SEED MYCOFLORA OF SOME BEANS

BY

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Affectionately dedicated to my grand parents
for their encouragement and good
wishes throughout my
educational
carrier.

CANDIDATE'S DECLARATION

I, hereby declare that the entire work embodied in this dissertation or any part thereof has not been previously submitted by me for a degree of any University.

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C E R T I F I C A T E

Shri Rajabhau Bhanudasrao Solanke has satisfactorily prosecuted his course of research for a period of not less than Four semesters and that the dissertation entitled "Seed Mycoflora of some Beans" submitted by him 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 dissertation or part thereof has not been previously submitted by him for a degree of any University.

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C E R T I F I C A T E

This is to certify that the dissertation entitled " Seed Mycoflora of Some Beans " submitted by Shri Rajabhau Bhanudasrao Solanke to the Marathwada Agricultural University, Parbhani, in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (Agriculture) in the subject of PLANT PATHOLOGY has been approved by the student's advisory committee after oral examination in collaboration with the external examiner.

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INTRODUCTION

Chapter 1 INTRODUCTION

Pulses constitute a group of crops of the legume family which, with the help of the bacteria in their root nodules, fix atmospheric nitrogen and improve the soil fertility. It is said that pulse crops have been the mainstay of Indian Agriculture, enabling the land to turnout reasonable quanties of grains.

Among pulses, the beans viz., Lima bean (Phaseolus lunatus L.), French bean (Phaseolus vulgaris L.), Cluster bean (Cyamopsis tetragonoloba (L.) Taub.) and Indian bean (Dolichos lablab L.) form an important part of Indian dietary. These beans supply vegetable protein, a essential adjucts to predominatly strachy diet of Indian people. They also supply carbohydrates and vitamins to human beings.

In Maharashtra pulses are grown over an area of 2.4 million ha with annual production of about 8.7 lakh tonnes. Out of which 20 per cent area is under beans. The beans are grown as green pod vegetable, fodder, green manure and mixed crop in Maharashtra State. It has been found that the average yield of beans are 4.07 lakh tonnes in Maharashtra State. There are several factors responsible for low yield. Amongst these, diseases are one of the factors responsible for poor yield. According to Edson and Wood (1939) the average losses from diseases to beans was estimated to 12 per cent. Some of the important fungal diseases like Powdery Mildew

(Erysiphe polygoni DC.), Bean rot (Phytophthora parasitica Dast.), Bean dry rot (Macrophomina phaseoli (Maubl.) Ashby), Leaf spot (Corcospora cruenta Sacc.) and (Cercospora canescens Ell. and Mart.), Rust (Uromyces appendiculatus (Pers.) Fries.), Anthracnose (Glomerella lindemuthiana Shear)are of regular occurrence. Amongst these some diseases are external or internal seed borne and play important role in seed germination and spread of the disease.

Since no much work with reference to external and internal seed mycoflora of beans, their effect on germination, spread of the disease and chemical control has been done in Maharashtra State. It was therefore, thought worthwhile to undertake detailed investigation on seed mycoflora of beans. The results of which are given in following pages.

CHAPTER 2

REVIEW OF LITERATURE

Chapter 2 REVIEW OF LITERATURE

Sundararaman (1936) isolated Macrophomina phaseoli from wilted wild horse gram (Dolichos biflorus) for the first time in Madras. He further reported that same strain was found to infect French bean (Phaseolus vulgaris) and black gram (P. mungo) in Madras. Kovacevski (1936) recorded Macrophomina phaseoli on french bean (Phaseolus vulgaris) for first time from Bulgaria. Andrus (1938) reported transmissibility of Macrophomina phaseoli through seeds in the lima bean (Phaseolus lunatus) in Georgia. The incidence of infection was 85 per cent in non-sterilized and 57 per cent in surface sterilized seeds respectively. McCormack (1939) found that Rhizoctonia sp. is responsible for a root rot of beans (P.vulgaris). Person (1944) observed that Rhizoctonia (corticium) solani is the agent of damping of beans in Louisiana. Snyder and Middleton (1945) described the diseases of lima bean (P. lunatus). They found that Rhizoctonia (corticium) solani, Fusarium solani f. phaseoli and Phythium lutimum were more severely affecting seedling and pods than Macrophomina phaseoli in California. Luttrell (1946) isolated pycnidial strain of Macrophomina phaseoli on agar media from the hypocotyl tissue of snap bean (P.vulgaris). Yu and Feng (1948) described the cultural and morphological features and proved the pathogenicity of Fusarium solani f. fabae and F. oxysporum f. fabae responsible for root rot and wilt respectively. They further stated that the beans,

(Phaseolus vulgaris), Pea and cowpea are the original host of Fusarium sp. Streets (1948) described root rot diseases of Guar (Cyamopsis tetragonoloba)due to Phymatotrichum omnivorum, Fusarium sp., Rhizoctonia sp., and Sclerotium rolfsii in Arizona. He further stated that Alternaria leaf spot may cause more or less extensive defoliation under abnormal humid condition. Wallace (1949) recorded Macrophomina phaseoli causing pod spot of lima bean (P. lunatus). Tisdale and Foster (1949) reported that in inoculation tests with Rhizoctonia and Trichoderma reduced emergence of snap bean (Phaseolus vulgaris) and lima bean (P. lunatus) in Florida. Miller (1949) identified seventeen genera of fungi isolated from garden bean (Phaseolus sp.), Tomato and Onion; the most common contaminants were Macrosporium, Rhizopus, Aspergillus and Periconia. He also observed that the incidence of Penicillium and Rhizopus was much lower than others. Swank (1951) gave a detailed account on symptoms, pathogenicity and morphology of Alternaria sp. causing leaf spot and die back of snap bean (Phaseolus vulgaris) from the central Florida. Srivastava and Mathur (1954) studied on the storage of field beans (Dolichos lablab) at different temperatures and observed that principal cause of disease during storage was Colletotrichum lindemuthianum producing pinkish or brownish spots on the seeds and mycelium on the pod at high humidities and Rhizopus nigricans which was very common at 39° to 42°F and above. They found optimum conditions for storage to be

32° to 35°F and 85 to 90 per cent relative humidity. Winstead and Hebert (1956) firstly recorded the symptom of Helminthosporium victoriae on tender green bean (P. vulgaris) pods. The symptoms were water soaked lesions about 1 mm in diameter, with black necrotic centres, dark brown to black, narrow streaks, 1 to 5 mm in length were also observed on stem petioles and veins. They also described morphological characters and proved pathogenicity. Rangaswami and Rao (1957) reported Alternaria blight of cluster beans in India. They proved pathogenicity and proposed a new name to be called as Alternaira cyamopsidis Rangaswami and Rao. Chatterjee (1958) reported dry rot of beans (Phaseolus vulgaris) primarily due to Fusarium solani f. phaseoli at Idaho Agricultural Experiment Station, Moscow. The pathogen penetrated directly through epidermal cells, through stomata on the hypocotyl and through wounds. He recorded 3 strains, distinguishable by cultural characters and degree of virulence. Huber and Finley (1959) isolated out of 64 bean (P. vulgaris) plants, 29 isolates of Fusarium solani f. phaseoli, 21 isolates of Gliocladium roseum and 14 mixture of these two fungi at Idaho Agricultural Experiment Station, Moscow. Further they sown the seeds in sand and soil artificially infected with G. roseum and F. solani f. phaseoli and found that disease caused by F. solani f. phaseoli was more severe in sand than in soil. Walker (1960) firstly observed two seed borne fungi (Rhizoctonia sp. and Sclerotinia sclerotiorum)

on french bean (P. vulgaris). He observed that the degree of seed infection is related to weather conditions during seed maturation. Chorin and Halfon (1962) noted infection of Phaseolus vulgaris seeds due to Corticium solani. Trujillo (1962) found for the first time Fusarium solani f. phaseoli in Hawaii causing dry cortical stem rot of Phaseolus vulgaris var. Hawaiian wonder. He further stated that disease was probably introduced from the Mainland seeds. Meyer (1963) reported new leaf spot disease of bean caused by Drechslera siccans Shoemaker Parasitica. The symptoms occurs on the lamina as almost circular spots 1-5 mm in diameter red brown with a fragile grey centre, bearing mycelium and soon falling away to leave a shot-hole. Perisic (1964) observed Macrophomina phaseoli (S. bataticola) a parasite of beans. Sowell (1965) described a leaf spot of Cyamopsis tetragonoloba caused by Alternaria cucumerina producing a characteristic concentric rings in the larger spots, distinct from the symptoms of anthracnose Colletotrichum dematium f. truncata. The pathogen is seed borne. It was similar to that reported on C. psoraloides in India as C. capsici f. cyamopsicola. Chand and Verma (1968) observed in India a leaf spot disease of Cyamopsis tetragonoloba caused by Curvularia lunata. They proved pathogenicity and described symptoms and morphology of fungus. Jain and Patel (1969) reported seed mycoflora of Guar, their role in emergence and vigour of seedlings and studied efficacy of fungicides. The seed mycoflora Aspergillus,

Rhizopus, Cephalosporium and Fusarium were the most frequent sp. associated with Guar seeds. Fusarium sp. and two isolates of Alternaria from the mycoflora were pathogenic, causing root rot and brown leaf spot respectively. Sood and Negodorn (1969) reported that in glass house inoculation Alternaria tenuis (A. alternata) caused small brown, irregular lesions which expand to become grey brown and oval, with concentric zones. The disease development was serve at 16°C less at 28°C and older plants were more susceptible than younger ones. Watanable (1972) observed that when bean seeds were sown in partially sterilized soils 18-23 per cent did not emerge, 7.5-14.5 produced diseased seedling which yielded Rhizoctonia slaeni or Colletotrichum lindemuthianum and sterilizer seeds yielded Alternaria, Fusarium, Colletotrichum, Chaetomium and Rhizoctonia. In artificial inoculation test C. lindemuthianum M. phaseoli and F. oxysporum showed very weak pathogenicity. He also reported the fungus Macrophomina phaseolina from commercial seeds and soil. Gupta and Saharan (1973) isolated from the lesions on bean seeds at Palampur, India. The fungi viz., Fusarium solani (76 per cent) Phyllosticta phaseolina (12 per cent) and Aspergillus flavus (10 per cent) and out of these first two caused 30 per cent and 20 per cent rotting respectively on inoculation to seeds. Deshmar and Khare (1973) observed paper towel method to be a rapid and easy technique for testing susceptibility of mung to Rhizoctonia bataticola (Taub.) Butler. Singh and Chohan (1973) reported the

seed mycoflora of Gaur (Cyamopsis tetragonolobe) and its effect on germination and growth of seedlings by blotter or agar plate methods. Bolkan et al. (1976) studied internally seed borne fungi associated with three soybean and three bean (Phaseolus vulgaris) cultivars and their control in central Brazil. Ellis et al. (1976) recorded internally seed borne fungi of bean (Phaseolus vulgaris) and reported twenty fungal genera.

Fungicidal control:

Leach and Houston (1944) gave brief note on the seed treatment of field and vegetable crops against diseases commonly found in California. Burchfield and McNew (1948) gave a seed treatment technique for pea, maize, lima bean (P. lunatus) with spergon and reported the results. Gould (1949) treated seeds of vegetable crops in Western Washington in economic proportion. The value of the treatment was primarily for seed protection against Fusarium spp., Rhizoctonia spp. and various un-identified Phycomycetes. Marsh (1950) reviewed the literature on soil application, seed dressing systemic and foliage fungicide and the application of certain materials on fruit vegetable and flower in Canada and U.S.A. Jacks (1951) studied the effect of several newly introduced seed protants on vegetable seeds under wide range of conditions. The seeds selected were Lettuce, Pea and French bean (P. vulgaris).

All gave satisfactory germination from the commercial aspect. The fungicides used were Cuporocide, Sperogan, Panogan, Thiram, Forbam, Dow 9B and 36 L obtained from Nagatuk Chemical Company, U.S.A. Jacks (1951b)studied a seed protectant Thiram for various vegetable seeds. He observed 10 per cent germination in each of seeds inoculated with Alternaria solani, Rhizoctonia solani and Botrysis cinerea when treated with Thiram on malt agar. Jacks (1953) further studied seed dis-infectants in Newzealand. The ability of seven fungicides including Panogan, Spergon and Thiram to eliminate fungi and bacteria on vegetable seeds was compared following several commonly used laboratory methods. Martin and Atkins (1954) carried out pot test to study the effect of Arasan, Dow 9B, Orthocide (Captan) 75, Phygon, Sperogon, Semesan, Thiram, Vercllo 512W and L.S.D. Ditman et al. (1955) treated combination of insecticide and fungicide to snap bean seeds (P. vulgaris) and Lima bean (P.lunatus.) He found that the performance of Captan and Thiram was more satisfactory than those of Chloranil and other fungicides. Devison and Vaughn (1957) tested three species of Fusarium causing root rot of Phaseolus vulgaris and found when seeds were treated with vanicides-65 and Crog, the desired degree of control. Bremer (1957) tested combination of fungicide and insectidde for the seed treatment of beans (P. vulgaris) and found that Captan and COBH, each with

dieldrin gave the best growth and organic mercury fungicide with lindance gave poor growth McEwen et al. (1957) observed relative effectiveness of various insecticides fungicides seed treatment on lima bean (P. lunatus.) Kefforal (1958) suggested control measures for seed borne disease of French bean (P.vulgaris). Lindstrom (1959) formulated the fungicidal dose and disinfection efficacy. Cruger (1961) gave a list of the diseases of various vegetables transmitted through seeds and controlled by Captan, quinoximebenzylhydra-zone and Thiram. The effect of these treatments on germination was noted and their application to particular vegetable is discussed. Sowell (1965) described the effect of seed treatment on seed borne pathogen of Guar (Cymopsis tetragonoloba). Agnihotri and Prasad (1970) reported efficacy of some fungicides against Colletotrichum capsici f. cyamopsicola (Pre. and Das.). Natti and Crosier (1971) treated seeds with Thiram 75 per cent, Benomyl 50 W, Thiram + Dieldrin and TBZ 60W alone and + Chloroneb and found that these fungicides were ineffective against root rot (Fusarium solani f. pahseoli) but in furrow spray with Benomyl, benomyl + Chloroneb and TBZ , control was observed. Boyadshiow (1972) gave brief account of seed treatment of garden bean (P.vulgaris) with fungicides and antibiotics against bacterial and fungus diseases. Crisan (1973)

studied some systemic fungicides on certain fungi infecting the bean (P.vulgaris). Stan and Reicu (1973) observed that seed treatment with 0.3- 0.5 per cent Vitavax gave good results against Rhizoctonia solani on bean (Phaseolus vulgaris Kaul (1973) gave the comparative effect of long storage after various treatment on the viability and mycoflora of bean (P. vulgaris) seeds found un-impored effect on viability of seeds. He tested five fungicides viz., Agrosan GN, Corsan, Captan, Tillex and Thiram and found effective control. Sirry et al. (1974) gave fungicidal seed-dressing of (P.vulgaris) in relation to Rhizoctonia root rot and the plant growth. Papavizas and Lewis (1975) studied the effect of seed treatment with fungicides on bean (P.vulgaris) in relation to bean root rot (Fusarium solani f. phaseoli). Russell and Musea (1977) evaluated potential seed treatment to control Fusarium solani f. phaseoli, the cause of foot and root rot of Phaseolus vulgaris.

Foregoing review indicates that no much work on seed mycoflora of beans with special reference to fungicidal seed treatment has been done in India, hence present work is undertaken.

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CHAPTER 3

MATERIALS AND METHODS

Chapter 3 MATERIALS AND METHODS

3.1: Isolation:

The seed samples of lima bean (Phaseolus lunatus L.), French bean (Phaseolus vulgaris L.), Cluster bean (Cyamopsis tetragonaloba (L.) Taub.) and Wal (Indian bean) (Dolichos lablab L.) were collected from the Department of Horticulture, Marathwada Agricultural University, Parbhani. An attempt was made to pick up those seeds from the mixture which appeared unhealthy. International rules for seed testing (ISTA) was followed throughout the studies. Four methods viz. agar plate method (Muskat, 1978); Standard blotter technique (deTempe, 1955), Moist sand method (Suryanarayana and Bhombe, 1961) and Rolled towel method (Deshker and Khare, 1973) were followed for isolation of seed fungi.

To isolate external and internal seed mycoflora associated with the seeds, from the sample of each bean, 400 seeds were selected at random and tested for seed mycoflora as given below.

Agar Plate method:

For isolation of internal seed borne fungi 200 seeds were selected and surface sterilized by dipping in 0.1 per cent Mercuric Chloride solution for 1½ to 2 minutes, then they were washed in three changes of sterile water

and five seeds were placed in petridish containing Potato dextrose agar. Similarly for isolation of external seed borne fungi 200 seeds were directly placed in Petridish containing sterile Potato dextrose agar. All Petridishes were incubated at (27-30C). Following method given by Leach (1962) Petridishes were exposed for 12 hours to Ultraviolet light and 12 hours to dark.. Observations were recorded for the growth of different organisms with the help of binocular microscope and again after 7 days second recording was made and mycelial fragments were transferred to Potato dextrose agar slants for further studies. The results of which are given in Table 1 to 4.

Blotter Method:

In this method for isolation of internal seed borne fungi 200 seeds were surface sterilized in 0.1 per cent mercuric chloride solution for 1½ to 2 minutes and washed in three changes of sterile water then ten seeds were placed equidistanty on three layers of moist blotters in Petridish. External seed borne fungi were isolated by placing 200 seeds directly on the Moist blotters in Petridish. All Petridishes were incubated at (27-30 C) and exposed to ultraviolet light and dark as described earlier. The seeds and seedlings were examined after five days with a binocular microscope, the percentage of individual seed mycoflora was recorded and

infected seeds were marked then again after 7 days final recording was made and mycelial fragments were transferred to Potato dextrose agar slants for further studies. The results of which are given in Table 1 to 4.

Sand Method:

Two hundred seeds were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 1½ to 2 minutes then washed in three changes of sterile water and placed on the moist sterile sand filled in tray. In each tray 50 seeds were placed. A second lot of 200 seeds was directly placed on the sterile moist sand filled in tray. These trays were then incubated at (27-30 C) and after 7 days, un-germinated seeds and seedlings were examined with a binocular microscope and percentage of individual organism was recorded. A second observation was recorded after 7 days and mycelial fragments were transferred to PDA slants for further studies. The results of which are given in Table 1 to 4.

Rolled towel method:

A lot of 200 seeds was surface sterilized in 0.1 per cent Mercuric chloride solution for 1½ to 2 minutes then washed in three changes of sterilic water and fifty seeds were placed on moist towel paper and covered with polythelene paper and rolled carefully so as not to disturb

the seeds from their place. A second lot of 200 seeds was directly placed on moist towel paper and covered with Polythelene paper and rolled carefully. The rolled towel papers were incubated at (27-30 C). Observations were recorded after 7 days, the seeds and seedlings were examined with a binocular microscope and percentage of the individual organism was recorded, second observation was recorded after 7 days and mycelial fragments were transferred to Potato dextrose agar slants and maintained for further studies. The results are given in Table 1-4.

3.2 Pathogenicity:

A single spore isolation was made from Potato dextrose agar slants and all cultures were brought into the pure form. One hundred apparently healthy seeds of each bean were selected for Pathogenicity test. Seeds were surface sterilized in 0.2 mercuric chloride solution for 1½ to 2 minutes then washed in three changes of sterile water. All seeds were rolled on actively sporulating cultures and were placed on moist sterilized sand in trays, on moist blotter paper in petridishes, on sterilized Potato dextrose agar petridishes and on moist towel paper then covered with polythelene paper. The material was incubated at (27-30 C) and exposed for 12 hours to light and 12 hours to dark.

Observations were recorded after 7 days for pre and post emergence of seedling rots and observations were continued up to 15 days. The culture of the fungus which was identical with original culture. Reisolations were made and same cultures were used throughout course of experimentation in given Table 6.

3.3 Evaluation of efficacy of different fungicides.

For evaluation of efficacy of different fungicides; seven fungicide viz. Thiram 0.2 (Tetra-methyl-thiuram disilphale); Bavistin 0.1 2(Methuxycarbamoyl Benzimidazole (50 per cent W.P.), Difolaton 0.2 N (1, 1, 2, 3-Tetrachloroethyl) sulfenyl-cis-4-cyclo-hexane- 1,2-discarboximide (80 per cent W.P.), Vitavax 0.2 (5-6-dihydro-2-methyl-1,4- oxathiin-3-carboxanilide (75 per cent W.P.) Captan 0.2-N (trichloronethyl thio-4-cyclohexene-1-2-discarboximide (83 per cent); M.B.C. 0.1 ([redacted]),Agrosan,GN 0.2 (Phenyl Mercury acetate + ethylmercury chloride) were used. Three hundred and fifty gram of healthy seeds of each bean was selected and out of them only 400 seeds were treated by each fungicide. Then these seeds were dried at room temperature. After drying, the seeds were placed in sterilized Potato dextrose agar in Petridish, Moist

blotter paper, sterilized moist sand in tray and Towel paper covered with polythelene paper rolled. Then material was incubated at (27 to 30 C) and exposed for 12 hours to light and 12 hours to dark. After 7 days the seeds and seedlings were examined with binocular microscope; the percentage of germination and infected seeds were recorded. After seed treatment seeds were stored. Three isolations were carried out at the interval of one month. The results are recorded in Table 8 to 13, 14 to 19, 20 to 25.

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CHAPTER 4

RESULTS

Chapter 4 RESULTS

4.1 Isolation of different mycoflora of beans.

The results given in table 1 to 4 indicate that the fungi isolated by agar plate, blotter paper, rolled towel and sand method were the species of Aspergillus, Alternaria, Fusarium, Curvularia Macrophomina, Rhizopus, Rhizoctonia, Helminthosporium, and Penicillium from the lima bean (Phaseolus lunatus L.), French bean (Phaseolus vulgaris L.), Val bean (Dolichos lablab L.) and Cluster bean (Cyamopsis tetragonoloba (L.) Taub.) seeds (Figure 1). These fungi were predominantly associated with bean seeds under study and hence they were used through out the experiment.

The results in Table 1 indicated that the germination percentage of seeds was found maximum in rolled towel (68 per cent), blotter (65 per cent) as compared to agar plate (60 per cent) and sand (63 per cent) in lima bean. It was also observed that the fungi Aspergillus sp., Fusarium sp., Macrophomina sp. and Rhizopus sp. were more prominant than Alternaria sp. Curvularia sp. Rhizoctonia sp. and Helminthosporium sp. in all the methods of isolation in lima bean.

The results in Table 2 indicated that the germination percentage of seeds was found maximum in rolled towel (64 per cent) and blotter paper (62 per cent) as compared to agar plate (58 per cent) and sand (60 per cent) in French bean seeds. The fungi Fusarium sp. Macrophomina sp. and

Aspergillus sp. were prominent than the Alternaria sp., Curvularia sp., Rhizopus sp., Rhizoctonia sp., Helminthosporium sp., (Drechslera sp.) and Penicillium sp. in all the methods. In general more number of fungi were found in agar plate and rolled towel than bloter and sand method in french bean seeds.

The results table 3 indicate that the germination percentage was more in rolled towel (55 per cent) and bloter paper (58 per cent) than agar plate (53 per cent) and sand (47 per cent) in wal bean seeds. It was also observed that the incidence of the fungal seed mycoflora viz., Fusarium sp. Aspergillus sp. Macrophomina sp. and Penicillium sp. was found maximum than Alternaria sp. Curvularia sp. Rhizoctonia sp. Rhizopus sp. and Helminthosporium sp. in all the methods in wal bean seeds.

The results table 4 indicate that the percentage of germination was more in rolled towel (70 per cent) and blotter (68 per cent) than agar plate (65 per cent) and sand (62 per cent) in cluster bean seeds. It was also found that the incidence of Aspergillus sp., Fusarium sp., Macrophomina sp., and Penicillium sp. was more than Alternaria sp., Curvularia sp., Rhizopus sp., Rhizoctonia sp., and Helminthosporium sp.

In general it was observed that agar plate method is superior than all other methods in yielding more number of fungi from the beans (Figure 2 to 7).

Table 1: Different mycoflora isolated from sterilized and unsterilized lima bean seeds

Fungi isolated	Agar		Blotter		Rolled towel		Sand	
	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.
Alternaria sp.	7	9	4	6	4	6	4	6
Aspergillus sp.	17	19	11	14	12	16	6	10
Fusarium sp.	21	22	6	10	14	18	5	6
Curvularia sp.	6	7	4	5	5	7	4	5
Macrophomina sp.	21	25	6	7	12	16	6	7
Rhizopus sp.	11	15	7	10	14	17	7	10
Rhizoctonia sp.	6	8	4	6	5	7	4	6
Helminthosporium sp.	5	6	3	5	4	6	3	5
Penicillium sp.	12	17	4	6	12	13	4	6
Germination %	60	52	65	60	68	64	63	60

Table 2: Different mycoflora isolated from sterilized and unsterilized French bean seeds

Fungi isolated	Agar		Blotter		Rolled towel		Sand	
	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.
Alternaria sp.	4	6	4	5	5	6	3	4
Aspergillus sp.	11	13	12	13	12	15	5	7
Fusarium sp.	16	19	12	14	13	16	6	7
Curvularia sp.	7	8	3	5	4	6	3	4
Macrophomina sp.	18	22	11	13	12	16	6	7
Rhizopus sp.	12	15	8	10	9	11	4	6
Rhizoctonia sp.	11	12	11	12	10	12	7	7
Helminthosporium sp.	4	5	4	6	4	6	4	5
Penicillium sp.	4	6	5	7	4	6	2	4
Germination %	58	52	62	55	64	60	60	56

Table 3: Different mycoflora isolated from sterilized and unsterilized Wal bean seeds

Fungi isolated	Agar		Blotter		Rolled towel		Sand	
	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.
Alternaria sp.	5	6	5	6	4	6	5	6
Aspergillus sp.	17	19	9	11	6	7	5	6
Fusarium sp.	12	14	11	13	7	9	6	7
Curvularia sp.	4	6	4	5	5	7	5	6
Macrophomina sp.	12	16	11	13	9	11	5	7
Rhizopus sp.	11	13	9	11	9	11	3	4
Rhizoctonia sp.	5	6	5	7	5	7	4	5
Helminthosporium sp.	4	5	4	6	5	6	5	7
Penicillium sp.	12	12	10	12	11	13	6	7
Germination %	53	47	58	54	55	45	47	42

Table 4: Different mycoflora isolated from sterilized and unsterilized Cluster bean seeds

Fungi isolated	Agar		Blotter		Rolled towel		Sand	
	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.
Alternaria sp.	4	6	4	6	5	6	2	4
Aspergillus sp.	9	14	19	14	8	10	6	8
Fusarium sp.	11	13	11	16	6	9	7	8
Curvularia sp.	5	7	5	6	4	5	3	5
Macrophomina sp.	11	13	11	12	7	9	10	11
Rhizopus sp.	8	9	8	14	6	7	5	6
Rhizoctonia sp.	5	7	5	6	4	6	3	4
Helminthosporium sp.	5	6	5	6	5	6	3	4
Penicillium sp.	11	13	11	13	8	9	7	11
Germination %	65	63	68	63	70	65	62	57

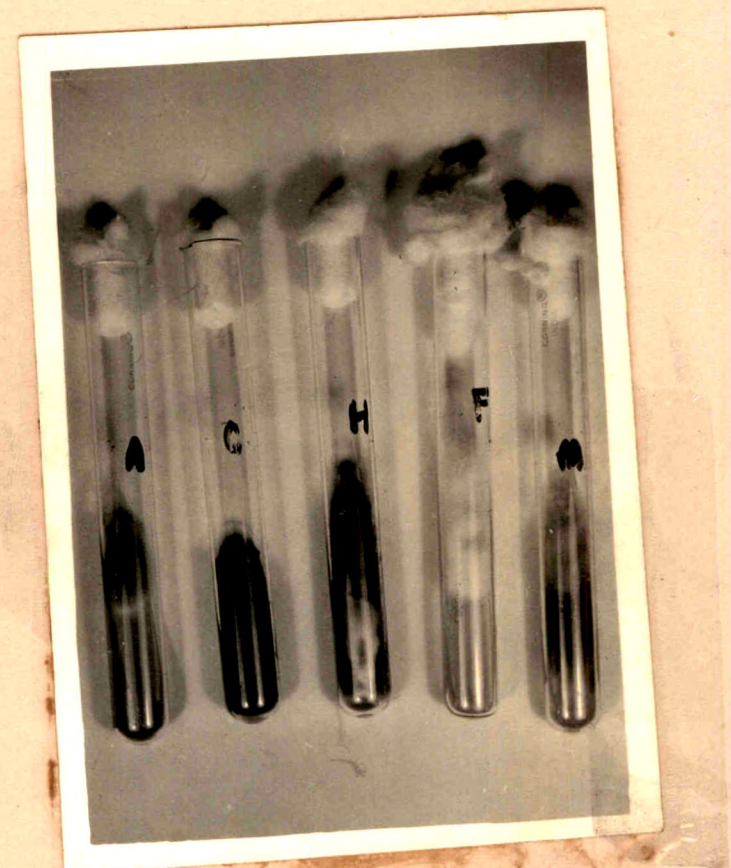
4.2 Effect of fungal inoculation on shoot and root length of beans.

The seeds of Lima bean, French bean, Wal bean and Cluster bean seeds were inoculated by rolling on the different sporulating cultures and these seeds were then planted on sterilized Potato dextrose agar, Blotter paper, Rolled towel and Sand. Adequate control was also maintained for each bean without inoculation of fungal cultures. (Figure 2-11)

The observations in Table 5 indicate that the length of shoot and root in control was maximum as compared with inoculated beans in all the methods. Secondly length of shoot and root was observed maximum in Rolled towel and Blotter method as compared with agar plate method and sand method.

It was also observed that the length of shoot and root was less in inoculated seeds with Helminthosporium sp., Curvularia sp. and Alternaria sp. as compared with Macrophomina sp. and Fusarium sp.

figure.1. Major seed mycoflora.

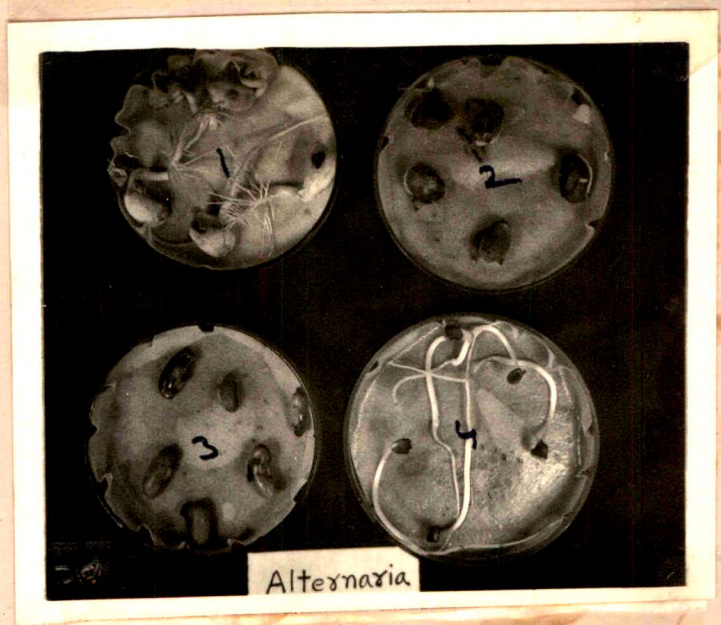


A = Alternaria sp ; C = Curvularia sp ;

H = Helminthosporium sp ; F = Fusarium sp ;

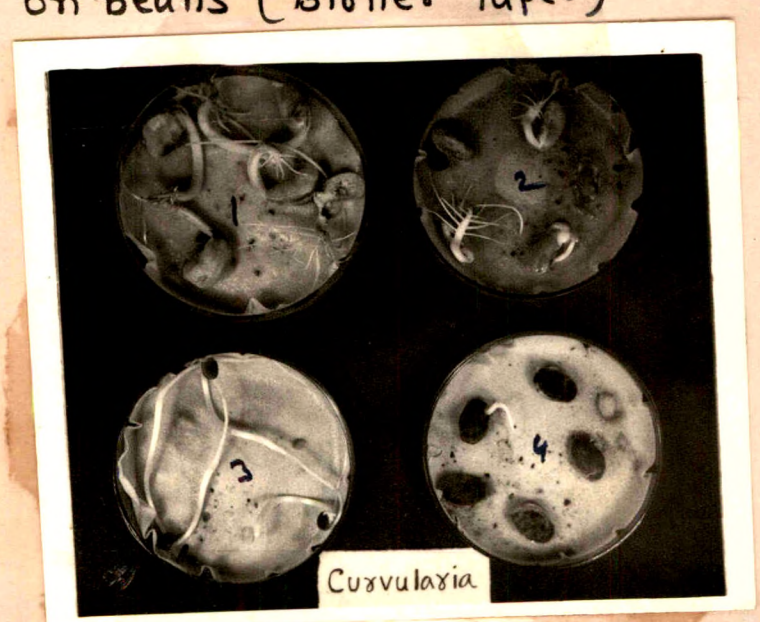
and M = Macrophomina sp .

Figure. 2. Effect of artificial inoculation on germination and incidence of infection on beans (Blotter method)



1) Lima bean
2) Wal bean
3) french bean
3) Cluster bean.

Figure. 3. Effect of artificial inoculation on germination and incidence of infection on beans (Blotter Paper)

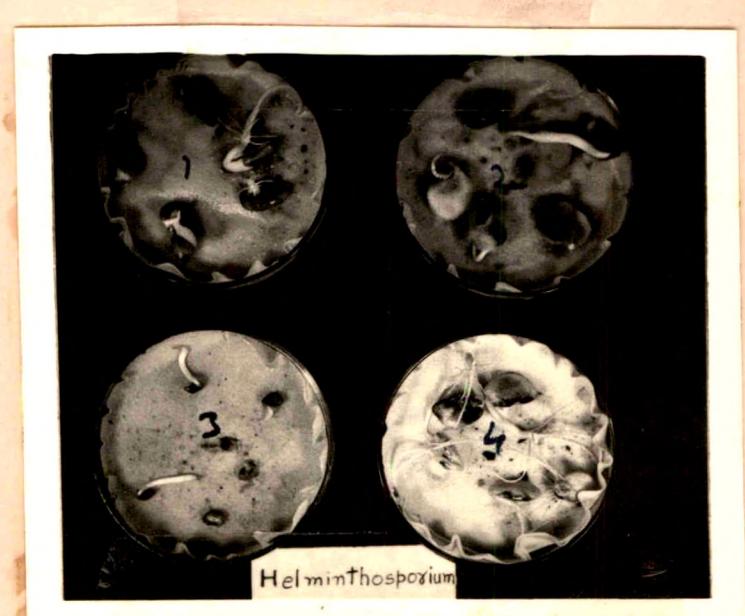


1) Lima bean
2) French bean
3) Cluster bean
4) Wal bean

figure. 4. Effect of artificial inoculation on germination and incidence of infection on beans (Blotter Paper)

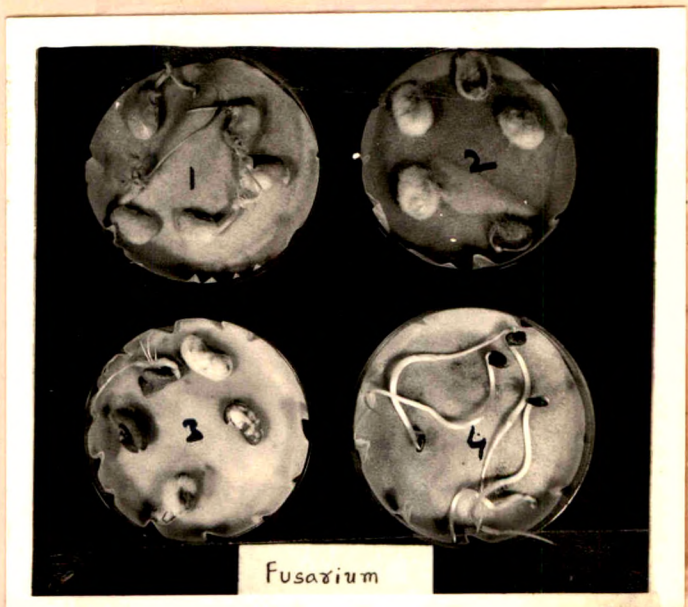
1) Wal bean 2) Lima bean
3) french bean 4) Cluster bean

figure. 5. Effect of artificial inoculation on germination and incidence of inflection on beans (Blotter Paper



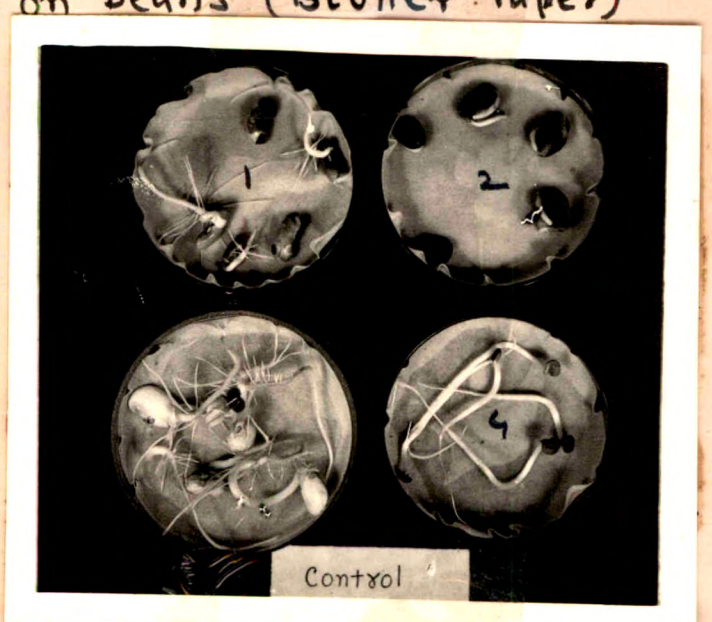
1) french bean 2) Wal bean
3) Cluster bean 4) Lima bean

figure. 6. Effect of artificial inoculation on germination and incidence of infection on beans (Blotter Paper)



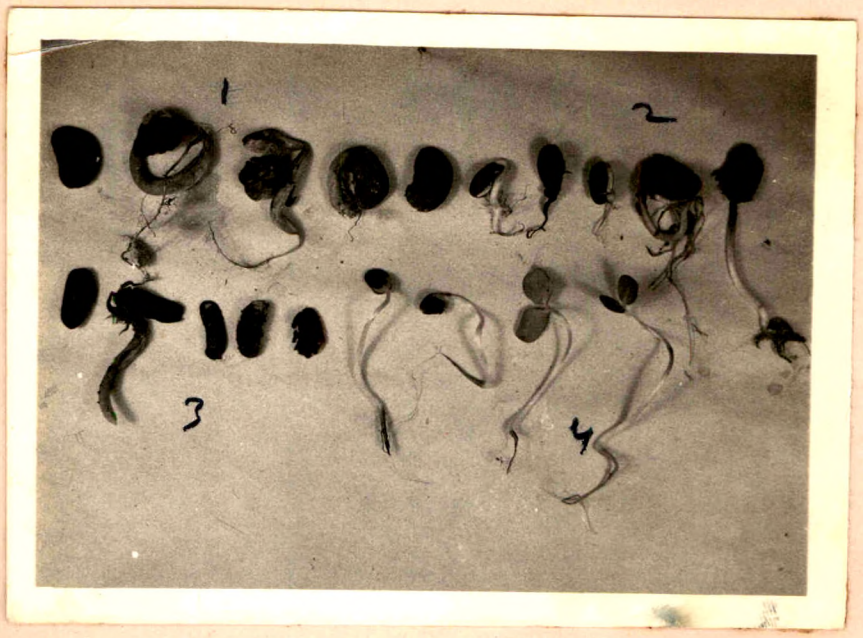
1) Lima bean 2) Wal bean
3) french bean 4) cluster bean

figure. 7. Effect of artificial inoculation on germination and incidence of infection on beans (Blotter Paper)



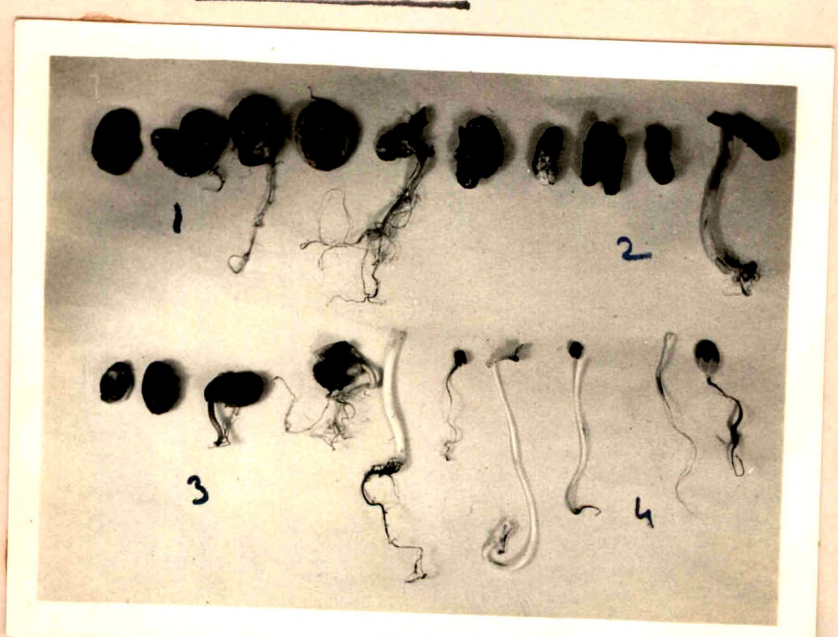
1) french bean 2) Wal bean
3) Lima bean 4) cluster bean

Figure. 8. Effect of artificial inoculation on germination and incidence of infection on beans (Rolled towel) Alternaria sp.



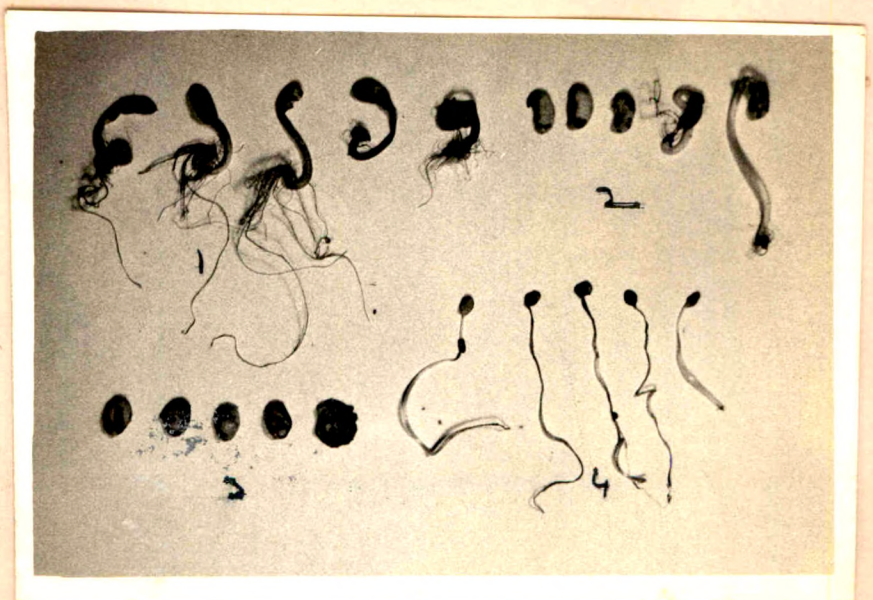
1) Lima bean
2) Walbean
3) French bean
4) cluster bean.

figure. 9. Effect of artificial inoculation on germination and incidence of infection on beans (Rolled towel) curvularia sp.



1) Wal bean
2) Lima bean
3) French bean
4) cluster bean.

Figure.10. Effect of artificial inoculation on germination and incidence of infection on beans (Rolled towel) *Fusarium* sp.



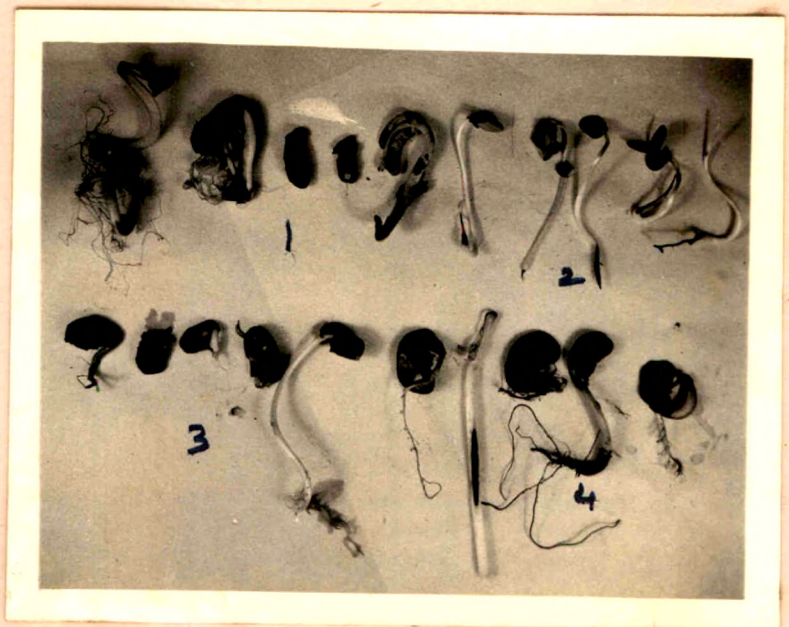
1) Lima bean
2) Wal bean
3) french bean
4) cluster bean

figure.11. Effect of artificial inoculation on germination and incidence of infection on beans (Rolled towel) *Helminthosporium* sp.

1) Lima bean
2) Wal bean
3) Cluster bean
4) french bean.

Figure . 12. Effect of artificial inoculation on germination and incidence of infection on beans (Rolled towel)

Macrophomina sp.



1) french bean
2) cluster bean
3) Wal bean
4) Lima bean

4.21 Effect of fungal inoculation on germination of bean seeds.

The surface sterilized seeds of bean were inoculated by rolling the seeds on the different sporulating cultures and these seeds were planted on sterilized and unsterilized moist sand.

The results in Table 6 indicate that Microphoming sp. found highly pathogenic on the seeds of lima bean and French bean resulting 75 per cent seeds and seedling rot. The Fusarium sp. deteriorated the germinability of the seeds of French bean to the extent of 69 per cent, thereby showing the association with the outer coat of the seeds. The fungi Culvularia sp., Alternaria sp. and Helminthosporium sp. multiplied superficially on Lima bean, French bean, Wal bean and Cluster bean seeds as they did not cause any appreciable seed or seedling rot. Artificially inoculated seeds of French bean, Lima bean, Wal bean and Cluster bean with different fungi produced relatively good seedling stand in unsterilized sand as compared to sterilized sand.

Table6: Effect of fungal inoculation on germination of bean seeds (Sand Method)

Host	Pathogen	% Pre-emergence rotting		% Post emergence rotting		% Normal seedling	
		S.S.	U.S.S.	S.S.	U.S.S.	S.S.	U.S.S.
Lima bean (*Phaseolus lunatus*)	*Macrophomina* sp.	40	43	39	32	21	25
	Fusarium sp.	32	30	28	23	40	47
	Curvularia sp.	22	28	27	31	51	49
	Alternaria sp.	25	30	27	20	48	50
	Helminthosporium sp.	27	29	30	38	43	33
	Control	15	16	13	14	72	70
French bean (*P. vulgaris*)	*Macrophomina* sp.	37	29	41	40	22	31
	Fusarium sp.	34	27	37	29	29	43
	Curvularia sp.	24	27	31	28	45	45
	Alternaria sp.	25	37	30	38	45	25
	Heminthosporium sp.	30	36	21	30	49	34
	Control	17	19	15	16	68	65
Wal bean (*Dolichos lablab*)	*Macrophomina* sp.	39	29	28	37	33	34
	Fusarium sp.	29	31	42	38	29	31
	Curvularia sp.	27	35	21	26	52	39
	Alternaria sp.	23	25	30	27	47	48
	Helminthosporium sp.	28	26	30	32	42	42
	Control	16	15	17	18	67	67
Clustor bean (*Cyamposis tetrogonoloba*)	*Macrophomina* sp.	35	34	32	30	33	36
	Fusarium sp.	27	28	29	27	44	45
	Curvularia sp.	22	21	27	31	51	48
	Alternaria sp.	31	29	40	39	29	32
	Helminthosporium sp.	27	24	35	30	38	46
	Control	10	12	5	7	85	81

Note: S.S. = Sterilized sand, U.S.S. = Unsterilized sand

4.3 Effect of fungicidal seed treatment on shoot and root length of beans.

For this purpose 400 surface sterilized seeds of each bean were treated with the fungicide, Thiram (0.2 per cent), Bavistin (0.1 per cent), Difolatan (0.2 per cent), Vitavax (0.2 per cent), Captan (0.2 per cent), MBC (0.1 per cent) and Agrosan GN (0.2 per cent) keeping adequate control.

The results in Table 7 indicate that fungicidal seed treatment resulted in increase of shoot and root length as compared to control. It was also observed that the length of shoot was maximum in Thiram (0.2 per cent) and MBC (0.1 per cent) as compared with other fungicides but in Vitavax only the root length was maximum as compared with all other fungicides treated. Decrease in shoot and root length was found in case of Difolatan (0.2 per cent).

Table 7: Ef

Name of beans	Agrosan GN		Control	
	oot length n cm	Shoot length in cm	Root length in cm	Shoot length in cm	Root length in cm
Lima bean	1.50	1.47	1.37	1.11	1.03
French bean	1.11	1.45	1.40	1.07	1.01
Wal bean	1.22	1.57	1.32	1.10	1.05
Cluster bean	1.20	1.02	1.00	1.00	1.01
Lima bean	7.23	8.33	7.72	5.60	5.00
French bean	6.06	6.27	5.44	6.12	4.90
Wal bean	6.06	7.17	6.29	5.47	4.91
Cluster bean	5.05	6.30	4.75	5.00	3.90
Lima bean	5.06	8.52	5.35	6.53	3.53
French bean	5.66	6.11	6.23	5.81	4.72
Wal bean	6.88	7.43	6.57	6.48	5.35
Cluster bean	4.70	5.77	4.88	4.69	4.01
Lima bean	5.15	6.75	5.01	5.99	4.85
French bean	4.48	7.11	5.15	5.10	4.75
Wal bean	5.10	6.79	5.70	5.10	4.65
Cluster bean	4.01	5.01	1.15	4.01	4.00

4.31 Effect of fungicidal seed treatment on mycoflora of beans.

The seeds of each bean was treated with seven fungicide viz. Vitavax (0.2 per cent), Difolatan (0.2 per cent), Agrosan GN (0.2 per cent, Thiram (0.2 per cent) Captan (0.2 per cent), MBC (0.1 per cent) and Bavistin (0.1 per cent) keeping one lot of each untreated bean seeds as control. The seeds were stored and then keeping one month's interval they were sown on agar plates, blotter paper and rolled towel paper.

The results given table 8 to 13 indicate that the germination percentage of all the seeds in fungicidal seed treatment was superior over control. It was also observed that the germination percentage was more in seed treated with Vitavax, Agrosan GN, Bavistin as compared to Difolatan, Thiram, Captan and MBC, but the fungal mycoflora was decreased in case of seeds treated with MBC, Bavistin, Vitavax and Agrosan GN as compared to Thiram, Captan and Difoltan in all the method. In general germination percentage found maximum in Blotter paper method and minimum in agar plate method and intermediate in rolled towel method. It is also concluded that blotter paper method is the best method for germination of treated seeds with fungicides MBC, Bavistin, Vitavax and Agrosan GN.

The results given in table 14 to 19 indicate that the germination percentage was maximum in blotter paper than agar and rolled towel in all the treated bean seeds. The germination percentage was also increased in bean seeds treated with Agrosan GN Vitavax and Bavistin, than Difolatan, Captan, Thiram and MBC in

all the methods. The number of fungi found were less in Bavistin, MBC, Vitavax and Agrosan GN as compared with Difolaten, Thiram and Captan in all method and in all the beans but increased as compared with seeds tested after one month.

The results given in table 20 to 25 indicate that the germination percentage was increased in blotter paper than agar plate and rolled towel in all treated bean seeds. The germination percentage was also increased in bean seeds treated with Agrosan GN, Vitavax and Bavistin than Difolatan, Thiram, Captan and MBC as compared to control. The number of fungi found increased in Bavistin, MBC, Vitavax and Agrosan GN than Thiram, Captan and Difolatan in all treated bean seeds in all the methods.

In general the seed mycoflora was found maximum after three months interval in treated bean seeds and also decreased the germination percentage than one month and two month stored treated seeds.

ɔpho- sp.	Rhizopus sp.	Rhizoctonia sp.	Helmin thosporium	Penicillium sp.
	-	-	-	1
	-	-	-	1
	-	-	-	-
	-	-	-	-
	-	-	-	2
	-	1	1	-
	-	-	-	-
	3	2	2	5
	-	-	1	1
	-	-	1	-
	-	-	-	1
	1	-	-	-
	-	-	-	2
	-	1	-	-
	1	-	1	1
	2	3	4	6

Table 9: Effect

Fungicides	ɔctonia	Helmin thosporium sp.	Penicillium sp.
Vitavax	-	-	-
Difolatan	-	-	1
Agrosan GN	-	1	-
Thiram	1	-	-
Captan	-	-	2
MBC	-	1	-
Bavistin	1	-	1
Control	3	4	5
Vitavax	-	-	-
Difolatan	-	-	1
Agrosan GN	-	-	1
Thiram	-	-	-
Captan	-	1	-
MBC	-	-	-
Bavistin	-	-	-
Control	3	2	4

:tonia	Helmin-thosporium sp.	Penicillium sp.
	-	1
	-	1
	-	-
	-	-
	-	2
	-	1
	-	1
	5	10
	-	-
	-	-
	-	1
	-	1
	-	1
	-	2
	-	-
	3	5

Rzoctonia sp.	Helmin-thosporium sp.	Penicillium sp.
-	-	-
-	-	-
-	-	1
-	-	-
-	-	1
-	-	1
-	-	-
3	3	8
-	-	2
-	-	-
-	-	1
1	1	-
-	-	-
1	1	-
-	-	1
4	4	6

Rhizoctonia sp.	Helminthosporium sp.	Penicillium sp
-	-	1
1	-	-
-	1	1
-	-	-
1	1	1
-	-	-
-	-	1
4	6	9
1	-	1
-	1	1
1	-	1
1	1	-
1	-	1
-	-	1
±	±	1
5	5	7

Table

Fungi	Rhizopus sp.	Rhizoctonia sp.	Helminthosporium sp.	Penicillium sp.
Vitav	-	1	-	2
Difol	1	1	-	1
Agros	1	-	1	-
Thira	1	-	2	-
Capta	2	1	1	2
NBC	2	-	-	1
Bavis	-	2	1	-
Contr	7	5	4	7
Vitava	2	1	-	1
Difola	-	2	-	-
Agrosa	2	-	1	2
Thiram	1	-	1	3
Captan	-	1	-	-
NBC	-	1	1	2
Bavist	1	2	-	1
Contro	6	4	4	6

Table 15: Ef

Fungicides	...bous	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitavax		-	-	3
Difolatan		1	1	-
Agrosan GN		-	-	1
Thiram		1	2	1
Captan		1	-	-
MBC		-	1	+
Bavistin		-	1	1
Control		4	3	5
Vitavax		1	-	1
Difolatan		-	-	-
Agrosan GN		-	1	-
Thiram		1	-	1
Captan		-	-	-
MBC		-	1	2
Bavistin		1	1	1
Control		5	3	6

Table 6: Effect

Fungicides	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitavax	1	-	-
Difolatan	-	1	2
Agrosan GN	-	-	3
Thiram	2	-	-
Captan	1	2	5
MBC	-	1	-
Bavistin	2	-	2
Control	3	5	8
Vitavax	1	-	2
Difolatan	2	1	1
Agrosan GN	-	2	2
Thiram	2	-	1
Captan	1	-	2
MBC	2	2	3
Bavistin	1	1	2
Control	4	5	7

Table 17

Fungicid	Zopus p.	Rhizoctonia sp.	Helminthosporium sp.	Penicillium sp.
Vitavax		1	-	2
Difolata		1	2	1
Agrosan		-	-	1
Thiram		1	-	3
Captan		2	1	2
MBC		-	2	1
Bavistin		-	1	2
Control		7	4	7
Vitavax		1	-	1
Difolata		1	-	1
Agrosan		1	-	-
Thiram		-	1	-
Captan		-	2	1
MBC		-	-	2
Bavistin		1	-	2
Control		3	2	5

Table 18: Effe

Fungicides	s	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitavax		-	-	2
Difolatan		2	1	2
Agrosan GN		-	-	1
Thiram		2	1	2
Captan		2	1	2
MBC		1	-	1
Bavistin		1	-	1
Control		7	5	7
Vitavax		1	1	2
Difolatan		1	-	2
Agrosan GN		1	-	-
Thiram		1	1	2
Captan		1	-	2
MBC		-	1	2
Bavistin		1	-	2
Control		4	4	9

Table

Fungic	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitava	1	2	2
Difola	-	-	3
Agrosa	-	1	1
Thiran	1	-	2
Captan	1	1	3
MBC	1	-	2
Bavist	-	-	1
Contro	4	5	7
Vitava	1	-	2
Difola	-	1	1
Agrosa	-	1	2
Thiran	1	-	1
Captan	1	-	2
MBC	-	-	2
Bavist	-	1	1
Contro	4	4	7

Table

Fungici	pus	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitavax		1	-	2
Difolat		-	2	3
Agrosan		-	-	-
Thiram		2	2	1
Captan		2	2	4
MBC		1	-	-
Bavisti		-	1	1
Control		5	4	7
Vitavax		2	1	-
Difolat		1	-	2
Agrosan		1	1	3
Thiram		2	2	4
Captan		-	1	3
MBC		1	1	2
Bavisti		1	1	2
Control		4	4	6

Table

Fungic	hizopus sp.	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitava	-	2	1	2
Difola	2	-	1	3
Agrosa	-	2	-	2
Thiram	1	-	2	2
Captan	2	1	2	3
MBC	4	1	2	2
Bavist	-	1	1	3
Contro	7	5	4	7
Vitava	-	-	1	2
Difola	2	-	-	-
Agrosa	-	1	1	-
Thiram	1	1	-	3
Captan	2	2	2	3
MBC	1	1	2	2
Bavist	1	2	1	2
Contro	5	2	4	5

Table 22 Ef

Fungicides	Rhizoctonia sp.	Helminthosporium sp.	Penicillium sp.
Vitavax	2	1	3
Difolatan	-	2	2
Agrosan GN	3	-	-
Thiram	-	2	4
Captan	4	-	4
MBC	-	-	3
Bavistin	2	2	2
Control	5	3	5
Vitavax	3	-	2
Difolatan	2	1	3
Agrosan GN	3	1	2
Thiram	2	1	2
Captan	2	2	4
MBC	1	1	3
Bavistin	2	1	2
Control	6	5	8

Table 23: Ef

Fungicides	s	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitavax		1	2	3
Difolatan		2	1	4
Agrosan GN		1	3	1
Thiram		2	2	5
Captan		3	2	4
MBC		2	1	4
Bavistin		1	2	3
Control		7	8	5
Vitavax		2	1	3
Difolatan		1	1	3
Agrosan GN		2	1	1
Thiram		1	1	4
Captan		2	-	3
MBC		2	1	3
Bavistin		-	1	2
Control		7	3	7

Table 24:

Fungicides	*Rhizoctonia* sp.	*Helminthosporium* sp.	*Penicillium* sp.
Vitavax	-	1	2
Difolatan	1	-	1
Agrosan GN	2	1	3
Thiram	-	-	2
Captan	1	-	1
MBC	-	2	2
Bavistin	2	1	3
Control	5	5	7
Vitavax	1	-	2
Difolatan	1	1	1
Agrosan GN	2	1	2
Thiram	-	2	3
Captan	-	1	2
MBC	1	3	1
Bavistin	-	2	1
Control	8	5	10

Table 25 Effec

Fungicides	Rhizocto-nia sp.	Helmin-thosporium sp.	Penicillium sp.
Vitavax	2	1	2
Difolatan	1	1	1
Agrosan GN	1	1	1
Thiram	2	2	2
Captan	1	-	2
MBC	2	1	1
Bavistin	1	2	3
Control	3	3	4
Vitavax	-	1	2
Difolatan	-	2	2
Agrosan GN	1	2	1
Thiram	1	2	2
Captan	2	-	2
MBC	2	1	2
Bavistin	-	1	2
Control	3	4	7

CHAPTER 5

DISCUSSION

Chapter 5 DISCUSSION

The seed mycoflora of Lima bean (*Phaseolus lunatus* L.), French bean (*Phaseolus vulgaris* L.), Cluster bean (*Cyamposis tetragonoloba* (L.) Taub.), and Wal bean (*Dolichos lablab* L.) has been studied using the methods viz., agar plate method, blotter paper method, sand method and rolled towel method in the present research project. The fungi *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Curvularia* sp., *Macrophomina* sp., *Rhizoctonia* sp., *Rhizopus* sp., *Helminthosporium* sp., (*Drechslera* sp. and *Penicillium* sp. were invariably obtained in different methods of isolation from all the beans under study.

The Germination of lima bean seeds was found maximum in rolled towel (68 per cent), blotter (65 per cent) as compared to agar plate (60 per cent) and Sand (63 per cent) and fungi *Aspergillus* sp., *Fusarium* sp., *Macrophomina* sp., and *Rhizopus* sp. were more prominent than the *Alternaria* sp., *Curvularia* sp., *Rhizoctonia* sp., *Helminthosporium* sp. (*Drechslera* sp.) and Penicillium sp. in all the methods of isolation.

Andrus (1938) reported transmissibility of *Macrophomina phaseoli* through seeds in the lima bean in Georgia. Person (1944) reported *Rhizoctonia solani* to be a agent of damping-off of beans in Louisiana. Snyder and Middleton (1945) found that *Rhizoctonia solani*, *Fusarium solani* f. *phaseoli* and *Phythium lutimum* were most severly affecting seedling of lima bean in California. Thus, the results obtained in present experiments are confirmed by Andrus (1938), Person (1944) Synder and Middleton (1945).

The germination of French bean seeds was found maximum in rolled towel (64 per cent), blotter (62 per cent) as compared to agar plate (58 per cent) and Sand (60 per cent). The fungi Fusarium sp., Macrophomina sp. and Aspergillus sp. were prominant than Alternaria sp., Rhizopus sp., Rhizoctonia sp Curvularia sp., Helminthosporium sp., (Drechslera sp.) and Penicillium sp. in all the methods. In general more number of fungi were found in agar plate and rolled towel than blotter and Sand method in French bean seeds.

Chatterjee (1958) reported dry rot of bean (Phaseolus vulgaris) primorily due to Fusarium solani f. phaseoli at Idaho Agricultural Experiment Station, Moscow. Huber and Finley (1959) isolated Fusarium solani f. phaseoli, Gliocladium roseum and found that in artificial inoculation test Fusarium solani f phaseoli was more serve in sand than in soil. Gupta and Sharan (1973) isolated Fusarium soloni, Phyllosticta phaseolina and Aspergillus flavus from the lesions on bean seeds at Palmapur (Himachal Pradesh). In present experiment, Fusarium sp. was prominently yieldod from French bean seeds in all the methods of isolation. The results pertaining to Fusarium sp. are in line with Chatterjee (1958), Huber and Finely (1958) and Gupta and Sharan (1973).

The germination of Wal bean seeds was found maximum in rolled towel (55 per cent) and blotter (58 per cent) as compared agar plate (53 per cent) and sand (47 per cent). The fungi Fusarium sp., Macrophomina sp., Aspergillus sp.,

and Penicillium sp. were found predominantly than Alternaria sp Curvularia sp., Rhizopus sp., Rhizoctonia sp. and Helminthosporium sp. (Drechslera sp.) in all the methods of isolation.

The result obtained or Rhizopus sp. in present experiment is in conformity with the report made by Srivastava and Mathur (1954).

The germination in Cluster bean was more in rolled towel (70 per cent) and blotter (68 per cent) than agar plate (65 per cent) and sand (62 per cent) in the present experiment. It was also found that the incidence of Aspergillus sp., Fusarium sp., Macrophomina sp.and Penicillium sp. were more than Alternaria sp., Rhizopus sp., Curvularia sp., Rhizoctonia s and Helminthosporium sp. in all the methods of isolation.

Sowell (1965) described leaf spot of Guar caused by Alternaria cucumerina, which produces characteristic concentric rings in the large spots, to be a seed borne pathogen. Jain and Patel (1969) reported seed mycoflora of Guar, their role in emergence and vigour of seedlings. The seed mycoflora, Aspergillus, Rhizopus, Cephalosporium and Fusarium were the most frequent sp. obtained by them. The result in present experiment also indicate that some fungi are common as were found by Sowell (1965) and Jain and Patel (1969).

The studies on effect of fungal inoculation on germination of bean seeds indicated that Macrophomina sp. was highly pathogenic to lima bean and French bean resulting 75 per cent seed and seedling rot. While Fusarium sp. deteriorated the germinability of the seeds of French bean seed to the extent of 69 per cent. The fungi Culvularia sp., Alternaria sp., and Helminthosporium sp. multiplied superficially on Lima bean, French bean, Wal bean and Cluster bean seeds. As they did not cause appreciable seeds and seedling rot.

Andrus (1938) reported transmissibility of Macrophomina phaseoli through seeds in the Lima bean (Phaseolus lunatus) in Georgia. The incidence of infection was 85 per cent in non-sterilized and 57 per cent in surface sterilized seeds respectively. In present experiment also low germination percentage was obtained due to inoculation of Macrophomina sp. in all the beans, this confirms the results obtained in fungal inoculation experiment.

The effect of fungal inoculation on shoot and root length of beans was studied and the results concluded that the shoot and root length in control was maximum as compared with inoculated beans in all the methods. The rolled towel and blotter methods were found to be superior over agar plate and sand method in increasing the length of shoot and root. In general, it is concluded that the fungal inoculation affects the normal germination and growth of seedlings.

The bean seeds were treated with seven fungicides viz. Thiram (0.2 per cent), Captan (0.2 per cent), MBC (0.1 per cent. Agrosan GN(0.2 per cent), Bavistin (0.1 per cent), Difolatan (0.2 per cent) and Vitavax (0.2 per cent) and it was observed that the fungal treatment resulted in increase of shoot and root length as compared to control. It was also observed that the length of shoot was maximum in Thiram and MBC as compared with other fungicides but in Vitavax only the root length was increased. However, decrease in shoot and root length was found in case of Difoltan.

Jacks (1961) reported satisfactory germination of vegetable seeds after fungicidal seed treatment. He further studied seed protectent Thiram for various vegetable seeds. He observed 10 per cent germination in each of seeds inoculated with Alternaria solani, Rhizoctonia solani and Botrysis cinera when treated with Thiram on malt ager. The results of seed treatment specially with Thiram indicated that there was increas in shoot length than all the fungicides. Thus the results on Thiram seed treatment are confirmed by Jacks (1951).

The effect of fungicidal seed treatment on mycoflora of beans was studied for three months, keeping one months interval and it was observed that the fungicide, Vitavax, Agrosan GN and Bavistin gave more percentage of germination as compared to Difolatan, Thiram, MBC and Captan but fungal mycoflora was decreased in case of seed treated with Bavistin, Vitavax, Agrosan GN and MBC as compared to Thiram, Captan and Difolatan in all the methods. In general germination percentage was found maximum in blotter, minimum in ager plate and intermediate in rolled towel method. It is also concluded that the blotter

method is the best method for germination of treated seeds with fungicides, MBC, Bavistin, Vitavax and Agrosan GN.

The results on treated bean seeds after two months indicate that the germination of bean seeds was maximum in blotter paper than agar and rolled towel in all the treated bean seeds. The germination was also increased in bean seeds treated with Agrosan GN, Vitavax and Bavistin than Difolatan, Captan, Thiram and MBC in all the methods and it was also found that the number of fungi were less in bean seeds treated with Bavistin, MBC, Vitavax and Agrosan GN as compared with Difolatan, Thiram and Captan in all the methods but increased as compared with seeds tested after one month.

The results on treated bean seeds after three months indicate that there was increase in germination percentage in treated bean seed with Agrosan GN, Vitavax and Bavistin than Difolatan, Thiram, Captan and MBC as compared to control. In general seed mycoflora was found minimum after three months interval in treated bean seeds and also decreased the germination percentage than one month and two months stored treated seeds.

Kaul (1973), found unimpaired effect on germination of the seeds treated with Agrosan GN, Captan, Thiram, during the storage for four years. This conform the result pertaining to Agrosan GN, Captan, Thiram inpresent Experiment.

Morphological characters of the different fungi obtained from various beans and used throughout experimentation were identified as given below. The results were also confirmed with the report from the Division of Mycology and Plant Pathology (IARI) New Delhi-110012.

I Lima bean (*Phaseolus lunatus* L.)

1 *Drechslera spicifera* (Bain.) Nicot.

2 *Fusarium semitectum* Berk. and Rav.

3 *Curvularia desmodii* Bharadwaj.

4 *Macrophomina phaseolina* (Tassi) Goid.

5 *Alternaria* sp.

II French bean (*Phaseolus vulgaris* L.)

1. *Drechslera Indica* Anahosur.

2 *Macrophomina phaseolina* (Tassi) Goid.

3 *Fusarium solani* (Mart.)Sacc.

4 *Curvularia desmodii* Bharadwaj.

5 *Alternaria* sp.

III Wal bean (*Dolichos lablab* L.)

1 *Macrophomina phaseolina* (Tassi) Goid

2 *Fusarium solani* (Mart.)Sacc.

3 *Curvularia desmodii* Bharadwaj.

4 *Drechslera Indica* Anahosur.

5 *Alternaria* sp.

IV Cluster bean (Cyamposis tetragonoloba (L.)Taub.)

1 Macrophomina phaseolina(Tassi)Goid

2 Fusarium semitectum Berk. and Rav.

3 Curvularia desmodii Bharadwaj.

4 Drechslera Indica Anahosur.

5 Alternaria sp.

CHAPTER 6

SUMMARY

Chapter 6 SUMMARY

The seed mycoflora of Lima bean (Phaseolus lunatus L.), French bean (Phaseolus vulgaris L.), Cluster bean (Cyamopsis tetragonoloba (L.) Taub.) and Wal bean (Dolichos lablab L.) were studied following agar plate, blotter paper, sand and rolled towel method in the present Research Project and fungi viz. Alternaria sp., Aspergillus sp., Fusarium sp. Curvularia sp., Macrophomina sp., Rhizoctonia sp., Rhizopus sp., Penicillium sp. and Helminthosporium sp., were invariably obtained.

The germination percentage was found maximum in rolled towel and blotter paper as compared to agar plate and sand method. In general more number of fungi were obtained in agar plate and rolled towel than blotter and sand method.

Seed inoculation with Helminthosporium sp., Curvularia sp., and Alternaria sp. to beans effected in decrease of shoot and root length as compared to control.

Fungicidal seed treatment resulted in increase of shoot and root length as compared to control. It was also observed that the length of shoot was maximum in Thiram and MBC while Vitavax resulted in increase of root length only.

Effect of fungicidal seed treatment on mycoflora of beans levealed that the germination percentage was increased with decrease in number of fungi in bean seeds treated with Agrosan GN, Vitavax and Bavistin than Captan, Thiram, Difolatan and MBC. Seed mycoflora was found maximum after three months

interval in treated bean seeds and also decreased the germination percentage than one month and two month stored treated seeds.

Morphological characters of the different fungi obtained from various beans and used throughout experimentation were identified as given below. The results were also confirmed with the report from the Division of Mycology and Plant Pathology (IARI) New Delhi-110012.

I Lima bean (*Phaseolus lunatus* L.)

1 *Drechslera spicifera* (Bain.)Nicot.

2 *Fusarium semitectum* Berk. and Rav.

3 *Curvularia desmodii* Bharadwaj.

4 *Macrophomina phaseolina*(Tassi) Goid

5 *Alternaria* sp.

II French bean (*Phaseolus vulgaris* L.)

1 *Drechslera Indica* Anahosur.

2 *Macrophomina phaseolina*(Tassi) Goid

3 *Fusarium solani* (Mart.)Sacc.

4 *Curvularia desmoddi* Bharadwaj.

5 *Alternaria* sp.

III Wal bean (*Dolichos lablab* L.)

1 *Macrophomina phaseolina*(Tassi)Goid

2 *Fusarium solani* (Mart.) Sacc.

3 *Curvularia desmoddi* Bharadwaj.

4 *Drechslera Indica* Anahosur.

5 *Alternaria* sp.

IV Clustern bean (*Cyamopsis tetragonoloba* (L.)Taub.)

1 *Macrophomina phaseolina*(Tassi)Goid.

2 *Fusarium semitectum* Berk. and Rav.

3 *Curvularia desmoddii* Bharadwaj.

4 *Drechslera Indica* Anahosur.

5 *Alternaria* sp.

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