STUDIES ON SEED MYCOFLORA OF *Phaseolus vulgaris* Linn.

BY

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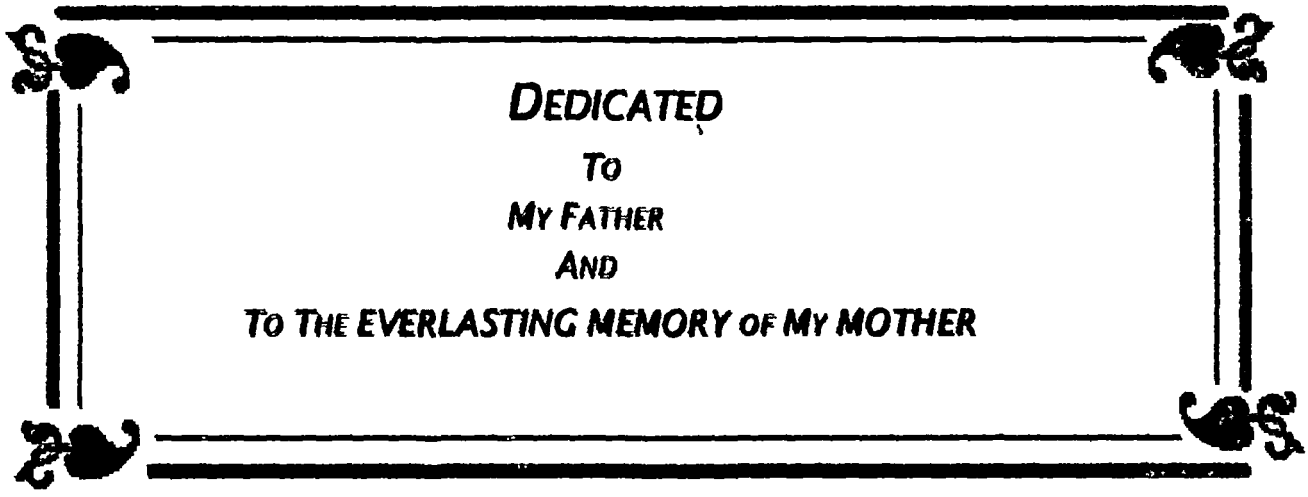
DEDICATED
TO
MY FATHER
AND
TO THE EVERLASTING MEMORY OF MY MOTHER

NORTH-EASTERN HILL UNIVERSITY

SHILLONG

CERTIFICATE

I, Atanu Bhattacharjee, do hereby declare that the subject matter of the thesis entitled **"Studies on seed mycoflora of *Phaseolus vulgaris* Linn."** is the record done by me and that the contents of this thesis did not form basis of the award of any previous degree or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Botany.

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CONTENTS

INTRODUCTION

Common bean (*Phaseolus vulgaris* Linn.) is an important leguminous crop that provides a large part of the daily protein requirement of the people residing in South America, the Caribbean, Africa and Asia. Common bean is a principle source of protein for more than 500 million people in Latin America and Africa; when consumed as snap beans, it is an important dietary source of vitamins and minerals in Asia. A vast stretch of land devoted to bean production in developing countries has increased steadily in the last couple of decades (CIAT, 1992). However, production of beans has not kept pace with the ever increasing population and it must increase to a level of 42% in Latin America and 72% in Africa by the year 2000 so as to satisfy the expected demand (Janssen, 1989). In China alone, the demand for snap beans was estimated to be 3.5 million tons in 1989 and 4.3 million tons is projected for the year 2000 (Yan, *et al.*1995). Bean production in developing countries is often on marginal land and few developing countries have significant reserves of arable land available for bean cultivation. An increased bean production would largely have to come through yield per hectare rather than expansion of area under cultivation. Average bean yields in most developing countries are below maximum yield potential, which indicates that substantial improvement in bean production could be realized by increasing yields per unit land area. Average yields of common beans are less than 1ton / ha in most developing countries (Laing *et al.*, 1984) The main reason for low yields are water

deficiency, high incidence of diseases, insects and use of inorganic fertilisers. However, there is a general dearth of information pertaining to the various aspects of bean cultivation.

P. vulgaris Linn. is the most widely grown of the five cultivated species of *Phaseolus*. The other four species are *P. coccineus, P. lunatus, P. polyanthus, P. acutifolius* and *P. latifolius. P. vulgaris* L. is also known as Dry, Field, French, Snap, Navy, or Kidney beans. Beans are usually grown in tropical countries for dry seeds and in temperate countries for dry seeds as well as for fresh pod consumption and for processing as frozen vegetables.

It is now well known that seeds play a vital role in the introduction of plant pathogens into new areas. The pathogens retain their potentiality till they are successful in finding a specific host for infection and subsequent disease development. Nowadays, host specific mutant strains are developing. Hence, the entire mankind faces an uphill task of saving the crop and subsequent increase in its production.

As early as 800-900 years ago, in summer, in the Eastern Mediterranean, man began to depend on seeds as a principal means of carrying his main crops, the cereals, from place to place and from season to season. The pathogens were carried with the seeds from the beginning, the earliest confirmed record of this association being from Jarmo, about 800 years ago (Stewart and Robertson, 1968). The nine major important crops of the world are wheat, rice, maize, barley, sorghum, sugar beet, common bean (*P. vulgaris*), soybean and groundnut, which represent the greater part of the food production of the world. Approximately 90% of all food crops grown on earth are propagated by seed (Neergaard, 1977). Hence, now it is pertinent to say that crops of

high yielding variety and disease resistance are the need of the hour to sustain the volatile population of the globe. But, then all these efforts could go in vain if the growers were not made aware to use the disease free and high yielding variety of seeds. Though the importance of obtaining quality seeds has been realised in the past, still, even in this 21st century, we cannot be completely satisfied with the extent of seed research. There is no doubt that the scientists all over the world has been working tirelessly and their efforts are reflected through the technological development of the seed industry.

Microorganisms exhibit their existence by harboring the seeds both externally as well as internally. The microorganisms become active under favorable environmental conditions such as light, temperature, moisture content etc. Among the microbes, fungi play a significant role in determining the quality and longevity of the seeds (Christensen and Lopez, 1963; Christensen and Kaufman, 1968 and Christensen and Mirocha, 1976). Hence, it becomes quite evident that it is necessary to test the health of seeds prior to sowing in order to ensure a better crop production.

Avoiding crop failures and the use of high yielding cultivers are the two main ways of boosting the crop production. But, even after using high yielding variety, the entire painstaking process may be futile if the microbes are able to invade the seed material. The disease caused by the microbes may be responsible for about 10% loss in major crops in India.

In the context of present day agriculture, sharing of seed and other propagative plant material has become essential for improving crop productivity. It is a known fact that India is one of the leading producers of many economically important crops, hence,

it is obvious that our country imports many varieties of seeds and plant materials to meet the demand of the ever-increasing population. So it is quite likely that along with the imported materials, some virulent strains of fungi may sneak in and cause devastating diseases, which may even take the form of an epidemic. It is due to this problem that imposition of plant quarantine have become essential and at present this law has been imposed in many countries. The main objective of this law is to prevent the entry of serious disease causing microbes into a country or a territory.

More and more attention is now being paid to the microbes causing disease and their ability to produce toxins. The deterioration in quality of seeds may be defined as, "the falling from a higher to a lower level in quality, character or vitality". It implies the impairment of vigour or usefulness (Gove, 1965).

The mould fungi cause extensive damage to the seeds. They grow well in grains and seeds during storage. They also may impair or retard the growth of seedlings. The impairment is caused mainly by the toxic metabolites secreted by the microbes. The quality of the seeds also deteriorate following the invasion by the microbes.

Temperature is another important factor that determines the type of microorganisms that will grow on the seeds during storage. At a temperature of about 25 ± 1^0C, almost all types of fungi are predominantly available. *Aspergillus flavus*, which produces the fungal metabolites, is the most predominant. It is also reported to be carcinogenous (Wogen, 1965). At low temperature, however, most fungi are found to be dormant. Above 50^0C, most fungi are killed. The deterioration of seeds may occur due to abrupt changes in the respiratory pathway and metabolic activities.

The seeds and their by-products are the major components of the diet of man and a large number of farm animals. At the global level, cereal grains contribute about 50% of the per capita energy intake (Fao, 1993).

The seeds remain viable for many years. Seeds of crop plants with low moisture content remain viable for longer time periods when stored under low temperature. The longevity of seed-borne pathogens can be independent of the seeds they inhabit and depends on the capability of the pathogen to remain viable as well as virulent from one season to the next in or on seeds. Pathogens may live longer than the seeds they colonize, for example *Colletotrichum lindemuthianum* remained active after the bean seeds, it colonised, lost their viability (Laing *et al.*, 1988). Neergaard (1984) generalised that (i) hyaline fungi with strong pigmentation and thick conidial walls were fairly long lived; (ii) fungi with fruiting bodies such as acervuli and pycnidia remained viable for long time periods, (iii) smut fungi lived long and (iv) fungi with thick walled resting mycelia survived the longest.

Airtight containers favour the survival of the seed-borne pathogens. Dry and cool weather provide an ambient condition for growth of seed-borne inoculum .The germination of *Phoma rabiei*, threshed after 5 months of storage at 25^0 to 30^0C, was found to be 50%. *C. graminicola* survived in maize kernels for more than 3 years at 4^0C. Advances in technology enhanced the ability of man to successfully store harvested plant products. Containers made of plant and animal materials were probably in widespread use during the last ice age; however, due to their perishable nature none of them survived. Basket making was highly developed art by 7000 BC. Earlier, baskets were used for gathering seeds, nuts, fruits and other vegetable materials and for storage

until their subsequent use. The ability to make pots of fried clay was an extremely significant advancement in storage technology and its appearance coinciding with the first domestication of grain bearing plants cannot be considered a mere chance. For the first time, it allowed the stored products to be sealed in containers that would significantly decrease the chance of loss due to fungal infestation. Another early technique developed for the storage of grains was underground pits and silos. During storage, respiration of the grain decreased the oxygen concentration and elevated the carbon dioxide level tremendously, thereby decreasing the chances of losses due to rodents and insects. Bernard in France conducted the first scientific studies on modified storage atmosphere in 1819.

The storage fungi may induce a decrease in germination percentage, important in malting barley and in seeds to be used for planting; discolored or otherwise damaged embryos or whole kernels, may help to determine the grade and price of the grains fit or unfit for consumption. Toxins constitute a health hazard for man and animals. They also cause heating, which is accompanied by drastic reduction in quality of seed or their complete spoilage (Christensen and Kaufman, 1965).

Forgac and Carll (1962) summarized information on a number of mycotoxins and animal disease caused by ingestion of foods and feeds invaded by fungi that produce toxins. Some of these mycotoxins present in common and widespread fungi result in severe illness and death of the consumers. The fact that fine quality seed is the need of the hour has been realised by the ancient growers since the time of cultivation. Though we are now in the 21st century, when we turn back to see the achievements, we find that the progress is still far from satisfactory. The scientists from all over the world

have been engaged in the seed research and some of their effort has been paid off as is seen in the advanced growth of seed industry.

There are a wide variety of microbes that are associated with the seeds. Under favourable conditions these microbes become active and cause severe damage to the field crops. Amongst these microorganisms, fungi play a significant role in determining the quality and longevity of seeds (Christensen and Kaufman, 1965 and Christensen and Mirocha, 1976). Hence, seed health testing and the use of healthy seeds are of great significance in improving the condition of the agricultural products.

It is known that many seed-borne fungi produce toxins, which at times may act as carcinogens. They also act as disease carriers, which may prove to be fatal to many crop plants. The change occurring in the seeds are not reversible and moreover, the qualities of the seeds degenerate even after full maturation.

While cleaning the seeds during storage, most of the unwanted materials are removed. However, some fungi still stay on and cause seed deterioration. The ideal temperature at which saprophytic and parasitic fungi can regain their germinating ability is 14^0C in cereals. The fungi included in this group are the species of *Alternaria, Chaetomium, Cladosporium, Fusarium, Penicillium, Aspergillus, Rhizopus* and *Helminthosporium.* These fungi are termed as storage fungi by Christensen and Kaufman (1965) which separates them from those of the field fungi, that may be present in the seed before or after the seed storage and destroy the seeds. The stored fungi may flourish under dry conditions. These fungi have the capability of attacking any crop under a favourable climatic condition.

The storage fungi also have the capability to alter the level of fatty acids and non-reducing sugars, induce a foul smell and offer discolourisation of seeds. Species of *Aspergillus* and *Penicillium* are the two most common fungi that cause extensive damage to the stored seeds throughout the world. The spoilage of the seeds takes place at 45^0C at 65-100% relative humidity. The activity of the fungi depends to a large extent on the physical condition, vitality and moisture content of the seed, temperature and relative humidity of the storage atmosphere.

The available stored materials and the storage tissue may be reduced by the decaying activity on seed by the saprophytic microorganisms thus making the crop susceptible and they may also affect the seedling germination. When the seeds from such seedlings are sown again, the microorganisms regain their growth under favorable conditions that may ultimately spoil the seed.

The toxic materials released from certain fungi affect the seedling growth severely. The appearance of the seedlings is also altered. The microorganisms affect the rise in temperature of the stored seeds (Panasenko, 1967).

The saprophytic mycoflora interrupts in the seed certification and indexing process, which determine the presence of the pathogens. The saprophytes are permitted to grow when the seeds are incubated and so they are the main reason for the difference in seed tests for the presence of plant pathogens (Limonard, 1967 and 1968).

In adverse field conditions, the mycelium of many fungi remain dormant and hence, are unable to impair seedling development (Mathur and Hansing, 1962).

Rise in temperature due to the excessive growth of fungi on stored seeds is usually noticed with 18% moisture content, in case of stored cereal seeds, the

temperature shot up from 17^0 to 43^0C (Gilman and Barron, 1930). Species of *Aspergillus* and *Penicillium* are the two main dominant fungi associated with the rise in temperature (Wallace and Sinha, 1962)

Annually a large quantity of crop is being lost or spoiled due to fungal infection both in terms of quality as well as quantity. The commercial value of the seeds gets depleted considerably and sometimes the grain is not fit for human consumption. The most common diseases encountered include (i) reduced size of the seed, (ii) discolourisation, (iii) *Fusarium* root rot, (iv) anthracnose, (v) necroses of seed and (vi) other physiological changes occurring in the seed.

Among the group of fungi, the species of *Fusarium* is the most dangerous customer to the crop plants. The common diseases caused by the pathogens include stunted plant growth, root rots and wilt. *Alternaria* in French bean causes leaf spots. The species of *Colletotrichum* is another successful pathogen, which establishes a firm hold on the leaf and pods of the crop. This pathogen is also responsible for decomposition of the seeds and the plant as a whole. The symptoms include dark brownish spots, which enter the pods and decompose it giving a bad odour. Considerable loss in yield of the crop is recorded every year due to such activity of the pathogen. Sometimes the loss in yield becomes difficult to ascertain.

With the infection of the seed, there seems to be some changes in their biochemical aspects The storage materials, enzymatic activity and respiratory pathway all get distorted from the normal range. Their sites and mode of action still need a careful and thorough attention.

Having a glance at the volatile population of the globe, it becomes an obvious fact that the demand for food supply has to be increased to keep pace with the increasing population. Hence, it is an imperative need to make an in-depth analysis of the seed production regarding its yield and quality. Seed pathology and its study has, therefore, become an interesting and a main thrust area of present agricultural research. Neergard (1977) defined seed pathology as, "the science and technology dealing with (i) seed-borne plant disease, (ii) seed diseases, (iii) the mechanism of their transmission, (iv) factor influencing their development, (v) techniques for their detection in seed, (vi) the method for preventing and controlling these diseases in the field and during seed storage and (vii) the assessment of seed-borne inoculum and seed diseases for seed certification schemes, quarantine, planting value and quality for consumption or processing".

ICAR, an important research agency is involved in extensive research work on seed and its development. In Northeast India, the ICAR is involved in developing hybrid seeds with care to ward off invasion by pathogens and its subsequent infection.

Considering the importance of fungi in causing the diseases in plants and reducing the yield of the field crops, the present investigation has been undertaken to generate important data for controlling the menace of fungal attack on bean seeds and increasing the yield for the ever- increasing population of the earth.

The following plan of work had been adopted to fulfill the desired goal of my research activity:

(1) Collection of different varieties of French bean (*Phaseolus vulgaris* Linn).

(2) Survey of seed borne fungi, their isolation and identification.

(3) Comparative study of different storage practices with respect to the incidence of fungi.

(4) Studies on the effect of certain seed-borne fungi on the germination of *Phaseolus vulgaris* Linn seeds

(5) Studies on the impact of seed leachates on the germination of spores of certain seed-borne fungi

(6) Studies on the effect of certain seed borne fungi in decomposition of the seed

(7) Studies on the influence of certain agro-chemicals on the seed-borne mycoflora.

(8) Screening of seeds for aflatoxins

(9) Control of certain pathogens by biological means.

REVIEW OF LITERATURE

The first report of a pathogen being carried along with seed came from Hellwig (1699) who suggested floatation technique to separate the ergot (*Cleviceps purpurea*) of rye from the seeds. Tillet (1755) gave the first demonstration of external seed transmission of a plant pathogen, as he showed that the spores were carried in smut balls mixed with seed The presence of seed mycoflora has been reported by workers like Padmanabhan (1949), Govindaswami (1955, 1957), Neergaard and Saad (1962), Christensen (1969), Lalrawna (1987) and Paul (1991). Different workers have studied different method of seed health testing. Doyer (1938) was the first to adopt the blotter methods as a process of seed health testing. Neergaard and Saad (1962) suggested that blotter and agar methods are both equally important and supplementary to each other. Limonard (1968) was the first to use deep-freezing in blotter method. The nutrients are released due to freezing which favours the growth of fungi on them. Agarwal *et al.* (1972) concluded that the blotter method proved better than the agar plate method for most of the fungi.

Meeting the demand of ever-increasing population of the globe has necessitated the people to exchange the seed materials from one country to another. So it is obvious that along with seed materials a large number of fungal species may sneak into a

country. The International Seed Testing Association (ISTA) was initiated in 1921 in Copenhagen, Denmark and established in 1924. From the year 1953, the rules and set of conditions were formally formulated as recommended. In India, Agarwal (1976), reviewed the progress of seed testing works and reported that the total number of samples tested has grown from 6,000 in 1962 to 2,00,000 by 1975.

Seed mycoflora has become an important area of research in present agricultural development. Almost all the seeds are harboured by fungi. The occurrence of seed mycoflora varies and depends on various factors like temperature, moisture content, relative humidity and oxygen availability (Nagel and Sameniuk, 1947). The difference between the field and the storage fungi is well elaborated by Christensen and Kaufman (1965 and 1969). Common field fungi are those which invade the seeds on developing stage of plants in the field and storage condition (Raghunathan and Majumdhar, 1968). The role of seed-borne pathogenic fungi in germination and disease of seedling is well established by Noble and Richardson (1968). Chirstensen and Kaufman (1969) have reported more than 150 fungal species from cereal grains. Christensen (1971) has advocated that storage fungi usually do not attack the seeds before harvest, but may be found on the seeds in very low percentage, thus providing the opportunity for the presence of the inoculum. A number of fungal species like *Acremonium, Diplodia, Monilia and Trichothecium* has been isolated from soybean seeds (Schneider *et al.*, 1971). The health condition of rice, wheat, blackgram and soybean has been studied by Agarwal *et al.* (1972). Isolation of soybean seed mycoflora has also been reported by Singh *et al.* (1973). The role of moisture content on the occurrence of fungi cannot be underestimated. Similar line of work has been made by Kanaujia and Singh (1975) on

stored seeds of *Avena sativa and Echinocola crussgali.* They concluded that high moisture content resulted in a high percentage of fungal infection with a corresponding decrease in the seed germination rate, during the study period of two years i.e., 1970 and 1971. There were reports of isolation of 27 fungal species from the seeds of broad beans collected from Imphal, India. The fungi present included the species of *Rhizopus, Mucor, Aspergillus, Chaetomium, Penicilium, Fusarium* etc, which considerably affected seedling germination. The elaboration of seedling disease influenced by fungal species of *Aspergillus, Cephalosporium, Fusarium, Pythium* and *Rhizoctonia* on maize has been made by Payek *et al* (1978).

The incidence of fungal species varies from season to season. Gupta *et al.* (1983) found maximum number of fungal species in rainy season followed by summer. The seed mycoflora of wheat from different locations of Saudi Arabia has been extensively studied by Abou-Heilah (1984), who found that agar plate recorded 19 fungal species and blotter 12 fungal species. The common fungi isolated were species of *Alternaria, Aspergillus, Drescheleria and Chaetomium.*

Tervet (1945) while studying on soybean found increased seed germinability with the culture filtrate of *A. fumigatus.* The heat stability of *F. moniliforme* toxin secreted in maize has been studied by Scott and Futrell (1970). Similar observation was made by Tripathi (1974) who found that toxin produced by *F. moniliforme* and *Colletotrichum graminicola* inhibited sorghum seed germination. An elaborate study on the activity of *A. niger, F. monilıforme, Helminthosporium sp.* and *Curvularia lunata* was made by Anahosur and Bidari (1974).

Extensive damage to stored grains of rice has been reported by Bedi and Dhaliwal (1970), Vidyasekaran *et al.* (1970) and Vaidehi and Ramarao (1978). Various fungi associated with the seeds produce toxic metabolites that are harmful for plant growth and it is reflected on their germination. The ability of *A. flavus* to significantly reduce germination and root-shoot length elongation in oil seeds has been reported by Mishra and Kanaujia (1973), Dublish and Pande (1976), Rai and Singh (1977) and Pande *et al.* (1982).

Singh *et al.* (1984) observed maximum suppression of germination by *Trichoderma viride* due to production of trichodermine by the fungus. Inhibition of root and shoot elongation of seedlings due to the application of pathogenic fungi was also observed. Maheswari *et al.* (1984) reported that the culture filtrates of *A. flavus, A. fumigatus, A niger, and F. moniliforme* significantly affected the seed germination. Maximum reduction in soybean seed germination by the culture filtrate of *F. semitectum, F equiseti* and *C. lunata* was noted by Singh *et al.* (1986). They recorded increased seed germinability by culture filtrates of *A. alternata, A. flavus, A. niger* and *Penicillium spp.* It was also inferred that root length had been increased by the culture filtrate of *C. lunata* and *A. alternata* but in contrast the root length decreased in presence of *Penicillium spp.* The culture filtrate of *Fusarium spp.* showed to have an inhibitory effect on the maize seed germination. The seedlings grown in its presence showed symptoms of brown spots with stunted growth.

The first report of aflatoxin contamination came from England in 1960.It was known as turkey 'x' disease as 10,0000 turkey poults died from feeds in which

groundnut served was contaminated with *A. flavus* (Asplin and Carnaghan, 1961). Aflatoxin production by *A. flavus* was reported by Sargeant *et al.* (1961) from peanuts.

The presence of number of toxic metabolites elaborated by fungi, which play vital role in many biochemical processes, has been reported by Turner (1971) and Keepler and Tu (1983). Aflatoxin production ability by the isolates of *A. flavus* has been reported by Diener and Davis (1969), Detroy *et al.* (1971) and Mehan and Cohan (1973). Uraguchi and Yamaazaki (1978) stated that "mycotoxin" is a term to designate such secondary metabolites that cause pathological abnormalities in man and warm-blooded animals.

The extraction and identification of aflatoxins from the oil cakes of sunflower, maize and husk showed that aflatoxin B_1 was present in high amount (80mg/Kg) in almost all the samples tested (Nandi, 1983). The level of aflatoxin in guava fruits was studied by Singh and Sinha (1983) who has reported the presence of *A. flavus* and *A. Parasiticus* which induced 0.563 and 0.257 ppm of aflatoxin respectively. Singh and Bedi (1985) worked with two commonly grown varieties of bread wheat, Duram wheat and *Triticales* and observed that none of them were totally immune to aflatoxin. The presence of aflatoxin in seeds of groundnut, maize, cotton, rice and wheat has been reported by Bedi and Cohan (1986). They could isolate 22 isolate of *A. flavus* and found that the isolated M-1 strain produced maximum amount of aflatoxin B_1.

On the basis of the amount of aflatoxin present in two varieties of sorghum, Reddy (1987) categorized different varieties into:

i) highly resistant (little or no toxin)

ii) moderately resistant (10 – 80 ppb)

iii) moderately susceptible (81 – 300 ppb)

iv) highly susceptible (more than 500 ppb)

Of the various factors influencing the fungal activity in stored grains, the moisture context, relative humidity, oxygen availability and temperature are the most important (Nagel and Semeniuk, 1947) Cahagnier and Paism (1974), while studying the efficiency of different containers with respect to the methods of storage found that none of the storage methods could prevent the occurrence of microorganisms. Milner *et al.* (1947) and Christensen and Gordon (1948) reported that a moisture level of 10 to 15 percent and relative humidity above 75 percent, a temperature range of 25^0C to 30^0C and oxygen concentration of about one percent seem to favour mould growth on the seed. The loss in the crop after harvest is mostly both in terms of monetary value and in man/hr (Stevens and Stevens, 1952) As a result of the fungal association with the seed, during improper storage, seed spoilage occurs due to various biochemical and physio-chemical changes in the seed The storage fungi are usually associated with the embryos of seeds and ultimately damage them (Christensen, 1955 and Kaufman, 1964). Loss in viability of the seeds is often associated with a change in the biochemical composition of the seeds. Goodman and Christensen (1952), Qasem and Christensen (1958, '60) and Bilgrami *et al.* (1976,'78) while experimenting on the germinability of the seeds after storage found 96% germination without fungal inoculation and no germination was noticed when the grain was pre-inoculated with fungi under same condition Majumder *et al.* (1961) while studying the storage of coffee beans in RCC bins found that with the change in diurnal temperature and humidity, there was a corresponding change in the temperature and relative humidity inside the bin The composition of fungal flora also differed to an extent

among the different containers of the coffee beans, primarily due to difference in temperature. Various other workers have indicated the significance of moisture content, relative humidity and temperature on the successful establishment of storage fungi on the seeds (Lopez and Christensen, 1967; Sharma and Sharma, 1984 and Malda *et al.* 1985).

Mehrotra (1983) emphasised the need for protecting the grains from fungal infection by keeping them in an environment where the seed is inactive but viable with least metabolic activity.

Due to the improper storage conditions, the dry weights of the seeds change due to fungal infestation. It has been noticed that with an increase in the storage period, there was a loss in the dry weight of rice and urad seeds (Bilgrami *et al.*, 1976). *A. flavus* and *C. pallescens* were found to be most active at that stage. Aquiero *et al.* (1978) found germination failure, root, coleoptile and stem rots and seedling blight of rice due to *F. moniliforme*, *C. lunata*, *Penicillium spp*, *Trichoconis padwickii* and *H. oryzae.* Significant effect of seed-borne mycoflora on the germination has been reported by Heeman *et al.* (1974), Kulik and Schoen (1982) and Fahim *et al.* (1985). Rati and Ramalingam (1974) have attributed the poor germination of seeds due to the rotting activities of storage fungi.

The term leaching has been used for the first time by Mann and Wallace (1925) who described leaching as the removal of mineral nutrients from seeds soaked in water. The importance of leachates due to their inhibitory effect on the germination and growth of fungal species are of considerable importance to the microbial population on the plant surfaces (Last and Deighton, 1965 and Blakeman, 1971). The seed leachates not only inhibit spore germination but also at times stimulate it. This has been reported by Barash

et al. (1964), Wang and Pickard (1971), Naim *et al.* (1976) and Kumar and Jalali (1985). The reduction in germination of various fungi was noted in chilli by Dhawale and Kodmelwar (1978) while studying the production of seed leachates in resistant and susceptible varieties of chickpea. They concluded that the organic substances released from the seeds play a vital role in controlling fungal population. The role of phenolic substances in inhibiting parasitic and bacterial invasion has been reported by Kraft (1973). The presence of some chemical substances in the leachates play a vital role in inhibiting fungal spore germination (Kandaswami *et al.* 1974).

From ages there has been a constant loss of crop due to the incidence of disease. This demands the control of the same. But it was only in the year 1885 that the discovery of Bordeaux mixture as a fungicide both for spraying as well as for seed treatment was made. The control of fungal diseases by directly treating the seeds with fungicide has been reported by Basak *et al.* (1984) and Bhatti *et al.* (1985). The control of *A. flavus* by the application of Bavistin at concentration from 2000 ppm to 3000 ppm has been reported by Devi and Polasa (1984). The inhibition of the mycelial growth of *Macrophoma magnifera* using Benlate, Captan, Dithane M-45 and Bevistan has been reported by Ekundayo (1984). The control of *Amaranthus* seed mycoflora using 0.2% Agrosan Gn and Ceresan has been studied by Sharma *et al.* (1980). The efficacy of Thiram and Vitavax against *C. capsici* has been reported by Grover and Bansal (1970). The contract of various seed-borne fungal diseases for bringing about unhealthy crops has been reported by Lalrawna (1987)

Though there is a marked loss in yield of crops due to the attack by different microorganisms, still many of the pathogens can be controlled biologically as well as chemically.

The biological control of fungal disease is lasting and enduring than the chemical control and the application of fungicide is not a permanent solution. It has been established that contaminated seeds may lead to severe disease development (Henry, 1931). The role of *Trichoderma viride* as an antagonist has been strongly supported by workers like Weindling (1934), Weindling and Emerson (1936) and Weindling and Fawcett (1936). *Chaetomium spp* has been shown to be antagonistic to seed-borne fungi such as *Fusarium spp,* which cause blight disease (Tveit and Wood, 1955). The significance of antagonistic effect of *Cladosporium spp* has been recognised by Bhatt and Vaughan (1963) and Warren (1972). The control of *Fusarium sp.* has been reported by Allison and Phillips (1963) in tomato and Long (1963) in cowpea. Lai and Bruehl (1968) studied the antagonism between *Cephalosporium gramineum* and isolates of *Trichoderma album, T. glaucum T. koningi* and *T. viride*. Reports of inhibition of one organism by the other have been reviewed by Dennis and Webster (1971) and Skidmore and Dickinson (1976). The colony interaction between an antagonist and test fungi has been reported by Srivastava *et al.* (1981). The control of *A. altenata, A. flavus, F. moniliforme* and *Alocladium atrum* by coating the seeds of rye (*Brasica juncea,* COSS.) with *Chactomium globosum* has been reported by Randhawa and Aulakh (1984). In one case Upadhyay and Rai (1987) observed that *A. flavus, A. niger, A terreus and P. citrinum* were antagonistic against *F. udum*. Paul (1991) reported *Cladosporium cladosporioides* and *T. viride* to be

antagonistic to maize seed-borne mycoflora such as *A. niger*, *F. moniliforme*, and *Rhizopus nigricans*.

MATERIALS AND METHODS

Two varieties of French bean (*Phaseolus vulgaris* Linn.) viz. Meghalaya and Manipur have been used for the present investigation. Both the varieties were procured from the farmers and stored for detailed investigations.

Sampling:

While drawing the samples from the bulk storage, International Rules for Seed Testing Association (ISTA 1966, 1976) was followed. The seeds of two varieties were collected from different regions of the storage lots (top, middle and lower regions). The samples thus collected were known as the primary samples. The primary samples were then mixed to make a final composite lot. The composite sample was then finally mixed thoroughly and reduced to a desired quantity. The final sample technically known as the submitted sample was than transferred into a sterile polythene bag and sealed. While performing the entire experimental task, care was taken to avoid fungal contamination.

The sealed polythene bags were then taken to the laboratory and stored at room temperature. Care was taken to avoid rats and mites. For the two-year survey of seed–borne mycoflora, fresh samples were collected each year soon after the harvest period and stored in the laboratory for each incumbent year.

Composition of media for the isolation of fungi:

A) Czapek's Dox Agar medium (Raper and Thom, 1949).

Agar	20.00g
$NaNO_3$	3.00g
KH_2PO_4	1.00g
$MgSO_4$	50 00g
KCl	0.50g
$FeSO_4.7H_2O$	0.01g
Sucrose	30.00g
Distilled. water	1.00Lit

B) Potato Dextrose-Agar Medium, (Lucy and Bridgmon, 1962).

Agar	20.00g
Potato	200.00g
Dextrose	20 00g
Distilled. water	1.00Lit.

C) Peptone Dextrose Rose Bengal Agar Medium (Martin, 1950)

Agar	20.00g
KH_2PO_4	1.00g
$MgSO_4.7H_2O$	0.50g
Peptone	5.00 g
Dextrose	10 00g
Rose Bengal (1%)	3.30ml
Distilled. water	1.00 Lit
Streptomycin	30.00 mg

D) Water-Agar Medium (for growth of seedling).

Agar	15.00g
Distilled. water	1.00 Lit

1. SURVEY OF SEED-BORNE FUNGI, THEIR ISOLATION AND IDENTIFICATION

The survey of seed-borne mycoflora of the two varieties of *P. vulgaris* Linn. was carried out for a period of two consecutive years. The survey was conducted on a seasonal basis, taking three seasons (winter, summer, and rainy) each year. The samples were stored in the laboratory and the working samples for periodical screening were drawn aseptically from the stock samples.

Methods for isolation of fungi:

The following three methods were used for isolation of fungi from *P. vulgaris* Linn. seeds:

(i) Standard Blotters Method:

Standard Blotters Method as recommended by International Seed Testing Association (ISTA, 1966) with a slight modification as suggested by Limonard (1966), known as the freezing method was followed. Three pieces of blotting papers (sterile) were placed in each sterile Petriplates (about 9cm diameter) moistened with sterile distilled water so that at least a little amount of water is left on the paper. Hundred seeds were randomly selected from each variety and four seeds were plated out equidistantly in each Petriplates. All the inoculation procedures were carefully done in a sterile Laminar flow chamber to avoid contamination .All the Petriplates were then frozen at -70^0C and subsequently incubated at 25 ± 1^0C for another 7 days under an alternate cycle of 12hr and 12hr light and darkness. The plates were observed on the eighth day with a stereo-binocular microscope. Infected seeds were marked and counted;

the percentage frequency of individual fungi was calculated. Various fungi occurring in the seeds were transferred to agar media (PDA) for confirmation and further investigation.

(ii) Agar Plate Method:

The various agar media used for isolation of fungi include Potato-Dextrose agar medium (PDA), Peptone Dextrose-Rose Bengal Agar medium, Czapek -Dox and Malt extract media

Sterilised media were poured aseptically into Petriplates and allowed to solidify. One hundred seeds were randomly selected from each variety and four seeds were placed equidistantly in a Petriplate containing PDA medium using a sterile forcep and needle. The transfer of sterile media to the Petriplates was carried out in sterile laminar flow chamber. The plates were then incubated at 25 ± 1^0C for seven days under an alternate cycle of light and darkness as in the previous method. Observation of the plates was done from the 5^{th} day, which continued till the 7^{th} day. The slow growing fungi were transferred to a fresh nutrient medium to avoid being overgrown by fast growing fungi. The infected seeds were counted and detected and the percentage incidence and relative abundance of individual fungi were determined.

(iii) Dilution plate method:

This method was employed for the quantitative estimation of fungi. One gram of seeds was taken in sterilised conical flask containing 99 ml sterile distilled water, and shaken thoroughly for 30 minutes using electrical shaker. 10 ml of this suspension was aseptically transferred to another sterilised 250 ml conical flask containing 90ml of sterile distilled water to get a suspension of 1:1000 dilution. 0.1 ml of this aliquot was pipetted out aseptically to a Petriplate containing potato-dextrose agar medium. The Petriplates were then gently and slowly rotated so that the inoculum was dispersed uniformly over the surface of the medium. Four

replicates were maintained for each sample .The plates were then incubated at 25 ± 1^0C for seven days. Observations were made on the 6^{th} day and the numbers of fungal colonies were counted.

Identification and isolation of fungal isolates:

The fungi isolated from the seeds were identified from the pure cultures maintained following the keys and manuals provided by different workers (Gilman, 1956; Subramanian, 1971; Barnett and Hunter; 1972 and Ellis, 1971and 1976). Various fungi thus isolated from the bean seeds by different techniques were purified by single sporing technique. The pure cultures of all the fungi thus obtained were maintained in replicates from time to time in test tubes in slants containing Czapeks dox Agar and Potato Dextrose Agar media, for further investigation.

The experimental results were expressed as an average of isolations done during the tenure of the survey. The relative abundance of individual fungi was calculated by the formula-

$$\% \text{ Relative abundance} = \frac{\text{Total no of individual species of fungus}}{\text{Total no. of all the species of fungi}} \times 100$$

2. COMPARATIVE STUDY OF DIFFERENT STORAGE PRACTICES WITH RESPECT TO THE INCIDENCE OF FUNGI

Seed sample:

In this case only one variety of the seed, i.e. the Meghalaya variety was used for the experiment.

Experimental containers:

The four types of storage containers used for the present investigation were (i) Gunny bag, (ii) Bamboo basket, (iii) Earthen pot and (iv) iron bin. The seed samples collected from the farmers were brought to the laboratory. The containers were first cleaned thoroughly and subsequently sterilised using 90% alcohol. Three kilograms of seeds was kept in each of the containers separately. The containers were properly sealed and kept in the laboratory at room temperature for a period of 12 months. Every precaution was taken to prevent the possible attack by rats and mites during this period.

Assessment:

The conditions of the seeds in various containers during the storage period of 12 months were tested periodically at 60 days interval using the following methods:

(i) Oven dried method for estimating the moisture content of the seeds:

The oven-dried method was used to determine the moisture content of the seeds. 5.0g of the seed sample was taken and subsequently dried in the oven at 105^0C for 24 hrs, and weighed till a constant weight was recorded. Three replicates were maintained for all the samples from each container. The percentage moisture content of the seed was calculated using the formula-

$$\% \text{ Moisture content} = \frac{\text{Loss in weight on drying (g)}}{\text{Initial weight of the sample (g)}} \times 100$$

(ii) Blotter method to test the germinability of the seeds:

In this method, three layers of blotting papers were kept in a sterile Petriplate and moistened with 10 ml of sterile distilled water. One hundred seeds were collected from all the containers and ten seeds were placed in each Petriplate. The plates were incubated at (20 ± 1^0C)

under the alternate cycle of light and dark. A stage had come when the seedlings had started to coil inside the Petriplates. It is at this stage that the plates were uncovered and on the 11th day, the plates were observed and the seedlings were removed one by one. The pre and the post emergence mortality of the seedlings were counted. The percentage germination of the seeds was calculated using the formula: -

$$\% \text{ Germination} = \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

Screening of seeds for incidence of fungal species:

The following methods were used for screening of seeds for the incidence of fungi:

(a) Standard Blotter method:

Freezing method of the standard Blotter technique, as described earlier was followed. Hundred seeds were randomly selected from different containers. Ten seeds were placed in each Petriplate containing water-moistened blotting papers. The plates were incubated following the process already described. After incubation for seven days, the plates were observed under a stereo binocular microscope. The numbers of infected seeds were counted and the fungi that appeared on the body of the seeds were identified.

(b) Agar plate method:

Potato-dextrose agar was used as nutrient medium. In this method hundred seeds from each container were placed following the same procedure described earlier. After incubation, the plates were examined, and the identification of all the fungi was made under a microscope. Suitability of the different containers, observation in respect of the incidence of fungi and the qualities of seeds was determined by:

(i) Determining the qualitative and quantitative incidence of the fungi during different periods of storage.

(ii) Comparing the percentage infection of the seeds at the initial stage with that of the percentage infection after storage.

(iii) Determining the fluctuation of moisture content of the seed in various containers during different periods of storage.

(iv) Determining the germinability of the seeds in various containers during and after storage.

The comparison of various containers with relation to the percentage infection of bean seeds was made with the statistical analysis (ANOVA)

3. STUDIES ON THE EFFECT OF CERTAIN SEED-BORNE FUNGI ON THE GERMINATION OF *Phaseolus vulgaris* Linn. SEEDS

Experimental Designs:

Two varieties of French bean (*Phaseolus vulgaris* Linn.) were used for assessing the effect of fungal extracts on seed germination and seedling growth.

Test fungi:

The four test fungi used in this experiment were: *Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum, Pythium intermedium* and *Trichoderma viride.*

Fungal extract preparation:

All the test fungi were cultured in sterilised Czapeks- Dox liquid medium in 250 ml Corning's conical flasks containing 150 ml liquid medium. After 10 days of incubation at room temperature (25°C±1°C), the culture filtrates were filtered first through four layers of cheesecloth and then through Whatman no.1 filter paper. One hundred surface sterilised seeds

were immersed in the culture filtrate for 24 hrs. The soaked seeds were then washed with sterilised distilled water and consequently used for the test.

(i) Pot culture experiment:

The field soil was first autoclaved at 20 P.S.I for 1hr and filled in sterilised pots made of plastic of diameter 15 cm. One hundred treated seeds from both the varieties were sown in the pots with 10 seeds in each pot. These pots were kept in a sterile place covered with polythene bags to avoid external contamination and subsequently kept in a sterile glass house for 15 days. All the test fungi were tested separately against both the varieties of bean seeds. One set of control i.e. without application of test fungi was maintained for both the varieties.

(ii) Test tube seedling symptom test:

The technique of Khare *et al.* (1977) was followed. One hundred test tubes of 30ml capacity were filled with 10 ml of water agar medium, plugged and autoclaved allowed to cool and subsequently solidified at a slight angle Hundred seeds treated with fungi were then sown aseptically, with one seed in each test tube. These test tubes were then incubated at 20 $\pm 1^{\circ}C$ for about 10 days under the cycle of light and dark. Finally, when the seedlings started reaching the rim of the test tubes, the plugs were removed from the rim. Thereafter the root-shoot length, percentage germination and dry weight of all the seedlings were measured. One control set of experiment with surface sterilised untreated seeds was performed for both the varieties.

4. STUDIES ON THE EFFECT OF SEED LEACHATES ON CERTAIN SEED-BORNE FUNGI

Seed samples:

Meghalaya and Manipur varieties of *Phaseolus vulgaris* Linn. seeds were used for the experiment.

Test fungi

The test fungi used in the study included *Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum, Pythium intermedium* and *Ttrichoderma viride.*

Preparation of seed leachates:

The seed leachates of *P. vulgaris* were prepared according to the method described by Saxena and Gupta (1982). Ten grams of seeds were surface sterilised by 0.1% mercuric chloride solution. The sterilised seed sample was then soaked in 100ml sterile distilled water in a 250ml flask for 36 hours. After soaking, the flask was shaken for 30 minutes using the electrical shaker and the remaining water was subsequently filtered. The filtrate was then concentrated to a final volume of approximately 5-8ml on a water bath at a temperature not exceeding 50^0C. This concentrated liquid is designated as "seed leachate". Its performance was tested against spores of the different test fungi.

Fungal spore germination:

The experiment on the germination of fungal spores in seed leachates was performed by hanging drop method in a cavity slide. The test fungi were taken and its single spore culture was grown on PDA medium. The fungal spores were collected from 10 days old cultures and spore suspensions were prepared in various seed leachates. The concentration of spores was adjusted to about 20-25 spores per microscopic field, under 150x magnification. A drop of spore suspension was placed on a clean micro-cover glass and subsequently placed on a cavity slide in an inverted position, so that the spores remain hanging in the cavity. A small amount of grease was applied on the edge of the cavity so as to prevent the evaporation of the extract and displacement of the cover glass. Three replicates were maintained for each sample. The slides were then kept in sterile Petriplates containing 10 ml sterile water to maintain the humidity of

the chamber. The entire set of the experiment was incubated at specified temperatures such as 15^0C, 20^0C, 25^0C and 30^0C. Control sets were maintained for each of the fungi using sterile distilled water as the medium. The slides were observed to note the germination of the spores at 2 hr, 5hr, 10 hr and 24 hrs of incubation.

The total germination and the percentage germination were calculated from the number of germinated spores counted from the observation of ten microscopic fields.

5. STUDIES ON THE IMPACT OF CERTAIN SEED-BORNE FUNGI ON THE DECOMPOSITION OF SEED

Both the Meghalaya and the Manipur varieties of *P. vulgaris* were used for the experiment. The test fungi were grown in pure cultures and maintained in test tubes, which were kept in a slanted position. The pure fungi thus obtained were then grown in Petriplates. The spores were then inoculated in a liquid medium. After 10 days of incubation, the spore suspensions were taken and its concentration was adjusted to approximately 4×10^4 spores/ml with the help of haemocytometer. 150 g of seeds was taken for each treatment. The sample thus weighed were surface sterilised by treating with 0.1% mercuric chloride solution for two minutes which was followed by several washings in sterile water. The seed samples were then cleaned with sterile blotting paper and subsequently dried in oven at 60^0C for 24 hrs. The samples were then shifted to 1000 ml flask and consequently incubated with 5 ml of the spore suspension of the test fungus. The flasks were then vigorously shaken. All the test fungi were assessed separately on both the varieties of *P. vulgaris* Linn. Both samples of seeds, after incubation were kept in a clean cloth bag and stored in a desiccator at 96% relative humidity maintained by a solution of sulphuric acid (CMI, 1974).

As a control set, another lot of both the varieties (150 g) after surface sterilization by 0.1% mercuric chloride was similarly kept without treatment with the test fungi. It was also kept in a desiccator at the same relative humidity (96%). Both the control and the treated samples were kept in the laboratory at room temperature for a period of six months. Thereafter assessment was made to see the effect of different fungi on the decomposition of the seeds, at a regular interval of 60 days by the chemical analysis of the same. The quantitative estimation of protein, sugars, starch, reducing and non-reducing sugars and fatty acids was carried out.

Estimation of starch

The estimation of starch content of bean seeds was carried out by the method suggested by Snell *et al.* (1961). The seeds were powered in a grinder.0.2 g of the sample was taken and 25 ml of 80% ethanol was added and stirred thoroughly. The solution was centrifuged after 5 minutes and the supernatant was decanted. The process of extraction was repeated by adding 30 ml of 80% hot ethanol to the residue and subsequently centrifuged. Decantation of the supernatant was again carried out and the residue obtained after centrifugation was taken for the estimation of starch. To the residue 5 ml of water was added and stirred thoroughly 6.5 ml of 52% perchloric acid (prepared by adding 270 ml of 70% percloric acid to 100 ml of distilled water) was added. The solution was stirred constantly for 10 minutes and after 15 minutes, 20 ml distilled water was added, centrifuged and the supernatant was collected. The entire process was repeated thrice. The supernatant was mixed thoroughly and the volume was raised to 100 ml. 10 ml of 0.1% anthrone reagent (prepared by adding carefully 760 ml of conc. H_2SO_4 to 330 ml water and 1g anthrone and dissolved using a magnetic stirrer) was added to the stock solution. It was then heated to 100°C for 12 minutes. With the appearance of blue green coloured solution it was cooled at room temperature and the optical density was read at 630

nm. Three replicates were maintained and the readings were compared with the standard curve prepared by the same process.

$$\% \text{ Starch} = \frac{\text{C (mg) X solution volume (ml)}}{\text{10 X aliquot (ml) X sample weight (g)}}$$

Where, C = starch obtained from the standard graph (mg)

Estimation of total sugars

The estimation of total sugar content was carried out by the procedure suggested by Bilgrami, *et al* (1979). The seeds were powered and to 0.2 g of the powered sample, 25 ml of 80% ethanol was added and stirred and the supernatant was centrifuged for 5 minutes. The process of extraction was repeated by adding 30 ml of 80% hot ethanol to the residue, which was subsequently centrifuged. The ethanol was then evaporated in an evaporating disc. The bottom residue was dissolved in 5 ml distilled water and it was from this stock that the total sugar was estimated. To 2 ml of this solution 0.14 ml of 80% aqueous phenol was added followed by the rapid addition of 5 ml conc.H_2SO_4.The solution was allowed to stand for 30 minutes after thorough mixing. The yellowed coloured solution was then read at optical density of 490 nm. The standard curve of glucose was prepared by the same process. Three replicates were used and the readings were compared with the standard curve. The total sugar content was expressed as mg/g of the sample.

Estimation of free fatty acids:

The free fatty acid content of the seeds was estimated by the method described in the official methods of analysis of the Association of Official Agricultural Chemists (1960). The

seeds were powdered in a grinder. Twenty grams of the powdered sample was taken into a 100ml flask; 50 ml of benzene was added and the flask was shaken periodically for 45 minutes. The flask was then tilted and the sample was allowed to melt and subsequently settled at an angle after few minutes. The extract thus obtained was filtered and then 25 ml of the filtrate was drawn and to this was added 25 ml of 0.04% alcohol-phenolphthalein solution. The entire mixture was then titrated against 0.0178 N KOH. Fat acidity was expressed in terms of mg KOH required to neutralize free fatty acids from 100 g of smashed seeds of *P. vulgaris* Linn.

Estimation of protein and soluble nitrogen:

Protein and soluble nitrogen were estimated by the Micro-Kjeldahl method using distillation apparatus. 100 mg of the sample was transferred to a 30 ml digestion flask. The boiling chips were added to the sample and digested till the solution became colourless. The digest was cooled and diluted with a small quantity of distilled ammonia free water and transferred to the distillation apparatus. The Kjeldahl flask was rinsed with small quantities of water. 5 ml boric acid solution with a few drops of mixed indicator with the tip of the condenser dipping below the surface of the solution was placed in a 100 ml conical flask. 10 ml of sodium hydroxide –sodium thiosulphate solution was added to the test solution in the apparatus. The distillation was carried out and ammonia was collected on boric acid. The tip of the condenser was rinsed and the solution titrated against the standard acid until the first appearance of violet colour (the end point). A reagent blank with an equal volume of distilled water was prepared. The titre volume of the blank was subtracted from that of the sample titre volume. The nitrogen content of the sample was calculated by using the formula-

$$\text{Ng/kg} = \frac{(\text{ml HCl} - \text{ml blank}) \times \text{normality} \times 14.01}{\text{Weight (g)}}$$

Non- protein Nitrogen:

100 mg of powdered material was extracted with ice cold 10% TCA (10ml). It was centrifuged and washed with TCA while all the supernatants were pooled to make a volume of 25 ml. An aliquot was taken and distilled as described earlier. It was titrated against the standard acid and the nitrogen content was calculated. This gave the percentage of non-protein nitrogen.

Protein nitrogen

Multiplying total nitrogen value with 6.25 gave the crude protein content which also included non-protein nitrogen. To get true protein content, the non-protein nitrogen was deducted from the total nitrogen and the value was multiplied with the factor.

Estimation of reducing and non-reducing sugars:

Reducing sugar content of *P. vulgaris* was estimated by the method described by Peach and Tracy (1955). 3.0g of the seeds were ground to powder. It was blended with 1.5 ml distilled water in a glass homoginiser. To this 0.2 ml of 0.3N barium hydroxide ($Ba(OH)_2$) and 0.2 ml of 5% zinc sulphate ($Zn(SO)_4$) were added and then mixed thoroughly. The entire mixture was then centrifuged. To 1ml of the supernatant 1 ml of alkaline copper reagent, which was prepared by dissolving 4g of $CuSo_4.5H_2O$, anhydrous Na_2Co_3 and 16g Na-K-tartarite in 1 litre water was added. The mixture was then heated in water bath for 15min and subsequently cooled. To this, 1ml of arsenomolybdate reagent (prepared by dissolving 25 g ammonium molybdate in 450 ml of distilled water and adding 3gm of $Na_2HSO_4 .7H_2O$ dissolved in 25 ml

of water, mixture and then placed at 37^0C for 24hr) was added and allowed to stand for few minutes till the effervescence had ceased to liberate.

6. STUDIES ON THE EFFECT OF CERTAIN AGROCHEMICALS ON SEED-BORNE PATHOGENS

Fungal flora:

The dominant seed fungi viz. *Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum, Pythium intermedium and Trichoderma viride* of *P. vulgaris* Linn. were selected as the test fungi.

Fungicides:

The fungicides used for the control of pathogens included Dithane M-45, Blitox-50, Topsin, and Indofil. The following were the experiments conducted to study the effect of fungicide on the dominant test fungi.

(i) Fungal spore germination

The efficiency of different fungicides on the fungal spore germination was studied at different concentration in the cavity slide by hanging drop method. 0.005%, 0.01%, 0.02%, 0.05%, 0.1%, 0.25% and 0.5%. of the concentration of the fungicides were prepared and with the help of a haemocytometer the number of fungal spore was adjusted to 20 spores per microscopic field. A drop of graduated spores and the fungicides of different concentrations were then placed in the cover slip and the slides were then kept in a plate containing sterile distilled water for 24 hrs in an incubator. Control set was maintained by using the fungal spores in sterile distilled water. The results thus obtained were expressed as the germination percentage. Five microscopic fields were taken for observation.

(ii) Effect on mycelial growth

The poisoned food technique was used at different concentrations of the fungicides.

Poisoned food technique:

Hundred milliliter of the PDA medium was taken in a sterile conical flask and mixed with different amounts of fungicides to get a media of 0.005%, 0.01%, 0.02%, 0.05%, 0.1%, 0.25% and 0.5% concentrations. The medium thus prepared was then poured in sterile Petriplates. It was allowed to cool and solidify. With the help of a sterile cork borer, a disc of 4mm diameter of each fungus was taken out from the pure culture and inoculated at the poisoned media. Control set was also maintained. The inference was drawn when the colonies started reaching the sides of the plates. The colony diameter was measured along the three axes and three replicates were maintained. The diameter of the fungi grown on poisoned media was compared with that of the control and the difference was expressed as the percentage inhibition as:

$$M\ (\%) = \frac{M_c - M_t}{M_c} \times 100$$

Where, M = Percentage inhibition

M_c= Colony diameter of control

M_t= Colony diameter of fungi on treated medium

7. SCREENING OF SEEDS FOR AFLATOXINS

Test for Blue Green Yellow Fluorescence

Bright greenish yellow fluorescence (BGYF) was taken as the criteria for detecting the aflatoxin contaminated samples (Fennel, *et al.* 1973). The samples were observed under long

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wave of ultra violet lamp. Samples giving typical Blue Green Yellow Fluorescence were studied for aflatoxin extraction.

Extraction of aflatoxins from seeds and their chemical confirmation:

The chemical extraction of aflatoxin was followed by the method of Thomas, *et al.* (1975). Fifty grams of sample were first powered. It was then blended in 250 ml methanol: water (60:40v/v) in an electric blender for 2 min. and the extract was filtered through Whatman No.1 filter paper. One hundred milliliter of the filtrate was taken in a separating funnel and to it 30 ml saturated sodium chloride and 50-ml hexane were added, shaken thoroughly and allowed to settle. The lower aqueous methanolic layer was taken to another seperating funnel and was subsequently extracted with 50 ml chloroform .The lower chloroform layer was drained off into a conical flask containing 5 g cupric carbonate. The mixture was agitated and cupric carbonate was allowed to settle down. The chloroform solution was decanted through a bed of Na_2SO_4.The chloroform solution was evaporated to dryness in a water bath. The residue left at the bottom was dissolved in 2 ml chloroform which was then used for chromatographic detection of aflatoxins.

Preparation of TLC plates:

36 g of silica gel was mixed with 72ml distilled water to form slurry. It was then coated (0.5 mm thick) in a glass plate of 20x20 cm, with an applicator. The plates were then dried at room temperature and activated in an oven at 120°C for 1hr. The plates were cooled before use.

Detection of aflatoxins by thin layer chromatography:

Fifty-milliliter aliquot (chloroform extract) was spotted on the TLC plate with the help of micropipette and run in a solvent, toluene: isoamylalcohol: methanol (90:32:2 v/v). The plate was then air-dried and observed under UV-lamp for detection of aflatoxin spots.

Chemical confirmation of aflatoxin B_1:

Chemical confirmation of aflatoxin B_1 was done by trifluoroacetic acid (TFA) as recommended by Stack and Pohland ((1975). After the chemical reaction with TFA, aflatoxin B_1 appeared as a blue fluorescent spot at a Rf about $1/4^{th}$ of that of aflatoxin B_2 on TLC plates.

Quantitative estimation of aflatoxins:

Spectrophotometer (Nabney and Nesbitt, 1965) was used for the quantitative estimation of aflatoxin B_1. The spots of aflatoxin B_1 on TLC plates were scrapped and subsequently extracted with 5ml cold methanol. It was then centrifuged at 3000 rpm for 15 minutes. The ultra-violet absorption spectrum of the methanolic solution was recorded in a spectrophotometer and the amount of aflatoxin present in the sample was calculated using the formula:

$$A = \frac{D \times M \times 10 \times 6}{E \times L \times 1000} \text{ mg/ml}$$

Where, A = Amount of aflatoxin present

D = Optical density

M = molecular weight of aflatoxin

E = molar extinction co-efficient

L = path length.

8. CONTROL OF CERTAIN PATHOGENS BY BIOLOGICAL METHODS

The biological control of the plant disease was explained by studying the effect of fungal metabolites on seed germination and colony interaction

(i) Fungal metabolites on seed germination.

The antagonist and the test fungi were grown in liquid culture medium of Czapek's Dox. After the growth of the fungal colonies, the liquid medium was filtered through cheesecloth and Whatman No.1 filter paper. The filtrate of the antagonist and the test fungi was then mixed in a definite proportion of 1:1,1:2 and 1:5. The seeds were then soaked in the different proportions of the antagonist and test fungi for 24 hrs. The seeds were then washed gently with sterile distilled water and subsequently placed on sterile moist blotters in Petriplates. The plates were then incubated at 25±1°C for 10days under light and dark of 12 hrs duration.

The seedlings from the Petriplates were then taken out slowly for the pot culture experiment. Then the number of pre- and post emergence mortality as well as the cases of successful germination were recorded. The length of roots and shoots was measured as the average length of roots and shoots of all the seedlings. The seedlings were then subjected to heat at 80^0C in hot air oven for 24 hrs. The dry weight of the seedlings was taken and expressed as the average dry weight of all the seedlings. The results were then compared with that of the control and expressed as the percentage change.

(ii) Colony interaction:

The colony interaction between the antagonistic and the test fungi was studied by placing the discs of pure culture of 4mm diameter about 3cms apart in a PDA medium. Three control sets were maintained. Control set was initiated by placing a block of 4mm in the center

of the media plate. The inference was drawn when no further growth or change in style of growth was noticed.

The result was expressed using the colony interaction model of Skidmore and Dickinson (1976). The germination of both the antagonistic and the test fungi was measured from the central loci at both sides i.e. towards and opposing each other. The breadth of inhibition zone, intermingled zone and percentage inhibition of radial growth (Fokkema, 1976), was recorded as-

$$\% \text{ Inhibition of radial growth} = \frac{r_1 - r_2}{r_1} \times 100$$

Where, r_1 = Radial growth of pathogenic fungus towards the side opposite to the colony of the antagonistic fungi.

r_2 = Radius of the antagonistic fungus towards the side opposite to the colony of the test fungus.

RESULTS

1. SURVEY OF SEED-BORNE FUNGI, THEIR ISOLATION AND IDENTIFICATION

The present investigation was carried out for two successive years (i.e. 1997 and 1998) in three different seasons viz., winter, summer and rainy for two varieties of *Phaseolus vulgaris Linn.* seeds.

Three methods i.e. dilution plate, agar plate and blotter methods were followed for the isolation of seed fungi from *P. vulgaris* L. Of the three methods, blotter method, proved to be the most effective in terms of the number of fungal species isolated followed by agar method. The dilution plate method was least effective in harboring the fungal species vis-a-vis the other two methods

In the Meghalaya variety, a total of 26, 28 and 28 fungal species were isolated by dilution plate, agar and blotter methods, respectively. Similar trend was observed in the Manipur variety, where a total of 28, 29 and 30 fungal species were obtained from the dilution plate, agar and blotter methods respectively

Maximum fungal species were isolated from the Manipur variety. In this variety, a total of 30, 29 and 26 fungal species were recorded, during rainy, summer and winter seasons respectively, whereas, the fungal species recorded from the Meghalaya variety during these three seasons were 28, 26and 23 respectively

Qualitatively, there was not much difference in the composition of fungal flora isolated from the seeds of both the varieties. A majority of the species isolated was common to both the

Table 1.1: The relative abundance of fungal species isolated during the study period of 1997 and 1998

MEGHALAY A VARIETY	1997										1998								
	Winter		Summer			Rainy					Winter		Summer			Rainy			
	D	A	B	D	A	B	D	A	B		D	A	B	D	A	B	D	A	B
Alternaria alternata	9.0	-	5.5	-	6.6	-	-	5.0	4.4		7.5	-	6.0	-	5.5	-	-	4.5	6.1
A.clavatus	-	18.6	5.8	-	5.2	-	-	6.2	3.8		6.8	9.0	7.3	-	4.2	-	-	4.0	-
A flavus	-	-	-	-	-	-	6.6	4.8	6.6		-	-	-	9.0	6.5	-	5.5	-	4.0
A niger	8.8	-	4.6	-	-	9.0	-	7.1	-		7.2	8.8	6.8	-	-	7.1	-	4.3	4.2
A tenuis	9.2	-	7.2	9 0	-	-	-	4.1	8.2		6 8	-	-	8.2	7.2	8.3	6 3	4.2	4.8
Botrytis sp	-	-	5.8	8.2	-	-	7.2	3.2	6.0		7.2	-	7.4	-	4.3	-	4.5	4.0	6.2
Cephalosporum acremonium	10.3	-	6.0	-	7.2	-	-	4	6.4		9.3	-	8.4	-	5.0	-	-	5.8	3.8
Cladosporium cladosporioides	9.0	-	-	-	8.0	-	5.6	6.1	4.2		6 8	-	-	-	6.3	6.5	6.2	5.2	2.5
C gresi	-	17.8	4 2	7.8	5.8	-	-	-	5.3		-	7.6	6.3	9.3	7.2	5.8	-	-	5.3
Chaetomium globosum	9.3	-	5.6	10.3	6.6	-	-	6.8	-		5.5	-	5.4	9.8	8.3	-	-	4.1	3.1
Colletotrichum	9.8	-	6.3	-	-	8.2	-	7.1	-		8.3	-	-	-	-	-	-	4 0	7.3
F moniliforme	-	-	-	11.2	-	-	4.8	6	-		-	-	-	10.2	-	-	4.8	3.8	3.3
F oxysporum	-	-	-	-	6.0	-	7	-	6.4		-	-	-	-	7.5	-	3.5	-	5.4
Mammaria echinobotryoides	-	19.2	-	-	7.2	7.8	5.8	-	-		-	6.8	-	-	5.4	8.2	6.1	5.5	-
Mucor sp	9.0	10.05	7.1	9.1	7.0	9.12	6.0	-	5.2		7.7	10.2	6.8	7.8	6.2	7.8	5.1	5.0	6.0
Penicillum chrysogenum	10.1	-	8.2	10.6	5.8	8.5	7.2	5.8	4.0		-	9.1	3.3	8.0	-	6.8	4.3	4 0	5.8
P expansum	-	-	5.0	9.3	-	10.2	-	6.2	-		-	11.01	7.8	-	-	6.0	-	4.5	-
P rubrum	-	-	6.1	-	-	-	-	6.4	3.7		-	-	-	-	-	-	5	3.8	4 1
Penicillum sp	-	18.1	-	-	6.1	11.2	-	4.2	2.3		-	8.0	6.0	-	6.5	7.0	-	2.5	5.4
Phoma pomogranatus	-	20.0	5.5	-	7.2	7.2	-	3.2	4.0		-	7.5	-	-	4.0	8.9	-	4.7	-
Phoma sp	-	-	-	-	5.4	-	6.0	5.5	4.4		-	-	-	-	3.2	9.0	3.8	5.3	4.2
Pythium intermedium	-	-	-	-	-	-	7.2	-	3.7		-	-	-	-	-	-	6.2	6.4	-
Pythium sp	-	-	-	-	-	-	6.8	4.2	2.5		6.4	10.0	-	-	-	-	7.1	4.3	3.8
Rhizopus nigricans	-	-	7.7	7.8	-	9.0	7.2	-	1.5		-	-	7.0	7.5	4.3	7.5	5.3	-	3.6
Rhizoctonia solani	-	-	8.2	-	-	7.8	5.2	-	4.0		-	8.5	5.4	10.3	-	6.2	6.1	-	4.8
Trichoderma harzianum	9.4	-	4.1	-	5.6	-	-	5.0	4.2		9.3	-	9.0	11.4	4.0	-	3.8	2.8	5.4
T.koningi	-	-	-	9.3	5 0	7.2	5.8	-	3.0		-	-	6.4	8.0	3.2	5.4	5.6	3.0	3.0
T viride	10.8	-	3.0	8.0	6.3	-	7.2	5 2	4.2		7.0	-	3.1	3.1	3.1	2.1	5.3	6.2	4.9
Sterile (I)	8 0	-	5.0	5.6	7.0	-	6.2	6.0	-		-	4.0	2.0	4.0	2.0	-	2.0	-	2.0
Brown mycelia (ii) White	4.5	-	2.0	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-

- Denotes absence

Table 1.2: The relative abundance of fungal species isolated during the study period of 1997 and 1998

MANIPUR VARIETY	1997									1998							
	Winter			Summer			Rainy			Winter			Summer			Rainy	
	D	A	B	D	A	B	D	A	B	D	A	B	D	A	B	D	A
Alternaria alternata	-	-	5.0	6.0	3.8	3.7	4.0	4.7	3.4	6.6	-	7.1	7.6	6.6	8.8	7.1	6.0
A.clavatus	-	7.0	5.0	-	3.0	3.5	-	3.2	3.0	6.3	8.3	7.0	8.0	5.2	7.4	-	5.8
A.flavus	6.7	-	5.2	-	-	4.0	3.8	4.1	4.0	5.8	-	6.5	-	-	-	6.8	7.2
A.glutaceus	7.5	7.2	-	5.0	3.0	3.6	3.5	3.5	3.0	5.0	8.0	5.0	-	-	-	-	-
A.ruber	-	-	-	-	4.2	-	3.0	4.0	4.0	4.0	-	7.14	-	-	8.0	8.1	6.1
A.tenusi	-	8.2	6.3	7.5	5.1	3.5	-	4.3	3.4	6.2	7.8	7.0	7.12	-	-	7.6	-
Botrytis sp	-	-	-	-	-	4.3	-	4.0	3.2	6.1	-	5.14	-	5.2	-	6.2	-
Cephalosporum acremonium	8.6	6.7	7.0	6.3	3.5	5.2	-	6.2	4.8	5.8	6.8	5.0	-	-	7.8	-	7.2
Cladosporium cladosoporioides	-	6.9	5.3	-	4.0	3.0	4.1	3.0	4.6	-	8.0	6.3	-	7.2	9.2	-	-
C. gresi	5.4	7.5	4.2	7.5	3.6	2.5	4.2	-	3.0	5.0	7.8	-	9.1	6.1	8.9	9.0	-
Chaetomium globosum	10.2	6.5	7.2	8	4.2	4.1	3.0	2.5	3.0	6.2	9.2	6.8	-	5.0	-	8.1	6.8
Colletotrichum lindemuthianum	7.3	-	4.2	-	2.5	3.8	3.1	5.0	3.5	-	-	5.12	6.7	5.2	9.4	-	7.5
F.moniliforme	-	7.0	-	-	4.3	3.3	-	5.0	3.6	-	9.2	-	87.12	7.1	-	7.2	-
Fusarium flocifferum	-	-	8.0	-	5.6	2.8	4.8	4.2	2.5	-	-	-	-	6.2	7.8	-	
F.oxysporum	-	-	-	-	3.3	4.3	4.0	-	3.0	-	-	7.0	-	5.3	-	-	8.2
Mammaria ecinobotryoides	8.5	-	5.6	-	4.0	-	3.5	-	-	-	-	-	-	-	8.2	9.3	6.0
Mucor sp	-	-	7.5	6.7	3.8	3.7	-	5.3	3.4	-	-	-	18.0	-	-	-	-
Penicillum chrysogenum	-	8.0	6.3	-	5.2	2.6	3.0	-	3.0	-	-	7.0	-	6.6	-	7.0	7.5
P.expansum	-	7.9	-	-	3.0	4.1	-	5.6	5.0	6.6	8.0	-	10.2	7.0	8.3	6.12	-
Penicillum sp	6.5	8.6	5.4	7.8	3.5	3.5	4.8	5.7	2.0	6.6	8.5	6.0	-	-	-	-	-
Phoma pomogranatus	-	-	-	-	-	3.0	5.0	-	3.0	-	-	-	-	-	-	-	6.2
Phoma sp	-	-	-	4.3	3.2	4.2	5.5	5.3	2.0	-	-	-	9.8	7.3	9.0	6.0	6.0
Pythium intermedium	-	-	-	-	-	2.2	3.0	6.3	3.4	-	-	-	-	-	-	7.2	5.8
Pythium sp	8.0	-	-	8.4	3.6	3.5	2.5	6.0	3.0	6.6	-	-	-	5.2	-	-	7.2
Rhizopus nigricans	-	6.3	-	-	-	3.6	-	-	4.0	-	-	-	-	-	-	8.5	-
Rhizoctonia solani	7.0	8.2	3.2	11.2	4.1	2.1	4.0	6.1	4.2	6.2	8.3	5.1	9.9	-	7.1	-	7.0
Trichoderma harzianum	6.5	-	4.0	10.9	4.0	3.7	2.0	5.5	2.8	5.8	-	5.0	-	-	-	-	-
T.koningi	-	10.0	5.2	-	4.8	3.0	3.0	4.0	3.7	-	7.5	-	-	-	-	-	-
T.viride	6.0	5.5	10.5	4.1	4.0	-	-	3.4	-	-	5.1	8.6	7.0	-	-	-	-
Sterile (I) Brown mycelia (ii) White	3.0	-	2.3	-	5.5-	4.2	-	-	-	-	2.0	-	-	6.2	7.02	-	-
	-	-	-	-		-	-	8.9	5.9	4.0	2.0	1.2	-	-	-	-	-

– Denotes absence

varieties. However, *Aspergillus alutaceus and Fusarium flocifferum* were restricted to the Manipur variety whereas, *Aspergillus niger* and *Penicillum rubrum* was observed in the Meghalaya variety only. The Meghalaya variety showed the dominance of fungal species like *Alternaria alternata, F. oxysporum* and *Pythium intermedium* whereas, the Manipur variety exhibited the predominance of seed fungi viz., *A. candidus, A. alternata, P.expansum* and *Rhizopus nigricans* (Tables 1.1 and 1.2)

2. COMPARATIVE STUDY OF THE DIFFERENT STORAGE PRACTICES WITH RESPECT TO THE INCIDENCE OF FUNGI

The two varieties viz., Meghalaya and Manipur of *Phaseolus vulgaris* Linn seeds were kept in different containers, viz., gunny bags, earthen pots, bamboo baskets and iron bins for a period of one year. On periodical inspection of the germination rate and occurrence of fungi in these two varieties stored in the containers, the following results were obtained:

The incidence of fungi on *P. vulgaris* Linn. during different seasons; viz., winter, summer and rainy revealed a total number of twenty eight fungal species by dilution plate, agar and blotter methods. Twenty-seven fungal species were isolated by blotter method; 23 fungal species were isolated by agar plate method and 21 fungal species were isolated by the dilution plate method. Rainy season recorded 28 fungal species, followed by 27 species in the summer season. Winter season recorded 23 fungal species. *Aspergillus flavus, Aspergillus niger, Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum, Penicillium expansum, Phoma pomogranatus, Pythium intermedium* and *Trichoderma viride* were found to be dominant at all the seasons and all the culture methods used (Table 2.1).

It was observed at the beginning (i.e. 0-day) of the study period that the percentage of infection was 65% in all the containers. It was also noticed that the earthen pots registered the

Table 2.1: The incidence of fungi on *Phaseolus vulgaris* Linn. seeds at different seasons.

FUNGAL SPECIES	WINTER			SUMMER			RAINY		
	D	A	B	D	A	B	D	A	B
Aspergillus alutaceus	+	+	-	+	+	+	+	+	+
A. candidus	+	-	+	-	+	+	-	-	+
A. clavatus	+	+	+	+	+	+	+	+	-
A. flavus	+	+	+	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+	+
A. ruber	-	+	-	-	+	+	-	+	+
A. tenusi	-	+	+	-	+	-	-	+	-
Alteranria alternaria	-	+	+	+	+	+	-	+	+
Cephalosporium acremonium	-	+	+	+	+	+	+	+	+
Chaetomium globosum	+	-	+	+	+	+	+	+	
Cladosporium cladosporioides	-	+	-	+	-	+	+	+	+
C. gressi	-	-	-	+	+	+	-	+	+
Colletotrichum lindemuthianum	+	+	-	+	+	-	+	-	+
F. moniliforme	+	+	+	+	+	+	+	+	+
F. oxysporum	+	+	+	+	+	+	+	+	+
Mammaria echinobotryoides	-	-	+	-	+	+	+	+	+
P. chrysogenum	-	+	-	+	+	+	+	+	
Penicillum expansum	+	+	+	-	+	+	+	+	+
Penicillum sp	-	-	+	-	-	-	-	+	+
Phoma medicaginis	+	+	-	+	+	+	+	+	+
P. pomogranatus	+	+	+	-	-	-	-	+	+
Phoma sp	-	-	-	+	+	+	+	+	+
Pythium intermedium	-	-	-	-	-	+	+	+	+
Pythium sp	+	-	+	+	+	+	-	+	+
Rhizopus nigricans	-	-	-	+	+	+	+	+	+
Rhizoctonia solani	-	-	-	+	+	-	-	-	+
Trichoderma viride	+	+	+	-	+	+	-	+	+
Trichoderma sp	-	-	+	+	+	+	+	+	+
Sterile mycelia									
(I) Brown	-	+	-	+	+	+	+	+	+
(ii)White	+	-	+	-	+	+	+	+	-

+ Denotes presence
- Denotes Absence

D = Dilution plate method
A = Agar method
B = Blotter method

Fig. 2.2: Percentage infection of *Phaseolus vulgaris* Linn. seeds stored in different containers during the storage period.

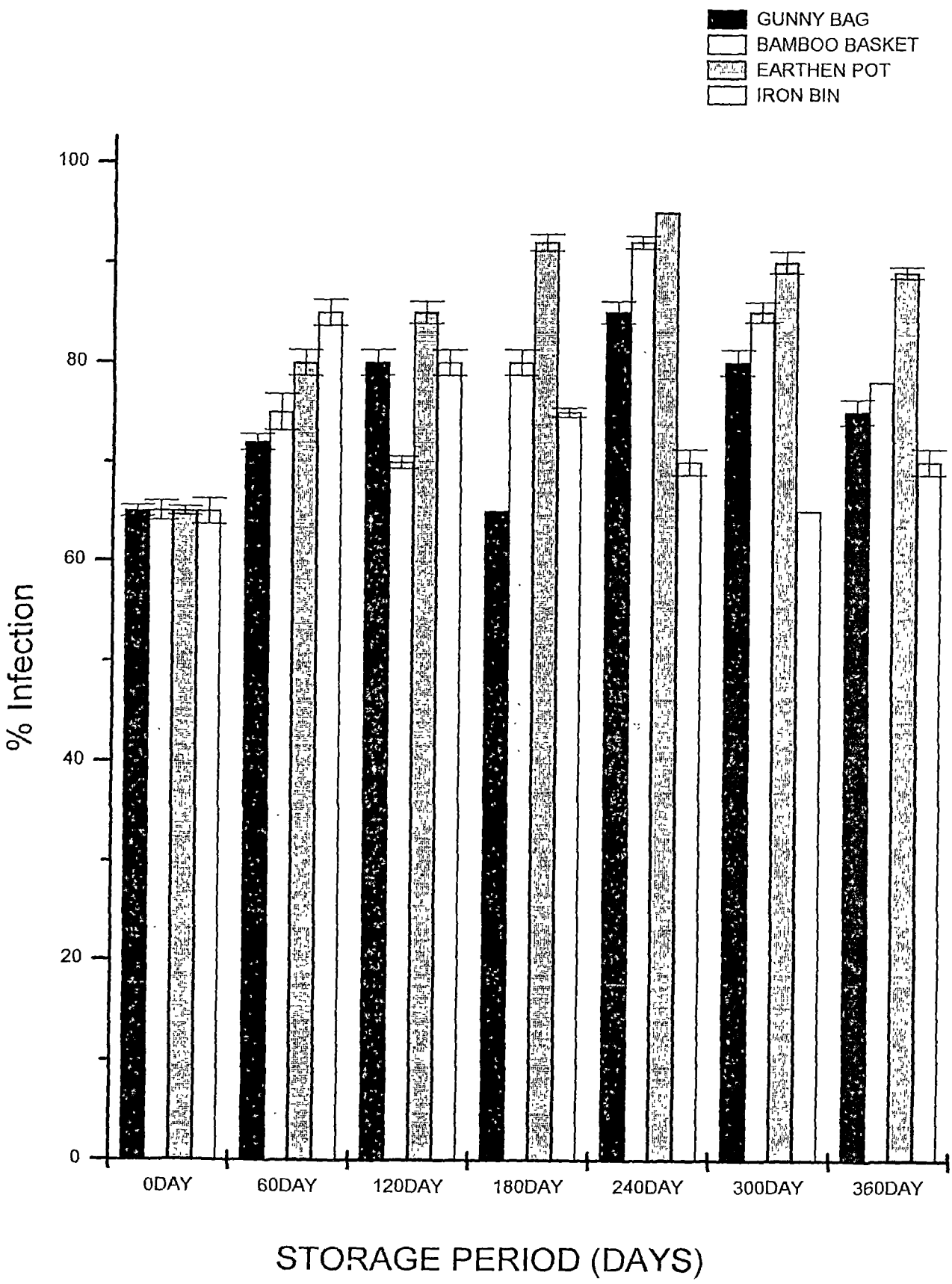

GUNNY BAG
BAMBOO BASKET
EARTHEN POT
IRON BIN
100
80
60
40
20
0
% Infection
0DAY
60DAY
120DAY
180DAY
240DAY
300DAY
360DAY
STORAGE PERIOD (DAYS)

Fig. 2.3: The percentage moisture content of *Phaseolus vulgaris* seeds stored in different containers during the study period.

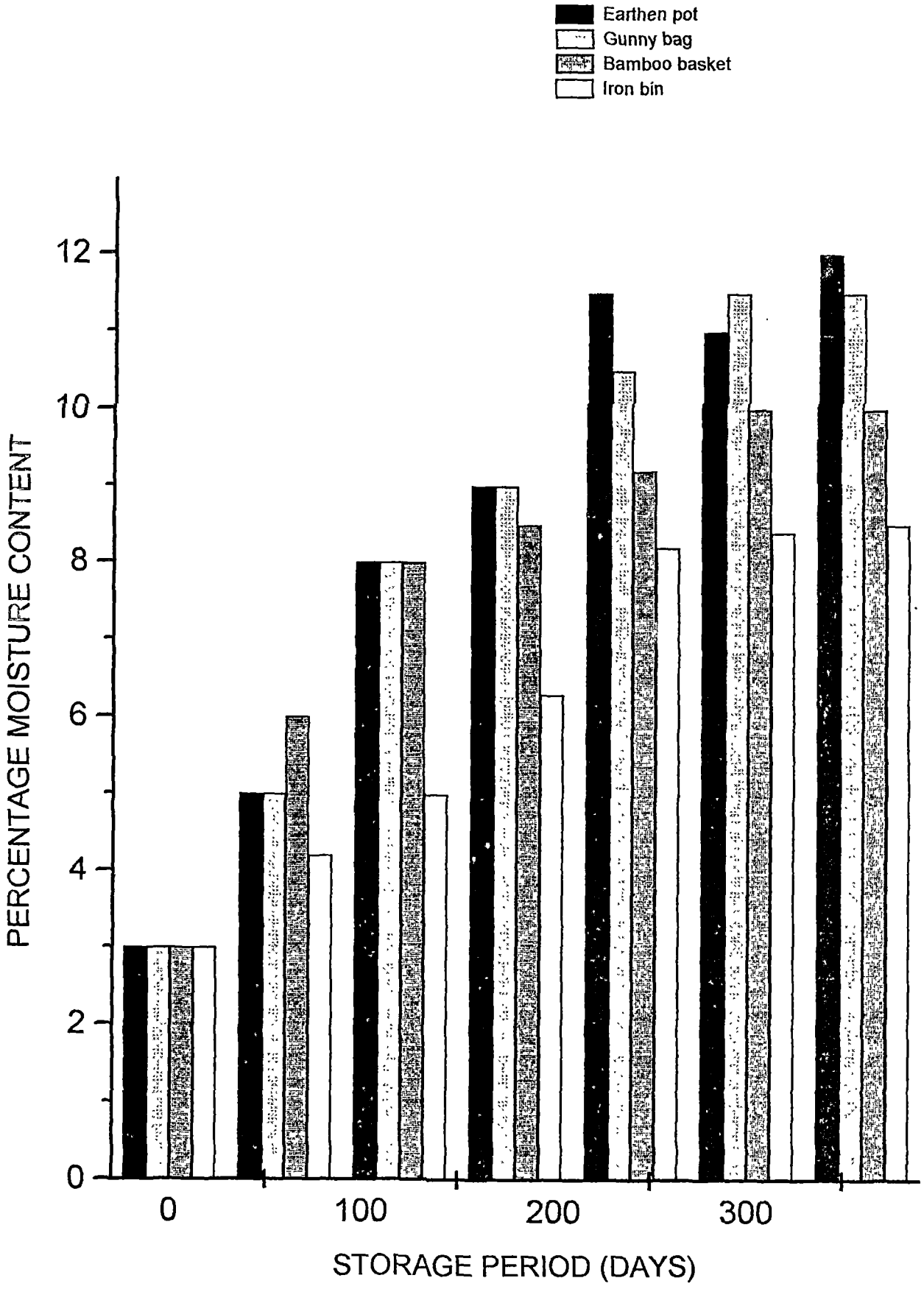

Earthen pot
Gunny bag
Bamboo basket
Iron bin
PERCENTAGE MOISTURE CONTENT
0
2
4
6
8
10
12
0
100
200
300
STORAGE PERIOD (DAYS)

Table 2.4: Germination percentage and mortality percentage of *Phaseolus vulgaris* Linn. seeds stored in different containers.

	GUNNY BAG			BAMBOO BASKET			EARTHEN POT			IRON BIN		
Storage (Days)	Emergence mortality(%)			Emergence mortality(%)			Emergence mortality(%)			Emergence mortality(%)		
	Pre	Post	FS (%)	Pre	Post	FS (%)	Pre	Post	FS (%)	Pre	Post	FS (%)
(0)	13	2	85	13	2	85	13	2	85	13	2	85
(60)	20	6	74 (12.941)	26	3	71 (16.470)	20	6	74 (12.941)	20	4	76 (10.588)
(120)	25	10	65 (23.529)	50	4	46 (45.882)	33	8	59 (30.588)	40	6	54 (36.470)
(180)	30	12	58 (31.764)	55	7	38 (55.294)	54	12	34 (60.00)	45	8	47 (44.705)
(240)	45	15	40 (52.941)	60	8	32 (62.35)	60	14	26 (69.411)	54	10	36 (57.647)
(300)	60	15	25 (70.588)	70	11	19 (77.647)	66	17	17 (80.00)	62	12	26 (69.411)
(360)	75	20	5 (94.112)	74	14	12 (85.882)	75	18	7 (91.764)	68	14	18 (78.823)

The figures in parenthesis indicate the percentage inhibition of germination over the initial germination

FS = Final Stand.

highest percentage of infection at each successive interval of 60 days of storage. At the end of 360 days, the total infection was 89% in earthen pots as compared to 78% in gunny bags and 75% in bamboo baskets. The least infection of 70% was recorded in iron bin container (Fig. 2.2).

The percentage moisture content of *P. vulgaris* seeds in containers like gunny bags, bamboo baskets, earthen pots and iron bins was assessed at an interval of 60 days for a total period of 360 days. It was observed that with the increase in time interval, there was a gradual increase in moisture content Among the containers, it was observed that the seeds in the earthen pots retained 12% moisture at the end of 360 days, in comparison to 11.05% in the case of gunny bags, 10.0% in bamboo baskets and 8.5% in iron bins (Fig. 2.3).

The germination of seeds of *P. vulgaris* Linn. during different periods of storage in different containers, brought to the fore the fact that on the first day, the pre-emergence and the post-emergence mortality were 13% and 2% respectively with the final stand of the crop being 85%. But at each successive interval of time, the pre and post-emergence mortality increased and the same trend was discernible in the case of the final stand. It was further observed that in case of seeds stored in gunny bags, the pre-emergence mortality was 20% after 60 days, which showed an increment to 75% at the end of 360 days. The initial percentage inhibition was 12.94%, which gradually increased to 94.11% at the end. In case of bamboo baskets, the pre and post- emergence mortality was 26% and 3% respectively at the end of 60 days, which rose to 74% and 14% respectively at the end of the study period. Similar trend was observed in the case of earthen pots, where the pre and post -emergence mortality were 20% and 6% respectively after 60 days. At the end of the experiment, the pre and post-emergence accounted for 75% and 18% mortality cases respectively (Table 2.4).

Table 2.5: Analysis of variance (ANOVA) to test the significance of variance of percentage fungal infection in *Phaseolus vulgaris* Linn. seeds stored in different containers.

	STORAGE CONTAINERS (IN PAIRS)	CALCULATED *F* VALUE
1.	Gunny bag and bamboo basket	0.53434(NS)
2.	Gunny bag and earthen pot	0.82609 *
3.	Gunny bag and iron bin	0.17618 (NS)
4.	Bamboo basket and earthen pot	2.01628 (NS)
5.	Bamboo basket and iron bin	1.25942 (NS)
6.	Earthen pot and iron bin	6.61933 *

NS = Non significant

* = Significance at 0.05 probability level.

The storage of seeds in earthen pots had a negative effect on the overall rate of germination. The impact of storage on seed was slightly better in bamboo basket followed by gunny bag. Seeds stored in iron bins exhibited a very low level of mortality. It is apparent from the findings that the germinability of the seeds in all the containers showed a reduction with the increase in storage period. Similar pattern was observed for the final stand (%) in all the cases investigated.

On performing the *F*- test to test the significance of variance of percentage infection in *Phaseolus vulgaris* Linn. seeds stored in different containers, insignificant variation in percentage fungal infection in *Phaseolus vulgaris* seeds was observed between gunny bag and bamboo basket, gunny bag and iron bin, bamboo basket and iron bin and between bamboo basket and earthen pot, while significant variation was observed between gunny bag and earthen pot and between earthen pot and iron bin at 0.05 level of significance (Table 2.5)

3. STUDIES ON THE EFFECT OF CERTAIN SEED-BORNE FUNGI ON THE GERMINATION OF *Phaseolus vulgaris* Linn. SEEDS

The mortality rate, percentage change and the final stand of the crop were obtained from the pot culture experiment using the Meghalaya variety of *P.vulgaris* seeds (Table 3.1). The control set recorded 95% in its final stand, which was the highest value. The lowest value of 78% of final stand was registered in case of seeds infected with *Alternaria alternata.* The seeds infected with *A. alternata* showed a very weak seedling growth, while seeds infected with *Colletotrichum lindemuthianum* showed small leaves, and thin and weak stem growth. In case of Manipur variety, too similar trend was observed (Table 3.2). Least final stand of 80% was recorded in the seeds treated with a culture of *A. alternata, C. lindemuthianum* and

Table 3.1: Effect of fungi on the germination of *Phaseolus vulgaris* Linn. seeds as seen in the pot culture experiment.

MEGHALAYA VARIETY

TEST FUNGI	EMERGENCE MORTALITY (%)		FINAL STAND(%)	CHANGE (%)	REMARK
	Pre	Post			
Alternaria alternata	16	2	78	17	+
Colletotrichum lindemuthianum	10	-	90	5	+++
Fusarium oxysporum	-	15	85	10	++
Pythium intermedium	12	7	79	16	++
Trichoderma viride	11	8	79	16	+
Control	-	5	95	-	+++

Table 3.2: Effect of fungi on the germination of *Phaseolus vulgaris* seeds as seen in the pot culture experiment

MANIPUR VARIETY

TEST FUNGI	EMERGENCE MORTALITY (%)		FINAL STAND(%)	CHANGE (%)	REMARK
	Pre	Post			
Alternaria alternata	18	2	80	-19	++
Colletotrichum lindemuthianum	14	6	80	-19	++
Fusarium oxysporum	8	-	92	-7	+++
Pythium intermedium	-	15	85	-19	++
Trichoderma viride	15	5	80	-14	++
Control	-	1	99	-	+++

+ = Poor
++ = Fair
+++ = Good

Table 3.3: Effect of fungi on the germination of *Phaseolus vulgaris* seeds as seen in the test tube seedling symptom test.

MEGHALAYA VARIETY					
TEST FUNGI	EMERGENCE MORTALITY(%) PRE-	POST-	FINAL STAND(%)	CHANGE(%)	REMARK
Alternaria alternata	-	-	-	100	-
Colletotrichum lindemuthianum	-	-	-	100	-
Fusarium oxysporum	15	07	78	22	++
Pythium intermedium	-	12	88	12	++
Trichoderma viride	14	07	79	21	++
Control	-	-	100	-	+++

Table 3.4: Effect of fungi on the germination of *Phaseolus vulgaris* seeds as seen in test tube seedling symptom test.

MANIPUR VARIETY					
TEST FUNGI	EMERGENCE MORTALITY (%) PRE	POST	FINAL STAND (%)	CHANGE (%)	REMARK
Alternaria alternata	-	-	-	100	-
Colletotrichum lindemuthianum	07	02	91	08	+++
Fusarium oxysporum	-	12	88	11	++
Pythium intermedium	05	02	93	06	+++
Trichoderma viride	-	-	-	100	+
Control	-	01	99	-	+++

+ = Poor
++ =Fair
+++ =Good

Table 3.5:The root-shoot length and dry weight of *Phaseolus vulgaris* seedlings as determined by test tube seedling symptom test.

MEGHALAYA VARIETY			
Test Fungi	Root (cm)	Shoot (cm)	Dry Weight (mg)
Alternaria alternata	-	-	-
Colletotrichum lindemuthianum	-	-	-
Fusarium oxysporum	13(+18.18%)	13((-22.5%)	0.15(-21.05%)
Pythium intermedium	16(+45.45%)	19(+11.76%)	0.10(-47.37%)
Trichoderma viride	01(-90.90%)	1.5(-91.17%)	0.03(-84.21%)
Control	11	17.00	0.19

The figures in parenthesis indicate the percentage change over the control.

Table 3.6: The root-shoot length and dry weight of seedlings as determined by the test tube seedling symptom test.

MANIPUR VARIETY			
Test Fungi	Root (cm)	Shoot (cm)	Dry Weight (mg)
Alternaria alternata	-	-	-
Colletotrichum lindemuthianum	0.5(-12.50%)	01(-93.75%)	0.03(85.00%)
Fusarium oxysporum	4.0(-50.00%)	05(-68.75%)	0.08(60.00%)
Pythium intermedium	-	-	-
Trichoderma viride	12(-50.00%)	16(0%)	0.22(10.0%)
Control	8(-)	16(-)	0.20

The figures in parenthesis indicates percentage change over the control

Plate 1(a): The control and *Fusarium oxysporum* infected seedlings of *Phaseolus vulgaris* in pot culture experiment.

(b): The control and *Pythium intermedium* infected seedlings of *Phaseolus vulgaris* in pot culture experiment

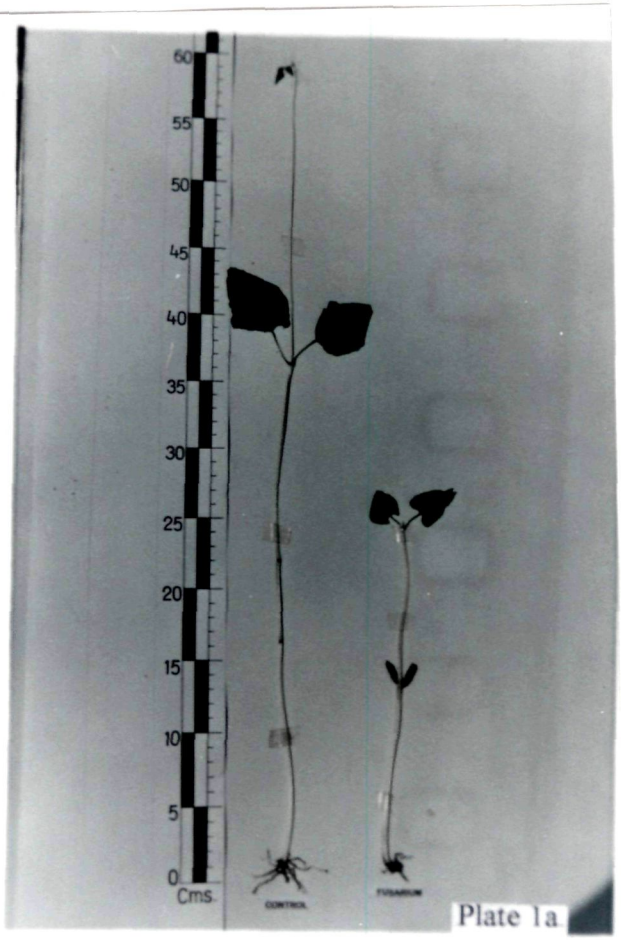


Plate 1a

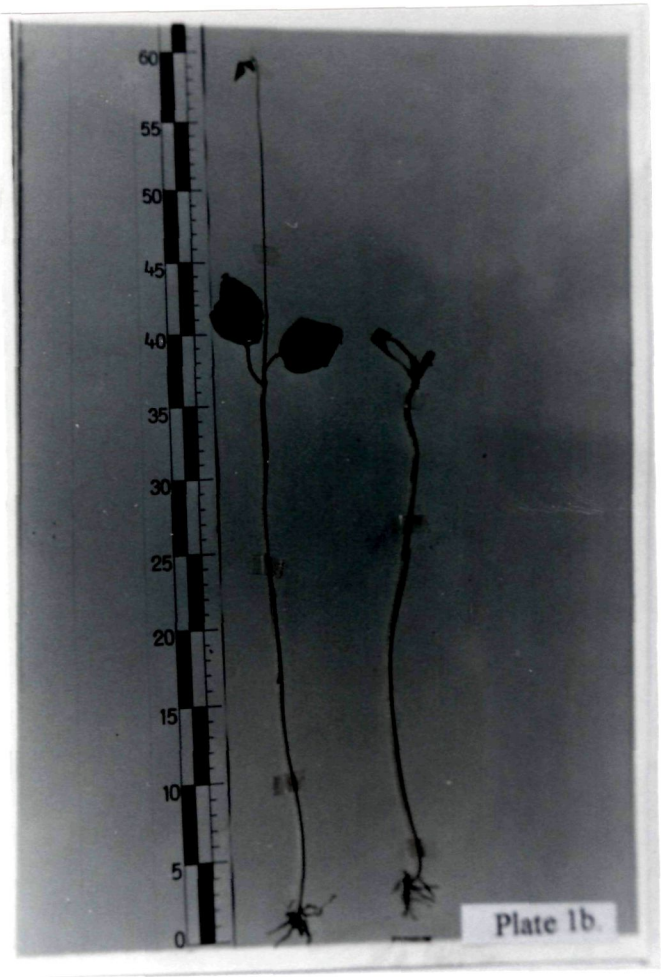


Plate 1b.

Plates 2(a, b, c and d): The control and treated seedlings of *Phaseolus vulgaris* in test tube seedling symptom test.

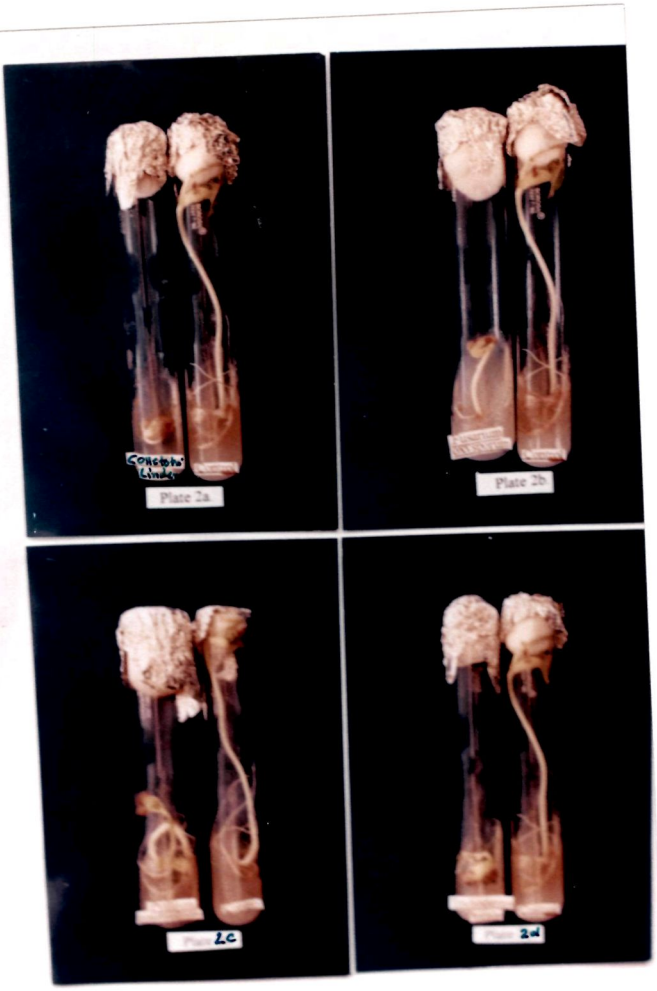


Plate 2a
Plate 2b
Plate 2c
Plate 2d

Trichoderma viride. *A. alternata* had a negative effect on the seedling growth as was seen from the reduction, curling and weakening of the leaves in its presence (Plate1a and b).

The test tube seedling symptom test of seeds of the Meghalaya variety was assessed and it was found that the germination was 100% in the final stand of the crop in case of the control set followed by *Pythium intermedium* infected seedlings with 88%. It was observed that seeds infected with *A. alternata* and *C. lindemuthianum* showed no germination or the emergence of radicals only were seen to sprout. *Fusarium oxysporum* exhibited a stunted growth of the seedlings with poorly developed leaves and the control could exhibit a perfect growth of the seedlings with well developed leaves and stems (Table 3.3) and (Plate 2 a) The same experiment was performed with the seeds of Manipur variety. It was observed that the control recorded a 99% in the final stand of the crop, whereas, *F.oxysporum* infected seeds registered 88% in terms of the final stand of the crop, which turned out to be the lowest value. It was further observed that *A. alternata* had a total inhibitory effect on the seed germination (Table 3.4) and (Plate 2 b).

The root and shoot length of the Meghalaya variety of *P. vulgaris* seedlings infected with the test fungi in the test tube seedling symptom test was studied. It was observed that the seeds infected with *P. intermedium* showed a root and shoot length of 16cm and 19cm respectively. The root-shoot length was also higher than that of the control, which recorded 11cm and 17cm respectively. A noticeable feature observed here is that *A. alternata* and *C. lindemuthianum* did not record any seedling growth. *T. viride* registered the least root and shoot length of 1.5 cm and 1.0 cm respectively. The control recorded a dry weight of 0.19 mg followed by *F. oxysporum* infected seedlings with 0.15mg (Table 3.5).

Table 3.7 The root-shoot length and the dry weight of seedlings as determined by pot culture experiment.

MEGHALAYA VARIETY			
Test Fungi	Root (cm)	Shoot (cm)	Dry Weight (mg)
Alternaria laternata	6.00(+20.00%)	36.00(-33.33%)	1.53(-20.31%)
Colletotrichum lindemuthianum	5.00(0.00%)	43.00(-20.37%)	1.92(0.00%)
Fusarium oxysporum	5.00(0.00%)	25.00(-53.70%)	0.96(-50.00%)
Pythium intermedium	2.00(-60.00%)	36.00(-33.33%)	1.50(-21.87%)
Trichoderma viride	5.00(0.00%)	48.00(-11.11%)	2.33(+21.35%)
Control	5.00	54.00	1.92

The figures in parenthesis indicate the percentage change over the control.

Table 3.8: The shoot-root and dry weight of *Phaseolus vulgaris* seedlings as determined by pot culture experiment.

MANIPUR VARIETY			
Test Fungi	Root (cm)	Shoot (cm)	Dry Weight(mg)
Alternaria laternata	10.5(-12.5%)	47.00(-21.67%)	1.20(-44.19%)
Colletotrichum lindemuthianum	6.0(-50.00%)	40.00(-33.33%)	1.90(-11.63%)
Fusarium oxysporum	5.0(-58.33%)	55.00(-8.33%)	2.39(+11.16%)
Pythium intermedium	0.4(-96.67%)	30.00(-50.00%)	2.10(-2.23%)
Trichoderma viride	8.0(-33.33%)	40.00(-33.33%)	2.92(+35.81%)
Control	12.0	60.00	2.15

The figures in parenthesis indicate the percentage change over the control.

Table.3.9: Analysis of variance (*t*-test) to see the significance of variance between the final stand percentage infested by various test fungi of both Meghalaya and Manipur variety of *Phaseolus vulgaris* Linn. seed in pot culture and test tube Seedling symptom test

EXPERIMENT	DEGREE OF FREEDOM	CALCULATED *t*- VALUE	TABLE VALUE	PROBABILITY LEVEL	SIGNIFICANCE
POT CULTURE	10	0 070973834	4.59	$\leq$0.001	NS
TEST TUBE SEEDLING SYMPTOM TEST	10	0 00603239	4.59	$\leq$0.001	NS

NS = Non significant

The root-shoot length of the Manipur variety is illustrated in table: 3.6. Highest shoot length of 16.0cm was recorded in case of the control set whereas lowest root and shoot length of 0.5cm and 1.0cm were recorded in seedlings infected with *C. lindemuthianum*. *A. alternata* and *P.intermedium* recorded no growth of the seedlings. *T. viride* infected seedlings recorded a dry weight 0.22mg while 0.20mg was recorded in the control set.

The root-shoot length and dry weight of the seedlings in the pot culture experiment of the Meghalaya variety revealed that the control recorded a root length of 5.0cm and a shoot length of 54.0cm, followed by *P. intermedium* and *A. alternata* infected seedlings with 36.0cm as shoot length. Highest root length of 6.0cm was observed in *A. alternata* infected seeds. *P. intermedium* infected seedlings recorded a lowest root length of 2.0cm. *T. viride* infected seedlings on the other hand registered a dry weight of 2.33mg followed by *C. lindemuthianum* and control set with 1.92mg. Least dry weight of 0.96mg was recorded in case of *F.oxysporum* infected seedlings. (Table 3.7).

The root-shoot length and dry weight of the seedlings in the pot culture experiment in case of the Manipur variety was investigated and it was found that the control recorded a root length of 12.0cm and a shoot length of 60.0cm, followed by *F. oxysporum* infected seedlings with 5.0 cm and 55.0cm as the root and shoot length respectively The lowest root and shoot length of 0.4cm and 30.0cm were recorded in case of *P. intermedium* infected seedlings. Dry weight of 2.39mg was recorded in seedlings infected with *F. oxysporum* and 2.92mg in case of *T. viride* infected seedlings (Table 3.8).

The data was processed by analysis of variance (*t*-test) and insignificant variation was observed between the final stand percentage of the seedlings of Meghalaya and Manipur

varieties of *Phaseolus vulgaris* Linn. in both pot culture and test tube seedling symptom experiments (Table 3.9)

4. STUDIES ON THE IMPACT OF SEED LEACHATES ON THE GERMINATION OF SPORES OF CERTAIN SEED-BORNE FUNGI

The germination of fungal spores in seed leachates of the two varieties (i.e.; Meghalaya and Manipur) of *P. vulgaris* Linn. treated at varying temperatures viz.; 15°C, 20°C, 25°C and 30°C was studied. It was observed that in case of the Meghalaya variety, there was no germination at 15°C. Maximum germination was observed at 25°C. The overall healthy spore germination of 80% was noticed in case of *Trichoderma viride* at 25°C, followed by *Pythium intermedium* with 75%. Least spore germination of 40.5% was recorded in case of *Colletotrichum lindemuthianum* (Table 4.1).

In case of the Manipur variety at 15°C, there was a negligible germination of the spores. At a temperature of 25^0C, *P.intermedium* and *T.viride* recorded a germination of 60.0%, followed by *C. lindemuthianum* with 50.5%. Lowest germination of 30.0% was recorded in case of spores of *F.oxysporum* at the same temperature. In both the varieties, the control sets showed a very good germination percentage. It was observed that at 25°C, all the test fungi showed a higher percentage of germination. Furthermore, it was observed that with increase in the temperature from 15°C to 30°C, the percentage germination also increased significantly at each successive stage (Table 4.2).

The data was processed by analysis of variance (ANOVA) and insignificant variation between fungal spore germination percentage in treated seeds and control with increase in temperature in case of both the varieties of *Phaseolus vulgaris* was observed (Table 4.3).

Table 4.1: Effect of seed leachates at different incubation temperature on the spore germination of certain seed-borne fungi.

MEGHALAYA VARIETY	PERCENTAGE SPORE GERMINATION AT DIFFERENT TEMPERATURES							
	15° C		20° C		25° C		30° C	
Fungal Species	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Alternaria alternata	-	12.0	10.0	25.0	35.0	55.0	25.5	55.0
Colletotrichum lindemuthianum	-	20.5	35.0	60.5	40.5	40.5	30.0	50.0
Fusarium oxysporum	-	10.0	35.0	55.5	55.0	65.5	50.0	60.0
Pythium intermedium	-	15.0	40	65.0	75.0	85.0	40.5	70.0
Trichoderma viride	-	30.0	45.0	60.5	80.0	100.00	30.0	80.0

- = Denotes no germination of fungal spores

Table 4.2: Effect of seed leachates at different incubation temperatures on the spore germination of certain seed-borne fungi.

MANIPUR VARIETY	PERCENTAGE SPORE GERMINATION AT DIFFERENT TEMPERATURES							
	15° C		20° C		25° C		30° C	
Fungal species	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Alternaria alternata	-	10 0	20.0	35.0	50.0	60.0	45.0	55.0
Colletotrichum lindemuthianum	-	15.5	30.0	50.0	50.5	65.0	20.0	70.0
Fusarium oxysporum	-	20.0	35.0	45.0	30.0	75.0	50.0	95.0
Pythium intermedium	-	15.0	15.0	40.5	60.0	25.0	80.0	60.5
Trichoderma viride	10.0	40.0	-	30.0	60.0	90.5	65.0	80.5

- = Denotes no germination of fungal spores.

Table 4.3: *F* test done to test the significance of variance between fungal spore germination percentage in treated and untreated seeds of two varieties of *Phaseolus vulgaris* Linn. seeds with increase in temperature.

VARIETIES	DEGREE OF FREEDOM	CALCULATED 't' VALUE	TABULATED 'T' VALUE	PROBABILITY LEVEL	SIGNIFICANCE
MEGHALAYA	6	0 036706208	5.96	≤ 0.001	NS
MANIPUR	6	0.062645958	5.96	≤ 0.001	NS

NS = Non significant.

5. STUDIES OF THE IMPACT OF CERTAIN SEED-BORNE FUNGI ON THE DECOMPOSITION OF THE SEED

Figs. 5.1A and B show that at the commencement of the experiment, the total protein content of *Phaseolus vulgaris* was twenty eight percent and twenty six percent in Meghalaya and Manipur variety respectively. With the increase in time interval, the protein content depleted in case of seeds infected with all the four test fungi. The Meghalaya variety, was adversely effected by *Alternaria alternata* and at the end of 180 days, the protein content was only 7.5%. On the other, hand *Colletotrichum lindemuthianum* and *Trichoderma viride* had lesser effect on protein depletion as compared to other fungi. Similar condition was noticed in the seeds of Manipur variety wherein, *Colletotrichum lindemuthianum* and *T. viride* registered 12.5% and 14.0% of protein respectively at the end of the study period.

Figs. 5.2 A and B depict the change in sugar content of Meghalaya and Manipur variety affected by different fungi. In the seeds of Meghalaya variety the total sugar content was 4.0 mg/g at the start of the experiment. At each successive interval of time, the sugar content of the seeds increased significantly. However there was not much variation in the level of increase of sugar in case of seeds infected by all the fungi. In the Manipur variety, similar trend was observed wherein, all the test fungi showed an increment in the sugar content of seeds at the end of 180 days.

Figs. 5.3 A and B shows the starch content of both the varieties of *P.vulgaris* seeds infected with the test fungi. There was a gradual reduction in the starch content in presence of the five test fungi in case of both the varieties. In the Meghalaya variety, maximum decrease in

Fig. 5.1 (A): The percentage protein content (%) of the *Phaseolus vulgaris* Linn. seeds of the Meghalaya variety during the storage period of six months.

(B): The percentage protein content (%) of the *Phaseolus vulgaris* Linn. seeds of the Manipur variety during the storage period of six months.

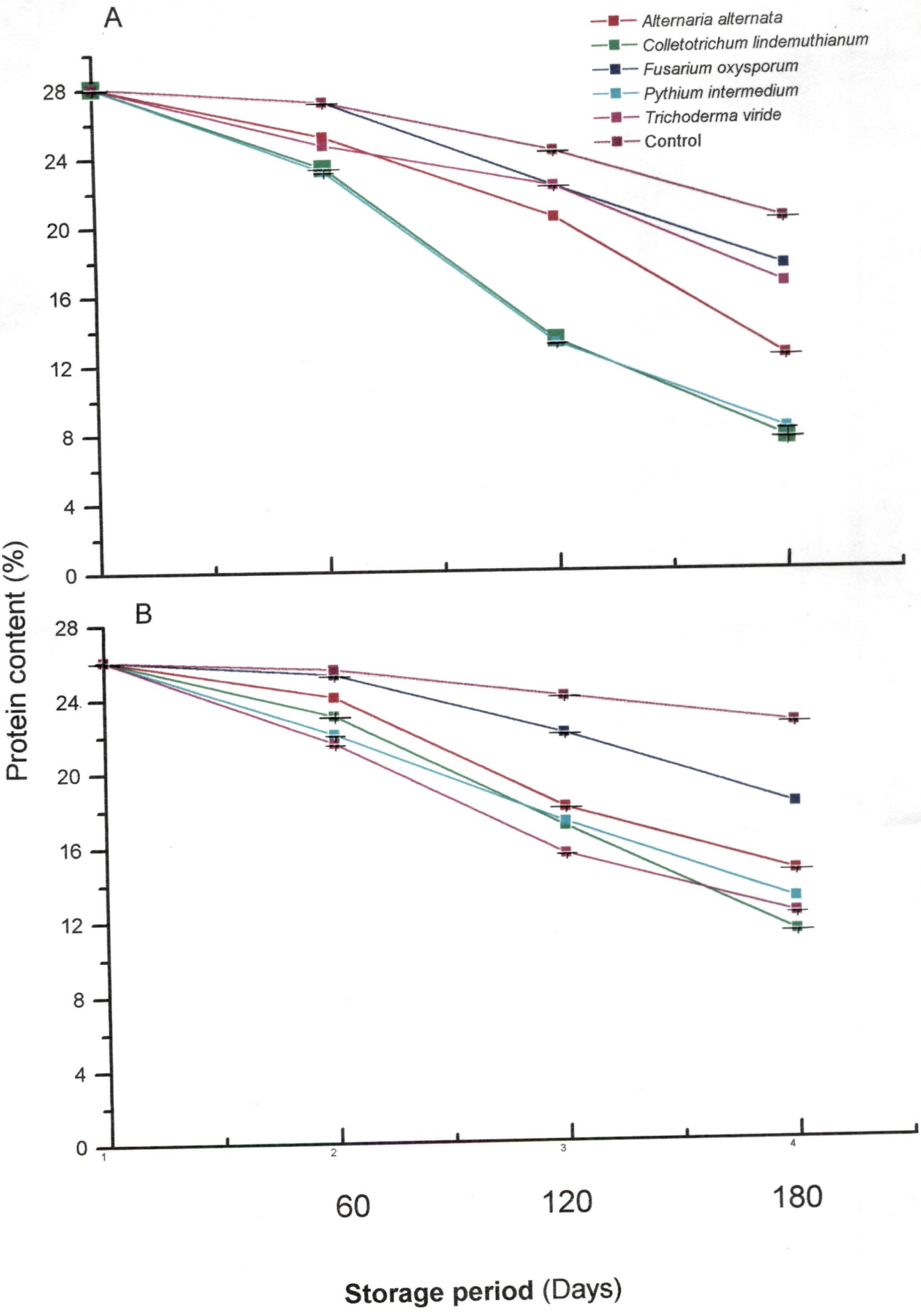

A
B
Alternaria alternata
Colletotrichum lindemuthianum
Fusarium oxysporum
Pythium intermedium
Trichoderma viride
Control
Protein content (%)
0
4
8
12
16
20
24
28
1
2
3
4
60
120
180
Storage period (Days)

Fig. 5.2 (A): The sugar content (mg/g) of the *Phaseolus vulgaris* Linn. seeds of the Meghalaya variety during the storage period of six months.

(B): The sugar content (mg/g) of the *Phaseolus vulgaris* Linn. seeds of the Manipur variety during the storage period of six months.

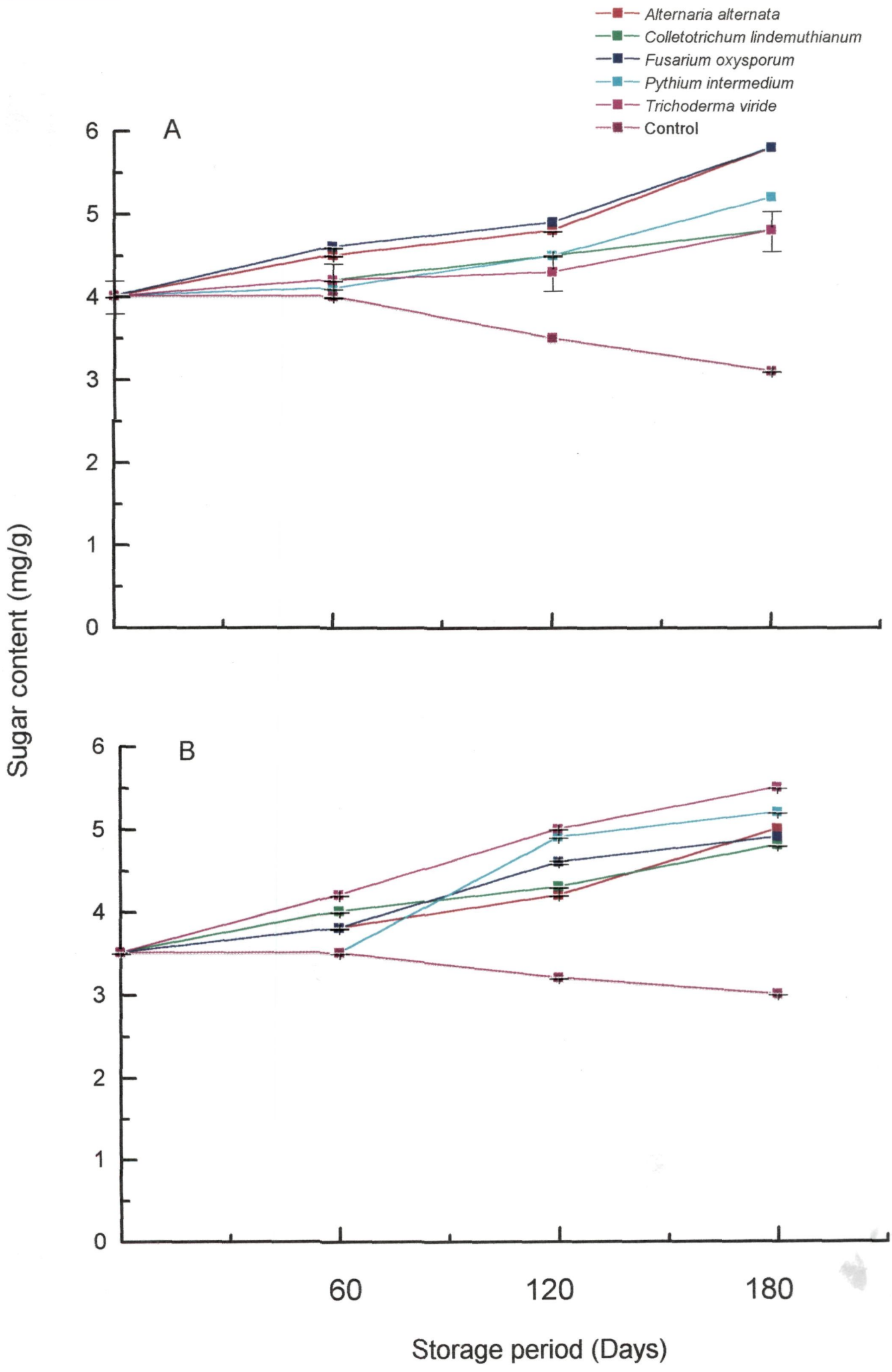

Alternaria alternata
Colletotrichum lindemuthianum
Fusarium oxysporum
Pythium intermedium
Trichoderma viride
Control
A
B
Sugar content (mg/g)
0
1
2
3
4
5
6
60
120
180
Storage period (Days)

Fig. 5.3 (A): The starch content (mg/g) of the *Phaseolus* vulgaris Linn. seeds of the Meghalaya variety during the storage period of six months.

(B): The starch content (mg/g) the *Phaseolus vulgaris* Linn. seeds of the Manipur variety during the storage period of six months.

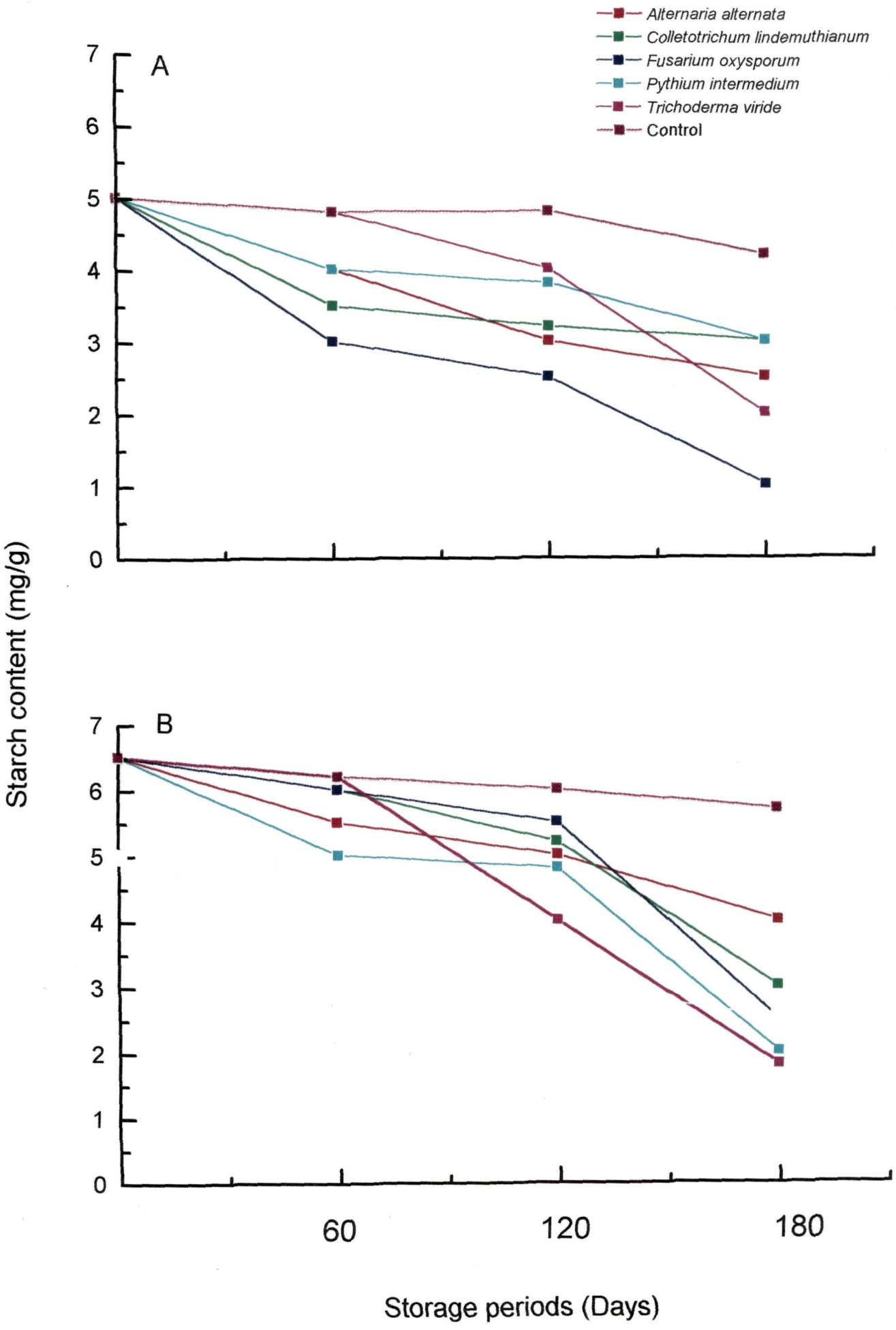

Alternaria alternata
Colletotrichum lindemuthianum
Fusarium oxysporum
Pythium intermedium
Trichoderma viride
Control
A
B
Starch content (mg/g)
0
1
2
3
4
5
6
7
60
120
180
Storage periods (Days)

Fig. 5.4 (A): The fatty acid content (mg/g) of the *Phaseolus vulgaris* Linn. seeds of the Meghalaya variety during the storage period of six months.

(B): The fatty acid content (mg/g) of the *Phaseolus vulgaris* Linn. seeds of the Manipur variety during the storage period of six months.

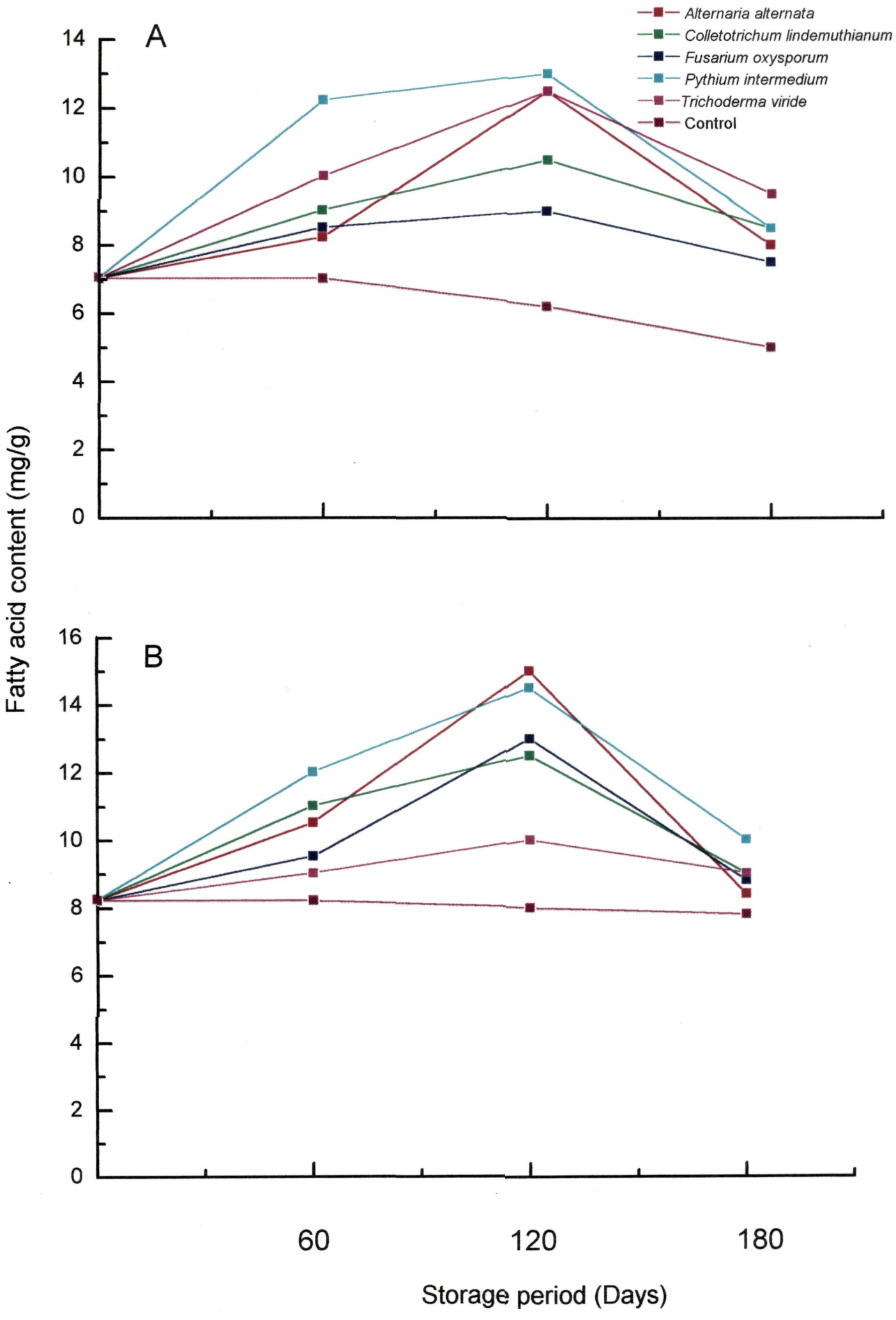

A
B
Alternaria alternata
Colletotrichum lindemuthianum
Fusarium oxysporum
Pythium intermedium
Trichoderma viride
Control
Fatty acid content (mg/g)
0
2
4
6
8
10
12
14
16
60
120
180
Storage period (Days)

Table- 5.5: The changes in the reducing and non-reducing sugar content of the *Phaseolus vulgaris* Linn. seeds during periods

MEGHALAYA VARIETY

FUNGI	STORAGE PERIOD (Days) 0		60		120		180	
	R	NR	R	NR	R	NR	R	NR
Alternaria alternata	2.2	2.3	2.8	2.0	3.4	1.3	4.0	1.0
Colletotrichum lindemuthianum	2.2	2.3	3.5	1.5	3.0	2.2	3.5	1.30
Fusarium oxysporum	2.2	2.3	3.0	1.7	2.50	2.10	3.0	1.60
Pythium intermedium	2.2	2.3	3.2	2.1	2.75	2.25	4.0	1.0
Trichoderma viride	2.2	2.3	3.5	2.0	2.90	2.10	3.2	2.0

Table 5.6: The changes in the reducing and non-reducing sugar content of the *Phaseolus vulgaris* Linn. seeds during storage

MANIPUR VARIETY

FUNGI	Storage period (Days) 0		60		120		180	
	R	NR	R	NR	R	NR	R	NR
Alternaria alternata	1.5	2.3	2.0	2.0	4.7	2.0	5.4	1.2
Colletotrichum lindemuthianum	1.5	2.3	2.0	2.0	3.0	0.50	3.20	0
Fusarium oxysporum	1.5	2.3	1.90	2.0	3.0	1.0	2.5	1.0
Pythium intermedium	1.5	2.3	2.3	1.90	3.2	1.2	3.0	0.80
Trichoderma viride	1.5	2.3	2.0	2.5	2.5	1.0	3.5	0.30

R = Reducing Sugar
NR = Non-reducing sugar

Table 5.7: Analysis of variance of starch, protein, sugar and fatty acid content in both the varieties of *Phaseolus vulgaris* seeds.

	Degree of freedom	Calculated value	Tabulated value	Probability	Significance
Starch					
Meghalaya variety	6	-0.42132681	5.96	≤.001	NS
Manipur variety	6	1.357155331	5.97	≤.001	NS
Protein					
Meghalaya variety	6	0.610811389	5.97	≤.001	NS
Manipur variety	6	2.575239593	2.45	≤0.05	S
Fatty acid					
Meghalaya variety	6	1.905859385	5.96	≤.001	NS
Manipur variety	6	0.14107387	5.96	≤.001	NS
Sugar					
Meghalaya variety	6	2.474208205	2.45	0.05	S
Manipur variety	6	3.76148412	2.45	0.05	S
			3.71	0.01	S

NS Denotes insignificance
S Denotes significance

starch content was noticed at the end of 180 days. *F. oxysporum* had the least effect on the level of change in the starch content.At each successive interval, the starch content decreased significantly.

From the Figs. 5.4 A and B, it is noticed that at the start of the experiment, the total fatty acid content was 7.0 mg/g in the Meghalaya variety of *P. vulgaris* seed which showed a marginal increase at the end of 60 days, whereas at the end of 120 days, the fatty acid increased significantly. Interestingly, at the end of 180 days, the fatty acid reduced appreciably. In the Meghalaya variety, *T. viride* infected seeds showed an increase in the fatty acid content to 9.5mg/ml at the end of 180 days as compared to *F. oxysporum* infected seeds which showed an increase of 1.2mg/g. In the Manipur variety, *F. oxysporum* infected seedlings showed a fatty acid content of 19.0mg/g. *P.intermedium* infected seedlings showed a marginal increase in the fatty acid content which registered 17.0 mg/g at the end of the experiment.

Tables 5.5 and 5.6 depict the changes in the reducing and non-reducing sugar content of the *P. vulgaris* Linn. seeds during different periods of storage in both the varieties. It was observed that, with the increase in storage period, the reducing sugars increased with a corresponding fall in non-reducing sugars in both the varieties of the bean seeds infected with all the five test fungi.

Analysis of variance (*t*-test) showed significant change in protein content (at 0.05 probability level) and sugar content (at 0.05 and 0.01 probability levels), but insignificant change in starch and fatty acid content was observed with increase in storage period in *P. vulgaris* seeds infested with the test fungi (Table 5.7).

6. STUDIES ON THE EFFECT OF CERTAIN AGROCHEMICALS ON THE SEED-BORNE FUNGI

In the study on the effect of agro-chemicals on seed-borne pathogens such as *Alternata alternata*, *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, *Pythium intermedium* and *Trichoderma viride*, the efficacy of four fungicides viz. Dithane M-45, Blitox-50, Topsin and Indofil were tested. Figs. 6.1 to 6.4 shows the spore germination of the test fungi at different concentration of the aforesaid fungicides. Maximum mycelial growth was observed in the set treated with the lowest concentration of 0.005% of the fungicide Dithane M-45 However, at 0.1% concentration, the growth of *P. intermedium* and *T. viride* were controlled. Again at 0.25%, concentration *C. lindemuthianum* and *F. oxysporum* were controlled. With the sole exception of *A. alternata*, the spore germination of all the fungal species was controlled at 0.25% concentration of the fungicide (Fig. 6.1A).

The efficacy of Blitox–50 was tested on the five test fungi. It was noticed that unlike the fungicide Dithane M-45, where most of the fungi could be controlled at 0.1% concentration, with the exception of *A. alternata* and *C. lindemuthianum* the germination of spores in this case was controlled at 0.25 % and 0.5% concentration of the fungicide respectively (Fig. 6.1B)

The efficacy of fungicide Topsin was screened and it was noticed that most of the test fungi were controlled at 0.25%, with the exception of *A. alternata*, which required 0.5% of the fungicide for its control. At 0.5% of the concentration of the fungicide, no germination was registered (Fig: 6.2A)

The efficacy of Indofil was tested and it was observed that most of the test fungi could be controlled at 0.25% concentration. However, the percentage spore germination of *F.*

Fig: 6.1 (A): The efficacy of fungicide, Dithane M-45 on the germination of spores of seed-borne fungi.

(B): The efficacy of fungicide, Blitox-50 on the germination of spores of seed-borne fungi.

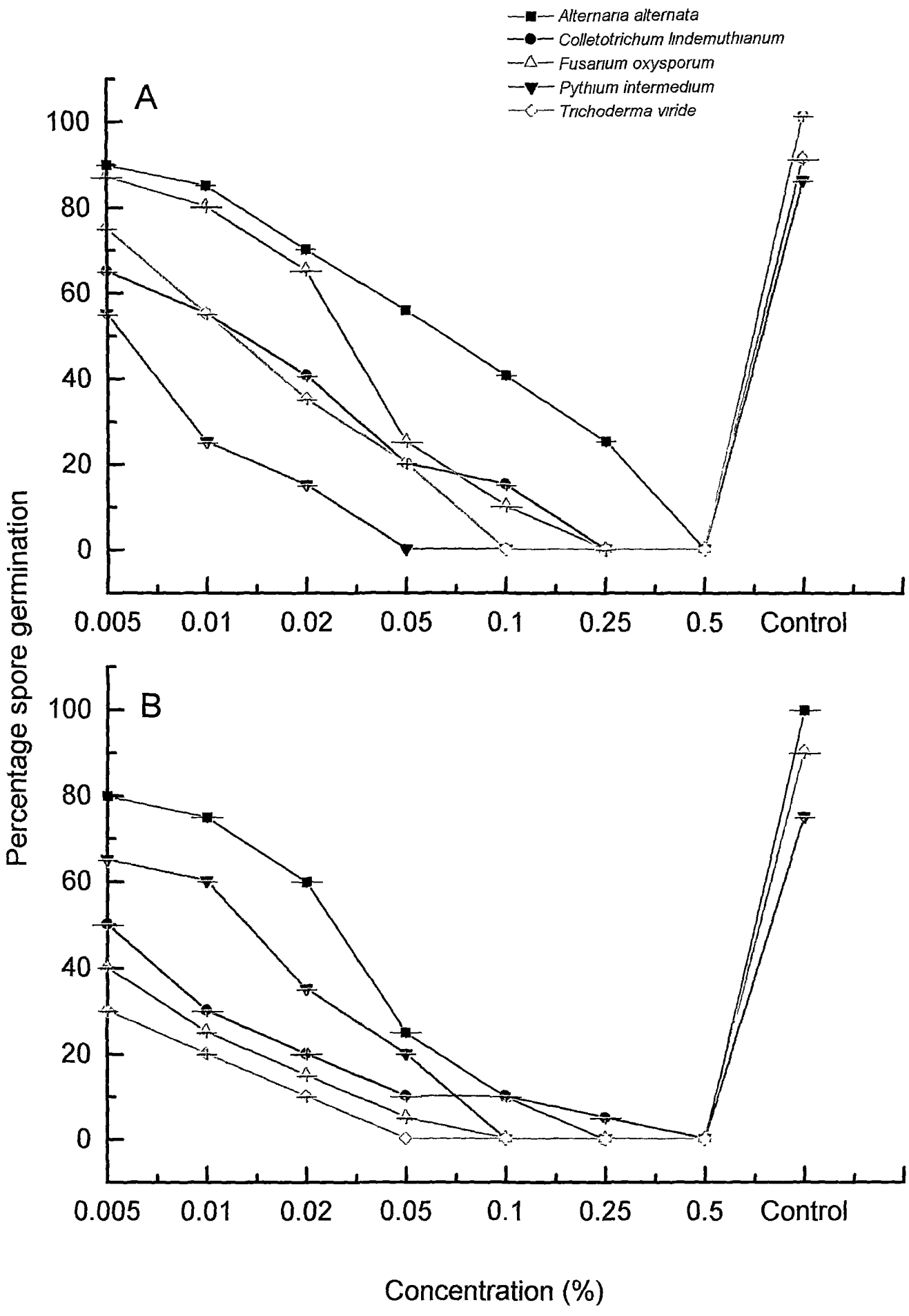

Alternaria alternata
Colletotrichum lindemuthianum
Fusarium oxysporum
Pythium intermedium
Trichoderma viride
A
B
100
80
60
40
20
0
0.005
0.01
0.02
0.05
0.1
0.25
0.5
Control
Percentage spore germination
Concentration (%)

Fig. 6.2 (A): The efficacy of fungicide, Topsin on the germination of spores of seed-borne fungi.

(B): The efficacy of fungicide, Indofil on the germination of spores of seed-borne fungi.

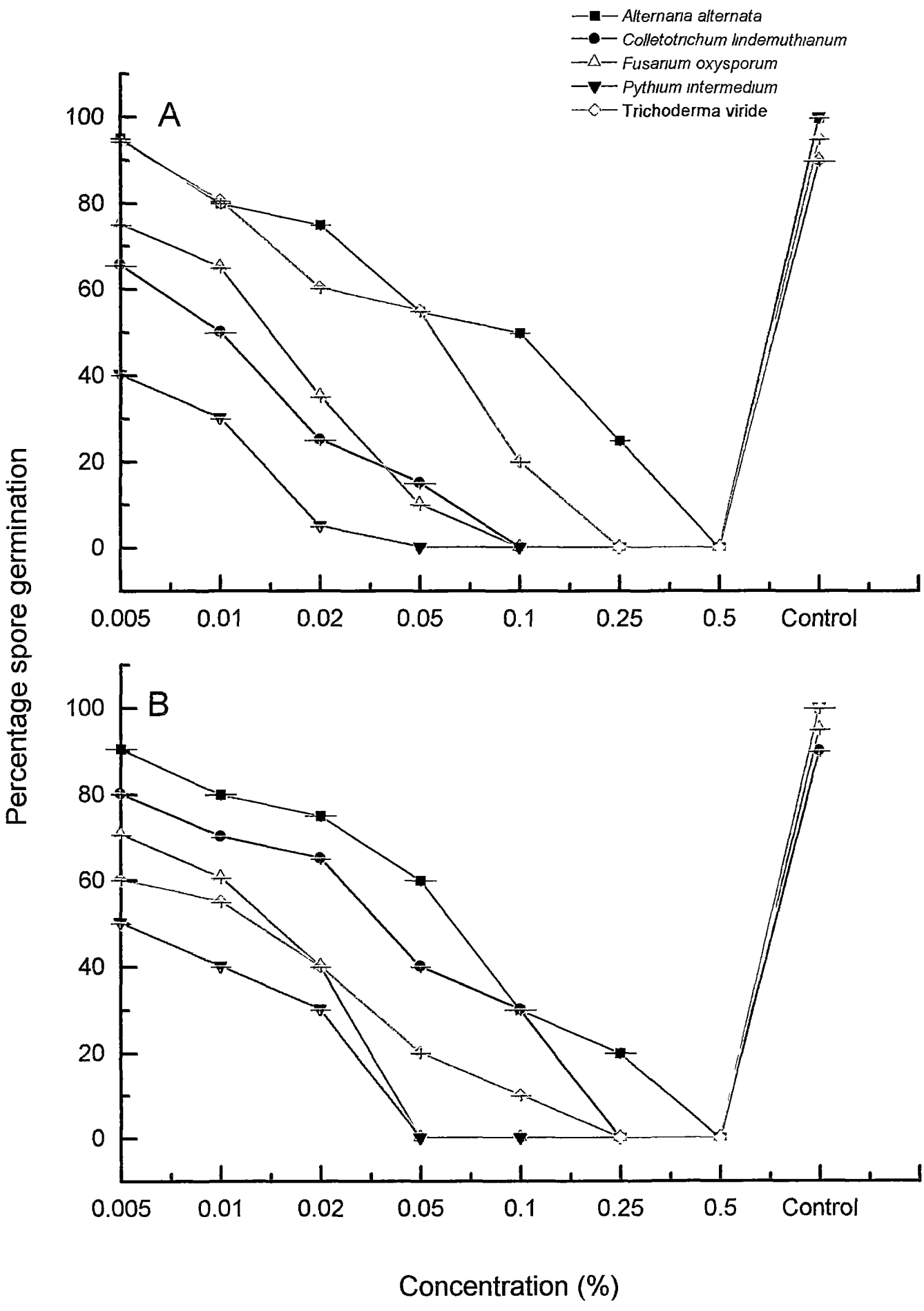

Alternaria alternata
Colletotrichum lindemuthianum
Fusarium oxysporum
Pythium intermedium
Trichoderma viride
A
B
100
80
60
40
20
0
0.005
0.01
0.02
0.05
0.1
0.25
0.5
Control
Percentage spore germination
Concentration (%)

Table 6.3: The mycelial growth of certain seed-borne fungi in response to the fungicide Dithane M-45.

	COLONY DIAMETER (cm) OF FUNGAL SPECIES IN DIFFERENT CONCENTRATION (%)							
FUNGI	0.005	0.01	0.02	0.05	0.1	0.25	0.5	CONTROL
Alternaria alternata	4.3	4.0	3.2	2.1	1.0	-	-	8.0
	(46.25)	(50)	(60)	(73.75)	(87.5)			
Colletotrichum	5.2	2.0	2.16	1.0	0.50	0.66	-	7.2
lindemuthianum	(27.77)	(72.22)	(70)	(86.11)	(93.05)	(90.83)		
Fusarium oxysporum	3.5	3.3	3.0	3.0	2.0	1.0	1.0	9.8
	(64.28)	(66.32)	(69.38)	(69.38)	(79.59)	(89.79)	(89.7)	
Pythium intermedium	7.3	3.5	2.16	0.50	5.0	-	-	8.4
	(13.09)	(58.33)	(70)	(40.81)	(40.81)			
Trichoderma viride	5.8	5.6	5.3	4.8	3.0	2.5	-	9.8
	(40.81)	(42.58)	(45.91)	(51.02)	(69.8)	(74.48)		

Table 6.4: The mycelial growth of certain seed-borne fungi in response to the fungicide Blitox-50

	COLONY DIAMETER (cm) OF FUNGAL SPECIES IN DIFFERENT CONCENTRATION (%)							
FUNGI	.005	.01	0.02	0.05	0.1	0.25	0.5	CONTROL
Alternaria alternata	4.3	4.0	3.2	3.2	2.1	1.0	-	9.0
	(52.22)	(55.5)	(64.44)	(64.44)	(76.66)	(88.88)		
Colletotrichum	5.3	3.6	1.0	-	-	-	-	10.0
lindemuthianum	(45.0)	(64.0)	(90.70)					
Fusarium oxysporum	-	-	-	-	-	-	-	8.0
Pythium intermedium	6.5	5.8	5.3	-	-	-	-	9.8
	(33.67)	(40.81)	(45.91)					
Trichoderma viride	8.1	5.3	1.3	1.0	1.0	.50	-	9.8
	(17.34)	(45.91)	(86.73)	(89.80)	(89.80)	(44.40)		

The figure in parenthesis indicates percentage change over the control
- = Denotes no colony growth

Table 6.5: The mycelial growth of certain seed-borne fungi in response to the fungicide of Topsin.

COLONY DIAMETER (cm) OF FUNGAL SPECIES IN DIFFERENT CONCENTRATION (%)								
FUNGI	0.005	0.01	0.02	0.05	0.1	0.25	0.5	CONTROL
Alternaria alternata	2.0 (73.3)	1.5 (80.0)	1.5 (80.0)	0.3 (96.0)	-	-	-	7.5
Colletotrichum lindemuthianum	-	-	-	-	-	-	-	6.5
Fusarium oxysporum	3.2 (64.4)	1.5 (83.3)	2.16 (76.0)	1.0 (88.8)	-	-	-	9.0
Pythium intermedium	7.4 (11.9)	3.0 (64.2)	-	-	-	-	-	8.4
Trichoderma viride	-	-	-	-	-	-	-	7.8

Table 6.6: The mycelial growth of certain seed-borne fungi in response to the fungicide of Indofil.

COLONY DIAMETER (cm) OF FUNGAL SPECIES IN DIFFERENT CONCENTRATION(%)								
FUNGI	0.005	0.01	0.02	0.05	0.1	0.25	0.5	CONTROL
Alternaria alternata	5.5 (26.66)	5.0 (33.33)	4.0 (46.6)	2.0 (73.32)	-	-	-	7.5
Colletotrichum lindemuthianum	6.5 (10.81)	6.0 (25.0)	3.2 (60.0)	2.0 (75.0)	-	-	-	8.0
Fusarium oxysporum	3.2 (64.4)	3.0 (66.56)	2.0 (77.77)	-	-	-	-	9.0
Pythium intermedium	4.5 (46.42)	4.0 (52.38)	4.0 (52.38)	2.2 (73.80)	-	-	-	8.4
Trichoderma viride	5.2 (35.0)	5.2 (35.0)	5.0 (37.5)	2.5 (68.75)	-	-	-	8.0

* The figures in parenthesis indicate the percentage change over the control.

- = Denotes no colony growth.

Plates 3(a, b and c): The effect of different concentrations of the fungicide Topsin on the mycelial growth of *Pythium intermedium.*

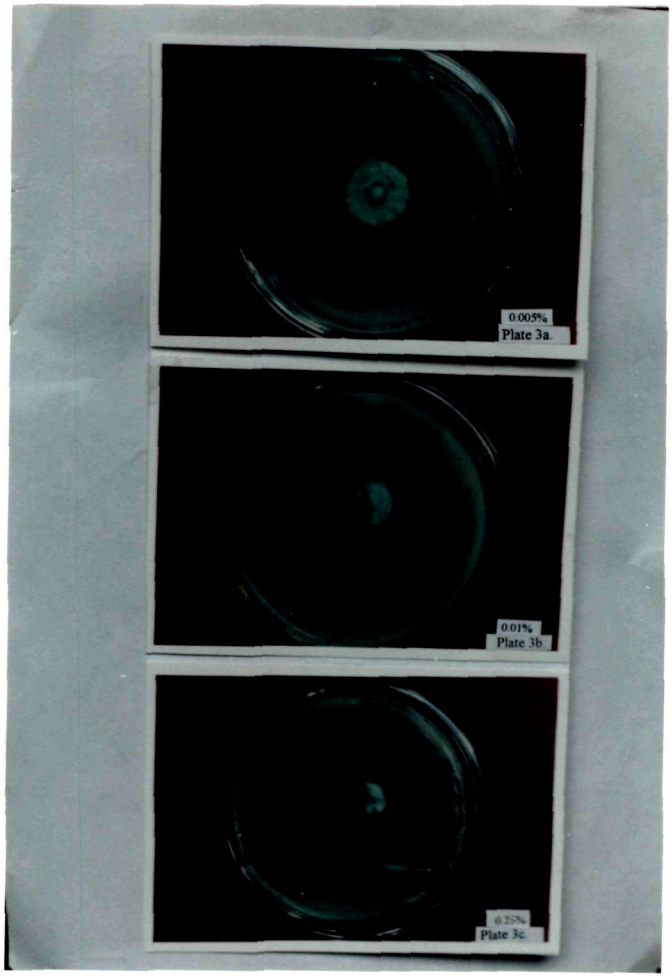
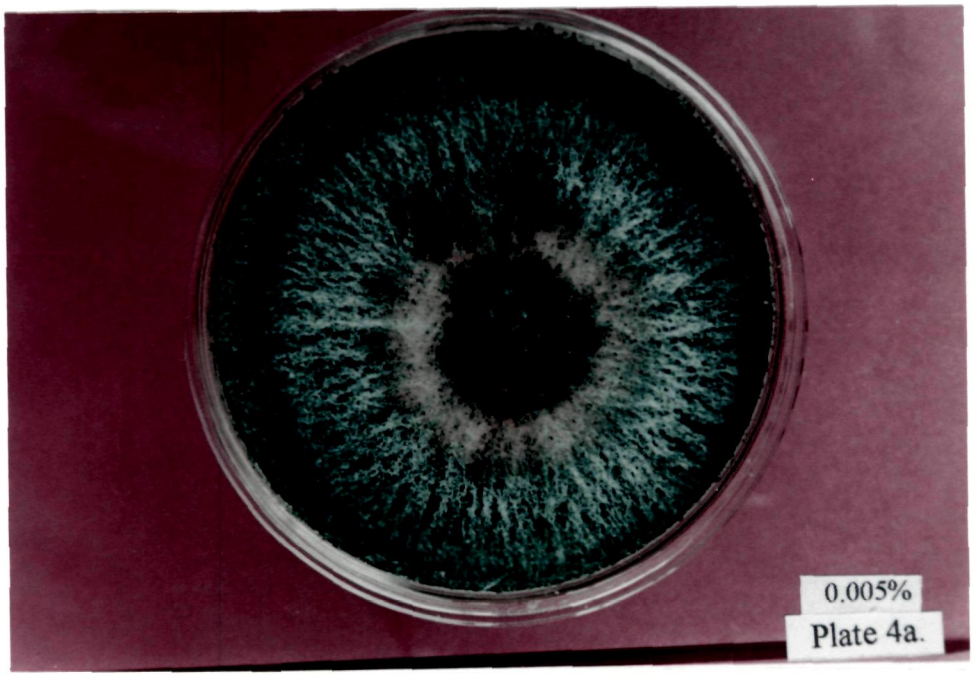
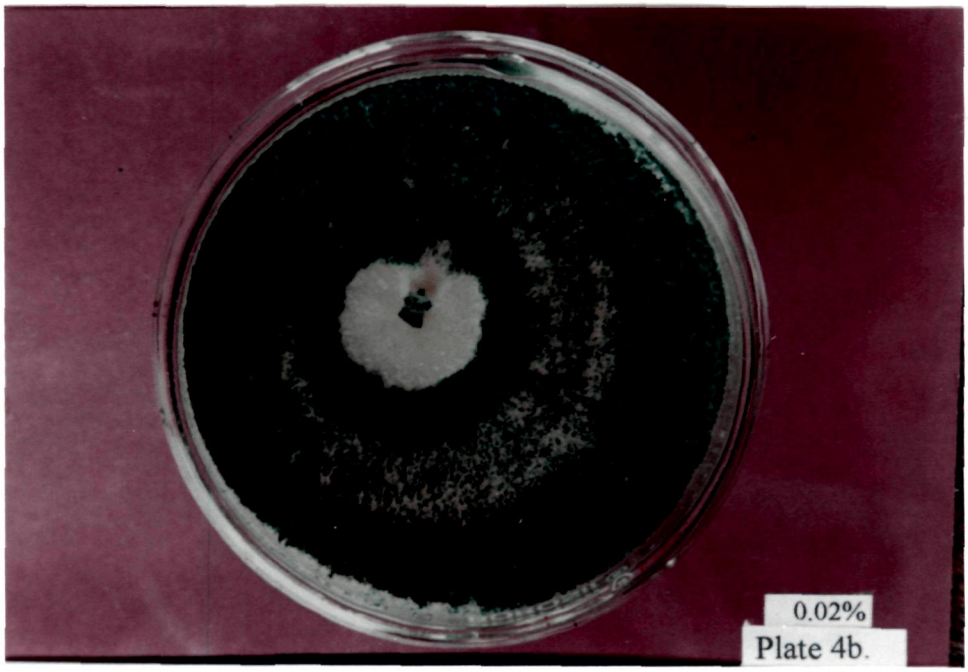

0.005%
Plate 3a.
0.01%
Plate 3b
0.25%
Plate 3c

Plate 4(a, b and c): The effect of different concentrations of the fungicide Blitox-50 on the mycelial growth of *Trichoderma viride*.


0.005%
Plate 4a.


0.02%
Plate 4b.

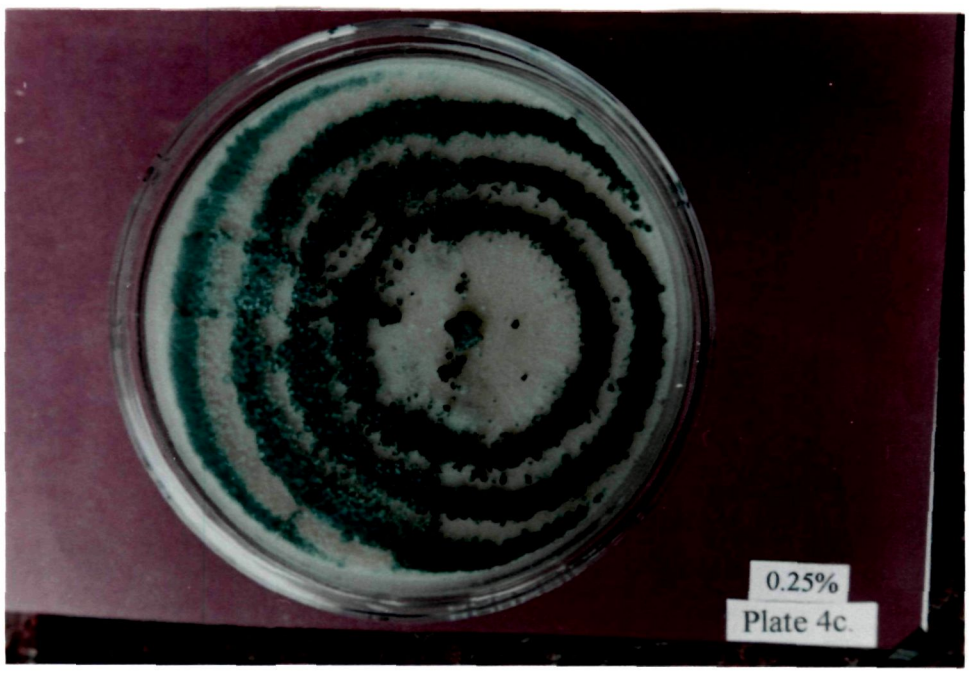
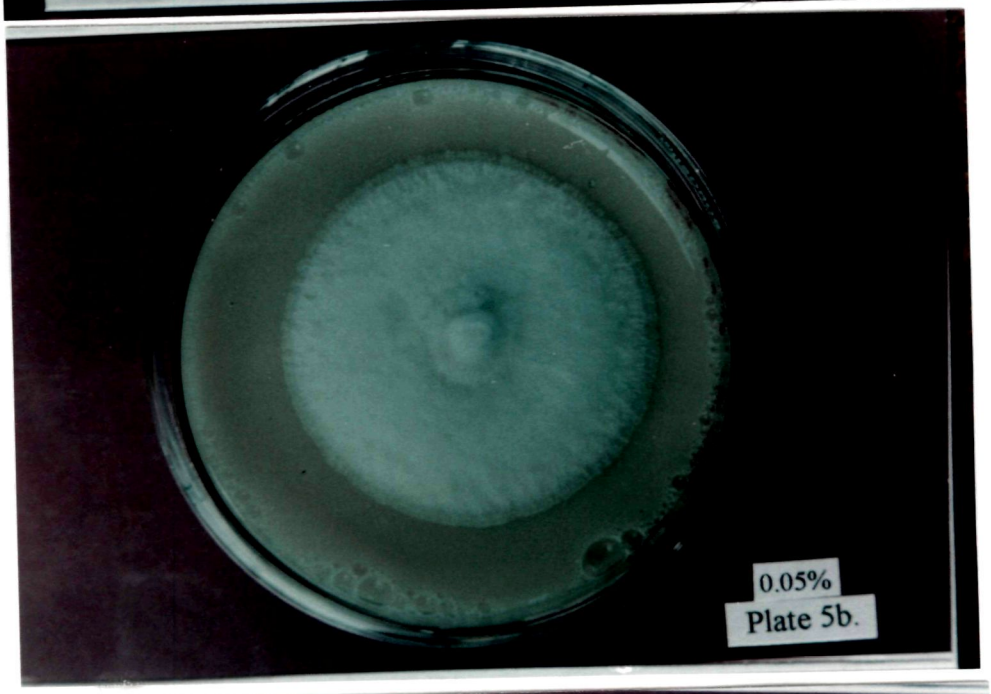
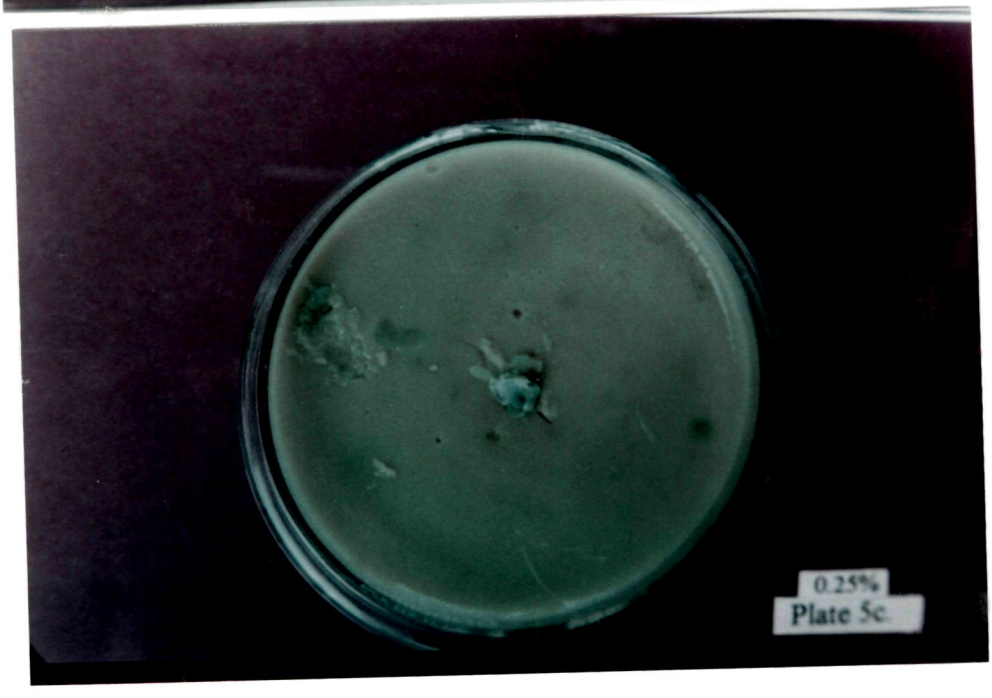

0.25%
Plate 4c.

Plate 5(a, b and c): The effect of different concentration of the fungicide Dithane M-45 on the mycelial growth of *Pythium intermedium*.


0.005%
Plate 5a.


0.05%
Plate 5b.


0.25%
Plate 5c.

oxysporum and *P. intermedium* were controlled at 0.05% concentration of the fungicide (Fig. 6.2B)

The mycelial growth of the five test fungi was assessed in the poisoned media using different fungicides at different concentrations (Tables 6.3-6.6). In the table 6.3, the mycelial growth of the fungi was recorded in Dithane M-45 poisoned fungal media. It was observed that the mycelial growth of all the test fungi were controlled at 0.5% concentration. It was also noticed that with a progressive increase in the concentration of the fungicide, the colony diameter showed a gradual decrease in its size (Plates 5a, 5b and 5c)

Table 6.4 shows the effectiveness of Blitox–50 on the test fungi. It was observed that it had a marked effect on fungi at low concentration. It had a very positive effect on *F. oxysporum*, where the fungus was controlled 0.005 % concentration. With the exception of *T. viride* and *C. lindemuthianum*, which were controlled at 0.25%, concentration of the fungicide, the rest of the fungal species were controlled at 0.05 % concentration (Plates 4a,4b and 4c)

The efficacy of fungicide Topsin on the mycelial growth of all the test fungi were tested and it was found that with the exception of *F. oxysporum* which was controlled at 0.05% concentration, the mycelial growth of all the other test fungi were controlled at 0.01% of the concentration (Table 6.5) and (Plates 3a, 3b and 3c).

The effectiveness of fungicide Indofil on the five test fungi revealed that it was not as effective as Bliotox – 50 or Topsin. The colony growth of most of the fungi was controlled at 0.1% with the exception of *F. oxysporum* which was controlled at 0.5 % concentration (Table 6.6).

Table 7.1: The BGYF test and the amount of aflatoxins present.

VARIETIES	BGYF	AFLATOXINS			AMOUNT OF AFLATOXINS mg/ml		
	B_1	B_2	G_1	G_2	B_1	B_2	G_1
1.MEGHALAYA	+	+	+	-	1.63	17.84	1.75
2 MANIPUR	+	+	-	-	0.836	20.2	-

+ Denotes present
- Denote absent

Table 7.2: The Wave length, molecular weight and molar co efficient extinction of aflatoxins present.

AFLATOXIN	WAVE LENGTH (nm)	MOL.WEIGHT (Dalton)	MOLAR CO-EFFICENT EXTINCTION
B_1	360	312	22,000
B_2	362	314	23,000
G_1	360	328	18,700
G_2	362	330	21,000

7. SCREENING OF SEEDS FOR AFLATOXINS

The presence of naturally occurring aflatoxins in varying amounts in bean seeds of both the varieties were revealed when the seeds were subjected to Blue Greenish Yellow Fluorescence Test (BGYF). The presence and the amount of aflatoxins are represented in the table: 7.1. It was observed that in the Meghalaya variety, aflatoxins B_1, B_2 and G_1 were present as compared to the Manipur variety, which had aflatoxins B_1 and B_2 only. Aflatoxin G_1 was absent in the Manipur variety and aflatoxins G_2 was absent in both the varieties. On further investigation, it was observed that in the Meghalaya variety, aflatoxin B_2 was present in greater amount (17.84 mg/ml), followed by aflatoxin G_1 with 1.75mg/ml. Least amount (1.63mg/ml) was registered in case of aflatoxin B_1. In the Manipur variety, (0.836 mg/ml) of aflatoxin B_1 and 20.2mg/ml of aflatoxin B_2 was recorded. The molecular weight and molar coefficient extinction of aflatoxins are presented in the table: 7.2

8. CONTROL OF CERTAIN PATHOGENS BY BIOLOGICAL METHODS

In case of Meghalaya variety of *Phaseolus vulgaris* Linn. seeds, *Trichoderma viride* was used as an antagonistic fungus, while *Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum and Pythium intermedium* were used as test fungi. The liquid pure cultures of antagonistic and test fungi were mixed at definite proportions of 1:1, 1:2 and 1:5 v/v (Tables 8.1). The percentage germination, the mortality rate, the final stand (%) and the percentage change were (%) recorded. The highest germination and final stand of 85% of the seeds was noted in the case of seeds treated with *T.viride* and *C. lindemuthianum* at 1:1 ratio. The lowest germination of 55% was registered in the seeds treated with the antagonist fungus (*T.viride*) and *P. intermedium*. The pre-emergence mortality of 45% was recorded in

Table 8.1: The germination percentage, mortality percentage and the final stand percentage of the crop of *Phaseolus vulgaris* seeds treated with antagonistic and test fungi in definite ratio.

MEGHALAYA VARIETY

TEST FUNGI	RATIO *Trichoderma viride*: TEST FUNGI	GERMINATION (%)	EMERGENCE MORTALITY (%)		FINAL STAND (%)	CHANGE (%)
			Pre	post		
Alternaria alternata	1:1	75	25	-	75	+7.142
Colletotrichum lindemuthianum	1:1	85	15	-	85	+21.428
Fusarium oxysporum	1:1	65	35	4	61	-12.857
Pythium intermedium	1:1	55	45	6	49	-30.00
Alternaria alternata	1:2	60	40	-	60	-14.285
Colletotrichum lindemuthianum	1:2	65	35	5	60	-14.285
Fusarium oxysporum	1:2	50	50	-	50	-28.571
Pythium intermedium	1:2	75	25	10	65	-7.142
Alternaria alternata	1:5	60	40	-	60	-14.285
Colletotrichum lindemuthianum	1:5	80	20	5	75	+7.142
Fusarium oxysporum	1:5	50.5	25	-	50.5	+29.285
Pythium intermedium	1:5	65	20	-	65	-7.142
Trichoderma viride	1:5	80	20	10	70	-

case of *P.intermedium* and *T.viride* mixed culture which was the highest value. The least seedling germination of 15% was observed in case of seeds pre-treated with *C.lindemuthianum* and *T.viride*. The minimum value of final stand of 49% was recorded in test fungus, *P. intermedium* mixed with the antagonist. The best available combination therefore, seems to be *T. viride: C. lindemuthianum*, where the germination percentage and the final stand were maximum.

In the ratio of 1:2, *T. viride* and *P. intermedium* pre treated seeds recorded the maximum seed germination of 75%. Seeds treated with a dual culture of *T. viridi* and *F. oxysporum* recorded a least germination of 50%. A maximum value of 65% in terms of final stand was observed in *T. viride* and *P. intermedium* pre-treated seeds. A minimum value of 50% was noted in case of the antagonistic fungus and *F. oxysporum* treated seeds. In the ratio of 1:5 between the antagonistic and test fungi, *T.viride* and C. *lindemuthianum* treated seeds showed a peak germination of 80% with pre-emergence mortality being as low as 20%. A final stand of 75% was observed in this case. On the other hand, in the mixed culture of *T.viride* and *F. oxysporum*, the seedlings registered the least germination of 50.5%, which was also the value of the final stand percentage.

The germination percentage, mortality percentage and the final stand of the crop were registered in the Manipur variety (Table 8.2). The ratio of 1:1 between the antagonistic and test fungi revealed the highest germination of 90% in case of *F. oxysporum* and *T.viride* treated seeds. A lowest germination of 70% was noted in seeds treated with *P.intermedium* and *T.viride*. No mortality case was encountered in this experiment. In the ratio of 1:2, the mixed culture of *T.viride* and *P. intermedium* recorded the highest germination of 85% while the final stand registered 80%. The least effective combination was observed in case of *A. alternata* and

Table 8.2: The germination percentage, mortality percentage and the final stand percentage of *Phaseolus vulgaris* seeds treated with antagonistic and test fungi in a definite ratio

MANIPUR VARIETY TEST FUNGI	RATIO *Trichoderma viride* :TEST FUNGI	GERMINATION (%)	EMERGENCE MORTILITY (%)		FINAL STAND (%)	CHANGE (%)
			Pre	post		
Alternaria alternata	1:1	75	10	-	75	+7.142
Colletotrichum lindemuthianum	1:1	80	15	20	60	-14.285
Fusarium oxysporum	1:1	90	-	-	90	+28.571
Pythium intermedium	1:1	70	25	-	70	28.571
Alternaria alternata	1:2	50	05	-	50	-28.571
Colletotrichum lindemuthianum	1:2	60	06	10	50	-50.00
Fusarium oxysporum	1:2	60	-	25	35	-7.142
Pythium intermedium	1:2	85	-	05	80	+28.571
Alternaria alternata	1:5	80	-	10	70	50.00
Colletotrichum lindemuthianum	1:5	90	10	-	90	+21.428
Fusarium oxysporum	1:5	50	-	15	35	-35.714
Pythium intermedium	1:5	65	05	20	45	-50.00
Trichoderma viride		80	-	10	70	

Table 8. 3: The root-Shoot length and dry weight of seedlings treated in different proportions of the antagonistic and test fungi.

MEGHALAYA VARIETY TEST FUNGI	RATIO *Trichoderma viride* :TEST FUNGI	ROOT LENGTH (cm)	SHOOT LENGTH (cm)	DRY WEIGHT (mg)
Alternaria alternata	1:1	12.5(-16.6)	10.5(+110.0)	0.111(-15.90)
Colletotrichum lindemuthianum	1:1	10.5(-30.0)	11.2(+124.0)	0.121(-8.33)
Fusarium oxysporum	1:1	17.0(+13 33)	8 5(+70.0)	0.132(+0.00)
Pythium intermedium	1:1	7.7(-48.66)	4.5(-10.0)	0.012(-90.90)
Alternaria alternata	1:2	8.5(-43.33)	6.5(+30.0)	0.121(-8.33)
Colletotrichum lindemuthianum	1 2	5.8(-61.33)	3.5(+30.0)	0.150(+13.63)
Fusarium oxysporum	1·2	17.5(+16.66)	11.5(+130.0)	0.142(+7.57)
Pythium intermedium	1·2	18.0(+20.00)	10.0(+100)	0.142(+7.57)
Alternaria alternata	1:5	7 5(-50.00)	6.0(+20.0	0.012(-90.90)
Colletotrichum lindemuthianum	1:5	19.0(26.66)	11.0(+120)	0.138(+4.54)
Fusarium oxysporum	1:5	18.0(+20.00)	5.0(0.00)	0.139(+5.30)
Pythium intermedium	1:5	10.2(-32 00)	7.0(+40)	0.110(-16.66)
Trichoderma viride		15.0	5.0	

The figure in parenthesis indicates the percentage change over the control.

T.viride treated seedlings which showed 50% germination and also the same was the case with the final stand. In the ratio of 1:5 between the antagonistic and test fungi, the highest germination of 90% was recorded in case of *C. lindemuthianum* and *T. viride* treated seeds. Least germination of 50% was noted in case of seeds treated with *T. viride* and *F. oxysporum*, which also recorded 35% in its final stand. The root-shoot length and the dry weight of the seedlings in different proportions of the antagonist and test fungi were measured in case of both the varieties (Tables 8.3 and 8.4). In the *Meghalaya* variety, the ratio of 1:1 between the antagonist *(T.viride)* and the test fungi (*C.lindemuthianum*), the root and shoot length were highest with 10.5cm and 11.2 cm respectively. The least root length of 7.7 cm was recorded in the seeds treated with equal proportions of *T.viride* and *P. intermedium*. *F.oxysporum* and *T.viride* treated seedlings recorded a dry weight of 0.132 mg. Least dry weight of 0.012 mg was registered in seeds treated with *P.intermedium* and *T.viride* In the ratio 1:2, *F. oxysporum* in association with the antagonistic fungi recorded a root length of 17.5cm and a maximum shoot length of 11.5 cm *P.intermedium* treated seeds registered *a* maximum root length of 18.0 cm and a dry weight of 0.142 mg. The ratio of 1:5 of the antagonist and test fungi revealed that *C.lindemuthianum* and *T.viride* treated seeds registered maximum root length of 19.0 cm. Least root length of 7.5 cm was recorded in *Alternaria alternata* and *T.viride* treated seeds in the same ratio. Seeds treated with one volume of *T.viride* and 5volumes of *F.oxysporum* recorded 0.139 mg in terms of dry weight, which was the maximum value. Least dry weight of 0.110 mg was recorded in case of seeds treated with *P.intermedium* and *T.viride*. In the control set (only *T.viride* treated seeds), the root and shoot length was 15.0 cm and 5.0 cm respectively and the dry weight was 0.132 mg (Table 8.3). The root-shoot length, dry weight of the seedlings,

Table 8.4: The Shoot-root length and dry weight of seedlings treated in different proportions of the antagonistic and test fungi.

MANIPUR VARIETY TEST FUNGI	RATIO *Trichoderma viride*: TEST FUNGI	ROOT LENGTH (cm)	SHOOT LENGTH (cm)	DRY WEIGHT in (mg)
Alternaria alternata	1:1	6.0(-42.85)	6.5(-31.57)	0.125(+1.62)
Colletotrichum lindemuthianum	1:1	5.8(-44.76)	8.0(-15.78)	0.123(+8.94)
Fusarium oxysporum	1:1	10.0(4.76)	7.5(-21.05)	0.112(-8.94)
Pythium intermedium	1:1	17.0(+61.90)	11.0(+15.78)	0.122(-0.813)
Alternaria alternata	1:2	15.0(+42.85)	10.0(+5.26)	0.111(-9.75)
Colletotrichum lindemuthianum	1:2	7.5(-28.57)	12.0(-26.31)	0.120(-2.43)
Fusarium oxysporum	1:2	22.0(+109.52)	7.10(-25.26)	0.111(-9.75)
Pythium intermedium	1:2	11.5(+9.52)	8.5(-10.52)	0.125(-1.63)
Alternaria alternata	1:5	8.5(-19.04)	10.5(+10.52)	0.100(-18.70)
Colletotrichum lindemuthianum	1:5	10.2(-2.85)	11.0(+15.78)	0.112(-8.94)
Fusarium oxysporum	1:5	4.2(-60.0)	6.3(-33.68)	0.106(-13.82)
Pythium intermedium	1:5	2.00(-75.00)	3.1(-30.00)	0.055(-21.00)
Trichoderma viride		9.5	10.5	0.123

The figure in parenthesis indicates the percentage change over the control.

pretreated with a definite proportion of the antagonist and test fungi in the *Manipur* variety of the seeds (Table 8.4) revealed the following results:

The dual culture of *T viride* with *P. intermedium* in the ratio 1:1 yielded a root length of 17.0 cm and a shoot length of 11.0 cm. Least shoot length of 6.5 cm was recorded in case of the association of *A.alternata* with *T.viride*. Least root length of 5.8 cm was observed in case of *C. lindemuthianum* and *T.viride* treated seeds. A maximum dry weight of 0.125 mg of the seedlings was observed in *A.alternata* and *T.viride* treated seeds and a least dry weight of 0.112 mg was registered in case of *F.oxysporum* and *T.viride* treated seeds. In the ratio of 1:2, a maximum shoot length of 12.0 cm of the seedlings was registered in the seeds pre treated with a dual mixture of *C. lindemuthianum* and *T. viride*. One parts by volume of *T.viride* and 2-parts by volume of *F.oxysporum* yielded a root length of 22 cm, which was the highest value. In case of the seeds treated with the 1:5 ratio of *T.viride* and *C. lindemuthianum*, the root and shoot length of seedlings was 10.2 cm and 11.0 cm respectively. Least root length of 2.0 cm was recorded in case of dual culture of *P.intermedium* and *T.viride.*

The colony interaction between the antagonistic fungus (*T. viride*) and the test fungi viz. *A. alternata, Colletotrichum lindemuthianum, Fusarium oxysporum* and *Pythum intermedium* revealed that *C. lindemuthianum* had formed a maximum interaction score of five, with the antagonist. The type of interaction was D i.e. there was a mutual interaction at a distance of less than 2 mm. No inhibition zone was observed here. This was followed by *Alternaria alternata* and *T. viride* with an interaction score of four, while their type of interaction was B_{ii}. They formed an intermingled zone of 0.75 cm. *F.oxysporum* and *P. intermedium* in presence of *T. viride* had an interaction score of three and B_{ii} was their type of interaction. They formed an intermingled zone of 0.80 cm and 0.65 cm respectively. *T. viride* inhibited the colonies of

Table 8.5: Colony interaction between the antagonistic and test fungi isolated from the *Phaseolus vulgaris* Linn. seeds.

INTERACTION	SCORE OF INTERACTION	TYPE OF INTERACTION	INTERMINGLED ZONE (CM)	INHIBITION ZONE (CM)	% INHIBITION OF THE COLONY OF PASSIVE FUNGI
Alternaria alternata and Trichoderma viride	4	B_{II}	0.75	-	25.0
Colletotrichum lindemuthianum and Trichoderma viride	5	D	-	0.65	32.0
Fusarium oxysporum and Trichoderma viride	3	B_{II}	0.80	-	50.0
Pythium intermedium and Trichoderma viride	3	B_{II}	0.65	-	62.0

- Denotes no inhibition zone.

Inhibition scores from 1 (mutually intermingling growth) to 5 (mutual interaction at a distance); based on Skidmore and Dickinson (1976).

Type of interaction B_I = Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below its colony.

B_{II} = Intermingling growth where the fungus under observation has ceased growth and is being overgrown by another colony.

C = Slight inhibition with a narrow demarcation line (1-2); D = Mutual inhibition at a distance of < 2mm

Plate 6(a, b and c): The antagonism between the antagonist, *Trichoderma viride,* and different test fungi.

Table 8.6 Analysis of variance for the effect of antagonistic and test fungi mixed in different pro on the final stand of the crop in the Manipur variety.

SOURCE OF VARIATION	DEGREE OF FREEDOM	CALCULATED *F* VALUE	TABULATED VALUE	PROABABILITY LEVEL	SIGNIFICANCE
Between the different proportions of the antagonistic and test fungi	$n_1=2$ $n_2=8$	0.0593411	18.5	$\leq .001$	NS
Between the fungal species	$n_1=2$ $n_2=8$	1.7950664	14.4	$\leq .001$	NS

Table 8.7 Analysis of variance for the effect of antagonistic and test fungi mixed in different pro the final stand of the crop in the Meghalaya variety.

SOURCE OF VARIATION	DEGREE OF FREEDOM	CALCULATED *F* VALUE	TABULATED VALUE	PROABABILITY LEVEL	SIGNIFICANCE
Between the different proportions of the antagonistic and test fungi	$n_1=2$ $n_2=8$	0.088923	18.5	$\leq .001$	NS
Between the fungal species	$n_1=2$ $n_2=8$	1.000615	14.4	$\leq .001$	NS

NS = Non significant

A. alternata Colletotrichum lindemuthianum, Fusarium oxysporum and *Pythium intermedium* by 25%, 32%, 50% and 62% respectively (Table 8.5) (and Plates 6a, 6b and 6c).The data was processed by analysis of variance (ANOVA) and insignificant variation in final stand percentage of seedlings between and within the different proportion of the antagonist (*T.viride*) to test fungi was observed (Tables 8.6 and 8.7)

DISCUSSION

1. SURVEYOF SEED-BORNE FUNGI, THEIR ISOLATION AND IDENTIFICATION

Throughout the study period a maximum number of fungal species could be isolated by blotter and the agar plate methods, which may be due to the availability of high moisture content in the blotter's plate and the availability of fungal nutrients in the agar plate. This agrees with the findings of Singh *et al.* (1984), Paul (1991) and Neergaard and Saad (1962) who concluded that the blotter and the agar plate methods are both valuable and supplementary to each other. Certain fungal species like *Aspergillus niger, Rhizopus nigricans, Penicillium spp, Fusarium oxysporum* and sterile mycelia dominated the agar plates. This finding corroborates with the work of Aulakh *et al.* (1976) and Paul (1991), mainly because they are the fast growing fungi.

There was a slight variation in seasonal occurrence of mycoflora. In both the Meghalaya and Manipur variety, maximum number of fungal species could be isolated during the rainy season, followed by summer and the least number of fungal species were isolated in the winter season. These differences in the number of fungal species during the different seasons could be due to the differences in the availability of ambient temperature and moisture condition favorable for the growth of the fungal species. This is in conformity to the findings of Reddy and Reddy (1983) and Paul (1991), who found that maximum number of fungal species could be isolated during the rainy season.

The fungal species such as *A. niger, Cladosporium cladosporioides, Colletotrichum lindemuthianum, F.oxysporum, Pythium intermedium* and *Trichoderma viride* were found to

be present in all the seasons of the year which may be due to their adaptability over a broad spectrum of nutritional and environmental conditions. A few species however, were found to be restricted to only one or two seasons. This result is substantiated by the work of Gilman and Semeniuk (1948), Campbell (1962), Bilgrami *et al.* (1979) and Lalrawna (1987).

Qualitatively, there was a slight difference in fungal species composition in both the Meghalaya and the Manipur varieties. The variation in the fungal species composition may be due to the variation in the physio-chemical properties, nutritional availability and harvesting condition of the seed varieties. This finding has been substantiated by the work of Lalrawna (1987) and Paul (1991), wherein, fungal species composition differed among the varieties of rice and maize seeds respectively.

It was observed that *Alternaria alternata, C. lindemuthianum, F. oxysporum, P. intermedium* and *T. viride* were the dominant fungi. This finding is in line with the work of Subbdiah (1982) and Paul (1991), while working on the seed mycoflora of maize. *Cephalosporium acremonium* and *C. cladosporioides* were observed to exhibit an increase in disease frequency with the storage period, which was later replaced by the more dominant fungi like *Aspergillus, Penicillium,* etc This finding corroborates with the work of Lal and Kapoor (1979), while studying the succession of fungi on wheat and maize seeds during storage period. It was also observed that the number of fungal species decreased gradually with increase in the storage period in both the varieties. This result correlates with the findings of Gupta *et al.* (1983), Reddy and Reddy (1983) and Vijaylakshmi and Rao (1985), which may be due to the varied fungal activity with the increase in storage period.

2. COMPARATIVE STUDY OF DIFFERENT STORAGE PRACTICES WITH RESPECT TO THE INCIDENCE OF FUNGI

The storage of bean seeds in different containers recorded maximum number of fungal species in the rainy season, followed by summer and winter seasons which may be due to the availability of moisture content and ideal temperature necessary for fungal growth in that season. This is in agreement with the findings of Paul (1991). Species of *Aspergillus* and *Penicillium* were the dominant fungal species in the present study, which corroborates the work of Whitney *et al.* (1993). A low rate of infection was detected even after six months of storage. This agrees with the work of Lalrawna (1987). The seeds stored in earthen pots showed a higher percentage of infection and low germinability, which could be due to the ability of the pots to retain maximum moisture favourable for the fungal growth. This correlates with the work of Jordi *et al.*, (1993) who worked on palas seeds. With the increase in storage period, the viability of the seeds decreased and the germinability reduced, which may be due to the interference of the fungi in the metabolic activity of the seeds and the consumption of stored food matter resulting in the abnormal or no growth of the seedlings.

In some isolated cases, poor germination and decaying of seeds were observed. This draws support from the findings of Kumar and Nema (1973) and Rati and Ramalingam (1974). Reduction in the germination can be explained, as put forward by Christensen and Kaufman (1965), that the fungal association changes the mitochondrial set up of the cell integrity and ultimately affect the viability of the seeds. The suppression of seed germination could be due to the release of fungal toxins thereby hampering the metabolic activities of the seed, whereby, there may be depletion of some metabolites and the

abolition of others. This is in conformity with the observation of Misra *et al.* (1969), Omokanye and Onifade (1993), Beganmi and Certellazzo (1996) and Vierira (1998) who worked with the seeds of *Centrosema pubescens* and *P. vulgaris* respectively.

Insignificant variation in percentage fungal infection in *Phaseolus vuilgaris* seeds was observed between gunny bag and bamboo basket, gunny bag and iron bin, bamboo basket and iron bin and between bamboo basket and earthen pot while significant variation was observed between gunny bag and earthen pot and between earthen pot and iron bin.

The inefficiency in moisture retaining capacity of gunny bag and bamboo basket, the total curtailation of moisture entry by iron bin and high moisture absorbing and retaining capacity of earthen pot could account for such results.

3. STUDIES ON THE EFFECT OF CERTAIN SEED-BORNE FUNGI ON THE GERMINATION OF *Phaseolus vulgaris* Linn. SEEDS

The present investigation reveals that the extracts of all the five test fungi viz., *Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum, Pythium intermedium and Trichoderma viride* had a negative impact on the overall germination and growth of *P. vulgaris* seeds. This finding agrees with the work of Dwivedi and Singh (1973), Dublish and Pande (1976) and Pande *et al.* (1982) who have reported the importance of fungal metabolites in reducing seed germination and sprouting. *F. oxysporum*, showed the characteristic yellowing of the leaves, stunted growth and leaf fall. *C. lindemuthianum* on the other hand, showed the characteristic brown spots on leaves and in pods. Kulik and Schoen (1982) made similar observation in sweet corn. The suppression of seed germination may be due to the toxic metabolites secreted by the fungus. This has been substantiated by the findings of Sinha and Prasad (1981) in mung seeds and Paul (1991) in maize seeds.

The root-shoot length and the dry weight of the bean seedlings were affected by the seed-borne pathogens. The extracts of *F. oxysporum* and *P. intermedium* adversely affected the seed germination and root-shoot length of the seedling, which is in conformity with the findings of Morsy *et al.* (1985) and Paul (1991), who worked on similar line with maize seeds and Siddaramaiah *et al.* (1980) and Singh and Gupta (1984) who worked on the effects of seed-borne fungi on seed germination and root-shoot growth of *Medicago sativa* L. The negative impact of seed-borne pathogens is well advocated by Brodnik (1975) who studied the toxic effects of the metabolites of *F. oxysporum* and *F. graminearum* on the maize seed germination and embryo growth. *P. intermedium* and *F. oxysporum* considerably reduced the average height, percentage germination and the dry weight. This correlates with the work of Mathur and Sehgal (1964). *C. lindemuthianum* on the other hand, had a positive effect on the seedling growth. This has support from works of Roy and Pandey (1971), Roy *et al.* (1971) and Lalrawna (1987).

Insignificant difference was found between the final stand percentage of *P.vulgaris* seedlings of both the varieties when grown in test tube and pots inoculated with different test fungi suggests that the seeds of both the varieties are equally susceptible to the test fungi.

4. STUDIES ON THE IMPACT OF SEED LEACHATES ON THE GERMINATION OF SPORES OF CERTAIN SEED-BORNE FUNGI

There was a considerable reduction in spore germination of the five test fungi treated with the seed leachates of *P. vulgaris*. This draws its support from Chaturvedi *et al.* (1974), Dhalwale and Kodmelwar (1978) and Johnson *et al.* (1997). The degree of inhibition varied with both the varieties of seeds. A similar trend was observed by Kumar and Jalali (1985) who had worked on chickpea. The inhibition of spore

germination could be mainly due to some chemical substances present in the seeds. The works of Ark and Thomson (1958) and Van Andel (1995) substantiates this. Kraft (1973) and Jalali (1976) reported that the phenolic compounds of the seed leachates impart a general resistance to parasitic and bacterial invasion. The optimum temperature of 25°C for fungal spore germination was also observed by Kapoor and Singh (1977) and Paul (1991).

There was insignificant change between the percentage fungal spore germination in treated seeds and control which shows that increase in temperature equally favoured the growth of fungi in both control and treated sets.

5. STUDIES ON THE IMPACT OF CERTAIN SEED-BORNE FUNGI ON THE DECOMPOSITION OF THE SEED

Analysis of the data (Tables 8.6-8.10) revealed that there was a progressive decline in the level of protein in the seeds due to fungal infestation. The level of reduction in protein content varied with the test fungi used. Similar result was reported by Bilgrami *et al.* (1979), Roy and Choubey (1984), Maheswari and Mathur (1985) and Lalrawna (1987). A slow decrease in the protein level at the initial stage suggested the least microbial activity at that stage. This draws support from the works of Jamaluddin *et al.* (1977); Sharma (1981) and Lalrawna (1987). It was further observed that the rate of reduction of the protein content varied among the different fungal species. This agrees with the findings of Roy and Choubey (1984) and Lalrawna (1987). The degradation of protein may take place with the help of fungal enzymes, as has been well advocated by Prasad *et al.* (1976) and Sinha and Prasad (1977).

The total sugar content showed a gradual increase in the diseased seeds. This is due to the hydrolysis of sucrose on release of amylase in the seeds by the fungi. This finding is

substantiated by the work of Bilgrami *et al.*, (1979). An increase in the reducing sugar with a corresponding fall in the non-reducing sugars was observed, which may be due to the hydrolysis of starch to simple sugars. This work is substantiated by the finding of Milner and Geddes (1946) in corn, Sharma (1973) in oil seeds and Lalrawna (1987) in rice seeds.

The level of fatty acid showed an increase at the end of 120 days of storage, which gradually decreased at the end of 180 days. The increase in fatty acid content was earlier reported by Lalithakumari *et al.* (1971), Charya and Reddy (1981), Lalrawna (1987) and Goodman and Christensen (1952). The increase in the fatty acid content may be because of the conversion of oil into fatty acids by the fungi. Exhaustion of oil contents is linked with the reduction of fatty acid content.

It was observed that *C. lindemuthianum* was most effective in increasing the level of fatty acids. This finding coincides with observation of Christensen (1952) and Maheswari *et al.* (1985). The total starch of the bean seeds showed a progressive fall, which may be due to conversion of starch into simpler compounds like glucose and fructose with the help of fungal hydrolytic enzyme. This work draws support from the works of Bilgrami *et al.* (1979).

Hence, it can be said that all fungi tested, played a vital role in the deterioration of common bean seeds. The capacity of different fungi to bring about changes differed according to their nutritional requirement and environmental changes. Tandon and Chaturvedi (1942) also reported that the strains of a species could differ widely in their utilization of seed contents. Similar observation was made by other workers like Winstead (1964); Hasize (1970) and Kapoor and Tandon (1970).

Significant variation in protein and sugar content with increase in storage period, while insignificant variation in case of starch and fatty acid suggest that the test fungi preferred the consumption of sugar and proteins to starch and fatty acids.

6. STUDIES ON THE EFFECT OF CERTAIN AGROCHEMICALS ON SEED-BORNE FUNGI

The four fungicides viz. Blitox-50, Topsin, Dithane M-45 and Indofil used in the present study showed an inhibitory influence on the mycelial growth and spore germination of the five test fungi viz. *Alternaria alternata, Colletotrichum lindemuthianum, Fusarium oxysporum, Pythium intermedium* and *Trichoderma viride*. Blitox-50 was effective against *C. lindemuthianum* and *F. oxysporum.* This is in agreement with the finding of Grewal (1982), Kumar and Singh (1985), Singh *et al.* (1985) and Lalrawna (1987). The Dithane M-45 fungicide was very effective against the fungal species of *P. intermedium,* C. *lindemuthianum* and *F. osysporum.* This can be correlated with the work of Sharma and Chaudhary (1975) and Lalrawna (1987). The efficacy of Dithane M-45 for control of seed seated infection has been studied by Dharam Vir *et al.*, (1970).

7. SCREENING OF SEEDS FOR AFLATOXINS

Both the seed varieties exhibited a positive response to the Blue Green Yellow Fluorescence Test (BGYF) which may be due to the influence exerted by the environmental factors such as oxygen availability, temperature, moisture content, type and nature of the fungal strains as well as the substrates which are known to play a dominant role in determining the extent of aflatoxin elaboration in the grains (Diener and Davis, 1969; Davis and Diener, 1970; Detroy, 1971 and Schroeder, 1974). In the present study, none of the seed varieties were immune to the aflatoxin elaboration in nature. This finding is in agreement with the work of Paul (1991), who worked on similar line with maize seeds.

8. CONTROL OF CERTAIN PATHOGENS BY BIOLOGICAL METHODS

The extracts of different test fungi and the antagonistic fungi mixed in different proportions, showed a better result in terms of seedling growth and survivability. This result agrees with the finding of Paul (1991) who found dual extracts of antagonistic and test fungi making discernible impact on maize seedlings. One of the reasons for the aforesaid result could be due to *Trichoderma viride* being a better antagonist and having a less bearing on the mixture with other fungi coupled with the competition between the fungi, if proper growth of the seedling is allowed. There were instances where the antagonistic fungi overgrew the other colonies as was observed in the case of *T. viride*, which showed a vigorous growth. There were also instances, where the antagonistic fungi formed inhibition zones probably due to the release of toxins from those fungi. This finding is substantiated by the work of Paul (1991).

The antagonistic fungi (*T. viride*) and the test fungi mixed in ratio of 1:5 yielded better result in terms of germinability of seeds and dry weight. This in agreement with the finding of Zheng *et al.* (1999) who worked with *T. viride* as antagonist on pea seeds. *T. viride* and *Colletotrichum lindemuthianum* mixed in 1:5 (V/V), showed better germinability of the seedlings. This is in conformity with the findings of Bhatrajas and Himani (1994).

The colony interaction between the antagonist and the test fungi revealed that there were cases where the two interacting fungi in culture media grew into one another having varying degree of intermingled zones. Several factors are involved in the microbial antagonism which include pH of the media, growth rate of the interacting fungi, competition for nutrients and space, composition of media and other physical factors. The occurrence of inhibition zone between the antagonist and pathogen on media is commonly considered as a result of production of antibiotics, pH changes and competition for nutrients. Mutual growth

of fungi in dual culture is seen only when they have similar growth rate and equal capacity of tolerance to antibiotics produced by each other, while over growth is achieved when one fungal species exhibits higher growth rate and good tolerance of antibiotics produced than the other fungi (Upadhyay and Rai, 1987 and Paul, 1991). In the present study *T.viride* played a very good role of an antagonist where it had suppressed the growth of other test fungi. This finding is supported by the work of Browen (1999). Mukhopadhyay (1987) reported that the most frequently studied fungi related to biological control are the species of *Trichoderma.*

Insignificant difference in final stand percentage of seedlings between different proportions of antagonist to test fungi suggest that the different ratios had similar effect on the final stand of the crop in both the varieties.

Information and data generated in the present study can prove to be useful to the farmers and cultivers at large and North-East in particular by educating them with different seed storage practices and controlling of fungal pathogens which can minimise their crop loss. Farmers can be provided with prior information to reduce the chances of incidence of fungal species in bean seeds by spraying of Blitox-50 fungicide. This would control the fungi at a very low concentration.

SUMMARY

Phaseolus vulgaris Linn. is one of the nine most important crops of the world and a major source of protein in the diet of many people globally. Considering its importance, and the general dearth of information pertaining to the various aspects of bean cultivation and its huge loss mainly due to fungal attack, the present investigation was carried out in an attempt to glean information that could provide some solution to stop the menace of fungal attack on this leguminous crop.

Two varieties of *Phaseolus vulgaris* Linn viz. Meghalaya and Manipur varieties were chosen for the survey of seed-borne mycoflora. Three techniques of isolation i.e., agar plate, blotter and the dilution plate methods were used. A total of twenty eight fungal species were isolated from the Meghalaya variety and thirty from the Manipur variety. In the Meghalaya variety, twenty eight fungal species could be isolated by blotter's method, twenty eight species by agar plate method and twenty six species by dilution plate method. In the Manipur variety, thirty fungal species were isolated by blotter's method, twenty nine species by agar plate method and twenty eight species by the dilution plate method. In the Meghalaya variety, twenty eight fungal species could be isolated during the rainy season, twenty six species during the summer season and twenty three fungal species during the winter season, whereas, in the Manipur variety, thirty species could be isolated during the rainy season, twenty nine species during the summer season and twenty six species during the winter season. Qualitatively, there was a slight difference in the composition of fungal flora isolated from the seeds of both the varieties. *Aspergillus alutaceus and Fusarium*

flocciferrum were restricted to the Manipur variety, whereas, *Aspergillus niger* and *Penicillium rubrum* were restricted to the Meghalaya variety.

The condition of the seeds stored in containers viz. earthen pots, bamboo baskets, gunny bags and iron bins revealed that none of the storage containers were totally effective or suitable for such purpose for none of these containers were free from fungal infestation. Among the storage materials, iron bin was better suited as compared to the other containers in terms of seed viability and less moisture retaining capacity. In contrast, the earthen pot proved to be the least effective storage material due to the presence of a large amount of moisture inside and thus providing an ambient condition for the development of large number of fungal colonies in the seeds. It was further observed that with an increase in the storage period, the viability or germinability of the seeds also showed a declining trend.

The pathogenecity of the five test fungi viz. *Alternaria alternata, Colletotrichum lindemuthianum, F. oxysporum, Pythium intermedium* and *Trichoderma viride* in the test tube seedling symptom test and pot culture experiment showed a marked reduction in the overall germination of the seeds in terms of both the root and shoot length. *C. lindemuthianum* showed the characteristic symptoms of brown spots on leaves and pods, which ultimately decayed the seedlings. *A. alternata* and *T. viride* showed the characteristic symptoms of leaf spots with pale and weaker seedlings having stunted growth.

The seed leachates were found to play the role of an inhibitor in the germination of fungal spores of species such as *A. alternata, C. lindemuthianum, F. oxysporum, P. intermedium* and *T. viride*. The extent of inhibition varied with the fungal species. The sugars and phenols detected in the leachates could possibly play a vital role.

There was a marked difference in the capacity of the test fungi viz., *A. alternata, C. lindemuthianum, F. oxysporum, P. intermedium* and *T. viride* to deteriorate the seed quality

in terms of protein, total sugars, reducing and non-reducing sugars, starch and fatty acids content. All the fungi were seen to cause a reduction in the dry weight of the seeds. There was a sharp decrease in the starch and non-reducing sugar content of the seeds. The reducing sugars, however, showed an increase in its content as the storage period was increased. The protein content of the seeds declined with a corresponding increase in soluble nitrogen during the study period of 180 days. There was a pronounced reduction in the starch content of the seeds with the progression of storage period. In case of the effect of the test fungi on the fatty acid content, it was noticed that there was a tendency towards an increase in its content at the start, but after attainment of a peak at a certain stage, gradual reduction was noticed.

The efficacy of four agrochemicals viz., Blitox–50, Dithane M–45, Topsin and Indofil tested against the mycelial growth and spore germination of pathogenic seed- borne fungi of *A. altenata*, C *lindemuthianum*, *F. oxysporum*, *P. intermedium* and *T. viride* revealed that all the test fungi were successfully controlled by all the fungicides though at different concentrations. Some fungicides particularly Topsin proved to be most successful in controlling both the mycelial growth and fungal spore germination The germination of spores of most of the fungi with the exception of *A. alternata* and *C. lindemuthianum* could be controlled at 0.1% concentration of the fungicide. The mycelial growth of *F. oxysporum* was controlled at 0 005% concentration of the fungicide.

The Blue Green Yellow Fluorescence Test (BGYF) revealed the presence of naturally occurring aflatoxins in seeds and showed a positive test in both the varieties. Aflatoxins B_1, B_2 and G_1 were present in the Meghalaya variety, whereas, aflatoxins B_1 and B_2 were found in the Manipur variety Aflatoxin G_2 was absent in both the varieties. In the Meghalaya variety aflatoxins B_1, B_2 and G_1 recorded 1 63 mg/ml, 17.84 mg/ml and 1.75

mg/ml respectively, in contrast to the Manipur variety where aflatoxins B_1 and B_2, recorded 0.836 mg/ml and 20.2 mg/ml respectively

The dual culture of antagonistic fungi, *T. viride* and the test fungi, A. *alternata*, *C. lindemuthianum*, *F. oxysporum*, *P. intermedium* mixed in different proportion of 1:1, 1:2 and 1:5 did not show any improvement in the overall germination of seedlings with the exception of *T. viride*. *F. oxysporum* mixed in 1 1 ratio, where 90% final stand of the crop was recorded. There was, however, a marked improvement in the length of root and shoot in some cases. *T. viride* showed antagonistic behavior with all the test fungi The varying zones of inhibition were observed with various fungi Maximum inhibition zone of 0.65 cm was observed in the dual culture of *T. viride* and *C. lindemuthianum*. Intermingled zones were also observed in dual cultures

BIBLIOGRAPHY

A.O.A.C. (1960). Official methods of analysis. The association of Agricultural Chemists, Benzamin Franklin Station, Washington, 832 pp.

Abou-Heilah, A.N. (1984) seed-borne fungi of wheat and their control by seed treatment. *Indian Pytopath.* **37**: 656-659.

Agarwal V.K., Mathur, S B and Neergard, P (1972) Some aspects of seed health testing with respect to seed-borne fungi of rice, wheat, blackgram, green gram and soybean in India *Indian Phytopath.* **25**: 91-100

Agarwal, R.L. (1976) Progress report of seed testing laboratories, *Seed Res.* **4**(1): 80-93.

Allison, C. C. and Phillips, D.V. (1963). In vivo selection of biotypes of *Cephalosporium* inhibitory to the development of tomato and cotton *Fusarium* with symptoms. *Phytopathology* **53**: 869 – 870.

Anahosur, K. H and Bidari, V. B. (1974) Role of the toxins of seed mycoflora in soybean seed spoilage. *Curr. Res.* **3**: 130 – 131.

Aquiero, V.M., Exconde O.R and Quintana I.J. (1978). Seed-borne fungi of rice and their response to seed treatment. Phillip *Agric* **49** (10): 871-878.

Ark, P. A. and Thomson, J. P. (1958). Antibiotic properties of the seed of wheat and barley. *Plant Dis. Reptr.* **42**: 959 – 962

Asplin, F. D. and Carnaghan, R. B. A. (1961). The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. *Vet. Rec.* **73**: 1215 – 1219.

Aulakh, K. S., Grewal, K. K. and Goul, R. K. (1976). Detection of seed-borne fungi of maize and their role in causing seed rot and seedling infections. *Indian Phytopath.* **29**: 241 – 245.

Barash. I.J.M. Klistewicz and Kosuge T. (1964). Biochemical factors affecting pathogenecity of *Botrytis cineria* on sunflower. *Phytopathology* **54**: 926 – 927.

Barnett, H. L. and Hunter, B. B. (1972). Illustrated genera of Imperfect Fungi. *2nd Ed., Burges Publ.*, Minneapolis, 241 pp.

Basak, A. B., Kasim, M. R., Hoque, M. N. and Biswas, A. P. (1984). Efficacy of fungicides on two seed-borne fungal infections on wheat. Chittagong Univ. *Stud. Part II, Sci.* **8**: 41 – 44.

Bedi, J. S. and Chohan, J. S. (1986). Effect of culture filtrate of toxigenic and non-toxigenic isolates of *Aspergillus flavus* on different crop plants and weeds. *Indian J. Mycol. Pl. Pathol.* **16** (1): 48 – 54.

Bedi, P. S. and Dhaliwal, D. S. (1970). Studies on the seed mycoflora of rice var. T. N. I. with special reference to its pathological significance. *F. Res. PAU, 7. Suppl.* 600 – 605.

Begnami, C.N. and Cortelazzo, A.L. (1996). Cellular alterations during accelerated aging of French bean seeds. *Seed Sci and Technol.* **24**: 295-303.

Bhatt, D. D. and Vanghan, E. K. (1963). Inter-relationships among fungi associated with strawberries in Oregon. *Phytopathology* **53**: 217 – 220

Bhatti, M. A. R., Randhawa, M. A. and Ali, S. (1985). Chemical control of fungi occurring on safflower *(Carthamus tinctorius)* Seed. Pak. *J. Sci. Ind. Res.* **28** (1): 31 – 32

Bilgrami, K. S., Prasad, T. and Sinha, R. K. (1979). Changes in nutritional components of stored seeds due to fungal association. Today and Tomorrow's Printers and Publishers. New Delhi, 80 pp.

Bilgrami, K.S., Sinha R. K. and Prasad, T. (1978). Effect of fungal flora on the seed contents of mung. *Indian Phytopath.* **31**: 476 – 479.

Bilgrami, S., Prasad, T., Jamaluddin and Roy A. K. (1976). Studies on the deterioration of some pulses by fungi. *Indian Phytopath.* **20** (4): 374 – 377.

Blakeman, J. P. (1971). The chemical environment of the leaf surface in relation to growth of pathogenic fungi. In: Ecology of Leaf Surface Microorganisms. (Eds. Preece, T. F. and Dickinson C. H.). Acad. Press, New York. 255 – 268 pp.

Block, R. J. and Schroeder, H. W. (1974). Influence of relative humidity on production of aflatoxin in rice by *Aspergillus parasiticus. Phytopathology.* **64**: 17 – 21.

Brodnik, T. (1975). Influence of toxic products of *Fusarium graminearum* and *F. moniliforme* on maize seed germination and embryo growth. *Seed Sci. Technol.* **3**: 691 – 694.

Campbell, W. P. (1962). Storage of treated moistened grains. *Canada Plant Dis. Survey,* **42**: 195 – 201.

Charya, M. A. S. and Reddy, S. M. (1981). Deterioration of mung *(vigna radiata)* seeds due to certain seed–borne fungi. *Indian J. Bot.* **4** (1): 80 – 82.

Chaturvedi, S. N., Muralia, R. N. and Siradhana, B. S. (1974). The influence of cumin seed exudates on fungal spore germination. *Plant and Soil.* **40**: 49 – 56.

Christensen, C. M. (1955) Grain storage studies 21. Viability and moldiness of commercial wheat in relation to the incidence of grain damage. *Cereal Chem.* **32**: 107 – 116.

Christensen, C. M. and Gordon, D. R. (1948). The mould flora of stored wheat and corn and its relation to heating of moist grain. *Cereal Chem.* **25**: 40 – 51.

Christensen, C. M. and Kaufman, H. H. (1965). Deterioration of stored grains by fungi. Ann. Rev. *Phytopath.* **3**: 69 – 84.

Christensen, C. M. and Kaufman, H. H. (1969). "Grain storage". Univ. of Minnesota Press, Minneapolis. 7 – 153 pp.

Christensen, C. M. and Kaufmann H. H (1969). Grain storage: The role of fungi in quality loss. Univ. of Mieensota Press, Minneapolis. **7**: 153 pp.

Christensen, C. M. and Lopez, L. C. (1963). Pathology of stored seeds. Proc. Int. Seed Test. Ass. **28**: 701 – 711.

Christensen, C. M. and Mirocha, C. J. (1963). Relation of relative humidity to the invasion of rough rice by *Aspergillus parasiticus. Phytopathology* **66**: 204 – 205.

Christensen, C.M. (1969). Influence of moisture content, temperature and time of storage upon invasion of rough rice by storage fungi. *Phytopathology* **59**: 145-148.

Christensen, C.M. (1971). Invasion of Sorghum seed by storage fungi at moisture contents of 13.5-15% and condition of samples from commercial bins. *Mycopath.Mycol.appl.***44**: 277-282.

Christensen, C.M. and Mirocha, C.J. (1976). Relation of relative humidity to the invasion of rough rice by *Aspergillus parasiticus. Pytopathology* **66**:204-205.

CIAT (1992). Trends in CIAT commodities. Working document III. CIAT, Cali. Colombia.

CMI, (1974). Plant Pathologist's Pocketbook, 1968. Lamport Gilbert Printers, Ltd. pp. 194 – 241 pp

Davis, N. D. and Diener, U. L. (1970). Environmental factors affecting the production of aflatoxin. In: *Toxic Microorganisms.* (Ed. M. Herzberg), Publ. By the UTN – Joint Panels on Toxic microorganisms and the U.S. Dept. of Interior. 43 – 47 pp.

Dennis and Webster, J. (1971b). Antagonistic properties of species groups of *Trichoderma* part *II.* Production of volatile antibiotics. *Trans. Brit. Mycol. Soc.* **57**: 41 – 48.

Detroy, R. W., Lillehoj, E. B. and Gieglar, A. (1971). Aflatoxin and related compounds. In: Mycobial Toxins (Eds. Ciegler, A., S Kadis and. Ajl, S. J.). Vol. VI. Acad. Press. New York, 3 – 178 pp.

Devi, G. Rama and Polasa, H. (1984). Inhibitory effect of bavistin (carbendazim) on growth and mycotoxin production by *Aspergillus* grown on maize. *Pesticides (Bombay)* **18** (12): 32 – 34.

Dharam Vir., Mathur, S. B. and Neergaard, P. (1970). Control of seed-borne infection of *Drechslera* spp. On barley, rice and oats with diathane M – 45. *Indian Phytopath.* **23**: 570 – 572.

Dhawale, S. D. and Kodmelwar, R. V. (1978). Studies on mycoflora of chilli seed. *Seed Res.* **6**: 23 – 30.

Diener, U. L. and Davis, N. D. (1969). Aflatoxin formation by *Aspergillus flavus. In: Aflatoxins.* (Ed. Goldblatt, L.A.), Acad. Press, New York, 13 – 54 pp.

Dublish, P. K. and Pande, P. C. (1976). Effect of culture filtrate of certain rhizosphere fungi on seed germination and seedling growth of *Phaseolus mungo* Linn, *Luffa cylindrical M Roem* and *Cucumis sativus* Linn. *Bangladesh J. Bot.* **5**: 39 – 42.

Dwivedi, R. S. and Singh, B. P. (1973). Effect of microflora on seed germination and seedling growth of *Lathyrus sativus*. *Acta. Bot. Indica.* **1**: 41 – 44.

Ekundayo, C. A. (1984). Effect of some fungicides on the mycelial growth and stomatal production of *Macrophoma magnifera*. *Microbios Lett.* 25 (97): 13 – 16.

Ellis, M. B. (1971). Dematiaceous hyphomycetes. CMI, Kew, Surrey, England. pp . 608.

Ellis, M. B. (1976) More Dematiaceous Hyphomycetes. CMI, Kew, Surrey, England 507 pp

Fahim, M. M., Barakat F. M., Attia, M. F and. Aly H. Y (1985). Fungi associated with stored sorghum grains and their role in grain deterioration, *Egyptian J. Phytopathology* **14**: 39 – 49.

FAO, (1993). Production Year Book. **47** :106-118.

Fennel, D. I., Lillehoj, E. B and Peterson, R. E. (1973). Bright–Greenish–Yellow Fluorescence and associated fungi in corn naturally contaminated with aflatoxin. *Cereal Chem.* **50**: 404 – 414.

Fischer, G.W. and Holton, C.S. (1957). Biology and control of smut fungi Ronald press, New York, 622 pp

Fokkema, N. J. (1976). Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. In: *Microbiology of Aerial Plant surfaces.* Eds. C. H. Dickinson and T. F. Preece. Pp. 487 – 506. Academic Press, London.

Forgacs, J. and Carll, W.T. (1962). Mycotoxicosis, *Adv.Vet. Sci.* **7**: 273 pp.

Gilman, J. C. (1956). A Manual of Soil Fungi (Revised Second Edition). Oxford and IBH Publishing Co.

Gilman, J. C. and Barron, D. H. (1930). Effect of molds on temperature of stored grain. *Plant Physiol.* **5**:565 – 573.

Gilman, J. C. and Semeniuk, G. (1948). Mold mycoflora in stored grain and its role in deterioration process. Trans. Amer. Assoc. *Cereal Chem.* **6**: 108 – 112.

Goodman, J. J. and Christensen, C. M. (1952). Grain storage studies XI. Lipolytic activity of fungi isolates from stored corn. *Cereal Chem.* **29**: 299 – 308.

Gove, P. B. ed. (1965). "Webster's Seventh New Collegiate Dictionary". G and C. Merriam Co., Springfield, Illinois.

Govindaswamy, C.V. (1955). Studies on seed-borne fungi of rice. *Madras Agric.*J. **42**: 56-65.

Grewal, J. S. (1982). Control of important seed-borne pathogens of chickpea *(Cicer arietinum). Indian J. Genet. Plant Breed.* **42** (3): 393 – 398.

Grover, R. K. and Bansal, R. D. (1970). Seed-borne nature of *Colletotrichum capsici* in chilli seeds and its control by seed dressing fungicides. *Indian Phytopath.* **23:** 664 – 668.

Gupta, P. G. and Rana, V. P. (1983). Effect of culture filtrate of some selected seed mycoflora on seedling growth of solanaceous crops. *Seeds and Farms* **7** and **8**: 11 – 13.

Gupta, P. K., Gupta, J. S. and Deo, P. P. (1983). Seasonal variations in mycoflora of mung *(Phaseolus aureus)* seeds and pulses collected from Agra mandi (India). *Indian J. Agric. Res.* **17**: 42 – 46.

Hasija, S. K. (1970). Physiological studies of *Alternaria citri* and *A. tennuis. Mycologia* **62**: 289 – 295.

Hellwig, L.C. (1699). Kurzes send-Schreiben Wegen des so genanndten Honig-Tauses, Welcher Sich am heurigen kornsehen lassen, und die grossen schwarzen korner (ins gemein Mutter-korn genanndt) hervorbracht, was davon Zu halten, wovon esentstanden, und ob es nutzlich, order schadlich sey.J.C. Bachmann, Langen saltza.

Henry, A. W. (1931). The natural microflora of the soil in relation to the foot-rot problem of wheat. *Can. J. Res. Sect. C.* **4**: 69 – 77.

Henry, A.W. and Campbell, J.A. (1938). Inactivation of seed-borne plant pathogens in the soil, *Can.J.Res.Sect.C.* **16** 331 pp.

Herman, G. E., Khan,A.A and Tao, K. L. (1974). Physiological changes in the early stages of germination of pea seeds induced by ageing and by infection by a storage fungus, *Aspergillus rubber*. *Can. J. Bot.* **54**: 39 – 44.

Holman, L. E. and Carter, D. G. (1952). Soybean storage in farm type bins: a research report, III. *Agr. Expt. Sta. Bul.* 553, 449 – 496 pp.

ISTA. (1966). International Rules for Seed Testing. Proc. *Int. Seed Test. Ass.* **31** (1): 1 – 152.

Jalali, B. L., Singh, G. and Grover, R K. (1976). Role of phenolics in bacterial blight resistance in cotton. *Acta. Phytopath. Acad. Sci. Hung.* **11**: 81 – 83.

Janseen, W. (1989). A socioeconomic perspective on earlines in beans.pp.135-155.*In*: S.Beebe (ed.). Current topics in breeding of common bean. Working Document S.Beebe 47. Bean program, CIAT, Cali. Colombia.

Kandaswamy, D., Kesavan, R., Ramaswamy, K. and Prasad, M. N. (1974). Occurrence of microbial inhibition in the exudates of certain leguminous seeds. *Indian J. Microbiol.* **14**: 25 – 30.

Kapoor, A. S. and Singh, B. M. (1977). Influence of some environmental factors on spore germination and infection of rice by *Pyricularia oryzae*. *Indian Phytopath.* **30**: 369 – 373).

Kapoor, K.J. and Tandon R.N. (1970). Changes in protein bound amino acids to tomato fruits under parthenogenesis.*Curr.Sci*.39: 146.

Kaufman, H.H. (1964). Consideration in grain storage. Cargill crop Bull.8-10 (Jan.23, 1964).

Keeler, F. and Tu, A.T. (1983). Plant and fungal toxins, Marcel Dekkar, Inc. New York and Basel, 934 pp.

Khare, M. N., Mathur, S. B and Neergaard, P. (1977). A seedling symptom test for detection of *Septoria nodurum* in wheat seed. *Seed Sci. and Technol.* **5**: 613 – 617.

Kraft, J. M. (1973). Seed coat colour and resistance in peas to *Fusarium* and *Pythium* root rot II. (Abstr.). Inter. Congress, *Plant Pathology* 944 pp.

Kulik, M. M. and Schoen, J. F. (1982). Germination, vigor and field emergence of sweet corn (*Zea mays spp.* Mays) seeds infected by *Fusarium moniliforme. Seed Sci. Technol.* **10** (3): 595 – 604.

Kumar, A. and Jalali, B. L. (1985). Differential aspects of seed leachates on seed-borne pathogens associated with chickpea *(Cicer arietinum). Indian Phytopath.* **38** (1): 99 – 103.

Kumar, K. and Singh, J. (1985). Effect of fungicidal seed treatment, duration and types of container on viability of sesame during storage. *Indian J. Mycol. Pl. Pathol.* **13** (3): 354 – 356.

Kumar, S. M. and Nema, K. G. (1973). Role of fungi associated with wheat seeds. *Jawaharlal Nehru Krishi Vishwavidyalaya res. J.* **7 (4)**: 277: 279.

Lai, P. and Bruehl, G. W. (1968). Antagonism among *Cephalosporium gramineum, Trichoderma* spp. and *Fusarium and culmorum. Phytopathology* **58**: 562 – 566.

Laing, D.R, Jones, P.J. and Davis, J.H.C. (1984). Common bean (*Phaseolus vulgaris* L.), pp.305-351.In. P.R.Goldsworthy and N M.Fisher (eds.). *The physiology of tropical crops.* Wiley, New York.

Laing,D.R., Kretchmer, R.J., Zuluaga, S. and Jones, P.G. (1983). Field bean pp. 227-248. In: IIRI (ed.). Potential productivity of field crops under different environments. IRRI, Las Banos, Philippines.

Lal, S. P. and Kapoor, J. N. (1979). Succession of fungi in wheat and maize during storage. *Indian Phytopath.* **32**: 101- 104.

Lalithakumari, D., Govindaswamy, C. V. and Vidhyasekaran, P. (1971). Effect of seed-borne fungi on the physico-chemical properties of groundnut oil. *Indian Phytopath.* **24**: 283 – 289.

Last, F. T. and Deighton, F. C. (1965). The non-parasitic microflora on the surface of living leaves. *Trans. Brit. Mycol. Soc.* **48**: 83 – 99.

Laxminarayan, C., Sinha, S. K., Ahuja, S. S., Consul, S. K., Singh, N. N., Payak, M. N., Nene, Y. L. and Renfro, B. L. (1966). Fungicidal seed treatments of maize in India. *Indian Phytopath. Soc. Bull.* **3**:18 – 26.

Limonard, T. (1966). A modified blotter test for seed health. Neth. *J. Pl. Path.* **72 (6)**: 319 – 321.

Limonard, T. (1967). Bacterial antagonism in seed health test. Neth. *J. Pl. Path.* **73**:1

Limonard, T. (1968). Ecological aspects of seed health testing. *Proc. Int. Seed Test. Ass.* **33** (3): 1pp.

Long, D. W. (1963). Inhibition of *Fusarium* wilt symptoms in cowpea by species of *Cephalosporium. Phytopathology* **53**: 881.

Lopez, L. C. and Christensen, C. M. (1967). Effect of moisture content and temperature on invasion of stored corn by *Aspergillus flavus. Phytopathology* **57** (6): 588 – 590.

Lucy, M. L. and Bridgmon, G. H. (1962). Potato Dextrose Agar prepared from dehydrated potatoes. *Phytopathology* **52**: 173.

Maheswari, R. K., Mathur, S. K. and Mathur, A. (1984). Effect of seed-borne fungi on seed viability and seedling vigour. *Indian Phytopath.* **37**: 713 – 714.

Maheswari, R. K., Mathur, S. K. and Mathur, A. (1985). Relative capacity of six seed-borne fungi of lobia in deterioration of lobia seeds *(Vigna sinensis Savi.). Acto Bot. Indica* **13**: 221 – 223.

Majumder, S. K., Natarajan C. P., Narasimhan K. S., Viraktamath C. S,. Balakrishnan R, Nair, D. S. Bhatia and. Subramanyan, V. (1961). Studies on the storage of coffee beans *III.* Bulk storage in bins. *Food Sci. (India)* **10**: 326 – 331.

Malda, J. A., Salgado, A. L. B. and Razera, I.C.J. (1985). Storage conditions and their effects on ambari hemp seeds (*Hibiscus cannabinus*). *Pesqui Agropecu Bras* **20** (1): 1 – 14.

Mann, C. E. T. and Wallace, T. (1925). The Effect of leaching with cold water on the foliage of the apple. *J. Pomol. Hort. Sci.* **4**: 146 – 161.

Martin, J. P. (1950). Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* **69**: 215 – 235.

Mathur, R. L. and Sehgal, S. P. (1964). Fungal mycoflora of seeds of Jower *(Sorghum vulgare),* its role in reduced emergence and vigour of seedlings and control. *Indian Phytopath.* **17**: 227 – 233.

Mathur, S. C. and Hansing, E.D. (1962). Root and shoot development of wheat, morphological characteristics of winter wheat. *Phytopathology* **52**: 20 pp.

Mehan, V. K. and Chohan, J. S. (1973). Aflatoxin B_1 producing potential of isolates of *Aspergillus flavus Link.*, ex Fries. from cotton, maize and wheat. *Mycopath. Mycol. Appl.* **49**: 263 – 274.

Mehrotra, B. S. (1983). The impact of fungal infestation of cereal grains in field and storage. In: Recent Advances in Plant Pathology. (Eds. A. Hussain, K. Singh, B. P. Singh and V. P. Agnihotri) Print House, (India), Lucknow. pp. 185 – 200.

Milner, M. and Geddes, W. F. (1946). Grain storage studies. III. The relation between moisture content, mold growth and respiration of soybeans. *Cereal Chem.* **23**: 225.

Milner, M., Christensen, C. M. and Geddes, W. F. (1947). Grain storage studies. VI. Wheat respiration in relation to moisture content, mold growth, chemical deterioration and heating. *Cereal Chem.* **24**: 182 pp

Mishra, A. B., Singh, S. P. and Sharma, S. N. (1969). Seed-borne fungi in certain *Triticum durum* and *T.aestivum* their pathogenicity and chemical control. *J. Appl. Sci.* **1**: 5 pp

Mishra, R. R. and Kanaujia, R. S. (1973). Studies on certain aspects of seed-borne fungi II. Seed-borne fungi of certain oil seeds. *Indian Phytopath.* **26**: 284 – 294.

Mukhopadhaya, A.N. (1987). Biological control of soil-borne plant pathogen by *Trichoderma spp. Indian J.Mycol. Pl. Pathol* **17** (1): I-X.

Nabney, J. and Nesbitt, B. F. (1965). A spectrophotometric method for determining the aflatoxins. *Analyst* **90**: 155 – 160.

Nagel, C. M. and Semeniuk, G. (1947). Some mould induced changes in shelled corn. *Plant Physiol.* **22**: 20 – 33.

Naim, M. S., Afifi, A. F. and El-Gindy, A. A. (1976). Effect of seed and root exudates of some crops as well as their constituents singly, on the spore germination of some soil micromycetes. *Indian Phytopath.* **29**. 412 – 417.

Neergaard, P. (1977). Seed pathology. The MacMillan Press ltd. 1187 pp.

Neergaard, P. and Saad, A. (1962). Seed health testing of rice – A contribution to development of laboratory routine testing methods.

Nusrath, M. and Nandi, S. (1983). Occurrence of aflatoxins and other mycotoxins in cattle feeds from Hyderabad. *Indian Phytopath.* **36** (1): 106 – 109.

Omokanye, A.T. and Onifade, O.S (1993). Effect of storage and scarification time on seed germination of centro (*Centrosema pubescens*). Seed research.**21 (1)**: 21-25

Padmanabhan, S.Y. (1949). Occurrence of fungi inside rice kernals.*Curr.Sci.***18**: 442-443.

Panasenko, V. T. (1967). Ecology of microfungi. *Bot. Rev.* **33**: 189.

Pandey, K. N., Pande, B. C., Saha, M. and Gupta, R. C. (1982). Effect of fungal metabolites on germination and sprouting of seeds of *Setaria italica* grown in Almora hills. *Indian Phytopath.* **35**: 136 – 138.

Payak, M. M., Sharma, R. C. and Kandhari, J. (1978). Maize diseases in India. *Seeds and Farms* **5**: 30 – 34.

Peach, K. and Tracey, M. V. (1955). Modern methods of plant analysis Vol. II. Springer-Verlag, Berlin. pp. 20 – 41.

Prasad, T., Bilgrami, K. S., Roy, A. K. and Jamaluddin. (1976). Deterioration of mung seeds by *Curvularia lunata. Seed Res.* **4** (2): 191 – 193.

Qasem, S. A. and Christensen, C. M. (1958). Influence of moisture content, temperature and time on the deterioration of stored corn by fungi. *Phytopathology* **48**: 544 – 549.

Qasem, S. A. and Christensen, C. M. (1960). Influence of various factors on the deterioration of stored corn by fungi. *Phytopathology* **50**: 703 – 709.

Raghunathan, A.N.and Mazumdar, S K. (1968). Problems of fungal disorders in stored grains. *Indian phytopath.*Soc.Bull.**4**: 43-51.

Rai, B. and Singh, D. B. (1977). Effect of fungal metabolites on seed mycoflora and seed germination of mustard. Symposium on Physiology of Microorganisms. Today and Tomorrow's Printers and Publishers, New Delhi, 325 – 430 pp.

Randhawa, H. S. and Aulakh, K. S. (1984). Reduction in seed-borne fungi of rye *(Brassica juncea)* by *Chaetomium globosum and Eppicoccuum purpurascens. Indian Phytopath.* **37**: 140 – 141.

Raper, K. B. and Thom, C. (1949). *Manuals of the Penicillia.* The Williams and Wilkins Co., Baltimore. pp. 875.

Raper, K. B. and Thom, C. (1949). Manuals of the Penicillia. The Williams and Wilkins Co., Baltimore, 875 pp.

Rati, E. and Ramalingam, A. (1974). Effect of *Aspergillus flavus* on the germinating seeds of some tropical crop plants. *Indian Phytopath.* **27**: 579 – 582.

Rati, E. and Ramalingam, A. (1974). Effect of *Aspergillus flavus* on the germination of seeds of some tropical crop plants. *Indian Phytopath.* **27**: 579 – 582.

Reddy, A. S. and Reddy, S. M. (1983). Influence of seed moisture on fungal succession on seed of sesamum *(Sesamum indicum). Seed Res. (New Delhi)* **40**: 549 – 550.

Roy, A. K. and Choubey, N. (1984). Changes in protein contents of bitter–gourd at different relative humidities by two pathogenic fungi. *Indian Phytopath.* **37**: 179 – 181.

Sargeant, K., Sheridan, A., O'Kelly, J. and Carnaghan, R. B. A. (1961). Toxicity associated with certain samples of groundnuts. *Nature* **191**: 1096 – 1097.

Schneider, R.W., Thapliyal, P.N. and Sinclair, J.B. (1971). Fungi associated with soybean seed from Indian. *Indian phyyopath.* **24**:792-794.

Scneider, E., Dietrich, R., Martlbauer, E., Usleber, E. and Terplan,G. (1991). Detection of aflatoxins, trichothecens, ochratoxinA and Zearalenone by test strip enzyme immunoassay: a rapid method for screening cereals for mycotoxins. *Food Agic.Immunol.* **3**: 185 pp.

Scott, G. E. and Futrell, M. C. (1970). Response of maize seedlings to *Fusarium moniliforme* and toxic material extracted from this fungus. *Plant Dis. Reptr.* **54**: 483 – 486.

Sekhawat, P. S. and Kothari, K. L. (1971). Amino acid composition of healthy and downy mildewed plants of opium poppy (*Papaver somniferum*). *Indian Phytopath.* **24**: 403 – 405.

Sharma, A. D., Thakur, V. and Munjal, R. L. (1980). Seed mycoflora of *Amaranthus caudatus*, its pathology and control. *Indian Phytopath.* **33**: 242 – 244.

Sharma, A. K. and Sharma, K. D. (1984). Effect of relative humidity and temperature on the spoilage of *Foeniculum vulgare* seeds during storage. *Agric Sci. Dig.* **4** (1): 25 – 28.

Sharma, J. R. and Basuchaudhary, K. C. (1975). Assessment of seed mycoflora of pearl millet and their control. *Indian Phytopath.* **28** 388 – 390.

Sharma, K. D. (1973). Microbial deterioration of certain oil seeds and their oils. Ph.D. Thesis, Agra Univ., Agra.

Siddaramaiah, A. L., Desai, S. A. and Hedge, R. K. (1980). Seed microflora of castor *(Ricinus communic)* and its control. Mysore *J. Agric. Sci.* **14** (4): 500 – 502.

Singh, A. and Sinha, K. K. (1983). Biochemical changes and aflatoxin production in guava fruits by *Aspergillus flavus* and *A. parasiticus. Indian Phytopath.* **36**: 365 – 366.

Singh, B. P. and Tandon, R. N. (1970). Post infection changes in sugar and organic acid contents of orange fruits.

Singh, O. V., Agarwal, V. K. and Nene, Y. L. (1973). Seed health studies in soybean raised in the Nainital Terai. *Indian Phytopath.* **26**: 260 – 267.

Singh, P. N. and Gupta, K. (1984). Seed-borne fungi of *Medicago sativa* L. Effect of culture filtrates of some isolates on seed germination and root shoot growth. *Seed Res.* **12** (1): 123 – 127.

Singh, P. N., Sindhu, I. R. and Singhal, G. (1984). Effect of fungal metabolites on seed germination and seedling growth of some cucurbits. *Geobios.* **11** (5): 212- 216.

Singh, P. P. and Bedi, P. S. (1985). Variation in aflatoxin production in important varieties of wheat and Triticales. *Indian Phytopath.* **38**: 773 – 774.

Sinha, M. K. and Prasad, T. (1977). Deterioration of arhar seeds by *Aspergillus flavus. Indian Phytopath.* **30**: 70 – 72.

Sinha, R. K. and Prasad, T. (1981). Effect of fungal metabolites on seed germination, mycrobial associations and seedling growth of mung. *Indian Phytopath.* **34**: 515 – 517.

Skidmore, A. M. and Dickinson, C. A. (1976). Colony interactions and hyphal interference between *Septoria nodorum* and Phylloplane fungi. *Trans. Brit. Mycol. Soc.* **66**: 57 – 64.

Snell, F.D., Snell, C.T. and Snell, C.A. (1961). Calorimetric methods of analysis vol. IIIA. D-von Nostrand company INC., Princetown, New Jersey, New york.22pp.

Srivastava, R. N. and Gupta, J. S. (1980). Studies of seed mycoflora of Pancy (*Viola tricolor* L.) and their control. *Seeds and Farms* **9**: 21 – 55.

Srivastrava, R.N. and Gupta, J.S (1981). Seed mycoflora from seed lots of Cosmos bipinnatus and their control, *Indian Phytopath.***34**: 383-385.

Stack, M. E. and Pohland, A. E. (1975). Collaborative study of a method for chemical confirmation of the identity of aflatoxin J.A.O.A.C. **58**: 110 – 113.

Stevens, N.E. and Stevens, R.B. (1952). Disease in plants. Botanica Waltman, Massa., U.S.A. 219 pp.

Stewart, R.B. and Robertson, W. (1968). Fungus spores from prehistoric potsherds. *Mycologia* **60**:701.

Subbdaiah, P. V., Shetty and Safeeulla, R. M. (1982). Incidence of seed-borne fungi in maize (*Zea mays* L.) and their significance. *Indian J. Mycol.* **22** (1): 57 – 60.

Subramanian, C. V. (1971). Hypomycetes: an account of Indian species except *Cercospora.* I.C.A.R. Publication.

Tervet, I. W. (1945). The influence of fungi on storage, on seed viability and seedling vigour of soybeans. *Phytopathology* **35**: 3 – 5.

Thomas, F., Eppley, M. and Trucksess, M.W. (1975). Rapid screening method for aflatoxins and zearalone in corn. *J. Ass. Offi. Anal. Chemists* **58** (1): 114 – 116.

Tillet, M. (1755). Dissertation sur la cause qui corrmpt et noircit les grains de bled dans les epis; et sur les, moyems de prevenir ces accidens. P. Brun, Bordeaux. (Transl.byH.B. Humphrey, Amer. *Phytopathol.soc.Phytopathol.Classics* **5**:1-191 (1937).

Tripathi, R. K. (1974). Head fungi of sorghum, phytotoxins and their effects on seed germination. *Indian Phytopath.* **27** (4): 499 – 501.

Turner, W. B. (1971). Fungal metabolites Academic Press. London, New York, 446 pp.

Tveit, M. and Wood, R. K. S. (1955). The control of *Fusarium* blight in oat seedlings with antagonistic species of *Chaetomium*. *Ann. Alppl. Biol* **43**: 538 – 552.

Upadhyay, R. S. and Rai, B. (1987). Studies on antagonism between *Fusarium udum.* Butler and root region microflora of pigeon. *Plant and Soil* **101**: 79 – 93.

Uraguchi, K. and Yamazaki, M. (1978). Toxicity, biochemistry and pathology of Mycotoxins. John Wiley and Sons, New York 288 pp.

Vaidehi, B. K. and Ramarao, P. (1978). Fungi from three varieties of rice seeds. *Indian Phytopath.* **25**: 547–550.

Vidyasekaran, C. L., Subramanian, C. V. and Govindaswami, C. V. (1970). Production of toxins by seed-borne fungi and its role in paddy seed spoilage. *Indian Phytopath.* **23:** 518 – 525.

Vieira, R.F., de Faria, M.N., Ramos, J.A., de O Vieira, C., Dozeles, S.M.L and deFreitas R.T.F (1998). Seed germination of six-grain legumes during storage at room conditions in Vicosa, Minas Gerais state, Brazil. *Seed Sci. and Technol.* **26**:489-499.

Vijayalakshmi, M. and Rao, A. S. (1985). Fungal infection of sunflower seeds under different conditions of storage. *Indian Phytopath.* **38 (2)**: 315 – 318.

Wallace, H. A. H. and Sinha, R. N. (1962). Fungi associated with hot spots in farm stored grains. Canad. Jour. *Plant Sci.* **42**: 130 – 141.

Wang, S. Y. S. and Pickard, J. A. (1971). Composition of the surface of the cotton bolls in relation to spore germination and resistance of *Diplodia gossypina* infection. *Phytopathology* **61**: 915.

Warren, R. C. (1972). The effect of pollen on the fungal leaf microflora of *Beta vulgaris* L. and on infection of leaves by *Phoma betae. Neth. J. Pl. Path.* **78**: 89 – 98.

Weindling, R. (1934). Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology* **24** : 1153 – 1179.

Weindling, R. and Emerson, O. H. (1936). The isolation of a toxic substance from the culture filtrate of *Trichoderma. Phytopathology* **26**: 1068 – 1070.

Weindling, R. and Fawcett, H. S. (1936). Experiments in the control of *Rhizoctonia* damping-off of *citrus* seedlings. *Hilgardia* **10**. 1 – 16.

Yan , X., Lynch, J.P. and Beebe, S.E.(1995). Genetic variation for phosphorus efficiency of common bean in contrasting soil types: I. Vegetative response. *Crop Sci.*35: 1086-1093.