STUDIES ON SEED MYCOFLORA OF BENGALGRAM
(*Cicer arietinum* L.)

By

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DEPARTMENT OF PLANT PATHOLOGY
SRI VENKATESWARA AGRICULTURAL COLLEGE
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
TIRUPATI - 517 502 (A.P.)

MARCH **2000**

CERTIFICATE

Miss A. KRISHNAVENI *has satisfactorily prosecuted the course of research and that the thesis entitled* **"STUDIES ON SEED MYCOFLORA OF BENGALGRAM (*Cicer arietinum* L.)"** *submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by her for a degree of any university.*

Date : 10-5-2000

Place : Tirupati

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(Dr. R. JOSHUA ANANDAM)
Major Advisor

CERTIFICATE

Miss A. KRISHNAVENI *has satisfactorily prosecuted the course of research and that the thesis entitled* "STUDIES ON SEED MYCOFLORA OF BENGALGRAM (*Cicer arietinum* L.)" *submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by her for a degree of any university.*

Date : 10-5-2000

Place : Tirupati

(Dr. R. JOSHUA ANANDAM)
Major Advisor

DECLARATION

I, **Miss. A. KRISHNAVENI,** hereby declare that the thesis entitled **"STUDIES ON SEED MYCOFLORA OF BENGALGRAM (*Cicer arietinum* L.)"** submitted to Acharya N.G. Ranga Agricultural University, for the Degree of Master of Science in Agriculture is the result of original work done by me. I also declare that the materials contained in this thesis have not been published earlier.

Date : 10.5.2000

(A. KRISHNAVENI)

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LIST OF SYMBOLS AND ABBREVIATIONS

At the rate of	@
Centimeter	cm
Critical Difference	CD
Degree Celisius	°C
Gram	g
Hour	h
Hectare	ha
Kilogram	Kg
Litre	l
Millilitre	ml
Micrometer	µm
ppm	Parts per million

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ABSTRACT

Title	:	**STUDIES ON SEED MYCOFLORA OF BENGALGRAM (*Cicer arietinum* L.)**
Author	:	**A. KRISHNAVENI**
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Mycoflora associated with ten varieties of bengalgram seeds viz., Annigeri, K-850, JG-62, GL-769, ICCV-10, BG-276, DCP 92-3, KWR-108, IPC 94-19 and IPC 94-37 includes *Alternaria* sp., *Aspergillus niger, A.flavus, Ascochyta rabiei, Botrytis cinerea, Colletotrichum* sp., *Curvularia lunata, Fusarium* sp., *Penicillium* sp. and *Rhizopus stolonifer*. Both agar plate and blotter methods were employed for the isolation of mycoflora. Agar plate method was found better in yielding maximum number of of fungi than blotter method. In both the methods, unsterilized seed yielded more number fungi of than sterilized seed. Among the ten fungi tested to study the effect on germination *in vivo*, *Alternaria* sp., *Ascochyta rabiei* and *Fusariun* sp. caused considerable reduction in percentage of germination and shoot and root length considerably.

Among the five fungicides tested by poison food technique, Carbendazim at all concentration and Captan at 1500 ppm were effective against *Alternaria* sp. Carbendazim inhibited the growth of *Ascochyta rabiei* at all concentrations while Thiram at 500 and 1500 ppm was effective against *Fusarium* sp.

Out of five plant extract tested, *Azadirachta indica* was most effective and inhibited the growth of *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp to a greater extent.

Introduction

CHAPTER - I

INTRODUCTION

Bengalgram (*Cicer arietinum* L.) is one of the major grain legume pulse crop of India and other semi-arid regions of the world. It is cultivated in parts of North India such as Punjab, U.P., Haryana and parts of Central and South India.

Among the major pulse crops chickpea contributes nearly 42-47 per cent of total pulse production. It is grown in an area of about 7541 ha with an annual production of 6127 tons in India. In Andhra Pradesh, it is grown in an area of about 120 ha with an annual production of 90 tons (Statistical Abstracts India, 1998).

It is an important source of protein in the largely cereal based diets in the semi-arid tropics. It has 17.1 per cent protein, 5.3 per cent fat, 61.2 per cent carbohydrates, 3.9 per cent fibre and 9.8 per cent moisture.

The association of mycoflora with seed and their role in deteriorating seed health and quality is an established fact. For successful cultivation of any crop, the seed must be sound and free from mycoflora which are likely to interfere with emergence, germination, and subsequent performance of the crop in the field. Bengalgram seeds are known to harbour several fungi which affect their health seriously, causing germination failures to complete death of seedling. Some seed borne pathogens produce toxic metabolites like aflatoxin which greatly hinders not only seed germination, but also vigour of seedling.

The use of fungicides and plant extracts in plant disease management was shown to be effective in the improvement of agriculture.

Keeping in view of all the above factors, studies on bengalgram seed mycoflora were undertaken with the following objectives.

1. To isolate the fungi associated with bengalgram seeds.

2. To study the effect of seed borne fungi on seed germination *in vivo*.

3. To study the effect of different fungicides and plant extracts on certain seed borne pathogens *in vitro*.

Review of Literature

CHAPTER - II

REVIEW OF LITERATURE

2.1 SEED BORNE FUNGI ASSOCIATED WITH BENGALGRAM

Seeds play a vital role for the healthy production of any crop. They are also known to carry microorganisms as contaminants both externally and internally. These microorganisms not only incite diseases but also reduce the viability and quality of the stored seeds. Among the microorganisms fungi are major cause of spoilage in stored grains and seeds. Numerous attempts have already been made to study the influence of seed borne microbes on the quality and viability of the seeds.

Gurha *et al.* (1981) isolated *Alternaria* sp. from infected bengalgram seeds by PDA (Potato Dextrose Agar) method. Chaturvedi and Tripathi (1987) reported that seventeen fungal species were isolated from chickpea and redgram. Among them, *Aspergillus flavus*, *Aspergillus niger* and *Fusarium oxysporum* were dominated.

Maden (1987) isolated *Ascochyta rabiei, Botrytis cinerea, Fusarium equiseti, F. moniliforme (Gibberella fujikuroi), F. oxysporum, F. sambucinum (G. pulicaris), F. solani, Macrophomina phaseolina, Rhizoctonia solani, Stemphylium spp. and Verticillium dahliae by* standard blotter method.

Raut and Somani (1988) isolated the fungus *Alternaria alternata* from diseased branches, pods and seeds of *Cicer arietinum* in Bhandara, Maharastra (India).

Amiruzzaman and Chaudhury (1989) from Bangladesh, isolated twenty four pathogens from the stored seeds. Among them *Alternaria* sp., *Cladosporium* sp. and *Curvularia* sp. were predominant.

Dwivedi (1989) detected fungi associated with surface sterilized seeds of *Cicer arietinum.* The pathogens isolated were *Aspergillus flavus, A.niger, Fusarium moniliforme (Gibberella fujikuroi)* and *Penicillium oxalicum.*

Simay (1989) checked seventy five chickpea seed samples for fungi and observed infection of *Alternaria alternata, Botrytis cinerea, Didymella pinodes (Mycosphaerella pinodes), Phoma pinodella, Ascochyta rabiei, Fusarium oxysporum, F. solani, Myrothecium roridum* and *Sclerotinia sclerotiorum.*

Abdeslam Hidden (1990) isolated *Aspergillus, Penicillium, Rhizopus, Phoma, Alternaria tenuis, Ascochyta pinodes, F. equseti, F. solani, Fusarium oxysporum* and *Phoma medicaginis* from four samples of chickpea seeds.

Dwivedi and Shukla (1990) obtained that seeds of chickpea cultivars from 12 locations in India and found association of 20 fungal species belonging to 12 genera (7 *Aspergillus spp.*, 3 *Fusarium* spp., and 2

Penicillium spp.) were found associated with the seeds. Of the seed borne pathogens and storage fungi identified were *Acremonium implicatum Aspergillus melleus*, *Aspergillus terreus*, *Aspergillus wentii*, *Bipolaris australiensis*, *Dreschlera specifera*, *Epicocum purpurascens*, *Penicillium puberulum* and *Scytalidium* sp.

Trapero - Casis *et al.* (1990) isolated *Pythium ultimum* var *ultimum* from rotten chickpea seeds, damped off seedlings and from soils in U.S. Pacific Northwest and Southern Spain by field and growth chamber tests.

Abdou *et al.* (1991) isolated *Ascochyta rabiei* from different parts of diseased chickpea plants and from seeds with concentric brown lesions and the fungus could remain viable for 4 years in stored infected seeds.

Ahmed and Singh (1991), from Bhagalpur, isolated *Alternaria*, *Cladosporium*, *Fusarium*, *Curvularia* from fresh seed and *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus terreus*, *Penicillium citrinum* and *Penicillium chrysogenum* in stored chickpea seeds.

Day and Singh (1994) reported that *Ascochyta rabiei* was found to be both externally and internally seed borne, while the external infection predominant in nature.

2.2 EFFECT OF SEED BORNE FUNGI ON SEED GERMINATION

Seeds play a vital role for the healthy growth of a crop. The importance of disease free and viable seeds in the crop production need not be over emphasised. The infected seeds germinate poorly and could be a major source of inoculum for many disease problems of the crops.

Halfon - Meiri and Aliza (1970) reported that chickpea seed infested with *Ascochyta rabiei* affected seed germination, seed formation and seed size. The growth of one month old seedlings from infested seed was slower than that of those from healthy seed.

Kamal and Verma (1979) stated that culture filtrates of *Alternaria alternata, Aspergillus flavus, A.nidulans, A.niger* and *Trichoderma viridi* showed adverse effect on seed germination, root (radicle) and shoot (plumule) length in redgram.

Chary and Reddy (1982) stated that the culture filterate of *Fusarium oxysporum* reduced the seed germination in *Vigna radiata*. Goel and Gupta (1986) reported that the seed inoculated with *Fusarium oxyporum* f.sp. *ciceri* reduced the shoot and root length in chickpea.

Tripathi *et al.* (1986) reported that chickpea seed infected with *Ascochyta rabiei* reduced the germinability of the seeds by 80.00 to 85.00 per cent.

Dwivedi and Shukla (1988) stated that culture filtrates of 20 fungi isolated from chickpea seed samples generally reduced germinability. The effect increased with the age of the culture. The greatest loss in germination was caused by 2 day old filtrate of *Aspergillus niger*. The filtrates also inhibited root and shoot length of seedlings.

Anwar *et al.* (1995) reported that 10 field fungi associated with soybean seed viz., *Alternaria atternata, Cercospora kikuchi, Colletotrichum truncatum, C.dematium, Fusarium equiseti, F.moniliforme. F.pallidoroseum, F.oxysporum, F.solani* and *Macrophomina phaseolina* caused root diseases and damping off and reduced the seed germination and seedling emergence.

Bhargava (1995) reported that culture filtrate of *Alternaria alternata* (causal agent of chickpea blight) inhibited seed germination besides reducing the plumule growth and blackening of seed in developing pods.

Sharma (1995) observed that fungal metabolities of three seed borne fungi viz., *Alternaria alternata*, *Cladosporium herbarum* and *Trichothecium roseum* reduced germination and caused rotting of soybean c.v.Bragg. Maximum seed rotting was caused by culutre filtrate of *C. herbarum*.

Jain *et al.* (1996) stated that among the metabolites from four *Aspergillus* species tested, metabolite obtained from *Aspergillus niger* caused maximum inhibition of seed germination (91.3 per cent) in chickpea.

2.3 EFFECT OF DIFFERENT FUNGICIDES AND PLANT EXTRACTS ON SEED BORNE FUNGI

2.3.1 Fungicides

Reddy and Kabebeh (1984) stated that seed treatment with Thiabendazole eliminated the pathogen *Ascochyta rabiei* from *Cicer arietinun* seed without any deleterious effect on germination.

Madhu Meeta *et al.* (1986) reported that only 4 out 13 fungicides evaluated as seed dressings for the control of *Botrytis cinerea* on *Cicer arietinum* were effective. Out of these, carbendazim was the best.

Reddy *et al.* (1986) reported that Calixin M (tridemorph + maneb) at 0.3 and 0.6 per cent concentration was very effective for complete control of *Ascochyta rabiei* in chickpea.

Tripathi *et al.* (1986) reported that seed treatment of *Cicer arietinum* with Calixin M (tridemorph + maneb), Calixin M + Thiram, Calixin M + Brassicol (Quintozene) and Carbendazim + Thiram eliminated the fungus *Ascochyta rabiei*.

Das (1987) stated that out of 8 seed treatment fungicides tested against *Fusarium oxysporum* f. sp. *ciceri* of *Cicer arietinum*, Agallol (2 methoxy ethyl mercury chloride) was found to be the best followed by Brassicol (Quintozene) which resulted in the highest yield.

Illyas and Bashir (1987) studied 9 fungicides with 7 different combinations and showed that the best control of seed borne fungi in *Cicer arietinum* was given by Calixin M (tridemorph) alone or in combination with Benlate (benomyl), Daconil (chlorothalonil) or Captan.

Singh and Singh (1990) stated that seed treatment of chickpea seed with combination of carbendazim and Thiram @ 3g/kg seed, gave 95-100 percent control of *Aschochyta rabiei*.

Trapero - Casis *et al.* (1990) reported that treating chickpea seeds with chemicals like Matalaxyl, Captan, benalaxyl and metalaxyl + thiabendazole were effective in controlling *Pythium* seed rot of bengalgram in both field and growth chamber tests.

Abdou *et al.* (1991) stated that, among the ten fungicides tested, benomyl (Benlate), tridemorph (Calixin) and thiabendazole inhibited *Ascochyta rabiei* at 10ppm, Thiram at 100 ppm. while chlorothalonil (Bravocarb) and Zineb prevented the growth at 1000 ppm.

Junaid Mukhtar *et al.* (1991) stated that seed treatment with Carbendazim gave the best results followed by PCNB (Qintozene) and Thiram for the control of *Sclerotium rolfsii* (*Corticium rolfsii*).

Chickpea cv C-727 seeds infected with *Aspergillus* spp., *Aspergillus niger, Fusarium* sp., *Alternaria* sp., *Rhizopus* sp. and *Ascochyta rabiei* were treated with Tilt (Propiconazole), Tecto-60, (Thiabendazole), Benlate (Benomyl), Daconil (Chlorothalonil) and Dithane M-45 (Mancozeb). Tilt, Tecto-60, Daconil, Benlate and Dithane M-45 reduced the recovery of total seed borne fungi by 98.8, 63.7, 59.2, 39.7 and 22.5 per cent respectively (Rauf *et al.*, 1991).

Zaidi *et al.* (1991) stated that treatment of chickpea seeds with 0.1 and 0.2 percent concentration of Benlate, Captan, Vitavax and Plantvax decreased the occurrence of fungal species and increased the percentage of germination.

Rauf *et al.* (1992) reported that control of *Ascochyta rabiei* was studied using different doses of Tilt (Propiconazole) *in vitro* studies. The fungus was almost totally eradicated by seed treatment with Tilt (1-2 ml/kg seed).

Malik *et al.* (1993) showed that out of 14 fungicides tested by poison food technique against *Botrytis cinerea*, complete inhibition was observed with Benlate and Tecto-60, while most of the other fungicides significantly inhibited growth of the fungus.

Singh *et al.* (1993) reported that Carbendazim at 100 µg/ml completely inhibited radial growth of *Fusarium oxyporum* f. sp. *ciceri*.

Singh *et al.* (1994) observed that to control chickpea seed borne infection of *Ascochyta rabiei*, carbendazim + TMTD (Thiram), Derosal (Carbendazim) and Bavistin alone were most effective.

DiazFranco and PerezGarcia (1995) stated that carboxin, benomyl and Captan were the effective fungicides against seed-borne *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina*, *Pythium ultimum*, *Verticillium albo-atrum* and *Sclerotium rolfsii* in Chickpea.

Shugha *et al.* (1995) reported that out of twelve fungicides evaluated against *Fusarium oxysporum* f. sp. *ciceri*, carbendazim and Thiram alone or in combination were highly effective in inhibiting the mycelial growth of the fungus *in vitro* condition.

Bidari *et al.* (1996) found that with Thiram, Captan, Dithane M- 45, Benlate and Bitertanol were the effective seed dressing against *Fusarium* spp. *Aspergillus flavus*, *Alternaria alternata*, *Botrytis* sp., *Curvalaria lunata (Cochilobolus lunatus)*, *Cladosporium* sp. and *Macrophomina phaseolina.*

Haware *et al.* (1996) observed that *Fusarium oxysporum* f.sp *ciceri* infection in the hilum of seeds of diseased *Cicer arietinum* and it was eradicated by seed treatment with Benlate T (benomyl + Thiram).

Khan *et al.* (1996) reported that Benlate followed by Calixin were effective against chickpea seed mycoflora viz., *Rhizopus stolonifer*, *Collectotrichum dematium*, *Fusarium oxysporum* and *Macrophomina phaseolina.*

2.3.2 Plant extracts

Singh *et al.* (1979) reported that aqueous leaf extract of garlic on *Fusarium oxysporum* f. sp. *ciceri*, and *Sclerotinia sclerotiorum* isolated from chickpea seed in liquid medium was greatly reduced by 7000 and 5000 ppm of the extract respectively.

Singh *et al.* (1980) stated that the growth of *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* was inhibited in liquid medium by extracts of leaf, trunk, bark, fruit pulp and oil of *Azadirachta indica*.

Bhowmick and Vardhan (1981) reported that the leaf extracts of *Cinamomum* and *Catharanthus* completely checked the radial growth of *Curvularia lunata. Azadirachta, Clerodendron, Phyllanthus* and *Vitex* were also effective in reducing the growth of the fungus to some extent.

Dharmapal *et al.* (1993) stated that aqueous extracts of neem leaf showed 80 - 100 per cent growth inhibition of *Rhizoctonia solani in vitro*. Lokesh *et al.* (1986) reported that unautoclaved extracts from four plants viz., *Parthenium hysterophorus*, *Thuja orientalis*, *Eukalyptus citridora* and *Melia azedarach* have inhibited the growth of *A. flavus*.

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

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Reddy and Reddy (1987) reported that antifungal activity of *Samanea saman* and *Peltophorum pterocarpum* completely inhibited the spore germination of *Drechslera specifera* and *Fusarium solani* while leaf extracts of *Agave americana*, *Impatiens balsamina* and *Cassia nodosa* completely inhibited *F. solani* spores but partially inhibited *Drechslera specifera* spores.

Naidu (1988) assayed the young and mature leaf extracts of *Cadiaeum variegatum* for antifungal activity and found that both the leaf extracts inhibited *Alternaria alternata*, *Fusarium oxysprum in vitro*, while old leaf extracts were effective against *Fusarium oxysporum.*

The essential oil of *Ocimum basilicum* at 1.5 ml/l completely inhibited the mycelial growth of 22 species of fungi including the mycotoxin producing species viz., *Aspergillus flavus* and *A. parasiticus* (Dube *et al.*, 1989).

Lakshmanan and Mohan (1989) reported that *in vitro* the water extracts of Garlic, *Bougainvillia* and *Azadirachta* were significantly effective in inhibitating the mycelial growth and sclerotia germination of *Thanatephorus cucumeris.*

Ajoene, a compound derived from garlic inhibited spore germination of *Alternaria solani, A. tenuissina, A. triticina, Alternaria* sp., *Collectotrichum* sp., *Curvularia* sp., *Fusarium lini*, *F. oxysporum*, *F. semitectum* and *F. udum* which cause serious diseases in some important crop plants in India (Singh *et al.*, 1990).

Volatile components of crude aqueous extracts of garlic bulbs inhibited germination of microconidia and hyphal extension of *Fusarium oxysporum* f. sp. *lycopersici* in axenic culture. (Tariq and Magee, 1990).

The effect of garlic, onion, ginger, *Ocimum sanctum* and *O. bacillicum* plant extracts on spore germination of *Phytophthora drechsleri* f. sp. *cajani* was studied by Chauhan and Singh (1991). Garlic and onion extracts were found good in inhibiting the spore germination at the concentration of 5000 and 10,000 ppm respectively.

Seethalakshmi (1991) reported that the plant extracts of Neem, Crotons, Parthenium and Bougainvillia were effective in controlling the growth of various fungi viz., *Colletotrictum musae*, *Botriodiplodia theobromae*, *Fusarium moniliforme, Gloeosporium musarum, Aspergillus niger, A. flavus* and *Rhizopus stolonifer*.

Tiwari *et al.* (1991) reported that the leaf extracts of *Piper beetle, Ocimum sanctum, Nyctanthes arboretritis* and *Citrus lemon* were effective in reducing the radial growth of *Pyricularia oryzae, Cochliobolus miyabeanus* and *Rhizoctonia solani in vitro*.

Yasmeen and Saxena (1992) reported that leaf extracts of *Diplazium esculantum* and *Microsporum punctatum* were effective in inhibiting the growth of *Rhizoctonia solani* and *Fusarium oxysporum* in chickpea seeds.

Aqueous extracts of fresh leaves and ethanolic extracts of dried and powdered leaves were made from eight plant species viz., *Cinnamomum zylanicum, Crysentia cuieta, Psidium guajava, Alangium salvifolium, Strychonos potatorum, S. nuxvomica, Thuja orientalis* and *Lagerstroemia* parviflora and antifungal activity was tested against six pathogens of rice viz., *Pyricularia oryzae, Rhizoctonia, Fusarium, Curvularia, Aspergillus*

flavus, and *A. niger* and the results were positive in many cases. The aqueous extracts of *P. guajava, S. potatorum, T. orientalis* and *L. parviflora* exhibited greater fungitoxic activity than the ethanolic extracts (Mishra *et al.*, 1992).

Antifungal activity of leaf extracts of *Azadirachta indica, Calotrophis gigantia, Catharanthus roseus, Eucalyptus sp., Parthenium hysterophorus* and *Pongamia pinnata* was tested against *Cerotelium fici* and *Cercospora mericola* causing leaf rust and leaf spot disease in mulberry respectively, *Azadirachta indica* was effective in inhibiting spore germination of *C. fici* by 91.2 per cent, whereas extracts of *Eukalyptus* sp. and *C. gigantia* proved highly toxic to *C. moricola* inhibiting conidial germination by 91.5 and 91.3 per cent respectively. Under field conditions also leaf extracts of *Eukalyptus* sp. and *C. gigantic* showed promising results. Extract of *P. pinnata* showed poor fungitoxicity against both the fungi tested (Sarvamangala *et al.*, 1993).

Singh *et al.* (1993) reported that the aqueous leaf extracts of medical plants viz., *Calotropis procera, Vitex negundo, Lantana camara, Azadirachta indica, Ficus religiosa, Ocimum sanctum, Thuja orientalis, Argemone mexicana, Achyranthes aspera, Datura fastuosa* and *Ricinus communis* were effective against *Botrydiplodia theobromae, Fusarium oxysporum, Helminthosporium specifera (Cochliobolus specifera), Curvularia lunata (Cochliobolus lunata), Aspergillus flavus* and *Trichothecium roseum*. Among the extracts *A. indica* and *O. sanctum* were most effective.

The fungitoxic effect of 15 plant extracts belonging to 12 families were tested against the wilt pathogen *Fusarium solani* by (Vimala *et al.,* 1993). Among the leaf extracts tested *Parthenium hysterophorus, Vinca rosea, Prosopis julifera, Lucaena leucocephala* and *Glyricidia maculate* showed recorded significantly lower wilt incidence of 26.66 to 62.22 per cent

The effect of 10 per cent alcoholic water extracts of fresh plant parts from twenty different species were studied on the development of powdery mildew (*Phyllactinia corylea*), leaf spot (*Pseudocercospora mori*) and leaf rust (*Cerotelium fici*) diseases in mulberry. Among these extract of *Adhatoda zeylanica* was most effective in decreasing the severity followed by extracts of *Azadirachta indica*, *Launea coromandelica* and *Oxalis corniculata* (Biswas *et al.,* 1995).

Gohil and Vala (1996) stated that the unsterilized phytoextracts of garlic (*A. sativum*) and soapnut (*S. trifoliate*) were found inhibitory to *Fusarium moniliforme*. The garlic extracts gave the best inhibition of *F. moniliforme* followed by soapnut extract, whereas all the sterilized phytoextracts failed to produce any inhibitory effect.

Jayasree (1996) reported that the leaf extracts of *Neem*, *Parthenium* and *Ocimum* were effective against *Colletotrichum gloeosporioides* and *Rhizoctonia* sp. isolated from pomegranate in both *in vitro* and *in vivo* studies.

Khan *et al.* (1996) stated that plant extracts of *Azadirachta indica, Calotropis procera, Cuscuta reflexa, Euphorbia pulcherrima* and *Solanum nigrum* were effective against *Rhizopus stolonifer, Colletotrichum dematium, Fusarium oxysporum* and *Macrophomina phaseolina* from chickpea seed.

Nagi Reddy (1996) tested the effect of ten plant extracts viz., *Azadirachta indica, Aloe vera, Strychonos nuxvomica, Ocimum sanctum, Allium sativum, Calotropis gigantia, Cassia auriculate, Parthenium hysterophorus, Datura metal* and *Sapindus indica* against *Colletotrichum gloeosporioides* and *Fusarium moniliforme*. Among them *Azadirachta indica, Aloe vera, Allium sativum* and *Datura metal* were found effective in both *in vivo* and *in vitro* studies.

Materials and Methods

CHAPTER - III

MATERIALS AND METHODS

The studies on seed mycoflora of bengalgram have been carried out during the year 1998-99 at S.V.Agricultural College, Tirupati. Laboratory and Pot culture studies were taken up in the Department of Plant Pathology, Sri Venkateswara Agricultural Collge, Tirupati, Chittoor District, Andhra Pradesh. Of the several methods of seed health testing used for detection of seed mycoflora, the agar plate method and standard blotter method (Neergaard and Saad, 1962) were employed in these investigations.

3. SELECTION OF SEED

For studying mycoflora associated with bengalgram seed ten varieties viz., Annigeri, K-850, JG-62, GL-769, ICCV-10, BG-276, DCP 92-3, KWR-108, IPC 94-19 and IPC 94-37 were used. Seed samples were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patencheru, A.P. and Indian Institute of Pulse Research, Kanpur, U.P.

3.1 LABORATORY TECHNIQUES

The general laboratory techniques followed during the investigations were according to those detailed by Rawlins (1933), Riker and Riker (1936) and Johnston and Booth (1983) with slight modifications whenever and wherever necessary for cleaning of glassware, preparation and sterilization of media, isolation and maintenance of fungal cultures, etc.

3.1.1 Cleaning of glassware

The glassware used in the experimental work was cleaned with washing soda and throughly rinsed in tap water, cleaning solution (Potassium dichromate + Sulphuric acid) was then poured in the glassware and kept for a day, after which it was drained off and cleaned thoroughly with several changes of tap water and finally with distilled water.

Cleaning solution for glassware :

Potassium dichromate	:	80.0 g
Sulphuric acid (Conc)	:	80.0 ml
Distilled water	:	1000 ml

3.1.2 Chemicals

The chemicals employed for all the experiments were of the analytical grade analar (BDH) or laboratory grade (Merck).

3.1.3 Water

In all the experimental studies double distilled water was used for both *in vitro* and *in vivo* studies.

3.1.4 Sterilization

Glassware viz., petriplates, pipettes, test tubes, etc. were sterilized at 160°C for 3 h in hot air oven. Potato Dextrose Agar (PDA) was autoclaved at 15 psi (121.6°C) for 15 minutes. Soils used for experimental work was sterilized at 20 psi (126.6°C) for one h. Blotter paper discs were sterilized in hot air oven at 60°C for 30 minutes after placing them one over the other in petridishes (9.5 cm) of Borosil make.

3.1.5 Surface sterilization of seed

Surface sterilization of seeds was done by 0.1 per cent mercuric chloride for 30 seconds. The seed was then washed with three changes of sterile water.

3.1.6 Media used

For isolation of seed mycoflora, the following media were employed.

a. Potato Dextrose Agar (PDA)

Potato	-	200.00 g
Dextrose	-	20.0 g
Agar	-	20.0 g
Water	-	1000.0 ml

b. Plain agar

Agar	-	20.0 g
Distilled water	-	1000.0 ml

3.2 ISOLATION OF SEED BORNE FUNGI

For isolation of seed borne fungi associated with bengalgram seed, the two standard methods viz., (1) Agar plate method (2) Standard blotter method (Neergaad and Saad, 1962) were employed.

3.2.1 Agar plate method

To isolate externally seed borne fungi of bengalgram, 50 seeds of each variety were taken and placed at the rate of 5 seeds in each petriplate containing Potato Dextrose Agar medium. Ten replications were maintained for each variety. The petriplates were incubated at 28 ± 1°C in the incubator for 7 days and observed every day for the development of fungi.

3.2.2 Blotter method (Neergard and Saad, 1962)

Two circular oven sterilized filter papers were placed in each of the petriplates and moistioned with sterile water ensuring that a slight excess of water was left on the filter paper. Totally 50 seeds from each variety were taken and 5 seeds per plate were placed. Then the petriplates were incubated in the incubator at 28 ± 1° C for 7 days and observed every day for the development of fungi.

The fungal colonies which were obtained by the above two methods were counted with the help of a stereobinocular microscope and the fungal hyphae were picked up from the seed and pure culture were maintained on agar slants.

The various fungal cultures obtained were identified by comparing with illustrations given by Barnett (1962), Gilman (1957) and Subramanian (1971). For identification of *Aspergillus* cultures, the *Aspergillus* manual by Thom and Raper (1945) was consulted.

3.3 EFFECT OF SEED BORNE FUNGI ON SEED GERMINATION *in vivo*

The various fungal isolates obtained by the above methods were tested for their effect on germination *in vivo*. For this purpose, variety K-850 was used.

3.3.1 Pot culture method

Conidial suspensions of different fungal isolates multiplied on PDA were taken. In that 30 surface sterilized seeds were soaked for 24 h. The inoculated seeds were then sown in lots of 10 in each pot filled with sterilized

soil. Controls with 10 uninoculated seeds were maintained. Three replications were maintained for each variety. The pots were watered daily with sterile water. Observations were recorded for germination upto 15 days. On the 15th day, seedlings were carefully uprooted, washed free of soil and the shoot and root lengths were recorded.

3.3.2 Preparation of conidial suspension

A bit of 7 day old culture of each fungus maintained on PDA medium was taken out with an inoculation needle and transferred to about 10 ml sterile water in a test tube. A spore load of 15 to 20 spores per low power microscopic field was maintained by adopting serial dilution plate technique.

3.4 EVALUATION OF DIFFERENT FUNGICIDES AND PLANT EXTRACTS ON SEED BORNE FUNGI *in vitro*

For this study, fungi which reduced both shoot and root length of bengalgram drastically, were selected. The effect of five fungicides viz., Captan, carbendazim, Calixin, metalaxyl and Thiram and five plant extracts viz., *Azadirachta indica, Mirabilis jalapa, Phyllanthus neruri, Solanum nigrum* and *Tamarindus indicus* were evaluated against the three test fungi viz., *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp. by poison food technique (Nene and Thapliyal, 1993).

3.4.1 Preparation of concentration of fungicides

For obtaining 1 ppm concentration 1 mg of pure solute/active ingredient (100 per cent pure chemical) was dissolved in one litre of water. The five fungicides selected were studied at three concentrations viz., 500, 1000 and 1500 ppm.

3.4.2 Poison food technique using fungicides

Potato Dextrose Agar (PDA) medium was prepared double the recommended strength and sterilized in an autoclave. A double strength PDA medium contains double the concentration of potato, dextrose and agar except water. For each fungicide, fungicidal solution was prepared double the test concentration, 10 ml of fungicidal solution was mixed throughly with 10 ml molten PDA medium in a sterilized petriplates under aseptic conditions and allowed for 5 - 10 min to solidify. Appropriate controls were maintained with distilled water. From seven day old culture plates 1.0 cm discs were cut from outer margin of vigorously growing fungus with sterilized cork borer and transferred to the centre of plates containing fungicidal medium. Controls were maintained by placing fungal discs in untreated plates. Three replications were maintained for each treatment. The whole procedure was carried out under aseptic conditions. The petriplates were then incubated in an incubator at 26 ± 1°C. The diameter of fungal colony was measured when the growth of the fungus in control was completely filled in the petriplates. The colony diameter in treated plates compared with control, was taken as measure of fungitoxicity. The growth of fungal colony in treated plates was determined by excluding 1.0 cm fungal inoculum disc from measured diameter of colony. The per cent inhibition was calculated by the following formula.

$$\text{Per cent inhibition} = \frac{\text{Diameter of colony in control plate} - \text{Diameter of colony in trated plate}}{\text{Diameter of colony in control plate}} \times 100$$

3.4.3 List of fungicides used *in vitro*

S.No.	Trade Name of the Fungicides	Chemical Name	Concentration (ppm)
1.	Captan 75 WP	N - Trichloromethyl thio-4-cyclohexane 1,2 dicarboximide	500 1000 1500
2.	Carbendazim 50 WP	Methyl - 2- benzimidazole Carbamate	500 1000 1500
3.	Calixin	N - tridecyl 2,6 dimethyl morpholine	500 1000 1500
4.	Metalaxyl	N (2,6 Dimethyl phenyl) N (Methoxy acetyl) - alanine methyl ester	500 1000 1500
5.	Thiram	Tetramethyl thiuram disulphide	500 1000 1500

3.4.4 Preparation of plant extracts

Five plant extracts were used in the experiment. The plant extract was prepared by grinding 1 g of the leaf material in mortar and pestle after adding 100 ml of distilled water and filtered through double layered muslin cloth.

3.4.5 Poison food technique using plant extracts

Similar procedure was adopted under poison food technique previously described. In place of fungicidal solution, plant extract was used.

4.6 List of plant extracts used *in vitro*

S.No.	Plant species	Concentration (%)	Plant part used
1.	*Azadirachta indica*	1.0	Leaf
2.	*Mirabilis jalapa*	1.0	Leaf
3.	*Phyllanthus neruri*	1.0	Leaf
4.	*Solanum nigrum*	1.0	Leaf
5.	*Tamarindus indicus*	1.0	Leaf

Results

CHAPTER - IV

EXPERIMENTAL RESULTS

4.1 Incidence of mycoflora on different varieties of Bengalgram Seed (Agar plate method)

To study the incidence of seed mycoflora on different varieties of bengalgram, 10 varieties viz., Annigeri, K-850, JG-62, GL-769, ICCV-10, BG-276, DCP 92-3, KWR-108, IPC 94-19 and IPC 94-37 were collected from International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patencheru, A.P. and Indian Institute of Pulse Research, Kanpur, U.P. and used to find out the incidence of mycoflora by agar plate method. The results are presented in Table 1.

The unsterilized seeds yielded more number of fungi than sterilized seeds in all the 10 varieties. Among the varieties K-850, Annigeri and ICCV-10 showed maximum number of 10 fungal species followed by GL-769, and JG-62 (9 fungal species in each variety). Rest of the varieties viz., BG-276, DCP 92-3, KWR-108, IPC 94-19 and IPC 94-37 yielded 8 fungal species. There was considerable difference in the number of fungi isolated from different varieties.

Among the fungi associated with bengalgram seeds, *Aspergillus niger* was the predominant in all the varieties to a great extent (54.6 per cent). The percentage of unsterilized seeds colonized by *A. niger* was 51.0 in Annigeri, 46.0 in K-850, 40.0 in JG-62, 37.0 in GL-769, 33.0 in ICCV-10, 34.0 in BG-276, 28.0 in DCP 92-3, 35.0 in KWR-108, 26.0 in IPC 94-19 and 30.0 in IPC 94-37.

TABLE 1. Incidence of seed mycoflora on different varieties of Bengalgram (Agar plate method)

S.No.	Name of the Fungus	Annigeri		K-850		JG-62		GL-769		ICCV-10		BG - 276		DCR-92-3		KWR-108		IPC-94-19		IPC-94-37	
		S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US
1.	*Alternaria sp.*	11	27	19	31	8	16	7	13	9	18	9	12	4	18	5	8	10	28	12	17
2.	*Aspergillus flavus*	21	48	27	43	18	27	19	22	15	47	13	38	16	45	11	32	12	21	18	32
3.	*Aspergillus niger*	38	51	28	46	15	40	12	37	18	33	17	34	16	28	14	35	11	26	17	30
4.	*Ascochyta rabiei*	17	21	12	26	12	16	11	14	9	10	5	7	13	21	5	16	12	27	8	23
5.	*Botrytis cinerea*	5	19	12	21	11	27	9	13	7	12	9	18	5	7	8	12	5	9	6	17
6.	*Colletotrichum* sp.	4	12	8	20	12	18	--	6	5	11	--	--	--	5	--	--	--	--	--	--
7.	*Curvularia lunata*	4	12	18	26	12	18	--	6	5	7	--	5	--	--	--	3	--	7	--	--
8.	*Fusarium* sp.	9	19	10	23	9	17	5	17	9	28	7	12	5	9	6	12	7	21	11	19
9.	*Penicillium* sp.	5	8	7	16	--	--	--	--	3	6	--	--	-	--	--	3	--	--	--	5
10.	*Rhizopus stolonifer*	7	16	14	19	4	12	6	8	12	17	9	21	13	24	8	12	6	10	13	18

S : Sterilized

US : Unsterilized

(All figures are percentage average of ten replications)

In case of sterilized seeds there was reduction in percentage of mycoflora. *Aspergillus flavus* was second in order of predominance in bengalgram seeds (52.5 per cent) and found to be associated to an extent of 48.0 per cent in Annigeri, 43.0 in K-850, 27.0 in JG - 62, 22.0 in GL - 769, 47.0 in ICCV-10, 38.0 in BG - 276, 45.0 in DCP 92-3, 32.0 in KWR-108, 21.0 in IPC 94-19 and 32.0 in IPC 94-37.

Ascochyta rabiei was observed on seeds to an extent of 21.0 per cent in Annigeri, 26.0 in K-850, 16.0 in JG-62, 14.0 in GL-769, 10.0 in ICCV-10, 7.0 in BG-276, 21.0 in DCP 92-3, 16.0 in KWR-108, 27.0 in IPC 94-19 and 23.0 in IPC 94-37.

Alternaria sp., was found to be associated with seed to an extent of 27.0 per cent in Annigeri, 31.0 in K-850, 16.0 in JG-62, 13.0 in GL-769, 18.0 in ICCV-10 12.0 in BG-276, 18.0 in DCP-92-3, 8.0 in KWR-108, 28.0 in IPC 94-19 and 17.0 in IPC 94-37.

Fusarium sp., was observed on seeds to an extent of 19.0 in Annigeri, 23.0 in K-850, 17.0 in JG-62, 17.0 in GL-769, 28.0 in ICCV-10, 12.0 in BG-276 9.0 in DCP 92-3, 12.0 in KWR-108, 21.0 in IPC 94-19 and 19.0 in IPC 94-37.

Botrytis cinerea colonized 19.0 per cent in Annigeri, 21.0 in K-850, 27.0 in JG-62, 13.0 in GL-769, 12.0 in ICCV-10, 18.0 in BG-276, 7.0 in DCP 92-3, 12.0 in KWR-108, 9.0 in IPC 94-19 and 17.0 in IPC 94-37.

Rhizopus stolonifer was yielded to an extent of 16.0 per cent in Annigeri, 19.0 in K-850, 12.0 in JG-62, 8.0 in GL-769, 17.0 in ICCV-10, 21.0 in BG-276, 24.0 in DCP 92-3, 12.0 in KWR-108, 10.0 in IPC 94-19 and 18.0 in IPC 94-37.

Curvularia lunata was found to be associated with seed to an extent of 12.0 per cent in Annigeri, 26.0 in K-850, 18.0 in JG-62, 6.0 in GL-769, 7.0 in ICCV-10, 5.0 in BG-276, 3.0 in KWR-108 and 7.0 in IPC 94-19.

Colletotrichum sp. was yielded to an extent of 12.0 per cent in Annigeri, 20.0 in K-850, 18.0 in JG-62, 6.0 in GL-769 11.0 in ICCV-10 and 5.0 in DCP 92-3.

There was no significant colonization of *Penicillium* sp. in all the varieties in both sterilized and unsterilized seeds.

4.2 Incidence of mycoflora on different varieties of Bengalgram seed (Blotter paper method)

Qualitatively similar fungi were obtained in both these methods (Agar and Blotter methods), but quantitatively agar plate yielded more number of fungi than blotter method (Table 2).

In blotter method also all the ten varieties yielded maximum colonies of *Aspergillus niger*. The percentage of seed colonized by *A. niger* was 49.0 in Annigeri, 38.0 in K-850, 32.0 in JG-62, 30.0 in GL-769, 34.0 in ICCV-10, 26.0 in BG-276, 24.0 in DCP 92-3, 33.0 in KWR-108, 24.0 in IPC 94-19 and 37.0 in IPC 94-37.

Aspergillus flavus was second in order of predominance among the fungi associated with the seed. The distribution of percentage of *Aspergillus flavus* in different varieties was 34.0 in Annigeri, 32.0 in K-850, 24.0 in JG-62, 27.0 in GL-769, 36.0 in ICCV-10, 33.0 in BG-276, 39.0 in DCP 92-3, 27.0 in KWR-108, 28.0 in IPC 94-19 and 31.0 in IPC 94-37.

TABLE 2. Incidence of Seed mycoflora on different varieties of Bengal gram (Blotter paper method)

S.No.	Name of the Fungus	Angeri		K-850		JG-62		GL-769		ICCV-10		BG - 276		DCP 92-3		KWR-108		IPC 94-19		IPC 94-37	
		S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US
1.	*Alternaria sp.*	7	21	13	28	7	13	5	12	6	10	3	8	4	12	3	5	--	5	--	6
2.	*Aspergillus flavus*	21	34	19	32	13	24	19	27	13	36	12	33	12	39	9	27	11	28	18	31
3.	*Aspergillus niger*	24	49	20	38	12	32	10	30	16	34	11	26	17	24	9	33	11	24	15	37
4.	*Ascochyta rabiei*	5	7	10	13	6	11	8	12	9	18	6	15	--	7	--	--	--	5	5	9
5.	*Botrytis cinerea*	--	5	8	12	4	5	9	11	4	6	5	9	5	13	9	12	--	--	--	--
6.	*Colletotrichum sp.*	--	--	9	19	5	11	--	--	5	7	--	--	--	--	--	--	--	--	--	--
7.	*Curvularia lunata*	5	7	5	10	--	--	--	--	--	--	--	--	--	--	5	9	--	7	--	--
8.	*Fusarium sp.*	7	12	8	14	11	18	5	7	5	14	7	16	5	9	--	7	--	--	5	12
9.	*Penicillium sp.*	--	--	5	7	--	--	--	--	--	5	--	9	-	--	3	5	--	--	--	--
10.	*Rhizopus stolonifer*	6	14	12	17	4	12	6	17	5	11	4	8	5	7	--	5	--	--	--	--

S : Sterilized

US : Unsterilized
(All figures are percentage average of ten replications)

The percentage of *Alternaria* sp. was 21.0 in Annigeri, 28.0 in K-850, 13.0 in JG-62, 12.0 in GL-769, 10.0 in ICCV-10, 8.0 in BG-276, 12.0 in DCP 92-3, 5.0 in KWR-108, 5.0 in IPC 94-19 and 6.0 in IPC 94-37.

Ascochyta rabiei was observed on seeds to an extent of 7.0 per cent in Annigeri, 13.0 in K-850, 11.0 in JG-62, 12.0 in GL-769, 18.0 in ICCV-10, 15.0 in BG-276, 7.0 in DCP 92-3, 5.0 in IPC 94-19 and 9.0 in IPC 94-37. Variety KWR-108 did not yield *Ascochyta rabiei*.

Fusarium sp. was found to be associated with 12.0 per cent in Annigeri, 14.0 in K-850, 18.0 in JG-62, 7.0 in GL-769, 14.0 in ICCV-10, 16.0 in BG-276, 9.0 in DCP 92-3, 7.0 in KWR-108 and 12.0 in IPC 94-37. IPC 94-19 did not yield *Fusarium* sp.

Botrytis cinerea was observed on seeds to an extent of 5.0 per cent in Annigeri, 12.0 in K-850, 5.0 in JG-62, 11.0 in GL-769, 6.0 in ICCV-10, 9.0 in BG-276, 13.0 in DCP 92-3 and 12.0 in KWR 108.

Rhizopus stolonifer was found to be associated to an extent of 14.0 per cent in Annigeri, 17.0 in K-850, 12.0 in JG-62, 17.0 in GL-769, 11.0 in ICCV-10, 8.0 in BG-276, 7.0 in DCP 92-3 and 5.0 in KWR-108. Two varieties viz., IPC 94-13 and IPC 94-37 did not yield *Rhizopus stolonifer*.

The occurrence of *Penicillium* sp., *Curvalaria lunata* and *Colletotrichum* sp. was minimum and the percentage of mycoflora ranged from 3.4 to 5.6.

4.3 Comparison of agar plate method and blotter paper method on the incidence of mycoflora

In the present investigations, both agar plate and blotter methods were employed to findout the effective one for obtaining large number of mycoflora both from sterilized and unsterilized seeds of bengalgram. The data obtained are presented in Table 3 (Fig. 1).

Out of the two methods tested, agar plate method yielded more number of fungi than blotter methods (Plate I). From the data it is evident that in both the methods, the incidence of mycoflora was more in the unsterilized seeds than in the sterilized seeds.

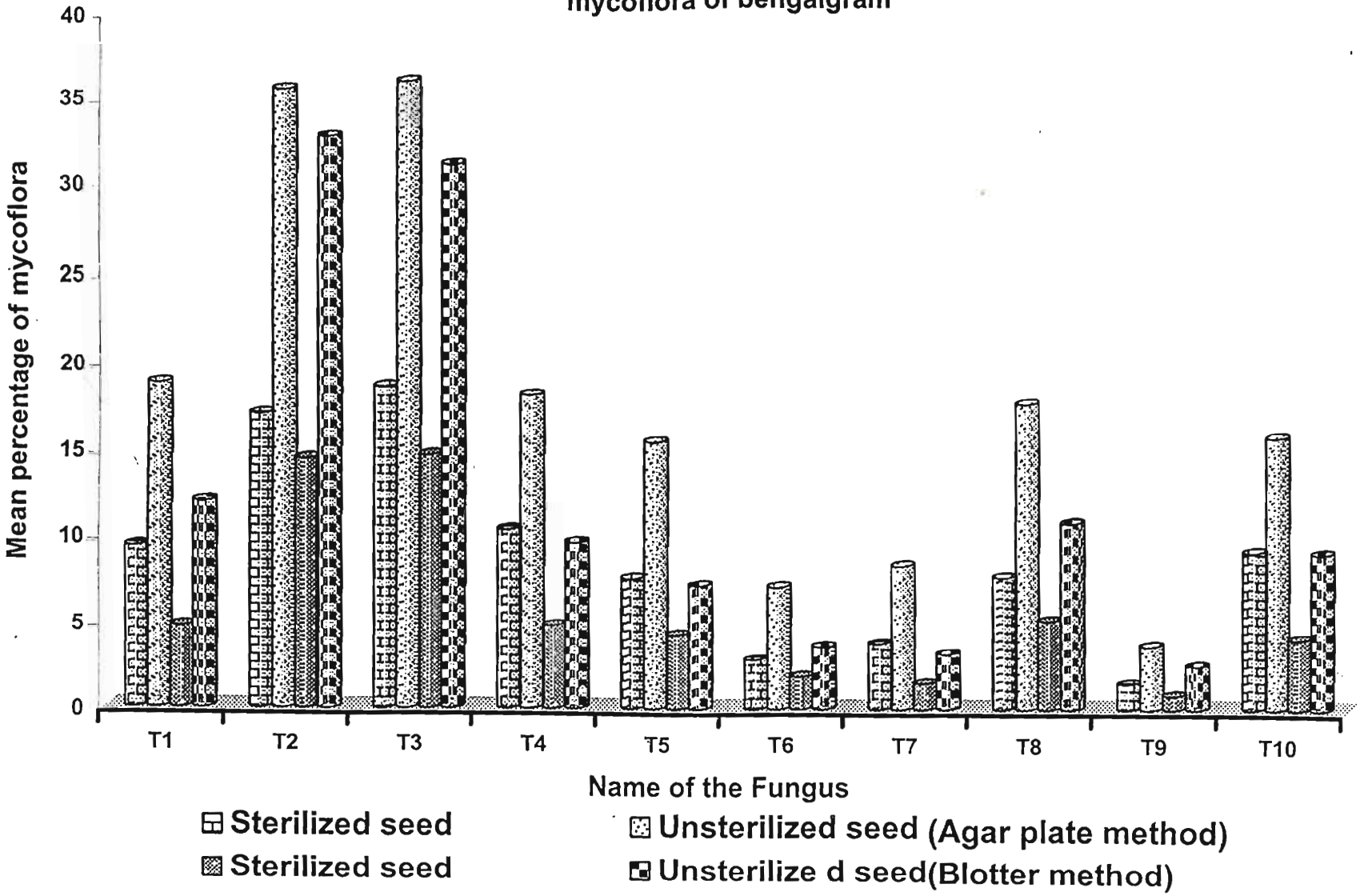
Among the fungi isolated, *Aspergillus niger* occurred abundantly in both sterilized (18.6 per cent) and unsterilized (36.0 per cent) seed in agar plate method, while in blotter method the percentage of colonies of *Aspergillus niger* were 14.7 and 31.1 per cent in sterilized and unsterilized seed respectively. Agar plate method recorded higher incidence of *Aspergillus niger* compared to blotter method.

The fungi encounted in agar plate method in unsterilized seeds were *Aspergillus flavus* (35.5 per cent), *Alternaria* sp. (18.8 per cent), *Ascochyta rabiei* (18.1 per cent), *Fusarium* sp. (17.7 per cent), *Rhizopus stolonifer* (15.7 per cent), *Botrytis cinerea* (15.5 per cent), *Curvularia lunata* (8.4 per cent) *Colletotrichum* sp. (7.2 per cent) and *Penicillium sp.* (3.8 per cent) in the order of predominance.

TABLE 3. Comparison of agar plate method and blotter method on the incidence of mycoflora of bengalgram

S.No.	Name of the Fungus	Agar plate method Mean percentage of mycoflora		Blotter paper method Mean percentage of mycoflora	
		Sterilized seed	Unsterilized seed	Sterilized seed	Unsterilized seed
1.	*Alternaria sp.*	9.4	18.8	4.8	12.0
2.	*Aspergillus flavus*	17.0	35.5	14.5	32.7
3.	*Aspergillus niger*	18.6	36.0	14.7	31.1
4.	*Ascochyta rabiei*	10.4	18.1	4.9	9.7
5.	*Botrytis cinerea*	7.7	15.5	4.4	7.3
6.	*Colletotrichum* sp.	2.9	7.2	1.9	3.7
7.	*Curvularia lunata*	3.9	8.4	1.5	3.3
8.	*Fusarium* sp.	7.8	17.7	5.3	10.9
9.	*Penicillium* sp.	1.5	3.8	0.8	2.6
10.	*Rhizopus stolonifer*	9.2	15.7	4.2	9.1

Fig.1 Comparison of agar plate and blotter methods on the incidence of mycoflora of bengalgram



T1: Alternaria sp. T2: Aspergillus flavus T3 : Aspergillus niger T4: Ascochyta rabiei T5: Botrytis cinerea T6: Colletotrichum sp. T7: Curvularia lunata T8: Fusarium sp. T9: Penicillium sp. T10: Rhizopus stolonifer

Plate I

In surface sterilized seeds also *Aspergillus niger* was the most predominant one (18.6 per cent) followed by *Aspergillus flavus* (17.0 per cent), *Ascochyta rabiei* (10.4 per cent), *Alternaria* sp. (9.4 per cent), *Rhizopus stolonifer* (9.2 per cent), *Fusarium* sp. (7.8 per cent), *Botrytis cinerea* (7.7 per cent), *Curvularia lunata* (3.9 per cent), *Colletotrichum* sp. (2.9 per cent) and *Penicillium* sp. (1.5 per cent).

In blotter method both sterilized and unsterilized seed yielded less number of colonies of fungi compared to agar plate method.

4.4 SYSTEMATIC ACCOUNT OF DIFFERENT SEED BORNE FUNGI

A brief morphological and taxonomic account of fungi detected from 10 Bengalgram varieties are given below.

4.4.1 *Aspergillus niger*

On PDA medium the growth of the fungus was rapid and the fungal colonies were blackish brown to almost black, mycelium partly superficial and hyphae were colourless.

Conidiophores mostly erect, straight, 1309.44 - 2301.75 (1720.47) µm long, 15.34-20.46 µm thick, enlarged into a spherical visicle at the tip 38.13-112.12 (78.23) µm thick in diameter.

Vesicle covered by closely packed clavate branches, 15.6-28.5 µm long 4.5-6.2 µm thick, phialides borne at the apex of the branches, flask shaped 7.5-10.2 µm long, 3.2-4.0 µm thick. Conidia borne in chains, globose,

echinulate, brown 2.98-3.98 (3.58) µm diameter. The above morphological characters are in line in those mentioned by Thom and Raper (1945) and Subramanian (1971) for *A. niger* (Plate II).

4.4.2 *Aspergillus flavus*

Fungal colonies on PDA medium were yellowish green in the early stages later altered by the disappearance of the green factor leaving shades of yellow brown. Conidial heads white in the formative phase, later turning to greenish in appearance.

Conidiophores arising from submerged hyphae 265.98 - 593.34 (459.12) µm long, 10.23 - 15.34 (11.66) µm thick, walls pitted, slightly rough, broadening upwards and enlarging into vesicle at the tip, 28.05 - 51.10 (38.17) µm in diameter dome like in shape.

Phialides borne directly on the vesicle, 9.1 - 12.1 µm long 3.3 - 5.6 µm thick. Conidia globose colourless to yellowish green, 3.01 - 6.01 (4.59) µm in diameter marked with pits and echinulated. The morphology of the fungus described above are in conformity in those described by Thom and Raper (1945) and was identified as *A. flavus* (Plte III & IV).

4.4.3 *Alternaria* sp.

Colonies usually olivaceous black on seeds. Conidiophores arising slightly at irregular intervals on hyphae, simple, straight or flexous, smooth with rounded apex, pale to olivaceous brown, 2-9 septate, 26.92 - 105.43 (56.47) µm long, 3.38 - 4.48 (4.35) µm thick.

Plate II : *Aspergillus niger* showing conidiophores, vesicles, sterigmata and conidia

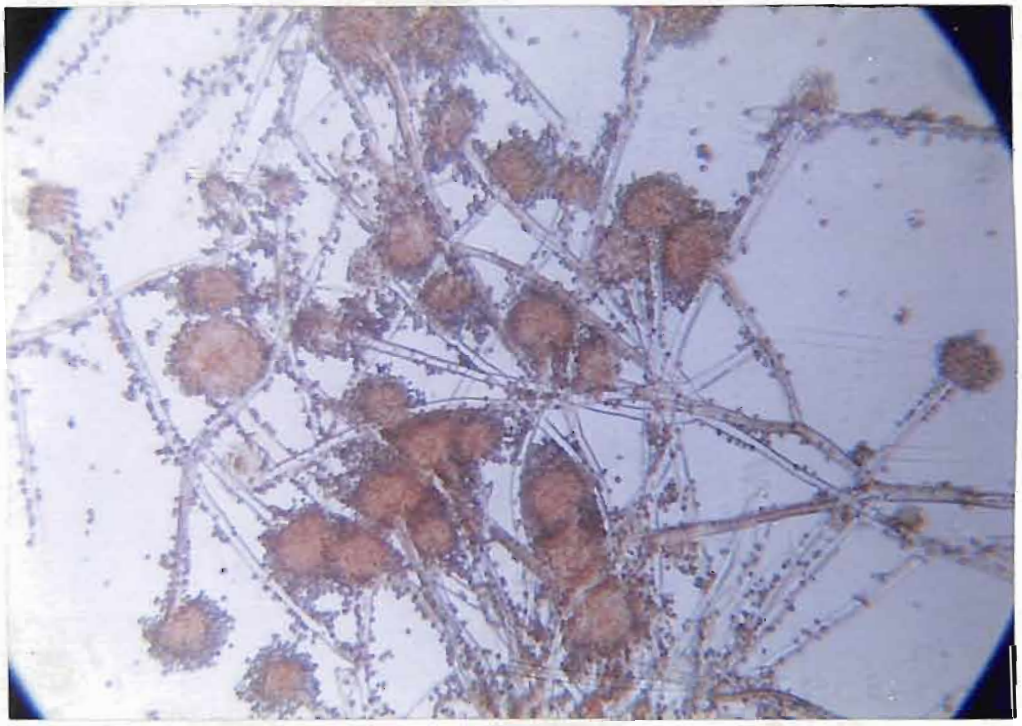


Plate II

Plate III : *Aspergillus flavus* showing conidiophores, vesicles, sterigmata and conidia (in low power)

Plate IV : *Aspergillus flavus* showing conidiophores, vesicles, sterigmata and conidia (in high power)

Plate III

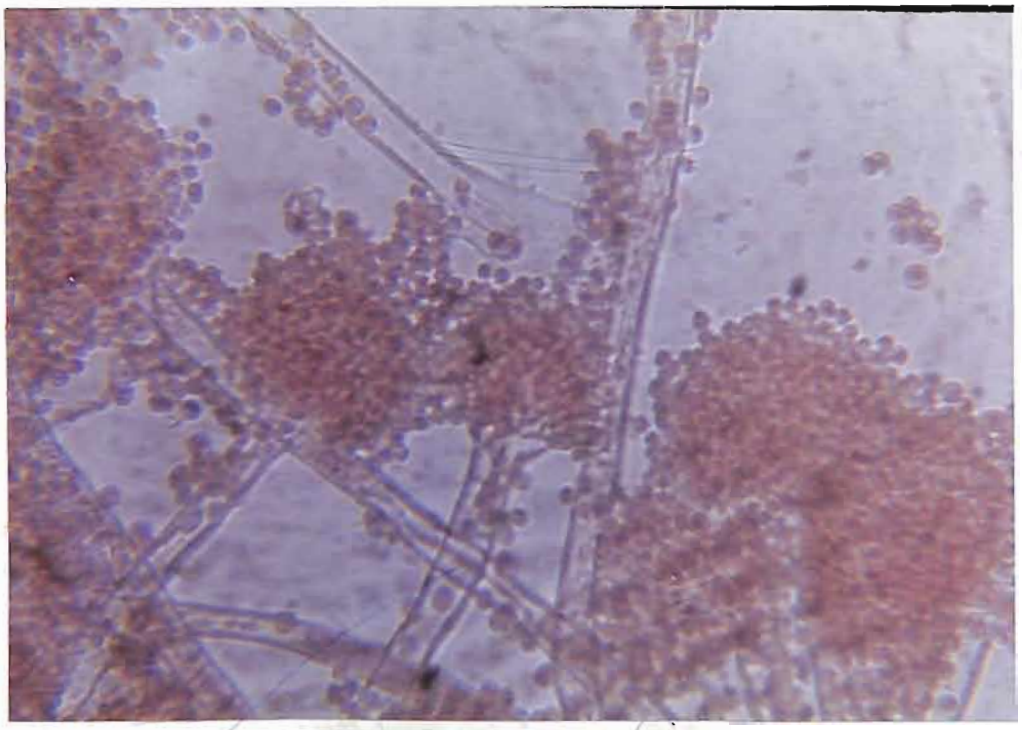


Plate IV

Plate VII : Conidia of *Botrytis cinerea*

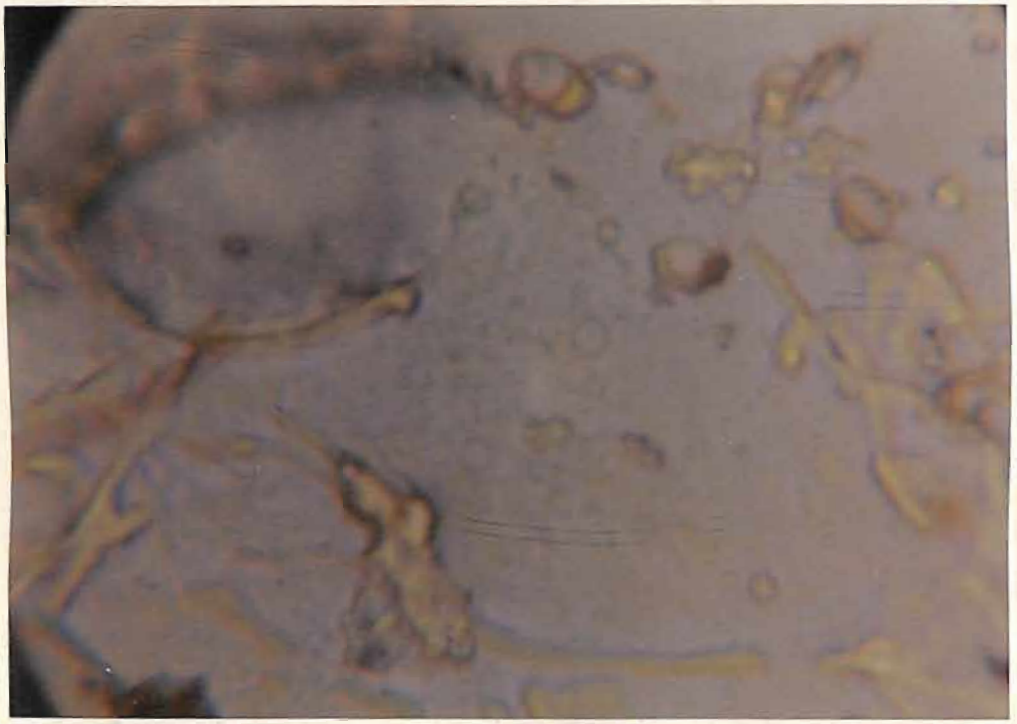


Plate VII

Conidia formed in long branched chains, obclavate, ovoid or ellipsoidal, often with a conical or cylindrical beak, 3-7 transverse septa with 1-2 longitudinal or oblique septa, 30.28 - 45.42 (36.63) µm long, 12.11 - 15.14 (13.62) µm thick (Plate V). The above features were similar to the characters described by Subramanian (1971).

4.4.4 *Ascochyta rabiei*

Conidia are hyaline, 1-2 celled, biguttulate, straight or slightly curved, oval to oblong, rounded at each end, and 6-16 x 3.4 - 5.6 µm. Most conidia are unicellular, and they may or may not be constricted at the septum (Plate VI).

The description of the conidia is in line with the conidia of *Ascochyta rabiei* described by Punithalingam and Holliday (1972) and Haware *et al.* (1986).

4.4.5 *Botrytis cinerea*

The colonies are white to light grey to greenish brown, spreading short distances around the infected seed, conidiophores are the seen at the tips and at intervals along the hyphae, appear to raise as pegs on the swollen ends of the hyaline branches. They are light grey nearly 2.0 mm long, 16-30 µm thick, and dichotomously branched. Conidiophores are brown at the base, becoming light brown towards the tip, with the ends of the branches often colourless, and bearing conidia in clusters. Conidia are produced on short denticles at the tips of the conidiophores. They are usually single celled, occasionally ellipsoidal or obovoidal. epiculate at the base, colourless to pale

Plate V : Conidia of *Alternaria* sp. showing both transverse and longitudinal septa

Plate VI : Conidia of *Ascochyta rabiei* (some are bicelled)

Plate V

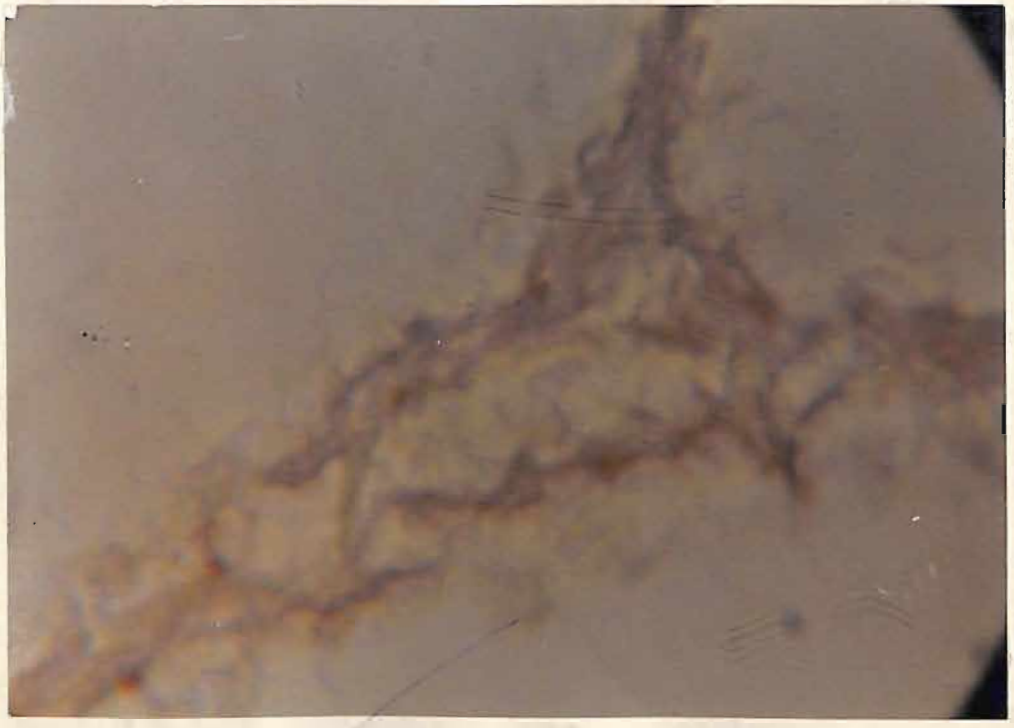


Plate VI

Plate VIII : Sickla shaped, septate macro conidia of *Fusarium* sp. (in low power)

Plate IX : Sickla shaped, septate macro conidia of *Fusarium* sp. (in high power)

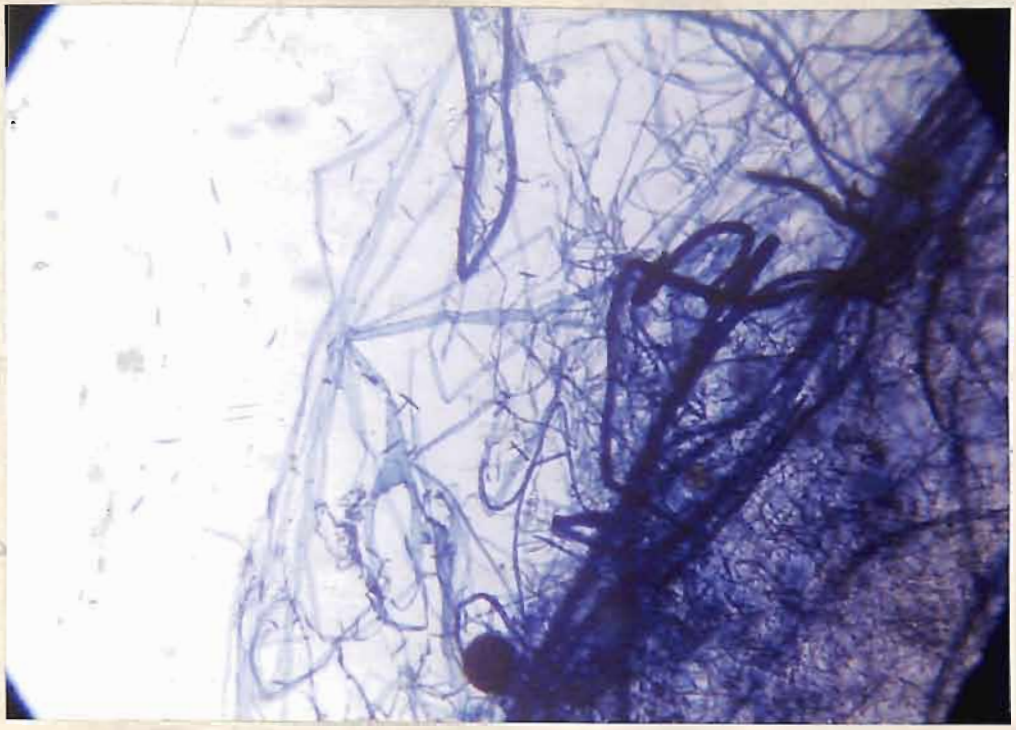


Plate VIII

Plate IX

(759.06) µm long, 10.23 - 30.69 (19.84) µm thick. Sporangia globose, olivaceous, with columella and hemispherical. Sporangiophore straight 3.51 - 8.02 (6.09) µm long, 3.51 - 6.01 (4.78) µm thick (Plate X). The morphological features of this isolate agreed with that of *Rhizopus stolonifer* (Gilman, 1957).

4.4.8 *Curvularia lunata*

Colonies on seed dark brown, stromatic with erect, stiff conidiophores and dark greyish horny stroma. Stroma long, often branched consisting of dark brown, compact, thick walled pseudoparenchymatous tissue, 460.35 - 2352.9 (1359.56) µm long, 132.91 - 255.75 (184.54) µm thick.

Conidia clavate, mostly curved, sometimes straight, 3 septate, smooth, third cell from base larger and darker, end cells hyaline, basal cell broadly conical, apical cell spherical, 15.92 - 31.84 (25.56) µm long, 7.96 - 14.93 (8.56) µm thick (Plate XI). The above features were similar to the characters described by Subramanian (1971) and hence that the species was identified as *Curvularia lunata*.

4.4.9 *Collectotrichum* sp.

The fungus on PDA appeared after 4 days with white fluffy growth and turned to light dark brown in colour. Mycelium was hyaline, branched, septate with oil globules. The conidia were on well developed hyaline conidiophores. Conidia are cylindrical with rounded ends. Some conidia were tapering towards one end measuring 7.67 - 19.52 µm in length and 3.75 - 5.70 µm in width (Mean 13.54 x 4.73 µm) with two oil globules (Plate XII).

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Plate X : *Rhizopus stolonifa* showing sporangiophore and sporangia

Plate XI : Conidiophore and conidia of *Curvularia lunata*

Plate X

3554

Plate XI

4.4.10 *Penicillium* sp.

Colonies on PDA are smooth velvety, dull yellowish green, vegetative hyphae coarse, thin walled, 4-15 (8-10) µm wide. Conidiophores coarse, comparatively short, arising from submerged hyphae or from basal mycelial felt, smooth, 30-100 x 4-5µ. Metulae variable in form and dimensions, commonly ranging from 15-30 x 4-6µ and bearing philalides invariable but always limited numbers. Phialides equally variable, 15-28 x 3.5 - 5.0 µ, producing chains of elliptical conidia. Conidia smooth, dull dark green in mass, varying in form and dimensions commonly 3.5 - 5.0 µm x 3.0 - 3.5 µm (Plate XIII).

The characters described above are in line with the characters described by Subramanian (1971).

4.5 EFFECT OF SEED-BORNE FUNGI ON SEED GERMINATION

The results (Table 4 and Fig. 2) indicated that the percentage germination of seeds was significantly reduced by some fungi.

There was gradual decrease in percentage germination of seeds soaked in spore suspensions over control. *Alternaria* sp. recorded 59.0 percent followed by *Ascochyta rabiei* (57.0 per cent) and *Fusarium* sp. (55.0 per cent) (Plates XIV, XV and XVI) which were on par with each other and significantly superior to the rest. Considerable reduction of germination percentage was noted in seeds soaked in spore suspension of *Botrytis cinerea* (46.0 per cent) and *Colletotrichum* sp. (38.0 per cent) and there was less

Plate X : ~~*Rhizopus stolonifa*~~ Colletotrichum sp. showing ~~sporangiophore~~ Conidia and sporangia

Plate XI : Conidiophore and conidia of ~~*Curvularia lunata*~~ Penicillium sp.

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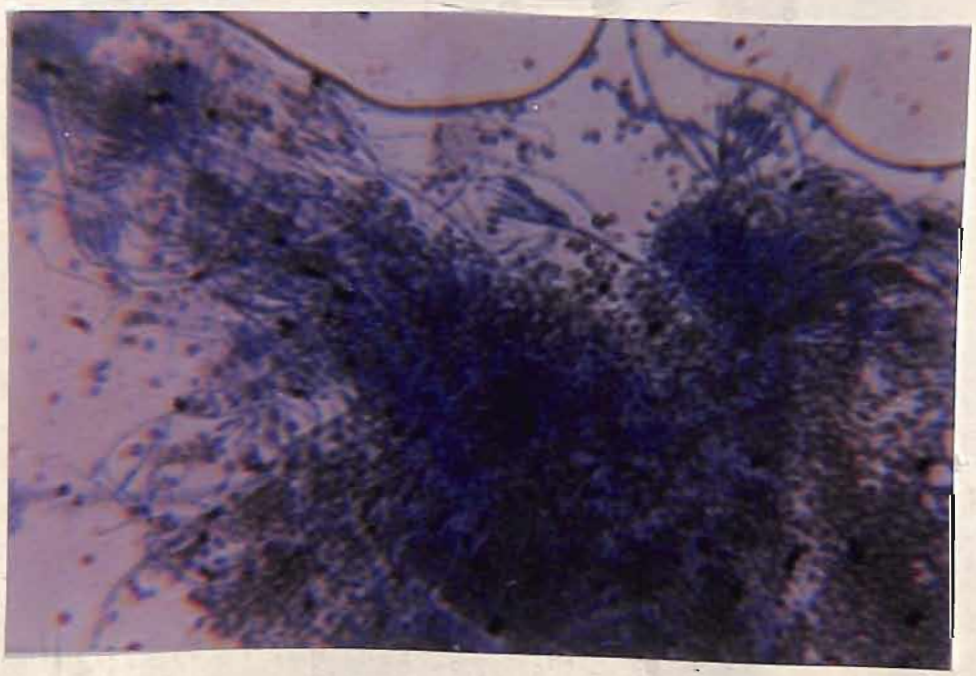


TABLE 4. Effect of seed borne mycoflora on germination of bengalgram

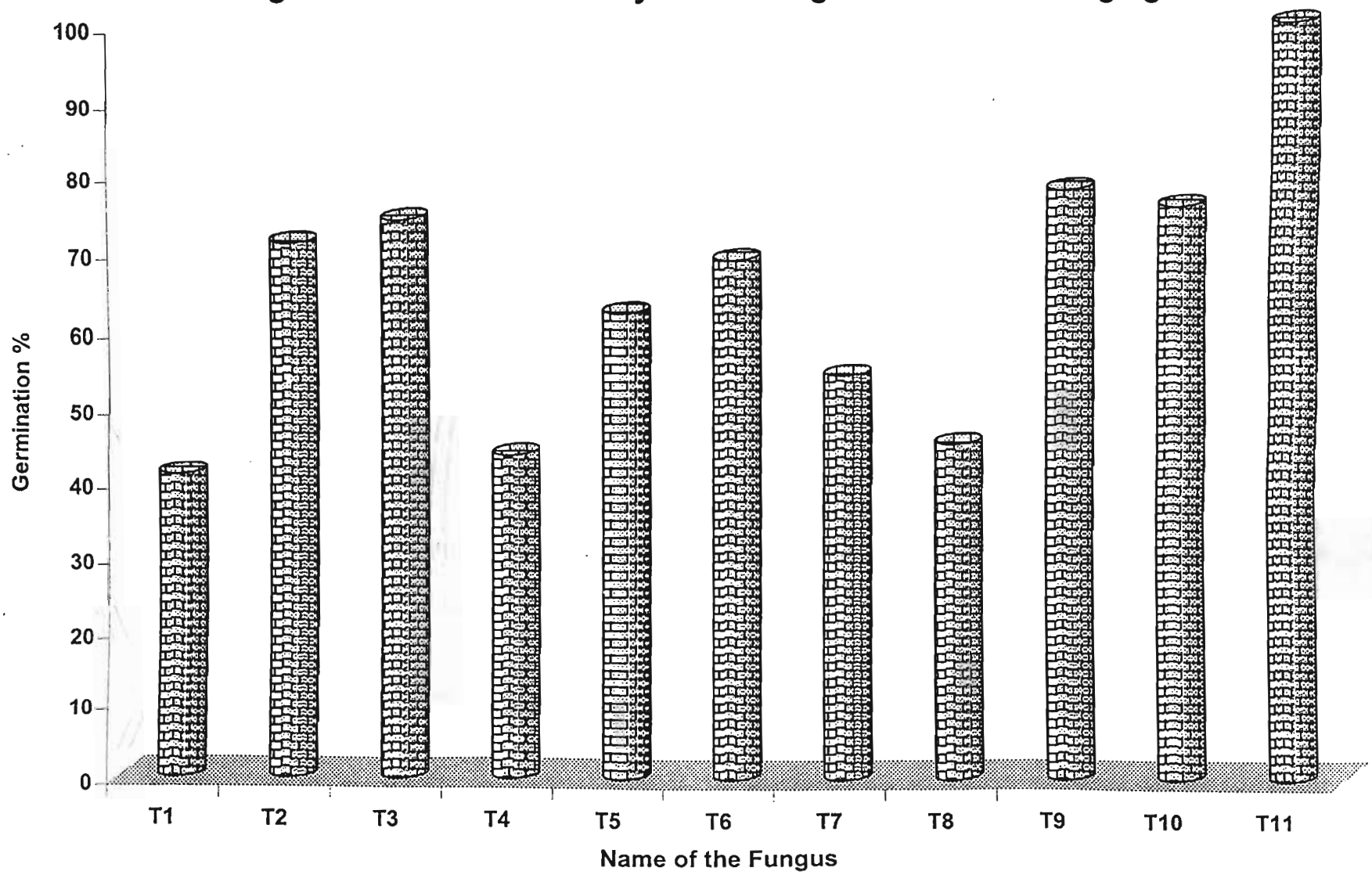
S.No.	Name of the Fungus	Germination (%)*	Percentage decrease over control
1.	*Alternaria sp.*	41.00	59.00 (50.18)
2.	*Aspergillus flavus*	71.00	29.00 (32.58)
3.	*Aspergillus niger*	74.00	26.00 (30.66)
4.	*Ascochyta rabiei*	43.60	57.00 (49.02)
5.	*Botrytis cinerea*	62.00	38.00 (38.06)
6.	*Colletotrichum* sp.	69.00	31.00 (33.83)
7.	*Curvularia lunata*	54.00	46.00 (42.71)
8.	*Fusarium* sp.	45.00	55.00 (47.87)
9.	*Penicillium* sp.	78.00	22.00 (27.97)
10.	*Rhizopus stolonifer*	76.00	24.00 (29.33)
11.	Control	100.00 (90.00)	

F - test at 5% level : Significant

C.D. at 5% : 2.47

* : Mean of value of three replications
(Figure in parenthesis are angular transformed values)

Fig. 2 Effect seed borne mycoflora on germination of bengalgram



T1: Alternaria sp. T2: Aspergillus flavus T3 : Aspergillus niger T4: Ascochyta rabiei T5: Botrytis cinerea
T6: Colletotrichum sp. T7: Curvularia lunata T8: Fusarium sp. T9: Penicillium sp. T10: Rhizopus stolonifer T11: Control

Plate XIV : *Alternaria* sp. showing reduction in germination

Plate XV : *Aschochyta rabiei* showing reduction in germination

Plate XIV

Plate XV

Plate XVI : *Fusariun* sp. showing reduction in germination

Plate XVI

percentage of germination reduction in case of seeds soaked in spore suspension of *Curvularia lunata* (31.0 per cent), *Aspergillus flavus* (29.0 per cent), *Aspergillus niger* (26.0 per cent), *Penicillium* sp. (22.0 per cent) and *Rhizopus stolonifer* (24.0 per cent) over control.

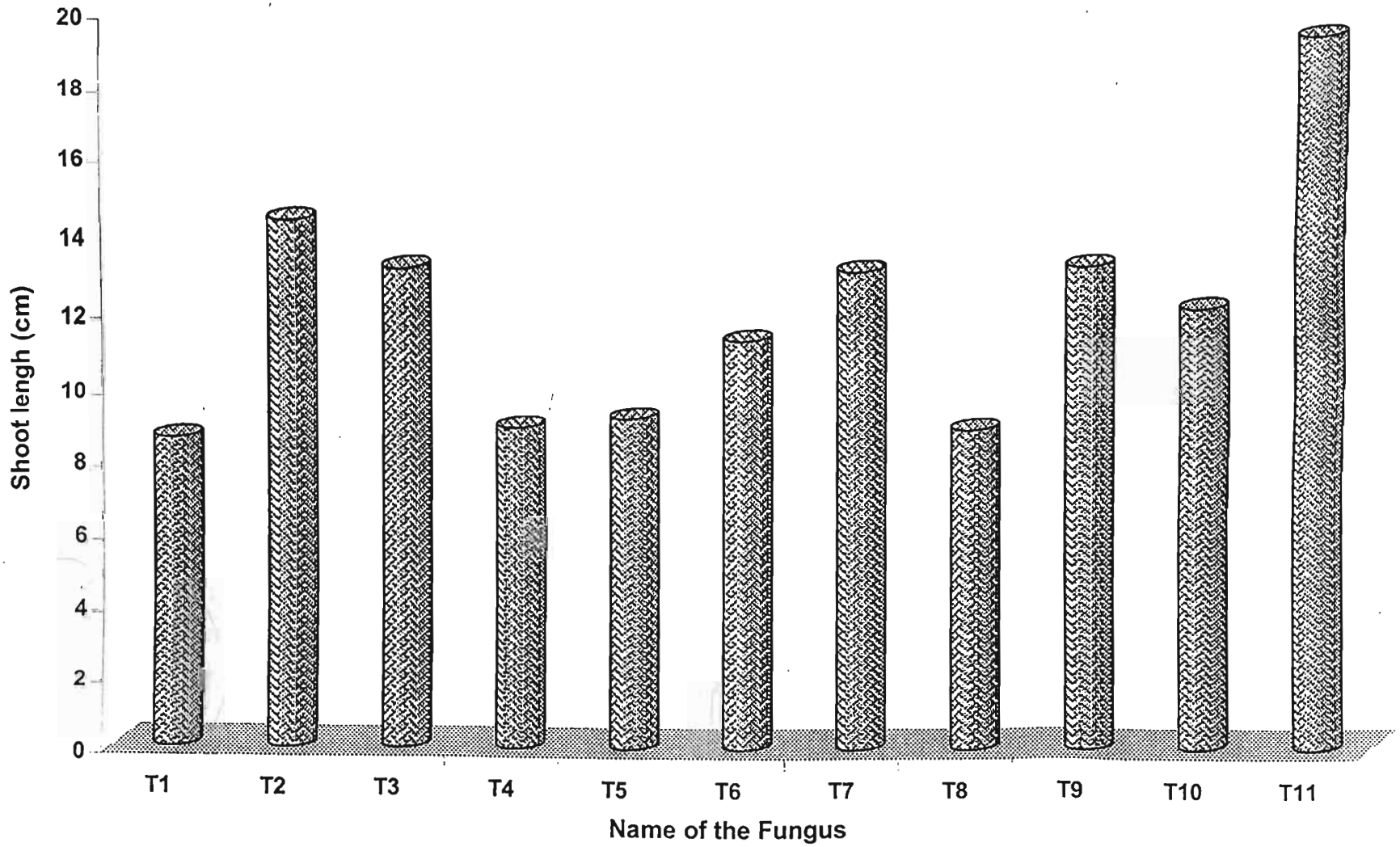
4.6 EFFECT OF SEED BORNE MYCOFLORA ON SEEDLING VIGOUR *in vivo* (SHOOT LENGTH)

The results *in vivo* studies (Table 5 and Fig. 3) indicated that *Alternaria* sp. reduced the shoot length drastically i.e. 8.53 cm compared to control (19.20 cm). This was followed by *Ascochyta rabiei* (8.84 cm) and *Fusarium* sp. (8.79 cm). The reduction in shoot length was 9.07, 11.21 and 13.15 cm by *Botrytis cinerea, Colletotrichum* sp. and *Penicillium* sp. respectively. The rest of the fungi namely *Curvularia lumata, Rhizopus stolonifer, Aspergillus flavus,* and *Aspergillus niger* have reduced the shoot length ranged from 12.11 - 14.25 cm.

4.7 EFFECT OF SEED BORNE MYCOFLORA ON SEEDLING VIGOUR *in vivo* (ROOT LENGTH)

The results (Table 6 and Fig. 4) indicated that maximum reduction in root length (4.42 cm) was noted in *Alternaria* sp. followed by *Fusarium* sp. (4.85 cm) and *Ascochyta rabiei* (5.40 cm) which were on par with each other. Significant reduction in root length was also noted when the seeds were treated with spore suspension of *Colletotrichum* sp., *Curvularia lunata* and *Penicillium* sp. and their length were 6.10, 6.27 and 6.66 respectively. Least reduction in root length was noted in the seeds which were treated with spore

Fig. 3: Effect seed borne mycoflora on seedling vigour of bengalgram (shoot length) *in vivo*



T1: Alternaria sp. T2: Aspergillus flavus T3 : Aspergillus niger T4: Ascochyta rabiei T5: Botrytis cinerea
T6: Colletotrichum sp. T7: Curvularia lunata T8 : Fusarium sp. T9: Penicillium sp. T10: Rhizopus stolonifer T11: Control

TABLE 6. Effect of seed borne mycoflora on seedling vigour of bengalgram (root length) *in vivo*

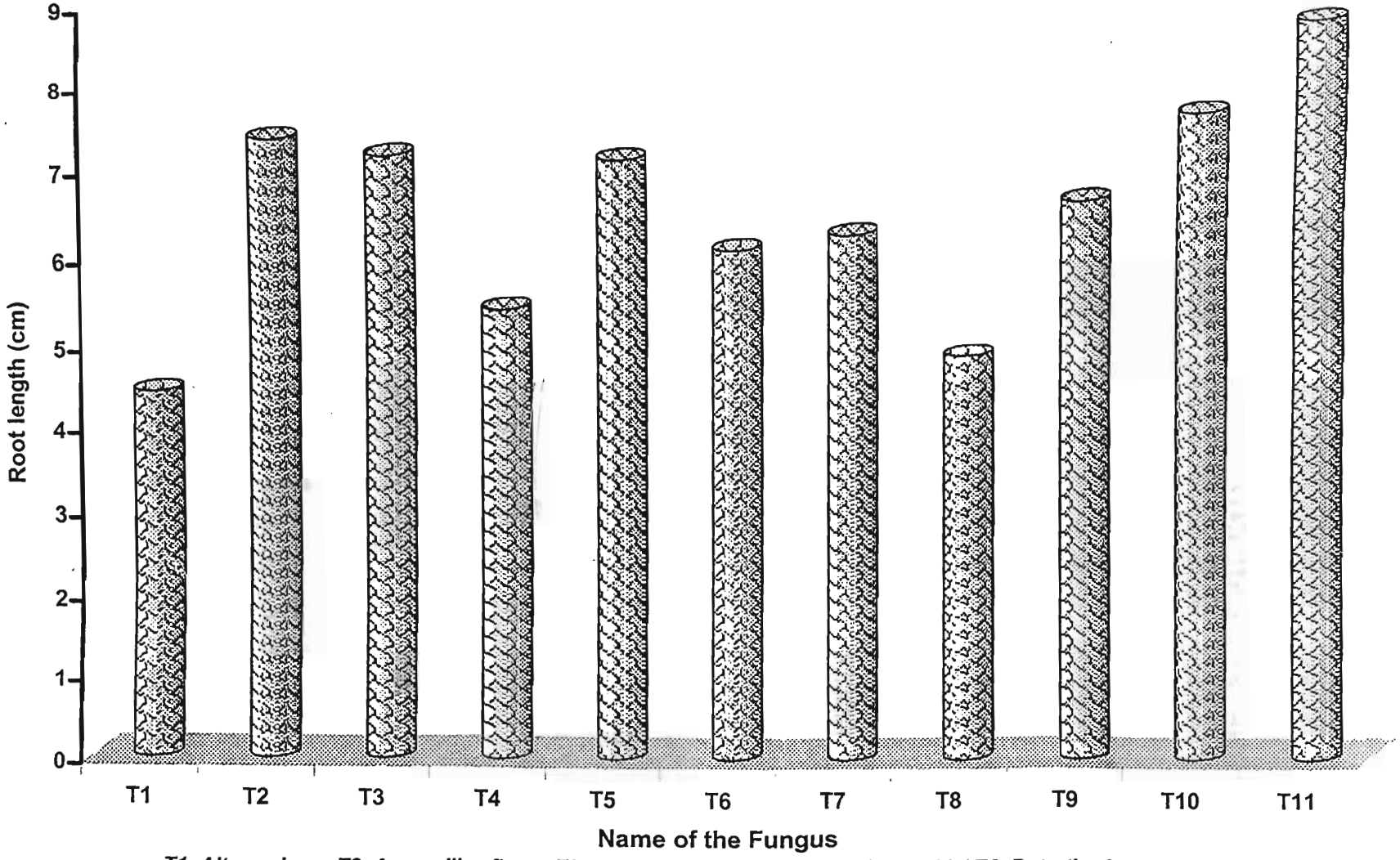
S.No.	Name of the fungus	Root length (cm)*	Percentage decrease over control
1.	*Alternaria sp.*	4.42	49.54 (44.71)
2.	*Aspergillus flavus*	7.33	16.32 (23.81)
3.	*Aspergillus niger*	7.15	18.37 (25.33)
4.	*Ascochyta rabiei*	5.40	38.35 (38.23)
5.	*Botrytis cinerea*	7.11	18.83 25.70)
6.	*Colletotrichum* sp.	6.10	30.36 33.46)
7.	*Curvularia lunata*	6.27	28.42 (32.20)
8.	*Fusarium* sp.	4.85	44.63 (41.90)
9.	*Penicillium* sp.	6.66	23.97 (29.27)
10.	*Rhizopus stolonifer*	7.66	12.55 (20.70)
11.	Control	8.76	

F - test at 5% level : Significant

C.D at 5% level : 1.36

* : Mean value of three reptications
(Figure in parenthesis are angular transformed values)

Fig.4 Effect of seed borne mycoflora on seedling vigour of bengalgram (root length) *in vivo*



T1: Alternaria sp. T2: Aspergillus flavus T3 : Aspergillus niger T4: Ascochyta rabiei T5: Botrytis cinerea
T6: Colletotrichum sp. T7: Curvularia lunata T8 : Fusarium sp. T9: Penicillium sp. T10: Rhizopus stolonifer T11: Control

suspension of *Botrytis cinerea, Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer* and their root length ranged from 7.11 - 7.66 cm compared to control (8.76 cm).

4.8 EFFECT OF DIFFERENT FUNGICIDES ON SEED BORNE FUNGI OF BENGALGRAM

Different fungicides namely captan, carbendazim, Calixin, metalaxyl and Thiram were evaluated against the seed-borne fungi of bengalgram to assess the efficacy of these fungicides at three concentrations viz., 500, 1000 and 1500 ppm by using poison food technique. The linear growth of fungus was measured and compared with the control.

4.8.1. Effect of different fungicides on linear growth of *Alternaria* sp.

The efficacy of various fungicides at different concentrations viz., 500, 1000 and 1500 ppm to control seed borne infection of *Alternaria* sp. was tested (Table 7 and Fig. 5).

The results revealed that all the fungicides reduced the growth of the fungus significantly at different concentrations over control. Among the fungicides tested, at 500 ppm carbendazim was more effective than others in which the percentage of inhibition was 62.61 followed by Thiram and Captan which reduced the growth of the fungus by 56.13 and 55.00 per cent respectively. Calixin and metalaxyl were least effective at 500 ppm in which the percentage of inhibition was 50.68 and 44.43. At 1000 ppm concentration, Carbendazim inhibited the growth of the fungus to an extent of 86.47 per cent followed by Thiram and Captan in which the percentage inhibition was 84.65

TABLE 7. Effect of different fungicides on *Alternaria* sp.

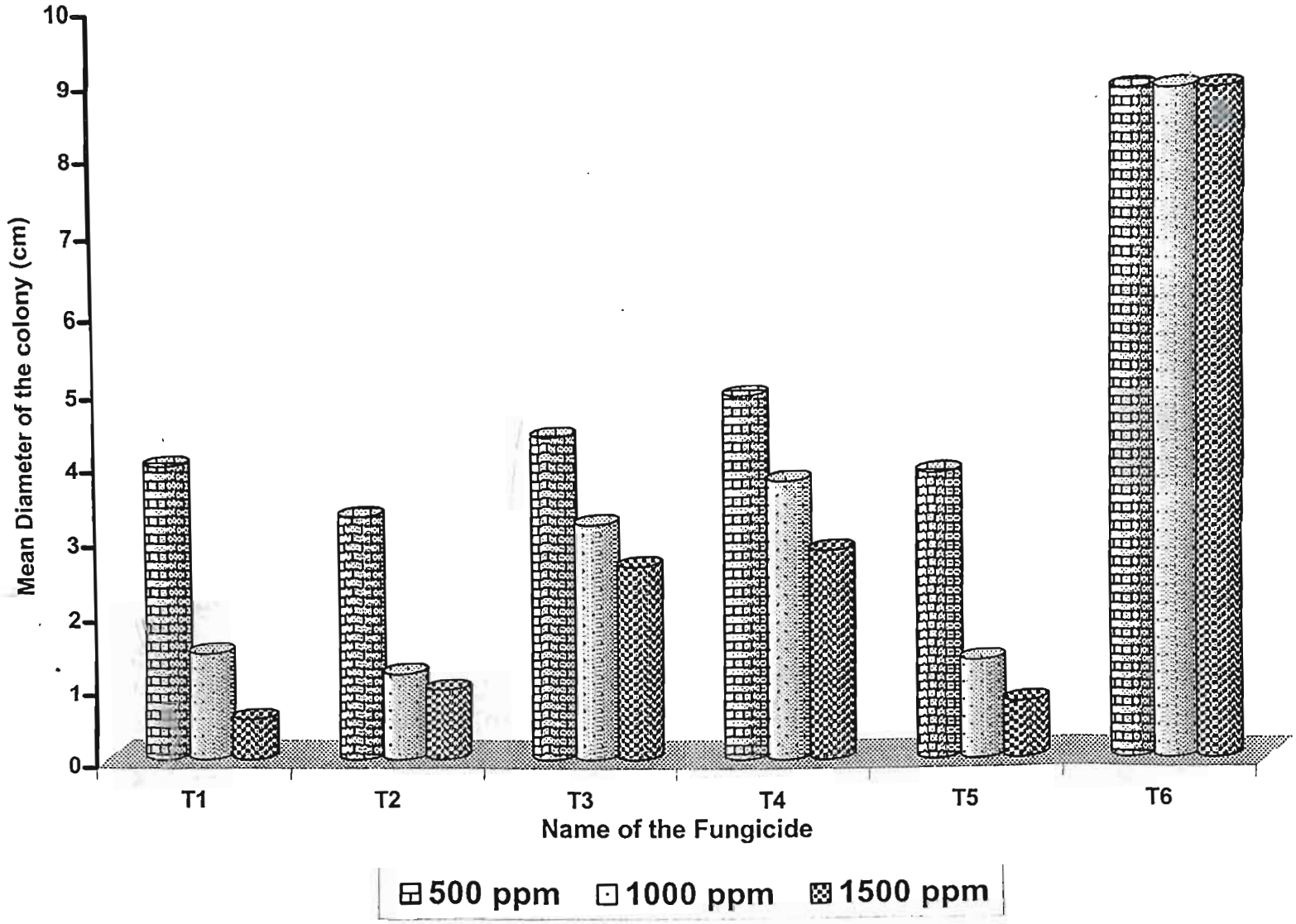
S.No.	Name of the fungicide	Conc. (ppm)	Mean diameter of colony (cm)*	Percent inhibition of growth
1.	Captan	500	3.96	55.00 (47.87)
		1000	1.46	83.40 (65.96)
		1500	0.55	93.75 (75.46)
2.	Carbendazim	500	3.29	62.61 (52.30)
		1000	1.19	86.47 (68.36)
		1500	0.97	88.97 (70.45)
3.	Calixin	500	4.34	50.68 (45.35)
		1000	3.18	63.86 (53.01)
		1500	2.62	77.22 (61.35)
4.	Metalaxyl	500	4.89	44.43 (41.78)
		1000	3.74	57.50 (49.31)
		1500	2.82	67.95 (35.49)
5.	Thiram	500	3.86	56.13 (48.50)
		1000	1.35	84.65 (66.89)
		1500	0.76	91.36 (72.84)
6.	Control		8.80	

F - test at 5% level : Significant

C.D. at 5% level : 0.77

* : Mean of three replication
(Figure in parenthesis are angular transformed values)

Fig. 5 Effect of different fungicides on *Alternaria*sp.



T1: Captan T2: Carbendazim 3: Calixin T4: Metalaxyl 5: Thiram T6: Control

Plate XVII : *Alternaria* sp. showing inhibition of growth when captan 1500 ppm was tested

Plate XVIII : *Aschochyta rabiei* showing inhibition of growth when carbendazim at 1000 ppm was tested

Plate XVII : *Alternaria* sp. showing inhibition of growth when captan 1500 ppm was tested

Plate XVIII : *Aschochyta rabiei* showing inhibition of growth when carbendazim at 1000 ppm was tested

Plate XVII

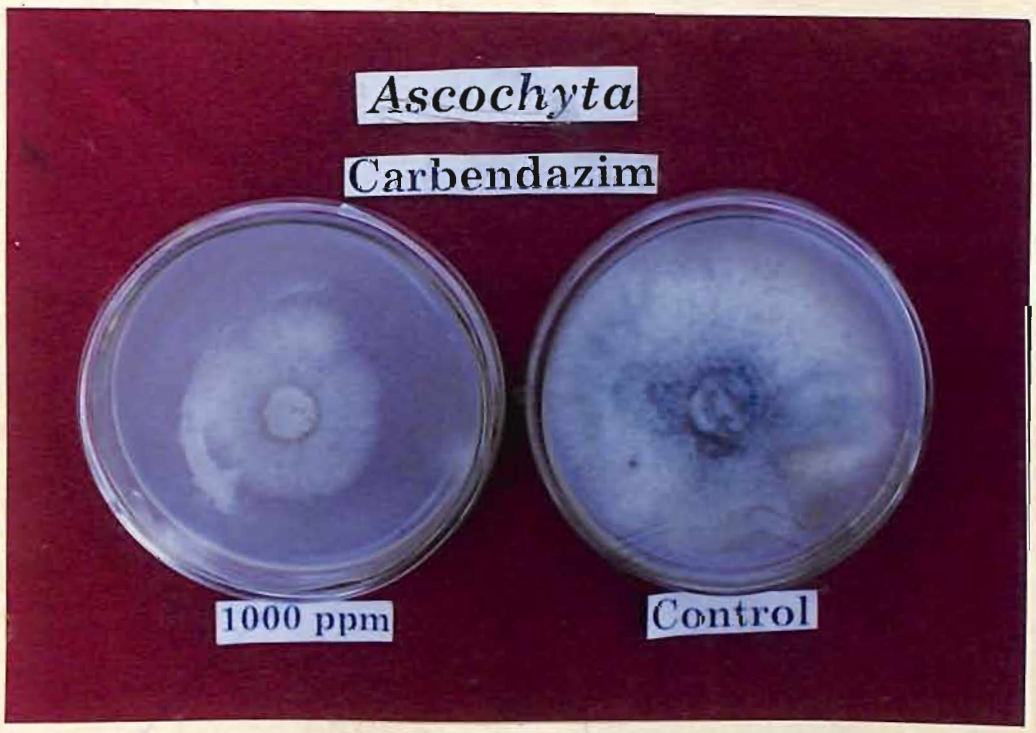


Plate XVIII

TABLE 8. Effect of differrent fungicides on *Ascochyta rabiei*

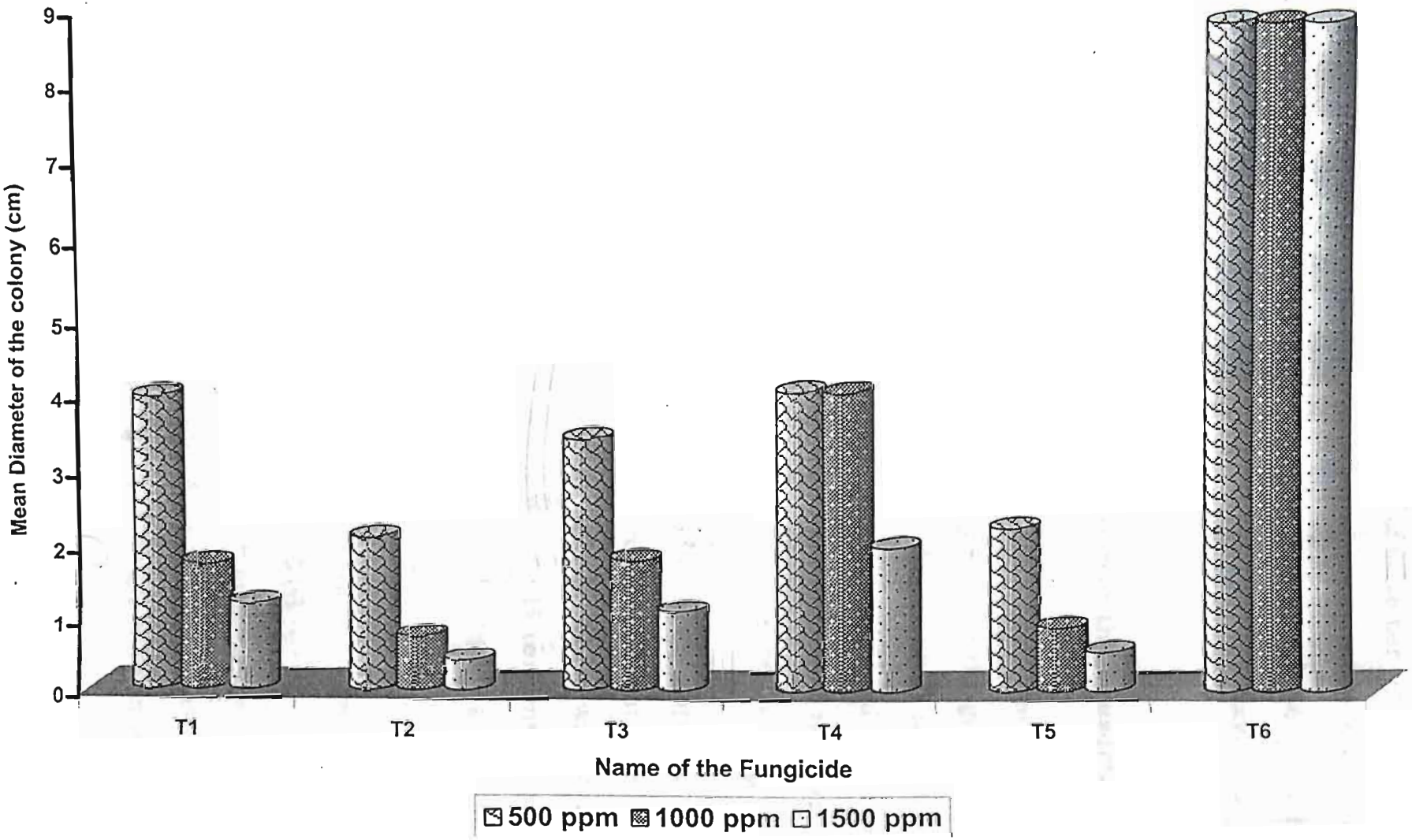
S.No.	Name of the fungicide	Conc. (ppm)	Mean diameter of colony (cm)*	Percent inhibition of growth
1.	Captan	500	3.97	54.36 (47.47)
		1000	1.75	79.88 (63.29)
		1500	1.21	86.09 (68.03)
2.	Carbendazim	500	2.11	75.74 (60.47)
		1000	0.75	91.37 (72.84)
		1500	0.41	95.28 (77.34)
3.	Calixin	500	3.41	63.90 (53.07)
		1000	1.81	79.19 (62.80)
		1500	1.10	87.35 (69.12)
4.	Metalaxyl	500	4.00	54.02 (47.29)
		1000	3.98	54.25 (47.41)
		1500	1.96	77.47 (61.62)
5.	Thiram	500	2.23	74.36 (59.54)
		1000	0.87	90.00 (71.56)
		1500	0.54	93.79 (75.46)
6.	Control		8.70	

F - test at 5% level : Significant

C.D. at 5% level : 0.27

* : Mean of three replications
(Figure in parenthesis are angular transformed values)

Fig. 6 Effect of different fungicides on *Ascochyta rabiei*



T1: Captan T2: Carbendazim 3: Calixin T4: Metalaxyl 5: Thiram T6: Control

At the concentration of 1500 ppm, Carbendazim was effective in reducing the growth of the fungus to an extent of 95.28 per cent followed by Thiram (93.79 per cent) and they were on par with each other. Calixin and Captan were reduced the growth of the fungus by 87.35 and 86.09 per cent respectively. Least per cent of inhibition was recorded by Metalaxyl (77.47 per cent).

4.8.3 Effect of different fungicides on linear growth of *Fusarium* sp.

From the Table 9 and Fig. 7, it is clear that Carbendazim at 500 ppm was more effective than others and significantly reduced the growth of the fungus to an extent of 75.95 per cent followed by Thiram (69.77 per cent) and Captan (64.04 per cent). Calixin and Metalaxyl were found to be least effective among the five fungicides at 500 ppm and they recorded the percentage of inhibition of growth by 41.79 and 41.12 respectively.

At 1000 ppm concentration also Carbendazim was superior to the others by reducing the linear growth of the fungus to an extent of 88.65 per cent folowed by Thiram in which the percentage of inhibition was 84.26 per cent. Captan reduced the growth to an extent of 76.17 per cent. Metalaxyl and Calixin were found to be least effective in inhibiting the growth of the fungus and recorded 60.89 and 54.71 per cent.

At the concentration of 1500 ppm, Thiram was the most effective in reducing the growth of fungus to an extent of 96.06 per cent (Plate XIX) followed by Carbendazim (91.57 per cent) and Captan (89.10 per cent) which were on par with each other. Calixin and Metalaxyl were least effective in inhibiting the growth of the fungus and the percentage of inhibition was only 72.80 and 68.65 per cent over control.

TABLE 9. Effect of different fungicides on *Fusarium* sp.

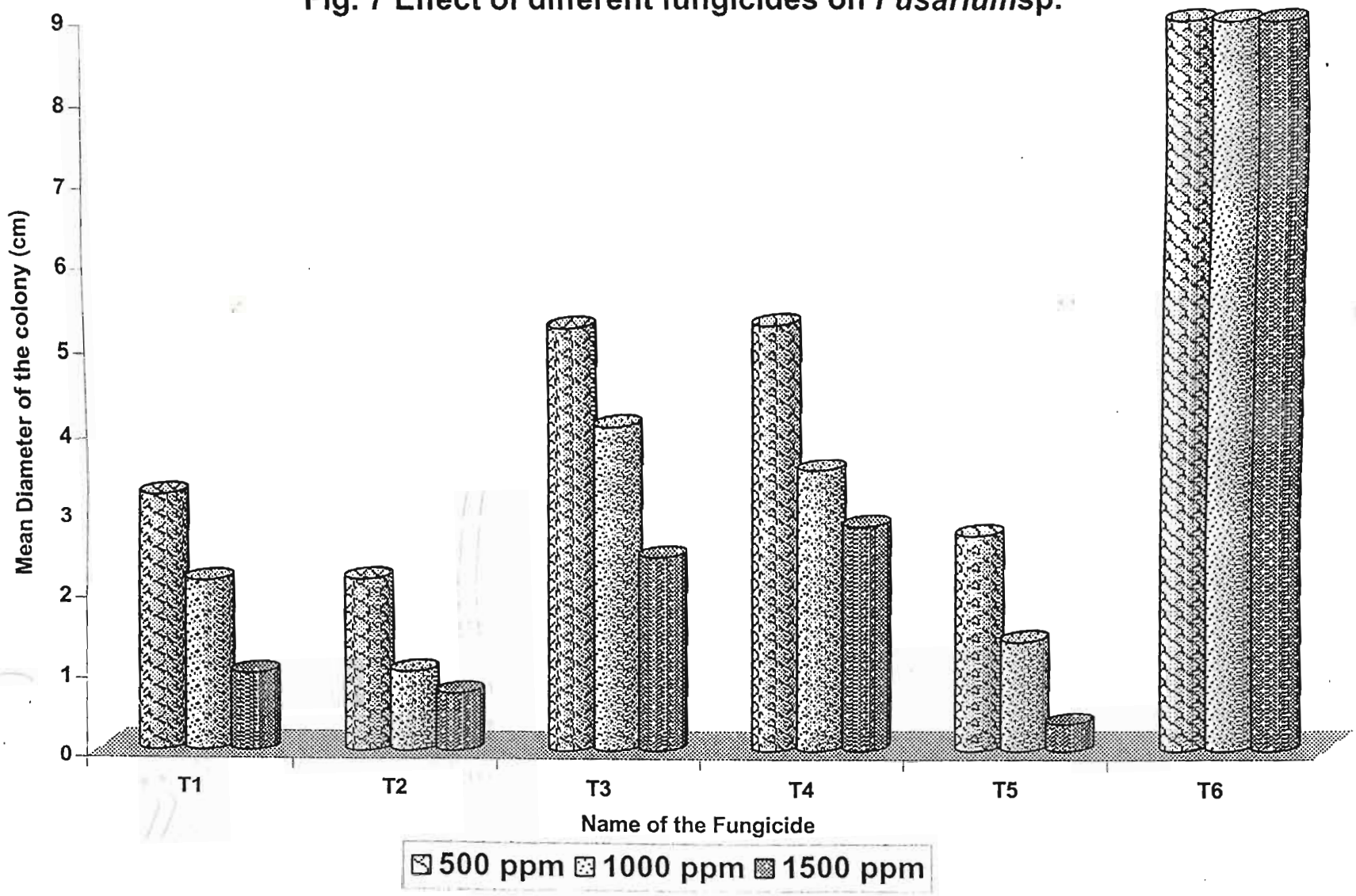
S.No.	Name of the fungicide	Conc. (ppm)	Mean diameter of colony (cm)*	Percent inhibition of growth
1.	Captan	500	3.20	64.04 (53.13)
		1000	2.12	76.17 (60.73)
		1500	0.97	89.10 (70.72)
2.	Carbendazim	500	2.14	75.95 (60.62)
		1000	1.01	88.65 (70.27)
		1500	0.75	91.57 (73.07)
3.	Calixin	500	5.18	41.79 (40.22)
		1000	4.03	54.71 (47.70)
		1500	2.42	72.80 (58.56)
4.	Metalaxyl	500	5.24	41.12 (39.87)
		1000	3.48	60.89 (51.24)
		1500	2.79	68.65 (55.92)
5.	Thiram	500	2.69	69.77 (56.60)
		1000	1.40	84.26 (66.58)
		1500	0.35	96.06 (84.26)
6.	Control		8.90	

F - test at 5% level : Significant

C.D. at 5% level : 0.28

* : Mean of three replications
(Figure in parenthesis are angular transformed values)

Fig. 7 Effect of different fungicides on *Fusarium*sp.



T1: Captan T2: Carbendazim 3: Calixin T4: Metalaxyl 5: Thiram T6: Control

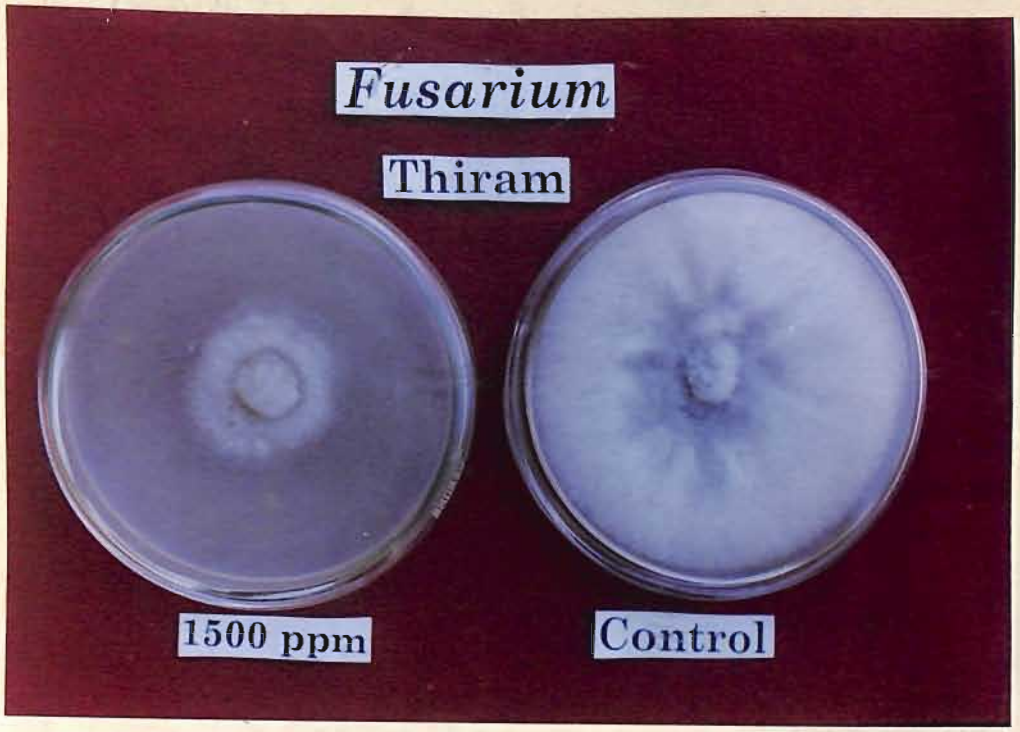

Fusarium
Thiram
1500 ppm
Control

Plate XIX

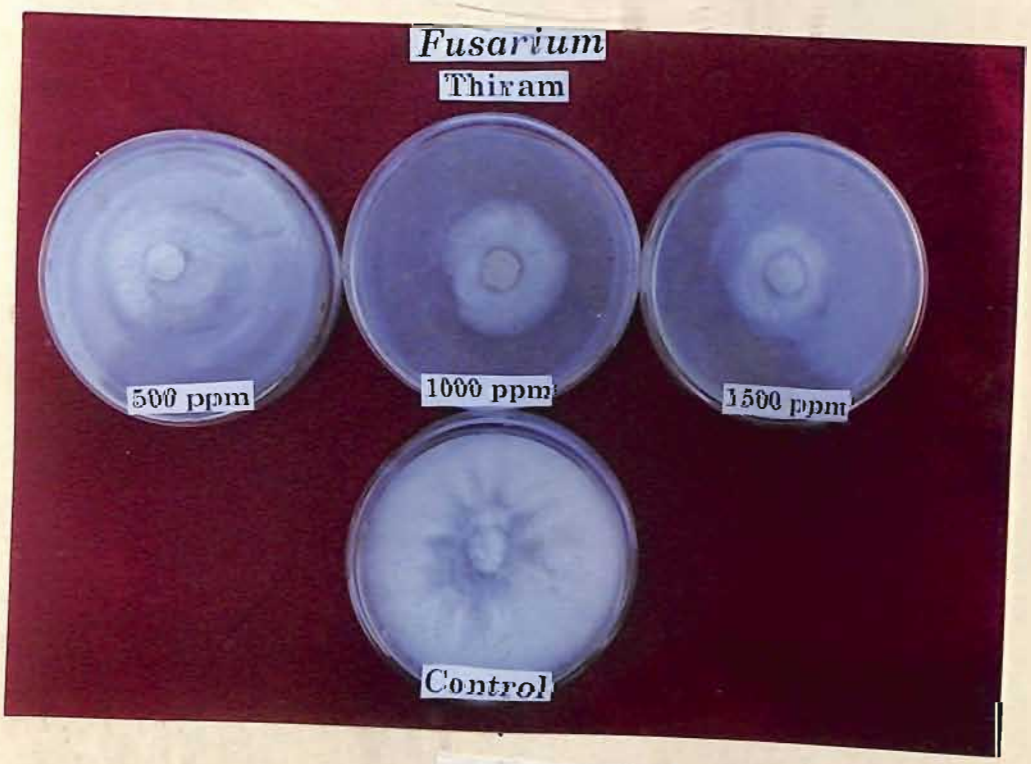

Fusarium
Thiram
500 ppm
1000 ppm
1500 ppm
Control

Plate XIX : *Fusarium* sp. showing inhibition of growth when Thiram at 1500 ppm was tested

Plate XX : Thiram showing effectiveness on *Fusarium* sp. at different concentrations

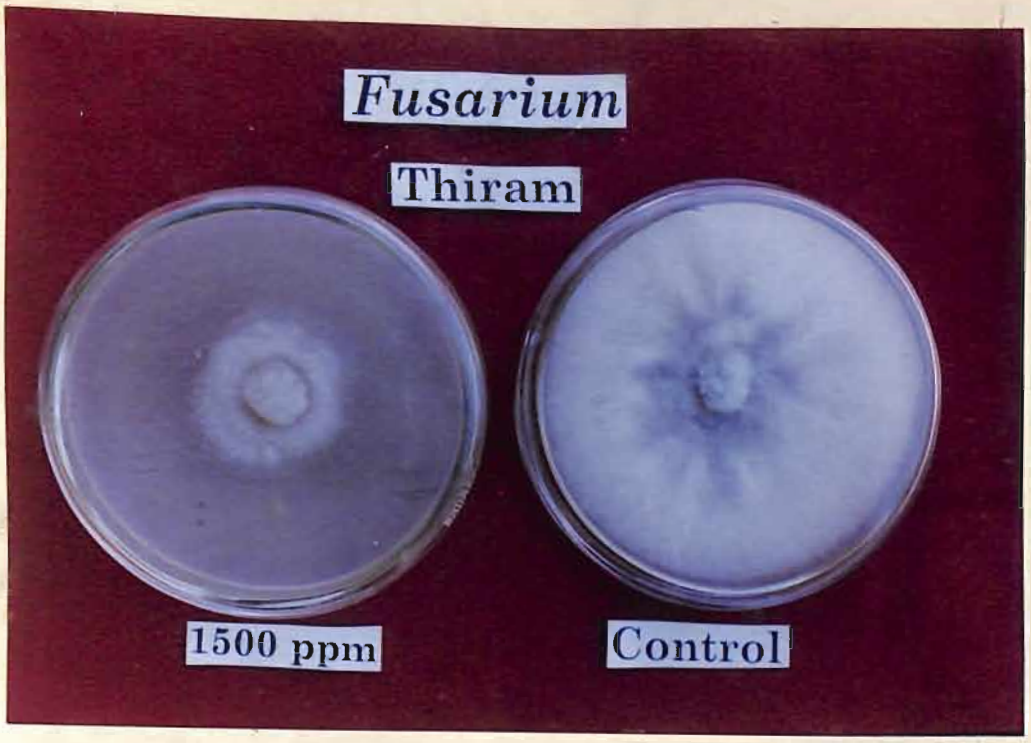


Plate XIX

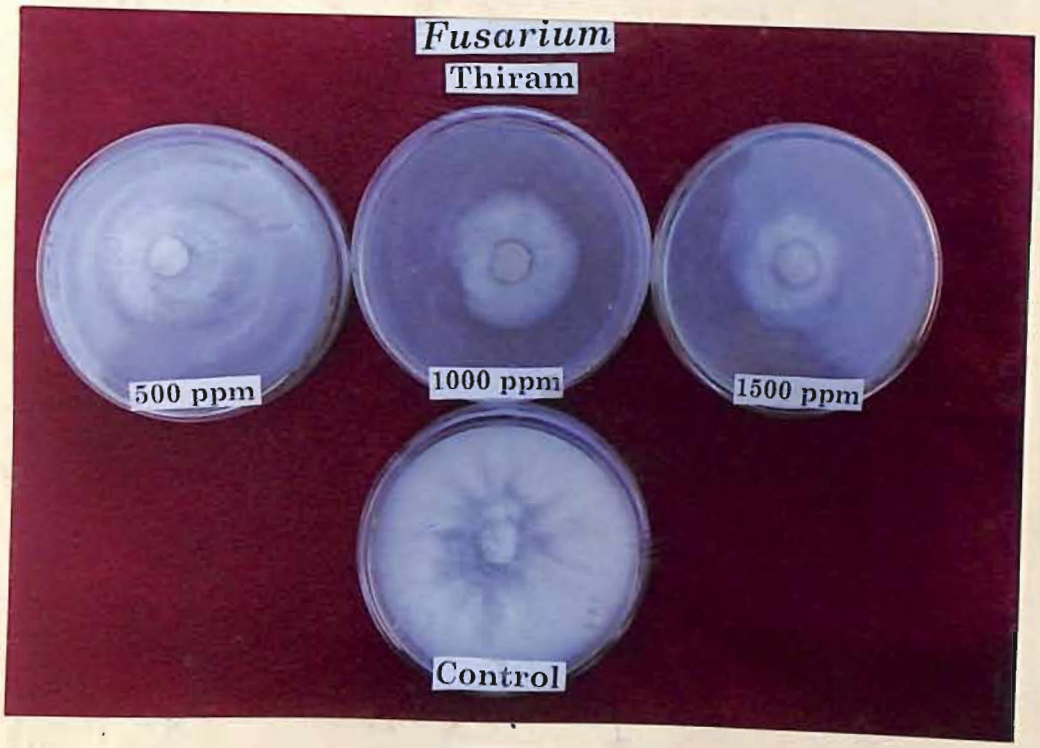


Plate XX

It is clear from the above data that Carbendazim and Thiram (Plate XX) at all concentrations reduced the growth of the fungus significantly compared to the other fungicides, while Metalaxyl was inferior to others in reducing the growth of the fungus at all concentrations.

4.9 EFFECT OF CERTAIN PLANT EXTRACTS ON LINEAR GROWTH OF CERTAIN SEED BORNE FUNGI OF BENGALGRAM

Poision food technique was used to findout the effect of five plant extracts on *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp. as these fungi appeared pathogenic.

4.9.1 Effect of certain plant extracts on *Alternaria* sp.

The data indicated (Table 10 and Fig. 8) that the plant extract viz., *Azadirachta indica* was the most effective in inhibiting the growth of fungus. The percentage of inhibition was 78.64 (Plate XXI). The next best were *Tamarindus indicus, Mirabilis jalapa* and *Solanum nigrum* in which the per cent inhibition was 61.72, 61.60 and 61.23 respectively, which were on par with each other. *Phyllanthus neruri* was found to be the least effective in reducing the growth of the fungus (45.55 per cent) over control.

4.9.2 Effect of plant extracts on *Ascochyta rabiei*

Poison food technique was employed to know the effect of certain plant extracts on the linear growth of *Ascochyta rabiei*. From the Table 11 and (Fig. 9) it is observed that the plant extract of *Azadirachta indica* was the

TABLE 10. Effect of different plant extracts on *Alternaria* sp.

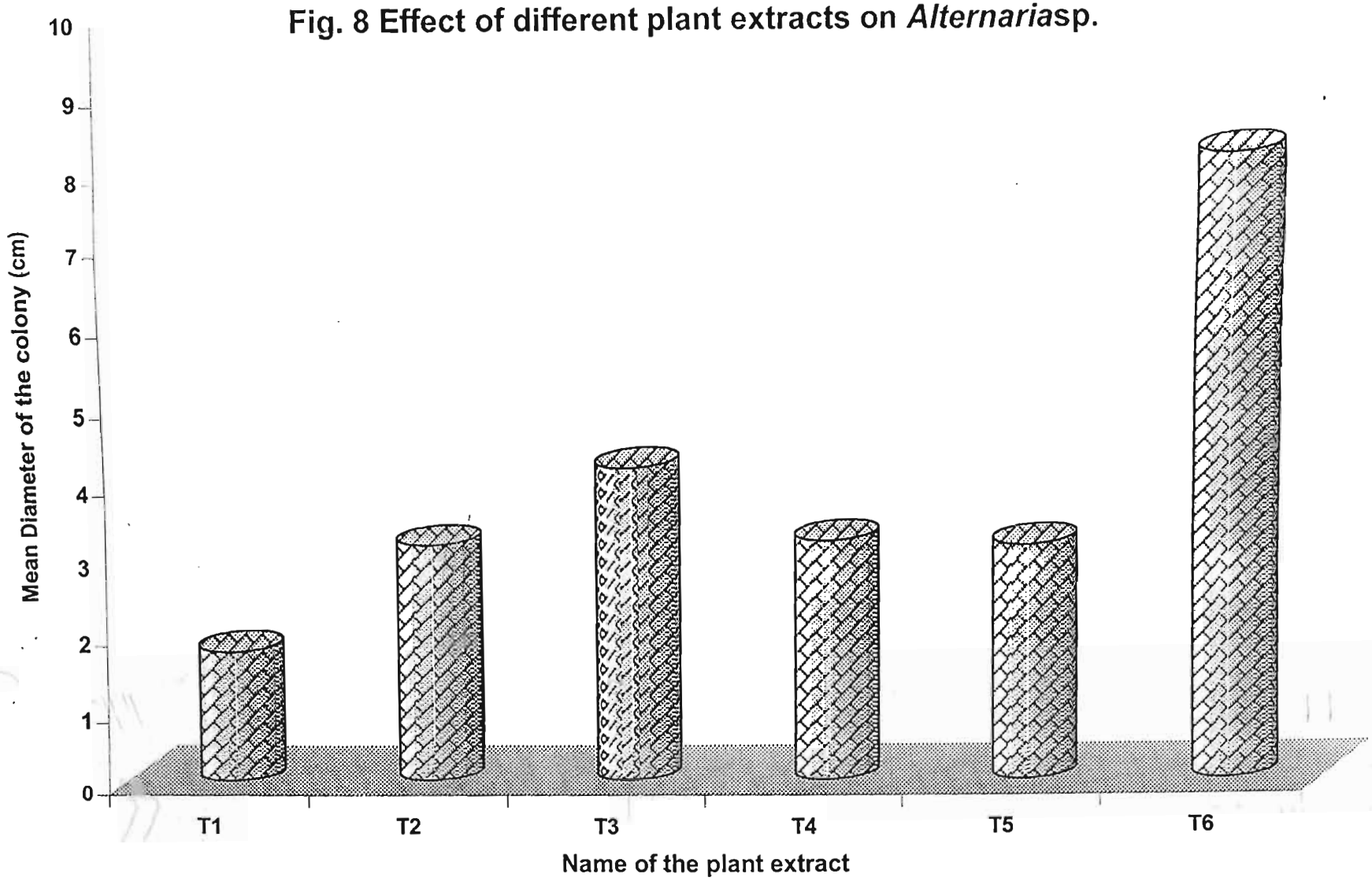
S.No.	Name of the plant Extract	Conc. (%)	Mean diameter of colony (cm)*	Percentage inhibition of growth
1.	*Azadirachta indica*	1.0	1.73	78.64 (62.44)
2.	*Mirabilis jalapa*	1.0	3.11	61.60 (51.71)
3.	*Phyllanthus neruri*	1.0	4.14	45.55 (42.42)
4.	*Solanum nigrum*	1.0	3.14	61.23 (51.47)
5.	*Tamarindus indicus*	1.0	3.10	61.72 (51.77)
6.	Control	--	8.10	

F - test at 5% level : Significant

C.D. at 5% level : 0.18

* : Mean of the three replication values (Figure in parenthesis are angular transformed values)

Fig. 8 Effect of different plant extracts on *Alternaria*sp.



T1: Azadirachta indica T2: Mirabilis jalapa T3: Phyllanthus neruri
T4: Solanum nigrum T5: Tamarindus indicus T6: Control

Plate XXI

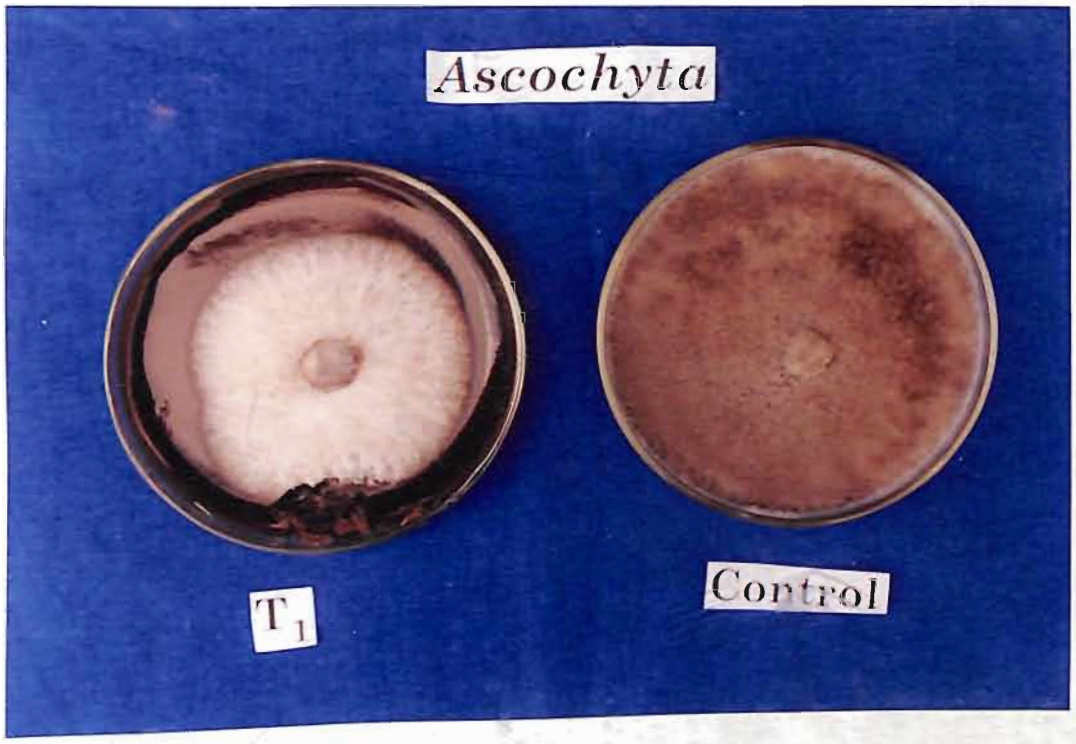


Plate XXII

TABLE 11. **Effect of different plant extracts on *Ascochyta rabiei***

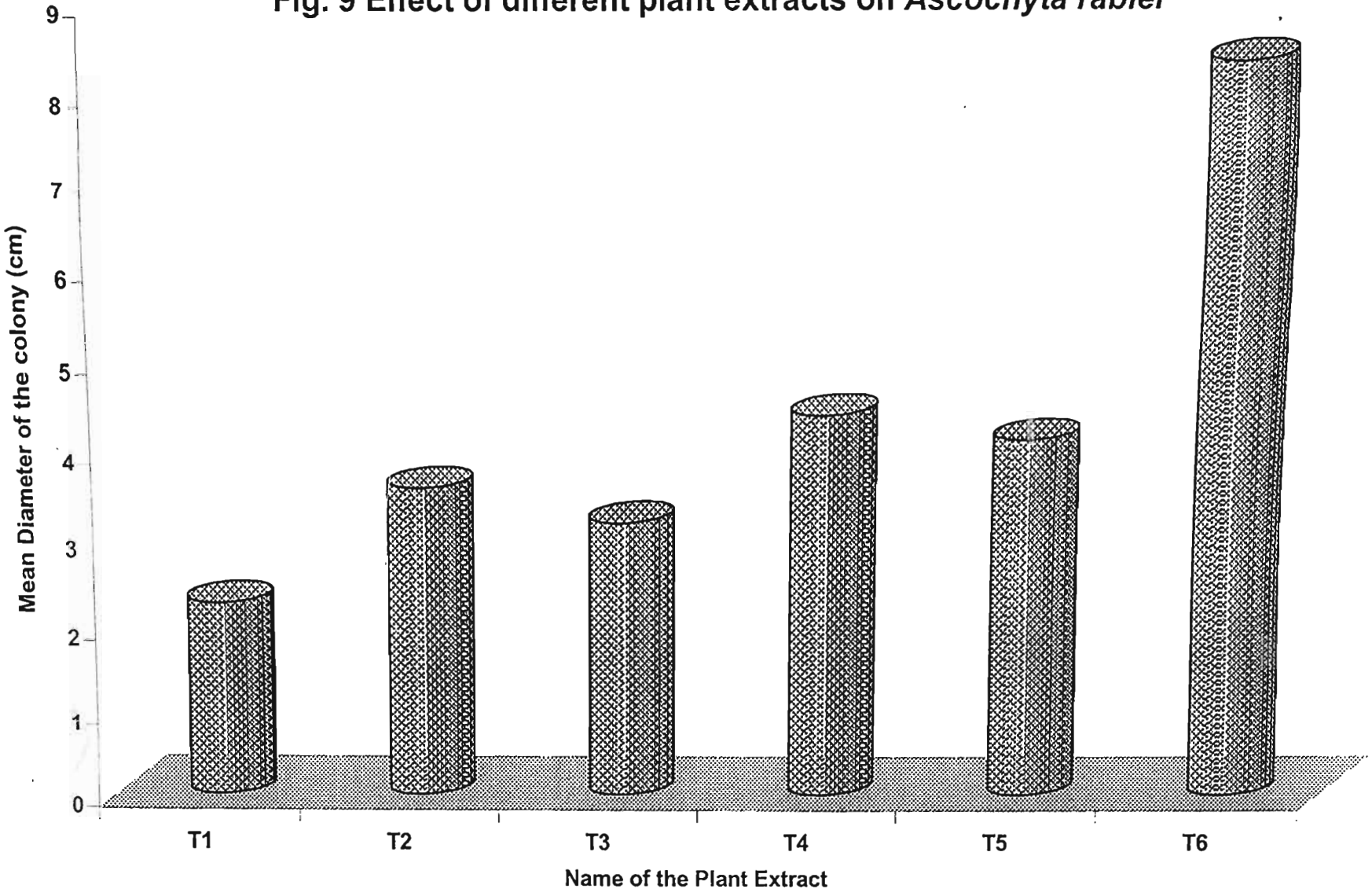
S.No.	Name of the plant Extract	Conc. (%)	Mean diameter of colony (cm)	Percentage inhibition of growth
1.	*Azadirachta indica*	1.0	2.25	72.89 (58.56)
2.	*Mirabilis jalapa*	1.0	3.55	57.22 (49.41)
3.	*Phyllanthus neruri*	1.0	3.15	62.04 (51.94)
4.	*Solanum nigrum*	1.0	4.38	47.22 (43.39)
5.	*Tamarindus indicus*	1.0	4.10	50.60 (45.34)
6.	Control	--	8.30	

F- test at 5% level : Significant

C.D. at 5% level : 0.66

* : Mean of the three replication values (Figure in parenthesis are angular transformed values)

Fig. 9 Effect of different plant extracts on *Ascochyta rabiei*



T1: Azadirachta indica T2: Mirabilis jalapa T3: Phyllanthus neruri
T4: Solanum nigrum T5: Tamarindus indicus T6: Control

most effective in which the percentage of inhibition was 72.89 over control (Plate XXII). Plant extracts of *Phyllanthus neruri* and *Mirabilis jalapa* inhibited growth by 62.04 and 57.22 per cent respectively. The extracts of *Tamarindus indicus* and *Solanum nigrum* were least effective as the reduction in growth was only by 50.60 and 47.22 per cent respectively and they were on par.

4.9.3 Effect of Plant Extracts on *Fusarium* sp.

The data from the Table 12 and (Fig. 10) indicated that all the plant extracts reduced growth of *Fusarium* sp. significantly over control. Among the five plant extracts, *Azadirachta indica* (Plate XXIII) and *Tamarindus indicus* were found to be superior to others in which the percentage of inhibition was 83.97 and 83.29 respectively. They were on par with each other. The per cent inhibition in the linear growth of the fungus by *Mirabilis jalapa, Solanum nigrum* and *Phyllanthus neruri* were 63.75, 58.40 and 54.31 respectively. In general, all the plant extracts showed significant reduction in the growth of *Fusarium* sp. (Plate XXIV).

TABLE 12. Effect of different plant extracts on *Fusarium* sp.

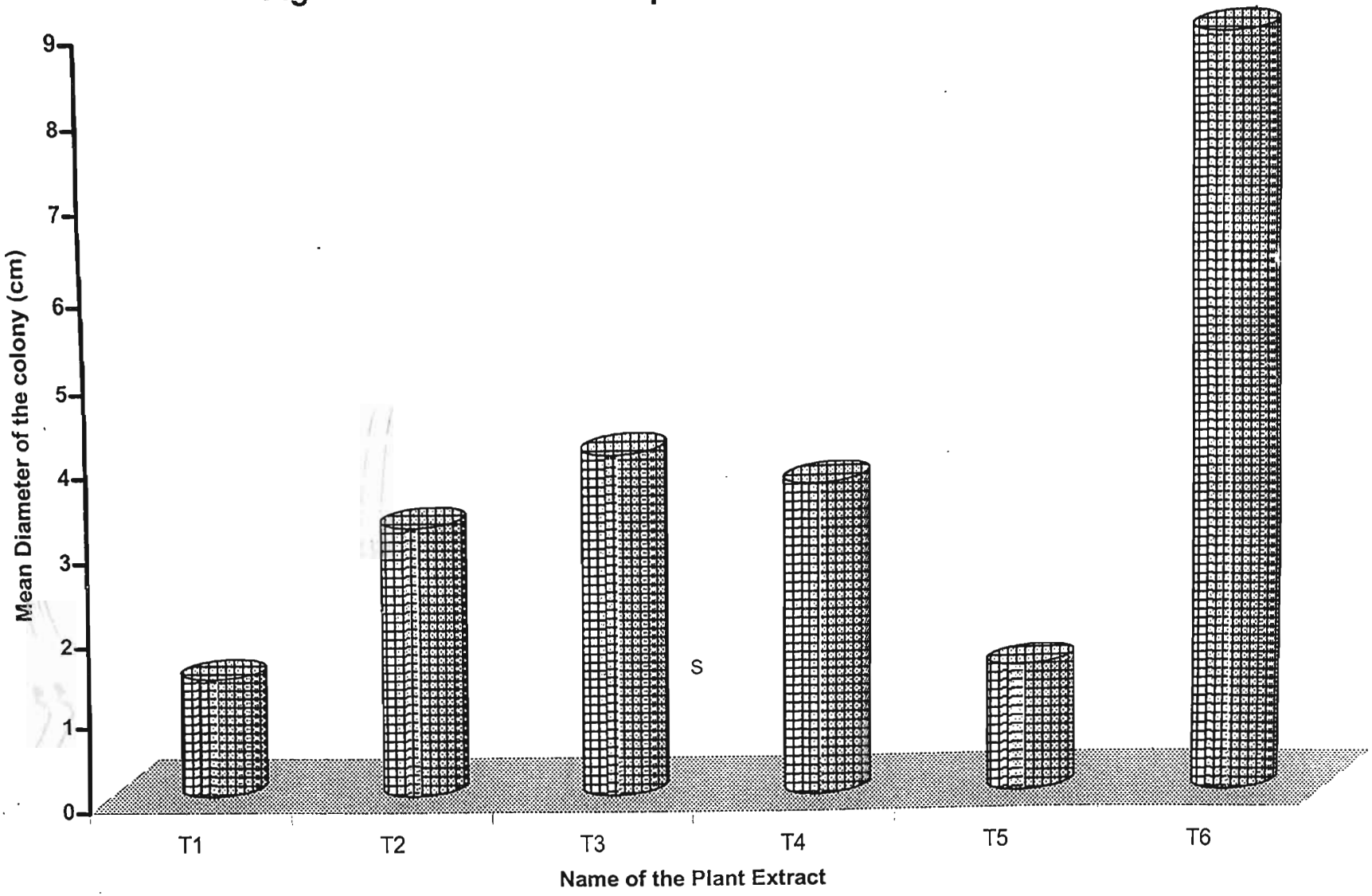
S.No.	Name of the plant Extract	Conc. (%)	Neam diameter of Colony (cm)	Percentage inhibition of growth
1.	*Azadirachta indica*	1.0	1.41	83.97 (66.74)
2.	*Mirabilis jalapa*	1.0	3.19	63.75 (52.95)
3.	*Phyllanthus neruri*	1.0	4.02	54.31 (47.47)
4.	*Solanum nigrum*	1.0	3.66	58.40 (49.89)
5.	*Tamarindus indicus*	1.0	1.47	83.29 (66.34)
6.	Control	--	8.80	

F - test at 5% level : Significant

C.D. at 5% level : 0.66

* : Mean of the three replication values
(Figure in parenthesis are angular transformed values)

Fig. 10 Effect of different plant extracts on *Fusarium*sp.



T1: Azadirachta indica T2: Mirabilis jalapa T3: Phyllanthus neruri
T4: Solanum nigrum T5: Tamarindus indicus T6: Control

Plate XXIII : T_1 Plant extract of *Azadirachta indica* showing inihibition of growth of *Fusarium* sp.

Plate XXIV : T_1-*Azadirachta indica*, T_2-*Tamarindus indicus*, T_3-*Mirabilis jalapa*, T_4-*Solanum nigrum* and T_5-*Phyllanthus neruri* showing reduction of growth on *Fusarium* sp.

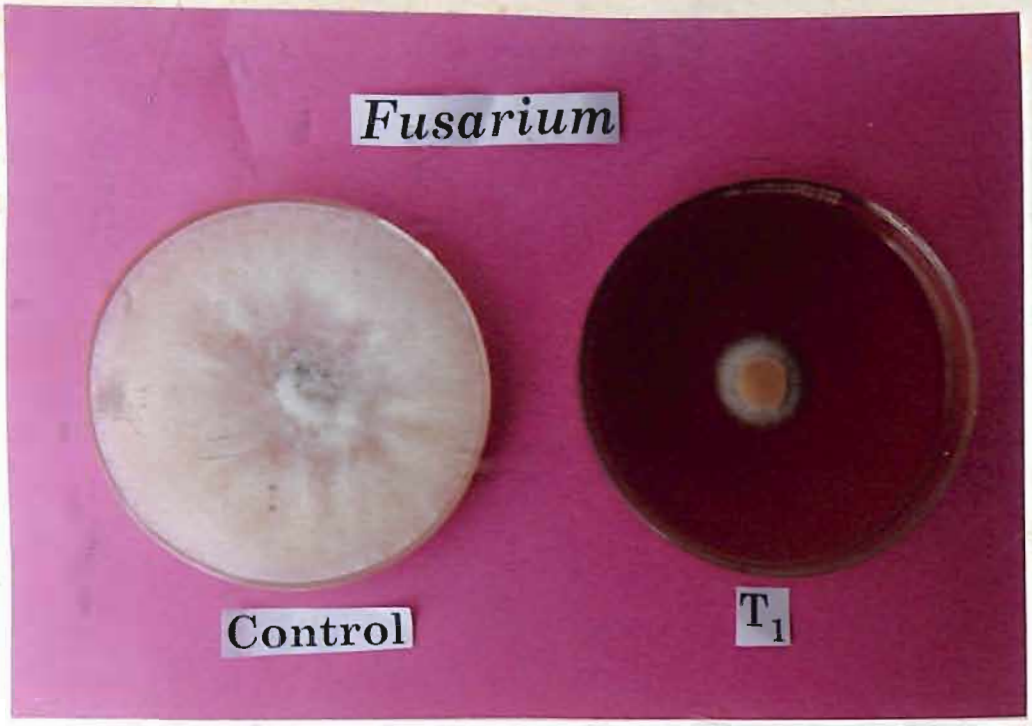


Plate XXIII

Plate XXIV

Discussion

CHAPTER - V

DISCUSSION

Bengalgram seeds are infected by various micro organisms, the extent of which varies according to season and locality. These microbes which are carried on the surface as well as inside the seed become active at the advent of favourable conditions, thereby causing heavy damage to the germinating seed and also can become a source of infection for a subsequent crop.

Investigation on mycoflora of bengalgram seed reported herein include dedtection of mycoflora on different varieties of bengalgram seed by using blotter and agar plate methods, effect on seed borne fungai on germination and seedling vigour, effect of certain fungicides and plant extracts on seed borne fungi.

Present investigation revealed that bengalgram seeds harboured many fungal species in and on the seeds of the varieties under study. Irrespective of methods of isolation and variety used, the mycoflora commonly associated with bengalgram seed samples were *Alternaria* sp., *Aspergillus flavus, A.niger, Ascochyta rabiei, Botrytis cinerea, Colletotrichum* sp., *Curvularia lunata, Fusarium* sp., *Penicillium* sp. and *Rhizopns stolonifer*. Several workers (Maden, 1987; Raut and Somani, 1988; Amiruzzaman and Chaudhury, 1989; Dwivedi and Shukla, 1990; Abdou *et al.*, 1991; Ahmed and Singh, 1991; Day and Singh, 1994) also recorded some of these fungi on bengalgram seed.

However some of the species like *Didymella pinodes, Phoma pinodella, Myrothecium roridum* and *Sclerotinia sclerotiorum* (Simay, 1989), *Ascochyta*

pinodes, *Phoma* sp and *Phoma medicaginis* (Abdeslam Hidden, 1990), *Acremonium implicatum*, *Bipolaris australinesis*, *Dreschlera specifera*, *Epicocum purpurascens* and *Scytalidium* sp. (Dwivedi and Shukla, 1990), *Pythium ultimum* var. *ultimum* (Trapero - Casis *et al.*, 1990), *Cladosporium* sp., *Aspergillus nidulans* and *Aspergillus tenneus* (Ahmed and Singh, 1991) and *Macrophomina phaseolina* (Khan *et al.*, 1996) were not encountered. The wide variation in occurrence of different fungi might be due to variation in the agroclimatic conditions under which the different varieties of chickpea were grown.

Choosing an efficient method for obtaining the maximum seed mycoflora is an important pre-requisite for this type of studies. Hence in the present studies, two of the well known methods viz., blotter and agar plate methods were tested for their efficacy. Of the two methods, agar plate method was found to be better in getting maximum per cent incidence of mycoflora than blotter method.

Several workers also have reported that agar plate method was superior to blotter method. Shakir and Mirza (1994) stated that agar plate method yielded greater number of fungi than the blotter method in chickpea seed.

Shah and Jain (1993) reported that the percentage of mycoflora isolated by agar plate was higher than blotter method on mustard. Solanke *et al.* (1993) also stated that agar plate method yielded more mycoflora than blotter method. But contrary to this Rani *et al.* (1978), Ravindranath and Indira (1979) and Bhale and Khare (1982) reported that blotter method was superior to agar plate method. This variation might be attributed to the

physico-chemical nature of the seed as well as agricultural practices and storage conditions used. Other possibility for such divergence might be attributed to a comparatively rapid growth of the saprophytes adhering to the seed surface and masking of slow growing pathogenic fungi.

Irrespective of the method of isolation and variety used unsterilized seeds gave more of mycoflora than sterilised seed throughout the investigation. Several other workers also indicated that unsterilized seed yielded more number of fungi than sterilized seed in different crops. (Shrotri *et al.*, 1983); (Srinivasulu *et al.*, 1994).

Studies on the isolation and identification of fungus associated with bengalgram include *Alternaria* sp., *Aspergillus flavus, A.niger Ascochyta rabiei, Botrytis cinerea, Colletotrichum* sp., *Curvularia lunata, Fusarium* sp., *Penicillium* sp., and *Rhizopus stolonifer*.

The isolated fungi were identified by comparing the morphological characters of each fungus earlier reported by Thom and Raper (1945), Gilman (1957), Subramanian (1971), Punithalingam and Holliday (1972), Ellis and Waller (1974), Laha and Grewall (1983) and Haware *et al.* (1986).

The results obtained with reference to the effect of seed mycoflora on germination clearly indicated that most of the fungi associated with bengalgram inhibited germination and reduced shoot and root length to a greater extent. Among the seed mycoflora, *Alternaria* sp. proved highly pathogenic followed by *Ascochyta rabiei* and *Fusarium* sp. which reduced the germination and shoot and root length.

Bhargava (1995) also reported that culture filtrates of *Alternaria alternate* casual agent of chickpea blight, inhibited seed germination, besides reduction in plumule growth with blackening of seed. *Ascochyta rabiei* (Halfon-Meiri and Aliza, 1970) and *Fusarium* sp. (Chary and Reddy, 1982) are also known to reduce the germination in chickpea and greengram respectively.

Mishra and Kanuja (1973) reported that the fungi would affect the seed germination directly either by lowering the viability of the seed, by making it nutritionally poor or by secreting certain mycotoxic substances unfavourable to the seed.

According to Christensen (1980), the pathogen might invade endosperm tissue immediately adjacent to the embryo. The first effect of this invasion would be weaking of embryo followed by death and discolouration. Vidyasekaran and Govindaswamy (1986) reported that the germination failure might be due to the exhaustion of the stored reserve food material in the seeds by invading fungi.

Regarding the effect of seed borne fungi on seedling vigour of bengalgram, majority of fungi adversely affected both shoot and root length. Among the fungi, *Alternaria* sp. caused most adverse effect in reducing the shoot and root length (8.53 and 4.42 cm) over control (19.2 and 8.76 cm). followed by *Ascochyta rabiei* and *Fusarium* sp. These observations are in accordance with the findings of Kamal and Verma (1979) and Goel and Gupta (1986) on redgram and chickpea respectively.

The other fungi which affected shoot and root length were *Aspergillus flavus*, *A.niger*, *Botrytis cinerea*, *Colletotrichum* sp., *Curvularia lunata*, *Penicillium* sp. and *Rhizopus stolonifer*.

The effect of five fungicides on three fungi viz., *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp. was studied. Captan, Carbendezim, Calixin, metalaxyl and Thiram were evaluated at three different concentrations viz., 500, 1000 and 1500 ppm *in vitro* by poison food technique. The results revealed that all the fungicides used were effective but the efficacy differed at different concentration.

Of the five fungicides at 500 ppm concentration tried against *Alternaria* sp. Carbendazim was most to be effective in inhibiting the growth of the fungus followed by Thiram and Captan. At 1000 ppm also carbendazim was found to be effective followed by Thiram and Captan compared to other fungicides. At 1500 ppm concentration, Captan was effective and inhibited the growth of the fungus to an extent of 93.75 per cent. Pieta and Patucha (1993) also observed that among other combination of fungicides for seed treatment, Thiram + carbendazim and Thiram + carboxin reduced the incidence of *Alternaria alternata* in soybean seed. The efficacy of Thiram and Captan in controlling several seed borne fungi which included *Alternaria* sp. was reported by Sundaresh *et al.* (1987) and Bidari *et al.* (1996).

The effect of the same five fungicides on *Ascochyta rabiei* indicated that all the fungicides reduced the growth of the fungus significantly at different concentrations over control. Among the fungicides, carbendazim was found most effective compared to other chemicals. Thiram, Calixin, Captan and metalaxyl also reduced good the growth of the fungus to some extent.

Eventhough metalaxyl decreased the growth of fungus, it was not as effective as other chemicals.

Similar results were also obtained by other workers. Singh and Singh (1990) stated that chickpea seed treated with combination of Carbendazim + Thiram gave 95 - 100 per cent control against blight fungus. Tripathi (1986) reported that seed treatment of bengalgram with Calxin M (tridemorph + maneb), Calixin M + Thiram and Bavistin + Thiram were effective against *Ascochyta rabiei*. Vir and Grewal (1975) found that Captan was most effective against *Ascochyta rabiei* in bengalgram.

In the present investigation with different fungicides in controlling seed borne *Fusarium* sp., carbendazim at 500 ppm was effective compared to others and significantly reduced the growth of the fungus. At 1000 ppm, Carbendezim was effective followed by Thiram, Captan, Metalaxy, and Calixin, but at 1500 ppm, Thiram was effective. metalaxyl was least effective at all concentrations in reducing the growth of the fungus.

Several workers also reported that the efficacy of different fungicides in inhibiting the growth of *Fusarium* sp. Singh *et al.* (1993) reported that Bavistin (carbendazim) completely inhibited radial growth of *Fusarium oxysporum* f. sp. *ciceri*. Gupta *et al.* (1997) stated that among several fungicides tested, carbendazim was the most effective and completely inhibited the growth of *Fusarium oxysporum* f. sp. *ciceri*. Shugha *et al.* (1995) reported that Carbendazim, Thiram and combination of both reduced the growth of *Fusarium oxysporum* f.sp. *ciceri*. Zaidi *et al.* (1991) reported that several fungicides including Captan found to be the most effective in reducing most of the fungal including *Fusarium oxysporum* f.sp. *ciceri*.

In the present investigation, it was found that *Azadirachta indica* also inhibited the growth of *Fusarium* sp. by 84.48 per cent followed by *Tamarindus indicus*. Both were on par with each other. Singh *et al.* (1980) found that oil of neem inhibited the growth of four fungi which included *Fusarium oxysporum* f.sp. *ciceri* in chickpea.

Seethalakshmi (1991) stated that among different plant entracts *Azadirachta indica* was more effective in inhibiting the radial growth of *Fusarium moniliforme*. Nagi Reddy (1996) also reported that among the ten plant extracts, *Azadirachta indica* was highly effective in inhibiting the linear growth of *Fusarium moniliforme*. These observations are in accordance with the results obtained in the present study. The effectiveness of *Azadirachta indica* may be attributed to the presence of certain antifungal ingredients '**Azadirachtin**' in the leaf.

Summary

CHAPTER - VI

SUMMARY

Ten bengalgram varieties viz., Annigeri, K-850, JG-62, GL-769, ICCV-10, BG-276, DCP 92-3, KWR-108, IPC 94-19 and IPC 94-37 were tested for seed mycoflora by agar plate and blotter methods. In order of predominance, the fungi detected by agar plate method were *Aspergillus niger, A.flavus, Ascochyta rabiei, Alternaria* sp., *Fusarium* sp., *Botrytis cinerea*, *Rhizopus stolonifer, Curvularia lunata, Fusarium* sp. and *Colletotrichum* sp. and by blotter method, *Aspergillus niger*, *A.flavus, Alternaria* sp., *Ascochyta rabiei*, *Fusarium* sp., *Botrytis cineria*, *Rhizopus stolonifer*, *Curvularia lunata, Colletotrichum* sp. and *Penicillium* sp. Agar plate method was found better than blotter method for detection of all fungi. Qualitatively same fungi were obtained in both the methods, but the difference was in the number of colonies. Irrespective of the variety, incidence of mycoflora was more in unsterilized than sterilized seeds. In both the methods, highest incidence of *Aspergillus niger* and *A.flavus* were recorded.

All the fungi isolated from bengalgram seed reduced the germination percentage considerably over control *in vivo*. Out of all the fungi, *Alternaria* sp. reduced maximum percentage of germination (59.00 per cent) over control followed by *Ascochyta rabiei* (57.00 per cent) and *Fusarium* sp. (55.00 percent). The effect of *Penicillium* sp. on percentage germination was negligible compared to other fungi.

Shoot and root lengths were adversely affected by *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp.

The effect of five fungicides viz., captan, carbendazim, calxin metalaxyl and Thiram was tested against three fungi viz., *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp. The fungicides were evaluated at 500, 1000 and 1500 ppm concentrations *in vitro* by poison food technique. Carbendazim was found the best among all at 500 and 1000 ppm concentration in inhibiting the growth of the *Alternaria* sp. while Captan at 1500 ppm concentration was the next best. Against *Ascochyta rabiei*, carbendazim was effective at all concentrations i.e. 500, 1000 and 1500 ppm, compared to other fungicides. Carbendazim at 500 and 1000 ppm and Thiram at 1500 ppm concentration were effective against *Fusarium* sp.,

The effect of five plant extracts viz., *Azadirachta indica, Mirabilis jalapa*, *Phyllanthus neruri, Solanum nigrum* and *Tamarindus indicus* on growth of three fungi viz., *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp.was studied by poison food technique was studied. *Azadirachta indica* extract was most effective on *Alternaria* sp., *Ascochyta rabiei* and *Fusarium* sp. which inhibited growth by 78.64, 72.89 and 84.43 respectively. *Phyllanthus neruri* was least effective in inhibiting *Alternaria* sp. and *Fusarium* sp. but its efficacy was better against *Ascochyta rabiei*.

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8554

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