	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0
	1000	0	0	0	0	0	0	0	0	0	0
Indofil 45	250	0.17	0.18	0.21	0.28	0.17	0.17	0.17	0.17	0.17	0.18
	500	0.15	0.15	0.16	0.17	0.15	0.16	0.15	0.14	0.15	0.17
	1000	0.04	0.04	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.05
Kabach 75 WP	250	0.26	0.26	0.28	0.31	0.28	0.3	0.29	0.31	0.31	0.34
	500	0.24	0.25	0.26	0.28	0.26	0.27	0.25	0.29	0.28	0.3
	1000	0.23	0.23	0.24	0.25	0.24	0.24	0.22	0.26	0.25	0.27
Control		0.27	0.28	0.31	0.31	0.28	0.32	0.32	0.32	0.31	0.36

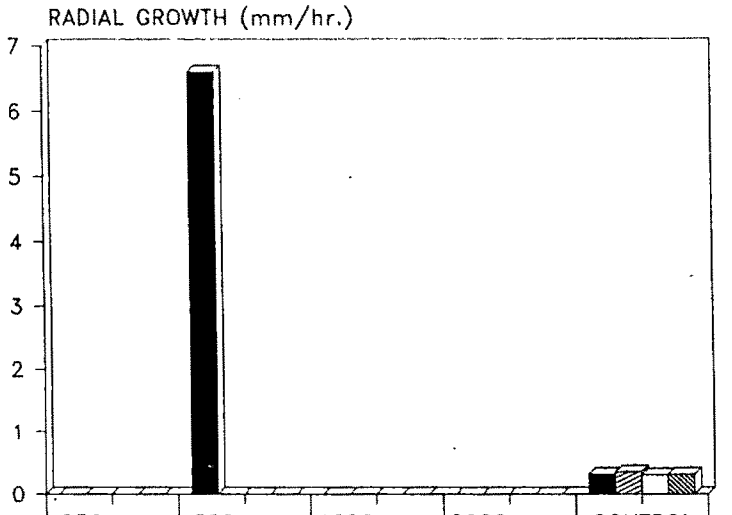
Table 4.9 (b) Effect of different fungicides on germination of sclerotia produced by four isolates of *A. niger*

Fungicide	Concentration (ppm)	Percentage of germination of sclerotia			
		Isolate 3	Isolate 16	Isolate 18	Isolate 23
Emisan6	250	0	6.6	0	0
	500	0	0	0	0
	1000	0	0	0	0
	2000	0	0	0	0
Blitox 50 WP	250	76.7	96.7	76.7	76.7
	500	73.3	86.7	50	56.7
	1000	46.7	60	43.3	36.7
	2000	36.3	46.7	33.3	33.3
Bavistin 50WP	250	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
	2000	0	0	0	0
Indofil 45	250	6.6	13.3	13.3	16.7
	500	0	10	0	0
	1000	0	0	0	0
	2000	0	0	0	0
Kabach 75 WP	250	100	100	100	100
	500	100	100	83.3	83.3
	1000	90	96.7	63.3	60
	2000	83.3	86.7	56.7	50
Control		97.98	100	96.6	96

Table 4.9 (c) Effect of fungicides on growth of four sclerotial isolates of *A. niger* from sclerotia

Fungicide	Concentration (ppm)	Radial growth in mm/hrs.			
		Isolate 3	Isolate 16	Isolate 18	Isolate 23
Emisan6	250	0.04	0.06	0.014	0
	500	0	0.04	0	0
	1000	0	0.019	0	0
	2000	0	0.01	0	0
Blitox 50 WP	250	0.13	0.19	0.18	0.14
	500	0.11	0.15	0.14	0.12
	1000	0.06	0.13	0.12	0.08
	2000	0.05	0.08	0.06	0.06
Bavistin 50WP	250	0	0	0	0
	500	0	0	0	0
	1000	0	0	0	0
	2000	0	0	0	0
Indofil 45	250	0.1	0.12	0.1	0.11
	500	0	0.08	0.06	0.07
	1000	0	0.06	0	0
	2000	0	0	0	0
Kabach 75 WP	250	0.23	0.3	0.25	0.29
	500	0.2	0.23	0.21	0.25
	1000	0.17	0.21	0.17	0.18
	2000	0.14	0.18	0.15	0.14
Control		0.32	0.36	0.31	0.32

Fig.18. Effect of Emisan 6 on the growth A.niger from sclerotia



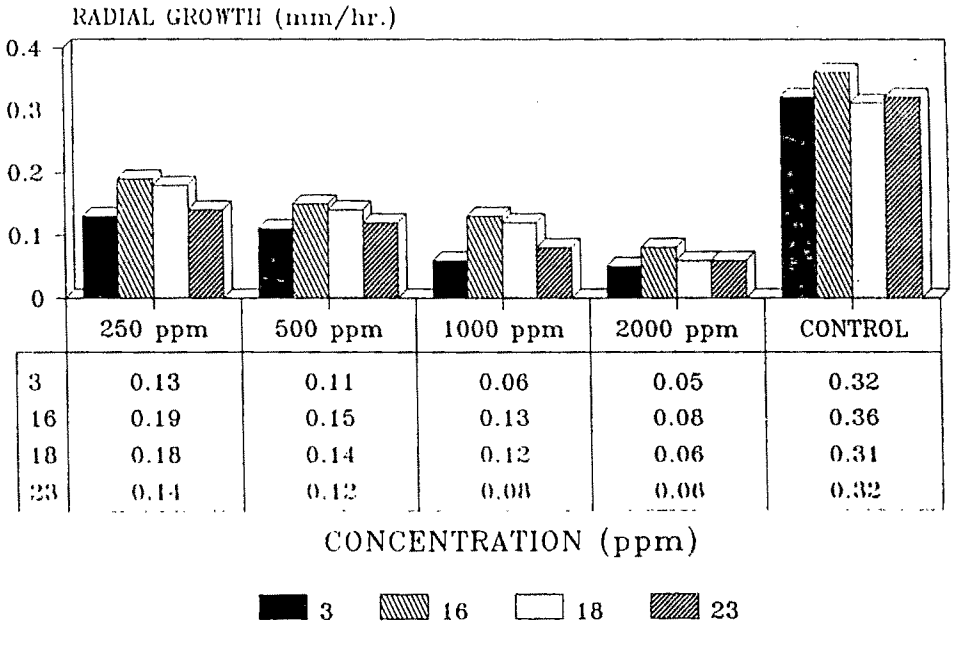
	250 ppm	500 ppm	1000 ppm	2000 ppm	CONTROL
3	0	6.6	0	0	0.32
16	0	0	0	0	0.36
18	0	0	0	0	0.31
23	0	0	0	0	0.32

CONCENTRATION (ppm)

3 16 18 23

(ISOLATES 3,16,18,23)

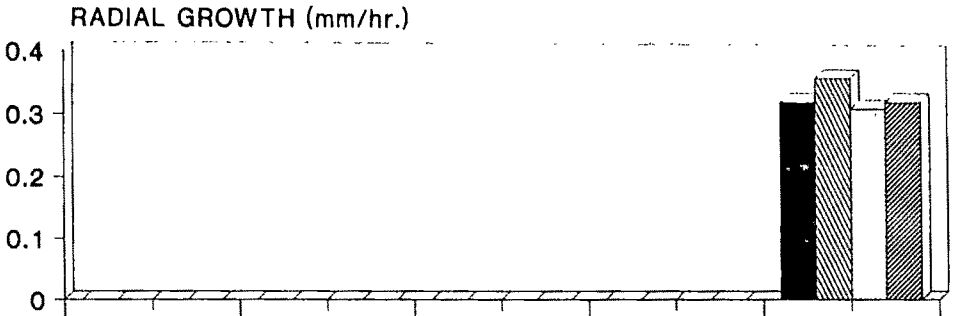
Fig.19. Effect of Blitox 50 WP on the growth of A.niger from sclerotia



	250 ppm	500 ppm	1000 ppm	2000 ppm	CONTROL
3	0.13	0.11	0.06	0.05	0.32
16	0.19	0.15	0.13	0.08	0.36
18	0.18	0.14	0.12	0.06	0.31
23	0.14	0.12	0.08	0.06	0.32

(ISOLATES 3,16,18,23)

Fig.20. Effect of Bavistin 50 WP on the growth of A.niger from sclerotia



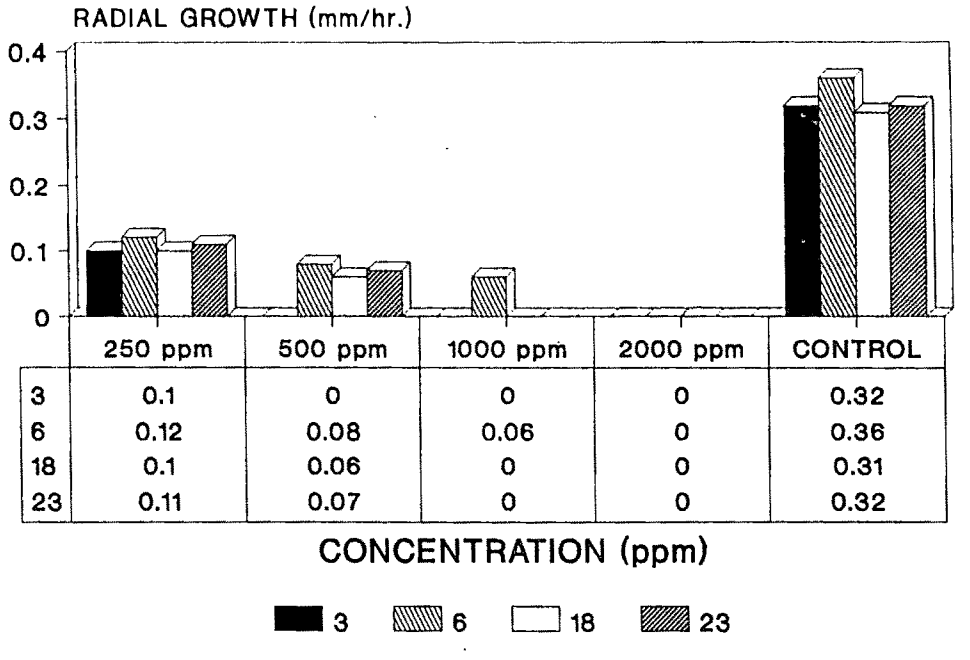
	250 ppm	500 ppm	1000 ppm	2000 ppm	CONTROL
3	0	0	0	0	0.32
16	0	0	0	0	0.36
18	0	0	0	0	0.31
23	0	0	0	0	0.32

CONCENTRATION (ppm)

3 16 18 23

(ISOLATES 3,16,18,23)

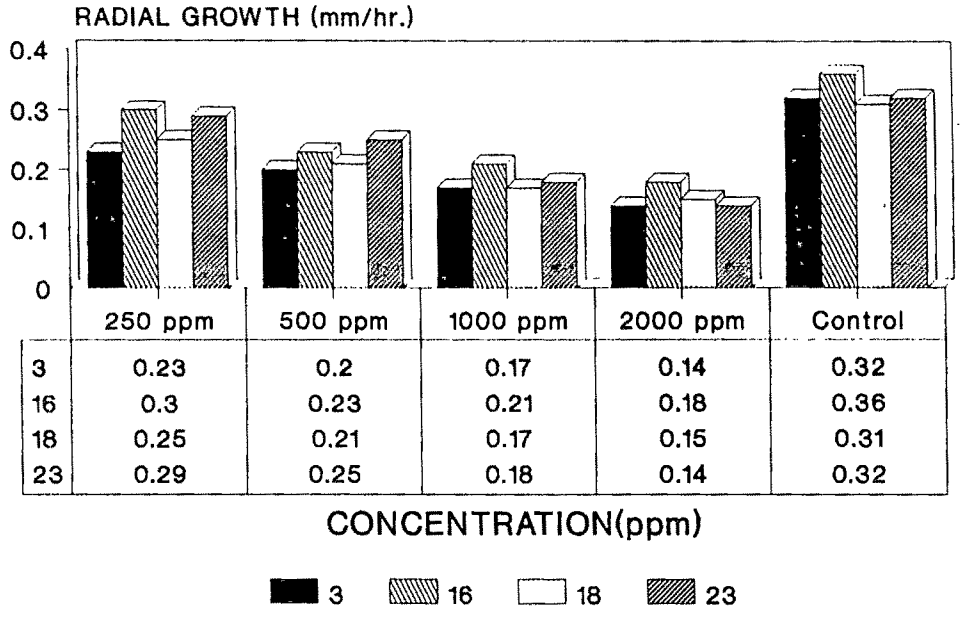
Fig.21. Effect of Indofil M-45 on the growth of A.niger from sclerotia



	250 ppm	500 ppm	1000 ppm	2000 ppm	CONTROL
3	0.1	0	0	0	0.32
6	0.12	0.08	0.06	0	0.36
18	0.1	0.06	0	0	0.31
23	0.11	0.07	0	0	0.32

(ISOLATES 3,16,18,23)

Fig.22. Effect of Kabach 75 WP on the growth of various isolates of A.niger from sclerotia



	250 ppm	500 ppm	1000 ppm	2000 ppm	Control
3	0.23	0.2	0.17	0.14	0.32
16	0.3	0.23	0.21	0.18	0.36
18	0.25	0.21	0.17	0.15	0.31
23	0.29	0.25	0.18	0.14	0.32

(ISOLATES 3.16,18,23)

From the data presented in table 4.9 (a), and Fig 18,19,20,21 and 22 it was observed that Emisan almost at all concentrations was effective in inhibiting the growth of all isolates of A. niger. Isolate 2 at 250 ppm and 500 ppm, Isolate 3 and 18 at 250 ppm and isolate 16 at all concentration of Emisan-6, showed some degree of tolerence.

Though higher concentrations reduced the growth rate, but Blitox 50WP at no concentration was effective in inhibiting the growth of any isolate of A. niger tested. Bavistin 50 WP at all concentrations completely inhibited the growth of all isoltes of A. niger. In case of Indofil M-45, though some degree of reduction in growth rate of all isolates of A. niger was observed over control but all isolates showed tolerence to some extent to all concentrations of Indofil M-45. All isolates showed fair degree of tolerence to Kabach 75 WP at all concentrations tested.

From the data presented in table 4.9(b), it was observed that Emisan 6 at all concentrations totally inhibited the germinations of sclerotia of all sclerotial isolates of A. niger tested except at 250 ppm concentration little percentage of germination was observed of isolate 16. Blitox 50WP at higher concentration somewhat reduced the germination percentage but at no concentration it could completely inhibited the germination. Bavistin at all concentrations totally inhibited the germination of sclerotia of all isolates. In case of Indofil M-45, little percentage of germination of sclerotia

of all isolates was observed at 250 ppm, but at higher concentrations it completely inhibited the germination of sclerotia of all isolates except isolate 16 where some degree oft germination was observed even at 500 ppm concentrations. Kabach 75WP at all concentration failed to inhibit the germination of sclerotia to any extent.

From the data present in table 4.9(c), it was observed only at 250 ppm concentration of Emisan slight growth of isolate 3,16, 18 was recorded from sclerotia. At all concentration, isolate 16 showed some degree of tolerence to Emisan. Isolate 16 totally failed to germinate and grow at any concentration of Emisan. Though some on degree of inhibition in growth rate was observed at higher concentration but at no concentration, Blitox 50 WP and Kabach 75 WP could inhibit the growth of sclerotial isolates of A. niger from sclerotia. Bavistin 50WP totally inhibited the growth of all sclerotial isolates of A. niger from sclerotia. Indofil M-45 also fairly reduced the growth rate at higher concentrations, though some degree of tolerence was recorded in case of all sclerotial isolates at lower concentration.

observed towards all concentrations Blitox 50 WP tested, but at 1000 ppm sparse mycelial growth of isolates 2,3,5,16,18 and 21 was

observed upto 120 hrs. of inoculation, after which slight whitish yellow immature conidial heads were observed at the centre region the fungal growth.

After 120 hrs. Isolate 24 of A. niger was found to produce whitish mycelium growth at the peripheral region of the inoculated plates in control. Black sporulation at the centre and growth circles in the rest part were observed. At 250 ppm concentration of indofit M-45, this isolate produced spare white mycelial growth at the peripheral region, whitish yellow sporulation at the sub peripheral region in a thin margin, and typical blackish sporulation at the centre and rest part of the growth. At 500 ppm, sparse whitish mycelial growth at peripheral region, whitish yellowish sporulation at the sub peripheral region, and blackish sporulation at the centre and sarounding region was observed. At 1000 ppm, sparse whitish mycelial growth of the peripheral region, blackish sporulation at the centre and whitish yellow sporulation between centre and peripheri was observed. And at 2000 ppm, sparse whitish mycelial growth at the peripheri and whitish yellow sporulation in the centre was observed. More or less similar effect on growth and sporulation of other isolates of A. niger was observed with increase in concentration of Indofil M-45.

4.10. Effect of culture filtrate on disease incidence

4.10.1. **Effect of seed soaking in culture filtrates of various isolates of A. niger on seed germination and subsequent growth of groundnut seedlings.**

Groundnut seeds (CV. JL -24) were soaked overnight in culture filtrates of ten isolates of A. niger separately. In control seeds were soaked with distilled water. Then soaked seeds were sown in autoclaved soil in earthen pots and kept in glass house. Effect on seed germination and on subsequent growth of the plant was recorded upto 45 days and results were tabulated in table 4.10.1.

The detail of the method followed has been given in chapter III of this mnauscript.

Table 4.10.1. Effect of seed soaking in pure culture filtrates of various isolates of A. niger on seed germination and subsequent growth on groundnut seedlings.

Isolates	Percentage of seed germination
2	* 43.3 (-56.17) +
3	76.6 (-22.46)
5	86.6 (-12.35)
16	33.3 (-66.29)
18	96.6 (-2.22)
20	40 (-59.51)
21	53.3 (-46.05)
22	70 (-29.15)
23	93 (-5.8)
24	30 (-69.63)
Control	98.8

* Results represent the average of observation on 40 plants, 10 from each of 4 pots per treatment.

\+ Values in parenthesis indicate the percentage reductions with reference to control.

From the data presented in table 4.10.1, it is observed that seed soaking with culture filtrate of most of the isolates drastically reduced the germination of groundnut seed over control. Severe reduction was observed in treatments with isolates 2,16, 20 and 24.

Seed soaking with culture filtrates not only reduced the germination percentage but also severe curvature of basal protion of stems, roots, (Plate 7) stunting of plant and crinkling of leaves were observed in different treatment. In control no such abnormalities were observed.

4.10.2. **Effect of culture filtrate of various isolates of A. niger on groundnut seedlings.**

To study the effect of culture filtrate on plants 10ml of pure culture filtrate was added to 100ml of nutrional medium (Hoogland solution) and upto root, portion 10 days old seedlings, developed in sterile sand, was dipped in that solution and kept under observation for 10 days. The observations were recorded in table 4.10.2. The detail of the method followed has been given in Chapter III of this manuscript.]

Table 4.10.2 : Effect of culture filtrate of various isolates of A. niger on groundnut seedlings.

Isolate	Percentage of plant death upto 10-days of observation.
2	66.7 (+138.2)
3	83.3 (+197.5)
5	100 (+257.14)
16	83.3 (+197.5)
18	100 (+257.14)
20	100 (+257.14)
21	50 (+78.6)
22	100 (+257.14)
23	66.7 (+138.21)
24	85.7 (+206.1)
Control	28

Results in table 4.10.2 shows that in treatments most of the plant were found to die within day 10 days of observation. 78.6 to 257.14% increase indeath of plant over control was observed. At first in the basal region of the plant necrotic lessions were produced, the gradual wilting of the plants was observed which lead to the death of plant. In control 28.1 plants were founds to die without producing any necrotic lession in the basal region (Plate).

4.10.3. **Effect of seed soaking with diluted culture filtrates of various isolates of A. niger on induced resistance against collar rot disease caused by respective isolate.**

To find out the role of culture filtrate in induction of resistance in groundnut plants against collar rot disease, groundnut seeds (CV. JL -24) were soaked overnight with diluted (5 times with sterile distilled water) culture filtrate of respective isolate of A. niger. Seeds were then sown in autoclaved soil kept in earthen pots and after 7 days of emergence seedlings were inoculated with inoculum of respective isolate grown in sand maize meal. In control seeds were soaked with distilled water. Disease incidence was recorded in Table 4.10.3 upto 45 DAS after which no increase in disease incidence was observed.

The detail of the method followed has been mentioned in Chapter III of this manuscript.

Table 4.10.3 : Effect of seed soaking with diluted culture filtrates of various isolates of A. niger on induced resitance against A. niger collar rot of groundnut.

Isolate	**Percentage of collar rotted plants**	
	Seedsoaking with distilled water	Seed soaking with culture filtrate
2	31.14 (33.9) *	23.07 (28.66)
3	33.33 (35.24)	17.39 (24.56)
5	36.66 (37.23)	7.69 (16.8)
16	58.62 (49.62)	21.42 (27.57)
18	27.58 (31.63)	3.44 (10.6)
20	33.3 (35.2)	6.6 (14.9)
21	23.3 (28.8)	12.5 (13.6)
22	42.3 (40.57)	15.38 (23.03)
23	17.85 (24.9)	7.14 (15.45)
24	62.3 (52.1)	23.42

C D 5% 1. For isolate 14.19

2. For treatment 5.19

3. For interaction 20.09

* Value in parenthesis is the angular transformed value.

From table 4.10.3, it is observed that direct effect of isolate is not significant, but the direct effect of treatment (i.e. seed soaking with distilled water/ culture filture) is significant i.e. seed soaking with culture filtrates significantly reduced the collar rot incidence in case of all isolates of A. niger tested.

The interaction effect is not significant i.e. the result show a non significant interation between isolates and treatment, indicating that the difference in isolate was not significantly affected by the treatment and that the treatment effect did not differ significantly with the isolate tested.

4.10.4 **Effect of seed soaking with diluted culture filtrate of A. niger (Isolate 16) in induction of resistance against collar rot caused by various isolates of A. niger**

To find out the role of culture filtrate of a Pathogenic isolate (16) of A. niger in induction of resistance in groundnut against collar rot disease, groundnut seeds (Cv. JL-24), were soaked overnight with dilated (5 times with sterile distilled water) culture filtrate of A. niger (isolate 16). Seeds were then sown in autoclaved soil kept in earthen pots and after 7 days of emergence seedlings were inoculated with inoculum of different isolates separately. In control seeds were soaked with distilled water. Disease incidence was recorded upto 45 days after which no increase

in disease incidence was observed and recorded in table 4.10.4. The detail of the method followed has been given in chapter III this manuscript.

Table 4.10.4 : Effect of seed soaking with diluted culture filtrate of A. niger (Isolate - 16) on induced registance against A. niger collar rot of groundnut.

Isolate	**Percentage of collar rotted plant**	
	Seed soaking with distilled water	Seed soaking with culture filtrate of isolate 16
2	31.33 (34.02) *	18.67 (25.58)
3	35 (36.27)	18.5 (25.47)
5	35 (36.27)	11.67 (19.95)
16	58.33 (49.2)	27.26 (31.46)
18	34 (35.67)	6.67 (14.94)
20	33.33 (35.24)	22.33 (28.2)
21	28 (31.95)	13 (21.13)
22	41.5 (40.11)	19.1 (25.91)
23	22 (27.97)	11 (19.37)
24	42.33 (40.57)	18.33 (25.33)

C D 5% 1. For isolate = 5.35

2. For treatment = 2.01

3. For interaction = 7.58

* The value in parenthesis is angular transformed value.

From table 4.10.4 it is observed, that the direct effect of isolate is significant. The direct effect of treatment is also significant i.e. seed soaking with dilulated culture filtrate of A. niger isolate 16 significantly reduced the collar rot incidence in case of all isolates. The interaction effect is also significant, i.e. the result show a significant interraction between isolate and treatment, indicating that the difference in isolate is significantly affected by the treatment and that the treatment effect was affected significantly with the isolates tested.

Results showed that seed soaking with dilulated culture filtrate of one isolate of A. niger induced resistance in groundnut plant against all isolates of A. niger tested.

4.11. Effect of seed soaking with growth regulators on disease susceptibility and growth characteristic and yield of groundnut.

To find out the effect of growth regulators on disease susceptibility and growth and yield of groundnut, seeds (CV. JL -24) were soaked in solutions of growth regulators at specific concentration e.g. Chitosan (1%, 0.3%, .1%), Cycocel (10^{-3}M, 10^{-4}M), I.A.A. (10^{-5}m), 2-4-D (10^{-6}M) and 2-4-5-T (10^{-6}M) for 12 hrs. before sowing in field, 7-day old culture of a virulent isolate of A. niger (isolate 16) grown in sand maize meal was mixed with double sterilized soil @ 1:1 to obtain the inoculum mixture. The field was

divided into four blocks of 8m x 2m size. Soaked seeds were sown in line (2m in length) at 5 cm apart and covered with inoculum mixture. Each line, placed 1m apart in each block represented each treatment. Disease incidence and growth characteristics and yield of plants was recorded in table 4.11

The detail method followed has been mentioned in Chapter III of this manuscript.

It is observed from the table 4.11 that seed soaking with different growth regulators at various concentrations could not reduce the incidence of seed rotting to significant extent except in case of chitosan at 0.3% conc where significant reduction over control was observed.

But all the treatments reduced the incidence of collar rotting significantly over control. Maximum reduction was observed in case of chitosan @ 0.1%. All these growth regulators appeared to be strong inducer of resistance in susceptible groundnut plant. Such induced resistance slowed down the progress of disease and reduced the expresssion of symptoms and disease index very significantly.

Table 4.11 Effect of seed soaking with growth regulators on disease susceptibility and growth characteristics and yield of groundnut

Treatment	Concen-tration	Seed rotting	Collar rotting	Growth Characteristics and yield					
				Fresh wt. (g)	Height (cm)	Root length (cm)	No. of leaflet	No. of nodules	Pod yield/ plant (g)
Control		32.5	25	201.8	40.2	27	357	605	21
I.A.A.	10-5 M	27.5	5	203.4	25	20.8	272	406	28.8
2-4-D	10-6 M	27.5	10	182	18.6	19.6	228	309	21
2-4-5-T	10-6 M	30	7.5	178.8	40.4	28.6	319	554	29.8
Cycocel	10-3 M	27.5	15	188.4	44	24	492	513	29.2
Cycocel	10-4 M	27.5	12.5	259.2	46	27.4	480	568	37.2
Chitosan	1.0%	30	5	240.8	34.6	32	330	886	29.2
Chitosan	0.3%	22.5	5	319.4	39	31.8	368	768	42.6
Chitosan	0.1%	25	2.5	174.8	39.4	25	327	527	25
Blank (No inoculation)			0	186.2	42.6	29.4	372	617	21.8
CD 5%		**9.17**	**8.41**	**25.16**	**4.6**	**4.15**	**62.59**	**22.21**	**5.36**

Treatment with some of the growth regulators affected the growth characteristics and yields of plants significantly. Seed Soaking with cycocel 10^{-4}M, Chitosan 1.0% and Chitosan 0.3% significantly increased the fresh weight of the plants over blank where no seed soaking and inoculation was done. Other treatments had no significant effect on fresh weight of the plants. Significant reduction in plant height was observed in treatment with I.A.A. (10^{-5}M), 2-4-D (10^{-6}M) and Chitosan (1.0%) than the blank. I.A.A. (10^{-5}M), 2-4-D (10^{-6}M), Cycocel (10^{-3}M) significantly reduced the no. of leaflet than the blank. Other treatments had no significant effect on no. of leaflets. All the treatments significantly reduced the no. of root nodules that the blank. Maximum reduction was observed in case of I.A.A. (10^{-5}M). Most of the treatments with growth regulators e.g. I.A.A. (10^{-5}M), 2-4-5-T (10^{-6}M), Cycocel (10^{-3} M), Cycocel (10^{-4}M), Chitosan (1.0% and 0.3%) significantly increased the yield/plant. Appreciably higher levels of yields were recorded with the treatment with Chitosan (0.1%) ,and Cycocel (10^{-4}M) where high level of induction of resistance were recorded.

CHAPTER - V

DISCUSSION

A. niger, the incitent of collar rot and seed rot of groundnut remain in soil seed and also is an airborne pathogen. It may be tolerent to fungicides, becomes virulent to different cultivars and genotypes (Chohan et al.1969, 1970, Chahal et al.1974, Dange and Srivastava 1985, Kirpal et al.1970, Mathur 1970, Pettit et al.1989). To acertain the pathogenecity of the isolates and disease reaction of different genotypes, a no. of genotypes or varieties were screened against two isolates of A. niger isolated from four agroclimatic zones of West Bengal out of which three varieties viz. EC 20998,C-421, C.No. 170 were found resistant against seed rot as well as collar rot against both isolate 16 and isolate 24 and had significantly less disease incidence as compared to other varieties. Varieties viz. 2848 and Faizpur 1-5, were found resistant against isolate 16 only and varieties 2809 and EC 133155 were found resistant against isolate 24 only both in respect of seed rotting and collar rotting. Some of the varieties, as mentioned earlier, showed resistance in respect of seed rotting only against single or both isolates. But they were found to be tolerant or susceptible to that isolates or both isolates in respect of collar rot incidence. Similarly, some of the varieties, which were found to be resistant in respect of collar rotting against single or both isolates, showed tolerance or susceptible reaction against that isolate or both isolates in

respect of seed rot incidence. This may be due to that in these varieties the gene for resistance to seed rot is different from the gene for resistance to collar rot. It is interesting to note that varieties viz. E 20998, C-421, C-No. 1780 found resistant to seed rot were also resistant to collar rot against both isolates and therefore resistance from the same source can be incorporated in the elite groundnut varieties simultaneously in breeding programme to develop high yielding and disease resistant varieties.

Seed rotting is more during the colder months and less during the warmer months of the year. On the other hand, collar rotting is more during warmer months and less during the colder months of the year. It may be due to temperature which plays an important role in growth and activity of the pathogen and also on the emergence and growth of the plant. During colder months seed germination and seedling emergence is delayed. Delay in emergence increased infection percentage of the disease (Ashworth etal. 1964; Chohan 1967, a, b, c). On the other hand growth and activity of A niger is more in warmer climate, which increased collar rot incidence. Chohan (1967) reported, at the temperature of 31 degree and 35 degree, the activity of both the host and pathogen was at its maximum. The quicker rate of germination of seed decreased seed rot, but it increased the collar rot attack because the pathogen remained active at the higher temperatures. Chohan and Kapoor (1967) stated that the crown rot disease

incidence of groundnut could not be controlled by a change in the date of showing because the mortality of seeds and seedlings caused by A niger was not materially affected.

Aspergillus niger continued to remain viable in soil even after one year of infestation though gradual reduction in infectivity of artificially infested soil was observed. Chohan (1967) analysed various soil factors influencing the survival of A niger, most important of them are relative humidity and presence of other organisms in soil.

Injury to the kernels and to the hypocotyl region has considerable bearing on the disease. So, possibility of disease is more where machine shelled kernels are used than carefully hand-shelled and hand-planted kernels. And extreme care should be taken during inter culture operations to avoid seedling injury to reduce disease incidence. Similar observations were also deserved by Ashworth et al. (1964), Morwood (1953), Gibson (1953) and Chohan (1969).

Moisture level of the soil played a significant role in seed rotting and collar rotting of groundnut caused by A niger, 25% moisture level reduced the seed rotting significantly with respect to 5% and 10% level. Again, 15% and 25% soil moisture level reduced the collar rotting significantly with respect to 5% and 10% moisture level. Both isolates of A. niger caused

significantly higher seed rotting and collar rotting with respect to control at all level of soil moisture. Maximum incidence of seed rotting and collar rotting were found at 10% and 5% moisture level respectively in case of both isolates. The results show a non-significant interaction between isolates tested and soil moisture level, indicating that the difference in isolate was not significantly affected by the soil moisture and the soil moisture effect did not significantly affected with the isolate tested. (Chohan 1965, 1967; Gibson 1953; Kang and Chohan 1966; Yi-Cheng - Lin 1982).

As regards enzyme production there is a close association between cell separation (maceration), cell death and electrolyte loss with PG enzyme activity maximum at pH 4.5) obtained from dialysed enzyme preparation of A. niger. There were large changes in permeability before protoplast death as assessed by inability to retain neutral red stain. Protoplast died well before separation of cell had completed. Similar findings have also been noted in in-vivo studies (Bisen 1978). Thatcher (1942). Lai et al. (1968) and Hall and Wood (1974) also reported similar findings.

The inhibition of cell death by the addition to macerating solution of certain solutes in plasmolysing concentrations, first demonstrated by Tribe (1955) and has been confirmed by Mount et al. (1970) and Hall and Wood (1974). They suggested that the cell death at higher plasmolysing concentration is retarded either

because plasmolysis by contracting surface membrane makes pectic substances less available to the enzyme or because in contracted state, the membrane delays movement of pectic enzymes to substrate within the cytoplasm. The present data confirms the above hypothesis.

Higher degree of disease incidence was recorded with isolate 24 and isolate 16 of A. niger with respect to other isolates. The invivo PG enzyme activity as well as amount of oxalic acid produced by those two isolates were somewhat higher than that of other isolates. A synergistic effect of oxalic acid and PG enzyme was observed in maceration of host tissue. Causal involvement of oxalic acid in pathogenesis of A niger on different hosts have been reported by different workers at different time. (Bateman and Beer 1965; Maxwell and Lumsden, 1970; Tanaka and Nonaka, 1977). It was observed that oxalic acid production by pathogens was a key factor in enabling this organism to attack living plants (Higgins 1927; Gibson, 1953, Kritzman, 1977) It may due to that oxalic acid enhanced the activity of PG enzyme by letting the susceptible tissues down to the optimum pH level for the enzyme (Bateman and Beer, 1965).

The control of soil borne plant pathogen by the treatment of the soil with Trichoderma sp. has been found by some worker to be effective (Hadar et al. 1979; Elad et al. 1982). T harzianum was found to inhibit the growth of the three pathogenic isolates of

A. niger (Lashin et al. 1989). In the current study also, T harzianum was found to inhibit the growth of two pathogenic isolates - isolate 16 (soil isolate) and isolate 24 (seed isolate) of A. niger by 63% and 58% respectively within 72 hours of inoculation in dual culture in Petridish.

Seed dressing with T. harzianum spore suspension, caused a considerable decrease in crown rot infection, at different levels of soil infestation with Aspergillus niger. Increased concentration of Trichoderma spores, decreased the crown rot percentage at different stages of growth.

The antagonistic effect of T. harzianum against A. niger could be distinguished from the reduction of crown rot infection on peanut plants grown in soil infested with both T. harzianum and A. niger. Similar results were obtained by Chohan (1968), Rai, Singh. Singh (1980), Lashin et al. (1989). Garren (1967) found T. viride to compete with the organism causing peanut pod rot. The soil and seed treatment with spores of Trichoderma sp were also found to be biologically effective against peanut pod rot incidence by Abo-Arkoub (1973) Abi ElAal (1973) , Hader et al. (1979) and Elad et al. (1982) and against peanut collar rot caused by F. oxysporum, R. salani and S. rolfsii by EL-sherif (1988).

Blitox 50 W.P (Cu-oxychloride 50%) was found not to reduce the disease incidence. In vitro also Blitox 50 was found not inhibit the growth and sporulation of A niger to any extent even at 1000 ppm. Roy (1980) found that A niger could solubilize copper compounds. Sundus and Raj (1988) reported that A. niger could tolerate cu-fungicides (Blitox-50) even at higher concentration.

Yield components were found to be increased due to treatment with Trichoderma and other fungicides except with Blitox 50. Similar stimulation of growth of plant was reported by Baker et al.(1984, 1988a), Baker & Cook (1988), Loper (1988), Salt (1978), Windham et al. (1986), Ahmed, Baker (1987a, 1988a), Baker (1989).

Growth stimulation by T. harzianum could be the result of production by the fungus of plant hormones, increased uptake of nutrients by the plant or the control of one or more subclinical pathogens.

Chohan (1968 and 1971) also observed that a Streptomyces sp. had highly antagonistic effect against A. niger in vitro. Espany et al.(1980) reported that Streptomyces sp. are antagonistic aginst Aspergillus sp.

The control of A. niger by T. harzianum may be due to mycoparasitism and\or antibiosis. During present study it has been found that hyperparasitism is characterised by frequent

coiling, penetration and hyphal growth of the parasite inside the conidiophores of Aspergillus niger CO_2 and Ethanol production by T. harzianum were considered responsible for growth and sprulation inhibition of A niger (Hutehingson and (Owan, 1972). Seed treatment with T. harzianum spores before storing has a significant role in reducing the crown rot incidence caused by A. niger.

When T. harzianum\Streptomyces sp. spore suspension was added into soil, the germination of sclerotia of A. niger was found to be reduced to a variable extent in various types of soil. When these sclerotia were observed under binocular, T. harzianum was found to colonise the sclerotia of A. niger. When the gernination of these sclerotia was tested, most of them failed to germinate and those who were gerninated were soon found to be colonised by T. harzianum. Streptomyces sp. was found to be predominant when bacterial isolation was done from germination inhibited scleratia of A. niger in case of treatment with Streptomyces sp.

Again, in case of both types of treatment i.e. with T. harzianum and with Streptomyces sp. it was observed that inhibition of germination of sclerotia was more in normal soil than in strilized soil. This may be due to the presence of other inhibitory or antagonistic organisms in normal soil. In normal course, with the passing of time sclerotia lose their germinability gradually. Ayers and Adam (1981) reported

mycoparasites softened and disintrigrated sclerotia of the host. Though Chet and Baker (1980) observed increased effectiveness of Trichoderma sp. in acid rather than alkaline soil, in present experiment better colonization of sclerotia of A. niger by T. harzianum was observed in neutral soil.

Salts of different heavy metals can induce resistance in susceptible groudnut plant (CV. JL-24) against collar rot disease caused by A. niger and most of these salts are known as effective phytoalexin inducers in plants. (Olsen, 1938; Byrde et al. 1953; Bouchereau and Atkin, 1950; Kent, 1941; Beckman, 1958; Gigante, 1935; Heggenen, 1942; Rodigin Krasnova, 1959; Carorascso et al. 1978; Kins and Pozsar, 1977; Covey, 1977; Balazas et al., 1978; Oguni et al., 1976; Keil et al. 1958; Carter and Wain 1964; Giri and Sinha, 1983 a,b; Sinha and Hait, 1982; Sinha and Giri, 1979;)

The reduction in mortality in some of these treatments seems to be of great scientific interrest and considerable practical significance.

It apears that wet-seed treatment with these salts, not normally used for plant protection and also have no or little fungitoxicity at the concentrations employed, can considerably limit the collar rot disease development in groundnut. There is little direct relationship between the concentrations of these chemicals and their protective effects. These observations

coupled with the fact that some test compounds show a fairly strong effect at rather dilute concentrations imply that in controlling disease these chemicals may have been based on the activation of its latent defence potential that normally remains suppressed in a compatible host pathogen interaction, this would mean induction of resistance. Nitrate, sulphate and chloride salts of some heavy metal, all known as phytoalxin inducers, when intially tested at 10^{-2} M to 10^{-6} M for seed soaking in glass house experiment, seedlings in most the treatments, developed resistance and showed reduced symptoms of collar rot as compared to untreated (Control) plants. Significant reduction in collar rotting varing from 29.7% to 79.9% over control was observed in case of the following chemicals at specific concentration $Ba(No_3)_2$, $ZnSO_4$, $ZnCl_2$, $HgCl_2$ in all the three tests concentrations (i.e 10^{-2}M to 10^{-4}M and $CuSO_4$ (10^{-3} M and 10^{-4} M), $CuCl_2$ (10^{-3}M and 10^{-4} M) and $Fecl_3$ (10^{-2} M and 10^{-3} M) concentrations.

With most of the chemicals tested, significant differences existed between the effects of three different concentrations. $Ba(No_3)_2$ $CuSO_4$, and $ZnCl_2$ appeared to induce strogest inhibitory effect at lowest concentration tested, while $ZnSO_4$, $MnSO_4$, $FeCl_3$ and $CuCl_2$ at intermediate concentration and BaSO4 and $HgCl_2$ at their highest concentretion. This provides another evidence against the causal involvement of the direct toxic effect of test chemicals, if any, in their protective effect against collar rot disease. During further studies with these effective treatment

under field condition, stroger effects were recorded with $HgCl_2(10^{-4}M)$, $Ba(NO_3)_2$ $(10^{-4}M)$ and $ZnSO_4$ $(10^{-4}M)$.

Analysing the results it was assumed that induction of resistance most possibly based on strengthening of host's innate defence through some induced changes in host metabolism. Studies to elucidate this point provided interesting information Significant reduction in PG enzyme activity were recorded in plant of effective seed treatment as compared to that in untreated plants and a positive correlation was found between reduced PG enzyme activity and reduced disease index. Greater reduction in collar rotting was recorded in treatment with $Ba(No_3)_2$, $(10^{-4}M)$, $ZnSo_4$ $(10^{-3}M)$, $BaSo_4$ $(10^{-2}M)$ and $HgCl_2$ $(10^{-4}M)$ varing from 53.5% to 63.5% as compared to control. Siminarly, percentage reductions of PG enzyme activity were also higher in these treatments varing from 63.43% to 72.7% as compared to control.

Decreased PG enzyme activity may be due to (a) metabolic changes in host issue or (b) suppression of fungal growth and consequent fall in enzyme production. Increased accumulation of phenol oxidation products may cause inhibition of PG enzyme activity.

To elucidate this point, an estimation of total phenol was made using tissue from both healthy plants and that from the affected collar region of both treatd and untreated plants. The level at endogenous phenol in healthy plants in $Ba(NO_3)_2$, $(10^{-4}M$, $FeCl_3$

(10^{-4}M), $MnSO_4$ (10^{-3}M) and $BaSO_4$ (10^{-2} M treatments were found to be either equal to or even slightly less than that of the control plants. In other treatments significant increase in phenol content over control was observed. Plant in all treatments along with the control (water) responded to inoculation by producing significantly greater amount of total phenol. In plants in control this increase was small or marginal. It seems that treated plants rapidly accumulated phenolic substances at the infection site withing a very short period, after being challenged by the pathogen. Such significant alteration in the host responses may have resulted from a conditioning effect of seed treatment on the susceptible cultivar. The increased accumulation of phenolics, for which evidence is available, and possible accumulation of phenol oxidation products like quinone, which could not be determined, might have been contributory factors leading to arrestation of fungal growth and suppression of PG activity - which results in drop in mortality.

Seed treatment with these heavy metal is expected to bring about some significant biochemical changes in the susceptible cultivar. Many of the treatments with heavy metal salts substantially reduced collar rot incidence in groundnut plant. One contributing factor may have been limitation of lesson size for which evidence are available. Analysis results on peroxidase activity show all the treatment responded to peroxidase activity as compared to control (water) significantly by 103.8% to 222.94%

highering of activity. Enhanced peroxidase activity is causally involved with lignin biosynthesis at its terminal phase. Enhanced peroxidase activity, associated with increased synthesis and deposition of lignin at the site of infection constitutes one of the important characteristic of incompatible host pathogen interaction and has been for some case induced resistance also (Vance et al. 1980).

Since some of the compounds tested in seed treatment substantially reduced disease development in groundnut plants, it was of interest to determine whether these affected various yield attributes. From results of analysis of growth attributes upto 75 DAS and yield attributes, it was evident that most of the successful treatments, significantly affected various growth and yield attributes of groundnut plant in different ways which ultimately resulted in significant increases in yield/plant.

Among the all fungicides tested Bavistin 50WP and Emisan 6 were found to be most promising in inhibiting the growth of various isolates of A. niger, Kumar and Khare(1970), Aulakh and Sunar(1970), Mathur & Sharma (1977) Gour and Ahmed (1983), Siddaramaiah et al. (1979), Shekhawat et al. (1986), Saha & Raj (1989), Chattopadyay and Raj (1991) reproted similar observations. Indofil M-45 showed an intermediate effect(Wangikar and Kodmelwar 1977). But Blitox 50 WP and Kabach 75 WP were found not to be efficient in inhibiting the growth of any isolate of

A. niger to any extent. Similar observation were also recorded by Roy (1980), Sundus and Raj (1989), Murphy and Levy (1989).

Though Indofil M-45 failed to inhibit the growth of the fungus completely but it adversely affected the sporulation and growth habits of the fungs. With the increase in concentration it remarkably reduced the sporulation and delayed the maturity of the conidia.

Many reports are available now to demonstrate that resistance can be effectively induced in many plants against their particular pathogen by previous inoculation with their avirulent races (Beauverie, 1901; Ray 1901) and with both related or unrelated agents (Matta 1980, Sinha 1976, 1984, Kuc 1982, 1983; Goodman, 1980; Hamilton, 1980). In present investigation seed soaking with culture filtrate drastically reduced the seed germination and diluted culture filtrate played an important role in inducing resistance in groundnut plant. Seed soaking with diluted culture filtrate of A. niger not only induced resistance in groundnut against the respective isolate of A. niger but a general resistance was induced against all isolates of A. niger tested. This indicates that seed soaking with diluted culture filtrate of A. niger brings about some changes in host physiology which leads to the production of some principles in the host which play an important role in induction resistance in groundnut plant against A. niger. Different isolates of A. niger differs in their

sensitivity towards the principles, produced in groundnut in response to seed soaking, so groundnut plant showed different degree of resistance against different isolates of A. niger tested.

Gowth regulators of diverse origin play an improtant role in induction of resistance in various plants (Sinha and Giri 1979; Sinha and Hait 1982; Akai 1955; Davis 1952; Bryd et al. 1953; Fawcett et al. 1955, 1957; Smalley 1962 Beckman, 1958; Sinha & Sengupta1986; Manibhusan Rao et al. 1987; Sumbali and Mehrotra, 1981; Hale et al . 1981; Soleman et al. 1988; Datrne; Lee 1990) In present studies, in most treatments with growth regulators no significant reducation in seed rotting was observed except with chitson at 0.3% but all the treatments with growth regulators, reduced the incidence of collar rotting significantly over control.

The above observations make one thing clear that, since there is no correlation between in vitro fungitoxicity of the test chemicals and the protection provided by them to groundnut plants over an extended period and quite often better protection is achieved with a lower concentration, it can be suggested that these chemical act as sensitizer and activate the dynamic defence potential of the host which normally remains suppressed in compatible host-pathogen interactions. Acting through induced changes in the metabolism of the susceptible host, these

chemicals appear to modify the host-pararite interactions in such a way as to result in an inhibition of symptom development and an expression of host resistance.

CHAPTER-VI

SUMMARY AND CONCLUSION

Investigation on various aspects of crown rot disease of groundnut caused by A. niger van Tieghem and its management was undertaken in the experiment. Given below is a summary of the experiments undertaken and observation recorded :

6.1. Varietal reaction to A. niger :

One hundred thirty eight varieties or germplasms of groundnut were screened against two isolates of A. niger (Isolate 16 and 24) in green house under artificial inoculation condition according to the method described by Dange et al. (1985) with some modification to find out the resistant sources which may be used in breeding programme to develop high yielding disease resistant cultivars.

In artificially inoculated soil surface sterilised seeds were sown. Rotted seeds were taken out regularly. Ten day old seedlings were inoculated at the basal region with respective isolate of A. niger. Disease scoring was done with the scale described by Chahal et al. (1974).

Among one hundred thirty eight germplasms or cultivars of groundnut, three showed immune reaction to isolate - 16 of A. niger, 29 cultivars were tolerent, 52 were moderately susceptible and 54 showed highly susceptible reaction. While in case of isolate - 24 of A. niger among 138 germplasms or cultivars, 32 showed tolerent reaction, 61 were moderately susceptible and the rest (45) were highly susceptible. None of the germplasm/cultivars showed imnune reaction to isolate 24 of A. niger.

Faizpur 1-5, C-No 1780 and Go- 053 were immune to isolate -16 of A. niger. In respect to both seed rot and collar rot variety 2848, Faizpur 1-5 showed resistant reaction to isolate-16 while variety 2809 and EC 133155 showed resistant reaction to isolate 24 and variety EC 20998, C-421 and C-No. 1780 were resistant to both isolates of A. niger.

Analysing the varietal reaction to both isolates of A. niger it can be concluded that the gene for resistance to seed rot may be different from the gene for resistance to collar rot. It is interesting to note that varieties viz EC 20998, C-421 and C-No.-1780 found resistant to both isolates in respect to both seed rot and collar rot and therefore resistance from same source can be incorporated in the elite groundnut varieties simultaneously in breeding programme to develop high yielding and disease resistant varieties.

6.2. **Relative incidence of crown rot disease of groundnut in different seasons**

To study the relative incidence of crown rot disease in different seasons, ten isolates of A. niger, isolated from soils of four agroclimatic zones of West Bengal (Isolate 2,3,5, 16, 18, 20, 21, 22, 23 and from infected seeds of groundnut (Isolate-24), were used against CV.-JL-24 of groundnut. Seeds were sown in inoculated soil with respective isolates in three different seasons (Jan-Feb., May-June & Oct-Nov.) under glasshouse condition. In field disease incidence against two virulent isolates of A. niger (e.s. isolate 16 & 24) was studied in three different seasons in two consecutive years.

It was observed that seed rotting was maximum during Jan.-Feb., intermediate during October-November and minimum during May-June in case of all isolates of A. niger both in glass house and field condition. Where as collar rotting was recorded maximum during May-June, intermediate during October-November and minimum during January-February in case of all isolates both in glass house and field condition. More or less similar results were obtained during both year of observation in field condition.

It may be due to temperature which plays an important role in growth and activity of the Pathogen and also on the emergence and

growth of the plant. During colder months seeds germination and seedling emergence is delayed. Delay in emergence increased infection percentage of the disease (Ashworth et al. 1964, Chohan 1967, a,b,c). On the other hand growth and activity of A. niger is more in warmer climate. The quicker emergence of seedling decreased seed rotting, but it increased the collar rot attack because the pathogen remained active at the higher temperature. So, it is rather difficult to control the disease through change in date of sowing.

6.3. **Relative Pathogenicity of different isolates of A. niger and Persistance of infectivity of sick soil** :

Groundnut cultivars JL-24 was screened against 10 isolates of A. niger (e.g. Isolate 2,3,5, 16,18,20,21, 22, 23 and 24) under artificial inculation in glass house condition to assess their relative pathogenicity. And persistance of infectivity of sick soil was assessed by sowing of surface sterilized seeds of groundnut (CV. JL-24) during May-June, 1990, October-November, 1990, February-March 1991 and May-June, 1991.

According to virulence the isolates were arranged 24> 16> 3> 18> 5> 22> 2> 23> 21> 20. It was observed that A. niger continued to remain viable in soil even after one year of artificial inoculation though gradual reduction in infectivity of sick soil was observed in all isolates A. niger. Among many other soil

factors probably relative humidity and presence of other organisms play an important role influencing the survival of A. niger.

6.4. **Effect of physical injury to seed and seedling on disease incidence.**

Surface sterilized groundnut seeds (CV.JL-24) were injured by needle scratching on the cotyledon and were sown in artificially inoculated soil kept in earthen pot under glass house condition. 7 day old seedlings developed from uninjured seed in autoclaved soil were injured at the hypocotyl region by needle scratching and inoculated by placing 0.5cm squares of conidial and mycelial mat on the injured hypocotyle. The experiment was conducted using isolate 16 and isolate 24 of A. niger separately.

It was observed that injury to the kernels and to the hypocotyl region of seedling has a considerable bearing on the disease. So, possibility of disease incidence is more where machine shelled kernels are used than carefully hand-shelled and hand-planted kernels. And extreme care should be taken during interculture operations to avoid seedling injury to reduce disease incidence.

6.5 **Effect of soil moisture on crown rot disease incidence of groundnut**.

Surface sterilized groundnut seeds (CV.JL-24) were sown in artificially inoculated soil, kept in earthen pot, inner side of which was covered with plastic sheet to avoid seepage of water. Measured quantity of water was given to each pot daily to maintain 5%, 10% and 25% moisture level by weight of the soil under glass house condition.

Moisture level of soil was found to play an important role in seed rotting and collar rotting of groundnut caused by A. niger. It was observed 25% moisture level reduced seed rotting significantly with respect to 5% and 10% level. Again, 15% and 25% soil moisture level reduced the collar rotting significantly with respect to 5% and 10% moisture level. Maximum incidence of seed rotting and collar rotting were recorded at 10% and 5% moisture level respectively in case of both isolates of A. niger used in the experiment.

6.6 **Production of hydrolytic enzymes by various isolates of A. niger (Both in vivo and in vitro)**.

(a) Invivo and in vitro production of PG and Cx enzymes by various isolates of A. niger.

Various isolates of A. niger (Isolates 2,3, 5, 16, 18, 20, 21, 22, 23 and 24) were maintained in PDA slants and were used for in vitro production of PG and Cx enzymes. For invitro production of enzymes Ammonium Osxalate medium was used with 1% pectin (Gupta and Gupta, 1967). For estimation of in-vivo enzyme production, 7-day old groundnut plants (CV.JL-24), grown in autoclaved sandy soil in earthen pots, were inoculated with isolate 16 and isolate 24 of A. niger separately at the collar region. Crude enzyme sample was prepared from infected tissue. Assay of PG enzyme was done with 1% sodium polypectate solution and for cellulase 1% CMC was used. Enzyme activity was estimated viscosimetrically.

It was observed that all isolates of A. niger tested, produced PG and Cx enzymes both in-vitro and in vivo condition to various extents. Their activity in in-vivo condition is lower than those displayed in in-vitro condition which indicated the complexicity and diversity of the factors operating in in-vivo condition. From this study it can be concluded that both pectolytic and cellulolytic enzyme are involved in pathogenesis and also that the Cx enzyme took active part in the breakdown of cellulose at the infected tissues and enhanced the effect of the pectolytic enzymes resulting in rapid decay.

(b) Cell death and cell disintegration and changes in permeability by hydrolytic enzymes produced by various isolates of A. niger.

Toxicity of the enzyme preparations of 10 isolates of A. niger was determined by plasmolytic method following Tribe (1955), based on the principle that only live protoplasts retain neutral red in plasmolysing solution. Maceration was assessed by the needle test. And permeability changes were measured by assessing increase in conductivity of solutions as electrolytes leaked out into them from tuber disks and was measured by using Systonics Direct Reading Conducitivity meter 303.

It was observed that there was a close association between cell separation (maceration), cell death and electrolyte loss with PG activity obtained from dialysed enzyme preparation of various isolates of A. niger. The speed of cell death and maceration was somewhat higher in more virulent isolates of A. niger (isolate 16 and isolate 24).

There were large changes in permeability before protoplast death as assessed by inability to retain neutral red stain. Protoplast died well before separation of cell had completed. The inhibition of cell death by the addition to maceration solutions of certain solutes in Plasmolysing

concentrations suggested that cell death of higher plasmolysing concentration is retarded either because plasmolysis by contracting surface membrane makes pectic substances less available to the enzyme or because in contracted state, the membrane delays movement of pectic enzymes to substrate within the cytoplasm.

6.7. **Role of Oxalic Acid on the collar rot disease incidence in groundnut caused by A. niger.**

To find out the role of Oxalic Acid in disease development the in-vivo production of Oxalic Acid by various virulent isolates of A. niger was estimated from crude enzyme preparations of various isolates of A. niger, collected from infected tissue of collar rotted plant, through titration with 0.02N Potassium Permanganate.

Higher degree of disease incidence was recorded with isolate 24 and isolate 16 of A. niger with respect to other isolates. The invivo PG enzyme activity as well as amount of Oxalic Acid produced by those two isolates were somewhat higher than that of other isolates. A synergistic effect of oxalic acid and PG enzyme was observed in maceration of host tissue.Causal involvement of Oxalic Acid in pathogenesis of A. niger on different host have been reported by different worker at different times. It was observed that Oxalic Acid production by Pathogens was a key factor in enabling the organism to attack

living plant. It may be due to that Oxalic Acid enhanced the activity of PG-enzyme by letting the susceptible tissues down to the optimum pH values for the enzyme.

6.8. **Biocontrol of A. niger and crown rot disease of groundnut.**

The control of soil borne plant pathogen by soil treatment with Trichoderma sp. has been found to be effective by some workers (Hadar et al. 1979 Elad et al. 1982). T. harzianum was found to inhibit the growth of three pathogenic isolates of A. niger (Lashin et al 1989). In the current study also T. harzianum was found to inhibit the growth of two pathogenic isolates e.g. isolate-16 (Soil isolate) and isolate-24 (Seed isolate) of A. niger by 63% and 58% respectively within 72 hours of inoculation in dual culture in Petridish.

Seed dressing with T. harzianum spore suspension at different concentration e.g. 0, 25, 100 and 250 x 10^{-6} spores/ml @ 20 ml/20g seeds, caused a considerable decrease in crown rot infection, at different levels of soil infestation with A niger. Increased concentration of Trichoderma spores decreased the crown rot percentage at different stages of growth.

The antagonistic effect of T. harzianum against A. niger could be distinguished from the reduction of crown rot infection on peanut plants grown in soil infested with both T. harzianum and

A. niger. Seed inoculation with T. harzianum and Streptomyces sp. separately was very efficient in reducing the seed rotting and pre emergence damping off of plants caused by A. niger.

To find out the efficacy of biocontrol agents in comparison to other seed treating chemicals a field experiment was conducted to control crown rot disease of groundnut. Seed treatment with Blitox 50 WP (Cu-oxychloride 50%) was found not to reduce the disease incidence to any considerable extent. In vitro also Blitox 50 WP was found not to inhibit the growth and sporulation of A. niger to any extent even at 1000 ppm. It may be due to solubilization of copper compounds by A niger (Roy, 1980; Sundus and Raj, 1988).

In all these treatment except in seed treatment with Blitox 50wp, yield components like number and weight of pod and seed per pod were found to be increased. Growth stimulation by T. harzianum could be the result of production by the fungus of plant hormones, increased uptake of nutrients by the plants or the control of one or more subclinical pathogens.

The control of A. niger by T. harzianum may be due to mycoparasitism and/or antibiosis. During present study it has been found that hyperparsitism is characterised by frequent coiling, penetration and hyphal growth of the parasite inside the conidiophores of A. niger CO_2 and Ethanol production by T.

harzianum were considered responsible for growth and sporulation inhibition of A. niger (Hutchingson and Cowan, 1972).

Seed treatment with T. harzianum spore suspension before storing was also found to have a significant role in reducing the crown rot disease incidence.

When spore suspension of T. harzianum and Streptomyces sp. was added to soil separately, the sclerotial germination of A. niger was found to be reduced to a variable extent in various types of soil. When these sclerotia were observed under binocular T. harzianum was found to colonise the sclerotia of A. niger. When germination of these sclerotia was tested, most of them failed to germinate and those who were germinated were soon found to be colonised by T. harzianum. Streptomyces sp. was found to be predominant when bacterial isolation was done from germination intibited sclerotia of A. niger in case of treatment with Streptomyces sp.

6.9. **Effect of seed treatment with various heavy metal salts on disease incidence** :

(a) **Fungitoxicity assay of heavy metal salts**

All these salts of heavymetal (e.g. Ba, Zn, Cu, Mn, Fe, Hg.) were initially screened at a range of three dilutions

for their fungi toxic effect if any, on sclerotial germination of the pathogen.

Both the pattern and percentage of sclerotial germination make it clear that none of the salt exhibited any discernible fungitoxic effect on the sclerotial germination at the dilutions screened, rather most of the test chemicals stimulated germination of sclerotia at their test dilutions.

(b) **Effect on disease incidence** :

Initially seeds of the susceptible groundnut cultivar were treated with 9 selected chemicals at three different dilutions. All these chemicals appeared to induce low to very high level of resistance in the susceptible groundnut plants as evident from disease index in glass house condition. Though there was a considerable reduction in percentage of seed rotting or pre-emergence damping off in the treatment over control, none of the test chemical at any dilution significantly reduced seed rotting or pre-emergence damping off over control. But a significant reduction in collar rotting varing from 29.7. to 79.9% over control was observed.

Some of the chemicals, which were found to have better resistance inducing potential in glass house condition, at a particular dilution, were screened and used for field experiment. Seeds were soaked in water for 24 hrs before soaking and subsequently inoculated with virulent isolate of A. niger (isolate-16) at an age of 10 days. A significant reduction by 30.3% to 63.6% in collar rotting was observed in all the treatments over control. Stronger effects were recorded with Mercuric chloride, Barium nitrate and Zinc sulphate, all at 10^{-4} M dilution.

There is little direct relationship between the concentrations of these chemicals and their protective effects.These observations coupled with the fact that some test compounds show a fairly strong effect at rather dilute concentrations imply that in controlling disease, these chemicals may have acted by conditioning susceptible groundnut plants for a more vigorous and dynamic defence response to the Pathogen.

Such conditioning of the host may have been based on the activation of its latent defense potential that normally remains suppressed in a compatible host pathogen interaction, this would mean induction of resistance.

(C) **Changes in host metabolism** :

A comparative estimation of biochemical changes in treated and untreated plants, before and after inoculation was carried out. Plants in all successful treatments appreciably suppressed the polygalacturonase activity and considerably higher level of phenol content after inoculation as compared to their control.

Decreased PG enzyme activity may be due to (a) metabolic changes in host tissue or (b) suppression of fungal growth and consequent fall in enzyme production. Increased accumulation of phenol oxidation products may cause inhibition of PG enzyme activity.

Regarding biochemical estimation, the peroxidase activities of both treated and untreated plants were measured, and a significant enhancement in peroxidase activities were recorded with all treatments.

Enhanced peroxidase activity is causally involved with lignin biosynthesis at its terminal phase. Enhanced peroxidase activity, associated with increased synthesis and deposition of lignin at the site of infection

constitutes one of the important characteristic of incompatible host pathogen interaction and has been for some cases induced resistance also.

(d) **Effect on growth characteristics and yield of plant**

Analysing the growth attributes upto 75 DAS and yield attributes, it was evident most of the successful treatments, possitively influenced the crop yield in various ways.

6.10 **Assay of various fungicides on growth of various isolates of A. niger.**

Among 5 fungicides tested Bavistin 50 wp and Emisan 6 were found to be most promising in inhibiting the growth of various isolates of A. niger in in-vitro condition. Indofil M-45 showed an intermediate effect. But Blitox 50 wp and kabach 75 wp were found not to be efficient in inhibiting the growth of any isolate of A. niger to any extent.

6.11. **Effect of culture filtrate of A. niger on groundnut**

Seed soaking with culture filtrate drastically reduced the seed germinability and diluted culture filtrate caused wilting and death of plant, but seed soaking with diluted culture filtrate played an important role in inducing resistance in groundnut

plant. Seed soaking with diluted culture filtrate of one isolate of A. niger only induced resistance in groundnut against the respective isolate of A. niger but a general resistance was induced against all isolates of A. niger tested. This indicates that seed soaking with diluted culture filtrate of A. niger brings about some changes in host physiology which leads to the production of some principles in the host which play an important role in induction resistance in groundnut plant against A. niger. Different isolates of A. niger differs in their sensitivity towards the principles, so groundnut plant showed different degree of resistance against different isolates of A. niger tested.

6.12. **Role of growth regulators in inducing resistance in groundnut plant against A. niger**

Seed (CV. JL-24) were soaked with 5 growth regulators separately e.g. chitosan (1%, 0.3%, 0.1%) cycocel (10^{-3}M, 10^{-4}M), IAA (10^{-5}M), 2-4-D (10^{-6} M) and 2-4-5-T (10^{-6}) for 12 hours before sowing in field. Seeds were sown in line and covered with soil-inoculum (1:1) mixture of A. niger (isolate-16).

In most treatments with growth regulators no significant reduction in seed rotting was observed. But a significant reduction in collar rot incidence was observed in all treatments over control.

Since there is no correlation between invitro fungitoxicity of the test chemicals and the protection provided by them to groundnut plant over an extended period and quite often better protection is achieved with a lower concentration, it can be suggested that these chemical act as 'sensitizer' and activate the dynamic defence potential of the host which normally remains suppressed in compatible host-pathogen interactions. Acting through induced changes in the metabolism of the susceptible host, these chemicals appear to modify the host-parasite interactions in such a way as to result in an inhibition of symptom development and an expression of host resistance.

CHAPTER - VII

FUTURE SCOPE OF RESEARCH

In the present study, investigation on various aspects of crown rot disease of groundnut caused by A. niger van Tieghem and its management was undertaken. Further research on following aspects will be helpful for effective management of the disease.

It is interesting to note that varieties viz. EC 20998, C-421, C-No-1780 found resistant to this disease. So, resistance from same source can be incorporated in the elite groundnut varieties simultaneously in breeding programme to develop high yielding and disease resistant varieties.

Promising results on biocontrol of crown rot disease of groundnut with T. harzianum and Streptomyces sp. have been obtained in the present investigation. But still further advancement is needed for development of a stable, cost effective and easy - to - apply biocontrol formulations for control of plant pathogens with introduced antagonists.

Some salts of heavy metals were found to induce resistance in groundnut plants effectively against crown rot disease. In the

context of hazardous consequences of continued and extensive use of expensive fungicides, the possibility of disease control through strengthening of host resistance by treatments with such cheap, simple chemicals with little or no toxicity, at least at the dose used, merits further exploration, particularly with respect to a tropical case crop like groundnut. This approach has been gaining ground in recent years. Further attempt to induce resistance in plants or to stimulate their natural defense mechanism by different treatments may be a worthwhile proposition and may provide a safer measure for plant disease control in the long run.

CHAPTER · VIII

BIBLIOGRAPHY

Abd EL-Aal, H.R. (1973) Seedling and pod-rot disease of groundnut in A.R.E. and its control. Ph. D. Thesis, Fac. of Agric., Al-Azhar Univ., Cairo, Egypt, P.90.

Abo- Arkoub, M.U.(1973) Seedling damping-off and pod-rot disease of cultivated peanut in EL-Tahrir province and its control. M.Sc. Thesis, Fac. of Agric., Cairo Univ., Egypt, P-55.

Agarwal, R.D. and Gupta, M.N. (1986) Studies on in-vivo enzyme production by Aspergillus niger and Rhizoctonia solani. Indian J. Mycol. Pl. Path., 16 (2): 224-225.

Agnihotri, J.P and Sharma, M.P. (1972) Efficacy of fungicides in controlling collar rot of groundnut (Arachis hypogaea L.) Acta Agronomica Academiae Scientiarum Hungaricae 21 (1/2): 222-225.

Ahmed, J.S. and Baker, R. (1987a) Rhizosphere competence of Trichoderma harzianum. Phytopath., 77 : 182-89.

Ahmed, J.S. and Baker, R.(1988a) Implications of rhizosphere - competence of Trichoderma harzianum. Canadian J. Microbiology, 34 : 229-34.

Akai, S. (1955) Chemotheraputic application of some compounds to rice plants and outbreak of Helminthosporium leaf spot. Univ. Kyto. Forsch. Pflakrankh. 5: 45.

Ashworth, L.J. Jr., Langley, B.C., Mian, M.A.W. and Wrenn, C.J., (1964) Epidemiology of a seedling disease of spanish peanut caused by Aspergillus niger. Phytopathology, 54: 1161-66.

Aulakh, K.S. and Chohan, J.S. (1974) Effect of seed treatment with fungicides during storage of groundnut in relation to collar rot incidence. Indian Phytopath., 27 (3) : 371-374.

Aulakh, K.S. and Sunar, M.S. (1970) Effect of seed treatment during storage of groundnut in relation to collar rot incidence. Proceedings of the 57th Indian Science Congress, Part III, P.P.624

Balazas, E., Sziraki, T. and Kiraly, Z. (1978) : Physiological Pl. Path., 11 : 29.

Barnett, H.L. and Binder, F.L. (1973) The fungal host parasite relationship. Ann. Rev. Phytopath., II : 273-92.

Basham, H.G. and Bateman, D.F. (1975) Relationship of cell death in plant tissue treated with a homogeneous endopectate lyase to cell wall degradation. Physiological pl. Path., 5 : 249-262.

Bateman, D.F. and Millar, R.I. (1966) Pectic enzyme in tissue degradation. Ann. Rev. Phytopath., 4 : 119-146.

Beauverie, M.M. (1901). Essais de L'immunisation des vegetaux centre les maladies cryptogamiques. Comptes Rendus Heldomadaires des Seances de L. Academic des Sciences 133;107-110 (cited by A. matta, Ann. Rev. Phytopath., 9 : 387-410, 1971)

Bryde, R.J.W., Crowdy, S.H. and Woodcock, D. (1953). Studies on systemic fungicide III. The activity of certain chlorine substituted - napthols and napthoxy-n-aliphatic carboxylic acid as systemic fungicide. Ann. appl. Biol. 40 : 152-165.

Bell, T.A., Ethells, J.L. and Jones, I.D. (1955). A method for testing cucumber salt stock brine for softening activity. U.S. Dept. Agric. Res. Serv. (Rep.) : 72-75.

Bhatia, D.S. and Chohan, J.S. (1970) Production of polygalactu - ronase by A. niger in-vitro and in-vivo. Indian J. Microbiology, 10 (1): 21-22.

Bisen, P.S. (1972) Studies on host parasite interactions in storage rot of apples. Ph.D. Thesis, Jabalpur Univ.

Bisen, P.S. (1978) Role of extracellular enzymes in soft rot of apple fruits caused by Aspergillus niger var. Teighem. Proc. Physiol. Pararitism (U.G.C. sponsored), Today Tommorrow, New Delhi.

Boosalis, M.G. and Mankau, R. (1965) Parasitism and predation of soil micro organisms. In Baker, K.F. and Snyder, W.C. (eds), Ecology of soil borne plant pathogens. Prelude to Biological Control, University of California press, Berkeley, California, pp. 374-391.

Bouchereau, P. and Atkins, J.G. Jr. (1950) Studies on the action of copper seed treatment for water planted rice. Phytopathology, 40 : 3.

Bray, H.G. and Jhorpe, W.V. (1954) Analysis of phenolic compounds of interest in metabolism. Methods Biochem. Anal., 1 :27-52.

Bryan, P.W. and Mc Gowan, J.c. (1945) Viridin : a highly fungistatic substance produced by Trichoderma Viride. Nature, 151 :144-45.

Bryde, R.J.W., Crowdy, S.H. and Woodcock, D. (1953) Studies on systemic fungicide III. The activity of certain chlorine substituted - napthols and napthoxy-n-aliphatic carboxylic acid as systemic fungicide. Ann. appl. Bio., 40: 152-165.

Burkowiez, A. and Goodman, R.N. (1967) The effect of the virulent and avirulent strains of Erwinia amylovora on the permeability of apple and pear tissue. Trans. Missouri Acad. Sci., 2 : 75-80.

Byrde, R.J.W., Fieldig, A.H., and Archer, S.A. (1973) The role of extracellular enzymes in the rotting of fruit tissue by Sclerotinia fructigena. In R.J.W. Byrde and C.V. Cutting (Eds.). Fungal pathogenicity and plant response : 39-62. Acad. Press, New York, London.

Carorasco, A., Boudet, A.M. and Mariago, G. (1978) Enhanced resistance to tomato plant to Fusarium by controlled stimulation of their natural phenolic production.

Physiol. Pl. Path., 12 : 225-232.

Carter, G.A. and Wain, R.L. (1964) Investigation on fungicides. IX. The fungitoxicity, phytotoxicity and systemic fungicidal activity of some inorganic salts. Ann. appl. Biol., 53 : 291-309.

Chang, Y.C., Chang, Y.C., Baker, R., Kleifeld, O. and Chet, I. (1986) Increased growth of plants in the presence of the biological control agent Trichoderma harzianum. Plant Disease, 70 : 145-48.

Chahal, A.S., Dutta, S., Chohan, J.S. (1974) Varietal resistance in groundnut (Arachis hypogaea L.) to collar rot (Aspergillus niger) in Punjab state. J. Res., Punjab Agril. Univ., 11(2) : 200-203.

Chet, I. and Baker, R.(1980) Induction of suppressiveness to Rhizoctonia solani in soil. Phytopath., 70 : 994-88.

Chet, I. and Baker R. (1981) Isolation and Biocontrol potential of Trichoderma hamatum from soil naturally suppressive to Rhizoctonia solani, Phytopath., 71 : 286-90.

Chet, I., Elad. A., Kalfon, Y., Hadar Y. and Katan, J. (1982) Integrated control of soil borne and bulb borne pathogens in Iris. Phytoparasitica, 10: 229-36.

Chi, C.C. (1960) Effects of Streptomyces and Trichoderma on Fusarium. Phytopath., 50: 631.

Chohan, J.S. (1965) Collar rot of groundnut (Arachis hypogaea L) caused by Asperigillus niger var. Tei ghem and A. pulvarulentus (Mc Alpine). Jhom. in Punjab J. Res., Punjab Agril. Univ., 2(1): 25-33.

Chohan,J.S., Rai, I.S.and Kapoor, S.P. (1966) Comparison of organo-nonmercuial and organo mercurial fungicides for the control of collar rot of groundnut. J. Res., Punjab Agril. Univ., 3 (4) : 406-409.

Chohan, J.S. and Kapoor, S.P. (1967) Cultural factors in relation to collar rot disease of groundnut, J. Res., Punjab Agril. Univ., 4 : 536-542.

Chohan, J.S. (1967) Survival of Aspergillus niger van. Tèi ghem and soil factors influencing the collar rot disease of groundnut. J. Res. (Ludhiana) 6 (3) : 634-640.

Chohan, J.S. (1968) Fungal diseases of groundnut in India. Review and future problems. Proceedings of the 1st Workshop Conference on Oilseeds (ICAR), 22nd to 24th April, 1968, pp. 53.

Chohan, J.S., Sandhu, R.S. and Dalal, J.L. (1969) Screening of world collection of groundnut germplasm against collar rot disease by Aspergillus niger van. Tèi ghem in Punjab. Indian J. Agril. Sci., 40 : 541-551.

Chohan, J.S., Sandhu, R.S. and Dalal, J.L (1970) Screening of world collection of groundnut (Arachis hypogaea L.) germplasm for resistance to collar rot disease caused by Aspergillus niger Van Tèighem in Punjab. Indian J. Agril. Sci., 40 (6) : 546-551.

Chohan, J.S. 1970 Antagonism of soil fungi and actinomycetes to Aspergillus niger var Tèighem. The causal agent of collar rot of groundnut in Punjab. J. Res., PAU, Ludhiana, VII (4): 561-566.

Chohan, J.S. (1971) Seed and soil-borne fungi, seed rot and seedling disease of groundnut and their control. Lab. dev. J. Sci. and Tech. 9B (1):10-16.

Cook, R.J., Baker and K.F. (1983) The Nature and Practice of Biological control of plant Pathogens. St. Paul, Minn: Am. phytopatho. Soc. pp. 539.

Covey, R.P. Jr. (1971). The effect of methionine on the development of apple powdery mildew. Phytopathology, 61 : 346.

Dange, S.R.S. and Saradave, M.R. (1985) Screening of selected groundnut cultivars against seed rot and collar rot caused by Aspergillus niger van Teighem. Agric. Sci. Digest 5(3): 138-140.

Dange, S.R.S. and Sara dava, M.R. (1985) Control of seed rot and collar rot of groundnut. J. Oilseed Res., 324-328.

Danielson, R.M. and Davey, C.B. (1973) The abundance of Trichoderma propagules and the distribution of species in forest soils. Soil Biol. Biochem., 5 : 485-94.

Davis, D. (1952) Chemotherapy of tobacco masaic virus - Phytopathology 42 : 465.

Davet, P., Martin, C. and Artigues, M.(1981) Aspects of biological control with Trichoderma harzianum. Agronomie 1 : 933-936.

Dehne, H.-W. .... "[Investigation on the influence of growth regulators on disease development]. Uritersuchugen Zur Beeinflussung de Krankheitsbereitschaft durch wachstumsregulatoren 1207-1213.

Dennis, C. and Webster, J. (1971) .....

..... Trans. British Mycol. Soc., 57 :363-369.

Desai, S.A., Siddaramaiah, A.L. and Hegde, R.K. (1981) Effect of culture filtrate of five species of fungi on seed germination and root and shoot elongation of niger. Mysore J. of Agric. Sci., 15 (1) : 48-50.

Devi. G.R. and Polasa, H. (1984) Inhibitory effects of Bavistin (Carbendazim) on growth and mycotoxin production by Aspergillus grown on maize. Pesticides, 18(12) : 32-34.

Dimov, A., Filipov, Kh. and Moskov, G. (1983) [Effect of groundnut seed treatment with fungicides and hydrophobization on germination and yield.] Rasteniev" dni Nauki, 20 (1) : 105-112.

Durrell, L.W. (1968) Hyphal invasion by Trichoderma Viride. Mycopatho. Mycol. Appl., 35 :138-44.

Elad, Y., Chet, I. and Henis, Y. (1981) Biological control of Rhizoctonia solani in strawberry fields by Trichoderma harzianum. Pl. Soil, 60: 245-54.

Elad, Y., Chet, I. and Henis, Y. (1982) Degradation of plant pathogenic fungi by Trichoderma harzianum. Canad. J. Microbiol., 28: 719-25.

Elad, Y., Chet. I. and Katan, J. (1980). Trichoderma harzianum : A biocontrol agent effective against Sclerotium rolfsii and Rhizoctonia soloni. Phytopath., 70 :119-21.

Elad, Y., Hadar, Y., Chet, I. and Henis, Y. (1982) Prevention, with Trichoderma harzianum Rifai aggr. of reinfestation by Sclerotium rolfsii Sacc. and Rhizoctonia solani kuhn of soil fumigated with methyl bromide and improvement of disease control in tomatoes and peanuts. Crop Prot., 1: 199-211.

Elad, Y., Hadar, Y., Hadar, E., Chet, I. and Henis, Y. (1981) Biological control of Rhizoctonia solani by Trichoderma harzianum in carnation. Pl. Disease, 65 : 675-77.

Elad, Y., Kalfon, A. and Chet, I. (1982) Control of Rhizoctonia soloni in cotton by seed-coating with Trichoderma spores. Pl. Soil, 66 : 279-81.

Elad, Y., Katan, J. and Chet, I. (1980) Physical, biological and chemical control integrated for soil borne diseases in potatoes. Phytopath., 70 : 418-22.

El-Sherif, Ebtim, M.M. (1983) Studies on the effect of certain pesticides on some soil fungi and fungal disease. Ph. D. Thesis, Fac. of Agric., Cairo University, pp. 79.

Espuny Tomas, J.M., Simon Pujol D.M., Congredo, F. and Suarez-Fernandez, G.S. (1980) Method to asssess antagonism of soil microorganism towards fungal spore germination. Soil Biol. Biochem., 12 (2): 197-198.

Espuny Tomas, J.M., Simon - Pujol, D.M., Congredo, F. and Suarez-Fernandez, G. (1982) Nature of antagonism of soil fungi by bacteria isolated from soil. Soil Biol. Biochem.,14 (6) : 557-560.

Fawcett, C.H.; Spancer, D.M. and Wain, R.L. (1955). Investigation on fungicides. I. Fungicidal and systemic fungicidal activity in certain arylonyal kene carbonylic acid. Ann. appl. Biol. 43 : 533-568.

Fawcett, C.H., Spancer, D.M. and Wain, R.L. (1957) Investigation on fungicide II. Aryloxy and arylthio-alkene carbonylic acid and their activity as fungicides and systemic fungides. Ann. appl. Biol. 45 : 158-176.

Feakin, S.D. (1967) Pest Control in groundnuts, pp.55-93. PANS manual No. 2, Ministry for Overseas Development, London.

Frank, Z.R. (1969) Localization of seed borne inocula and combined control of Aspergillus & Rhizopus rots of groundnut seedlings by seed treatment. Israel J. Agric. Res., 19(3) : 109-114.

Gange, S., Antoun, H. and Richard, C. (1985). Inhibition of plant pathogenic fungi by bacteria isolated from soil and rhizosphere of leguminous plants. Canadian J. Microb., 31 (a) : 856-860.

Gajdzinski, M.and Mroczkiewicz, A. (1983) [Toxicity of imidazole derivatives towards selected Ascomycetes and Deuteromycetes]. Toxizitat von Imidazolderivatan generuber ausgewa hlten Pilzen aus den kalssen Ascomycetes und Deuteromycetes. Material und Organismen, 18 (3) : 183-194.

Garibaldi, A., and Bateman, D.F. (1971) Pectic enzymes produced by Erwinia chrysanthemi and their effects on plant tissue. Physiol. Plant Path., 1 : 25-40.

Garibaldi, A. and Bateman, D.F. (1971) Pectic enzyme produced by Erwinia chrysanthemi and their effects on plant tissue. Physiol. Plant Path., 1 : 25-40.

Garren, K.H. (1967) Relation of several pathogenic organisms and the competition of Trichoderma viride to pod breakdown. Pl. Disease Reporter, 51 : 601.

Ghewande, M.P., Pandey, R.N., Shukla, A.K. and Misra, D.P. (1984) Toxicity effect of culture filtrates of Aspergillus flavus on germination of seed and seedling growth of groundnut. Indian Botanical Reporter, 3 (2) : 107-111.

Gibson, I.A.S. (1953) Crown rot, a seedling disease of groundnut caused by Aspergillus niger II. An anomalous effect of organo-mercurial seed dressings. Trans. British Mycol. Soc. 36 : 324-334.

Gibson, I.A.S. (1953) Crown rot, a seedling disease of groundnut caused by Aspergillus niger. Trans. British Mycol. Soc. 36: 198-209.

Gibson, I.A.S. (1953) Crown rot, a seedling disease of groundnut caused by Aspergillus niger. Trans. British Mycol. Soc., 36 : 198-209.

Gigante, R.(1935) .....
.....Boll. Staz. Patol. Veg. Roma, 15 : 471.

Giri, D.N. and Sinha, A.K. (1983a) Effects of heavy metal salts on susceptibility of rice seedlings to brown spot disease. Ann. appli. Biol., 103 : 229-235.

Gokulapalan, C. and Nair, M.C. (1984) Antagonism of a few fungi and bacteria against Rhizoctonia solani Kuhn. Indian J. Microb., 24 (1): 57-58.

Goodman, R.N. (1980) Defenses triggered by previous invaders bacteria. In Plant Disease Advanced treatise, edited by J.G. Horsfall and E.B. Cowling, Vol.5, pp. 305-317, Academic Press, New York.

Gour, R.B. and Ahmed. S.R. (1983) Screening of fungicides against leaf spots and crown rot diseases of groundnut and to study the effect of date of sowing on tikka development. Indian J. Pl. Protection, 11 : 27-30.

Gupta, A.A., and Sengupta, A.K. (1983) Synthesis and fungicidal properties of some 3- (substituted sulphamidophenyl) phodanines and their benzylidene derivatives. Indian J. Agric. Sci. 53 (5) : 358-360.

Gupta, M.N. and Gupta, S.C. (1967) Studies in pectic enzymes of parassitic fungi. VIII. Factors affecting the production of polygalacturonase and pectin-estarase enzymes by Fusarium orthoceras. Mycopath. Mycol. Appl., 29 :193-200.

Gupta, R.C., Upadhyay, R.S. and Rai, B. (1979).....??

Mycopathologia, 67 : 147-151.

Gupta, V.K. and Chohan, J.S. (1967) Seed-borne fungi and seed-health testing in relation to seedling disease in groundnut. Proceedings of the 10th International Workshop on Seed Pathology, I.A.R.I, New Delhi (ICAR and International Seed Association) 11th -13th January, pp. 15-18.

Hadar, Y., Chet, I. and Henis, Y. (1979) Biocontrol of Rhizoctonia solani damping off with wheat bran culture of Trichoderma harzianum. Phytopathology, 69 : 64-68.

Hadar, Y., Harman, G.E. and Taylor, A.G. (1984) Evaluation of Trichoderma Koningiill and T. harzianum from New York soils for biological control of seed rot caused by Pythium spp. Phytopathology, 74: 106-10.

Haider, M.M., Sulaiman, E.D. and Daw Wood, R.K. (1986) Effect of culture filtrate of 5 species of fungi and their mixture on seed germination and seedling development of safflower. J. Biol. Sci. Res., 17 (1) : 141-150.

Hale, M.G., Moove, L.D. and Orcutt, D.M. (1981). Effect of gibberellin A-3, and 2-4-D on plant and root exudate lipids and susceptibility to Pythium myriotylum. Soil biology and Biochemistry. 13(5) : 395-399.

Hall, J.A. and Wood, R.K.S. (1974) Permeability changes in tissue and other effects of cell-separation solutions from rots caused by Corricium praticola and Erwinia atroseptica. Ann. Bot., 38 : 124-140.

Hall,J.A. and Wood, R.K.S. (1974) Permeability changes in tissue and other effects of cell-separation :solutions from soft rots caused by Corticum praticola and Erwinia atroseptica. Ann. Bot., 38 : 124-140.

Hamilton, R.I. (1980). Defenses triggered by previous invaders : Viruses. In Plant Disease Advanced treatise, edited by J.G. Horsfall and E.B. Cowling, Vol.5, pp. 279-303, Academic Press, New York.

Hancock, J.G., Millar, R.L. and Lorbeer, J.W. (1964) Pectolytic and cellulolytic enzymes produced by Botrytis alii, B. cinerea and B. squamosa in vitro and in vivo. Phytopath., 54: 928-931.

Harman, G.E., Chet, I. and Baker, R. (1980)/. Trichoderma hamatum effects on seed and seedling disease induced in radish and pea by Pythium spp. or Rhizoctonia solani. Phytopath., 70 : 1167-72.

Harman, G.E., Chet, I. and Baker, R. (1981) Factors affecting Trichoderma hamatum applied to seeds as a biocontrol agent. Phytopath., 71 : 569-72.

Harrison, P.G. (1985) Fungicidal influence on peanut seed germination. Tech. note, Dept. of Primary Production, Northern Territory, Australia, No. 41, pp.4.

Heggeness, H.G. (1942) Effect of borax application on the incidence of rust on flax. Pl. physiol., 17 : 143.

Henis, Y., Ghaffar, A. and Baker, R. (1979) Induction in soils of suppressiveness to Rhizodonia solani. Phytopath. 69 : 1164-1169.

Henis, Y. AND Papavizas, G.C. (1983) Factors affecting germinability and susceptibility to attack of sclerotia of Sclerotium rolfsii by Trichoderma harzianum in field soil. Phytopath., 73 : 1469-74.

Hutchinson, S.A. AND Cowan, M.E. (1972) Identification and Biological effects of volatile metabolites from cultures of Trichoderma harzianum. Trans. British Mycol. Soc. 59 : 71-77.

Jayanta Saha and Raj, S.K. (1989) Effects of fungicides on the seed microflora of groundnut (Arachis hypogaea L.) Indian J. Mycol. Res., 27 (2) : 183-189.

Jochems, S.C.J. (1926) Aspergillus niger op Katjang tanah. De Indische Culteren (Teysmanhia), 11: 325-326.

Joffe, A.Z. (1968) Mycoflora of surface - sterilized groundnut kernels. Pl. Disease Reporter, 52 : 608-611.

Joffe, A.Z. (1969) The Mycoflora of groundnut rhizosphere, soil and geocarposphere on light, medium and heavy soil and its relations to A. flavus. Mycopath. Mycol. Appl., 37 : 150-160.

Jordon E.G. and Erb, K. (1976) Mycologia, 68 : 920-924

Kang, M.S. and Chohan, J.S. (1966) Some pathogenic fungi from groundnut seeds in Punjab. J. Res., Punjab Agric. Univ. 3 : 158-61.

Katan, J. (1981) Solar heating (solarization) of soil for control of soil borne pests. Ann. Rev. Phytopath., 19 : 211-36.

Keil, H.L., Frohlich, H.P. and Van Hook, J.C. (1958) chemical control of cereal rust. I. Protective and eradicative control of rye leaf rust in the green house with various chemical compounds. Phytopath. 48 : 652.

Kenney, D.S, and Couch, T.L. (1981) Mass production of biological agents for plant disease, weed and insect control. In Papavizas, G.C. (ed). Biological Control in Crop Production, Totowa, NJ: Allanheld, Osmun : pp 143-50.

Kent, N.L. (1941) Absorption, translocation and ultimate fate of lithium in the wheat plant. New Physiol., 40: 241.

Kirpal S. Aulakh and R.S. Sandhu. (1970) Reaction of groundnut varieties against Aspergillus niger. Plant Disease Reporter, 54 (4) : 337-338.

Kiss, S.A. and Pozsar, B.I. (1977). Resistance induced by magnesium nutrition. Acta Agron Acad. Sci. Soc. Hung., 26: 156-163.

Kocaturk, S. and Maden, S. (1977) [Studies on the protective action of certain chemicals against seed borne fungus disease of groundnut.] Yerfistiginda tohumla tasinan hastalik etmeni funguslara karsi muthtelif ilaclarin koruyucu etkilen uzerinde calismalar. Zirai Mucadele Arastirma Yilligi. No. 11. 95-96.

Kodmelwar, R.V., Wangikar, P.D. and Shukla, V.N. (1977) In vitro evaluation of dithiocarbamate fungicides. J. Maharashtra Agric. Univ., 2 : 50-51.

Kohl, J. and Schlosser, E. Decay of sclerotia of Botrytis cinerea by Trichoderma sp. at low temperatures. J. Phytopath., 125 (4) : 320-326.

Kommendahl, T. and Windels, C.E. (1981) Introduction of microbial antagonists to specific courts of infection : Seeds, seedlings and wounds. In Papavizas. G.C. (ed). Biological Control in Crop Production, Totowa, NJ : Allanheld Osmun : pp. 227-48.

Krishnaiah, J., Satyaprasad, C., Singh, T.G., Jhirupathaiah, V. and Dave, B. (1985) Protection of Banana fruits against food-grade fruit coatings. Indian J. Hort., 42(1/2) : 136-138.

Kuc, J. (1982) Plant-immunization mechanism and practical implications. In Active Defense Mechanism in Plants. Edited by R.K.S. Wood, pp. 157-178, Ptenum Press, New York.

Kuc. J. (1983) Induced systemic resistance in plant to diseases caused by fungi and bacteria. In the Dynamics of Host defense, edited by J.A. Bailey and B.J. Deverall, pp. 191-221, Academic Press, Toranto.

Kulfinski, F.B. and Pappelis, A.J. (1980) A note on the effect of a product of Aspergillus niger on nuclei of Allium cepa. Cytologia, 45 (3): 477-480.

Kumar, S.M. and Khare, M.N. (1970) Efficacy of seed dressing fungicides in controlling Aspergillus blight of groundnut. Mysore J. Agric. Sci., 4 (3) : 349-352.

Kumar, K., Singh, Jitendra, and Saksena, H.K. (1983) Seed borne fungi of table pea; their pathogenicity and control. Indian Phytopath., 36 (4) : 716-718.

Lal, T.B. and Jayamma, K.K. (1978) Evaluation of seed dressing fungicide mixtures against Aspergillus niger, the incitant of collar rot of groundnut. Indian J. Pl. Protection, 6(1) : 15-18.

Lalithakumari, D., Govindaswamy, C.V. and Vidyasekaran, P. (1972) Isolation of seed borne fungi from stored groundnut seeds and their role on seed spoilage. Madras Agric. J., 59(1): 1-6.

Lande, H.L., Van DE, Misser, R.S.S. and Frankel, L.T. (1986) The effect of seed treatment on hand-shelled and machine-shelled peanut. Surinaamse Landbouw, 34(1-3): 54-58.

Lashin, S.M., El - nasr, H.I.S, El-nagar, M.A.A. and Nojal, M.A. (1989) Biological control of Aspergillus niger, the causal organism of peanut crown rot by Trichoderma harzianum. Annals Agric. Sci. (Cairo) 34 (2) : 795-803.

Lai, M.T., Weinhold, A.R., and Hancock, J.G. (1968) Permeability changes in Phaseolus aureus associated with infection by Rhizoctonia solani. Phytopathology, 58 : 240-245.

Lee, H.S. (1990). [Effect of pre-sowing seed treatment with GA-3 and IAA on flowering and yield components in groundnut]. Korean Journal of Crop Science (1990). 35 (1). 1-9.

Lewis, J.A. and Papavizas, G.C. 1980. Integrated control of Rhizoctonia fruit rot of cucumber. Phytopathology 70 : 85-89.

Lewis, J.A. and Papavizas, G.C. (1987) Reduction of inoculum of Rhizoctonia solani in soil by germlings of Trichoderma hamatum. Soil Biol. Biochem., 19 : 195-201.

Lisansky, S.G. (1985) Production and commercialization of pathogens. In Hussey, N.W. and Scopes, N.(ed.). Biological Pest Control. Blandford Press, Poole, UK, pp. 210-218.

Loper, J.E. (1988) Role of fluorescent siderophore production in biological control of Pythium ultimum by a Pseudomonas fluorescens strain. Phytopath., 78 : 166-72.

Manibhuson Rao, K., Waheeta, A., Sreenivasaprasan, S. and Chitralekha, R.S. (1987). Induced resistance in rice to sheath blight. Current Science, 56: 486-489.

Marsh, R.W. (1977). Systemetic fungicides. London, Longman. pp-401.

Mathur, R.L. and Sharma, L.C. (1970) Observation on performance of groundnut varieties with respect to crown rot disease (A. niger) in Rajasthan. Indian Phytopath., 23 (1): 143-144.

Mathur, R.L. and Sharma, L.C. (1971). Field assay of seed dressing fungicides for germination of groundnut seed. Madras Agricultural Journal 58(3) : 199-202.

Mathur, R.L. and Sharma, K.B. (1977). Bioassay of fungicides against Aspergillus niger Var. Teighem - Pesticides. 11 (2): 55.

Matta, A. (1980) Defenses triggered by previous diverse invaders. In Plant Disease : Advance Treatise, Edited by J.G. Horsfall and E.B. Cowling, Vol. 5. pp. 345-361, Academic Press, New York.

Mixon, A.C. (1980) Comparison of pod and seed screening methods on Aspergillus sp. infection of peanut genotypes. Peanut Sci. 7 (1) : 1-3.

Morwood, R.B. (1953) Peanut emergence and crown rot investigation. Queensland J. Agric. Sci. 10: 222-36.

Morwood, R.B. (1945) : Peanut disease. Qd. agric. J. 51 : 266-271.

Mount, M.S.; Bateman, D.F. and Basham, H.G. (1970) Induction electrolyte loss, tissue maceration and cellular death of potato tissue by an endo-polygalacturonate transeliminase. Phytopath., 60 : 924-931.

Munnecke, D.E. (1972) Factors affecting the efficacy of fungicides in soil. Annual Review Phytopathology, 10: 375-98.

Murphy, R.J. and Levy, J.F. (1983). Production of copper oxalate by some copper tolerent fungi. Transaction of the british Mycological Society; 81(1) : 165-168.

Narain, A. and Mohanty, A.P.(1984) Bacterial antagonists of some phytopathogenic fungi. Indian J. Mycol. Pl. Patho., 13 (1): 28-31.

Natarajan, S., Narayanaswamy, P. and Kandaswamy, T.K. (1983). Control of post emergence root rot and collar rot disease of groundnut. In the proceedings of the National Seminar on management of diseases of oilseed crops. Madurai, India, T.N. Agric. Univ : 29-30.

Nelson, E.B. and Hoitink, H.A.J. (1983) The role of micro organisms in the supervision of Rhizoctonia solani in container media amended with composted hardwood bark. Phytopathology, 73: 274-78.

Nema, K.G., Jain, A.G. and Asthana, R.P. (1955) Further studies on Aspergillus blight of groundnut seedlings, its occurrence and control. Indian Phytopath., 8 : 23-31.

Nene, Y.L. and Thapliyal P.N. 1979. fungicides in Plant Disease control (2nd end.) Oxford and IBH Publishing Co., New Delhi, p-507.

Norton, D.C. (1954) Antagonism in soil between Macrophomina phaseoli and selected soil inhabiting organisms. Phytopath., 44: 522-524.

Oguni, I., Suzuki, K. and Uritami I. (1976) Terpenoid induction in sweet potato root by cyclic-3,5-1-adenosine monophosphates. Agric. Biol. Chem., 40 : 1251-1252.

Old, K.M. and Wong, J.N.F. (1976)

Soil Biol. and Biochem., 8 :285-292.

Olsen, C. (1939) The employment for water culture experiments of distilled water containing traces of copper. C.V. Trav. Lab. Carlsbers. Ser. Chin., 23 : 37-44.

Omprakash and Siradhana, B.N. (1978) Effect of toxins on tomato seedlings produced by four species of Aspergillus isolated from maize grains. Madras Agric. J., 65 (II): 752.

Papavizas. G.C., ed. 1981 Biological control in crop production. Totowa, NJ: Allanheld, Osmun, pp. 461.

Papavizas, G.C. and Lewis, J.A. (1981) Introduction and augmentation of microbial antagonists for the control of soil borne plant pathogens. Papavizas G.C. (ed). Biological Control in Crop Production. Totowa, NJ. Allanheld, Osmun, pp 305-22.

Papavizas, G.C., Lewis, J.A., and Abd-El Moity, T.H. (1982) Evaulation of new biotypes of Trichoderma harzianum for tolerence to benomyl and enhanced biocontrol capabilities. Phytopath., 72 : 126-32.

Papavizas, G.C. and Lewis, J.A. (1983) Physiological and biological characteristics of stable mutants of Trichoderma viride resistant to MBC fungicides. Phytopath., 73: 407-11.

Papavizas, G.C., Dunn, M.T., Lewis, J.A. and Beagle Ristaino, J. (1984) Liquid fermentation technology for experimental production of biocontrol fungi. Phytopath., 74 : 1171-75.

Papavizas, G.C. 1985 Trichoderma and Gliocladium : biology, ecology and potential for biocontrol. Annual Rev. Phytopath. 23 :23-54.

Park, D. (1965) In, "Ecology of soil-borne plant pathogens" (Ed. K.F. Baker and W.C. Snyder). John Murray, London, pp. 82 - 97.

Paul, A.K. and Banerjee, A.K. (1984) Antifungal actinomycetes in soils of West Bengal. Hindustan Antibiotics Bulletin, 20 (1/2): 18-22.

Perrin, D.R. and Cruickshank, I.A.M. (1965) Studies on phytoalexin. VIII. Chemical stimulation of pisatin formation in Pisum sativum L. Aust. J. Bid. Sci., 18: 803-816.

Pettit, R.E., Azaizeh, H.A., Taber, R.A., Szerszen, J.B. and Smith, O.D. (1989) Screening groundnut cultivars for resistance to Aspergillus flavus, A. parasiticus and aflatoxin contamination. In Aflatoxin contamination of groundnut. Proceedings of the International Workshop, 6-9 Oct. 1987. ICRISAT Centre, India (Edited by Mc Donald, D, Mehan, V.K.F, Patancheru, A.P. 502324, India.

Purss, G.S. (1960): Further studies on the control of preemergence rot and crown rot of peanut. Qd. J, agric. Sci. 17, 1-14.

Postlethwait, S.N. and Curtis, R.W. (1959) Histology of malformation produced on bean plants by culture filtrate of A. niger. Am. J. Bot., 46 : 31-35.

Qadri, S.M.H., Srivastava, K.J., Bhonde, S.R., Pandey U.B. and Bhcgchandani P.M. (1982). fungicidal bioassay against some important pathogen of onion. Pesticides, 16(11): 11-16.

Rai, B. Singh, V.N. and Singh, D.B. (1980) Trichoderma viride as mycoparasite of Aspergillus spp. Pl. Soil. 57 (1): 131-135.

Ranjan Chattopadhyay; Raj, S.K. (1991) Soil moisture and rhizosphere microflora of groundnut (A. hypogaea L) developed from fungicide tested seeds. Journal of Mycopathological Research, 29(1) : 83-86.

Rao, A.S. (1962) Fungal populations in the rhizosphere of peanut (Arachis hypogaea L.) Pl. Soil, 17: 260.

Ray, A.K. (1979) Solubilization of copper compounds by Aspergillus niger. Indian Phytopath. 32 (4) : 609-611.

Ravindar, V. and Lingaiah, p. (1984). Effect of amide group ligands and their metal complexes on pathogenic fungi. Current Science, India. 53 (19): 1032-1034.

Reddy, G.R., Reddy A.G.R. and Rao, K.C. (1991). Effect of different seed dressing fungicides against certain seed-borne fungi of groundnut. Journal of oilseed Research. 8 (1) : 79-83.

Reddy, M.M., Reddy, S.M., Srihari, S. and Raju, N.A. (1985). Antifungal activity of phenyl hydrazones of some o-hydroxyaldehydes and ketones. Pesticides, 19(8) : 38-39.

Reiss, J. (1970) [Untersuchungen uber die phytotoxizitat von Penicillium expansum, Aspergillus niger and Rhizopus nigricans] Investigations on the phytoxicity of P. expansum, A. niger and R. stolonifer. Phytopath., Z. 69 (1): 78-82.

Richardson, L.T. (1954) The persistence of thiram in soil and its relationship to the microbiological balance and damping off control. Canadian. J. Bot., 32: 335-46.

Rodgers, P.B. (1989) Potential of biological control organisms as a source of antifungal compunds for agrochemical and pharmaceutical products development. Pesticide Sci. 27 (2) : 155-164.

Rodigin, M.N. and Krasnova, T.M. (1959) Seed treatment microelements against brown rust of wheat. Abstr. In. Rev. Appl. Mycol., 39 : 405.

Ross. A.F. (1961a). Local acquired resistance to plant virus infection in hypesensitive hosts. Virology, 14: 329-339.

Ross, A.F. (1961b) Systemic acquired resistance induced by localized virus infections in plants. Virology, 14 : 340-358.

Roy, A.K. (1979 and Rubi. 1980). Solubilization of copper compounds by Aspergillus niger. Indian Phytopathology 32(4): 604-611.

Roy, J. (1901) Cultures et formes attences des maladies cryptogamiqmes des vegetaux. Compt. Rend. Acad. Sci. 133 : 307-309. (Cited by R.J. Allen in Plant Pathology) Ann advanced Treatise, ed. J.G. Horsfall and A.E. Dimond, 1 : 435-467, 1959.

Ryan, C.A. (1978). Trend biochemical Science. 3 : 148-50.

Salt, G.A. (1978) The increasing interest in minor pathogens. In Schippers, B. and Garm, W. (eds), Soil borne Plant Pathogens. Academic Press, New York, pp. 289-312.

Sanford, G.B. (1956) Factors influencing formation of sclerotia by Rhizoctonia solani. Phytopath., 46: 281-284.

Shekhawat, K.S., Verma, G.P. and Pathak, V.N. (1986). Effect of seed dressing fungicides on collar rot of groundnut caused by Aspergillus niger. Summa Phytopathologica. 12 (3-4) : 207- 216.

Sharma, M.P. and Agnihotri, J.P. (1969). Seed mycoflora of groundnut (Arachis hypogaea L.) with special reference to Aspergillus niger var. Teighem, the causal agent of collar rot. Bull, Grain Tech. 7, 205-216.

Sharma, M.P., Mathur, R.L. and Dalela, G.C. (1973). Check crown rot in groundnut. Indian farming. 23 (7): 24-25.

Sharma, S., Mishra, B.P. and Sharma, R.K. (1986) Effect of some antifungal compounds to combat the post-harvest Aspergillus rot of Baramasi Lemon.. Progressive Horticulture. 18 (1/2): 71-72.

Siddaramaiah, A.L., Prasad, K.S.K. and Hegde, R.K. (1979). Effectiveness of seed dressing chemicals against crown rot of groundnut. Pesticides, 13 (1) : 28-29.

Siddaramaiah, A.L., Desai, S.A. and Hegde, R.K. (1981). Effect of few fungicides on crown rot and afla-root disease of groundnut. Mysore Journal of Agricultural Science 15(1) : 44-47.

Sidhu, G.S. and Chohan, J.S. (1971). The field scale testing of different fungicides for controlling collar rot of groundnut in Punjab. J. Res. Ludhiana, 8 (2): 211-213.

Singh, D.B. (1984). Effect of mycostatin of phylloplane mycoflore of mustard and barley. Iranian Journal of Plant Pathology, 20 (1/4) : 22-26.

Singh, J.P. Sushil Sharma; Yamadagni, R. (1985). Control of post harvest black mould of grapes. Indian Phytopathology 38 (3) : 531-532.

Singh, S.P. and Jha, K. (1989). Indolinone derivaties as potential antimicrobial agents. Zentralbhalf fur Mikrobiologie. 144(2) : 105-109.

Sinha, A.K. (1976). Induced resistances as a factor in plant disease control. Indian Biol., 9 : 1-16.

Sinha, A.K. (1984) A new concept in plant disease control. Sci. and Cult., 50 : 181-186.

Sinha, A.K. (1984). A new concept in plant disease control. Science and culture. 50 : 181-186.

Sinha, A.K. and Giri, D.N. (1979). An approach to control brown spot of rice with chemicals known as phytoalexin inducers. Currrent Science. 48 : 782-784.

Sinha, A.K. and Hait G.N. (1982) Host sensitization as a factor in induction of resistance in rice against Dreschslera by seed treatment with phytoalexin inducers. Trans. British Mycol. Soc., 79 : 213-219.

Sinha, A.K. and Sengupta.T.K. (1986). Use of unconvensional chemicals in the control of blast rice. In proceedings of the Second International Conference. On plant protection in the tropics. 17-20. March, Genting Highlands, Malayasia.

Smalley, F.B. (1962). Prevention of Dutch elm disease by treatments with 2,3,6 - trichlorophenyl acetic acid. Phytopathology 52 : 1090-1091.

Sneh, B., Humble, S.J. and Lockwood, J.L. (1977) _____

Phytopath., 67 : 622-628.

Srivastava, N., Bahadur, S., Verma, H.M. and Khan, M.A.A. (1984). Synthesis of new 5, 3/5 and 2- substituted (1,3,4(- oxadiazoles and their related products as potential antifungal and antiviral agents. Current Science, India, 53(5) : 235-239.

Soleman, N.K., Mikhail, M.S., Habb, K.K. and Khalil, E.M. (1988). Response of broad bean plants infected with Rhizoctonia solani to application of growth regulators and calcium. Egyptian Journal of Phytopathology 20(1) : 1-12.

Stack, J.P. and Pettit, R.E. (1984) Colonization of organic matter substrates in soil by Aspergillus flavus. Proc. Am. Peanut Res. Educ. Soc., 16 : 45.

Sumbali, G. and mehrotra, R.S. (1981). Reduction of post horvest fruit decay by antibiotics and growth regulators. National Academy Science letter. 4. (9): 353-356.

Sumbali, G. and Mehrotra, R.S. (1983, recd 1986). Control of post harvest diseases of temperate fruits by chemicals and treated wrapers. Indian Phytopathology. 36(2) : 270-273.

Sundus, D.K.; Raj, S.K. 1988. Pathogenicity of fungicide tolerent isolates of Aspergills niger on groundnut seedlings. Indian J. Mycol. Res. 26 : 95-98.

Thatcher, F.S. (1942) Further studies of osmotic and permeability relations in parasitism. Canad. J. Res. C. 20: 288-311.

Tomlinson, J.A. and Webb, M.J.W. (1958) Control of turnip and Cabbage mildew (Erysiphe polygoni Dc.) by Zinc. Nature, 181 :1352.

Tribe, H.T. (1955) Studies on the physiology of parasitim. XIX. On the Killing of the plant cells by enzymes from Botrytis cinerea and Bacterium aroideae. Ann. Bot.,19 : 351-368.

Tronsmo, A. and Dennis, C. (1978) Effect of temperature on antagonistic properties of Trichoderma sp. Trans. British Mycol. Soc., 71: 469-474.

Tyagi, D.K. (1986). Effect of ammonia or associated surface mycoflora, seed germination and seedling growth of Abelmoschus esculentus L. Current science, India, 55(3): 154-155.

Upadhyay, R.S. (1979) Ecological studies on Fusarium udum Butler causing wilt disease of pigeon-pea. Ph.D. Thesis, Banaras Hindu University, Varanasi, U.P., India.

Van Andel, O.M. (1966). Amino acids and plant diseases. Annual Review of Phytopathology. 4 : 349-368.

Venkataraman, G.S. and Rajyalakshmi, B. (1971). Interaction between pesticides and soil micro organisms. Indian J. exp. Bio. 9(4) : 521-522.

Vishunavat, K. and Shukla, P. (1981) Screening of culture filtrate of seed mycoflora of lentil upon seed germinability and seedling length. Indian Phytopath., 34 (4) : 510-512.

Wadibhasme, S.S., Ingole, O.V. and Wangikar, P.D. (1991). Chemical control of bud blight of groundnut caused by Aspergillus niger. Bioved 2(2) : 125-126.

Walker, H.L., and Connick, W.J. Jr. (1983) Sodium alginate for production and formulation of mycoherbicides, Weed Sci., 31 : 333-38.

Wangikar, P.D. and Kodmelwar, R.V. (1977). Screening of various fungicides against Aspergillus niger (seedling blight of groundnut and Fusarium oxysporum f. sesami (wilt of sesame). Pesticides 11(2) : 41-42.

Weindling, R. (1932) Trichoderma lingorum as a parasite of other soil fungi. Phytopath., 22: 837-45.

Wells, H.D, Bell, D.K. and Jaworksi, C.A. (1972) Trichoderma harzianum - a biocontrol for Sclerotium rolfsii. Phytopath. 62 : 808.

Whitehead, M.D. and Thirumalachor, M.J. (1971). Effect of aureofungin spray on the control of fungal disease and Aspergillus flavus infestation in peanuts. Hindustan Antibiotic Bull. 13 (3-4) : 79-80.

Wicklow, D.T. and Wilson, D.M. (1986) Germination of Aspergillus flavus in a Georgia maize field, Trans. British Mycol. Soc., 87 : 651.

Widden, P. and Scattolin, V. (1988) Competitive interaction and ecological strategies of Trichoderma sp. colonizing spruce litter. Mycologia, 80 (6) : 795-803.

Windham, M.T., Elad, Y. and Baker, R. (1986) A mechanism for increased plant growth induced by Trichoderma spp. Phytopath. 76 : 518-21.

Wood, R.K.S.(1960) Pectic and Cellulolytic enzymes in plant disease. Am. Rev. Plant Physiology, 11 : 299-322.

Wu, W.S, (1982) Seed treatment by applying Trichoderma spp. to increase the emergence of Soybeans. Seed Sci. Technol., 10 : 557-63.

Yarwood, C.E. (1959). Predisposition. Plant physiology vol. 1 (eds, T.G. Horsfall and A.E. Dimond) P-521, New York, Academic Press.

YI, Sheng-Lin (1982) Factors influencing the development of peanut crown rot caused by Aspergillus niger and measures for disease management. J. Agric. Res., China, 31 (2): 144-154.