STUDIES ON SEED FUNGI OF MAIZE—BIOCHEMICAL CHANGES IN RELATION TO FUNGAL INFECTION

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C E R T I F I C A T E

I certify that the thesis entitled "Studies on seed fungi of maize - biochemical changes in relation to fungal infection" submitted by Mr. Makhan Chandra Paul for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

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(M.C. Paul)

C O N T E N T S

Introduction

Fungi and bacteria emerged with land plants from the Paleozoic seas more than 330 million years ago, and evolved in form and physiology as the land flora developed. With the appearance of the seed plants in the Jurassic and particularly after angiosperms became the dominant flora in the Crutaceous, atleast 130 million years ago, seed became the principal means of plant reproduction. Some fungal pathogens are thought (Buller, 1950) to have developed mechanisms for seed transmission about this time and it is probable that many other seed-pathogen relationships also developed very early. Because of the extremely long period during which the highly varied means of seed transmission of plants pathogens have evolved, host-parasite interactions are as expected both complex and effective.

Now it is an established fact that seeds play the key role in the introduction of plant pathogens into new areas. The pathogens remain viable till they find a specific host for infection and disease development. More new strains of the pathogens are being evolved in the nature, which are disseminated as host-specific pathogens and cause extensive damage to the plant population. The subject of seed pathology is, therefore, of concern to man in his unending quest for more and better food.

As past 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. Although plant pathogens were undoubtedly carried with the seed from the beginning, the earliest confirmed

record of this association is from Jarmo, about 8000 years ago (Stewart and Robertson, 1968).

The nine major important crops of the world are wheat, rice, maize, barley, sorghum, sugarbeet, commonbean, soybean and groundnut, which represent the greater part of the food production in the world. About 90 per cent of all food crops grown on earth are propagated by seed (Neergaard, 1977). Therefore, it is amply clear that the success of global food production for the ever increasing population of the world, greatly depends on the speed with which good quality seed of high yielding and disease resistant varieties are produced and used by the growers.

The importance of good quality seed has been realised by the ancient growers ever since the cultivation of crop was taken up systematically. But, even at the door step of the 21st century, one cannot be fully satisfied with the present status of seed research; its improvement and preservation in a proper scientific manner. Seed scientists and technologists all over the world have been engaged in developing technologies for better quality seed, and the success they achieved is evident from the spectacular growth of the seed industry.

From technological view point, the green revolution in India in the sixties and seventies, made considerable contribution in increasing agricultural production in the country. This increase was the result of various factors, one of the most important being the creation and development of an organised seed industry to support agricultural development. Since then the seed industry

has developed rapidly. Inspite of this, only about 20 per cent of the required seeds for cereals and pulses are produced and marketed by the organised public and private seed sector. The balance comes from traditional sources such as the part of the produced saved by the farmers as seed for sowing. Agricultural production can be improved if small and marginal farmers get good quality seeds of improved varieties and other vital input. The role of extension agencies in convincing the farmers about the advantages of using these seeds is important. Though agricultural universities, ICAR institutes and state departments of agricultural are trying to reach out to an increasing number of farmers, progress has been slow owing to the vastness of our country.

With the imposition of seed act, it has been made compulsory to sell seeds of certified quality only. The new seed policy of India, which has come into effect from October, 1988, has liberalised the import of seeds. It also envisages reduction in import duty on machines/equipments for seed production and processing, not manufactured in the country or for which technological upgradation is necessary.

Seeds carry a wide range of microorganisms externally or internally and these organisms become active at the advent of favourable conditions and cause extensive damage to the seeds and severe disease on crops raised from them. Amongst these microorganisms fungi play a significant role in determining the quality and longevity of the seeds (Christensen and Lopez, 1963;Christensen and Kaufman, 1968; Christensen and Mirocha,

1976). Therefore, in order to improve the agricultural products, seed health testing and use of healthy seeds are of great importance.

Exchange of seed and other progative plant materials at international level is very important in the present day agriculture to improve quality of crops and thereby augment food production. India being one of the major crop producing countries in the world is fully aware of it and for the last few years thousands of seed samples are being introduced from all over the world. Associated with these introductions the dangers of introducing some of the very serious plant pathogens hitherto not recorded from India as also more virulent strains or races of the known pathogens is quite possible. Even a single serious pathogens, if introduced along with imported seed, can set at rest the benefits of introducing high yielding varieties. This problem has led to the imposition of plant quarantine regulations. Plant quarantine legislation is currently in force in various countries and territories to prevent introduction of important seed-borne diseases and pests.

Studies of seed mycoflora have greatly increased in the recent past in view of their importance as disease carrier, as deteriorating agents and also as toxin producers. Deterioration of seed can 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). It can also be explained as an irreversible degenerative change in the quality of a seed after it has reached its maximum quality level during

seed development and maturation, and microorganisms involved with the seeds, are one of them.

At harvest seed lots contain much extraneous materials, most of which are removed by cleaning. However, certain microorganisms are not removed, and they cause or hasten seed deterioration. Saprophytic and parasitic seed-borne fungi e.g. *Alternaria, Chaetomium, Cladosporium, Fusarium, Helminthosporium, Rhizopus,* species of *Aspergillus* and *Penicillium* etc. remain dormant during seed storage unless seed moisture content increases greatly e.g. to over 14 per cent in cereals. These fungi, termed as "Storage fungi" by Christensen and Kaufman (1965) to differentiate them from "Field fungi", which are present in or on seeds prior to storage, can destroy stored seeds, because they can grow under limited moisture conditions where field fungi cannot grow. Certain moulds, not usually present in or on seeds at harvest, can carry out their life cycle on stored seeds. In fact many of these storage fungi are actually osmophillic and grow best under relatively dry conditions. These storage fungi can attack almost any kind of seed under favourable environmental condition since these mould can grow on most organic materials.

Invasion of seed by storage fungi may result in loss of viability, increase in free-fatty acid, decrease in non-reducing sugar, development of musty odours, and discolouration. Deterioration can occur in a few days when seeds are stored under unfavourable conditions. Species of *Aspergillus* and *Penicillium* cause maximum damage to the stored seeds which commonly occur throughout the world. These fungi invade and destroy seeds at 4-

45°C and 65-100 per cent relative humidity. Their activity is largely determined by the physical condition, vitality and moisture content of the seed and the ambient temperature and relative humidity of the storage area. Cereals seeds prove to be the most vulnerable for these fungi. *A.amstelodami,A.chevalieri, A.repens, A.restrictus* and *A.ruber* grow in seeds with a moisture content of 13.2 per cent. The major fungi found in cereal seeds above 15 per cent moisture content are *A.candidus, A.flavus, A.ochraceus, A.tamarii* and *A.versicolor* (Christensen, 1957). *Penicillium* spp. though not as common as *Aspergillus* spp. are often found in cereal seed, particularly in lots with a moisture content above 16 per cent and stored at relatively low temperatures (Christensen and Kaufman, 1965).

Saprophytic microorganisms on the seeds may partly or wholly decay the seed. This reduces the volume of storage tissue and the amount of stored materials available to the developing seedlings, thus increasing their susceptibility to diseases. Metabolic products of these microorganisms may also affect seedling growth. Finally when the seeds are planted, the saprophytes resume growth in the favourable condition of the soil, and may then decay the seed completely.

The mould fungi cause extensive damage to the seeds. The seedling emergence is severly reduced due to the decay of the seed before or after plantation. Seedling growth is affected by toxicity of metabolic products of the microorganisms and seed appearance is markedly affected. Storge of seed is effected due to rise in temperature by the microorganisms on it (Panasenko, 1967).

Utilisation of the seed as food is reduced because of toxic metabolic products from microorganisms growing on them. The carcinogenic aflatoxin from *Aspergillus flavus* is an example of this condition. Seed indexing to determine the pathogens present, or certification, is hampared markedly by the presence of saprophytic microflora, particularly when seeds are incubated to permit the pathogens to develop. These saprophytes on seed are considered to be one of the prime factors in variability in comparative seed tests for the presence of pathogens (Limonard, 1967, 1968).

Discolouration of the seeds occur due to the fungal association with the seed during storage (Christensen and Kaufman, 1969). Seeds which change colour show low germinability both in labortory and field. West and Harris (1963) evaluated some of the physiological and biochemical differences between normal and off-colour alfalfa seeds. They found that off colour seeds had lower respiration rates, lower rates of root-shoot growth, and higher shoot-root ratios than normal seeds. It has been found that discoloured seeds are more susceptible to fungal invasion than the normal seeds (Isely, 1957).

Discoloured seeds indicate the association of saprophytic and parasitic fungi and reflect their vitality and viability which are taken into consideration for grain consumption and industrial purposes. Although in many cases blemishes in seed have no real affect on the quality of seed for sowing, they usually depreciate its market value. White streaks in maize, often radiating from the embryo, have been found due to infection of *Fusarium*

moniliforme and *Cephalosporium acremonium*. Black streaks or specks beneath the pericarp of maize may be caused by *Physalosporazeae*, grey ear rot (Ullstrap, 1961).

Discolouration due to spores and mycelium of saprophytic fungi is another degrading factor of utmost importance. The disorder is caused sometimes by profuse growth of pathogens such as *Drechslera sorokiniana* in different cereals, *D.teres* in barley, *D. oryzae* in rice (Kondo and Okamura, 1927) as well as by common saprophytes such as *Alternaria tenuis* and *Cladosporium*, to a less extent by *Curvularia* spp. particularly in rice. This disorder because of shooty appearance, may depreciate their market value heavily.

Deep penetrating rots and necroses reduce the viability of seeds,their longevity in storage and emergence in field. Seeds with dormant mycelium in the embryo show less resistance to severe adverse field conditions such as excessively deep sowing or excessive high seeding rate, germination and seedling development being prevented (Mathur and Hansing, 1962).

Extensive growth of the stored fungi on the seed cause considerable increase in temperature. Temperature of stored cereal seeds with 18 per cent moisture content was raised from 17°C to 43°C due to the fungal infection (Gilman and Barron,1930). Wallace and Sinha (1962) found the fungi associated with the rise in temperature of seeds stored on the farm, and they were mainly species of *Aspergillus* and *Penicillium*. The temperature may rise even upto 53°C in winter. Christensen (1957)

stated that "heating is likely to be the final and violent effect of mould invasion of the seed, not an indication of beginning deterioration".

Every year considerable loss is being incurred due to fungal infection of seed both qualitatively and quantitatively. Both saprophytes and parasites lower the quality of seeds and seriously depreciate the commercial value of seeds,particularly of grain meant for consumption. The following types of diseases and disorders are encountered, often in combination : (i) seed abortion, (ii) shrunken seeds, reduced seed size, (iii) seed rot, (iv) sclerotisation of seed, (v) seed necroses, (vi) seed discolouration, (vii) reduction or elimination of germination capacity, (viii) physiological alteration in seed.

Logan (1974) has reported loss upto 50 per cent due to cob rot in some farms. *Fusarium moniliforme* is extremely widespread and in some countries, in seeds, particularly uncertified ones, infection may be 100 per cent as revealed by laboratory testing. In addition to stalk rot this pathogen causes seed rot, wilt and stunt, and may be difficult or impossible in the field to identify infected, including slightly stunted plants. There is little doubt that such infections make depreciation of yield difficult to estimate.

Deteriorated seeds show many biochemical changes in their constitution. Some of them are changes in respiratory and synthetic pathways, storage compounds and enzyme activity. But still the primary causes of seed deterioration, the sites and nature and sequence of biochemical reactions involved remain

unsolved. Efforts have been made to correlate biochemical changes with more visible manifestation of deterioration such as in germinability, seedling growth or yield.

Fungal infected deteriorated seeds show significant changes in major food reserves. Increase in acidity is one of the many changes that is associated with the deteriorated seeds (Milner and Geddes, 1946; Milner *et al.* 1947 and Sorger-Domenigg *et al.* 1955). Milner *et al.* (1947) showed that the increase in free fatty acid is due to the microorganisms associated with the deteriorated seeds. It has also been shown that increased fat acidity and loss of germination accompanied growth of moulds on wheat. They showed that when wheat seeds were stored in a nitrogen atmosphere which inhibited fungal growth, neither fat acidity nor germinability changed.

The development of fat acidity in seeds has been shown to accompany death. Germination declined 8 per cent and fat acidity increased 14 units when soybeans were stored at 8 to 9 per cent moisture content for 700 days. With increased seed moisture content, germination dropped rapidly and fat acidity increased sharply (Holman and Carter, 1952).

Maize is one of the most important cereals of the world. With its world average yield of 27 - 28 q/ha maize ranks first among cereals and is followed by rice, wheat and millets. In terms of world average, India's position is fifth, whereas with regard to production it ranks eleventh.

The name maize is derived from the South American Indian Arawak

name "mahiz". Maize is also known by the name "Indian corn" and in America simply as "corn". In India maize was introduced from America at the beginning of 17th century.

In India maize is an important cereal and it occupies the second place after rice. From tassel to root, the maize plant is valuable. The stalk, leaves, silk, cob and kernels all have commercial value,that of kernel being the largest. Maize is used mainly as a staple human food, a feed for live stock and as a raw material for many industrial products.

Both the area and production of maize in India have steadily increased during the past three decades. During 1985 - 1986, maize cultivation was done in 5.8 million hectares, with a production of 6.86 million tonnes. But in comparison to the world average yield, India's average is too low, which is only 11.72 q/ha.

Under the auspices of the all India co-ordinated maize improvement scheme, eleven high yielding hybrids and six composites have been released for cultivation in various regions of the country. Seeds of these hybrid and composite varieties are being multiplied and sold through the National Seed Corporation, the Tarai Development Corporation and various state agencies.

No crop is free from diseases, maize is subject to as many as 112 diseases on a global basis and in India 35 of them have been recorded. Different types of maize diseases are (i) seed - rot and seedling blight, (ii) foliar diseases, (iii) downy mildews, (iv) stalk rots and wilts, (v) rusts, (vi) smuts, (vii) cob and

ear rots. Other than these, moulds cause extensive damage to the maize seeds during storage. Payak *et al.* (1978) extensively studied the maize diseases in India. They reported that the seed rot and seedling diseases are caused by species of *Fusarium, Rhizoctonia, Aspergillus, Pythium, Cephalosporium* etc. The poor seeds show meagre germination, pre-emergence seed rot, post-emergence wilting and ultimately seedling collapse.

Losses from cornsmut are likely to occur wherever corn is grown. Greyish white galls that are filled with black spores are produced on various parts of the plant. As the plant matures, the gall membrane ruptures and the spores are exposed and diffused into the atmosphere, and ultimately to the soil. The spores remain viable for long time, germinate to produce sporidia which are ready to infect a fresh corn plant. It is essential to cut off the infected parts of the plant to minimise the infection. Chemicals commonly used to control other cereal smuts are not effective in the control of corn smut. Plant breeders have developed strains that have moderate resistance.

Corn rots are caused by a number of organisms and infect the corn plant at various stages of growth. Some of the most common are *Gibberella, Fusarium, Diplodia, Nigrospora. Gibberella* causes a seedling blight, and rots of stalk, ear and kernel. If the infection is early, they produce small ears with chaffy kernels. *Gibberella* ear rot is favoured by wet weather at silking time. Infection starts at the tip of the ear and proceeds downwards to the base.

Species of *Fusarium* cause extensive damage by ear rot. This

fungus frequently enters the ear, infected kernels develop pinkish discolouration on their caps. *F. moniliforme* is one of the most important pathogens of maize which causes ear rot, kernel rot, stalk rot and seedling blight. Mode of action of *F. moniliforme* is probably caused by a toxin which inhibited root growth (Futrell and Kilgore, 1969).

Dhanraj and Mathur (1965) reported that *Cephalosporium acremonium* causes ear rot of maize in Eastern India (West Bengal and Sikkim) which is found on both local and hybrid varieties. They found no external symptoms, but when husks were removed, white fluffy mycelium was revealed on the entire suface of the ears and was particularly dense in the grooves between the kernel rows.

Fungi associated with seed cause considerable damage to the seeds in various ways. In case of severe infections the quality of seed gets deteriorated and sometimes the grains are not suitable for human and animal consumption. The fungi involved produce metabolites that are poisonous, sometimes fatal to man and animal. Production of these mycotoxins depends on species or strain of the fungus and on the ecological conditions, in particular food source, temperature and humidity.

Aflatoxicosis, in man and animal, is caused by a series of toxins, including the most commonly encountered aflatoxin B_1, B_2, G_1 and G_2 which are produced by *Aspergillus flavus, A. parasiticus* and others. These fungi are found extensively on the stored seeds and are responsible for the poisonous "mouldy grain". Apparently healthy seeds may cause severe diseases in man

and animal due to the presence of toxins in them. The sudden outbreak of hepatitis in Rajasthan and Gujarat in October, 1974, affecting man and dogs was found to be due to the consumption of spoiled grains which were heavily contaminated with *Aspergillus* spp.

Bilgrami, *et al.* (1980) recorded changes in the qualitative and quantitative pattern of amino acids, organic acids, sugars, starch, protein, nitrogen and calorie value of maize seeds due to infestation with *Aspergillus parasiticus.* Recent studies also revealed the deterioration of seeds by storage fungi (Vijayalakshmi and Rao, 1985 and Meheswari and Mathur, 1985).

In view of the importance of seeds in the world food supply and the hazardous impact of seed-borne microorganisms on the health and quality of the seeds, seed pathology has become an important line of research in the agricultural development. Neergaard (1977) defined seed pathology as "the science and technology dealing with (i) seed-borne plant diseases, (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".

Maize is one of the most important staple crop of North-Eastern India. In terms of acreage of maize cultivation it comes after rice and wheat. During the year 1985-1986, 94.2 thousand hectares

of land was under maize cultivation, with a total production of 104.3 thousand tonnes. The average yield per hectare in the region is 1107 kg which is marginally lower than the national average. The agroclimatic condition of the region is suitable for maize cultivation. The yield could be substantially increased if high yielding hybrid / composite cultivars replace the local cultivars.

The Indian Council of Agricultural Research (ICAR) for the North-Eastern Region, with the headquater in Shillong, is doing important work in the selection of cultivars suitable for different altitudes of this region.

In this region the sowing period of maize is generally during last week of April to first week of May and harvesting is done in the month of August. The region being a hilly one, three types of agricultural systems are generally practised for the cultivation of maize. The systems commonly practised are slash and burn type of shifting agriculture (jhum), valley land and terrace land agriculture. Shifting agriculture locally known as "jhum" is extensively practised by the tribal people of the region.

In the states of North-Eastern India a few varieties of maize both local and improved ones are grown for human consumption. Much work has to be done to provide surveys of the incidence, distribution and intensity of seed-borne diseases which fluctuate in this respect from locality to locality and year to year. This kind of survey is indispensible for seed-borne diseases control and for the primary step to take decision as to what disease problems should be given priority in the development of research.

To examine the viability, intensity of resistance and susceptibility of different varieties of maize seeds against the seed-borne fungi, this research plan has been under taken and studies were carried out under the following heads:

1. Assessment of saprophytic and pathogenic seed-borne fungi of hybrid and local varieties of maize.
2. Effect of seed leachates on certain seed-borne fungi.
3. Effect of fungal extracts of dominant pathogenic and saprophytic fungi on seed germination and seedling growth of maize.
4. Effect of commonly used seed dressing agro-chemicals on the seed-borne mycoflora.
5. Studies on the production of aflatoxin in maize seeds.
6. Studies on the control of seed-borne disease using certain saprophytic fungi of seed and chemotherapic techniques.
7. Effect of fungal infection on nutritive value of maize seeds in terms of starch and lipid contents with dominant pathogenic fungi.

Review of Literature

Early planters must often had noted that certain lots of seed tended to produce a poor crop, and many have speculated how this came about. Fracastoro (1546) came close to this mark when he postulated "germs of contagion" that spread from apple to apple or grape to grape. The belief that seeds carry some harmful agents was strengthened through the works of Wellwig, Micheli, Needham and Tillet by the middle of the eighteenth century. These studies were very remarkable because they antedated the demonstration (Prevost, 1807) that fungi were able to infect a plant and cause disease, and disproof of the idea of spontaneous generation (Pasteur, 1861).

The first report of a pathogen being carried along with seed came from Hellwig (1699) who suggested floatation to seperate the ergot (*Claviceps purpurea*) of rye from the seeds. Micheli (1723) gave the first clear demonstration of seed transmission of a plant pathogen and the first instance of accompanying seed transport of a spermatophyte. Needham (1743, 1745) was the first worker to show the internal seed transport of a plant pathogen and positive demonstration of this type of seed transmission came 30 years later (Roffredi, 1775). Tillet (1755) gave the first demonstration of external seed transport and transmission of a plant pathogen, as he showed that the spores were carried in smut balls mixed with seed. The book "The diseases of the cultivated crops: their causes and their control" was the first published text by Kuhn (1858) (after Walker, 1969) in which fungi were regarded as casual factor in plant diseases. The author also

gave much attention to the improvement of seed treatment method for cereals.

Studies on seed mycoflora have greatly increased in the recent past in view of their importance as disease carriers, as deteriorating agents and also as toxin producers. There are quite a few reports on seed-mycoflora from various parts of the world (Cherewick 1954, Iizuka 1957, 1958, Neergaard and Saad, 1962, Christensen, 1969). Padmanabhan (1949, 1957), Govindaswami (1955) and many others have reported seed mycoflora from India.

Milner and Geddes (1946) demonstrated that spontaneous heating in soyabean, stored at humidity levels favourable to mould growth is directly and positively correlated with increase of respiration: this increase was associated with increase in growth of *Aspergillus glaucus* and *A.flavus*. The impact of storage fungi on the respiration and heating of grain has been substantiated by the works of Carter (1950) and Hummel *et al.* (1954) on wheat.

Though people became aware of the deleterious organisms associated with the seed and their role in seed spoilage, much work could not be done during the early part of this century due to the lack of adequate techniques. Subsequently, with the rapid development of new tools for investigation, the last quarter of century saw rapid increase in volume of new informations on plant diseases. There arose a great hope in disease control with the introduction of Bordeaux mixture in 1885 and growing attention was given to new fungicides for spraying of foliar and for treatment of seed.

Storage fungi affect the germinability of the seed as some of them invade the embryos. Field and King (1962) found that pea seeds infected with *Aspergillus* spp. (*A.flavus, A.candidus*, and *A.ruber*) lost germination capacity within six months, whereas uninfected seeds maintained a germination percentage of 95. Maize, wheat and sorghum retained a germination percentage of 90-95 throughout the experimental period of moisture content favourable to growth of storage fungi, whereas infected samples lost their germinability within a few weeks or months (Christensen and Lopez, 1963).

Christensen and Kaufmann (1965, 1969) made a distinction between two ecological categories of fungi that invade seeds, field fungi and storage fungi. Field fungi are those that invade seeds on the developing plants in the field, often in the maturing seed and after the crop is cut and swathed but before it is threshed. They may be pathogens or saprophytes. Common field fungi are *Alternaria tenuis, Cladosporium herbarum, Curvalaria* spp, *Eppicoccum purpurescens, Fusarium* spp., and in some seeds the pathogenic fungi *Fusarium moniliforme* and *Verticillium albo-atrum* (Malone and Muskett, 1964). Noble and Richardson (1968) studied the pathogenic fungi of seeds, reduction in germination and diseases of the seedlings or growing plants. The field fungi usually remain dormant during the storage period of the seeds, because these fungi cannot grow under conditions of less relative humidity. On the contrary the storage fungi are able to grow without free water and on media with a high osmotic pressure. Most of the storage fungi are species of *Aspergillus* and

Penicillium which are active at relative humidities ranging from 70 to 90 percent. It has been established that storage fungi usually do not attack the seed before harvest (Christensen and Kaufmann, 1969; Christensen, 1971), but they may be found on the seed in very low percentages, often below 1 per cent, thus providing for the presence of inoculum of storage fungi (Qasem and Christensen, 1958). Warnock and Preece (1971) found those storage fungi not only as contaminants but also as dormant mycelium within the tissues of pericarp or seed coat.

The specific composition of fungal flora in storage condition is highly dependent on the water content of the seed and very small variation in the moisture percentage may change the flora substantially, qualitatively as well as quantitatively. Christensen and Lopez (1963) worked extensively on the low limits of moisture content which would permit invasion of seeds of stored cereals such as wheat, barley and maize. There are five phases in the developing mycoflora in stored seeds (Clarke *et al.* 1967; Clarke, *et al.* 1969). In the first phase the predominant fungi are *Alternaria, Cladosporium* and *Fusarium.* These fungi decrease during the second phase and are replaced in the third by yeast, sometimes accompanied by *Penicillium roqueforti.* In the fourth phase typical storage fungi occur, and by spontaneous heating of the grain the growth of the thermotolerant and thermophillic fungi of the first phase is promoted such as *Absidia* spp., *Mucor pusillus* at about 30°-35°C, *Aspergillus fumigatus* at about 40°C and *Humicola lanuginosa* and *Micropolyspora faeni* at 50°-60°C (Mulinage and Chesters, 1970; Lacey, 1971).

Along with the development of modern agriculture, people started exchanging seeds from one country to another. Associated with these introductions the danger of introducing some of the very serious plant pathogens hitherto not recorded, started. An account of some of the economically important pathogens intercepted on imported seeds and seedling materials are available (Nath and Lambat, 1971; Kumar, 1984). Um, *et al.* (1983) isolated 25 fungal species from 27 seed samples from five countries in Korea. Muthaiyan *et al.* (1984) gave an account of fungi intercepted from 253 consignments of seed, plant and planting materials during 1982 and 1983 at the plant Quarantine and Fumigation station in Madras, India.

Seed Health Testings:

The International Seed Testing Association (ISTA) was initiated in 1921 in Copenhegan, Denmark and formally organised in 1924. But at that time it did not adopt a set of rules for testing seeds until 1931 (Anonymous, 1931). Minor revisions have been made at the triennial Congress and major revisions were made in 1953 and 1965 (Annomymous, 1966). Since 1953, each section of the International Rules has been designated either as prescription or recommendation.

Other than ISTA, there are some more organisations directly or indirectly related with the seed health testing of various crops, viz. Food and Agricultural Organisation (FAO), European and Mediterranean Plant Protection Commission (IAPSC), Plant Protection Committee for the South East Asia and Pacific Region

(SEAPRPC), Near - East Plant Protection Commission (NEPPC) etc.

Seeds are being tested under controlled laboratory conditions or on plants grown in the field for variety verification according to specific procedures. With available research data these procedures have been adjusted as much as possible to yield results that compare most favourably with field-planting value. Now uniform methods have been developed for determining seed quality, and when the work is done by trained personnel it must yield results with a high degree of accuracy regardless of where or when the tests are made (Linehan, 1960; Clark, 1961). Justice *et al.* (1952) published a comprehensive manual on testing agricultural and vegetable seeds, including identification of crop and weed seeds. Barton's "Bibliography of seeds" (1967) has been a great asset in seed research and planning. Much of the literature in English language, directly applicable to seed technology, has been published in (a) The proceedings of the Associations of Official Seed Analysts (USA and Canada) and (b) The proceedings of the International Seed Testing Association.

Owing to the great importance of seed-borne fungi, extensive work has been done in this regard in the recent past. In India there has been a growing interest in promoting the activities of seed health testing. A training course of seed pathology was organised in New Delhi in May 1971, a symposium on seed pathology in Nagpur in January 1974, and a Summer School on Seed Pathology, plant quarantine amd storage in Bangalore during March to April, 1974 (Neergaard, 1975). Agarwal (1976) reviewed the progress of seed testing works in India and reported that the total number of

samples tested has grown from 6,000 in 1962 to 2,00,000 by 1975.

Different methods of seed health testing have been studied by various workers and their efficacy and reliability is recorded. Blotter method is one of the most convenient and efficient method of seed health testing. Doyer (1938), a pioneer in seed pathology research, was the first to adopt blotter method in seed health testing. Later on, the usual blotter method has been modified by various workers according to their needs. Some such methods are: rolled paper towel method, 2,4-D method; deep freezing method. Neergaard (1956) was the first to introduce the use of 2,4-D (the common herbicide) in the blotter test, which retards seed germination and the growth of the seedling, as a result the seeds are not displaced and recording of fungi occuring on the seeds became easier. Limonard (1968) was the first to use deep-freezing in blotter method. Deep-freezing kills the seed and its nutrients are released for the fungi to grow on them. Neergaard and Saad (1962) concluded from their study on the comparative methods of seed health testing that the blotter and the agar plating methods are both equally valuable and supplementary to each other. In a comparison of four laboratory methods for the detection of fungi, de - Tempe (1962) found that the blotter test in light gives the most reliable results. Agarwal *et al.* (1972) concluded that the blotter method proved better than the agar plate method for most of the fungi. Singh *et al.* (1974) in evaluating the efficacy of seed health testing techniques, concluded that the deep-freezing method of blotter technique is the most suitable for routine seed-health testing. Many other workers (Lima *et al.* 1982; Jorgensen, 1982;

Paiva *et al.* 1984) found that deep freezing method is reliable for routine seed health testing.

Seed Mycoflora:

Mycoflora on seed vary according to the mode of storage. There are many factors which determine the occurrence and activity of mycoflora on the seed. Amongst these, temperature, moisture content and relative humidity and oxygen availability are most important (Nagel and Sameniuk, 1947). A moisture level of about 10-15% in the grain, relative humidity above 75% and a temperature range of 25°-30°C with oxygen concentration of about 1% seem to favour mould growth on the seed (Milner *et al.* 1947; Christensen and Gordon, 1948).

The involvement of fungi in seed spoilage is well established. Some classes or genera are frequent in seed, others occur only occasionally and some are not found at all. Again some fungi are easily detected, others do occur but cannot be revealed by conventional testing procedures. The extent to which fungi occur in seeds depends on their capability to survive under extreme dry conditions of seed as a carrier. To survive in seed, most fungi must be able to endure dehydration, yet there are two ecologically distinct groups of fungi forming a contrast regarding survival and longevity in seed : hydrophillic fungi and xerophillic fungi. Most seed borne-fungi are either Fungi Imperfecti or Ascomycetes that occur on seed in their asexual state. Consequently, these fungi are usually identified on seed in the imperfect state.

Extensive work has been done on seed mycoflora throughout the world. Apart from the seeds having visible symptoms, almost all seeds which look apparently healthy also contain a rich fungal flora (Padmanabhan, 1949). It is very difficult to find seeds without any mycoflora (Rahugunathan and Majumdar, 1968). After studying the interaction of grain moulds, Delhate (1968) has stated that the first coloniser keeps its dominant position; the weak ones susceptible to inhibition are short lived and are replaced by the strong or the dominant ones. Christensen (1969) has pointed out that the shifting predominance of any one species over other occuring on the kernels depends on the interaction of unknown ecological factors. Much of the work on seed mycoflora has been done on the cereals and more than 150 fungal species have been listed from the cereal grains alone (Christensen and Kaufmann, 1969). Jain and Patel (1969) screened castor seed for external and internally seed-borne fungi using both agar plate and the blotter method. They found that 37% of the surface sterilised seeds were internally contaminated, *Alternaria*, *Curvularia* and *Aspergillus* spp. being the predominant ones. According to them both the agar plate and blotter methods are supplementary to one another. *Penicillium*, *Rhizopus*, *Nigrospora* and *Hormiscium* were detected by agar plate method but not by sterilised blotter technique, and reverse was true to *Stachybotrys*, *Memnoniella*, *Chaetomium* and *Stemphylium*.

Gupta and Chohan (1970) studied the surface and internally borne seed mycoflora of groundnut collected from different places of Punjab (India). Schneider *et al.* (1971) found eight new genera

of fungi not previously reported to be associated with soybean seed in India. The new fungi recorded from surface sterilised soybean seeds were species of *Pythium, Botrytis, Cladosporium* and *Chaetophoma* and the other four fungi from non-sterilised soybean seeds were *Acremonium, Diplodia, Monilia* and *Trichothecium*. Vaidehi and Ramarao (1972) collected rice samples from All India Coordinated Rice Improvement Project, Hyderabad (India) to study the mycoflora associated with them. The important field fungi isolated were *Curvularia, Helminthosporium, Fusarium, Nigrospora, Trichocorus, Alternaria, Cladosporium*. Khare *et al.* (1972) screened Khesari *(Lathyrus sativus)* for external and internal seed-borne fungi and isolated *Alternaria, Aspergillus, Chaetomium, Colletotrichum, Curvularia, Fusarium, Helminthosporium* and *Mucor*. Out of these *Alternaria, Colletotrichum* and *Fusarium* were found to be more harmful causing seed and seedling rot.

Singh *et al.* (1973) reported 19 fungal species from soybean seed. According to them few fungal species were recorded for the first time occuring on soybean seed, viz. *Alternaria tenuis, Aspergillus niger, A. tamaril, Chaetomium brassiliense, C. erectum, Penicillium cyclopium, Rhizopus arrhizus* and *Trichoderma* spp. A concise list of host and pathogens that have been studied in the International Comparative Seed Health Testing Schemes during the period from 1958 to 1974 and in the 14th International Workshop on Seed Pathology connected with these scheme is given by Neergaard (1975). Sharma and Basuchoudhury (1975) studied the seed mycoflora of 7 seed samples of pearl-millet in the Varanasi Region of India. They have isolated a total of 15 fungal species

and *Aspergillus flavus* was found to be the most common species and was isolated from almost all the seed samples varying in percentage from 2.5 to 36.5. Studies conducted in different parts of the world reveal that Safflower *(Carthamus tincotrious* L.) seed harbours various fungi (Ali *et al.* 1979) which bring about changes in its oil, protein and carbohydrate contents (Singh *et al.* 1974). Kanaujia and Singh (1975) studied the effect of moisture content on the occurrance of fungi on some stored seeds, *Pennisetum tryphoides, Echinochola crussgali, Setaria glauca* and *Avena sativa* were the common fungi isolated during the years 1970 and 1971. They found that the higher moisture level of the seeds caused appreciable reduction in the seed germination and thereby causing higher infection percentage in these seeds. The dominance of the Deuteromycetes especially aspergilli may be due to their wide range of distribution and greater tolerance to various limiting factors.

Sharma *et al.* (1980) isolated 25 fungal species from *Amaranthus caudatus* seed. Agarwal *et al.* (1972) studied the status of seed health of rice, wheat, blackgram, greengram and soyabean grown in India. They isolated a large number of fungi from the seed, among the common ones were *Alternaria, Cephalosporium, Cercospora, Drechslera, Fusarium, Phoma* etc. Recent studies have revealed that *Helminthosporium nodulosum, Fusarium solani, Curvularia lunata* and *Sclerotium bataticola* affected the germination of safflower seed. Srivastava and Gupta (1982) collected seed samples of cosmos from 5 different localities of India and studied fungi occuring on them. They could find a

total of 19 and 22 fungi at low (20 ± 2°C) and high (28 ± 2°C) temperatures respectively. Their number in individual samples ranged between 10-19. Out of these *A.flavus, A.niger, A.sydowii, Alternaria alternata, Chaetomium* spp., *Cladosporium* spp., *Drechslera* spp., *Fusarium equiseti, F. oxysporum* and *Myrothecium verucaria* were intercepted from all the seed samples without any exception. Basak and Mridha (1983) studied the seed mycoflora of rice collected from different locations of Bangladesh. The fungi commonly occuring on those seed samples were species of *Alternaria, Aspergillus, Chaetomium, Drechslera, Penicillium, Curvularia, Fusarium, Nigrospora, Trichoconis* and *Rhizopus*. There was a variation in the percentage incidence of fungi in rainy, winter and summer seasons as reported by Gupta *et al.* (1983) from a study of mycoflora of mung seed. They found maximum average percengage incidence of fungi in the rainy season and least in the summer. Kumar *et al.* (1983) found eight fungal species associated with the bold and apparently healthy looking seeds of table pea. Shrivelled and abnormal looking seeds carried five more fungal species in addition to those present on healthy looking seeds. *A.flavus, F.oxysporum* and *F.solani* were the first predominant fungal species amongst the fungi isolated. Abou-Heilah (1984) collected seeds of 12 locally cultivated varieties of wheat from different areas of Saudi Arabia and studied the mycoflora associated. He could isolate 12 fungal species by blotter method and 19 fungal species by agar plate method. The most common fungi present on these varieties were species of *Alternaria, Aspergillus, Drechslera, Chaetomium* and *Ullocladium atrium*. Association of various mycoflora of wheat

seed has been reported by workers from all over the world (Bhowmik, 1969; Flannigan, 1970 and 1978, Nath Ram and Lambat, 1971; Agarwal *et al.* 1972; Bateman, 1979).

In a comprehensive survey of fungi associated with food grains stored in silos in Iraq, Sulaiman and Husain (1985) isolated 28 species of fungi belonging to storage moulds and 33 species of field fungi. They found that species of *Aspergillus* and *Penicillium* were the most dominant ones. Singh and Singh (1986) reported 27 fungal types and 2 sterile forms from the seeds of broad beans collected from different markets of Imphal and its suburbs (India). They found that the seed-borne fungi isolated affect germinability of the seed. The common fungi isolated were *Rhizopus, Mucor*, species of *Chaetomium, Aspergillus, Penicillium, Cladosporium, Alternaria, Fusarium*, etc. Bhadraiah and Ramarao (1987), studied mycoflora associated with stored sorghum seed of rabi and kharif season using dilution plate, agar plate and blotter method of isolation. They isolated more number of fungal species from kharif than rabi seed. Of the 52 fungal species isolated from sorghum seeds, *Alternaria alternata, Aspergillus flavus, A. niger, A. sydowii, A. terreus, Curvularia lunata, C. pallescens, Penicillium purpurrescens, P.italicum* and *Fusarium solani* were dominant. They also noticed a decline in field fungi and increase in storage fungi during the storage.

Maize seed mycoflora:

During a disease survey of maize in 1964, Dhanraj and Mathur (1965) found maize ear rot caused by *Cephalosporium acremonium* in West Bengal and Sikkim (India) on both local and hybrid

varieties; when the husks were removed white fluffy mycelium was found on the entire surface of some of the ears and was particularly dense in the grooves between the kernal rows. Cahagnier and Poison (1974) studied the mycoflora of maize grains in relation to various methods of storage and concluded that none of the storage methods prevented the development of microorganisms. However, the choice of storage will determine the composition of the mycoflora. Bhatt *et al.* (1977) reported species of *Alternaria, Aspergillus, Drechslera, Fusarium, Nigrospora, Penicillium, Rhizopus, Trichothecium* as contaminants of corn from Rajasthan (India). Vaidehi and Ramarao (1978) studied the mycoflora associated with five samples of maize seeds collected from two districts of Andhra Pradesh (India) and found that the fungal members vary from sample to sample. Of the 20 species isolated, *Aspergillus* and *Pencillium* were the most dominant, each being represented by 6 species. *Aspergillus flavus, A. niger, A. terreus,* and *Penicillium rubrum* were common to all the five samples. Payek *et al.* (1978) made an elaborate study on the maize diseases in India. They found that maize seed rot and seedling disease are incited by species of *Fusarium, Rhizoctonia, Aspergillus, Pythium, Cephalosporium* etc. and when the infected seeds are sown, they produce poor crop, pre-emergence seed rot, post-emergence "damping off" collar rot and collapse of seedlings.

Pelhate (1981) studied the mycoflora of unhusked maize seed and classified the seed-borne fungi as field fungi *(Fusarium* spp. and *Eppicoccum purpurescens),* intermediate fungi (*Cladosporium* spp., Mucorales and *Trichoderma* spp.) and storage fungi (*Penicillium*

spp.). There are many other reports on the seed mycoflora of maize from various parts of the world (El-kady *et al.* 1982; Singh and T. Ji, 1980; Christensen, 1980; Salgodo and Carvalho, 1980; Ilag, 1973; Kanaujia, 1974; Kim *et al.* 1984). Handoo and Aulakh (1982) studied the seed mycoflora of 34 maize samples collected from 13 different agroclimatic regions of India using the washing test method. *Alternaria alternata, Curvularia lunata, C.pallescens,* species of *Drechslera* and *Fusarium* were some of the predominant fungi recorded from various samples, Subbaiah *et al.* (1982) recorded the seed mycoflora of maize of local and hybrid varieties from Mysore (India) using different methods of seed health testing. They could isolate 29 fungal species representing 15 genera on 18 samples of 5 different cultivars. *Alternaria tenuis, Aspergillus flavus, A.niger, A. sydowii, Cephalosporium acremonium, Curvularia lunata, Drechslera hawaiiensis, D. rostrata, Fusarium moniliforme, F. semitectum, Penicillium* spp. and *Trichothecium roseum* occured commonly on seeds of all the cultivars.

Bedi and Jhooty (1986) collected 4 lots of maize grains, each from 6 cultivars from different locations in Punjab and studied the incidence of seed-borne *Aspergillus flavus* and resistance to its invasion in maize cultivars. Singh *et al.* (1987) made a systematic study on the detection and survey of seed-borne mycoflora of corn in tribal localities of Rajasthan. When the dry seeds were inspected they could find seeds with (i) black streaks (ii) black discolouration (iii) golden brown streaks (iv) microsclerotia and (v) fungal spores lodged in basal caps. In

the incubation test they observed 51 parasitic and saprophytic fungal species. *Botryodiplodia theobromae, Cephalosporium acremonium, Drechslera maydis, Fusarium moniliforme, Nigrospora oryzae,* and *Trichothecium roseum* were the important pathogenic fungi. Dominant saprophytes were species of *Aspergillus, Curvularia, Penicillium, Paecilomyces, Rhizopus* and *Trichothecium.*

Seed leachates: Mann and Wallace (1925) used the term "leaching" for the first time to describe the removal of mineral nutrients from leaves soaked in water. Leachates contain components available as nutrients by microorganisms and may also contain substances which inhibit their germination and growth. They are of considerable importance to the microbial population of plant surfaces (Last and Deighton, 1965). It has been proved by many workers that deteriorated seeds leach out more materials than of vigorous and sound seeds (Ching and Schoolcraft, 1968; Takayanagi and Murakami, 1968; Abdul-Baki and Anderson 1970; Bradnock and Mathews 1970). The seed coat plays an important role in regulating the physiological activity of the seed mycoflora as it is the common site of infection by the microorganisms (Ark and Thomson, 1958; Srivastava and Mishra, 1971; Mishra and Kanaujia, 1973; Charya and Reddy, 1980). Protein degradation of the host tissues has been observed to take place following infection by different fungi (Jalali and Suryanarayana, 1971, 1972), which may partially explain the susceptible host cultivars. Mishra and Kanaujia (1973) reported that seed leachates of 7 oil crops inhibited the conidial germination of *Rhizopus nigricans, Mucor hiemalis, Chanephora cucurbitarum, Aspergillus flavus,*

Penicillium spp., *Curvularia lunata* and *Fusarium nivale*. Chaturvedi *et al.* (1974) studied the cumin seed leachates on fungal spore germination and found that two types of substances are leached out of cumin seed at different timings. In the beginning an inhibitory substance(s) and later on between five and seven hours another substance which appear to act as promoter of spore germination. They also found that with the increase in dilution the inhibitory effect diminished and at the lowest concentration the spore germination was significantly not different from that of control in case of *Curvularia lunata* and *Alternaria solani*. In the presence of inhibitor the protrusion of the germ tube was high but the tips become bulbous and deformed, they observed. Regarding the possible cause of reduction in spore germination of various fungi by seed leachates, it appears that some chemical components of the leachates interfere with the spore germination (Kandaswami *et al.* 1974). Kuc (1966); Kraft (1973) and Jalali *et al.* (1976) observed that phenolic compounds present in the leachates impart general resistance in higher plants to the parasitic and bacterial invasion. Dhawale and Kodmelwar (1978) also observed reduction in the spore germination of various fungi induced by seed leachates of chilli.

Naim *et al.* (1976) studied the role of seed and root exudates of cotton, maize and broad bean, as well as their constituents each singly, on spore germination of *Fusarium solani*, *Alternaria alternata*, *Trichoderma aureoviride* and *Humicola fuscoatra*. In general seed and root exudates of the experimental plants

stimulated spore germination of the above mentioned fungi differently. Different effects may be attributed to the variations in amino acids, sugars and growth regulators present in the exudates. According to them the detection of growth promoters in the experimental exudates indicated the presence of substances of indole nature, as well as gibberellin and gibberelline like substances. The substances exerted stimulatory effect on the fungal spore germination. Interaction between abiotic and biotic environment is mediated to a larger extent by the occurrence of seed leachates in relation to seed-borne diseases (Takayanagi and Murakami, 1968).

Leachates obtained after different durations of soaking produced both inhibitory and stimulatory effects on the spore germination of the seed mycoflora (Saxena and Gupta, 1982). They recorded that the leachates of *Vigna* seed retarded the spore germination of *Aspergillus flavus, Alternaria alternata, Ascotricha chartarum, Colletotrichum* spp. and *Trichothecium roseum.* The stimulation of the spore germination of the seed fungi was found to be 1.9 to 16.3%. They could find 6 sugars, 12 amino acids and 5 organic acids in the leachates of *Vigna* seed.

Ghewande *et al.* (1984) studied the spore germination of *Aspergillus niger* and *A.flavus* in seed coat leachates of 36 groundnut varieties. They found that some of the leachates were inhibitory while others were stimulatory. Maximum inhibition of *A.niger* (89.7%) and *A.flavus* (74.4%) was observed in seed coat leachates of Jyoti and TMV-12 variety respectively. Kumar and Jalali (1985) noted certain variations between resistant and

susceptible varieties of chick pea in the inhibitory influence of spore germination. According to them the organic components of the seed leachates play an important role in imparting resistance or susceptibility to the host plants. The concentration gradients of these organic factors in host tissues have been considered to play a definite role in conferring disease resistance.

Fungal extract on seed germination and seedling growth:

Most of the mycoflora associated with the seeds of crop plants produce metabolites which are hazardous for the seed health as well as for the health of the consumers. The fungal metabolites are of considerable importance in reducing seed germination and sprouting (Humphreys-Jones and Waid, 1963; Christensen and Kaufmann, 1965; Lalithakumari *et al.* 1972; Dwivedi and Singh, 1973; Dublish and Pande, 1976; Rai and Singh, 1977; Pande *et al.* 1982). Diener and Davis (1966) and Harman and Pfleger (1974) have studied the role of microorganisms on plant growth and found that the secretions of various fungi are toxic to the plants. Seed-borne fungi of rice play a significant role in the deterioration of stored seed as well as cause extensive damage (Padmanabhan, 1949, 1957, 1974; Govindaswami, 1955; Vidyasekaran *et al.* 1970; Vaidehi and Ramarao, 1972; Bedi and Dhaliwal, 1970).

Seed germination and seedling growth of various crops undergo inhibition and stimulation due to the involvement of microorganisms as they secrete some toxic materials or phytohormones as well as due to the ability of roots to absorb various nutrients from the soil and removal of germination

inhibitors supposed to be present in the seed coat (Gibson, 1953; Humphreys-Jones and Waid, 1963; Subramanian *et al.*, 1964; Reddy and Bagyraj, 1969; Kamal and Sinha, 1974).

Tervet (1945) found the increased seed germinability of soybean with the culture filtrate of *A.fumigatus*. Vishunavat and Shukla (1981) found similar results with lentil seed.

Armolik *et al.* (1965) studied the role of microorganisms in the deterioration of stored barley seeds and noticed that *Fusarium moniliforme* reduced the germinability more rapidly than the species of *Aspergillus* and *Penicillium*. Scott and Futrell (1970) reported that *F.moniliforme* toxin is toxic to maize and they found it to be heat stable. Similar observations were made by Tripathi (1974) who found that toxins produced by *F.moniliforme* and *Colletotrichum graminicola* which inhibited sorghum seed germination is thermostable and therefore not proteinaceous in nature. Tripathi (1973, 1974) reported the inhibition of seed germination to varying degrees by the head moulds of sorghum. Bhagavat and Pedgoankar (1973) reported the loss in viability of sorghum seeds due to the activity of *A.niger, F. moniliforme, Helminthosporium tetramera* and *Curvularia lunata*. Similarly toxins of the seed-borne fungi are reported to reduce the seed germination, root-shoot elongation in soybean (Anahosur and Bidari, 1974), Paddy (Vidyasekaran *et al,* 1970), and in oil seed (Mishra and Kanaujia, 1973). Mishra and Kanaujia (1973) observed remarkable decrease in the germinability and root-shoot elongation of some oil seeds due to the effect of the culture filtrate of *A.flavus*. Sinha and Prasad (1981) prepared the

culture filtrate of the mycoflora of mung seed and studied its effect on seed germination which was found to be inhibitory. Gupta and Rana (1983) found that the culture filtrates of *A.niger, A. flavus, A. sydowii, Alternaria tenuis, Curvalaria lunata* and *Memnomiella echniata* inhibited root growth in some solanaceous crops. Singh *et al.* (1984) found that the culture filtrates of the fungi *Aspergillus nidulans, Chaetomium globosum, Drechslera australiensis, Myrothecium roridum* and *Trichoderma viride* had an adverse effect on germination percentage and seedling growth of the cucurbits (*Luffa cylindrica, L. acutangula* and *Citrullus vulgaris*). They observed maximum inhibition of 68.5% with *C.vulgaris*, 83% with *L.cylindrica* and 81.3% with *L.acutangula* by the metabolites of *T.viride*. Similar observations have been made by many workers (Humphreys-Jones and Waid, 1963; Dwivedi and Singh, 1973; Anahosur, 1976; Yeh and Sinclair, 1980; Hepperly and Sinclair, 1981). The maximum inhibition by *T.viride* observed by Singh *et al.*, (1984) may be due to trichodermine secreted by the fungus, which is toxic and harmful to the seed germination (Humphreys-Jones and Waid, 1963; Dennis and Webster, 1971; Kamal and Verma, 1979). Singh *et al.*, (1984) also observed that the test fungi inhibited root and shoot elongation of the seedlings. Singh and Gupta (1984) studied the effect of culture filtrates of some seed-borne fungi of *Medicago sativa* L. on the seed germination and root-shoot growth. They found *Fusarium moniliforme, T.viride* and *T.roseum* to be severe seed-borne pathogens as they produced upto 25.1% seedling mortality. The maximum reduction in germination percentage of seed was observed in 30 day-old and boiled culture filtrate of

F.moniliforme and minimum reduction was observed in the culture filtrate of *Drechslera halodes*. Similar observations in other crop seeds with different seed-borne fungi have also been reported by Kanta (1982). Maheswari *et al.* (1984) reported that the culture filtrates of *Aspergillus flavus, A.fumigatus, A.nidulans, A. niger, A. terreus* and *F.moniliforme* adversely affected the seed germination, root-shoot length to varying degrees. The metabolites of *A.nidulans* were most effective in inhibiting the seed germination by 38.76%, root length by 82.8% and checked the plumule completely. Singh (1984) reported increased root-shoot length of wheat seedlings by the culture filtrates of *Penicillium chermesinum, Aspergillus amstelodami* and *Penicillium oxalicum*.

Singh *et al.* (1986) found maximum reduction in soybean seed germination by the culture filtrate of *Fusarium semitectum* (44.72%) followed by *F.equiseti* (51.1%) and *Curvularia lunata* (62.5%). They have recorded a substantial increase in seed germinability in the culture filtrates of *Alternaria alternata, Aspergillus flavus, Penicillium* spp., and *A.niger*, root length was incresed by the culture filtrate of *Curvularia lunata, Alternaria alternata* and decreased by *Penicillium* spp., *Fusarium equiseti, F. semitectum, A. niger* but all the fungi reduced the shoot length significantly.

Kumar *et al.* (1987) stated that the culture filtrate of *Fusarium* showed differential effect on germination of 4 cultivars of maize seeds; the culture filtrate showed considerable inhibition in seed germination and root-shoot growth. The seedlings showed

characteristic browning symptoms with stunted growth and a total collapse of the seedlings within 20 days.

Control measures:

A programme of spraying or dusting the seed crops is directed against three major groups of pests: fungi, bacteria and virus vectors. The primary incentive is, of course, to avoid yield loss and thus to be of direct benefit to the farmers. Control measures received impetus with the introduction of Bordeaux mixture in 1885 and growing attention was given to new fungicides for spraying of foliage and for treatment of seed. Copper carbonate was the first dry-treating material used in the control of wheat smut (Darnell-Smith, 1910, 1915, 1917). Modern era of fungicide and seed treatment started with the introduction of the organic mercury compounds. Since World War II there has been a vigorous development of non-mercurial organic fungicides applicable to both seed and growing plants. The first fungicides of this type appeared after 1930's when, in 1934,Tisdale and Williams took out U.S. patent rights for thiram, a derivative of dithiocarbamic acid. Then followed ferbam and later zineb. Other organic compounds from this period are chloranil and dichlone, captan and various polychlorobenzenes.

Now hundreds of compounds are available for crop protection as sprays, dusts, fumigants, systemics and other categories of remedies. Reference is made to descriptions and discussions of this variety of chemicals and their effects as protectants or eradicants in handbooks, monographs and reviews such as those published by Horsfall (1945, 1956); Horsfall and Dimond (1951);

Sharvelle (1961); Rose (1963); Torgeson (1967, 1969); Commonwealth Mycological Institute (1968); Nene (1971) and Martin (1972, 1973).

Hoppe (1957) got good control of *C.acremonium* and *F.moniliforme* using captan at lower doses. Thiram has been mentioned by many workers to give excellent control of *C.acremonium* and *F.moniliforme* with seed treatment (Olimpieva, 1964; Fahim *et al.* (1970).

Tarr (1958) reported that thiram and agrosan GN were found suitable fungicides against *Aspergillus niger, A.flavus* and *Rhizopus* spp. Sreekantiah and Mathur (1961) have observed the effectiveness of organomercurial fungicides against the pea seed-fungi. Dharam vir *et al.* (1970) reported that seed treatment with dithane M-45 at 0.3% of seed weight gave complete control of seed-borne infection of *Drechslera sativa, D. oryzae* and *D.avenae* in barley, rice and oats respectively, and seed dressed with dithane M-45 gave higher germination counts as compared to untreated ones. When thiram and captan were applied as dry seed dresser, they gave best control of *Aspergillus niger* (Sidhu and Chohan, 1971).

Perwaiz *et al.* (1971) found copper sandoz and Bordeaux mixture to be most effective in the control of *Alternaria* fruit rot of chilli, but Narain and Panigrahi (1971) found ziram to be the most effective against *C.capsici.* Harware and Joshi (1974) recommended the use of benlate and dithane M-45 for ginger rhizome treatment for the control of rhizome rot and seedling infection caused by *Fusarium oxysporum.* Ellis *et al.* (1975) found

that the effectiveness of captan is due to its ability to move into deeper layers of seed coats. Sharma and Basuchoudhury (1975) tested the effects of different fungicides on the spore germination of pearlmillet seed mycoflora, *Curvularia lunata, Drechslera rostrata, Alternaria alternata, Fusarium equiseti, Cephalosporium acremonium* and found diflotan and thiram to be most successful in inhibiting spore germination of all the fungi at the concentration of 20 ppm.

Raju and Lal (1977) screened 25 systemic and non-systemic fungicides against two maize seed mycoflora viz. *Cephalosporium acremonium* and *F.moniliforme* by poisoned food technique and found only 5 fungicides, viz. TCMBT, TBZ, captan, duter and brestanol to be effective against both the fungi. Jharia *et al.* (1977) observed that the chilli seed treated with thiram + captan (1:1) - 0.3% was highly effective in checking the pre-and post-emergence losses and mortality at adult stage.

Sharma *et al.* (1980) tested 0.2% agrosan GN and ceresan against the common seed-borne fungi of *Amaranthus* and found that these two fungicides were effective in checking the *Amaranthus* seed mycoflora. Grover and Bansal (1970) reported thiram and vitavax to be most effective under *in vitro* studies against *Colletotrichum capsici*, the seed-borne fungus of chilli, but in pots only thiram and some other fungicides were found satisfactory.

Patil (1980) tested 7 fungicides on groundnut seeds to see their effects on the seed mycoflora and germination percentage of the

seed. He found that all the fungicides were effective over control, but bavistin proved superior showing highest mean percentage germination to the extent of 81.35, followed by thiram (74%), agrosan GN (69.7%) and brassicol (68.5%).

Reddy and Subbayya (1981) reported that benlate and vitavax treatments assumed complete freedom of the blackgram *(Phaseolus mungo* L.) seeds from fungal colonisation and recorded 96-100% germination followed by thiram and captan. Similar results as observed by them regarding the efficacy of benlate and vitavax as seed dresser for blackgram were reported by Jain and Khare (1972) and with thiram and captan by Agarwal *et al.* (1972).

Devi and Polasa (1984) reported that lower concentration of bavistin (25-100 ppm) enhanced the growth of *Aspergillus* spp; a concentration of 500-1000 ppm was needed for 100% inhibition of growth of the fungi in liquid media, whereas 2000-3000 ppm was required to obtain comparable results when fungi were grown on maize seed.

Singh and Agarwal (1986) reported the improved germination and reduced occurrence of *Fusarium moniliforme* in pearlmillet seeds when treated with captan, thiram, emisan, bavistin. However, bavistin was found to be the most suitable one in the complete eradication of the seed infection of *F.moniliforme* and gave maximum germination.

Biological Control:-

Fungicides (lime sulfur, Bordeaux mixture, mercuric chloride, formaldehyde) were of a non-specific or broad spectrum character

until 1940. Development of compounds that affected specific metabolic processes increased effectiveness and by the 1960s these had largely replaced the earlier materials. Many species have become resistant to the chemicals used as fungicides. Under field conditions the wheat smut, *Tilletia foetida*, has developed resistance to hexachlorobenzene in Australia, *Helminthosporium avenae* to organomercurials in Scotland, Ireland, Netherlands, New Zealand, New York, and perhaps Austria (Baker, 1972), and powdery mildews and other fungi to benomyl (Schroeder and Provvidenti, 1969). More and more examples of fungicide resistant plant pathogens are being discovered.

Disease control by fungicide is not a permanent one and needs repeated applications. But on the contrary, biological control, when effective usually is more enduring. Sometimes the use of fungicides causes an increase in the occurrence of disease as the fungicides may reduce the number or effectiveness of the antagonists. Understanding the limitation of the use of fungicide, much attention is being drawn in favour of biological control. According to Freeman (1981) when one biotic agent acts upon another in such a manner to limit its population then a state of biological control operates. While stressing the importance of biological control Mukhopadhyay (1987) said, "Anyone who considers biological plant protection to be merely the control of pathogens by biological means is taking for too narrow a view. I regard biological plant protection as too important for its needs to be met merely or chiefly from the ecologically motivated substitutions of chemically plant protection".

It has been established that contaminated seed sown in disinfected soil may lead to more severe development of diseases than contaminated seed sown on untreated soil, where antagonistic soil organisms have not been eliminated. *Drechslera sorokiniana*, which is very sensitive to the effect of other fungi, developed vigorously in sterilised soil but could be almost completely suppressed when a little unsterilised soil was added (Henry, 1931). *Trichoderma viride* has been reported by Weindling and co-operators, and other workers as being strongly antagonistic to many fungi including seed-borne parasites and to be able to produce specific antibiotics (Weindling, 1934; Weindling and Emerson, 1936; Weindling and Fawcett, 1936).

Tveit and Wood (1955) found that seed-borne *Fusarium* spp. causing blight of oat seedlings were controlled by treating grains with certain isolates of *Chaetomium* spp. Write (1956) reported that dusting seeds with spores of *Trichoderma viride* and *Pencillium frequentus* gave some protection to mustard seedlings from infection by *Pythium* spp.

Cephalosporium inoculated to host of *Fusarium oxysporum* prior to inoculation of this pathogen has been shown to have an inhibitory effect on development of the vascular *Fusarium*. *Cephalosporium* prevented symptoms of *Fusarium* wilt in tomato (Allison and Phillips, 1963; Roy and Patel, 1963), in cotton (Allison and Phillips, 1963), in cowpea (Long, 1963). Chang I-pin and Kommedahl (1968) found that coating corn kernels with *Chaetomium globosum* and sowing them in soil amended with *Fusarium roseum* f.

sp. *cerealis* "graminearum" resulted in control of seedling blight; kernels coated with either of the two mycoflora gave results in the fields as good as results from treating kernels with either captan or thiram. Lai and Bruehl (1968) studied the antagonism between *Cephalosporim gramineum* and isolates of *Trichoderma album, T.glaucum, T. koningi* and *T.viride.* Various reports are available on studies dealing with mycoparasitic reactions (Barnett and Binder 1973; Dennis and Webster, 1971c; Upadhyay and Rai, 1983), inhibition of one organism by the other due to volatiles (Dennis and Webster, 1971b; Skidmore and Dickinson, 1976) and non-volatile metabolites (Dennis and Webster, 1971a; Skidmore and Dickinson, 1976) fungistasis (Lockwood, 1977) and the role of staling growth products in microbial interaction (Park, 1964) mainly in view of possible biological control of plant pathogens. Srivastava *et al.*(1981) studied the colony interactions and mycoparasitism between *Acremonium furcatum* and 10 species of *Aspergillus* in dual cultures and found that *F.furcatum* overgrew the colonies of all the 10 species of *Aspergillus* studied.

Randhawa and Aulakh (1984) observed that coating rye (*Brassica juncea* Coss) seeds with *Chaetomium globosum* caused a drastic reduction in the percentage of seed yielding *Alternaria alternata, Aspergillus flavus, F. moniliforme,* and *Ulocladium atrum.* There was complete elimination of *Cladosporium cladosporioides, Penicillium utrinum, Rhizopus nigricans, Stachybotrys atra* and *Trichothecium roseum.* Seeds coated with *Epicoccum purpurascens* proved still more effective which caused a reduction of 54.5% of *A.brassicae,* the major pathogen. The

antagonistic role of *Cladosporium* spp. has been recognised by several workers (Newhook, 1957; Bhatt and Vaughan 1963; Warren, 1972). Upadhyay and Rai (1987) found that *A. flavus, A.niger, A. terreus and Penicillium citrinum* were antagonistic against *Fusarium udum*.

Aflatoxin: Uraguchi and Yamazaki (1978) stated that "mycotoxin" is a term to designate such secondary metabolites which cause pathological abnormalities in man and warm blooded animals.

Bilgrami (1986) while delivering 38th Mundkar Memorial lecture stated that "fungi elaborate hundred toxic metabolites (Turner 1971, Keeler and Tu, 1983) which play crucial roles in various biological and biochemical interactions". Production of these mycotoxins depends on species or strain of the fungus and on the ecological conditions for its development, in particular food source, temperature and humidity. Aflatoxicosis in man and animals, is caused by a series of toxins, including the most commonly encountered aflatoxin B_1, B_2, G_1 and G_2 which are produced by *A.flavus, A.parasiticus* and other related species. The first report of aflatoxin contamination was from England in 1960, known as `turkey X disease' (Blount, 1961) as 100000 turkey poults died from feed in which one of the constitutents was groundnut invaded by *A.flavus* (Asplin and Carnaghan, 1961).

Sargeant *et al.* (1961) for the first time reported aflatoxin production by *A.flavus* grown on sterile peanuts and in Czapeck's solutions. Since then various workers have reported toxin production by the isolates of *A.flavus* (Diener and Davis, 1969;

Detroy *et al.* 1971; Mehan and Cohan, 1973).

Different grains are being affected by *Aspergillus flavus* and the strains from tropical and subtropical regions produce more aflatoxin (Diener and Davis, 1969). Due to consumption of contaminated food, the animal products may also be affected. Ruminants affected by aflatoxin excrete in milk a toxin derivative of it, M-toxin, which is just as harmful as aflatoxin (Wogan, 1966; Schoental, 1967). It is now established that consumption of aflatoxin contaminated maize may lead to several liver disorders (Krishnamachari *et al.*, 1975, 1977). Karki *et al.* (1979) evaluated 17 cereal samples from Nepal and pointed out the aflatoxin contamination in raw and paraboiled rice. Nusrath and Nandi (1983) studied the cattle feed samples (collected from different parts of Hyderabad City, India) for the extraction and identification of aflatoxins and other toxins. They found aflatoxin B_1 in almost all the ingradients of food samples in variable amounts, the highest being 80 *fg*/kg, and B_2 was found in the oil cakes of sunflower and maize and also in the husk. Many other workers have reported the danger of aflatoxin in cattle feed and its ingradients (Loosemoore *et al.* 1964; Horrocks *et al.* 1965; Fulsounder and Skukla, 1978; Jagadish and Veeranarayana, 1979). Singh and Sinha (1983) reported the production of aflatoxin by *A.flavus* and *A.parasiticus* in guava fruits which was found to be 0.563 and 0.257 ppm respectively.

Bhadraiah and Ramarao (1984) studied 4 varieties of sorghum and aflatoxin B_1 and G_1 were detected in three varieties during the kharif season. Acharya *et al.* (1984) studied the production of

aflatoxin in raw and paraboiled rice, rice crisp, puffed rice and flattened rice and found puffed rice yielding maximum aflatoxin B_1.

Singh and Bedi (1985) investigated the toxin producing potential of the 10 most commonly grown varieties of bread wheat, duram wheat and triticales and found none immune to aflatoxin production. Many reports are available regarding the occurrence of aflatoxin in wide range of commodities with varying concentrations (Shotwell and Hesseltine, 1983; Lillehoj *et al.* 1983; Nandy and Haggblom, 1984; Jeswal, 1986).

Bedi and Cohan (1986) isolated 22 isolates of *A.flavus* Link ex. fries from the seeds of groundnut, maize, cotton, rice and wheat and screened them for their ability to produce aflatoxin. They found that the isolate M-1 produced maximum amount of aflatoxin B_1 (13.2 mg/50 ml of culture filtrate).

Reddy (1987) found 11 out of 13 varieties of sorghum susceptible for aflatoxin production and on the basis of the amount of aflatoxin B_1 produced, different varieties were categorised into (i) highly resistant (with little or no toxin), (ii) moderately resistant (10-80 ppb) (iii) moderately susceptible (81-300 ppb) and (iv) highly susceptible (more than 300 ppb). In another experiment he reported that out of 54 isolates of *A.flavus* screened for aflatoxin production, only 18 were toxigenic. Majority of isolates (13) produced aflatoxin B_1 whereas aflatoxin B_2 and G_1 were produced by 8 and 4 isolates respectively.

Biochemical changes: Gove (1965) defined deterioration as "the falling from a higher to a lower level in quality, character, or vitality. It implies the impairment of vigor or usefulness. It is an irreversible degenerative change in quality of a seed after it has reached its maximum quality level." The association of fungi with seed under poor storage conditions results in the deterioration of the seed. Fungi bring about various biochemical and physico-chemical changes leading to the final reduction of their quality and quantity. The general type of losses are loss of viability, discolouration, loss of nutritive value, production of toxins and loss in milling yields (Rahugunathan and Majumder, 1968; Vidhyasekaran and Govindaswami, 1968; Christensen and Kaufmann, 1969).

The role of fungi in seed deterioration has been studied by a number of workers all over the world (Goodman and Christensen, 1952; Qasem and Christensen, 1960; Lalithakumari *et al.* 1971; Bilgrami *et al.* 1976, 1978; Prasad *et al.* 1976). Qasem and Christensen (1958) stored corn of 17.18% moisture content for two years at 15°C, one set free of storage fungi and the other inoculated with storage fungi. The sample free of fungi retained a germination of 96%, while germination of samples inoculated with fungi was reduced to zero. Similar observations were made on wheat and maize by Papavizas and Christensen (1960) and Christensen (1964). Fields and King (1962), Mathur and Sehgal (1964), Siddaramaiah *et al.* (1980), Kumar and Nema (1973) and Rati and Ramalingam (1974) also reported the loss in germinability due to the association of storage fungi on the

stored seed. Maize, wheat and sorghum retained a germination percentage of 90-95 throughout the experimental period at a moisture content favourable to growth of storage fungi, whereas infected samples lost their germinability within a few weeks or months (Christensen and Lopez, 1963). Studies concerning the constitutional losses incurred due to the fungal association in stored materials have received attention of many workers (Lalitha Kumari *et al.* 1971; Vidhyasekaran *et al.* 1973; Harman *et al.* 1974; Prasad *et al.* 1976; Reddy *et al.* 1984; Roy and Choubey, 1984; Maheswari and Mathur, 1985). Singh and Sinha (1982) reported that the fungal association causes considerable loss to the food contents by changing the level of some of their vital chemical compounds. Bhargava and Arya (1983) gave a review of the biochemical changes, such as changes in sugars, amino acids, ascorbic acid and certain other changes by different fungi on some important fruit during pathogenesis. Nema (1983) reported that the inoculation of apple with *Alternaria alternata* reduced the concentration of sugar.

Changes in fatty acids:

One of the changes associated with deterioration of seed in general, and of oily seeds, in particular is increase in their acidity (Zeleny and Coleman, 1938, 1939; Milner and Geddes, 1946; Milner *et al.* 1947; Sorger-Domenigg *et al.* 1955). Milner *et al.* (1947) showed that this specific type of deterioration is caused by microorganisms. They showed that increased fat acidity and loss of germination accompanied growth of moulds on wheat. They stated that when wheat seeds were stored in a nitrogen atmosphere

which inhibited growth, neither fat acidity nor germinability changed. Christensen and Kaufmann (1969) also reported the role of storage fungi in fatty acid production.

Vidhyasekaran and Govindaswami (1968) studied the role of seed-borne fungi in paddy seed spoilage and recorded an increase in fatty acid production due to storage fungi.

Sharma (1973) also reported that out of 20 fungi studied *A.niger* caused maximum increase in fatty acid contents in castor and cotton seeds. Other workers also have reported the increase in fatty acid due to fungal infection of various crops during storage: corn (Sauer and Christensen, 1968), groundnut (Lalitha Kumari *et al.* 1971), mung (Charya and Reddy, 1981).

Maheswari *et al.* (1985) studied the role of six seed-borne fungi in the deterioration of *Lobia* seed and found that *A.flavus* and *A.fumigatus* were responsible for a maximum increase in fatty acid content (33.33%), followed by *A.niger* and *A.nidulans*.

Singh *et al.* (1986) observed that wheat grains stored under different storage conditions had considerably less lipid contents as compared with those in the fresh wheat samples with a maximum of 21.9% reduction. They also stated that the total amount of free fatty acid was considerably more in the samples stored under different storage conditions as compared with that in fresh samples.

Changes in Starch: Starch is one of the major food materials which is being depleted due to the fungal involvement in the

seeds. There are some reports of starch depletion in different types of seeds: mung (Vidhyasekaran and Kandaswami, 1972), arhar (Sinha and Prasad 1977), paddy (Bilgrami *et al.*, 1979). Allen (1942) had reported the marked fall in starch contents of infected tissues of wheat and Mirocha and Zaki (1966) reported for barley. Sinha and Prasad (1977) reported that the starch content of arhar seed infested with *A.flavus* exhibited a considerable decrease as compared to the content of unifested seeds and after 15 days of incubation 57.9% of degradation was noticed. There was a quantitative depletion of starch in mung and urad seed by *A.flavus* at different relative humidity (Sinha, 1979). Parmat *et al.* (1983) studied the biochemical analysis of leaves, stem and roots of *Cichorium intybus* infested by *Alternaria cichorium* and reported that there was a 45% reduction in starch contents of the host. Sinha *et al.* (1981) studied the changes in starch contents of arhar seed by infesting the seeds with *A.flavus, A.niger, Alternaria alternata, F.moniliforme, Curvularia lunata, Drechslera hawaiiensis, Rhizopus stolonifer.* They observed a decrease in the starch content in general with *A.flavus* and *A.niger* being responsible for maximum decrease. Fungi are very selective in the utilization of starch, which can be said on the basis of observation made by Tandon and Chandra (1962). Hasija (1970) and Singh and Tandon (1971) found fair and moderate utilization of starch contents by various fungi *in vitro.* Vidhyasekaran and Kandaswami (1972) reported that the association of *Cercospora* and powdery mildews in *Phaseolus aureus* resulted in considerable damage to the starch content.

Materials and Methods

STUDY MATERIALS : Three varieties of maize seeds were selected for the present study. Out of the three varieties two were hybrid, viz. Vijay and VL-16, and third one was locally cultivated variety, viz. Local White. The seeds of the varieties Vijay and Local White were procured from the ICAR complex located at Barapani near Shillong and VL-16 was collected form VPKAS, Almora, UP.

The samples were collected from the bulk storage maintained by these two research organisations. While collecting the samples from the bulk storage, International Rules for Seed Testing (ISTA, 1966, 1976) were followed. Several individual samples from different parts of the bulk (top, middle, bottom and side) were drawn with hands. The primary samples drawn from parts of the bulk were then mixed to make a composite sample. The composite sample, after thorough mixing, was reduced to the desired quantity. The final sample, technically called the submitted sample was then transferred to sterile polythene bags and sealed. During sampling care was taken to avoid any external contamination by cleaning the hands and other equipments used for sampling with 90% alcohol. The submitted samples of different varieties were then taken to the laboratory and stored in sterile gunny bags at room temperature. Care was taken to avoid fungal contamination, invasion by mites and rats during storage. For the two year survey of seed-borne mycoflora fresh samples were collected for each year soon after the harvest and stored in the laboratory for each incumbent year. However, samples for other

experiments were collected only once and stored in the laboratory during the whole period of investigation.

Nutrient Media used : The following nurient media were used during the whole period of investigation.

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

Potato	--------	200g
Dextrose	--------	20g
Agar	--------	20g
Water	--------	1000ml

Potato tubers were scrubbed clean and cut into small pieces. 200gm. of sliced potato were taken and rinsed rapidly in running water, boiled for one hour until it became soft. It was then meshed and filtered through muslin cloth. Agar was added to the filtrate and dissolved, dextrose was added and stirred to dissolve. The mixture solution was made upto 1 lit. by adding water and autoclaved.

(2) Water Agar Medium.

Agar	--------	15g
Water	--------	1000ml

Agar was dissolved in 1 lit. of tap water and autoclaved.

(3) Czapek's Dox Agar Medium (Raper and Thom, 1949).

$NaNO_3$	--------	3.0g
KH_2PO_4	--------	1.0g
$MgSO_4.7H_2O$	--------	0.5g
KCl	--------	0.5g

$FeSO_4.7H_2O$	----------	0.01g
Sucrose	----------	30.00g
Agar	----------	20.00g
Distilled water	----------	1000.00ml

(4) Peptone-Dextrose-Rose Bengal Agar Medium (Martin, 1950)

Agar	----------	20.00g
KH_2PO_4	----------	1.00g
$MgSO_4.7H_2O$	----------	0.5 g
Peptone	----------	5.00g
Dextrose	----------	10.00g
Rose Bengal(%)	----------	3.3ml
Distilled water	----------	1000.0ml
Streptomycin	----------	30.0mg

(5) SMKY Liquid Medium (Diener and Davis, 1966).

Sucrose	----------	200.0g
$MgSO_4.7H_2O$	----------	0.5 g
KNO_3	----------	30.0 g
Yeast Extract	----------	7.0 g
Distilled water	----------	1000.0ml

Sterilization of different nutrient media was carried out by autoclaving at a pressure of 15 p.s.i. for 20 minutes. Glasswares and other such equipments were sterilized either by autoclaving or by heating in a hot air oven at a temperature of 105°C for 24 hours.

I.SURVEY OF SEED-BORNE MYCOFLORA

Survey of seed-borne mycoflora of the three varieties of maize was carried out for a period of two consecutive years, 1987 and

1988. Survey was conducted on a seasonal basis, taking three seasons (winter, summer and rainy) each year. Working samples for periodical screening were drawn aseptically from the stock samples stored in the laboratory.

Methods for isolation of fungi : The following methods were followed isolation of fungi from maize seeds throughout the period of investigation.

(i) Standard Blotter method : Standard blotter method as recommended by International Seed Testing Association (ISTA, 1966) with a slight modification as suggested by Linonard (1966), known as the freezing method was followed. Three pieces of sterile blotting papers were placed in a sterilized petridish (9.5 cm. diameter) and moistened with sterile distilled water, so that little surplus of water was left on the surface of the paper. 400 seeds were randomly selected from each of the variety and 10 seeds were plated equidistantly in each petridish. All the platings were done in a sterile Laminar Flow chamber to avoid external contamination. The petridishes were then incubated at 20 ± 1°C for three days. The plates were then frozen overnight at 10°C and subsequently incubated at 25 ± 1°C for another seven days under an alternate cycle of 12 hr/12hr light and darkness provided by daylight fluorescent tube light. The plates were observed on the eighth day with a stereo-binocular microscope. Infected seeds were marked and counted, the percentage frequency of occurrence of individual fungi was calculated. Compound microscope was used for identification of various fungi. Various fungi that occur on the seeds were transferred to a nutrient PDA medium for confirmation of characters and for further

investigations.

(ii) Agar Plate Method : The International Rules for Seed Testing (ISTA, 1966) was followed. The nutrient medium used for isolation of fungi was Potato Dextrose Agar. Sterilized medium was poured aseptically into petridishes and allowed to cool and settle down. Four hundred seeds of maize were randomly selected from each variety and 10 seeds each were spaced out in a petridish containing PDA medium, using a sterile forcep. The pouring and platings were carried out in a sterile Laminar Flow chamber. The plates were then incubated at 25 ± 1°C for seven days under alternate cycle of 12hr/12hr light and darkness as in the blotter method. Observation of the plates were made from the fifth day and continued till the seventh day. Slow growing fungi were transferred to a fresh nutrient medium to avoid being over grown by fast growing fungi. Infected seeds were counted and the percentage incidence of individual fungi was determined.

(iii) Dilution Plate Method : 1 gm of seed was taken in a 250 ml conical flask containing 99 ml sterile distilled water and shaken thoroughly for 30 minutes using electrical shaker. 10 ml of this washing was aseptically transferred to another 250 ml conical flask containing 90 ml of sterile distilled water to get a suspension of 1:1000 dilution. 1 ml of this aliquot was pippetted aseptically to each petridish containing PDA nutrient medium. Sterilized fresh pippette was used for each sample. The petridishes were rotated gently in a swirlimg motion so that the inoculum was dispersed uniformly over the surface of the medium. Five replicates were maintained for each sample. The whole

process of inoculation was carried out in a sterilized Laminar Flow chamber. The plates were then incubated at 25 ± 1°C for five days. Observations were made from the sixth day.

Identification and purification of fungal isolates:
Fungi isolated from the seeds were identified from the pure cultures maintained following the keys and manuals provided by various workers (Gilman, 1956; Subramanian, 1971 and Barnett and Hunter, 1972). Various fungi isolated from maize seeds by different techniques were purified by single sporing technique. Pure cultures of various fungi were raised and maintained in tube slants containing Czapek's Dox Agar and Potato Dextrose Agar media. Some fungi isolated and purified were used for further investigations.

Interpretation of results : The experimental results were expressed as an average of isolations done during the two years of survey. The percentage frequency of occurrence of individual fungus was calculated using the formula

$$\% = \frac{\text{Total number of individual species of fungus}}{\text{Total number of all the species of the fungi}} \times 100$$

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

Experimental materials :
Seed samples : Seeds of the three varieties of maize (Local White, Vijay and VL-16) were used for this experiment.
Test fungi: The following six fungi isolated from maize seed were used as test fungi : *Alternaria alternata, Aspergillus flavus,*

Fusarium moniliforme, Penicillium expansum, Rhizopus nigricans and *Trichoderma viride.*

Preparation of seed leachates : The seed leachates of maize were prepared according to the method described by Saxena and Gupta (1982). 10 gm of seeds was surface sterilized by 0.1% mercuric chloride solution. The sterilized seed sample was then soaked in 100 ml sterile distilled water in 250 ml flask for 36 hours. After soaking, the flask was shaken for 30 minutes using electrical shaker and the water remaining therein was subsequently filtered. The filtrate was concentrated to a final volume of 5-8 ml on a water bath at a temperature not higher than 50°C. The concentrated liquid was designated as "Seed leachate". Its activities were tested against spores of various fungi and also the chemical analysis for qualitative estimation of sugars and phenols present in the leachates was performed.

Fungal spore germination : The germination of fungal spores in seed leachates was conducted in a cavity slide by hanging drop method. Single spore cultures of the test fungi were 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 the 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 little amount of grease was applied on the edge of the cavity to prevent the displacement of the micro-cover glass. Three

replicates were maintained for each sample. The slides were then kept in sterile petridish containing 10 ml of sterile water to maintain the humidity of the chamber. The whole set was incubated at specified temperatures such as 15°C, 20°C, 25°C and 30°C. Control sets were also maintained for each fungi using sterile distilled water as the medium. Observation of the slides for the germination of spores was made at 2 hr, 5hr, 10hr, and 24 hr of incubation. The number of germinated spores counted from observation of ten microscopic fields was recorded as the total germination and the percentage germination was calculated. The percentage spore germination in various seed leachates was compared with that of the control and the percent inhibition/stimulation was calculated as :

$$G_I = \frac{G_1 - G_2}{G_1} \times 100$$

where G_I = Inhibition / stimulation of spore germination (%)

G_1 = Spore germination on control (%)

G_2 = Spore germination on leachates (%)

Chromatographic analysis of seed leachates :

The seed leachates were chromatographically analysed for the presence of sugars and phenols.

Detection of sugars : For detection of sugars unidirectional paper chromatography (Block, *et al.* 1955) was followed. 25 µl of the seed leachate was loaded on the paper chromatogram along with the standard solutions of sugars. The solvent system used for

running the chromatograms was n-butanol : acetic acid : water (4:1:5 V/V). Aniline - phthalate solution (prepared by dissolving 1.66 gm phthalic acid in 100 ml water saturated butanol to which was added 0.99 ml aniline) was used for developing the spots of sugars. After spraying, the chromatogram was kept at 105°C for 5 minutes. Identification of sugars was done by comparing the Rf values of the spots with the known samples. Intensities of the spots were graded by visual observations in different catagories and marked as +++, ++, + and -(absent).

Detection of phenols : Detection of phenols was carried out by unidirectional paper chromatography using n-butanol : acetic acid : water (4:1:1 V/V) as the solvent system. Diazotised sulphanilic acid was used as the spray reagent for developing the spots. The reagent was prepared by dissolving 50 gm of sulphanilic acid in 250 ml of 10% KOH, allowed to cool and 200 ml of 10% $NaNO_2$ was added. Then this mixture was added drop by drop to 120 ml of conc HCl (a mixture of 80 ml conc HCl and 40 ml water). Precipitated diazonium salt was filtered with ice water. The precipitate was washed with ethanol followed by ether and then dried in air, the dried salt was stored at 2-4°C. 50 mg of the salt was mixed with 20 ml of 20% Na_2CO_3 for spraying. After spraying the chromatogram was dried at room temperature. Phenols appeared as deep yellow,light red and in other similar patterns. Identification was done by comparing the Rf values of the spots with the known standard samples which was run side by side.

III. STUDIES ON THE EFFECT OF FUNGAL EXTRACTS ON SEED GERMINATION AND SEEDLING GROWTH OF MAIZE

Experimental materials :

Seed materials : Three varieties of maize seeds (Local White, Vijay and VL-16) were used as the seed materials for this experiment.

Fungal materials : six dominant fungi isolated from maize seeds viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Penicillium expansum, Rhizopus nigricans* and *Trichoderma viride* were used as the test fungi and fungal extract was prepared from these six fungi.

Preparation of fungal extract : The six test fungi were grown in sterilized Czapek's liquid medium in 250 ml corning's conical flasks containing 150 ml medium. After 18 days of incubation at room temperature (24 ± 1°C), the culture filtrates were filtered first through four layers of cheese cloth and then through whatman No.1 filter paper.

One hundred surface sterilized seeds (treated with 0.1% mercuric chloride solution for oNe minute and subsequently with water) were immersed in the culture filtrate for 24 hrs. The soaked seeds were then washed with sterilized distilled water and subsequently used for the germination test.

Germination test : The effect of different fungal extracts on the germination and seedling growth of maize was studied by the following two methods :

(i) Test tube seedling symptom test : The technique of Khare, *et al.* (1977) was followed. 100 rimless test tubes of 30 ml capacity were filled with 10 ml of water agar medium, plugged and autoclaved; allowed to cool and solidify at a slight angle. 100 treated seeds were then sown aseptically, one seed in each test tube.The test tubes were then incubated at 20 ± 1°C for 10 days under the cycle of light/darkness. When the seedlings started reaching the rim of the test tubes, the pluggs were removed. All the six fungi were treated seperately against all the three varieties of maize seeds. One control set of experiment with surface sterilized, untreated seeds was also performed for each variety following the same procedure.

(ii) Pot culture experiment : Garden soil was autoclaved at 20 p.s.i. for 1 hr and filled in sterilized plastic pots of 12 cm diameter. 100 treated seeds from each vaiety were sown in the pots, 20 seeds in each pot. The pots were covered with polythene bags to avoid external contamination and subsequently kept in a glass house for 15 days. All the six fungi were tested separately against all the three varieties of maize seeds. One control set was also maintained for each variety.

Observation : Observation of the experiment was made on the 11th day in case of test tube seedling symptom test and on the 16th day in case of pot culture experiment. The general appearence of the seedlings was noted and any sign of disease symptom was observed. Then the seedlings were carefully removed with the roots keeping intact. The number of pre- and post-emergence mortality was counted and expressed as the percentage mortality.

The number of successful germination was counted and expressed as the final stand of the crop. The roots were carefully cleaned and the length of shoots and roots was measured and expressed as the average length of shoots and roots of all the seedlings. The seedlings were then carefully wrapped with paper and dried in oven at 100°C for 48 hrs. The dry weight of the seedlings was taken and expressed as the average dry weight of all the seedlings. The percentage seed germination, the average shoot/root length and the average dry weight of the diseased seedlings were compared with that of the controls and the difference was expressed as the percentage change over the control.

IV STUDIES ON THE EFFECT OF CERTAIN SEED DRESSING AGRO-CHEMICALS ON THE SEED-BORNE MYCOFLORA

Experimental materials :

Fungicides: The fungicides selected for this experiment were : agrosan GN (Ethyl mercury and acetate), bavistin (2-methoxy-carbomyl-benzamidazole), benomyl (50% methyl-1-(butylcarbamoyl)-2-bezimidazole carbamate), blitox-50 (copper-oxychloride), captan (N-trichloro-methyl-thio-tetra hydrophthalamide, 50 percent), dithane M-45 (zinc-manganese ethylene bis-di-thiocarbonate), thiram(tetramethyl thiurum disulphide) and thiram+captan.

Fungal materials : Six dominant seed-borne fungi of maize, viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Penicillium expansum, Rhizopus nigricans* and *Trichoderma viride* were selected as the test fungi.

To study the effect of the fungicides on the six seed-borne fungi, the following two experiments were conducted :

(i) Fungal spore germination : The efficacy of different fungicides on the germination of fungal spores was studied at different concentrations in cavity slides by hanging drop method. Different concentrations of the fungicides, viz. 50 ppm, 100 ppm, 200 ppm, 1000 ppm, 2500 ppm and 5000 ppm were prepared. Spore suspensions of different fungi were prepared in the various concentrations of the fungicides. The concentration of the spores was adjusted to 20-25 spores per microscopic field at 150x magnification. A drop of the spore suspension was placed on a cavity slide in an inverted position so that the spores remain hanging in the cavity. The slides were then kept in a sterile petridish containing 10 ml sterile water to maintain the humidity of the chamber. The whole set was incubated at 25°C for 24 hrs. Three replicates were maintained for each fungus and for each concentration. Control sets were also kept for each fungus using sterile distilled water. The results were expressed as the percentage of germination, taking ten microscopic fields of observations in each case.

(ii) Effect on mycelial growth: The effect of the fungicides on the mycelial growth of the six fungi was tested at different concentrations by using the poisoned food technique. Ten days old pure cultures of the fungi were used for all the experiments and the nutrient medium used was Potato Dextrose Agar.

Poisoned food technique: To prepare fungicidal-potato dextrose

agar medium, 100 ml of the medium was taken in a sterile conical flask and mixed with different amount of the fungicide to get a medium of 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm, 2500 ppm, and 5000 ppm concentrations. The poisoned medium thus prepared was poured into sterile petridishes, allowed to cool and solidify. Five replicates were kept for each concentration. Control set (without fungicide) was also maintained for each fungus.

Sterile cork borer of 4 mm diameter was used to punch the fungal discs from the pure culture. The fungal discs were then placed in the middle of the petridish containing the poisoned nutrient medium. The inoculated plates were then incubated at 25 ± 1°C for seven days. In case of fast growing fungi the plates were observed when the rim of the colony of the control set reached the side of the plates. The colony diameter was measured along three axes and the average of three measurements was recorded as the colony diameter. The colony diameter of the test fungi grown on the poisoned nutrient media was compared with that of the control and the difference was recorded as the inhibition of the fungicide on mycelial growth. It was expressed as the percentage inhibition and calculated as :

$$M_i = \frac{M_c - M_t}{M_c} \times 100$$

where M_i = Inhibition of mycelial growth (%)

M_c = Colony diameter of control

M_t = Colony diameter of fungi on treated medium.

V. STUDIES ON THE PRODUCTION OF AFLATOXIN IN MAIZE SEEDS

Seed samples: Three samples of maize seeds representing the three varieties of maize, viz. Local White, Vijay and VL-16 were screened for aflatoxins.

Observation of samples under ultra violet lights (BGYF test) : Bright greenish yellow flourescence (BGYF) was taken as the criterion for distinguishing the aflatoxin contaminated samples (Fennel, *et al.* 1973). The samples were observed under long wave ultra violet lamp. Samples giving typical BGYF were selected for the extraction of the aflatoxins.

Extraction of aflatoxins from the grains and their chemical confirmation : The chemical extraction of aflatoxin was done by the method of Thomas, *et al.* (1975). 50 gm powdered samples were blended in 250 ml methanol : water (60:40 V/V) in an electric blender for 2 minutes and the extract was filtered through whatman No. 1 filter paper. 100 ml of the filtrate was taken in a separating funnel, to it 30 ml saturated NaCl and 50 ml hexane were added, shaken thoroughly and allowed to settle. Then the lower aquous methanol layer was taken to another separating funnel and was extracted with 50 ml chloroform. The lower layer of chloroform was drained off into a conical flask containing 5 gm 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 on 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 : Glass plates of 20x20 cm. were used for this purpose. 36 gm silica-gel-G was mixed with 72 ml distilled water to form a slurry. The clean glass plates were uniformly coated with the slurry (0.5 mm thick) with the help of applicator. The plates were dried at room temperature and then activated in an oven at 120°C for 1 hr. The plates were cooled before use.

Detection of aflatoxins by thin layer chromatography : 50 µl aliquot (chloroform extract) was spotted on the TLC plate with the help of micropippette and run in the solvent toluene: isoamylalcohol: methanol (90:32:2 V/V). The plate was then air dried and observed under UV-lamp for aflatoxin spots.

Chemical confirmation of aflatoxin B_1 : Chemical confirmation of aflatoxin B_1 was done by trifluoroacetic acid (TFA) as suggested by Stack and Pohland (1975). After reaction with TFA, aflatoxin B_1 appeared as a blue fluorescent spot at an Rf about 1/4th of that of aflatoxin B_1 on TLC plates.

Quantitative estimation : The quantity of aflatoxin B_1 was determined by spectrophotometer (Nabney and Nesbitt, 1965). The spots of aflatoxin B_1 on TLC plates were scrapped and subsequently extracted with 5 ml cold methanol. Then it was centrifuged at 3000 rpm for 15 minutes. The ultraviolet absorption spectrum of the methanolic solution was recorded in spectrophotometer and the amount of aflatoxin present in the sample was calculated according to the following formula :

$$\frac{D \times M \times 10^6}{E \times L \times 1000} \quad \mu g / ml$$

where, D = Optical Density

M = Molecular weight of aflatoxin

E = Molar Extinction Co-efficient

L = Path Length.

Screening of *Aspergillus flavus* isolates for aflatoxin producing ability : *Aspergillus flavus* was isolated from different varieties of maize seeds by standard blotter method (ISTA, 1966). Various isolates of *A. flavus* were purified and maintained in PDA slants and subsequently screened for aflatoxin producing ability. Aflatoxin producing potential of *A. flavus* isolates was tested in SMKY liquid medium (Diener and Davis, 1966). 25 ml of the medium was taken in 250 ml conical flask, autoclaved and inoculated with 0.5 ml spore suspension of *A. flavus* isolates. The flasks were incubated at 28 ± 1°C for 10 days. The flasks were shaken every day during the whole period of incubation. On the 11th day each flask was thoroughly shaken and then filtered through whatman No. 1 filter paper. The aflatoxins were extracted from the culture filtrate with chloroform following the same method described earlier. Qualitative analysis was done by chromatography on TLC plates and quantitative estimation of aflatoxin B_1 was done by spectrophotometric method.

Screening of maize seeds for aflatoxin production by *A. flavus*: Three varieties of maize seeds, viz. Local White, Vijay and VL-16 were screened for their potentiality to aflatoxin production. 25 gm seeds of each variety were taken in 250 ml conical flask and

soaked in distilled water for 4 hrs. Each set was run in triplicates. The flasks were autoclaved at 15 p.s.i. for 10 minutes and subsequently inoculated with 0.5 ml spore suspension of a toxigenic strain of *A. flavus*. The concentration of the spores was adjusted to approximately 4 x 10^4 spores / ml with the help of haemocytometer. The flasks were incubated at 28 ± 1°C for 11 days. On the 12th day the infested seeds were dried at 60°C for 48 hrs and subsequently powdered. Aflatoxins were extracted and estimated by the methods described earlier.

VI. STUDIES ON THE CONTROL OF SEED-BORNE DISEASES OF MAIZE

Biological control:

Fungal materials: Three saprophytic fungi, viz. *Cephalosporium acremonium, Cladosporium cladosporioides* and *Trichoderma viride,* were selected as the antagonistic fungi. The test fungi selected for the experiment were *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme* and *Penicillium expansum.*

Seed materials: All the three varieties of maize (Local White, Vijay and VL-16) were used for this experiment.

Biological control of seed-borne diseases was studied under three categories of experiments :

(i) Pelleting the seeds with the saprophytic fungi :

Surface disinfected maize seeds were coated with the spores of *C. acremonium, C. cladosporioides* and *T. viride* by rolling the seeds over 8 days old sporulating pure cultures of the respective fungi. The seeds thus coated were plated on moist blotter with 10 seeds per plate distributed uniformly. The whole operation was

conducted in the Laminar Flow chamber to avoid external contamination. The plates were incubated at 25 ± 1°C for 10 days under the 12 hr cycle of light / darkness. One control set was maintained for each variety of seeds.

(ii) Fungal metabolites on seed germination :

The antagonistic fungi and the test fungi were grown on sterilized Czapek's liquid medium. After 16 days of incubation at room temperature (24 ± 1°C), the culture filtrates were filtered through cheese cloth and then through whatman No. 1 filter paper, autoclaved and diluted with sterile distilled water in 1:5 ratio. The diluted cultures of the antagonists and test fungi were mixed in 1:1, 1:2 and 1:5 ratios, and the seeds were soaked in the mixed culture filtrates for 24 hrs. Control sets were maintained using only the culture filtrates of the antagonistic fungi. The soaked seeds were washed thrice with sterile distilled water and plated on the moist blotter with 10 seeds per plate. The whole operation was conducted in the Laminar Flow chamber. The plates were then incubated at 25 ± 1°C for 10 days under the 12 hr cycle of light/darkness.

Observations: Observation of these two experiments was made on the 11th day. For the first experiment, seeds were examined for the occurrence of seed mycoflora, but the fungi occuring on seeds with which they were coated were not recorded.Then for both the experiments, the seedlings were carefully removed one by one with the roots intact. The number of pre- and post- emergence mortality and the number of successful germination was also recorded and the percentage was calculated. The length of roots

and shoots was measured and expressed as the average length of roots and shoots of all the seedlings. The seedlings were carefully wrapped with paper and dried in hot air oven at 100°C for 48 hrs. The dry weight of the seedlings was taken and expressed as the average dry weight of all the seedlings. The percentage seed germination, the average shoot/root length and the average dry weight of the treated seedlings were compared with that of the control and the difference was expressed as the percentage change over the control.

(iii) Colony interactions: Colony interactions between various fungi were studied in the dual cultures by placing 4 mm discs of the antagonistic and the test fungi approximately 3 cm apart on 100 mm petridish containing PDA. Each treatment was replicated 5 times, controls were maintained by placing a single 4 mm block of each fungus in the middle of the petridish containing PDA. The assessments were made when the fungi had achieved an equilibrium and there was no further alteration in the growth pattern.

Interactions were assessed using the colony interaction model of Skidmore and Dickinson (1976). The growth of both the fungi in a plate was measured from their central loci at both sides i.e. towards and opposing each other. The parameters used for the assessment of colony interaction were the breadth of inhibition zone, intermingled zone and percent inhibition of radial growth which was calculated as follows (Fokkema, 1976) :

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

where r_1 = radial growth of pathogenic fungus towards the opposite side

r_2 = radius of the pathogenic fungus towards the test fungus

The same can be calculated for the inhibition of the test fungi also.

Chemical control:

Seed materials: Three varieties of maize seeds, viz. Local White, Vijay and VL-16 were used for the experiment.

Fungal materials: Six dominant seed-borne fungi of maize, viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Penicillium expansum, Rhizopus nigricans* and *Trichoderma viride* were selected as the test fungi.

Fungicides: The fungicides selected for this experiment were : agrosan GN (Ethyl mercury and acetate), bavistin (2-methoxy-carbomyl-benzamidazole), benomyl (50% methyl-1-(butylcarbamoyl)-2-bezimidazole carbamate), blitox-50 (copper-oxychloride), captan (N-trichloro-methyl-thio-tetra hydrophthalamide, 50 percent), dithane M-45 (zinc-manganese ethylene bis-di-thiocarbonate), thiram(tetramethyl thiurum disulphide) and thiram+captan.

In one line of experiment unsterilized seeds of all the three varieties of maize were shaken separately with the fungicide at the rate of 0.25 per cent of seed weight for about 30 minutes, then stored for 24 hrs. One hundred seeds of each treatment as well as control were plated on moist blotter and incubated at 25

± 1°C under 12 hr cycle of light / darkness.

In the other line of experiment, the surface sterilized seeds of all the three varieties of maize were contaminated with the spore suspensions of the six test fungi seperately. The seeds thus contaminated with fungal cultures were treated with the recommended doses of the fungicides by shaking the seeds vigorously by hand for 30 minutes in flasks. The seeds so treated were then placed on sterilized moist blotters. Surface sterilized seeds contaminated with fungal spores served as control.

Observations: Observations were made on the 8th day for detection of percentage seed infection, number of pre- and post- emergence mortality, number of successful germination and final stand expressed in percentage. The length of shoots and roots was measured and expressed as average length of shoots and roots of all the seedlings. The percentage seed germination, the average shoot/root length of the treated seedling were compared with that of the control and the difference was expressed as the percentage change over the control.

VII. STUDIES ON THE EFFECT OF FUNGAL INFECTION ON NUTRITIVE VALUE OF MAIZE SEEDS

Experimental materials :

Fungal materials : five dominant pathogenic fungi of maize, viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Penicillium expansum,* and *Trichoderma viride* were selected as the test fungi.

Seed materials: Three varieties of maize seeds, viz. Local White, Vijay and VL-16 were used for the experiment.

Spore suspensions of ten days old cultures of the test fungi were prepared and the surface sterilized maize seeds were artificially infested aseptically in flasks with the spore suspensions. All the five fungi were treated seperately for all the three varieties of maize seeds. The treated flasks were incubated at room temperature for 10, 20 and 30 days in alternating cycles of 12 hrs light (NUV) and darkness for the growth of fungal organisms over the seeds. Control was maintained by adding 3 ml sterilized distilled water instead of spore suspension. Quantitative estimations of fatty acid and starch were performed on the 10th, 20th and 30th day of incubation. Dry weight of 100 seeds was also taken along with the estimation of fatty acids and starch.

Determination of dry weight : 100 seeds were randomly selected and dried in hot air oven at 100°C for 24 hrs and then placed in a dessicator charged with calcium chloride for about 30 minutes and weighed. The dry weight of the control set was compared with that of the treated seeds and it was expressed as the percentage change over the control. It was calculateed using the formula :

$$\% \text{ loss of dry weight} = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 = Dry weight of the control set

W_2 = Dry weight of the treated set

Estimation of fatty acid : 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 by a hand operated grinder. 20 gm powdered sample was taken into a 100 ml flask; 50 ml benzene was added and the flasks were shaken periodically for 45 minutes. Then the flasks were tilted and allowed the meal to settle at an angle for few minutes. The extract was filtered and 25 ml of the filtrate was drawn into 25 ml of 0.04 % alcohol phenolphthalein solution. This mixture was titrated against 0.0178 N KOH. Fat acidity was expressed in terms of mg KOH required to neutralise free fatty acids from 100 gm of maize flour.

Estimation of starch : The starch content of maize seeds was estimated by the procedure given by Snell, *et al.* (1961). The seeds were powdered with a hand operated grinder. To 0.2 gm of the powdered sample, 25 ml of 80 % ethanol was added and stirred thoroughly. After 5 minutes it was centrifuged and the supernatant was decanted. Extraction was repeated by adding 30 ml hot ethanol (80%) to the residue and centrifuged. The supernatant was again decanted and the residue obtained after centrifugation was taken for estimation of starch.

5 ml distilled water was added to the residue and stirred up thoroughly. To dissolve the starch, 6.5 ml of 52 % perchloric acid (prepared by adding 270 ml of 70 % perchloric acid to 100 ml of distilled water) was added. Constant stirring for 10 minutes was done and after 15 minutes, 20 ml distilled water was added,

centrifuged and the supernatant was collected. Extraction was repeated thrice. Finally the supernatants were mixed together and the volume was made upto 100 ml. 1 ml of this stock solution was developed with 10 ml of 0.1 % anthrone reagent (prepared by adding carefully 760 ml of conc. H_2SO_4 to 330 ml water and to the mixture was added 1 gm anthrone and dissolved using magnetic stirrer) heated at 100°C for 12 minutes. The bluish green solution was cooled at room temperature rapidly and optical density was read at 630 nm. Four replicates were used and the readings were compared with the standard curve of starch prepared by the same procedure. The starch content of the seeds was expressed as percentage and it was calculated as :

$$\% \text{ starch} = \frac{C_{(mg)} \text{ X Solution volume (ml)}}{10 \text{ x aliquot (ml) x Sample weight (gm)}} \text{ x } 100$$

where C = mg starch obtained from the the graph.

Results

I. SURVEY OF SEED-BORNE MYCOFLORA

The results of the survey of seed-borne mycoflora of three varieties of maize, viz. Local White, Vijay and VL-16 are presented as follows:

(1) Comparison of different techniques of isolation with respect to the incidence of fungi (Table 1 and figure 1.4).
(2) Variations in the incidence of fungi on the seeds at different seasons of the year (Table 1.3 and figures 1.2 & 1.4).
(3) Variations in the incidence of fungi on the seeds of different varieties of maize (Table 1.2 and figure 1.1 & 1.3).
(4) Qualitative assessment of fungal flora of maize seeds in various seasons (Table 1.4a, b & c and figures 1.5a, b & c).

(1) Different techniques of isolation: Table 1.1 depicts the average of all isolations of fungal species done during the two years of survey using the three methods of isolations, viz. dilution plate method, agar plate method and blotter method. A total of 32 species of fungi belonging to 16 genera was isolated from the three varieties of maize seeds. Blotter method of isolation resulted to maximum number of fungal species followed by agar plate method and dilution plate method. A total of 28 fungal species under 13 genera was isolated by blotter method, 26 species belonging to 13 genera were isolated by agar plate method and 20 species belonging to 9 genera appeared in the dilution plate method.

Table 1.1 Incidence of fungal flora on maize seeds detected by different methods of isolation.

Fungi	Dilution Plate	Agar Plate	Blotter Method
Alternaria alternata (Fries)Keissler	+	+	+
A. tenuis Nees.	-	-	+
Aspergillus candidus Link	+	+	+
A. clavatus Desm.	-	+	+
A. flavus Link	+	+	+
A. niger Van Tiegh.	+	+	+
A. sydowii (Bainer & Sart.) Thom & Charch	+	+	-
A. terreus Thom	+	+	+
A. ruber (Bremer) Thom & Raper	+	-	+
Cephalosporium acremonium Corda	+	-	+
Chaetomium globosum Kunze	-	+	+
Cladosporium cladosporioides (Fres) de Vries	+	+	+
Curvularia lunata (Wakker) Boed	+	-	+
Drechslera maydis (Nisikado) Subram.& Jain	+	+	-
Fusarium equiseti (Corda) Sacc.	+	-	+
F. moniliforme Scheld.	+	+	+
F. oxysporum Schl. ex Fr.	-	+	+
F. semitectum Berk. & Rav.	-	+	+
Nigrospora oryzae (Berk & Br.) Petch	-	-	+
Paecilomyces varioti Bainier	-	+	-
Penicillium chrysogenum Thom.	+	+	+
P. citrinum Thom.	+	+	+
P. expansum Link ex Fries.	+	+	+
P. oxalicum Thom.	-	+	+
P. rubrum Stoll	+	+	+
Penicillium spp.	+	+	+
Phoma spp.	-	+	-
Rhizopus nigricans Ehernb	+	+	+
Trichoderma viride Pers ex Fries	-	+	+
Trichothecium roseum (Pers) Link ex Fries	-	+	+
Verticillium albo-atrum Rienke & Berth.	-	+	+
Sterile mycellia	+	+	+

+ = present
- = absent

Alternaria alternata, Aspergillus candidus, A. flavus, A. niger, A. terreus, Cladosporium cladosporioides, Fusarium moniliforme, Penicillium chrysogenum, P. citrinum, P. expansum, P. rubrum, Penicillium spp., *Rhizopus nigricans* and sterile mycelia were isolated by all the three techniques. *Paecilomyces varioti* and *Phoma* spp. were detected only by agar plate method whereas *Alternaria tenuis* and *Nigrospora oryzae* were isolated by blotter method only. *Aspergillus sydowii* and *Drechslera maydis* occurred only on dilution plate and agar plate methods but could not be detected on the blotter method during the whole period of investigation.

From the table 1.1 it is evident that both the agar plate and blotter method of isolation are essential for the detection of seed-borne mycoflora. Yet the blotter method was found to be advantageous than agar plate method as some fungi having faster growth rate overgrow other slow growing fungi in the agar plate method and thus rendering some disadvantage in recording the seed mycoflora. The dilution plate method mostly results in the detection of surface mycoflora which are saprophytic in nature like species of *Alternaria, Aspergillus, Cephalosporium acremonium, Cladosporium cladosporioides, Curvularia lunata, Drechslera maydis, Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp.

(2) Seasonal variations in the incidence of fungal flora: Table 1.3 and figures 1.2 & 1.4 depict the incidence of fungal flora on maize seeds in three different seasons, viz. rainy, summer and winter, using the three methods of isolation. The results have

been expressed as an average of all the isolations done for the three varieties (Local White, Vijay and VL-16) of maize during the two years of survey.

It is evident from table 1.3 that both rainy and summer seasons yielded 31 fungal species each under 15 genera and winter season yielded 25 fungal species under 10 genera. Rainy and summer seasons recorded mainly the field fungi along with other seed-borne pathogenic fungi. The prominent field fungi recorded were *Alternaria* spp., *Aspergillus* spp., *Cephalosporium acremonium, Cladosporium cladosporioides, Curvularia lunata, Drechslera maydis, Fusarium* spp., *Penicillium* spp., *Trichoderma viride, Verticillium albo-atrum* etc. Most of the field fungi were replaced by the storage fungi in the winter season like *Aspergillus flavus, A. niger, A.ruber, A. terreus, Penicillium* spp. etc. Other than these variations, most of the fungal species occurred in all the three seasons though their percentage frequency of occurrence varied considerably in different seasons.

(3) **Variations of fungal species between different varieties of maize:** The average incidence of fungal species on the three varieties of maize seeds, viz. Local White, Vijay and VL-16 has been shown in the table 1.2 and figure 1.1. Amongst the three varieties, Local White yielded maximum number of fungal flora followed by VL-16 and Vijay. 30 fungal species belonging to 16 genera were isolated from the seeds of Local White variety; VL-16 recorded 28 fungal species under 13 genera and Vijay yielded 26 species under 13 genera. *Alternaria alternata, Aspergillus* spp., *Fusarium* spp., *Penicillum* spp. and *Rhizopus nigricans* were the

Table 1.2 Incidence of fungal flora on different varieties of maize seeds.

Fungi	LW			VJ			VL-16		
	D	A	B	D	A	B	D	A	B
Alternaria alternata	+	+	+	-	+	+	+	+	+
A. tenuis	-	-	+	-	-	-	-	-	+
Aspergillus candidus	+	+	+	-	-	+	-	-	+
A. clavatus	-	-	+	-	-	+	-	+	+
A. flavus	+	+	+	+	+	+	+	+	+
A. niger	-	+	+	+	+	+	-	+	+
A. sydowii	+	-	-	-	-	-	+	+	-
A. terreus	+	+	+	-	-	-	-	-	-
A. ruber	-	-	+	-	-	+	+	-	-
Cephalosporium acremonium	-	-	+	+	-	-	+	-	+
Chaetomium globosum	-	+	-	-	+	+	-	-	-
Cladosporium cladosporioides	+	-	+	+	-	+	+	+	-
Curvularia lunata	-	-	+	+	-	+	+	-	+
Drechslera maydis	-	+	-	+	+	-	-	-	-
Fusarium equiseti	-	-	-	-	-	+	+	-	+
F. moniliforme	-	+	+	+	+	+	+	+	+
F. oxysporum	-	+	+	-	-	+	-	+	+
F. semitectum	-	-	-	-	-	+	-	+	-
Nigrospora oryzae	-	-	+	-	-	-	-	-	-
Paecilomyces varioti	-	+	-	-	+	-	-	+	-
Penicillium chrysogenum	-	-	+	-	+	+	+	-	+
P. citrinum	+	+	+	+	+	-	-	+	+
P. expansum	+	+	+	-	+	+	+	+	+
P. oxalicum	-	+	+	-	-	+	-	+	+
P. rubrum	+	+	+	+	-	+	+	-	-
Penicillium spp.	+	+	+	-	+	-	-	-	+
Phoma spp.	-	+	-	-	-	-	-	+	-
Rhizopus nigricans	+	+	+	+	+	+	-	+	+
Trichoderma viride	-	-	+	-	+	+	-	+	+
Trichothecium roseum	-	+	-	-	+	-	-	-	+
Verticillium albo-atrum	-	+	+	-	-	-	-	+	-
Sterile mycellia	+	+	+	+	+	-	-	+	+

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

+ = present
- = absent

Table 1.3 Incidence of fungi on maize seeds during different seasons.

Fungi	Rainy			Summer			Winter		
	D	A	B	D	A	B	D	A	B
Alternaria alternata	+	+	+	-	+	+	-	+	+
A. tenuis	-	-	+	-	+	+	-	-	+
Aspergillus candidus	+	-	+	+	-	+	+	+	+
A. clavatus	-	+	+	-	+	+	-	+	+
A. flavus	+	+	+	+	+	+	+	+	+
A. niger	-	+	+	-	+	-	+	+	+
A. ruber	+	-	+	+	-	+	-	+	+
A. sydowii	+	+	-	-	+	-	-	+	+
A. terreus	-	-	+	+	-	+	+	+	+
Cephalosporium acremonium	+	-	+	-	-	+	-	-	-
Chaetomium globosum	-	-	-	-	+	+	-	+	+
Cladosporium cladosporioides	+	+	+	+	-	+	-	-	-
Curvularia lunata	+	-	+	+	-	+	-	-	-
Drechslera maydis	+	+	+	+	+	-	-	-	-
Fusarium equiseti	-	-	+	-	-	+	+	-	-
F. moniliforme	+	+	+	+	+	+	-	+	+
F. oxysporum	-	+	+	-	-	+	-	+	+
F. semitectum	-	+	+	-	-	+	-	-	-
Nigrospora oryzae	-	-	+	-	-	+	-	-	-
Paecilomyces varioti	-	+	-	-	+	-	-	-	-
Penicillium chrysogenum	+	-	+	-	-	+	+	-	+
P. citrinum	+	-	-	+	+	+	+	+	+
P. expansum	-	+	+	+	+	+	+	+	+
P. oxalicum	-	+	+	-	+	+	-	-	+
P. rubrum	+	-	+	+	-	+	+	+	+
Penicillium spp.	+	-	+	-	+	-	+	+	+
Phoma spp.	-	+	-	-	+	-	-	+	-
Rhizopus nigricans	+	+	+	+	-	+	+	+	+
Trichoderma viride	-	+	+	-	+	+	-	-	+
Trichothecium roseum	-	-	+	-	-	-	-	+	+
Verticillium albo-atrum	-	+	+	-	+	+	-	+	+
Sterile mycellia	+	+	+	+	+	+	+	+	-

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

+ = present
- = absent

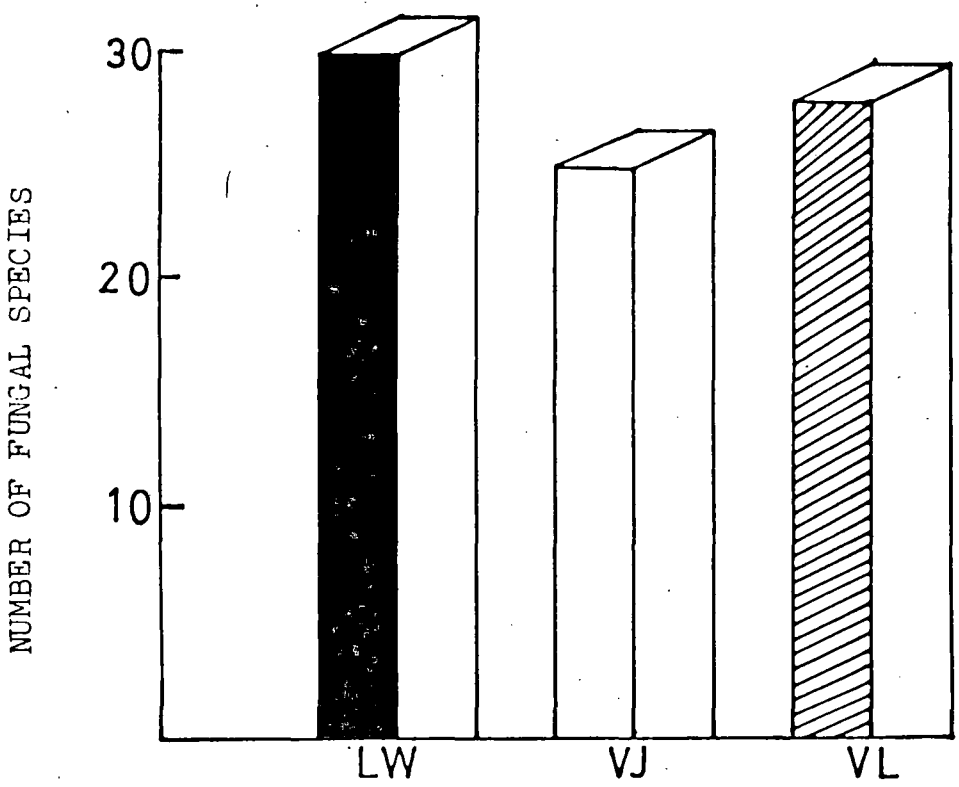


Fig. 1.1 Total number of fungal species isolated from different varieties of maize seeds. LW= Local White, VJ= Vijay and VL= VL-16.

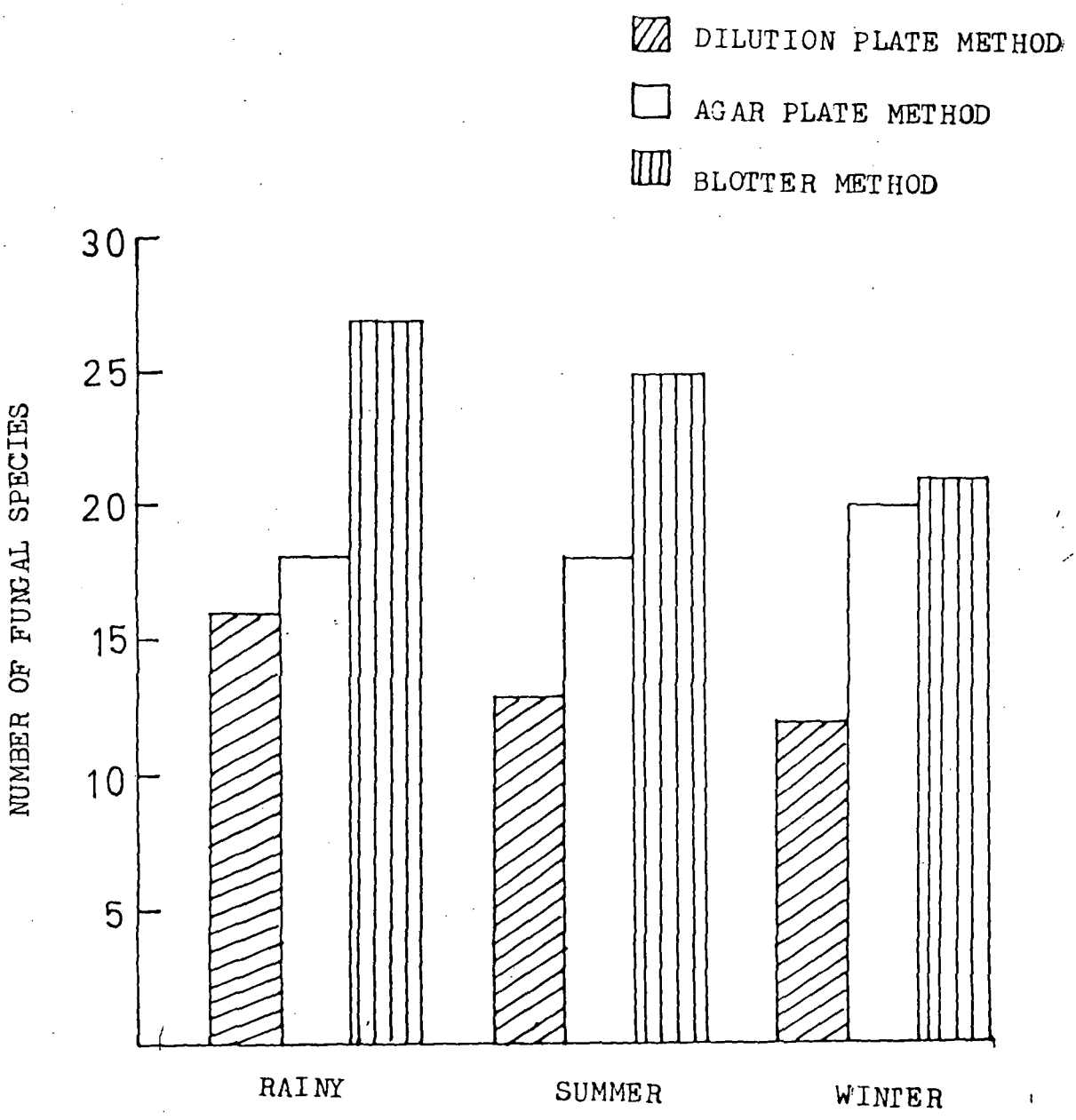


Fig. 1.2 Total number of fungal species isolated during different seasons using different techniques of isolation.

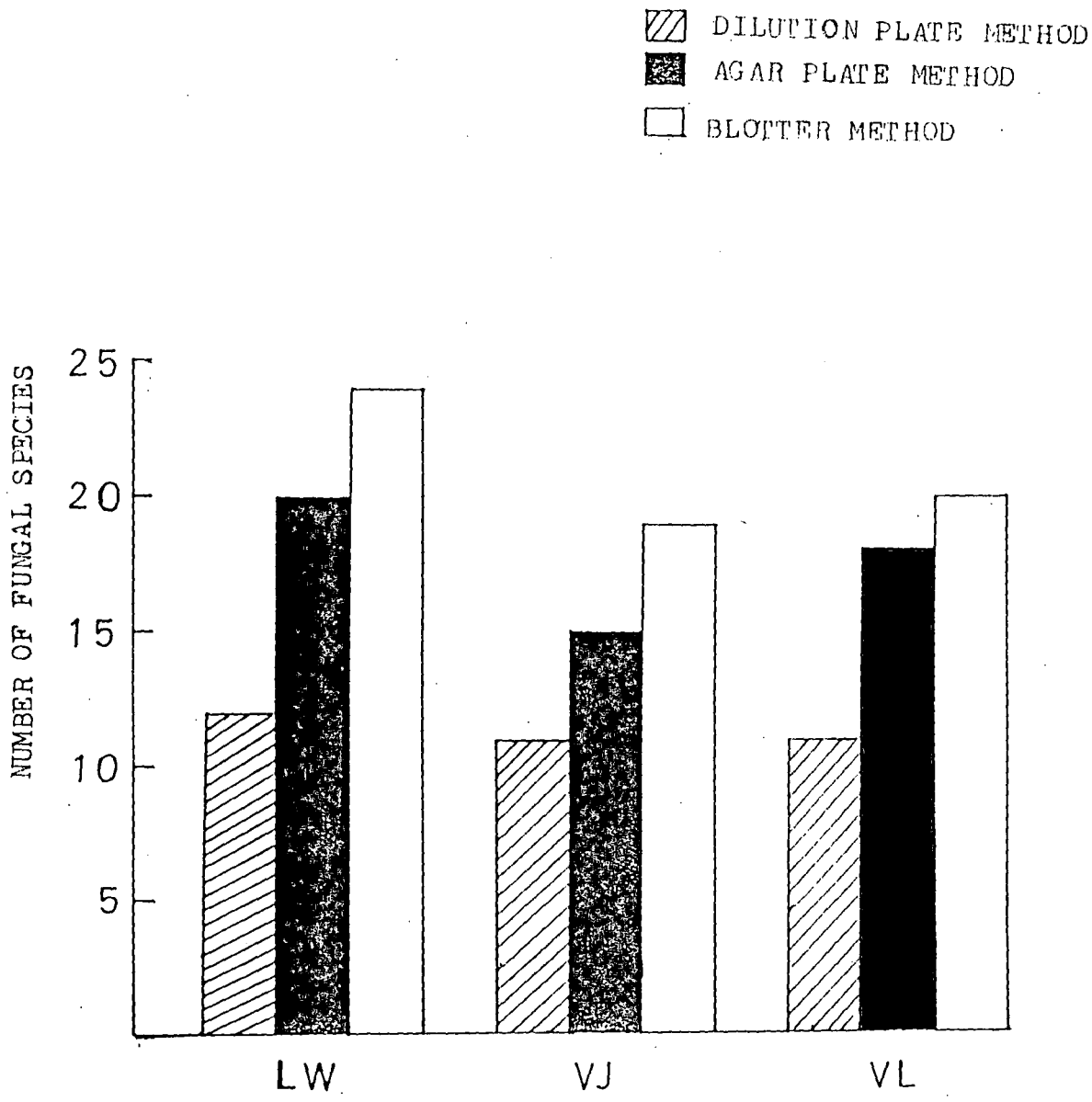


Fig. 1.3 Total number of fungal species isolated using different methods of isolation. LW - Local White, VJ - Vijay and VL - VL-16.

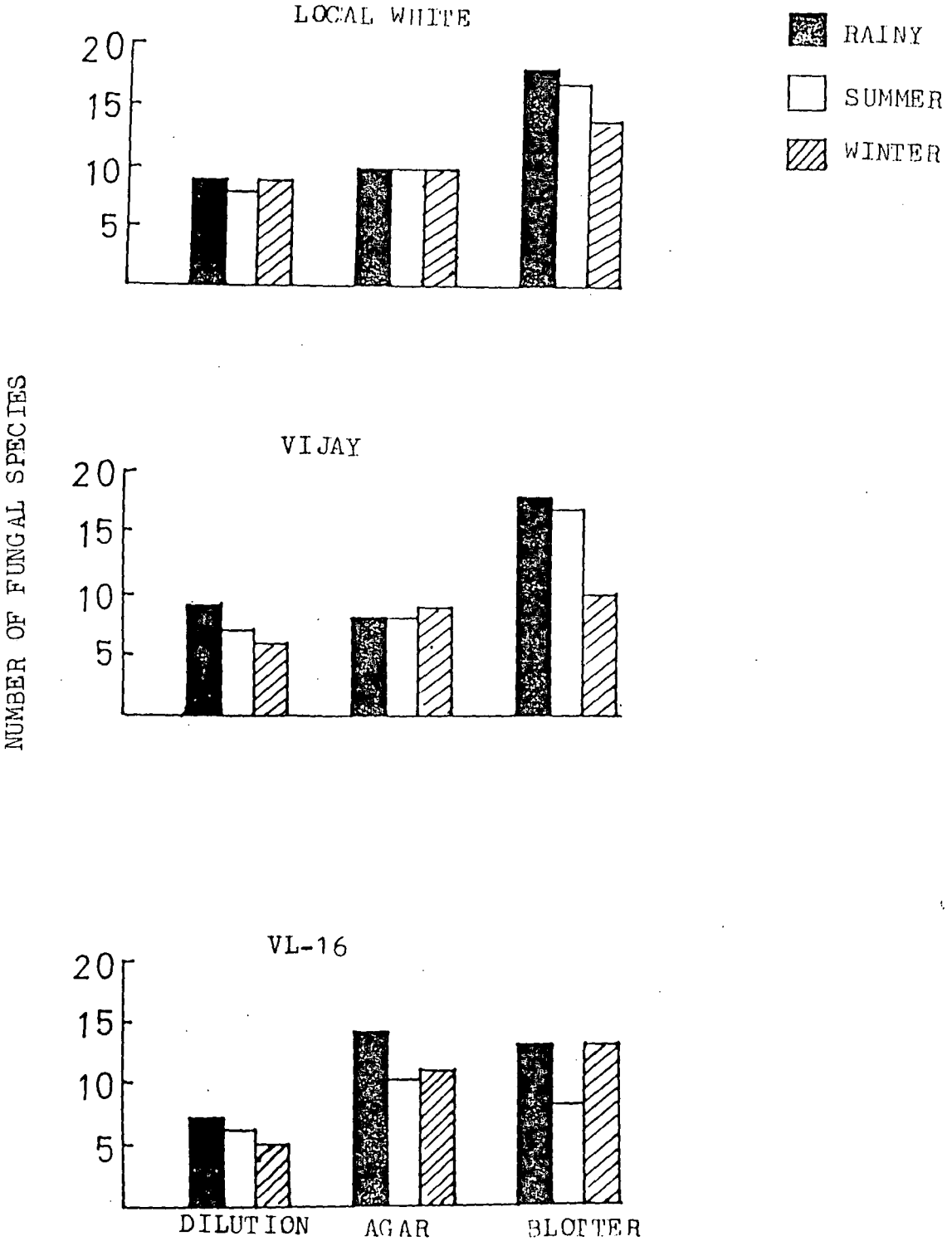


Fig. 1.4 Total number of fungal species isolated using different methods of isolation during different seasons of the year.

dominant fungi found on all the varieties of maize seeds. Some other less dominant fungal species, viz. *Cladosporium cladosporioides, Cephalosporium acremonium, Chaetomium globosum, Curvularia lunata, Paecilomyces varioti, Trichoderma viride, Trichothecium roseum* and sterile mycelia were also recorded from all the three varieties. Few fungal species were detected only from Local White but not from Vijay and VL-16, viz. *Aspergillus terreus, Chaetomium globosum* and *Nigrospora oryzae. Alternaria tenuis, Aspergillus sydowii* and *Phoma* spp. could not be isolated from the variety Vijay but occurred in the other two varieties though with a less frequency. The only two fungal species which could not be detected on the seeds of the variety Local White were *Fusarium equiseti* and *F.semitectum.* Most of the fungi were isolated by the blotter method followed by agar plate and dilution plate methods.

(4) Quantitative assessment of fungal flora: The average percentage frequency occurrence of fungal species (based on agar plate and blotter method) on the three varieties of maize seeds during different seasons (rainy, summer and winter) of the year has been depicted in the tables 1.4a,b & c and figures 1.5a,b&c.

Blotter method was found to be more suitable than the agar plate method in isolating the fungal flora from the seeds of the variety Local White in the three seasons (table 1.4a). Blotter method yielded 18, 17 and 14 fungal species during the rainy, summer and winter periods respectively, whereas agar plate yielded only 11,10 and 10 species respectively. Similar results were also observed in the variety Vijay (table 1.4b), where

Table 1.4a Percentage frequency of occurrence of fungi on maize seed (Variety : Local White).

Fungi	Rainy		Summer		Winter	
	Agar	Blotter	Agar	Blotter	Agar	Blotter
Alternaria alternata	6.5	3.4	11.3	-	-	4.5
A. tenuis	-	-	-	-	-	2.2
Aspergillus candidus	-	-	-	2.8	9.0	4.5
A. clavatus	-	2.7	-	3.8	-	-
A. flavus	33.8	17.8	33.5	23.1	-	27.2
A. niger	-	-	-	-	-	13.6
A. ruber	-	3.4	-	3.1	6.7	4.5
A. sydowii	-	-	-	-	-	-
A. terreus	-	1.1	-	1.4	14.2	4.1
Cephalosporium acremonium	-	3.1	-	2.8	-	-
Chaetomium globosum	-	-	2.9	-	2.2	-
Cladosporium cladosporioides	-	4.2	-	3.5	-	-
Curvularia lunata	-	6.5	-	3.1	-	-
Drechslera maydis	3.8	8.1	4.7	-	-	-
Fusarium equiseti	-	-	-	-	-	-
F. moniliforme	-	11.2	10.1	16.4	-	-
F. oxysporum	14.2	3.8	-	2.8	15.7	-
F. semitectum	-	-	-	-	-	-
Nigrospora oryzae	-	2.3	-	3.5	-	-
Paecilomyces varioti	3.2	-	-	-	-	-
Penicillium chrysogenum	-	-	-	-	-	4.1
P. citrinum	-	-	-	-	-	4.5
P. expansum	-	13.9	16.7	7.0	23.3	17.0
P. oxalicum	15.3	4.6	7.1	3.8	-	-
P. rubrum	-	-	-	10.8	9.0	4.9
Penicillium spp.	-	-	-	-	6.7	4.1
Phoma spp.	2.1	-	3.5	-	-	-
Rhizopus nigricans	4.9	6.5	-	5.6	-	-
Trichoderma viride	-	4.2	-	3.8	-	2.6
Trichothecium roseum	8.7	-	-	-	8.2	-
Verticillium albo-atrum	3.8	1.5	2.9	2.4	-	1.5
Sterile mycelia	3.2	0.77	6.5	-	4.5	-

Table 1.4b Percentage frequency of occurrence of fungi on maize seed (variety : Vijay).

Fungi	Rainy		Summer		Winter	
	Agar	Blotter	Agar	Blotter	Agar	Blotter
Alternaria alternata	-	6.4	-	3.9	3.7	3.7
A. tenuis	-	2.1	3.2	1.5	-	-
Aspergillus candidus	-	-	-	6.2	-	3.2
A. clavatus	-	5.7	-	7.4	-	2.7
A. flavus	25.6	21.2	16.8	13.7	24.6	-
A. niger	-	-	25.3	-	22.8	25.9
A. ruber	-	-	-	4.3	-	6.4
A. sydowii	-	-	-	-	-	-
A. terreus	-	-	-	-	-	-
Cephalosporium acremonium	-	-	-	-	-	-
Chaetomium globosum	-	-	-	3.5	4.3	4.6
Cladosporium cladosporioides	-	3.9	-	2.7	-	-
Curvularia lunata	-	5.7	-	3.5	-	-
Drechslera maydis	6.5	3.2	7.1	-	-	-
Fusarium equiseti	-	4.3	-	3.9	-	-
F. moniliforme	21.3	11.5	-	15.3	16.0	-
F. oxysporum	-	2.5	-	-	-	-
F. semitectum	-	5.7	-	2.7	-	-
Nigrospora oryzae	-	-	-	-	-	-
Paecilomyces varioti	3.2	2.5	4.5	-	-	-
Penicillium chrysogenum	-	-	-	-	-	16.6
P. citrinum	-	-	16.8	-	17.9	-
P. expansum	10.9	9.3	-	11.4	-	19.4
P. oxalicum	15.8	2.8	-	-	-	9.7
P. rubrum	-	3.9	-	7.8	-	7.4
Penicillium spp.	-	-	18.1	-	-	-
Phoma spp.	-	-	-	-	-	-
Rhizopus nigricans	7.6	5.7	-	4.7	5.5	-
Trichoderma viride	8.7	2.5	7.7	3.9	-	-
Trichothecium roseum	-	-	-	-	1.2	-
Verticillium albo-atrum	-	-	-	2.7	-	-
Sterile mycelia	-	-	-	-	3.7	-

Table 1.4c Percentage frequency of occurrence of fungi on maize seed (Variety : VL-16).

Fungi	Rainy		Summer		Winter	
	Agar	Blotter	Agar	Blotter	Agar	Blotter
Alternaria alternata	-	8.4	6.0	6.4	2.6	1.2
A. tenuis	-	2.1	-	-	-	-
Aspergillus candidus	-	3.7	-	5.2	-	7.3
A. clavatus	5.0	-	5.1	-	10.0	4.7
A. flavus	19.7	19.0	21.5	18.2	25.3	-
A. niger	7.6	-	15.5	-	16.9	24.6
A. ruber	-	-	-	-	-	-
A. sydowii	2.5	-	4.3	-	2.1	-
A. terreus	-	-	-	-	-	-
Cephalosporium acremonium	-	4.2	-	-	-	-
Chaetomium globosum	-	-	-	-	-	-
Cladosporium cladosporioides	3.1	-	-	-	-	-
Curvularia lunata	-	6.3	-	-	-	-
Drechslera maydis	-	-	-	-	-	-
Fusarium equiseti	-	5.2	-	-	-	-
F. moniliforme	18.4	16.4	17.2	15.8	-	3.8
F. oxysporum	5.0	3.7	-	7.0	-	3.0
F. semitectum	2.5	-	-	-	-	-
Nigrospora oryzae	-	-	-	-	-	-
Paecilomyces varioti	3.1	-	-	-	-	-
Penicillium chrysogenum	-	-	-	10.4	-	7.7
P. citrinum	-	-	16.3	5.8	11.1	12.5
P. expansum	10.8	-	-	30.5	19.0	19.4
P. oxalicum	5.0	-	-	-	-	-
P. rubrum	-	-	-	-	-	-
Penicillium spp.	-	13.7	-	-	-	6.9
Phoma spp.	-	-	6.0	-	3.1	-
Rhizopus nigricans	12.1	8.4	-	-	4.7	5.1
Trichoderma viride	-	4.7	-	-	-	1.7
Trichothecium roseum	-	3.7	-	-	-	1.2
Verticillium albo-atrum	2.5	-	4.3	-	1.5	-
Sterile mycelia	1.9	-	3.4	-	3.1	-

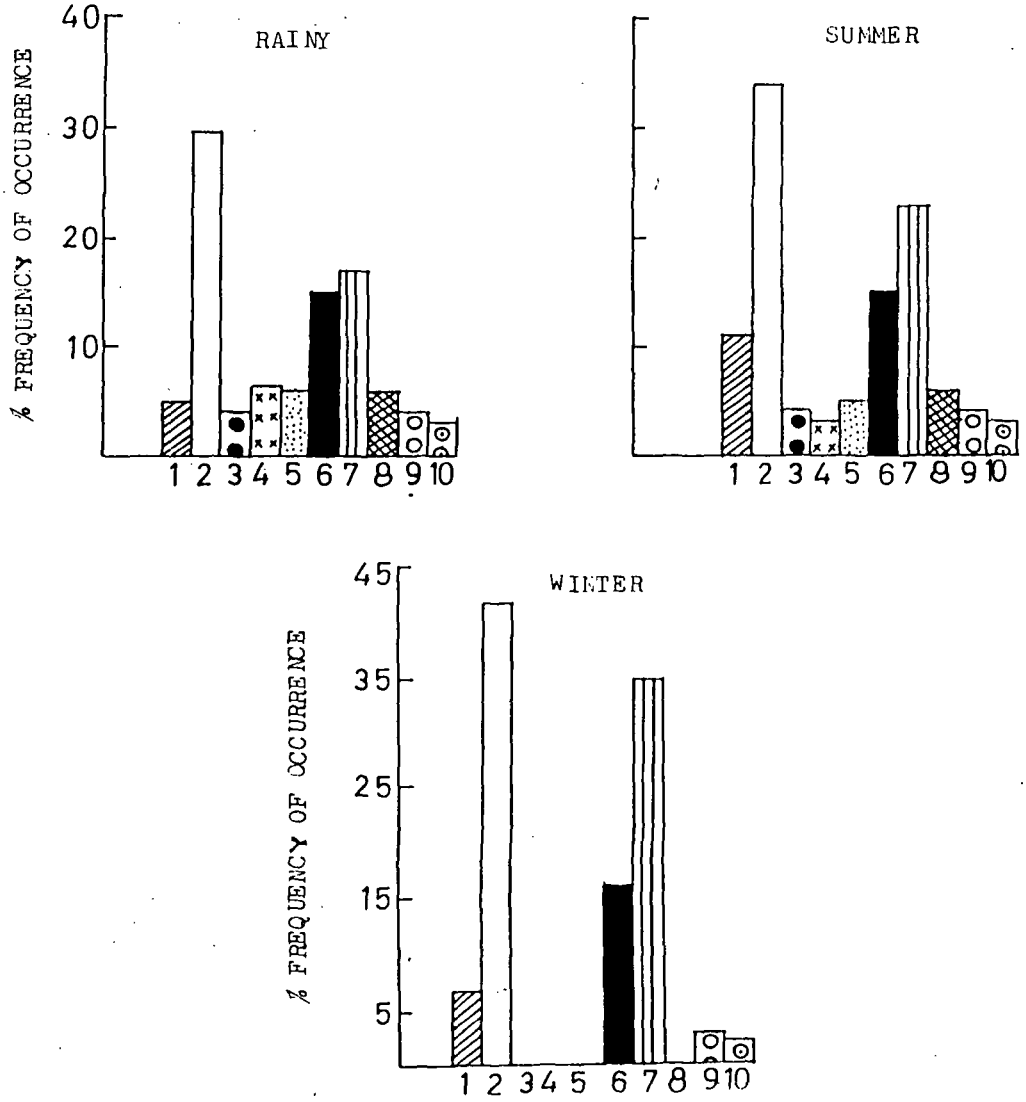


Fig. 1.5a Average percentage frequency of occurrence of certain fungal species on maize seeds during different seasons of the year. (Variety - Local White). 1 - A.alternata, 2 - A. flavus, 3 - C.cladosporioides, 4 - C.lunata, 5 - D.maydis, 6 - F.moniliforme, 7 - P.expansum, 8 - R.nigricans, 9 - T.viride and 10 - V.albo-atrum.

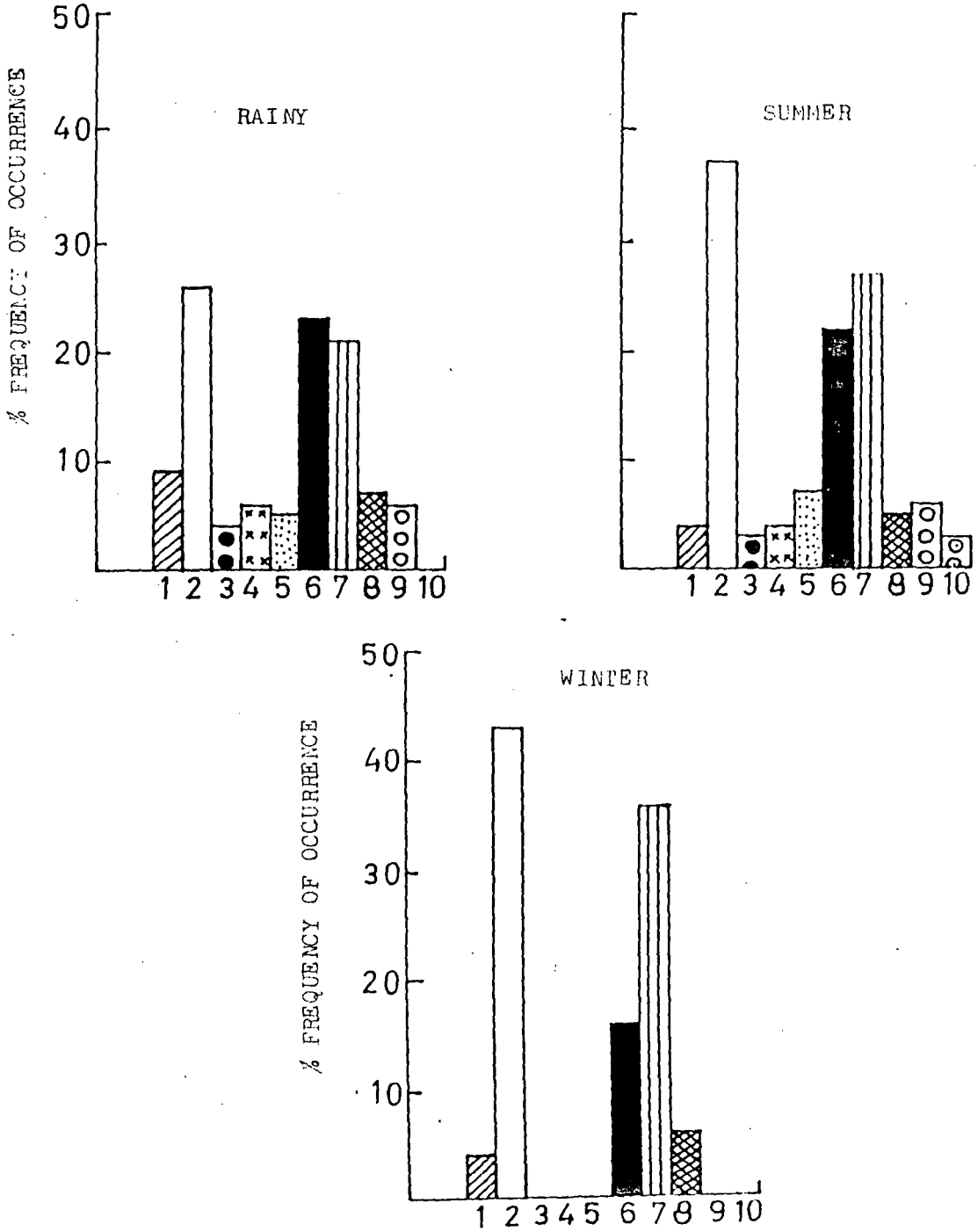


Fig. 1.5b Average percentage frequency of occurrence of certain fungal species on maize seeds during different seasons of the year. (Variety - Vijay). 1- A.alternata, 2- A. flavus, 3- C.cladosporioides, 4- C.lunata, 5- D. maydis, 6- F.moniliforme, 7- P.expansum, 8- R.nigricans, 9- T. viride and 10- V.albo-atrum.

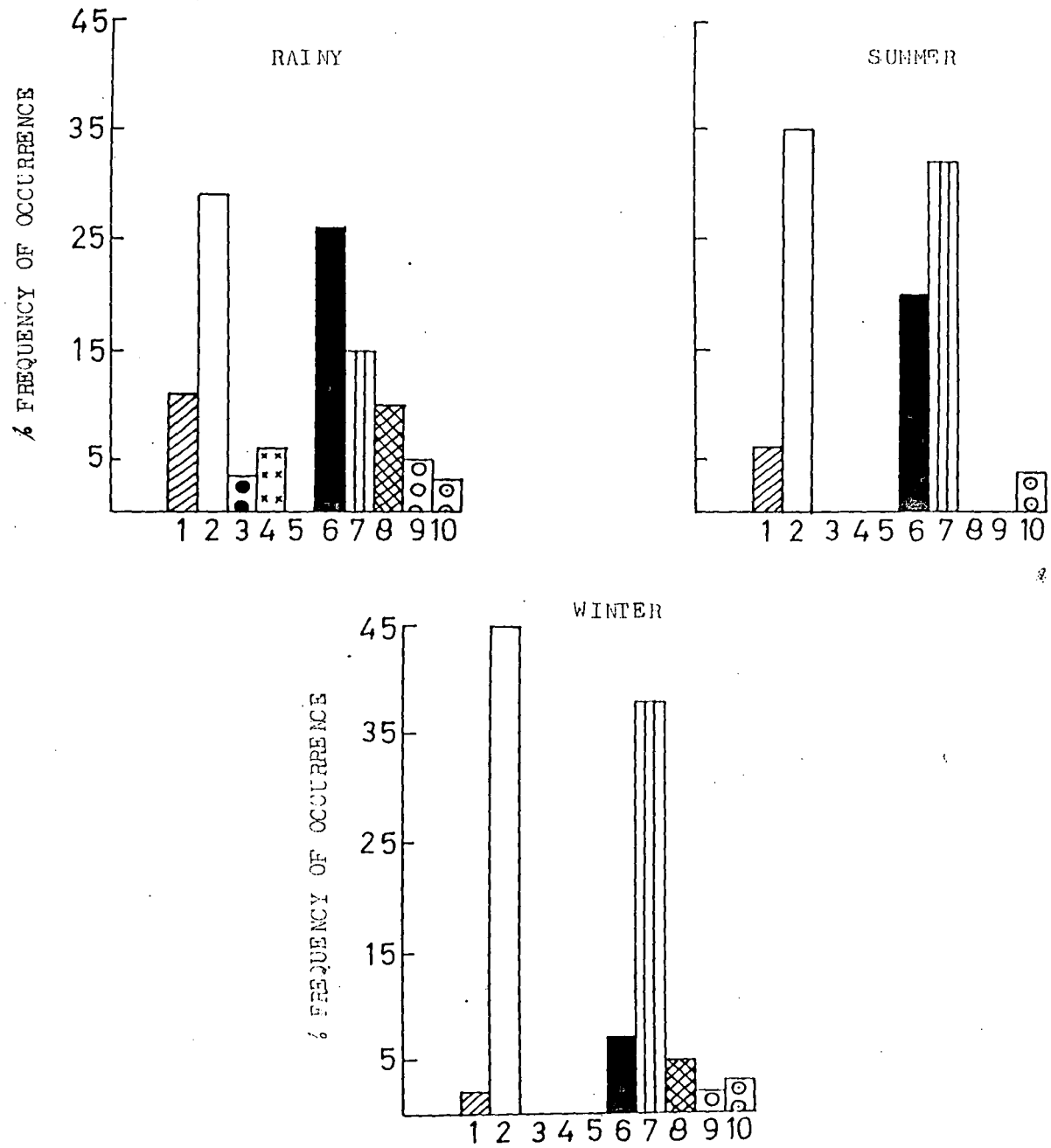


Fig. 1.5c Average percentage frequency of occurrence of certain fungal species on maize seeds during different seasons of the year. (Variety- VL-16). 1- A.alternata, 2- A.flavus, 3- C.cladosporioides, 4- C.lunata, 5- D.maydis, 6- F.moniliforme, 7- F.expansum, 8- R.nigricans, 9- T.viride and 10- V.albo-atrum.

blotter method recorded for more number of fungal species than the agar plate method. On the contrary, in the variety VL-16, agar plate method recorded more fungal species than the blotter method in the rainy and summer seasons (table 1.4c).

Though there was a total of 32 fungal species isolated from all the three varieties, yet, only a few species viz. species of *Aspergillus*, *Fusarium* and *Penicillium* dominated throughout the year. The percentage frequency of occurrence of the storage fungi increased along with the increase in storage time (figures 1.5a,b&c). The percentage frequency of surface-borne field fungi, viz. *Cephalosporium acremonium*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Drechslera maydis*, *Verticillium albo-atrum* etc. decreased along with time. Above fungi were not recorded during the winter season. A few fungal species like *Chaetomium globosum*, *Nigrospora oryzae*, *Paecilomyces varioti*, *Phoma* spp. and *Trichothecium roseum* were found to occur less frequently in comparison to other fungal forms and were restricted to one or two varieties only. Amongst the species of *Aspergillus*, *A. flavus* and *A. niger* were most dominant and percentage frequency of *A.niger* was maximum in the winter season. *Penicillium expansum* was found to be more frequent than other species of *Pencillium* and the percentage frequency was also found to be more during the winter season.

II. EFFECT OF SEED LEACHATES ON CERTAIN SEED-BORNE MYCOFLORA

Six important seed-borne fungi of maize, viz. *Alternaria alternata*, *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium expansum*, *Rhizopus nigricans* and *Trichoderma viride* were tested

against the seed leachates of three varieties of maize, viz. Local White, Vijay and VL-16 and the results are presented in the figures 2.1 to 2.3.

Figures 2.1a and 2.1b show the effect of seed leachates of three varieties of maize on the percentage spore germination and percentage inhibition of spore germination of the six seed-borne fungi during 24 hours of incubation at 25°C. It is evident from the tables and figures that the seed leachates of all the three varieties of maize exerted inhibitory effect on the spore germination of all the six fungi tested. The degree of inhibition, however, varied for different fungi. The germination of the spores of *F.moniliforme* and *P. expansum* was most effected followed by *T. viride, R. nigricans, A. flavus* and *A.alternata.* Maximum inhibition of spore germination was noticed in *F.moniliforme* treated with the seed leachates of the variety Vijay which was 78.4%; the minimum effect of the seed leachates on the spore germination was noticed also in *F.moniliforme* treated with the seed leachates of the variety VL-16. The results show that the leachates of Local White is effective against *F.moniliforme, P. expansum* and *T. viride* having inhibited the spore germination by 73.9%, 62.6% and 57.7% respectively,whereas in the cases of *A. alternata, A. flavus* and *R.nigricans* the inhibition of spore germination was less than 50%. Similarly the leachates of the variety Vijay were found to be most effective against *F. moniliforme, P. expansum* and *T. viride* with 78.4%, 71.3% and 47.9% inhibition of spore germination respectively, whereas it was comparatively less effective against *A. alternata, A. flavus* and *R.nigricans.* The leachates of the variety VL-16

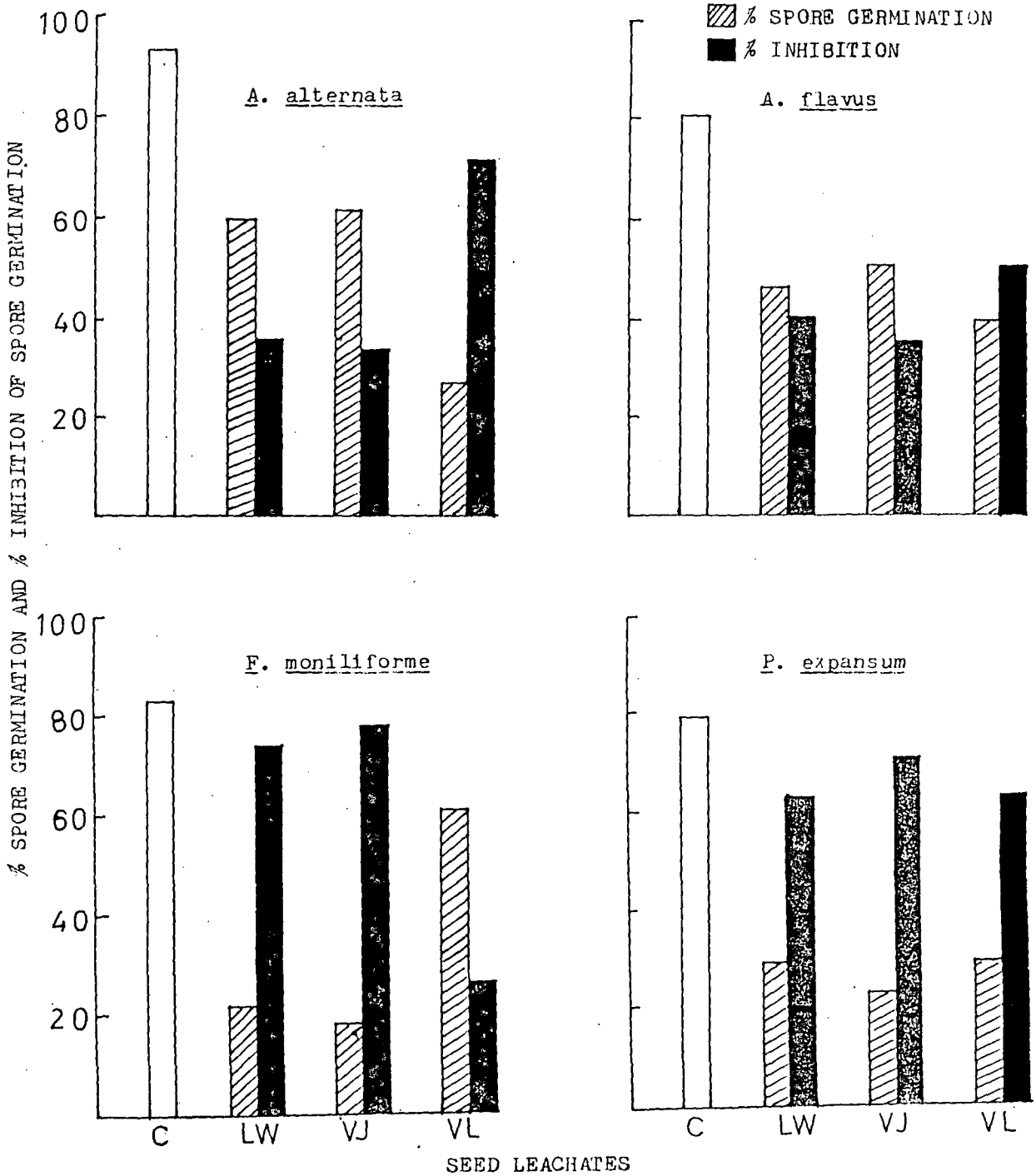


Fig. 2.1a Effect of seed leachates of different varieties of maize on the spore germination of certain seed-borne fungi. C - Control, LW - Local White, VJ - Vijay and VL - VL-16.

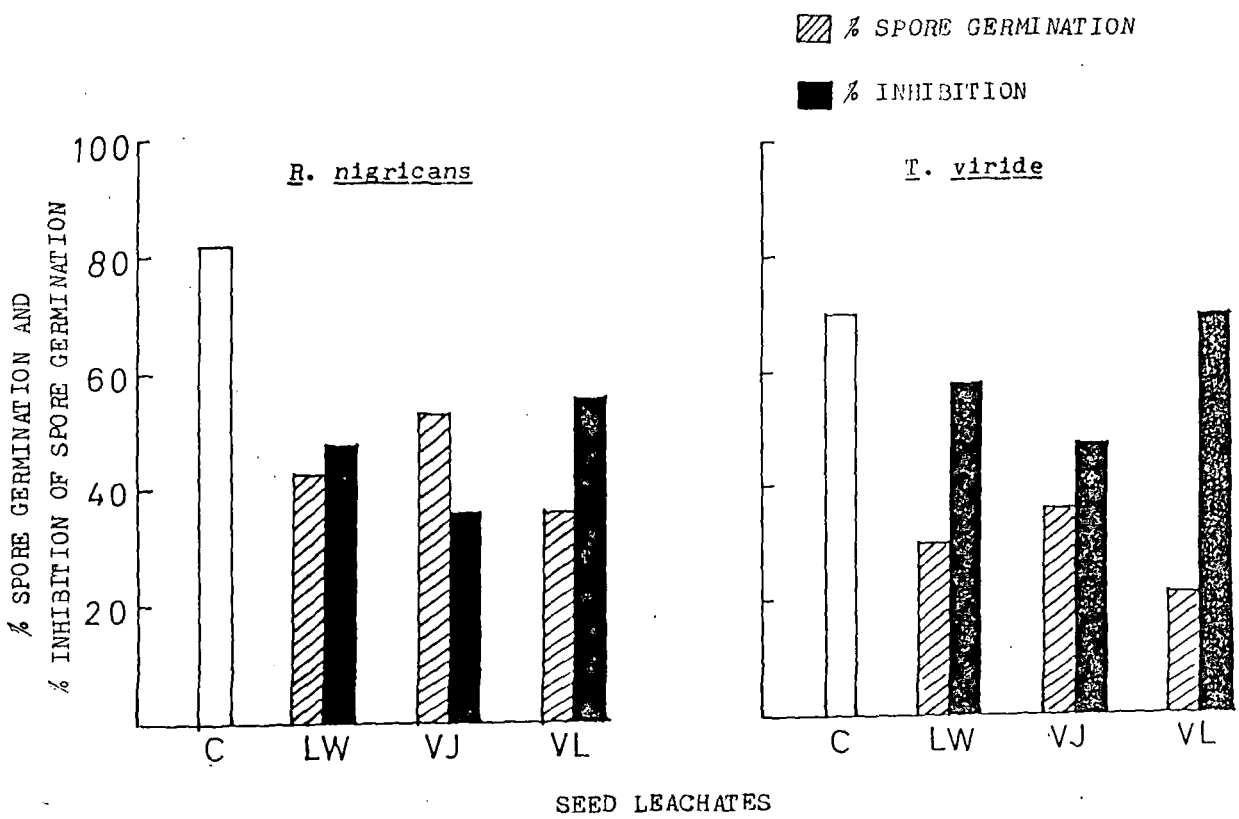


Fig. 2.1b Effect of seed leachates of different varieties of maize on the spore germination of certain seed-borne fungi. C - Control, LW-- Local White, VJ - Vijay and VL - VL-16.

was found to be the most effective amongst all the fungi tested, which inhibited the spore germination of all the fungi to a great extent except that of *F. moniliforme*. Statistical analysis (two way analysis of variance) showed that there was a significant difference in the spore germination between the varieties (F=17.596**) but not between the fungal species (F=1.874 NS).

Figures 2.2a and 2.2b depict the effect of the seed leachates of different varieties of maize (Local White, Vijay and VL-16) under different temperatures of incubation for a period of 24 hours on the spore germination of six seed-borne fungi of maize, viz. *A. alternata, A. flavus, F. moniliforme, P. expansum, R. nigricans* and *T. viride*. It is clear from the table and figures that with the increase in temperature there was a rise in the spore germination and maximum spore germination was observed at 25°C. It has been observed that the spore germination of all the fungi was inhibited considerably at 15°C. The spores of *F. moniliforme* and *T. viride* treated with the leachates of all the varieties failed to germinate at all at 15°C and even at higher temperatures, viz. 20°C, 25°C and 30°C germination was inhibited to a considerable extent. *A. alternata, A. flavus, P. expansum* and *R. nigricans* showed rapid increase in spore germination when the temperature was raised from 15°C to 20°C. In almost all the cases there was a fall in spore germination when the temperature was raised from 25°C to 30°C.

Two way analysis of variance showed that there was significant difference in spore germination between different temperatures

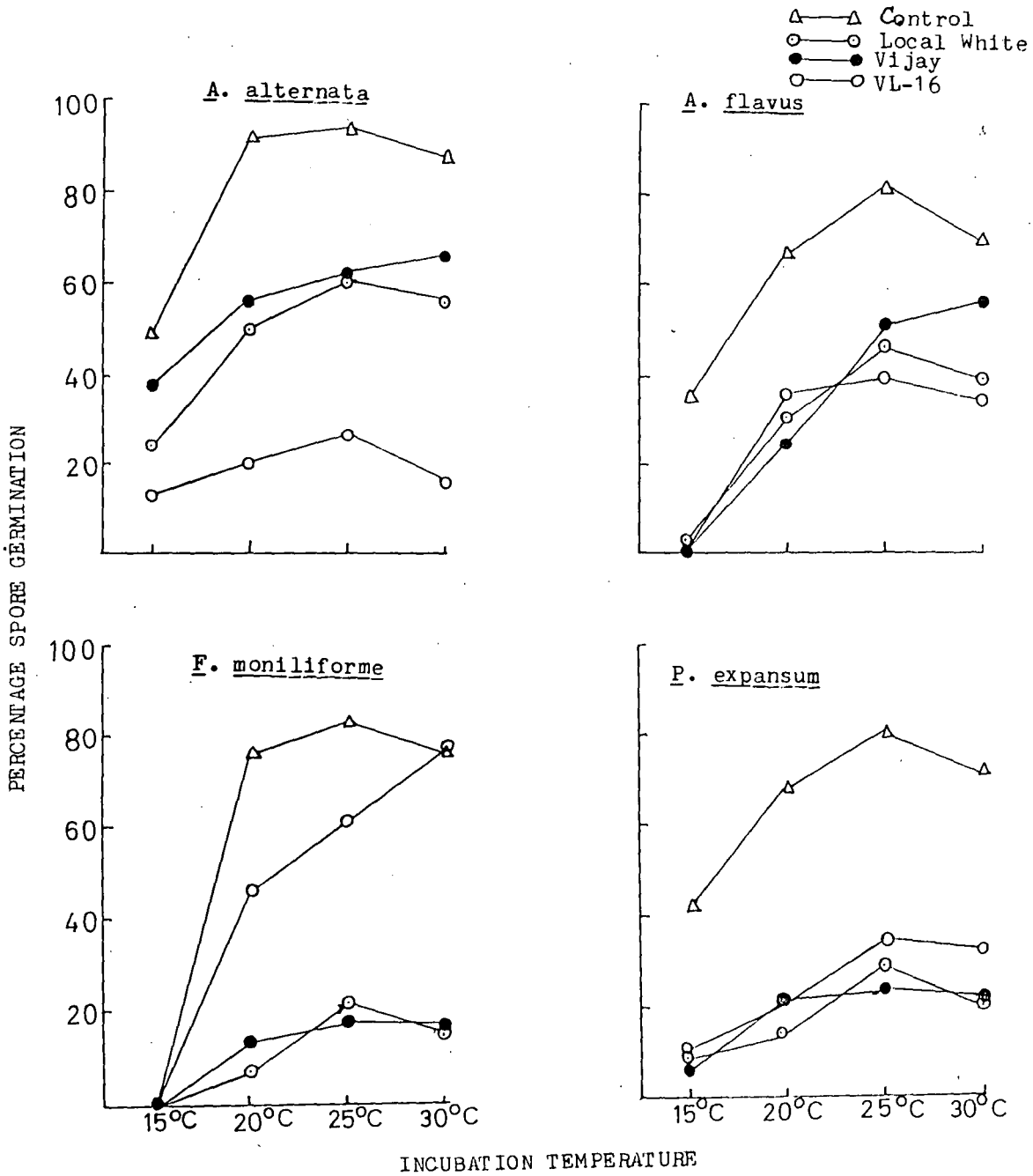


Fig. 2.2a Effect of seed leachates of different varieties of maize under different temperatures of incubation on the spore germination of certain seed-borne fungi.

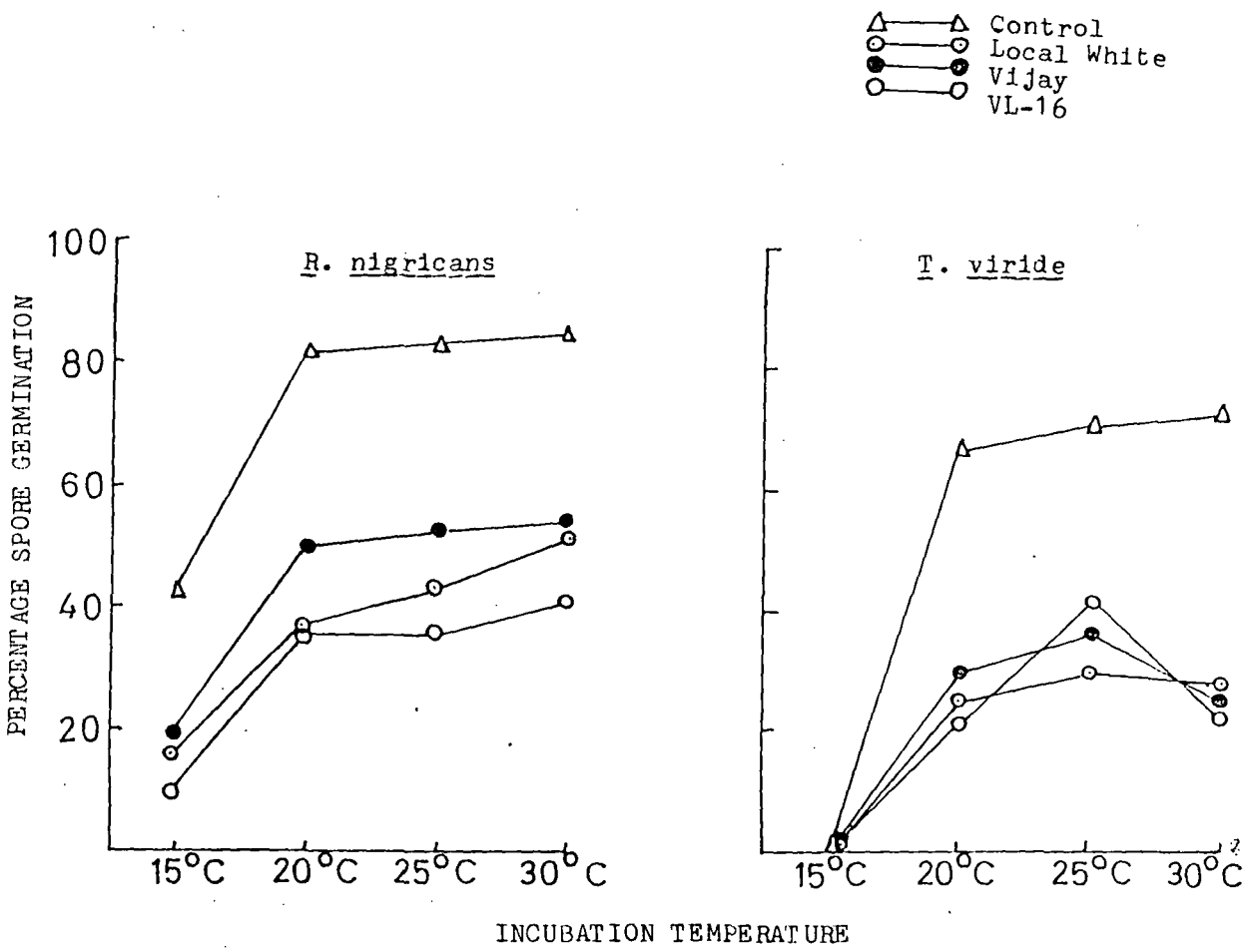


Fig. 2.2b **Effect** of seed leachates of different varieties of maize under different temperatures of incubation on the spore germination of certain seed-borne fungi.

(F=30.345**, 22.619** and 12.397** for Local White, Vijay VL-16 respectively) as well as between the species (F=20.784**, 21,719** and 3.215* for Local White, Vijay and VL-16 respectively).

Figures 2.3a and 2.3b depict the effect of different incubation periods, viz. 2 hours, 5 hours, 10 hours and 24 hours, at 25°C, on the spore germination of the test fungi. It has been observed that the spores of *A.alternata*, *R. nigricans* and *T. viride* treated with the leachates of the variety Local White at 2 hours incubation had 4.6%,5.1% and 7.6% spore germination respectively whereas spores of *A. flavus*, *F. moniliforme* and *P.expansum* could not germinate at all. All the fungi showed a marginal increase in the spore germination after 5 hours of incubation except *A. alternata* which had 41.5% germination. During the period of 10 to 24 hours there was considerable increase in the spore germination with *A.alternata* having the maximum germination percentage of 59.7. None of the fungal spores treated with the leachates of the variety Vijay could germinate at 2 hours of incubation. *A.alternata* and *R.nigricans* had 26.3% and 26.5% spore germination whereas *A.flavus*, *P. expansum*, *F. moniliforme* and *T. viride* had only 10.5%, 3.6%, 4.9% and 8.9% germination respectively at 5 hours of incubation. Along with the increase in the incubation period the germination percentage also increased with *A. alternata* having the maximum germination of 62.2%.

Other than the spores of *R.nigricans*, no other fungal spores could germinate at 2 hours incubation period which were treated

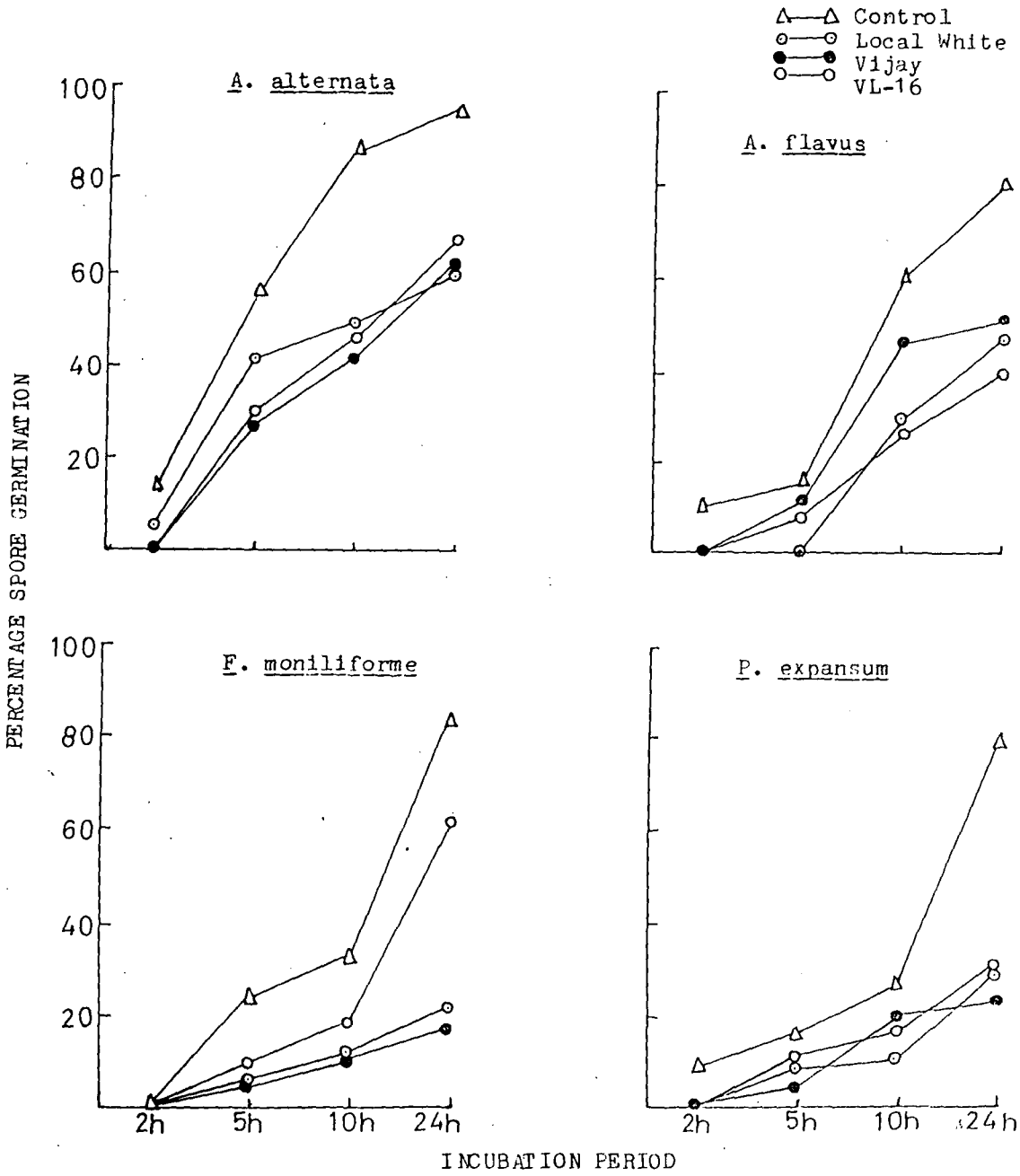


Fig. 2.3a Effect of seed leachates of different varieties of maize under different incubation periods on the spore germination of certain seed-borne fungi.

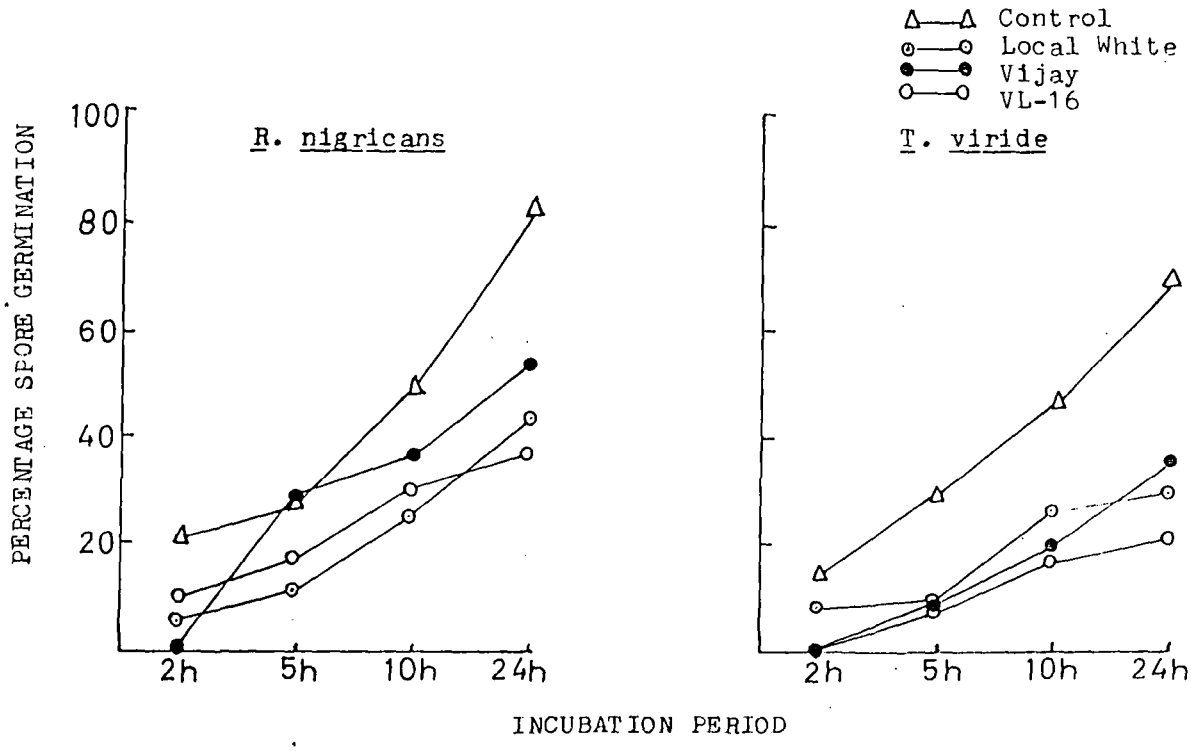


Fig. 2.3b Effect of seed leachates of different varieties of maize under different incubation periods on the spore germination of certain seed-borne fungi.

with the leachates of the variety VL-16. In this case also *A. alternata* had the maximum germination of 65.5% at 24 hours of incubation, followed by *F. moniliforme* (61.3%). Statistical analysis showed that the spore germination differed significantly between different incubation periods (F=21.223**, 24.678** and 22.328** for Local White, Vijay and VL-16 respectively), as well as between the fungal species (F=6.255**,5.301** and 3.66* for Local White, Vijay and VL-16 respectively).

Sugars and phenols composition of the seed leachates: The sugars and phenols composition of the seed leachates of all the three varieties of maize are shown in the table 2. Five sugars, viz. fructose, glucose, maltose, ribose and sucrose were detected from the leachates of the three varieties of maize; of these, fructose, glucose and sucrose were found to be present in all the varieties of maize. Maltose was not detected in the variety Local White and ribose was spotted only in the leachates of the variety Local White. Altogether five phenols, viz. caffeic acid, chlorogenic acid, dinitrophenol, metacresol and resorcinol were detected in the leachates of different varieties of maize, of which chlorogenic acid and resorcinol were detected in all the varieties. Caffeic acid was found to be present in the varieties Vijay and VL-16, but dinitrophenol was spotted only in VL-16. The leachates of the varieties Local White and VL-16 did not show the spots of matacresol which was observed only in the variety Vijay. Thus the variety Local White showed the presence of 2 phenols and Vijay and VL-16 showed 4 phenols each.

Table 2 Sugar and Phenol composition of maize seed leachates.

Sugar	Local White	Vijay	VL-16
Fructose	+	+	+
Glucose	+	+	+
Maltose	-	+	-
Ribose	+	-	-
Sucrose	+	+	+
Phenol			
Caffeic acid	-	+	+
Chlorogenic acid	+	+	+
Dinitrophenol	-	-	+
Metacresol	-	+	-
Resorcinol	+	+	+

+ = present

- + absent.

III. EFFECT OF FUNGAL EXTRACT ON SEED GERMINATION AND SEEDLING GROWTH

The effect of the fungal extract of six seed-borne fungi, viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Penicillium expansum, Rhizopus nigricans and Trichoderma viride* on the germination of maize seeds, root-shoot length and dry weight of the seedlings was tested by two methods, viz. test tube seedling symptom test and pot culture experiment. The results are depicted in the tables 3.1 to 3.4.

It has been observed that the culture filtrate of all the test fungi yielded poor seedlings in comparison to the control in all the varieties tested by seedling symptom test and pot culture experiment. Seeds treated with the extract of *A. flavus, F. moniliforme, P. expansum* and *T. viride* yielded seedlings with brown necrotic patches on the leaves in all the varieties and the growth was stunted in almost all the cases. Extract of *A.alternata* and *R. nigricans* did not show significant change in the final stand of the seedlings. Extract of *A. flavus* and *T. viride* initiated first, yellowing and then falling of the leaves in the variety Local White in both the seedling symptom test and pot culture experiment. In all the three varieties, extract of *F. moniliforme* caused maximum damage to the seedlings with stunted growth, falling of leaves, paler and weaker seedlings.

It has been observed from the experiment that the extract of all the test fungi reduced the germinability and final stand of the seedlings of all the varieties tested than that of the control

(tables 3.1 & 3.2). The extract of all the test fungi had marked influence on the pre-emergence rather than post-emergence mortality. In the test tube seedling symptom test (table 3.1) the highest percentage reduction in the final stand was observed in the variety Vijay treated with the extract of *T. viride* which had 32.29% reduction over control. Minimum percentage reduction of final stand was also noticed in the variety Vijay treated with the extract of *A. alternata* which had only 10.4% reduction over control. In the other two varieties the percentage reduction in the final stand varied from 12 to 32. Extract of *F. moniliforme* and *T. viride* was found to be most harmful for seed germination and final stand of the maize seedlings. Statistical analysis showed that there was no significant change in the final stand between the varieties (F=0.042 NS), but found to be significant between the fungal species (F=5.706**).

In the pot culture experiment also the extract of all the fungi reduced the final stand of the maize seedlings in all the varieties, except a solitary case of promotion in the variety Local White treated with the extract of *R. nigricans* which showed a 3.52% increase over control. This experiment also showed that the extract of *F. moniliforme* and *T. viride* was most toxic to the seed and caused considerable reduction in the final stand of the seedlings in all the varieties with a maximum of 38.63% reduction in the variety Vijay treated with *T. viride*. Extract of *A.alternata* was found to be least effective with a minimum of 4.7% reduction over control as observed in the variety Local White. In this case also there was no significant change in the final stand between the varieties (F=1.665 NS) but found to be

Table 3.1 **Effect of the fungal extract of selected maize seed mycoflora on the germination of maize seed (determined by test tube seedling symptom test).**

Variety : Local White

Fungi	% germination	Mortality%		Final stand%	% Change
		pre-emergence	post-emergence		
Alternaria alternata	75	25	5	70	-30.00
Aspergillus flavus	78	22	2	76	-24.00
Fusarium moniliforme	75	25	5	70	-30.00
Penicillium expansum	85	15	-	85	-15.00
Rhizopus nigricans	90	10	10	80	-20.00
Trichoderma viride	90	10	5	85	-15.00
Control	100	-	-	100	-
				Variety : Vijay	
A. alternata	86	14	-	86	-10.41
A. flavus	89	11	4	85	-11.45
F. moniliforme	78	22	6	72	-25.00
P. expansum	76	14	-	76	-20.83
R. nigricans	84	16	3	81	-15.62
T. viride	70	30	5	65	-32.29
Control	96	4	-	96	-
				Variety : VL - 16	
A. alternata	80	20	-	80	-20.00
A. flavus	88	12	-	88	-12.00
F. moniliforme	75	25	5	70	-30.00
P. expansum	80	20	-	80	-20.00
R. nigricans	78	22	5	73	-27.00
T. viride	70	30	2	68	-32.00
Control	100	-	-	100	-

Table 3.2 Effect of fungal extract of selected maize seed mycoflora on the germination of mazie seed (determined by pot culture experiment).

Fungi	% germination	Mortality%		Final stand %	% Change
		pre-emergence	post-emergence		
A. alternata	85	15	4	81	-04.70
A. flavus	72	28	6	66	-22.35
F. moniliforme	70	30	10	60	-29.40
P. expansum	82	18	8	74	-12.94
R. nigricans	88	12	-	88	+03.52
T. viride	80	20	4	76	-10.58
Control	90	10	5	85	-
					Variety : Vijay
A. alternata	80	20	10	70	-20.45
A. flavus	85	15	8	77	-12.50
F. moniliforme	70	30	12	58	-34.09
P. exapansum	75	25	7	68	-22.72
R. nigricans	80	20	10	70	-20.45
T. viride	66	34	12	54	-38.63
Control	90	10	2	88	-
					Variety : VL - 16
A. alternata	82	18	6	76	-15.55
A. flavus	80	20	7	73	-18.88
F. moniliforme	73	27	10	63	-30.00
P. expansum	82	18	10	72	-20.00
R. nigricans	72	28	5	67	-25.55
T. viride	74	26	15	59	-34.44
Control	90	10	-	90	-

significant between the fungal species (F=5.386**).

The results of the effect of fungal extract on the shoot-root length and dry weight of the seedlings are depicted in the tables 3.3 & 3.4. In the test tube seedling symptom test it was observed that the extracts of almost all the fungi retarded the shoot and root length of the seedlings except in the variety Vijay treated with *A. alternata* and *A. flavus* where the seedlings were found to be taller than those of the control. The average dry weight of the seedlings has also been found to be reduced in all the cases except two cases of enhancement in the variety Vijay treated with *P. expansum* and *R.nigricans*.

T. viride was found to be the most effective in reducing the shoot length of the seedlings of all the varieties, but maximum inhibition of shoot length was observed in the variety Local White treated with the extract of *A. alternata* which was found to be 49.44%. Extract of *A. alternata, A. flavus, F. moniliforme* and *T. viride* was found to be very effective in reducing the root length in the variety Local White with 41.23%, 37.91%, 30.33% and 41.7% reduction respectively, whereas the inhibition of root length due to the fungal extract was not very effective in the variety Vijay. In the variety VL-16, maximum inhibition was caused by *P.expansum* (39.25%) followed by *T. viride* (34.93%). The role of the fungal extract in the percentage change of the average dry weight of the seedlings over control was found to be not very prominent, the maximum reduction being 19.04% in the variety VL-16 treated with *A. flavus*. Only two cases of enhancement of dry weight was noticed in the variety Vijay

Table 3.3 Effect of the extract of selected maize seed mycoflora on the shoot-root length and dry weight of maize seedlings (determined by test tube seedling symptom test).

Variety : Local White

Fungi	Length in cm.		Dry weight (mg)
	Shoot	Root	
A. alternata	9.1 (-49.44)*	12.4 (-41.23)	0.169 (-07.65)
A. flavus	13.2 (-26.66)	13.1 (-37.91)	0.171 (-06.55)
F. moniliforme	13.8 (-23.33)	14.7 (-30.33)	0.159 (-13.11)
P. expansum	15.3 (-15.00)	17.9 (-15.16)	0.175 (-04.37)
R. nigricans	15.3 (-15.00)	16.0 (-24.17)	0.174 (-04.91)
T. viride	12.0 (-33.33)	12.3 (-41.70)	0.168 (-08.19)
Control	18.0 -	21.1 -	0.183 -
			Variety : Vijay
A. alternata	14.3 (+06.71)	19.4 (+07.77)	0.168 (-05.61)
A. flavus	14.1 (+05.22)	18.4 (+02.22)	0.159 (-10.67)
F. moniliforme	12.3 (-08.20)	17.2 (-04.44)	0.169 (-05.05)
P. expansum	11.2 (-16.41)	16.6 (-07.77)	0.197 (+10.67)
R. nigricans	12.3 (-08.20)	15.2 (-15.55)	0.193 (+08.42)
T. viride	11.2 (-16.41)	15.3 (-15.00)	0.152 (-14.60)
Control	13.4 -	18.0 -	0.178 -
			Variety : VL-16
A. alternata	11.3 (-09.6)	13.1 (-21.08)	0.162 (-14.28)
A. flavus	9.5 (-24.0)	15.0 (-09.63)	0.153 (-19.04)
F. moniliforme	10.6 (-15.2)	12.9 (-22.28)	0.161 (-14.81)
P. expansum	9.9 (-20.8)	10.0 (-39.75)	0.168 (-11.11)
R. nigricans	11.0 (-12.0)	11.1 (-33.13)	0.157 (-16.93)
T. viride	9.4 (-24.8)	10.8 (-34.93)	0.162 (-14.28)
Control	12.5 -	16.6 -	0.189 -

* Figures in parenthesis indicate percentage change over the control

Table 3.4 Effect of the fungal extract of selected maize seed mycoflora on the shoot-root length and dry weight of maize seedlings (determined by pot culture experiment).

Variety : Local White

Fungi	Length in cm				Dry Weight (mg)	
	Shoot		Root			
A. alternata	10.8	(-33.74)*	14.8	(-28.50)	0.166	(-10.27)
A. flavus	9.7	(-40.49)	13.2	(-36.23)	0.176	(-04.86)
F. moniliforme	9.3	(-42.94)	12.7	(-38.64)	0.151	(-18.37)
P. expansum	14.8	(-09.20)	16.9	(-18.35)	0.177	(-04.32)
R. nigricans	15.2	(-06.74)	15.3	(-26.08)	0.179	(-03.24)
T. viride	10.2	(-37.42)	14.6	(-29.46)	0.164	(-11.35)
Control	16.3	-	20.7	-	0.185	-
					Variety : Vijay	
A. alternata	12.8	(+02.4)	17.6	(-03.29)	0.169	(-01.77)
A. flavus	12.6	(+00.8)	18.5	(+01.64)	0.158	(-08.13)
F. moniliforme	7.4	(-40.8)	13.1	(-28.02)	0.148	(-13.95)
P. expansum	8.9	(-28.8)	14.2	(-21.97)	0.156	(-09.30)
R. nigricans	11.4	(-08.8)	16.5	(-09.34)	0.166	(-03.48)
T. viride	7.6	(-39.20)	13.4	(-26.37)	0.144	(-16.27)
Control	12.5	-	18.2	-	0.172	-
					Variety : VL - 16	
A. alternata	11.2	(-08.19)	13.5	(-14.55)	0.183	(-06.63)
A. flavus	11.6	(-04.91)	8.4	(-46.83)	0.146	(-25.51)
F. moniliforme	10.2	(-16.39)	9.3	(-41.13)	0.159	(-18.87)
P. expansum	12.1	(-00.81)	11.1	(-29.74)	0.179	(-08.67)
R. nigricans	9.3	(-23.77)	10.1	(-36.07)	0.151	(-22.95)
T. viride	9.9	(-18.85)	9.4	(-40.50)	0.158	(-19.38)
Control	12.2	-	15.8	-	0.196	-

* Figures in parenthesis indicate percentage change over the control.

treated with *P.expansum* and *R. nigricans* which were found to be 10.67% and 8.42% respectively.

All the three parameters, viz. shoot length, root length and dry weight of the seedlings were statistically analysed and found that shoot length had no significant change between the varieties (F=1.075 NS) as well as between the fungal species (F=0.551 NS); there was significant difference in root length between the varieties (F=7.271**) but not between the fungal species (F=1.652 NS); change in the dry weight of the seedlings was found to be non-significant both between the fungal species (F=2.571 NS0) and between the varieties (F=1.524 NS).

The effect of the fungal extract on the root-shoot and dry weight was found to be more pronounced in the pot culture experiment than that of test tube seedling symptom test (table 3.4). In this experiment also there was a marked reduction in the shoot length due to the fungal extract except two cases of marginal promotion in the variety Vijay treated with the extract of *A. alternata* and *A.flavus*. In the variety local White, the extract of *A. alternata, A. flavus, F. moniliforme* and *T. viride* caused considerable reduction in the shoot length with the percentage inhibition of 33.74, 40.49, 42.94 and 37.42 respectively but *P. expansum* and *R. nigricans* were found not to be very effective in reducing the shoot length in this variety. In the variety Vijay, *F. moniliforme* caused maximum reduction in the shoot length (40.8%) followed by *T. viride* (39.2%) but there was 2.4% and 0.8% promotion in the shoot length due to the extract of *A. alternata*

and *A. flavus* respectively in this variety. Extract of *R. nigricans* was found to be the most effective in the variety VL-16 with 23.77% reduction in shoot length followed by *T. viride* (18.85%). Except a single case of marginal promotion in the root length in the variety Vijay treated with *A. flavus* (1.64%) there was considerable reduction in the root length of the seedlings of all the varieties treated with the extract of the test fungi. Extract of *F. moniliforme* and *T. viride* was harmful for root elongation in all the varieties; the percentage inhibition caused by *F. moniliforme* was 38.64, 28.02 and 41.3 in the varieties Local White, Vijay and VL-16 respectively, whereas *T. viride* induced an inhibition of 29.46%, 26.37% and 40.5% respectively, but the maximum inhibition of shoot elongation was caused by *A. flavus* in the variety VL-16 with a percentage inhibition of 46.83. In the pot culture experiment also there was reduction in the dry weight of the seedlings of all the varieties treated with the extract of the test fungi. *F. moniliforme* caused a percentage reduction of 18.37, 13.95 and 18.87 in dry weight in the varieties Local White, Vijay and VL-16 respectively, whereas *T. viride* was responsible for a reduction of 11.35%, 16.27% and 19.38% respectively. The maximum reduction in the dry weight was caused by the extract of *A. flavus* (25.51%) in the variety VL-16.

In this experiment also there was no statistically significant change in the shoot length both between the varieties (F=1.685 NS) and between the fungal species (F=2.061 NS); but the root length showed significant variations both between the varieties (F=16.853**) and between the fungal species (F=4.64*); the dry

weight of the seedlings had significant variations between the fungal species (F=3.221*) but not between the varieties (F=2.391 NS).

IV. EFFECT OF CERTAIN SEED-DRESSING AGRO-CHEMICALS ON THE SEED-BORNE MYCOFLORA OF MAIZE

The six test fungi, viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Pencillium expansum, Rhizopus nigricans* and *Trichoderma viride* were tested against different concentrations (50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm, 2500 ppm and 5000 ppm) of eight fungicides, viz. agrosan GN, bavistin, benomyl, blitox-50, captan, dithane M-45, thiram and thiram+captan for their spore germination and colony growth. The results of the experiments are presented in the tables 4.1 and 4.2 and figures 4.1 and 4.2.

Spore germination: It is evident from the results that all the eight fungicides tested exhibited inhibitory influence on the spore germination of the test fungi in all the concentrations used. Almost all the fungicides were most effective at the concentrations of 2500 ppm and 5000 ppm where there was total inhibition of spore germination except a few isolated cases (tables 4.1a to 4.1h and figures 4.1a to 4.1f).

Table 4.1a reveals that agrosan GN was the most effective against *P.expansum, R. nigricans* and *T. viride* where 100 percent inhibition of spore germination was observed at the concentration of 1000 ppm, whereas *A. alternata, A. flavus* and *F. moniliforme* showed certain amount of tolerance towards this fungicide.

Bavistin was found to be most effective against *F. moniliforme* where spores failed to germinate even at the concentration of 500 ppm (table 4.1b) and none of the fungal spores could germinate at the concentration of 2500 ppm. Benomyl did not prove to be very effective against the fungi tested (table 4.1c) though *A. flavus* and *T. viride* were inhibited at the concentration of 2500 ppm. It was least effective against *A. alternata* and *F. moniliforme* which germinated even at the highest concentration of 5000 ppm(26.9% and 37.5% respectively). It is clear from the results in table 4.1d that blitox-50 was not very effective in the total inhibition of spore germination of the test fungi where spores of *A. alternata* and *T. viride* could germinate even in the highest concentration (5000 ppm) though in a small percentage; only *F. moniliforme* was completely inhibited at the concentration of 2500 ppm. Captan could inhibit the spore germination of *A. alternata, P. expansum* and *T. viride* at 1000 ppm, but *F. moniliforme* was less effected and could germinate even at the concentration of 2500 ppm (table 4.1e). Dithane M-45 was found to be very effective against all the test fungi (table 4.1f) except *A. alternata* whose spore germination could not be inhibited completely even at the highest concentration (5000 ppm). Dithane M-45 proved to be most toxic to *R. nigricans* which showed 89.8% inhibition of spore germination at a concentration as a low as 200 ppm. Table 4.1g indicates that spores of *A. alternata* and *F. moniliforme* were inhibited to the extent of 89.3% and 86.4% respectively by thiram at the concentration of 200 ppm and 100% inhibition was observed at 5000 ppm, whereas it was found to be least effective against *A. flavus* which could germinate even at

Table 4.1a Effect of different concentrations of agrosan GN on the spore germination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	76.1 (23.9)*	63.2 (36.8)	50.3 (49.7)	26.3 (73.7)	14.2 (85.8)	5.2 (94.8)	—
Aspergillus flavus	79.5	65.1 (18.1)	49.3 (37.9)	36.7 (53.8)	19.5 (75.4)	7.5 (90.5)	—	—
Fusarium moniliforme	82.0	56.7 (30.8)	41.5 (49.3)	27.9 (65.9)	15.6 (80.9)	8.9 (89.1)	—	—
Penicillium expansum	82.2	37.6 (54.2)	30.5 (62.8)	17.8 (78.3)	10.7 (86.9)	—	—	—
Rhizopus nigricans	78.0	56.7 (27.3)	36.5 (53.2)	21.7 (72.1)	5.6 (92.8)	—	—	—
Trichoderma viride	90.4	62.3 (31.0)	42.7 (52.7)	26.3 (70.9)	7.5 (91.7)	—	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

- => Total inhibition of spore germination.

Table 4.1b Effect of different concentrations of bavistin on the spore germaination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	96.1 (3.9)*	80.2 (19.8)	52.6 (47.4)	25.3 (74.7)	15.4 (84.6)	—	—
Aspergillus flavus	79.5	70.3 (11.5)	56.3 (29.1)	31.4 (60.5)	6.5 (91.8)	—	—	—
Fusarium moniliforme	82.0	36.7 (55.2)	10.5 (87.1)	5.2 (93.6)	—	—	—	—
Penicillium expansum	82.2	69.3 (15.6)	59.6 (27.4)	41.3 (49.7)	25.7 (68.7)	9.7 (88.1)	—	—
Rhizopus nigricans	78.0	59.1 (24.2)	40.3 (48.3)	29.5 (62.1)	9.5 (87.8)	—	—	—
Trichoderma viride	90.4	51.8 (42.6)	29.3 (67.5)	10.7 (88.1)	3.8 (95.7)	—	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

- => Total inhibition of spore germination.

Table 4.1c Effect of different concentrations of benomyl on the spore germaination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	92.1 (7.9)*	86.4 (13.6)	81.3 (18.8)	70.2 (29.8)	60.3 (39.7)	46.3 (53.7)	26.9 (73.1)
Aspergillus flavus	79.5	46.5 (41.5)	31.7 (60.1)	21.4 (73.0)	17.7 (77.7)	5.6 (92.9)	—	—
Fusarium moniliforme	82.0	73.5 (10.3)	70.7 (13.7)	65.3 (20.3)	60.6 (26.0)	56.3 (31.3)	49.7 (39.3)	37.5 (54.2)
Penicillium expansum	82.2	75.3 (8.3)	57.8 (29.6)	49.3 (40.0)	25.7 (68.7)	19.7 (76.0)	12.5 (84.7)	—
Rhizopus nigricans	78.0	65.8 (15.6)	56.7 (27.3)	49.5 (36.5)	30.5 (60.8)	15.7 (79.8)	6.3 (91.9)	—
Trichoderma viride	90.4	75.7 (16.2)	62.3 (31.0)	46.5 (48.5)	22.3 (75.3)	5.6 (93.8)	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

- => Total inhibition of spore germination.

Table 4.1d Effect of different concentrations of blitox-50 on the spore germaination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	69.6 (30.4)*	60.1 (39.9)	45.2 (54.8)	36.1 (63.9)	29.5 (70.5)	20.1 (79.9)	10.5 (89.5)
Aspergillus flavus	80.5	59.3 (26.3)	46.1 (42.7)	35.3 (56.1)	21.3 (73.5)	10.3 (87.2)	6.7 (91.6)	—
Fusarium moniliforme	83.0	56.7 (31.6)	39.1 (52.8)	19.3 (76.7)	10.2 (87.7)	5.6 (93.2)	—	—
Penicillium expansum	82.4	50.8 (38.3)	39.6 (51.9)	26.5 (67.8)	19.7 (76.0)	11.5 (86.0)	4.7 (94.2)	—
Rhizopus nigricans	76.0	65.5 (13.8)	55.7 (26.7)	37.8 (50.2)	21.7 (71.4)	15.5 (79.6)	5.6 (92.6)	—
Trichoderma viride	90.7	76.7 (15.4)	56.3 (37.9)	44.5 (50.9)	31.9 (64.8)	19.8 (78.1)	10.5 (88.4)	3.6 (96.0)

* => Figures in parenthesis indicate percentage inhibition of spore germination

- => Total inhibition of spore germination.

Table 4.1e Effect of different concentrations of captan on the spore germaination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	20.1 (79.9)*	16.3 (83.7)	10.1 (89.9)	5.1 (94.9)	—	—	—
Aspergillus flavus	79.5	62.3 (21.6)	45.1 (43.2)	26.5 (66.6)	16.5 (79.2)	5.5 (93.0)	—	—
Fusarium moniliforme	82.0	69.6 (15.1)	51.5 (37.1)	37.3 (54.5)	21.5 (73.7)	15.6 (80.9)	10.3 (87.4)	—
Penicillium expansum	82.2	51.3 (37.5)	37.6 (54.2)	20.5 (75.0)	9.8 (88.0)	—	—	—
Rhizopus nigricans	78.0	59.7 (23.4)	47.8 (38.7)	29.5 (62.1)	20.6 (73.5)	10.5 (86.5)	—	—
Trichoderma viride	90.4	62.7 (30.6)	59.1 (34.6)	20.3 (77.5)	11.1 (87.7)	—	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

\- => Total inhibition of spore germination.

Table 4.1f Effect of different concentrations of dithane M-45 on the spore germination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	67.3 (32.7)*	50.1 (49.9)	46.2 (53.8)	31.6 (68.4)	26.5 (73.5)	18.9 (81.1)	10.6 (89.4)
Aspergillus flavus	79.5	56.3 (29.1)	39.5 (50.3)	27.6 (65.2)	16.5 (79.2)	—	—	—
Fusarium moniliforme	88.0	62.4 (29.0)	32.7 (62.8)	27.5 (68.7)	19.6 (77.7)	7.9 (91.0)	—	—
Penicillium expansum	82.4	70.5 (14.4)	59.6 (27.6)	32.5 (60.5)	19.7 (76.0)	10.3 (87.5)	—	—
Rhizopus nigricans	76.2	49.5 (35.0)	31.4 (58.7)	7.7 (89.8)	—	—	—	—
Trichoderma viride	92.5	56.7 (38.7)	39.5 (57.2)	17.7 (80.8)	—	—	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

\- => Total inhibition of spore germination.

Table 4.1g Effect of different concentrations of thiram on the spore germaination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	46.6 (53.4)*	30.2 (69.8)	10.7 (89.3)	—	—	—	—
Aspergillus flavus	86.0	71.5 (16.9)	65.3 (24.0)	56.3 (34.5)	45.7 (46.8)	31.6 (63.2)	20.1 (76.6)	5.1 (94.0)
Fusarium moniliforme	90.5	51.5 (43.0)	26.9 (70.2)	12.3 (86.4)	—	—	—	—
Penicillium expansum	82.2	63.3 (22.9)	56.3 (31.5)	42.1 (48.7)	34.1 (61.8)	16.5 (79.9)	7.5 (90.8)	—
Rhizopus nigricans	76.5	42.4 (44.5)	31.7 (58.5)	22.2 (70.9)	17.1 (77.6)	10.5 (86.2)	2.6 (96.6)	—
Trichoderma viride	96.2	76.1 (20.8)	56.3 (41.4)	39.5 (58.9)	15.7 (83.6)	9.6 (90.0)	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

- => Total inhibition of spore germination.

Table 4.1h Effect of different concentrations of thiram+captan on the spore germination of certain seed mycoflora of maize.

Fungi	Spore germination (%)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	100	16.2 (83.8)*	10.5 (89.5)	5.6 (94.4)	—	—	—	—
Aspergillus flavus	79.5	26.5 (66.6)	15.7 (80.2)	9.5 (88.0)	3.5 (95.5)	—	—	—
Fusarium moniliforme	82.0	56.3 (31.3)	37.2 (54.6)	10.5 (87.1)	—	—	—	—
Penicillium expansum	82.2	46.5 (43.4)	29.3 (64.3)	18.8 (77.1)	9.2 (88.8)	—	—	—
Rhizopus nigricans	78.0	50.2 (35.6)	35.7 (54.2)	21.8 (72.0)	9.9 (87.3)	—	—	—
Trichoderma viride	90.4	36.5 (59.6)	21.7 (75.9)	12.8 (85.8)	9.8 (89.1)	—	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

- => Total inhibition of spore germination.

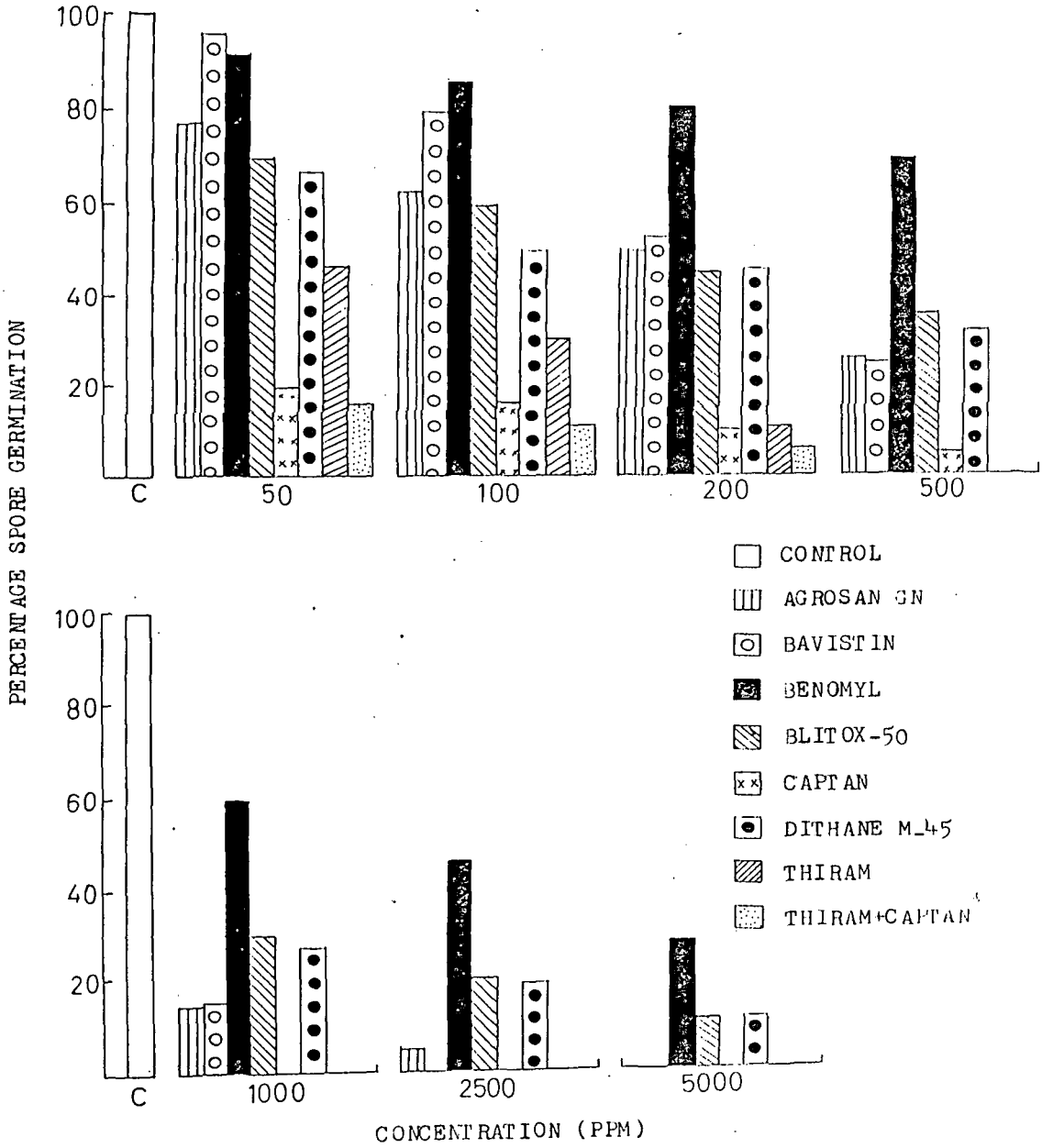


Fig. 4.1a Showing change in the spore germination of A. alternata due to the treatment of different fungicides in different concentrations.

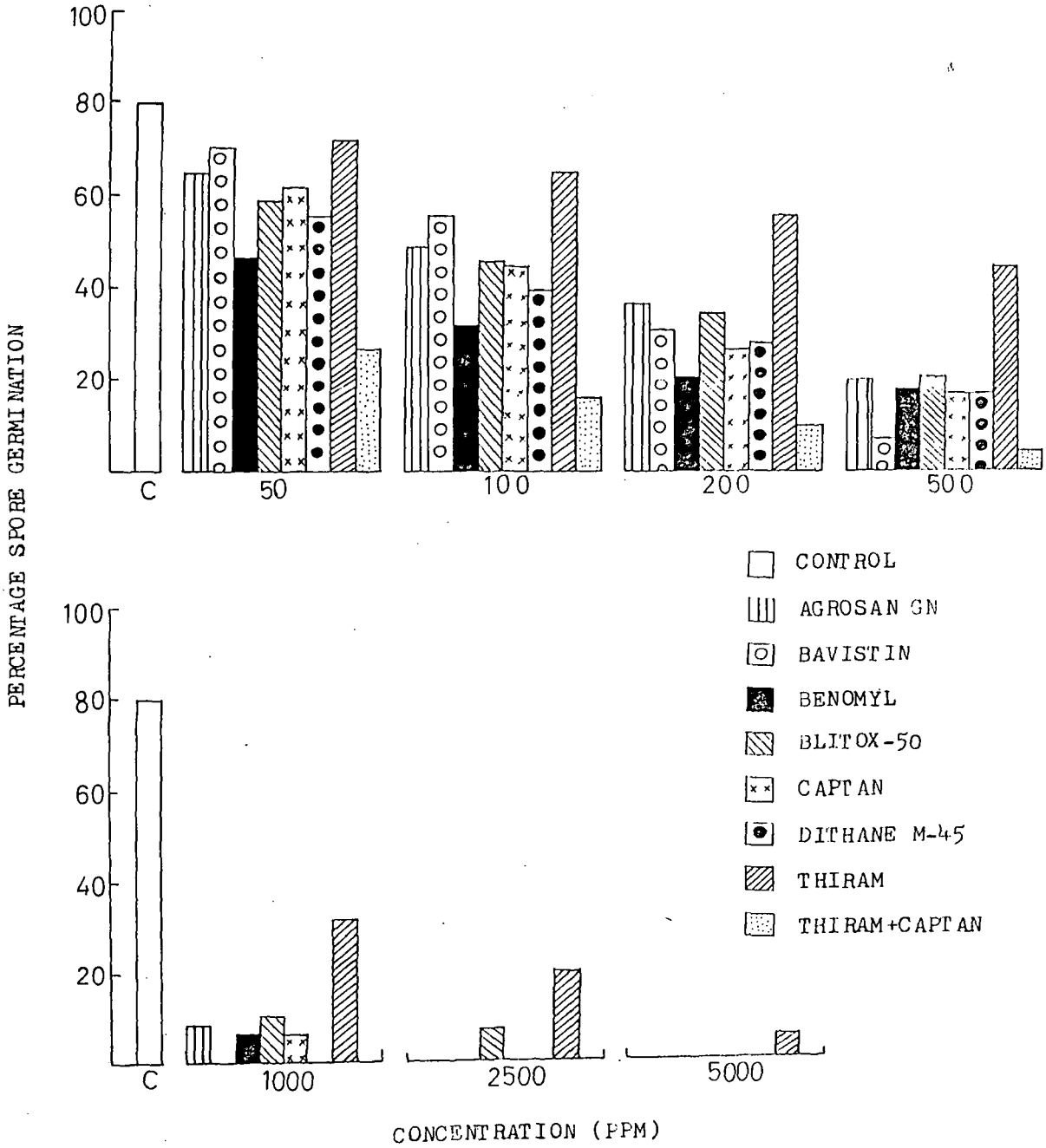


Fig. 4.1b Showing change in the spore germination of A. flavus due to the treatment of different fungicides in different concentrations.

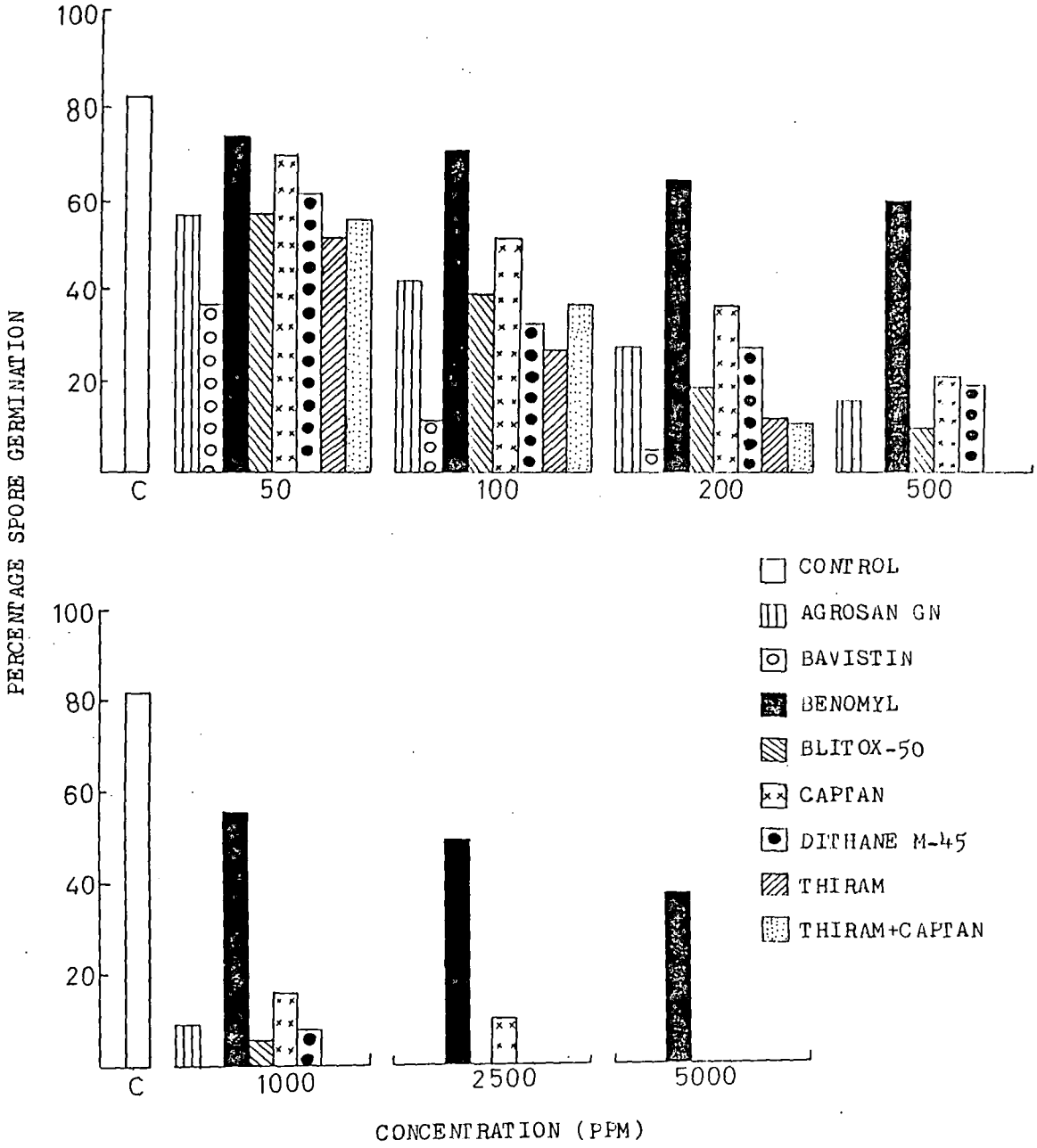


Fig. 4.1c Showing change in the spore germination of F. moniliforme due to the treatment of different fungicides in different concentrations.

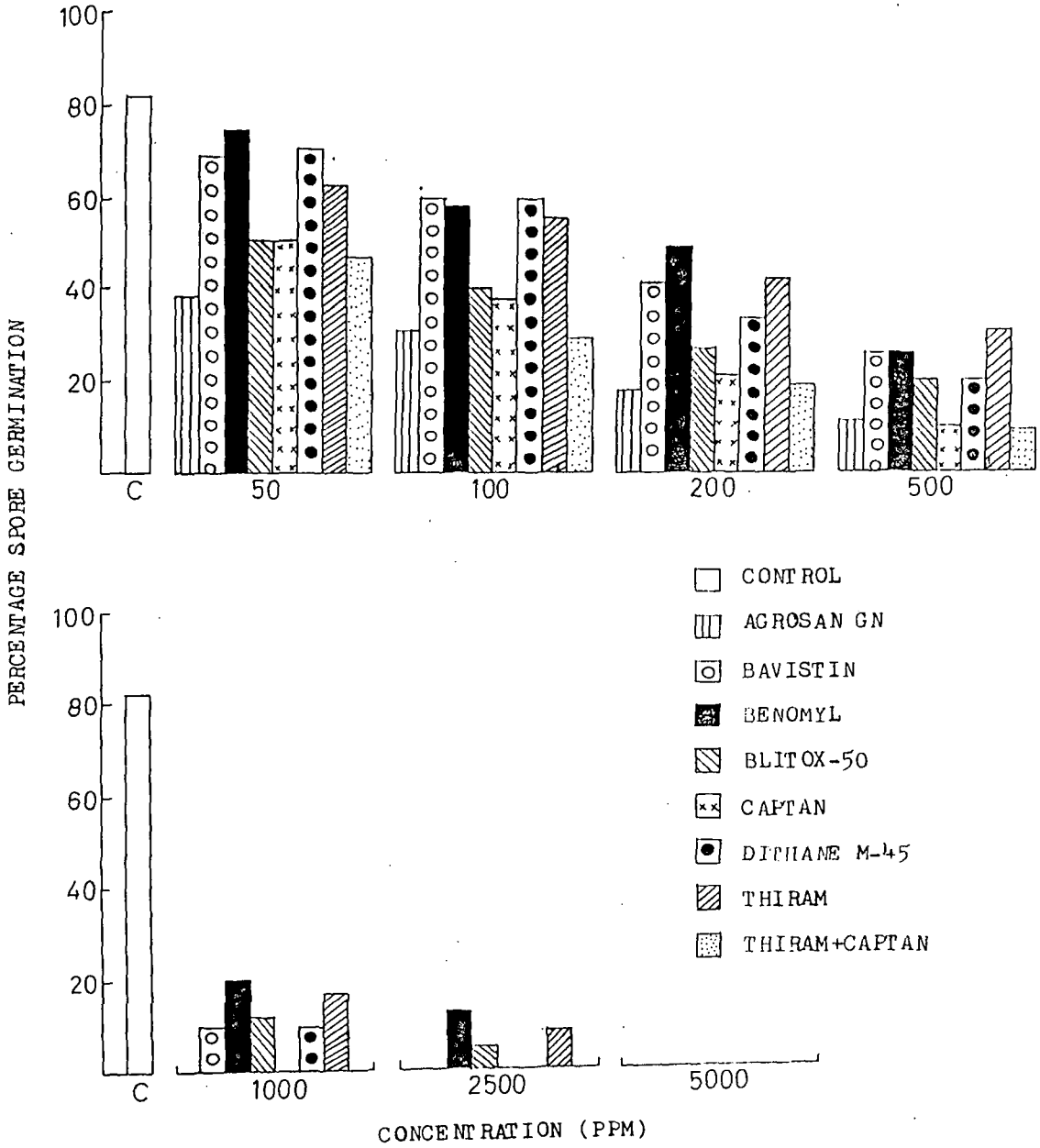


Fig. 4.1d Showing change in the spore germination of P. expansum due to the treatment of different fungicides in different concentrations.

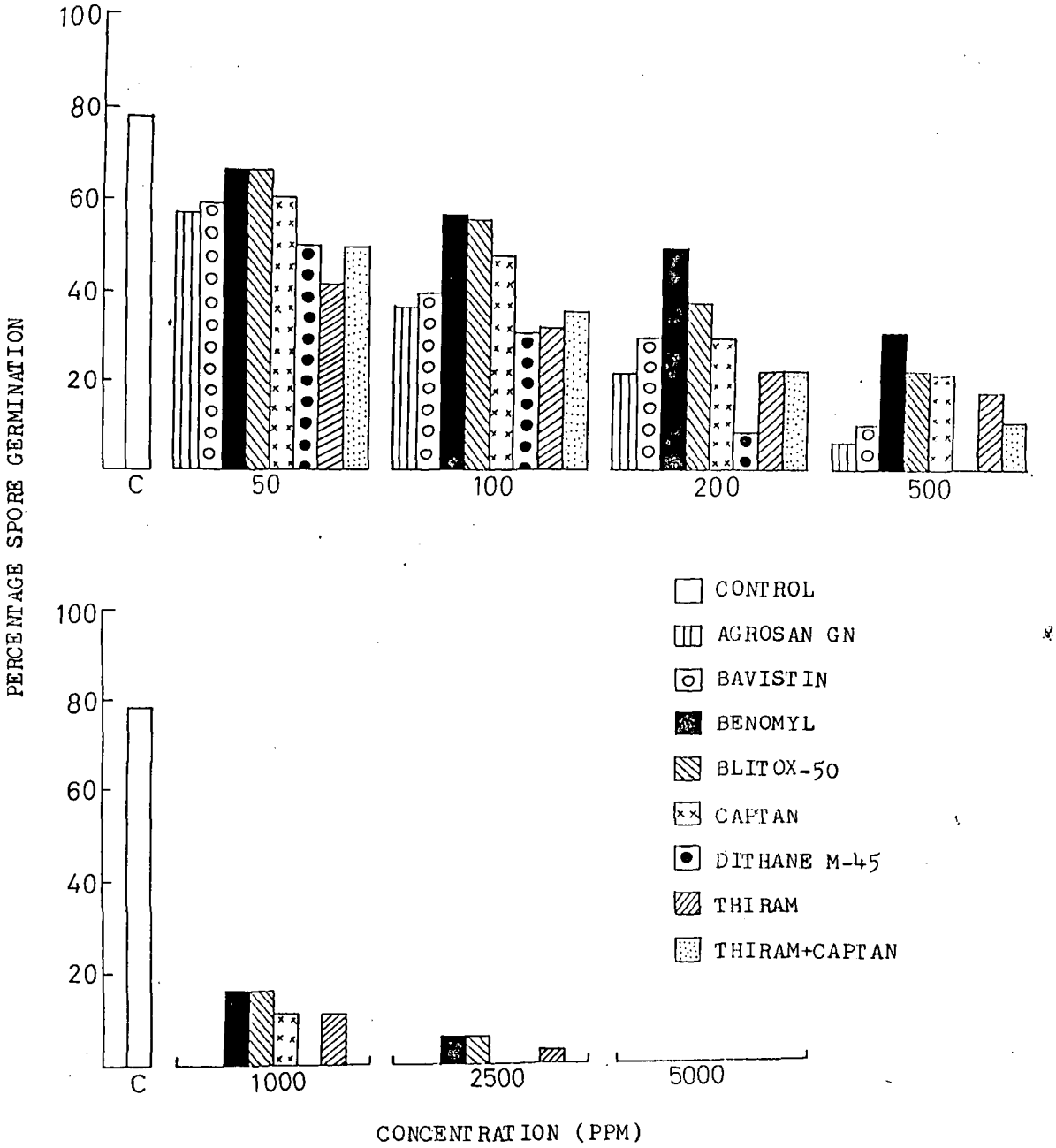


Fig. 4.1e Showing change in the spore germination of R. nigricans due to the treatment of different fungicides in different concentrations.

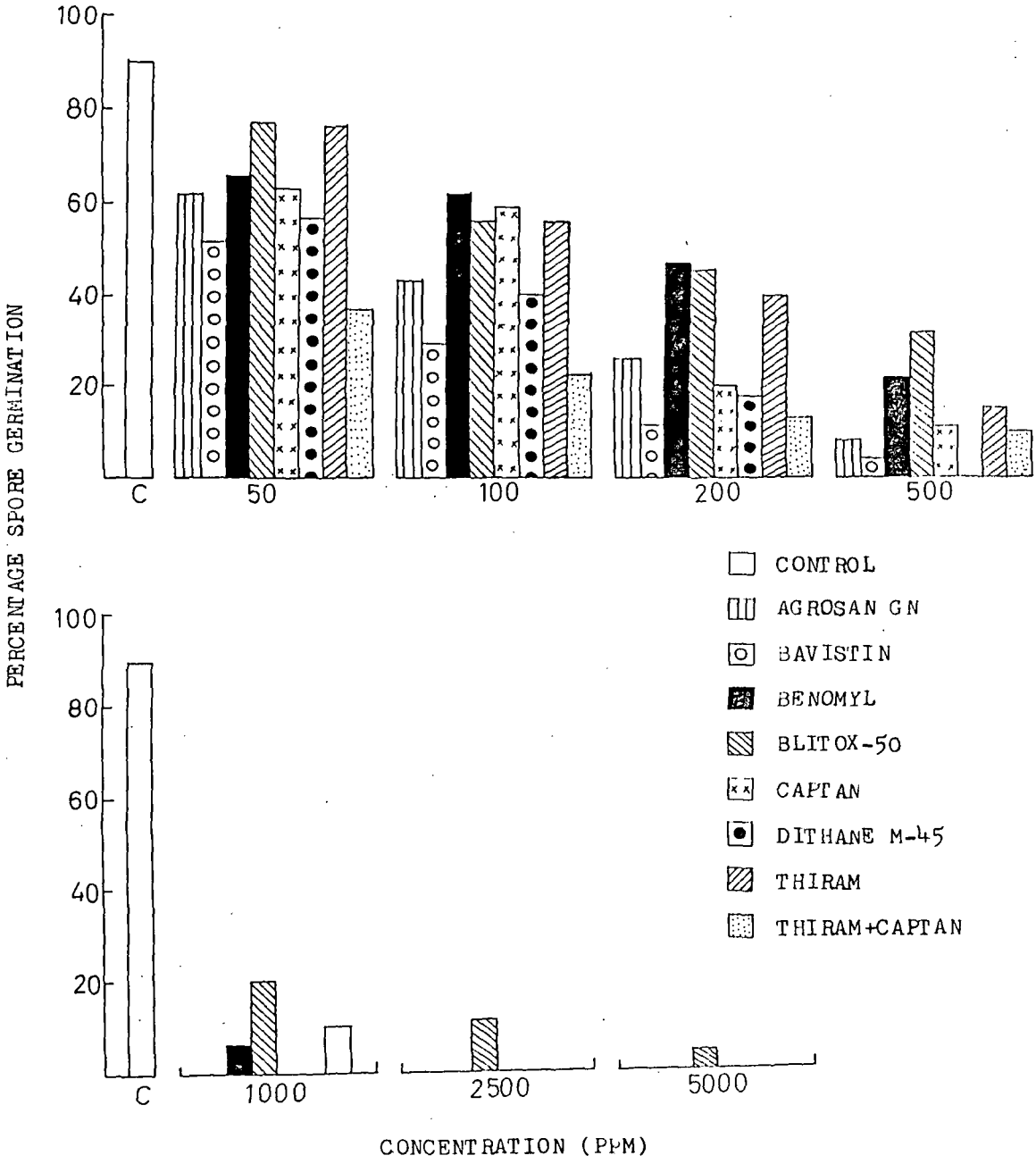


Fig. 4.1f Showing change in the spore germination of T. viride due to the treatment of different fungicides in different concentrations.

the highest concentration (5000 ppm). The combined effect of thiram and captan on the spore germination was more pronounced than when used individually (table 4.1h). None of the fungal spores could germinate at the concentration of 1000 ppm; *A. alternata* and *F. moniliforme* had 100% inhibition at 500 ppm. Thus it is evident that thiram+captan combination was the most effective fungicide in inhibiting the spore germination of all the test fungi, followed by dithane M-45 and captan.

Spore germination of the six test fungi in different concentrations of the fungicides was statistically analysed by calculating the F value which was found to be highly significant between different concentrations of the fungicides (F=178.782**, 59.989**, 47.238**, 291.337**, 72.921**, 157.808**, 63.339** and 94.955** for agrosan GN, bavistin, benomyl, blitox-50, captan, dithane M-45, thiram and thiram+captan respectively), and also between the fungal species (F=11.651**, 8.117**, 26.3**, 26.097**, 3.571**, 14.625** and 9.317** for agrosan GN, bavistin, benomyl, blitox-50, captan, dithane M-45 and thiram respectively). Thiram+captan did not show significant change between the fungal species (F=1.727 NS).

Colony growth: Nutrient media (PDA) were poisoned with different concentrations of the fungicides and the six test fungi were grown on the treated media, the control being maintained on pure PDA medium. The diameter of the fungal colony in the treated samples was compared with that of the control and the results in the form of average diameter of fungal colony and the percentage inhibition of growth are presented in the tables 4.2a to 4.2h and

figures 4.2a to 4.2f. It has been observed that all the fungicides tested stopped the mycelial growth of the test fungi at the concentration of 5000 ppm except those of *A. alternata* and *R. nigricans* treated with blitox-50.

Agrosan GN inhibited the mycelial growth of *R. nigricans* and *T. viride* at the concentration of 1000 ppm and that of *P. expansum* at 2500 ppm (table 4.2a). Bavistin proved to be very effective in checking the mycelial growth of all the test fungi with *A. flavus, F. moniliforme* and *T. viride* being checked completely at the concentration of 50 ppm (table 4.2b); only *R. nigricans* had some growth at 2500 ppm. The inhibition of mycelial growth was noticed in all the test fungi treated with benomyl but it could not check the growth completely. There was some growth even at the concentration of 2500 ppm and 5000 ppm (table 4.2c). Similar pattern was noticed in the case of blitox-50 which could inhibit the mycelial growth of *R. nigricans* at 1000 ppm, *A. alternata* and *F. moniliforme* at 2000 ppm, but the growth of *T. viride* could not be checked completely even at the highest concentration of 5000 ppm (table 4.2d). *P. expansum* was found to be very sensitive to captan whose growth was arrested completely at 500 ppm (table 4.2e), followed by *A. alternata* at 1000 ppm, *A. flavus* and *F. moniliforme* at 2500 ppm and *R. nigricans* and *T. viride* could be checked only at 5000 ppm. Dithane M-45 exhibited total inhibition of mycelial growth of the test fungi at the concentration of 2500 ppm, but the growth of *R. nigricans* and *T. viride* was effectively checked at 500 ppm (table 4.2f). It caused percentage inhibition of 78.4, 72.4 and 20.3 for *A. flavus, F. moniliforme* and *P. expansum* respectively at the

Table 4.2a Effect of different concentrations of agrosan GN on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	52.5 (18.6)*	49.3 (23.5)	40.4 (37.3)	31.5 (51.1)	26.7 (58.6)	19.3 (70.0)	—
Aspergillus flavus	60.8	51.3 (15.6)	46.6 (23.3)	38.1 (37.3)	30.1 (50.4)	21.1 (65.2)	19.6 (67.7)	—
Fusarium moniliforme	69.7	63.2 (9.3)	52.2 (25.1)	46.6 (33.1)	33.4 (52.0)	27.4 (60.6)	11.6 (83.3)	—
Penicillium expansum	54.5	47.4 (13.0)	39.9 (26.7)	26.3 (51.7)	20.5 (62.3)	12.3 (77.4)	—	—
Rhizopus nigricans	92.6	63.7 (31.2)	47.7 (48.4)	24.4 (73.6)	12.3 (86.7)	—	—	—
Trichoderma viride	72.3	52.4 (27.5)	42.9 (40.6)	31.7 (56.1)	16.8 (76.7)	—	—	—

* => Figures in parenthesis indicate percentage inhibition of spore germination

\- => Total inhibition of spore germination.

Table 4.2b Effect of different concentrations of bavistin on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	59.7 (7.4)	42.2 (34.5)	36.8 (42.9)	19.7 (69.4)	12.3 (80.9)	—	—
Aspergillus flavus	60.8	31.3 (48.5)	18.6 (69.4)	11.2 (81.5)	—	—	—	—
Fusarium moniliforme	69.7	45.6 (34.5)	30.3 (56.5)	10.5 (84.9)	—	—	—	—
Penicillium expansum	54.5	50.3 (07.7)	42.7 (21.6)	32.3 (40.7)	27.6 (49.3)	19.6 (64.0)	—	—
Rhizopus nigricans	92.6	69.9 (24.5)	57.3 (38.1)	36.3 (60.7)	24.5 (73.5)	16.3 (82.3)	11.5 (87.5)	—
Trichoderma viride	72.3	49.6 (31.3)	30.3 (58.0)	20.5 (71.6)	—	—	—	—

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

- => Total inhibition of mycelial growth.

Table 4.2c Effect of different concentrations of benomyl on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	59.1 (08.3)*	49.3 (23.5)	38.2 (40.7)	27.5 (57.3)	20.3 (68.5)	16.3 (74.7)	11.1 (82.7)
Aspergillus flavus	60.8	50.4 (17.1)	42.1 (30.7)	30.5 (49.8)	26.3 (56.7)	20.4 (66.4)	17.3 (71.5)	—
Fusarium moniliforme	69.7	56.4 (19.0)	40.2 (42.3)	31.1 (55.3)	20.7 (70.3)	12.4 (82.2)	—	—
Penicillium expansum	54.5	50.7 (06.9)	46.3 (15.0)	40.4 (25.8)	32.1 (41.1)	27.7 (49.1)	19.6 (64.0)	—
Rhizopus nigricans	92.6	76.7 (17.1)	66.8 (27.8)	53.7 (42.0)	41.1 (55.6)	26.8 (71.0)	20.4 (77.9)	14.5 (84.3)
Trichoderma viride	72.3	65.9 (08.8)	59.7 (17.4)	42.5 (41.2)	32.1 (55.6)	29.8 (58.7)	14.6 (79.8)	—

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

- => Total inhibition of mycelial growth.

Table 4.2d Effect of different concentrations of blitox-50 on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	58.3 (09.6)*	46.7 (27.5)	30.8 (52.2)	16.7 (74.1)	11.3 (82.4)	—	—
Aspergillus flavus	60.8	56.1 (07.7)	50.2 (17.4)	44.3 (27.1)	38.7 (36.3)	26.5 (56.4)	20.1 (40.7)	—
Fusarium moniliforme	69.7	58.3 (16.3)	40.1 (42.4)	29.7 (57.3)	19.3 (72.3)	10.1 (85.5)	—	—
Penicillium expansum	54.5	50.4 (07.5)	49.2 (09.7)	38.7 (28.9)	29.5 (45.8)	23.7 (56.5)	12.6 (76.8)	—
Rhizopus nigricans	92.6	69.9 (24.5)	47.8 (48.3)	20.3 (78.0)	12.5 (86.5)	—	—	—
Trichoderma viride	72.3	65.3 (09.6)	45.3 (37.3)	40.7 (43.7)	36.9 (48.9)	29.8 (58.7)	17.3 (76.0)	12.5 (82.7)

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

\- => Total inhibition of mycelial growth.

Table 4.2e Effect of different concentrations of captan on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	52.3 (18.9)*	40.7 (36.8)	20.6 (68.0)	11.2 (82.6)	—	—	—
Aspergillus flavus	60.8	49.6 (18.4)	39.8 (34.5)	27.3 (55.0)	20.4 (66.4)	16.3 (73.1)	—	—
Fusarium moniliforme	69.7	55.4 (20.5)	26.7 (61.6)	22.3 (68.0)	16.5 (76.3)	10.4 (85.0)	—	—
Penicillium expansum	54.5	40.2 (26.2)	31.5 (42.2)	19.6 (64.0)	—	—	—	—
Rhizopus nigricans	92.6	70.4 (23.9)	68.6 (25.9)	40.5 (56.2)	37.7 (59.2)	26.9 (70.9)	13.8 (85.0)	—
Trichoderma viride	72.3	62.4 (13.6)	53.7 (25.7)	36.4 (49.6)	29.8 (58.7)	20.4 (71.7)	13.4 (81.4)	—

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

- => Total inhibition of mycelial growth.

Table 4.2f Effect of different concentrations of dithane M-45 on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	56.7 (12.0)*	38.3 (40.6)	29.7 (53.9)	16.3 (74.7)	—	—	—
Aspergillus flavus	60.8	46.7 (23.1)	31.3 (48.5)	20.4 (66.4)	17.3 (71.5)	13.1 (78.4)	—	—
Fusarium moniliforme	69.7	60.1 (13.7)	49.7 (28.6)	39.5 (43.3)	26.6 (61.8)	19.2 (72.4)	—	—
Penicillium expansum	54.5	51.1 (06.2)	46.4 (14.8)	44.5 (18.3)	30.5 (44.0)	20.3 (34.2)	—	—
Rhizopus nigricans	92.6	57.4 (38.0)	32.6 (64.7)	16.5 (82.1)	—	—	—	—
Trichoderma viride	72.3	46.3 (35.9)	27.4 (62.1)	16.7 (76.9)	—	—	—	—

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

- => Total inhibition of mycelial growth.

Table 4.2g Effect of different concentrations of thiram on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	56.8 (11.9)*	35.2 (45.4)	12.1 (81.2)	—	—	—	—
Aspergillus flavus	60.8	49.1 (19.2)	37.8 (37.8)	30.3 (50.1)	26.4 (56.5)	20.7 (65.9)	12.1 (80.0)	—
Fusarium moniliforme	69.7	40.2 (42.3)	29.5 (57.6)	11.7 (83.2)	—	—	—	—
Penicillium expansum	54.5	51.8 (04.9)	43.2 (20.7)	40.5 (25.6)	33.4 (38.7)	29.4 (46.0)	19.5 (64.2)	—
Rhizopus nigricans	92.6	70.3 (24.0)	65.9 (28.8)	40.2 (56.5)	36.9 (60.1)	20.7 (77.6)	13.5 (85.4)	—
Trichoderma viride	72.3	66.4 (08.1)	62.6 (13.4)	51.7 (28.4)	38.8 (46.3)	20.9 (71.0)	15.8 (78.1)	—

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

- => Total inhibition of mycelial growth.

Table 4.2h Effect of different concentrations of thiram+captan on the colony growth of certain seed mycoflora of maize.

Fungi	Average diameter of fungal colony (mm)							
	Concentration in ppm							
	Control	50	100	200	500	1000	2500	5000
Alternaria alternata	64.5	46.2 (28.3)*	23.7 (63.2)	—	—	—	—	—
Aspergillus flavus	60.8	39.6 (34.8)	26.8 (55.9)	19.6 (67.7)	12.1 (80.0)	—	—	—
Fusarium moniliforme	69.7	56.6 (18.7)	43.5 (37.5)	21.1 (69.7)	13.4 (80.7)	—	—	—
Penicillium expansum	54.5	31.6 (42.0)	24.3 (55.4)	—	—	—	—	—
Rhizopus nigricans	92.6	72.4 (21.8)	62.3 (32.7)	35.6 (61.5)	30.1 (67.4)	12.5 (86.5)	—	—
Trichoderma viride	72.3	59.7 (17.4)	48.5 (32.9)	38.8 (46.3)	22.8 (68.4)	14.5 (79.9)	—	—

* => Figures in parenthesis indicate percentage inhibition of mycelial growth

- => Total inhibition of mycelial growth.

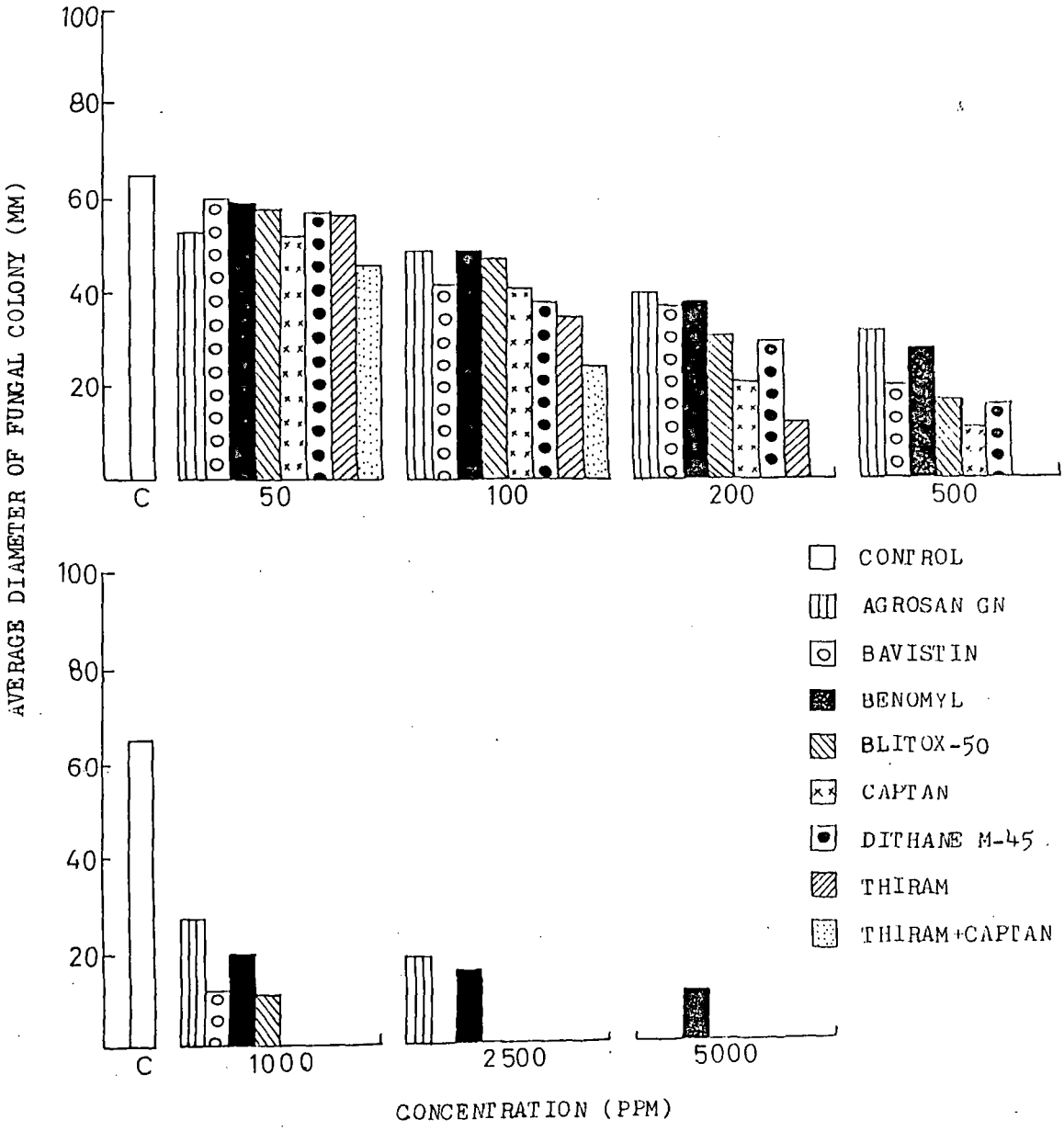


Fig. 4.2a Showing change in the average diameter of the colony of A. alternata due the treatment of different fungicides in different concentrations.

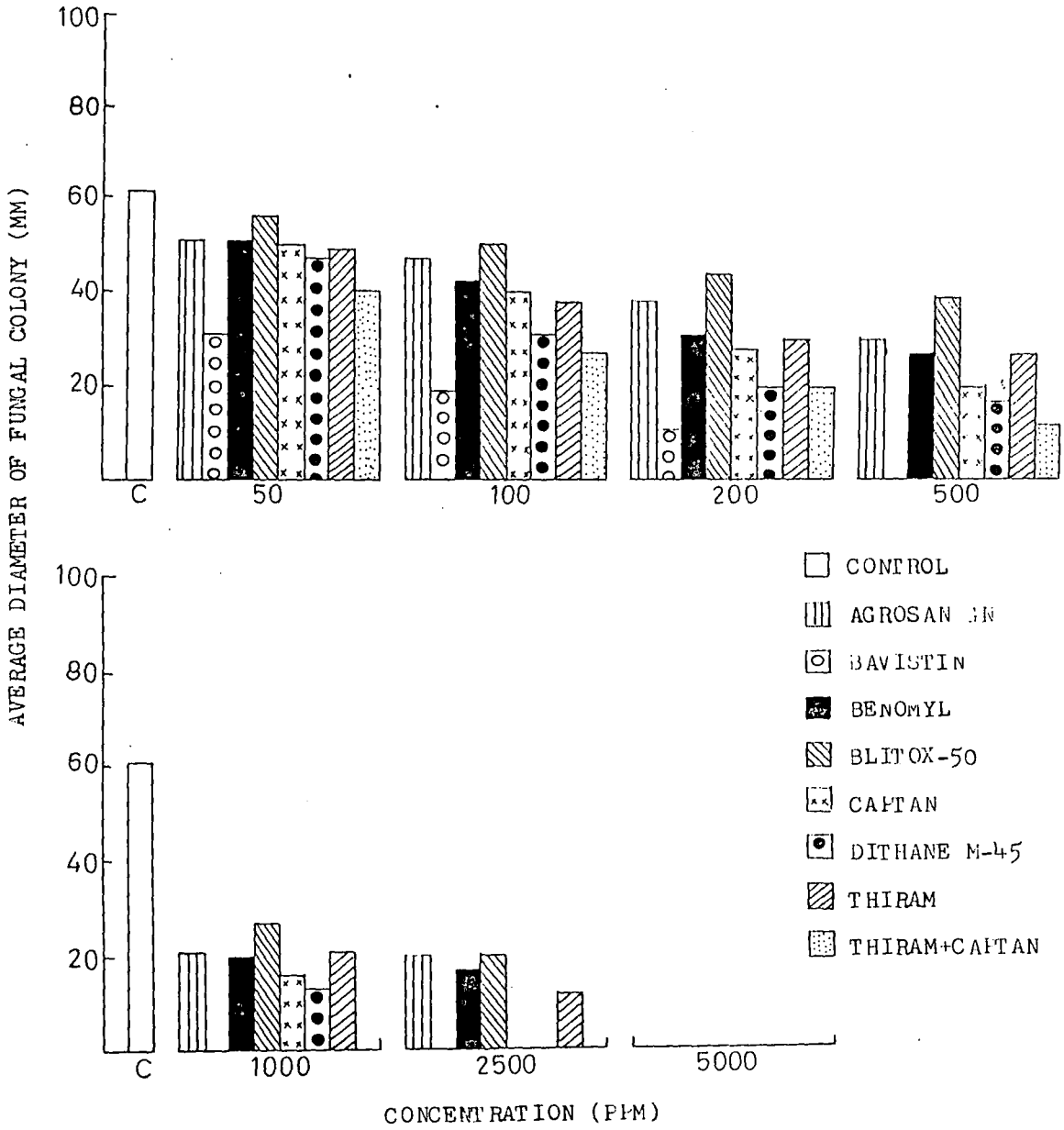


Fig. 4.2b Showing change in the average diameter of the colony of A. flavus due the treatment of different fungicides in different concentrations.

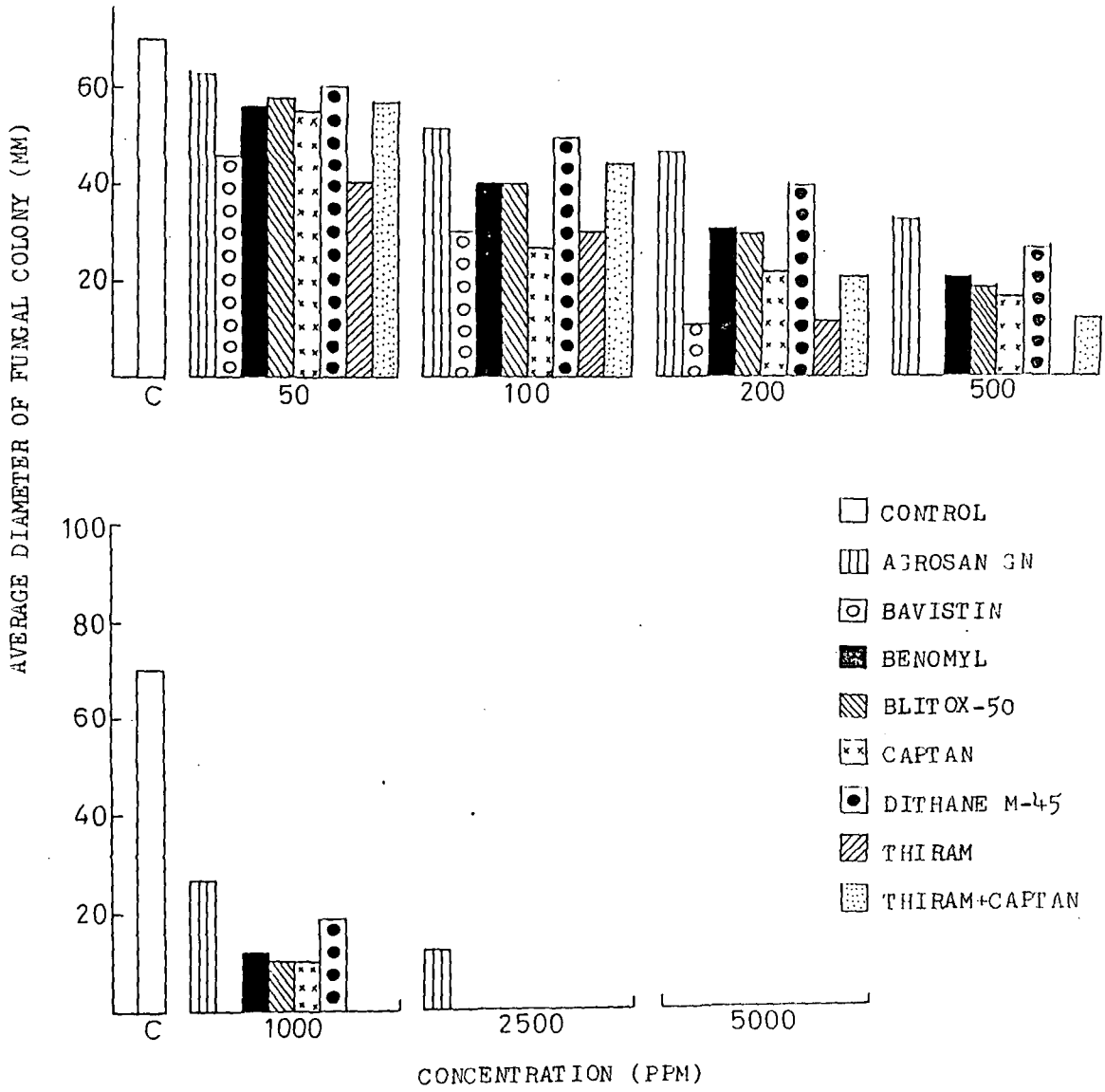


Fig. 4.2c Showing change in the average diameter of the colony of F. moniliforme due the treatment of different fungicides in different concentrations.

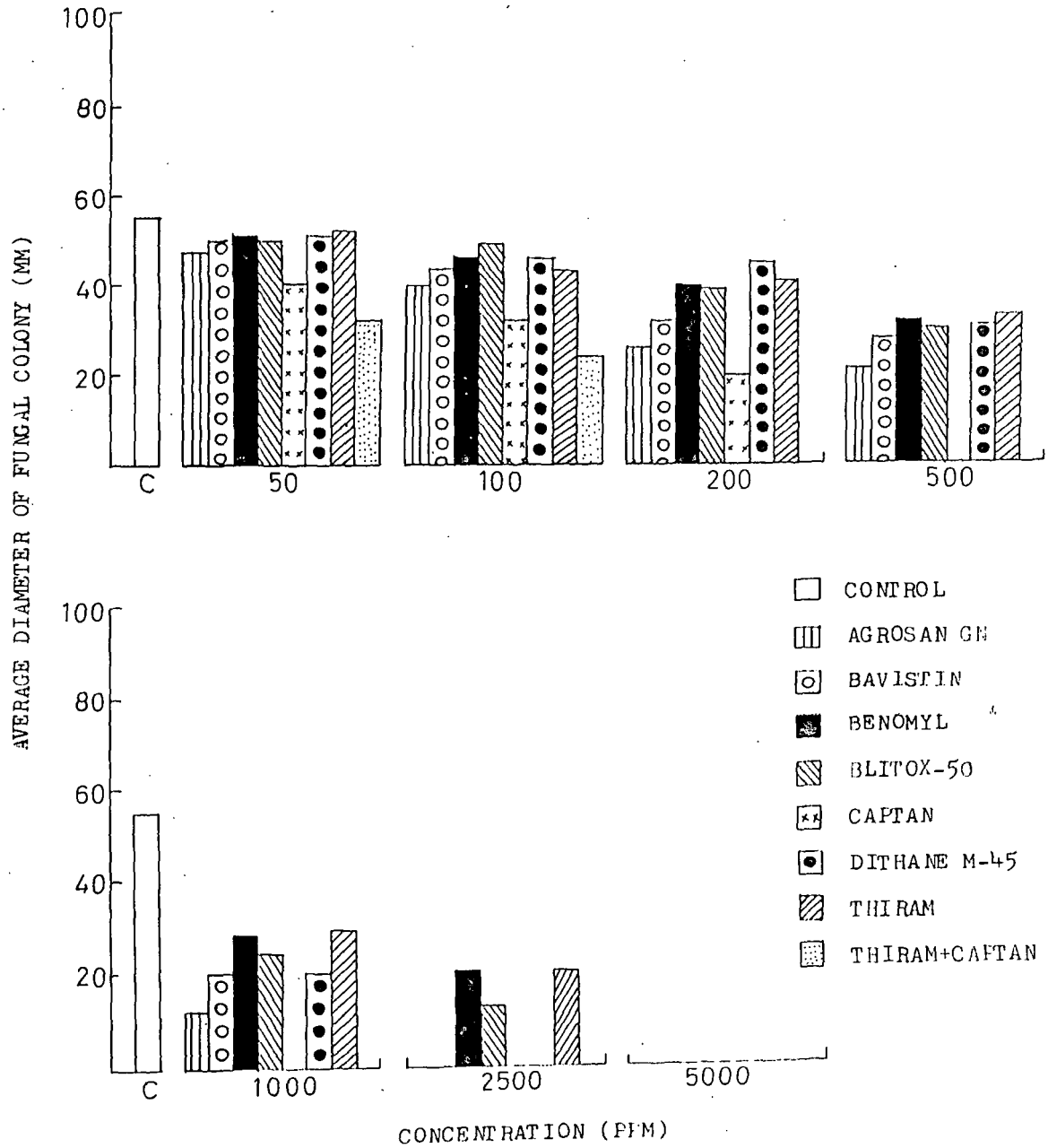


Fig. 4.2d Showing change in the average diameter of the colony of P. expansum due the treatment of different fungicides in different concentrations.

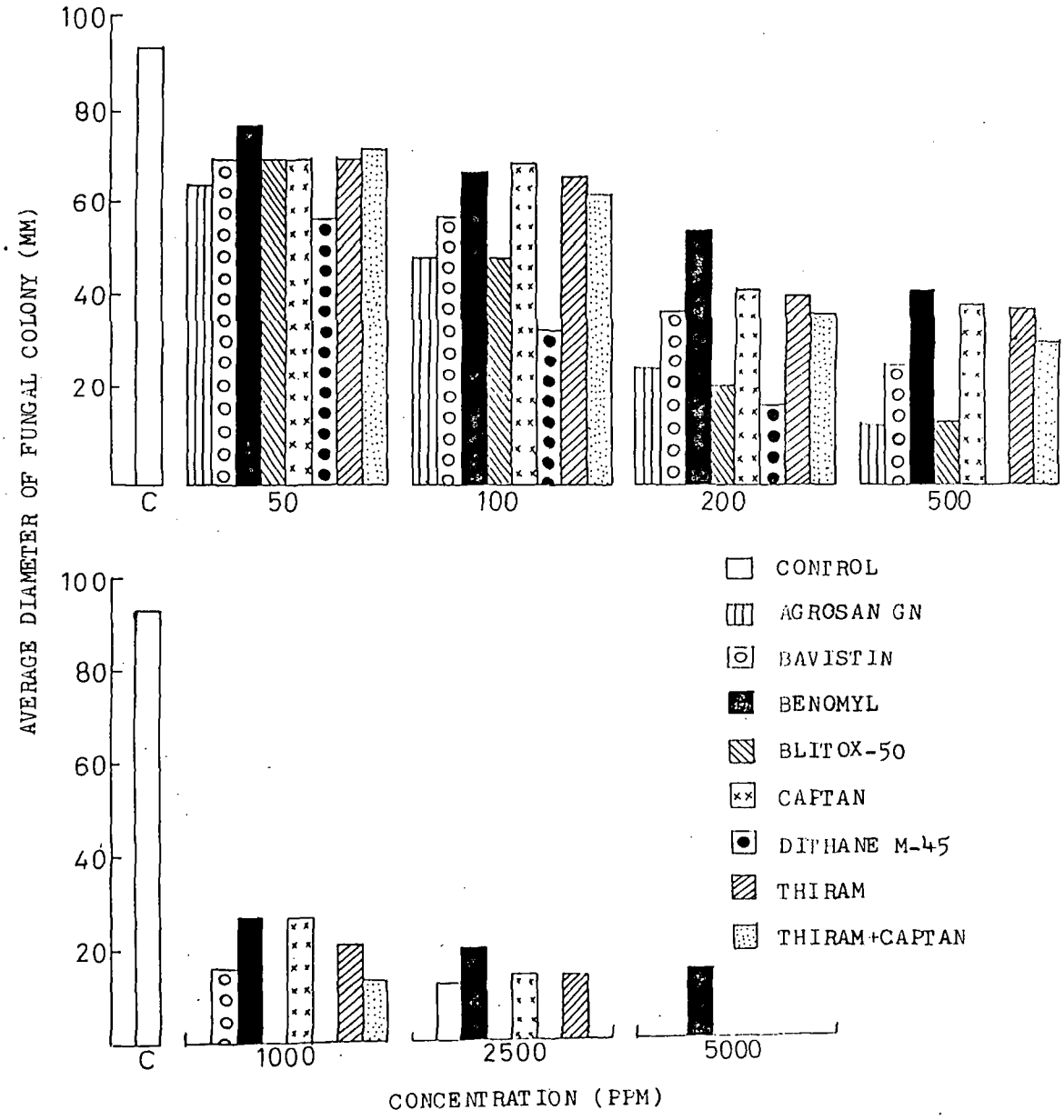


Fig. 4.2e Showing change in the average diameter of the colony of R. nigricans due the treatment of different fungicides in different concentrations.

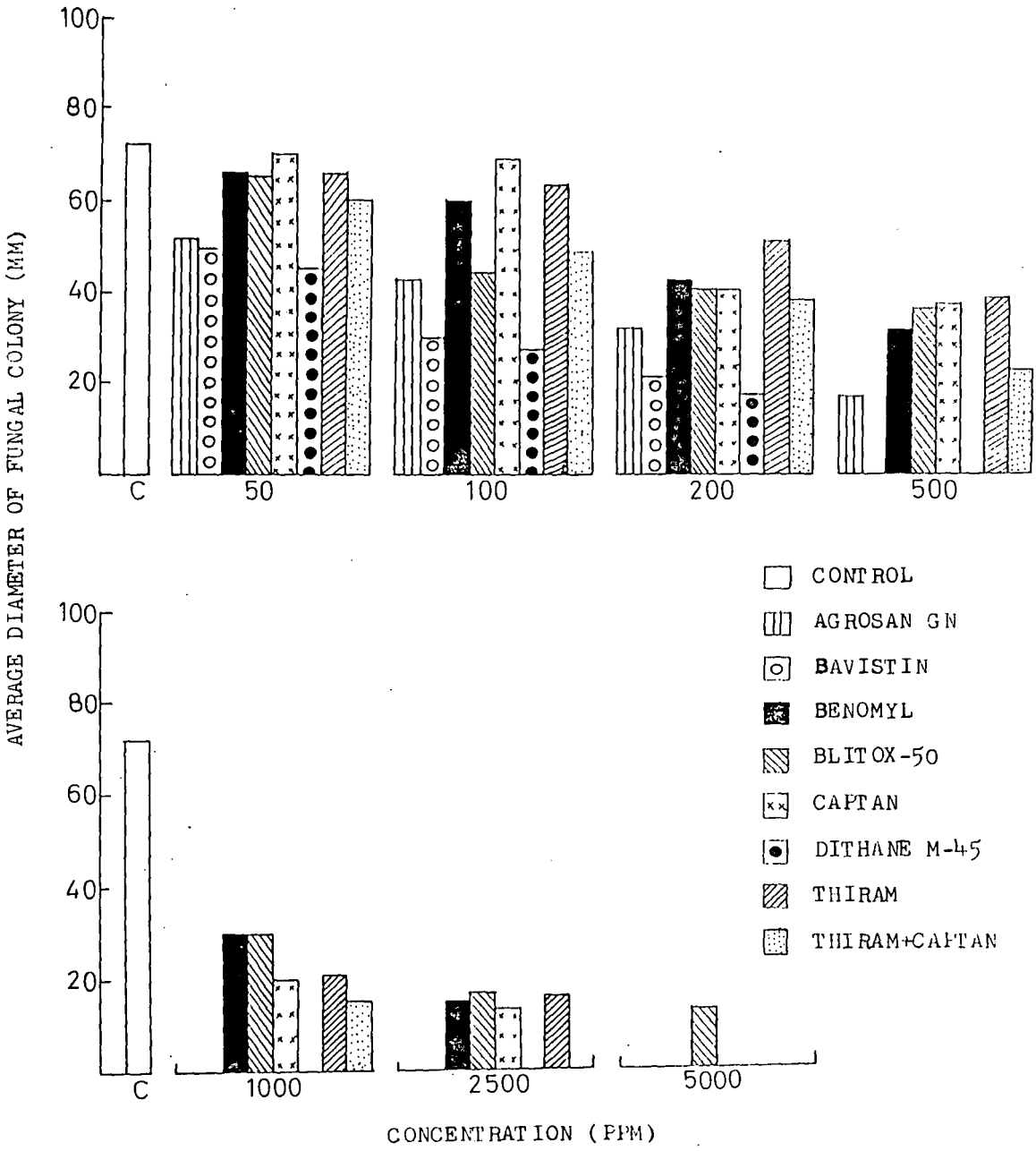


Fig. 4.2f Showing change in the average diameter of the colony of T. viride due to the treatment of different fungicides in different concentrations.

concentration of 1000 ppm. Thiram proved to be very toxic for the growth of *A. alternata* and *F. moniliforme* whose growth was completely inhibited at the concentration of 500 ppm (table 4.2g) whereas the growth of other test fungi could be checked only at the highest concentration of 5000 ppm. In this experiment also the combined effect of thiram and captan was found to be most successful in inhibiting the mycelial growth of all the test fungi (table 4.2h). The growth of *A. alternata* and *P. expansum* was most effected and there was total inhibition at a concentration as low as 200 ppm. *A. flavus* and *F. moniliforme* had 80% and 80.7% inhibition respectively at 5000 ppm and 100% at 1000 ppm; *R. nigricans* and *T. viride* could be checked completely at 2500 ppm.

Two way analysis of variance showed that there was significant difference in the colony growth of the test fungi between different concentrations of the fungicides (F=55.617**, 66.593**, 92.769**, 49.002**, 103.004**, 52.178**, 43.38** and 67.93** for agrosan GN, bavistin, benomyl, blitox-50, captan, dithane M-45, thiram and thiram+captan respectively). Except a single case, there was significant change in the colony growth between the fungal species in all the fungicides (F=3.2*, 9.947**, 12.812**, 2.557*, 19.355**, 9.936** and 12.062** for agrosan GN, bavistin, benomyl, blitox-50, captan, thiram and thiram+captan respectively).

V. SCREENING OF MAIZE SEEDS FOR AFLATOXIN

Three varieties of maize seeds, viz. Local White, Vijay and VL-16

were tested for the detection of *Aspergillus flavus* on the seeds and aflatoxin elaboration in natural condition as well as in the laboratory. Different isolates of *A. flavus* were also tested for their potentiality to produce aflatoxin in the synthetic medium. The results of the experiments are presented in the tables 5.1 to 5.3.

Results from table 5.1 shows that all the samples of the maize seed were contaminated with *A. flavus* and the variety Vijay showed maximum percentage occurrence of incidence (24), followed by Local White (18) and VL-16 (17).

In the BGYF test under long wave length ultra violet lamp, all the seed samples showed positive fluorescence though the intensity was very faint in all the samples. Table 5.1 indicates that all the samples showed the spots of aflatoxin B_1 and spots of aflatoxin B_2 were observed only from the variety Vijay. In the quantitative analysis of the natural occurrence of aflatoxin B_1 on the maize seed samples, VL-16 yielded the maximum of 664 ppb followed by Vijay (460 ppb) and Local White (325 ppb).

It is clear from the table 5.2 that a total of 22 isolates of *A. flavus* was isolated from different samples of maize seeds of which 9 isolates were from the variety VL-16, 7 from Vijay and 6 from Local White. Out of 22 isolates, only 10 were found to be toxigenic which yielded aflatoxin B_1, B_2 and G_1 in the synthetic medium (SMKY). Aflatoxin G_2 could not be found from any one of the samples. Isolates from all the varieties yielded aflatoxin B_1; aflatoxin B_2 was found from the isolates obtained from the

Table 5.1 Occurrence of *Aspergillus flavus* on maize seeds and natural incidence of aflatoxins.

Varieties	% occurrence of *A. favus*	BGYF test	Aflatoxins				Amount of aflatoxin B_1 (ppb)
			B_1	B_2	G_1	G_2	
Local White	18	+	+	-	-	-	325
Vijay	24	+	+	+	-	-	460
VL - 16	17	+	+	-	-	-	664

Table 5.2 Aflatoxin producing potentiality of *A. flavus* isolates obtained from maize seeds.

Varieties	Number of *A. flavus* isolates		Aflatoxin				Range of aflatoxin B_1 conc. (ppm)
	screened	positive to aflatoxin	B_1	B_2	G_1	G_2	
Local White	6	3	+	-	+	-	2.5 - 10.4
Vijay	7	4	+	+	-	-	4.6 - 12.7
VL - 16	9	3	+	+	+	-	1.6 - 16.5

Table 5.3 **Aflatoxin production by the isolates of *A. flavus* on different varieties of maize seeds.**

Varieties	Aflatoxins				Concentration of aflatoxin B_1 (ppm)
	B_1	B_2	G_1	G_2	
Local White	+	-	+	-	13.9
Vijay	+	+	-	-	10.8
VL-16	+	+	+	-	11.5

varieties Vijay and VL-16, whereas aflatoxin G_1 was yielded by the isolates of Local White and VL-16. Table 5.2 also shows the quantitative analysis of aflatoxin B_1; isolates from the variety Local White yielded 2.5 to 10.4 ppm of aflatoxin B_1, isolates from the variety Vijay had the range of 4.6 to 12.7 ppm and a concentration range of 1.6 to 16.5 ppm was obtained from the isolates of VL-16. Table 5.3 shows the results of aflatoxin elaboration by different samples of maize seeds artificially inoculated with a toxigenic isolate *A. flavus*. It has been observed that all the samples had the potentiality to produce aflatoxin under laboratory conditions and aflatoxin B_1 was the most common in all the samples. Two varieties, viz. Local White and Vijay yielded two types of aflatoxin each, viz. aflatoxin B_1, G_1 and B_1, B_2 respectively, but the variety VL-16 yielded three aflatoxin, viz. aflatoxin B_1, B_2 and G_1. Aflatoxin G_2 could not be spotted from any one of the samples. Table 5.3 also depicts the quantity of aflatoxin B_1 produced by *A. flavus* on the maize seeds of different varieties and it has been found that the variety Local White yielded the maximum of 13.9 ppm followed by VL-16 (11.5 ppm) and Vijay (10.8 ppm).

VI. CONTROL OF SEED-BORNE DISEASES OF MAIZE

A. Biological control: Biological control of the seed-borne diseases of maize was studied under 3 different experiments, viz. (i) coating the seeds with saprophytic fungi, (ii) fungal metabolites on seed germination and seedling growth and (iii) colony interaction of the seed-borned fungi. The results of the experiments are depicted in the tables 6.1 to 6.9 and figures 6.1 to 6.3.

(i) Seed coating with saprophytic fungi

Table 6.1 depicts the results of the effect of coating of the maize seeds with 3 saprophytic fungi, viz. *Cephalosporium acremonium, Cladosporium cladosporioides* and *Trichoderma viride* which show the occurrence of 6 prominent seed-borne fungi, viz. *Alternaria alternata, Aspergillus flavus, Fusarium moniliforme, Pencillium expansum, Rhizopus nigricans* and *Trichoderma viride* and the final stand of maize seedlings. It has been observed that all the 3 saprophytic fungi were effective in reducing the incidence of seed fungi in all the three varieties of maize. *T. viride* was found to be the most dominant one which inhibited the occurrence of all the seed-borne fungi except *F. moniliforme* in all the varieties. *C. acremonium* coated seeds of the variety Local White showed the occurrence of only *F. moniliforme* and *T. viride* whereas *C. cladosporioides* treated seeds showed the presence of *A. flavus*, F. moniliforme, P. expansum *and* T. viride. C. acremonium coated seeds yielded only *F. moniliforme* in the variety Vijay whereas seeds coated with *C. cladosporioides* showed the presence of *A. flavus, F. moniliforme and* T. viride. *C. acremonium* coated seeds of the variety VL-16 could not prevent the occurrence of *F. moniliforme, R. nigricans* and *T. viride*, whereas *C. cladosporioides* could prevent the occurrence of *A. alternata, P. expansum* and *R. nigricans*.

The effect of fungal coating on seed germination and final stand of the seedlings was found to be not very remarkable except *T. viride* treated samples of the variety VL-16, where the final stand of the seedlings was found to be reduced by 41.66%. In

general, there was both promotion and reduction in the final stand due to the saprophytic fungi. In the variety Local White, the final stand was reduced by 4%, 8% and 10% due to *C. acremonium*, *C. cladosporioides* and *T. viride* respectively, whereas, in the variety Vijay there was a promotion of 7.8% and 5.2% by *C. acremonium* and *C. cladosporioides* respectively and a reduction of 2.6% by *T. viride*, *C. acremonium* enhanced the final stand by 4.16% and *C. cladosporioides* reduced it by 2.08% in the variety VL-16.

The results of the effect of saprophytic fungi on the shoot-root length and dry weight of the seedlings are presented in the table 6.2. In general there was both promotion and reduction in the shoot-root length and dry weight of the seedlings raised from the seeds coated with the three saprophytic fungi. In the variety Local White, all the three fungi reduced the shoot length with a maximum of 36.11% by *C. acremonium*, followed by *C. cladosporioides* (9.72%) and *T. viride* (1.38%). In the variety Vijay, *C. cladosporioides* increased the shoot length by 96.6% whereas *C. acremonium* and *T. viride* reduced it 6.6% and 13.33% respectively, *C. acremonium* was responsible for 70.58% increase in shoot length in VL-16, but *T. viride* decreased it by 9.8%. *C. acremonium* and *T. viride* promoted the root length by 24.13% and 14.65% respectively in the variety Local White, whereas, *C. cladosporioides* initiated a reduction of 6.89%. In the variety Vijay, *C. cladosporioides* and *T. viride* enhanced the root length by 47.12% and 10.34% respectively, but *C. acremonium* did not show any effect on the root length. *C. cladosporioides* and *T. viride*

Table 6.1 Effect of maize seed treatment with saprophytic fungi on the occurrence of seed mycoflora and germination.

Variety : Local White

Seed treated with	Fungi recovered						Mortality%		Final stand %	% Change
	Alt	Asp	Fus	Pen	Rhi	Tri	pre-emergence	post-emergence		
Cephalosporium acremonium	-	-	+	-	-	+	4	-	96	- 4
Cladosporium cladosporioides	-	+	+	+	-	+	5	3	92	- 8
Trichoderma viride	-	-	+	-	-	-	8	2	90	- 10
Control (without fungi)	+	+	+	+	+	+	-	-	100	-
									Variety : Vijay	
Cephalosporium acremonium	-	-	+	-	-	-	12	6	82	+7.8
Cladosporium cladosoporioides	-	+	+	-	-	+	8	12	80	+5.2
Trichoderma viride	-	-	+	-	-	-	18	8	74	-2.6
Control (without fungi)	-	+	+	-	+	+	10	14	76	-
									Variety : VL-16	
Cephalosporium acremonium	-	-	+	-	+	+	-	-	100	+4.16
Cladosporium cladosporioides	-	+	+	-	-	+	6	-	94	-2.08
Trichoderma viride	-	-	+	-	-	-	26	18	56	-41.66
Control (without fungi)	+	+	+	-	+	-	4	-	96	-

Table 6.2 Effect of maize seed treatment with saprophytic fungi on shoot- root length and dry weight of the seedlings.

Variety : Local White

Seeds coated with	Ave. length in cm		Ave. dry weight (mg)	
	shoot	Root		
Cephalosporium acremonium	4.6 (-36.11)*	14.4 (+24.13)	0.125	(+02.45)
Cladosporium cladosporioides	6.5 (-09.72)	10.8 (-06.89)	0.157	(+28.68)
Trichoderma viride	7.1 (-01.38)	13.2 (+14.65)	0.120	(-01.63)
Control (without fungi)	7.2 -	11.6 -	0.122	-
			Variety : Vijay	
Cephalosporium acremonium	2.8 (-06.66)	8.7 -	0.153	(+06.25)
Cladosporium cladosporioides	5.9 (+99.66)	12.8 (+47.12)	0.141	(-02.08)
Trichoderma viride	2.6 (-13.33)	9.6 (+10.34)	0.160	(+11.11)
Control (without fungi)	3.0 -	8.7 -	0.144	-
			Variety : VL - 16	
Cephalosporium acremonium	8.7 (+70.58)	14.8 (+14.72)	0.159	(-10.67)
Cladosporium cladosporioides	5.5 (+07.84)	12.5 (-03.10)	0.155	(-12.92)
Trichoderma viride	4.6 (-09.80)	6.0 (-53.48)	0.186	(+04.49)
Control (without fungi)	5.1 -	12.9 -	0.178	-

* Figures in parenthesis indicate percentage change over control.

were responsible for 3.1`% and 53.48% reduction in root length in VL-16, whereas, *C. acremonium* initiated a promotion of 14.72%.

The role of the saprophytic fungi on the dry weight of the seedlings was found to be not very remarkable, though, there was both increase and decrease of varying degrees. *C. cladosporioides* increased the dry weight by 28.68% in the variety Local White and *T. viride* was responsible for an increase of 11.11% in the variety Vijay, and in other cases the percentage change of dry weight over the control was found to be not very remarkable.

(ii) **Fungal metabolites:** The metabolite of *T. viride* was mixed with the metabolite of *A. alternata, A. flavus, F. moniliforme* and *P. expansum* in 3 different ratios, viz. 1:1, 1:2 and 1:5 and tested against seed germination, final stand, shoot-root length and dry weight of the seedlings the results of which are depicted in the tables 6.3 to 6.8 and figures 6.1 to 6.3.

Table 6.3 shows that the metabolites, in general, increased the seed germination and final stand of the seedlings of the variety Local White in comparison to the control where metabolite of only *T.viride* was used. There were 4 instances where seed germination was reduced, 3 in the 1:1 ratio *(A.alternata, F. moniliforme* and *P. expansum)* and one in the 1:2 ratio *(F.moniliforme)*. The 2 cases of reduction in the final stand were due to the metabolites *F. moniliforme + T. viride* in the ratio of 1:1 (2.85%) and *A. alternata + T.viride* in the 1:2 ratio (25.71%). The maximum increase in the final stand was noticed with *A. alternata + T.*

viride in the 1:5 ratio (35.71%), followed by *A. flavus + T. viride* and *P. expansum + T. viride* in the 1:5 ratios with 24.28% increase each, and in other cases the percentage increase was not very significant.

The effect of fungal metabolites in the variety Vijay was found to be similar to that of Local White (table 6.4), where the maximum seed germination was 82% and a minimum of 70% in comparison to 72% in the control. Maximum increase in the final stand was noticed in *A. flavus+T.viride* (1:1 ratio) and *P. expansum + T. viride* (1:5 ratio) where it was found to be 20.58% in both the cases, followed by 17.64% by *P. expansum + T. viride* (1:1 ratio), *A. flavus + T. viride* (1:2 ratio), *A. flavus + T. viride* (1:5 ratio) and in others the increase was not very remarkable. Metabolites of *P. expansum + T. viride* in the ratio 1:2 was found to be deletorious for maize seedlings where a decrease of 23.52% was noticed.

In the variety VL-16 too, there was, in general, enhancement of seed germination and final stand except 3 cases of reduction in seed germination and 2 instances of fall in the final stand (table 6.5). Maximum seed germination was noticed with *A. alternata + T. viride* in the 1:5 ratio (86%) in comparison to 72% in the control, and the minimum seed germination was due to the metabolites of *F. moniliforme + T. viride* (1:2 ratio) which showed a germination of 68%. The maximum increase in the final stand was due to the treatment of *A. alternata + T. viride* in the 1:5 ratio (24.63%) followed by *P. expansum + T. viride* in the 1:5 ratio (13.04%) and in others the increase was less than 12%.

Table 6.3 The combined effect of the metabolites of *T. viride* and certain pathogenic fungi of maize seed on the seed germination and final stand of the seedlings. (Variety : Local White)

Fungi	Ratio	germination %	Mortality %		Final stand%	% change
	Trich : Test fungi		Pre-emergence	Post-emergence		
Alternaria alternata	1:1	72	28	-	72	+02.85
Aspergillus flavus	1:1	80	20	4	76	+08.57
Fusarium moniliforme	1:1	70	30	2	68	-02.85
Penicillium expansum	1:1	70	30	-	70	-
Alternaria alternata	1:2	90	10	38	52	-25.71
Aspergillus flavus	1:2	84	16	8	76	+08.57
Fusarium moniliforme	1:2	76	24	-	76	+08.57
Penicillium expansum	1:2	80	20	5	75	+07.14
Alternaria alternata	1:5	95	5	-	95	+35.71
Aspergillus flavus	1:5	90	10	3	87	+24.28
Fusarium moniliforme	1:5	82	18	6	76	+08.57
Penicillium expansum	1:5	87	13	-	87	+24.28
Control (only *Trichoderma viride*)	-	80	20	10	70	-

Table 6.4 The combined effect of the metabolites of *T. viride* and certain pathogenic fungi of maize seed on the seed germination and final stand of the seedlings. (Variety : Vijay)

Fungi	Ratio Trich : Test fungi	germination %	Mortality % Pre-emergence	Mortality % Post-emergence	Final stand%	% change
Alternaria alternata	1:1	70	30	4	66	-02.94
Aspergillus flavus	1:1	82	18	-	82	+20.58
Fusarium moniliforme	1:1	70	30	10	60	-11.76
Penicillium expansum	1:1	80	20	-	80	+17.64
Alternaria alternata	1:2	75	25	58	70	+02.94
Aspergillus flavus	1:2	78	22	-	78	+14.70
Fusarium moniliforme	1:2	70	30	-	70	+02.94
Penicillium expansum	1:2	72	28	20	52	-23.52
Alternaria alternata	1:5	76	24	5	71	+04.41
Aspergillus flavus	1:5	80	20	3	77	+13.23
Fusarium moniliforme	1:5	74	26	8	66	-02.94
Penicillium expansum	1:5	82	18	-	82	+20.58
Control (only *Trichoderma viride*)	-	72	28	4	68	-

Table 6.5 The combined effect of the metabolites of *T. viride* and certain pathogenic fungi of maize seed on the seed germination and final stand of the seedlings. (Variety : VL -16)

Fungi	Ratio T.V: Test fungi	germination	Mortality % Pre-emergence	Mortality % Post-emergence	Final stand %	change %
Alternaria alternata	1:1	72	28	8	64	-07.24
Aspergillus flavus	1:1	75	25	5	70	+01.44
Fusarium moniliforme	1:1	70	30	8	62	-10.14
Penicillium expansum	1:1	82	18	-	82	+18.84
Alternaria alternata	1:2	80	20	4	76	+10.14
Aspergillus flavus	1:2	70	30	-	70	+01.44
Fusarium moniliforme	1:2	68	32	10	58	-15.94
Penicillium expansum	1:2	82	18	5	77	+11.59
Alternaria alternata	1:5	86	14	-	86	+24.63
Aspergillus alternata	1:5	80	20	2	78	+13.04
Fusarium moniliforme	1:5	76	24	6	70	+01.44
Penicillium expansum	1:5	78	22	2	76	+10.14
Control (only *T. viride*)	-	72	28	3	69	-

T.V. = *Trichoderma viride*

Statistical analysis (two way analysis of variance) showed that there were no significant variations in the final stand of the maize seedlings between different ratios (*T. viride*: test fungi) of fungal metabolites (F=3.619 NS, 0.481 NS and 2.087 NS for Local White, Vijay and VL-16 respectively) as well as between the test fungi (F=0.344 NS, 1.087 NS and 3.37 NS for Local White, Vijay and VL-16 respectively).

The F values were also calculated for the variations in the final stand between the varieties and between the test fungi for the three different ratios (1:1, 1:2 and 1:5). Except a single case, there was no significant variations in the final stand between the varieties (F=0.2 NS, 0.058 NS and 6.478* for the ratios of 1:1, 1:2 and 1:5 respectively) and in no case there was significant variations between the test fungi (F=3.924 NS, 0.292 NS and 4.234 NS for the ratios of 1:1, 1:2 and 1:5 respectively).

It is clear from the results in table 6.6 and figures 6.1 and 6.2 that the fungal metabolites had marked influence on the shoot-root length of the maize seedlings. In almost all the cases there was enhancement of shoot-root length and the metabolites of *A. alternata* + *T. viride* in the 1:5 ratio was responsible for the maximum increase of 70.17% over control and highest inhibition of shoot length was initiated by *F. moniliforme* + *T. viride* in the 1:5 ratio (43.85%). The only other instance of shoot length inhibition was noticed in the seedlings treated with the metabolites of *F. moniliforme* and *T. viride* in the 1:2 ratio. Table 6.6 also shows that the fungal metabolites acted as both

inhibitor and promoter of root length of maize seedlings. The maximum promotion in root length was noticed in the seedlings treated with the metabolites of *A. flavus + T. viride* in the 1:1 ratio (23.89%) and the maximum in the 1:2 ratio was due to the metabolites of *F. moniliforme + T. viride* (55.97%). The effect of the metabolites on the dry weight of the seedlings was found to be less pronounced in comparison to the changes in shoot-root length. Except 3 cases of increase, there was a fall in dry weight in all the cases and the maximum reduction in dry weight was due to the metabolites of *A. flavus + T. viride* in the 1:2 ratio with percentage reduction of 10.44. The combined effect of the metabolites of *Trichoderma* and certain test fungi on the shoot length, root length and dry weight of the maize seedlings was statistically analysed (two way analysis of variance) and it has been found that only root length showed significant variations between different ratios (F=10.53*) but not shoot length and dry weight (F=1.646 NS and 0.626 NS respectively). Only shoot length showed significant variations between the test fungi (F=6.025*) but not root length and dry weight (F=1.796 NS and 1.272 NS respectively).

In the variety Vijay (table 6.7 and figures 6.1 and 6.2), there was a reduction in the shoot length in almost all the cases except *P.expansum + T. viride* in the 1:1 ratio (+6.94%) and *P. expansum + T. viride* in the 1:2 ratio with no change at all. The metabolites of *A. flavus + T. viride* in the 1:2 ratio was responsible for maximum reduction in shoot length (47.22%) and the minimum reduction was due to *F. moniliforme + T. viride* in the 1:1 ratio (11.11%). On the contrary, there was an increase

in root length in all the cases except a single instance of reduction due to *A. flavus + T. viride* in the 1:2 ratio (21.42%). Metabolites of *P. expansum + T. viride* in the 1:1 ratio was responsible for the maximum increase in the root length (51.58%), however, considerable increase in the root length was noticed in all the cases of 1:5 ratio category. The results show that (figure 6.3) the metabolites were responsible for reduction in dry weight in all the cases except a single case of increase (4.24%) due to the treatment of the metabolites of *A. alternata + T. viride* in the 1:1 ratio. The maximum decrease in dry weight was due to *F. moniliforme + T. viride* in the 1:5 ratio (22.42%) and a minimum of 1.81% reduction due to *P. expansum + T. viride* in the 1:2 ratio was noticed.

Statistical analysis (two way analysis of variance) showed that there was significant variations in root length and dry weight of the seedlings between different ratios (F=5.85* and 13.343** respectively but not shoot length (F=.218 NS). There was significant variation between the test fungi for shoot length only (F=5.201*) but not for root length and dry weight of the seedlings (F=.403 NS and 2.067 NS respectively).

Table 6.8 shows that the fungal metabolites proved to be harmful for both shoot and root elongation as well as dry weight of the seedlings of the variety VL-16. Except a single case of increase, there was a reduction of shoot length in all the treatments with the maximum inhibition of 77.01% caused by the metabolites of *F. moniliforme + T. viride* in the 1:2 ratio. In all the cases there was reduction in the root length, the maximum

Table 6.6 The combined effect of the metabolites of *T. viride* and certain pathogenic fungi of maize seed on seedling growth. (Variety : Local White)

Fungi	Ratio T.V.: Test fungi	Length in cm		Dry weight in mg.
		Shoot	Root	
Alternaria alternata	1:1	8.0(+40.35)*	17.2(+08.17)	0.122(-08.95)
Aspergillus flavus	1:1	9.2(+61.40)	19.7(+23.89)	0.122(-08.95)
Fusarium moniliforme	1:1	7.0(+22.80)	17.0(+06.91)	0.126(-05.97)
Penicillium expansum	1:1	8.4(+47.36)	17.0(+06.91)	0.131(-02.23)
Alternaria alternata	1:2	8.9(+56.14)	05.0(-68.55)	0.124(-07.46)
Aspergillus flavus	1:2	6.1(+07.01)	13.5(-15.09)	0.120(-10.44)
Fusarium moniliforme	1:2	3.3(-42.10)	07.0(-55.97)	0.143(+06.71)
Penicillium expansum	1:2	7.6(+33.33)	14.7(-07.54)	0.135(+00.74)
Alternaria alternata	1:5	9.7(+70.17)	17.3(+08.80)	0.129(-03.73)
Aspergillus flavus	1:5	8.0(+40.35)	17.4(+09.43)	0.132(-01.49)
Fusarium moniliforme	1:5	3.2(-43.85)	15.4(-03.14)	0.121(-09.70)
Penicillium expansum	1:5	7.2 (+26.31)	17.3(+08.80)	0.139(+03.73)
Control (*Trichoderma viride* only)	-	5.7 -	15.9 -	0.134 -

* Figures in parenthesis indicate percentage change over control.
T.V. *Trichoderma viride*

Table 6.7 The combined effect of the metabolites of *T. viride* and certain pathogenic fungi of maize seed on seedling growth.(Variety : Vijay)

Fungi	Ratio T.V.: Test fungi	Length in cm Shoot	Root	Dry weight in mg.
Alternaria alternata	1:1	4.7(-34.72)*	13.0(+03.17)	0.172(+04.24)
Aspergillus flavus	1:1	4.1(-43.05)	17.0(+34.92)	0.152(-07.87)
Fusarium moniliforme	1:1	6.4(-11.11)	14.5(+15.07)	0.149(-09.69)
Penicillium expansum	1:1	7.7(+06.94)	19.1(+51.58)	0.150(-04.24)
Alternaria alternata	1:2	4.6(-36.11)	13.7(+08.73)	0.155(-06.06)
Aspergillus flavus	1:2	3.8(-47.22)	9.9(-21.42)	0.154(-06.66)
Fusarium moniliforme	1:2	5.7(-20.83)	12.9(+02.38)	0.158(-04.24)
Penicillium expansum	1:2	7.2 -	12.6 -	0.162 -
Alternaria alternata	1:5	4.5(-37.50)	18.2(+44.44)	0.145(-12.12)
Aspergillus flavus	1:5	5.6(-22.22)	18.4(+46.03)	0.139(-15.75)
Fusarium moniliforme	1:5	6.3(-12.50)	15.8(+25.39)	0.128(-22.42)
Penicillium expansum	1:5	5.5(-23.61)	17.2(+36.50)	0.137(-16.96)
Control (*Trichoderma viride* only)	-	7.2 -	12.6 -	0.165 -

* Figures in parenthesis indicate percentage change over control.
T.V. *Trichoderma viride*

Table 6.8 The combined effect of the metabolites of *T. viride* and certain pathogenic fungi of maize seed on seedlings growth. (Variety:VL-16)

Fungi	Ratio T.V.: Test fungi	Length in cm Shoot	Root	Dry weight in mg.
Alternaria Alternata	1:1	7.1(-18.39)*	14.2(-17.91)	0.173(-04.94)
Aspergillus flavus	1:1	7.3(-16.09)	12.5(-27.74)	0.179(-01.64)
Fusarium moniliforme	1:1	7.3(-16.09)	13.6(-21.38)	0.180(-01.09)
Penicillium expansum	1:1	6.5(-25.28)	12.7(-26.58)	0.160(-12.08)
Alternaria alternata	1:2	4.5(-48.27)	11.0(-36.41)	0.167(-08.24)
Aspergillus flavus	1:2	6.9(-20.68)	13.2(-23.69)	0.167(-08.24)
Fusarium moniliforme	1:2	2.0(-77.01)	4.1(-76.30)	0.180(-01.09)
Penicillium expansum	1:2	6.0(-31.03)	12.1(-30.05)	0.164(-09.89)
Alternaria alternata	1:5	5.2(-40.22)	11.6(-32.94)	0.145(-20.32)
Aspergillus flavus	1:5	7.4(-14.94)	13.4(-22.54)	0.140(-23.07)
Fusarium moniliforme	1:5	6.3(-27.58)	12.7(-26.58)	0.128(-29.67)
Penicillium expansum	1:5	8.8(+01.14)	15.9(-08.09)	0.137(-24.72)
Control (*Trichoderma viride* only)	-	8.7 -	17.3 -	0.182 -

* Figures in parenthesis indicate percentage change over control.
T.V. = *Trichoderma viride*

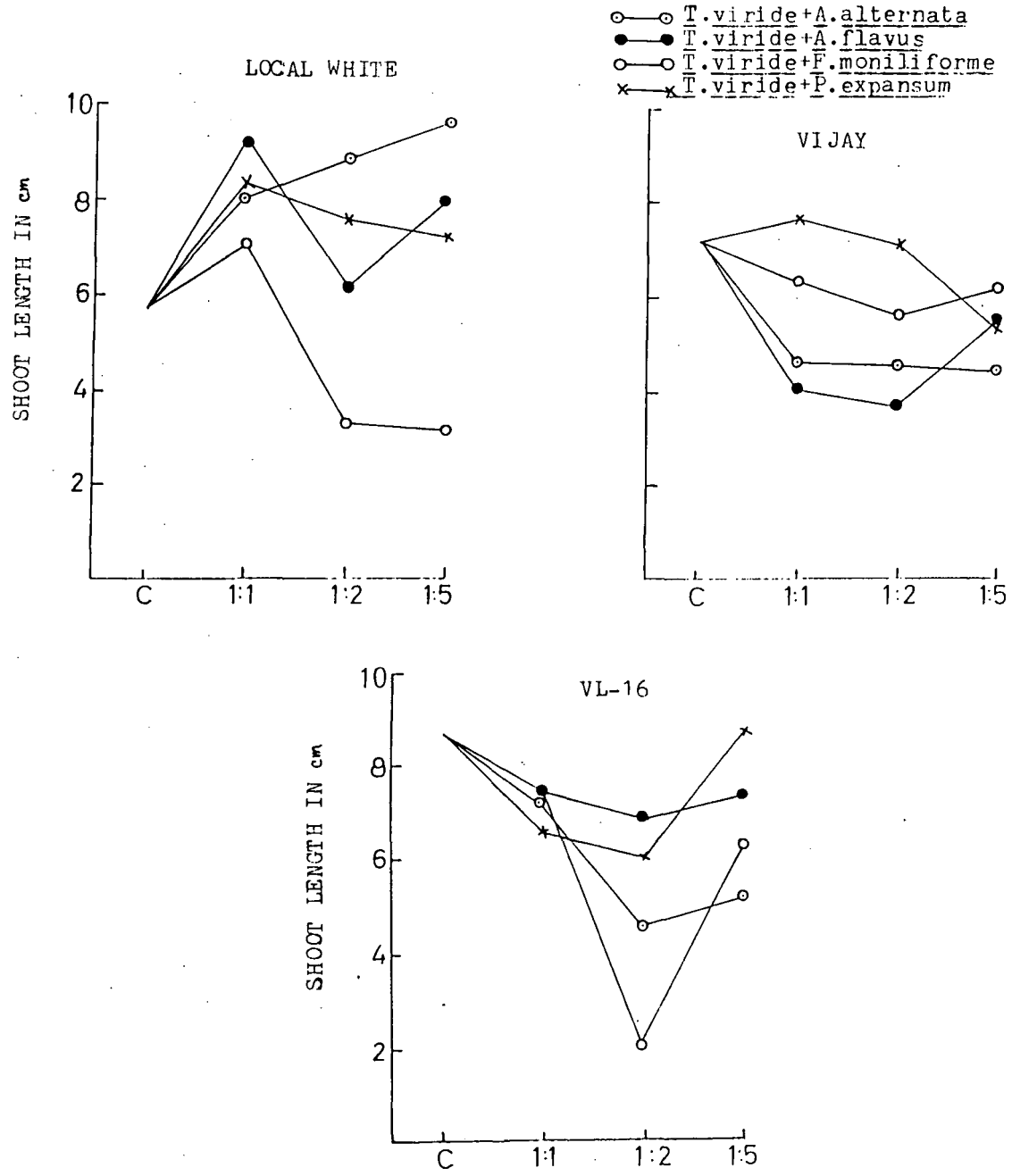


Fig. 6.1 The combined effect of the metabolites of Trichoderma viride and certain pathogenic fungi of maize seeds in 3 different ratios (1:1, 1:2 and 1:5) on the shoot length of maize seedlings. C - Control (extract of T.viride only).

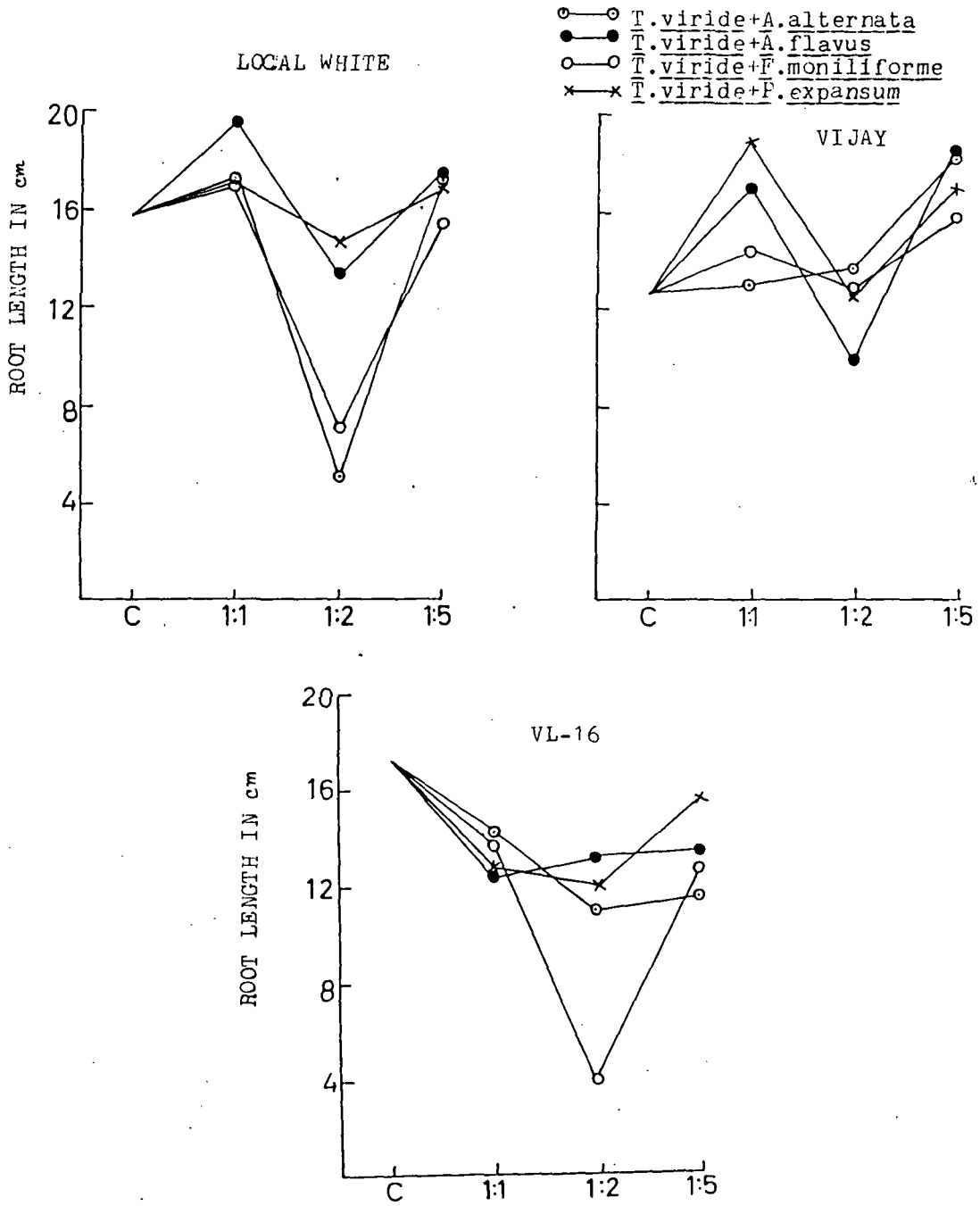


Fig. 6.2 The combined effect of the metabolites of Trichoderma viride and certain pathogenic fungi of maize seeds in 3 different ratios (1:1, 1:2 and 1:5) on the root length of maize seedlings. C - Control (extract of T.viride only).

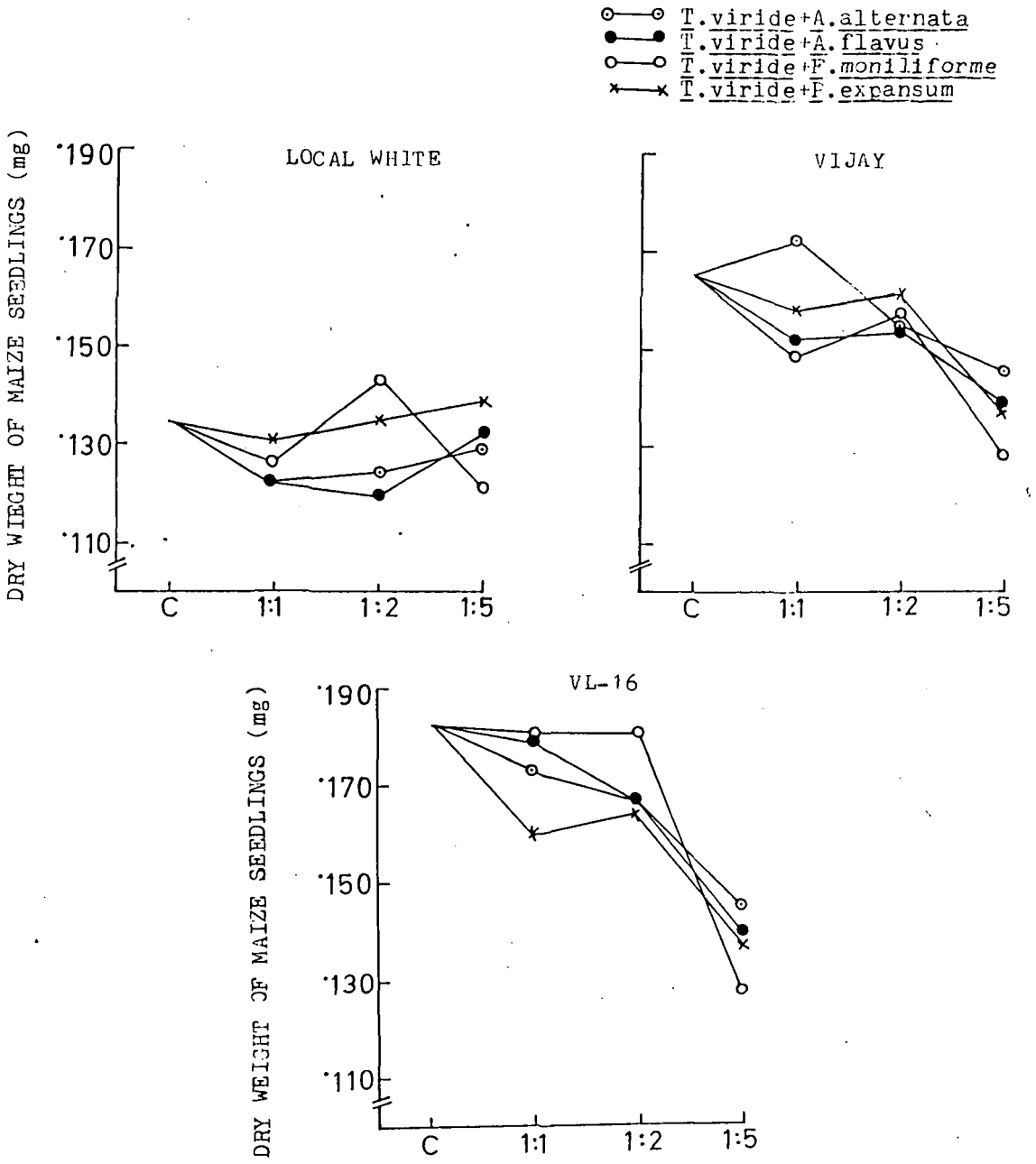


Fig. 6.3 The combined effect of the metabolites of Trichoderma viride and certain pathogenic fungi of maize seeds in 3 different ratios (1:1, 1:2 and 1:5) on the dry weight of maize seedlings. C - Control (extract of T.viride only).

being initiated by the metabolites of *F. moniliforme + T. viride* in the 1:2 ratio with a reduction of 76.3%. Metabolites of all the test fungi had inhibitory effect on the dry weight of the seedlings. It has been observed that the metabolites of all the test fungi in the 1:5 ratio were very effective in reducing the dry weight with a maximum of 29.67% reduction *(F.moniliforme + T. viride)*.

Statistical analysis (two way analysis of variance) showed that neither shoot length nor root length had significant variations between different ratios (F=3.008 NS and 1.993 NS respectively) as well as between the test fungi (F=1.592 NS and .98 NS respectively). Dry weight showed significant variation between different ratios (F=23.080**) but not between the test fungi (F=.814 NS).

F-values were also calculated for the variations in shoot length, root length and dry weight between the varieties and between the test fungi for the 3 ratios (1:1, 1:2 and 1:5). It has been found that the shoot length did not have significant variations between the varieties in all the 3 ratios of 1:1, 1:2 and 1:5 (F=3.694 NS, .811 NS and .720 NS respectively) as well as between the test fungi (F=.294 NS, 1.646 NS and .534 NS respectively). Variations in root length was found to be statistically significant between the varieties in the ratios of 1:1 and 1:5 (F=5.327* and 12.647** respectively), but insignificant in the 1:2 ratio (F=.942 NS), but variations between the test fungi was found to be insignificant in all the 3 ratios of 1:1, 1:2 and 1:5 (F=.537 NS, 1.223 NS and 1.818 NS respectively). Variations in

the dry weight were found to be statistically significant between the varieties for the ratios of 1:1 and 1:2 (F=25.425** and 48.259** respectively) but not for the ratio of 1:5 (F=3.745 NS). Variations in dry weight between the test fungi were found to be insignificant in the ratios of 1;:1 and 1:2 (F=.214 NS and 3.265 NS respectively) but significant in the 1:5 ratio (F=6.634*).

Dual cultures: Table 6.9 and plate I show the results of colony interactions of certain seed-borne fungi of maize, which were expressed as interaction score, type of interaction, intermingling and inhibitory zone. The interaction scores and type of interaction of the dual cultures were designated following the model of Skidmore and Dickinson (1976), where interaction scores were counted from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance). And the types of interaction were designated as A, Bi, Bii, C and D, where A=> Mutually intermingling growth where both the fungi grew into one another without any microscopic sign of interaction; Bi=> Intermingling growth where the fungus being observed is growing into the opposed fungus either above or below its colony; Bii=> 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 mm); D= Mutual inhibition at a distance of >2 mm.

It is clear from the table and plate that there were 6 instances where the interacting fungi exhibited mutual inhibition at a distance. Amongst these,the dual cultures of *A. flavus* - *C.*

acremonium, *A. flavus* - *C. cladosporioides* and *A. flavus* - *F. moniliforme* had interaction score of 5 each and the type of interaction being `D', the other three dual cultures, viz. *A. alternata* - *C. acremonium*, *A. alternata* - *C. cladosporioides* and *C. acremonium* - *T. viride* had the interaction score of 4 each with `D`, `C' and `D' type of interaction respectively. Dual culture of *A. flavus* - *T. viride*, *A. alternata* - *F. moniliforme* and *F. moniliforme* - *T. viride* showed interaction score of 3 each and the type of interaction being `Bi', 4 dual cultures, viz. *A. alternata* - *T. viride*, *C. acremonium* - *F. moniliforme*, *C. cladosporioides* - *F. moniliforme* and *C. cladosporioides* - *T. viride* showed interaction score 2 and `Bii' interaction type. 7 dual cultures exhibited intermingling growth with a maximum of 1.5 cm of intermingling zone (*F. moniliforme* - *T. viride*), followed by the dual cultures of *A. alternata* - *F. moniliforme* (1.2 cm), *C. cladosporioides* - *F. moniliforme* (1.0 cm) and others having intermingling zone less than 1 cm. 6 dual cultures exhibited inhibition of one fungi by the other with a definite zone of inhibition, the maximum being 1.0 cm in the dual cultures of *A. flavus* - *C. cladosporioides*, where *C.cladosporioides* being inhibited by *A. flavus*, *C. acremonium* was also inhibited by *A. flavus* with an inhibition zone of 0.9 cm; a minimum of 0.3 cm of inhibition zone was noticed in the dual cultures of *A. alternata* - *C. cladosporioides*. Table 6.9 also shows the percentage inhibition of the comparatively passive fungi by the dominant ones, where *T. viride* was found to be the most dominant fungi which inhibited the colony of *A. alternata*, *F. moniliforme*, *C. acremonium*, *A. flavus* and *C. cladosporioides* by 71.42%, 60%, 50%,

Table 6.9 Colony interactions between different maize seed mycoflora on agar plates.

Interaction between	Interaction score*a	Type*b	Intermingled zone (cm)	Inhibition zone (cm)	% inhibitio of the colony of passive fungi
Aspergillus & Cephalosporium	5	D	-	0.90	22.22
Aspergillus & Cladosporium	5	D	-	1.00	50.00
Aspergillus & Fusarium	5	D	-	0.60	40.00
Aspergillus & Trichoderma	3	B_i	0.20	-	50.00
Alternaria & Cephalosporium	4	D	-	0.40	33.33
Alternaria & Cladosporium	4	C	-	0.30	25.00
Alternaria & Fusarium	3	B_i	1.20	-	16.66
Alternaria & Trichoderma	2	B_{ii}	0.80	-	71.42
Cephalosporium & Fusarium	2	B_{ii}	0.60	-	33.33
Cephalosporium & Trichoderma	4	D	-	0.40	50.00
Cladosporium & Fusarium	2	B_{ii}	1.00	-	42.85
Cladosporium & Trichoderma	2	B_{ii}	0.60	-	28.57
Fusarium & Trichoderma	3	B_i	1.50	-	60.00

*a = Scores from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance); based on Skidmore and Dickinson (1976).

*b = A => Mutually intermingling growth where both the fungi grew into one another without any microscopic sign 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} => Intermingiling 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 mm); D => Mutual inhibition at a distance of > 2 mm.

Plate I.A Dual cultures showing antagonism between :
left : *Aspergillus* and *Cephalosporium*
right: *Fusarium* and *Cephalosporium*

Plate I.B Dual cultures showing antagonism between :
left : *Alternaria* and *Cephalosporium*
right: *Trichoderma* and *Cephalosporium*

Plate I.C Dual cultures showing antagonism between :
left : *Aspergillus* and *Cladosporium*
right: *Fusarium* and *Cladosporium*

PLATE I.A

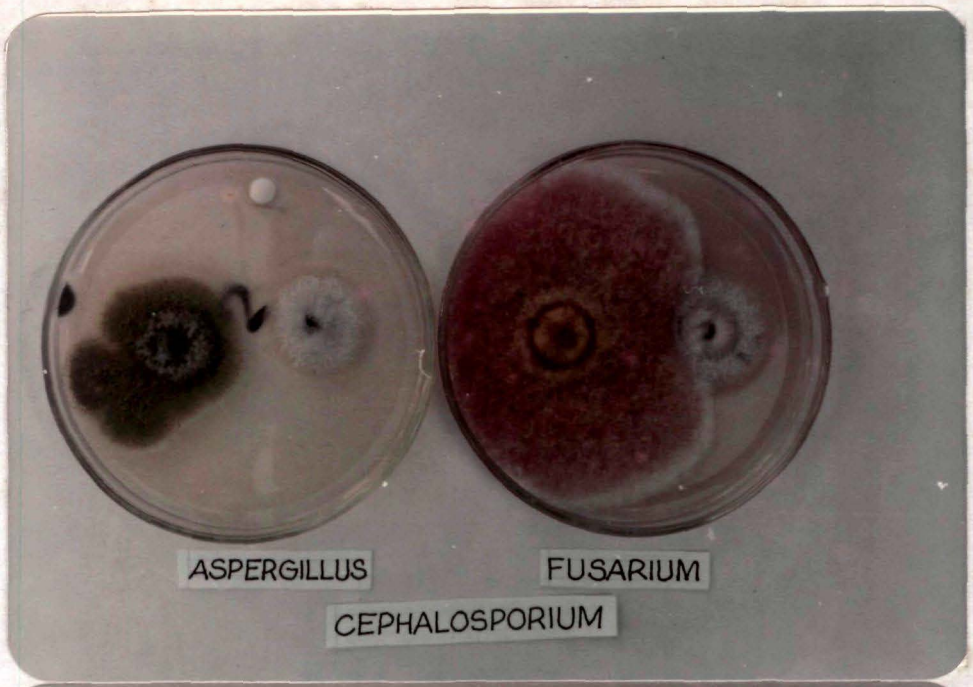


PLATE I.B

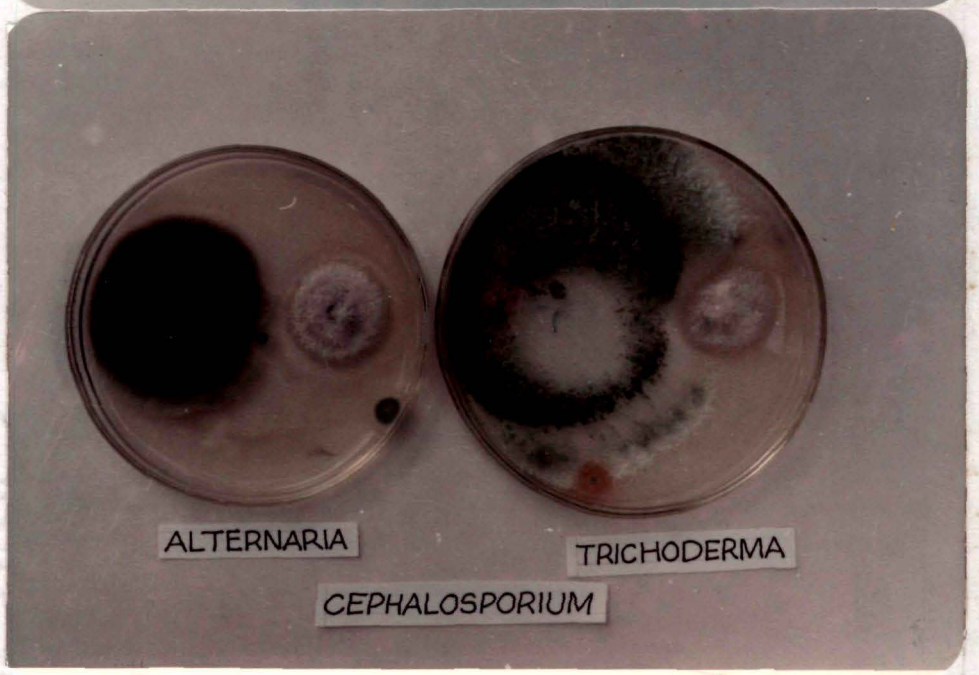


PLATE I.C

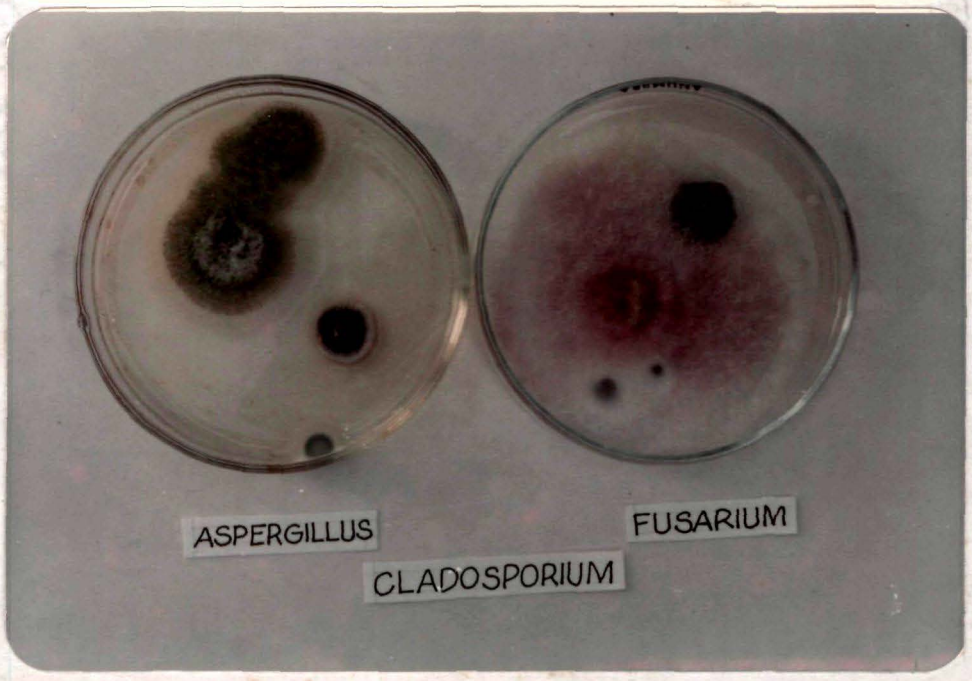


Plate I.D Dual cultures showing antagonism between :
left : *Trichoderma* and *Cladosporium*
right: *Alternaria* and *Cladosporium*

Plate I.E Dual cultures showing antagonism between :
left : *Alternaria* and *Fusarium*
right: *Aspergillus* and *Fusarium*

Plate I.F Dual cultures showing antagonism between :
left : *Cephalosporium* and *Fusarium*
right: *Trichoderma* and *Fusarium*

PLATE I.D

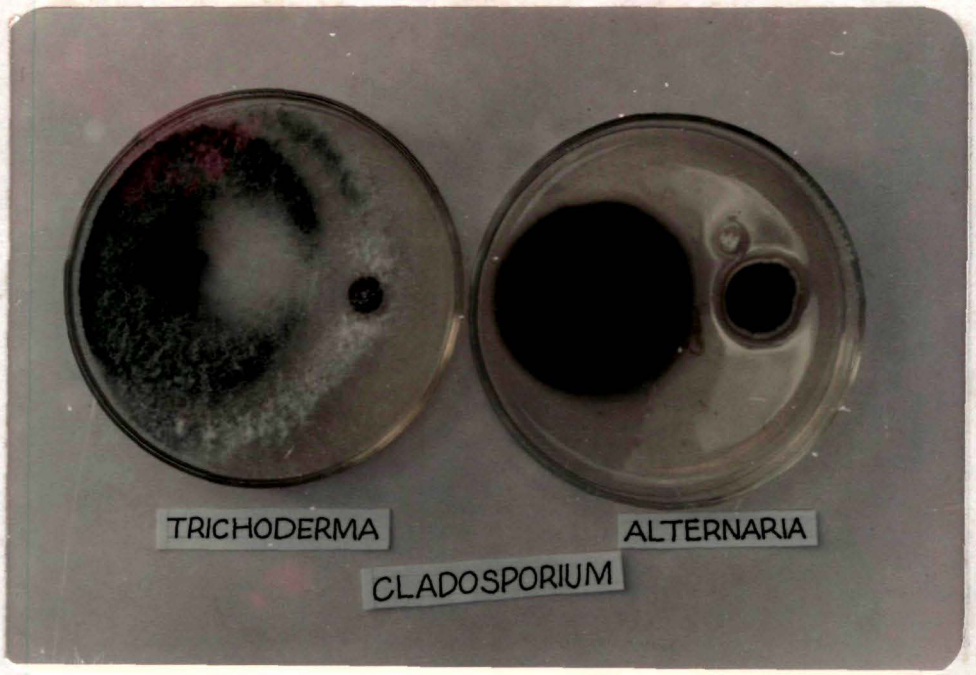


PLATE I.E

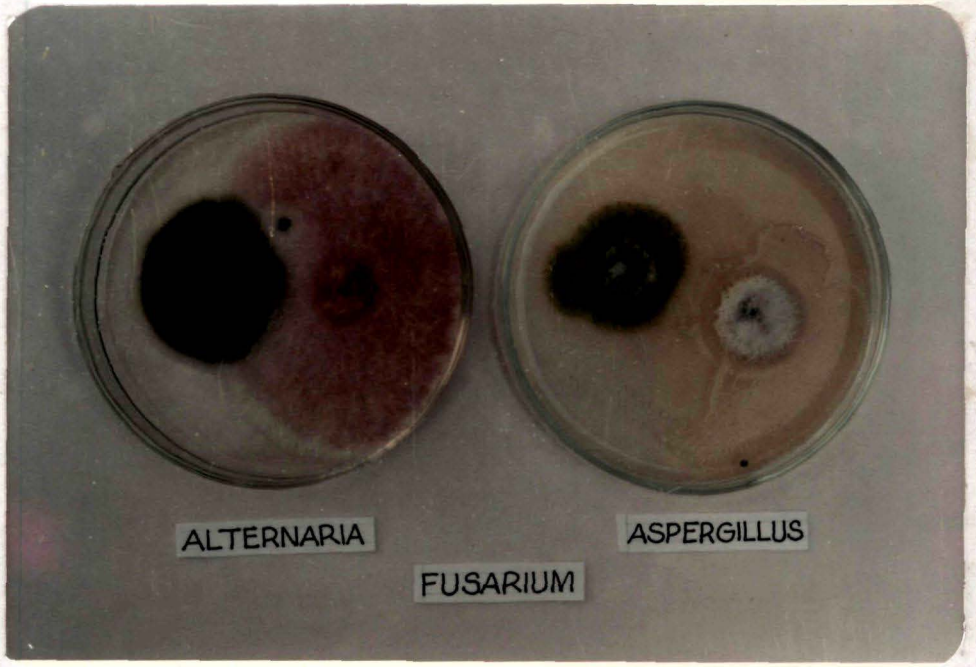


PLATE I.F

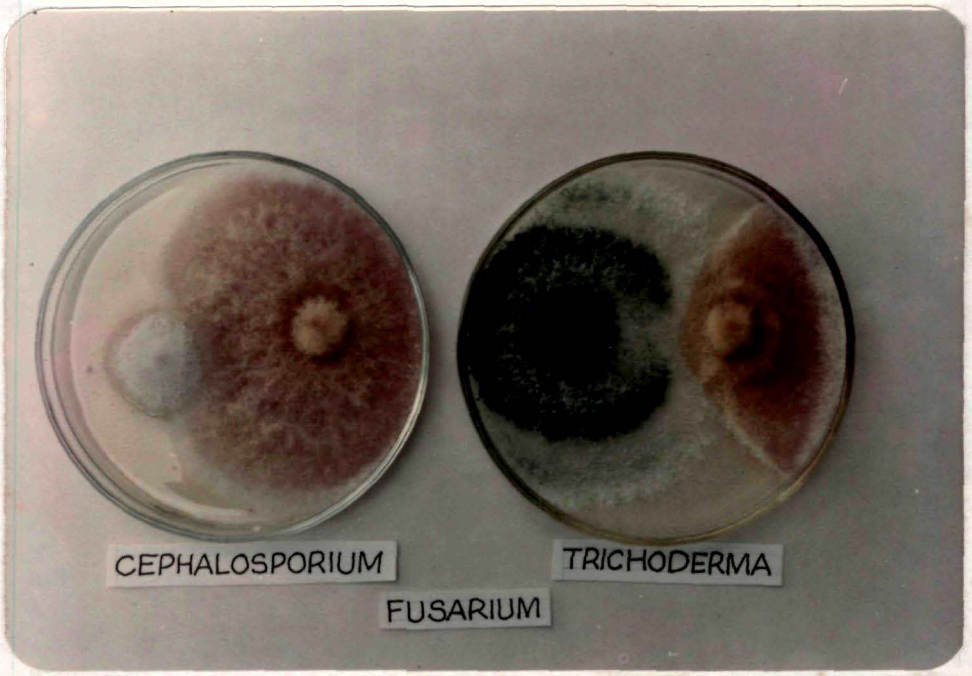


Plate I.G Dual cultures showing antagonism between :
left : *Aspergillus* and *Trichoderma*
right: *Alternaria* and *Trichoderma*

Plate I.H Dual cultures showing antagonism between :
left : *Cephalosporium* and *Trichoderma*
right: *Fusarium* and *Trichoderma*

PLATE I.G

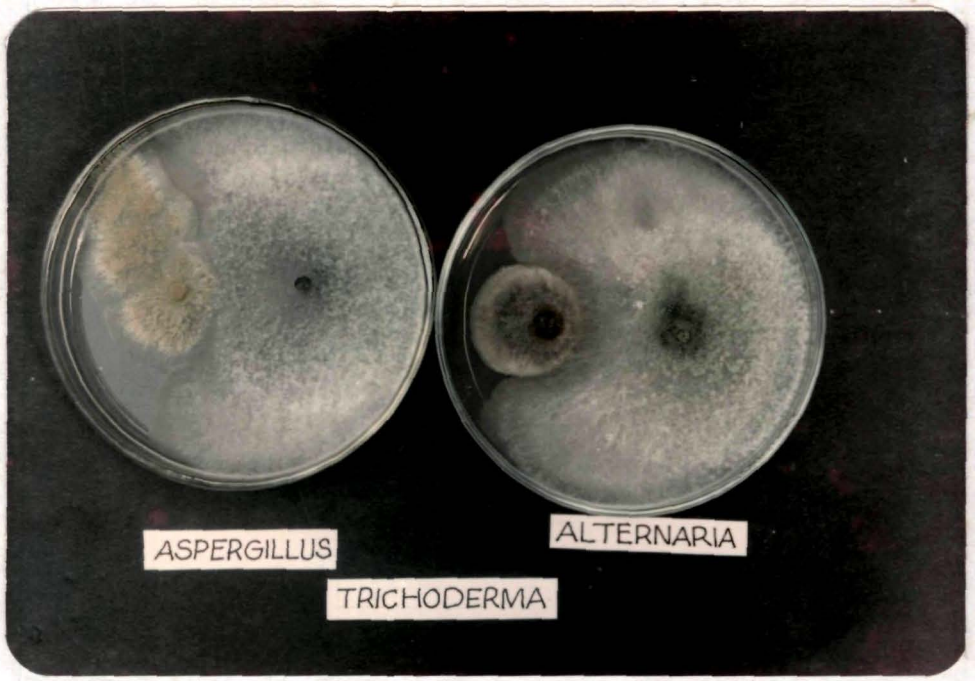
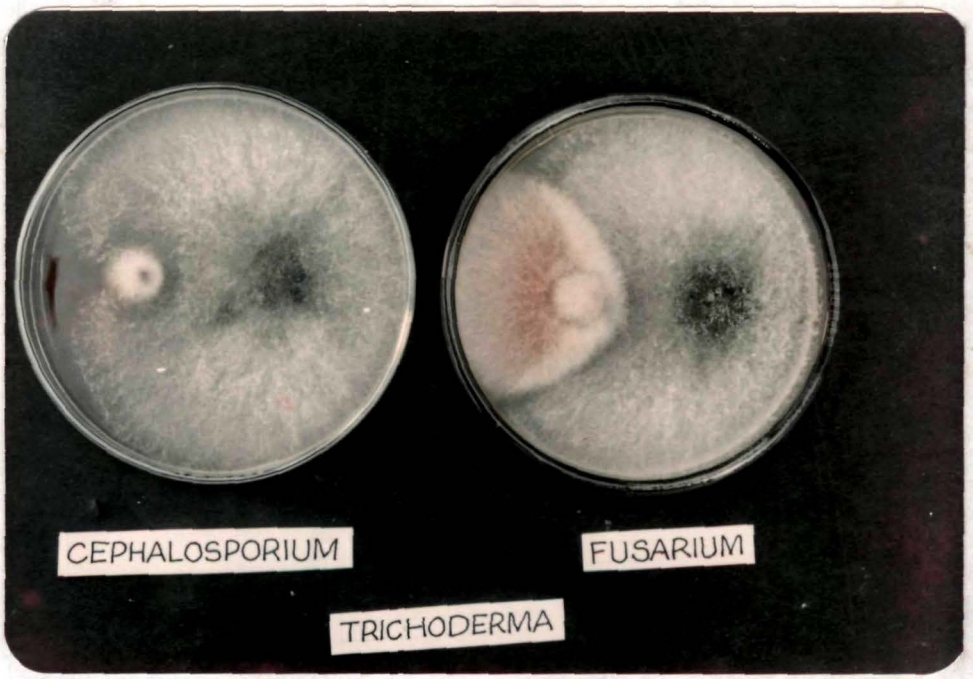


PLATE I.H



50% and 28.57% respectively.

B. Chemical Control:

The results of the effect of different fungicides on the maize seed germination and seedling growth are presented in the tables 6.10 to 6.12 and figures 6.4 to 6.7.

It is clear from the results in table 6.10 that all the fungicides markedly reduced the incidence of fungal flora on the maize seeds of all the varieties. The combined effect of thiram and captan was found to be most successful in reducing the occurrence of fungi on the treated seeds which was 2%, 2% and 3% in the varieties of Local White, Vijay and VL-16 respectively, compared to 46%,, 50% and 39% seed infection in the untreated samples of the three varieties respectively. Other than thiram + captan, dithane M-45, thiram, agrosan GN and captan were also found to be effective for the variety Local White with percentage infection of 3,4,6 and 7 respectively; benomyl treated seeds of this variety had maximum incidence of fungi with 14% infection. In the variety Vijay, agrosan GN comes next to thiram + captan in checking the seed mycoflora with only 3% infection, followed by thiram (5%), dithane M-45 (6%), bavistin (8%), captan (9%). Blitox-50 was found to be least effective with 16% seed infection. Agrosan GN comes ahead of thiram + captan in checking the fungal flora with only 2% seed infection; thiram, dithane M-45 and captan also were found to be very effective in controlling the fungal flora with 3%, 4% and 6% seed infection respectively.

Table 6.10 also shows that the fungicides treated seeds of all the varieties had better percentage of final stand over control (figure 6.4). Thiram + captan treated seeds of the variety Local White and Vijay had the maximum final stand of 96% each in comparison to 85% and 72% respectively in the untreated seeds. In the variety VL-16, agrosan GN treated seeds had the maximum final stand of 94% compared to 88% in the control sets. The effect of the fungicides on the percentage change over control in final stand was found to be not very significant in the varieties Local White and VL-16, where 12.94% was the maximum increase over control due to thiram + captan treatment in Local White, and no change at all in the benomyl treated seeds of the variety VL-16.

To way analysis of variance showed that there was no significant variations in the final stand of the maize seedlings between varieties (.558 NS), but showed significant variation between the fungicides (F=3.489*).

Table 6.11 and figures 6.5 to 6.7 show the result of the effect of the fungicides on the shoot-root length and dry weight of the seedlings. There was an increase in the shoot length of all the seedlings except the seedlings raised from thiram treated seeds of the variety Local White. Captan was found to be the most effective in increasing the shoot length of the seedlings of the variety Local White(33.33%), followed by benomyl (28.98%), thiram + captan (21.73%), bavistin (18.84%), dithane M-45 (13.04%), blitox - 50 (8.69%) and agrosan GN (7.24%). A maximum of 65.21% enhancement in the shoot length was noticed in the seedlings of the variety Vijay treated with benomyl, followed by bavistin

(54.34%), thiram + captan (50%), agrosan GN(41.3%), thiram (34.78%), dithane M-45 (26.08%), blitox-50 (15.21%) and captan (10.86%). Thiram + captan was found to be most effective in shoot elongation in the variety VL-16 with 62.06% increase over control and blitox- 50 was least effective with 6.89% increase.

All the fungicides were found to be very effective in increasing the root length of the seedlings of all the varieties (Table 6.11). Though thiram was responsible for a decrease in the shoot length in the variety Local White, it almost doubled the root length with 97.54% increase over control. Thiram + captan also showed similar effect with 95.9% increase, dithane M-45 and captan were responsible for 52.45% and 49.18% increase respectively over control in this variety. In the variety Vijay, thiram + captan and thiram were found to be most successful in increasing the root length with 77.52% and 64.04% increase respectively over control, but blitox-50 was least effective with only 4.49% increase. In the variety VL-16 too, thiram + captan exhibited a maximum of 53.03% increase in root length over control. Dithane M-45, bavistin, agrosan GN and thiram also had marked influence on the root length with 38.63%, 34.84%, 26.51% and 25.75% increase respectively.

The influence of the fungicides on the dry weight of the seedlings was found to be not very remarkable compared to the change in shoot and root length of the seedlings. It is clear from the results in the table 6.11 that in almost all the cases there was an increase in the dry weight of the seedlings treated with different fungicides except 4 instance of decrease, viz.

Table 6.10 Effect of different fungicides on the germination of maize seed.

Variety : Local White

Fungicides	% seed infection	Mortality		Final stand%	% change
		Pre-emergence	Post-emergence		
Agrosan GN	6	8	2	90	+ 05.88
Bavistin	9	11	-	89	+ 04.70
Benomyl	14	6	-	94	+ 10.58
Blitox-50	12	11	3	86	+ 01.17
Captan	7	6	6	88	+ 03.52
Dithane M-45	3	3	5	92	+ 08.23
Thiram	4	8	-	92	+ 08.23
Thiram + Captan	2	4	-	96	+ 12.94
Control	46	10	5	85	-
					Variety : Vijay
Agrosan GN	3	6	-	94	+ 15.27
Bavistin	8	8	2	90	+ 25.00
Benomyl	12	4	10	86	+ 19.44
Blitox - 50	16	10	3	87	+ 20.83
Captan	9	10	-	90	+ 25.00
Dithane M - 45	6	8	-	92	+ 27.77
Thiram	5	5	3	92	+ 27.77
Thiram + Captan	2	4	-	96	+ 33.33
Control	50	20	8	72	-
					Variety : VL - 16
Agrosan GN	2	4	2	94	+ 06.81
Bavistin	9	10	-	90	+ 02.27
Benomyl	16	12	-	88	-
Blitox - 50	10	6	5	89	+ 01.13
Captan	6	10	-	90	+ 02.27
Dithane M - 45	4	10	1	89	+ 01.13
Thiram	3	8	-	92	+ 04.54
Thiram + Captan	3	7	-	93	+ 05.68
Control	39	10	2	88	-

Table 6.11 Effect of different fungicides on the shoot-root length and dry weight of maize seedlings.

Variety : Local White

Fungicides	Length in cm		Dry Weight (mg)
	Shoot	Root	
Agrosan GN	7.4 (+07.24)*	15.2 (+24.59)	0.140 (+03.70)
Bavistin	8.2 (+18.84)	14.6 (+19.67)	0.139 (+02.96)
Benomyl	8.9 (+28.98)	15.0 (+22.95)	0.148 (+09.62)
Blitox-50	7.5 (+08.69)	14.5 (+08.85)	0.131 (-02.96)
Captan	9.2 (+33.33)	18.6 (+52.45)	0.151 (+11.85)
Dithane M-45	7.8 (+13.04)	18.2 (+49.18)	0.141 (+04.44)
Thiram	6.1 (-11.59)	24.1 (+97.54)	0.149 (+10.37)
Thiram + Captan	8.4 (+21.73)	23.9 (+95.9)	0.158 (+17.03)
Control	6.9 -	12.2 -	0.135 -
			Variety : Vijay
Agrosan GN	6.5 (+41.30)	12.3 (+38.20)	0.153 (+06.25)
Bavistin	7.1 (+54.34)	9.6 (+07.86)	0.149 (+03.47)
Benomyl	7.6 (+65.21)	10.4 (+16.85)	0.140 (-02.77)
Blitox-50	5.3 (+15.21)	9.3 (+04.49)	0.150 (+04.16)
Captan	5.1 (+10.86)	11.6 (+30.33)	0.146 (+01.38)
Dithane M-45	5.8 (+26.08)	12.2 (+37.07)	0.149 (+03.47)
Thiram	6.2 (+34.78)	14.6 (+64.04)	0.152 (+05.55)
Thiram + Captan	6.9 (+50.00)	15.8 (+77.52)	0.159 (+10.41)
Control	4.6 -	8.9 -	0.144 -
			Variety : VL-16
Agrosan GN	8.1 (+39.65)	16.7 (+26.51)	0.188 (+06.81)
Bavistin	7.6 (+31.03)	17.8 (+34.84)	0.180 (+02.27)
Benomyl	6.5 (+12.06)	14.5 (+09.84)	0.177 (+00.56)
Blitox -50	6.2 (+06.89)	13.8 (+04.54)	0.172 (-02.27)
Captan	7.6 (+31.03)	14.4 (+09.09)	0.170 (-03.40)
Dithane M-45	8.5 (+46.57)	18.3 (+38.63)	0.186 (+05.68)
Thiram	8.6 (+48.27)	16.6 (+25.75)	0.181 (+02.84)
Thiram + Captan	9.4 (+62.06)	20.2 (+53.03)	0.189 (+07.83)
Control	5.8 -	13.2 -	0.176 -

* Figures in parenthesis indicate percentage change over control.

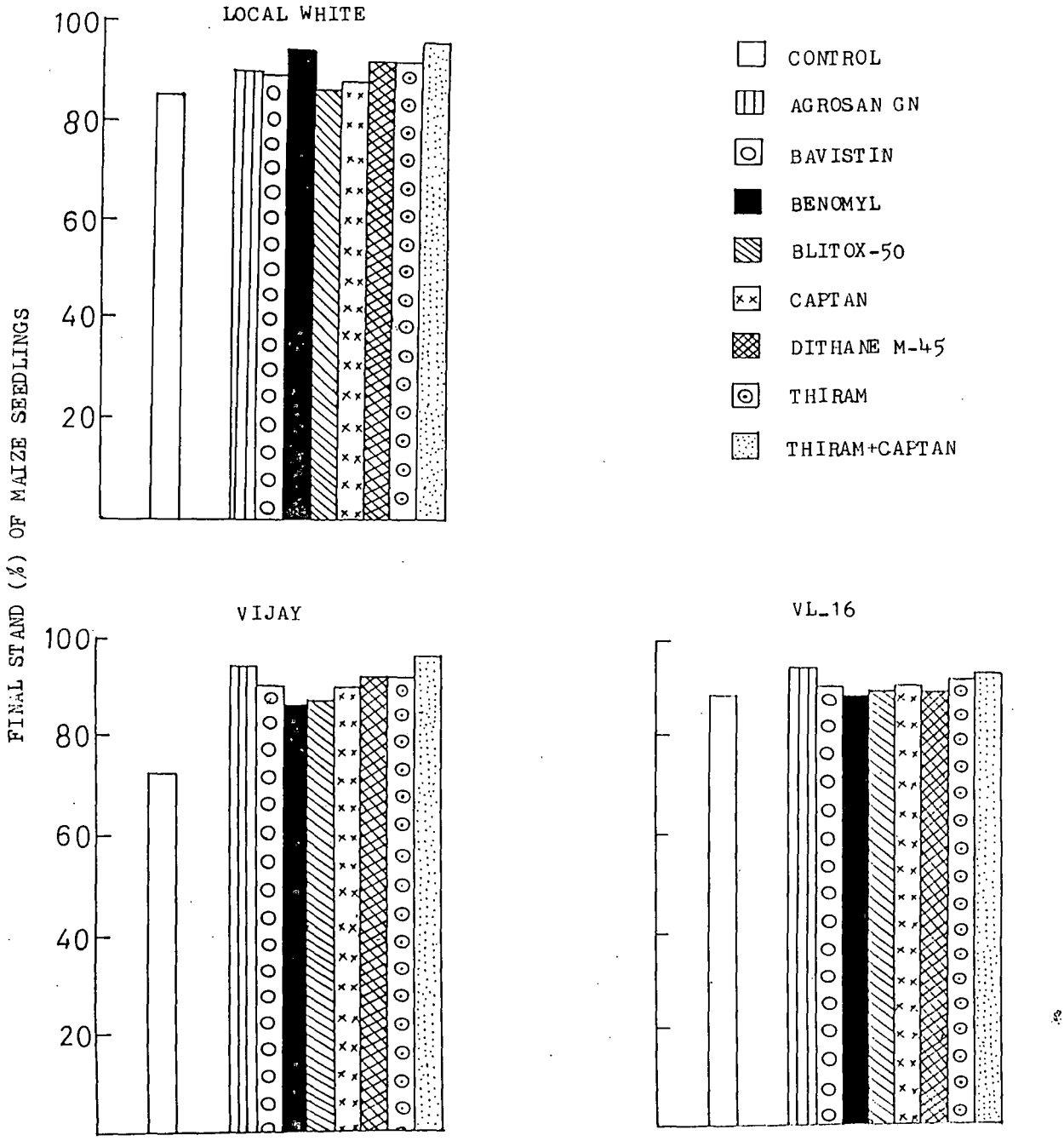


Fig. 6.4 Showing change in the final stand of maize seedlings of different varieties due to the treatment of different fungicides.

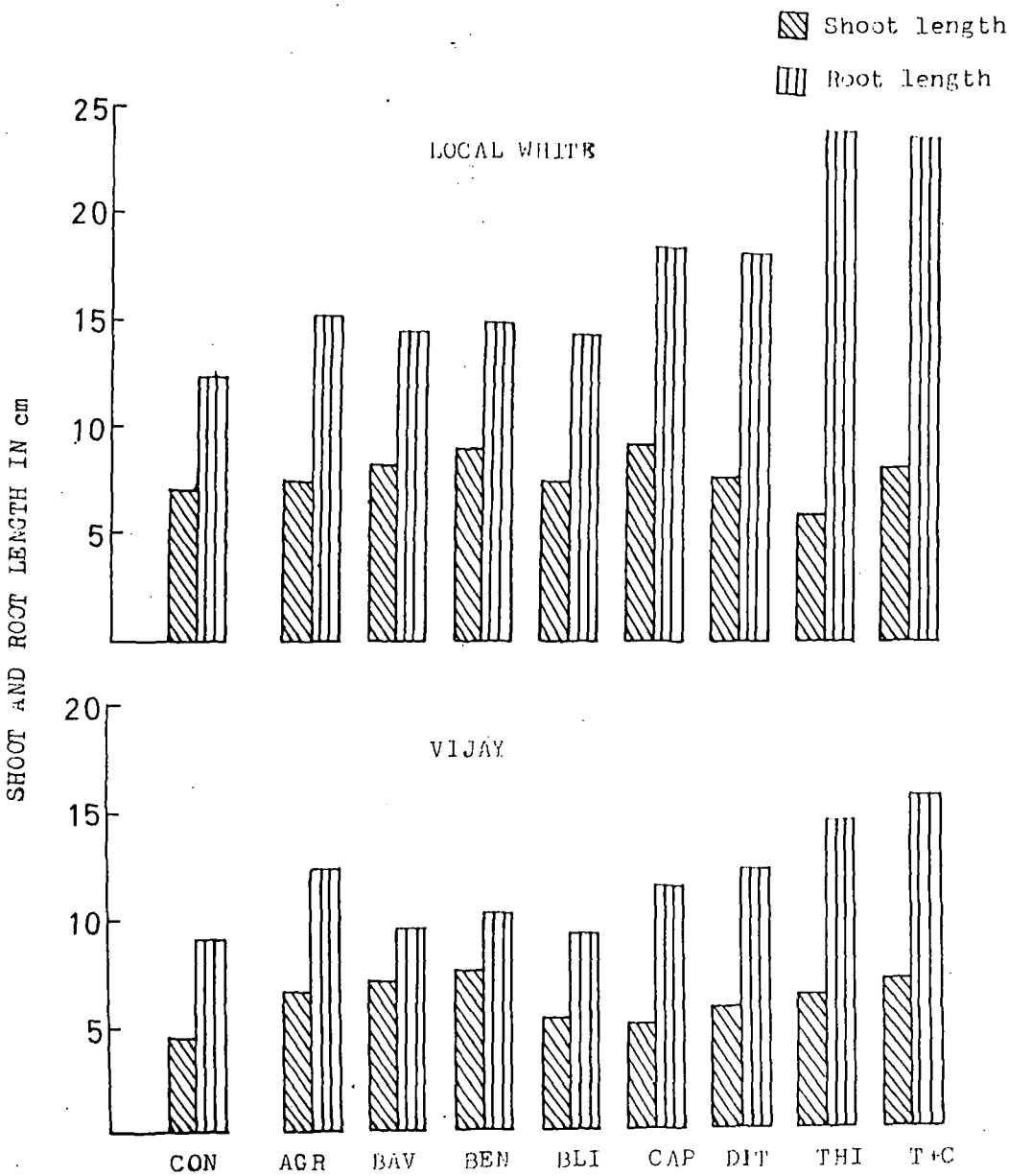


Fig. 6.5 Effect of different fungicides on the shoot and root length of the maize seedlings of the varieties Local White and Vijay. CON- Control, AGR- agrosan GN, BAV- bavistin, BEN- benomyl, BLI- blitox-50, CAP- captan, DIT- dithane M-45, THI- thiram and T+C- thiram+captan.

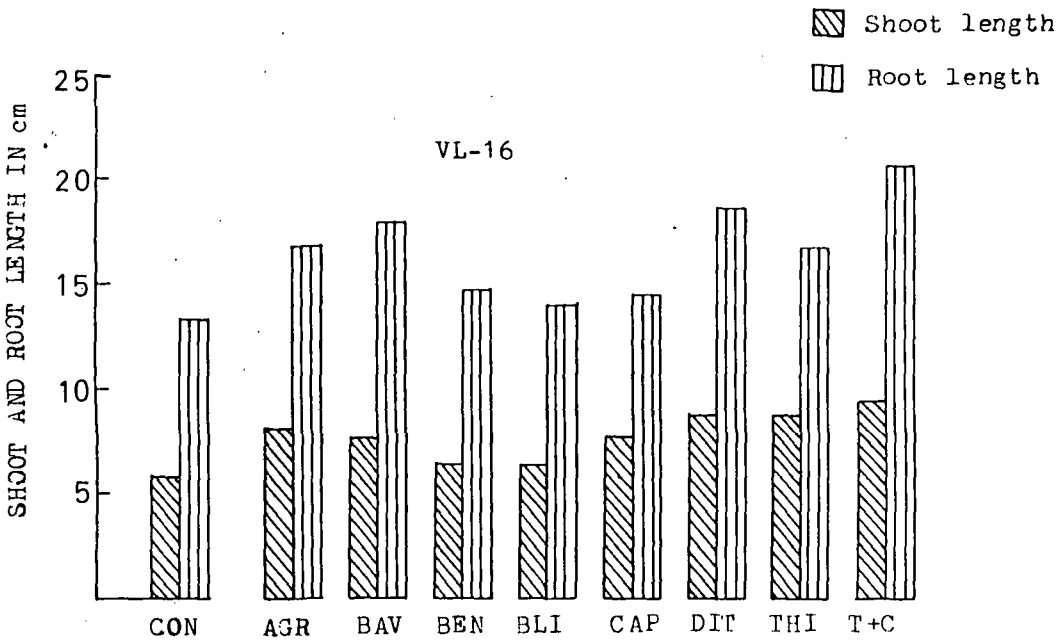


Fig. 6.6 Effect of different fungicides on the shoot and root length of the maize seedlings of the variety VL-16. CON- control, AGR- agrosan GN, BAV- bavistin, BEN- benomyl, BLI- blitox-50, CAP- captan, DIT- dithane M-45, THI- thiram and T+C- thiram+captan.

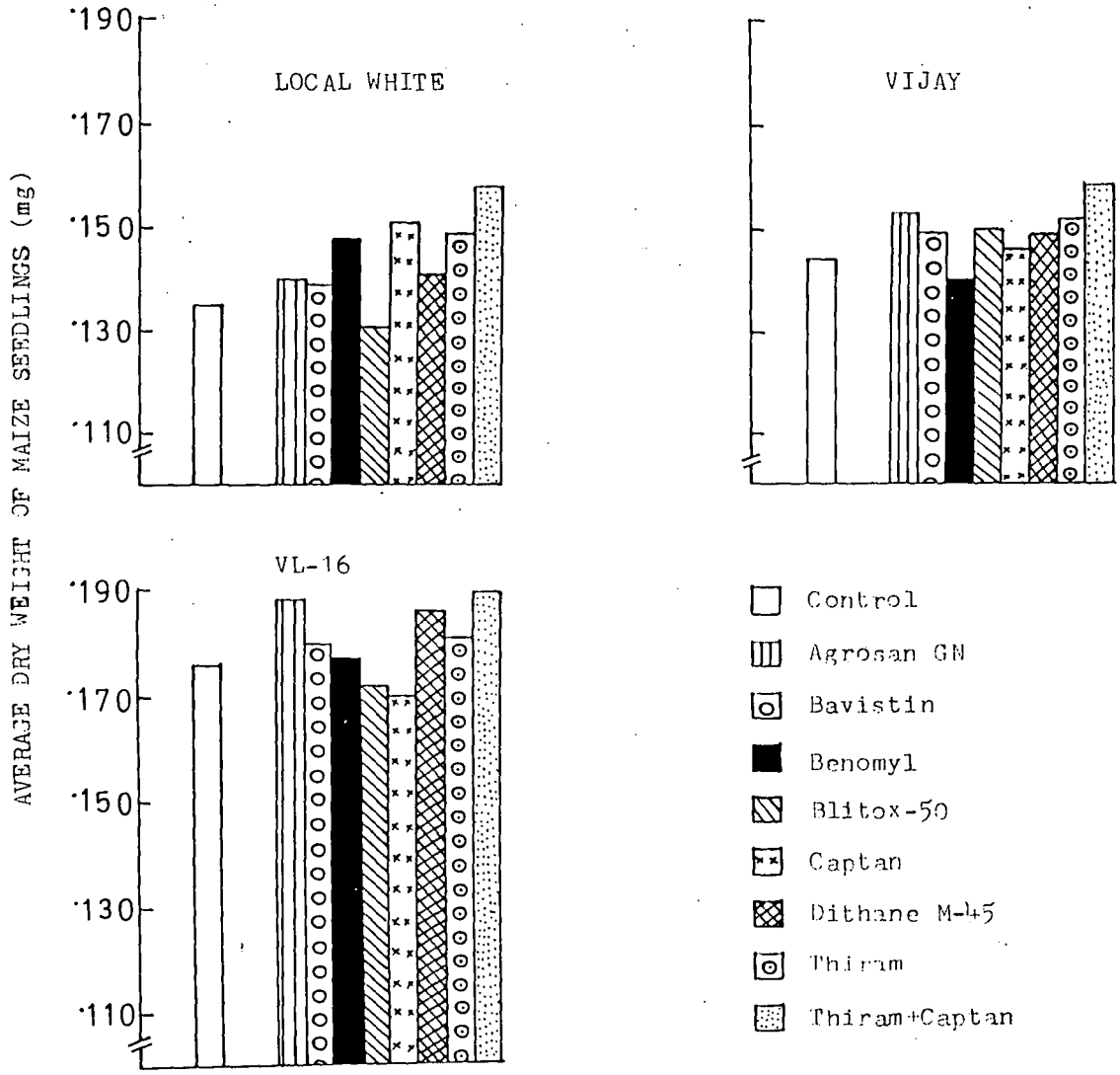


Fig. 6.7 Effect of different fungicides on the average dry weight of the maize seedlings of the varieties Local White, Vijay and VL-16.

blitox-50 treated sample of Local White (2.96%), benomyl treated samples of Vijay (2.77%) and blitox-50 (2.27%) and captan (3.4%) in the variety VL-16. A maximum of 17.03% increase in dry weight was observed in the thiram + captan treated samples of Local White, followed by captan (11.85%), thiram (10.37%) and other fungicides showed less than 10% increase. In the variety Vijay, only thiram + captan treated samples showed more than 10 percent increase in dry weight (10.41%) and the rest of the fungicides had poor influence on the dry weight of the seedlings. No fungicide could increase the dry weight of the seedlings of the variety VL-16 by more than 10%.

The variations in the shoot length, root length and dry weight of the maize seedlings due to the treatment by the fungicides were statistically analysed (two way analysis of variance). It has been found that there were significant variations in the shoot length, root length and dry weight between the varieties ($F=8.596^{**}$, 25.992^{**} and 100.993 respectively) but variations between the fungicides were found to be significant only for root length and dry weight ($F=7.193^{**}$ and 2.584^{*} respectively) and not the shoot length ($F=1.849$ NS).

Table 6.12 shows the results of the experiment in which the maize seeds were treated with both fungi and fungicides and expressed as percent recurrence of the fungi and percent seed germination. It is clear from the results that all the fungicides sharply reduced the occurrence of the fungi on the seeds with which they were treated.

Table 6.12 Efficacy of different fungicides in controlling different mycoflora associated with maize seed.

Fungi/Fungicide	Control		Agrosan GN		Bavistin		Benomyl		Blitox-50		Captan		Dithane M-45		Thiram		Thiram + Captan	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
A. alternata	79	74	5	86	16	90	17	86	13	82	8	92	5	87	4	92	3	96
A. flavus	86	69	7	90	4	92	3	94	8	81	16	80	2	90	6	90	2	95
F. moniliforme	72	62	2	90	0	96	8	89	6	90	16	82	6	89	4	92	4	91
P. expansum	74	66	3	70	6	72	10	69	8	70	2	94	3	78	3	86	3	80
R. nigricans	90	71	2	77	4	78	8	74	10	71	3	87	0	92	3	88	3	90
T. viride	92	64	4	72	2	82	10	70	12	62	5	82	1	88	2	90	8	86
Variety : Vijay																		
A. alternata	66	70	3	88	6	92	11	90	10	86	4	94	3	89	2	90	1	92
A. flavus	80	61	8	84	8	86	9	92	20	79	3	86	9	86	0	86	2	90
F. moniliforme	69	63	4	86	3	80	3	84	5	92	14	80	3	91	2	90	1	89
P. expansum	60	70	0	88	2	92	2	74	9	76	5	92	1	86	1	92	0	88
R. nigricans	92	76	0	80	6	86	12	90	12	78	0	90	2	85	2	89	2	92
T. viride	86	59	4	78	7	79	3	75	10	70	3	80	4	82	1	86	6	90
Variety : VL-16																		
A. alternata	70	69	4	86	8	89	10	82	12	79	6	94	4	90	2	90	5	90
A. flavus	85	72	5	80	9	80	2	89	18	76	4	90	2	88	1	92	1	92
F. moniliforme	80	69	2	76	6	87	8	82	8	80	11	82	1	85	2	86	0	88
P. expansum	71	65	1	79	2	82	3	88	5	86	2	88	4	84	1	85	2	90
R. nigricans	90	70	0	80	10	80	4	90	3	88	1	92	0	91	2	90	5	87
T. viride	88	55	6	72	9	76	12	79	16	74	6	82	6	80	3	80	4	85

A = % Seed yielding fungi, B = % Seed germinated.

A. alternata treated seeds of the variety Local White were best controlled by thiram + captan where the recurrence of *A. alternata* was only 3%, thiram, agrosan GN, dithane M-45 and captan treated seeds also showed good control of *A.alternata* with 4%, 5%, 5% and 8% of seed yielding the fungi. Benomyl and bavistin were less effective in controlling *A. alternata* where 17% and 16% respectively of seed infection was observed. *A.flavus* was best controlled by the treatment of thiram + captan and dithane M-45 where only 2% seed showed the occurrence of the fungi, benomyl and bavistin also effectively checked the fungus with only 3% and 4% infection respectively. Though bavistin was found to be most effective against *F. moniliforme* where no seed yielded this fungus after the treatment,other fungicides too, except captan, effectively checked the reccurrence of the fungus with less than 10% seed infection compared to 72% seed infection in the control sets. *P.expansum, R.nigricans* and *T.viride* were also effectively controlled by all the fungicides used where the reccurrence of the fungi varied from 0 to 12% only compared to 74%, 90% and 92% seed infection respectively in the control sets. In the variety Vijay, most of the fungicides, viz.agrosan GN, bavistin, dithane M-45, thiram and thiram + captan were found to be effective in checking the fungi tested, with less than 10% seed infection in each case. Captan treated seeds also showed good control of the test fungi except *F. moniliforme* which reccured in 14% of the seeds. Blitox - 50 was found to be less effective in controlling the test fungi compared to other fungicides in this variety.

In the variety VL-16 too, all the fungicides except blitox-50 showed effective control of the test fungi where the range of infection was between 0 to 12% and blitox-50 treated seeds showing infection between 3-18%.

The results of the effect of fungi and fungicides on seed infection and seed germination were statistically analysed (two way analysis of variance) and it was found that there was significant variations in seed infection between the fungicides in all the three varieties (F=192.068**, 134.967** and 251.088** for Local White, Vijay and VL-16 respectively), variations between the fungal species were insignificant for Local White and Vijay (F=1.045 NS and 1.983 NS respectively) but significant for VL-16(F=2.626*). Seed germination had significant variations both between the fungicides (F=9.647**,14.259* and 26.399** for Local White, Vijay and VL-16 respectively and between the fungal species (F=6.919**,4.823** and 10.84** for Local White, Vijay and VL-16 respectively).

VII EFFECT OF FUNGAL INFECTION ON THE NUTRITIVE VALUE OF MAIZE SEEDS

Figures 7.1 to 7.10 depict the results of the effect of fungal infestation of maize seeds on the nutritive value of the seeds in terms of dry weight, fatty acid and starch contents. Dry weight of 100 infested and unifested seeds was taken at an interval of 10 days for a period of 30 days and the results are presented in the figures 7.1 and 7.2. The results show that there was a gradual fall of dry weight during the incubation period of 30

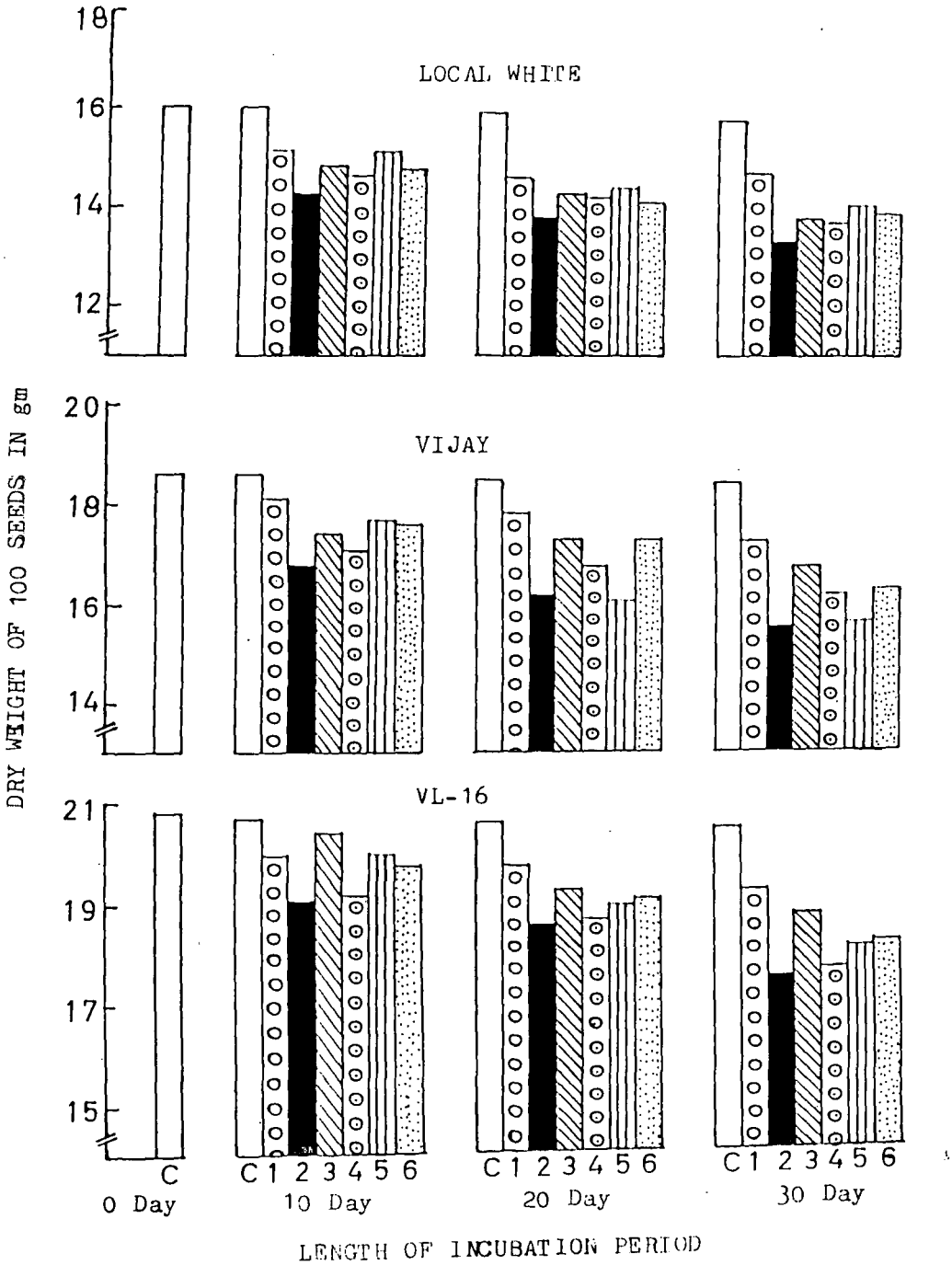


Fig. 7.1 Change in the dry weight of 100 seeds of maize due to fungal infestation. C- Control, 1- Alternaria alternata, 2- Aspergillus flavus, 3- Fusarium moniliforme, 4- Penicillium expansum, 5- Rhizopus nigricans and 6- Trichoderma viride.

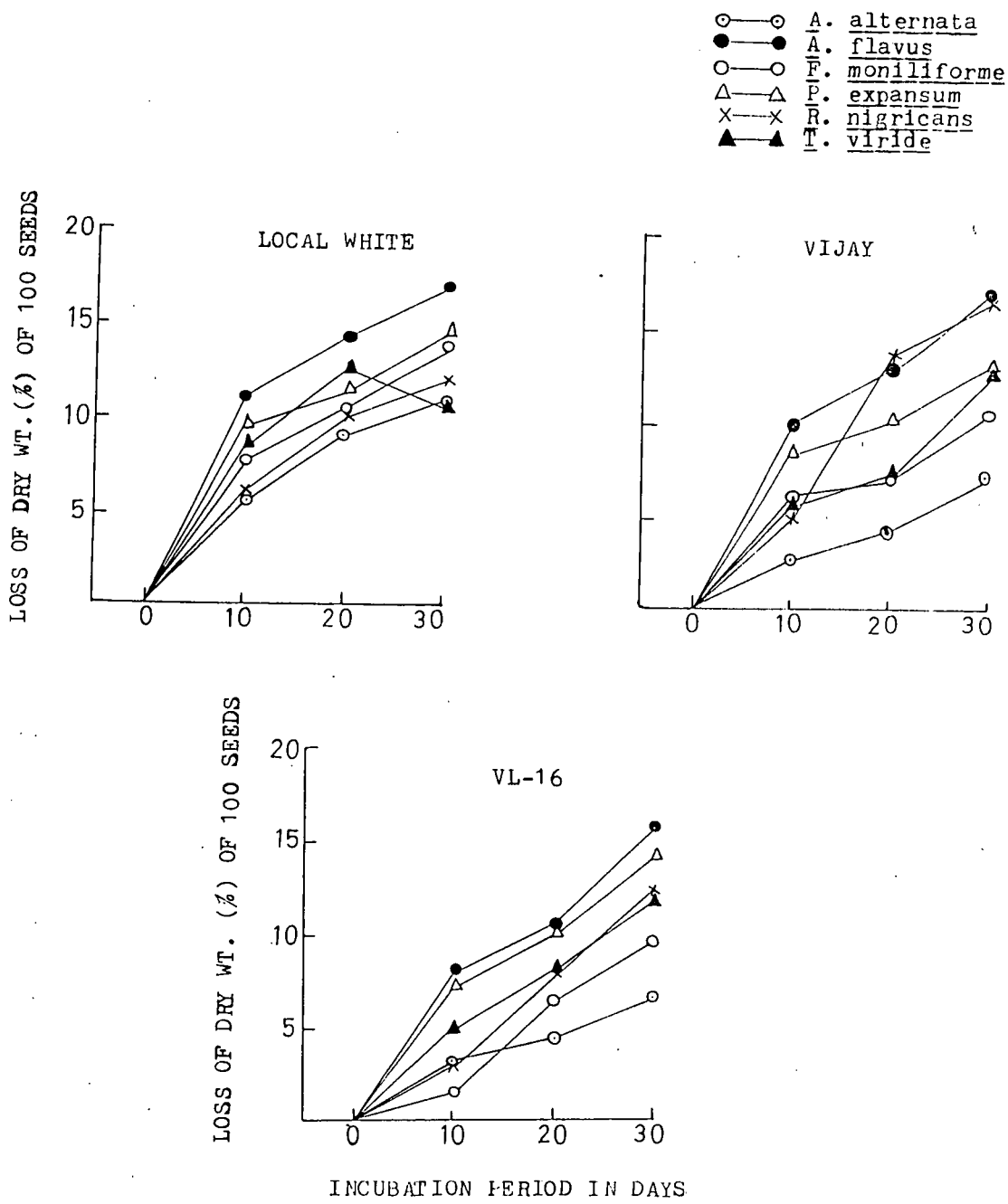


Fig. 7.2 Showing percentage loss of dry weight of maize seeds due to fungal infestation.

days in both the treated and control samples, however, the loss of dry weight in the treated samples was far more pronounced in comparison to that of the control. In all the three varieties *Aspergillus flavus* caused maximum reduction in dry weight with 17.13% in Local White, 17.23% in Vijay and 15.94% in VL-16 after 30 days of incubation. There was a maximum of 13.49% reduction in dry weight after 20 days of incubation with *Rhizopus nigricans* in the variety Vijay. Other than *A. flavus* and *R. nigricans, Fusarium moniliforme, Penicillium expansum* and *Trichoderma viride* were also found to be effective in reducing the dry weight of the seeds in all the varieties. *F. moniliforme* initiated 13.57%,10.7% and 9.69% reduction in the varieties Local White, Vijay and VL-16 respectively, *P. expansum* was responsible for 14.64%, 13.35% and 14.6% reduction respectively and the percentage reduction due to *T. viride* was found to be 10.7%, 12.62% and 12.62% respectively. There was marginal decrease in dry weight of the uninfested seeds (control) with only 1.21% in the variety Local White, 1.47% in Vijay and 1.48% in VL-16 after 30 days of incubation.

Statistical analysis (two way analysis of variance) showed that there were significant variations in the dry weight of the maize seeds between the incubation periods (F=31.669**, 23.466** and 28.498** for Local White, Vijay and VL-16 respectively) as well as between the fungal species (F=7.425**, 5.955** and 6.052** for Local White, Vijay and VL-16 respectively).

Figures 7.3 to 7.6 depict the results of the effect of fungal infection on the fatty acid contents of maize seeds during

different periods of incubation. The figures show that both the infested and uninfested (control) seeds exhibited increase in fatty acid contents along with progress of time and maximum increase was noticed after 30 days of incubation, however, uninfested seeds (control) showed little change in the fatty acid content in comparison to the treated samples.

During the initial phase of incubation the rise in fat acidity level was found to be less pronounced than that of the last phase of incubation where a sharp increase was noticed in all the three varieties. *A. flavus* was responsible for maximum increase in fat acidity level in the varieties of Local White and Vijay with 53.5% and 69.2% increase respectively, whereas, *P. expansum* caused a maximum increase of 74.2% in the variety VL-16. In the variety Local White, *A. flavus* was followed by *P. expansum* (42.8%), *F. .moniliforme* (32.1%), *A. alternata* and *T. viride* (28.5%) and *R. nigricans* (25.0%) in increasing the fat acidity level of the seed. Seeds of the variety Vijay showed remarkable increase in fat acidity level after 30 days of incubation by all the test fungi, where *A. flavus* was followed by *P. expansum* and *T. viride* (57.6%), *F. moniliforme* and *R. nigricans* (46.1%) and *A. alternata*(42.3%),whereas in the variety VL-16 *P.expansum* was followed by *A. flavus* (65.7%), *F. moniliforme* (40%), *R. nigricans* (37.1%), *A. alternata* (34.2%) and *T. viride* (31.4%). The uninfested (control) seeds showed an increase of 6.6%, 15.3% and 14.2% in the varieties of Local White, Vijay and VL-16 respectively.

Statistical analysis (two way analysis of variance) of the

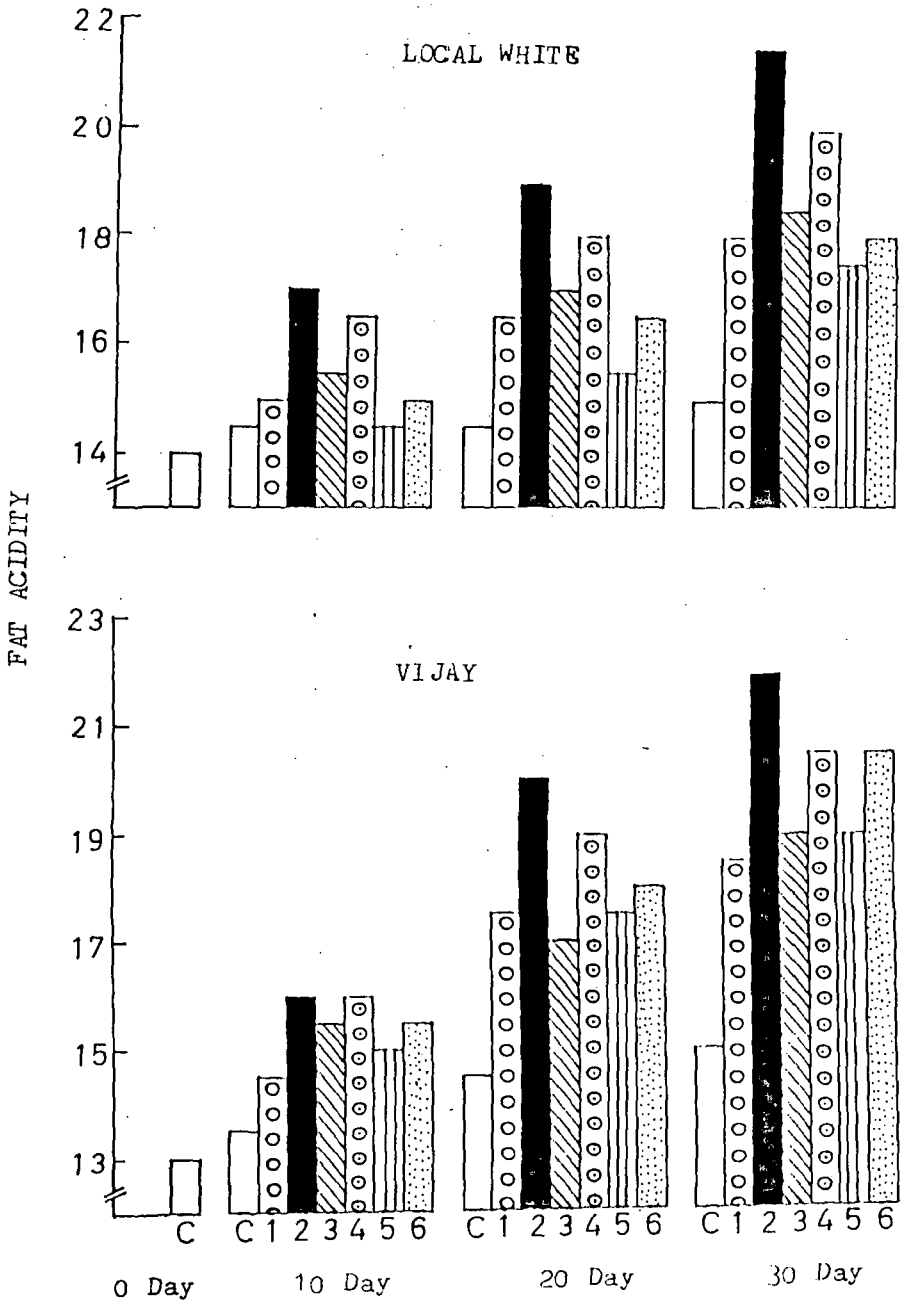


Fig. 7.3 Change in the fatty acid contents of maize seeds due to fungal infestation. C- Control, 1- A.alternata, 2- A.flavus, 3- F.moniliforme, 4- P.expansum, 5- R.nigricans and 6- T.viride.

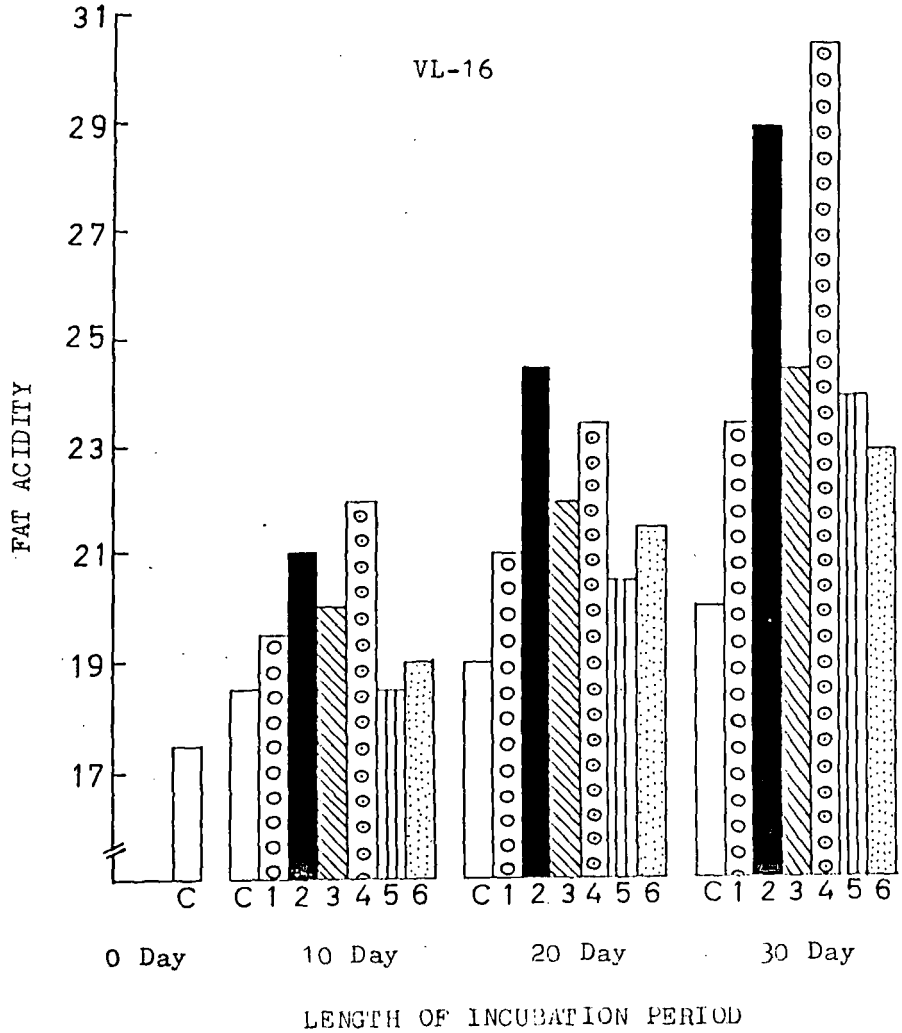


Fig. 7.4 Change in the fatty acid contents of maize seeds due to fungal infestation. C- Control, 1- A.alternata, 2- A.flavus, 3- F.moniliforme, 4- P.expansum, 5- R.nigricans and T.viride.

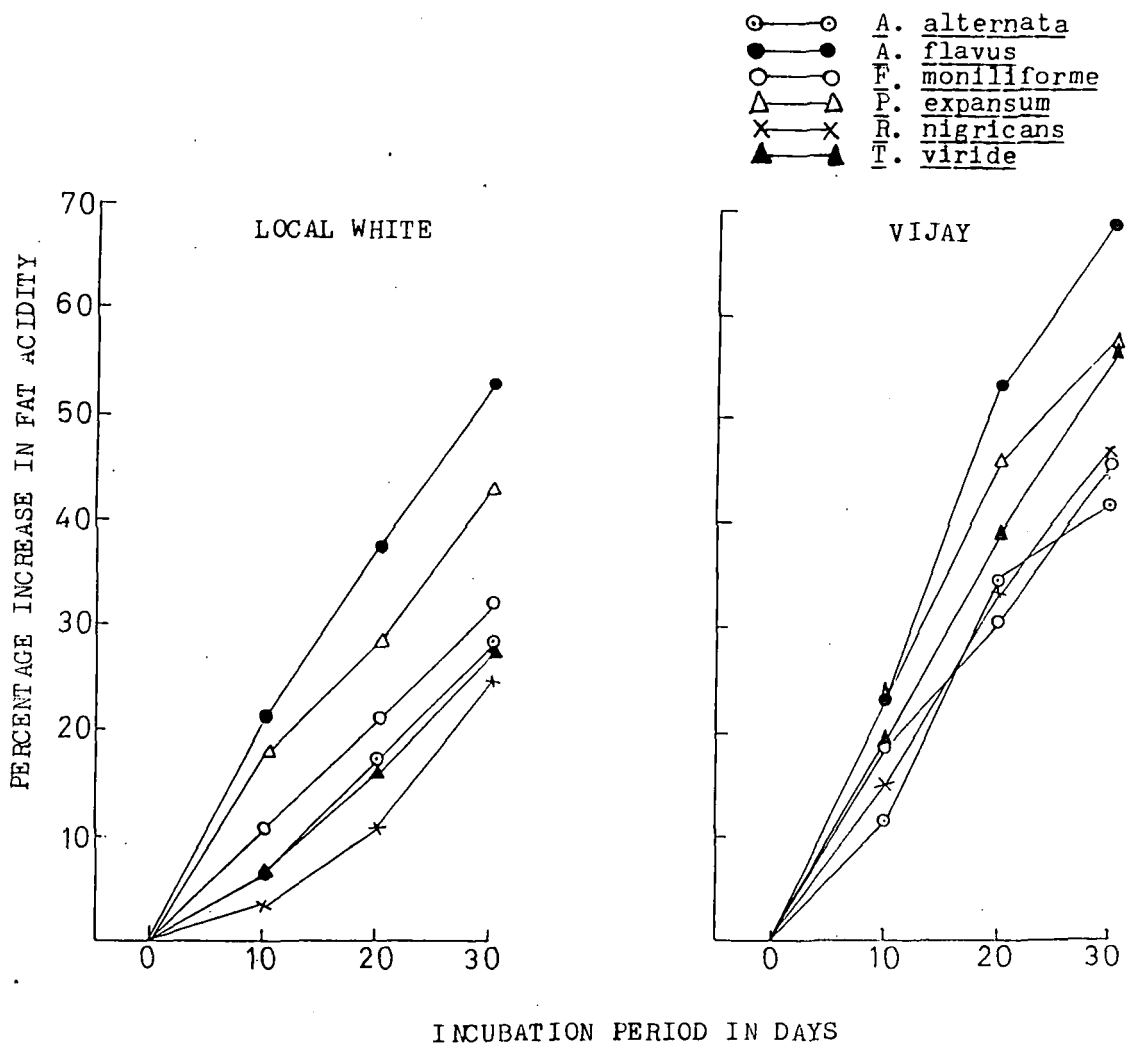


Fig. 7.5 Showing percentage increase in the fatty acid contents of maize seeds due to fungal infestation.

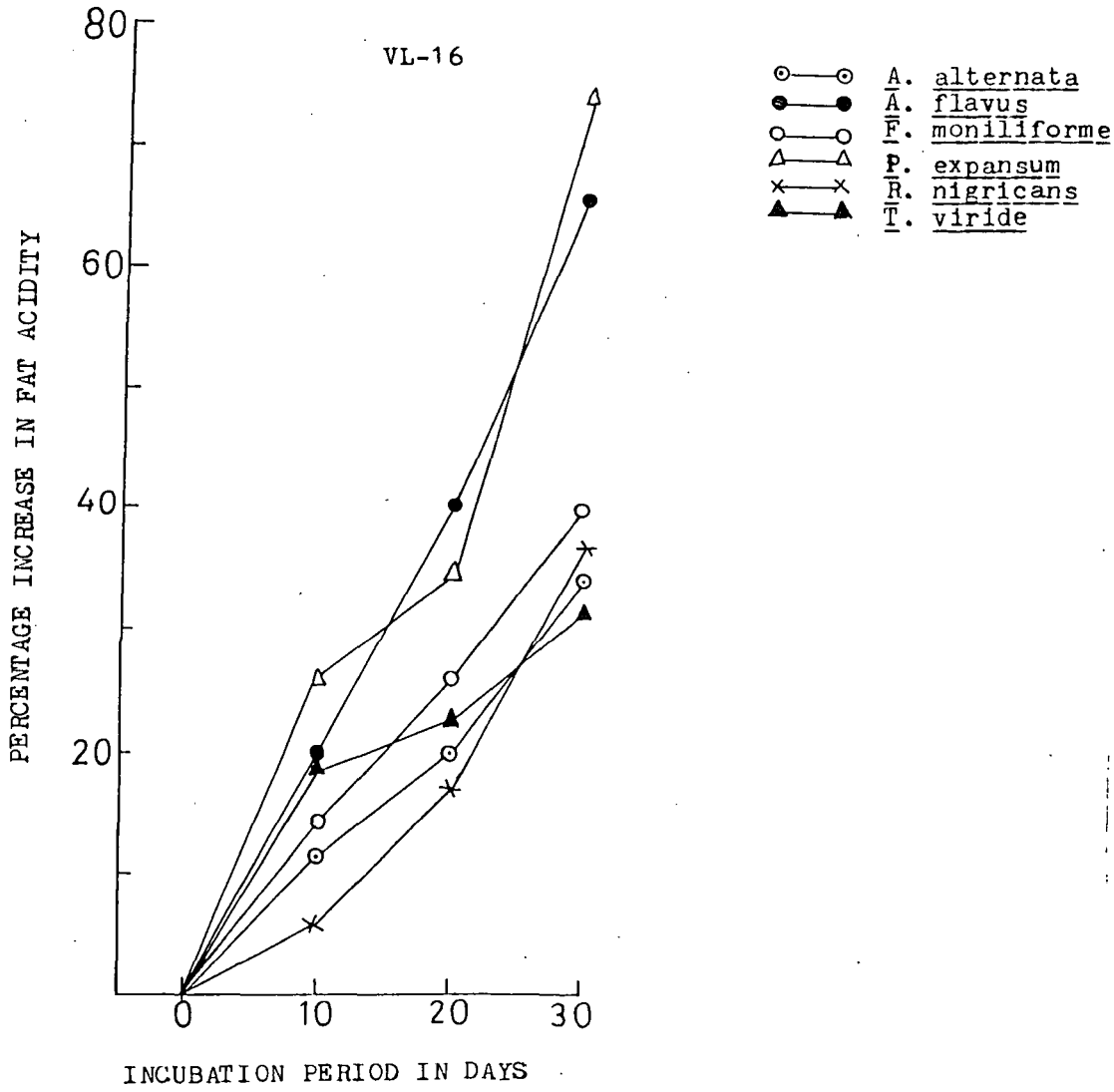


Fig. 7.6 Showing percentage increase in the fatty acid contents of maize seeds due to fungal infestation.

variations in fatty acid contents of maize seeds due to fungal infestation showed that there were significant variations between the incubation periods (F=4.287*, 52.122** and 28.388** for Local White, Vijay and VL-16 respectively). Significant variations between the fungal species were noticed only for Vijay and VL-16 (F.5.622** and 4.489** respectively) but not for Local White (F=1.558 NS).

The results of the effect of fungal infestation on the starch content of maize seeds are depicted in the figures 7.7 to 7.10. It is clear from the figures that all the test fungi were responsible for depletion of starch contents of the seeds in all the three varieties. During the initial phase of incubation, in general, the starch depletion was slow, but became pronounced after 30 days of incubation period. *A. flavus, P. expansum* and *T. viride* were found to be prompt in reducing the starch content of the seeds of the variety Local White, where 18.8%, 14.8% and 14.8% reduction respectively was noticed after only 10 days of incubation period. After 30 days of incubation, *A. flavus* caused a maximum depletion of 44.1%, followed by *P. expansum* (39.5%), *R. nigricans* (37.3%), *T.viride* (36.7%), *F.moniliforme* (36.1%) and *A. alternata* (25.3%).

In the variety Vijay, *A. flavus* was responsible for a maximum reduction of 48.5%, followed by *T. viride* (48.3%), *P. expansum* (42.6%), *R. nigricans* (39.0%), *F. moniliforme* (32.0%) and *A. alternata* (27.5%). There was early depletion of starch in the seeds of the variety VL-16, where 8.1% to 23.3% reduction was noticed after only 10 days of incubation with the different test

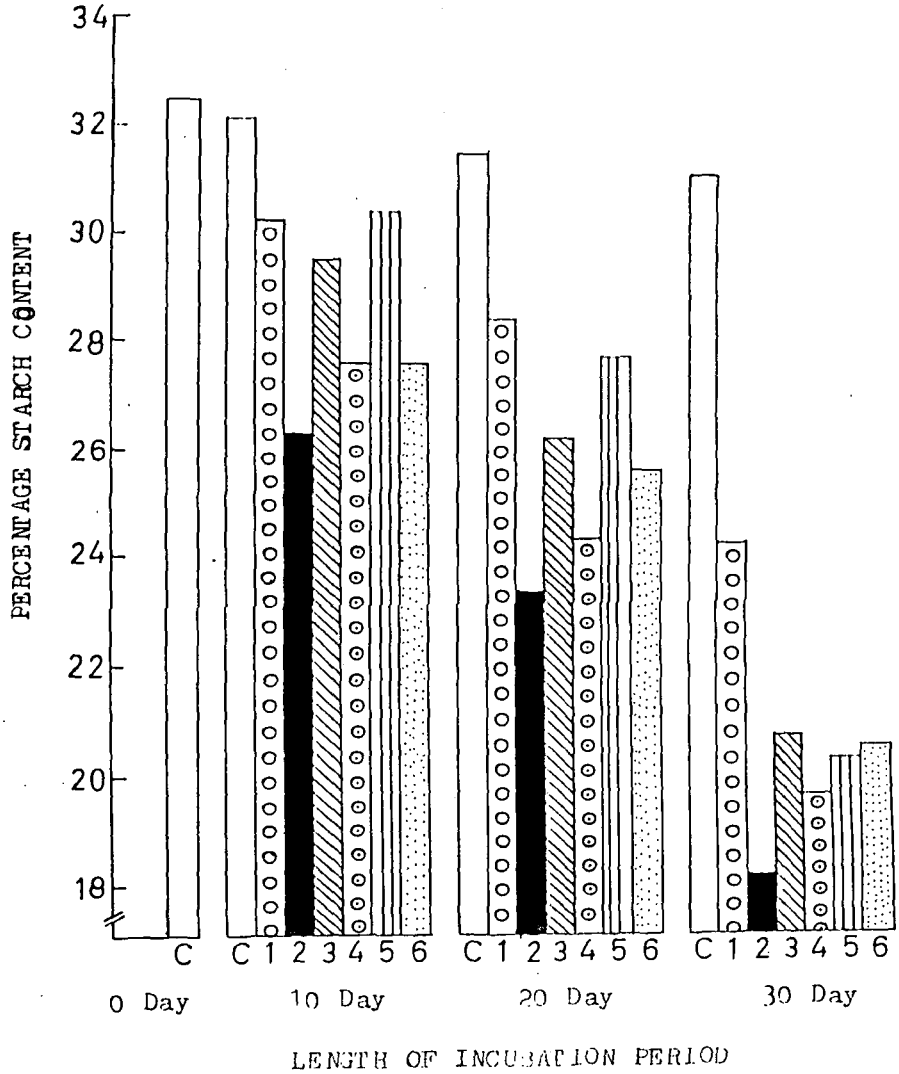


Fig. 7.7 Change in the starch contents of maize seeds due to fungal infestation. (Variety:Local White). C- Control, 1- A.alternata, 2- A.flavus, 3- F.moniliforme, 4- F.expansum, 5- R.nigricans and 6- T.viride.

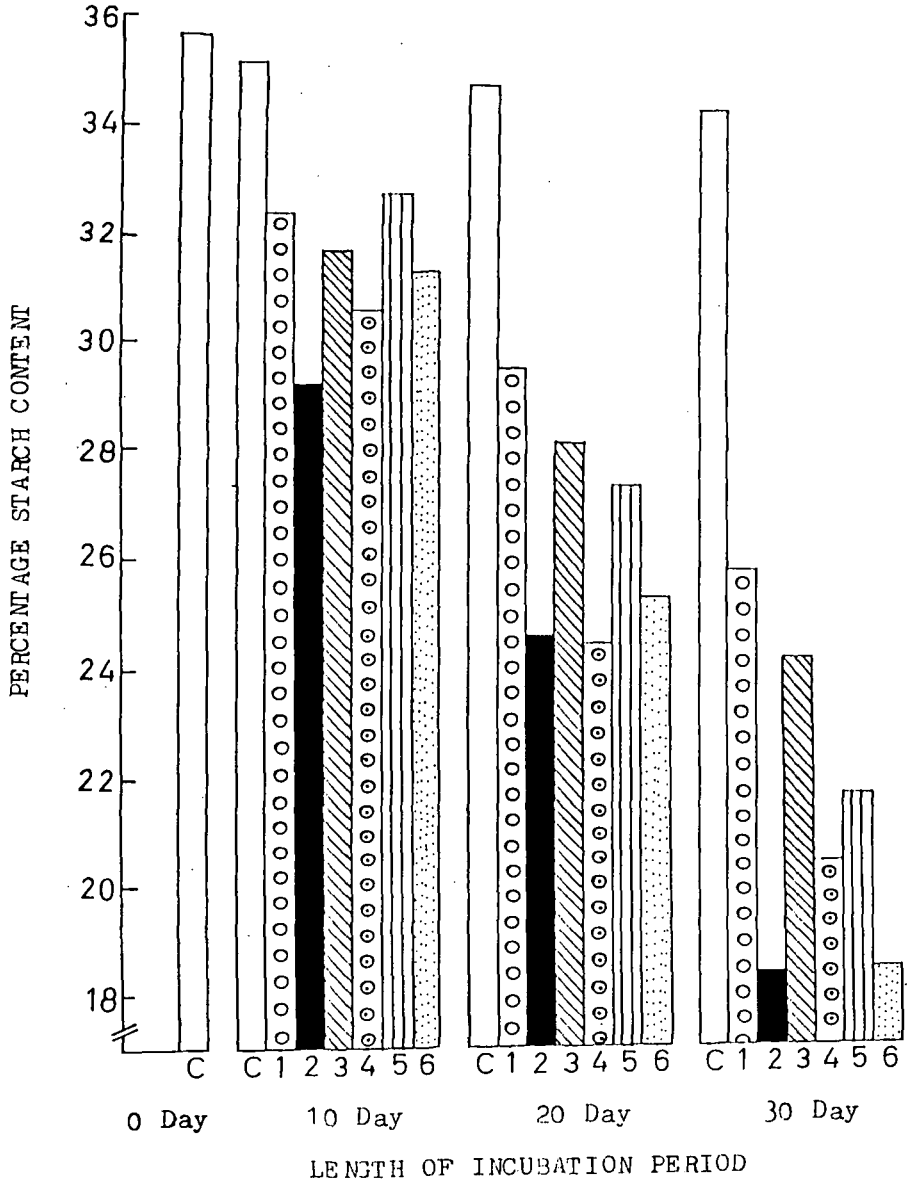


Fig. 7.8 Change in the starch contents of maize seeds due to fungal infestation. (Variety:Vijay). C- Control, 1- A.alternata, 2- A.flavus, 3-F. moniliforme, 4- P.expansum, 5- R.nigricans and 6- T.viride.

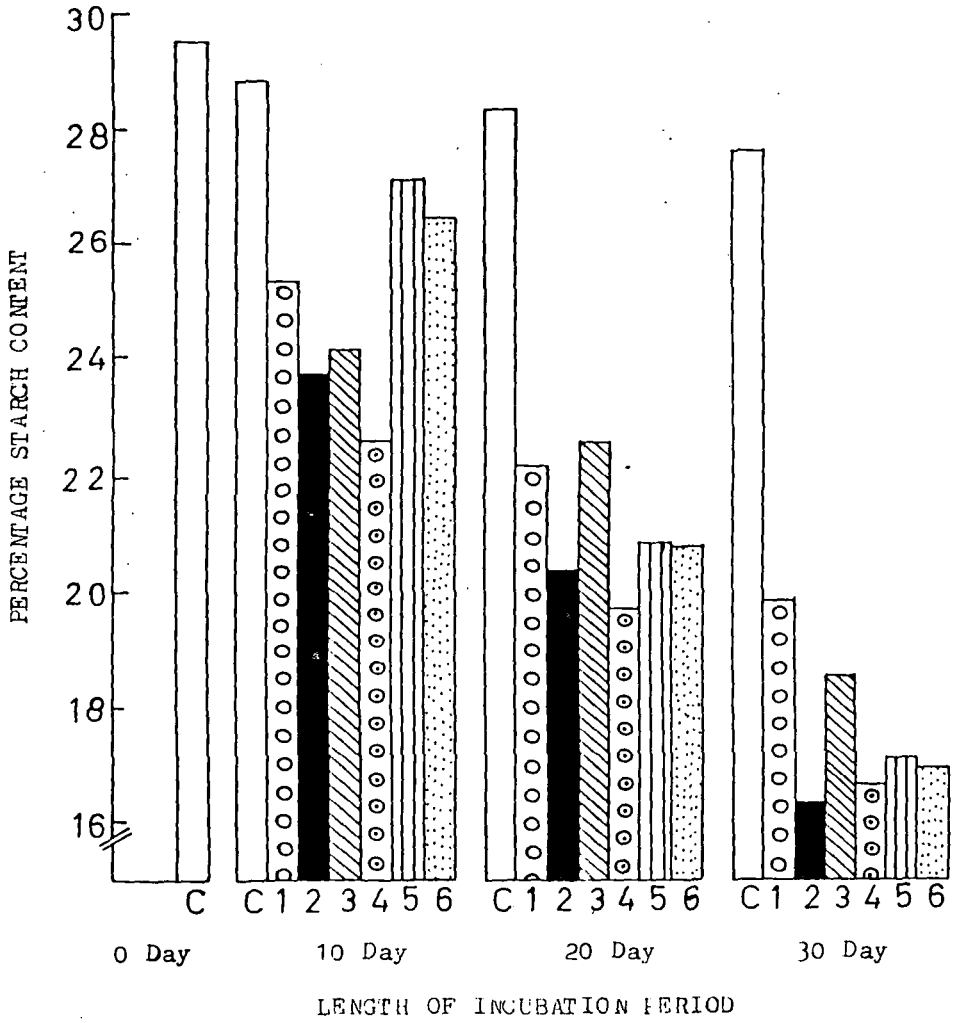


Fig. 7.9 Changes in the starch contents of maize seeds due to fungal infestation. (Variety:Vijay). C- Control, 1- A.alternata, 2- A.flavus, 3- F.moniliforme, 4- P. expansum, 5- R.nigricans and 6- T.viride.

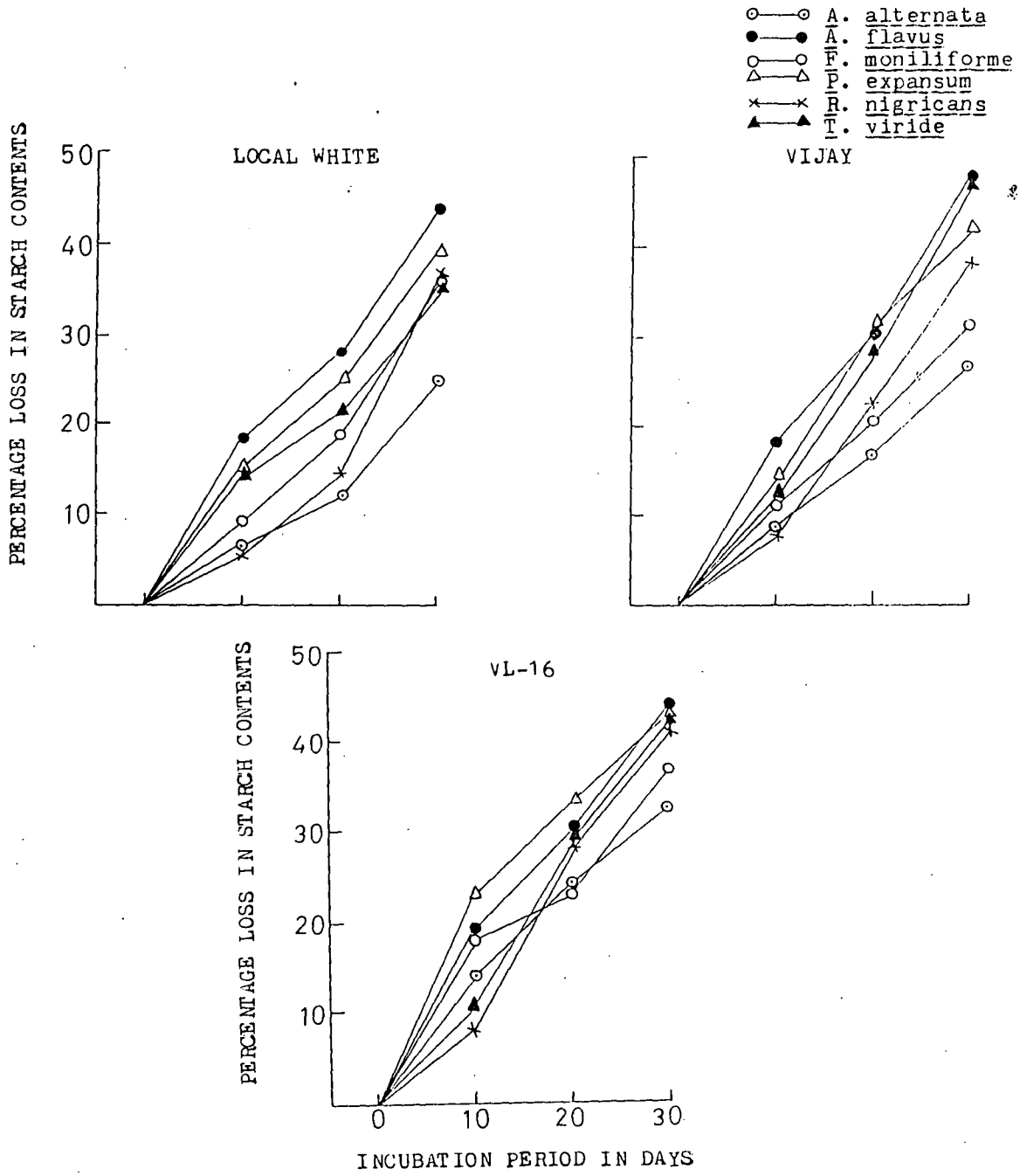


Fig. 7.10 Showing percentage loss in the starch contents of maize seeds due to fungal infestation.

fungi. After 30 days of incubation, *A. flavus*, initiated a maximum reduction of 44.7% followed by *P. expansum* (43.7%), *T. viride* (42.7%), *R. nigricans* (42.0%), *F. moniliforme* (37.2%) and *A. alternata* (32.8%). Untreated (control) seeds of the varieties of Local White, Vijay and VL-16 showed very little change in starch content with only 4%,3.6% and 6.4% depletion respectively after 30 days of incubation period.

Variations in the starch content were found to be statistically significant between the incubation periods (F=35.15**, 10.733** and 39.346** for Local White, Vijay and VL-16 respectively) but variations between the fungal species were significant only for Local White, Vijay and VL-16 (F=5.244** and 5.018** respectively) and not for Vijay (F=2.253 NS).

General Discussion

Detection of seed mycoflora was carried out using three methods of isolations, viz. blotter method, agar plate method and dilution plate method. Out of these, blotter method was found to be the most successful in detecting the fungal flora which recorded highest number of fungal species from the maize seeds. In terms of number of fungi isolated, blotter method was followed by agar plate method and dilution plate method, which is in agreement with the findings of other workers like de Tempe (1962), Agarwal *et al.* (1972), and Singh *et al.* (1973). Deep freezing method of the blotter technique was recommended by Singh *et al.* (1974a) as the most useful method for routine testing of seed health. Similar conclusion was drawn by many other workers (Lima, *et al.* 1982; Jorgensen, 1982 and Paiva *et al.* 1984). In the agar plate method certain fast growing fungi, eg. *Aspergillus* spp., *Fusarium* spp., *Pencillium* spp., *Rhizopus* spp. and some sterile mycelia dominated the plates supressing the growth of other fungi which is in agreement with the findings of Aulakh, *et al* (1976) who found that agar plate method does not give correct picture because of the chances of contamination and overgrowth by some fast growing fungi over comparatively slow growing ones. Neergaard and Saad (1962) concluded that the blotter method and the agar plate method are both valuable and supplementary to each other. Further, both agar and blotter methods have been selected as standard procedures as per International rules for seed Testing (ISTA, 1966) by International seed Testing Association after an extensive comparative seed health testing schemes. In

the present investigation too, it has been revealed that both agar plate method and blotter methods are necessary for the complete detection of seed mycoflora. The dilution plate method was found to be useful for the detection of surface mycoflora which are saprophytic in nature like species of *Alternaria, Aspergillus, Cephalosporium acremoninium, Cladosporium cladosporioides, Curvularia lunata, Drechslera maydis, Fusarium* spp., *Penicillium* spp. and *Rhizopus* spp. These are mostly field fungi whose frequency of occurrence gradually went down along with storage time.

Seasonal variations of maize seed mycoflora was observed during rainy, summer and winter periods of the year. There was a gradual fall in the number of fungal species on the seeds as the storage period increased from rainy to winter (table 1.3). Gupta, *et al.* (1983) also had earlier observed that there was a variation in the percentage incidence of fungi on mung seeds in rainy, summer and winter seasons and reported the maximum average percentage incidence of fungi in the rainy season and least in the summer. Decline in the number of fungal species during storage has also been observed by other workers (Reddy and Reddy, 1983; Shree, 1984 and Vijaylakshmi and Rao, 1985). It is evident from the tabulated results that there was a reduction in the incidence of field fungi and an increase in the storage fungi during the winter periods. The storage fungi were mainly *Aspergillus flavus, A. niger, A. ruber, A. terreus, Penicillium* spp. and *Fusarium* spp. The reduction of field fungi and increase of storage fungi during storage has also been reported by Schroeder and Sorensen (1961), Christensen (1969) and Nandi, *et*

al.(1982). Succession of seed mycoflora may be due to their different role in deteriorating the quality of seeds favoured by different seasons as the storage period increased. Bhikane and Mukadam (1982) observed variation in species content and intensity of fungal attack due to the influence of temperature and other conditions. Neergaard and Saad (1962) pointed out that storage for a few months under laboratory conditions may change the composition of the seed mycoflora considerably. As the results of the present investigation show, some of the fungal species were encountered in all the seasons of the year (table 1.3). Such fungi have wide range of nutrient requirements and have capacity of tolerance for varying environment. Those which were restricted to a particular season reflect greater sensitivity to the storage condition. Fungi of rare occurrence reflect their more demanding nature for nutrients and environmental conditions as suggested by Bilgrami, *et al.* (1979). The continuous occurrence of some species in all the seasons and reduced frequency or absence of some other species of fungi may be due to unfavourable storage conditions or lack of competitive ability (Gilman and Semeniuk, 1948, Campbell, 1962).

Qualitative variation of fungal species was observed in different varieties during different seasons of the year. Altogether 32 fungal species belonging to 16 genera were detected from the three varieties of maize seeds,of which a maximum of 30 fungal species were detected from the seeds of the variety Local White, followed by 28 from VL-16 and 26 from the variety Vijay (table 1.2). The variation in the number and type of fungi associated

with different types of seeds is well advocated. The fungal composition differed possibly due to physico-chemical nature of the seeds, agricultural operations, storage, climatological conditions of the locality during harvesting and sampling (Neergaard,1970). Vaidehi and Ramarao (1978) studied the mycoflora associated with five samples of maize seeds collected from two districts of Andhra Pradesh (India) and found that the fungal members vary from sample to sample. Of the 20 species isolated, species of *Aspergillus* and *Penicillium* were the most dominant which substantiate the present findings.

Quantitative assessment of fungal species on maize seeds revealed that species of *Alternaria, Aspergillus, Fusarium, Penicillium* and *Rhizopus* were dominant in all the three varieties of maize seeds. *Cladosporium cladosporioides, Cephalosporium acremonium, Chaetomium globosum, Curvularia lunata, Paecilomyces varioti* and *Trichoderma viride* were also recorded from all the varieties though with a less frequency of occurrence (table 1.4). Subbdaiah, *et al.* (1982) studied the seed mycoflora of maize of local and hybrid varieties from Mysore (India) and could isolate 29 fungal species representing 15 genera on 5 different cultivars. They observed that *Alternaria tenuis, Aspergillus flavus, A. niger. A. sydowii, Cephalosporium, Curvularia, Drechslera* spp., *Fusarium moniliforme, F. semitectum, Penicillium* spp. and *Trichothecium roseum* were the common fungi occurring on all the cultivars. The present investigation is in agreement with their findings. It has also been noticed in the present investigation that the percentage frequency of occurrence

of *Cephalosporium acremonium, Cladosporium cladosporioides, Curvularia lunata, Drechslera maydis, Verticillium albo-atrum* decreased along with storage period and these fungi were absent during the winter season. This explains their saprophytic nature. Such fungi are predominantly field fungi and are replaced later on by the dominant storage fungi. Similar observations were also made by Lal and Kapoor (1979) while studying the succession of fungi on wheat and maize seeds during storage. They reported that the field fungi comprising of the species of *Alternaria, Drechslera, Curvularia, Botryodiplodia, Cephalosporium, Nigrospora, Epicoccum* and *Fusarium* were abundant at the time of harvest but the frequency of occurrence declined almost to nil after five months of storage. The general decline of these fungi is due to the development of storage fungi as under the ecological conditions prevailing during storage the latter can thrive better. The tabulated results also indicate that some fungi were detected only once or rarely during the whole period of investigation. Inconspicuous seed-borne fungi, may, however, produce disease in the field ranging from scarcely detectable symptoms to quite destructive epithytotics (Neergaard, 1970) and therefore cannot be ignored.

Results from the figure 2.1 show that there was considerable reduction in spore germination of the six test fungi treated with the seed leachates of maize. Inhibitory effect of seed leachates on the fungal spore germination has been reported by Mishra and Kanaujia (1973), Chaturvedi, *et al.* (1974), Dhawale and Kodmelwar (1978) and others. The degree of inhibition exerted by the leachates of different varieties of maize seeds varied with

fungal species. Statistical analysis has revealed that the seed leachates of different varieties of maize seeds had significant effect on the spore germination of the test fungi. Kumar and Jalali (1985) also reported certain variations between resistant and susceptible varieties of chickpea in the inhibitory influence on spore germination. In the present investigation it has been found that *A.flavus* and *R.nigricans* were less effected compared to *A. alternata, F. moniliforme, P.expansum* and *T.viride*. This explains the constant association of *A. flavus* and *R. nigricans* with the maize seeds. Regarding the possible cause of reduction in spore germination of fungi due to the seed leachates, it appears that some chemical components of the leachates interfere with the spore germination. These substances have been suggested to be acting as defensive agents against seed infecting organisms (Ark and Thomson, 1958; Srivastava and Mishra, 1971; Kandaswamy, *et al.* 1974). In the present investigation, analysis of the seed leachates has shown the presence of sugars and phenols which may have some role in spore germination of the test fungi. Kraft (1973) and Jalali *et al.* (1976) reported that phenolic compounds of the seed leachates impart general resistance to parasitic and bacterial invasion. The sugar level hypothesis propounded by Horsfall and Dimond (1957) has been supported by the fact that growth regulating substances can decrease or increase the sugar contents of the host tissues and thus alter susceptibility or resistance according to expectations (Prabhu and Swaminathan, 1968). Several amino acids have also been reported to inhibit spore germination or mycelial growth of several fungi (Van Andel, 1969). Comparison of different incubation temperatures showed

that maximum spore germination occurred at 25°C for most of the fungi tested (figure 2.2). A temperature of 15°C was found to be too low and 30°C more than optimum for spore germination. Bhargava (1970) and Kapoor and Singh (1977) also indicated that optimum temperature for fungal spore germination lies between 25°C to 30°C.

It is evident from the results in tables 3.1 to 3.4 that the extract of all the test fungi had adverse effect on seed germination of maize and also on the final stand of the seedlings. Extracts of *A. flavus, F. moniliforme, P. expansum* and *T.viride* induced characteristic disease symptoms on the seedlings. Many other workers like Humphreys-Jones and Waid (1963), Christensen and Kaufman (1965), Lalithakumari *et al.* (1972), Dwivedi and Singh (1973); Dublish and Pande (1976), Rai and Singh (1977) and Pande *et al.* (1982) have also reported the importance of fungal metabolites in reducing seed germination and sprouting. In the present study it has been observed that the extracts of *A. flavus* and *T. viride* initiated first yellowing and then falling of the leaves of the maize seedlings, and extract of *F. moniliforme* caused maximum damage to the seedlings of all the varieties with stunted growth, falling of the leaves, paler and weaker seedling. Kulik and Schoen (1982) reported the role of *F. moniliforme* associated with the seeds of sweet corn which was found to be significantly correlated with field emergence. Mishra *et al.* (1969) has assigned the toxins produced by the seed-borne fungi as the possible agent for suppression of seed germination. Christensen and Kaufman (1965) and Tripathi (1974)

have reported that various fungi secreted exotoxins causing reduced seed germination and plant growth. Similar observation was made by Scott and Futrell (1970) who reported that *F. moniliforme* toxin is toxic to maize. The reduction in seed germinability as observed in the present study, may be due to some pathogenic metabolites secreted by the test fungi in the culture media (Curtis, 1958). In agreement with the present findings, Sinha and Prasad (1981) also found that the extract of mung seed-fungi had inhibitory effect on the seed germinability.

Singh and Gupta (1984) studied the effect of culture filtrates of some seed-borne fungi of *Medicago sativa L.* on the seed germination and root-shoot growth. They found *F. moniliforme, T. viride* and *T. roseum* to be severe seed-borne pathogens as they produce upto 25.1% seedling mortality. They also observed that the culture filtrate of *T.viride* caused maximum percentage inhibition of root followed by *F. moniliforme* and least by *A. flavus*. The present findings also showed similar results of reduction in average root-shoot length and dry weight of the seedlings due to fungal extract. It has been observed that the extract of *T. viride* was most effective in reducing the shoot length of the seedlings of all the varieties (tables 3.3 and 3.4). Extract of *A. alternata, A. flavus, F. moniliforme* and *T. viride* was found to be quite effective in reducing the root length of the treated samples. The maximum inhibition of seedling growth by the extract of *T. viride* may be attributed to the trichodermine secreted by the fungus which is toxic and harmful to seed germination. Singh *et al.* (1984) observed similar results when they tested the culture filtrate of

A.nidulans, Chaetomium globosum, Drechslera australiensis, Myrothecium roridum T. viride against the cucurbits. Maheswari *et al.*(1984) too reported that the culture filtrate of *A. flavus, A. fumigatus, A. nidulans, A. niger, A. terreus* and *F. moniliforme* adversely affected the seed germination, root-shoot length to varying degrees which is in agreement with the present findings. It has been observed in the present study that the extract of *A. alternata* and *A. flavus* had promotory effect on the root-shoot length of the seedlings of the variety Vijay (tables 3.3 and 3.4). Singh (1984) had also reported the increase in root-shoot length of wheat seedlings by the culture filtrate of *Penicillium chermesinum, Aspergillus amstelodemi* and *P.oxalicum.* Singh *et al.* (1986) also reported the increase in root length of soybean seedlings due to the culture filtrate of *Curvularia lunata* and *A. alternata.* Sinha and Prasad (1981) reported that culture filtrate of *A. flavus, A. niger* and *Penicillium* spp. had phytotoxic effects on all the three varieties of mung seed by reducing the percentage germination of seed and poor seedling growh.

The pronounced adverse effect of the extract of *F. moniliforme* on seed germination and root-shoot length is well advocated. Brodnik (1975) studied the toxic effect of the metabolites of *F. graminearum* and *F. moniliforme* on maize seed germination and embryo growth. Similarly other workers like Futrell and Kilgore (1969) have concluded that the mode of action of *F.moniliforme* was caused by a toxin which inhibited the root growth.

It is amply clear from the tabulated results that the fungicides used in the present investigation exhibited an inhibitory influence on spore germination and mycelial growth of the test fungi (tables 4.1 and 4.2). The inhibitory capability of the fungicides varied with fungal species, which was found to be statistically significant. It is well documented that the fungicides are toxic against fungi, however, toxicity of the fungicides varies depending on the chemical formulation, species of fungus which is being treated, and also the climatic and edaphic factors at the application site. General tendency of dose dependent decrease in spore germination was directly related with the concentration of fungicides used. Agrosan GN was found to be more effective against *P.expansum*, *R. nigricans* and *T. viride* compared to other test fungi (table 4.1a). Kumar *et al.* (1983) also had earlier reported that agrosan GN, bavistin and captan showed broad spectrum effect and eliminated all the fungi from pea seeds. Khare *et al.* (1972) observed that agrosan GN and thiram were most effective in checking the growth of the seed fungi of khesari. Bavistin was found to be effective against all the test fungi on both spore germination and mycelial growth. The efficacy of bavistin against seed-borne fungi has been reported by many workers (Grewal, 1982; Kumar and Singh, 1985). Bavistin was reported to exert total inhibition of growth and toxin production by *Aspergillus* spp. (Devi and Polasa, 1984). All these observations are in agreement with the present findings. Benomyl and blitox 50 was found to be less effective against the test fungi compared to other fungicides as observed

in the present investigation. Malhan *et al.* (1975) had also reported the ineffectiveness of benomyl against some plant pathogenic fungi, particularly *A. triticina* and *F.oxysporum f. vasinfectum.* The ineffectiveness of blitox-50 against *A. flavus* has also been reported by Bansal and Sobti (1988). In their study of comparison of four fungicides on the growth of *F. solani, Sclerotium rolfsii, A. solani, Helminthosporium oryzae, C. lunata* and *Cerospora personata.* Bagchi and Das (1968) found that blitox-50 had poor capability to check the growth of these fungi. Captan was found to be quite effective against all the test fungi particularly against *A. alternata, P.expansum* and *T.viride.* Earlier Bhowmik (1974) had also observed that captan and dithane M-45 were very effective in the inhibition of spore germination and mycelial growth of *Alternaria.* The efficacy of captan against various seed-borne fungi has been reported by many other workers (Sidhu and Cohan, 1971; Agarwal *et al.* 1972; Ellis *et al.* 1975; Raju and Lal 1977; Reddy and Subbayya, 1981; Singh and Agarwal, 1986). In the present investigation dithane M-45 was found to be very effective against almost all the test fungi except *A.alternata* (table 4.1f). Dithane M-45 was found by Sharma and Basuchoudhury (1975) to be effective in the control of many seed-borne fungi. Abou-Heilah (1984) reported that bavistin and dithane M-45 totally inhibited the growth of some fungi, while their behaviour varied with other species of fungi. Dharam Vir, *et al.* (1970) have established the superiority of dithane M-45 and ceresan for control of seed-borne diseases where infection is deep seated. During the present investigation thiram was found to be very effective against *A. alternata* and *F.moniliforme*

in both spore germination and mycelial growth. According to Burchfield (1960) compounds such as thiram, captan and dichlone are among the best to check the seed-borne fungi. Parveen and Prakash (1981) found that out of the three fungicides evaluated against *C.lunata, F. equiseti, C. oxysporum* and *A.humicola*, thiram was most effective. The efficacy of thiram against seed-borne fungi has also been reported by Siddarmaiah, *et al.* (1980). Many other workers had earlier reported the efficacy of thiram against seed-borne fungi (Sharma and Basuchoudhury, 1975; Patil, 1980; Reddy and Subbayya, 1981; Singh and Agarwal, 1986). In the present study it has been observed that the combined effect of thiram and captan was most effective against all the test fungi compared to any other individual fungicide. The superior efficacy of thiram + captan has also been reported by Jharia, *et al.* (1977) while studying the control of fungal diseases of chillies.

It has been observed in the present study that all the seed samples of the three varieties of maize, were contaminated with *A. flavus* and showed positive response to BGYF test. While screening the seeds for aflatoxin elaboration in nature, it was found that all the seed samples had the presence of aflatoxin. Aflatoxin B_1 was found to be present in all the seed samples and B_2 in only one sample, whereas aflatoxin G_1 and G_2 were not detected on the seeds under natural conditions. Devi and Polasa (1982) examined the maize samples used as poultry feed for mould isolation and toxin production. They found that 28% of the maize samples are contaminated with three mycotoxins and among these the aflatoxin B_1, B_2 and G_1 were found to be above the tolerance

level i.e. 34, 40 and 105 ppb respectively. Bhadraiah and Ramarao (1984) studied four varieties of sorghum and aflatoxin B_1 and G_1 were detected in three varieties during the kharif season. Kumari and Sinha (1987) screened 25 samples of mung seeds, out of which 15 were found to be naturally contaminated with different components of aflatoxin and the concentration of aflatoxin B_1 was upto 2800 ppb . Mishra (1977) reported 35.5% aflatoxin contamination in maize grains from terai regions of Nainital U.P. (India). Daradhiyar (1980) reported high percentage of aflatoxin in stored maize grains. There are other reports of aflatoxin contamination in pulses (Wogan, 1968; Habish, 1972; Pons, *et al.* 1972; Vora, 1978, Sinha, 1980). Natural contamination of aflatoxin is known to be greatly influenced by the environmental factors (Davis and Diener, 1970; Boller and Schroeder, 1974). Oxygen availability, temperature, moisture content, type and nature of the fungal strains as well as the substrates are known to play dominant role in determining the extend of aflatoxin elaboration in the grains (Diener and Davis, 1969; Detroy, *et al.* 1971). Bilgrami and Sinha (1983) reported the natural occurrence of aflatoxin on a wide range of agricultural and industrial comodities used as food and feed. Jones *et al.* (1981) opined that the development of aflatoxin in field corn is influenced by factors that increase plant stress, particularly during the pollination and grains filling stage of development.

While studying the aflatoxin producing potentiality of *A.flavus* on artificial liquid media it was observed that out of 22 isolates of *A.flavus* screened, 10 isolates produced aflatoxin B_1,

and B_2 and G_1 but not G_2 (table 5.2). Aflatoxin B_1 was produced in varying quantities ranging from 1.6 to 16.5 ppm. Kumari and Sinha (1987) found that out of 124 isolates of *A. flavus* from mung seed, 46 produced aflatoxin in the liquid media in varying concentration. Reddy (1987) screened 54 isolates of *A.flavus* from sorghum seeds for aflatoxin production, and found that aflatoxin B_1 was produced by 13 isolates, whereas aflatoxins B_2 and G_1 were produced by 8 and 4 isolates respectively. Subrahmanyam and Rao (1974) reported that out of 240 isolates of *A.flavus* only 72 produced aflatoxin. Of these, 16 isolates produced aflatoxin B_1 + G_1 and others produced B_1 + B_2 + G_1 + G_2.

Roy, *et al.* (1988) reported that out of 50 isolates of *A. flavus* obtained from different drug plants, 21 isolates were found to be toxigenic. Amongst these, 12 isolates had the potentiality to produce aflatoxin B_1 only, 7 had both B_1 and B_2 and only 2 produced B_1, B_2 and G_1. The aflatoxin producing potentiality of different isolates of *A.flavus* varied from 1.118 ppm to 4.85 ppm. The variation in the aflatoxin production by different isolates of *A.flavus* may be due to the variation in the toxin producing potentiality of different strains.

It is evident from the tabulated results (table 5.3) that none of the maize seed samples under study were immune to aflatoxin elaboration when artificially inoculated with a toxigenic strain of *A.flavus*, however, the amount of aflatoxin B_1 produced varied with different seed samples. Bilgrami *et al.*(1982) screened 15 varieties of maize seeds for aflatoxin production by artificial inoculation of *A.parasiticus* (NRRL-3240) and found aflatoxin B_1

production by all the varieties in varying quantities. The variation is possibly due to certain inhibitors present inside the seeds. Nagarajan and Bhat (1972) reported one maize variety to be resistant to aflatoxin production by *A.parasiticus* under artificial condition. They identified the inhibitory factors as a protein of low molecular weight. Singh and Bedi (1985) reported that none of the wheat and triticates showed immune reaction to aflatoxin production when artificially inoculated with a toxigenic strain of *A.flavus* and maximum of 4 ppm of aflatoxin B_1 was detected. Similar observation was also made by Gaur and Siradhana (1984) after studying the aflatoxin producing potentiality of *A. flavus* on maize seeds. They could detect a maximum of 12.588 µg/g of aflatoxin B_1 from the maize seed. All these report are in agreement with the present findings.

The tabulated results (tables 6.1 and 6.2) show that the antagonistic fungi could not check the occurrence of some of the harmful fungi on the seed thereby affecting seed germination and seedling growth adversely in some of the cases. The reccurrence of *F.moniliforme* and *T.viride* in all the cases and *A. flavus* and *P.expansum* in some of the cases imparted their characteristic effects on seed germination and seedling growth. Out of three antagonistic fungi (*C.acremomium, C. cladosporioides* and *T.viride*), *T.viride* caused maximum damage to the seed germination and seedling growth. Similar results have also been reported by other workers. Aulakh *et al.* (1976) reported that the presence of *A.flavus, A. niger, F. moniliforme* and *Penicillium* spp. may be the reason for low germination in maize in the field. These

pathogens are known to cause serious seed damage and seedling infection to various crops (Harris and Ellett, 1945; Tervet, 1945; De Tempe, 1964). Bulter (1947) reported the occurrence of *A.flavus, A.niger* and *F.moniliforme* in causing rotting of individual grains on cobs in maize. *Penicillium* spp. are reported to cause weakening of seedlings of maize and sometimes killing them (Soluta, 1969). Zhukova (1957) found 10-20% reduction in seed germination under field condition due to *Penicillium* spp. Schroeder (1966) reported 100% reduction in stand when seeds of maize inoculated with *P.oxalicum* were sown. The effect of *T.viride* on emergence of seedlings and the production of herbage in perennial grass was of interest as this fungus has been employed as an antagonist in the biological control of soil-borne diseases on many occasion (Wood and Tveit, 1955). However, Simmonds and Ledingham (1937) found *Trichoderma* to be pathogenic to wheat seedlings, Edwards (1940) reported its pathological effects on maize seedlings. After coating the cosmos seeds with *Cladosporium oxysporum,* Srivastava and Gupta (1981) observed that the fungus acts as a mild pathogen which caused 15-20% seed and seedling mortality. Though the seed-fungi in general imparted adverse effect on seed germination and seedling growth, few instances of promotion in seed germination and root-shoot elongation and dry weight was noticed. This may be due to the fact that the seeds in these cases were less affected and had no effect on germination and seedling growth. There are some reports which indicate that seed infection, unless severe, has little effect on germination or on the vigour of young seedlings (Warmke and Schenck, 1971). The results of the

present investigation on the influence of the antagonistic fungi used on seed germination and seedling growth are too erratic to indicate any benefit to maize plant (tables 6.1 and 6.2).

It has been observed in the present investigation that the extract of *A.alternata, A. flavus, F. moniliforme* and *P.expansum* when mixed with the extract of *T. viride* in different ratios (1:1, 1:2 and 1:5) showed better seed germination compared to the control where extract of only *T.viride* was used. Different ratios did not show any statistically significant difference in the final stand of the seedlings, neither the test fungi showed any significant variations in the final stand, thereby indicating similar results which has been obtained in the previous experiment of fungal extract and seed germination (chapter 3). The combined effect of the metabolites of the test fungi and *T.viride* on the root-shoot length and dry weight showed similar results where there were no significant variation between different ratios as well as between the test fungi except in one or two isolated cases. The results are too erratic to be recommended for the biological control of seed-borne diseases of maize.

It is clear from the tabulated results (table 6.9) that there were seven cases where the two interacting fungi in the culture media grew into one another having varying degree of intermingling zones and six cases where one interacting fungi inhibited the growth of other interacting fungi having varying degree of inhibition zones. There are several factors involved in the microbial antagonism which include pH of the media, growth

rate of the interacting fungi, competition for nutrients and space, competitive colonization potential for fungi, nature and chemical composition of growth media, ionic or nutrient imbalance caused due to nutrient impoverishment and physical factors. The occurrence of inhibition zone between the antagonist and pathogen on agar medium is commonly considered as a result of the production of antibiotics, pH changes and nutrient competition. Mechanical obstruction of the growth and hyphal interference are other main phenomena of antagonism. A mutual growth of fungi in dual cultures is possible when both the fungal species show equal growth rate, equal competition and equal capacity of tolerance to antibiotics produced by each other. The over growth is achieved when one fungal species exhibits higher growth rate, higher capacity of antibiotic production and good tolerance capacity against antibiotics produced than the other fungus (Upadhyay and Rai, 1987). In the present investigation *T.viride* was found to be the most dominant one inhibiting the growth of other fungi in the culture plate (table 6.9 and plate - I). There are many reports regarding the use of *Trichoderma* spp. as an agent of biological control. Mukhopadhyay (1987) reported that the most frequently studied fungi in relation to biological control are species of *Trichoderma*. *Trichoderma* spp. have long been known as effective antagonists against plant pathogenic fungi and is the focus of much recent research. He also opined that the mechanism of action in reducing plant disease is unknown, however, antibiosis and mycoparasitism may be involved simultaneously, at different time, or act independently as indicated by various studies in cultures *in vitro*. Knowledge of their mode of action

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could be very useful in the most effective management of the biological control system in the field.

Chemical treatment of seeds as a mean of protection against seed-borne fungi has met with considerable success in raising healthy crops (Leukel, 1948, 1953; Laxminarayan *et al.* 1966; Raju and Lal, 1977; Grewal, 1982; Raut *et al.* 1983; Pal, 1984; Mathur and Sehgal, 1964). Recent workers have been using various fungicides for the control of seed-borne fungi by treating seeds directly with the fungicides (Swarup, 1970; Basak *et al.* 1984; Bhatti *et al.* 1985; Shrotri *et al.* 1985). In the present investigation it has been observed that all the fungicides tested checked the seed-borne fungi and improved seed germination and seedling growth compared to control (tables 6.10 and 6.11). Amongst all the fungicides, thiram + captan gave best results in terms of seed-borne fungi, final stand of the seedlings, root-shoot length and dry weight of the seedlings. Jharia *et al.* (1977) also reported best results with thiram + captan for seed germination and average yield of chillies. They also found thiram, captan, dithane M-45 and benomyl to be useful in seed treatment. Srivastava and Gupta (1980) reported similar results with agrosan GN, dithane M-45 and others which reduced the seed fungi of pancy (*Viola tricolor L.*) significantly besides improving seed germinability. Kumar *et al.* (1983) reported that agrosan GN, captan and bavistin showed broad spectrum effect and eliminated all the fungi from pea seed and improved germination to the extent of 90-97% as compared to 60% obtained in untreated seeds. Singh and Agarwal (1986) observed that captan, thiram, emisan and bavistin besides improving the seed germination of pearlmillet,

increased the radical length significantly compared to control, however, they did not find significant change in plumule length from that of the control.

Table 6.12 shows the results of the experiments where seeds were treated with both the test fungi and the fungicides and it is evident that all the fungicides effectively reduced the reccurrence of the fungi on the seeds and improved the germination percentage compared to the control. In a similar type of experiment Reddy and Subbayya (1981) found that captan, thiram and benomyl checked the reccurrence of the fungi on black gram (*Phaseolus mungo* L.) seed and recorded 96-100% germination. Similar results were also obtained by Jain and Khare (1972) and Agarwal *et al.* (1972). Patil (1980) found captan, agrosan GN, thiram and bavistim to be effective in checking the reccurrence of the species of *Alternaria, Aspergillus, Fusarium, Penicillium* and improving the germination percentage of groundnut. Sidhu and Chohan (1971) reported the effectiveness of thiram and captan when applied as dry seed dresser and gave best control of *A.niger.* Thiram and agrosan GN were found to be suitable fungicides against *A.flavus, A.niger* and *Rhizopus* spp. as reported by Tarr (1958).

Several seed-borne fungi are reported to cause considerable loss in the seed contents (Goodman and Christensen, 1952; Lalithakumari *et al.* 1971; Vidhyasekaran *et al.* 1973). The spoilage of the seeds in storage become more pronounced under improper storage conditions which provide ideal environment for the rapid colonization of the seeds by storage microorganisms

(Christensen and Kaufman, 1969). In the present investigation it has been observed that there was a continuous and gradual decline in the dry weight of maize seeds due to fungal infestation in storage (figure 7.1 and 7.2). The loss in weight may be attributed to the hydrolysing effect of fungal organisms as evidenced by starch depletion as suggested by Vidhyasekaran and Govindaswamy (1968), Vidhyasekaran *et al.* (1973) and Bilgrami *et al.* (1979) on rice, Vidhyasekaran and Kandaswami (1972) on mung, Sinha and Prasad (1977) on arhar, Anahosur and Patil (1983) on sorghun.

Results from the figures 7.3 to 7.6 show that there was considerable increase in fat acidity in the seeds due to fungal infestation during the storage period. This is in support of the earlier reports of Sauer and Christensen (1968) in corn, Vidhyasekaran and Govindaswami (1968) in paddy seeds, Lalithakumari *et al.* (1971) in groundnut oil, Charya and Reddy (1981) in mung. Goodman and Christensen (1952) reported that four species of fungi isolated from mouldy corn caused an increase in fatty acids and the amount of fatty acids produced varied with the species of the fungus. They also opined that the fatty acids were a product of fungus lipase activity upon corn oil and none of the fungi caused any increase in fatty acids in the meal free of oil. In the present ivestigation also similar result was noticed where the change of fatty acid varied significantly (statistically) between different species of fungi (figure 7.3). It has been noticed in the present investigation that *A. flavus* caused maximum increase in fatty acid content of maize seeds.

This is in conformity with earlier reports of Goodman and Christensen (1952) and Maheswari *et al.* (1985). Sharma (1973) reported that out of 20 fungi tested, *A.niger* caused maximum increase in fatty acid contents of castor and cotton seeds.

Results from the figures 7.7 to 7.10 show that there was a gradual decline in the starch contents of the seeds infested with the test fungi during the storage period. Reduction in starch contents in infested tissues has earlier been reported and attributed to starch hydrolysing enzymes, beta-amylase which converts starch into simple sugars (Schipper and Mirocha, 1968, 1969b; Vidhyasekaran and Kandaswami, 1972; Harris, 1976). The present observation regarding the reduction in the starch contents gets further substantiated by the report of Allen (1942) in wheat, Microcha and Zaki (1966) in barley. In the present study *A.flavus* was found to be most effective in reducing starch contents which is in agreement with the findings of Sinha and Prasad (1977) in arhar seeds. Schipper and Mirocha (1969a) has suggested that the metabolites obtained due to starch degradation play an active role during pathogenesis by supplying nutrients for the fungal growth. Baker (1965) and Wu (1973) also gave similar opinion regarding the depletion of starch stating that starch is utilised by the fungus as respiratory substrate.

Summary

Survey of seed-borne mycoflora of maize of both local and hybrid varieties was carried out using three techniques of isolation, viz. agar plate method, blotter method and dilution plate method. A total of 32 fungal species belonging to 16 genera was isolated from the three varieties of maize seeds. Blotter method of isolation resulted to maximum number of fungal species (28) followed by agar plate method (26) and dilution plate method (20). The number of fungal species gradually got reduced as the storage period progressed. Winter period exhibited lesser number of fungal species compared to rainy and summer periods where the field fungi were found to be replaced by the storage fungi. In the varietal variation of fungal species, there was not much variation in the number and composition of fungal species. Maximum number of fungi was isolated from the variety Local White (30), followed by VL-16 (28) and Vijay (26). During the study period, species of *Aspergillus, Fusarium* and *Penicillium* were found to be the most dominant in all the varieties. The percentage frequency of occurrence of the field fungi, viz. *Cephalosporium acremonium, Cladosporium cladosporioides, Curvularia lunata, Drechslera maydis, Verticillum albo-atrum* etc. decreased along with the progress of storage time and gradually got replaced by the species of *Aspergillus, Fusarium* and *Penicillium*.

The seed leachates of the three varieties of maize were found to be inhibitory to the spore germination of six dominant maize seed fungi, viz. *Alternaria alternata, Aspergillus flavus, Fusarium*

moniliforme, Penicillium expansum, Rhizopus nigricans and *Trichoderma viride*. The degree of inhibition varied with different fungi and with different varieties of maize. *F. moniliforme* and *P. expansum* were affected most followed by *T. viride, R. nigricans, A. flavus* and *A. alternata*. Chemical analysis of the seed leachates showed the presence of five sugars and five phenols which are supposed to have some role in the change of spore germination.

The extract of the six test fungi, viz. *A. alternata, A. flavus, F. moniliforme, P. expansum, R. nigricans* and *T. viride* caused reduction in the percentage seed germination, average shoot-root length and dry weight of almost all the maize seedlings. Extracts of *A. flavus, F. moniliforme, P. expansum* and *T. viride* induced characteristic disease symptoms with paler and and weaker seedlings having stunted growth of the seedlings of all the varieties of maize. The extracts of *F. moniliforme* and *T. viride* proved to be the most harmful compared to other fungi where there was maximum decrease in shoot-root length and dry weight of the seedlings. The pathogenic influence of the fungal extract was found to be almost similar in both the test tube seedling symptom test and the pot culture experiment.

The six dominant seed-borne fungi of maize, viz. *A. alternata, A. flavus, F. moniliforme, P. expansum, R. nigricans* and *T. viride* were tested against 8 different agrochemicals, viz. agrosan GN, bavistin, benomyl, blitox-50, captan, dithane M-45, thiram and thiram + captan for their spore germination and mycelial growth. All the 8 fungicides tested were found to be effective in the

inhibition of spore germination and mycelial growth of all the test fungi, however, the inhibitory capability of the fungicides varied with fungal species. In general, maximum inhibition of spore germination and mycelial growth was noticed at the concentrations of 2500 ppm and 5000 ppm of all the fungicides, though there were some instances of 100 percent inhibition of spore germination and mycelial growth at the concentrations of 500 ppm and 1000 ppm. In both the experiments of spore germination and mycelial growth, thiram + captan proved to be the most effective one against all the test fungi.

All the three varieties of maize seeds screened were found to be contaminated with *A. flavus* to varying degree. All the seed samples were positive to BGYF test and aflatoxin B_1 was detected from the seed samples of all the varieties in natural conditions. Out of 22 isolates of *A. flavus* isolated from different seed samples of maize, 10 were found to be toxigenic and yielded aflatoxin B_1, B_2 and G_1 in the synthetic liquid medium. The samples of all the varieties of maize under study were potential producers of aflatoxin when artificially inoculated with a toxigenic strain of *A. flavus*. The types of aflatoxin produced and the amount of aflatoxin B_1 elaborated varied with different varieties of maize seeds.

Seed samples coated with the three saprophytic antagonistic fungi, viz. *Cephalosporium acremonium*, *Cladosporium cladosporioides* and *Trichoderma viride* had some effect on the occurrence of some of the common seed-borne fungi of maize but, in general, failed to improve the seed germination and seedling

growth. The combined effect of the metabolites of *T. viride* with those of *A. alternata, A. flavus, F. moniliforme* and *P. expansum* in differnt ratios (1:1, 1:2 and 1:5) proved to be not effective in the improvement of seed germination and seedling growth of maize. Colony interactions of some of the important seed-borne fungi of maize showed *T. viride* to be the strongest antagonistic fungus which could inhibit the growth of other test fungi in the dual cultures. *A. flavus* was also found to be antagonistic to *C. acremonium* and *C. cladosporioides* having prominent inhibition zone of 0.90 cm and 1.00 cm respectively. Cultures with intermingled growth were also noticed with a maximum intermingled zone of 1.50 cm in the dual cultures of *F. moniliforme* and *T. viride*.

The 8 agrochemicals used in one of the previous experiments were also used as seed dressers and it has been found that all the fungicides improved the seed germination and seedling growth of maize. Shoot-root length and dry weight of the seedlings were promoted in almost all the cases. When the seeds were treated with both the fungi and fungicides, there was drastic reduction in the percentage occurrence of the fungi on the seeds in all the cases and germination percentage of the treated seeds increased compared to the control.

The relative capacity of the six dominant seed-borne fungi of maize, viz. *A. alternata, A. flavus, F. moniliforme, P. expansum, R. nigricans* and *T. viride* in deteriorating the nutritive value of maize seeds in terms of dry weight, fatty acid and starch contents was determined. All the test fungi were found to reduce

the dry weight of the seeds of all the varieties with *A. flavus* recording the maximum reduction of 17.13% in the variety Local White, 17.23% in Vijay and 15.94% in VL-16 after 30 days of incubation. There was a gradual increase in the fatty acid content of the seeds in all the cases with a maximum increase of 53.5% and 69.2% due to the infestation by *A. flavus* in the variety Local White and Vijay respectively, but *P. expansum* was responsible for the maximum increase of 74.2% in the variety VL-16. Substantial reduction in the starch contents of the seeds due to fungal infestation was noted and the extent of starch depletion varied with fungal species and maize varieties. In this case also *A. flavus* proved to be the most effective fungus in the depletion of starch in all the three varieties with 44.1%, 48.5% and 44.7% in the varieties of Local White, Vijay and VL-16 respectively.

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* Original not seen.