STUDIES ON SEED MYCOFLORA OF SOYBEAN
(*Glycine max* (Linn.) Merri.)

By

S. KHAYUM AHAMMED

B.Sc., (Ag.)

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

MASTER OF SCIENCE IN AGRICULTURE

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

DECEMBER, 1998

CERTIFICATE

Mr. S. KHAYUM AHAMMED has satisfactorily prosecuted the course of research and that the thesis entitled "**STUDIES ON SEED MYCOFLORA OF SOYBEAN (*Glycine max* (Linn.) Merri.)**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any University.

Date : 9-12-98

Place : Tirupathi

(Dr. R. JOSHUA ANANDAM)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitl ed **"STUDIES ON SEED MYCOFLORA OF SOYBEAN *(GLYCINE MAX* LIMNN. MERRI)"** submitted in partial fulfilment of the requirements of the degree of Master of Science in Agriculture of Acharya N. G. Ranga Agricultural University, Hyderabad is a record of the bonafied research work carried out by **Mr. S. KHAYUM AHAMMED.** under my guidance and supervision. The subject of the thesis has been approved by the Students' Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of the investigations have been duly acknowledged by the author of the thesis.

R. Joshua Anandam

(DR. R. JOSHUA ANANDAM)
Chairman of the Advisory Committee

Thesis approved by the Student Advisory Committee

Chairman	:	(Dr. R. JOSHUA ANANDAM) Associate Professor Dept. of Plant Pathology S.V.Agricultural College, Tirupati	R. Joshua Anandam
Member	:	(Dr. A. V. RAMANA REDDY) Associate Professor Dept. of Plant Pathology S.V.Agricultural College, Tirupati	A. Ramana Reddy
Member	:	(Dr. A. SUDHAKARA RAO) Plant Pathologist RARS, Tirupati.	(A. SUDHAKARA RAO)
Member		(Dr. K. HARIBABU) Associate Professor Dept. of Horticulture S.V. Agricultural College, Tirupati.	

DECLARATION

I, **Mr. S. KHAYUM AHAMMED** *, hereby declare that the thesis entitled* **"STUDIES ON SEED MYCOFLORA OF SOYBEAN (*Glycine max*(Linn.) Merri.)"** *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 : 4/2/99

(S. KHAYUM AHAMMED)

CONTENTS

LIST OF ILLUSTRATIONS

LIST OF PLATES

LIST OF TABLES

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
Microgram	µg
Molar	M
Micrometer	µm
Parts per million	mg kg^{-1} (ppm)
Parts per billion	ppb

ACKNOWLEDGEMENT

At the out set, I thank god for his abundant love, grace blessing upon me, in every stride of my career and also for successful completion of my research work.

By the grace of the Almighty I have tried to put all efforts to fulfill the task assigned to me by the learned members of the Advisory Committee. Fervently and modestly, I extol the genuine and constant encouragement, worthy guidance and every suggestive help offered to me by the chairman of my Advisory Committee ***Dr.R.Joshua Anandam,*** *Associate Professor, Department of Plant Pathology, S.V.Agricultural College, Tirupati, right from the initiation of the work to the ship-shaping of the manuscript. The present work bears at every stage, the impression of his wise counsel and concrete suggestions, careful and reasoned criticism and meticulous attention to the details. I take it as a great privilege and pride to have had an opportunity of working under his unending inspiring and indomitable spirit.*

My deepest admiration and heartful thanks to ***Dr.A.V.Ramana Reddy,*** *Associate Professor, Department of Plant Pathology, S.V.Agricultural College, Tirupati for his keen interest, acquiescence, benign attitude valuable suggestions, and talented guidance have lead to the present investigation to the final shape.*

My deep sense of gratitude is due to ***Dr.A.Sudhakar Rao,*** *Plant Pathologist, RARS, Tirupati for his judicious guidance, constructive criticism, generous help rendered during my research work.*

My sincere thanks to ***Dr.K.Hari Babu,*** *Associate Professor, Department of Horticulture, S.V.Agricultural College, Tirupati as a member of the Advisory Committee for his help and encouragement during my research work.*

It gives me immense pleasure in extending my deep sense of reverence and gratitude to the other members of the Department, ***Dr.K.Sitaramaiah,*** *Professor and Head,* ***Dr.V.K.Krishna Rao,*** *Associate Professor,* ***Dr. S.V.Ram Krishna Rao,*** *Associate Professor,* ***Dr. N.P. Eswara Reddy,*** *Assistant Professor, Department of Plant Pathology, S.V.Agricultural College, Tirupati for their generous suggestions, timely help and encouragement during this course of work.*

My deepest admiration and heartful thanks to ***Dr. K. Subramanyam Reddy,*** *Associate Professor and incharge head of the Department of Statistics and Mathematics, S.V.Agricultural College, Tirupati. I am very much greatful to* ***Dr.S.R.Viswanath,*** *soybean breeder, GKVK, Bangalore providing the seeds for my research work. I place on record my sincere thanks and express my indebtness to* ***Sri.A.Mohan Rao,*** *Assistant Professor, GKVK, Bangalore, for his untiring efforts in helping me in all respect of bring this thesis to a shape.*

I greatly acknowledge with thanks to ***Sri. Kodanda Ramaiah*** *for his help and encouragement during my course of work.*

I express my deep sense of gratitude and regards to my parents ***Sri.S.Nazeeruddin*** *and* ***Smt.S.Ashia begum*** *who have given me life*

and taught the concepts of life. They have been a fountain of inspiration throughout my life without whose help in every walk of life, this work would not have been possible. I owe an encompassing debt to my endearing brother ***S.Sayum Ahammed,*** *brother-in-law* ***S.Habeeb,*** *sister* ***S.Shameem Siddiqua,*** *uncle* ***S. Mahaboob basha*** *and relatives for boundless love, indefatigable efforts; incessant encouragement and passionate forbearance through out my study which enable me to reach upto this level.*

I thanks with immense pleasure to express my gratitude to my colleagues ***Rao, Tirumal, Ravi,*** *Swamy and Seniors* ***Mathi Reddy, Lux*** *and* ***Vemana*** *and Juniors* ***MK, Sreenu, Syam, Sundaram, Sakthi, Krishnaveni*** *and other seniors* ***Rafi, Chakri, Khadri, Royal, Chenchu, Chowdary, Raju, Subbu.***

I felt elated to express my bountiful thanks to my affectionate and beloved friends, ***Sasidhar, Zee, Basha, Hari, Shyam, Venkat, Prasad, Boss, Vamsi, Jak, Chandra, Subbu, Vijay, Bala^2, Uppu, Break, Sobhan, Anil, Ramakrishna, Kalai, Bharat, Yousaf, Chand, Shameer, Ramprasad, Babu*** *and my juniors* ***Hum, Prakash, Bore, Gopi, Rajsekhar, Rajesh, Mouli, Prasad, Ashok, Ajay, Ramakrishnan, Sahoo, Praveen, Sudhakar, Abbas, Somu, Murugan, Bhaskar, Saravanan, Gurunath, Sagar, Rajan, Chitti, Bharat, Anise, Heku, Palmurii, Musallaiah, Subodh.***

I am very much grateful to ***Visweswaraiah*** *for taking such a nice photographs.*

My thanks to ***Doraswamy, Govind, Srinivasaulu, Eswaraiah, Chenchayya*** *for their assistance in the lab during my study period.*

My thanks to library staff for providing all the necessary facilities required for the preparation of thesis.

The financial assistance rendered by the Government of Andhra Pradesh and Acharya N.G.Ranga Agricultural University in the form of stipend during the course of study is greatly acknowledged.

I wish to express my thanks to ***Hemanth*** *for putting in their best effort in bringing out this thesis in the most presentable form.*

(S. KHAYUM AHAMMED)

ABSTRACT

Name	:	**S. KHAYUM AHAMMED**
Title of the thesis	:	**STUDIES ON SEED-MYCOFLORA OF SOYBEAN (*Glycine max*(Linn.) Merri.)**
Degree	:	**MASTER OF SCIENCE**
Faculty	:	**AGRICULTURE**
Department	:	**PLANT PATHOLOGY**
Major Advisor	:	**Dr. R. JOSHUA ANANDAM**
University	:	**ACHARYA N.G.RANGA AGRICULTURAL UNIVERSITY**
Year of Submission	:	**1998.**

ABSTRACT

Soybean is a promising pulse crop since it contains higher percentage of protein and oil. It contains 20-24 per cent oil and 40-42 per cent protein. Mycoflora associated with ten varieties of soybean seed viz., PK-1162, Bragg, PK-1029, MACS-450, MACS-129, MACS-34, Monetta, JS-HS-90-91, Harade and KHSB-2 includes ***Alternaria sp., Aspergilus flavus, A.niger, Curvularia lunata, Fusarium sp., Rhizoctonia sp., Rhizopus stolonifer*** and ***Penicillium*** sp. Both agar plate and blotter methods were employed for the isolation of mycoflora. Agar plate method was found better in yielding maximum number of colonies of fungi than blotter method. In both the methods, unsterilized seed yielded more number of colonies than sterilized seed. Among the eight fungi tested to study the effect of germination ***in vivo, Fusarium sp., Rhizoctonia sp.*** and ***Alternaria sp.*** have shown greater effect in reducing the percentage of germination and shoot and root length considerably.

Among the five fungicides tested by poison food technique, thiram at all concentration and captan at 2000 ppm were effective against ***Rhizoctonia sp.*** captan inhibited the growth of ***Fusarium*** **sp.** at all concentrations while celest at 500 and 1000 ppm were effective against ***Alternaria sp.***

Out of the ten isolates of ***Aspergillus flavus*** screened for aflatoxin production, Isolate - IV produced large amounts compared to others, but there was no aflatoxin production when isolates II, VI and X were used. There was maximum reduction in shoot and root lengths in the five prominent varieties when the seeds were soaked in culture filtrate of isolate IV containing large amount of aflatoxin.

INTRODUCTION

CHAPTER - I

INTRODUCTION

Soybean (*Glycine max*(Linn) Merri.) has recently been introduced on a large scale for cultivation in India. it is a promising pulse crop since it contains higher percentage of protein and oil. Soybean seed contain 20-24 per cent oil and 40-42 per cent protein. Soybean protein is rich in the valuable amino acids like lycine (5 per cent) in which most of the cereals are deficient. It is also a good source of vitamin-B complex, thiamin and riboflavin. Soya based foods contain low carbohydrate and are considered suitable to diabetic patients. The enhanced utilisation of soybean will not only over come the problem of malnutrition and protein deficiency, but also prevent particularly in weaker section of the society.

Soybean is grown in an area of about 2.821 million ha with an annual production of 2.275 million t in India (Bhatnagar and Tiwari 1993). It is largely grown in the States of Madhya Pradesh, leads in acreage 3.0 million ha as well as production of 2.32 million t accounting for about 80 per cent share and the rest by Maharastra, Rajasthan, Uttar Pradesh, Tamilnadu. In India there is a still a great potential in the state like Andhra Pradesh, Karnataka, Bihar because India has been among the top three countries with the Argentia and Brazil.

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 germination, emergence and subsequent performance of the crop in the field. Soybean 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 synthetic fungi toxic chemicals in plant disease control was shown to be effective in the improvement of agriculture by way of disease management. Aflatoxin contamination has been encountered in several agricultural commodities including soybean.

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

1. To isolate the fungi associated with soybean seeds
2. To study the effect of seed borne fungi on seed germination *in vivo*.
3. To study the effect of certain fungicides on seed-borne fungi *in vitro*.
4. To study the effect of aflatoxin produced by *Aspergillus flavus* on soybean seeds.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 SEED BORNE FUNGI ASSOCIATED WITH SOYBEAN

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.

Schneider and Thaplial (1971) detected fungi associated with Soybean seeds (Var. Bragg and Clark - 63) from plots near Jabalpur, Madhya Pradesh. The seeds frequently yielded species of ***Cercospora, Rhizoctonia, Aspergillus*** *and* ***Fusarium.*** Several genera like ***Pythium, Botrytis, Cladosporium, Chetophoma, Acremonium, Diplodia, Monilia, Tricothecium,*** which were not detected previously from Soybean seeds, were detected.

Singh ***et al.*** (1973) carried out seed health studies on Soybean raised in the Nainital Tarai. They found 19 species of fungi and bacterium (***Bacillus).*** Out of these, ***Alternaria tenuis, Aspergillus niger, A. temarii, Chaetomium brassiliense, C. erectum,***

Penicillium cyclopium, Rhizopus arrhizus, Trichoderma *sp. and* ***Bacillus*** *sp.* appeared to be new record on cv. Clark -63 gave higher counts of fungi than Bragg. The blotter method proved to be superior over agar plate method.

Sundaresh and Hiremath (1978) studied seed mycoflora of soybean seed in Karnataka and isolated ***Rhizoctonia*** and ***Alternaria*** from surface sterilized seed and ***Aspergillus, Fusarium Curvularia, Penicillium*** and ***Cladosporium*** from unsterilized seeds of 6 cultivars.

Mengistu and Sinclair (1979) found 38 genera of fungi and ***Bacillus subtilus*** associated with soybean seed lots of 16 cultivars grown in Ethiopia and plated on Potato Dextrose Agar (PDA). Out of them ***Alternaria tenuissima, Aspergillus flavus, A. niger, Chaetomium funicola, Cladosporium herbarum, Colletotrichum dematium var. truncata, Fusarium oxysporum, Penicillium, Phomopsis*** were new record for world.

Zad (1979) isolated fungi from 12 cvs. of soybean from different parts of Iran. ***Alternaria*** spp., ***Ascochyta pinodella, Cercospora kikuchi, Colletotrichum truncatum, Diaporthe phaseolorum, Fusarium sp., Macrophomina phaseolina, Peronospora manshurica*** and ***Verticillium*** sp., were predominant.

Karunakar ***et al.*** (1980) reported 12 fungal spp. associated with soybean seed samples of 2 cvs. Among them ***Alternaria*** sp., ***Fusarium moniliforme, Cercospora*** sp. ***Colletotrichum*** sp. and ***Macrophomina phaseolina*** were predominant.

Singh ***et al.*** **(1983)** carried out ***in vitro*** tests using a modified blotter method, the pH of the water influenced the isolation of ***Alternaria tenuis, Aspergillus flavus, A.niger, Botrytis cinera, Chaetomium indicum Curvularia lunata, Drechsclera tetramera, Fusarium oxysporum, Macrophomina phaseolina, Memnoniella echinata, Penicillium chrysogenum, Trichothecium roseum*** associated with soybean seeds.

Muthegowda ***et al.*** (1987) isolated 38 fungi from soybean seed. Deuteromycetes fungi ***Aspergillus*** and ***Penicillium*** were predominent.

Sharma (1987) reported that samples of soybean seed from farmers field had a very high incidence of some important fungi which included species of ***Alternaria, Fusarium, Trichothecium*** and ***Cladosporium.***

Agarwal and Gupta (1989) studied the seed mycoflora of 200 seeds of 16 soybean varieties from Jabalpur, (M.P), Jungarh, Navsari (Gujarat), Parbhani, Akola and Pune (Maharashtra). They detected ***Macrophomina phaseolina*** on all seeds except on those from Akola or on cultivars

Bragg and DS-74-42. ***Colletotrichum dematium*** was not detected in any of the seed samples from Navsari nor on seeds of cultivars of AMSS-21, Kalitur, MACS-13, MACS-41 and UPSM-19. ***Colletrotrichum dematium*** seed infection was maximum on cultivars Bragg and JS-75-19. ***Fusarium*** sp. and ***Aspergillus flavus*** was found in all the seed samples.

Weidenborner and Hindorf (1989) studied the seed-borne mycoflora of soybean, bean, pigeon pea, cotton and pods of groundnut by transferring to 10% sodium chloride-malt extract agar. The storage fungi of ***Aspergillus glaucus, A. flavus, A. ochraceous*** and ***A. niger*** occurred on both seeds and pods. Field fungi were less frequently detected on each sample than storage moulds. Of the fungi only ***Cladosporium*** was present on both seeds and pods.

Yum and Park (1989) isolated 20 genera of fungi from soybean seed samples of which 17 were identified. The major pathogens were ***Cercospora kikuchi, Colletotrichum truncatum, Diaporthe phaseolorum*** var. ***sojae***, ***Fusarium*** sp., ***Macrophomina phaseolina.*** Other fungi identified were ***Chaetomium, Alternaria, Penicillium, Myrothecium verrucaris, Epicoccum, Nigrospora, Cladosproium, Curvularia, Drechsclera, Monilia, Rhizoctonia*** and ***Spaceloma glycines.***

Tripathi and Singh (1991) studied the mycoflora of seed samples of soybean cultivars T49, Bragg, and Lee from 5 different locations in Uttar Pradesh using standard blotter and agar plate methods and identified 16 fungal species viz., ***Alternaria alternata, A.tenussima, Aspergillus flavus, A. niger, A. nidulans, A. terreus, A. sydowi, Penicillium oxalicum, Fusarium oxysporum, F. moniliforme, Mucor racemosus, M. subtilissimus, Curvularia lunata, Rhizopus arrhizus, Myrothecium roridum*** and ***Nigrospora oryzae.***

Khattak ***et al.*** (1993) isolated ***Alternaria alternata, Alternaria tenussima. Arthobotrys*** sp., ***Aspergilus flavus, A.niger, Cladosporium cladosporoides, Colletotrichum trun-catum, Curvularia lunata, Drechslera australiensis, Fusarium oxysporum, Nigrospora*** sp., ***Penicillium sp., Rhizoctonia solani, Rhizopus*** sp., ***Septocylindrium*** sp. and ***Trichothecium*** sp. from soybean seed by agar plate method.

Murthy and Raveesha (1996) studied mycoflora of soybean in Karnataka and identified 38 species of fungi out of which ***Aspergillus, Penicillium*** and ***Rhizopus nigricans*** were the most commonly occurring storage fungi and ***Alternaria, Chaetomium, Colletotrichum, Cladosporium, Diaporthe, Fusarium, Macrophormina, Myrothecium, Phoma*** and ***Trichothecium*** were the most commonly occurring field fungi.

Goulart (1997) reported seed-borne fungi viz., ***Phomopsis*** spp., ***Colletrotrichum truncatum, Cercospora kikuchi, C.sojina, Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium semitectum, Aspergillus*** spp. and ***Penicillum*** spp. on soybean seeds in Brazil.

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.

Ellis ***et al.*** (1979) isolated 35 genera of fungi from soybean seeds, out of which 19 genera significantly reduced soybean germination ***in vitro***. The percentage of seed germination were greater when inoculated seeds were planted in sterile sand than ***in vitro*** germination on PDA. ***Phomopsis*** sp., ***Sclerotium rolfsii, Botryodiplodia, Theobroma, Colletotrichum dematium, Macrophomina phaseolina*** and ***Cephalosporium gregatum*** were most pathogenic and reduced the seed germination.

Singh ***et al.*** (1986) isolated 8 fungi from soybean seed. In the tests with 8 fungi associated with the seed, the maximum reduction in seed

germination was observed with the culture filterates of ***Fusarium semitectum*** followed by ***F. equiseti*** and ***Curvularia lunata***. All the fungi reduced shoot length but the effect on root length was variable.

Tripathi and Singh (1991) conducted tests of culture filterates of 16 fungal species isolated from 30 seed samples from various locations in Uttar Pradesh. The metabolites of ***Aspergillus flavus*** and ***A. niger*** were the most damaging to germination of soybean seed and to elongation of seedling roots.

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

Sharma (1995) observed fungal metabolites of three seed-borne fungi viz., ***Alternaria alternata, Cladosporium herbarum*** and ***Trichothecium roseum*** which reduced the germination and caused the rotting of soybean c.v Bragg. Maximum seed rotting was caused by culture filterate of ***C.herbarum***.

2.2 EFFECT OF DIFFERENT FUNGICIDES ON SEED-BORNE FUNGI

Singh ***et al.*** (1974) observed that thiram and terraclor super-X at 0.5% significantly increased seedling emergence after 6 months of seed storage. They reported that terrachlor super-X completely eliminated, and captan, PCNB and thiram reduced the incidence of ***Aspergillus flavus, A. niger, Colletotrichum truncatum, Macrophomina phaesolina, Penicillium cyclopium, Phoma*** sp. and ***Rhizopus arrhizus.***

Ellis ***et al.*** (1975) stated that the seed treatment with captan, thiram and benomyl at 0.016, 0.033, and 0.033 g a.i. / 20 g of soybean increased germination ***in vitro.***

Patil and Mayee (1977) reported that vitavax among the systemics reduced pre-emergence and post-emergence death, while the non-systemics were more specific. Dithane M-45 and agrosan G.N. controlled pre-emergence and post-emergence death of ***Sclerotium rolfsii*** of soybean.

Ganacharya (1979) reported that captan, thiram and derosal (Carbendazim) at 3.45 and 2 g/kg of soybean seed significantly increased emergence to 99.69, 98.33 and 96.56% respectively.

Sundaresh *et al.* (1987) reported that seed treatment with thiram and dithane M-45 gave good control of seed-borne fungi including *Aspergillus, Penicillium, Fusarium, Macrophomina, Alternaria* and *Rhizopus* and increased germination by 18-28 per cent *in vitro* and 22-23 per cent *in vivo*.

Pardeshi *et al.* (1989) used seven fungicides viz., mancozeb, bavistin, thiram, vitavax, captan, sulphur and difolatan against 5 cultivars (Monetta, JS-72-44, JS-2 MACS-13 and Bragg) of soybean for seed treatment. All fungicides (except vitavax on Bragg and difolatan on JS-2, increased germination percentage. Thiram, carbendazim and difolatan were most effective. Root and shoot length, vigour index (percentage germination X root length + shoot length) were increased most effectively after application of captan followed by thiram, sulphur and mancozeb.

Setty *et al.* (1991) observed that Captan increased germination of soybean seeds by 10 per cent *in vitro* and 21 per cent *in vivo*. Mancozeb was more effective which increased germination by 23 and 40 per cent *in vitro* and *in vivo* respectively. Germination was low in untreated seed.

Shah *et al.* (1992), studied the efficacy of seven fungicides viz., benlate, bayton, captan, dithane-M.45, orthocide, vitavax and topsin

against 15 seed-borne fungi in soybean seed. All the fungicides reduced the incidence of fungal pathogens, but benlate followed by captan were found to be the best.

Chung and Ju (1993) reported in Korea that treatment of soybean seed with benoram (20 per cent benomyl+20 per cent thiram) improved seedling emergence rate and increased length of the hypocotyl.

Pieta and Patucha (1993) observed that seed treatment with fungicides like triademenol + imazalil, isofenphos + thiram, Iprodione, thiram + carbendazim and thiram + carboxin reduced the incidence of ***Phoma exiqua*** var. ***exiqua*** and ***Alternaria alternata*** on soybean seeds.

Vitti ***et al.*** (1993) observed high incidence of ***Cercospora kikuchi, Colletotrichum dematium var. truncata, Penicillum sp.*** and ***Aspergillus sp.*** in soybean seeds. They reported that thiabendazole, captan and benomyl were effective against all the fungi, except *C. truncatum. In vitro* tests revealed that benomyl and thiabendazole were highly effective against *C. truncatum.*

Peshney ***et al.*** (1994) isoloated ***Aspergillus flavus Aspergillus niger, Penicillium sp., Fusarium semitectum*** and ***Rhizoctonia bataticola*** from seeds of different varieties of soybean in Maharastra.

A. flavus, A. niger and *Penicillium sp.,* occurred with higher frequency than *Rhizoctonia bataticola*. Thiram 0.3 per cent as seed dresser was more effective against *Aspergillus* and *Penicillum* while captan 0.3 per cent and carbandezim 0.1 per cent were effective against *Fusarium semitectum* and *Rhizoctonia bataticola* respectively.

Ali *et al.* (1995) reported nine fungal species viz., *Alternaria alternata, Fusarium moniliforme, F. oxysporum, F. solani, Macrophomina phaseolina, Aspergillus flavus, Myrothecium roridum, Chaetomium sp.,* and *Aspergillus sp.* from 20 soybean seed samples collected from grain markets in Punjab and Pakistan. Of the 9 species *M. phaseolina* was the most pathogenic in germination tests. Topsin-M and benomyl were effective in controlling *M. phaseolina*.

Goulart *et al.* (1995) evaluated the effectiveness of seed treatment for the control of seed-borne fungi of soybean in Brazil. All the fungi tested reduced the incidence of pathogens associated with seeds compared with the control. The best control of *Colletotrichum truncatum* was obtained with carboxin + thiram, tolyl fluamid M-100, thiobendazole + thiram and tolylfluanid M-75. The same fungicides also controlled *Cercospora kikuchii* varying from 77 to 100 per cent. *Fusarium semitectum, Aspergillus* sp. and *Penicillium* sp. were controlled with thiabendazole + thiram, tolylfluanid M and pencycuron.

Picinini and Fernandes (1996) studied the effect of 9 fungicides and fungicide mixture in controlling the seed borne pathogens in Brazil. Among the fungicides tested captan gave good control of ***Phomopsis***, while thiobendazole + thiram and carbendazim gave 100 per cent control of ***Cercospora kikuchii***.

Solanke ***et al.*** (1997) stated that response of soybean seed i.e MACS-13 and PK-472 to ***Fusarium moniliforme, Aspergillus flavus*** and ***A. niger*** inoculation were less in thiram dry seed treatment than carbendazim, captan, captafol, mancozeb and thiram + carbendazim and untreated control.

2.4 EFFECT OF AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS* ON SOYBEAN SEEDS

Aflatoxin contamination has been encountered in several agricultural commodities and more so in pulses. Aflatoxins are carcinogenic metabolities produced chiefly by *A. flavus* group of fungi in nature.

Hesseltine ***et al.*** (1966) grew the strains of ***Aspergillus flavus*** on six major agricultural commodities in stationary and shake cultures. Strain 2999 produced the most aflatoxin B_1 on sorghum, peanuts, soybeans and rice where as strain 3000 produced the most on wheat and corn, and strain 3145 produced 50-90% less aflatoxin than the other strains in all cases in stationery culture.

Wogan (1968) observed in food samples collected from various parts of the world, particularly from Africa and Asia. Aflatoxins were detected at biologically significant levels in a wide spectrum of commodities including barley, cassava, corn, cotton seed, cowpea, millets, peanuts, peas, rice, sesame, sorghum, soybeans, sweet potatos and wheat.

Shot Well ***et al.*** (1968) detected aflatoxin in the poorer grades of samples of commercial oat, wheat, corn, soybean and grain sorghum seed.

Hensarling ***et al.*** (1983) reported that aflatoxins were not produced by ***Aspergillus flavus*** SRRC-1000 on unautoclaved meal. Addition of zinc (as $ZnSO_4$) to autoclaved meal inhibited aflatoxin production and supplementation with sodium phytate relieved this inhibition. Addition of sodium phytate alone promoted production of aflatoxin.

According to the Wilson and Bell (1984) maize and groundnut samples were inoculated with 14 isolates of ***Aspergillus flavus*** and ***A. parasiticus*** while soybean samples were inoculated with four isolates of ***A. flavus*** and two isolates of ***A. parasiticus*** produced B_1, B_2, G_1 and G_2, while ***A. flavus*** isolates produced aflatoxin B_1 and B_2. The mean aflatoxin B_1 production was approximately 10 times greater on groundnut seed than on maize seed and more aflatoxin G_1 was produced on soybean seed than aflatoxin B_1.

Farag (1990) stated that ***Aspergillus flavus*** changed the chemical composition protein, lipid, carbohydrate and crude fibre of sesamum and soybean seed in growth medium. Tests on the effects on various media on aflatoxin production revealed that Indole Acetic Acid (IAA) increased production more than gibberellic acid.

Fernandez ***et al.*** (1991) investigated natural ocurrence of aflatoxin contamination on soybean in Argentina. He reported that aflatoxin levels in positive samples were low, ranging from trace to 36 fg/kg of total aflatoxin. Levels of aflatoxin produced by 3 isolates of ***Aspergillus flavus*** were dependent both on the toxigenic potential of the fungal isolates and on the variety of soybean. Under laboratory conditions variety Prata was the most susceptible and variety SRF450 the least susceptible to aflatoxin formation.

El-Kady and Youssef (1993) assayed 100 soybean samples for the growth of fungi at 2 incubation temperatures (28°C and 45°C). A total of 73 species and 8 varieties belonging to 32 genera were isolated ***Aspergillus flavus***, ***A. fumigatus, A. niger*** and ***A. alutaceus*** were predominated at 28° C and ***Aspergillus fumigatus*** was the dominant species at 45°C. The seeds were assayed for aflatoxin production by TLC and aflatoxin was detected in 35 per cent of soybean seed samples (5-35 fg/kg).

MATERIALS AND METHODS

CHAPTER - III

MATERIALS AND METHODS

Laboratory and pot culture studies were taken up in the Department of Plant Pathology at Sri Venkateswara Agricultural College, Tirupati of Chittoor District, Andhra Pradesh (India). Standard international procedures for seed testing were employed throughout the investigations.

3. SELECTION OF SEED :

For studying the seed mycoflora associated with soybean cultivars viz., Bragg, PK-1162, PK-1029, MACS-450, MACS-129, MACS-34, Monetta, JS-HS-90-91, Hardae and KHSB-2, were used. Seed samples were obtained from 'Soybean Scheme", Gandhi Krishi Vignan Kendra (GKVK), Bangalore.

3.1 CHEMICALS :

The chemicals used were both of analytical and laboratory grades. Double distilled water was used for the preparation of media and also in blotter method.

3.1.1 Cleaning of glassware :

The glassware used in the experimental work was cleaned with washing soda and thoroughly rinsed in tap water. Cleaning solution (Sulphuric acid + Potassium dichromate solution) was then poured in the

glassware and kept for a day, after which they were drained and cleaned thoroughly with several changes of tap water and finally with distilled water.

3.1.2 Sterilization :

Glassware viz., petriplates, pipettes, test tubes etc., were sterilized at 160°C for 2 hrs minutes in hot air oven. Nutrient agar media were autoclaved at 15 PSI. (121.62°C) for 15 minutes. Soil used for experimental work was sterilized at 20 PSI (126.6°C) for one h. Blotter paper circles 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 Corning make.

3.1.3 Surface sterilization of seed :

Surface sterilization of seeds was done by 1:1000 Mercuric chloride for 30 sec. The seed was washed with three changes of sterilised water.

3.1.4 Media used :

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

a. Potato dextrose agar (PDA) :

Potato	: 200.0 g
Dextrose	: 20.0 g
Agar	: 15.0 g
Distilled water	: 1000 ml

b. Plain agar :

Agar : 15.0 g

Distilled water : 1000 ml

For studying aflatoxin production, yeast extract-sucrose medium was used.

a. Yeast extract - Sucrose medium (Dowson, 1959)

Yeast extract : 10.0 g

Sucrose : 10.0 g

Agar : 15.0 g

Tap water : 1000 ml

pH 6.8 - 7.2

3.2 Isolation of seed-borne fungi

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

3.2.1 Agar plate method

To isolate externally seed borne fungi of soyabean, 50 seeds of each variety were taken and placed at the rate of 10 seeds in each petriplate containing Potato Dextrose Agar (PDA) medium. Five replications were maintained for each variety. The petriplates were

incubated at 28 ± 1°C in an incubator for 7 days and observed every day for development of seed borne fungi.

3.2.2 Blotter method (Neergaard and Saad, 1962)

Two circular oven-sterilized filter papers were placed in each of the petriplates and moistened with sterile water ensuring that a slight excess of water was left on the filter papers. Totally 50 seeds per each variety was taken and 10 seeds per plate were placed. Then the petriplates were incubated in an incubator at 28 ± 1°C for 7 days and observed every day for the development of seed-borne fungi.

The fungal colonies which were obtained by the above two methods were counted with the help of a stereo-binocular microscope and the fungal hyphae were picked up from the seed and pure cultures were maintained in agar slants.

The various fungal cultures obtained were tentatively identified by comparing with illustrations given by Barnett (1962), Gilman (1957) and Subramanian (1971) for identification of ***Aspergillus*** cultures, the manual by Raper and Fennel (1965) 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, variety "Bragg" was taken and studied.

3.3.1 Pot culture method :

Conidial suspensions of different fungal isolates multiplied on PDA were taken. In each suspension 30 surface sterilized seeds were soaked for 24 h. The treated seeds were sown in lots of 10 in each pot filled with sterilized soil. Three replications were maintained for each treatment (fungal isolate). The plots were watered daily with sterile water. Observations were recorded for germination upto 15 days. On 15th day, seedlings were carefully uprooted, washed free of soil and the shoot and root lengths were recorded. Controls with 10 seeds for each treatment were maintained.

3.4 EVALUATION OF DIFFERENT FUNGICIDES ON SEED-BORNE FUNGI *IN VITRO*

Three fungi which reduced both shoot and root length of soy bean drastically, were selected. The effect of five fungicides viz., Captan, Thiram, Celest, Metalaxyl, Carbendazim was evaluated in three fungi by "Poison food technique".

3.4.1 Preparation of concentration of fungicides :

For obtaining 1 ppm concentration 1 mg of pure solute / active ingradient (100 per cent pure chemical) was dissolved in one litre of water/PDA. The five fungicides selected were studied at three concentrations viz., 500, 1000 and 2000 ppm.

3.4.2 Poison food technique :

For each treatment 60 ml of PDA medium was taken in 250 ml conical flask and sterilized. To this medium required concentration of fungicide was added and mixed thoroughly and poured into 3 petri plates for maintaining replications and allowed to solidify. From seven days old culture plates 1.0 cm discs were cut from outer margin with sterilized cork borer and transferred to the centre of the plates containing fungicides. Control was maintained by placing fungal discs in untreated plates and incubated at room temperature (28-32°C). The whole procedure was carried out under aseptic conditions. The diameter of fungal colony was measured when the growth of the colony in control plate was complete. The colony diameter in treated plates compared with control, was taken as measure of fungi toxicity. The growth of fungal colony in treated plates was determined by excluding 1.00 cm fungal inoculum disc from measured diameter of colony. The percent inhibition was calculated by the following formula :

$$\frac{\text{Diameter of colony in control plate} - \text{Diameter of colony in treated plate}}{\text{Diameter of colony in control plate}} \times 100$$

3.4.3 Evaluation of fungicides *in vitro* :

To test the relative efficacy of different fungicides against three fungi viz., ***Alternaria, Rhizoctonia*** and ***Fusarium*** **sp.** experiments were conducted with 5 fungicides at different concentrations. In all the

experiments suitable controls were maintained and all the treatments were replicated thrice. The poison food technique as described by Nene and Thapliyal (1979) was followed.

3.4.4 List of fungicides used *in vitro* :

S. No.	Trade name of the fungicides	Chemical name	Concentrations (ppm)
1.	Captan 75 WP	N-Trichloromethylthio-4-cyclohexane-1,2-dicarboximide	500 1000 2000
2.	Thiram	Tetramethyl thiuram disulphide	500 1000 2000
3.	Celest	4-(2,2-difluoro-1,3-benzo dioxal-4-yl)-1H-pyrrole-3-carbonitrile	500 1000 2000
4.	Metalaxyl	N-(2,6-Dimethylphenyl)-N-(methoxy acetyl)-alanine methyl ester	500 1000 2000
5.	Carbendazim 50 WP	Methyl-2-benzimidazole carbamate	500 1000 2000

3.5 STUDY OF THE EFFECT OF AFLATOXIN PRODUCED BY *ASPERGILLUS FLAVUS* ON SOYABEAN SEEDS :

In the present experiment the ability of ***Aspergillus flavus*** to produce aflatoxin was studied. For this 10 different isolates of ***Aspergillus flavus*** obtained from soybean seed, were screened for aflatoxin production by the method reported by Van Walbeek ***et al.,*** (1968) with slight modifications.

Conidial suspension of each isolate was prepared by adding sufficient quantity of sterile water. One ml of each isolate was added to 250 ml conical flask containing 25 ml of "yeast extract-sucrose" medium and the flasks were incubated at room temperature (28-32°C) for 7 days. Three replications were maintained for each isolate. After 7 days, the culture filtrate of 3 flasks of each isolate was pooled up in a glass beaker and 150 ml of boiling (60°C) chloroform were added to the same. The mixture was added in a separating funnel. After shaking the funnel for 2 minutes, the lower chloroform layer was taken and the upper fraction was discarded. The chloroform layer so collected was passed through a column containing anhydrous sodium sulphate in order to remove traces of moisture. The chloroform extract was filtered. The residue was dryness by keeping on water bath. The residue was redissolved in 5 ml of chloroform and spotted on Thin Layer Chromatography (TLC) plates coated with silicagel along with standard aflatoxin which was obtained from College of Veterinary Science, Tirupati. Then these plates were developed in a solvent systems of chloroform: methanol : acetic acid : water (78 : 10 : 10 :2). After development these TLC plates were observed under UV lamp. Bright florescent spots were observed at the same Rf as that of standard which indicated the presence of aflatoxin.

The standard used contains 10 µg/ml of aflatoxin and total volume is 2.5 ml i.e 25 µg of aflatoxin. The intensity ratings were recorded as below :

No of aflatoxin	—
Trace of aflatoxin	+
Small amounts of aflatoxin	++
Moderate amounts of aflatoxin	+++
Large amounts of aflatoxin	++++

The brightest spot produced by the extract of the isolate indicated the maximum production of aflatoxin. The isolate producing maximum amount of aflatoxin was thus selected and used throughout the studies.

3.5.1 Effect of aflatoxin on seedling vigour of soybean

The seeds of 5 varieties viz., Bragg, Monetta, Hardae, PK-1029, KHSB-2 were soaked for 24 h in the culture filtrate of the isolate which produced maximum amount of aflatoxin for 24 h for each variety 10 seeds were taken and were incubated and studied by "blotter method", controls soaked in sterile water were maintained for each variety. Three replications for each variety were maintained. The seeds were surface sterilized with 0.1 per cent mercuric chloride before soaking. After 7 days the root and shoot lengths were recorded and compared with controls.

RESULTS

CHAPTER - IV

RESULTS

4.1 INCIDENCE OF MYCOFLORA ON DIFFERENT VARIETIES OF SOYBEAN SEED (AGAR PLATE METHOD) :

To study the incidence of seed mycoflora on different varieties of soybean, 10 varieties viz., Bragg, PK-1162, PK-1029, MACS-450, MACS-129, MACS-34, Monetta, JS-HS-90-91, Hardae and KHSB-2 were collected from GKVK campus, Bangalore 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 PK-1162, MACS-450, (Plate I), MACS-34, JS-HS-90-91, Hardae and KHSB-2 (Plate II) recorded maximum number of 8 fungal species followed by Bragg (Plate III), PK-1029, MACS-129 and Monetta in which 7 fungal species were present in each variety. Quantitatively, there was considerable difference in the number of fungi isolated from different varieties.

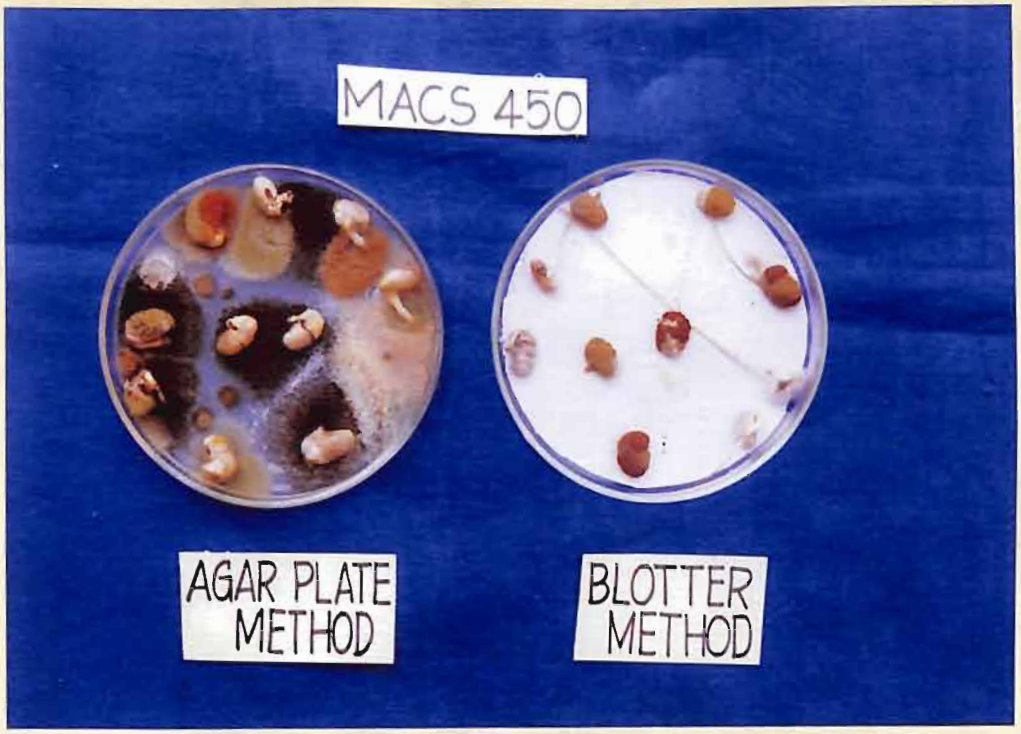
Among the fungi associated with soybean seeds ***Aspergillus flavus*** was the most predominant in all the varieties (63.2 per cent). The percentage of unsterilized seeds colonized by ***A. flavus*** was 36.0 in

TABLE - 1 : Incidence of seed-mycoflora on different varieties of soybean (Agar Plate Method)

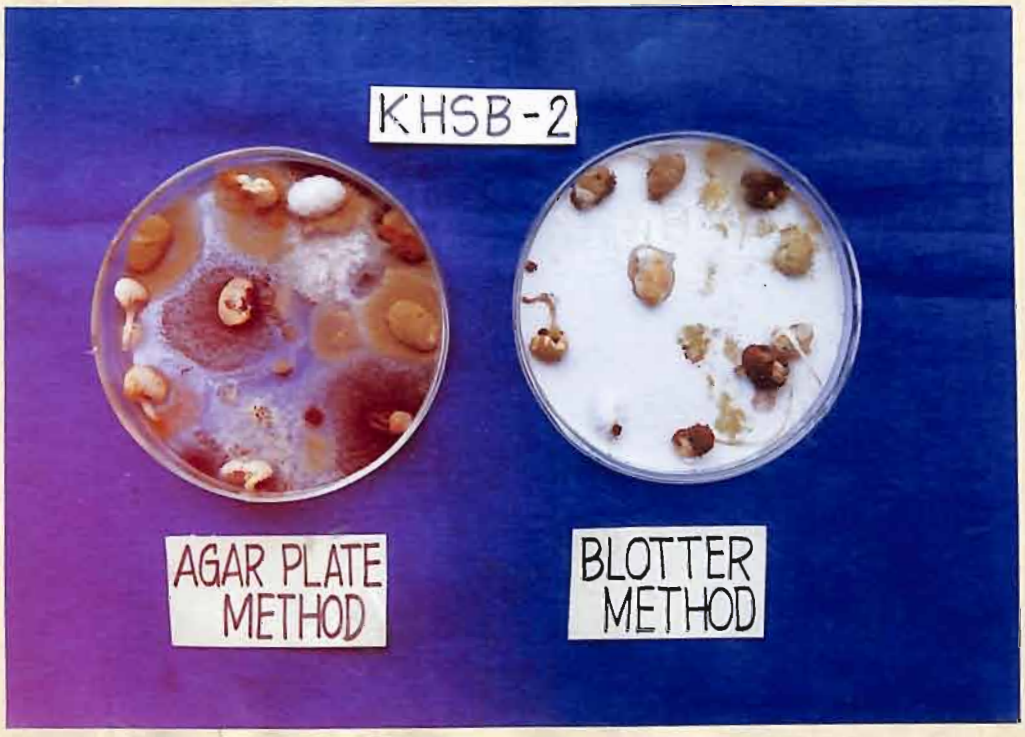
S. No	Name of the fungi	PK 1162		Bragg		PK 1029		MACS-450		MACS-129		MACS-34		Monetta		JS-HS-90-91		Hardae		KHSB-2	
		S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US
1	*Alternaria* sp.	6	24	16	24	4	8	10	20	8	23	14	29	18	26	6	22	—	10	28	38
2	*Aspergillus flavus*	22	36	28	42	18	41	18	46	12	40	15	48	16	45	16	54	20	60	15	40
3	*Aspergillus niger*	13	31	18	37	15	33	8	28	14	30	8	16	15	35	—	16	8	18	10	32
4	*Curvularia lunata*	7	29	12	28	14	32	12	28	16	20	14	30	10	28	3	8	7	10	26	30
5	*Fusarium* sp.	12	18	8	16	12	20	13	15	18	30	10	26	—	8	8	26	6	19	16	34
6	*Rhizoctonia* sp.	10	25	12	24	15	27	3	10	6	15	6	18	14	35	4	17	15	27	6	23
7	*Rhizopus stolonifer*	6	18	4	12	6	8	6	17	10	26	10	25	9	19	5	22	8	14	10	20
8	*Penicilliumsp*	6	18	—	—	—	—	6	20	—	—	12	24	—	—	12	26	4	15	4	18

S : Sterilized
US : Unsterilized

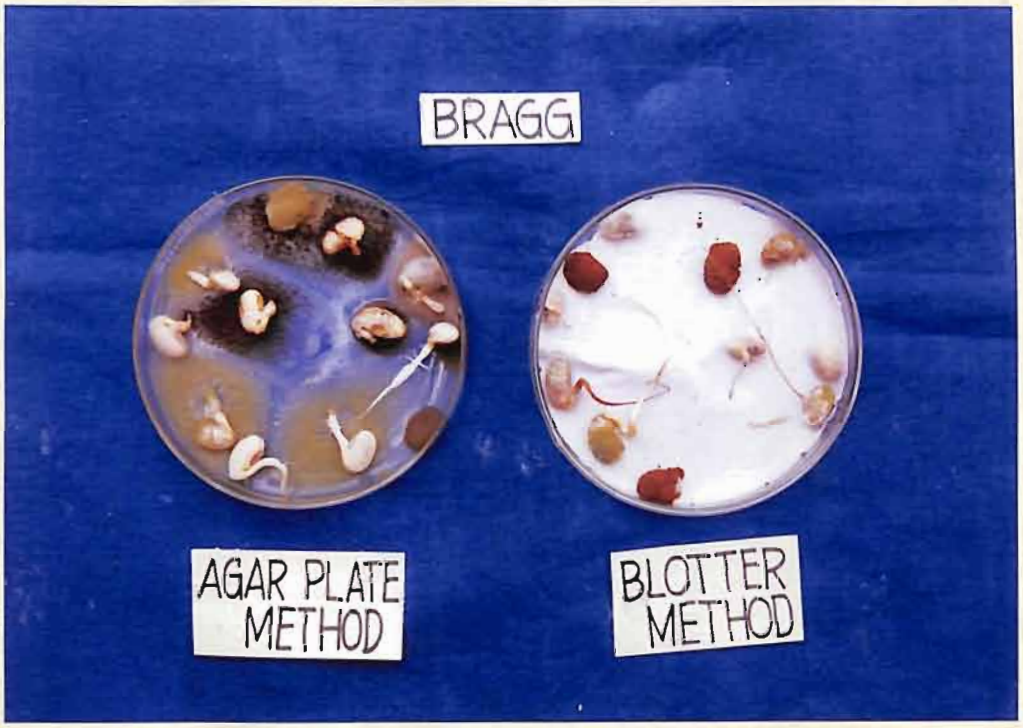
All figures are percentage average of five replications



i MACS-450 of soybean variety showing colonization of mycoflora (Agar plate and blotter method)



ii KHSB-2 of soybean variety showing colonization of mycoflora (Agar plate and blotter method)



iii Bragg of soybean variety showing colonization of mycoflora (Agar plate and blotter method)

PK-1162, 42.0 in Bragg. 41.0 in PK-1029, 46.0 in MACS-450, 40.0 in MACS-129, 48.0 in MACS-34, 45.0 in Monetta, 54.0 in JS-HS-90-91, 60.0 is Hardae and 40.0 in KHSB-2. In case of sterilized seed there was reduction in percentage of seed colonized by mycoflora.

Aspergillus niger was second in order of predominance in soybean seeds (38.5 per cent) and found to be associated to an extent of 31.0 per cent in PK-1162, 37.0 in Bragg, 33.0 in PK-1209, 28.0 in MACS-450, 30.0 in MACGS-129, 16.0 in MACS-34, 35.0 in Monetta, 16.0 in JS-HS-90-91, 18.0 in Hardae, and 32.0 in KHSB-2.

Curvularia lunata was observed on seeds to an extent of 29.0 per cent in PK-1162, 28.0 in Bragg, 32.0 in PK-1029, 28.0 in MACS-450, 20.0 in MACS-129, 30.0 in MACGS-34, 28.0 in Monetta, 8.0 in JS-HS-90-91, 10.0 in Hardae and 30.0 per cent in KHSB-2. This occupied the third place in order of occurrence yielding a total percentage of 36.4

Fusarium sp., was found to be associated with 18.0 per cent in PK-1162, 16.0 in Bragg, 20.0 in PK-1029, 25.0 in MACS-450, 30.0 in MACS-129, 26.0 in MACS-34, 26.0 in JS-HS-90-91, 19.0 in Hardae and 34.0 per cent in KHSB-2. In the variety Monetta, ***Fusarium*** sp. was not recorded in sterilized seeds and only 8 per cent was observed in unsterilized seeds.

Rhizoctonia sp. was found to be associated to an extent of 25.0 per cent in PK-1162, 24.0 in Bragg, 27.0 in PK-1029, 10.0 in MACS-450, 15.0 in MACS-129, 18.0 in MACS-34, 35.0 in Monetta, 18.0 in JS-HS-90-91, 27.0 in Hardae and 23.0 in KHSB-2.

Alternaria sp. colonized 24.0 per cent in PK-1162 and Bragg, 8.0 in PK-1029, 20.0 in MACS-450, 23.0 in MACS-129, 29.0 in MACS-34, 26.0 in Monetta, 22.0 in JS-HS-90-91, 10.0 in Hardae and 38.0 per cent in KHSB-2.

Rhizopus stolonifer was yielded to an extent of 18.0 per cent in PK-1162, 12.0 in Bragg, 8.0 in PK-1029, 17.0 in MACS-450, 26.0 in MACS-129, 25.0 in MACS-34, 19.0 in Monetta, 22.0 in JS-HS-90-91, 14.0 in Hardae and 20.0 in KHSB-2.

There was no significant colonization of ***Penicillium*** sp. in all the varieties in both sterilised and unsterilised seed.

4.2 INCIDENCE OF MYCOFLORA ON DIFFERENT VARIETIES OF SOYBEAN SEED (BLOTTER METHOD) :

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

TABLE - 2 : Incidence of seed-mycoflora on different varieties of soybean (Blotter Paper Method)

S. No	Name of the fungi	PK 1162		Bragg		PK 1029		MACS-450		MACS-129		MACS-34		Monetta		JS-HS-90-91		Hardae		KHSB-2	
		S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US
1	*Alternaria* sp.	—	16	12	20	—	—	8	23	4	12	10	18	15	23	4	18	—	16	20	34
2	*Aspergillus flavus*	16	24	10	32	10	38	3	45	3	33	5	42	9	38	9	46	5	42	6	36
3	*Aspergillus niger*	8	18	12	22	8	24	2	24	7	25	—	4	16	30	18	28	12	32	16	28
4	*Curvularia lunata*	—	16	8	14	—	15	3	14	9	23	8	18	7	16	3	15	4	14	8	15
5	*Fusarium* sp.	6	10	4	12	8	14	12	23	18	28	12	26	10	22	—	20	—	4	—	8
6	*Rhizoctonia* sp.	8	16	10	18	12	22	—	4	—	6	—	—	12	28	8	16	12	24	6	18
7	*Rhizopus stolonifer*	4	12	8	20	—	4	12	24	—	24	8	30	14	26	12	23	6	12	10	22
8	*Penicilliumsp*	—	8	13	16	—	—	—	6	—	—	—	—	—	4	—	6	4	10	4	12

S : Sterilized
US : Unsterilized

All figures are percentage average of five replications

In blotter method also, all the ten varieties yielded maximum colonies of ***Aspergillus flavus***. The percentage of seed colonized by ***A. flavus*** was 24.0 in PK-1162, 32.0 in Bragg, 38.0 in PK-1029, 45.0 in MACS-450, 33.0 in MACS-129, 42.0 in MACS-34, 38.0 in Monetta, 46.0 in JS-HS-90-91, 42.0 in Hardae and 36.0 per cent in KHSB-2.

Aspergillus niger was second in order of predominance among the fungi associated with the soybean seed. The colonies were present to an extent of 33.4 per cent in all the varieties. The distribution of percentage of ***Aspergillus niger*** in different varieties was 18.0 in PK-1162, 22.0 in Bragg. 24.0 in PK-1029, 24.0 in MACS-450, 25.0 in MACS-129, 4.0 in MACS-34, 30.0 in Monetta, 28.0 in JS-HS-90-91, 32.0 in Hardae and 28.0 per cent in KHSB-2.

Rhizopus stolonifer which was third in order of predominance found to be associated to an extent of 12.0 per cent in PK-1162, 20.0 in Bragg, 4.0 in PK-1029, 24.0 in MACS-450, 24.0 in MACS-129, 30.0 in MACS-34, 26.0 in Monetta, 23.0 in JS-HS-90-91, 12.0 in Hardae and 22.0 per cent in KHSB-2.

The percentage of ***Alternaria*** sp. was 16.0 in PK-1162, 20.0 in Bragg. 23.0 in MACS-450, 12.0 in MACS-129, 18.0 in MACS-34, 23.0 in Monette, 18.0 in JS-HS-90-91, 16.0 in Hardae and 34.0 per cent in KHSB-2. The variety PK-1029 did not yield ***Alternaria*** species.

The colonies of species of ***Fusarium*** were found to occur to an extent of 10.0 per cent in PK-1162, 12.0 in Bragg, 14.0 in PK-1029, 23.0 in MACS-450, 28.0 in MACS-129, 26.0 in MACS-34, 22.0 in Monetta, 20.0 in JS-HS 90-91, 4.0 in Hardae and 8.0 per cent in KHSB-2. The occurrence of ***Penicillium sp., Curvularia lunata*** and ***Rhizoctonia*** sp. was minimum and the percentage mycoflora ranged from 8.3 to 22.0

4.3 COMPARISON OF AGAR PLATE AND BLOTTER METHODS ON THE INCIDENCE OF MYCOFLORA OF SOYBEAN

Agar plate method against blotter method :

In the preliminary studies, both agar plate and blotter methods were employed to find out the effective one for obtaining large number of mycoflora both from sterilized and unsterilized seeds of soybean. For this experiment var KHSB-2 seed was used as this variety yielded more number of seed mycoflora (Plate II). The data obtained are presented in Table-3, Fig.-1.

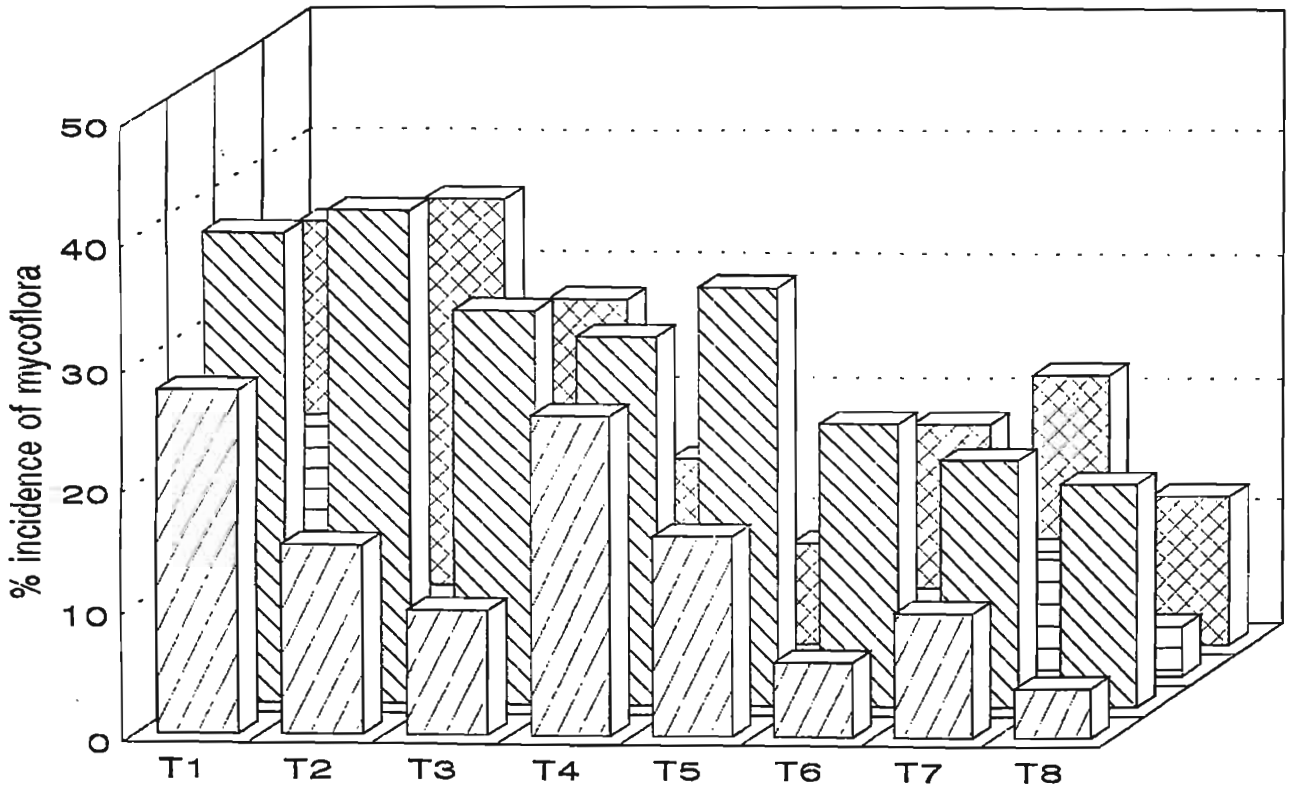
From the data it is evident that in both the methods, the incidence of mycoflora was more in the unsterilized seeds than in the sterilized seeds.

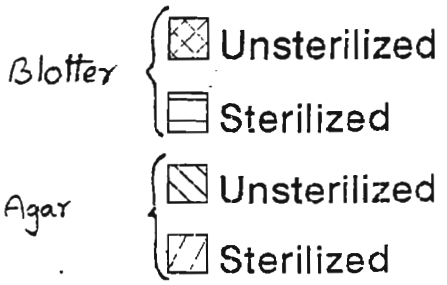
Among the fungi isolated ***Aspergillus flavus*** occurred abundantly in both sterilized (15 per cent) and unsterilized (40.0 per cent) seed in agar plate method while in blotter method the percentage of colonies of

TABLE - 3 : Comparison of agar plate and blotter methods on the incidence of mycoflora of soybean

S.No	Name of the fungi	Agar plate method		Blotter method	
		Percentage of mycoflora on sterilized seed	Percentage of mycoflora on unsterilized seed	Percentage of mycoflora of sterilized seed	Percentage of mycoflora on unsterilized seed
1	*Alternaria* sp.	28	38	20	34
2	*Aspergillus flavus*	15	40	6	36
3	*Aspergillus niger*	10	32	16	28
4	*Curvularia lunata*	26	30	8	15
5	*Fusarium* sp.	16	34	—	8
6	*Rhizoctonia* sp.	6	23	6	18
7	*Rhizopus stolonifer*	10	20	10	22
8	*Penicillium* sp.	4	18	4	12

FIG-1: COMPARISON OF AGAR PLATE AND BLOTTER METHODS ON THE INCIDENCE OF MYCOFLORA





T1-Alternaria Sp.
T2-Aspergillus flavus
T3-Aspergillus niger
T4-Curvularia lunata
T5- Fusarium Sp.
T6-Rhzioctonia SP.
T7-Rhziopus stolonifer
T8-Penicillium Sp.

A.flavus was 6.0 and 36.0 per cent in sterilized and unsterilized seed respectively. Agar plate method recorded higher incidence of ***A.flavus*** compared to blotter method.

The other fungi isolated from surface sterilized seeds in agar plate method were ***Alternaria*** sp. (28.0 per cent), ***Curvularia lunata*** (26.0 per cent), ***Fusarium*** sp. (16.0 per cent). ***A. flavus*** (15.0 per cent) ***A. niger*** and ***Rhizopus stolonifer***, (10.0 per cent)., ***Rhizoctonia*** sp. (6.0 per cent), and ***Penicillium*** sp. (4.0 per cent) in the order of predominance.

In surface sterilized seed, ***Alternaria*** sp. was the most predominant one (20.0 per cent) followed by ***Aspergillus niger*** (16.0 per cent), ***Rhizopus stolonifer*** (10.0 per cent). ***A. flavus, Curvularia lunata, Rhizoctonia*** sp., and ***Penicillium*** sp. ranged from 4-7 per cent respectively in blotter method.

4.4 SYSTEMATIC ACCOUNT OF DIFFERENT SEED BORNE FUNGI :

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

4.4.1 *Aspergillus flavus :*

Fungal colonies on PDA medium were yellowish green in the early stages later altered by the disappearance of the green factor leaving

iv Conidiophores, sterigmata and Conidia of *Aspergillus flavus*

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, 28.05-51.1 (38.17) µm is 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 (Plate IV).

4.4.2 *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 (172.47) µm long, 15.34 - 20.46 µm thick, enlarged into a spherical vesicle 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 (Plate V).

v Conidiophore, sterigmata and Conidia of *Aspergillus niger*. Conidial heads of *Aspergillus niger*

4.4.3 *Rhizopus stolonifer*

On seeds the fungus covered completely with plenty of sporangiophores and sporangial heads. Stolons creeping, showing curves and touching the substrate, raised up from the substrate and implanted by stout rhizoids. Sporangiophores usually in groups of 2 to 3, measuring 378.51-1432.2 (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 VI).

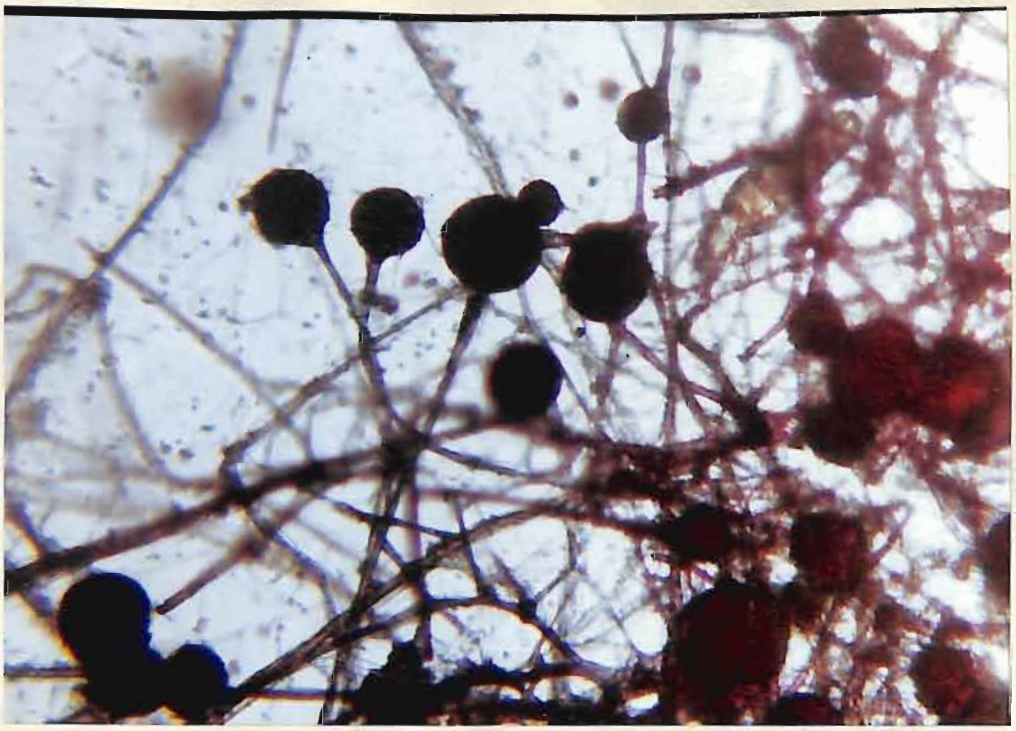
4.4.4 *Rhizoctonia* sp.

Sclerotia jet black, minute, smooth externally composed of anastomosed black hyphae, interior light to dark brown, composed of free thick-walled cells. ***Sclerotia*** variable in shape, globose, oval, oblong, elliptical, curved or even forked, varying in size, produced abundantly in the infected host tissues, 24.6 x 21.2 - 151.7 x 31.4 µm (Plate VII).

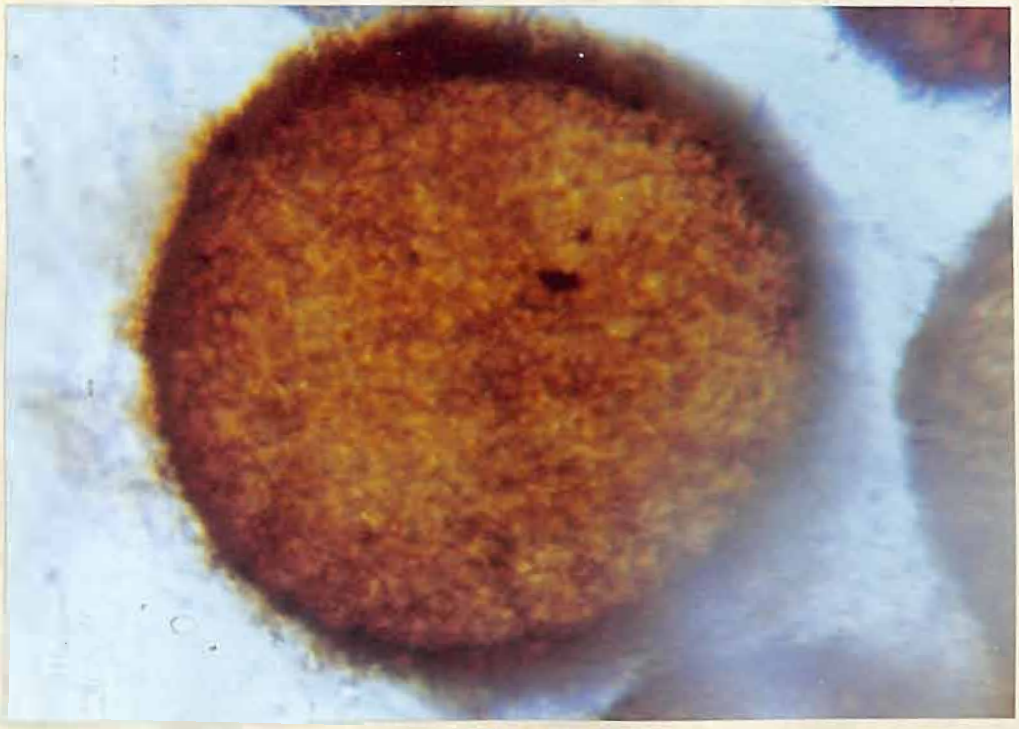
4.4.5 *Curvularia lunata* :

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.



vi Sporangiophores with columella and Rhizoids of ***Rhizopus stolonifer***



vii Sclerotial bodies of *Rhizoctonia* sp.

Conidia clavate, mostly curvesd,some times 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 VIII).

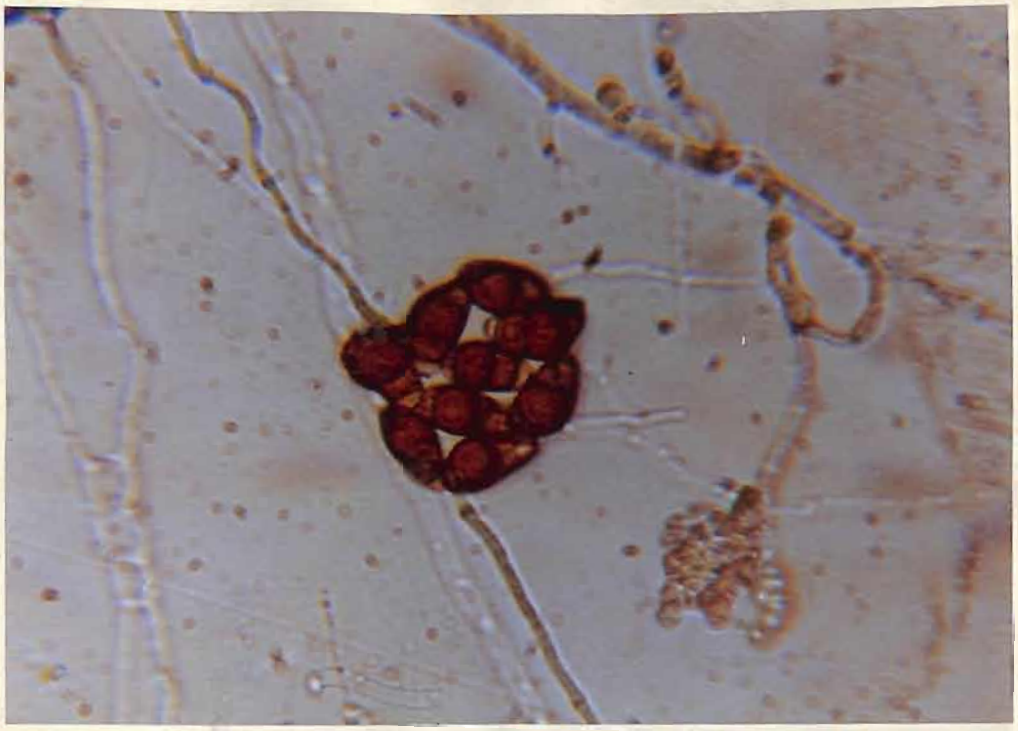
4.4.6 *Alternaria* sp.

Colonies usually olivaceous black on seeds, conidiophores arising singly at irregular intervals on hyphae, simple, straight or flexuous, 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.

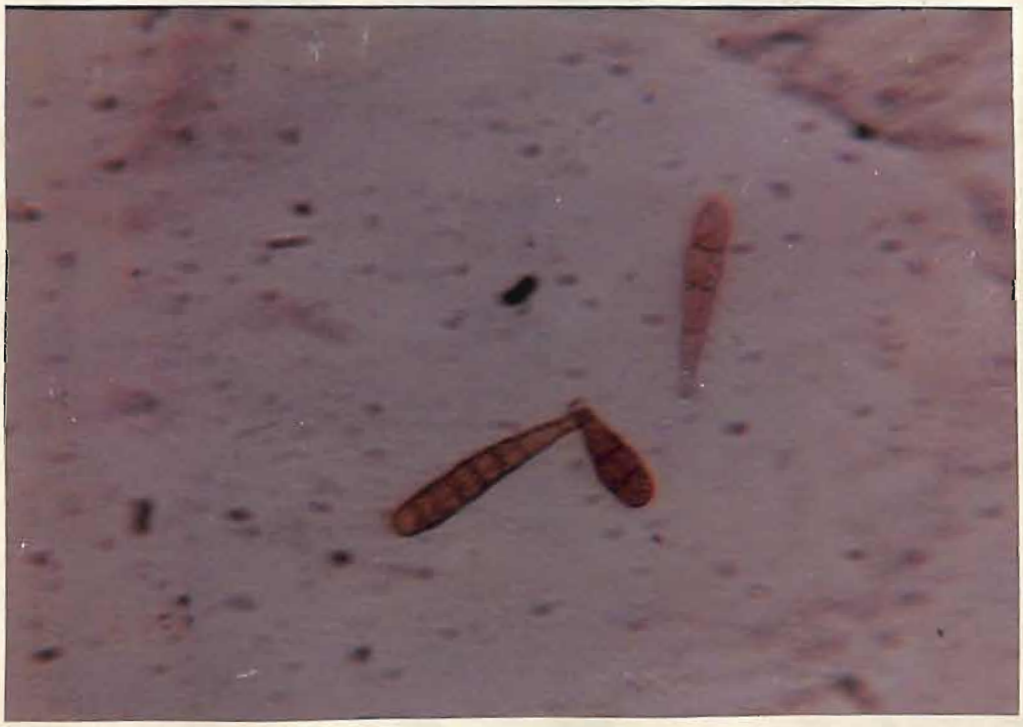
Conidia formed in long branched chains, obclavate, ovoid or ellipsoidal, often with a conical or cylindrical beak, 3-7 transverse septa with 1-2 longitudinal or oblique septa, 30.28-45.42 (36.63) µm long, 12.11-15.14 (13.62) µm thick (Plate IX).

4.4.7 *Penicillium* sp.

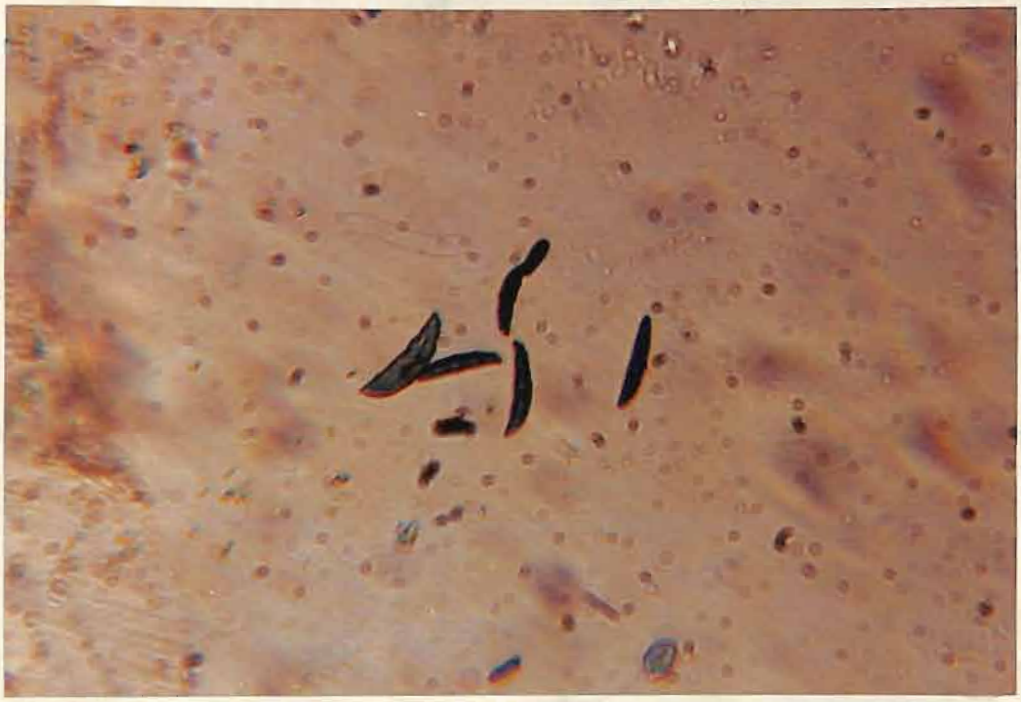
Colonies on PDA are smooth, velvety, dull yellowish green, vegetative hyphae coarse, thin walled, 4-5 µm wide. Conidiophores coarse, comparatively short, arising from submerged hyphae or from basal mycelial felt, smooth, 30-100 x 4-5 µm. Metulae variable in from and dimensions, commonly ranging from 15.30 x 4-6 µm and bearing phialids in variable but always limited numbers. Phialids equally variable, 15.28 x 3.5-50 µm producing chains of elliptical conidia. Conidia



viii Conidiophore with conidia of *Curvularia lunata*



ix Conidia of *Alternaria* sp.



x Macro conidia of *Fusarium* sp.

smooth, dull, dark green in mass, varying in form and dimensions commonly 3.5-5.0 µm x 3-3.5 µm.

4.4.8 *Fusarium* sp.

This is some what slow growing fungus as PDA medium. The aerial mycelium was white to light pinkish in colour. The conidia were seen in loosely arranged heads. Both micro and macro conidia were observed. macro conidia were sickle shaped, narrow at both the ends, hyaline, thin walled and 2-5 septate. The microconidia were oval, one or two celled and measured 4.0 - 11.0 x 2.0 - 3.0 µ (Plate X).

0 - septate 6-12 x 2-3 µm

1 - septate 9-23 x 2-3 µm

3 - septate 21-41 x 2.5 - 4.5 µm

5 - septate 33-50 x 3.5 - 4.5 µm

4.5 EFFECT OF SEED-BORNE FUNGI ON SEED GERMINATION

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

There was gradual decrease in percentage germination of seeds soaked in spore suspension over control ***Fusarium*** **sp.** recorded 62.0 per cent followed by ***Rhizoctonia*** sp. (58.0 per cent) which were on par with each other and significantly superior to the rest. Considerable

TABLE - 4 : Effect of Seed-borne mycoflora on germination *in vivo*

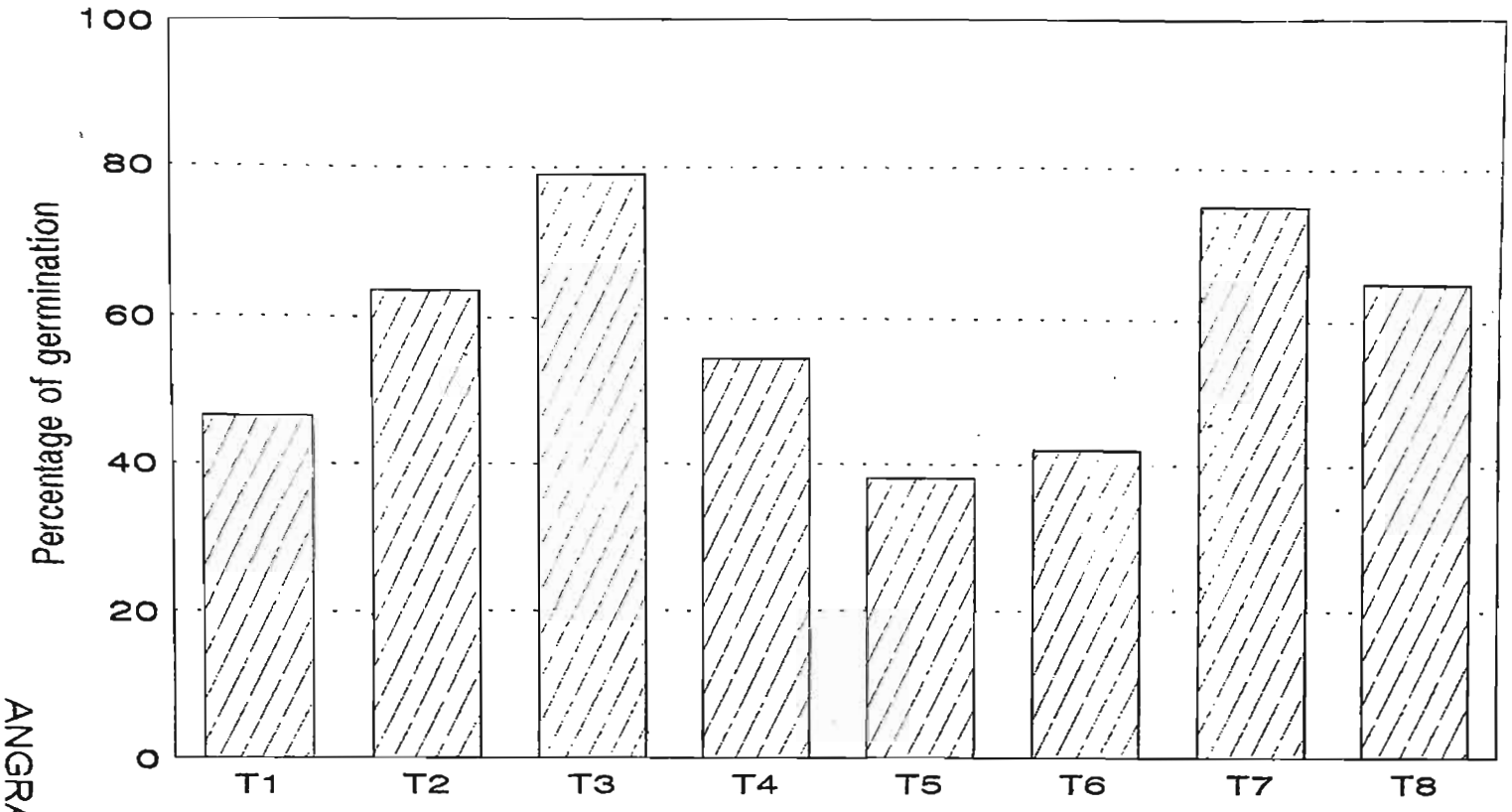
S. No	Name of the fungi	Germination* (%)	Percentage decrease over control
1	*Alternaria* sp.	46.6 (43.05)	53.4
2	*Aspergillus flavus*	63.6 (52.89)	36.7
3	*Aspergillus niger*	79.0 (62.72)	21.0
4	*Curvularia lunata*	54.6 (47.64)	45.4
5	*Fusarium* sp.	38.0 (38.05)	62.0
6	*Rhizoctonia* sp.	42.0 (40.39)	58.0
7	*Rhizopus stolonifer*	75.0 (60.00)	25.0
8	*Penicillium* sp.	65.0 (53.73)	35.0
9	Control	100 (90.00)	—

CD at 5% : 2.370

* : Mean of three replication

(Angular values in paranthesis)

FIG-2: EFFECT OF SEED-BORNE MYCOFLORA ON GERMINATION IN VIVO



T1-Alternaria Sp.
T2-Aspergillus flavus
T3-Aspergillus niger
T4-Curvularia lunata
T5- Fusarium Sp.
T6-Rhizoctonia Sp.
T7-Rhizopus stolonifer
T8-Penicillium Sp.

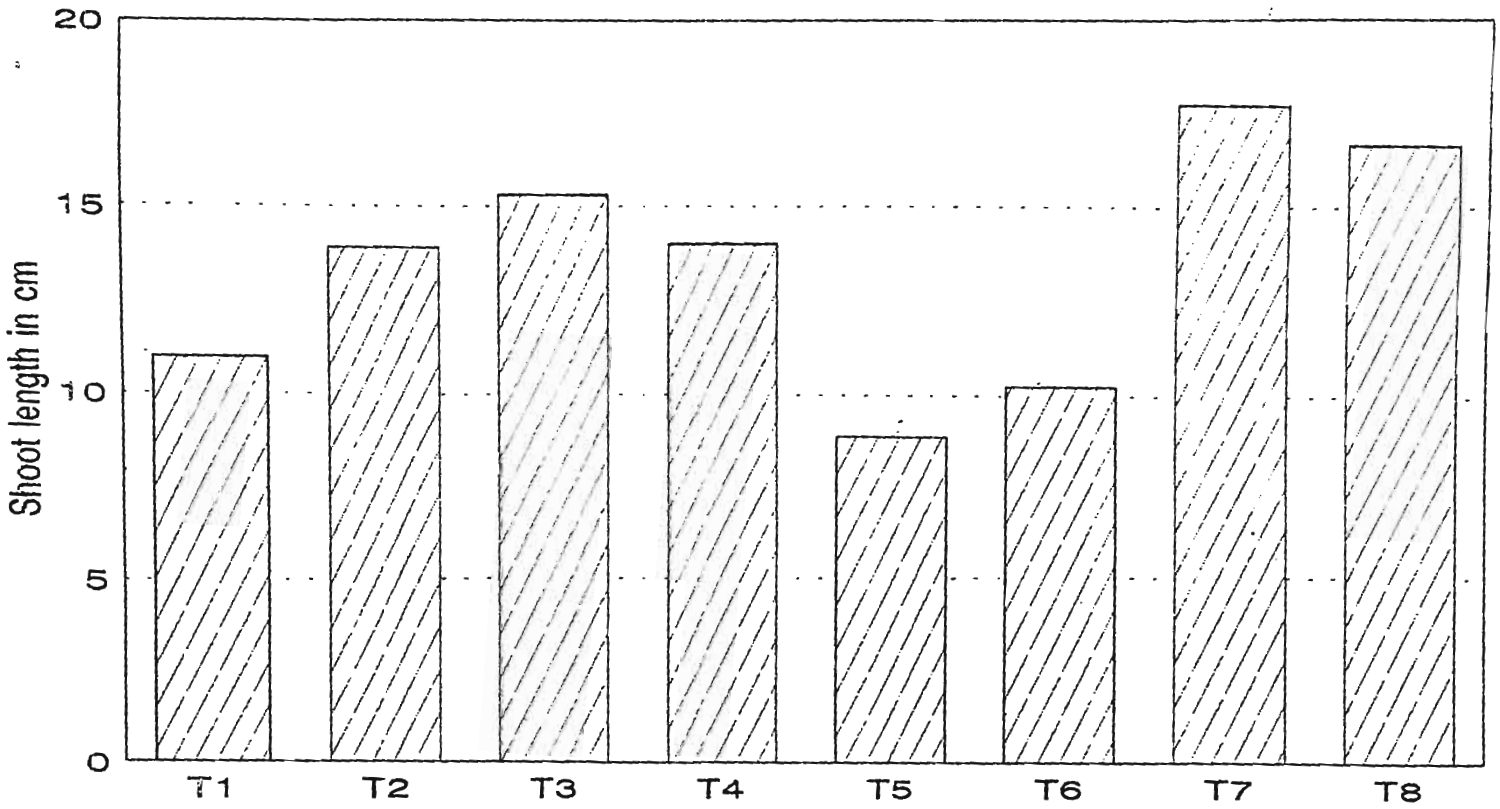
TABLE - 5 : Effect of seed-borne mycoflora on seedling vigour (shoot length) *in vivo*

S. No	Name of the fungi	Shoot length (cm)*	Percentage decrease over control
1	*Alternaria* sp.	11.00	51.54
2	*Aspergillus flavus*	13.93	38.63
3	*Aspergillus niger*	15.30	32.59
4	*Curvularia lunata*	14.03	38.19
5	*Fusarium* sp.	8.85	61.01
6	*Rhizoctonia* sp.	10.25	54.84
7	*Rhizopus stolonifer*	17.25	24.00
8	*Penicillium* sp.	16.70	26.43
9	Control	22.70	

CD at 5% level 0.383

* Mean of 3 replications

FIG-3: EFFECT OF SEED-BORNE MYCOFLORA ON SEEDLING VIGOUR IN VIVO (SHOOT LENGTH)



T1-Alternaria Sp.
T2-Aspergillus flavus
T3-Aspergillus niger
T4-Curvularia lunata
T5- Fusarium Sp.
T6-Rhizoctonia Sp.
T7-Rhizopus stolonifer
T8-Penicillium Sp.

reduction of germination percentage noted in seeds soaked in spore suspension of ***Alternaria*** sp. (53.4 per cent) and ***Curvularia lunata*** (45.40 per cent), and there was less percentage of germination reduction in case of seeds soaked in spore suspension of ***Aspergillus flavus*** (36.7 per cent) and ***Penicillium*** sp. (35.0 per cent) which were on par with each other. Least reduction in percentage of germination of seeds was obtained when they were soaked in spore suspension of ***Rhizopus stolonifer*** (25.0 per cent) and ***A. niger*** (21.00 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, Fig. 3) indicate that ***Fusarium*** sp. reduced the shoot length drastically i.e 8.85 cm compard to control (22.70 cm) (Plate XI). This was followed by ***Rhizoctonia*** sp. (10.25 cm) (Plate XII) and ***Alternaria*** sp. (11.00 cm) (Plate XII). The shoot lengths in ***Aspergillus flavus*** and ***Curvularia lunata*** were 13.93 cm and 14.03 cm which were on par with each other. The shoot lengths recorded in the case of ***A. niger, Penicillium*** sp. and ***Rhizophus stolonifer*** were 15.30, 16.70 and 17.25 cm respectively.

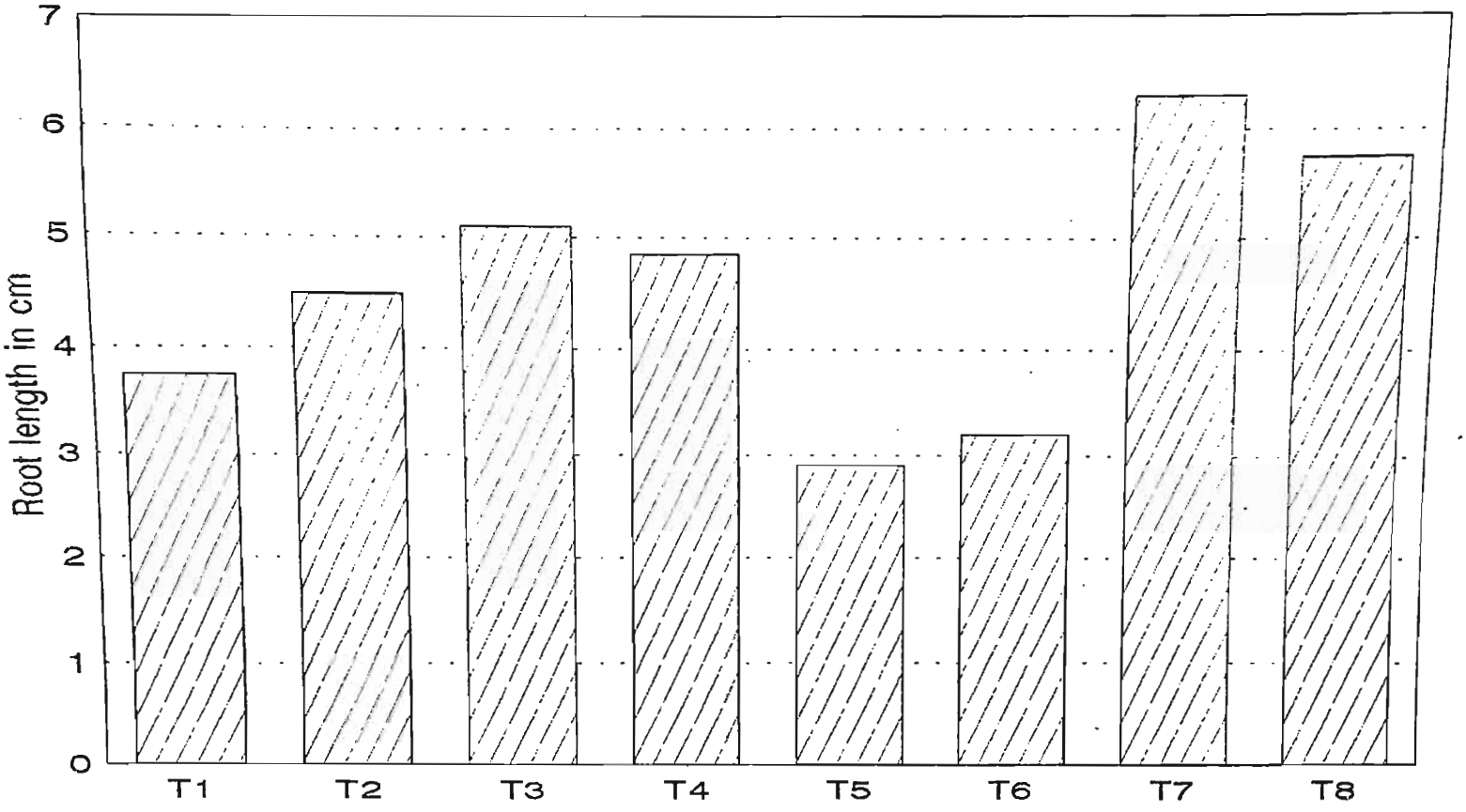
4.7 EFFECT OF SEED-BORNE MYCOFLORA ON SEEDLING VIGOUR *IN VIVO* (ROOT LENGTH)

The results (Table 6, Fig. 4) indicate that the maximum reduction in root length was noted in ***Fusarium*** sp. which was 2.90 cm followed

TABLE - 6 : Effect of seed-borne mycoflora on root length *in vivo*

S. No	Name of the fungi	Root length (cm)*	Percentage decrease over control
1	*Alternaria* sp.	3.75	47.62
2	*Aspergillus flavus*	4.50	37.15
3	*Aspergillus niger*	5.10	28.77
4	*Curvularia lunata*	4.85	32.26
5	*Fusarium* sp.	2.90	59.49
6	*Rhizoctonia* sp.	3.20	55.30
7	*Rhizopus stolonifer*	6.30	12.01
8	*Penicillium* sp.	5.75	19.69
9	Control	7.16	
	CD at 5% level	0.171	
	*	Mean of 3 replications	

FIG-4: EFFECT OF SEED-BORNE MYCOFLORA ON SEEDLING VIGOUR IN VIVO (ROOT LENGTH)



T1-Alternaria Sp.
T2-Aspergillus flavus
T3-Aspergillus niger
T4-Curvularia lunata
T5- Fusarium Sp.
T6-Rhizoctonia Sp.
T7-Rhizopus stolonifer
T8-Penicillium Sp.

by ***Rhizoctonia*** sp. (3.20 cm) and ***Alternaria*** sp. (3.75 cm). Significant reduction in root length was also noted in ***Aspergillus flavus, Curvularia lunata,*** and ***A.niger*** treated seeds and their lengths were 4.50 cm, 4.85 cm and 5.10 cm respectively. There was least reduction in root length in the seeds treated with ***Penicillium*** sp. and ***Rhizophus stolonifer*** which were 5.75 cm and 6.30 cm respectively.

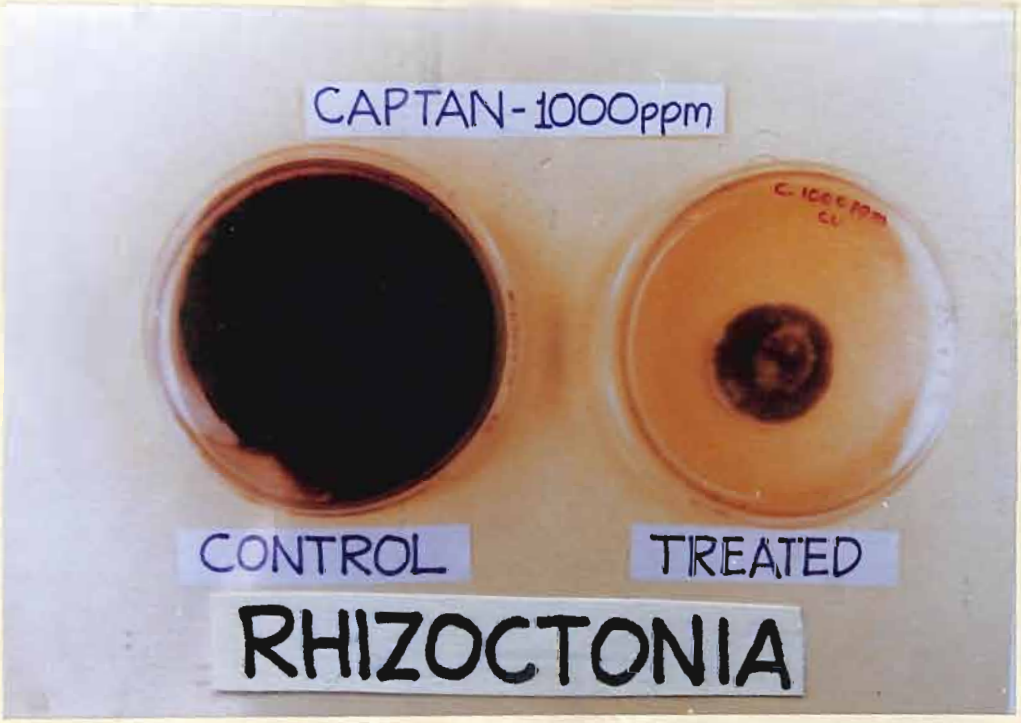
4.8 EFFECT OF DIFFERENT FUNGICIDES ON SEED-BORNE FUNGI OF SOYBEAN

Different fungicides namely bavistin, captan, celest, metalaxyl, thiram were evaluated against the seed-borne fungi of soybean to assess the efficacy of these fungicides at three concentrations viz., 500, 1000, 2000 ppm by using poisoned 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 *Rhizoctonia* sp.

The efficacy of various fungicides at different concentrations viz., 500, 1000 and 2000 ppm to control seed-borne ***Rhizoctonia*** sp. was tested. (Table 7. Fig. 5)

The results revealed that all the five fungicides reduced the growth of the fungus significantly at different concentrations over control.



xiv *Rhizoctonia* sp. showing inhibition of growth when Captan 2000 ppm was treated.

Among the fungicides tested at 500 ppm concentration, Thiram found to be superior to others by inhibiting the growth of the fungus to an extent of 65.27 per cent, followed by celest and captan which inhibited the growth to an extent of 61.88 and 61.37 per cent respectively and were on par with each other. Bavistin and metalaxyl was found to be least effective in reducing the growth of the fungus by 58.33 and 56.25 per cent respectively over control.

Among the same fungicides which were tested at 1000 ppm, thiram was found to be superior over all the five fungicides in which the percentage inhibition was 87.69 followed by celest which inhibited the growth of the fungus to an extent of 83.27 per cent. Bavistin and metalaxyl controlled the growth of fungus to an extent of 79.85 and 75.00 per cent respectively. Captan was found to be the least effective in reducing the linear growth of fungus upto 72.25 per cent over control.

At 2000 ppm concentration captan was effective compared to others and reduced the linear growth of the fungus to an extent of 94.62 per cent (Plate XIV) followed by thiram (93.54 per cent) (Plate - XV) which were on par with each other. The percentage inhibition of the fungus was 90.99 by using bavistin. Celest and metalaxyl was found least effective in which the inhibition per cent was 85.99 and 83.44 respectively over control (Table 7).

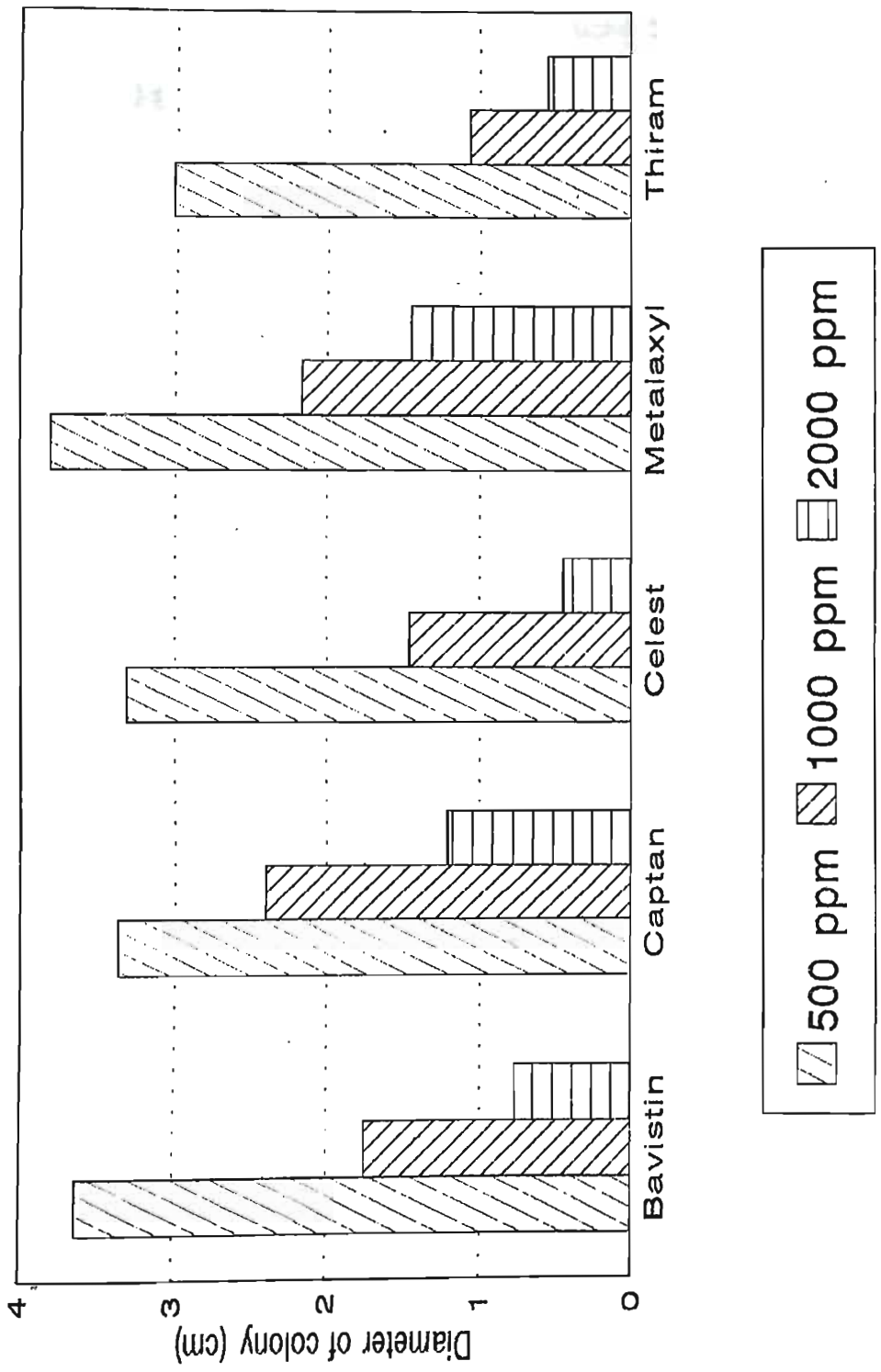
TABLE - 7 : Effect of different fungicides on *Rhizoctonia* sp.

S. No	Name of the Fungicide	Conc. (ppm)	Mean diameter of the colony (cm)*	Percent inhibition of growth
1	Bavistin	500	3.62	58.33
		1000	1.75	79.85
		2000	0.78	90.99
2	Captan	500	3.36	61.37
		1000	2.41	72.25
		2000	0.46	94.62
3	Celest	500	3.31	61.88
		1000	1.45	83.27
		2000	1.21	85.99
4	Metalaxyl	500	3.80	56.25
		1000	2.17	75.00
		2000	1.44	83.44
5	Thiram	500	3.02	65.27
		1000	1.07	87.69
		2000	0.56	93.54
	Control		8.70	

CD at 5% : 0.141

* : Mean of 3 replications

FIG-5: EFFECT OF DIFFERENT FUNGICIDES ON RHIZOCTONIA Sp.

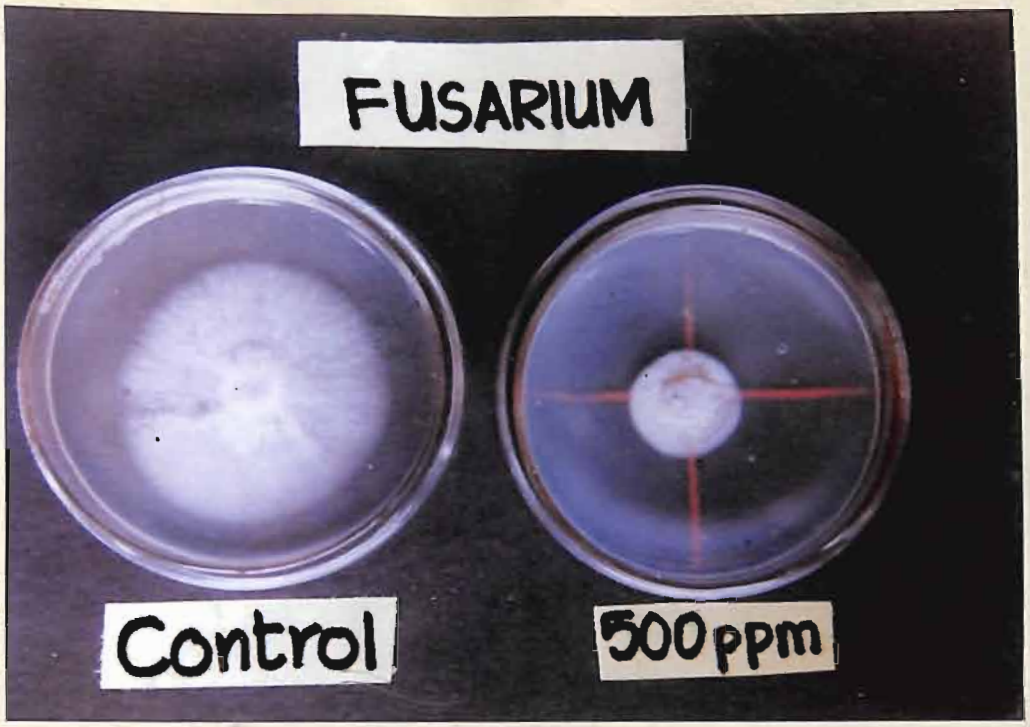


4.8.2 Effect of different fungicides on linear growth of *Fusarium* sp.

From the Table-8 (Fig. 6) it is clear that captan at 500 ppm was more effective than others and significantly reduced the growth of the fungus to an extent of 61.33 per cent followed by thiram (59.33 per cent) (Plate XVI) which were on par with each other. Bavistin reduced the growth to an extent of 46.72 per cent. Metalaxyl and celest was found least effective in inhibiting the growth of fungus. The per cent of inhibition was 31.84 and 28.33 per cent respectively in metalaxyl and celest respectively.

At 1000 ppm concentration captan was superior by reducing the linear growth of fungus by 76.59 per cent (Plate XVII) followed by thiram (73.92 per cent) and bavistin (72.69 per cent) which were on par with each other. Metalxyl and celest reduced the growth of the fungus by 52.47 and 45.69 per cent respectively and were on par with each other.

At the concentration of 2000 ppm also Captan was the most effective in reducing the growth of the fungus to an extent of 96.28 per cent (Plate XVII) followed by Thiram (91.78 per cent) and Bavistin (89.45 per cent) which were on par with each other. Celest and Metalxyl was least effective in inhibiting the growth of the fungus by 68.75 and 62.50 per cent respectively and they were on par with each other (Table-8).



xvi Captan effective on *Fusarium* sp. at 500 ppm concentration

xvii Captan effective on *Fusarium* sp. at 1000 and 2000 ppm concentration

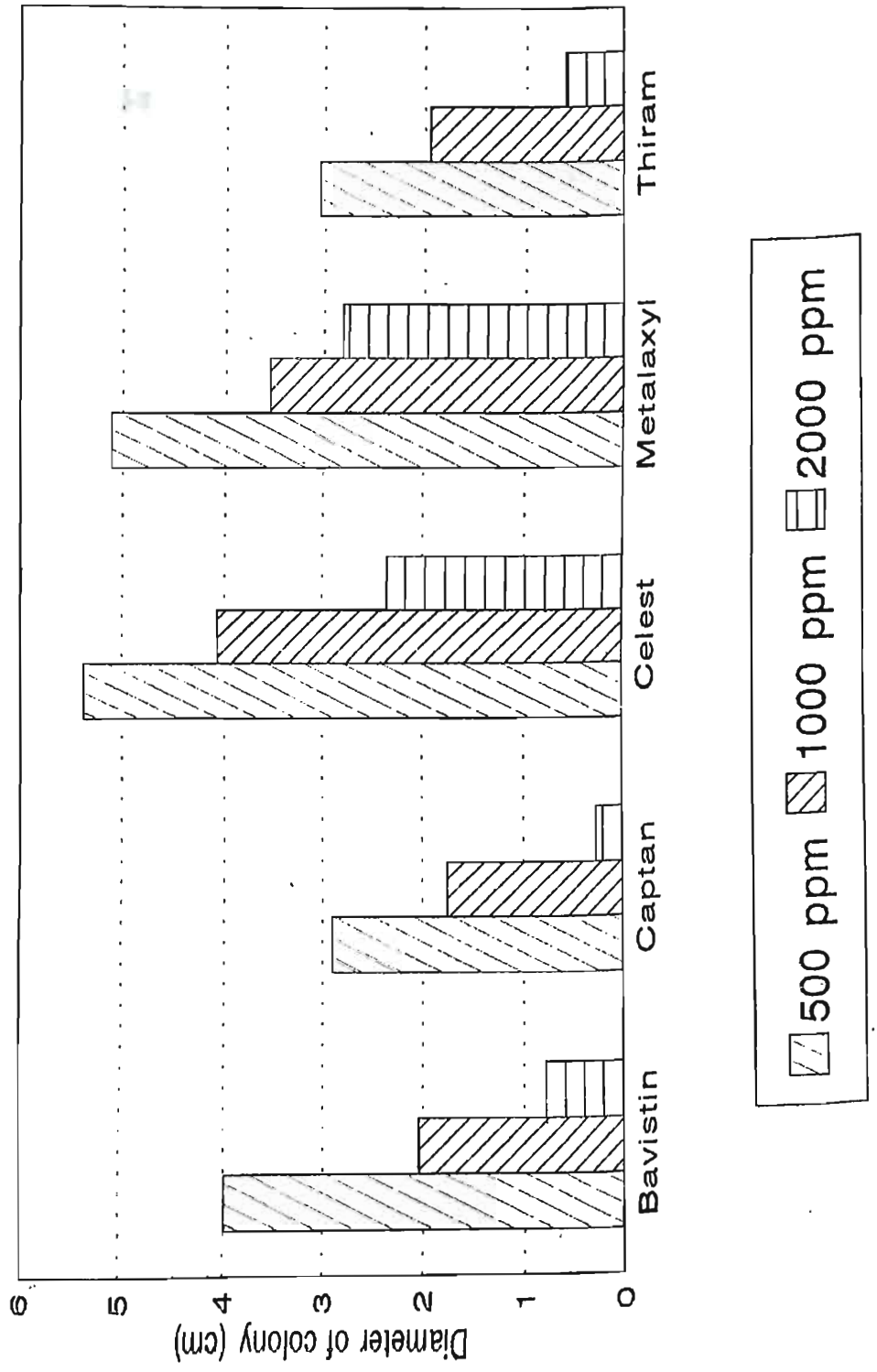
TABLE - 8 : Effect of different fungicides on *Fusarium* sp.

S. No	Name of the Fungicide	Conc. (ppm)	Mean diameter of the colony (cm)*	Percent inhibition of growth
1	Bavistin	500	3.99	46.72
		1000	2.04	72.69
		2000	0.79	89.45
2	Captan	500	2.90	61.33
		1000	1.75	76.59
		2000	0.27	96.28
3	Celest	500	5.37	28.33
		1000	4.07	45.69
		2000	2.34	68.75
4	Metalaxyl	500	5.11	31.84
		1000	3.56	52.47
		2000	2.81	62.50
5	Thiram	500	3.05	59.33
		1000	1.95	73.92
		2000	0.61	91.78
	Control		7.50	

CD at 5% : 0.829

* : Mean of 3 replication

FIG-6: EFFECT OF DIFFERENT FUNGICIDES ON FUSARIUM Sp.



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

From the data presented in Table-9 (Fig.-7) it is evident that all the fungicides significantly controlled the growth of the fungus at different concentrations. At 500 ppm celest was more effective than others in which the percentage of inhibition was 62.33 followed by thiram and captan which reduced the growth of fungus by 59.30 and 54.82 per cent respectively. Metalaxyl and bavistin were least effective at 500 ppm in which the percentage of inhibition was 51.93 and 45.55 over control.

At 1000 ppm concentration celest inhibited the growth of the fungus to an extent of 87.92 per cent followed by thiram and captan in which the percentage inhibition was 83.77 (Plate XVIII) and 73.50 and the latter two fungicides were on par with each other. Metalaxyl reduced the growth of fungus to an extent of 69.25 per cent and Bavistin is found to be the least effective.

At 2000 ppm concentration, thiram and captan were effective and controlled the growth of the fungus to an extent of 94.25 and 93.33 per cent respectively. Celest and metalaxyl inhibited the growth of the fungus to an extent of 91.23 and 87.44 respectively. Bavistin was found to be least effective (66.43 per cent) compared to other fungicides (Table-9)

xviii *Alternaria* sp. showing inhibition of growth when thiram at 1000 ppm was treated

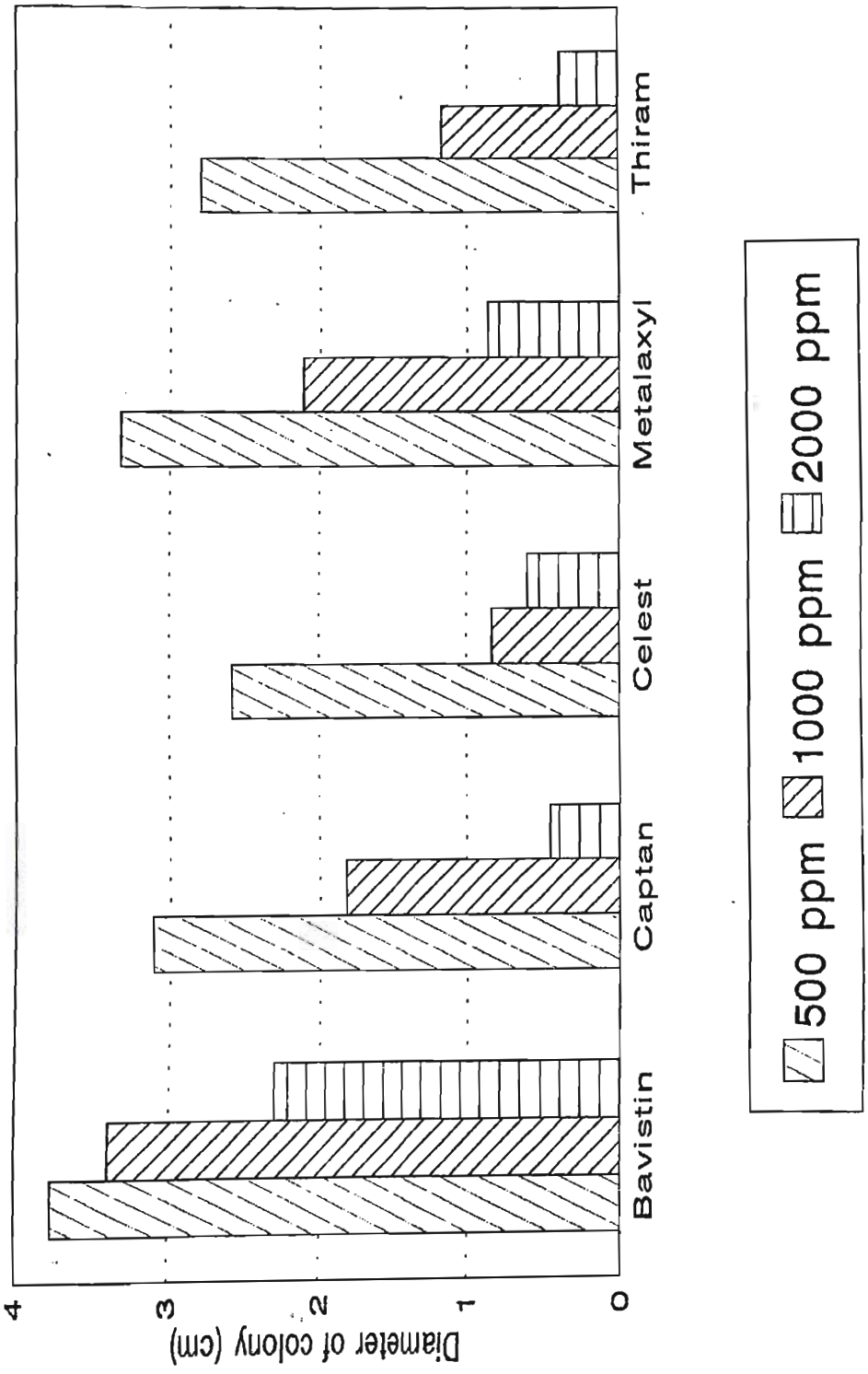
TABLE - 9 : Effect of different fungicides on *Alternaria* sp.

S. No	Name of the Fungicide used	Conc. (ppm)	Mean diameter of the colony (cm)*	Percent inhibition of growth
1	Bavistin	500	3.75	45.55
		1000	3.38	50.97
		2000	2.31	66.43
2	Captan	500	3.11	54.82
		1000	1.82	73.50
		2000	0.46	93.33
3	Celest	500	2.59	62.33
		1000	0.83	87.92
		200	0.60	91.23
4	Metalaxyl	500	3.31	51.93
		1000	2.12	69.25
		2000	0.86	87.44
5	Thiram	500	2.80	59.30
		1000	1.17	83.77
		2000	0.39	94.25
	Control		6.90	

CD at 5% : 0.122

* : Mean of three replications

FIG-7: EFFECT OF DIFFERENT FUNGICIDES ON ALTERNARIA Sp.



4.9 EFFECT OF AFLATOXINS PRODUCED BY *ASPERGILLUS FLAVUS* ON SOYBEAN SEEDS

The strains of ***Aspergillus flavus*** were isolated from 10 varieties of soybean seeds. These strains were grown on "yeast extract - sucrose" medium and aflatoxin was extracted from these ten strains.

From the Table 10 it is clear that the isolate - IV produced large amount of aflatoxin which was brightest and was equal to that of standard aflatoxin. Isolate III, V, VII, IX, produced moderate amount of aflatoxin, where as I and VIII produced only small amount of aflatoxin, Aflatoxin production was found to be completely absent in isolates II, VI and X.

4.9.2 Effect of aflatoxin on shoot length of soybean *in vitro*

Isolate IV which produced the maximum amount of aflatoxin was selected and used to study the effect of aflatoxin on five popular varieties of soybean viz., Bragg, Monettea, Hardae, PK-1029 and KHSB-2.

The results (Table 11, Fig. 8) revealed that among the varieties Monetta showed the highest percentage of decrease in shoot length (55.36) over control, followed by Bragg (48.29 per cent). In the variety Hardae the percentage decrease of shoot length over control was 45.52 followed by PK 1029 (43.07 per cent). Least percentage (42.64) of decrease was found in hybrid KHSB-2 due to aflatoxin treatment.

TABLE - 10: Intensity rating of aflatoxin obtained from different isolates of *Aspergillus flavus*

S.No	Isolates	Rating obtained
1	Isolate I	++
2	Isolate II	—
3	Isolate III	+++
4	Isolate IV	++++
5	Isolate V	+++
6	Isolate VI	—
7	Isolate VII	+++
8	Isolate VIII	++
9	Isolate IX	+++
10	Isolate X	—
11	Standard aflatoxin	++++

No aflatoxin : —
Traces of aflatoxin : +
Small amount of aflatoxin : ++
Moderate amount of aflatoxin : +++
Large amount of aflatoxin : ++++

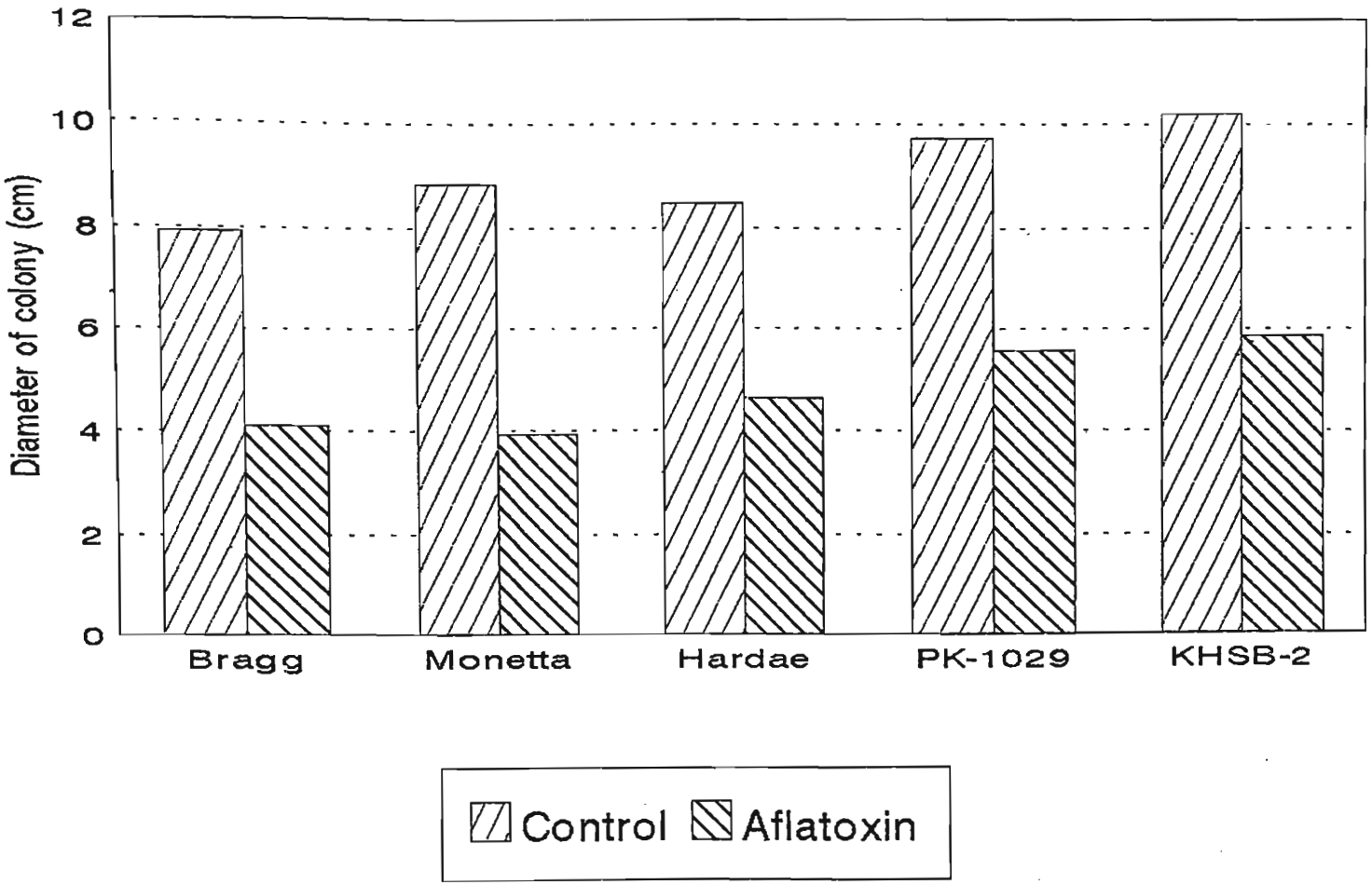
TABLE - 11 : Effect of Aflatoxin on shoot length of soybean *in vitro*

S.No	Variety	Shoot length		Percent decrease over control
		Control*	Aflatoxin*	
1	Bragg	7.93	4.10	48.29
2	Monetta	8.85	3.95	55.36
3	Hardae	8.50	4.63	45.52
4	PK-1029	9.75	5.55	43.07
5	KHSB-2	10.20	5.85	42.64

* Mean of three replications

Factors	SE	CD
Main treatment	0.0159	0.0473**
Sub treatment	0.0251	0.0749**
MT x ST interaction	0.0355	0.1059**

FIG-8: EFFECT OF AFLATOXIN ON SHOOT LENGTH OF SOYBEAN IN VITRO



4.9.3 Effect of aflatoxin on root length of soybean *in vitro*-

In varieties of soybean viz., Bragg, Monetta, Hardae, PK-1029, and KHSB-2 root length was also recorded and data are presented in Table-12 (Fig. 9).

In Bragg variety there was highest percentage decrease in root length, i.e 61.73 over control followed by Monetta (61.11 per cent). In the varieties Hardae percentage decrease over control was 57.80 percent. KHSB-2 and PK-1029 showed least percentage decrease of root length viz., 50.62 and 49.91 respectively over control.

TABLE - 12: Effect of Aflatoxin on root length of soybean *in vitro*

S.No	Variety	Root length		Percent decrease over control
		Control*	Aflatoxin*	
1	Bragg	5.75	2.20	61.73
2	Monette	6.30	2.45	61.11
3	Hardae	5.20	2.19	57.80
4	PK 1029	6.15	3.08	49.91
5	KHSB-2	5.63	2.78	50.62

* Mean of three replications

Factors	SE	CD
Main treatment	0.0292	0.0866**
Sub treatment	0.0461	0.1370**
MT x ST interaction	0.0653	0.1937**

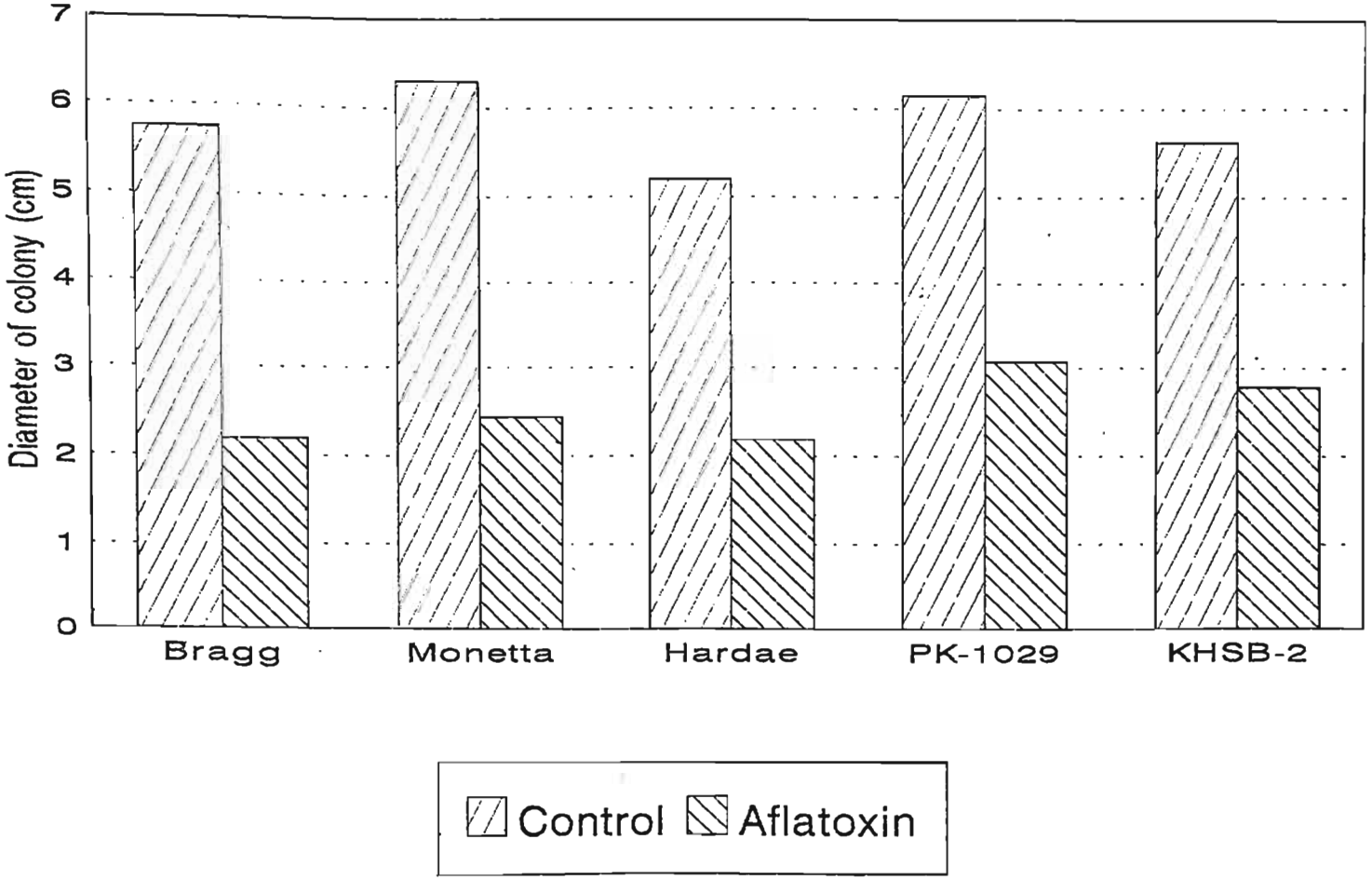
TABLE - 12: Effect of Aflatoxin on root length of soybean *in vitro*

S.No	Variety	Root length		Percent decrease over control
		Control*	Aflatoxin*	
1	Bragg	5.75	2.20	61.73
2	Monette	6.30	2.45	61.11
3	Hardae	5.20	2.19	57.80
4	PK 1029	6.15	3.08	49.91
5	KHSB-2	5.63	2.78	50.62

* Mean of three replications

Factors	SE	CD
Main treatment	0.0292	0.0866**
Sub treatment	0.0461	0.1370**
MT x ST interaction	0.0653	0.1937**

FIG-9: EFFECT OF AFLATOXIN ON ROOT LENGTH OF SOYBEAN IN VITRO



DISCUSSION

CHAPTER - V

DISCUSSION

Soybean seeds are affected by various 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, there by causing heavy damage to the germinating seed and subsequent crop.

Investigation on mycoflora of soybean reported herein include isolation and incidence of mycoflora on different varieties of soybean seed using blotter and agar plate methods, effect on seed-borne mycoflora on germination and seedling vigour, effect of certain fungicides on the seed borne fungi and effect of aflatoxin produced by ***Aspergillus flavus*** on soybean seed.

Present investigation revealed that soybean seeds harboured many fungal species in and on the seeds of the varieties under study. Irrespective of method of isolation and variety used, the mycoflora commonly associated with soybean seed samples are ***Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Penicillium sp., Curvularia lunata, Fusarium sp., Rhizoctonia sp., and Alternaria sp.*** Several workers (Schneider and Thaplial, 1971; Singh ***et al.,*** 1973; Sundaresh and Hiremath, 1978; Singh ***et al.*** 1983; Muthegowda ***et al.*** 1987;

Sharma, 1987; Tripathi and Singh, 1991; Murthy and Raveesha, 1996) have also obtained the same fungi from soybean seed.

The species like ***Cercospora kikuchi, Pythium, Botrytis, Cladosporium, Cheatophoma, Acremonium, Diplodia, Monilia, Tricothecium, Cheatomium, Colletotrichum truncatum, Phomopsis, Ascochyta pinodella, Diaporthe, Myrothecium, Epicocum, Nigrospora, Monilia, Spaceloma, Arthobotrys, Septocylindricum, Sclerotinia*** which were reported by Schneider and Thaplial (1971), Mengistu and Sinclair (1979), Zad (1979), Singh ***et al.*** (1983), Karunakar ***et al.*** (1980), Sharma (1987), Yum and Park (1989) and Goulart (1997) as frequent contaminants of soybean seeds were not encountered in the present studies. The wide variation in occurrence of different species have been due to variation in the agro-climatic conditions under which the different varieties of soybean were cultivated and the seeds collected.

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

Khattak ***et al.*** (1993) reported that the percentage of mycoflora isolated by agar plate was higher than blotter method in soybean. Solanke ***et al.*** (1997) also stated that agar plate method yielded more mycoflora than blotter method.

But contrary to this, Singh ***et al.*** (1973) reported blotter method as superior compared to agar plate method. This 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 seed gave more number of mycoflora than the sterilized seed. These results are in agreement with the findings of Sundaresh and Hiremath, (1978) in soybean seed mycoflora.

Studies on the isolation and identification of fungus associated with soybean include ***Aspergillus flavus, A. niger, Alternaria sp., Curvularia lunata, Fusarium sp., Rhizocotnia sp., Rhizopus stolonifer, Penicillium*** **sp.**

The isolated fungi were identified by comparing the conidia, spore or sporangiophores as the case may be with the earlier reports published by Thom and Raper (1945), Gilman (1957) and Tandon (1968), Subramanian (1971) and Mehrotra and Aneja (1990).

The results obtained with reference to the effect of seed mycoflora on germination clearly indicated that most of the fungi associated with soybean seed inhibited growth germination percentage and reduced shoot and root growth to a greater extent. Among the seed mycoflora ***Fusarium*** **sp.** proved highly pathogenic followed by ***Rhizoctonia*** sp. and ***Alternaria*** sp. in reducing the percentage germination of soybean seed. Anwar ***et al.*** (1995) also reported that among several fungi tested ***Fusarium*** sp. and ***Alternaria*** sp. were also responsible in reducing percentage germination of soybean seed.

Vidhyasekaran and Govindaswamy (1968) reported that the germination failure might be due to the exhaution of the stored reserve food material in the seeds by invading fungi. According to Christensen (1980), the pathogen might invade endosperm tissue immediately adjacent to the embryo. The first effect of this invasion would be weakning the embryo followed by death and discolouration.

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.

Regarding the effect of seed-borne fungi on seedling vigour of soybean, majority of fungi adversely affected both shoot and root growth. Among the fungi, ***Fusarium*** sp., was found to be highly infective in reducing the shoot and root length followed by ***Rhizoctonia*** and **Alternaria sp.** The other fungi which affected shoot and root length include ***Aspergillus flavus***, ***A. niger***, ***Penicillium*** sp. ***Rhizopus stolonifer*** and ***Curvularia lunata***. These observations are in accordance with finding of Singh ***et al.,*** (1986).

The effect of five fungicides on three fungi viz., ***Alternaria*** sp., ***Rhizoctonia*** **sp.** and ***Fusarium*** sp. was studied. Bavistin, captan, celest, metalxyl and Thiram were evaluated at three different concentrations viz., 500, 1000, 2000 ppm ***in vitro*** by poisoned food technique. The results revealed that all the fungicides used were effective but the efficacy differed at different concentrations.

Out of the five fungicides at 500 ppm and 1000 ppm concentration, thiram was found to be effective to control the linear growth of the ***Rhizoctonia*** species when compared to bavistin, captan, metalxyl and celest. At 2000 ppm concentration captan was found to be better than all other fungicides.

Dharamvir ***et al.*** (1964) reported that parzate and vapam were effective against ***Rhizoctonia*** sp. ***in vitro***. Singh ***et al.*** (1974) reported that captan, PCNB and thiram reduced the incidence of ***Macrophomina phaseolina***.

Indramalhan ***et al.*** (1975) reported that benomyl was found to be highly toxic to ***Rhizoctonia bataticola*** and some other fungi by poison-food technique.

Ramadoss and Sivaprakasam (1986) evaluated the fungicides on the linear growth of ***Macrophomina phaseolina*** and reported that bavistin at 100, 250, 500 and 1000 ppm concentration was found effective.

Udepurkar (1991) evaluated different fungicides viz., captan, thiram, dithane M-45 and bavistin alone and captan, thiram and dithane M-45 in combination with bavistin on ***Rhizoctonia bateticola*** by poison-food technique. All the fungicides tested were found to be effective against the fungus.

In the present investigation with different fungicides in controlling seed-borne *Fusarium* sp. captan at 500 ppm was effective compared to others and significantly reduced the growth of the fungus. At 1000 and 2000 ppm captan was the most effective in inhibiting the growth of the

fungus followed by thiram and bavistin. Celest and metalaxyl was the least effective at all concentrations in reducing the growth of the fungi.

Peshney ***et al.*** (1994) reported that captan 0.3 per cent and carbendazim 0.1 per cent were effective against ***Fusarium semitectum***.

Pandey and Vyas (1995) evaluated a newly released organometallic copper complex fungicide "Kaarmaar" against ***Fusarium oxysporum in vitro*** and found that "Kaarmar" was highly effective at 200 ppm concentration.

Regarding the effect of same fungicides on ***Alternaria*** sp., all the fungicides, reduced the growth of the fungus significantly at different concentrations over control. Celest at 500 ppm, 1000 ppm concentration and thiram 2000 ppm concentration were found to be good compared to bavistin and metalaxyl.

Sundaresh ***et al.*** (1987) reported that seed treatment with thiram and dithane M-45 gave good control of seed-borne fungi which included ***Alternaria*** and other fungi. Pieta and Patucha (1993) also observed that among other combinations of fungicides for seed treatment, thiram + carbendazim and thiram + carboxin reduced the incidence of ***Alternaria alternata*** on soybean seed.

While testing the ability of isolates of ***Aspergillus flavus*** obtained from then varieties of soybean to produce aflatoxin, it was found that isolate-IV produced large amounts of aflatoxin. Isolate III, V, IV, VII and IX produced moderate amounts of aflatoxin. Aflatoxin was absent in the isolates II, VI and X. Considerable variation in toxin production was seen among the ten varieties tested. The possible cause of variation in production of aflatoxin among different isolates may be due to hard seed-coat in some varieties which impedes the penetrability of the fungus thus resulting in low toxic production.

Schrooder and Hein (1967) stated that enough moisture content in seed and temperature (25-35°C) favoured the production of good amount of aflatoxin.

Reddy and Subbayya (1985) reported aflatoxin production in blackgram seed and indicated that the amount of aflatoxin production was varied by different isolates. High amount of aflatoxin production was obtained by isolates I and IV while it was moderate in isolate II. Isolate III proved to be non-toxic. This suggests that isolates of ***Aspergillus flavus*** vary in their ability to produce aflatoxin.

Effect of aflatoxin in shoot and root lengths of soybean was studied ***in vitro***. It was observed that variety Monetta showed highest per cent of decrease in shoot length over control followed by Bragg. Least percentage of decrease in shoot length was recorded in KHSB-2.

Regarding the effect of aflatoxin on root length, it was observed that there was highest percentage of decrease in root length in the variety Bragg followed by Monetta and Hardae. KHSB-2 showed least percentage decrease of root length.

Kuldeep Verma ***et al.*** (1976) revealed that aflatoxin extracted from ***A. flavus*** have been found reduced the percentage germination and shoot and root length and allantoinase (allantoin-amidohydrolase) activity of germinating groundnut, mungbean and black gram.

Earlier workers (Sharma and Gupta, 1980) also reported reduction in germinability of seed, root and shoot lengths by toxins produced by ***Aspergillus*** sp.

The possible reason of reduction in shoot and root lengths is that aflatoxin in plants inhibit mitosis, germination and chlorophyll synthesis and cause chromosomal aberrations (Viswanadhan and Venkatasubramanian, 1971). Similarly Schoental and White (1965) reported that aflatoxin caused albinism or chlorophyll deficiency and inhibited seed germination.

SUMMARY

CHAPTER VI

SUMMARY

The soybean varieties viz., PK-1162, Bragg, PK-1029, MACS-450, MACS-129, MACS-34, Monetta, JS-HS-90-91, Hardee and KHSB-2 were used for the incidence of mycoflora by agar plate and blotter methods. In order of predominance the fungi isolated by agar plate method were ***Aspergillus flavus, A.niger, Curvularia lunata, Fusarium sp., Alternaria sp., Rhizoctonia sp., Rhizopus stolonifer*** and ***Penicillium sp.*** By blotter method, ***Aspergillus flavus, A.niger, Rhizopus stolonifer, Alternaria sp., Fusarium sp., Rhizoctonia sp., Curvularia lunata, Penicillium sp.*** were found to be present in order of predominance. Agar plate method proved to be better than blotter method for all fungi by yielding more number of colonies. 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 flavus*** and ***A.niger*** was recorded.

All the fungi isolated from soybean seed reduced the germination percentage considerably to varying degrees over control ***in vivo***. Out of all the fungi, ***Fusarium sp.*** reduced the maximum percentage of

germination (62.00) over control followed by ***Rhizoctonia*** sp. (58.0 per cent) and ***Alternaria*** sp. (53.40 per cent). The effect of ***A. niger*** on percentage germination was negligible (21.00) compared to others.

Shoot length and root lengths were adversely affected by ***Fusarium sp., Rhizoctonia sp.*** and ***Alternaria sp.***

The effect of five fungicides on three fungi viz., ***Fusarium sp., Rhizoctonia sp.*** and ***Alternaria sp.*** was studied. Bavistin, Captan, Celest, Metalaxyl, Thiram were evaluated at three concentration viz., 500, 1000 and 2000 ppm.

Thiram was found to be the best of all the fungicides at different concentrations (500, 1000 and 2000 ppm) in inhibiting the growth of the ***Rhizoctonia*** sp., while Captan at 2000 ppm concentration was the next best.

On ***Fusarium*** sp., Captan was effective at all concentrations i.e. 500, 1000 and 2000 ppm compared to others. Celest at 500 and 1000 ppm and Thiram and Captan at 2000 ppm concentration were found to be effective against ***Alternaria sp.***

The ability of isolates of ***Aspergillus flavus*** obtained from ten varieties of soybean to produce aflatoxin was studied. It was found that

Isolate IV produced large amount of aflatoxin, while isolates III, V, VII, IX produced moderate amount of aflatoxin. Aflatoxin production was not observed by the isolates II, VI and X.

There was maximum reduction of shoot and root lengths of five prominent varieties viz., Bragg, Monetta, Hardae, PK-1029 and KHSB-2 where the seed were soaked in culture filtrate of isolate IV when containing large amount of aflatoxin.

LITERATURE CITED

LITERATURE CITED

Agarwal S C and Gupta R K 1989 Fungal pathogens detected on soybean seeds grown in different locations. Seed Research 17: 208-210.

Ainsworth G L 1961 Dictionary of fungi. Commonwealth mycological institute, England pp. 547.

Ali M I, Doger M A and Ahmed R 1995 Seed-borne fungi of soyabean and their chemical control. Pakistan Journal of Phytopathology 7: 160-162.

*Al-Kassim M Y 1996 Seed-borne fungi of some vegetables in Saudiarabia and their chemical control. Arab-Gulf-Journal of Scientific research 14: 705-715.

Anwar S A, Abbas S F, Gill M M, Rouf C A, Mahmood S and Butta A R 1995 Seed borne fungi of Soybean and their effect on seed germination, Pakistan Journal of Phytopathology 7: 184-190.

Barnett 1962 Illustrated genera of imperfect fungi. Burges Publishing Company pp 225.

Bhatnagar P S and Tiwari S P 1990 Production and productivity constraint analysis. Technology for increasing soybean production in India NRCS Technology Bulletin No. 1, Indor pp. 2-3.

Bhatnagar P S and Tiwari S P 1993 Augementing soybean production and oil situation in India. Research and development of oil seeds, NOVOD Board, Gurgon, India pp. 19-21 and 119.

Carvalho N M and Jacinto C M R 1979 Time of fungicidal treatment of soyabean seeds. Cientifica 7: 261-265.

Christensen C M 1980 Research on storage moulds in grains, seeds and their products. Plant Disease 64: 1067-1070.

Chung K W and Ju J I 1993 Effect of fungicide benoram seed treatment on germination, growth and yield in summer type soybean. Korean Journal of Crop Science 38: 166-173.

de Tempe J 1953 The blotter method of seed health testing. Procedings of International Seed test association 28: 133-151.

Dharama Vir K D, Pathak and Mathur S B 1964 Evaluation of certain fungicides for the control of ***Rhizoctonia bataticola*** and ***Selerotium rolfsii***. Indian Phytopathology 17: 154-156.

Dowson WJ 1959 Plant disease due to bacteria, Cambridge University Press, UK pp. 232.

Ellis M A, Ilyas M B and Sinclair J B 1975 Effect of three fungicides on internally seed-borne fungi and germination of soybean seed. Phytopathology 65: 553-556.

Ellis M A, Paschal E H, Powell P E and Tenne F D 1979 Internally seed-borne fungi of soybean in puerto Rico and their effect on seed germination. Tropical agriculture 56: 171-174.

El-Kady I A and Youssef M S 1993 Survey of mycoflora and mycotoxins in Egyptian Soybean seed. Journal of basic microbiology 33: 371-378.

Farag R S 1990 Effects of fungal infection and agrochemicals on the chemical composition of some seeds and aflatoxin production. Bulletin of faculty of Agriculture, University of Cairo 41: 43-61.

Fernandez Pinto V E, Vamonde G, Brizzio S B and Apro N 1991 Aflatoxin production in Soybean varieties grown in Argentina Journal of food protection 54: 542-545.

Gillman 1957 A manual of soil fungi. pp. 227.

Goulart A C P, Paiva F de A and Andrede P J M 1995 control of soybean seed-borne fungi by fungicide treatments. Summa - phytopathologia 21: 239-244.

*Goulart ACP 1997 Fungi in soybean seed: detection and importance. Documents EMBRAPA-Cento-de-pesquisa-Agropecularia do-oesto 11: 58.

Hall R and Xue A G 1995 Effectiveness of fungicidal seed treatments applied to smooth or shrivelled soyaean seeds contaminated by ***Diaporthe phaseolorum***. Phytoprotection 76: 47-56

Hasseltine C W, Shotwell J J, Ellis J J and Stubblefield R D 1966. Aflatoxin formation by ***Aspergillus flavus***. Bacteriological review 30: 795-805.

Hensarling J P, Jack T J, Lee L S and Ciegler A 1983 Production of aflatoxin on soybean and cotton seed meal. Mycopathologia 83: 125-127.

Karmakar S, Subuddi S and Bandyopadhyay B 1980 Studies on seed mycoflora of soybean seeds. Indian Journal of Microbiology 20: 236-238.

Indramalhan P D, Tyagi P D and Grover R K 1975 Laboratory evaluation of benomyl against some plant pathogenic fungi. Indian Phytopathology 28: 76.

Kenneth B Raper and Dorothy I Fennel 1945 The genus aspergillus 147-196.

Khattak I, Hussain S, Ali A and Shah S J A 1993 Efficacy of various methods employed for the study of seed borne microflora of soybean. Sarhad Journal of Agriculture 9: 45-48.

Kuldeep Verma V, Sareen K and Singh R 1976 The effect of aflatoxin on Allantoinase activity of germinating seeds. Journal of Research 13: 99-104.

Mehrothra R S and Aneja K R 1990 An introduction to mycology. Wiley Eastern Ltd. pp. 330.

Mengistu A and Sinclair J B 1979 Seed-borne microorganisms of Ethiopian grown soybean. Plant Disease Reporter 63: 616-619.

Mishra R R and Kanuja R S 1973 Studies on certain aspects of seed-borne fungi. II. Seed-borne fungi of certain oil-seeds. Indian Phytopathology 26: 284-294.

Murthy Y L K and Raveesha K A 1996 Seed mycoflora of soybean in Karnataka. Plant Disease Reporter 11: 78-82.

Muthagowda, Sullia S B and Gowda M 1987 Seed mycoflora of cowpea, field bean and soybean. Acta Botanica indica 15: 165-169.

Nene Y L and Thapliyal P N 1993 Fungicides in plant disease control. Oxford and IBH Publishing Co. pp. 531.

Om Vir Singh, Agarwal V K and Nene Y L 1973 Seed health studies on soybean raised in the Nainital Tarai. Indian Phytopathology 26: 260-267.

Pardeshi V F, Reddy VG and Nalwandikar PK 1989 Effect of different fungicides on seedling vigour and seed viability in soybeen. Journal of Maharastra Agricultural Universities 14: 33-36.

Pereira L A G, Costa N P, Almeida A M R and Silva C M 1981 Effect of interaction of chemical treatment of Soybean seeds and Vigour level Fitopatologia Brasileira 6:: 159-163.

Kuldeep Verma V, Sareen K and Singh R 1976 The effect of aflatoxin on Allantoinase activity of germinating seeds. Journal of Research 13: 99-104.

Mehrothra R S and Aneja K R 1990 An introduction to mycology. Wiley Eastern Ltd. pp. 330.

Mengistu A and Sinclair J B 1979 Seed-borne microorganisms of Ethiopian grown soybean. Plant Disease Reporter 63: 616-619.

Mishra R R and Kanuja R S 1973 Studies on certain aspects of seed-borne fungi. II. Seed-borne fungi of certain oil-seeds. Indian Phytopathology 26: 284-294.

Murthy Y L K and Raveesha K A 1996 Seed mycoflora of soybean in Karnataka. Plant Disease Reporter 11: 78-82.

Muthagowda, Sullia S B and Gowda M 1987 Seed mycoflora of cowpea, field bean and soybean. Acta Botanica indica 15: 165-169.

Nene Y L and Thapliyal P N 1993 Fungicides in plant disease control. Oxford and IBH Publishing Co. pp. 531.

Om Vir Singh, Agarwal V K and Nene Y L 1973 Seed health studies on soybean raised in the Nainital Tarai. Indian Phytopathology 26: 260-267.

Pardeshi V F, Reddy VG and Nalwandikar PK 1989 Effect of different fungicides on seedling vigour and seed viability in soybeen. Journal of Maharastra Agricultural Universities 14: 33-36.

Pereira L A G, Costa N P, Almeida A M R and Silva C M 1981 Effect of interaction of chemical treatment of Soybean seeds and Vigour level Fitopatologia Brasileira 6:: 159-163.

Peshney N L, Mahant S F and Ninawe B N 1994 Detection of chemical control of seed borne fungal pathogens of soybean. Journal of soils and corps 4: 131-135.

Picinini E C and Fernandes J M C 1996 Fungicides efficiency in controlling soybean seed borne pathogens. Fitopatologia- Brasileira 21: 492-495.

*Pieta D and Patucha 1993 Fungi infecting soybean seeds and effect of some fungicides on seed treatment. Biuletyn-Warzywniczy 40:101-109.

Ramadoss S and Sivaprakasam K 1986 effect of fungicides and insecticides on the linear growth of ***Macrophomina phaseolina.*** Abstracts of papers presented in the seminar on the management of soil-borne diseases of crop pants TNAU 16-17.

Ravikumar C H, Kulkarni G N, Vyakapnal B S and Shashidhara S D 1987 effect of fungicides and insecticides on storability of soyabean genotypes. Plant pathology news letter 5(1-2):11.

Reddy M R S and Subbayya J 1985 Aflatoxin production in black gram seed. Seed research 13: 125-126.

Setti T A S, Jagannatha V and Rao K B 1991 effect of chemical seed treatment againest seed-borne fungi on germination of soybean. Current research UAS Bangalore 20: 86-87.

Schoental R and white A F 1965 Aflatoxin and albinism in plants. Nature 205: 57-58.

Schrooder H W and Hein H 1967 Microbial toxins. Applied microbiology 15: 441.

Shah S J A, Hussain S, Ali A and Khattak I 1992 effect of different seed dressing fungicides on germination and yield components of soybean. Sarhad Journal of agriculture 8: 679-682.

Sharma A K 1995 Effect of seed metabolites of some seed-borne fungi on seed mycoflora and seed germinations of soybean. Journal of Hill-research 8: 165-168.

Sharma J P and Gupta J S 1980 studies on stored seed mycoflora of moth bean and effect of their filterates on germination and seedling vigour. Seed research 8: 163-168.

Shotwell O L, Hesseltine C W, Burmeister H R, Kwolek W F, Sharma G M and Hell H H 1968 Surveys of cereal grains and soybeans for the presence of aflatoxin. Cereal chemistry in press.

Sinclair J B 1981 Fungicide sprays for the control of seed-borne pathogens of rice soybean and wheat. Seed science and Technology 9: 597-705.

Singh O V, Agarwal V K and Nene Y L 1974 Influence of fungicidal seed treatment on the mycoflora of stored Soyabean seed and seedling emergence. Indian Journal of agriculture sciences 43: 820-824.

Singh V, Sinhar R K P and Sinha S K 1986 Effect of culture filterates of soybean mycoflora of soybean upon seed germinability and seedling length . Indian Journal of Mycology and plant pathology 16: 192-193.

Solanke R B, Kore S S and Sudewad S M 1997 Detection of soyabean seed-borne pathogens and effect of fungicides. Journal Maharastra Agriculture Universities 22 168-170.

Subramanian C V 1971 Hypomycetes. Indian Counsil of Agricultural research, New Delhi pp. 930.

Sundaresh H N and Hiremath P C 1978 Seed mycoflora of soybean in Karnataka. Current research 10: 178-179.

Sundaresh H N and Hiremath P C 1982 Effect of Chemical seed treatment on germination and yield of soybean in Karnataka. Pesticides 16: 22

Sundaresh H N, Ranganathaiah K G, Janardhan A and Viswanath S R 1987 Chemical seed treatment againest seed-borne fungi in soybean. Current research UAS Bangalore 16: 110-111.

Thomson T E and Raper K B 1945 A manual of ***Aspergilli*** pp. 373.

Tripathi D P and Singh B R 1991 Metabolites of soybean seed mycoflora affecting seed viability and seedling vigour. Madras Agricultural Journal 78: 133-135.

Udepurkar M 1991 Control of ***Rhizoctonia bataticola*** through physical, chemical and biological methods. Indian Phytopathology 44: 15.

Van Walbeek W, Scott P M and Thatcher F S 1968 Mycotoxins from seed-borne fungi. Canadian Journal of Microbiology 14: 131-137.

Vidhyasekaran P and Govindaswamy C V 1986 Role of seed-borne fungi in paddy seed spoilage III. Production of carbon-di-oxide, fatty acids and reducing sugars. Indian Phytopathological Society Bulletin 4: 71-78.

Viswanathan and Venkatasubramanian 1971 Toxicity abstract, 2nd International Symposium, Plant Pathology, New Delhi.

Vitti A J, Carvalho M L, Menten J O M 1993 Effect of chemical treatment on the performance of soybean seeds. Ecosistema 18: 75-83.

Wahid A, Javed M S and Idrees M 1995 Chemical control of ***Fusarium*** root rot, wilt and coller rot of soybean. Pakistan Journal of Phyto Pathology 7: 21-24.

Wan Zainum Nik 1980 Seed-borne fungi of soybean and their control. Pertanika 3: 125-132.

Weidenborner M and Hindorf H 1989 Fungi isolated from protein enriched seeds and pods with special emphasis on the genus ***Aspergillus***. Seed Science and Technology 17: 383-390.

Wilson D M and Bell D K 1984 Aflatoxin production by ***Aspergillus flavus*** and ***A. parasiticus*** on visibly sound rehydrated peanut, corn and soybean seed. Peanut science 11: 43-45.

Wogan G N 1968 Aflatoxin risks and control measures fed. proc. 27: 932-938.

Yum K J and Park E W 1989 Occurrence and distribution of soybean seed-borne fungi in Korea. Korean Journal of Plant Pathology 5: 287-293.

Zad J 1979 Mycoflora of soybean. Iranian Journal of Plant Pathology 15: 42-47.

* Originals are not seen