BIO-DETERIORATION OF IMPORTANT CUCURBITACEOUS SEEDS DUE TO MYCOFLORA

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Bio-deterioration of important Cucurbitaceous seeds due to mycoflora" is a bonafide record of research work done by me during the course of research work and 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 entitled "Bio-deterioration of important Cucurbitaceous seeds due to mycoflora" is a record of research work done independently by Smt. Saleena George 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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SALEENA GEORGE

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Introduction

INTRODUCTION

One of the accepted methods for vegetable production is the use of improved seeds which are free from seed borne diseases and pests. For laying down health standards against seed borne diseases of vegetables, it is necessary to have background information such as the mycoflora associated with the seed materials and their nature and extent of damage in natural as well as controlled conditions. Earlier, Suryanarayana and Bhombe (1961) and Suryanarayana et al. (1961) have studied the seed mycoflora of certain common Indian vegetable seeds. In Kerala, high relative humidity prevails in most part of the year, resulting bio-degradation of vegetable seeds especially cucurbitaceous seeds during storage.

Seed borne fungi constituted the main group of pathogen which reduce germinability of seed. The damage results in pre-emergence killing, seedling blight and diseased seedlings usually caused during seed germination process. When seed germination begins, the fungi which remain quiescent during seed dormancy become active, kill seeds and produce diseased seedlings. The severity of fungus injury depends on the environmental condition during storage as well as the locality of fungi in the seed. Some fungi kill the seed before germination, probably while the seed is still attached to the mother plant. It can be assured that damage caused by the pathogen to the seed germination potential is caused by metabolites secreted by the fungi.

The principal purpose of storing seeds is to preserve planting stocks from season until the next. Barton (1941) found that seeds of high initial viability resisted unfavourable storage condition better than similar seeds of low initial viability. The most obvious pre-harvest factor affecting seed viability and storability is weather, especially seasonal change.

Relative humidity and temperature are the most important factors determining the storage life of seeds. Seeds attain a rather specific and characteristic moisture content when subjected to given levels of atmospheric humidities. This characteristic moisture content is referred to as equilibrium moisture content. Equilibrium moisture content, for a particular kind of seed at a given relative humidity, tends to increase as temperature decreases and as deterioration progresses. Thus the maintenence of seed moisture content during storage is a function of relative humidity and to a lesser extent of temperature.

Moisture content of seeds during storage is the most influencial factor affecting seeds longevity. Although it is important to reduce seed moisture content to a safe level for storage, it is also important to be aware of possible adverse effect of low moisture content. Very dry seeds are susceptible to mechanical breakage resulting in physical breakage or fracturing of essential seed parts which make the seed vulnerable to fungal attack and reduced storage potential.

The vigour of seeds at the time of storage is an important feature, that affects their storage life. Vigour and viability cannot always be differentiated in storage experiments, especially in seed lots that are rapidly deteriorating.

Deterioration of stored seeds by fungi is controlled principally by drying the seeds to a safe moisture content prior to storage. Most storage fungi cannot invade seeds that are in moisture equilibrium with 65 per cent relative humidity or lower. One of the traditional practice of the farmers in Kerala, is to protect the seeds by smearing wood ash before storing. Several fungicides are also used to combat the fungal infection in seeds.

Cucurbitaceous vegetables are extensively cultivated and used in this State. Since not much studies have been undertaken in this vegetable seeds with regards to storage. It is decided to perform the present study.

Even though a lot of literature are available in seed mycoflora of different vegetable seeds and other seeds, works on mycoflora of cucurbitaceous seeds in respect of their nature, the loss caused, the role of these organisms in different environmental and storage intervals and their control are lacking. Furthermore, most of the cucurbitaceous vegetables are grown in Kerala for seed purpose only during summer and so preserving these seeds for one year to the next season is

of prime importance. The infection with field as well as storage fungi will deteriorate its germination and may kill the germinating seeds and seedlings as pre and post-emergence rot. Therefore, the present study was conducted with the following objectives.

1. To assess the role of seed borne mycoflora on bio-deterioration of important cucurbitaceous seeds,
2. To find out the variation in seed mycoflora of stored seeds in different periods of the year.
3. To assess the role of different humidity levels on the viability of seed,
4. To assess the role of seed mycoflora on pre and post-emergence rotting of seedlings, and
5. To evolve a suitable management practice to avert the bio-deterioration of seeds.

Review of Literature

REVIEW OF LITERATURE

Seeds are the principal means of plant reproduction and has been recognised as one of the vital inputs for maximising agricultural production. Viable and vigorous seeds of improved/high yielding varieties act as catalyst for realizing the potentials of other costly inputs viz., fertilizer, irrigation and plant protection materials.

Bio-deterioration of seeds in storage is one of the major factors affecting the quality of seeds throughout the season. Several studies have been carried out to find out the factors on bio-degradation due to different micro-organisms and storage conditions. Large number of investigations have also been undertaken to reduce the bio-degradation of seeds by different fungicidal treatment and improving the storage conditions.

Mycoflora associated with vegetable seeds

Seed borne fungi constitute the main group of pathogen which reduce the viability of seeds. Good number of works have been conducted on this subject.

Prevost (1807) proved scientifically for the first time that spores present on the surface of seeds are responsible for smut disease. Ramnat (1937) was one of the earliest scientist to understand the importance of seed borne fungi.

Groves and Skolko (1946) isolated Trichocladium asperum from pumpkin (Cucurbita pepo), pea (Pisum sativum) and

broad bean (Vicia faba). But they did not find any significance in causing disease. Skolko and Groves (1948) isolated Chaetomium funicolum from pea, cucumber (Cucumis sativum) and tomato (Lycopersicon esculentum).

Walker (1952) reported that beans were usually attacked by Rhizoctonia spp. and Sclerotinia sclerotiarum which were internally seed borne. Suryanarayana and Bhombe (1961) reported that the fungi like Alternaria spp., Aspergillus spp., Fusarium sp., Helminthosporium spp., Colletotrichum sp. and Phomopsis spp. infect the seed and cause pre and post-emergence death of vegetable seedlings. Kerling et al. (1967) reported the seed borne infection of Fusarium solani sp., Cucurbita from Cucurbita ficifolia.

Jain and Patel (1969) reported that Aspergillus, Rhizopus, Cephalosporium and Fusarium were the most frequent spp. associated with Guar (Cyampsis psoraloides) seeds. They found that Fusarium and two isolates of Alternaria were pathogenic, causing root rot and brown leaf spot of Guar. Khandelwal and Prasada (1970) carried out investigations on the mycoflora of cucumber seeds and obtained Aspergillus sp., Rhizopus sp., Cladosporium sp., Cylindrocephalum sp., Helminthosporium sp., Alternaria sp., Fusarium sp., Curvularia sp., Penicillium sp., Botrytis sp. and Verticillum spp.

Nath et al. (1970) reported Macrophomina phaseolina, Cercospora kikuchii, Colletotrichum truncatum, Myrothecium roridum, Botryodiplodia palmarum, Fusarium equiseti, Fusarium

moniliformae and F. solani from the seeds of mung bean (Phaseolus aureus). Goel and Mehrotra (1972) isolated Macrophomina phaseolina from roots and seeds of Abelmoschus esculentus. Superficial contamination was found in 30-45% of seeds of a commercial variety and deep seated infection in 12-22%. Singh and Chohan (1973) isolated nine fungi from Methra and four fungi from Kasurimethi seeds and found that Alternaria alternata caused 5 per cent post-emergence death of methra seedlings. Puttoo and Sohi (1974) reported the seed borne nature of Rhynchosporium sp., Fusarium solani, Geotrichus candidus, Paceilomyces varioti and Phoma glomerata from egg plant (Solanum melongena).

Gangopadhyay and Sharma (1976) reported that Rhizoctonia solani and Fusarium oxysporum are seed borne fungi and are seed borne fungi and are responsible for spongy rot in pumpkin. Kaniyasu and Kishi (1977) studied the seed transmission of bottle gourd (Lagenaria sicerares) and found that 2.5 per cent of commercial seed lots were infected with F. oxysporum sp. Langenaria. Bilgrami et al. (1976) reported the occurrence of Aspergillus flavus, A. niger, Penicillium sp. Alternaria alternata, Fusarium semitectum, Curvularia lunata, Helminthosporium hawaiiense and Cladosporium sp. with seeds of mung and urd in storage.

Saxena and Sinha (1977) investigated the seed borne fungi of Vigna mungo (Black gram) and found the occurrence of Ascochyta chartarum, Colletotrichum truncatum and Fusarium

semitectum. Saxena and Sinha (1978) reported 28 fungi as adherent on the surface of green gram and black gram seeds. They also reported extra embryonial infection in black gram and green gram with Aspergillus niger, A. flavus and Alternaria alternata.

Sesan and Dumitras (1979) reported Colletotrichum lindemuthianum, Fusarium sp., Phoma phaseoli, Rhizoctonia solani and Botrytis cinera from 38 seed samples of Phaseolus vulgaris. Pangtey and Sinha (1980) confirmed the seed borne nature of Colletotrichum capsici and Phoma medicagini after direct pathogenicity test in Horsegram.

Naseema (1981) isolated Aspergillus flavus, A. niger, A. ochraceus, Rhizopus stolonifer, Chaetomium macrosporum, Botryodiplodia theobromae and Colletotrichum langenarium from bitter gourd (Momordica charantia), A. niger, R. stolonifer, Cephaliophora irregularis and Fusarium solani from pumpkin (Cucurbita pepo) and A. flavus, A. niger, R. stolonifer from snake gourd (Trichosanthus anguina) while studying the mycoflora of vegetable seeds.

According to Mehrotra and Wadhwani (1981) soyabean seed yielded 27 fungi, of which Aspergillus sp. and Penicillium sp. constituted about 41 and 51 per cent respectively. Saxena and Gupta (1981) isolated Ascochyta charatanum, Colletotrichum truncatum, F. oxysporum and F. semitectum from the seed coat and cotyledons of green gram and black gram. Vidhyasekaran and Kandaswamy (1981) reported F. oxysporum, F. semitectum,

F. moniliforma, Macrophomina phaseoli and Aspergillus flavus as seed borne pathogens of okra.

Das and Narain (1982) recorded Aspergillus spp., M. phaseolina, Chaetomium spp., Curvularia lunata, F. semitectum, Fulvia fulvum, Geotrichum candidum, Penicillum chrysogenum, Alternaria alternata and Phoma multistroata from the seeds of bitter gourd, ridge gourd (Luffa acutaqula), pea, tomato and okra. Nair (1982) observed Aspergillus niger, Rhizopus nigricans, Penicillum variable, Cladosporium herbarum, Curvularia pallesceus, Paceilomyces varioti, Nigrospora oryzae, Chaetomium aterrinum, C. dolichotrichus, C. globosum, Cunninghamella verticillata and species of Coprinus on cucurbitaceous vegetable seeds.

Singh and Singh (1982) reported that M. phaseolina is a serious pathogen which infect the seed both internally and externally. Kabeere and Taligoola (1983) studied the mycoflora and deterioration of soyabean seeds and found Aspergillus glaucus, A. cchraceus and Rhizopus spp. from seeds of soyabean. Jenkins and Whner (1983) reported the occurrence of Fusarium oxysporum on Cucumis sativus seed stocks from North Carolina.

Maholay and Sohi (1982) reported the occurrence of Botryodiplodia theobromae on the seeds of bottle gourd and squash and found that the pathogen survived for 12 months in seed of bottle gourd and 7 months in squash. Palodhi and Sen (1983) reported the seed borne nature of F. oxysporum,

F. solani and F. moniliforme in five cucurbitaceous crops.

Furgal and Wegrzyeka (1984) reported the Fusarium sp., Ascochyta spp. and Alternaria terreus were the main pathogenic fungi isolated from the seeds of pea. Lee (1984) isolated Alternaria alternata, Arthrobhytis sp., Aspergillus spp., Cephalosporium sp., Cladosporium sp., Cylindrocarpon sp., F. equiseti, F. moniliforme, F. semitectum, F. solani, Penicillium sp. and Rhizopus as saprophytic fungi and Cercospora kikuchii, Colletotrichum tunicatum, Diaportha phaseolinum var. Sojae and F. oxysporum as pathogenic fungi from soyabean seeds. Maholay and Sohi (1983) isolated Macrophomina phaseolina from the seeds of bottle gourd, musk melon and squash. They also found that inoculation with this fungus had no effect on the germination of squash and bottle gourd seeds. However, musk melon seeds showed 20 to 30% pre-emergence mortality in blotter and soil tests respectively. This fungus survived for 21 months in bottle gourd, squash and musk melon seeds. Fakir and Mridha (1985) reported that Colletotrichum demetum and M. phaseolina were seed borne pathogen, which cause die-back disease in Hibiscus esculentus. Kononkov and Dudina (1987) reported Aspergillus glaucus, A. versicolor, A. candidus and Penicillium sp. from non-sterilised seeds of tomato, carrot, radish and cucumber. Adisa and Aborisadi (1987 observed that A. flavus,

Botryodiplodia theobromae and Penicillium digitatum were the most predominant fungi isolated from germinating seeds of okra.

Prasad and Prasad (1987) studied the seed mycoflora of different cultivars of dolicos lab lab seed. Freshly harvested seed yielded 35 spp., of fungi, of these most of them were Dematiaceous and some Fusaria. They did not observe much difference in the fungi associated with the stored seed and freshly harvested seed. The A. niger suppressed the seed germination while A. chevalieri, A. flavus, A. candidus, A. niveus and Alternaria alternata caused staining and necrosis of cotyledons of 23 to 37% of seeds which resulted in twisting of cotyledons and first few leaves to a range of 19 to 27 per cent.

Germination of discoloured seeds of Capsicum annum was considerably reduced. The most commonly occurring seed borne fungi on chilli were Colletotrichum, Cladosporium, Alternaria, Drechslera and Curvularia spp. These fungi affected root elongation more adversely than shoot elongation (Adiver et al. 1987). Abdul Hafez (1988) reported that Aspergillus, Penicillium, Rhizopus, Mucor and Fusarium were the predominated fungi among 22 genera isolated from chickpea, broad bean and lentil seeds. He also found that A. niger, A. flavus, A. nidulans, A. terreus, A. flavus vr. columaris, P. chrysogenum, P. citrinum, P. funiculosum, R. stolonifer and F. miniliforme were frequently associated with the seeds.

Sandhu and Sharma (1988) found that seed borne infection by Ascochyta pisi varied from 6.6% in the pea cultivar Bonneville to 33.3% in lincoln. Germination was adversely affected and was only 5% in lincoln seeds infected by the fungus. Starndberg (1988) detected Alternaria dauci from infested carrot seed when incubated on moisture filter paper with carrot leaf extract or with water.

Vaughan et al. (1988) studied the routes of entry of Alternaria sp. into soyabean seed coat and found that the fungus penetrated seed coat tissue either by direct penetration through the cuticle or through microphyle.

The causal organism of the charcol fruit rot of musk melon, Macrophomina phaseolina and the fruit rot organism Rhizectonia solani of musk melon were also found on the seeds of musk melon (Maholay, 1988).

Weidenborner and Hinderf (1989) isolated A. glavicus, A. flavus, A. ochraceus and A. niger from seeds and pods of pegion pea. They also reported that field fungi were less frequently detected in these seeds than storage moulds. Of the field fungi only Cladosporium was present on both seeds and pods.

Effect of relative humidity, moisture content and storage periods on seed deterioration and seed mycoflora

Seeds lose their viability in storage greatly due to fungal activity. Moisture content of seeds is an important factor which encourages fungal growth. According to Indian

minimum seed certification standards (1971), the optimum moisture content is 7 per cent for cucurbitaceous seeds. Seeds with high moisture content could not be stored for longer periods. Storing of seeds in large quantities at low moisture level itself may not be feasible and economical as seeds absorb ambient moisture continuously from surroundings. Coleman and Fellow (1925) reported that when relative humidity was raised from 65 to 85 per cent the moisture content of cereal seeds increases rapidly and this was due to the absorption of moisture from air. High moisture content allowed growth of mycoflora resulting in rapid deterioration of seeds. Kechler (1938) observed that Aspergillus spp. require various moisture content for its growth. According to Gosh (1951) the development of mould depends more on the relative humidity and storage temperature than the moisture content of the grain.

Quasem and Christensen (1958) found that when samples of maize seeds were stored for 6 to 9 months, those with poorest germination had high percentage of fungal infection, especially by Aspergillus flavus and Penicillum sp. Fields and King (1962) studied the influence of storage fungi on deterioration of stored cowpea seeds. They found that high quality cowpea seed inoculated with Aspergillus flavus, A. ruber, A. candidus, A. restictus and A. amstelodami and stored under controlled temperature (10-30°C) and Relative humidity of 75 to 92 per cent for 2 to 8 months decreased the germination moderately to drastically. Invasion by storage fungi proceeded

seed deterioration. A.flavus was the most pathogenic one among the Aspergillus species studied. They also reported that an increase in the moisture content of seeds or an increase in storage temperature increased the rate of fungal invasion and a correspondingly decreased germination.

Christensen and Lopez (1963) reported that stored seeds were susceptible to invasion by fungi growing on equilibrium with RH 65-98% which reduce germinability and cause various biochemical changes. According to Majumder et al. (1965) 85 per cent relative humidity could cause visible mould growth on seeds of rice, wheat, sorghum, bengal gram, green gram, groundnut, cumin and coriander. Kudrina (1967) studied the changes in the mycoflora of vegetable seeds during storage and found that at RH greater than 70 per cent and temperature ranging from 10 to 20°C the bacteria were more predominant on the seeds of onion (Allium cepa), cucumber, carrot (Daucus carota) and tomato in addition to fungi like Penicillium, Aspergillus and Mucor.

Christensen and Kaufman (1968) observed that Aspergillus sp. and Penicillium sp. proliferated on rice grain within moisture content of 12 to 16 per cent while Rhizopus sp. required 16 to 22 per cent moisture content.

Majumder and Venugopal (1969) studied the effect of moisture content on the production of clestothecial bodies on cereal grain.by Aspergillus repense, A. amstelodomi, A. chevalieri and A. ruber. They observed that these fungi

produced clestothecial bodies on grains when the moisture content was at least 15 to 15.5 per cent. A positive correlation between moisture content and growth of storage fungi on sorghum was noticed by Burrough and Saucer (1971).

Kulik (1973) inoculated the seeds of cabbage, cucumber, okra, onion, pepper, radish, salsify, spinach and turnip with spores of A. amstelodami and A. flavus and stored at 85 per cent relative humidity at 22-25°C for 30 days. He observed that only a small number of seeds were invaded by these fungi in the case of cabbage, cucumber radish and turnip while more seeds were invaded by these organism in the case of okra, onion, pepper, salsify and spinach. Fungus invasion, however, did not reduced germinability.

Christensen (1973) reported that storage fungi such as Aspergillus sp. and Penicillium sp. did not invade the seed to any serious extend before harvest, but reduced germinability and caused other damage to seeds in storage. Though these fungi were not effectively controlled with seed treatment fungicides, its invasion was avoided by storing the seeds at low moisture and temperature condition.

Skvortsova et al. (1975) studied the association of microflora of vegetable seeds under different methods of storage. They found that Cladosporium and Alternaria were replaced by Penicillium and Aspergillus species when the seeds were stored below the critical moisture content. The highest number of microorganisms was isolated from the seeds stored

at 15°C while the lowest was from the seeds stored at 25°C. Bilgrami et al. (1976) studied the deterioration of pulses seed by fungi and reported that a number of fungi were found associated with the seeds of mung (Phaseolus aureus) and urd (P. mungo) in storage. Aspergillus flavus was the most predominant one and this fungus caused a reduction in weight and changed the constituents resulting in marked deterioration of the seed.

Harrison (1978) while studying the role of seed borne infection in the epidemiology of Botrytis fabae on field beans found that this organism was not detected after 9 months of storage. The fungus was isolated from testa, cotyledons, hypocotyl, the base of pumule and top of root from most of the seedlings soon after sowing. Aggressive infection occurred when the level of inoculum was high and more inoculums were required at 10°C than at 20°C.

According to Saxena and Gupta (1979) most of the field fungi associated with the seeds of Vigna radiata persisted as long as 120 days in storage at 45 to 75 per cent RH and temperature 6.2 to 29.6°C. They found that the field fungi were replaced by storage fungi after 120 days of storage at RH 87 per cent and temperature 28°C.

Konokov et al. (1980) studied the microflora and seed germinability of seeds of carrot, cabbage, raddish, tomato and cucumber on storage. They found that at 45 per cent RH the initial microflora gradually died while the germinability

was unchanged. At 30 and 50 per cent RH infestation by Aspergillus fumigatus was the lowest and by A. flavus highest, after 18 weeks of storage. They also observed that there are no decline in germinability after 18 weeks of storage at 30 to 50 per cent RH but the germination declined after 12 weeks of storage at 95 per cent RH.

Prasad and Narayan (1980) reported that, seed rot of Kulthi (Dolichos biflorus) occurred in seeds stored at high RH.

Coelha et al. (1982) conducted a study with soyabean seeds stored at three different environmental conditions. They stored six soyabean varieties at 28°C, at 59, 66 and 72 per cent RH and reported that the mean initial and final germination percentage were 63 and 67, 73 and 77 and 68 and 73 respectively. They also conducted a field study with these seeds stored for six months and obtained a germination percentage of 16, 25 and 21, respectively. They concluded that there was no significant difference in germination among the varieties stored at different humidity levels.

Nandi et al. (1982) studied the deterioration of oil seeds in storage. They used two cultivars of sesamum, two species of mustard and one species of linseed and stored under 80 and 90 per cent RH and 20 and 30°C. They observed that in all cases both seed moisture and fungal infection were higher at 80 per cent RH at 20°C than in other treatments. There was a gradual decrease in infection by field fungi with concomitant

increase by storage fungi. A reduction in seed germination occurred as the storage period increased. The highest loss of germinability was when the seeds were stored at 90 per cent RH and at 30°C. Mallick and Nandi (1982) reported that in rice with seasonal fluctuations grain moisture changed, germinability decreased and fungal infection increased. A gradual decrease in field fungi was found to be accompanied with corresponding increase in storage fungi.

According to Onesirosan (1982) there was little loss in germinability when cowpea seeds with 12.5 per cent moisture content was stored at 8, 20 and 30°C for 20 weeks while germination of seeds with 15.5 per cent moisture stored at 8 and 20°C was good but fell to 75 per cent at 30°C. Seeds with 18.5 % moisture content stored well only at 8°C while there was a rapid fall in germination when it was stored at 20°C and 30°C.

Gorecki and Jagielski (1983) studied the storage quality of pea, field bean and yellow lupin seeds and found that seeds of all these crops, stored at 90 per cent RH exhibited a marked decrease in germination capacity and vigour after only two month storage and seeds of yellow lupin senesced faster than those of field beans and pea seeds.

Kabeere and Taligoola (1983) conducted a study where soyabean seed samples of cultivars Clark, S38 and CHF maintained for three months at 23-25°C were stored separately for 10 months in laboratory at 23-25°C and in cold room at

5°C. They found that as the moisture content increased germination decreased with increased storage period at room temperature. Seeds in cold storage constantly had higher germination than those stored in laboratory. Clark seeds were heavily invaded by Rhizopus spp. and bacteria. With increased storage period the number of seeds with field fungi decreased except for Aspergillus glaucus and A. ochraceus. The heaviest fungal invasion occurred after 10 months of storage and this coincided with the period of highest moisture content and sharpest decrease in germination, suggesting that storage of fungi caused a decrease in germination of seeds. Percentage of dead/rotten seeds covered with Rhizopus sp. and bacteria increased as the storage period increased, indicating that the micro-organism may also have contributed to loss of seed viability.

Maholay and Sohi (1983) reported that Macrophomina phaseolina causing seed rot of bottle gourd, squash and musk melon survived for 21 months in all the three host seeds.

Thai (1983), working with seeds of maize, sorghum and soybeans found that moisture content of seeds increased with increasing storage time when they were stored at high RH. Under controlled environmental condition at 20°C and 50 per cent RH the germination percentage was not much reduced after 18 months of storage and was 87.3, 77.3 and 52.6 for maize, sorghum and soyabean respectively.

Vishunavat and Shukla (1983) conducted a study on the

effect of different temperature, humidity and period of storage upon prevalence of seed mycoflora of lentil and observed that Aspergillus flavus, A. niger, A. terreus, A. fumigatus, A. sydowi and Penicillium oxallicum appeared on the seed after six months of storage and continued to prevail upto 12 months. Rhizopus arrihzus and Fusarium oxysporum were observed at all relative humidity levels in storage periods of 6 to 12 months. Gupta and Gupta (1984) studied the deterioration of mung beans by fungi. They isolated thirtyfour fungi from the stored seeds of two cultivars of mung beans during twentyfour months of storage. Sharma and Gupta (1984) noticed the fungal deterioration of moth bean (Phaseolus oconitifolius) seeds during storage. They isolated 28 fungi from the deteriorated seeds and these seeds were found very poor in germination. Maude and Bambridge (1985) reported that redbeet seed clusters retained a high level of germination when stored for 13 years at 10°C and 50 per cent RH in seed stored. Seed infection of Pleospora betaes declined from 27.5 to 4.5 per cent over the same period.

Rupe and Ferriss (1986) reported that seed infection rate by Phomopsis spp. was linearly related to water content between 35 and 19 per cent. At water content 19 per cent, no seed infection was observed on seed.

Komaraiah and Reddy (1986) found that weight loss by seed borne fungi in stored seeds increased with increasing relative humidity, but reverse was true for germination.

Kononkov and Dudina (1987) studied the effect of fungi on vegetable crop seed namely radish., tomato, carrot, cucumber and cabbage stored in conditions of increased air relative humidity and temperature. Aspergillus glaucus, A. versicolar and Penicillium spp. were the fungal species detected on non-sterile seeds during storage. Each species fungi dominated within a definite range of seed moisture content. Germination of sterilised seeds were reduced by higher relative humidity and temperature. The presence of fungi also accelerated in high relative humidity, thus accelerated the process of seed damage.

Doijode (1987) reported that the seed viability was reduced to a great extent by keeping cauliflower seed at 90 per cent RH for six days at 40°C. He also recommended that storage of cauliflower seed at low temperature and low moisture/RH was desirable for getting good viable seeds.

Rao and Reddy (1987) observed the incidence of storage fungi on sorghum seeds increased with an increase in the relative humidity of the storage environment over a period of one month. Seed deterioration was accelerated with higher relative humidity. The percentage seed germination was inversely proportional to the relative humidity in storage.

Saxena et al. (1987) studied the vegetable seed deterioration due to storage and found that seeds of cabbage, radish, cauliflower, pea and okra showed a decrease in germination with length of storage.

Singh and Yadav (1987) reported that when green gram seeds with moisture content of 12, 14 and 16 per cent were stored in air tight condition upto six months, germination of seeds was unaffected during the first 2 months in seeds with 12 per cent moisture content, but decreased later. At 16 per cent moisture content germination was only one per cent after six months of storage. Seeds which were stored at 27°C under 50-60 per cent RH showed higher germination upto six months of storage.

Wetty et al. (1987) while studying the influence of moisture content, temperature, length of storage and seed germination of tall fescue and perennial rye grass seeds found that seeds when stored for 18 months at 10-30°C and 11.5 to 95 per cent RH,temperature, moisture content of seed and time intract to influence germination.

Subramaniya et al. (1988) reported that high storage temperature of 37°C and high relative humidity of 75-87 per cent favoured colonization of sorghum seeds by storage fungi. They also observed that high temperature and relative humidity decreased the seed quality even after the fungicide treatment.

Evaluation of seed dressing fungicide on seed borne fungi

Seed treatment with fungicide is the one of best method for controlling seed borne mycoflora and to improve seed germination. Seed treatment with fungicide not only controls seed borne pathogens, but also control soil borne pathogens by forming inhibition zone around the seed.

Ramnat (1637) mentioned the value of seed treatment with sodium chloride for the control of stinking smut. Schulthesis (1761) first suggested the use of copper sulphate on wheat seed against stinking smuts. Prevost (1807) proved scientifically that seeds treated with copper sulphate solution gave good germination and inhibited the spore germination of smut disease. In India, Ozanne (1885) tried copper sulphate against smut disease in sorghum seed for the first time. Darnell-Smith (1917) introduced copper carbonate as a dust seed treatment for wheat. Hilson (1925) used organomercurials for the first time in India for the control of sorghum smut through seed treatment. Ramamurthy (1933) stated that foot rot of paddy was successfully controlled by dry seed dressing with organomercurials. Cunningham and Sharvelle (1940) introduced chloronil as a practical organic seed protectant.

Jacks (1951) reported that Thiram (0.2%) treatment of lettuce seeds inoculated with one of the following fungi, viz., Alternaria solani, Rhizactonia solani and Botrytis cinerea gave 100 per cent germination.

Veenenboz (1955) showed thiram containing fungicide could be used effectively controlling seed borne fungi of peas and beans. Sanchez (1956) found that captan at the rate of 2 g/kg seed was the best for the control of damping off and seed decay in pea and bean. Aycock (1958) reported that seed treatment of watermelon with seedox, panogen and emmi gave effective control of Colletotrichum langanarium which

causes anthracnose disease. Christensen and Lopez (1963) reported that growth of storage fungi is not inhibited by the seed treatment fungicides in seeds with moisture content in equilibrium with RH 65.85 per cent.

Ashworth et al. (1964) showed that for the control of Aspergillus niger associated with groundnut, thiram and captan (0.3%) were more effective than mercury seed dressings. Effective control of Pythium sp. and Fusarium sp. by captan treatment in peas was reported by Harper (1964).

Wales and Somers (1968) reported Difoltan (0.25%) was the best for the control of Aspergillus flavus in groundnut, in field and in storage. Frank (1969) conducted laboratory and field trials and proved that 3 parts of 75 per cent captan + 1 part of 75 per cent PCNB at the rate of 3 g/kg seed was effective in controlling Aspergillus and Rhizopus rot of groundnut seedlings. Efficacy of various seed dressing fungicide against different seed borne fungi of cucumber was studied by Khandelwal and Prasada (1970). They found that seed treatment with agrosan GN and baileaves completely checked the growth of all seed borne fungi of cucumber seed. Nakamura et al. (1972) observed that seed dressing with organomercurials, severely affected the germination of eggplant, beats and cabbage. He also opined that organosulphur dust are more suitable than mercury containing ones for the storage of treated vegetable seeds.

In a comparative study on the viability and mycoflora of bean seeds after various treatments, it was found that, seeds treated with Captan and Thiram at 0.2% gave effective control of the mycoflora while leaving germination unimpaired during storage for four years (Kaul, 1973). Singh et al. (1974) reported that seed treatment with Thiram and Tenechlor super X at 0.5% significantly increased the seedling emergence of soyabean after a six months of storage and also reduced the incidence of Aspergillus flavus, A. niger, Macrophomina phaseoli, Penicillium cyalopium, Phoma sp. and Rhizopus arrhizus. Suhay (1975) pointed out that seed treatment of green gram with Ceresan, Agallol, Captan and Thiram reduced the fungi associated with it and improved the germination of seeds. Frolova (1976) showed excellent control of Didymella bryoniae in cucumber with 0.2% Thiram treatment.

Gabrelson and Mulanax (1977) observed effective erradication of seed borne Phoma lugans in crucifers with benzimedazole fungicide.

Sorade and Kadam (1977) reported that seed treatment with thiram gave the best control of Helminthosporium sp. on eggplant seeds.

Mercar and Kisyombe (1978) attempted to find out the effect of various seed dressing fungicide on controlling the fungal flora of groundnut kernals and observed that seed treatment with Thiram increased the crop yield as well as inhibited the microflora of groundnut kernals except A.flavus.

Sesam and Dumitras (1979) noticed that seed treatment with various fungicides like Delsan F, Delsan a, Thiram and Thiophanate-methyl effectively controlled the fungal flora of Kidney bean. Sharma et al. (1980) found that, out of ten fungicides namely Ceresan dry, Agrosan G.N., Benlate, Vitavax, Dithane M.45, Brassicol, Hexathir, Captan, Panoctine and Panoram tested against seed mycoflora of Amaranthus caudatus, 0.2 per cent Agrosan G.N. and Ceresan proved to be effective in controlling almost all common seed borne fungi.

Vidhyasekaran et al. (1980) observed that Bavistin in combination with Thiram effectively controlled most of the fungal flora of brinjal seeds in storage and effectively preserved the viability and vigour of the seedlings after 24 months of storage. Oladiran and Okusanva (1980) reported that seed treatment with Thiram, Captan and Captafol at 200 ppm gave effective control of pre-emergence damping-off of cowpea caused by Pythium aphanidermatum and Sclerotium rolfsii. Seed treatment with 100 ppm Captafol, Benomyl and Thiabendazole gave best control of Colletotrichum lindemuthianum in cowpea (Barros et al., 1981). Kore and Solanke (1981) conducted a study on the effect of fungicide on seed mycoflora and longevity of seeds of Dolicos lablab and they observed that seed treatment with Agrosan G.N., Vitavax, Carbendazim improved the germination and Agrosan G.N., Captan and Thiram eliminated the fungal flora.

Naseema (1981) studied the role of woodash as a vegetable

seed preservative and reported that the germination percentage of Ash treated bitter gourd, Snake gourd, cucumber were preserved upto 33.3%, 100% and 55% respectively after 3 months of storage.

Vishunavat and Shukla (1982) revealed that seed treatment with Thiram and Bavistin eliminated all fungi associated with lentil seeds except Alternaria alternata and Rhizopus arrhizus.

In a study conducted by Zote and Mayee (1982) on the influence of fungicidal treatment on seed borne fungi of mung bean in storage, it was seen that seed treatment with Bavistin followed by Thiram and Dithane M.45 improved the germination of seeds as well as inhibited the fungal growth. Singh et al. (1982) pointed out that seed treatment with Bavistin and Thiram at 0.25% gave excellent protection to wheat seeds from Aspergillus and Penicillium spp. for more than 275 days. But Bavistin alone was effective only for 30 days.

Reddy et al. (1982) found that Calixin - M gave complete control of seed borne infection by Ascochyta rabei on chickpea at 0.3 and 0.6% concentrations.

Siddaramaiah et al. (1982) reported that seed treatment with Thiram, Ferban, and Agallol were efficient in controlling the seed mycoflora of linseed. They also noticed that Thiram and Dithane M.45 increased the seed germination of about 80-90% against 40% in the control. Singh and Agarwal (1984)

reported that seed treatment with Bavistin was effective in controlling the purple strain discolouration of Soyabean and caused by Cercospora kikuchi and also gave maximum germination. According to Bhattim et al. (1984) seed treatment with Calixin-M (Tridemorph) and Captan at 0.1% gave 60% eradication of the fungi in chickpea. They also found that seed treatment with these fungicides increased the germination as well as seedling vigour chickpea. Kumar and Jitendra Singh (1984) reported that seed treatment with Bavistin at 2 g/kg seed, eliminated all the fungi associated with sesame seeds during storage except Aspergillus sesami, Curvularia lunata and Preslase tatramora.

Pandey and Gupta (1984) studied the effect of fungicidal treatment on seed mycoflora and germination of Satraria italica and reported that of the five fungicides tested against seed mycoflora and germination, Agrosan G.N. and Difolatan showed the best result in inhibiting the fungi of Setraria italica seed. Singh et al. (1984) found that Bavistin and Bavistin + Thiram were the effective fungicides for controlling the storage fungi of wheat. Kumar and Srivastava (1985) stated that seed borne fungi of Pigeon pea were effectively reduced by seed treatment with Agrasan G.N., Bavistin, Difolatan, Captan, Vitavax and Dithane M.45. Wu and Lee (1985) evaluated 13 fungicides against the seed borne pathogen Phomopsis sojae of Soyabean and stressed that Bavistin performed best in controlling this fungus.

Wheat seeds treated with Captan, Emisan, Thiride and Vitavex at 2 g/kg were stored in various container for 6 months and are found to be highly effective in protecting seed viability. It is also noticed that Thiride gave the best control of seed borne organism.irrespective of containers (Randhawa et al., 1985).

Seed treatment of corn seed with benomyl, Captafol, Captan carbondazim maneb, Chlorothalonil thiabendazol at 750 and 1125 ppm a.i. at 85% relative humidity, showed a significant difference in the growth of mycoflora as compared to untreated ones,(Moreno et al., 1985).

Robfrti et al. (1985) reported that seed treatment with Captafol, Carboxin and Thiram gave best result in controlling Colletotrichum lindemuthianum in bean (Phaseolus vulgaris). Kumar and Patnaik (1985) conducted a study on seed borne nature of Alternaria alternata in Pegion pea and its control. They found that dry seed treatment with Bavistin and Captafol were effective in controlling the pathogen as well as improving the seed germination by 8.5 to 24.5%. Maude and Bambridge (1985) observed a high level of germination of red beet seed when stored for 13 years at 10°C and 50% relative humidity. They also noticed that seed treatment with Thiram before storage improved the germination and reduced infection of Pleospora betae.

Peresypkin and Pidoplianko (1985) found that root rot infection in winter wheat caused by Cercosporella sp. and

Fusarium sp. was reduced by seed treatment with Bavistin and Thiram respectively. Shekhawat et al. (1986) applied seeds with carbondazin, benomyl, carboxin and ethyl mercury fungicide 2-2.5 g/kg seed for controlling pre and post-emergence collar rot of groundnut. Prasad and Prasad (1987) reported that seed treatment with Emisan 6 improved the seed germination of Coriander seeds even after six months of storage.

Hegde and Hiremath (1987) reported that seed treatment with Captan, Ceresan dry Thiram and Bavistin @ 2.5-5 g/kg were effective in controlling the mycoflora of cowpea seeds and seedling vigour. They also noticed that the fungicides retained their efficacy even after 90 days of storage of seeds.

Agnihortri and Sharma (1987) observed a reduction in wilt infection by Fusarium oxysporum sp. cumina with seed treatment of Bavistin at 0.2 mg/kg seed.

Control of Rhizoctonia solani causing root rot in pea with seed treatment using Benomyl and Captafol was noticed by Chauhan and Duban (1987). Ahmad (1987) found that seed dressing with Captan, Thiram and Carbandazim eliminated most of the seed borne fungi of mungbean.

Ramadoss and Sivaprakasan (1987) tested the effect of fungicides and insecticides as seed treatment on the inhibition of Macrophomina phaseolina and viability of cowpea seed during storage and observed that of the 3 fungicides tested Carbendazim produced the maximum inhibition.

Sajita and Indrahooda (1987) observed control of Fusarium solani and Pythium aphanidermatum causing damping off of chilli and tomato could be actively controlled by seed treatment with MEMC, Captan and Captafol. Bolteber (1988) tested eight fungicidal preparation as seed dressing agents against Botrytis allii causing storage rot in onion and found that the combined preparation containing Benomyl, Carbendazim and Thiram was effective in preventing rotting during 7 months storage.

Perana and Joi (1988) reported that among the five fungicides tested Thiram gave the best control of seed borne infection of Colletotrichum capsici in Capsicum fruticens.

Sandhu and Sharma (1988) observed that effective control of seed borne infection of Ascochyta pisi on pea seeds with Thiram 2.5 g/kg seed. Subramaniya et al. (1988) reported that seed treatment with Thiram was effective in controlling the seed mycoflora of Sorghum. However, Bavistin was less effective against Curvularia lunata, Dreschslera bawainenas and Alternaria alternata.

Dwivedi and Shukla (1989) conducted a comparative evaluation of fungicides on mycoflora of green gram seeds reported that Captan and Thiram completely eliminated the mycoflora associated with the seeds.

Materials and Methods

MATERIALS AND METHODS

Collection of seeds

Three cucurbitaceous seeds viz., bitter gourd var. Priya (*Momordica charantia* L.), Pumpkin var. Ambili (*Cucurbita moschata* Poir) and cucumber var. Mudicode Local (*Cucumis melo* L.) developed and produced by the Department of Olericulture, College of Horticulture, Vellanikkara and Agricultural Research Station, Mannuthy were used for the investigation. After collection of seeds the germination percentage and moisture content of the seeds were estimated.

Determination of germination percentage of seeds

Blotter method and pot culture experiment were employed to test the germination of the seeds.

Blotter Method (ISTA, 1966)

Ninety randomly selected seeds of each vegetable were used for the germination test. The seeds were placed at equidistance on sterilised moistioned filter paper kept in sterilised petri dishes. In each petri dish fifteen seeds were kept and the dishes were incubated at room temperature. Six replications were maintained. Observation on the germination of seeds were taken daily for 15 days and the germination percentage was calculated.

Pot culture experiment

The seeds were planted in nine inch earthen pots filled with top soil collected from the field. Ten seeds were planted

at equidistance in each pot. Nine replications were maintained. The pots were watered daily and were exposed to natural atmospheric conditions. Observations on the number of seeds germinated were taken daily for three weeks and germination percentage was calculated.

Determination of moisture content

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

$$\text{Percentage of moisture} = \frac{M_1 - M_2}{M_1} \times 100$$

where

M_1 = weight of seed before drying

M_2 = weight of seed after drying

Determination of Mycoflora associated cucurbitaceous seeds at different intervals of storage

The fungi associated with the three cucurbitaceous vegetable seeds were estimated by standard blotter method (de Tempe, 1953).

Ten seeds of each cucurbitaceous vegetable were placed at equidistance in petridishes lined at the top and bottom with sterilized moist filter paper and incubated at room

temperature. Ten replications (100 seeds) were maintained for each type of seed. Observations on the number of seeds associated with fungi were made from fifth to the fifteenth day, and the percentage of mycoflora associated cucurbitaceous seeds was calculated. The same experiment was repeated using surface sterilised seeds. Surface sterilisation of the seeds was carried out by dipping the seeds for two minutes in 0.1 per cent mercuric chloride solution followed by three washings in sterile water. These experiments were carried out immediately after the collection of seeds and after every three months for a period of one year.

Mycoflora associated with the seed

Agar plate method (ISTA, 1966) was used to find out the mycoflora associated with the seed. Surface sterilised and unsterilised seeds were plated on potato dextrose agar medium (PDA). Surface sterilisation was carried out by dipping in 0.1 per cent mercuric chloride solution for two minute, washed in three changes of sterile water. The seeds were plated at the rate of five seeds per petridish. The petridishes were incubated at room temperature and examined daily for the growth of the fungi upto 15 days. The fungi were isolated and purified by single spore isolation or hyphal tip method. The fungi were identified based on their morphological characters. The identity was further confirmed through the curtsy of Director, Commonwealth Mycological Institute, U.K.

Mycoflora associated with different parts of seeds

To find out the mycoflora associated with seed coat, endosperm and embryo of the three cucurbitaceous seeds component plating technique was adopted. This experiment was carried out immediately after the collection of seeds. The seeds were soaked in sterile distilled water for six to eight hours. The seeds were then dissected aseptically using sterile needle and forceps. The separated seed coat, endosperm and embryo were washed in 0.1 per cent mercuric chloride solution for two minutes, washed in three changes of sterile water and placed on potato dextrose agar (PDA) medium at the rate of five bits per petri dish. Three replications were maintained. Unsurface sterilised and surface sterilised seeds were also plated in PDA medium. The dishes were incubated at room temperature and were examined daily for the growth of the fungi upto 15 days. The fungi found growing on seed coat, endosperm and embryo were isolated and purified by single spore and or hyphal tip method and identified.

Mycoflora fungi associated with the stored cucurbitaceous seeds at different intervals of storage

Three cucurbitaceous seeds namely, bitter gourd, pumpkin and cucumber were packed in small cloth bags and stored in tin boxes for 12 months. Each cloth bag contained 100 seeds. These stored seeds were tested for the presence of fungi just before storage and after every three months interval for a period of one year. Hundred seeds of each cucurbitaceous seeds were surface sterilised with 0.1 per cent mercuric

chloride solution and plated in PDA medium and 50 seeds were plated in PDA medium without surface sterilisation. The fungi grown from both surface sterilised and unsterilised seeds were isolated and identified.

Viability and moisture content of cucurbitaceous seeds at different intervals of storage

The seeds of three cucurbitaceous seeds were packed in cloth bags and stored in tin boxes for a period of one year. The germination of the stored cucurbitaceous seeds were tested employing blotter method and pot culture method experiment. The moisture content of seeds was determined by air oven method. These experiments were carried out just before storage of seeds and after every three months interval for a period of one year.

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

The seeds of three cucurbitaceous vegetables were treated with six different fungicides and wood ash. The following are the treatments.

Treatments	Concentration
T_1 Bavistin 50 WP (Methyl-2-benzimidazol carbamate)	0.1%
T_2 Thiride 75 WP (Tetramethyl thiuram disulphide)	0.3%
T_3 Captafol 80 WP (Cis N 1,1,2,2, Tetra chloro-ethylthio 4 cyclohexene 1,2, dicarboximide)	0.1%
T_4 Calixin 50 E.C. (N-tridecyl 2,6 dimethyl morpholine)	0.1%

Treatments	Concentration
T_5 Aureofunginsol	0.2%
T_6 Emisan 3 WP (Methoxy ethyl mercury chloride)	0.1%
T_7 Wood ash	
T_8 Control	

The seeds were dipped in respective fungicidal suspensions for half an hour and dried in shade before storage. Wood ash was used as a smear on seed. A set of treated and control seeds were packed in cloth bags and kept in tin boxes at room temperature and stored for one year. Another set of treated as well as untreated seeds were packed in cloth bags and kept at different humidity levels of 66.8, 75.6, 82.9 and 92.9 per cent in separate desiccator and stored for 3, 6, 9 and 12 months. Different levels of humidity were maintained inside the desiccator using different concentrations of sulphuric acid as follows (CMI Plant Pathologist Pocket Book, 1968).

Sulphuric acid per cent	Relative humidity per cent at 25°C inside the desicator
15	92.9
25	82.9
30	75.6
35	66.8

After 3, 6, 9 and 12 months of storage samples were drawn and their germination percentage was estimated by both blotter and pot culture method.

Influence of internally seed borne mycoflora on pre and post-germination rotting of cucurbitaceous vegetable seeds

To study the effect of fungi isolated from the surface sterilised seeds on the pre and post-germination rotting of seeds, 100 surface sterilised seeds of each vegetable were inoculated separately by rolling them on the surface of actively sporulating or non-sporulating culture of the different fungi isolated from the different vegetable seeds was estimated by blotter method and the percentage of pre and post-germination rotting was calculated.

The following fungi were used for inoculating the cucurbitaceous seeds.

a. Bitter gourd

1. Acremonium sp.
2. Alternaria alternata
3. Aspergillus amstelodami
4. A. flavus
5. Botryodiplodia theobromae
6. Chaetomium globosum
7. Cladosporium cladosporioides
8. Corynespora cassicola
9. Fusarium oxysporum
10. F. solani
11. Helminthosporim sp.
12. Humicola gresia
13. Nigrospora sacchari
14. Paceilomyces varioti
15. Rhizopus stolonifer
16. Hyaline non-sporulating fungi
17. Dark coloured non-sporulating fungi

b. Pumpkin

1. Alternaria alternata
2. Aspergillus flavus
3. A. fumigatus
4. A. niger
5. A. ochraces
6. A. terreus
7. Botryodiplodia theobromae
8. Chaetomium globosum
9. Fusarium oxysporum
10. F. solani
11. Humicola gresia
12. Paecilomyces varioti
13. Rhizopus stolonifer
14. Hyaline non-sporulating fungus

c. Cucumber

1. Alternaria alternata
2. Aspergillus amstelodami
3. A. chevaleieri
4. A. flavus
5. A. flavipes
6. A. fumigatus
7. A. terreus
8. Chaetomium globosum
9. Cladosporium cladosporioides
10. Fusarium oxysporum
11. F. solani
12. Humicola gresia
13. Rhizopus stolonifer
14. Hyaline non-sporulating fungus

Statistical analysis

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

Results

RESULTS

Seeds of three cucurbitaceous vegetables used in this study viz., bitter gourd (Momordica charantia L.), pumpkin (Cu curbita moschata Poir) and cucumber (Cucumis melo L.) were collected from the Department of Olericulture, College of Horticulture, Vellanikkara and Agricultural Research Station, Mannuthy. Immediately after the collection of the seeds, and just before the experiment the germination percentage and moisture contents were assessed as described in Materials and Methods. The data are presented in Table 1.

It was evident from the table that all the three types of seeds used in the study had a moisture content within the permissible limit and the germination percentage was high above the standard fixed for vegetable seeds.(Hand book of Minimum Seed Certification Standards, 1971).

Mycoflora associated with the cucurbitaceous seeds

The fungi associated with three cucurbitaceous vegetables namely bitter gourd, pumpkin and cucumber were estimated as described in Materials and Methods.

The unsterilised and surface sterilised whole seeds were kept in sterile moist chambers and in PDA plates to assess the percentage of seeds associated with mycoflora and the data are presented in Table 2.

The data revealed that all the unsterilised whole seeds had fungal association at different storage periods. The

Table 1. Germination percentage and moisture content of cucurbitaceous seeds

Sl. No.	Name of seed	Germination (per cent)		Moisture content (per cent)
		Blotter method	Pot culture experiment	
1	Bitter gourd	88	80	7.4
2	Pumpkin	82	78	7.3
3	Cucumber	90	88	7.6

Table 2. Percentage of mycoflora associated cucurbitaceous seeds at different intervals of storage

Sl. No.	Name of cucurbitaceous seed	Before storage	After 3 months storage	After 6 months storage	After 9 months storage	After 12 months storage
1	Bitter gourd					
	a) Unsterilised	100	100	100	100	100
	b) Surface sterilised	27	30	31	31	32
2	Pumpkin					
	a) Unsterilised	100	100	100	100	100
	b) Surface sterilised	30	32	32	33	35
3	Cucumber					
	a) Unsterilised	100	100	100	100	100
	b) Surface sterilised	20	20	21	21	22

surface sterilised whole seeds showed considerably less percentage of fungal association and there was a slight increase in the number of infected seeds with the increase in storage period. Before storage, only 27 per cent of the surface sterilised whole seeds of bitter gourd were found to be associated with mycoflora. After 3 months of storage, a 3 per cent increase was noticed and later the increase was considerably less. Similar trend was observed in the case of pumpkin seeds also. Initially 30 per cent of pumpkin seeds yielded the fungal growth and another 5 per cent increase was noticed after one year of storage. But in the case of cucumber seeds, initial percentage of fungal association was only 20 and later it increased to 22 per cent after one year of storage.

The observations revealed that all the unsterilised seeds tested were associated with mycoflora either externally or internally. But after the surface sterilisation of the seeds, the percentage of seeds associated with mycoflora was considerably reduced. Therefore, it could be reasonably presumed that more than 70 per cent seeds were free from invasion of seed pathogen. After one year of storage a maximum increase of 5 per cent was observed in the case of pumpkin and 2 per cent in the case of bitter gourd and cucumber seeds.

Mycoflora associated in different parts of seeds

Before storage, the infection percentage of the mycoflora on different parts of the seeds namely seed coat,

endosperm and embryo was assessed for fungal association.

The fungi isolated from unsterilised and surface sterilised whole seeds and from different parts of these three vegetable seeds were identified and their percentages of occurrence are presented in Table 3. Altogether 34 species of fungi were found associated with the unsterilised seeds. Of these, eleven species belonged to the genus Aspergillus, four species to Penicillium, two species to Fusarium and one each to Absidia, Acremonium, Alternaria, Botryodiplodia, Chaetomium, Cladosporium, Corynespora, Curvularia, Cunninghamella, Helminthosporium, Humicola, Nigrospora, Paecilomyces, Rhizopus and Syncephalastrum. Two fungi were not identified and they belonged to mycelia sterilia which did not produce spores.

1. Bitter gourd

The unsterilised whole seeds of bitter gourd were found associated with 27 species of fungi. Of these, ten species viz., Absidia corymbifera, Aspergillus chevalieri, A. fumigatus, A. flavipes, A. nidulans, A. terrus, Cunninghamella elagans, Curvularia lunata, Penicillium citrinum and P. herquei were found to be purely external on the seed coat. Surface sterilised whole seed and seed coat yielded 17 species of fungi. They were, Acremonium species, Alternaria alternata, Aspergillus amstelodami, A. flavus, Botryodiplodia theobromae, Chaetomium globosum, Cladosporium cladosporioides, Corynespora cassicola, Fusarium oxysporum, F. solani, Helminthosporium sp., Humicola gresia, Nigrospora sacchari, Paecilomyces varioti,

Table 3. Mycoflora associated with different parts of seeds (in percentage)

Sl. No.	Name of fungi	Bitter gourd					Pumpkin					Cucumber				
		US	SS	Seed coat	Endo-sperm	Embryo	US	SS	Seed coat	Endo-sperm	Embryo	US	SS	Seed coat	Endo-sperm	Embryo
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Absidia corymbifera (Cohn) Saco & A. Troth	5	-	-	-	-	4	-	-	-	-	4	-	-	-	-
2	Acremonium sp.	4	3	3	1	-	-	-	-	-	-	-	-	-	-	-
3	Alternaria alternata Fr. Keissler	5	4	4	2	-	16	6	6	-	-	5	3	3	-	-
4	Aspergillus amstelodami (Margin)	14	1	1	-	-	-	-	-	-	-	10	2	2	-	-
5	A. caesiellus Saito	-	-	-	-	-	14	-	-	-	-	18	-	-	-	-
6	A. chevalieri (Margin) Thom & Church	16	-	-	-	-	-	-	-	-	-	16	2	2	-	-
7	A. flavus Lebic Ex. Fr.	26	10	10	5	3	26	8	8	4	4	20	6	6	3	2
8	A. flavipes Bainier & Sartory	18	-	-	-	-	15	-	-	-	-	18	5	5	-	-
9	A. fumigatus Fres	15	-	-	-	-	12	10	10	5	-	16	6	6	2	-
10	A. melleus Yukawa	-	-	-	-	-	14	-	-	-	-	12	-	-	-	-
11	A. nidulans (Eidam)	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(contd.)

Table 3 contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
12	A. niger van Tiegh	-	-	-	-	-	18	4	4	-	-	18	-	-	-	-
13	A. ochraces Welham	-	-	-	-	-	20	6	6	2	-	18	-	-	-	-
14	A. terreus Thom	14	-	-	-	-	20	5	5	1	-	18	5	5	-	--
15	Botryodiplodia theobromae Pat	4	4	4	-	-	4	2	2	-	-	-	-	-	-	-
16	Chaetomium globosum Kunze	3	3	3	-	-	10	5	5	-	-	3	3	3	-	-
17	Cladosporium cladosporioides (Pres)	4	4	4	3	-	4	-	-	-	-	3	2	2	1	-
18	Corynespora cassiçola Berk & M.A. Curls Wei	4	2	2	-	-	-	--	-	-	-	-	-	-	-	-
19	Cunnighamella elegans Leud.	3	-	-	-	-	3	-	-	-	-	3	-	-	-	-
20	Curvularia lunata (Walker) (Boedei)	12	-	-	-	-	10	-	-	-	-	8	-	-	-	-
21	Fusarium oxysporum Schl exfr. Sacc.	8	6	6	2	1	8	5	5	1	-	8	5	5	2	1
22	Fusarium solani (Mart) Sacc.	12	11	11	4	2	11	10	10	6	4	10	8	8	4	2
23	Helminthosporium sp. Link Ex.Fr.	4	2	2	-	-	-	-	-	-	-	-	-	-	-	-

(contd.)

Table 3 contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
24	Humicola gresia Eraacn.	3	2	2	-	-	8	3	3	-	-	3	2	2	-	-
25	Nigrospora sacchari Speg. Mason	3	3	3	-	-	-	-	-	-	-	-	-	-	-	-
26	Paecilomyces varioti Bain	4	3	3	-	-	6	5	5	2	-	-	-	-	-	-
27	Penicillium citrinum Thom	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-
28	Penicillium purpurogenum	-	-	-	-	-	15	-	-	-	-	-	-	-	-	-
29	Penicillium herquei Bainier & Sartory	16	-	-	-	-	12	-	-	-	-	15	-	-	-	-
30	Penicillium pinophilum Hedgock	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-
31	Rhizopus stolanifer Fr. Lind	12	10	10	2	-	18	11	11	4	-	16	8	3	-	-
32	Syncephalastrum racemosum (Cohu) Sachart.	-	-	-	-	-	8	-	-	-	-	8	-	-	-	-
33	Hyaline non-sporulating fungus	5	4	4	1	-	5	1	1	-	-	5	1	-	-	-
34	Dark coloured non-sporulating fungus	6	4	4	-	-	4	-	-	-	-	4	-	-	-	-

US = Unsterilised
SS = Surface sterilised

Rhizopus stolonifer and two species of non-sporulating unidentified fungi. Here, on the surface sterilised seeds, Aspergillus spp. and Fusarium spp. were predominant. All the fungi isolated from the surface sterilised seeds were found on the seed coat of the seed. In the endosperm, only eight species of fungi were found associated. They were Acremonium species, Alternaria alternata, Aspergillus flavus, Cladosporium cladosporioides, Fusarium oxysporum, Fusarium solani, Rhizopus stolonifer, and hyaline sterile fungi. Here also Aspergillus and Fusarium species were the predominating ones. In embryo, only three species of fungi were found associated and all of these were also found in seed coat and endosperm. The fungi harboured in the embryo were Aspergillus flavus, Fusarium oxysporum and Fusarium solani.

2. Pumpkin

The unsterilised whole seeds of pumpkin yielded 25 species of mycoflora while in the surface sterilised seeds 14 species of fungi were found. The results indicated that 11 species of fungi externally contaminated the seed coat. The predominant ones were Aspergillus, Penicillium and Rhizopus species. The fungi noticed were Absidia corymbifera, Aspergillus caesiellus, A. flavipes, A. melleus, cladosporium cladosporioides, Cunninghamella elegans, Curvularia lunata, Penicillium purpurogenum, Penicillium herquei, Syncephalastrum racemosum and dark coloured sterile mycelial fungus.

In the seed coat, 14 species of fungi were found associated and the same fungi were also found in the surface sterilised whole seeds. The fungi associated with seed coat were Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. ochraces, A. terrus, Botryodiplodia theobromae, Chaetomium globosum, Fusarium oxysporum, F. solani, Humicola gresia, Paceilomyces varioti, Rhizopus stolonifer and hyaline non-sporulating fungus. Among these, Rhizopus, Aspergillus and Fusarium species were the predominant fungi in the seed coat.

Eight species of fungi were isolated from the endosperm of pumpkin. The predominating fungi were Fusarium solani, Aspergillus flavus and Rhizopus stolonifer. The other seed-borne pathogens were Aspergillus fumigatus, A. ochraces, A. terrus, Paceilomyces varioti and Fusarium oxysporum.

Only two species of fungi namely Aspergillus flavus and Fusarium solani were found in the embryo. Both of these fungi caused 4 per cent infection in the embryo. These fungi were also found in the seed coat and endosperm of pumpkin seeds.

3. Cucumber

Twentyfive species of fungi were found associated with unsterilised whole seeds. Of these, the 11 species which were not found in surface sterilised whole seeds were Absidia corymbifera, Aspergillus caesiellus, A. melleus, A. niger,

A. ochraces, Curvularia lunata, Cunninghamella elegans, Penicillium herquei, P. pinophilum, Syncephalastrum racemosum and dark coloured sterile fungus.

Fourteen species of seed-borne pathogens were isolated from the seed coat of cucumber seeds. The predominating fungi were Fusarium solani and Rhizopus stolonifer. Other fungi observed were Aspergillus flavus, A. fumigatus, A. flavipes, A. amstelodami, A. chevalieri, A. terreus, Fusarium oxysporum, Alternaria alternata, Chaetomium globosum, Cladosporium cladosporioides, Humicola gresia and hyaline sterile fungus. Of these 14 species, six species were found in the endosperm and they were Aspergillus flavus, A. fumigatus, Cladosporium cladosporioides, Fusarium oxysporum, F. solani and Rhizopus stolonifer. In the embryo, three species of fungi were found associated and the predominant infection was caused by Aspergillus flavus and Fusarium solani. Fusarium oxysporum also caused infection in the embryo. These fungi were also found in the seed coat and endosperm of the cucumber seeds.

In all the three vegetable seeds the maximum species of fungi were found in the seed coat. But the number got reduced in the internal parts of the seeds namely endosperm and embryo. No new pathogen was found in the internal part of the seed.

The study revealed that out of the 23 species of seed borne mycoflora isolated from the seeds of three vegetables namely, bitter gourd, pumpkin and cucumber, eight species were found in all the seeds. They were Alternaria alternata,

Aspergillus flavus, Chaetomium globosum, Fusarium oxysporum, F. solani, Humicola gresia, Rhizopus stolonifer and hyaline non-sporulating fungus.

Two seed borne pathogen namely, Paceilomyces varioti, and Botryodiplodia theobormae were found in the seed coat of bitter gourd and pumpkin whereas Aspergillus amstelodami and Cladosporium cladosporioides were found in the seed coat of bitter gourd and cucumber. The seed pathogens Aspergillus fumigatus and A. terreus were observed in the seed coat of cucumber and pumpkin.

The seed pathogens namely, Acremonium sp., Corynespora cassicola, Helminthosporium sp., Nigrospora sacchari and dark coloured sterile mycelial fungus were restricted to the seed coat of bitter gourd. Other two seed pathogens Aspergillus niger and A. ochraces were found only in the seed coat of pumpkin. Aspergillus chevalieri and A. flavipes were found associated only with the seed coat of cucumber seeds.

Twelve species of seed pathogens were isolated from the endosperm of seeds of three cucurbitaceous crops. The four seed pathogens namely, Aspergillus flavus, Fusarium oxysporum; F. solani and Rhizopus stolonifer were found in the endosperm of all the three vegetable seeds. Aspergillus fumigatus and was restructed to the endosperm of pumpkin and cucumber while Cladosporium cladosporioides was found only in the endosperm of bitter gourd and cucumber. Acremonium sp., Alternaria alternata and hyaline non-sporulating fungi were restricted

to the endosperm of bitter gourd alone. Aspergillus ochraces A. terreus and Paceilomyces varioti were seen only in the endosperm of pumpkin.

Three seed pathogens were found to cause infection of the embryo of the vegetable seeds tested. Of these Aspergillus flavus and Fusarium solani were found in the three types of vegetable seeds while Fusarium oxysporum was found only in the bitter gourd and cucumber seeds.

Mycoflora associated with the stored cucurbitaceous seeds

The stored seeds were tested for the association of different species of fungi at 3 months interval for one year. In the study no new fungus other than those present before storage was found in the surface sterilised and unsterilised seeds of bitter gourd, pumpkin and cucumber during different periods of storage.

1. Bitter gourd

The fungi isolated from both unsterilised and surface sterilised seeds stored for a period of one year were examined at an interval of 3 months and observations are presented in Table 4.

The propagules of Aspergillus chevalieri and Aspergillus nidulans were viable after 3 months of storage but they did not appear after six months of storage. Absidia corymbifera, Aspergillus flavipes, Cunninghamella elegans and Penicillium herquei were viable on the surface of the seeds only upto six

Table 4. Fungi isolated from bitter gourd seeds at different intervals of storage

Sl. No.	Name of fungi	Before storage		After 3 months storage		After 6 months storage		After 9 months storage		After 12 months storage	
		US	SS	US	SS	US	SS	US	SS	US	SS
1	2	3	4	5	6	7	8	9	10	11	12
1	Absidia corymbifera	+	-	+	-	+	-	-	-	-	-
2	Acremonium sp.	+	+	+	+	+	+	+	+	+	+
3	Alternaria alternata	+	+	+	+	+	+	+	+	+	+
4	Aspergillus amstelodami	+	+	+	+	+	+	-	-	-	-
5	A. Chevalrieri	+	-	+	-	-	-	-	-	-	-
6	A. flavus	+	+	+	+	+	+	+	+	+	+
7	A. flavipes	+	-	+	-	+	-	-	-	-	-
8	A. fumigatus	+	-	+	-	+	-	+	-	+	-
9	A. nidulans	+	-	+	-	-	-	-	-	-	-
10	Botryodiplodia theobromae	+	-	+	-	+	-	+	-	+	-
11	Botryodiploidia theobromae	+	+	+	+	+	+	+	+	+	+
12	Chaetomium globosum	+	+	+	+	+	+	+	+	+	+

(contd.)

Table 4 contd.

1	2	3	4	5	6	7	8	9	10	11	12
13	Cladosporium cladosporioides	+	+	+	+	-	-	-	-	-	-
14	Corynespora cassicola	+	+	+	+	+	+	+	+	+	+
15	Cunninghamella elegans	+	-	+	-	+	-	-	-	-	-
16	Curvularia lunata	+	-	+	-	+	-	+	-	+	-
17	Fusarium oxysporum	+	+	+	+	+	+	+	+	+	+
18	Fusarium solani	+	+	+	+	+	+	+	+	+	+
19	Helminthosporium sp.	+	+	+	+	+	+	+	+	+	+
20	Humicola gresia	+	+	+	+	+	+	+	+	+	+
21	Nigrospora sacchari	+	+	-	-	-	-	-	-	-	-
22	Paceilomyces varioti	+	+	+	+	+	+	+	+	+	+
23	Penicillium citrinum	+	-	+	-	-	-	+	-	+	-
24	Penicillium herquei	+	-	+	-	+	-	-	-	-	-
25	Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+
26	Hyaline sterile mycelium	+	+	+	+	+	+	+	+	+	+
27	Dark coloured sterile mycelium	+	+	+	+	+	+	+	+	+	+

US = Unsterilised

SS = Surface sterilised

months of storage, while the propagules of Aspergillus fumigatus, A. terreus, Curvularia lunata and Penicillium citrinum were viable even after one year of storage.

Out of the seventeen internally seed born pathogen isolated during the study, the fungus Nigrospora sacchari was found only before the storage. Viability of the propogules of Cladosporium cladosporioides was lost within six months of storage, while that of Aspergillus amstelodami was lost only within nine months of storage. All other seed born pathogens were viable in the seed even after 12 months of storage.

2. Pumpkin

Pumpkin seeds stored were examined for the mycoflora at 3 months intervals for a period of 12 months and the results are presented in Table 5. Of the 25 fungi isolated from the pumpkin seeds, 11 were found purely external. Of these, Aspergillus melleus was found viable only after 6 months of storage. The viability of propogules of Absidia corymbifera, Aspergillus flavipes, Cunninghamella elagans, Penicillium herquei and Syncephalostrum racemosum was observed upto six months of storage on the surface of the seeds. The other external fungi like Curvularia lunata, Paceilomyces varioti, Penicillium purpurogenum and hyaline sterile mycelial fungus were found viable even after 12 months of storage.

Out of the 4 species of internally seed borne pathogens isolated from surface sterilised pumpkin seeds, the fungi

Table 5. Fungi isolated from pumpkin seeds at different intervals of storage

Sl. No.	Name of fungi	Before storage		After 3 months storage		After 6 months storage		After 9 months storage		After 12 months storage	
		US	SS	US	SS	US	SS	US	SS	US	SS
1	2	3	4	5	6	7	8	9	10	11	12
1	Absidia corymbifera	+	-	+	-	+	-	-	-	-	-
2	Alternaria alternata	+	+	+	+	+	+	+	+	+	+
3	Aspergillus caesiellus	+	-	+	-	+	-	+	-	+	-
4	A. flavus	+	+	+	+	+	+	+	+	+	+
5	A. flavipes	+	-	+	-	+	-	-	-	-	-
6	A. fumigatus	+	+	+	+	+	+	+	+	+	-
7	A. melleus	+	-	+	-	+	-	-	-	-	-
8	A. niger	+	+	+	+	+	+	+	+	+	+
9	A. ochraces	+	+	+	+	+	+	+	+	+	+
10	A. terreus	+	+	+	+	+	+	+	+	+	+
11	Botryodiplodia theobromae	+	+	+	+	+	+	+	+	+	+
12	Chaetomium globosum	+	+	+	+	+	+	+	+	+	+
13	Cladosporium cladosporioides	+	-	+	-	-	-	-	-	-	-
14	Cunninghamella elegans	+	-	+	-	+	-	-	-	-	-

Table 5 contd.

1	2	3	4	5	6	7	8	9	10	11	12
15	Curvularia lunata	+	-	+	-	+	-	+	-	+	-
16	Fusarium oxysporum	+	+	+	+	+	+	+	+	+	+
17	Fusarium solani	+	+	+	+	+	+	+	+	+	+
18	Humicola gresia	+	+	+	+	+	+	-	-	-	-
19	Paceilomyces varioti	+	+	+	+	+	+	+	+	+	+
20	Penicillium purpurogenum	+	-	+	-	+	-	+	-	+	-
21	Penicillium herquei	+	-	+	-	+	-	-	-	-	-
22	Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+
23	Syncelpalostrum racimosum	+	-	+	-	+	-	-	-	-	-
24	Hyaline sterile mycelium	+	+	+	+	+	+	+	+	+	+
25	Dark coloured sterile mycelium	+	-	+	-	+	-	+	-	+	-

US = Unsterilised
SS = Surface sterilised

Humicola gresia was found viable upto 9 months of storage. But the other 13 fungi remained viable in the seed even after 12 months of storage.

3. Cucumber

Cucumber seeds were stored for one year and the mycoflora associated with them were examined at 3 month intervals and the observations are recorded in Table 6.

Out of the 25 fungi isolated from the stored cucumber seeds, 12 species were found purely external. Of these, Syncephalastrum racemosum, Cunninghamella elegans and Absidia corymbifera were found viable only upto 3 months of storage. Aspergillus melleus, A. ochraces and Penicillium herquei were viable upto six months of storage whereas Aspergillus niger, A. caesiellus, Curvularia lunata, Penicillium pinophilum and hyaline sterile mycelial fungus were found viable even after 12 months of storage.

The internally seed pathogens like Aspergillus chevaleiri, A. flavipes and Cladosporium cladosporides were found viable in the seed only upto 3 months of storage. But Humicola gresia remained active on the seed even after six months of storage, but it was not present after 9 months of storage. All other nine internally seed borne pathogens namely, Alternaria alternata, Aspergillus flavus, A. fumigatus, A. terreus, Chaetomium globosum, Fusarium oxysporum, F. solani, Rhizopus stolonifer and dark coloured non-sporulating fungus were found viable upto 12 months of storage.

Table 6. Fungi isolated from cucumber seeds at different intervals of storage

Sl. No.	Name of fungi	Before storage		After 3 months storage		After 6 months storage		After 9 months storage		After 12 months storage	
		US	SS	US	SS	US	SS	US	SS	US	SS
1	2	3	4	5	6	7	8	9	10	11	12
1	Absidia corymbifera	+	-	+	-	-	-	-	-	-	-
2	Alternaria alternata	+	+	+	+	+	+	+	+	+	+
3	Aspergillus amstelodami	+	+	+	+	+	+	+	+	+	+
4	A. caesiellus	+	-	+	-	+	-	+	-	+	-
5	A. chevaleieri	+	+	+	+	-	-	-	-	-	-
6	A. flavus	+	+	+	+	+	+	+	+	+	+
7	A. flavipes	+	+	+	+	-	-	-	-	-	-
8	A. fumigatus	+	+	+	+	+	+	+	+	+	+
9	A. melleus	+	-	+	-	+	-	-	-	-	-
10	A. niger	+	-	+	-	+	-	+	-	+	-
11	A. ochraces	+	-	+	-	+	-	-	-	-	-
12	A. terreus	+	+	+	+	+	+	+	+	+	+
13	Chaetomium globosum	+	+	+	+	+	+	+	+	+	+
14	Cladosporium cladosporioides	+	+	+	+	+	-	-	-	-	-

Table 6 contd.

1	2	3	4	5	6	7	8	9	10	11	12
15	Cunninghamella elagans	+	-	+	-	-	-	-	-	-	-
16	Curvularia lunata	+	-	+	-	+	-	+	-	+	-
17	Fusarium oxysporum	+	+	+	+	+	+	+	+	+	+
18	Fusarium solani	+	+	+	+	+	+	+	+	+	+
19	Humicola gresia	+	+	+	+	+	+	-	-	-	-
20	Penicillium herquei	+	-	+	-	+	-	-	-	-	-
21	P. Pinophilium	+	-	+	-	+	-	+	-	+	-
22	Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+
23	Syncephalostrum racemosum	+	-	+	-	-	-	-	-	-	-
24	Dark coloured sterile mycelium	+	-	+	-	+	-	+	-	+	-
25	Hyaline sterile mycelium	+	+	+	+	+	+	+	+	+	+

US = Unsterilised

SS = Surface sterilised

Viability and moisture content of cucurbitaceous seeds at different intervals of storage

The viability and moisture content of cucurbitaceous seeds at different periods of storage were determined and the data are presented in Table 7.

It was evident from the data that as the storage period increased there was a decrease in germination percentage in all the three cucurbitaceous seeds. Bitter gourd, pumpkin and cucumber seeds recorded a germination percentage of 77, 76 and 80 respectively after three months of storage when tested with blotter method. The percentage of germination of these seeds in pot culture experiment was less compared to that in blotter test and it was 70 in bitter gourd, 78 in cucumber and 65 in pumpkin seeds.

The moisture content of these seeds after 3 months of storage was more when compared with moisture content before storage. Bitter gourd seed recorded a moisture content of 10.2 per cent while those of pumpkin and cucumber seeds were 9.8.

After six months of storage a further loss of viability was recorded in bitter gourd and pumpkin seeds, the germination percentages being 66 and 63 respectively when tested in blotter technique. Cucumber seeds did not show any further reduction in germination percentage (80). In pot culture experiment, a similar trend was observed. Here, the bitter gourd seeds showed a germination percentage of 56 while that

Table 7. Germination percentage and moisture content of cucurbitaceous seeds at different intervals of storage

Sl. No.	Name of seed	Before storage			After 3 months of storage			After 6 months of storage			After 9 months of storage			After 12 months of storage		
		Germination (%)		Moisture content	Germination (%)		Moisture content	Germination (%)		Moisture content	Germination (%)		Moisture content	Germination (%)		Moisture content
		B	P		B	P		B	P		B	P		B	P	
1	Bitter gourd	88	80	7.4	77	70	10.2	66	56	9.66	40	33	9.2	26	15	7.2
2	Pumpkin	82	78	7.3	76	65	9.8	63	53	9.42	33	26	8.9	10	8	7.8
3	Cucumber	90	83	7.6	80	78	9.8	80	70	9.61	68	62	9.5	66	56	7.3

B = Blotter method

P = Pot culture method

of pumpkin seed was 53. For cucumber seeds, the germination percentage was 70. A slight decrease in the moisture content was noticed for the three cucurbitaceous seeds after six months of storage as compared to that of 3 months storage. The percentage moisture recorded was 9.66 in bitter gourd, 9.42 in pumpkin and 9.61 in cucumber seeds.

After nine and 12 months of storage there was a substantial reduction in the germination percentage in bitter gourd and pumpkin seeds. The reduction was more pronounced in the latter. Though, cucumber seeds also showed a reduction in percentage of germination during the above periods, it was not much pronounced. Nine months after storage, the bitter gourd seeds showed a germination percentage of 40 in blotter technique while in pot culture experiment it was 33. Germination percentage of 33 and 26 were noticed after nine months of storage for pumpkin seeds in blotter technique and in pot culture experiment, respectively. After nine months of storage the cucumber seeds recorded a lesser germination percentage of 68 in blotter technique and 62 in pot culture experiment.

In general, the moisture content of seed after 9 months storage was comparatively lower than that of 6 months storage. The values were 9.2 per cent in bitter gourd, 8.9 per cent in pumpkin and 9.5 per cent in cucumber seeds.

Germination percentages of 26 in blotter method and 18 in pot culture experiment were noticed in the case of bitter gourd seeds after 12 months of storage. The pumpkin

seeds recorded a marked reduction in germination after 12 months of storage and the germination percentage was 10 and 8 respectively in blotter method and pot culture experiment. However, the cucumber seeds did not show much reduction in germination as compared to bitter gourd and pumpkin seeds, the germination percentage being 66 in blotter method and 56 in pot culture experiment. It was evident that as the storage period increased there was a decrease in the germination percentage of cucurbitaceous seeds and the decrease was much pronounced in bitter gourd and pumpkin seeds compared to that of cucumber seed.

The moisture content of seeds decreased further and the values after 12 months of storage for bitter gourd, pumpkin and cucumber were 7.2, 7.8 and 7.3 per cent respectively.

Effect of seed treatments on the viability of cucurbitaceous seeds at different storage periods and humidity levels

Bitter gourd

The treated seeds were evaluated for their germination before storage. In blotter method, Calixin treated seeds showed the minimum germination compared to the untreated seeds and the reduction in germination was 23 per cent (Table 8). Ash treated seeds also showed eight per cent reduction in germination compared to the untreated seeds. In pot culture experiments, the seeds showed more germination compared to untreated seeds except Calixin and Ash treated seeds. But the reduction in ash treated seeds was very meagre, of two per cent

Table 8. Germination percentage of treated seeds of bitter gourd stored for 3 months at different humidity levels in blotter and pot culture experiment

Treatments	Germination percentage											
	Blotter method						Pot culture experiment					
	Before storage	Normal storage	Humidity level (Per cent)				Before storage	Normal storage	Humidity level (per cent)			
			66.8	75.6	82.9	92.9			66.8	75.6	82.9	92.9
T_1 Bavistin	90	80	80	60	0	0	85	76	70	56	0	0
T_2 Thiride	88	88	88	60	0	0	82	86	86	54	0	0
T_3 Captafol	88	86	90	60	0	0	82	80	86	57	0	0
T_4 Calixin	65	60	65	53	0	0	60	56	60	50	0	0
T_5 Aureofunginsol	82	75	76	40	0	0	82	70	72	36	0	0
T_6 Emisan	90	88	90	60	0	0	86	83	88	53	0	0
T_7 Ash	80	80	82	48	0	0	78	76	80	40	0	0
T_8 Control	88	77	80	44	0	0	80	70	70	40	0	0

while a drastic reduction of 20 per cent was noticed in Calixin treated seeds.

Three months after storage, under normal condition, seeds treated with Thiride and Emisan recorded the maximum percentage of germination of 88 in blotter method and were on par with other treatment except Calixin which showed minimum germination of 60 per cent (Table 8). In pot culture experiment, Thiride treated seeds showed the maximum percentage of germination of 86 and was on par with Emisan, Captafol, Bavistin and Ash treated seeds. The minimum germination of 56 per cent was observed in Calixin treated seed.

When the treated seeds were stored for three months at 66.8 per cent humidity level and tested for the germination by blotter method the maximum germination of 90 per cent was noticed with Captafol and Emisan treatments and they were on par with other seed treatments except Calixin. Minimum percentage of germination of 65 was recorded with Calixin treatment. Significant difference in germination percentage was observed in pot culture experiment also with the maximum of 88 per cent in seeds treated with Emisan and was on par with Captafol, Ash, Thiride and Aureofunginsol treated seeds. The minimum germination of 60 per cent was noticed with Calixin treated seeds. When compared to the normal storage conditions, seeds stored in 66.8 per cent humidity level showed a better germination percentage in all the treatments both in blotter

and in pot culture methods except Bavistin where six per cent reduction were observed in pot culture method.

After three months of 75.6 per cent humidity level a reduction in the germination of bitter gourd seed was noticed. In blotter method, the maximum germination percentage of 60 was observed when the seeds were treated with Bavistin, Captafol, Thiride and Emisan and was on par with Calixin, Ash and untreated seeds. Aureofunginsol treated seeds showed the minimum germination percentage of 40. In pot culture experiment the maximum germination of 57 per cent was noticed in Captafol treated seeds and which was on par with all other treatments except Aureofunginsol treatment. The minimum germination of 36 per cent was noticed in Aureofunginsol treated seeds.

At 82.9 per cent and 92.9 per cent humidity levels bitter gourd seeds did not show any germination after three months of storage revealing an adverse effect of high humidity on the germination of bitter gourd seeds.

Bitter gourd seeds were kept for six months at different humidity levels and data are given in Table 9. Under normal condition, the maximum germination in blotter test was in Thiride treated seeds (84 per cent) closely followed by Emisan treatment. The minimum germination of 63 per cent was in Calixin and Aureofunginsol seed treatments. In pot culture experiment Thiride treated seeds recorded significantly higher germination of 76 per cent. The minimum germination of 56

Table 9. Germination percentage of treated seeds of bitter gourd stored for 6 months at different humidity levels in blotter and pot culture experiment

Treatments	Germination percentage								
	Blotter method					Pot culture experiment			
	Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
		66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1 Bavistin	73	72	15	0	0	70	63	10	0	0
T_2 Thiride	84	86	58	0	0	76	80	50	0	0
T_3 Captafol	70	74	58	0	0	66	70	50	0	0
T_4 Calixin	63	64	50	0	0	58	62	46	0	0
T_5 Aureofunginsol	63	69	0	0	0	56	60	0	0	0
T_6 Emisan	83	76	30	0	0	70	72	26	0	0
T_7 Ash	73	76	16	0	0	56	72	10	0	0
T_8 Control	66	70	0	0	0	56	62	0	0	0

per cent was observed in Aureofunginsol, Ash and untreated seeds. Bitter gourd seeds stored for six months at 66.8 per cent humidity level showed significantly higher germination of 86 per cent in Thiride treated seeds in blotter method and was on par with rest of the treatments except Calixin which showed the minimum germination of 64 per cent. In pot culture experiment also Thiride treated seeds showed the maximum germination of 80 per cent. The minimum germination of 60 per cent was in Aureofunginsol treatment. When the seeds were stored under 66.8 per cent humidity level in almost all the treatments, the germination percentage was a slightly high when compared to normal storage condition except in Bavistin.

Significant difference among the treatments on the germination percentage was noticed when bitter gourd seeds were stored for six months at 75.6 per cent humidity. In blotter test, Thiride and Captafol treated seeds recorded higher germination of 58 per cent and were on par with that of Calixin. None of the seeds treated with Aureofunginsol germinated. All other treatments showed comparatively poor germination. Similar trend was noticed when germination of bitter gourd seeds was tested in pot culture also. Here all the treatments showed comparatively poor germination. In this humidity level, 50 per cent germination was observed in seeds treated with Thiride and Captafol followed by 46 per cent in Calixin. Very poor germination was observed in Bavistin and Ash treated

seeds (10 per cent). Compared to normal storage and storage under 66.8 per cent humidity level, the germination percentage at 75.6 per cent humidity level was drastically low both in blotter method and in pot culture experiments.

After six months of storage at 82.9 and 92.9 per cent humidity level, none of the treated seeds germinated.

When the seeds were kept for nine months under normal conditions, the maximum and minimum germination were noticed in Thiride (76 per cent) and Aureofunginsol (36 per cent) treated seeds respectively in blotter method (Table 10). In pot culture experiment, similar trend was observed with maximum germination in Thiride treated seeds (70 per cent) and minimum in Aureofunginsol treatment (32 per cent).

The viability of bitter gourd seeds stored for nine months at 66.8 per cent humidity was assessed in blotter method. The result revealed that the maximum germination percentage was (80 per cent) in Thiride and minimum in Aureofunginsol treated seeds (40 per cent). In pot culture experiment also, a higher germination of 77 per cent was observed in Thiride treated seeds. The minimum germination of 35 per cent was in Aureofunginsol treated seeds.

At 75.6 per cent humidity level after 9 months of storage Thiride treatment gave the maximum germination of 50 per cent in blotter method and which was on par with Emisan and Calixin treatments. Ash treated seeds showed no

Table 10. Germination percentage of treated seeds of bitter gourd stored for 9 months at different humidity levels (in blotter and pot culture experiment)

Treatments		Germination percentage								
		Blotter method					Pot culture experiment			
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	60	60	10	0	0	50	54	4	0	0
T_2	Thiride	76	80	50	0	0	70	77	46	0	0
T_3	Captafol	53	63	26	0	0	50	60	10	0	0
T_4	Calixin	38	48	40	0	0	36	40	38	0	0
T_5	Aureofunginsol	36	40	6	0	0	32	35	4	0	0
T_6	Emisan	54	50	43	0	0	46	44	40	0	0
T_7	Ash	38	48	0	0	0	36	42	0	0	0
T_8	Control	40	45	8	0	0	33	40	0	0	0

germination. The Captafol, Bavistin, Aureofunginsol and untreated seeds showed comparatively poor germination with the minimum of six percentage of germination in Aureofunginsol treated seeds. The pot culture experiment also revealed a similar trend. Though, poor in germination, the seeds treated with Thiride showed significantly higher germination percentage of 46 and this treatment was on par with Emisan and Calixin. Untreated seeds and Ash treated seeds showed no germination. Captafol, Bavistin and Aureofunginsol treated seeds showed very poor germination. Here also a decrease in germination was noticed in pot culture experiment compared to blotter method. In both methods the germination per cent was very less when compared to the normal storage and storage under 66.8 per cent humidity level.

None of the bitter gourd seeds was viable at 82.9 and 92.9 per cent humidity level after nine months of storage.

Thiride treated bitter gourd seeds stored for a period of 12 months under normal storage condition recorded a maximum germination percentage of 66 in blotter method and a minimum of 26 per cent in Ash, Calixin and untreated seeds. Similar trend was observed in pot culture experiment also (Table 11).

At 66.8 per cent humidity level, the bitter gourd seeds retained comparatively good viability even after 12 months of storage. Significantly higher germination percentage (73 per cent) was noticed in blotter test in Thiride treated seeds.

Table 11. Germination percentage of bitter gourd seeds stored for 12 months at different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage								
		Blotter method					Pot culture experiment			
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	40	45	10	0	0	26	50	2	0	0
T_2	Thiride	66	73	45	0	0	58	68	32	0	0
T_3	Captafol	36	60	20	0	0	33	55	26	0	0
T_4	Calixin	26	36	10	0	0	23	33	6	0	0
T_5	Aureofunginsol	33	40	2	0	0	28	35	0	0	0
T_6	Emisan	46	53	25	0	0	43	48	21	0	0
T_7	Ash	26	38	0	0	0	23	36	0	0	0
T_8	Control	26	33	0	0	0	18	26	0	0	0

The minimum germination of 33 per cent was noticed in untreated seeds. Similar trend was noticed in pot culture experiment also. Compared to the normal storage condition, higher germination percentage was obtained when the treated seeds were stored at 66.8 per cent humidity level after 12 months of storage both in blotter and pot methods.

When seeds were kept for 12 months at 75.6 per cent humidity level maximum germination of 45 per cent was noticed in Thiride treated seeds and it differed significantly with all other treatments. Untreated seeds and ash treated seeds failed to germinate at the above humidity level. Very poor germination was observed in Aureofunginsol (two per cent), Calixin (ten per cent) and Bavistin (ten per cent) in treated seeds. Very poor germination was observed at 75.6 per cent humidity level when compared to normal and 66.8 per cent humidity level after 12 months storage.

At higher humidity levels of 82.9 and 92.9 per cent none of the bitter gourd seeds germinated.

Pumpkin

Immediately after seed treatment the germination percentage of pumpkin seed was determined by blotter method and pot culture experiment (Table 12). In blotter method, Thiride treated seeds recorded the maximum germination percentage of 92 closely followed by Captafol and Emisan treatments. Calixin treated seeds showed the minimum germination of

Table 12. Germination percentage of treated seeds of pumpkin stored for 3 months under different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage											
		Blotter method						Pot culture experiment					
		Before storage	Normal storage	Humidity level (per cent)				Initial	Normal	Humidity level (per cent)			
				66.8	75.6	82.9	92.9			66.8	75.6	82.9	92.9
T_1	Bavistin	80	80	78	40	0	0	76	75	72	38	0	0
T_2	Thiride	92	86	92	60	0	0	88	82	86	50	0	0
T_3	Captafol	90	90	90	54	0	0	86	82	85	48	0	0
T_4	Calixin	70	66	66	36	0	0	68	62	64	28	0	0
T_5	Aureofunginsol	80	80	80	36	0	0	70	70	70	30	0	0
T_6	Emisan	90	86	92	38	0	0	86	80	88	36	0	0
T_7	Ash	84	80	82	36	0	0	86	70	79	33	0	0
T_8	Control	82	76	78	36	0	0	78	65	72	33	0	0

70 per cent. All the treatments except Aureofunginsol, Bavistin and Calixin showed higher germination than the control both in blotter test and in pot culture experiment. In pot culture experiment also the maximum germination percentage of 88 was recorded in seeds treated with Thiride, followed by Captafol, Emisan and Ash treatments. A minimum of 68 per cent germination was recorded with Calixin seed treatment.

Three months after storing of seeds under normal conditions, Captafol treated seeds showed significantly higher germination of 90 per cent in blotter method and this treatment was on par with other treatment except Calixin, which showed the minimum germination percentage of 66. In pot culture experiment Thiride and Captafol treated seeds recorded the maximum germination percentage of 82 after three months of storage under normal condition. The minimum germination of 62 per cent was in seeds treated with Calixin. All the treatments except Calixin showed higher germination than the control both in blotter method and in pot culture experiment.

After three months of storage of 66.8 per cent humidity level, maximum germination in blotter method was observed in Thiride and Emisan treated seeds (92 per cent) and these treatments were on par with Captafol treatment, while seeds treated with Calixin recorded the minimum germination of 66 per cent. In pot culture experiment, the maximum germination of 88 per cent was recorded in seeds treated with Emisan which was closely followed by Thiride and Captafol. Here also

Calixin treated seeds recorded the minimum germination of 64 per cent. When compared to the normal storage all the treatments had an increased germination percentage when the seeds were stored under 66.8 per cent humidity level except in Bavistin treated seeds which showed slight decrease both in blotter and in pot culture methods after three months.

In general, pumpkin seeds kept at 75.6 per cent humidity level for a period of three months showed comparatively lesser germination in blotter as well as pot culture experiment than the normal and 66.8 per cent humidity level. In blotter test a maximum germination of 60 per cent was noticed in Thiride treated seeds and this treatment differed significantly with all other treatments except Captafol treatment. The minimum germination percentage of 36 was observed for Aureofunginsol, Calixin, Ash and untreated seeds. In pot culture experiment also maximum germination of 50 per cent was observed in seeds treated with Thiride closely followed by Captafol. Here, seeds treated with Calixin recorded the minimum germination of 28 per cent.

The seed stored for 3 months under 82.9 and 92.9 per cent humidity levels did not germinate.

Under normal storage condition after six months of storage the maximum germination in blotter method was observed in Emisan treated seeds (80 per cent) (Table 13). Minimum germination of 53 per cent was observed in Aureofunginsol treated seeds. Similar trend was observed in pot culture experiment

Table 13. Germination percentage of treated seeds of pumpkin stored for 6 month under different humidity levels in blotter and pot culture experiment.

Treatments		Germination percentage								
		Blotter method					Pot culture experiment			
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	66	72	10	0	0	53	66	8	0	0
T_2	Thiride	78	78	46	0	0	74	76	0	0	0
T_3	Captafol	70	78	0	0	0	65	72	0	0	0
T_4	Calixin	57	60	0	0	0	50	53	0	0	0
T_5	Aureofunginsol	53	60	30	0	0	50	56	26	0	0
T_6	Emisan	80	86	10	0	0	74	78	8	0	0
T_7	Ash	56	62	0	0	0	50	56	0	0	0
T_8	Control	63	66	12	0	0	53	60	0	0	0

also. Here the maximum germination percentage of 74 was observed in seeds treated with Emisan and Thiride and the minimum of 50 per cent in Calixin, Aureofunginsol and Ash treatments.

A maximum of 86 percentage of germination was observed in Emisan treated seeds in blotter method after six months of storage at 66.8 per cent humidity level. The minimum germination of 60 per cent was observed in seeds treated with Calixin and Aureofunginsol. Similar trend was observed in pot culture experiment also. A better germination percentage was observed when the seeds were stored at 66.8 per cent humidity level compared to the normal storage in all the treatments both in blotter and in pot culture experiment.

At 75.6 per cent humidity level after six months of storage pumpkin seeds showed comparatively poor germination in blotter method. Thiride treated seeds showed the maximum germination (46 per cent) and the minimum in Bavistin and Emisan treatment (10 per cent). Seeds treated with Captafol, Calixin and Ash did not show any germination, while in pot culture experiment, all the treatments except Bavistin, Aureofunginsol and Emisan showed loss of germination. Among them, Aureofunginsol treated seeds recorded the maximum germination of 26 per cent and a minimum of eight per cent in Emisan and Bavistin treatments. When compared to the normal storage and under 66.8 per cent humidity level a drastic reduction of germination has been observed both in blotter method and in pot culture experiment.

There was no germination under 82.9 per cent and 92.9 per cent humidity after six months of storage.

Pumpkin seeds stored for nine months under normal storage condition recorded the maximum percentage of germination of 76 in Thiride treated seeds in blotter method (Table 14). The minimum germination of 30 per cent was observed in ash treated seeds. Similar trend was observed in pot culture also. Thiride treated seeds showed the maximum germination of 72 per cent. The minimum germination of 26 per cent noticed in ash and untreated seeds.

Result of study by blotter method on the germination of pumpkin seeds stored for nine months at 66.8 per cent humidity level revealed that the maximum germination of 78 per cent was in Thiride treated seeds which was on par with Captafol and Emisan treatments. The minimum germination of 50 per cent was in Aureofunginsol, Ash and untreated seeds. In pot culture experiment, the maximum germination of 74 per cent was observed in Thiride treated seeds, and the minimum of 32 per cent in untreated seeds. A better germination percentage was observed when the seeds were stored under 66.8 per cent humidity level than the normal for a period of nine months both in blotter and in pot culture experiment. Observation on the germination by blotter method of the seeds stored at 75.6 per cent humidity level for nine months revealed that only Thiride treated and untreated seeds germinated. Thiride treated seeds showed 45 per cent germination. In pot culture experiment only

Table 14. Germination percentage of treated seeds of pumpkin stored for 9 months under different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage								
		Blotter method					Pot culture experiment			
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	60	67	0	0	0	50	60	0	0	0
T_2	Thiride	76	78	45	0	0	72	74	36	0	0
T_3	Captafol	68	74	0	0	0	62	68	0	0	0
T_4	Calixin	53	58	0	0	0	48	46	0	0	0
T_5	Aureofunginsol	36	50	0	0	0	28	45	0	0	0
T_6	Emisan	62	70	0	0	0	50	60	0	0	0
T_7	Ash	30	50	0	0	0	26	43	0	0	0
T_8	Control	33	50	6	0	0	26	32	0	0	0

Thiride treated seeds gave 36 per cent germination and all other treated and untreated seeds were found non-viable.

There was no germination of seeds at 82.9 per cent and 92.9 per cent humidity level after nine months of storage in all the treatments.

In blotter method, the maximum germination percentage of pumpkin seeds stored for 12 months under normal storage was in Thiride treated seeds (70 per cent), and the minimum in untreated seeds (10 per cent). Similar trend was observed in pot culture also, with a maximum germination percentage of 67 in Thiride treated seeds and a minimum of eight in untreated seeds (Table 15).

After 12 months of storage at 66.8 per cent humidity level, the germination test by blotter method revealed that Thiride treated seeds had the maximum of 72 per cent germination. The minimum germination of 33 per cent was in ash treatment. In pot culture experiment the maximum germination was observed in Thiride treated seeds (70 per cent) and the minimum (20 per cent) in untreated seeds. Increased germination percentage was noticed at 66.8 per cent humidity level after 12 months of storage compared to the normal storage condition both in blotter as well as pot culture experiments.

At 75.6 per cent humidity level after 12 months of storage, none of the seeds germinated except the Thiride treated seeds when tested by both blotter method and pot culture experiment. Here, the Thiride treated seeds showed

Table 15. Germination percentage of treated seeds of pumpkin stored for 12 months under different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage									
		Blotter method					Pot culture experiment				
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)			
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	26	46	0	0	0	16	46	0	0	0
T_2	Thiride	70	72	32	0	0	67	70	29	0	0
T_3	Captafol	60	66	0	0	0	53	60	0	0	0
T_4	Calixin	23	36	0	0	0	16	30	0	0	0
T_5	Aureofunginsol	26	35	0	0	0	16	30	0	0	0
T_6	Emisan	50	65	0	0	0	40	58	0	0	0
T_7	Ash	18	33	0	0	0	16	30	0	0	0
T_8	Control	10	36	0	0	0	8	20	0	0	0

very poor germination percentage of 32 and 29 in blotter and pot culture experiment respectively.

No germination was observed in any treatment at 82.9 and 92.9 per cent humidity levels after 12 months of storage.

Cucumber

The germination of the treated cucumber seeds was evaluated before storage both by blotter test and pot culture experiment. The results of blotter test revealed that the maximum (98 per cent) and minimum germination (63.per cent) were with Thiride and Calixin treated seeds respectively (Table 16). Similar trend was observed in pot culture experiment also, where only the Calixin and Aureofunginsol treated seeds and untreated control gave below 80 per cent germination.

After three months of storage under normal conditions, maximum germination in blotter test was observed in Thiride treated seeds (99 per cent). Seeds treated with Calixin showed the minimum germination of 60 per cent. Superiority of Thiride was again established in pot culture studies which showed maximum germination (97 per cent) and the Calixin treated seeds showed significantly lower percentage of germination (52 per cent).

When seeds were stored for three months at 66.8 per cent humidity level, 100 per cent germination was recorded in Thiride treated seeds under blotter method. A minimum germination of 65 per cent was observed in Calixin treated seeds. In pot

Table 16. Germination percentage of treated seeds of cucumber stored for 3 months under different humidity levels in blotter and pot culture experiment

Treatments	Germination percentage											
	Blotter method						Pot culture experiment					
	Before storage	Normal storage	Humidity level (per cent)				Before storage	Normal storage	Humidity level (per cent)			
			66.8	75.6	82.9	92.9			66.8	75.6	82.9	92.9
T_1 Bavistin	85	80	88	55	0	0	80	76	86	40	0	0
T_2 Thiride	98	99	100	78	0	0	94	97	98	75	0	0
T_3 Captafol	96	86	90	58	0	0	87	80	86	46	0	0
T_4 Calixin	63	60	65	53	0	0	60	52	58	33	0	0
T_5 Aureofunginsol	81	70	80	53	0	0	76	68	78	36	0	0
T_6 Emisan	93	84	93	80	0	0	88	79	87	75	0	0
T_7 Ash	83	79	83	53	0	0	80	78	80	36	0	0
T_8 Control	90	80	81	53	0	0	72	70	68	36	0	0

culture experiment the maximum germination of 98 per cent was observed in Thiride treated seeds. Lowest germination of 58 per cent was noticed in Calixin treated seeds. Compared to normal storage condition the treated seeds stored at 66.8 per cent humidity level showed a better percentage of germination both in blotter method and in pot culture experiment.

Three months after storing seeds at 75.6 per cent humidity level, Emisan treated seeds showed significantly higher germination (80 per cent) in blotter test and this treatment was on par with Thiride treated seeds. The lowest germination of 53 per cent was noticed in Calixin, Aureofunginsol, Ash and untreated seeds. Emisan and Thiride treated seeds recorded a maximum of 75 per cent germination in pot culture experiment while Calixin treated seeds showed the lowest germination of 33 per cent. The seeds stored under 79.6 per cent humidity showed a reduction in germination percentage both in blotter test and in pot culture studies.

Under high humidity levels of 82.9 and 92.9 per cent none of the seeds in the different treatments showed any germination after three months of storage both in blotter method and pot culture experiment.

After storing the seeds for six months under normal storage conditions and tested for germination by blotter method, the maximum germination of 80 per cent was observed in Thiride, Captafol and Emisan treated seeds (Table 17). The minimum germination (56 per cent) was noticed in Calixin treated seeds.

Table 17. Germination percentage of treated seeds of cucumber stored for 6 months under different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage								
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)			
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	78	80	30	0	0	62	77	25	0	0
T_2	Thiride	80	90	70	0	0	78	82	68	0	0
T_3	Captafol	80	84	0	0	0	73	80	0	0	0
T_4	Calixin	56	50	10	0	0	46	45	6	0	0
T_5	Aureofunginsol	76	74	40	0	0	63	64	20	0	0
T_6	Emisan	60	90	76	0	0	78	86	70	0	0
T_7	Ash	76	72	20	0	0	66	62	10	0	0
T_8	Control	76	72	16	0	0	68	62	0	0	0

In pot culture experiment a maximum germination of 78 per cent was recorded in Thiride and Emisan treated seeds. The lowest germination was recorded in Calixin treated seeds (46 per cent).

Six months after storing the cucumber seeds at 66.8 per cent humidity level the maximum germination of 90 per cent in blotter method was observed in Thiride and Emisan treated seeds and was on par with Bavistin and Captafol treated seeds. The minimum germination was noticed in Calixin treated seeds (50 per cent). In pot culture experiment, the maximum percentage of germination was noticed in Emisan treated seeds (86 per cent) and was on par with Thiride, Captafol and Bavistin treated seeds. The minimum germination (45 per cent) was noticed in Calixin treated seeds. In general there was an increase in germination when the seeds were stored at 66.8 per cent humidity level than the normal storage condition.

Seeds which were kept for six months under 75.6 per cent humidity level showed a maximum germination of 76 per cent in Emisan treatment when tested in blotter method and this was on par with Thiride treatment. The minimum of 10 per cent germination was noticed in Calixin treated seeds. No germination was noticed in Captafol treated seeds. In pot culture experiment captafol treated and untreated seeds showed no germination. A maximum germination of 70 per cent was observed in Emisan treated seeds and it was on par with Thiride treated seeds. Drastic reduction of the germination percentage was noticed when the seeds were stored under 75.6 per cent humidity

for six months, both in blotter test and in pot culture experiment compared to the normal and 66.8 per cent humidity level storage. But this reduction was comparatively less in the case of Thiride and Emisan treated seeds.

The seeds kept at 82.9 and 92.9 per cent humidity level for six months did not show any germination when tested in blotter and pot culture.

The seeds were stored for nine months under normal storage condition and the germination was tested in both blotter and pot culture experiment and the results are presented in Table 18. In blotter test the maximum germination was observed in Thiride treated seeds (74 per cent) which was on par with all the other treatments except Calixin treatment. The lowest germination (50 per cent) was recorded in Calixin treated seeds. Similar trend was observed in pot culture experiment also. Maximum germination (72 per cent) was recorded in Thiride and minimum in Calixin treated seeds (44 per cent).

After storing the seeds under 66.8 per cent humidity level for nine months, the germination study in blotter test showed the maximum germination of 88 per cent in Thiride treated seeds. The minimum germination percentage of 46 was in Calixin treated seeds. While in pot culture experiment a maximum germination of 83 per cent was noticed in Thiride treated seeds followed by Captafol treated seeds (76 per cent). The minimum germination (43 per cent) was recorded in Calixin treated seeds.

Table 18. Germination percentage of treated seeds of cucumber stored for 9 months at different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage								
		Blotter method					Pot culture experiment			
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	70	70	10	0	0	60	66	6	0	0
T_2	Thiride	74	88	60	0	0	72	83	58	0	0
T_3	Captafol	70	84	0	0	0	66	76	0	0	0
T_4	Calixin	50	46	0	0	0	44	43	0	0	0
T_5	Aureofunginsol	62	64	30	0	0	56	60	16	0	0
T_6	Emisan	70	70	50	0	0	60	66	46	0	0
T_7	Ash	73	78	16	0	0	63	63	10	0	0
T_8	Control	72	78	7	0	0	62	60	0	0	0

Under 75.6 per cent humidity level after nine months of storage there was no germination in Captafol and Calixin treated seeds in blotter method. Here the maximum germination was (60 per cent) in Thiride treated seeds which was on par with Emisan treated seeds. Minimum germination was noticed in untreated seeds (7 per cent). In pot culture experiment no germination was recorded in Captafol, Calixin and untreated seeds. The highest germination percentage of 58 was noticed in Thiride treated seeds which was on par with Emisan treated seeds. A minimum germination of six per cent was noticed in Bavistin treated seeds. When the seeds were stored under 75.6 per cent humidity for nine months the reduction of germination was very high in all the treatments both in blotter and pot culture experiment when compared to the normal and 66.8 per cent humidity level storage, but the reduction of germination in treatments of Thiride and Emisan was not much as that of other treatments.

None of the seeds showed germination under high humidity levels of 82.9 and 92.9 per cent.

When seeds were kept for 12 months under normal storage condition the maximum germination in blotter method was recorded in Thiride treated seeds (70 per cent) which was on par with all the treatments except Aureofunginsol and Calixin seed treatment (Table 19). The minimum germination (45 per cent) was observed in Calixin treated seeds. In pot culture experiment the maximum germination of 68 per cent was observed in

Table 19. Germination percentage of treated seeds of cucumber stored for 12 months at different humidity levels in blotter and pot culture experiment

Treatments		Germination percentage								
		Blotter method					Pot culture experiment			
		Normal storage	Humidity level (per cent)				Normal storage	Humidity level (per cent)		
			66.8	75.6	82.9	92.9		66.8	75.6	82.9	92.9
T_1	Bavistin	62	68	0	0	0	60	60	0	0	0
T_2	Thiride	70	83	56	0	0	68	73	50	0	0
T_3	Captafol	67	80	0	0	0	60	70	0	0	0
T_4	Calixin	45	43	0	0	0	35	38	0	0	0
T_5	Aureofunginsol	50	60	10	0	0	45	56	6	0	0
T_6	Emisan	67	60	10	0	0	58	60	10	0	0
T_7	Ash	60	60	10	0	0	50	60	8	0	0
T_8	Control	66	80	7	0	0	56	60	0	0	0

Thiride treated seeds. The minimum germination was observed in Calixin treated seeds (35 per cent).

Cucumber seeds stored for 12 months at 66.8 per cent humidity level, maintained a good germination in almost all the treatments. Thiride treated seeds showed a maximum germination of 83 per cent closely followed by Captafol and untreated seeds. The minimum germination (43 per cent) was noted in Calixin treated seeds, when tested in blotter test. In pot culture experiment Thiride treated seeds showed the maximum germination (73 per cent) and was on par with Captafol treated seeds. A minimum of 38 per cent germination was noticed in Calixin treated seeds. In general keeping the seeds at 66.8 per cent humidity level was found beneficial as it favoured the increase in germination compared to the normal storage condition.

Twelve months after storing at 75.6 humidity level Thiride treated seeds showed maximum germination of 56 per cent in blotter test and it differed significantly from the other treatments. Here no germination was observed in Captafol, Bavistin and Calixin treated seeds. Very poor germination was noticed in Aureofunginsol, Emisan and Ash treated seeds (10 per cent) and seven per cent in untreated seeds. Similar trend was noticed in pot culture experiment also with a maximum germination of 50 per cent in Thiride treated seeds. Emisan, Ash Aureofunginsol treated seeds showed poor germination of 10, 8 and 6 per cent respectively. No germination was recorded in Bavistin, Captafol, Calixin and untreated seeds.

Under high humidity levels of 82.9 and 92.9 per cent none of the seeds in the different treatments showed any germination after 12 months of storage both in blotter method and in pot culture experiment.

Influence of internally seed borne mycoflora on pre and post-germination rotting of cucurbitaceous seeds

The influence of the mycoflora isolated from the internal parts of the cucurbitaceous seeds were studied in relation to pre and post-germination rotting by blotter technique as described in materials and methods.

Bitter gourd

From bittergourd seed seventeen species of mycoflora were isolated from the internal parts. All the isolates were tested for their role on the pre and post-germination rotting and the results are presented in Table 20.

Among the seed borne mycoflora, Fusarium solani was the most virulent and it caused eight per cent pre-germination and 32 per cent post-germination rotting. Nine per cent of the seed did not germinate due to mixed infection of different mycoflora. Inoculation of Fusarium oxysporum also resulted in 10 and 24 pre and post-germination rotting respectively. Here eight per cent of seed showed mixed infection. Rhizopus stolonifer infection resulted in six and 15 per cent pre and post germination rotting and 15 per cent of seeds failed to germinate due to mixed infections. Aspergillus flavus inoculation of seeds resulted in 20 per cent pre-germination and

Table 20. Effect of various internally seed borne mycoflora on the pre and post-germination rotting of bitter gourd seeds

Name of pathogens	Pre-emergence rotting (per cent)	Post-emergence rotting (per cent)	Multiple infection (per cent)	Seedlings survived (per cent)
Acremonium sp	-	-	8	92
Alternaria alternata	12	8	6	74
Aspergillus amstelodami	-	-	10	90
A. flavus	20	6	7	67
Botryodiploidia theobromae	-	6	9	85
Chaetomium globosum	6	-	10	84
Cladosporium cladosporioides	14	-	6	80
Corynespora cassicola	-	-	12	88
Fusarium oxysporum	10	24	8	58
Fusarium solani	8	32	9	51
Helminthosporium sp.	-	-	12	88
Humicola gresia	-	-	11	89
Nigrospora sacchari	-	-	12	88
Paceilomyces varioti	-	-	8	92
Rhizopus stolonifer	6	15	15	64
Hyaline non-sporulating fungus	5	-	12	83
Dark coloured non-sporulating fungus	2	-	16	82

six per cent post-germination rotting. Seven per cent germination failure was also noticed due to the mixed infection. Alternaria alternata caused 12 and eight per cent pre and post-germination rotting apart from six per cent mixed infection. Cladosporium cladosporioides inoculated seeds showed 14 per cent pre-germination rotting and six per cent mixed infection. Botrydiplodia theobromae caused six per cent post-emergence rotting with nine per cent multiple infection. Whereas Chetomium globosum gave six per cent pre-germination rotting with 10 per cent multiple infection. The hyaline and dark coloured sterile mycelial fungi caused five and two per cent post-emergence rotting independently with twelve and sixteen per cent mixed infection. The internally seed borne pathogen like Acremonium sp., A. amstelodami, Corrynespira cassicola, Helminthosporium sp., Humicola gresia, Nigrospora sacchari, Paceilomyces varioti did not show independent infection and seed inoculated with these organism showed eight to twelve per cent germination failure due to multiple infection.

Pumpkin

Fourteen seed pathogens were isolated from the seed coat, endosperm and embryo of pumpkin seed. These fungi were tested for their effect on pre and post-germination rotting of seeds and the data are tabulated in Table 21.

The data revealed that the test fungi decreased the germination per cent of the seed. Fusarium solani caused 20 per cent pre-germination and 25 per cent post-emergence

Table 21. Effect of various internally seed borne mycoflora on the pre and post-germination rotting of pumpkin seeds

Name of pathogens	Pre-emergence rotting (per cent)	Post-emergence rotting (per cent)	Multiple infection (per cent)	Seedlings survived (per cent)
Alternaria alternata	15	12	8	65
Aspergillus flavus	20	8	20	52
A. fumigatus	10	15	2	73
A. niger	2	2	10	86
A. ochraces	10	5	5	80
A. terreus	8	3	10	79
Botryodiplodia theobromae	-	8	10	82
Chaetomium globosum	6	-	10	84
Fusarium oxysporum	18	12	20	50
Fusarium solani	20	25	14	46
Humicola gresia	-	-	12	88
Paceilomyces varioti	6	-	14	80
Rhizopus stolonifer	12	18	15	55
Hyaline non-sporulating fungus	-	3	15	82

rotting. Fourteen per cent mixed infection were also observed in Fusarium solani inoculated seeds, resulting a poor survival of the seedlings. Fusarium oxysporum showed 18 per cent pre-germination rotting and 12 per cent post-germination rotting. It also caused 20 per cent mixed infection. Aspergillus flavus and Rhizopus stolonifer caused 20 and 12 per cent pre-germination and eight and 18 per cent post-germination rotting. Twenty and 15 per cent mixed infection was noticed in this treatment. Inoculation of Alternaria alternata was resulted in 15 and 12 per cent pre and post-germination rotting along with eight per cent mixed infection. Aspergillus fumigatus inoculated seeds showed 10 and 12 per cent pre and post-germination rotting in addition to the two per cent mixed infection. Aspergillus terreus and Aspergillus ochraceus caused ten and eight per cent pre-germination rotting, five and three per cent post-germination rotting with five and 10 per cent mixed infection, whereas Paceilomyces varioti and Chetomium globosum gave six per cent each pre-germination rotting with 10 and 14 per cent multiple infection. Hyaline non-sporulating fungus and Botryodiplodia theobromae caused only three and eight per cent germination rotting with fifteen and ten per cent multiple infection. Aspergillus niger and Humicola gresia were found to be the weaker pathogens.

Cucumber

From the cucumber seed fourteen species of internally seed borne mycoflora were isolated. All the fungi were tested

for their pathogenicity and pre and post-germination rotting. The results are presented in Table 22.

Among the fourteen seed borne pathogen Fusarium solani was found to be the most severe pathogen and caused 18 per cent pre-germination and 32 per cent post-germination rotting. Twelve per cent seed did not germinate due to the mixed infection. The final stand of the seedlings after the artificial inoculation of this fungus was only 38 per cent. Fusarium oxysporum was also showed high percentage of infection with 15 and 30 per cent pre and post-germination rotting apart from the 14 per cent mixed infection. Rhizopus stolonifer caused seven and 18 per cent pre and post-germination rotting and 14 per cent seeds did not germinate due to mixed infection. Aspergillus flavus infected a total 51 per cent of the seeds of which 25 per cent seeds were affected by pre-germination rotting and 16 per cent post-germination rotting apart from the ten per cent mixed infection. Inoculation of Alternaria alternata resulted in ten and six per cent pre and post-germination rotting and 14 per cent mixed infection. Aspergillus flavipes caused three and two per cent pre and post-germination rotting part from 14 per cent mixed infection. Six and two per cent pre and post-germination rotting was resulted due to infection of Cladosporium cladosporioides and eight per cent seeds were not germinated due to mixed infection. Aspergillus fumigatus and Chaetomium globosum caused two per cent pre-germination rotting and 12 per cent mixed infection. The

Table 22. Effect of various internally seed borne mycoflora on the pre and post-germination rotting of cucumber seeds

Name of pathogens	Pre-germination rotting (per cent)	Post-germination rotting (per cent)	Multiple infection (per cent)	Seedlings survived (per cent)
Alternaria alternata	10	6	14	70
A. amstelodami	-	-	12	88
A. chevaleieri	-	-	14	86
A. flavus	25	16	10	49
A. flavipes	3	2	14	81
A. fumigatus	2	-	12	86
A. terreus	-	-	13	87
Chaetomium globosum	2	-	12	86
Cladosporum cladosporioides	6	2	8	84
F. oxysporum	15	30	14	41
F. solani	18	32	12	38
Humicola gresia	-	-	13	87
Rhizopus stolonifer	7	18	14	61
Hyaline non-sporulating fungus	-	-	15	85

seed-borne pathogens like A. chevalieri, A. amstelodami, A. terreus, Humicola gresia and hyaline non-sporulating fungus did not show independent infection and caused only mixed infection of the 14, 13 and 15 per cent respectively.

Discussion

DISCUSSION

Seeds are not only the source of life from one cropping season to the next but also unwittingly carrying the disease germs from one season to other. Seeds which are sound when harvested may during processing and storage be invaded by a variety of fungi. The fungal pathogen either contaminates the seed externally (externally seed borne) or may also occur internally (internally seed borne). Apart from these, saprophytic fungi also may grow on the seed surface under favourable conditions and affect the seed health and thereby reduce germination.

The seeds of three cucurbitaceous vegetable viz., bitter gourd, pumpkin and cucumber used in the present study were found to be of high quality with good germination percentage. The moisture content of the three vegetable seeds ranged from 7.3 to 7.6 per cent, which is within the permissible limit prescribed for seed certification standards.

Low germination capacity of vegetable seed, is expected due to the association of different fungi with the seeds. The three types of cucurbitaceous seeds were found to be associated with mycoflora. The result of the study showed that all the unsterilised seeds of the three types were associated with mycoflora either externally or internally. The mycoflora association on the surface sterilised whole seeds was considerably less than that of unsterilised seeds and it ranged from 20-30 per cent just prior to storage. Therefore, it

could be reasonably assumed that 70-80 per cent seeds were associated with superficial contaminants. The percentage of internally infected seeds increased with storage. This increase ranged from 27 to 32 per cent in bitter gourd, 30 to 35 per cent in pumpkin and 20 to 22 per cent in cucumber seeds within one year under normal storage conditions. The percentage of internally infected seeds will increase under normal storage conditions and this findings are supported by the earlier workers (Quasem and Christensen, 1958; Christensen and Lopez, 1963 and Christensen, 1973).

Some of the fungal pathogen which occur an external contaminants are also found internally. In the present investigation, an attempt was made to estimate the internally seed borne fungal pathogen on different parts of seed namely seed coat, endosperm and embryo. The maximum fungal association was found on the seed coat of pumpkin (30 per cent) followed by bitter gourd (27 per cent) and the minimum in cucumber (20 per cent). Fungal invasion was considerably less in the internal parts of seeds. Endosperm infection of ten, eight and six per cent was observed in seeds of pumpkin, bitter gourd and cucumber respectively. The infection percentage was further less in embryo with the maximum in pumpkin embryo (eight per cent) followed by bitter gourd (six per cent) and cucumber (five per cent). This indicated that most of the fungi were either surface contaminants or established in the seed coat when the conditions were favourable. Some fungi might have adhered to the seed coat while harvesting and

processing. Most of them were incapable of entering into the internal tissue of the seed.

Altogether 34 species of fungi were isolated from the unsterilised seeds of bitter gourd, pumpkin and cucumber (Table 3). Of these, eleven species were of Aspergillus, four of Penicillium and two of Fusarium, one species each of Absidia, Acremonium, Alternaria, Botryodiplodia, Chaetomium, Cladosporium, Cunninghamella, Corynespora, Curvularia, Paecilomyces, Rhizopus, Syncephalastrum, Humicola, Nigrospora and Helminthosporium. Two fungi which did not sporulate on seeds or in potato dextrose agar medium were not identified.

Of these, 34 species, 27 were found associated with unsterilised seeds of bitter gourd. Out of the 27 species, 10 were purely external on the seed coat and the predominant ones among them were species of Aspergillus and Penicillium. Seventeen species of fungi were associated with the surface sterilised seeds of bitter gourd. Among these Fusarium solani Aspergillus flavus and Rhizopus stolonifer were the predominant ones. All these fungi which were associated with the surface sterilised seeds were also found in the seed coat. Only eight species of fungi were found associated with endosperm of bitter gourd. As in the case of seed coat, Aspergillus flavus and Fusarium solani were the predominant fungi infecting endosperm. Only three species of fungi were isolated from the embryo and they were Aspergillus flavus, Fusarium oxysporum and F. solani.

From the unsterilised seeds of pumpkin 25 species of fungi were isolated but the surface sterilised seeds had only 14 species which clearly indicated that eleven species of fungi were superficial contaminants on the seed coat. Here also species of Aspergillus and Penicillium were the predominant external contaminants. The predominant fungi on surface sterilized seeds were Rhizopus stolonifer, Aspergillus fumigatus, Fusarium solani and Aspergillus flavus. These fungi were also found in the seed coat. Eight species of fungi were found in the endosperm of the pumpkin seed, which were also seen in the seed coat. The predominant ones were Fusarium solani, Aspergillus fumigatus, A. flavus and Rhizopus stolonifer. In the embryo, only Aspergillus flavus and Fusarium solani were observed.

The unsterilised seeds of cucumber yielded 25 species of fungi while the surface sterilised seeds had only 14. These fungi were found in the seed coat also. The predominant fungi found associated with seed coat were species of Aspergillus and Fusarium. Among these A. fumigatus, A. flavus, F.oxysporum showed maximum association with the seed coat. From the endosperm of cucumber six species of fungi were isolated. Of these six species, Aspergillus fumigatus and Fusarium solani recorded maximum percentage of association with the endosperm. Only three species of fungi viz., A. flavus, F. solani and F. oxysporum were found to infect the embryo.

It is interesting to note that the maximum number of

mycoflora were associated with the seeds as external contaminant in all three vegetable seeds. The species of fungi and their percentage of infection on surface sterilised seeds and seed coat were the same for all the three types of vegetable seeds. When we consider the different part of the seed, the seed coat has the maximum infection followed by endosperm and the minimum in embryo. The predominant internally seed borne pathogens were species of Aspergillus and Fusarium in all the three seeds. Similar observations were made by Khandelwal and Prasada (1970) in their studies on the mycoflora of cucumber seed. The findings of Naseema (1981), Mehrothra and Wadhwani (1981), Saxena and Gupta (1981) and Prasad and Prasad (1987) on the seed mycoflora of vegetable seeds, soyabean, green gram and black gram, and dolichos lablab respectively were also in conformity with the present observation.

The viability of fungal propogules associated with cucurbitaceous vegetable seeds varied depending upon the storage period. During the storage many of the mycoflora associated with the seed lost their viability.

At the end of 12 months of storage as many as 18 species of fungi were found associated with bitter gourd seeds. Of these, four were observed on seed surface as external contaminants. In pumpkin at the end of 12 months of storage 17 species of fungi were viable. Among them, five species were external contaminants. In cucumber seed 15 species of fungi

were found viable, at the end of 12 months of storage. Of these, five were associated with the seed surface as external contaminants. The viability of the seeds reduced on storage is due to several factors. One of the important factors is the fungal flora associated with the seed. From the study it is clear that even after one year of storage, a large number of fungal flora is seen viable on or in the seeds. The study also revealed that survival ability of internally seed borne fungi is far better than that of the fungi observed on the surface of the seed. The result of the present study is in conformity with those of Harrison (1978) and Vishunavet and Shukla (1983). Aspergillus flavus, Chaetomium globosum, F. oxysporum, F. solani and Rhizopus stolonifer were observed inside all the three vegetable seeds even at the end of 12 months of storage. This indicates that under local conditions the above mentioned fungi may be responsible for reducing the viability of seed.

The viability of seeds changes depending upon several factors. One of the important factors which affects viability of seed is the moisture content. Moisture content of the seed stored under normal storage condition fluctuated at different periods of observation. In all the vegetable seeds, the moisture content increased and reached the maximum at the end of 3 month of storage, which gradually decreased and at the end of 12 month of storage it was almost equal to that of the initial moisture content (before storage).

Seeds lose their viability on storage by the activities of fungi. Moisture content of the seed is an important factor which encourages the fungal growth. The viability and moisture content of the cucurbitaceous seeds under different storage period were studied. At the beginning, the moisture content and germination percentage of all the three vegetable seeds were within the permissible limits. As the moisture content of the seed increased it gave a congenial condition for the fungi to multiply on the seeds. This in turn affected the germination of the seed as shown in Table 7. The increase in the moisture content of the seed may be due to the high atmospheric humidity during the monsoon period. Eventhough at the end of 6, 9 and 12 months of storage there was a gradual reduction in the moisture content, germination percentage decreased. The fungi which were active in the seed when the moisture content was high, might have damaged the seed even before the moisture content reduced later. The reduction in germination of the seed may occur by different ways such as production of toxins, disintegration of tissues or by changes in the constituents of the seed due to fungal activity. Once the mycoflora damaged the seeds due to its activity under high moisture content, the damage cannot be made good. The loss of seed viability of relatively high moisture content due to increased mycofloral activity resulting in the production of toxic metabolites and changes in the constituents of the seed had been well established (Field and King, 1962; Kabeera and Taligoola,1983; Saxeena et al.,1987; Naseema, 1981 and Nair,1982).

In Kerala high relative humidity prevails in most part of the year resulting in biodegradation of vegetable seeds especially cucurbitaceous seeds during storage. Seed borne mycoflora and improper storage conditions are often responsible for rapid deterioration in the viability of most of the vegetable seeds during storage. The germination percentage of vegetable seeds stored under different humidity levels under different periods showed marked variation. The initial germination of bitter gourd was 88 per cent in blotter method and 80 per cent in pot culture experiment. But this was reduced to 26 and 18 per cent after 12 month of storage. Eventhough the pumpkin and cucumber seeds also showed similar trend both in blotter and pot culture experiments, the reduction in germination during storage was much pronounced in pumpkin and bitter gourd compared to cucumber seeds. The result of the study indicated that all the vegetable seeds deteriorated during storage under normal storage condition. So it can be presumed that under normal storage conditions all the three vegetable seeds could be stored only for a period of six months with the minimum required germination percentage.

When seeds were kept under different humidity levels, the seeds kept at 66.8 per cent humidity level showed the best germination and it was slightly better than the normal condition both in blotter and in pot culture methods. When the humidity level in the storage condition increased from

66.8 per cent the germination percentage reduced considerably and it was more pronounced when the storage period increased. At 75.6 per cent humidity level, no germination was observed after six months of storage in bitter gourd but only 12 per cent germination was observed in blotter method in pumpkin. In cucumber at this level of humidity and storage period, 16 per cent germination was observed in blotter method while in pot culture experiment there was no germination. After nine months of storage also, pumpkin and cucumber seeds showed six and seven per cent germination at 75.6 per cent humidity level in blotter but none of the seeds germinated in pot culture experiment. After 12 months of storage there was a complete failure of germination of all the vegetable seeds both in blotter and in pot culture method except cucumber seeds which gave seven per cent germination in blotter technique.

None of the vegetable seeds germinated at higher humidity level of 82.9 and 92.9 per cent at different periods of observation. The result of the study clearly indicated that the viability of the three cucurbitaceous vegetable seeds deteriorated at higher humidity levels in storage and the best humidity level for storage is 66.8 per cent. At this humidity level the seeds showed better germination compared to the normal storage condition.

Coleman and Fellow (1925) observed that as the relative humidity was raised from 65 to 85 per cent, the moisture content of the seeds increased rapidly due to the absorption of

moisture which resulted in rapid growth of mycoflora on the seeds leading to its quick deterioration. Quasem and Christensen (1958) reported that the germination percentage of maize seed drastically decreased at the relative humidity level of 75 to 92 per cent. Kudrina (1967) noticed poor germination of vegetable seeds due to increased mycoflora growth at relative humidity higher than 70 per cent. Saxeena and Gupta (1979) also observed considerable reduction in germination of Vigna radiata seeds stored at 75 per cent relative humidity for more than 120 days. Results of the present investigation are supported by findings of the above workers. Therefore, it can be clearly concluded that storage period and humidity levels have marked influence on the viability of the seeds. To maintain the viability of the three vegetable seeds during storage, 66.8 per cent humidity level is the best. High humidity level, even 75 per cent, adversely affected the viability of these seeds during storage.

Seed treatment with fungicides is one of the best methods for controlling seed borne mycoflora and to improve seed germination. Seed treatment with fungicide not only controls seed borne pathogen but also controls soil borne pathogen forming inhibition zone around the seed. However, the effect of various seed treatment materials affects the germination of the seeds. In some cases, increase/decrease of the germination percentage of seed as a result of treatment may be exhibited either immediately after the treatment or after

several days/months of storage. Thus, before recommending any material for seed treatment its effects on the germination of the seed over an extended period of time should be studied. The effect of seed treatment material is also governed by the condition under which the treated seeds are stored.

Three cucurbitaceous seed viz., bitter gourd, pumpkin and cucumber were treated with six different fungicides and wood ash and kept under varying levels of humidity for a period of 12 month and its effects on germination of the seeds were studied. The germination percentage of all the treated seeds except Calixin treated seeds ranged from 78 to 90 per cent just after fungicidal treatment in bitter gourd both in blotter and pot culture experiment. However, germination of the seed, treated with Calixin was between 60-65 per cent. Almost a similar pattern was observed with pumpkin and cucumber seed also. When the treated seeds of bitter gourd and pumpkin were stored for a period of three months under normal conditions, the various treatments except Calixin did not show any significant differences from one another. The germination of Calixin treated seed was significantly lower than that of the control. Almost a similar result was observed with cucumber seeds also. However, in this case, both Calixin and Aureofunginsol were on par and inferior to other treatments. The higher germination of 88 per cent in blotter method and 86 per cent in pot culture method was

observed in Thiride treated seeds of bitter gourd and the corresponding figures for cucumber were 99 and 97 per cent. In pumpkin, highest germination of 90 per cent in blotter and 82 per cent in pot culture method were obtained in Captafol treatment.

The superiority of thiride for all the cucurbitaceous seeds was also noticed at the end of six months under normal storage condition. In general, after six months of storage, germination of all the cucurbitaceous seeds was less in Calixin treatment. Aureofunginsol treated bitter gourd and pumpkin seeds also showed poor germination compared to the control and it was almost similar in Calixin treatment. However, Aureofunginsol supported better germination in cucumber seeds at the end of six months compared to the Calixin.

At the end of nine months under normal storage condition the bitter gourd seeds treated with Aureofunginsol gave the least germination while in pumpkin the least germination was noticed in ash treated seeds. However, Calixin treatment gave the least germination in cucumber seeds. The superiority of Thiride over all other treatments in giving good germination was also evident at the end of nine months of storage. There was a marked reduction in germination in bitter gourd, after 12 months of storage. None of the treatments except Thiride supported more than 50 per cent germination at the end of 12 months in bitter gourd seeds. In pumpkin, as well as in cucumber also, the superiority of Thiride over other

treatments was pronounced. Among the various chemicals tried the Calixin treated bitter gourd seeds had the least germination at the end of 12 months. Similar result was observed with pumpkin and cucumber seeds. However, in pumpkin the least germination of less than 10 per cent was noticed in untreated seeds.

In general, as the humidity increased there was a reduction in germination. This trend was noticed when the seeds were examined at 3, 6, 9 and 12 months intervals. The seeds kept at a humidity of 82.9 per cent and above failed to germinate when stored for, three months and more. None of the fungicides had also showed any influence to retain the germination of these vegetable seeds at these higher humidity levels.

All the treatments except Calixin and Aureofunginsol supported more than 80 per cent germination in bitter gourd seed at the end of three months storage under 66.8 per cent humidity level. The least germination of 60 per cent at this humidity level was noticed in Calixin treatment. Similar results were obtained in pumpkin and cucumber also. In general, Thiride, Captafol and Emisan were found to be the better chemicals for maintaining a good germination of cucurbitaveous seeds after 3 months of storage at 66.8 per cent humidity level.

After six months of storage under 66.8 per cent humidity level, the least germination was noticed in seeds which were

treated with Calixin while the best was that treated with Thiride or Emisan in all the three cucurbitaceous seeds. At the end of nine month at 66.8 per cent humidity level also Thiride treated cucurbitaceous seeds supported the maximum germination percentage of more than 70 per cent, while least was with Aureofunginsol in bitter gourd and pumpkin seeds. In cucumber, Calixin treatment showed the least germination. The superiority of Thiride in maintaining the germination of seeds kept at 66.8 per cent humidity level was evident even at the end of 12 months of storage. Captafol was also on par with Thiride in the seed germination.

As the percentage humidity in the atmosphere in which the seeds were stored increased from 66.8 to 75.6 there was a marked reduction in germination of the seeds in the various treatments. At the end of three months at 75.6 per cent humidity level the least germination in bitter gourd was noticed with Aureofunginsol treatment while the fungicide like Bavistin, Captafol,Thiride and Emisan did not differ significantly from one another and gave comparatively good germination. For pumpkin and cucumber seeds, the minimum germination was with Aureofunginsol and Calixin treatment. The maximum germination for pumpkin was with Thiride and Captafol treatments while for cucumber it was with Emisan and Thiride.

At the end of six months at 75.6 per cent humidity level untreated seeds of bitter gourd and seeds treated with

Aureofunginsol failed to germinate. The germination was less than 16 per cent in seeds treated with ash or Bavistin. Thiride, Captafol and Calixin treated seeds supported more than 46 per cent germination. Pumpkin seeds treated with Calixin, Captafol and ash failed to support the germination after 6 months of storage at 75.6 per cent humidity level. Eventhough Thiride treated pumpkin seed gave 46 per cent germination in blotter method, no germination was noticed in pot culture experiment. In pot culture experiment, only Aureofunginsol, Emisan and Bavistin treated seeds germinated In cucumber, the maximum germination was noticed in Emisan treatment and the minimum was with Calixin treatment.

At the end of nine months at a humidity level of 75.6 per cent, a higher germination percentage was observed in bitter gourd seeds treated with Thiride, Emisan and Calixin while seeds treated with wood ash failed to germinate. Bavistin and Aureofunginsol treated seeds and control showed less than 10 per cent germination. None of the treated pumpkin seeds germinated at the end of nine months except Thiride treated seeds (45%), while in cucumber seeds only Captafol and Calixin treated seeds failed to germinate at the end of nine months. The highest germination was observed in seeds treated with Thiride and Emisan.

At the end of 12 months of storage at 75.9 per cent humidity level, control seeds as well as ash treated seeds of bitter gourd did not support any germination. Here also

the maximum germination was observed in Thiride treated seeds. In pumpkin, only Thiride treatment supported the germination. In cucumber seeds, Thiride treatment gave maximum germination at the end of 12 months while Bavistin, Captafol and Calixin treated seeds failed to germinate. Germination percentage in all other treatments was less than 10 per cent.

It is clear from the study that in general, among the various seed treatments with the fungicides, Thiride, Emisan and Captafol were found to be the most effective to get higher germination when treated seeds were stored for different periods of time in varying humidity levels. Calixin treatment had an adverse effect on the germination in all the three vegetable seeds tested and it cannot be recommended as a seed dressing fungicide for vegetables. The superiority of Thiride as a good seed dressing fungicide has been well established and the findings of the earlier workers like Ash Worth et al. (1964), Singh et al. (1974), Frolova (1976), Sarode and Kadam (1977), Mercae and Kisyombe (1978), Siddaramaiah et al. (1982) are in conformity with the result of the present investigation. Captafol was also found to be a good seed dressing fungicide and it can be very safely used in vegetable as a seed dressing fungicide. The efficacy of Captafol as a vegetable seed dressing fungicide has been reported by Lukem (1969). Captafol as a seed dressing fungicide was also reported by Wales and Somer (1968), Oladiran and Okusanva (1980) and Barros et al. (1981). The result of the present study is also supported by the

findings of the earlier workers. Emisan is also found to be an effective seed dressing fungicide and a number of workers (Suhay, 1975; Sharma et al., 1980; Pandy and Gupta, 1984 and Prasad, 1986) had established the efficacy of this fungicide as an effective seed dresser. Due to health hazards, the use of this fungicide is restricted. The systemic fungicides like Bavistin and Calixin behaved irratically. Calixin gave adverse effect on germination during the early stages of storage. Therefore, this two systemic fungicides cannot be considered as effective seed dresser. The findings of Singh et al. (1982), Reddy et al. (1982) and Singh and Agarwal (1984) showed that Bavistin and Calixin can be used as good seed dresser for non-cucurbitaceous seed. But in the present investigation, the Bavistin and Calixin were not found effective compared to Thiride, Captafol and Emisan for cucurbitaceous seeds. The wood ash is a traditional seed dresser. However in the present study it is not found to be very effective in keeping the germination of the seeds during storage. Naseema (1981) had reported wood ash as a good vegetable seed dresser upto 3 months but the findings of the present investigation did not agree with her observations and even after three months storage at normal storage condition it was on par with untreated check. In general, the germination percentage of seeds stored at a humidity level of 75 per cent and above, was comparatively poor. At this higher humidity level, even with fungicidal treatment the seeds

could not be stored for longer period of time because the germination percentage was far below the standard germination prescribed for the cucurbitaceous seeds. Out of the 23 fungi isolated from the internal parts of the three types of cucurbitaceous vegetable seeds, eight of them namely Alternaria alternata, Aspergillus flavus, Chaetomium globosum, Fusarium oxysporum, F. solani, Humicola gresia, Rhizopus stolonifer and hyaline non-sporulating fungus were found in all the three vegetable seeds studied. Of these, Aspergillus flavus, Fusarium oxysporum and F. solani were found to be more virulent pathogens. Khadelwal and Prasada (1970) established the high pathogenicity of Aspergillus spp. and Fusarium spp. especially in pre-emergence rotting on different variety of Cucumis sativus on artificial inoculation. The findings of Suryanarayana and Bhombe(1961) also support the above observation.

Rhizopus stolonifer was one of the common seed pathogens isolated from the seed coat and endosperm of all the three vegetable seeds, which caused pre and post-emergence rotting. However, the extent of rotting in this case was comparatively less than that observed in seeds inoculated with Aspergillus flavus, F. oxysporum and F. solani. The pathogenicity of this mucrale fungus was reported by Khandelwal and Prasada (1970) and Kabeera and Taligoola (1983). The cellulolytic fungus Chaetomium globosum caused only pre-emergence rotting. Sinha and Khara (1977) established the pathogenicity of

Chaetomium species in cowpea seeds. Kanapathipillai (1978) also found the weak pathogenicity of this fungus. All other seed borne pathogens tested, were found to be either very weak in their pathogenicity or not able to do any damage to the seeds and seedlings.

From the present investigation it is clear that the two seed borne pathogens namely, Fusarium solani and Aspergillus flavus were found in all the internal part of the seeds and they were the most virulent seed borne pathogens. Rhizopus stolonifer a common seed pathogen found in the internal part of the seed except embryo can also do much damage to the seeds. Other pathogenic seed borne fungi are Alternaria alternata, Chaetomium globosum, Humicola gresia and hyaline sterile fungus can also do some damages to the seeds. All other internal seed borne fungi did not cause much damage to the seeds on artificial inoculation.

Summary

SUMMARY

The study "Bio-deterioration of important cucurbitaceous seeds due to mycoflora" was conducted during 1987 at College of Horticulture, Vellanikkara. Three cucurbitaceous vegetable seeds, viz., bitter gourd, pumpkin and cucumber were collected from Department of Olericulture, College of Horticulture and Agriculture Research Station, Mannuthy.

The seeds were examined for their initial germination and moisture content. The germination percentage of all the three seeds ranged from 80 to 90 in blotter method and 78 to 88 in pot culture method. The moisture content of the seeds was between 7.3 to 7.6 per cent. The germination percentage and moisture content were within the permissible limits of seed certification.

Mycoflora associated with the stored cucurbitaceous seeds were estimated by blotter method at an intervals of three months for a period of one year. The unsterilised seeds of these vegetables were found associated with mycoflora throughout the period of investigation. Mycoflora associated with surface sterilised seeds was less than that of unsterilised seeds.

Altogether 34 species of fungi were found associated with the unsterilised seeds of the three cucurbitaceous seeds. Among these Aspergillus sp., Penicillium sp. and Rhizopus sp. were the predominating ones.

Twenty three species of internally seed borne pathogens were isolated from the three vegetable seeds. The predominant fungi were Fusarium solani, Aspergillus flavus and Rhizopus stolonifer.

The maximum fungal infection was observed in the seed coat with 17 species in bitter gourd, 14 species each in pumpkin and cucumber, respectively. Of these, 8 species were found in the seed coat of all the three types of vegetables. They were Alternaria alternata, Aspergillus flavus, Chaetomium globosum, Fusarium oxysporum, F. solani, Humicola gresia, Rhizopus stolonifer and hyaline non-sporulating fungus.

Twelve species of seed borne fungi were isolated from the endosperm of three cucurbitaceous seeds with eight species each in bitter gourd and pumpkin, six species in cucumber. Of these four species of fungi namely, Aspergillus flavus, Fusarium oxysporum, F. solani and Rhizopus stolonifer were found in the endosperm of all the three vegetable seeds.

Three seed pathogens were found to cause infection of the embryo of all the three vegetable seeds. Of these Aspergillus flavus and Fusarium solani were found in all the three types of vegetable seeds while Fusarium oxysporum was found only in the bitter gourd and cucumber seeds.

Viability of fungal propogules associated with cucurbitaceous vegetable was studied for one year. It was found that even at the end of 12 months storage, 18 species of fungi were

viable in bitter gourd, 17 species of fungi in pumpkin and 15 species in cucumber seeds. The moisture content of the stored seeds fluctuated during different period of storage. In all the three vegetable seeds, the moisture content increased and reached the maximum after three months of storage (during South West Monsoon). Then it gradually decreased and it was equal to that of initial moisture content.
The germination percentage of all the vegetable seeds gradually decreased with the increase in storage period.

The germination percentage of the three vegetable seeds stored under different humidity levels of 66.8, 75.6, 82.9 and 92.9 per cent was studied at an interval of three months for a period of one year. The result of the study clearly indicated that the seeds could be stored for longer period of time at the humidity level of 66.8 per cent. At 82.9 per cent and above none of the seeds show any germination at different periods of observation.

The effects of seed treatments and humidity level on the germination percentage of the three vegetable seeds were studied. Six fungicides viz., Bavistin, Thiride, Captafol, Calixin, Aureofunginsol and Emisan and wood ash were used to treat the seeds. The treated seeds were incubated under normal storage condition as well as at different humidity levels of 66.8, 75.6, 82.9 and 92.9 per cent for a period of one year and germination percentage of seeds were recorded after every three months. The germination percentage of all the treated seeds except Calixin treated seed ranged from 78 to 90 per cent

just after fungicidal treatment in bitter gourd. Almost similar result was observed with pumpkin and cucumber seeds also.

When the treated seeds were stored for 3 months under normal condition, the highest germination was supported by Thiride treatment. The germination of Calixin treated seeds was significantly lower than that of the control in all the three cucurbitaceous seeds. The superiority of Thiride for all the cucurbitaceous seeds was also noticed at the end of six months under normal storage condition. Calixin and Aureofunginsol treated seeds supported the minimum germination. At the end of 9 months Thiride treated seeds showed the maximum germination in all the three vegetable seeds. Aureofunginsol, Ash and Calixin treated seeds gave the minimum germination in bitter gourd, pumpkin and cucumber respectively. The maximum germination of all cucurbitaceous seeds was obtained in Thiride treated seeds and the minimum in Calixin treated seeds after 12 months of storage.

Under 66.8 per cent humidity level after 3 months of storage all the treatments except Calixin and Aureofunginsol supported more than 80 per cent germination. After six months of storage, the minimum germination was noticed in Calixin treated seed while maximum was with those treated with Thiride and Emisan in all the three vegetable seeds. At the end of 9 months of storage Thiride treated seeds supported the maximum germination. The minimum germination in bitter gourd and

pumpkin was with Aureofunginsol treatment while that in cucumber was with Calixin treatment. At the end of 12 months of storage Thiride treated seeds showed the maximum germination. Aureofunginsol, Ash and Calixin treated seeds showed the minimum germination in bitter gourd, Pumpkin and Cucumber respectively.

There was a marked reduction in germination of seeds in the various treatments under 75.6 per cent humidity level. Thiride, Captafol and Emisan treated seeds showed the maximum germination and the minimum in Calixin and Aureofunginsol treated seeds in all the seeds after 3 months of storage. After six months of storage Thiride, Captafol and Calixin treated seeds supported maximum germination in bitter gourd and the minimum in wood ash and Bavistin treated seeds. In pumpkin the maximum germination was with Thiride treatment and the minimum was with Bavistin treatment. Emisan treated seeds of cucumber showed the maximum germination while the minimum was observed in Calixin treated seeds. At the end of 9 months Thiride, Emisan and Calixin supported the maximum germination in bitter gourd, Bavistin and Aureofunginsol showed the minimum germination. None of the pumpkin seeds except those treated with Thiride was germinated. In cucumber highest germination was observed in Thiride and Emisan treated seeds and the minimum in Bavistin treated seeds. At the end of 12 months only Thiride treated seeds supported the maximum germination. Germination percentage in all other treatments was less than 10 per cent.

At higher humidity level even with fungicidal treatment the seeds did not germinate at all.

The present study showed that four seed borne pathogen viz., Fusarium solani, F. oxysporum, A. flavus and Rhizopus stolonifer were the most virulent pathogens which cause severe pre and post-germination rotting to the seeds of three cucurbitaceous crop.

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BIO-DETERIORATION OF IMPORTANT CUCURBITACEOUS SEEDS DUE TO MYCOFLORA

By

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ABSTRACT OF A THESIS

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ABSTRACT

The study "Bio-deterioration of important cucurbitaceous seeds due to mycoflora" was conducted at College of Horticulture, Vellanikkara.

The objectives of this study were to find out the role of seed borne mycoflora on the bio-deterioration of important cucurbitaceous seeds, the variation in the seed mycoflora in different periods of the year, to assess the role of different humidity levels on the viability of seeds and to evolve a suitable management practice to prevent the bio-deterioration of seeds.

The study revealed that the unsterilised seeds were found associated with mycoflora throughout the period of investigation. Mycoflora associated with surface sterilised seeds was less than that of unsterilised seeds.

The maximum fungal association was found on the seed coat, then in the endosperm and least in the embryo in all the three vegetable seeds.

Twentyseven species of fungi were found associated with bitter gourd seeds. Ten of them were external contaminants. From pumpkin 25 species of fungi were isolated and 11 species were found as external contaminants. Twentyfive species of fungi were obtained from the cucumber seed of which 11 species were found to be externally seed borne. The predominating externally seed borne fungi were Aspergillus sp., Penicillium sp. and Rhizopus sp. while among the internally

seed borne fungi the predominant ones were Fusarium solani, Aspergillus flavus and Rhizopus stolonifer.

Eighteen species of fungi were found viable at the end of 12 months storage in bitter gourd, 17 species in pumpkin and 15 species in cucumber seeds. Internally seed borne fungi showed more survival ability than the externally contaminant fungi.

The study showed that seeds could be stored for longer period of time at the humidity level of 66.8 per cent. Storage under humidity levels of 82.9 and above was found not suitable for cucurbitaceous seeds.

Among the various seed dressing fungicides, Thiride, Emisan and Captafol were found to be the most effective to get higher germination when treated seeds were stored for different periods in varying humidity levels. Calixin treatment had an adverse effect on the germination in all the three vegetable seeds tested and it cannot be recommended as a seed dressing fungicide.

Seed borne fungi were found to cause pre and post-germination rotting of seed. Fusarium solani, F. oxysporum, Aspergillus flavus and Rhizopus stolonifer were the virulent pathogens which caused the maximum pre and post-germination rotting in the seeds and thereby reducing the germination.