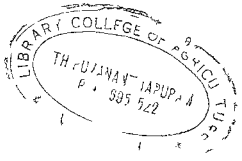
BIO-DETERIORATION OF IMPORTANT VEGETABLE SEEDS DUE TO MYCOFLORA II



By

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THESIS

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Faculty of Agriculture
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Department of Plant Pathology
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DECLARATION

I hereby declare that this thesis entitled "BIO-DETERIORATION OF IMPORTANT VEGETABLE SEEDS DUE TO MYCOFLORA-II" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis is a record of research work done independently by Kum.S. Ambika under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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C O N T E N T S

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Introduction

INTRODUCTION

Seeds are the main propagative unit of crop plants. Maintenance of high seed germination and vigour from harvest till planting is the most important activity in raising a good crop. Seeds are practically worthless if they fail to give adequate plant stand in addition to healthy and vigorous plants. The principal purpose of storing seeds of economic plants is to preserve planting stocks from one season to the next. Good seed storage is therefore a basic requirement in seed production.

Seeds are the most important means of perpetuation of plant pathogenic fungi (Agarwal and Sinclair, 1987). Fresh seeds are associated with different types of field and storage fungi which grow and multiply on the seed as long as food and moisture are adequate and temperature is not unfavourable. As the storage period is increased, the micro-ecological conditions of the seed are disturbed and the environmental set up becomes less favourable to a number of fungi due to depletion of food level and moisture supply. Under these conditions, storage fungi develop and multiply on the seed. The fungi affect seed germination directly either by lowering viability of seed by making it nutritionally poor or by secreting certain mycotoxic substances deleterious to seed (Mishra and Kanaujia, 1973).

The most obvious factor affecting seed viability and storability is weather, especially seasonal changes. Since seeds are hygroscopic, when stored, seed moisture content and environmental humidity are closely correlated. Seed moisture content is a major factor influencing seed longevity. A rise in humidity in store as well as presence of damaged seeds in seedlot permit rapid development of saprophytic fungi introduced into the store by the seeds.

Deterioration of stored seeds by fungi is controlled principally by drying seed to a safe moisture level and storing these seeds under moisture and temperature conditions unfavourable for growth of storage fungi. It is difficult to obtain such conditions in tropical rainy areas for technical and economic reasons. Therefore, it is important to find alternative methods of control. Specific chemicals for control of all these fungi have not been developed.

In Kerala, high relative humidity prevails in most part of the year resulting high biodegradation of vegetable seeds. Seeds of the previous season will be stored for raising the crop in the next season. Hence, it is very important to find out a suitable management practice to keep the seed in a viable condition at least for a year. Therefore, the present study was undertaken with the following objectives.

1. To assess the role of seed-borne mycoflora and bio-deterioration of important vegetable seeds (bhindi, cowpea and dolichos bean).
2. To study the influence of seasons on the association of seed-borne mycoflora of stored vegetable seeds.
3. To find out the effect of humidity on viability of vegetable seeds.
4. To investigate the role of seed mycoflora on germination of seeds.
5. To find out the effect of fungicides to minimise fungal bio-deterioration of stored vegetable seeds.

Review of Literature

REVIEW OF LITERATURE

The seed is a fertilized mature ovule consisting of an embryonic plant and having a protective seed coat. Deterioration of seeds can occur at any time from seed to seed. The deterioration may occur due to micro-organisms like fungi, bacteria and viruses, insects, birds and other vertebrate animals. Seed enzymes may also play a role in deterioration. Of these, the biodeterioration due to infection by microorganisms is having its own importance which can render the seeds unfit for any of the basic uses. Christensen and Sauer (1982) had given a review on the involvement of fungi in seed deterioration. The role of bacteria, fungi and viruses in seed deterioration was reviewed by Harman (1983).

Fungi associated with seeds

Transmission of plant pathogens through seeds was first documented by du Tillet (1775) who showed that stinking or hill bunt of wheat (Triticum aestivum L.) was caused by a poisonous substance contained in the dust sticking on the seed surface. In 1807, Prevost proved that stinking bunt was caused by a parasitic fungus, Tilletia caries (DC.) Tul. Frank (1883) demonstrated the

internally seed-borne nature of Colletotrichum lindemuthianum (Sacc. & Magn.) Br. & Cav. in bean (Phaseolus vulgaris L) seeds. In soybean (Glycine max(L) Merr.) Diaporthe phaseolorum var. sojae Nits., Sclerotinia sclerotiorum (Lib.) de Bary and Colletotrichum truncatum (Schw.) Andrus & Moore are internally seed-borne and may inhibit field emergence (Nicholson et al., 1972 and Wallen and Seaman, 1962). Sharma and Sohi (1975) found that tomato (Lycopersicon esculentum Mill) seeds infected with Phytophthora parasitica (Dastur) Waterh. either fail to germinate or if they germinate, seedlings are killed by the pathogen.

Fungi associated with leguminous seeds

Jain and Patel (1969) reported that Fusarium Link ex Fr. and two isolates of Alternaria Nees ex Wallr. from the mycoflora of guar (Cyamopsis psoraloides DC) seeds were pathogenic causing root rot and brown leaf spot respectively. Singh and Mathur (1974) isolated Sclerotium rolfsii Sacc. from bean seeds and established its pathogenicity. Bilgrami et al., (1976) reported the occurrence of Aspergillus flavus Link, A. niger Van Tiegh., Penicillium sp Link ex.Fr. Alternaria alternata (Fr.) Keissler., Fusarium semitectum Berk. and Rav. Curvularia lunata (Wakker) Boedijn.,

Helminthosporium hawaiiense Boug. and Cladosporium sp Link ex Fr. with the seeds of mung (Phaseolus aureus Roxb) and urad (Phaseolus mungo L) in storage among which the frequency of occurrence of Aspergillus flavus was the highest.

Seed transmission of Botrytis cinerea Pers. ex. Fr., unidentified species of Botrytis Pers. ex. Fr. and Sclerotinia Fuckel and several sterile fungi were noted in gram (Cicer arietinum L) for the first time by Cother (1977). Sinha and Khare (1977) observed that Macrophomina phaseolina (Tassi) Goid and Fusarium equiseti (Corda.) Sacc. in cowpea (Vigna unguiculata (L) Walp) seeds was intraembryonal and mycelium was present in cotyledons, plumule and radicle. Sinha and Khare (1977) isolated Aspergillus sp. Mich. ex.Fr., A. Flavus, A. niger van Tiegh., Chaetomium sp. Kunze ex Fr. Curvularia verrucosa Tandon and Bilgrami ex Ellis., Fusarium equiseti and Macrophomina phaseolina from cowpea seeds. Saxena and Sinha (1977) investigated the seed-borne fungi of black gram (Vigna mungo (L) Hepper) and found the occurrence of Ascochyta chartarum, Colletotrichum truncatum and Fusarium semitectum. These fungi were reported to be seed-borne in several leguminous crops causing disease in the field.

According to Singh and Chohan (1977a) fungi found associated with gram seeds included Cladosporium cladosporioides (Fres.) de Vries. (19.0%), Curvularia clavata Jain. (52%), Fusarium equiseti (19.0%), Penicillium cyclopium westling (18.0%), Pleospora infectoria Fuckel (41%), Rhizopus arrhizus Fisher (5%) and Trichothecium roseum (Pers.) Link ex Fr. (10%). Singh and Chohan (1977b) observed fungi like Aspergillus niger, Curvularia sp. Boedijn, Fusarium equiseti, F. oxysporum Schlecht, Penicillium crustosum Thom and Phoma glomerata (Corda) Wallenw & Hochapfel. to be associated with seeds of black gram.

Botryodiplodia theobromae Pat. is internally seed-borne in horse gram (Dolichos biflorus Linn.) and caused seed rot and seedling blight (Maholay and Sohi, 1977). Diaz Polanco et al., (1978) confirmed seed transmission of Rhizoctonia solani Kuhn. in cowpea and stated that damage was greater within the first 30 days of plant growth. Sawhney and Aulakh (1980) isolated fungi like Alternaria alternata, A. longissima, Ascochyta pisi Lib., Aspergillus flavus, A. niger, Cephalosporium Corda sp., Cladosporium cladosporioides, Curvularia lunata (Wakker) Boedijn., C. pallescens Boedijn, Drechslera hawaiiensis (Bugnic.) Subram. & Jain ex M.B.Ellis, D. tetramera Mckinney,

Fusarium moniliforme Sheldon, Penicillium sp., Rhizopus sp Ehrenb. ex Corda and Trichothecium roseum Link. ex.Fr. from seeds of pea (Pisum sativum L). Except Curvularia lunata, C. pallescens and Rhizopus sp. all other fungi were pathogenic causing seed rots and seedling infections under artificial inoculation. Vishunavat and Shukla (1980) isolated 25 species of fungi from lentil (Lens esculenta Moench) seeds of which Alternaria alternata, A. tenuissima, Aspergillus flavus, A. niger, Fusarium equiseti, F.oxysporum and Rhizoctonia bataticola (Taub.) Butler were observed the external and internal seed tissues.

Pangtey and Sinha (1980) confirmed the seed-borne nature of Colletotrichum capsici (Syd.) Butler and Bisby and Phoma medicaginis Malbr. & Roum after direct pathogenicity tests in horse gram and found that both fungi were constantly associated with the seeds in the field and in storage. Ibrahim and Owen (1981) isolated four species of Fusarium from rotted bean roots and naturally contaminated seeds of broad bean (Vicia faba L). The four species appeared to be carried by the seed mostly as surface contaminants and among these only Fusarium oxysporum caused root rot. Siddaramaiah et al., (1981) found that Trichothecium roseum was internally and externally seed-borne in dolichos bean (Dolichos lablab L.) and they isolated the fungus from the seed coat, cotyledons and embryo.

In a survey of 214 samples of commercial pea seed by Ali et al., (1982), 90, 72, 31 and 24 per cent were found infected with the four fungi viz., Mycosphaerella pinodes (Berk & Blox) Vesterg., Macrophomina phaseolina, Phoma medicaginis var. pinodella and Fusarium oxysporum respectively. Only 10 per cent of the samples were free from infection. Kanapathipillai (1982) isolated the following fungi Curvularia sp., Drechslera sativum Pamm, King & Bakke, Diplodia sp. Fr., Chaetomium globosum Kunze ex. Fr., Pestalotiopsis sydowina Steyaert, Aspergillus sp and Fusarium sp from two seed lots of dolichos bean and cowpea. Nigrospora sacchari Zimm. and Phyllosticta sp. pers. ex. Desm. were confined to dolichos bean while Colletotrichum Corda sp. and Macrophomina phaseolina were found only in cowpea.

Gill et al., (1983) studied the mycoflora of some forty species of leguminous seeds. Aspergillus elevatus, A. flavus, A. fumigatus Fres., A. giganteus Wehmer, A. niger and A. oryzae (Ahlburg.) Cohn were the most common pathogens. Rhizopus spp were found only in ground nut (Arachis hypogaea L), pigeon pea (Cajanus cajan(L) Millsp), dolichos bean and cowpea. Nik (1983) isolated Botryodiplodia theobromae, Colletotrichum dematium (Pers. ex Fr) Grove, Diaporthe phaseolorum Nitsch, Fusarium spp, Macrophomina phaseolina,

Myrothecium roridum Tode ex Fr, Phoma sorghina (Secc.) Beerene and Pestalotia de Not. sp. from seeds of soybean and mung bean.

Saxena (1984) reported that fungal infection of green gram (Phaseolus aureus Roxb) and black gram seeds in various regions of Uttar Pradesh varied from 4-12 per cent, most damage being caused by Fusarium oxysporum and F. semitectum followed by Colletotrichum truncatum and Ascochyta chartarum. Of the 25 fungal species found associated with the seeds of winged bean (Psophocarpus tetragonolobus (L) DC), Aspergillus spp were predominant (Thite, 1984). Nik and Lim (1984) observed that Colletotrichum dematium f. sp truncatum (Pers. ex Fr.) Grove was the most prevalent of 13 fungal species detected in seeds or 11 soybean cultivars. Mycelia occurred in all the three layers of seed coat while acervuli were present in the pallisade layer alone. Tylkowska (1984) isolated fungi from bean seeds of 13 cultivars of which Alternaria tenuis Auct. was found in 87.7 to 98.1 per cent of the samples, Colletotrichum in 45 to 63.1 per cent and Fusarium spp in 45 to 78.1 per cent. Other pathogenic fungi isolated were Botrytis cinerea Pers., Pleospora herbarum Pers. Rabenh, Rhizoctonia solani and Sclerotinia sclerotiorum. Czyzewska (1984) isolated Fusarium solani f. sp. pisi (Jones.) Snyder and Hansen,

F. oxysporum f. sp. pisi (Linford) Snyder & Hansen, F. culmorum (W.G.Sm.) Sacc., F. avenaceum (Fr.) Sacc., F. equiseti, F. sambucinum Fuckel and F. sporotrichoides Sherb from infected plants and seeds of pea.

As reported by Abdel-Hafez (1984), the most frequent genera of fungi from seeds of pea, broad bean, lentil, lupin (Lupinus termis Forsk) and pea were Aspergillus (16 spp+2 var), Penicillium (14 spp), Rhizopus (1 sp) and yeast followed by Fusarium (3 sp) Mucor (4 Sp), and Drechslera (3 sp). Among these Aspergillus niger, A. flavus, P. citrinum Thom., Rhizopus stolonifer (Ehrarb. ex.Fr.) Lind, Fusarium moniliforme, Mucor hiemalis Wehmeyer and Drechslera spicifer (Bain.) Nicot. were the most prevalent species.

Barros and Menezes (1985) while studying the seed-borne fungi associated with 34 cultivars of cowpea reported that Fusarium oxysporum was found in 46.7 per cent of the samples, Phomopsis sp in 28.9 per cent, Macrophomina phaseolina in 28.7 per cent, Botryodiplodia theobromae in 14.5 per cent, Fusarium semitectum in 9.3 per cent, Fusarium equiseti in 3.4 per cent, Diplodia sp. in 3.3 per cent and phoma sp. in 2.3 per cent.

Colletotrichum lindemuthianum causing anthracnose was located in the seed coat, cotyledons and embryo of cowpea. Transmission studies showed that infected seeds serve as a primary source of inoculum for the spread of the disease (Prasanna, 1985). Mathur and Tyagi (1985) found Choanephora cucurbitarum (Berk. and Rav.) Thaxt. as the cause of a serious pod rot of moth bean (Vigna aconitifolia), mung bean, black gram and cowpea. According to Umechuruba (1985) the most commonly isolated pathogen from cowpea seeds was Aspergillus flavus. Pea leaf and pod spot caused by Ascochyta pisi, A. pinodella Jones and Mycosphaerella pinodes B. & Blox. is transmitted by infected seed or infected plant remains in the soil (Bedlan, 1985).

Kumar and Patnaik (1985) observed Alternaria alternata over a range of 5 to 20 per cent in 10 of 12 samples of pigeon pea from different locations. Pre-treatment of seeds with chlorine reduced the recovery of the pathogen. Component plating of seed parts showed that the fungus was always present in the seed coat of infected seeds and was never recovered from the cotyledons or embryonal axis. Pathogenic fungi isolated by Nitsche and Cafati (1985) were Rhizopus stolonifer, Rhizoctonia solani, Botrytis cinerea and Fusarium oxysporum from bean seeds.

Botrytis cinerea was naturally seed-borne at a rate of 8 to 18.5 per cent in three gram cultivars (Grewal and Laha, 1986). Singh and Sinclair (1986) found that Cercospora kikuchii Mats and Phomopsis sp in soybean can invade the embryo, endosperm and seed coat tissues. Phompsis sp is more aggressive than Cercospora kikuchii and may cause severe damage to embryo and seed coat tissues. Seed-borne fungi of pea seeds included Aspergillus niger, Fusarium solani, Drechslera tetramera and Rhizoctonia bataticola (Kumar et al., 1986).

Germination failure of legume seeds due to mycoflora

Nath et al., (1970) studied the seed-borne fungi of mung bean and found that Fusarium equiseti, Macrophomina phaseolina and Botryodiplodia theobromae caused seed rot and seedling blight. Rati and Ramalingam (1974) tested the effect of Aspergillus flavus on the germinating seeds of ground nut, tamerind (Tamerindus indica L.), pea, cotton (Gossypium hirsutum L), dolichos bean, cowpea, lima bean (Phaseolus vulgaris L) and found that Aspergillus flavus was one of the most important pathogens of the germinating seeds. Four types of infection viz., seed rot, non-emergence of cotyledons, cotyledonary infection and plumule infection were recorded.

According to Sinha and Khare (1977) cowpea seeds with severe infection of Macrophomina phaseolina and Fusarium equiseti rotted completely and the pathogens finally grew on the seed surface. Maholay and Sohi (1977) showed that Botryodiplodia theobromae caused seed rot and seedling blight in horse gram. Suryanarayana (1978) stated that in cowpea seeds, infection by Macrophomina phaseolina and Colletotrichum lindemuthianum caused failure of germination and seedling blight. In pea seeds infected by Alternaria sp dark patches were found on the seed coat and cotyledons. The radicle of these seedlings were blighted and the seeds showed reduction in germination upto 32 per cent.

All the fungi recovered from surface disinfected seed lots of pigeon pea, Alternaria tenuissima (Nees ex.Fr.) Wiltshire, Cladosporium sp, Fusarium semitectum, Botryodiplodia theobromae and Phomopis sp. reduced in vitro seed germination (Ellis and Smith, 1978).

Sawhney and Aulakh (1980) showed that fungi like Alternaria alternata, A. longissima Deighton & Macgarvie, Ascochyta pisi, Aspergillus flavus, A. niger, Cephalosporium sp, Cladosporium cladosporioides, Drechslera hawaiiensis, D. teramera, fusarium moniliforme, Penicillium sp. and Trichothecium roseum were pathogenic on seeds of peas and

caused seed rots and seedling infections under artificial inoculation. Yeh and Sinclair (1982) found that Cercospora kikuchii inhibited soybean seed germination on potato dextrose agar, moist cellulose pads and sand. Singh and Srivastava (1984) found that Macrophomina phaseolina was capable of causing seed rot, blighting of roots or leaves, dry root rot and stem necrosis in moth bean. Lee (1984) observed that germination was reduced in soybean seeds infected by Colletotrichum truncatum, Diporthe phaseolorum var, sojae and Fusarium oxysporum.

Alternaria alternata, Fusarium sp, Aspergillus chevalieri and A. niger invaded seeds of two soybean cultivars, viz., Clark and Wood Worth. Seed germination decreased gradually with increasing storage period in both inoculated and uninoculated seeds with all fungi tested (Morsy et al., 1985). Prasanna (1985) reported that germination percentage of cowpea seeds decreased with an increase in seed infection by Colletotrichum lindemuthianum which caused seed rot and seedling mortality.

Fungi associated with Malvaceous seeds

Ribeiro et al. (1971) reported seed transmission of Fusarium solani f. sp. hibisci which infects bhindi (Abelmoschus esculentus (L) Moench.) only. Goel and Mehrotra (1972)

isolated Rhizoctonia bataticola from seeds of bhindi and found that this pathogen caused root and collar rot. Robbs et al., (1972) reported Fusarium oxysporum f. sp. vasinfectum as the cause of wilt of bhindi and that the pathogen is seed transmitted.

Tai Luang Huan and Musa Bin Jamil (1975) studied the seed-borne pathogens of bhindi and found that Rhizoctonia sp and Choanephora cucurbitarum caused crop loss upto 20 per cent in a diseased plot. Vidhyasekaran and Kandaswamy (1980) isolated Fusarium oxysporum, F. semitectum, F. moniliforme, Macrophomina phaseolina and Aspergillus flavus from seeds of bhindi.

Naseema (1981) recorded Fusarium oxysporum, Nectria haematococca Berk. & Br. and Botryodiplodia theobromae on seeds of bhindi while studying the seed mycoflora of vegetable seeds. Pizzinatto et al. (1984) found that Alternaria sp, B. theobromae, Colletotrichum sp., Fusarium oxysporum, Rhizoctonia sp. and Verticillium sp were associated with the seeds of cotton (Gossypium spp.L).

According to Tanaka and Paolinelli (1984) the dominant fungi isolated from cotton seeds were Fusarium sp., F. moniliforme, Chaetomium sp., Aspergillus sp and Trichothecium roseum.

Fakir and Mridha (1985) found that Colletotrichum dematium and Macrophomina phaseolina causing die-back of bhindi, were seed transmitted.

When delinted surface sterilized cotton seeds were assessed for the presence of filamentous fungi, Klich (1986) noted that there were no consistent differences in the fungal flora among the cultivars examined. Only Alternaria spp, Colletotrichum gossypii, Fusarium equiseti and F. semitectum were present in more than ten per cent of the seeds. Adisa and Aborisade (1987) found that among the seed mycoflora of bhindi, Aspergillus flavus, Botryodiplodia theobromae and Penicillium digitatum were predominant.

Germination failure of Malvaceous seeds due to mycoflora

Goel and Mehrotra (1972) showed that Rhizoctonia bataticola caused root and collar rot in bhindi. Robbs et al. (1972) reported that Fusarium oxysporum f. sp. vasinfectum caused wilt of bhindi. Vidhyasekaran and Kandaswamy (1980) found that F. oxysporum caused wilting and Macrophomina phaseolina induced root rot while F. semitectum, F. moniliforme and Aspergillus flavus reduced seed germination and plant vigour in bhindi.

Naseema (1981) observed that storage fungi such as Aspergillus flavus, A. niger and Rhizopus stolonifer caused

inhibition of germination of bhindi seeds. Seed inoculation with Rhizopus sp, A. niger, and A. flavus reduced germination and vigour in cotton, depending on the abundance of the fungus inoculum (Lima et al., 1984).

Zhou (1984) studied the causes of death of cotton seedlings and found that 30 to 50 per cent of the seedlings were affected by fusarial wilt, resulting in 24 to 30 per cent mortality. On old nursery beds wilt incidence was 35.5 per cent and seedling mortality was 27.5 per cent compared with values of 5.5 and 1.05 per cent respectively on new nursery beds.

Effect of relative humidity on seed mycoflora and germination in storage

Christensen and Lopez (1963) stated that stored seeds are susceptible to invasion by fungi growing in equilibrium with relative humidity (RH) of 65 to 90 per cent which reduce germinability and cause various biochemical changes. The growth of storage fungi is not inhibited by fungicidal treatment in seeds with moisture contents in equilibrium with 65 to 85 per cent relative humidity.

According to Kulik (1973), when seeds of cabbage (Brassica oleracea var. capitata L.) cucumber, bhindi, onion (Allium cepa), pepper (Capsicum annuum L), radish (Raphanus sativus L), salsify, spinach (Spinacea oleracea) and turnip

(Brassica campestris var. rapa L) were inoculated with spores of Aspergillus amstelodami or A. flavus and stored at 85 per cent relative humidity at 22 to 25°C for 30 days, these fungi invaded only a small number of seeds of cabbage, cucumber, radish and turnip. Though more number of seeds of okra, onion, pepper, salsify and spinach were invaded by either or both A. amstelodami and A. flavus the fungal invasion did not appear to cause a decline in germinability. Saxena and Gupta (1979) reported that most of the field fungi associated with the seeds of green gram and black gram persisted as long as 120 days in storage at a RH of 45 to 78 per cent and temperature of 6.2 to 29.6°C.

Kononkov et al., (1980) while studying the changes in the microflora and seed germinability of vegetable crops like (Beet (Beta vulgaris L), carrot (Dacus carota L), Parsnip (Pastinaca sativa L), radish, tomato and cucumber) on storage found that at 45 per cent RH the initial microflora gradually died, while seed germinability was unchanged. At 75 per cent and 85 per cent RH the initial microflora was replaced by storage moulds and bacteria and seed germinability was reduced. The best storage conditions are thus a RH of 45 per cent and temperature of 3 to 5°C temperature. Seenappa et al. (1980) reported that healthy intact seeds of

red pepper were susceptible to Aspergillus halophilicus which invaded by forming appressoria and infection pegs. When peppers were stored under high RH, the predominant species changed completely. A flavus and A. ochraceus predominated at 85 per cent RH and A. flavus alone at 95 per cent RH. Prasad and Narayan (1980) observed that in dolichos bean most seed rot occurred in seeds stored at high RH. Zainun and Hasbullah (1982)found that infestation of mung bean with A. flavus, A. fumigatus and A.parasiticus was more than 80 per cent after 18 weeks of storage at 95 per cent RH. Maximum infestation of 90 per cent by A. niger was obtained after nine weeks at 95 per cent RH. At 30 and 50 per cent RH infestation of A.fumigatus was the lowest and by A. flavus was highest after 18 weeks. Seeds stored at 30 and 50 per cent RH showed no decline in germinability even after 18 weeks, but at 95 per cent RH after 12 weeks germination declined.

Nandi et al., (1982) studied the deterioration of oil seeds (two cultivars of sesame, mustard (Brassica sp L) and linseed (Linum usitatissimum L.) in storage and found that infestation of field fungi decreased and of storage fungi increased and the germination per cent decreased with increasing length of storage. They also stated that lowest

germination was obtained when the seeds were stored at higher RH and temperature. Onesirosan (1982) showed that in cowpea, seeds with 12.5 per cent moisture content stored at 8°C, 20°C and 30°C for 20 weeks there was little loss in germination. Cowpea seeds with 15.5 per cent moisture content stored well only at 8°C, whereas at 20°C and 30°C there was rapid loss in germination.

Minor and Paschal (1982) reported that percentage germination of soybean seeds stored at 30°C and 80 per cent RH decreased slowly during the first four weeks of storage, after which decrease was rapid and very little germination was obtained after 10 weeks. When lentil seeds were stored at different relative humidities (75, 82 and 92 per cent). A. flavus, A. niger, A. terreus, A. fumigatus, A. sydowvi and Penicillium oxallicum appeared after six months of storage and continued to prevail upto 12 months. Rhizopus arrhizus and Fusarium oxysporum were observed at all RH levels in storage periods of six to twelve months (Vishunavat and Shukla, 1983).

Dange and Patel (1984) studied the effect of relative humidity and storage period on fungal invasion and viability of ground nut seeds and stated that unshelled seeds of groundnut stored at 62, 76, 85 or 93 per cent RH suffered

greater invasion by A. niger, A. flavus and particularly by Rhizopus sp at higher RH. At 62 per cent RH seed viability was not reduced. Storage at 85 per cent RH for 120 days resulted in complete loss of viability, while at 93 per cent RH for 90 days, highest germination obtained was five per cent. Vyas and Nene (1984) reported that germination percentage of thiram treated gram seeds decreased with increase in RH in storage.

Sharma and Gupta (1984) suggested that low seed moisture, temperature and relative humidity were suitable for safe and long term storage of moth bean. Maheswary and Mathur (1985) observed that seeds of cowpea were more severely affected under conditions of higher temperature and relative humidity during storage by the fungus A.flavus. Kononkov and Dudina (1986) studied the fungi on vegetable crop seeds (tomato, carrot, radish, cucumber and cabbage) stored under conditions of high relative humidity and temperature and found that A. glaucus. A. versicolor, A. candidus and Penicillium sp. were the fungal species detected on non-sterile seeds during storage. Weight loss by seed-borne fungi in stored methi (Trigonella foenumgraecum L.) increased with increasing relative humidity but germination was decreased (Komaraiah and Reddy, 1986). The incidence of storage fungi

in sorghum (Sorghum vulgare) increased with increase in the relative humidity of the storage environment over a period of one month, but the incidence of field fungi decreased over this period. Seed deterioration was accelerated with high relative humidity. The percentage seed germination was inversely proportional to the relative humidity of the storage vessel (Rao and Reddy,1987). Moreno et al., (1987) treated barley (Hordeum vulgare) seeds with fungicides and stored at RH 75, 80 and 85 per cent at 26°C and found that there was almost no difference on seed viability between treated and untreated seeds when stored upto 210 days at RH of 75 per cent and upto 150 days at RH of 80 and 85 per cent.

Effect of duration of storage on germination and seed mycoflora

Quasem and Christensen (1958) reported that when samples of maize (Zea mays L.) was stored commercially for six to nine months, those with the poorest germination were found to have high percentages of fungal infection, especially by A. flavus and Penicillium sp. Harrison (1978) stated that Botrytis fabae was common in commercial seed stocks of field bean but was not detected after storage of nine months. The fungs was isolated from the testa, cotyledons, hypocotyl, the base of the plumule and the top of the root from most

seedlings soon after sowing although they appeared healthy, but later the proportion from which it was isolated decreased.

Kuniyasu (1980) established that commercial seed samples of bottle gourd (Lagenaria siceraria(Molina.) Sandl) were two to five per cent infected by Fusarium oxysporum f. sp. lagenaria. The pathogen was still viable in stored seeds after two to five years.

According to Mallick and Nandi (1982), rice (Oryza sativa L.) grain moisture changed with seasonal fluctuation, germinability decreased and fungal infection increased. A gradual decrease in field fungi was found to be accompanied with concomitant increase in storage fungi. Kabeere and Taligoola (1983) observed that moisture content increased and germination percentage decreased in soybean with the increase of storage period at room temperature and the prolonged storage decreased the field fungi.

Maholay and Sohi (1983) established that Macrophomina phaseolina causing seed rot of bottle gourd, squash (Cucurbita pepo L.) and musk melon (Cucumis melo L) survived for 21 months in all the three hosts. Vanangamudi et al., (1986) noted that none of the seed treatments prevented loss in germinability of dolichos bean seeds during

storage. But treated seeds produced seedlings with increased root length.

Vishunavat and Chaube (1986) showed that in pot experiments gram seeds infected with Ascochyta rabiei (Pass.) Labr. gave a germination rate of 65 per cent and seedling infection of two per cent when seeds were stored at room temperature, compared with 78 per cent and 48 per cent respectively for seed from cold storage.

General seed treatment for controlling seed-borne pathogens

Kaul (1973) reported that captan and thiram at 0.2 per cent gave effective control of the mycoflora of bean. Shirsat and Kale (1976) found that seed treatment with Rovral, Mildothane, Brassicol and Dithane M-45 were the best for increasing seedling vigour and germination in chickpea (Cicer arietinum). Patil and Mayee (1977) reported that Dithane M-45 and Agrosan GN controlled pre-emergence and post-emergence deaths respectively in soybean.

Sarode and Kadam (1977) conducted fungicide tests against a Helminthosporium sp consistently isolated from brinjal (Solanum melongena L) seeds in which seed treatment with thiram was proved as the best control. Sinha and Khare (1977a) reported that thiram, Difolatan and ceresan

dry were excellent in controlling seed-borne infection of Macrophomina phaseolina and Fusarium equiseti on cowpea in pots and field. Mercer and Kisyombe (1978) showed that thiram had a strong inhibitory effect on the kernel flora of groundnut with the exception of Aspergillus flavus.

Sesan and Dumitras (1979) tested some fungicides against seed-borne fungi in bean among which Delson F, Delsene C, thiram and thiophanate-methyl were found to be the most effective. Diaconu (1979) reported that Topsir M-70 at 1.5 to 2 g/kg was effective as a seed treatment in the control of Pythium sp and Rhizoctonia solani in cucumber.

Sharma and Sohi (1980) reported that when cowpea seeds were treated with different fungicides like Bavistin, NF-44 (thiophanate-methyl), NF-48 (thiophamine), Calixin and Benlate and sown in pots, appreciable antifungal property could be detected upto 21 days after germination. Oladiran and Okusanya (1980) found that thiram + Benzene-hexachloride, captafol, captan, thiram and fentin hydroxide gave effective control of pre-emergence damping off caused by Pythium aphanidermatum and Sclerotium rolfsii but not post-emergence damping off in cowpea.

According to Rao et al., (1980) Dithane M-45 when treated on pre-soaked seeds of bhindi at 100-150 ppm promoted

growth particularly at the lower doses and shorter duration treatments. Barros et al., (1985) reported that captafol at 100 ppm and benomyl and thiabendazole each at 500 ppm gave the best control of Colletotrichum lindemuthianum in cowpea. Treatment of dolichos bean seeds with Agrosan GN, Vitavax, MBC (Carbendazim) and Bavistin improved germination more than Difolatan, thiram and captan (Kore and Solanke, 1981). Reddy and Subbayya (1981) conducted seed treatment tests with five fungicides in black gram and found that benlate, thiram, Ceresan and Vitavax were highly effective against Macrophomina phaseolina.

Vishunavat and Shukla (1982) tested 11 fungicides (Bavistin, Benlate, Vitavax, Plantvax, Dithane M-45, thiram, Aretan, Agrosan GN, Ceresan dry, captan and Brassicol each at 0.2 per cent except Dithane M-45 and Ceresan dry which were at 0.3 per cent against seed mycoflora of lentil and found that only captan eliminated all the fungi, viz., Alternaria spp. Aspergillus flavus, A. niger, A. sydowi, A. terreus, A. nidulans, Curvularia lunata, Fusarium oxysporum and Rhizopus arrhizus associated with seeds. Thiram controlled all except Alternaria alternata and Bavistin was effective against all except Rhizopus arrhizus. Karwasra and Mohinder Singh (1982) observed that the most effective seed treatment chemicals in cluster bean (Cyamopsis tetragonoloba L.Taub)

were Agrosan GN, Captan, Dithane Z-78, and Dithane M-45. Sohi and Kalra (1982) reported that Bavistin, Benlate and NF-44 completely inhibited the growth of Myrothecium roridum causing ring rot of tomato.

Thiram and Dithane M-45 resulted in 80-90 per cent seed germination in linseed compared with 40 per cent germination in the control (Siddaramaiah et al., 1982). Sharma and Jain (1984) found that Bavistin and MBC can be used against seed-borne Fusarium spp. Konde et al., (1984) conducted in vitro tests with 10 pesticides against 23 seed-borne fungi of pearl-millet (Pennisetum typhoides) and found thiram, Dithane M-45, Aureofungin, Brassicol and Agrosan GN (all at 100 ppm) to be effective. Singh and Agarwal (1984) reported that seed treatment with Bavistin gave maximum germination of soybean seeds infected with the purple stain fungus, Cercospora kikuchii. Gupta et al. (1984) recommended Agrosan GN, Dithane Z-78 and Dithane M-45 as seed protectants of red gram (Cajanus cajan L. Millsp) and lentil.

As reported by Kumar and Srivastava (1985) seed-borne fungi of pigeon pea viz., Aspergillus candidus, Botrytis cinerea, Cladosporium cladosporioides, Colletotrichum graminicola, Curvularia pallescens, Fusarium semitectum, Penicillium rubrum, Rhizopus arrhizus and R. nigricans were

controlled by seed treatment with Agrosan GN, Bavistin, Difolatan, Captan, Vitavax and Dithane M-45.

Yehia and Hassan (1985) found that Tecto TBZ (thiabendazole), Vitavax 200 (Carboxin + thiram) and Topsin M-70 (thiophanate-methyl) were effective as seed treatment fungicides against root rot disease of broad bean caused by Fusarium solani. Roberti et al. (1985) reported that seed treatment of bean with captafol and carboxin followed by thiram and benomyl gave the best results of control of seed-borne fungi in the glass house. In the field thiram was the most effective, captafol also gave good results. Wu and Lee (1985) found that Bavistin performed the best among 13 fungicides tested in vivo against the pod and stem blight pathogen of soybean, Phomopsis sojae.

Peresypkin and Pidoplichko (1985) found that Bavistin reduced incidence of Cercosporella herpotrichoides and thiram that of Fusarium sp. on winter wheat. Saifudinova (1985) reported that seed treatment with thiram reduced infection of cucumber by Rhizoctonia sp and Fusarium sp. from 59.2 per cent (untreated) to 16.7 per cent and on tomato from 33.2 per cent to 13 per cent. Of the seven fungicides tested by Mali and Joi (1985) for the control of seed mycoflora of chilli (Capsicum annuum L) Difolatan, thiram and

Vitavax were the most effective against colony growth and sporulation of Alternaria alternata, Colletotrichum capsici, Curvularia lunata, Drechslera rostrata and Macrophomina phaseolina.

Chauhan (1986) reported that control of Rhizoctonia solani and Macrophomina phaseolina was best and germination maximum when cotton seeds were treated with Bavistin, followed by quintozene. Chopra and Sharma (1986) observed that three formulations of carbendazim among six fungicides used as seed treatments of cotton gave the best reductions of pre-and post-emergence mortality caused by Rhizoctonia solani. Best control of Fusarium oxysporum f. sp.vasinfectum in cotton was achieved by seed treatment with Bavistin followed by agallol.

Control of the storage mycoflora and germination failure

Singh et al. (1974) observed that thiram and Terrachlor super X (23% PCNB + 6% terrazole (5-ethyl - 3 trichloro methyl 1, 2, 4 - thiadiazole) both at 0.5 per cent significantly increased seedling emergence after six months of seed storage in soybean. Grewal et al., (1981) studied the effect of fungicides viz., captan 0.2%, thiram 0.2%, Aretan 0.2%, Vitavax 0.2%, Bavistin 0.1%, Blitox 0.3%, Demosan 0.1% and Brassicol 0.2%) on seed-borne fungi of pea

under different storage conditions like tightly corked glass bottles, polythene bags and paper bags in the refrigerator at about 7-10°C. They stated that the seeds should be treated with Dithane M-45 or Vitavax and stored in air tight containers.

According to Zote and Mayee (1982), seed treatment improved germination of mung bean seed during storage. The best results were obtained with Bavistin followed by thiram and Dithane M-45. Singh et al. (1984) stated that seed treatment with Bavistin + TMTD (thiram) at 0.25 per cent gave excellent protection to wheat seeds previously inoculated with Aspergillus and Penicillium spp. for more than 275 days whereas Bavistin alone was effective for only 30 days.

Kumar and Singh (1984) found that best results of viability were achieved by storing sesame (Sesamum indicum L.) seeds treated with Bavistin at 2 g/kg seed, eliminating all fungi except Aspergillus sesami, Curvularia lunata and Drechslera tetramera. When wheat seeds treated with fungicides like captan, Emisan, Thiride and Vitavax at 2 g/kg were stored in various containers for six months, all the fungicides were found to be highly effective in protecting

seed viability but Vitavax was not effective to check deterioration of seedling vigour (Randhawa *et al.*, 1985).

Germination of naturally infected coriander (*Coriandrum sativum* L) seed treated with Emisan - 6 for six months was 90.5 per cent compared with 77.66 per cent with paradichlorobenzene (Prasad, 1986).

Materials and Methods

MATERIALS AND METHODS

Collection of seeds

Three vegetable seeds viz., bhindi (Abelmoschus esculentus (L.) Moench) var. Pusa Sawani, cowpea (Vigna unguiculata (L.) Walp) var. Kanakamony and dolichos bean (Dolichos lablab L.) var. Pusa. Early prolific obtained from the Department of Olericulture, College of Horticulture, Vellanikkara were used for the investigations. The germination percentage, moisture content and associated mycoflora were studied. Blotter method and pot culture experiment were employed to test germination of seeds. Air oven method was used to determine moisture content and agar plate method to study associated mycoflora.

Blotter method

Ninety randomly selected seeds of each vegetable were used for the germination test. The seeds were placed equidistant apart on sterilised petridishes lined with filter papers. In each petridish fifteen seeds were kept and the filter paper lining the petridishes were moistened with sterile water and incubated at room temperature. Six replications were maintained. Observations on germination of seeds were taken daily for 15 days and the per cent of germination was calculated.

Pot culture experiment

Ten seeds were sown in nine inch earthen pot filled with top soil at equal distance. Nine such pots were maintained as replications. The pots were watered daily and kept under natural conditions. Observations on the number of seeds germinated were taken daily for three weeks and germination per cent was calculated.

Ungerminated seeds were collected, washed thoroughly with tap water and surface sterilised with 0.1 per cent mercuric chloride for two minutes followed by washing in three changes of sterile water and plated in potato dextrose agar medium. The petridishes were incubated at room temperature and examined for the growth of fungi for 15 days. Each fungus developed was isolated, purified and identified.

Moisture content

Moisture content of the seeds were determined by air oven method (ISTA, 1966). Approximately five grams of seeds were kept in a crucible and placed in an air oven maintained at 130°C and dried until two consecutive constant weights were obtained. The moisture per cent was calculated using the formula,

$$\text{Moisture content} = \frac{M_2 - M_3}{M_2 - M_1} \times 100 \text{ per cent}$$

M_1 - Weight of crucible

M_2 - Weight of crucible + seed before drying

M_3 - Weight of crucible + seed after drying

Enumeration of mycoflora from seed

Agar plate method (ISTA, 1966) was used to find out mycoflora associated with seed. Surface sterilised and unsterilised seeds were plated on potato dextrose agar (PDA) medium. Surface sterilisation was carried out by dipping seeds for two minutes in 70 per cent ethanol followed by washing in two changes of sterile water. The seeds were plated at the rate of five seeds per petridish. The Petri dishes were incubated at room temperature and examined daily for growth of fungi upto 15 days. Fungi were isolated and purified by single spore isolation or hyphal tip method. These enumerations were carried out immediately after collection of seeds and after every three months for a period of one year.

Enumeration of mycoflora from different parts of seed

To find out mycoflora associated with seed coat, endosperm and embryo, the following procedure was adopted. Seeds were soaked in sterile distilled water for six to eight hours. The seeds were then dissected aseptically using sterile needles and forceps. The separated seed coat,

endosperm and embryo were washed in 0.1 per cent mercuric chloride solution for two minutes followed by washing in three changes of sterile water and plated on PDA medium at the rate of five bits per Petridish with three replications. The Petridishes were incubated at room temperature and examined daily for growth of fungi upto 15 days. The fungi were isolated, purified and identified.

Enumeration of mycoflora associated with stored seeds at different intervals of storage

The seeds of bhindi, cowpea and dolichos bean were packed in small cloth bags separately and stored in tin boxes for 12 months. These stored seeds were tested for presence of live fungi just before storage and after every three months interval for a period of one year with hundred seeds taken each time. Fifty seeds were surface sterilised with 70 per cent ethanol and plated on PDA medium. The remaining fifty seeds were plated on PDA medium without surface sterilisation. Fungi developing on the seeds were isolated, purified and identified. This procedure was followed separately for each vegetable seed.

Effect of seed-borne fungi on germination of seeds

The seeds of the three test vegetables viz., bhindi, cowpea and dolichos bean were used to determine the effect of

Seed-borne fungi on germination of seeds. The fungal spore suspension was made in sterile distilled water to a concentration of 50 or 60 spores under the low power of the miscroscope. Seeds were surface sterilized by washing in 0.1% mercuric chloride solution followed by three changes of sterile water and then soaked for 12 hours in five ml of the respective fungal spore suspension. After 12 hours, the seeds were tested for their germination by blotter method. In control plates, the seeds were surface sterilised with 0.1% mercuric chloride solution followed by washing in three changes of sterile water. Observations on the number of seeds germinated were recorded on every third day for a period of 15 days and the per cent inhibition in germination over control was calculated in each case. The fungi which were isolated from the respective vegetable seeds were used to test their pathogenicity.

Effect of seed treatment on viability of seeds stored for different periods under different humidity levels

The seeds were treated with six different fungicides.

Sl. No.	Name of Commercial formulation	Chemical name	Concentration
1.	Bavistin 50 WP	Methyl benzimidazol-2-ylcarbamate	0.1%
2.	Thiride 75 WP	Tetramethylthiuramdisulphide	0.3%
3.	Captafol 80 WP	N-(1,1,2,2-tetrachloroethyl-thio)-3a,4,7,7a-tetrahydrophthalimide	0.1%
4.	Dithane M-45	Complex of zinc and maneb containing 20% manganese and 2.5% zinc	0.3%
5.	Topsin M-70 WP	1,2-di-(3-methoxycarbonyl-2-thioureido) benzene	0.1%
6.	Emisan 3 WP	Methoxyethylmercury chloride	0.1%

Seeds were dipped in fungicidal suspension for half an hour and dried in shade before storage. Untreated seeds served as control. Treated seeds and untreated seeds were taken in cloth bags and different sets were stored in tin boxes under normal storage conditions and separate desiccators under different humidity levels of 66.8, 75.6, 82.9 and 92.9% so as to take samples at three months interval for a period of one year. Samples were drawn at three, six, nine and twelve months storage and their germination percentages were assessed by blotter method and pot culture experiments.

Different humidity levels were maintained inside the desiccator using different concentrations of sulphuric acid with water (v/v) as follows (CMI Plant Pathologists pocket book, 1968).

Sulphuric acid (%)	Relative humidity (%) at 25°C inside the desiccator
15	92.9
25	82.9
30	75.6
35	66.8

The seeds were kept inside the desiccators and the edges of the desiccators were sealed with greese to make it airtight. Separate sulphuric acid preparations and sealing of desiccator were made for taking seeds at three months interval. At three months interval their germination per cent were assessed.

Statistical analysis

Data on germination percentage of seeds were analysed using chi-square analysis.

RESULTS

Seeds of the three vegetables namely, bhindi (Abelmoschus esculentus (L) Moench), Cowpea (Vigna unguiculata (L.) Walp) and dolichos bean (Dolichos lablab L.) used for these investigations were obtained from the Department of Olericulture, College of Horticulture, Vellanikkara. The moisture content and germination percentage of the seeds before storage were determined as described in the materials and methods. Data are presented in Table 1.

It was observed that bhindi seeds had the lowest germination percentage, 70 in blotter method and 68 in pot culture experiment. The germination percentage of cowpea seeds were 100 and 98 and that of dolichos bean 89 and 86 per cent respectively in blotter method and pot culture experiments. The moisture content was 9.43 per cent in bhindi, 8.66 in cowpea and 8.3 per cent in dolichos bean.

Mycoflora associated with seeds

Fungi associated with the seeds were studied as mentioned in the materials and methods.

The whole seed, surface sterilized and unsterilized were plated on potato dextrose agar (PDA) medium and different

Results

Table 1. Moisture content and germination percentage of vegetable seeds

Crop	Moisture percentage	Germination percentage	
		Blotter method	Pot culture
Bhindi	9.43	70	68
Cowpea	8.66	100	98
Dolichos bean	8.30	89	86

Germination percentage was rounded to the nearest whole number in all the germination studies.

species of fungi were isolated and presented in Tables 2, 3 and 4.

Bhindi

Eighteen species of fungi were isolated from the unsterilized seeds. They are Absidia corymbifera, Acremonium sp, Aspergillus flavus, A. niger, A. fumigatus, Alternaria sp, Botryodiplodia sp, Chaetomium brasiliense, Fusarium solani, F. oxysporum, F. pallidoroseum, Paecilomyces variotii, Penicillium sp, P. implicatum, Rhizopus sp, Syncephalastrum racemosum a black sterile mycelium and a white sterile mycelium. The surface sterilized seeds yielded 11 species of fungi. These 11 species were also found on unsterilized seeds, but seven species namely, Acremonium sp, A. fumigatus, B. sp. F. oxysporum, F. pallidoroseum, F. solani and the black sterile mycelium were found only on unsterilized seeds (Table 2).

Cowpea

Unsterilized seeds yielded 20 species of fungi when plated on PDA while only 13 species were obtained from the surface sterilized seeds. The fungi isolated from unsterilized seeds were Acremonium sp, Alternaria sp., Aspergillus flavus, A. fumigatus, A. niger, Botryodiplodia sp, Chaetomium brasiliense, Cladosporium herbarum, Cochliobolus geniculatus, Eurotium chevalieri, Fusarium solani, F. oxysporum,

Table 2. Mycoflora associated with bhindi seeds

	Unsterilized		Sterilized
1	Absidia corymbifera (Cohn) Sacc. & A. Trotter	1	Absidia corymbifera
2	Acremonium sp. Link ex Fr.	2	Aspergillus flavus
3	Aspergillus flavus Link ex. Fr.	3	A. niger
4	A. niger Van Tiegh	4	Alternaria sp
5	A. fumigatus Fres.	5	Chaetomium brasiliense
6	Alternaria sp. Nees ex Wallr.	6	Paecilomyces variotii
7	Botryodiplodia sp. Sacc.	7	Penicillium implicatum
8	Chaetomium brasiliense Batista & Pontual	8	P. sp.
9	Fusarium solani (Mart.) Sacc.	9	Rhizopus sp.
10	F. oxysporum Schlecht.	10	Syncephalastrum racemosum
11	F. pallidoroseum (Cooke) Sacc.	11	White sterile mycetium
12	Paecilomyces variotii Bainier		
13	Penicillium implicatum Biourge		
14	P. sp. Link ex Fr.		
15	Rhizopus sp. Ehrenb ex Corda		
16	Syncephalastrum racemosum Cohn ex Schroter		
17	Black sterile mycelium		
18	White sterile mycetium		

F. pallidoroseum, Helminthosporium sp, Periconia saraswatipurensis, Penicillium sp, Rhizopus sp, Syncephalastrum racemosum, a white sterile mycelium and a black sterile mycelium. Surface sterilized seeds yielded 13 species of fungi. Aspergillus flavus, A. niger, Absidia corymbifera, Acremonium sp, Alternaria sp, Botryodiplodia sp, Chaetomium brasiliense, Cladosporium herbarum, Fusarium oxysporum, Humicola fuscoatra, Periconia saraswatipurensis, Syncephalastrum racemosum and white sterile mycelium. Fungi like Aspergillus fumigatus, Eurotium chevalieri, Cochliobolus geniculatus, Fusarium solani, F. pallidoroseum, Helminthosportum sp, Penicillium sp, Rhizopus sp. and the black sterile mycelium were found only on the unsterilized seeds whereas Absidia corymbifera and Humicola fuscoatra were restricted only to the surface sterilized seeds (Table 3).

Dolichos bean

The fungi isolated from unsterilized and surface sterilized seeds of dolichos bean are presented in Table 4. Absidia corymbifera, Alternaria sp, Aspergillus flavus, A. niger, Botryodiplodia sp, Chaetomium strumarium, Cladosporium herbarum, Fusarium solani, F. oxysporum, F. pallidoroseum, Rhizopus sp, Syncephalastrum racemosum, Trichoderma pseudokoningii, black sterile mycelium and white sterile

Table 3. Mycoflora associated with cowpea seeds

	Unsterilized		Sterilized
1	Acremonium sp	1	Absidia corymbifera
2	Alternaria sp	2	Acremonium sp
3	Aspergillus flavus	3	Alternaria sp
4	A. fumigatus	4	Aspergillus niger
5	A. niger	5	A. flavus
6	Botryodiplodia sp	6	Botryodiplodia sp
7	Chaetomium brasiliense	7	Chaetomium brasiliense
8	Cladosporium herbarum Pers. Link Fr.	8	Cladosporium herbarum
9	Cochliobolus geniculatus Nelson	9	Fusarium oxysporum
10	Eurotium chevalieri Mangin	10	Humicola fuscoatra
11	Fusarium solani	11	Periconia saraswatipurensis
12	F. oxysporum	12	Syncephalastrum racemosum
13	F. pallidoroseum	13	White sterile mycelium
14	Helminthosporium sp. Link ex Fr.		
15	Periconia saraswatipurensis Bilgrami		
16	Penicillium sp		
17	Rhizopus sp		
18	Syncephalastrum racemosum		
19	White sterile mycelium		
20	Black sterile mycelium		

Table 4. Mycoflora associated with dolichos bean seeds

	Unsterilized		Sterilized
1	Absidia corymbifera	1	Alternaria sp
2	Alternaria sp	2	Aspergillus niger
3	Aspergillus flavus	3	Botryodiplodia sp
4	A. niger	4	Chaetomium strumarium
5	Botryodiplodia sp	5	Cladosporium herbarum
6	Chaetomium strumarium Rai, Tewari & Mukerji) P. Cannon	6	Fusarium solani
7	Cladosporium herbarum	7	F. oxysporum
8	Fusarium solani	8	F. pallidoroseum
9	F. oxysporum	9	Nodulisporium gregarium (Berk. & M.A.Curtis) Meyer.
10	F. pallidoroseum	10	N. sp
11	Rhizopus sp.	11	Periconia saraswatipurensis
12	Syncephalastrum racemosum	12	Trichoderma pseudokoningii
13	Trichoderma pseudokoningii Rifai	13	Black sterile mycelium
14	Black Sterile mycelium	14	White sterile mycelium
15	White sterile mycelium		

mycelium were isolated from unsterilized seeds. Fungi isolated from surface sterilized seeds were Alternaria sp, Aspergillus niger, Botryodiplodia sp, Chaetomium strumarium, Cladosporium herbarum, Fusarium solani, F. oxysporum, F. pallidoroseum, Nodulisporium gregarium, N. sp. Periconia saraswatipurensis, Trichoderma pseudokoningii, a black sterile mycelium and a white sterile mycelium.

Mycoflora associated with different parts of the seed

Qualitative studies on seed-borne mycoflora before storage was conducted on different parts of the seed namely seed coat, endosperm and embryo according to the method described in the materials and methods and results are presented in Table 5, 6 and 7.

Bhindi

The maximum mycoflora was observed in the seed coat and 16 species of fungi were isolated. This includes Absidia corymbifera, Acremonium sp, Alternaria sp, Aspergillus flavus, A niger, A. fumigatus, Chaetomium brasiliense, C. globosum, Fusarium solani, F. oxysporum, F. pallidoroseum, Paecilomyces variotii, Rhizopus sp, Syncephalastrum racemosum, a white sterile mycelium and a black sterile mycelium.

Eight species of fungi namely Absidia corymbifera Alternaria sp, Aspergillus flavus, A. fumigatus, Fusarium solani, Paecilomyces variotii, Rhizopus sp. and a white sterile mycelium were isolated from the endosperm.

Alternaria sp, Fusarium oxysporum and a hyaline mycelium without any spore were the fungal flora isolated from the embryo of bhindi seed (Table 5).

Cowpea

Seventeen species of fungi were isolated from the seed coat of cowpea. Among these three species were Aspergillus, A. flavus, A. niger and A. amstelodami. Others were Alternaria sp Botryodiplodia sp, Chaetomium sp, C. brasiliense, Cochliobolus geniculatus, Fusarium oxysporum, F. semitectum, Helminthosporium sp Periconia saraswatipurensis, Penicillium sp, Rhizopus sp, Syncephalastrum racemosum, a black sterile mycelium and a white sterile mycelium.

The endosperm of cowpea harbours 14 species of fungi which contain Aspergillus flavus, A. fumigatus, A. amstelodami, Botryodiplodia sp, Cochliobolus geniculatus, Chaetomium sp. Eurotium chevalieri, Fusarium oxysporum, Humicola fuscoatra, Penicillium implicatum, Rhizopus sp., Trichoderma pseudokoningii a black sterile mycelium and a white sterile mycelium.

Table 5. Mycoflora associated with different parts of bhindi seed

	Seed coat		Endosperm		Embryo
1	Absidia corymbifera	1	Absidia corymbifera	1	Alternaria sp
2	Acremonium sp	2	Alternaria sp	2	Fusarium oxysporum
3	Alternaria sp	3	Aspergillus flavus	3	White sterile mycelium
4	Aspergillus flavus	4	A. fumigatus		
5	A. niger	5	Fusarium solani		
6	A. fumigatus	6	Paecilomyces variotii		
7	Chaetomium brasiliense	7	Rhizopus sp		
8	C. globosum	8	White sterile mycelium		
9	Fusarium solani				
10	F. oxysporum				
11	F. pallidoroseum				
12	Paecilomyces variotii				
13	Rhizopus sp				
14	Syncephalastrum racemosum				
15	White sterile mycelium				
16	Black sterile mycelium				

Five species of fungi were isolated from the embryo of cowpea. They were Alternaria sp, Aspergillus flavus, Fusarium oxysporum, Penicillium implicatum and a white sterile mycelium (Table 6).

Dolichos bean

On isolation 17 species of fungi were found on the seed coat of dolichos bean. They were Absidia corymbifera, Alternaria sp, Aspergillus flavus, A. niger, Botryodiplodia sp, Chaetomium sp, C. strumarium, Cladosporium herbarum, Fusarium solani, F. oxysporum, F. semitectum, Penicillium sp, Rhizopus sp, Syncephalastrum racemosum, Trichoderma pseudokoningii black sterile mycelium and white sterile mycelium.

The endosperm of dolichos bean was infected with six species of fungi which include one black and one white sterile mycelium. Others were Alternaria sp., Aspergillus flavus, Botryodiplodia sp. and Cochlibolus geniculatus (Table 7). Fusarium oxysporum and one white sterile mycelium were found infecting the embryo of dolichos bean.

Of the three seeds, the maximum number of fungal species were found on cowpea (22) followed by dolichos bean (18) and bhindi (16). In all the cases, the maximum fungi were found on the seed coat. The least number of fungal species

Table 6. Mycoflora associated with different parts of cowpea seed

	Seed coat		Endosperm		Embryo
1	Alternaria sp	1	Aspergillus flavus	1	Alternaria sp
2	Aspergillus flavus	2	A. fumigatus	2	Aspergillus flavus
3	A. niger	3	A. amstelodami	3	Fusarium oxysporum
4	A. amstelodami	4	Botryodiplodia sp.	4	Penicillium implicatum
5	Botryodiplodia sp.	5	Cochliobolus geniculatus	5	White sterile mycelium
6	Chaetomium brasiliense	6	Chaetomium sp		
7	C. sp	7	Eurotium chevalieri		
8	Cochliobolus geniculatus	8	Fusarium oxysporum		
9	Fusarium oxysporum	9	Humicola fuscoatra		
10	F. semitectum	10	Penicillium implicatum		
11	Helminthosporium sp	11	Rhizopus sp		
12	Periconia saraswatipurensis	12	Trichoderma pseudokoningii		
13	Penicillium sp	13	Black sterile mycelium		
14	Rhizopus sp	14	White sterile mycelium		
15	Syncephalastrum racemosum				
16	Black sterile mycetium				
17	White sterile mycelium				

Table 7. Mycoflora associated with different parts of dolichos bean seed

	Seed coat		Endosperm		Embryo
1	Absidia corymbifera	1	Alternaria sp	1	Fusarium oxysporum
2	Alternaria sp	2	Aspergillus flavus	2	White sterile mycelium
3	Aspergillus flavus	3	Botryodiplodia sp		
4	A. niger	4	Cochliobolus geniculatus		
5	Botryodiplodia sp	5	White sterile mycelium		
6	Chaetomium strumarium	6	Black sterile mycetium		
7	C. sp				
8	Cladosporium herbarum				
9	Fusarium solani				
10	F. oxysporum				
11	F. semitectum				
12	Penicillium sp				
13	Rhizopus sp				
14	Syncephalastrum racemosum				
15	Trichoderma pseudokoningii				
16	Black sterile mycelium				
17	White sterile mycelium				

were isolated from the embryo, two from dolichos bean, three from bhindi and five from cowpea.

Mycoflora associated with ungerminated seeds in the pot culture experiment

The fungi associated with ungerminated seeds in the pot culture experiment was studied as explained in the materials and methods.

Bhindi

14 Species of fungi were isolated from the ungerminated bhindi seeds. This includes Absidia corymbifera Alternaria sp., Botryodiplodia sp, Cochliobolus geniculatus, Fusarium sp, F. solani, F. oxysporum, F. pallidoroseum, Humicola fuscoatra, H. grisea, Paecilomyces variotii, Syncephalastrum racemosum, a black sterile mycelium and a white sterile mycelium (Table 8).

Cowpea

Ungerminated cowpea seeds when plated on PDA yielded 11 species of fungi. They were Botryodiplodia sp. Cochliobolus geniculatus, Fusarium sp, F. solani, F.oxysporum, F. pallidoroseum, Humicola fuscoatra, Periconia saraswatipurensis, Syncephalastrum racemosum, a black sterile mycelium and a white sterile mycelium (Table 8).

Table 8. Fungi isolated from ungerminated seeds in the pot culture experiment

	Bhindi		Cowpea	Dolichos bean
1	Absidia corymbifera	1	Botryodiplodia sp	Absidia corymbifera
2	Alternaria sp	2	Cochliobolus geniculatus	Alternaria sp
3	Botryodiplodia	3	Fusarium solani	Botryodiplodia sp
4	Cochliobolus geniculatus	4	F. oxyosporum	Cochliobolus geniculatus
5	Fusarium sp	5	F. pallidoroseum	Fusarium solani
6	F. solani	6	F. sp	F. oxysporum
7	F. oxysporum	7	Humicola fuscoatra	F. pallidoroseum
8	F. pallidoroseum	8	Periconia saraswatipurensis	F. sp
9	Humicola fuscoatra	9	Syncephalastrum racemosum	Humicola fuscoatra
10	H. grisea	10	Black sterile mycelium	Periconia saraswatipurensis
11	Paecilomyces variotii	11	Black sterile mycelium	Syncephalastum racemosum
12	Syncephalastrum racemosum			White sterile mycelium
13	Black sterile mycelium			
14	White sterile mycelium			

Dolichos bean

Twelve species of fungi were isolated from the ungerminated seeds of dolichos bean. Of these four were Fusarium, F. solani, F. oxysporum, F. pallidoroseum and one unidentified species of the genus Fusarium. Others were Absidia corymbifera, Alternaria sp., Botryodiplodia sp. Cochliobolus geniculatus, Humicola fuscoatra, Periconia saraswatipurensis, Syncephalastrum racemosum and white sterile mycelium (Table 8).

Effect of seed-borne fungi on the germination of seeds

Most of the seed-borne fungi were found to cause inhibition in germination of the respective vegetable seeds from which they were isolated.

Bhindi

When bhindi seeds were dipped in the spore suspension of Absidia corymbifera and Paecilomyces variotii there was 30 per cent inhibition in germination when compared to control. Acremonium sp. and Aspergillus niger had no effect on the germination of bhindi seeds. Maximum inhibition of 70 per cent was by Fusarium solani. There was 60 per cent inhibition by Syncephalastrum racemosum, 47 per cent by

Fusarium pallidoroseum, 25 per cent by Penicillium sp. and 20 per cent inhibition by Aspergillus fumigatus and Fusarium oxysporum. The inhibition in germination by Aspergillus flavus, Chaetomium brasiliense, Botryodiplodia sp, Rhizopus sp and Alternaria sp were less than 19 per cent (Table 9).

Cowpea

There was 100 per cent inhibition in germination of cowpea seeds when the seeds were dipped in the spore suspension of Aspergillus flavus, A. niger and A. fumigatus. Inhibition ranged from 60 to 90 per cent by Acremonium sp, Eurotium chevalieri, Penicillium sp, Cladosporium herbarum, Alternaria sp and Botryodiplodia sp. Ten per cent or less inhibition was caused by Humicola fuscoatra, Fusarium pallidoroseum and Chaetomium brasiliense (Table 9).

Dolichos bean

Aspergillus flavus, A. niger, chaetomium strumarium, Fusarium pallidoroseum and Nodulisporium gregarium had no effect on the germination of dolichos bean seeds. Maximum inhibition in germination was seen when the seeds were dipped in the spore suspension of Botryodiplodia sp (50 per cent) and Cochliobolus geniculatus (40 per cent). In all other cases inhibition was 30 per cent or less (Table 9).

Table 9. Effect of seed-borne fungi on germination of seeds (Figures indicate the per cent inhibition of germination over control).

Sl. No.	Name of fungi	Percentage inhibition of germination		
		Bhindi	Cowpea	Dolichos bean
1	Absidia corymbifera	30	-	27
2	Acremonium sp	0	60	-
3	Alternaria sp	7	80	30
4	Aspergillus flavus	18	100	0
5	A. niger	0	100	0
6	A. fumigatus	20	100	-
7	Botryodiplodia sp.	10	90	50
8	Chaetomium brasiliense	15	4	-
9	C. strumarium	-	-	0
10	Cladosporium herbarum	-	80	4
11	Cochliobolus geniculatus	-	40	40
12	Eurotium chevalieri	-	60	--
13	Fusarium solani	70	27	10
14	F. oxysporum	20	33	3
15	F. pallidoroseum	47	8	0
16	Humicola fuscostra	-	7	-
17	Helminthosporium sp.	-	10	-
18	Nodulisporium sp	-	-	-
19	N. gregarium	-	-	0
20	Paecilomyces variotii	30	-	-
21	Penicillium sp	25	64	-
22	Periconia saraswatipurensis	-	40	-
23	Rhizopus sp.	10	30	12
24	Syncephalastrum racemosum	60	47	28
25	Trichoderma pseudokoningii	-	32	22

Mycoflora associated with the seeds after different periods of storage

Bhindi

The association of fungal flora was noticed on both surface sterilized and unsterilized seeds during the storage periods. There was a gradual reduction in the number of fungi associated with unsterilized seeds in the storage periods, but the fungal species associated with surface sterilized seeds showed a slight increase upto six months of storage after which it decreased. Some fungi like Rhizopus sp. and Aspergillus flavus were present throughout the storage on both unsterilized and surface sterilized seeds. Fungi like Aspergillus niger, A. fumigatus and F. oxysporum were present throughout the storage either or both on unsterilized and surface sterilized seeds. Absidia corymbifera was detected upto nine months on both sterilized and unsterilized seeds, Chaetomium brasiliense and Syncephalastrum racemosum upto six months. Acremonium sp was present initially on unsterilized seeds but was not detected after the storage, even after three months. Botryodiplodia sp., Fusarium solani and black sterile mycelium were detected after three months of storage but not detected later on (Table 10).

Table 10. Mycoflora associated with bhindi seeds after different storage periods

	Name of fungi	Storage									
		Initial		3 months		6 months		9 months		12 months	
		US	SS	US	SS	US	SS	US	SS	US	SS
1	Absidia corymbifera	+	+	+	+	+	+	+	+	-	-
2	Acremonium sp.	+	-	-	-	-	-	-	-	-	-
3	Aspergillus flavus	+	+	+	+	+	+	+	+	+	+
4	A. niger	+	+	+	+	+	+	+	-	+	-
5	A. fumigatus	+	-	+	-	+	-	+	+	+	+
6	Alternaria sp.	+	+	+	-	-	+	-	+	-	-
7	Botryodiplodia sp.	+	-	-	+	-	-	-	-	-	-
8	Chaetomium brasiliense	+	+	+	+	+	+	-	-	-	-
9	Fusarium solani	+	-	+	-	-	-	-	-	-	-
10	F. oxysporum	+	-	-	+	-	+	-	+	-	+
11	F. Pallidoroseum	+	-	-	+	-	+	-	+	-	-
12	Paecilomyces variotii	+	+	+	-	+	+	+	+	-	-
13	Penicillium sp.	+	+	+	+	-	+	-	-	-	-
14	Penicillium implicatum	+	+	+	+	-	+	-	-	-	-
15	Rhizopus sp	+	+	+	+	+	+	+	+	+	+
16	Syncephalastrum racemosum	+	+	+	+	+	+	-	-	-	-
17	Black sterile mycelium	+	-	+	-	-	-	-	-	-	-
18	White sterile mycelium	+	+	+	+	-	+	-	+	-	-
	Total number of fungi	18	11	14	12	8	13	6	9	4	4

US - Unsterilized
SS - Surface sterilized

\+ Presence
\- absence

Cowpea

Stored cowpea seeds were examined for the presence of fungi after different intervals of storage. It could be seen that the number of fungal species associated with the unsterilized and surface sterilized seeds decreased as the storage period was increased. Aspergillus flavus and Rhizopus sp. were present throughout the storage on both sterilized and unsterilized seeds. Absidia corymbifera was detected upto 9 months on sterilized seeds. Acremonium sp. was detected upto three months of storage but later on it was not detected. Alternaria sp. was detected upto 3 months and A. niger upto 9 months. Helminthosporium sp. and Humicola fuscoatra were not detected even after three months of storage (Table 11).

Dolichos bean

The fungi isolated from unsterilized and surface sterilized seeds of dolichos bean after different periods of storage are presented in Table 12. It could be observed that Rhizopus sp., Aspergillus flavus and A. niger were present throughout the storage either or both on unsterilized and surface sterilized seeds. Absidia corymbifera and Chaetomium strumarium which were present initially on seeds was not detected after storage while Cladosporium herbarum,

Table 11. Mycoflora associated with cowpea seeds after different storage periods

Name of fungi	Storage periods									
	Initial		3 months		6 months		9 months		12 months	
	US	SS	US	SS	US	SS	US	SS	US	SS
Absidia corymbifera	-	+	-	+	-	+	-	+	-	-
Acremonium sp	+	+	+	-	-	-	-	-	-	-
Alternaria sp.	+	+	+	+	-	-	-	-	-	-
Aspergillus flavus	+	+	+	+	+	+	+	+	+	+
A. fumigatus	+	-	-	-	-	-	-	+	-	-
A. niger	+	+	+	+	+	-	+	-	-	-
Botryodiplodia sp.	+	+	+	+	-	-	+	-	-	-
Chaetomium brasiliense	+	+	+	+	+	-	-	-	+	-
Cladosporium herbarum	+	+	-	-	-	-	-	-	-	-
Cochliobolus geniculatus	+	-	+	-	-	-	-	-	-	-
Eurotium chevalieri	+	-	+	-	-	-	-	-	-	-
Fusarium oxysporum	+	+	-	+	-	-	+	+	+	+
F. Pallidoroseum	+	-	+	-	-	-	+	-	-	-
F. solani	+	-	+	-	-	-	+	-	-	-
Helminthosporium sp.	+	-	-	-	-	-	-	-	-	-
Humicola fuscoatra	-	+	-	-	-	-	-	-	-	-
Periconia saraswatipurensis	+	+	-	+	-	-	-	-	-	-
Penicillium sp	+	-	+	-	+	-	-	-	-	-
Penicillium implicatum	-	-	+	-	-	-	-	-	-	-
Rhizopus sp	+	-	+	+	+	+	+	+	+	+
Syncephalastrum racemosum	+	+	-	-	+	-	-	-	-	-
White sterile mycelium	+	+	+	+	-	+	-	+	+	+
Black sterile mycelium	+	-	+	-	-	-	-	-	-	-
Total number of fungi	20	13	15	10	5	4	6	4	5	4

US - Unsterilized
SS - Surface sterilized
\+ presence
\- absence

Nodulisporium sp., N. gregarium, Periconia saraswatipurensis, Trichoderma pseudokoningii and black sterile mycelium survive upto three months of storage, not beyond that. Alternaria sp. upto 12 months and white sterile mycelium survive upto nine months. In general, there was a reduction in the number of fungi over the storage periods.

Germination studies in storage at different humidity levels

The seeds of three vegetables collected were stored under four different humidity levels, 66.8, 75.6, 82.9 and 92.9 apart from normal storage condition which served as control. The maximum germination percentage was observed in seeds stored at the humidity level of 66.8 per cent in all the periods of storage and the germination percentage was decreased as the time of storage was increased.

Bhindi

Blotter

When the seeds were stored under normal laboratory conditions, 60 per cent germination was seen after three months storage but only 45 per cent germination was obtained after 12 months storage. But there was three to eight per cent increase in germination when the seeds were stored at the humidity level of 66.8 per cent. The viability of seeds was lost after six months storage under the high humidity levels

Table 12. Mycoflora associated with dolichos bean seeds after different storage periods

Name of fungi	Storage period									
	Initial		3 months		6 months		9 months		12 months	
	US	SS	US	SS	US	SS	US	SS	US	SS
Absidia corymbifera	+	-	-	-	-	-	-	-	-	-
Alternaria sp.	+	+	+	-	+	-	+	+	+	-
Aspergillus flavus	+	-	+	+	+	-	+	+	+	+
A. niger	+	+	+	-	+	-	+	-	+	-
Botyodiplodia sp.	+	+	+	+	-	-	+	-	-	-
Chaetomium strumarium	+	+	-	-	-	-	-	-	-	-
Cladosporium herbarum	+	+	-	+	-	-	-	-	-	-
Fusarium oxysporum	+	+	-	-	+	+	-	+	-	+
F. pallidoroseum	+	+	+	+	-	-	+	-	-	-
F. solani	+	+	+	+	-	-	+	-	-	-
Nodulisporium gregarium	-	+	-	+	-	-	-	-	-	-
N. sp	-	+	-	+	-	-	-	-	-	-
Periconia saraswatipurensis	-	+	-	+	-	-	-	-	-	-
Rhizopus sp	+	-	+	+	+	+	+	+	+	+
Syncephalastrum racemosum	+	-	-	+	-	+	-	-	-	-
Trichoderma pseudokoningii	+	+	-	+	-	-	-	-	-	-
Black sterile mycelium	+	+	+	+	-	-	-	-	-	-
White sterile mycelium	+	+	+	+	-	+	+	+	-	-
Total number of fungi	15	14	9	13	5	2	7	4	4	3

US - Unsterilized
SS - Surface sterilized

\+ presence
\- absence

of 82.9 and 92.9 per cent but in that condition there was 36 and 24 per cent germination respectively after three months storage. There was no significant difference between different storage periods when the seeds were kept under normal storage condition and at the humidity level of 66.8 per cent. At the end of three months, there was no significant difference in germination percentage between seeds kept under normal condition, at 66.8 and 75.6 per cent humidity levels, but they differed significantly from that at 82.9 and 92.9 per cent humidity. After six months storage germination percentage of seeds kept at 66.8 per cent humidity and under normal storage condition did not differ significantly from one another but differed significantly from other humidity levels. At the humidity level of 75.6 per cent the deterioration of seeds was rapid, only 20 per cent germination after six months, it again reduced to two per cent after nine months. The data clearly indicate that the viability of seeds decreased when the humidity level in storage was increased and the decrease was much pronounced when the storage period was prolonged (Table 13).

Pot culture

Almost the same trend was observed in the germination of bhindi seeds in pots also. There was complete failure of

Table 13. Percentage germination of bhindi seeds in different periods of storage under different humidity levels in blotter and pot culture

	Blotter					Pot culture			
	Normal storage	Humidity levels (per cent)				Normal storage	Humidity levels (per cent)		
		66.8	75.6	82.9	92.9		66.8	75.6	22.9	92.9
3 months	60	63	48	36	24	52	56	44	24	20
6 months	54	62	20	0	0	52	54	6	0	0
9 months	50	58	2	0	0	50	50	0	0	0
12 months	45	52	0	0	0	40	48	0	0	0

germination of the seeds when they were kept even at 75.6 per cent humidity for nine months. At this level of humidity, the germination percentage was very less even after six months storage (six per cent). At the end of three months there was no significant difference in germination between the seeds kept under normal storage condition and at the humidity levels of 66.8 and 75.6 per cent. Germination per cent of seeds kept at 66.8 per cent humidity and under normal storage condition were on par after six, nine and 12 months storage also. There was complete failure of germination after six months at the humidity levels of 82.9 and 92.9 per cent (Table 13).

Cowpea

Blotter

The maximum germination (98 per cent) was seen in seeds kept at the humidity level of 66.8 per cent after three months (Table 14). Under normal storage, the per cent germination was less than that at 66.8 per cent humidity after three months storage. The germination per cent of seeds kept at 66.8 per cent humidity and normal storage condition were on par and differ significantly from all other treatments after three months storage. There was complete loss of viability after six months at the humidity levels of 82.9 and 92.9 per cent. After six months the germination

Table 14. Percentage germination of cowpea seeds in different periods of storage under different humidity levels in blotter and pot culture

	Normal storage	Blotter				Normal storage	Pot culture			
		Humidity levels (per cent)					Humidity levels (per cent)			
		66.8	75.6	82.9	92.9		66.8	75.6	22.9	92.9
3 months	78	98	54	50	48	88	90	52	46	44
6 months	76	90	40	0	0	64	74	20	46	44
9 months	48	88	20	0	0	48	70	3	0	0
12 months	42	84	0	0	0	28	60	0	0	0

per cent of seeds kept under normal storage condition and at the humidity levels of 66.8 and 75.6 per cent differ significantly from one another. The same trend was seen after nine months also. At the end of 12 months there was complete failure of germination of seeds kept at 75.6 per cent humidity. The seeds kept at 66.8 per cent humidity and under normal storage conditions differ significantly from one another (Table 14).

Pot culture

At the end of three months, the germination per cent of seeds in pots showed the same pattern, as that under blotter technique. After six months the germination per cent of seeds kept at 66.8 per cent humidity and under normal storage condition were on par and they differ significantly from the germination per cent of seeds kept at 75.6 per cent humidity. Storage for nine months at the humidity level of 75.6 per cent reduced the germination per cent to three, which differed significantly from the seeds kept at 66.8 per cent humidity and under normal storage conditions. There was complete failure of germination after six months at the humidity levels of 82.9 and 92.9 per cent and after 12 months at the humidity level of 75.6 per cent (Table 14).

Dolichos bean

Blotter

When dolichos bean seeds stored under different humidity levels were subjected to germination studies, it was observed that there was not much difference between the seeds kept at 66.8 per cent humidity and under normal storage conditions, the difference ranged from two to 13 per cent (Table 15).

There was complete loss of viability when the seeds were stored at 82.9 and 92.9 per cent humidity for nine months. At the end of three months maximum germination of 93 per cent was in seeds stored at 66.8 per cent humidity and minimum, 48 per cent in seeds kept at 92.9 per cent humidity. The germination per cent of seeds kept at 66.8 per cent humidity, at 75.6 per cent humidity and under normal storage conditions did not differ significantly after three months. After six months, normal storage condition and humidity level of 66.8 per cent were on par and differed significantly from all other treatments. After 12 months 85 per cent germination was obtained in seeds stored at 66.8 per cent humidity and 73 per cent under normal storage condition which were significantly different from one another.

Table 15. Percentage germination of dolichos bean seeds in different periods of storage under different humidity levels in blotter and pot culture

	Blotter					Pot culture				
	Normal storage	Humidity levels (per cent)				Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9		66.8	75.6	22.9	92.9
3 months	87	93	83	70	48	75	86	60	50	20
6 months	88	90	10	7	0	70	80	7	6	0
9 months	75	88	10	0	0	63	70	6	0	0
12 months	73	85	0	0	0	57	63	0	0	0

Pot culture

Humidity level of 66.8 per cent and normal storage condition were found to be the best when the germination studies were conducted by pot culture experiment after three months storage. Only seven per cent germination was seen after six months of storage at the humidity level of 75.6 per cent. The other observations were similar to that obtained in the blotter test. Complete failure of germination was noted after six months at 92.9 per cent humidity, after nine months at 82.9 per cent humidity and after 12 months at 75.6 per cent humidity (Table 15).

Germination percentage of fungicide treated seeds after different periods of storage under normal storage condition

Bhindi

Blotter

After three months under normal storage conditions untreated seeds gave only 60 per cent germination, but it did not differ significantly from fungicide treated seeds where the germination percentage ranged from a maximum of 76 per cent in Emisan treatment to a minimum of 72 per cent in Topsin M-70. When the germination per cent of fungicide treated seeds and untreated seeds were studied after a storage period of six months, also there was no significant difference between treated and untreated seeds. Maximum germination

percentage of 70 was obtained in Thiride, captafol and Emisan treatments and minimum 54 per cent in untreated control after six months. The same trend was noticed after nine months, where captafol gave the highest germination percentage (70) and lowest in untreated control (50). At the end of twelve months all the treated seeds gave significantly better germination percentage when compared to control (Table 16).

Pot culture

When the seeds were stored for three months under normal storage condition, maximum germination percentage of 70 was obtained in Thiride and captafol treatment while untreated seeds gave only 52 per cent germination. But there was no significant difference between treated seeds and control. A similar trend was noticed after six and nine months storage. At the end of six months captafol treatment gave the maximum germination percentage (68) while the minimum (52 per cent) was in untreated control. When the storage period was increased to nine months, the highest germination percentage was in captafol and Dithane M-45 treatments (66 per cent) while the lowest (50 per cent) was in control. After 12 months storage all the fungicide treatments except Thiride was on par with control (Table 16).

Table 16. Germination percentage of fungicide treated bhindi seeds after different periods of storage under normal conditions

	Blother				Pot culture			
	Month							
	3	6	9	12	3	6	9	12
Bavistin	74	68	62	60	65	62	58	54
Thiride	73	70	69	68	70	66	59	62
Captafol	75	70	70	70	70	68	66	60
Dithane M-45	73	68	62	60	68	64	66	54
Topsin M-70	72	68	66	60	60	56	52	54
Emisan	76	70	68	66	68	64	62	60
Control	60	54	50	45	52	52	50	40

Cowpea

Blotter

After three months storage Thiride, captafol and Emisan treatments were on par and superior to all other treatments. Thiride treated seeds gave the maximum germination percentage of 98 while the minimum 78 was in untreated seeds. At the end of six months there was no significant difference between treated seeds and control, the lowest germination percentage of 76 was in control and the highest 88 per cent in captafol and Emisan. There was no significant difference between Dithane M-45, captafol, Bavistin and Thiride treatments after nine months storage but they were superior to control, Topsin M-70 and Emisan. A different trend was observed after storage for 12 months, where Topsin M-70 treatment was on par with control while all other treatments were significantly better than control. The variation in germination percentage ranged from 42 per cent (control and Topsin M-70) to 74 per cent (captafol) (Table 17).

Pot culture

When the seeds were stored for three months under normal storage conditions, there was no significant difference between treated seeds and control. The variation in germination percentage was from 88 (Control) to 95

Table 17. Germination percentage of fungicide treated cowpea seeds after different storage periods under normal storage condition

	Blotter				Pot culture			
	Month							
	3	6	9	12	3	6	9	12
Bavistin	80	86	80	68	95	82	72	60
Thiride	98	86	78	66	95	86	72	60
Captafol	96	88	84	74	95	86	80	62
Dithane M-45	88	86	86	64	92	82	80	54
Topsin M-70	88	80	64	42	94	80	60	38
Emisan	96	88	62	60	92	84	82	60
Control	78	76	48	42	88	64	48	28

(Bavistin, Thiride and captafol). At the end of six months all the treated seeds gave significantly better germination percentage when compared to control, but there was no significant difference among the different fungicidal treatments. The minimum germination percentage of 64 was in control and maximum 86 per cent in Thiride and captafol treatments. After nine months all the fungicides except Topsin M-70 were on par and significantly better than control. The lowest germination percentage of 48 was in control and the highest, 82 in Emisan. A similar trend was observed after 12 months also, minimum 28 per cent in control and maximum 62 per cent in captafol treatment (Table 17).

Dolichos bean

Blotter

When the seeds were stored under laboratory conditions for three months, maximum germination percentage was in Thiride treatment (100) and minimum was in control and Dithane M-45 (87 per cent). Thiride treatment was significantly superior. There was no significant difference between treated seeds and control after six months. Minimum germination percentage of 80 was in Topsin M-70 treatment and maximum, 92 in Thiride and captafol treatments after six months. At the end of nine months the highest germination percentage of 92 was in captafol treatment and the lowest

untreated control (75 per cent). There was no significant difference among the different fungicidal treatments, Topsin M-70 treatment was on par with control and significantly inferior to all other treatments after storage for 12 months (Table 18).

Pot culture

In the pot culture experiment, there was no significant difference between treated and untreated seeds when the storage period was increased. In all cases minimum germination was in untreated seeds (Table 18).

Effect of seed treatment on the viability of vegetable seeds at different storage periods and humidity levels

Bhindi

Blotter

In general, the germination per cent of bhindi seeds treated with fungicides was better than the untreated control. Similarly as the relative humidity was increased, a reduction in the germination was observed in all the treatments.

Three months

When seeds were stored under laboratory conditions the per cent germination of bhindi seeds treated with fungicides did not differ significantly from untreated seeds. When the seeds were stored at a constant humidity of 66.8 per cent, there was no significant difference with different fungicidal

Table 18. Germination percentage of fungicide treated dolichos bean seeds after different storage periods under normal storage condition

	Blotter				Pot culture			
	Month							
	3	6	9	12	3	6	9	12
Bavistin	90	86	84	82	80	72	70	60
Thiride	100	92	88	86	86	73	73	60
Captafol	96	92	92	90	83	73	68	63
Dithane M-45	87	87	85	84	83	76	67	60
Topsin M-70	90	80	78	70	76	70	67	63
Emisan	88	88	82	84	77	73	68	63
Control	87	88	75	73	75	70	63	57

treatments. However, the percent of germination was slightly better than the seeds stored under laboratory conditions. A general reduction in germination in all the fungicidal treatments was noticed at the end of three months when the seeds were stored at a humidity of 75.6 per cent. But the treatments did not differ significantly from one another. In all the treatments as the humidity was increased from 75.6 to 82.9 per cent there was a gradual reduction in the germination percentage. Untreated control was on par with Dithane M-45, Topsin M-70 and Bavistin. The highest germination percentage of 58 was noticed in Thiride treated seeds eventhough it did not differ significantly from all other fungicides except Dithane M-45. The germination percentage showed a drastic reduction as the humidity was increased to 92.9 per cent (Table 19). However, there was no significant difference in the germination with difference in the fungicides. Topsin M-70 with germination percentage of 30 exhibited the least germination in various fungicide treated seeds and it was on par with control (24 per cent).

Six months

Under normal storage conditions, constant humidity levels of 66.8 and 75.6 per cent treatment with fungicides showed no significant difference in the germination percentage when compared with untreated seeds. At the humidity level of

Table 19. Germination percentage of treated bhindi seeds stored for three months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	74	77	60	52	40
Thiride	73	75	62	58	50
Captafol	75	75	64	56	45
Dithane M-45	73	75	56	40	40
Topsin M-70	72	74	56	42	30
Emisan	76	78	64	56	44
Control	60	63	48	36	24

82.9 per cent two per cent germination was obtained in captafol and Emisan treated seeds, but in all other treatments including control, the germination was nil. At the highest humidity level of 92.9 per cent the viability of seeds was completely lost in all the treatments including control. In general fungicidal treatment could not retain the viability of seeds at higher humidity levels of 82.9 and 92.9 per cent as the storage period was increased (Table 20).

Nine months

Viability was completely lost after nine months storage at humidity levels of 82.9 and 92.9 per cent (Table 21). No significant difference in germination was observed between treated and untreated seeds at the humidity level of 66.8, 75.6 and when the seeds were kept at room conditions. Under normal storage condition maximum germination percentage of 70 was obtained in captafol treated seeds and minimum 50 per cent in the untreated control. The respective values for 66.8 per cent humidity were 72 and 58 per cent. At 75.6 per cent humidity none of the treatments showed more than 12 per cent germination.

Twelve months

The viability of seeds were completely lost when the seeds were stored above 75.6 per cent humidity. At 66.8 per cent

Table 20. Germination percentage of treated bhindi seeds stored for six months at different humidity levels in blotter method

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	68	70	20	0	0
Thiride	70	72	42	0	0
Captafol	70	72	46	2	0
Dithane M-45	68	70	38	0	0
Topsin M-70	68	69	20	0	0
Emisan	70	72	44	2	0
Control	54	62	20	0	0

Table 21. Germination percentage of treated bhindi seeds stored for nine months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	62	64	8	0	0
Thiride	69	70	10	0	0
Captafol	70	72	12	0	0
Dithane M-45	62	62	6	0	0
Topsin M-70	66	68	4	0	0
Emisan	68	70	8	0	0
Control	50	58	2	0	0

humidity no significant difference in germination was obtained among treated and untreated seeds. Thiride, captafol and Emisan treated seeds exhibited 70 per cent germination. Under normal storage condition maximum germination percentage of 70 was obtained in captafol treated seeds and minimum in control (45 per cent). There was no significant difference in the germination percentage when the seeds were treated with different fungicides. But all the treatments were significantly better than control (Table 22).

Bhindi

Pot culture

In general, the germination of bhindi seeds in soil was less than the germination obtained in blotter method under laboratory conditions, but the trend was same. Bhindi seeds failed to germinate when they were stored for six months and above under humidity level of 82.9 per cent and above.

Three months

There was no significant difference in germination between captafol, Thiride and control under normal storage conditions, eventhough a minimum germination of 52 per cent was observed in control and the maximum of 70 per cent in

Table 22. Germination percentage of treated bhindi seeds stored for twelve months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	60	62	0	0	0
Thiride	68	70	0	0	0
Captafol	70	70	0	0	0
Dithane M-45	60	60	0	0	0
Topsin M-70	60	62	0	0	0
Emisan	66	70	0	0	0
Control	45	52	0	0	0

Thiride and captafol treatments. Under 66.8 per cent and 75.6 per cent humidity also there was no significant difference between the treatments. But in both the cases minimum germination of 56 and 44 per cent respectively were noticed in untreated seeds. At 82.9 per cent humidity there was 20 per cent reduction in the germination in control compared to the seeds kept at 75.6 per cent humidity, while the difference in germination of fungicide treated seeds under these varying humidity conditions ranged from 16 (Dithane M-45) to six per cent (Thiride). There was no marked difference in germination among the seeds treated with different fungicides, but all the fungicide treated seeds except Dithane M-45 and Topsin M-70 were significantly better than the control (Table 23). When the humidity was increased to 92.9 per cent the reduction in germination noticed in different treatments compared to that observed at 82.9 per cent humidity ranged from two per cent in Dithane M-45 to 14 per cent in captafol. All the treatments except Topsin M-70 (26 per cent) was significantly better than the control (20 per cent).

Six months

There was no significant difference among the different treatments at the end of six months when seeds were stored under normal conditions, with 52 per cent germination in

Table 23. Germination percentage of treated bhindi seeds stored for three months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	65	70	58	50	40
Thiride	70	72	60	54	48
Captafol	70	72	62	54	40
Dithane M-45	68	68	54	38	36
Topsin M-70	60	66	48	38	26
Emisan	68	70	62	48	42
Control	52	56	44	24	20

control and 68 per cent in captafol. A similar pattern was noticed when the seeds were stored at 66.8 per cent humidity. At 75.6 per cent humidity there was considerable reduction in germination compared to that observed at 66.8 per cent humidity. A maximum of 54 per cent reduction in germination was observed in Topsin M-70 (58 per cent to four per cent) and Dithane M-45 (64 per cent to 10 per cent) treated seeds, when the germination at 66.8 and 75.6 per cent humidity were compared. Captafol and Thiride were best and significantly superior to all other treatments. Emisan, Bavistin and Dithane M-45 were on par and better than control and Topsin M-70. With further increase in humidity in storage complete loss of viability was noticed (Table 24).

Nine months

After storage for nine months under normal storage conditions, the variation in germination among treatments ranged from two per cent to sixteen per cent when compared to control (Table 25). The lowest germination percentage of 50 was obtained in control and highest in captafol and Dithane M-45 (66 per cent) treated seeds. However there was no significant difference among the treatments. A similar pattern was seen when the seeds were stored at 66.8 per cent humidity also. No remarkable difference in the germination was noticed

Table 24. Germination percentage of treated bhindi seeds stored for six months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	62	64	18	0	0
Thiride	66	68	36	0	0
Captafol	68	70	40	0	0
Dithane M-45	64	64	10	0	0
Topsin M-70	56	58	4	0	0
Emisan	64	62	22	0	0
Control	52	54	6	0	0

Table 25. Germination percentage of treated bhindi seeds stored for nine months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	58	60	8	0	0
Thiride	59	64	10	0	0
Captafol	66	68	14	0	0
Dithane M-45	66	58	10	0	0
Topsin-M-70	52	56	2	0	0
Emisan	62	68	15	0	0
Control	50	50	0	0	0

among the different treatments compared to the seeds stored at normal storage conditions. There was a drastic reduction in germination percentage when the seeds were stored at the humidity level of 75.6 per cent and no germination in untreated seeds. Among the fungicide treated seeds maximum germination (15 per cent) was obtained in Emisan treated seeds and minimum (two per cent) in Topsin M-70 treated seeds. A further increase in humidity in storage to 82.9 and 92.9 per cent resulted in complete germination failure (Table 25).

12 months

There was no considerable reduction in germination among treatments when seeds were stored under normal conditions and at 66.8 per cent humidity between periods of nine months and twelve months. However when the seeds were stored at 75.6 per cent humidity and above, all seeds failed to germinate. No significant difference in germination was there between control (40 per cent) and all other fungicidal treatments except Thiride (62 per cent) under normal conditions. The germination in all the treatments did not differ significantly when seeds were stored at the humidity level of 66.8 per cent (Table 26).

Table 26. Germination percentage of treated bhindi seeds stored for twelve months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	54	58	0	0	0
Thiride	62	66	0	0	0
Captafol	60	66	0	0	0
Dithane M-45	54	58	0	0	0
Topsin M-70	54	60	0	0	0
Emisan	60	68	0	0	0
Control	40	48	0	0	0

Cowpea

Blotter

In general, humidity level of 66.8 per cent was found to be the best for storage of cowpea seeds since more than 90 per cent germination was observed even after 12 months. The seeds in different treatments failed to germinate after six months of storage when the humidity level was more than 82.9 per cent.

Three months

When the seeds were stored under laboratory conditions maximum germination of 98 per cent was noticed in Thiride treatment and minimum 78 in untreated seeds. Thiride, captafol and Emisan treatments were on par and superior to all other treatments. The variation between treatments and control ranged from two(in Bavistin) to 20 (in Thiride) per cent. At 66.8 per cent humidity there was no considerable variation among the treatments and 100 per cent germination was obtained with the treatment of Bavistin, Thiride and captafol. Germination in all the treatments were on par with control. Eventhough a reduction in germination was noticed in all the treatments when they were stored at 75.6 per cent humidity compared to 66.8 per cent, it was highly significant (44 per cent reduction) in control, Dithane M-45 (34 per cent),

Topsin M-70 (17 per cent) and Bavistin (15 per cent). The highest germination of 96 per cent was noticed in seeds treated with Thiride. The germination of cowpea seeds at 82.9 per cent humidity was only four per cent less compared to those obtained at 75.6 per cent humidity in control (54 to 50) while it was 24 per cent in Dithane M-45 (64 to 40) and 23 per cent (91 to 68) in Emisan treated seeds while the least germination was in Dithane M-45 and it was on par with control. Maximum germination percentage of 87 was observed in seeds treated with Thiride, as observed in seeds stored at 82.9 per cent humidity. At 92.9 per cent humidity, the least germination was noticed in seeds treated with Dithane M-45 (36 per cent) which was inferior to all other treatments (Table 27).

Six months

The difference in the germination of fungicide treated cowpea seeds at the end of six months did rot differ significantly from that observed at the end of three months when the seeds were stored under normal laboratory conditions. The germination among different treatments including control also did not differ significantly (Table 28). In general, the germination percentage of the seeds stored at 66.8 per cent humidity was better than those stored under laboratory

Table 27. Germination percentage of treated cowpea seeds stored for three months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	80	100	85	80	64
Thiride	98	100	96	87	58
Captafol	96	100	95	78	60
Dithane M-45	88	98	64	40	36
Topsin M-70	88	93	76	67	42
Emisan	96	95	91	68	63
Control	78	98	54	50	48

Table 28. Germination percentage of treated cowpea seeds stored for six months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	86	94	60	0	0
Thiride	86	100	64	0	0
Captafol	88	100	70	0	0
Dithane M-45	86	96	62	0	0
Topsin M-70	80	92	56	0	0
Emisan	88	94	80	0	0
Control	76	90	40	0	0

conditions. The variation in the germination in different treatments was almost on par with those observed under similar conditions at the end of three months of storage. Hundred per cent of the seeds germinated in Thiride and captafol treatments while in control, there was 90 per cent germination. When the germination percentage of the seeds stored at 75.6 per cent humidity was compared with the seeds stored at 66.8 per cent humidity, a marked reduction was noticed in the seeds stored at 75.6 per cent humidity. A maximum of 50 per cent difference in the germination of seeds stored at 66.8 and 75.6 per cent humidity was observed in control (90 to 40 per cent) while the minimum of 14 per cent was in Emisan (94 to 80 per cent). All the fungicide treated seeds gave better germination percentage compared to control and treatment with Emisan was superior to all other treatments except captafol (70 per cent).

Nine months

The variation in the germination percentage of cowpea seeds after nine months under normal storage conditions with that observed at the end of six months ranged from zero (Dithane M-45) to 28 per cent in control (76 to 48 per cent). Germination percentage of seeds treated with Thiride, Bavistin, captafol and Dithane M-45 ranged from 78 to 86 per cent and they did not differ significantly from one another,

but they were significantly better than control, Topsin M-70 and Emisan. At 66.8 per cent humidity the difference in the germination percentage between the various treatments after six months and nine months ranged from zero (Emisan and Bavistin) to 10 per cent (Topsin M-70). Seeds treated with Topsin M-70 gave a germination percentage of 82 which was inferior to all other treatments including control at 66.8 per cent humidity. All other treatments did not differ significantly from one another. At 75.6 per cent humidity the highest germination percentage of 50 was observed in Emisan treated seeds which was significantly better than all other treatments. The germination percentage in all other treatments including control varied from 20 (control and Topsin M-70) to 30 per cent (captafol) which in turn were on par to one another (Table 29).

Twelve months

The germination percentage of cowpea seeds after storage for 12 months under normal storage conditions ranged from 42 per cent (control and Topsin M-70) to 74 per cent (captafol). All the fungicides excepting Topsin M-70 supported a higher germination percentage than control. There was no marked difference in the germination percentage of seeds stored at 66.8 per cent humidity after twelve months compared to those after nine months under similar conditions. All the

Table 29. Germination percentage of treated cowpea seeds stored for nine months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	80	94	26	0	0
Thiride	78	96	27	0	0
Captafol	84	96	30	0	0
Dithane M-45	86	92	26	0	0
Topsin M-70	64	82	20	0	0
Emisan	62	94	50	0	0
Control	48	88	20	0	0

treatments gave more than 80 per cent germination. The lowest germination was observed in Topsin M-70 (80 per cent) and the highest in Thiride and captafol (94 per cent). The difference among the treatments were not highly significant. A sudden decrease in germination was noticed after 12 months in seeds kept at 75.6 per cent humidity. None of the seeds treated with Bavistin, Topsin M-70 and control germinated, while the seeds treated with Emisan gave 20 per cent germination. All other treatments gave less than five per cent germination (Table 30).

Cowpea

Pot culture

The pattern of germination of cowpea seeds kept under different humidity levels and stored for varying periods, in soil was almost similar to that observed when the seeds were allowed to germinate by blotter method.

Three months

There was no significant difference in the germination of cowpea seeds under different treatments after three months under normal storage conditions. The germination rate varied from 88 per cent (control) to 95 per cent (Bavistin, Thiride and captafol). A similar trend was noticed in seeds kept at a fixed humidity of 66.8 and more than 90 per cent

Table 30. Germination percentage of treated cowpea seeds stored for twelve months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	68	92	0	0	0
Thiride	66	94	2	0	0
Captafol	74	94	4	0	0
Dithane M-45	64	90	3	0	0
Topsin M-70	42	80	0	0	0
Emisan	60	90	20	0	0
Control	42	84	0	0	0

germination was obtained in the different treatments. As the humidity was increased to 75.6 per cent the germination percentage decreased. When the germination percentage at 75.6 per cent humidity was compared with that at 66.8 per cent humidity, the maximum reduction of 38 per cent was noticed in control (90 to 52 per cent) (Table 31). Among the fungicides Thiride gave 87 per cent germination while the lowest was in Bavistin (62 per cent) which in turn was on par with control. At 82.9 and 92.9 per cent humidity the percentage germination obtained in various treatments was similar to that obtained at 75.6 per cent humidity with control and Bavistin giving the lowest germination. The performance of all other fungicides were on par with one another.

Six months

All the fungicide treated cowpea seeds gave a germination of 80 per cent or more after storage for six months under normal storage conditions. There was no significant difference in germination among the fungicidal treatments. Control with 64 per cent germination was significantly inferior to the other treatments. The reduction in germination in control compared to that observed after three months was 24 per cent (88 to 64). However, among the

Table 31. Germination percentage of treated cowpea seeds stored for three months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	95	95	62	56	48
Thiride	95	97	87	67	65
Captafol	95	98	81	68	64
Dithane M-45	92	98	81	66	62
Topsin M-70	94	96	81	62	60
Emisan	92	98	79	72	68
Control	88	90	52	46	44

fungicidal treatments Topsin M-70 treatment gave a maximum reduction of 14 per cent (94 to 80).

A similar trend was observed when the seeds were stored at 66.8 per cent humidity and 75.6 per cent humidity. The germination percentage was only 20 per cent in untreated seeds while it ranged from 40 (Topsin M-70) to 58 per cent (Dithane M-45) in the fungicide treated seeds when they were stored at 75.6 per cent humidity (Table 32).

Nine months

The reduction in the germination of cowpea seeds under normal storage conditions after nine months compared to six months was only marginal in all the fungicidal treatments except Topsin M-70 where 20 per cent reduction was noticed (80 to 60 per cent). In the control a reduction of 16 per cent (64 to 48) was observed. There was no significant difference among the various fungicides except Topsin M-70 which was inferior. A similar pattern was seen in seeds stored at a fixed humidity of 66.8 per cent. Under this condition all the treated seeds supported 80 per cent or more germination while in control 70 per cent germination was noticed (Table 33). Further increase in humidity to 75.6 per cent drastically reduced the germination. Eventhough all the fungicide treated cowpea seeds except Topsin M-70 germinated

Table 32. Germination percentage of treated cowpea seeds stored for six months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	82	92	44	0	0
Thiride	86	94	45	0	0
Captafol	86	92	56	0	0
Dithane M-45	82	94	58	0	0
Topsin M-70	80	90	40	0	0
Emisan	84	88	56	0	0
Control	64	74	20	0	0

Table 33. Germination percentage of treated cowpea seeds stored for nine months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	72	90	20	0	0
Thiride	72	88	20	0	0
Captafol	80	92	25	0	0
Dithane M-45	80	92	22	0	0
Topsin M-70	60	80	4	0	0
Emisan	82	88	35	0	0
Control	48	70	3	0	0

significantly better than the control, the maximum germination percentage observed was only 35 per cent in Emisan while in all others it was less than 25 per cent. When the germination of cowpea seeds after nine months at 75.6 per cent humidity was compared with that after six months, the reduction noticed was highly significant in Topsin M-70 where a reduction of 36 per cent (40 to 4) was noticed.

Twelve months

After storage for 12 months under normal storage conditions more than 54 per cent germination was obtained in all the treatments except Topsin M-70 (38 per cent) and control (28 per cent) (Table 34). There was no significant difference between Topsin M-70 treated seeds and untreated seeds, while all other treatments were on par. At 66.8 per cent humidity all the treatments were significantly superior to the control except Topsin M-70 treatment. Under this condition, Bavistin, captafol and Dithane M-45, treatments supported 90 per cent germination while the least germination among the treated seeds was in Topsin M-70 (76 per cent). When the seeds were stored at 75.6 per cent humidity only six per cent germination was obtained in Emisan. All the other seeds failed to germinate.

Table 34. Germination percentage of treated cowpea seeds stored for twelve months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	60	90	0	0	0
Thiride	60	88	0	0	0
Captafol	62	90	0	0	0
Dithane M-45	54	90	0	0	0
Topsin M-70	38	76	0	0	0
Emisan	60	86	6	0	0
Control	28	60	0	0	0

Dolichos bean

Blotter

The viability of dolichos bean seeds was retained when the seeds were stored for more than 12 months under normal storage conditions and at a fixed humidity of 66.8 per cent. The seeds lost their viability at the end of nine months when they were stored at a humidity of 82.9 per cent and above.

Three months

Cent per cent germination of dolichos bean seeds was observed after three months in Thiride treatment under normal storage conditions. All other treatments including control supported more than 87 per cent germination and were on par (Table 35). When the seeds were stored at 66.8 per cent humidity the germination ranged from 93 to 97 per cent and there was no significant difference among the treatments. At 75.6 per cent humidity also more than 80 per cent germination was observed in all the treatments and difference among treatments were not appreciable. Bavistin, Thiride and captafol supported 93 per cent germination. At 82.9 per cent humidity the treatments Bavistin, Thiride, captafol and Emisan were on par giving a germination above 87 per cent while the treatments Dithane M-45, Topsin M-70 and control with a germination of 60 to 70 per cent was inferior to the above treatments. At 92.9 per cent humidity

Table 35. Germination percentage of treated dolichos bean seeds stored for three months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	90	97	93	90	70
Thiride	100	97	93	90	65
Captafol	96	97	93	90	73
Dithane M-45	87	93	80	70	60
Topsin M-70	90	93	80	60	57
Emisan	88	93	90	87	67
Control	87	93	83	70	48

the fungicidal treatments did not differ significantly from one another. The least germination of 48 per cent was noticed in control and the highest germination of 73 per cent was observed in captafol treatment.

Six months

There was no significant difference in the germination of seeds when they were stored under laboratory conditions or at a humidity of 66.8 per cent. In both these cases the germination was more than 80 per cent in the various treatments (Table 36). At 75.6 per cent humidity, however, there was considerable reduction in the germination in all treatments compared to that observed at 66.8 per cent and 75.6 per cent humidity after storage for three months. A maximum reduction in germination of 80 per cent was noticed in seeds treated with Dithane M-45 and control (90 to 10 per cent) while the minimum 57 per cent was in Bavistin (90 to 33 per cent). The highest germination of 33 per cent was noticed in Bavistin and Thiride treated seeds while the lowest of 10 per cent was in Dithane M-45 and control. At 82.9 per cent humidity the minimum germination of three per cent was observed in Emisan treated seeds which was on par with all other treatments except Thiride (23 per cent) and captafol (17 per cent). A further increase in humidity to 92.9 per cent completely inhibited

Table 36. Germination percentage of treated dolichos bean seeds stored for six months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	86	90	33	13	7
Thiride	92	94	33	23	17
Captafol	92	96	27	17	7
Dithane M-45	87	90	10	9	7
Topsin M-70	80	88	17	10	7
Emisan	88	90	17	3	0
Control	88	90	10	7	0

the germination of seeds in control and seeds treated with Emisan. The Thiride treated seeds with a germination percentage of 17 was significantly better than all other treatments.

Nine months

Under normal storage conditions, at the end of nine months, seeds exposed to all treatments including control supported more than 75 per cent germination and this was almost similar to the germination rate observed under similar conditions after six months. The highest germination of 92 per cent was noticed in captafol treatment (Table 37). Almost similar germination rate was noticed when the seeds were stored at 66.8 per cent humidity also. However, there was no significant difference among the various treatments. Seeds treated with Bavistin, Dithane M-45, Emisan and control showed 88 per cent germination. At 75.6 per cent humidity there was a sudden decrease in the germination and there was no significant difference among the various treatments. Germination ranged from 24 (Emisan) to 10 per cent (Control).

Twelve months

There was practically no difference in the germination of seeds at the end of twelve months in the various treatments compared to that observed at the end of nine months for similar

Table 37. Germination percentage of treated dolichos bean seeds stored for nine months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	84	88	20	0	0
Thiride	88	90	20	0	0
Captafol	92	94	16	0	0
Dithane M-45	85	88	16	0	0
Topsin M-70	78	86	13	0	0
Emisan	82	88	24	0	0
Control	75	88	10	0	0

treatments under normal storage conditions. The variation in germination among the different treatments ranged from 70 per cent (Topsin M-70) to 90 per cent (captafol). There was no significant difference among the treatments at the end of 12 months, when the seeds were stored at a constant humidity of 66.8 per cent. The least germination of 77 per cent was recorded in Topsin M-70 treated seeds and the highest of 92 per cent in captafol. Seeds treated with Topsin M-70 and untreated seeds failed to germinate when they were incubated for 12 months at a constant humidity of 75.6 per cent. All other fungicidal treatments did not differ significantly from one another and the germination percentage was 10 per cent or less (Table 38).

Dolichos bean

Pot culture

Seeds of dolichos bean failed to germinate in soil after nine months storage under a fixed humidity of 82.9 per cent and above.

Three months

There was no significant difference in the germination of dolichos bean seeds when the seeds were incubated under normal storage conditions or at a fixed humidity of 66.8 per cent. The highest germination under normal storage condition was observed in Thiride (86 per cent) while at 66.8 per cent

Table 38. Germination percentage of treated dolichos bean seeds stored for twelve months at different humidity levels in blotter method.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	82	86	6	0	0
Thiride	86	88	10	0	0
Captafol	90	92	10	0	0
Dithane M-45	84	86	3	0	0
Topsin M-70	70	77	0	0	0
Emisan	84	86	10	0	0
Control	73	85	0	0	0

humidity, maximum germination (90 per cent) was in Emisan and captafol treated seeds. The reduction in the germination in seeds stored at 75.6 per cent humidity compared to that in 66.8 per cent humidity, was maximum in the control (86 to 60 per cent) and minimum in Bavistin (89 to 79 per cent). Captafol, Emisan and Bavistin supported a significantly higher germination rate than the untreated control. At the humidity level of 82.9 per cent the fungicides Thiride and Topsin M-70 were on par with the control while other treatments did not differ significantly from one another. At 92.9 per cent humidity all the fungicide treatments showed a germination of more than 50 per cent and significantly better than the control where the rate of germination observed was only 20 per cent (Table 39).

Six months

The germination percentage of the seeds under normal storage conditions at the end of six months varied only from 70 to 76 per cent and did not differ significantly from one another. Even at 66.8 per cent humidity, there was no difference among the treatments, however the germination percentage ranged between 77 (Topsin M-70) and 88 (Bavistin and Thiride). A marked reduction in the germination rate was observed when the seeds were stored at 75.6 per cent humidity. A maximum reduction of 73 per cent was observed in control (80 to 7) while the minimum of 55 per cent in Bavistin treated

Table 39. Germination percentage of treated dolichos bean seeds stored for three months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	80	89	79	77	67
Thiride	86	89	68	63	59
Captafol	83	90	88	80	68
Dithane M-45	83	87	77	70	50
Topsin M-70	76	83	65	60	54
Emisan	77	90	88	83	65
Control	75	86	60	50	20

seeds (88 to 33). The fungicides Topsin M-70 and Dithane M-45 which supported a germination of 15 and 18 per cent respectively were on par with the untreated control. The germination percentage in all other treatments varied from 25 to 33 per cent, but there was no significant difference among them. Eventhough a maximum germination of 33 per cent was observed in Bavistin at 75.6 per cent humidity it failed to support any germination when the seeds were stored at 82.9 per cent humidity. The germination percentage was less than 10 per cent in seeds treated with Topsin M-70, Emisan and control. When the humidity was increased to 92.9 per cent, the germination was decreased to zero per cent in all excepting Thiride and captafol treated seeds where the germination percentage was 17 and seven per cent respectively (Table 40).

Nine months

When the seeds were stored under normal storage conditions, after nine months, the germination of the seeds varied from 63 in control to 73 per cent in Thiride treated lot. However there was no significant difference among the treatments. At 66.8 per cent humidity the germination of all the seeds was marginally better than the one which was kept at normal conditions except in captafol where there was a five per cent reduction in germination. Under this condition,

Table 40. Germination percentage of treated dolichos bean seeds stored for six months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	72	88	33	0	0
Thiride	73	88	29	13	17
Captafol	73	86	25	17	7
Dithane M-45	76	80	18	10	0
Topsin M-70	70	77	15	7	0
Emisan	73	87	26	4	0
Control	70	80	7	6	0

all the treatments including control was significantly better than captafol. At 75.6 per cent humidity the germination per cent of seeds was decreased considerably and it ranged from six per cent in control to 21 per cent in Emisan. There was no significant difference among the various treatments (Table 41).

Twelve months

Seeds kept under normal storage conditions showed a germination between 57 (control) to 63 (captafol, Topsin M-70 and Emisan) per cent even after 12 months, but there was no significant difference among the treatments. In all the cases the germination percentage was higher when it was stored at 66.8 per cent humidity where the germination rate ranged from 63 to 80 per cent. The treatments, Bavistin, Thiride,captafol and Emisan gave 80 per cent germination and it was significantly superior to control. A further increase in humidity to 75.6 per cent, drastically reduced the germination of seeds. The seeds failed to germinate in untreated control and in Topsin M-70 while in other treatments the germination percentage ranged from two (Dithane M-45) to nine (captafol) per cent (Table 42).

Table 41. Germination percentage of treated dolichos bean seeds stored for nine months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.9
Bavistin	70	82	10	0	0
Thiride	73	80	12	0	0
Captafol	68	63	13	0	0
Dithane M-45	67	73	13	0	0
Topsin M-70	67	70	10	0	0
Emisan	68	70	21	0	0
Control	63	70	6	0	0

Table 42. Germination percentage of treated dolichos bean seeds stored for twelve months at different humidity levels in pot culture experiment.

Fungicides	Normal storage	Humidity levels (per cent)			
		66.8	75.6	82.9	92.7
Bavistin	60	80	3	0	0
Thiride	60	80	7	0	0
Captafol	63	80	9	0	0
Dithane M-45	60	77	2	0	0
Topsin M-70	63	70	0	0	0
Emisan	63	80	6	0	0
Control	57	63	0	0	0

Discussion

DISCUSSION

Seeds play a vital role for the healthy growth and higher production of a crop. They sometimes may carry fungal pathogens which cause heavy yield losses. The damages include reduced seed germination pre-and post-emergence killing and seedling blight. The severity of loss depends on the environmental conditions during storage as well as the loci of infection by the fungus in the seed. Seeds of high initial viability can resist unfavourable storage conditions better than similar seeds of low initial viability. Moisture content of the seed is one of the most important factor influencing seed viability during storage.

Seeds of bhindi, cowpea and dolichos bean were assessed for their initial germination and moisture content. They were found to be good as the germination percentage and moisture content were within the limits prescribed for seed certification (Table 1).

Association of mycoflora on the seed surface

A study on the association of mycoflora on the surface of the three vegetable seeds namely, bhindi, cowpea and dolichos bean were conducted during 1988-89 at the College of Horticulture, Vellanikkara.

A total of 18 species of fungi were isolated from bhindi seeds (Table 2). A perusal of literature revealed that among these, eight fungi namely Aspergillus flavus, Fusarium oxysporum, F. semitectum (Vidhyasekaran and Kandaswamy, 1980), F. solani (Ribeiro et al., 1971). Aspergillus niger, Rhizopus sp., Botryodiplodia theobromae (Naseema, 1981) and Penicillium sp. (Adisa and Aborisade, 1987) were reported earlier. Out of 22 species of fungi isolated from cowpea seeds (Table 3), eight species of fungi namely, Aspergillus sp, A. flavus, A. niger and Chaetomium sp. (Sinha and Khare, 1977), Fusarium sp. (Kanapathipillai, 1982), A. fumigatus and Rhizopus sp. (Gill et al., 1983) and Botryodiplodia (Barros et al., 1985) were reported earlier. From dolichos bean seeds a total of 18 fungi were isolated in the present study (Table 4). Among these the seed-borne nature of Aspergillus sp and Fusarium sp (Kanapathipillai, 1982), A. flavus, A. niger and Rhizopus sp. (Gill et al, 1983) were recorded earlier.

All the fungi isolated from the seed surface need not be pathogenic. Some of them might have been atmospheric contaminants. When these seeds were stored for longer periods under high humidity levels, even the nonpathogenic fungi were seen growing on the surface and causing reduction in germination of seeds.

Mycoflora associated with different parts of the seed

Different fungi could be observed either externally on the seed coat or internally in the endosperm or in embryo. On the seed coat, it could be seen either in the vegetative state (mycelia) or as spore. The fungi which were present on the surface of the seed surface may be pathogenic or contaminant. But those which were present inside the seed were mostly pathogenic in nature which may caused reduction in germination percentage or affected the growth and vigour of the plant.

Bhindi

Sixteen species of fungi were observed on the seed coat of bhindi (Table 5). Besides from the seed coat, several fungi were also observed in endosperm and embryo of seeds. From bhindi, eight species of fungi were isolated from the endosperm while three species were isolated from the embryo. Among the different fungi, only Alternaria sp and unidentified white sterile mycelium were present on embryo, endosperm and seed coat. This indicates that the above two fungi have a close relationship with bhindi seeds. Alternaria sp. was established as a pathogen of bhindi by Docea and Coroianu (1982). It is evident that Alternaria sp is a common fungus which causes the deterioration of bhindi

seeds. Fusarium oxysporum was isolated from the seed coat and from embryo while it was not observed in the endosperm. The association of fungi in embryo generally hampers the germination and growth of the seed. Vidhyasekaran and Kandaswamy (1980) reported that F.oxysporum could cause wilting in bhindi. But the association of fusarial wilt pathogen in seed has not been studied in detail by previous workers. Since the fungus is seen in association with embryo of the seed it indicates that it may affect the germination and growth of the plant. Detailed investigation on this line is necessary to find out the role of this fungus in the deterioration of seed and vigour of the plant.

Cowpea

A total of 17 species of fungi were isolated from the seed coat of cowpea (Table 6). The endosperm of cowpea yielded 14 species of fungi while five species were isolated from embryo. Aspergillus flavus, Fusarium oxysporum, Penicillium implicatum and an unidentified white sterile mycelium were present both on embryo and endosperm of cowpea and so it may affect germination of the seed. A.flavus when artificially inoculated caused complete inhibition of germination (Table 9). Studies of Rati and Ramalingam (1974) on seeds of cowpea also showed the pathogenic nature of A. flavus.

Dolichos bean

Six species of fungi were isolated from the endosperm and two from the embryo of dolichos bean (Table 7). Only the white sterile mycelium was isolated from the seed coat, endosperm and embryo. Fusarium oxysporum was isolated from the seed coat and embryo but was not isolated from the endosperm. The white sterile mycelium and F. oxysporum may have pathogenic effect on the seed. The pathogenic effect of white sterile mycelium was not worked out because it failed to sporulate in the culture medium. F. oxysporum eventhough was isolated from the embryo and seed coat it caused only three per cent inhibition in germination on artificial inoculation (Table 9). Thus it is clear that the mere presence of a fungus on embryo need not cause considerable reduction in germination. According to Prasanna (1985), in seeds with deep seated infection where the fungus is present in endosperm or embryo, fungal growth were not observed on the agar plates even though they were observed when isolations were made from the separated embryo and endosperm. This difference may be attributed to the lack of uptake of water by intact seeds on agar medium and consequent failure of the fungus to grow out of the seed.

Association of mycoflora on the seed surface need not necessarily be due to an interaction between the fungus

and the seed. If the fungi are observed either in embryo or endosperm it shows that these fungi have a close association with the seed. This association can be beneficial or detrimental to the germination of the seed and growth of the plant. Further study on this line is essential to throw more light on the exact nature of relationship of the internally seed-borne mycoflora.

Failure of germination due to mycoflora

The inhibitory effect of seed mycoflora on the germination of seeds was in general pronounced when tested under laboratory conditions.

Bhindi

Eventhough Alternaria sp was isolated from the seed coat, endosperm and embryo, it did not markedly influence germination of bhindi seeds (Table 9). Fusarium oxysporum which was present on the embryo and seed coat reduced the germination. The maximum reduction in germination was observed when the seeds were dipped in the spore suspension of F. solani, a fungus found in the endosperm and seed coat. F. pallidoroseum which was observed only on the seed coat also reduced the germination considerably. These observations clearly indicate that F. spp are responsible for the reduction in seed germination when they are present either on the seed coat or inside the seed. The inhibitory nature

of chemicals produced by Fusarium sp. is well documented (Heinz Kern, 1972). The reduction in seed germination by Fusarium spp was reported earlier by Naseema (1981) and Robbs et al. (1972).

Cowpea

Cent per cent inhibition of germination of cowpea was observed when the seeds were exposed to Aspergillus flavus, A. niger and A. fumigatus (Table 9). Of these, A. flavus was isolated from the embryo and endosperm and A. fumigatus from the endosperm. The inhibitory nature of A. spp was established earlier by Naseema (1981). Cladosporium herbarum, Alternaria sp. and Botryodiplodia sp. caused 80 to 90 per cent inhibition. The inhibitory nature of B. sp was recorded by Nath et al. (1970) and Naseema (1981). Suryanarayana (1978) observed the inhibitory nature of Alternaria sp. on pea seed. Thus the present finding is in confirmity with that of earlier workers.

Dolichos bean

Dolichos bean seed germination was not influenced by the presence of most of the fungi found on the seed surface. The maximum reduction in germination was noticed when the seeds were dipped in the spore suspension of Botryodiplodia sp. and Cochliobolus geniculatus (50 and 40 per cent respectively) (Table 9). Both these fungi were

isolated from the endosperm. There was no reduction in germination when the seeds were dipped in the spore suspension of Aspergillus spp. Nath et al. (1970) observed B. theobromae to be responsible for seed rot in mungbean while Ellis and Smith (1978) stated that B. theobromae reduced seed germination in pigeon pea. The same fungus caused seed rot and seedling blight in horse gram (Maholay and Sohi, 1977).

In the present investigation most of the fungi isolated from dolichos bean seeds did not decrease germination. The inability of the fungi to penetrate into the thick seed coat of dolichos bean seeds may be the possible reason for this. Aspergillus flavus is a principal group of fungus producing aflatoxin (Blount, 1961) which in turn may affect the germination of seeds. In the present investigation 100 per cent inhibition in germination of cowpea seeds was observed when they were dipped in the spore suspension of A. flavus, A. niger and A. fumigatus (Table 9). Hence aflatoxin produced by Aspergillus spp may be the cause for suppression of germination of seeds. This finding is in confirmity with Nair (1982) who reported that reduced percentage of germination of cucurbitaceous seeds was due to the aflatoxins produced by species of Aspergillus. The findings of Joffe (1969), Schroeder (1969),

Lalithakumari *et al.*(1972), Kang *et al.* (1971) and Thomson and Mehdy (1978) clearly indicated that *A.flavus* group are the principal producers of aflatoxin. The studies of Naseema (1981) showed that maximum aflatoxin production was by the isolate of *A. flavus* obtained from cucurbitaceous seeds while the least quantity was produced by isolates from bhindi, brinjal and cowpea. Amaranthus isolate was in between these extremes. This clearly shows that *Aspergillus* sp. behaved differently as far as aflatoxin production was concerned. In the present study *A. flavus* present in dolichos bean and *A. niger* and *A. fumigatus* present on bhindi did not cause marked reduction of the germination which may be due to the inability of these fungi to produce toxic metabolites inhibitory to germination of seeds.

Mycoflora associated with the seeds after different periods of storage

The association of different fungi with the seed showed a varying pattern depending upon the period of storage. The fungi isolated from the seed without surface sterilization are assumed to be both externally as well as internally seed-borne. Whereas those isolated from seeds which are surface sterilized with alcohol are assumed to contain only the fungus present on endosperm and embryo.

In bhindi, Aspergillus flavus, A. fumigatus, Fusarium oxysporum and Rhizopus sp. were found to be associated with the seed for a period of 12 months (Table 10). All these fungi were observed either on the endosperm or in the embryo (Table 5). Absidia corymbifera, Alternaria sp., Paecilomyces variotii and the white sterile mycelium which were isolated from the endosperm or embryo were found on sterilized seeds upto nine months of storage. Botryodiplodia sp. and the black sterile mycellium which were observed initially could be isolated only upto three months of storage. They were present only on the surface of the seed. Thus the survival of the fungi is better when they were present internally either in the embryo or endosperm rather than on the seed coat. Maude (1972) also reported similar findings.

From cowpea seeds, Aspergillus flavus, Fusarium oxysporum, Rhizopus sp. and white sterile mycelium were isolated from the endosperm or embryo and they survived even after storage for 12 months (Table 11). Chaetomium brasiliense was isolated from the unsterilized seeds after 12 months storage, but it was not seen on the endosperm or embryo. Penicillium implicatum and Alternaria sp though isolated from the embryo, survived only for three months.

Fusarium oxysporum and the white sterile mycelium were isolated from the embryo of dolichos bean (Table 7). Of these, the white sterile mycelium was detected upto nine months of storage and F. oxysporum upto 12 months (Table 12). Alternaria sp. survived upto nine months, Aspergillus flavus upto 12 months, Botryodiplodia sp. upto nine months and black sterile mycelium upto three months. All these were isolated from the endosperm. Rhizopus sp. and Aspergillus niger though detected after 12 months storage was not present on embryo or endosperm.

Fungus survival depends on the host species. The longevity of seed-borne pathogens can be independent of the seeds they inhabit and depends upon the capability of the pathogen to remain viable as well as virulent from one season to the next in or on the seeds.(Agarwal and Sinclair, 1987). Ascochyta pisi survived in broad bean seeds for nine years with survival varying among seed lots (Sprague, 1929). The present study indicated that the survival of the seed-borne pathogens depends on apart from the storage condition, whether they are present inside or outside the seedcoat. Further studies on this aspect are required to get more information.

Germination studies in storage at different humidity levels

The relative humidity content of the atmosphere where the seeds are stored plays an active role in their viability. Variation in the chemical composition of the seeds are known to be responsible at least partly for the amount of moisture absorbed by them even under identical conditions of storage (Barton, 1961). The moisture content of the seed in turn will influence the mycoflora. Seed deterioration leading to loss of germination progressed very rapidly at a higher humidity, when the seeds were stored at fluctuating temperature conditions. Similar observations are also reported by Harrington (1973).

Bhindi

When the seeds were stored under normal condition, 60 per cent germination was seen after three months of storage but only 45 per cent germination was obtained after 12 months storage. There was three to eight per cent increase in germination when the seeds were stored at the humidity level of 66.8 per cent. The viability of seeds was completely lost after six months storage under the humidity levels of 82.9 and 92.9 per cent and after 12 months at the humidity level of 75.6 per cent (Table 13).

In cowpea maximum germination of 98 per cent was recorded in seeds kept at the humidity level of 66.8 per cent

after three months (Table 14). Under normal storage conditions the germination percentage was slightly less than that at 66.8 per cent humidity after three months storage. There was complete loss of viability after six months at the humidity level of 82.9 and 92.9 per cent and after 12 months at the humidity level of 75.6 per cent.

When dolichos bean seeds stored under different humidity levels were subjected to germination studies, it was observed that there was not much difference between the seeds kept at 66.8 per cent humidity and under normal storage condition, the difference being ranged from two to 13 per cent. There was complete loss of viability when the seeds were stored at 92.9 per cent humidity for six months, at 82.9 per cent humidity for nine months and 75.6 per cent humidity for 12 months (Table 15).

The reduction in germination of seeds stored under increasing humidity percentage was established by various workers with different types of seed (Nandi et al., 1982; Dange and Patel, 1984; Komaraiah and Reddy, 1986 and Rao and Reddy, 1987). The fungi detected on seeds during storage under varying humidity conditions are mainly storage moulds group like species of Aspergillus, Penicillium and Rhizopus. These fungi were found to cover the seeds after nine months

of storage under high humidity which in turn cause deterioration of the seeds. The results of the present studies are in confirmity with the findings of the above workers.

Effect of fungicidal treatment on the viability of vegetable seeds at different intervals of storage

The widely used control practice against seed-borne diseases is treatment of seeds with fungicides. The protective coating thus provided around the seed coat, act as a barrier, to ward off attack by both seed-borne and soil-borne microorganisms once the seed is planted.

Bhindi

In bhindi, no significant difference was noticed between treated and untreated seeds after three, six and nine months of storage when the germination per cent was assessed by blotter method as well as by pot culture experiment (Table 16). But Emisan, captafol and Thiride gave better germination in blotter method. In pot culture experiment Thiride, captafol and Dithane M-45 were better. All fungicidal treatments gave better germination than control after storage for 12 months.

Cowpea

When germination percentage of treated cowpea seeds was examined after three months storage, Thiride, captafol and Emisan were found to be superior than all other treatments in blotter method, whereas in pot culture experiment there was no significant difference between treatments and control. At the end of six months there was no significant difference among the treatments in blotter method but in pot culture experiment all fungicides were better than control. Dithane M-45, captafol, Bavistin and Thiride were superior to control after nine months in the blotter test while in pot culture experiment, Topsin M-70 and control were on par and significantly inferior to all other treatments. After storage for twelve months, Topsin M-70 and control were on par and significantly inferior to all other treatments in blotter method and pot culture experiment (Table 17).

In dolichos bean, Thiride was found to be superior to all other treatments including control in blotter method after three months of storage whereas in pot culture, all treatments were on par with control. There was no significant difference among treatments after six months in blotter method and in pot culture experiment after storage exceeding six months (Table 18).

In general Thiride was found to be good as a seed treating fungicide in bhindi, cowpea and dolichos bean. Nene and Thapliyal (1979) opined that thiram is one of the most effective seed protectants and compared to mercuries, it is least phytotoxic. The effectiveness of Thiride as a seed treating fungicide is well documented (Kaul, 1973; Sarode and Kadam, 1977; Sinha and Khare, 1977, Mercer and Kisyombe, 1978 and Siddaramaiah et al., 1982).

Effect of seed treatment on the viability of vegetable seeds at different storage periods and humidity levels

Bhindi

The germination percentage of bhindi seeds treated with fungicides was better than the untreated control. As the humidity was increased a reduction in germination was obtained in all the treatments.

The germination percentage at the end of three months was maximum in seeds treated with Emisan and kept at the humidity level of 66.8 per cent while the least was in untreated control at the humidity level of 92.9 per cent when tested by blotter method. There was no significant difference in the germination percentage of treated and untreated seeds when they were stored at a constant humidity of 66.8 and 75.6 per cent in blotter method and put culture

experiment. At 82.9 per cent humidity, Thiride, captafol and Emisan were better than all other treatments including control in blotter method while in pot culture, captafol, Thiride, Bavistin and Emisan were better than Dithane M-45, Topsin M-40 and control. At the humidity level of 92.9 per cent in both blotter method and pot culture all the fungicides except Topsin M-70 were better than control.

When bhindi seeds were stored for six months and above at the humidity level of 66.8 per cent, there was no significant difference among treatments both in blotter method as well as pot culture experiment. At the humidity level of 75.6 per cent fungicidal treatment was on par with control in blotter method while in pot culture experiment captafol and Thiride were superior after six months and Emisan and captafol after nine months of storage. Germination was completely lost after six months at humidity levels of 82.9 per cent and above.

The efficacy of fungicide was not pronounced upto 75.6 per cent humidity. This may be due to the fact that at a humidity level of 75.6 or less the growth of fungi on the seed surface was less compared to that at higher humidities. At higher humidity levels of 82.9 and 92.9 per cent, the germination percentage decreased due to the abundant growth of the fungus on the seed surface and due to its toxic effect

on the seed. Fungicides like Thiride, captafol and Emisan checked the growth of the fungus to some extent thereby increasing germination of the seeds. The efficiency of the fungicide was better expressed at the highest humidity level of 92.9 per cent. Among the fungicides tested Topsin M-70 was the least effective at 92.9 per cent humidity while at 82.9 per cent humidity both Topsin M-70 as well as Dithane M-45 were ineffective. Kuiper (1974) reported that prolonged storage of seeds treated with maneb results in lesser seed germination. He also stated that excessive heat or moisture in the storage may cause decomposition of maneb. The reduction in the residual fungitoxicity of Dithane M-45 at high humidity level has been well documented (Nene and Thapliyal, 1971). In the present study Topsin M-70 was found to be ineffective as a seed treating fungicide but Diaconu (1979), Sesan and Dumitras (1979) reported that Topsin M-70 was the most effective fungicide for seed treatment in cucumber and kidney bean. Thiride was found to be effective in checking the fungus growth and enhancing the seed germination.

Cowpea

The cowpea seeds stored at 66.8 per cent humidity for three months gave 90 per cent or more germination in all treatments including control in blotter method as well as

in pot culture experiment. At 75.6 per cent humidity Thiride, captafol and Emisan were better while Dithane M-45 was least effective in blotter method. In pot culture experiment all the fungicides except Bavistin was superior to control. Highest germination was observed in Thiride treated seeds at the humidity level of 82.9 per cent in blotter method wereas Dithane M-45 was least effective. Bavistin was significantly better than control in the blotter method but it was not effective in pot culture experiment. Almost a similar trend was noticed at 92.9 per cent humidity in pot culture experiment.

At the end of six months all treatments supported 90 per cent or more germination at 66.8 per cent humidity in blotter method. At 75.6 per cent humidity all the treatments were better than control in blotter method as well as pot culture experiment.

After storage for nine months at the humidity level of 66.8 per cent all fungicides were found to be better than control in blotter method. Among these Thiride and captafol were the most promising. In pot culture all fungicides except Topsin M-70 were significantly better than control. At 75.6 per cent humidity Emisan was significantly superior to all other treatments including control in blotter method.

In pot culture experiment all the fungicides except Topsin M-70 were superior to control and were on par.

When cowpea seeds were stored for 12 months at the humidity level of 66.8 per cent all the fungicides tested did not differ significantly and supported more than 80 per cent germination in blotter method while in pot culture experiment all the treatments except Topsin M-70 were significantly superior to control. More thar 75 per cent germination was noticed in all the seeds treated with different fungicides.

Thus in cowpea also fungicide treatment was better than control. Thiride was found to be superior in the blotter method, while, Thiride, captafol and Dithane M-45 were effective in pot culture. The above findings are in confirmity with that of earlier workers (Sinha and Khare, 1977; Oladiran and Okusanya, 1980).

Dolichos bean

In general, captafol, Bavistin and Thiride gave better germination percentage in dolichos bean. With increase in humidity a decrease in germination was observed. The seeds lost their viability after storage for nine months at the humidity level of 82.9 per cent and above.

After three months storage at the humidity level of 66.8 per cent no significant difference was noted between treated seeds and control in blotter method as well as in pot culture experiment. At the humidity level of 75.6 per cent Dithane M-45 and Topsin M-70 treated seeds gave germination percentage less than control and the efficacy of other fungicides was also not pronounced compared to control. In pot culture experiment, captafol, Emisan, Bavistin and Dithane M-45 were superior to control. When the humidity level was increased to 82.9 per cent, Bavistin, Thiride, captafol and Emisan were on par and superior to all other treatments including control in blotter method. In pot culture experiment all fungicides except Thiride and Topsin M-70 were significantly superior to control. There was significant difference between treatments and control at 92.9 per cent humidity in the blotter method, while in pot culture, captafol, Bavistin, Emisan, Thiride and Topsin M-70 were on par and superior to control.

After storage for six months there was no significant difference between treated seeds and control both in blotter method and pot culture experiment at the constant humidity level of 66.8 per cent where all the treatments gave more than 77 per cent germination. At 75.6 per cent humidity Bavistin, Thiride and captafol were better than control and

Dithane M-45. Germination percentage in all the treatments were less than 33 per cent. At 82.9 and 92.9 per cent humidity levels, germination percentages were considerably reduced. At 82.9 per cent humidity Thiride gave maximum of 33 per cent germination in blotter method and in pot culture maximum germination percentage of 17 was in captafol treatment. At 92.9 per cent humidity there was no germination in Emisan and control in blotter method and in all treatments except Thiride and captafol in pot culture experiment.

At the constant humidity level of 66.8 and 75.6 per cent, after nine months, there was no significant difference between treatments and control in blotter method and pot culture experiment. All treatments supported more than 86 per cent germination at the humidity level of 66.8 per cent in blotter and more than 70 per cent in pot culture experiment. At 75.6 per cent humidity germination was drastically reduced and it was less than 25 per cent in blotter method and 21 per cent in pot culture experiment.

At the end of twelve months, there was no significant difference between fungicidal treatment and control in blotter method while in pot culture experiment, all treatments were better than control at the humidity level of 66.8 per cent. At 75.6 per cent humidity none of the seeds germinated in

in the treatments with Topsin M-70 and control in both blotter method and pot culture experiments.

The efficacy of captafol was the better pronounced in this investigation for controlling the seed mycoflora associated with dolichos bean. This is in agreement with findings of Kumar and Srivastava, 1985 and Roberti et al.,1985.

Seed deterioration leading to loss of germination progressed very rapidly at higher humidity and temperature levels (Harrington, 1973). At higher humidity, seed moisture provided conditions favourable for spread and establishment of fungi which eventually caused excess heating. At high humidity level of 80 per cent and above invasion by storage fungi was much more severe as these fungi are known to be very active under such conditions (Nandi et al., 1982). Invasion of seeds by storage fungi lead to an escalating process involving increase in respiration, water content and temperature, resulting in damage to the embryo and eventually affecting seed germination to a great extent (Christensen, 1973; Neergaard, 1977). Loss of viability of stored seeds are associated with the rate of respiration during storage. The moisture content of the seeds could increase by respiration of microflora on the seeds, especially storage fungi,

when heavy invasions occurred (Kabeere and Taligoola, 1983). In the present study also, in seeds stored at high humidity, there was considerable reduction in germination of seeds. At higher humidity levels there was drastic reduction even after storage for three months. Gorecki and Jagielski (1982) also reported that seeds of pea, field bean and yellow lupin at 90 per cent relative humidity exhibited a marked decrease in germination capacity and vigour after only two months storage.

Summary

SUMMARY

"Bio-deterioration of important vegetable seeds due to mycoflora-II" was studied during 1988-89 at the Department of Plant Pathology, College of Horticulture, Vellanikkara. The three vegetable seeds used were bhindi var. Pusa sawani, cowpea var. Kanakamony and dolichos bean var. Pusa Early Prolific collected from the Department of Olericulture, College of Horticulture, Vellanikkara.

The seeds were examined for their initial germination, moisture content and associated mycoflora. The germination percentages were 70 and 68 for bhindi, 100 and 98 for cowpea and 89 and 86 for dolichos bean in blotter method and pot culture experiment respectively. The moisture content were 9.43 per cent in bhindi, 8.66 per cent in cowpea and 8.3 per cent in dolichos bean. The germination percentages and moisture content were within the permissible limits of seed certification. When isolations were made by agar plate method 18 and 11 species of fungi were found on the unsterilised and surface sterilised seeds of bhindi respectively. Twenty species of fungi were isolated from unsterilised cowpea seeds while 13 species were recorded

from the surface sterilised seeds. The corresponding figures for dolichos bean was 15 and 14 respectively.

Seed coat, endosperm and embryo of the seeds were dissected out and isolations were made on PDA. Sixteen species of fungi were obtained from the seed coat, eight species from endosperm and three species from the embryo of bhindi seeds. Seventeen, 14 and five species of fungi were associated with the seed coat, endosperm and embryo of cowpea respectively. In dolichos bean the corresponding figures were 17, six and two.

Ungerminated seeds in the pot culture experiment were collected and examined for the associated mycoflora by the agar plate method. Fourteen species of fungi were isolated from the ungerminated seeds of bhindi, 11 from cowpea and 12 from dolichos bean.

The inhibitory effect of seed mycoflora on the germination of seeds was studied and it was found that Fusarium oxysporum and Syncephalastrum racemosum caused maximum inhibition in germination of bhindi. In cowpea Aspergillus flavus, A. niger and A. fumigatus caused cent per cent inhibition. Most of the fungi did not cause considerable reduction in germination in dolichos bean and

maximum inhibition in germination was by Botryodiplodia sp (50 per cent).

Survival of mycoflora during storage was assessed and it was observed that in bhindi Aspergillus flavus, A. niger, A. fumigatus, Fusarium oxysporum and Rhizopus sp. persisted even after storage for 12 months. In cowpea Aspergillus flavus, Chaetomium brasiliense, Fusarium oxysporum, Rhizopus sp. and a hyaline mycelium without any spore were isolated after 12 months storage. Aspergillus flavus, A. niger, Fusarium oxysporum and Rhizopus sp. were found after storage for twelve months in dolichos bean.

Investigations on the viability of seeds during storage at different humidity levels showed that maximum germination was in seeds stored at the humidity level of 66.8 per cent in all the three seeds. In bhindi and cowpea viability was completely lost after storage for six months at the humidity levels of 82.9 per cent and above and after 12 months at the humidity level of 75.6 per cent. In dolichos bean, there was complete loss of viability after six months at the humidity level of 92.9 per cent, after nine months at the humidity level of 82.9 per cent and after 12 months at the humidity level of 75.6 per cent.

Effect of fungicides on the viability of seeds during storage under normal storage conditions were studied and t

was found that in general fungicide treated seeds gave better germination percentage than control. In bhindi, there was no significant difference between treatments and control in both blotter method and pot culture experiment after three, six, nine and twelve months storage. But after 12 months storage, all treatments were found to be better than control in both blotter method and pot culture experiment.

In cowpea, after three months storage Thiride, Captafol and Emisan treatments were superior to all other treatments in blotter method, while in pot culture, there was no significant difference between treatments and control. At the end of six months there was no significant difference between treatments and control in blotter while in pot culture, all fungicidal treatments were better than control. Germination studies after nine months storage showed that Dithane M-45, captafol, Bavistin and Thiride were superior than all other treatments in blotter method and Emisan, captafol, Dithane M-45, Bavistin and Thiride were significantly better than control in pot culture experiment. After storage for 12 months captafol, Bavistin, Thiride, Dithane M-45 and Emisan were significantly superior in blotter method while captafol, Bavistin, Thiride, Emisan and Dithane M-45 were significantly better than control in pot culture experiment.

In dolichos bean, thiride was found to be superior after three months of storage in blotter method, all treatments were on par with control after storage exceeding six months in blotter method and in all storage periods in pot culture.

The efficacy of fungicides at different humidity levels after different storage periods were investigated and it was concluded that seeds should be stored at the humidity level of 66.8 per cent. At this level of humidity there was no significant difference between fungicidal treatments and control after three, six, nine and twelve months storage in the case of bhindi in both blotter as well as pot culture.

In cowpea, after three months at the humidity level of 66.8 per cent Bavistin, Thiride, captafol, Dithane M-45 and control were better than Topsin M-70 and Emisan in blotter method and in pot culture all fungicides were better than control. At the end of six months Thiride and captafol were significantly superior than control in blotter method while in pot culture all fungicidal treatments gave germination percentage better than control. When the storage period was increased to nine months Topsin M-70 treatment was found inferior and all other treatments

were on par in blotter method while in pot culture, all fungicidal treatments were better than control. Study of germination percentage after 12 months showed that Dithane M-45 treatment was significantly inferior in the blotter method while in pot culture Bavistin, captafol, Dithane M-45, Thiride and Emisan were on par and significantly better than control.

In dolichos bean at 66.8 per cent humidity, there was no significant difference between treatments and control after three and six months storage in both blotter method and pot culture experiment. At the end of nine months all the fungicidal treatments were better than control in blotter method and in pot culture the germination percentage of captafol treated seeds was inferior. After storage for 12 months there was no significant difference between treated and untreated seeds in blotter method while in pot culture, Bavistin, Thiride, captafol and Emisan treatments were superior.

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* Originals not seen

BIO-DETERIORATION OF IMPORTANT VEGETABLE SEEDS DUE TO MYCOFLORA II

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ABSTRACT OF A THESIS

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ABSTRACT

"Bio-deterioration of important vegetable seeds due to mycoflora-II" was studied at the Department of Plant Pathology, College of Horticulture, Vellanikkara during the year 1988-89. Three vegetable seeds viz., bhindi (Abelmoschus esculentus (L.) Moench), cowpea Vigna unguiculata (L.) Walp) and dolichos bean (Dolichos lablab L.) were used for the investigations.

The objectives of the investigations were 1. To assess the role of seed-borne mycoflora and bio-deterioration of important vegetable seeds (bhindi, cowpea and dolichos bean) 2. To study the influence of seasons on the association of seed-borne mycoflora of stored vegetable seeds 3. To assess the effect of relative humidity on the viability of vegetable seeds 4. To study the role of seed mycoflora on the germination of seeds and 5. To find out the effect of fungicides to minimise the fungal bio-deterioration of stored vegetable seeds.

The initial germination per cent and moisture content of the seeds were within the permissible limits of seed certification. On isolation by agar plate method 18 and 11 species of fungi were found on the unsterilized and surface sterilized seeds of bhindi. From cowpea 20 species of fungi were isolated from the unsterilized seeds while 13 species were recorded on

the surface sterilized seeds. The corresponding figures for dolichos bean were 15 and 14 respectively.

From bhindi, 16 species of fungi were obtained from the seed coat, eight from endosperm and three from embryo. The separated seed coat, endosperm and embryo of cowpea yielded 16, 14 and five species of fungi. Seventeen, six and two species of fungi were found on the seed coat, endosperm and embryo of dolichos bean. Ungerminated seeds of bhindi yielded 14 species of fungi, cowpea 11 and dolichos bean 12 species respectively when isolations were made by agar plate method.

Maximum inhibition in germination of seeds was caused by Fusarium oxysporum in bhindi, Aspergillus flavus, A. fumigatus and A. niger in cowpea and Botryodiplodia sp in dolichos bean. After storage for 12 months, A. flavus, A. fumigatus, F. oxysporum and Rhizopus sp was isolated from bhindi seeds, A. flavus, Chaetomium brasiliense, F. oxysporum, R. sp. and a hyaline mycelium without any spore from cowpea and A. flavus, A. niger, F. oxysporum and R. sp. from dolichos bean seeds.

Among the different humidity levels, maximum germination was in seeds stored at the humidity level of 66.8 per cent in all the three seeds. Captafol and Thiride can be used for keeping the viability of bhindi, cowpea and dolichos bean seeds under normal storage conditions. Humidity level of 66.8 per cent

and treatment with captafol, Emisan or Thiride will keep the viability of bhindi seeds, humidity of 66.8 per cent and treatment with Thiride, captafol or Bavistin in cowpea, humidity level of 66.8 per cent and treatment with captafol, Thiride, Bavistin or Emisan were good for storage of dolichos bean seeds.