INTRODUCTION

Seed is the basic unit in crop production technology. It plays an important role in the healthy crop production. The seeds carry a heavy load of microorganisms which are capable of causing severe diseases and considerable loss of the yield. These microorganisms enter into the seedcoat; cotyledons and embryonic parts of the seed from field and also proliferate the infection during the ill storage condition. Microorganisms with the seeds cause several types of abnormalities like seed damage, reduction in germination, seed-discoluration, seed-biodeterioration, seed-poisioning etc. Such infected seeds have poor in quality for consumption as well as seed industry.

It is revealed from the literature that fungi associated with the seeds utilize seed contents and produce toxins in the seeds. Such seeds are known as biodeteriorated seeds. Such seeds are also carry diseases to the next generation. In order to control such diseases it is very important to observe the seed health condition which includes percent seed germiability and percent seed mycoflora. Workers in the field of pathology have recommended various types of chemical treatments for the control of seed-borne diseases. It is also observed that use of deadly poisonous chemicals has been found to create many side effects on environment, soil and health of the life on the earth. Now a days a new approach to control the seed-borne fungi is being developed by using biopesticides. Such type of disease
management is becoming more popular and eco-friendly. Considering the importance of the fact the present topic ‘Studies on biocontrol of seed-borne pathogens of some crops’ has been selected for the research.

The first part of research is fully concentrated on the collection of seed samples from the field, market places, and store houses from different districts of Marathwada region of the Maharashtra state. These seeds were employed to detect the external and internal seed-borne fungi associated with the seed by using standard blotter paper method and agar plate method as recommended by ISTA (1966).

A comparative account of seed mycoflora of naturally abnormal non-treated seeds with treated seeds by surface sterilizer (HgCl₂) and seed treated with neem leaf extracts were studied in detail. Seed categorically representing cereals like jowar (Sorghum vulgare L.) and Bajra (Pennisetum americanum (L.) K. Schum.) legumes like pea (Pisum sativum L.) and Gram (Cicer arietinum L.), oil seeds like Groundnut (Arachis hypogaea L.) and Safflower (Carthamus tinctorius L.), vegetable seeds like Tomato (Lycopersiccom esculentum Mill.), Brinjal (Solanum melongena L.), spices like Dhania (Coriandrum sativum L.) and Jeera (Cuminum cyminum L.) showed significant variation in qualitative and quantitative seed mycoflora. The seeds treated with neem leaf extracts in case of vegetable seeds and seeds of spices found to be very promising to control the seed-borne fungi.

Among the total seed mycoflora of the crops studied for isolation dominant fungi namely Alternaria alternata, Aspergillus flavus, Curvularia
lunata, Fusarium roseum, Penicillium notatum were frequently occurred on almost all types of seeds. Hence for further studies these five fungi were used.

A large number of Angiospermic plants were screened to know their antifungal activities. Plants with different groups, family, characters and active principles were employed for screening with the dominant seed-borne fungi. It was interesting to note that out of the 151 plants screened, plants like Azadirachta indica A. Juss., Aegle marmelos (L.) Corr., Datura stramonium L., Jatropha curcas L., Lantana camera L., Ocimum sanctum L., Polyalthia longifolia (Sonn.) Thw., Tridex procumbens L., Catharanthus roseus (L.) G. Don., Vitex negundo L., showed very promising results to check the growth of fungi. However, Azadirachta indica A. Juss. leaf extract appears to be most effective followed by aqueous leaf extract of Datura stramonium and Ocimum sanctum against growth of fungi. Effect of different plant part like stem, flower, seeds, roots were also studied. However the results showed that aqueous neem leaf extract at 10 gm / 100 ml concentration was considered to be an ideal to use as a biopesticide.

In order to understand the nutritional influence of the chemicals on these pathogens the studies were carried out on growth and sporulation of the fungi under the influence of various carbon sources, nitrogen sources, phosphorus sources and sulphur sources. The results would be highly helpful in order to predict the pathogenicity; degree of the pathogens and
nutritional values of the seeds. These results were found to be highly promising on this aspect.

Similarly, effect of antibiotics and fungicides which are traditionally used by the farmers are also studied. The degree of inhibition of pathogens with the use of these chemicals was compared with the degree of inhibition that we have studied by using biocontrol agents in the present research work.

The second part of research work is on toxin production. Out of the total botanicals screened for their ability against fungal growth. Ten botanicals are further studied for their inhibitory activity against the five fungi. Leaf extracts of botanicals namely *Aegle marmelos* (L.) Corr., *Azadirachta indica* A. Juss., *Datura stramonium* L., *Jatropha curcas* L., *Lantana camera* L., *Ocimum sanctum* L., *Polyalthia longifolia* (Sonn.) Thw., *Tridex procumbens* L., *Catharanthus roseus* (L.) G. Don. and *Vitex negundo* L, were studied against the test fungi. Test fungi growing in the presence of botanicals produced comparative less amount of toxin in the culture media. When these culture filtrates were used to study the degree of toxin production in case of the fungi, it was observed that in the presence of some botanicals degree of toxin production by these fungi was found to be reduced considerably. Experiments regarding the effect of toxin on percent seed germination, effect on growth of root and shoot has been worked out in the present investigation.
The role of hydrolytic enzymes produced by pathogens has been stated in the degrading of the storage chemicals of the seeds. Therefore the studies on leaf extracts of different plants on the production of hydrolytic enzymes of the pathogens were carried out. It was observed that the plant extracts were found to be significantly useful.

While studying the biological control against commonly occurring seed-borne fungi it was essential to pinpoint the actual plant part and active principal content in it and how it is inhibitory to the fungi, effect of different plant parts were studied extensively. Effect of leaf extract, stem extract, flower extract, seed extract, root extract were employed against growth of fungi and the results are very much promising. Effect of plant products was also used as biological control. Along with higher plants, plants from lower group like Algae, Bacteria were also studied for their inhibitory activity. Presently *Trichoderma* is commonly used in Agricultural management to control the parasitic and non-parasitic fungi. Hence it was also studied in detailed to know the antagonistic activity of different species of *Trichoderma* with the test fungi. Botanicals can inhibit the growth of fungi. But to control totally systematic fungicides along with botanicals are strongly recommended. Uses of less degree of fungicides and increased doses of biopesticides will be the suggestive measures to the farmers to control seed-borne fungi.
REVIEW OF LITERATURE

Seeds are the important carriers of plant pathogens, like fungi, bacteria, viruses, mycoplasma and insects etc. It is observed from the literature that microbes during their association with seeds both in the field as well as in the storage cause different abnormalities in the seeds (Neergaard, 1977). The most prominent abnormalities in the seeds are seed rot, necrosis, discoloration, seed coat cracks, and loss in germinability have been reported by most of the workers. Similarly, associated microorganisms with the seeds utilise and reduce the storage chemicals and toxify the nutrients present in the seeds.

It is clear from the studies on seed pathology carried out in the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad that seeds of the crops studied, Jowar (Panchal, 1984), Legumes (Bhikane, 1988), oil seeds (Sandikar, 1990), Pea (Sonavane, 2000) and vegetable seeds (Bharaswadkar, 2003), that number of fungi occur on these seeds prominently. The abnormal seeds are considered to be poor in quality for consumption purposes as well as for seed industries.

SEED MYCOFLORA OF DIFFERENT CROPS

A) Cereals

1) Jowar (*Sorghum vulgare* pers.)

The first systematic work on seed health testing of jowar has been made by Leukel and Martin (1943). They have reported several fungi
associated with abnormal seeds. Similarly Basuchaudary (1973) isolated ten fungi and three actinomycetes by blotter test in which *Drechslera rostrata*, *D. turcica*, *Alternaria tenuis*, *Fusarium moniliforme* were in maximum counts. Castor (1977) observed blacken jowar grains in the field due to *Curvularia lunata*. Similarly Williams and Rao (1980) observed that pinkish discoloured jowar grains show *Fusarium moniliforme* and *F. semitectum* and blackish grains showed *Curvularia lunata* with *Phoma sorghima* on the seed surface. Panchal (1984) isolated species of *Alternaria*, *Curvularia*, *Aspergillus*, *Dreschsleria* and *Penicillium*, which caused mix type of discolouration to the five local varities cultivated in Marathwada region.


2) Wheat (*Triticum aestivum* L.)

Kietreiber (1972) reported wheat seeds in Canada were found to be infected dominantly by *Drechslera sorokiniana* which caused dark brownish spots at the pointed end of the seeds. He also observed that the
seeds were severely infected with *Alternaria tenuis* at milky stage about a month before harvest while seed discolouration took place later at yellow ripening stage of the crop. Shashi and Kappor (1979) recorded that *Fusarium moniliforme* was found to be predominant on the seeds of different wheat varieties at the time of harvest.

3) **Bajra (Pennisetum typhoides Burm)**

Young *et. al.*, (1947) reported *Helminthosporium rostrata* as a dominating seed-borne pathogen. While Rao and Saleem (1954) reported *Curvularia* sp. as a dominating fungus. Whereas, Mathur *et. al.*, (1960) isolated *Curvularia peniseti* and *C. lunata* from abnormal discoloured seeds of bajra from the field. Fance and Patil (1971) recorded the presence of *Aspergillus flavus*, *Chaetomium globosum* and *Trichothecium roseum*. While Sharma and Basuchoudhary (1975) have identified 15 fungal species namely, *Aspergillus flavus*, *A. niger*, *A. nidulans*, *Alternaria alternata*, *Ascotricha* sp., *Cephalosporium acremonium*, *Chaetomium* sp., *Curvularia geniculata*, *C. lunata*, *Drechslera rostrata*, *F. equiseti*, *Penicillium spinulosum* and *Rhizopus stoniifer* isolated from bajra seeds. Mathur (1979) recorded maximum counts of *Alternaria alternata*, *Dreschlera specifer*, *F. fusarioides* and *Curvularia penniseti* from abnormal seeds of bajra. Recently, Bodke and Wadje (2004), reported 18 fungi associated with grains of bajra local varieties.
4) **Rice (Oryza pativa L.)**

The first ever recorded report on seed discoloration in rice was by Hemmi *et al.*, (1931). They found prominent reddish pigmentation in the seed coat infected with *Fusarium moniliforme*. Whereas Fazli and Schroeder (1966) observed a typical reddish brown, oval spots on seed coat of rice infected with the pathogenic fungus, *Helminthosporium oryzae*. The fungus was reported to remain in seed coat and endosperm of the infected and discoloured seeds. Similarly, susceptible rice varieties have been found to be attacked by number of pathogenic as well as saprophytic moulds.

Similarly Waghmare *et al.*, (1988) studied seed mycoflora in random samples of flood affected paddy, collected from both standing crop and threshing floors in Nellore and isolated *A. flavus, A. fumigatus, A. nidulans, A. niger, Chaetomium globosum, Cladosporium herbarum, Curvularia lunata, Drechslera oryzae, Fusarium oxysporum, Penicillium digitatum, P. pyrporogenum, P. tardum, Rhizopus stolonifer, Sarocladium oryzae, Trichoconis padwickii.*

5) **Maize (Zea mays L.)**

Dhanraj and Mathur (1965) in their studies found that infection of maize cobs with *Cephalosporium acremonium* had resulted into whitish, mycelial growth streaks on the growing seeds in the cob. Whereas Summer (1966) reported presence of *F. moniliforme* and provided evidence in favour of disease transmission from seed to seedling. Prasad and Pathak (1987) found *Fusarium semitectum, F. oxysporum* and other seed-borne fungi with
maize seeds. Whereas Ravishankar et. al., (2002) reported different fungi such as *Verticillium alboatrum*, *Trichoderma harzianum*, *Sclerotium rolfsii*, *Botryodiplodia* sp., *Fusarium moniliforme* etc., on maize seeds.

B) Pulses

1) Gram (*Cicer arietinum* L.)

Seed-borne pathogens from gram seeds were studied by different workers. Suhag (1973) recorded presence of *Alternaria tenuis*, *Aspergillus niger*, *Cladosporium fulvum*, *Fusarium* sp., *Curvularia lunata*, *Rhizoctonia bataticola*, *Rhizopus nigricans* and *Verticillium* sp. Jamil Khan et. al., (1974) reported *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium moniliforme* and *F. semitectum* on gram seeds from Pakistan. Whereas Iqbal Singh and Chohan (1975) noted association of pathogens such as *Cladosporium cladosporiodes*, *Curvularia clavata*, *Penicillium cyclopium*, *Fusarium equiseti*, *F. moniliforme*, *F. semitectum*, *Pleospora infectoria*, *Rhizopus arrhizus* and *Trichothecium* species. Similarly, Haware and Rajeshwari (1978) recorded presence of *Fusarium oxysporum* f. sp. *Ciceri* from the seeds of different cultivars of Gram. While Kamal and Verma (1978) reported 16 fungi from gram seeds of Var. T21, and maximum counts were of species of *Alternaria*, *Aspergillus* and *Trichoderma*. Similarly, Singh et. al., (1981) showed that the presence of *Alternaria tenuis*, *Aspergillus flavus*, *A. nidulans*, *A. niger*, *Helminthosporium tetramera* and *Rhizopus arrhizus* on abnormal gram seeds. Whereas, Kumhar et. al., (1987) isolated 11 seed-borne fungi from stored abnormal

2) **Green gram (Phaseolus aureus L.)**

Seed-borne fungi which are associated with these seeds were studied by several workers. Ramnath *et. al.*, (1970) noted presence of necrotic type of discolouration as well as the presence of *Fusarium semitectum* on the abnormal green gram seeds. Agrawal *et. al.*, (1972) isolated different fungi such as *Alternaria longissima*, *Cercospora kikuchii*, *Chaetomium* species, *Colletotrichum lindenmuthianum*, *C. truncatum*, *Curvularia lunata*, *Drechslera tetramera*, *Fusarium equiseti*, *F. moniliforme*, *F. semitectum*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Melanospora* sp., *Periconia* sp. and *phoma* species. Similarly Suhag (1973) noted the association of seed-borne fungi *Alternaria tenuis*, *Aspergillus nidulans*, *Cladosporium fulvum*, *Curvularia lunata*, *Fusarium* sp., *Rhizoctonia bataticola*, *Rhizopus nigricans* from the green gram seeds. Nik (1983) reported 15 fungi from abnormal moong seeds. Similarly, Bikane (1988) has recorded presence of fungi like *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Chaetomium globosum*, *Cladosporium herbarum*, *Curvularia lunata*, *C. pallescens*, *Drechslera tetramera*, *Fusarium moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Phoma exigua*, *Rhizoctonia solani* and *Rhizopus stolonifer* from green gram seeds.
Recently, Gachande (2001) isolated *Aspergillus flavus*, *A. niger*, *Dreschlera tetramera*, *F. moniliforme*, *F. roseum*, *Macrophomina phaseolina* and *Rhizoctonia solani* from mung seeds.

**3) Soybean (Glycine max L.)**

It is the most important source of plant proteins in the human diet. Singh *et. al.*, (1973) isolated species of *Aspergillus*, *Alternaria*, *Rhizoctonia*, *Fusarium*, *Phoma* and *Chaetomium* from abnormal soybean seeds. Similarly, Sundaresh and Hiremate (1978) isolated species of *Aspergillus*, *Curvularia*, *Cladosporium*, *Fusarium*, *Penicillium*, *Rhizoctonia* and *Alternaria* from soybean seeds. Ellis *et. al.*, (1979) reported the dominance of *Phoma* sp., *Sclerotinum rolfsii*, *Lasiodiplodia*, *Colletotrichum dematium*, *Macrophomina phaseolina* and *Cephalosporium gregatum* from abnormal soybean seeds. Similarly, Agarwal (1981) recorded the 19 fungi like species of *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Helminthosporium*, *Macrophomina*, *Monilia*, *Penicillium*, *Phoma*, *Rhizopus*, *Trichoderma* and *Verticillium*. While, Kheterpal and Lambat (1981) showed the association of *Fusarium*, *Peronospora manohurica*, *Ascochyta soieda*, *Colletotrichum dematum*, *Myrothecium verrucaria*, *Phomopsis soyae* and *Rhizoctonia solani*, from soybean germplasm imported from U.S.A. (America). While, Muthe Gowda and Sullia (1987) recorded the sp. of *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Macrophomina*, *Mucor*, *Penicillium syncephalastrum* and *Trichothecium*, from abnormal seeds of soybean. Tribhuwan Singh (1992) isolated seed-
borne pathogens from seeds of soybean grown in Rajasthan and recorded the 65 fungal species which are saprophytic and pathogenic one. While Tripathi and Singh (1993) studied the association of 16 fungi from soybean seeds. Whereas, Thippeswamy et. al., (2002) reported the relationship between essential oil treatment on seed mycoflora and seedling, quantity of some oil seed crop sp. Recently, Sah and Patil (2004) studied the presence of seed-borne fungi on soybean seeds such as *Fusarium oxysporum*, *Alternaria tenuis* and *Penicillium digitatum* are most common pathogens.

**4) Pea (Pisum sativum L.)**

Pea is one of the important crops commonly used as vegetable, which have also been reported the association of seed mycoflora. Which was studied by different workers. Haware (1971) studied the seed mycoflora of 5 varieties of pea with species of *Aspergillus*, *Rhizopus*, *Mucor* and *Fusarium* are dominantly on the pea seeds. Sawhney and Aulakh (1980) isolated the seed-borne pathogens from abnormal pea seeds and these are *Alternaria alternata*, *A. longissima*, *Ascochyta pisi*, *Aspergillus flavus*, *A. niger*, *Cephalosporium* sp., *Cladosporium cladosporioides*, *Curvularia lunata*, *C. pallescens*, *Drechslera hawaiensis*, *D. tetramera*, *Fusarium moniliforme*, *Penicillium* sp., *Rhizopus* sp. and *Trichoderma roseum* etc. Whereas, Rupinder et al.,(1992) studied the fungi like species of *Fusarium*, *Rhizoctonia bataticola*, *Phoma* sp., *Alternaria alternata*, *Aspergillus flavus*, *A. niger* from abnormal seeds of pea.
Recently, Sharma et al., (2004) isolated 17 species of microorganisms which are pathogenic in nature from abnormal pea seeds.

c) Oil seeds

1) Sunflower (*Helianthus annus* L.)

Oil seeds are highly economically important crops. Mukewar and Sen (1979) observed the incidence of *Aspergillus niger*, *Penicillium* sp., *Stachyobotrys* sp., *Alternaria alternata*, *A. zinnae*, *Verticillium alboatrum* and *Phoma exique*. Shrotri et. al., (1983) also reported 35 seed-borne pathogens. Vijayalaxmi and Rao (1985) detected 38 moulds and Roberts et. al., (1986) observed the 98 fungal species from different varieties of sunflower seeds. While Ataga and Akyeshic (1986) showed the maximum number of fungi like *Alternaria tenuis*, *Curvularia lunata*, *Fusarium moniliforme* and *M. phaseolina* from sunflower seeds. Zad (1990) studied the association of 20 seed-borne fungal species from sunflower seeds. Whereas Shahanz and Gaffer (1991) reported the incidence of fungi such as *Aspergillus flavus*, *A. niger*, *Alternaria alternata*, *Rhizoctonia solani*, *Fusarium moniliforme*, *F. solani*, *F. semitectum* and *Cochilobolus specifer* on sunflower seeds from Pakistan. Recently Umatale (1995) studied the seed mycoflora from different varieties (LS11, 21, MH-9, MSFH-8, KBSH-1, E-48414, SS-56, LDMRSH-1, Morden) which are cultivated in Marathwada region.
2) **Groundnut (Arachis hypogea L.)**

The seed mycoflora of groundnut have been studied by several workers. Welty and Cooper (1967) recorded an unidentified species of *Fusarium* from stored groundnut abnormal seeds. Gupta and Chauhan (1970) detected the moulds such as *Aspergillus niger*, *A. fumigatus*, *M. Phaseolina*, *Fusarium oxysporum*, *Rhizopus arrhizus*, *Neocosmospora vasinpect*, *Paecilomyces varioti*, *Alternaria tenuis*, *Penicillium* sp. and *Curvularia* sp. are the maximum count. While, Anonymous (1973) found that the *Macrophomina phaseolina* on seeds from infected pods showed seed discolouration. Singh and Ghewande (1980) detected the moulds like *Aspergillus niger*, *Macrophomina phaseolina*, *Sclerotium rolfsii* from seeds of groundnut. Chauhan and Mall (1981) isolated seed mycoflora from the discoloured seeds. And they stated that the major role in seed discoloration was found to be due to the association of *Aspergillus flavus*.

Abnormal seed mycoflora was studied by Sobti and Sharma (1988) and they isolated *Aspergillus flavus*, *A. sydowii*, *A. niger*, *A. wentii*, *Macrophomina phaseolina*, *Trichothecium roseum*, *Fusarium* sp. and *Actinocnucor elegans* are the maximum count. Similarly, Ramkrishna and Kolte (1988) showed the association of fungi like *Rhizoctonia solani*, *Sclerotium rolfsii*, *Trichoderma pseudokoningi* and *Fusarium semitectum* from abnormal seeds. Whereas, Chavan and Danai (1993) showed the presence of species of *Aspergillus*, *Macrophomina*, *Fusarium*, *Trichoderma* and *Spicaria* from abnormal seeds. Recently, Umatale (1995) showed an
incidence of fungi from different varieties (LGN-1, LGN04, LGN-5, IGCS-11, SB-11) of groundnut seeds.

3) Sesame (*Sesamum indicum* L.)

It is most important oil seed crop, utilised both for medicinal and edible purposes. Seed mycoflora of sesame were studied by several workers. Choudhary (1945) isolated the *Cercospora sesami* from the sesame seeds. Later on Stone and Jones (1960) recorded *Corynespore cassicola* from the seeds. Similarly, Meiri and Sobel (1963) studied the association of fungi like *M. phaseolina* which is dominant. Similarly, Noble and Richardson (1968) identified 7 fungi. Mishra and Kanujia (1973) detected the *F. nivale* and unidentified *Fusarium* species from sesame abnormal seeds. Similarly, Verma and Daftri (1974) showed the presence of *M. phaseoli*. Mathur and Kabeera (1975) observed the maximum count of *Alternaria sesami* from the seeds of Uganda. Similarly, Kushi and Khare (1979) showed the presence of fungi like *Macrophomina phaseolina*, *Corynespora cassicola*, *Alternaria sesami*, *Fusarium oxysporum*, *F. cauiseti* and species of *Phoma*, *Cephalosporium*, *Aspergillus*, *Penicillium*, *Memonoiella*, *Rhizopus*, *Haplo sporangium*, *Chaetomium*, *Curvularia* and *Botrytis*. Whereas, Singh and Ghewande (1980) isolated the dominant fungi like *Phytophthora parasitica* var. *Sesami*, *Macrophomina phaseolina*, *Alternaria* sp. from the abnormal sesame seeds/capsules/cotyledons. Similarly, Jani and Siddiqui (1981) recorded 17 fungal species from sesame seeds. Lateron Yu (1981) found the pathogenic moulds from
sesamum seeds from Korea. Whereas, Vaidehi and Lalitha (1985) detected 27 fungi from abnormal sesame seeds. Whereas, Wu (1988) reported 10 seed moulds, from sesame seeds from Taiwan.

4) Mustard (*Brassica compestris var. Sarson*) Prain:

It is cultivated as oil seed crop in Assam, Haryana, Punjab, U.P., Bihar and West Bengal. It is also grown in Marathwada region of Maharashtra state. Singh and Ghewande (1980) detected the seed-borne pathogens from the abnormal seeds with dominant fungus was *Alternaria brassicae*. Vishnuvat *et. al.*, (1985) found that the abnormality, gray discolouration and shrivelled appearance of seeds due to the presence of *Alternaria brassicae*. While Jain *et. al.*, (1982) isolated the seed mycoflora of abnormal mustard seeds with dominant fungi like *Alternaria brassicae, Aspergillus flavus, Drechslera austreliansis, Fusarium oxysporum, F. roseum, Myrothecium roridum* and species of *Cladosporium, Penicillium* and *Phoma* etc. Whereas Sinha *et. al.*, (1988) observed the different samples of *Brassica campestris* Linn and *B. juncea* Coss. from different parts of Bihar. And they obtained 96 and 85 isolates of *A. flavus*, from these mustard seeds respectively.

Gupta and Basuchaudhary (1994) studied range of infection of seed-borne fungi from different seed samples of Mustard seeds, and they found that *Alternaria alternata* is the most common pathogen along with the presence of *Alternaria brassicicola, Alternaria sp., Aspergillus flavus,*
Cladosporium, Cladosporioides, Curvularia lunata, Fusarium sp., Penicillium sp., Rhizopus sp., (Ulocladium consortiales)

5) Castor (Ricinus communis L.)

Seed mycoflora of castor was studied as early as in 1948 by Singh indicating that Aspergillus niger as seed-borne in nature. Lateron Jain and Patel (1968) published a detail account of their studies on seed-borne fungi of castor. They found species of Alternaria, Stachybotrya, Aspergillus, Fusarium, Cladosporium, Rhizopus, Penicillium, Chaetomium, Nigrospora, Helminthosporium, Curvularia, Hormiscium, Stemphyllum and Mammoniella to be associated with the seeds of castor.

E) Vegetables

1) Brinjal (Solanum melongena L.)

Brinjal or Egg plant is the most common vegetables in South East Asian countries including in India, Bangladesh, China and Japan. Fruit and seed shows several pathogens which causes damages to the fruits. Kadow (1934) found that the severe damage in the production of brinjal was due to Verticillium wilt transmitted through seeds. Porter (1943) isolated Phoma vexans from infected fruits of brinjal.

Bujdoso and Szurkej (1977) noted that Verticillium dahliae, Alternaria solani, Colletotrichum altramentarium are the seed borne pathogens on brinjal seeds. While, Choudhary and Hasija (1979) observed the soft rot of brinjal due to Phomopsis vexans. Similarly, Vidyasekaran et al., (1980) found that Aspergillus flavus, A. niger, A. glaucus, Fusarium
moniliforme and Helminthosporium tetramera are the seed borne pathogens of abnormal seeds of brinjal. Datar and Ashtaputre (1984) recorded the Phoma vexans, causes the phomopsis blight of different brinjal varieties and Solanum species. Whereas, Tyagi and Chauhan (1985) showed an infection of brinjal seeds by Alternaria solani.


2) Chilli (Capsicum annum L.)

It forms a part of Indian diet. The fruits (dry or raw) are used in daily food. Suryanarayana and Bhombe (1961) isolated the fungal flora of crop and observed the dominant seed mycoflora like Aspergillus flavus, Alternaria sp., and Phomopsis sp., etc.

Abnormal seed mycoflora were studied by Dhwale and Kodmelwar (1978) and recorded the association of Alternaria alternata, Colletotrichum capsici, Fusarium sp., Penicillium sp., and Cladosporium sp., from stored abnormal chilli seeds. Dwivedi et. al.,(1982) detected fungi like Aspergillus flavus, Rhizopus stolonifer, Fusarium moniliforme and Cladosporium cladosporioids from abnormal seeds of chilli. Whereas Deena and
Basuchaudhary (1984) noted that *Colletotrichum capsicum*, *Alternaria alternata*, *Fusarium* sp., and *Aspergillus* sp. are the abnormal seed borne fungi which causes the fruit rotting, discolouration, losses in seed viability and seedling mortality in nursery bed. Whereas, Tripathi *et al.*, (1984) recorded *A. flavus*, *A. niger* with 18 associated other fungi from stored abnormal seeds and fruits. Similarly, Riberio and Bolkan (1985) observed the seed moulds of market chilli seeds. Mishra and Rath (1986) recorded *Fusarium* spp., from post harvest decay of chillies. Gupta and Basuchaudhary (1987) studied the seed samples of vegetables (chilli) from Sikkim and found the *Alternaria alternata*, *Colletotrichum dematium* are found in dominance on seed. Whereas, Padaganur and Naik (1991) also studied the saprophytic as well as pathogenic seed moulds. Asalmol *et al.*, (2001) detected seed moulds from Akola from Chilli seeds. Dharamsingh and Maheshwari (2002) showed the seed moulds from abnormal chilli seeds which are responsible for poor vigour, low seedling emergence in the field.

Durairaj (1956) recorded the seed borne fungus of *Colletotrichum capsici*. Smith and Crossan (1958) detected *Colletotrichum capsici*, *C. piperitum* which causes fruit rot. Whereas, Rai (1971) found that *Colletotrichum capsici* causes the fruit rot of chillies. While, Datar and Ghule (1985) recorded the *Aspergillus flavus* as dominant pathogen from chilli seeds which causes the fruit rot diseases in Marathwada region.
Sujathabai (1992) recorded the presence of *Alternaria tenuis* from fruit rot of chilli

3) **Tomato (Lycopersicon esculentum Mill).**

It suffers from several diseases due to seed-borne pathogen infections. Haymaker (1928) noted *Fusarium lycopersici* causes the wilt of tomato. While Boyd (1935) detected *Phytophthora infestans* causes the late blight of tomato. Where as, Wellmann (1939) recorded the wilting of plant due to *Fusarium* sp. Sokhi and Sohi (1972) isolated *Rhizoctonia solani* from fruit rot of tomato. While, Jamaluddin *et. al.*, (1974) noted the post harvest decay of fruit and it caused by *Cyclindrocladium scoparium*. Similarly, Khanna and Chandra (1975) isolated *Fusarium moniliforme* from post harvested fruit rot of tomato. Mehta *et. al.*, (1975) found that the fruit rot caused by *Alternaria solani* and *A. tenuis* which are seed-borne pathogens.

According to Thakur and Chenulu (1970) three species of *Rhizopus* causes the diseases in storage and market tomato seeds. Similarly, Ramkrishnan *et. al.*, (1971) observed the species of *Alternaria* from tomato abnormal seeds. Similarly, Orlova *et. al.*, (1982) studied the external as well as internal seed-borne pathogens from seeds, fruits of tomato. Franceschini *et. al.*, (1982) studied the pathogenicity of *Alternaria alternata* f.sp. *Lycopersici*. While, Chandra *et. al.*, (1983) reported the presence of *Fusarium oxysporum, F. solani*. Besri (1978) identified and isolated the presence of pathogenic fungi such as *Fusarium oxysporum* f. sp. *Lycopersici* and *Verticillium dahliae* from tomato abnormal seeds.
Similarly Roy (1981) studied the association of fungi of abnormal tomato seeds. While, Datar and Mayee (1981) stated the yield loss is caused due to *Alternaria solani*.

Silva *et. al.*, (1991) recorded the 78 fungi from abnormal seeds of tomato. Similarly, Jagadish Kumar and Lokesh (1999) studied and isolated the seed moulds of 8 varieties from abnormal tomato seeds. Whereas, Bhatt *et. al.*, (2000) recorded the seed-borne nature of *Alternaria alternata*. Recently, Rangpal and Sher Singh (2001) recorded the presence of *Alternaria solani* from abnormal tomato seeds.

**4) Okora (Bhendi) (*Abelmoschus esculentus* L.)**

It is used as vegetable, grown in Assam, Haryana, Maharashtra, Punjab, Orissa, U.P. and West Bengal. Bhendi suffers from seed-borne pathogens which causes an enormous losses. Grover and Singh (1970) recorded the wilt of Okara by *Fusarium oxysporum*. Similarly, Gangopadhyay and Kappor (1977) also recorded the same.

Whereas, Gupta and Basuchaudhary (1987) studied the seed samples of Bhendi from Sikkim. Similarly Singh *et. al.*, (1988) noted that fungal diseases are due to seed-borne pathogens which are responsible for poor quality and low yield (20-30%). Plant losses every year in most Okara growing areas of the country. Vijay Kumar (1996) isolated fungi like *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Colletotrichum* sp., *Curvularia lunata*, *Drechslera* sp., *Fusarium moniliforme*, *Penicillium* sp. from discoloured abnormal Okara seeds.
f) Medicinal plant seeds:

Neem (Azadirachta indica) commonly known as Margosa and it has reputed value for its medicinal use. Sinniah et. al., (1983) collected Aspergillus tamarii, A. niger, A. quircinus, sp. of penicilium i.e. P. citrinum from the neem seeds. While, Saxena (1985) identified and isolated seed mycoflora of Eucalyptus grandis and E. tereticornis and showed 30 and 16 fungi respectively. And 16 fungi are internally seed-borne in nature. Whereas, Roy and Veenitia Kumari (1991) showed the seed moulds from Acacia nilotica, Caesalpinea crista and Nelumbo nucifera and detected the 5 species of Aspergillus and they stated that the A. flavus was found to be dominantly. Recently, Dhake (1995) studied the seed microorganisms of Azadirithica indica (A. Juss) and he found the 17 fungal species belonging to 9 different genera.

g) Spices and condiments

Dwivedi et. al., (1973) studied and isolated the seed mycoflora from coriander abnormal seeds. Whereas, Prasad (1979) studied the seed microorganisms of coriander abnormal seeds and he found that 06 fungi such as Aspergillus flavus, A. candidus, Curvularia lunata, C. pallescens, A. tenuis, Cladosporium oxysporum, etc. While Shrivastava R.K. (1985) recorded the presence of seed-borne fungi from seeds of coriander, Fennel and Fenugreek. And these seeds shows the heavy population of species of Aspergillus, Rhizopus, Fusarium, Macrophomina, Curvularia, etc. While, Khulbe et. al., (1991) isolated seed mycoflora as Didymella lycopersici,
Diaporthe phaseolorum from seedling, leaves fruits from seed coat, inner seed tissues of red pepper and bell pepper of kumaun himalaya. Whereas, Rastogi et. al., (1992) studied the occurrence of seed moulds of cumin (Cuminum cyminus L.) growing in Rajasthan and they found 54 fungal species and 29 genra.

Prasad et. al., (1988) observed the physico-chemical changes in food reserve of coriander seeds due to storage moulds, like Aspergillus flavus, Curvularia pallescens, C. lunata. Recently, Chavan (2002) studied deterioration of fibre due to seed-borne pathogen of fennel. And he found that the seeds infected with Fusarium oxysporum reported maximum loss in crude fibre than Aspergillus flavus. Whereas, Aspergillus niger, Penicillium funiculosum shows considerable loss and Alternaria alternata, Curvularia lunata and Helminthosporium tetramera shows moderate deterioration of crude fibres.

h) Ornamental seeds:

The seed mycoflora have been studied by several workers. Gupta and Shrivastava (1981) from 09 samples of cosmos seeds. Whereas, Shrotri and Shrivastava (1983) recorded the presence of seed moulds from marigold (Tagetes erecta L.) seeds.
BIOLOGICAL CONTROL

Biopesticides and their use in the control of plant diseases has become a modern trend in agriculture, which is termed as biological control. Biological control of various plant pathogens using different bioagents has been attempted and found effective. Such biocontrol agents are found to be belonging to different groups of plants and microbes besides, which have been reported to be highly potent, economical and ecofriendly for the successful control of plant pathogens.

In general, the management of plant diseases mainly based upon conventional method, such as use of toxic chemicals. However, concerns increased due to problems due to pesticid residues in food and environment. Due to these facts, an alternative method like Biological control of plant diseases to be convenient. It differs fundamentally from the conventional chemical control of plant diseases. It manipulates an environment around the crop plants which favour the beneficial micro-organisms that contribute to plant health and vigour. In fact method is not new, rather used indirectly from ancient time. The use of cow dung on prunned surface and green manuring are the indigenous methods to exploit resistant microflora to overcome cankers and soil borne disease problem. The success of agrobacterium radiobacter used for biocontrol of *Aspergillus tumefaciens* causing crown gall of stone fruits and other plants are the first examples for large scale application of biocontrol agents.
Microorganisms play an important role in biological control of plant diseases. The strains of genera *Bacillus*, *Pseudomonas* and *Enterobacter* and fungal genera *Trichoderma* and *Gliocladium* have shown biocontrol activity against damping off disease in several crops (Hader *et al.*, 1983, Adams, 1990).

1) **Leaf extracts as biopesticide**

Walker *et al.*, (1929) reported inhibition of spore germination of *Colletotrichum* sp., with leaf extract of *Allium cepa*. Kumar and Nene (1968) showed that the leaf extract of *Cleome isocandra* inhibits growth of several fungi including the test fungus *Helminthosporium maydis*. Besides the leaf extracts; the root, stem, flower and seeds extracts of this plant also inhibited growth of the fungi completely. Shekhawat and Prasad (1971) recorded that out of 41 plant species tested the leaf extracts of 13 plants and flowers of 04 plants showed antifungal activity against *Alternaria tenuis* (from beans) *Curvularia penniseti* (from bajra) and *Helminthosporium* sps. Whereas Kolte and Shinde (1973) studied the influence of plant extract (*Crotalaria juncea* L.), mung (*Phaseolus radiatus* L.), Urid (*Phaseolus mungo* L.) *P. aconitifolius* and Dhanicha (*Sesbania* sp.) which inhibited growth and sclerotial production in *Macrophomina phaseolina*. Mukadam *et al.*, (1976) investigated the antifungal activities in deproteinised leaf extracts of weeds and non weeds. Similarly, Misra and Dixit (1976) records the fungicidal spectrum of the plant and leaf extract of *Allium sativum* Linn., which completely checked growth of *Absidia spinosa, Alternaria*
tenuis, Curvularia lunata and Helminthosporium sp., and Fusarium nivale and he observed activity of the active principle of Clematis gonriana protoanemonin and commercial fungicides (Bilox-50, Dithan-Z-78, Ziram) and they found that the leaf extract was found to be 27.7 and 55.4 times more active than the fungicides.

Advesh Narian and Satapathy (1977) reported that leaf extract of Vinca rosea was more antifungal than the extract of its flower, stem and root-against Helminthosporium nodulosum, Sclerotium rolfsii, Pestalotia sp., Fusarium oxysporum, Collectotrichum sp. and Aspergillus niger. Similarly, Pandey (1982) reported that leaf extracts of Datura alba and Cannabis sativa effectively reduced the seed mycoflora of Eleusine coracana in storage conditions. While, Prasad and Ojha (1986) studied and found that the leaf extract of Adhatoda vasica, Andrographis paniculata, Azadirachta indica, Catharanthus roseus, Cinnamomum camphora, Ocimum sanctum, Plumbagao zeylanica, Strychnos nux-vomica, Lantana camera and Vitex negundo were active against Fusarium equiseti, Fusarium semitectum and Curvularia lunata which cause post harvest decay of cucurbits. Natarajan and Lalithakumari (1987) recorded antifungal activity of leaf extract of Lowsonia inermis on Dreschlera oryzae. Ghewande (1987) studied effect of extracts of Azadirachta indica, Lawsonia alba, Pongamia glabra and Tridex procumbens, along with fungicides for the control of the late leaf spot (Phaeoisariopsis personata) and rust (Puccinia arachidis) diseases of groundnut crop. Whereas, Shetty et. al., (1989)
reported the leaf and fruit extracts of *Azadirachta indica* were useful in the control of *Trichoconiella padwickii* the seed-borne fungus in paddy. Kishore *et al.*, (1989) studied antifungal activity of 20 plants but only *Seseli indicum* exhibited absolute inhibition for *Rhizoctonia solani* at 1500 ppm. Khan and Kumar (1990) found that the leaf extract of *A. indica* checked the seed mycoflora of wheat. Similarly, Sinha and Saxena (1990) recorded the latex of *Euphorbia hirta* provided protection to tomato from *Aspergillus niger* causing fruit rot disease.

Chakraborty *et al.*, (1991) recorded antifungal activity of aqueous extracts of leaves of *Cymbopogan pendulas, Canabis sativa* and *Lantana camera* against *Colletotrichum camelliae, Alternaria solani* and *Curvularia lunata*. Similarly, Tewari and Nayak (1991) used leaf extracts of 4 plant species viz., *Piper betle, Ocimum sanctum, Nyctanthes arboritris* and *Citrus limon* were effective in reducing the radial in vitro growth of *Pyricularia oryzae, Cochliobolus miyabeanus* and *Rhizoctonia solani*.

Dubey and Dwivedi (1991) reported the leaf extract of *Acacia arabica, Allium cepa, Allium sativum* plants were fungitoxic to *Macrophomina phaseolina*. While, Datar and Qureshi (1991) found that the leaf extract of *Polyalthia longifolia* was most effective in reducing mycelial growth of *Curvularia lunata*, which was followed by *Parthenium hyesterophorus, Eucalyptus citriodora, Punica granatum, Tabernaemontana coronaria, Calotropis procera* and *Ipomea carnea* and bulb extract of *Allium cepa* was more active than *Allium sativum*. However,
Ramesh et al., (1991) studied the ethanol extract of leaves of *Croton sparsifloras* was antifungal to plant pathogenic fungi *Pyricularia oryzae*, *Dreschlera oryzae*, *Alternaria tenuis*, *Rhizoctonia solani*, *Sarocladium oryzae*, *Colletotrichum capsici*, *C. coffeum* and *Fusarium javanicum*.

However, Wahegaonkar and Sahasrabundhe (2000) studied that leaf extracts of *Ocimum sanctum*, *Azadirachta indica*, and *Catharanthus roseus* controlled the surface mycoflora of some seeds. Ghangaoankar and Mukadam (2001) stated that the extract of leaves, stem and bark of neem tree are highly inhibitory to the fungi of onion bulbs. Similarly, Kamble and Bhale (2001) showed that ethanol extracts of *A. indica*, *Polyalthia longifolia*, *Sericarpus anacardium* and *Commiphora stocksiana* has antifungal activity against *Fusarium udum* wilt. Recently, Babu et al., (2001) used fresh leaves of 25 different plants and found that except *vitex negundo* all the plants reduced mycelial growth of *Alternaria solani*. Similarly, Varshney (2001) recorded that diluted *A. indica* and *Tagets erecta* leaf extracts found more antifungal than *Lantana camera*, *Pinus roxburghii* against stripe disease of barley caused by *Dreschlera gramineae*. Sharma et al., (2002) reported antifungal activity of plant extracts of *Aloe barbadensis*, *Datura stramonium*, *Zingiber officinale*, *Murraya koenigii*, *A. indica*, *Brassica juncea*, *Mentha piperita* oil against *Aspergillus flavus*, *Fusarium oxysporium* f.sp. *pisi*, *Macrophomina phaseolina*, *Alternaria alternata* and *Rhizoctonia solani* which are found on pea seeds. Whereas Chaudhary and Bhansali (2002) studied the effect of different
concentrations of *Lantana camera* leaf extract on spore germination of *Physcomitrium japonicum* and found maximum inhibition due to leaf extract followed by extracts of stem and root. Similarly Kamble and Gangawane (2003) noticed that some medicinal plant extracts act as biopesticides and among these 11 plant extracts were tested against *Aspergillus flavus* and *Rhizoctonia bataticola* groundnut which cause pod rot. Hiwale and Kamble (2004) used 10% leaf extracts of *Salvia aegyptiaca* L., *Melia azaderach* L., *Ocimum sanctum* L., in the management of fruit rot of *Cucumis sativus* as biopesticides. Recently, Sharma *et al.*, (2004) studied the petroleum ether fruction of extract from dried leaves of *Polyalthia longifolia* for antifungal activity against *Alternaria solani*. Devi and Jadhav (2005) studied the effect of neem, and *Parthenium* leaf extract in combination with chemicals (2, 4-D and BHC) for the control of sugary disease of sorghum.

2) **Stem/bark extracts as biopesticides**

Kolte and Shinde (1973) recommended the stem extracts of *Crotalaria juncea*, *Phaseolus radiatus*, *P. mungo*, *P. aconitifolius* and *Sesbania* sp., have inhibitory potency against growth and sclerotial production in *Macrophomina phaseolina*. While Umalkar *et al.*, (1978) suggested that the antifungal activities in the extracts of bark of *Acacia nilotica*. Similarly, Tiwari (1997) isolated volatile constituent from bark of *Cinnamomum zeylanicum* and found the mycelial growth of *Aspergillus flavus* and *A. niger* was completely inhibited. However, Ghangaonkar and
Mukadam (2001) tested the bark extracts of neem against *Alternaria pori*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum* and *Macrophomina phaseolina* and found antifungal activity maximum in the extracts. Simultaneously, Chaudhary and Bhansali (2002) studied the effect of *Lantana camera* stem extracts on spore germination of *Physcomitrium japonicum*. Tripathi *et al.*, (2002) screened the bark of *Acacia niotica* for its fungitoxicity against *Penicillium italicum*.

Gawai (2004) stated that the effect of stem extracts of *Lantana camera* and *Curcuma longa* was found significant on growth of 2 fungal pathogens of cabbage and tomato i.e. *Alternaria solani* and *A. brassicae*. The stem extract of *Curcuma longa* was found to be more inhibitory for the growth of *A. solani* than to *A. brassicae*.

3) **Root extracts as biopesticides**

Kolte and Shinde (1973) suggested that the root extracts of *Crotalaria juncea, Phaseolus radiatus, Phaseolus mungo, Phaseolus aconitifolius* and *Sesbania* sps., inhibited growth and sclerotial production in *Macrophomina phaseolina*. Whereas Charya *et al.*, (1979) studied and found that root extracts of *Lawsonia inermis* and *Prosopis juliflora* are toxic against spore germination of *Dreschlera rostrata* and *Curvularia lunata*. While, Gourinath and Manoharachary (1991) found root extract of *Eucalyptus lanceolatus* was least toxic to the spore germination of phytopathogens. Jambhale *et al.*, (2002) prepared biosticides from roots of different plants for controlling fungal diseases, viral and insects.
4) Flower extracts as biopesticides:

Shekhawat and Prasad (1971) reported that flower extracts of 04 plants showed strong antifungal activity against *Alternaria tenuis*, *Curvularia penniseti* and *Helminthosporium* sp. While Charya *et al.*, (1979) tested flower extracts of 48 species of medicinal plants against spore germination of *Dreschlera rostrata* and *Curvularia lunata*. They reported that the flower extract of only *Rosa chinensis* exhibited complete inhibition of spore germination.

Recently Selvamani and Latha (2005) studied the antimicrobial potency of flowers of *Cassia alata* against number of bacterial and fungal organisms by disc diffusion method and found that ethanol extract at a concentration of 25 and 50 mg / disc showed significant activity.

5) Seed extracts as biopesticides

Abdullaera (1962) found that seed extract of *Allium sativum* and *A. cepa* exhibited toxicity against *Fusarium oxysporum*, *Rhizoctonia solani* and *Verticillium dehalae*. Pandey *et al.*, (1982) tested the seed extracts of 32 plants and found that extracts of soybean, lentil, *Leonotis nepetafolia*, *Paspalum scrobiculatum* and *Peltophorum pterocarpum* exhibited absolute toxicity against *Alternaria alternata* and *Aspergillus niger*. On the contrary, Kulkarni and Deshpande (1987) stated that exudates from groundnut (var. K4-11) seeds stimulated spore germination of *Aspergillus niger* but inhibited germination of *A. flavus* and *Rhizoctonia stolonifer*. 

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Harit and Rathee (1996) screened seed extracts of *Trichosanthes anguinea, T. bracteata, T. cucumerina* and *T. diocia* prepared in petroleum ether and found that the unsaponified matter possesses antifungal properties. While Waghamare and Mukadam (1997) studied the inhibitory nature of seed extracts against species of *Fusarium* and found that the extracts of *Abrus prectorious, Phyllanthus embilica; Semicarpus anacardium, Terminalis chebula* and *Acacia cocina* have highly inhibitory action to *Fusarium* species. Nidiry (1998a) showed that ethyl acetate and methyl alcohol extracts of *Cimullus nilgaris* seeds exhibited higher activity against the mycelial growth of *Colletotrichum gloeosporioioides* than petroleum ether extract. While, Juneja and Patel (2002) studied the antifungal properties of powder of custard apple seed (1 %), black pepper seed (1 %), mint leaves (5 %) and orange peels (3 %) which controlled seed mycoflora during storage.

Recently, Sonawane (2002) investigated that seed extracts at 10 % (aquous) concentration affected growth of fungi. The growth of *Alternaria alternata* was inhibited due to chawali (*Vigna unguniculata*) seeds, *Aspergillus flavus* due to by Gram (*Cicer arietinum*), moth bean (*Phaseolus aconitifolius*), Green gram (*Phaseolus aureus*) seeds. *Fusarium roseum* due to chawali (*Vigna unguniculata*) and *Helminthosporium tetramera* inhibited due to moth bean (*Phaseolus aconitifolius*) and chawali (*Vigna unguniculata*).
6) Plant products as biopesticides

Ali et al., (1992) conducted in vitro evaluation of certain neem products against post harvest fruit rotting fungi of tomato and found useful. While Dhanpal et al., (1993) reported that the neem products have 80 to 100 % growth inhibitory potency for Rhizoctonia solani and Phytophthora meadii which cause root rot disease of Elettaria cordamomum. Thangavely et al., (1995) tested 4 products like Neem gold, Margocide 20 E.C. Hinosen, Replin R.D.-9 and found that neem formulations Repelin RD – 9 proved significantly superior for inhibiting spore germination of rice blast disease. Chabra et al., (1999) reported that the fungicides and Bio neem (Fortune and Nimbicidinie (each @ 0.3 %)) significantly reduced the intensity of early blight and gave more yield of tomato fruits. Maximum yield was observed in Bio-neem (57.20 %) followed by Fortune (51.27 %), Karachi (46.11 %), Dithane, Nimbicidinie (95.11 %) etc.

Recently Awach and Ellis (2002) stated that the 3 plant powders ( Ocimum gratissimum, clove and Syzygium aromaticum) only Syzygium powder gave 100 % protection for 2 months to groundnut seeds against storage fungi and Ocimum gratissimum gave 4 months protection to groundnut seeds. Rawal et al., (2004) showed the effect of Achook, Neem basisine as neem formulations and vitavax with Trichoderma viride combined seed treatment were found highly fungitoxic / effective for better management of seed borne pathogenic mycoflora of fennel (Foeniculum vulgare Mill.)
Recently Singh et al., (2005) used 5 neem products (Achook, Neem gold, Neemta, Repelin and neem oil) against *Alternaria alterata* *in vitro* at 5, 1.0, 2.0 and 5 % concentration. Among these, Neemta appeared best followed by neem oil, neem gold, Repelin and Achook in the reduction of mycelial growth, inhibition in germination of spores was highest by Achook followed by Neemata, Neem gold, Neem oil and Repelin.

7) **Oils as biopesticides**

A large number of essential oils for antifungal activities have been screened against several pathogenic fungi. However, studies regarding their characterization and fungitoxic properties have received little attention. Several workers confined their investigations in vitro fungitoxic studies of the oils and little attention has been paid to find out their in vivo applicability for the control of fungal diseases. Maruzella and Lignori (1959) have screened 51 essential oil against some storage fungi. While Haerdtl (1962) reported essential oils of *Eucalyptus* sp. was fungitoxic to *Aspergillus niger*. Whereas Dogygich (1971) exhibited the oil of basil, coriander and fennel to be active against *Alternaria tenuis*, *Aspergillus oryzae* and *Penicillium chrysogenum*. However Banerjee and Nigam (1977) tested the essential oil of rhizome of *Curcuma augustifolia* to some storage fungi. Simultaneously, Sawhney et al.,(1977) showed essential oils of *Cymbopogan citratus*, *C. martini*, *C. winteramus*, *Ocimum basilicum*, *O. gratissimum*, *Mentha arvensis* having fungitoxicity against *Penicillium notatum*. While Thind and Dahiya (1977) reported the essential oils of A.
*indica, Allium sativum* possessed strong antifungal activity against *Aspergillus fumigatus* and *Penicillium italicum*.

Misra and Dixit (1978) stated that the essential oil of *Inula racemosa* having antifungal ability against *Fusarium moniliforme* and *Colletotrichum capsici*. While Chaturvedi (1979) exhibited the *Adenocalymna allicea* oil showed broad spectrum of antifungal activity against 13 storage fungi. Whereas, Grover and Rao (1979) have been reported the moderate activity of essential oil of *Psoralea corylifolia* against *Aspergillus flavus, A. niger, Rhizopus stolonifer*. Similarly, Lahanya and Rao (1979) observed fungitoxic activities of *Cyperus scariosus* and *Ocimum basilicum* oils against *Aspergillus fumigatus, A. niger* and *Penicillium italicum*. And the oil of *Cyperus scariosus* was found to be most biological activity.

Similarly, Garg and Oswal (1982) collected the oil from *Chloroxylon swietenia* and tested against *Aspergillus oryzae* and *A. terreus*. Singh *et al.*, (1983) showed the efficacy of *Mentha arvensis* var. *Piperascens* oil against 16 storage fungi. Tripathi *et al.*, (1983) found the inhibition action of *Alpinia galangal* oil against 26 storage fungi and oil showed broad anti fungal spectrum at 0.4 percent and 0.6 percent concentration. However Singh and Vays (1984) found that the oil cake of mustard (*Brassica compestris*), *Linum usitatissimum* (Linseed), *Ricinus communis* (Castor), *A. indica* (Mahua) inhibits the mycelial growth of (betelnine) *Phytophthora parasitica* var. *Piperrina*. Similarly Kola *et al.*, (1984) screened the essential oils of *Zingiber* sp. Lemon grass, Palmarosa and *Mentha* sp.
against *Aspergillus parasiticus*. While, Pathak and Dixit (1984) screened essential oils of *Glossocardia bosvallia* against *Phytophthora prasitica*, *Botryodiplodia theobromae*, *Fusarium solani* and *Rhizopus nodolaorus*. Whereas, Adisa (1985) recommended that mature kernel oil avoids infection by *R. oryzae*, *Curvularia lunata* and *Phoma sorghina* (soft rot pathogens) and *Fusarium equisetii* (dry rot pathogens). However Batra and Mehta (1985) isolated essential oil from the seeds of *Argyeria speciosa* and tested against *Geotrichum condidum*, *Alternaria solani*, *Helminthosporium* sp. and *Colletotrichum dematum*. Simultaneously Maiti et al., (1985) showed the essential oils of *Mentha piperata*, *M. citrata*, *Cymbopogoan pendulus* having antifungal activity against *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Dreschlera oryzae* and *D. sorokiana*. While Dixit (1986) suggested that oils of several plants tested against *Aspergillus flavus* and *A. niger* and found *Ocimum gratissimum* oil to be the most effective. While Yadav (1986) stated that the inhibition of growth of *Aspergillus flavus* and *A. niger* by the oils of *Lepidagathis hyalina*.

Siddiqui and Garg (1987) isolated oils from *Artabotrys odoratissimus* and tested against 8 storage fungi i.e. *Trichoderma viride*, *C. lunata*, *Rhizopus* sp., and *Chaetomium* species. However, David et al., (1988) screened oils of leaves of *Vitex negundo* against *Trichoderma viride*, *Fusarium* sp., and *Colletotrichum* species. While, Naseem and Lanjewar (1989) suggested that seed treatment with neem oil is effective against *Aspergillus niger*. Simultaneously, Farog et al., (1989) screened oils of
some species (thyme cumine, clove, caraway, rosemary, sage) against *Aspergillus parasiticus* and found the complete inhibition of fungus and aflatoxin production by them. Similarly, Onawunmi (1989) showed the oil of lemon grass to be strong fungitoxic against *Aspergillus fumigatus*.

Narayana *et al.*, (1980) tested *Cinnamomum zeylanicum* oil against *A. fumigatus*, *A. niger* and *Rhizome* sp. Renu *et al.*, (1980) exhibited that *Cestrum diurnum* oil was found to be inhibitory to the growth of 21 fungi at 0.75 concentration. While, Asthana and Singh (1981) showed fungicidal activity of *Ocimum adsecendens* oil at its minimum concentration of 200 ppm. Whereas, Bhargava *et al.*, (1981) tested *Ocimum canum* oil against 13 storage fungi. Chandra and Dixit (1981) found that *Ageratum conyzoides* oil having fungitoxicity against *Colletotrichum capsici* and *Penicillium italicum*. Rao and Prasad (1981) tested *Artemisia pallens* and *A. vulgaris* oils against 7 storage fungi at 0.2 percent concentration. Kishore *et al.*, (1982) studied the fungitoxic spectrum of the oil of *Chenopodium ambrosioides* against 15 storage fungi.

Similarly, Mishra *et al.*, (1993) used essential oils of *Apium graveolens* and *Cumimum cuminum* against 29 fungi. Similarly Srivastava *et al.*, (1993) screened the Palmarosa oil and Eucalyptus oils obtained from *Cymbopogan martini* and *Eucalyptus citriodora* against *Aspergillus* sp., *Fusarium* sp., *Curvularia* sp., and *Cladosporia* sp.

Zambonelli *et al.*, (1996) studied the effect of essential oils of *Thymus vulgaris* L. *Mentha pipertia* L. against the pathogenic fungi.
Pattnaik et al., (1996) observed the antibacterial and antifungal activity of 10 oils in vitro. Simultaneously Sharma and Chaudhary (1996) studied the fungitoxic activity of oil against pathogens of beetle vine crops and the oil inhibited the growth of *Colletotrichum piperis* and *Sclerotium rolfsii* completely at 500 ppm.

Ndounga and Ouamba (1997) showed the oil of *Ocimum gratissimum* having strong activity against microorganisms and *Ocimum basilicum* having moderate activity.


Sahasrabudhe et al., (2000) studied the antimicrobial activity of castor (*Ricinus communis* L.) mustard (*Brassica compestris* L.), clove (*Eugenia caryophyllus*), *Eucalyptus* sp. oil against different pathogens. Similarly, Hussain et al., (2001) reported that the two essential oils citral and piperitone rich oil obtained from lemon grass and *Cymbopogan jawarcusa* inhibited the radial growth of *Fusarium solani, Rhizoctonia*
solani, Curvularia lunata, Phoma sorghina with 0.1 \% oil concentration. While, Singh et al., (2001) noticed that the fungi toxicity of Eucalyptus and garlic oils against Phytophthora infestans, Cyperous menthe and lemon grass oil against Rhizoctonia solani, Penicillium digitatum, and Inula racemosa against A. solani, Fusarium oxysporum, R. solani etc. Certainly, Meleo et al., (2001) tested the antifungal activity of monoterpenoid essential oil evaluated for Fusarium oxysporum.

Recently, Sonawane (2002) studied the anti fungi toxicity of essential oil and medicinal concentration oil (0.5 \%) against pathogenic fungi. All the oils inhibited the growth of Alternaria alternata, clove oil and lemon grass oil inhibited the growth of Aspergillus flavus where Curvularia lunata and Fusarium roseum showed moderate inhibition as like Alternaria alternata, Castor oil inhibited the growth of Helminthosporium tetramera.

Mehta (2005) found that the oil of Spilanthus ciliate having antimicrobial and antifungal activity against pathogenic fungi. Yang et al., (2005) screened the chemical composition and antimicrobial activity of the essential oils of Crhysanthemum indicum against 15 microorganisms.

8) Bacteria as Biocontrol Agents:

The biocontrol by using bacteria may also provide plant protection through induction of host plant defence mechanisms, elimination of plant trigger pathogen development or competition for nutrition or by production of antagonistic compounds such as antibiotics, siderophores, cyanide and hydrolytic enzymes. Effective bacterial biocontrol agents with the activity
against phytopathogenic fungi often synthesize a variety of antifungal metabolites and enzymes (Gutlerson et al., 1990). Isolates of *Pseudomonas, Bacillus, Erwinia* and *Serratia* are some of the bacteria used as (important) biocontrol agents. Kamla Nalini (2000) has reported that *Pseudomonas chlorosaphis* was an effective biocontrol agent against *Curvularia lunata, Alternaria* sp., *Rhizoctonia solani* and *Pyricularia oryzae*.

However, Singh et al., (1981) found the antagonistic effect of cotton phylloplane bacteria against *X. campestris pv. Malvacearum in vitro* and *in vivo*. Whereas, Gnanamanickam (1987) suggested the plant pathogens like *Fusarium oxysporum* f. sp. cubense (Panama wilt pathogen of banana), *Sarocladium oryzae* (sheath rot pathogen of rice), *Sclerotium rolfsii* and *Rhizoctonia solani* (stem and root pathogens of groundnut) were controlled by using bacterial strains i.e. *Pseudomonas florescence*.

Whereas Turner and Backman (1991) stated that bacterization of peanut rhizosphere with *Bacillus subtilis* decreased the root rot incited by seed borne *Rhizoctoia solani*. However, Mc. Manus et al., (1993) reported the inhibition of *Tilletia laevis* teliospore germination and suppression of common blunt of wheat by *Pseudomonas florescence*. Whereas Lazzaretti et al., (1994) observed the *Rhizoctonia solani, Sclerotinia sclerotionum, Bipolaris sorokiniana* and *Pyricularia oryzae* found in association with bean and wheat seeds were inhibited by the metabolites of *Bacillus subtilis*. 
Other pathogens like *Alternaria tenuis*, *Macrophomina phaseolina*, *S. rolfsii*, *Fusarium solani* were inhibited by *Bacillus subtilis*.

Hokeberg *et al.*, (1997) controlled the seed borne diseases of cereals caused by *Pyrenophora teres* and *Tilletia tritici* by using pseudomonas. Rangaeshwaran and Prasad (2000) screened 300 rhizosphere bacteria against *Sclerotium rolfsii* (causal organism of root rot / collar rot in sunflower). They found 11 rhizobacteria antagonistic to *Sclerotium rolfsii* like *Pseudomonas fluorescens*, *Pseudomonas* sp., *P. putida*, *Streptomyces* sp., *Bacillus pantothenticus* and *Alcatigenes odorans*, *P. putida* completely inhibited *Sclerotium rolfsii* under dual culture.

9) **Fungi as biocontrol agents:**

Most of the plant diseases are caused by fungi belonging to only 50 genera. In many countries, restrictions are now imposed for use of chemical fungicides (including pesticides) to protect food quality and environment. In recent years, the need to develop disease control measures as an alternative to chemical control, the biological control using fungi antagonistic against fungi pathogens has gained considerable attention and appears viable supplement or alternative to chemical control. New advances in formulation technology has produced fungal biomass for use as bio-control (mycotixins), insecticides and herbicides. Similar technology for the production of biocontrol microorganisms effective against plant pathogen is in its infancy.
Among the fungi the species of *Trichoderma* are the most important biocontrol agents because they control various root diseases caused by a wide range of fungal pathogens (Alagarsamy *et al.*, 1987; Mathivanan *et al.*, 2000). Similarly, Weindling (1932) reported for the first time, the potential of *Trichoderma* as an effective biocontrol agent against soil borne fungal pathogens. Later several researchers across the world have demonstrated the control of a wide range of plant pathogens using different species of *Trichoderma*.

Similarly, Mukhopadhyay (1985) firstly reported of biocontrol methods for control of tobacco damping off by *Trichoderma* i.e. *harzianum*. Whereas, Raguchander (1993) stated that dry root rot in mungbean caused by *Macrophomina phaseolina* was reduced by the application of biocontrol agent *Trichoderma viride*. Similarly Sethuraman and Murthuswamy (1994) observed the efficacy of antagonistic fungi *Trichoderma* sp. and *Laetiseria arvalis* against the *Macrophomina phaseolina* in the sesamum (*Sesamum indicum*). Simultaneously, Theradi *et al.*, (1994) conducted the experiment to test/check the different carrier of *Trichoderma* for the control of root rot of urdbean (*Vigna mungo* L.) caused by *Macrophomina phaseolina*. Similarly, Pushapavati and Chandrasekhararao (1999) tested the *Trichoderma* spp. i.e. *T. viride, T. harzianum* against *Sclerotium rolfsii* the incitant of groundnut stem rot.

Kore and Chavan (2000) observed the efficacy of *Trichoderma* species in the management of safflower charcoal rot disease. *T. hamatum*
was found more effective and inhibits the growth of *Macrophomina phaseolina*. Similarly, D’Souza *et al.*, (2001) screened *T. harzianum* against major fungal pathogens of betal vine ice *Phytophthora parasitica, Colletotrichum capsici, Sclerotium rolfsii,* and *Rhizoctonia solani*. While Anith and Manomohandas noted that combined application of *T. harzianum* and *Alcaligenes* sp. (bacterial strain) significantly reduced the incidence of *Phytophthora capsici* induced nursery rot disease of black pepper. Whereas, Gupta *et al.*, (2002) studied the antagonistic properties of *Penicillium* sp. against different fungi viz., *Fusarium, Curvularia, Pestalotiopsis, Aspergillus, Hemilica* and a gram positive bacterium.

Recently, Swami and Mukadam (2004) observed the efficacy of *T. viride* against the tomato fungi (*Alternaria solani, Geotrichum candidum, Phytophthora* sp., *Fusarium oxysporum, Aspergillus niger* and *Rhizopus stolonifer*.) Similarly Patale (2005) showed the antagonistic potency of *T. viride, T. harzianum* and *T. hamatum* against *Aspergillus niger, A. flavus, Rhizoctonia* sp., *Rhizopus* sp., and *Mucor* sp. Ukey *et al.*, (2004) suggested that by seed treatment and foliar sprays of *T. viride*, major diseases of cotton such as root rot (*Rhizoctonia solani*), wilt (*F. oxysporum, f. sp. *vasinfectum*), bacterial blight (*Xanthomonas axonopodis pv. Malvaceanum*), leaf spots (*Myrothecium roridum*), and *Alternaria macrospore* were significantly controlled eco-friendly.
MATERIAL AND METHODS

A) Studies on seed-borne fungi

1) Collection of seed samples

Seed samples were collected by the method described by Neergaard (1977). Accordingly random samples of different varieties of seeds were collected from fields, store houses, market places and seed companies. A composite samples of each variety was prepared by mixing the individual samples together, preserved in cloth bags at laboratory conditions at room temperature during the studies.

2) Detection of seed mycoflora

The seed mycoflora was isolated by using standard moist blotter method (SBM) and Agar plate methods (APM) as recommended by International Seed Testing Association (ISTA 1966); De Tempe (1970), Neergaard (1977) and Agarwal (1976).

a) Standard blotter method (SBM)

A pair of white blotter papers of 8.5cm diameter was jointly soaked in sterile distilled water and were placed in pre-sterilized petriplates of 10cm diameter. Ten seeds of test samples per petriplates were placed at equal distance on the moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25±2°C under diurnal conditions for 07 days.
b) Agar plate method (APM)

In this method, pre-sterilized corning glass petriplates of 10cm diameter were poured with 15ml of autoclaved potato dextrose agar (PDA) medium. On cooling the medium, ten seeds per petriplates of the test sample were placed at equal distance aseptically. Incubation conditions and other details were same as described for the blotter method.

In order to isolate only internal mycoflora, seeds were pre-treated with 0.1% solution of mercuric chloride for two minutes and subsequently thoroughly washed thrice with sterile distilled water and placed on agar plates. Seeds without any such pre-treatment were employed for the total seed mycoflora.

3) Identification of seed-borne fungi

The fungi occurring on each and every seed in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of seed-borne fungi was made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals. Pure cultures of these fungi were prepared and maintained on potato dextrose agar (PDA) slants.
4) Composition of media used in isolation

I) Potato Dextrose Agar (PDA)

Peepd potato – 200gm, Dextrose 20g, Agar 20 gm and distilled water 1000ml, pH 5.6. Peeled potatoes were boiled until soft and pass through muslin cloth. Then dextrose was added in it and final volume of solution was made up to 1000ml. In this solution agar was added, pH was adjusted to 5.6.

II) Glucose nitrate agar (GNA)

Glucose – 10gm, KNo3 – 2.5gm, KH2OPO4 – 1.0gm, MgSO4.7H2O – 0.5g, Agar – 20 gm and distilled water 1000 ml, pH – 5.6.

III) Czapek Dox Agar (CZA)

Sucrose – 30g, NaNO3 – 2.0g, K2HPO4 – 1.0gm, MgSO4.7H2O – 0.5g, KCl – 0.5 gm, FeSO4.7H2O – 0.01gm, Agar – 15 gm and distilled water 1000 ml, pH – 5.6.

IV) Martin's Rose Bengal Agar (RBA)

Glucose – 10gm, Peptone – 5.0gm, K2HPO4 – 1.0gm, MgSO4.7H2O – 0.5g, Rose Bengal – 0.0001 gm, Agar – 20 gm and distilled water 1000 ml, pH – 5.6.

B) Biological control:

1) Use of plant / Plant parts extracts

Fungitoxicity of plant extracts was studied by the poisoned food technique described by Nene and Thapliyal (1993). Glucose nitrate medium was prepared in flasks and sterilized. To this medium, the requisite quantity
of the plant extract was added. Plant extract was prepared by collecting fresh plant parts, washed thoroughly in distilled water and grinned in distilled water. The plant extract was thoroughly mixed by stirring. The medium was then autoclaved at 15 lb pressure for 20 minutes. After cooling the medium, fungi were inoculated in aseptic condition and incubated for 6 day at room temperature. Suitable checks were kept where the fungi were grown under the same conditions on glucose nitrate without plant extract. Mycelial growth and sporulation of the test fungi was measure after harvesting. The mycelial growth of the fungi compared with check, was taken as a measure of the fungitoxicity.

The use of algae, essential oils, gum, latex for biocontrol of yrdy fungi, the same method was implemented were the plant extract was replaced by algae, essential oil, gum and latex.

2) Use of antagonistic microorganisms

Antagonistic potential of *Trichoderma viride* against 5 test fungi was studied by dual culture method used by Sudhamoy Mandal *et al.* (1999). An agar disc (5mm) containing mycelium of *Trichoderma viride* was inoculated at the centre of PDA poured petriplates and culture discs of the test fungi were placed at the centre of the plate. Petriplates were incubated for a week at 25±1°C. Plates without antagonists served as control. Two replicates were kept for each treatment and observations on colony diameter (mm) and formation of inhibition zone were recorded.
C) Studies on Phytotoxins:

1) Production of phytotoxins:

The test fungi isolated from different seeds were grown on G.N. medium. 25ml of the medium was added in 100ml conical flasks and autoclaved at 15lbs pressure for 15min. On cooling, flasks were inoculated separately with 1ml of spore suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated at 25±2°C for six days and were harvested by filtering their contents through whatman No.1 filter paper. The filtrates were collected in presterlized bottles and termed as crude toxin preparations. These preparations were tested for their toxicity.

2) Assay method:

The toxicity of culture filtrate was determined by using following methods.

a) Seed germination method:

Surface sterilized hundred seeds of each variety were soaked in crude toxin preparation for 24 hours. They were then placed on moist blotter in petriplates. Seed soaked similarly in freshly prepared uninoculated liquid medium served as control. Percent germination or percent inhibition of germination, root and shoot length of seedlings were measured after 7 days of incubation at room temperature.
b) Wilting of shoots:

Shoots of jowar grown in the field were used for the test. Freshly prepared 5ml culture filtrate was taken in a vial in which shoot was dipped to the level of culture filtrate and incubated for 24 hours at room temperature. Shoots kept in freshly prepared sterile medium served as control. The wilting symptoms caused due to the culture filtrates were recorded.

c) Composition of media used for phytotoxin production:

Glucose nitrate medium:

Glucose 10g, KNO$_3$ 2.5g, KH$_2$PO$_4$ 1.0g, and MgSO$_4$.7H$_2$O 0.5g, dissolved in 1000ml distilled water.

D) Production of hydrolytic enzymes

1) Amylase

a) Production of Amylase

Production of amylase(s) was studied by growing the fungi in liquid medium containing starch 1000-10g, KNO$_3$ 2.5gm, KH$_2$PO$_4$ 1.00gm and MgSO$_4$.7H$_2$O 500mg, pH of the medium was adjusted at 5.5. twenty five ml of the medium was poured in 100ml conical flasks autoclaved and inoculated separately with 01 ml spore suspension of the fungi which were grown for 7 days on PDA slants. Unless otherwise stated, the flasks were incubated for 6 days at 25 ± 1°C with diurnal periodicity of light. On 7$^{th}$ day, the flasks were harvested by filtering the contents through Whatman...
filter No.1. The filtrates were collected in presterilized bottles and termed as crude enzyme preparation.

b) Assay (Cup-plate method)

Determination of amylase activity was done with the help of cup-plate method which was adopted by Singh and Saxena (1982), where 20ml of starch agar assay medium (soluble starch – 10gm, Na₂HPO₄ – 2.84gm, NaCl – 0.35gm, Agar agar 20gm, distilled water 1000ml and pH 6.9) was poured in each petriplate. On solidification of the medium, a cavity (08 mm diameter) was made in the centre with the help of a cork borer (No.4) and was filled with 1ml culture filtrates (crude enzyme preparation) of the test fungi. The plates were incubated at 28°C for 24 hours, then they were flooded with Lugol’s iodine solution as an indicator. A clear, non blue, circular zone obtained surrounding the central cavity, diameter of the zone was measured (mm) as the amylase activity zone. Similar procedure followed for the control except pouring of culture filtrates in the central cavity instead of the activity enzyme.

c) Composition of media used for Amylase production

i) Starch nitrate medium

Soluble starch 10gm, KNO₃ 2.5gm, KH₂PO₄ 1gm, MgSO₄.7H₂O 0.5gm, dissolved in 1000ml distilled water.
2) **Protease**

   a) **Production**

   Production of protease(s) was made by growing the fungi on liquid medium containing glucose 10gm, gelatine 10g, dipotassium hydrogen phosphate 1.0g, MgSO$_4$.7H$_2$O 500mg and distilled water 1000ml pH of the medium was adjusted at 5.5. Twenty five ml of medium was poured in 100ml Erlenmeyer conical flasks and autoclaved at 15lbs pressure for 20 min. the flasks on cooling were inoculated separately with 01 ml standard spore/mycelial suspension of test fungi prepared from 7 days old cultures grown on PDA slants. The flasks were incubated for 6 days at 25 ± 1°C with diurnal periodicity of light. On 7$^{th}$ day, the flasks were harvested by filtering the contents through Whatman’s filter No.1. The filtrates were collected in the presterilised bottles and termed as crude enzyme preparation.

   b) **Assay (cup-plate method)**

   Determination of protease(s) activity was done with the help of cup-plate method, adopted by Hislop *et.al.*, (1982) and Rajamani (1990). A basal medium was prepared by adding D/W=1000 = 20gm (W/V) agar and 10gm (W/V) gelatin. pH of the medium was adjusted at 5.6 with Mcllvaine’s buffer. Then it was sterilized at 15lbs pressure for 15 min. About 15ml of the medium was poured in presterilized petriplates under aseptic conditions. On solidification 6mm diameter cups/cavities were made in the centre of each of the agar plate with a sterilized cork borer (No.4). The cups/cavities were filled carefully with about 0.5ml of culture filtrate
(crude enzyme preparation). The plates were incubated at 25°C for 24 hours. Then the plates were flooded with 15% mercuric chloride in 7N HCl. After 10 min of standing, a clear transparent zone indicated the hydrolysis of gelatin by extra cellular proteolytic enzymes, whereas the rest of the region of the petriplates became opaque due to the coagulation of gelatin (protein) by mercuric chloride. Diameter of the clear zone was used as measure (mm) of protease activity, while non appearance of clear zone considered absence of protease(s) in the culture filtrates.

c) Composition of media used for protease production

Various synthetic media were employed for the production of protease(s) in the preliminary experiment. Composition of the media is given below.

i) Glucose gelatin medium

Glucose 10gm, Gelatin 10gm, K$_2$HPO$_4$ 1gm and MgSO$_4$.7H$_2$O 500mg, dissolved in 1000ml distilled water.

3) Lipase

a) Production:

Production of lipase was studied by growing the five tested fungi on liquid medium at 5.6 pH containing oil 10g, KNO$_3$ 2.5g, KH$_2$PO$_4$ 1g, MgSO$_4$ 0.5g and distilled water 1000 ml.

Further details are similar to that described for studied on amylase production. The C.F. termed as crude lipase preparations.
b) **Assay (Cup-plate method):**

Determination of lipase activity was done with the help of cup-plate method which was adopted by Sierra (1957). The medium contains Difcopeptone 10g, NaCl 5g, CaCl$_2$.2M$_2$O – 0.1g and agar agar 20g per liter and 10 ml lipid substrate sorbiton monolaurate (Tween – 20) presterilised was added to it. pH of the medium was adjusted to 6.0. 15ml of the medium were poured in each petriplates on solidifying the medium, (No.4) a cavity (8mm diameter) was made in the centre with the help of cork borer and was filled with 0.1ml of enzyme preparation in triplicates. They were incubated at 28oC after 24 hours, a clear circular zones were observed surrounding the central cavity. Diameter of the zone was measured (mm) as lipase activity. Similar procedure was followed for the control except the pouring of autoclaved enzyme preparation in the central cavity instead of the active enzyme.
EXPERIMENTAL RESULTS

PART – I

Effect of seed treatments on seed mycoflora

Seeds of different crops like cereals, pulses, oil seeds, vegetables, spices and condiments growing in the Marathwada region were used for the isolation of seed mycoflora by using standard blotter paper method and agar plate methods as recommended by ISTA (1966).

In order to know the effect of seed treatment, the seeds were treated with HgCl$_2$ and Neem leaf extract separately before plating. The mycoflora of seeds without any treatment served as control and the results are given in table No. 1 to 10 and plate Nos. 2 and 3.

A) Cereals

It is clear from the results of seed mycoflora of cereals like jowar (Sorghum vulgare L.) and Bajra (Pennisetum americanum L.) given in table No. 1 & 2 that, the seeds of both the crops yielded twenty one and twenty fungi respectively. On treatments of HgCl$_2$ the seed mycoflora was found poor in incidence. Similarly, the seeds treated with neem leaf extract effectively reduced the incidence of seed mycoflora in both the crops.

Untreated jowar seeds yielded five species of Aspergillus, three species of Alternaria, followed by two species of Curvularia, Drechslera, Fusarium and Rhizopus. Whereas, untreated bajra seed shows five species of Aspergillus, three species of Alternaria, two species of Curvularia, Drechslera, Fusarium and Rhizopus in dominance.
Effect of neem leaf extract also shows similar type of association of fungi in jowar and bajra. However, it shows poor incidence of the pathogens. Similarly, seed treated with HgCl₂ yielded fourteen species of fungi in jowar and twelve species of fungi in case of bajra with very less incidence of fungi.

B) Pulses

The seed mycoflora of pulses like pea (*Psium sativum* L.) and Gram (*Cicer arietinum* L.) showed association of fifteen and thirteen fungi respectively. Seed treated with HgCl₂ and neem leaf extract showed poor incidence of fungi in both the crops (Table 3 and 4).

Untreated pea seeds yielded four species of *Aspergillus*, and *Fusarium* which are in dominant whereas, gram seed also showed similar type. However the percent incidence varies.

Seed treated with aqueous neem leaf extract of pea yielded fifteen fungi and seeds of gram yielded only thirteen fungi. While, Pea seed treated with HgCl₂ yielded eleven fungi and gram seeds yielded ten fungi only. However, the percent incidence of the seed mycoflora is poor as compared with the untreated seeds.

C) Oil seeds

Effect of seed treatments with HgCl₂ and aqueous neem leaf extract was observed in case of oil seeds like groundnut (*Arachis hypogaea* L.) and safflower (*Carthamus tinctorius* L.). The results are summarized in the table 5 and 6.
It is clear from the results given in the table that groundnut seeds yielded eighteen fungi which are dominated by five species of *Aspergillus*, four species of *Alternaria*, three species of *Fusarium* and two species of *Trichoderma*, whereas, safflower seeds yielded seventeen fungi with similar type of incidence in all the fungi.

Seeds treated with aqueous leaf extract of groundnut showed association of thirteen fungi which are dominated by *Alternaria alternata*, *Fusarium oxysporum*, *Curvularia lunata*, *Fusarium moniliformae*, *Alternaria tenuis* and *Aspergillus fumigatus*. Whereas, safflower seed, treated with aqueous neem leaf extract showed fifteen fungi which are dominated by *Alternaria alternata*, *Alternaria candidus*, *A. carthami*, *Aspergillus flavus*, *A. fumigatus*, *A. candida*. However, seeds treated with HgCl₂ in case of groundnut yielded twelve fungi and that of safflower yielded ten fungi with poor incidence.

D) Vegetables

Comparative account of seed mycoflora of treated and non-treated vegetable seeds of Tomato (*Lycopersicom esculentum* Mill) and brinjal (*Solanum melongena* L.) was isolated and the results are given in table no. 7 and 8.

It is observed from the results that, the untreated seeds of tomato showed nineteen fungi and brinjal yielded seventeen fungi. Both the seeds showed maximum incidence of *Aspergillus niger*, *A. flavus*, *Fusarium*
oxysporum followed by *Alternaria alternata*, *Curvularia lunata*, *Rhizopus* sp. *Drechslera* sp. and *Trichoderma viride*.

Seeds treated with neem leaf extract showed comparatively less incidence of fungi in both the crops. Seeds treated with HgCl\textsubscript{2} yielded eleven fungi in both the crops with poor association.

E) Spices and condiments

In order to study the seed mycoflora of spices and condiments, available in the market and fields of this region, seed mycoflora of Dhania (*Coriandrum sativum* L.) and Jeera (*Cuminum cyminum* L.) were isolated and the results are summarised in the table No. 9 & 10.

It was interesting to note that Dhania seeds yielded fifteen fungi from which include four species of *Aspergillus*, three species of *Fusarium*, two species of *Alternaria* and *Rhizopus*. However, treated seeds with neem leaf extract yielded comparatively less incidence of fungi.

Untreated seeds of jeera yielded thirteen pathogens which are dominated by four species of *Aspergillus* and *Fusarium*, two species of *Alternaria* and *Rhizopus* whereas, seeds treated with neem leaf extract showed association of four species of *Aspergillus*, two species of *Alternaria*, and *Rhizopus*. However, seed treated with HgCl\textsubscript{2} yielded only seven pathogens with very poor incidence.
EFFECT OF BOTANICALS AND NUTRITIONAL FACTORS

a) Physical factors

1) Effect of incubation period on fungal growth and sporulation

Five test fungi were studied for their growth on nutrient medium along with neem leaf extract incubated upto 15 days at room temperature. The results are summarized in table 13.

It is observed from the results that all the five fungi started their mycelium growth on 3rd day of incubation whereas sporulation started only in *Penicillium notatum*. However, in the presence of neem leaf extract all the fungi expressed maximum both growth and sporulation from 7th to 10th day while on 15th day the growth was found to be stagnant in all the fungi studied.

2) Effect of pH on fungal growth and sporulation

In order to understand the toxicity of neem extract the fungi were grown at eight different pH values at room temperature and the results are given in table 14.

The observations recorded in table clearly indicated that at 2.5 pH very meagre mycelium growth of *Aspergillus flavus* and *Penicillium notatum* was observed. The mycelium growth and sporulation were moderate at 3.5 to 4.5 pH in case of all the fungi. Whereas, maximum growth and sporulation took place from 6.5 to 7.5 pH in all fungi. Whereas, increase in pH from 7.5 to 9.5 mycelium growth and sporulation gradually reduced.
3) **Effect of temperature on fungal growth and sporulation**

Fungi were grown at six different temperatures along with aqueous neem leaf extract. It was clear from the table 15 that, at 10°C only *Aspergillus flavus* started its mycelium growth. All the test fungi were grown maximum at 25 to 30°C along with maximum sporulation.

4) **Effect of light on fungal growth and sporulation**

Effect of continuous light condition against fungal growth was observed in the presence of neem leaf extract in nutrient medium and the results are given in table 16.

It was seen from the results that continuous dark illuminated showed maximum sporulation whereas, alternate dark and light condition shows maximum mycelium growth and sporulation in all tested fungi. It was interesting to note that in continuous light *Alternaria alternata, Fusarium roseum* and *Aspergillus flavus* shows less mycelium growth as compared with *Curvularia lunata* followed by *Penicillium notatum*. 


b) **Nutritional factors**

1) **Effect of neem leaf extract with different carbohydrates sources:**

In order to understand the nutritional influence on the toxicity of neem leaf extract along with five different carbohydrate sources were tested separately against five seed-borne pathogens. Whereas, neem leaf extract alone served as control. The results are summarised in the table 17 and Fig.1.

It is clear from the results that, neem leaf extract along with CMC was found highly influential for all the test fungi. Whereas, neem leaf extract along with sucrose also proved toxic to *Alternaria alternata*, *Aspergillus flavus*, *Fusarium roseum* and *Penicillium notatum*. Similar observations were noted in case of fructose for *Aspergillus flavus* and *Curvularia lunata*, maltose for *Aspergillus flavus*, *Fusarium roseum* and *Penicillium notatum*.

2) **Effect of neem leaf extract with different nitrogen sources:**

The nutritional influence on the toxicity of neem leaf extract was studied along with ten different nitrogen sources for five different seed-borne fungi. The results are summarised in the table 18 and Fig. 2.

Ten nitrogen sources at 0.5% concentration belong to nitrate, nitrites and organic category were studied. The toxicity of neem leaf extract along with potassium nitrate, ammonium nitrate and urea was found highly effective against *Alternaria alternata*, whereas, with potassium nitrate, ammonium nitrate, ammonium sulphate, peptone and casein also inhibited
growth of *Curvularia lunata*. However, all the tested nitrogen sources do not nullify the toxicity of neem leaf extract in case of *Aspergillus flavus*, *Fusarium roseum* and *Penicillium notatum*. The mycelial growth and sporulation were moderate to maximum as compared it with neem leaf extract.

3) **Effect of neem leaf extract with different phosphorus sources**

Five phosphorus sources at 0.1% concentration along with neem leaf extract tested for the mycelium growth and sporulation and the results are summarised in the table 19 Fig. 3.

It is clear from the results that, disodium hydrogen phosphate and calcium phosphate along with the neem leaf extract did not check the mycelium growth and sporulation in all the test fungi. Whereas, the toxicity of neem leaf extract along with sodium phosphate proved inhibitory in all the test fungi.

4) **Effect of neem leaf extract with different sulphur sources**

In order to know the influence of neem leaf extract along with the sulphur sources on the growth of seed-borne pathogens, six sources at 500 ppm concentration were tested and the results are summarised in the table 20 and Fig. 4.

It is clear from the results that, neem leaf extract along with potassium sulphate, ammonium sulphate and sodium sulphate proved inhibitory to *Alternaria alternata*, ammonium sulphate and sodium sulphate proved inhibitory to *Curvularia lunata*, potassium sulphate and sodium
sulphate proved inhibitory to *Fusarium roseum*, whereas, neem leaf extract along with any sulphur sources are unable to control the growth of *Aspergillus flavus* and *Penicillum notatum*.

5) **Effect of neem leaf extract with different salts**

In order to understand the effect of macro-elements required for the mycelium growth and sporulation, five different salts sources at 0.05 concentration along with neem leaf extract were tested and the results are summarised in table 21.

It is interesting to note that all the fungi grew slow in magnesium chloride but did not sporulate. However, *Aspergillus flavus*, *Penicillum notatum* did not respond for the toxicity of neem leaf extract along with the salts. Whereas, sodium chloride and potassium chloride proved inhibitory for mycelial growth and sporulation in case of *Fusarium roseum*.

6) **Effect of neem leaf extract with different vitamins**

Vitamins play an important role for growth in micro-organisms therefore six vitamins at 100 ppm concentration were used to test their influence on the growth of fungi and the results are summerised in table 22.

It is clear from the results that neem leaf extract along with ascorbic acid, pyridoxine and nicotinic acid inhibited the growth of *Alternaria alternata*. Whereas, other fungi did not show any effect of neem leaf extract with vitamins.

7) **Effect of neem leaf extract with different antibiotics**
Five different antibiotics at 100 ppm concentration along with the neem leaf extract were studied to know the antifungal behaviour of neem leaf extract against five seed-borne pathogens, the results are given in table 23.

It is observed from the results that, ampicillin and terramycin along with neem leaf extract stimulated the mycelium growth and sporulation in all fungi. However, Grisonin, Hostacyclin and Streptomycin along with the leaf extract inhibited the growth of *Alternaria alteranta* and *Fusarium roseum*. Whereas, the growth of *Aspergillus flavus*, *Penicillium notatum* moderately inhibited due to antibiotics.

8) **Effect of neem leaf extract with different fungicides**

Comparative behaviour of well known fungicides along with neem leaf extract is studied against five seed-borne fungi and the results are summerised in the table 24.

It is clear from the table 9 that, out of eight fungicides at 100 ppm concentration bavistin inhibited the *Fusarium roseum* and *Penicillium notatum*. While, thiride inhibited *Alternaria alternata* and *Curvularia lunata* completely.

The effect of neem leaf extract in comparison with fungicide sources do not show any effect in *Aspergillus flavus*, *Penicillium notatum*. However, *Curvularia lunata*, *Fusarium roseum* inhibited the growth and sporulation moderately in all fungicides.
PART – II

A) BIOLOGICAL CONTROL

1) Effect of leaf extracts of botanicals

Botanicals are known to possess antifungal chemicals therefore to know the actual effect of extracts of different plant parts an aqueous 10% concentration extracts were tested against seed-borne pathogens. The fungi were grown in nutrient medium without botanicals as control. The results are given in table 25, Fig. 5.

It clearly indicates that all the leaf extracts showed inhibition at more or less degree and all the five tested fungi showed maximum retardation of growth in *Azadirachta indica* by *Datura stromanium* and *Ocimum sanctum*. *Alternaria alternata* also showed moderate inhibition due to *Polyalthia longifolia* followed by *Aegle marmelos*, *Tridex procumbens* and *Jatropha curcas*.

*Curvularia lunata* was inhibited by *Vitex negundo*, followed by *Polyalthia longifolia*, *Tridex procubens*, and *Aegle marmelos*, *Fusarium roseum* due to by *Polyalthia longifolia* followed by *Jatropha curcas*, *Aegle marmelos*, and *Tridex procubens* whereas, *Penicillium notatum* was inhibited due to *Polyalthia longifolia* followed by *Aegle marmelos*, *Lantana camera* and *Tridex procumbens*. While, *Aspergillus flavus* was inhibited only by *Polyalthia longifolia* and *Tridex procumbens*. 
2) **Effect of stem extracts**

In order to know the toxicity of stem extracts, 10 botanicals at 10% concentrations were tested against the five fungi for sporulation and growth. The nutrient medium without stem extracts of botanicals served as control. The results are summarized in table 26 and Fig. 6.

It was interesting to note that *Azadirchta indica*, *Datura stramonium* and *Ocimum scantum* were proved inhibitory to all the test fungi except *Penicillium notatum*. However, it was observed from the results that *Alternaria alternata* also inhibited its growth in *Aegle marmelos*, *Curcularia lunata* also inhibited in *Polyalthia longifolia*, *Fusarium roseum* in *Aegle marmelos* and *Tridex procumbens* and *Penicillium notatum* in a *Tridex procumbens* and *Vitex negunda*. It was interesting to note that *Aspergillus flavus* and *Penicillium notatum* do not inhibited considerably in rest of tested botanicals.

3) **Effect of Root extract**

Fresh root extracts at 10% concentration were tested to know their effect on pathogens. Nutrient media without root extracts served as control and the results are summarized in table 27 and Fig. 7.

The results in table clearly indicates that all the five tested fungi inhibited the mycelium growth and sporulation considerably in root extracts of *Azadirachta indica*, *Datura stramonium* and *Ocimum scantum*. However, extract of *Polyalthia longifolia* also inhibited the growth of *A alternaria alternata*, *Curvularia lunata* and *Fusarium roseum* whereas, all other root
extracts do not show the inhibitory nature to control the mycelium growth and sporulation of the tested fungi as compared with the fungi growing in nutritional medium.

4) **Effect of flower extracts**

Inhibitory efficiency of flower extracts is studied against five seed-borne pathogens. The nutrient medium without flower extracts served as control and the results are summerised in table 28 and Fig. 8.

The results clearly indicates that, *Azadirachta indica* is proved inhibitory for mycelium growth and sporulation in all the fungi. Whereas *Alternaria alternata* also inhibited by *Ocimum scantum* followed by *Datura stramonium*. Similarly *Curvularia lunata* inhibited by *Datura stramonium* followed by *Polyalthia longifolia* and *Fusarium roseum* inhibited by *Ocimum scantum*. Whereas, flower extracts of other plants proved stimulatory to the mycelium growth and sporulation of the pathogens.

5) **Effect of seed extracts**

Seed extracts of seven plants are used at 10% (aqueous) concentration against the growth of fungi and the results are given in table 29 and Fig. 9.

It is interesting to note that, *Penicillium notatum* do not show inhibition with any seed extracts. Whereas, *Aegle marmelos* inhibited the growth of *Alternaria alternata* and *Fusarium roseum*. Similarly *Azadirachta indica* inhibited the growth of *Curvularia lunata* and *Fusarium*
However, other seed extracts proved more stimulatory against the growth of fungi.

6) Effect of essential oils

In order to know the effect of essential and medicinal oils on growth of fungi. Fungi were grown on nutrient medium along with 1ml of oil sample and the results are summarised in table 30.

It is clearly seen from the table that, clove oil, camphor oil, eucalyptus oil, tulsi oil is proved highly inhibitory to all the mycelium growth and sporulation of five test fungi. However, *Alternaria alternata* also inhibited by castor oil, *Aspergillus flavus* and *Curvularia lunata* inhibited by black pepper and castor oil, *Fusarium roseum* inhibited by camphor oil and black pepper oil. However, other oils do not proved its inhibitory effect on the growth of fungi.

7) Effect of latex and gum:

In order to study the antifungal activities of latex of six plants were tested on fungal growth and sporulation at 5 ml by mixing with nutrient agar. The nutrient agar without latex served as control and the results are summarized in table 31.

It is observed from the results that the five tested pathogens growing without latex in the nutrient media showed maximum growth and sporulation and the fungal growth checked by *Calatropis procera* followed by *Euphorbia hirta* and *Ficus benghalensis*. 
In order to observe the toxicity of plant product (as gum) against the five tested seed borne fungi at 5 gm along with nutrient media. The media without gum act as control and the results are summarised in table 32.

It is seen from results that the five tested pathogens growing without gum extracts in nutrient media showed maximum growth and sporulation. However the fungal growth checked by gum of *Azadirachta indica* followed by *Acacia nilotica* and *Ficus benghalensis*. However *Acacia chundra*, *Mangifera indica* and *Moringa oleifera* showed considerably inhibitory for growth.

8) **Effect of neem leaf extract in different solvents on growth and sporulation**

Effect of different solvent was used to extract the bioactive compounds of neem leaf extracts. For which 10 gm fresh leaves were extracted in different solvents and the volume was made upto 100 ml in which equal amount of nutrient medium was added and fungi are allowed to grow and the mycelium growth and sporulation was recorded in table 33 and plate 8.

It was interesting to note that all the fungi grown in a aqueous medium showed maximum growth and sporulation in all tested fungi. However, solvent like Diethyl ether, Acetone for *Alternaria alternata*, Acetone, Diethyl ether and Methanol for *Aspergillus flavus*; Acetone and Diethyl ether for *Fusarium roseum* proved maximum inhibitory. Whereas, *Penicillium notatum* moderately affected its growth in different solvents.
Similarly Hexane proved stimulatory for the growth and sporulation of *Aspergillus flavus, Fusarium roseum* and *Penicillium notatum*.

9) **Effect of *Trichoderma* species on fungal growth**

Antagonistic effect of four species of *Trichoderma* was studied against the growth of five seed-borne pathogens and the results are summarized in the table 34 and plate 9.

It is noted from the results that all the species of *Trichoderma* proved inhibitory for the growth of test fungi. However, the inhibition against *Aspergillus flavus* was very less with the *Trichoderma* sp. whereas, *Alternaria alternata* was greatly inhibited by *Trichoderma* (local species). *Curvularia lunata, Fusarium roseum* and *Penicillium noatum* were maximum inhibited by *Trichoderma viride* followed by *Trichoderma harzianum*.

10) **Effect of algal extracts on fungal growth**

In order to know the effect of algal extracts in toxin production at 1 gm along with nutrient media is used for fungal growth. The nutrient media without algal extract act as control and the results are summarised in table 35.

It is seen from the results that the five tested fungi growing without algal extracts in the nutrient media showed maximum growth and sporulation. However, spirogyra extract showed stimulatory inhibition in all tested fungi, whereas, chara and sytoncma extract showed considerably
inhibition in case of *Alternaria alternata* and moderately by cladophora species.

In case of *Aspergillus flavus* chara and cladophora inhibits considerably and syctonema shows moderately inhibition, while in case of *Curvularia luata* and *Penicillium notatum* cladophora, syctonema followed by chara and cladophora followed by syctonema, chara respectively. In case of *Fusarium roseum* syctonema extract inhibited maximum growth and sporulation..

11) **Effect of different media on fungal growth**

In order to know the effect of nutritional media on the growth of fungi three different media were tested against the growth of fungi and the results are summarised in the table 36.

It is observed from the table that all tested fungi grows vigoursly in glucose nitrate media, whereas, maximum sporulation were also reported in the glucose nitrate media. Whereas, all tested fungi grows moderately in rose Bengal and Czapek-Dox medium.

B) **TOXIN AND ENZYMES OF FUNGI**

a) **Effect of botanicals on toxin production of fungi**

i) **Seed germination**

The fungal pathogens are known to produce toxin which affects the seed germination and also produce several abnormalities in the seeds. To control this behaviour of fungi eco-friendly commonly available 10
botanicals were tested against five seed borne pathogens for their ability to inhibit the toxin and the results are summarized in table 37 and plate 7.

It was noted from the results that leaf extract of *Azadirachta indica* followed by *Datura stramonium* and *Ocimum scantum* was successfully helpful for maximum germinations of seeds in all the culture filtrate of test fungi. This was happened because of botanicals produces anti-fungal toxins which control the toxicity of fungi and allow to seed germination. However, other botanicals like *Aegle marmelos, Jatropha curcas, Polylalthia longifolia*, and *Lantana camera* also produced anti-toxins compounds due to which moderate seed germination taken place. However, seed germination in the culture filtrate of *Pinicillium notatum* less effects of the toxins.

**ii) Shoot length**

The fungal toxin also affects the shoot elongation in the germinated seeds of the crops. Therefore the effects of ten plant extracts were tested against five pathogens for their ability to produce toxins. The toxin produced by fungi in glucose nitrate medium was the control.

The seeds treated with the culture filtrate of the medium in plant extracts and non plant extract (control) were tested for germination of jowar seeds. The length of shoot was measured and the results are summarized in table 38.

It is clear from the results that, the culture filtrate of all the five fungi retarded shoot length to a significant level. It was maximum in case of
Curvularia lunata, followed by Fusarium roseum, Alternaria alternata, Aspergillus flavus and Penicillium notatum. It was interesting to note that the pathogenic fungi were inhibited for the production of toxins in leaf extract of most of the plants. In case of Alternaria alternata, Azadirachta indica, Aegle marmelos and Ocimum sanctum proved inhibited the toxin production in Alternaria alternata due to which the seeds of jowar shows maximum shoot length.

Similarly, the toxin inhibition in case of Aspergillus flavus was due to Azadirachta indica and Datura stromonium and Ocimum plant extracts, while in case of Curvularia lunata, it was due to Aegle marmelos, Azadirachta indica and Datura stromonium. Whereas similar observations were recorded for Fusarium roseum, in case of Ocimum sanctum and Penicillium notatum with the extract of Azadirachta indica, Ocimum sanctum and Datura strominum.

iii) Root growth

In order to know the affect of botanicals in toxin production in relation to root length, ten botanicals at 10 % concentration alongwith the medium was used for fungal growth. The culture filtrate was further utilized for the seed germination and the variation in the root length were recorded in the table 39.

It is clear from the results that fungal growth without botanicals in the nutrient media showed minimum root length in all the fungi. However, fungi growing with extracts of Azadirachta indica, Datura stromonium and
Ocimum scantum showed maximum elongation of root length, whereas, extracts of Jatropha curcas and Aegle marmelos, Polyalthia longifolia, Tridex Procumbens in case of Curvularia lunata, Aegle marmelos and Polyalthia longifolia in case of Penicillium notatum proved more toxic which also shows moderate reduction in the growth of roots.

iv) Seedling growth

The effect of culture filtrate of fungi on the abnormalities of the seedlings and vascular disturbances were studied for which seedling of jowar (Sorghum vulgare L.) plant are growing in the field were used which have 5 to 8 mm in length. Roots are deep in the test tube which containing 10 ml of fresh culture filtrate and the experiment was set at room temperature. Seedling in fresh water served as control and the results are summarized in the table 40.

It was clear from the results that maximum root were rot in Fusarium roseum, Penicillium notatum and Aspergillus flavus followed by Curvularia lunata and Alternaria alternata. The shoot abnormalities were reported on 3rd, 5th and 7th day. Seedling kept in case of culture filtrate of Aspergillus flavus, Curvularia lunata, Fusarium roseum showed only leaf curling on 3rd day whereas, culture filtrate of Penicillium notatum shows wilting and curling. Whereas, culture filtrate of Alternaria alternata shows 1st leaf chlorosis, whereas on 5th day leaf curling was reported in all the fungi along with wilting and chlorosis. Whereas, on 7th day almost all the seedling kept
in culture filtrate shows similar wilting and curling, whereas, seedling kept in control was appear normal.
b) **Effect of botanicals on hydrolytic enzyme activity**

Seed-borne pathogens are known to produce extra cellular hydrolytic enzymes which are responsible for biodeterioration of the seeds. Hence, fungi are grown in nutrient medium along with botanicals and effect of botanicals on enzymatic activity are studied.

i) **Effect of leaf extracts on Amylase production**

Fungi are grown on starchy seeds produces amylolytic enzyme. To check this activity of the fungi, five fungi are grown in starch-nitrate medium with 10% leaf extracts of botanicals. Whereas, fungi only grown in starch-nitrate medium acts as control and the results are summarised in table 41 and plate 10.

It is seen from the results that, fungi growing in *Azadirachta indica* check the amylase production considerably followed by the fungi growing in *Ocimum sanctum* and *Datura stramonium*. However, other botanicals proved to be inhibitory for amylase activity as compared with the fungi growing in nutrient medium.

ii) **Effect of leaf extracts on protease activity**

Fungi growing in legume and pulses are responsible for protease production. However, to know the role of botanicals in inhibition of protease production are studied by growing the fungi in gelatine nitrate medium along with the botanicals. Whereas, the medium without botanicals served as control and the results are shown in table 42 and Fig. 10.
It is clear from the results that, protease production by *Alternaria alternata* inhibited in *Azadirachta indica* and *Lantana camera* followed by *Datura stramonium* and *Aegle marmelos*. Whereas, protease activity of *Curvularia lunata* check by the *Azadirachta indica* and *Ocimum sanctum* followed by *Datura stramonium*, *Aegle marmelos*. It is of *Fusarium roseum* is by *Azadirachta indica* followed by *Datura stramonium* and *Ocimum sanctum* followed by *Polyalthia longifolia*. Whereas, *Penicillium notatum* checked by *Azadirachta indica*, *Datura stramonium* and *Ocimum sanctum*. However, other botanicals also proved to be inhibitory to the protease activity of enzyme of fungi.

**iii) Effect of leaf extracts on lipase production**

In order to know the role of botanicals in inhibition of lipase production are studied by growing the fungi in oil medium along with the botanicals. Whereas, the medium without botanicals served as control and the results are summarised in table 43 and Fig. 11.

It is clear from the results that lipase production was checked by *Azadirachta indica* considerably followed by *Ocimum sanctum* and *Datura stramonium*. However other botanicals proved to be inhibitory for lipase activity as compared with the fungi growing in nutrient medium.
DISCUSSION

Intensive agriculturing is the motto of twenty first century of the world. India being the country with large area of agriculture activities therefore all the attempts regarding intensive agriculturing are very essential to meet the need of food supplies to ever increasing load of population. The seeds of crops with high yielding, varieties free from the pathogens, is an important aspect for intensive agriculturing. It is clear from the literature that seeds in the most of the crops are found associated with variety of pathogens including fungi, bacteria, virus, nematodes. If such infested seeds are used for sowing purposes it may result in the crop loss due to seviour disease incidence. Fungi are the prominent microorganisms which are carried by crop seeds and such fungi are known as seed-borne fungi or seed mycoflora. In the present work studies were carried out to understand mycoflora load of different crops belonging to cereals, pulses, oil seeds, vegetables, spices and condiments. Similarly attempts have been also made to find out biocontrol measures. The results are discussed here.

It is understood from the results summarized in the table (1 to 10) that the maximum seed mycoflora of cereals yielded twentyone fungi, pulses – fifteen fungi, oilseeds – eighteen, vegetables – nineteen, spices and condiments – fifteen fungi. Among the fungi recorded from cereals *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Penicillium notatum, Drechslera tetramera*; pulses – *Alternaria*
tenuis, Aspergillus niger, A. flavus, C. lunata, F. roseum, P. notatum; Oilseeds – Alternaria alternata, Aspergillus niger, A. flavus, A. ustus, Fusarium oxysporum, F. roseum, P. notatum, Rhizopus nigricans, Vegetables – A. alternata, Aspergillus flavus, F. oxysporum, Drechslera tetramera, Trichoderma viride; Spices and condiments – Alternaria tenuis, Aspergillus flavus, A. niger, A. fumigatus, Fusarium semitectum, Penicillium and Rhizopus sp. were found to be dominative. Similar types of reports about the incidence of fungi were reported in case of cereals (Bhale and Khare, 1982), Navi et al., (1999), Patil and Pandule (2001); pulses (Deo and Gupta, 1980), Chavan and Danai, 1993, Sonawane, 2000, Gachande, 2001); Oilseeds (Sandikar, 1990, Chavan and Danai, 1993; Umatale, 1995); Vegetables – (Patil et al., 2000, Datar et al., 1992, Bharaswadkar, 2003, Vidyaskaran et al., 1980), Spices and condiments – (Srivastava, 1985, Prasad, 1981, Chavan, 2002) have been made. This clearly indicates that seeds irrespective of the crop showed association of seed borne fungi. Isolation and identification of seed mycoflora was kept as the major aspect of the studies and at the same time control of seed borne fungi due to seed treatment at the time of seed plating was an important aspect of the study. Therefore the seeds treated with different plant extracts whenever used for isolation of seed mycoflora. It was found that there was significant reduction in the incidence of fungi in the plates. This clearly indicates that use of plant extracts as seed treatment for the control of seed borne fungal pathogens would be most useful and eco-friendly way of controlling plant
diseases. Similar type of inhibitory effect of plant extracts on the incidence of seed borne fungi or on the activities of the fungi have been reported by many workers as Khanna and Chandra (1976); Bhowmick and Vardhan (1981); Reddy (1987); Shenoi et al. (1993); Ansari (1995), Dubey (1998); Kurucheve and Padmavati (1998).

An extensive screening of plant extracts nearly 151 grows naturally or cultivated were used for preparation of plant extracts and tested against seed mycoflora of different crops and the results are given in table 11. The results are highly useful that most of the plants significantly inhibited incidence of fungi on the seeds. Among such plant extracts of *Aegle marmelos* (L.) Corr., *Azadirachta indica* A. Juss., *Catharanthus roseus* (L.) G. Don., *Datura stramonium* L., *Jatropha curcas* L., *Lantana camara* L., *Ocimum scantum* L., *Polyalthia longifolia* (Sonn) Thw., *Tridex procumbens* L., *Vitex negundo* L., proved promising significant results. This clearly gives an idea for sustainable agriculture in spite of killing the useful of microflora of soil with the use of toxic chemicals in the form of fungicides and pesticides naturally biodegradable plant extracts can be the better answer. Hence the present study can be highly useful to the farmers, to use of plant extracts prepared in own fields with no expenditure for the control of seed borne diseases in the crops by treating the seeds with plant extracts at the time of sowing. Similar type of results regarding the use of plant extracts for the control of plant pathogenic fungi have been reported by different workers such as Mishra and Tiwari (1992); Shivpuri et. al.,

**Physical factors**

Effect of physical factors like incubation period, temperature, pH and light (Tables 13 to 16) were studied.

It was observed that while growing the pathogens in nutrient medium physical factors affects the metabolic activities, in the presence of NLE. On 3rd day incubation period except *Penicillium notatum* none of the fungi showed sporulation. It was observed that due to presence of NLE maximum growth occurred on 15th day of incubation. It clearly indicates that toxicity of NLE was responsible to delay the growth and sporulation of test fungi. Similar type of observations were reported by Charya and Reddy (1982) where the optimum incubation period more than week in case of *Fusarium oxysporum, Rhizoctonia solani* and *Phoma exigua*.

Experiments were carried out at eight different pH values and the observations clearly indicates that the maximum growth of fungi at 5.5 pH value. Similarly all fungi grows maximum at 25°C to 35°C. Intensity of different light also studied and the observations showed that alternate light and dark light intensity was ideal for the growth of fungi. Various workers reported their views on fungal growth against physical factors. Patil and Shashtri (1982) says that 20°C to 30°C was ideal temperature for the growth of pathogens.
Looking into the promising inhibitory effect of neem leaf extract on incidence of seed-borne fungi. The affectivity of neem leaf extract was further studied in the laboratory on growth, sporulation, toxin and enzyme production of certain selected seed-borne fungi.

It was found the effect of various nutrient sources like carbohydrate, nitrogen sources, vitamin sources, phosphorus sources, mixed with neem leaf extract. It is clear from the result summarized in the table 17 that the presence of carbohydrate like glucose, sucrose reduces the toxicity of neem leaf extract for inhibition of fungal growth. At the same time in the presence of CMC (carboxymethyl cellulose) like carbohydrate the toxicity of neem remain constant. This clearly indicates that seed borne fungi in the presence of sugars like glucose, sucrose did not respond to the toxicity of neem plant. But in their absence the toxicity is significant. Stimulation of growth in the presence of different sugars may be the point of consideration for decrease in the toxicity of neem. Stimulatory effect of sugars on growth have been reported by several workers. Charya and Reddy (1982) reported that only glucose source stimulates among the source of carbohydrates, the growth and enzyme production, whereas, Chavan (1993) reported that Aspergillus ruber, A. glaucus, Sphaerica violcia, Trichoderma viride and Fusarium dimerum grow luxuriantly in presence of glucose and fructose. But they are totally inhibited growth and sporulation in CMC which clearly indicates that the incapability of cellulose production by the fungi.
NLE at 10% concentrated mixed with 10 different nitrogen sources, five different phosphorus sources and six different sulphur sources showed great variation in the fungal growth (Tables 19 and 20). Among nitrogen sources *Aspergillus flavus* did not respond to the toxicity of NLE whereas, other fungi grow moderately. Among phosphorus sources sodium dihydrogen phosphate and calcium phosphate sources were stimulatory even in the presence of NLE against the five tested fungi. Among the six different sulphur sources were studied and the results clearly indicates that fungi in the presence of NLE reduces the mycelium growth moderately but the sporulation was increased. This type of work was supported earlier by several workers.

Patil and Shastri (1982) supported the variation in the requirements of nitrogen sources in case of *Alternaria alternata*. Similarly Gachande (2001) reported dia sodium phosphate, Ammonium phosphate proved to be completely inhibitory in *Fusarium oxysporum* and *Macrophomina phaseolina*. He also noted that sodium thiophosphates inhibitory in *Alternaria alternate* and Ammonium thiosulphate in case of *Macrophomina phaseolina*, whereas MgSO$_4$ supported the enzyme activity in *Aspergillus flavus*, *Curvularia lunata* and *Drechslera tetramera*.

In the presence of neem leaf extract mixed with different supplementary sources like vitamins, antibiotics, fungicides and salts (tables 21 to 24), this clearly indicates that NLE inhibited the growth of *Alternaria alternata* in presence of ascorbic acid, pyridoxine and nicotinic acid.
Whereas, *Aspergillus flavus* and *Penicillium notatum* showed stimulatory growth and sporulation in presence of NLE mixed with vitamin sources.

At the same time in presence of antibiotics sources Ampicillin, Terramycin showed stimulatory growth of *Alternaria alternata* and *Curvularia lunata*, whereas other antibiotics sources like Grisonin, Streptomycin, Hostacyclin inhibited the growth of *Alternaria alternata* and *Fusarium roseum* in presence of NLE. Such type of observation have been reported by Waghmare (1996) in case of seed-borne *Fusarium* sp..

Looking into the promising inhibitory effect of NLE mixed with different fungicides (table 24) it was also found that the NLE mixed with bavistin proved inhibitory for growth and sporulation of *Fusarium roseum* and *Penicillium notatum* and thiride proved inhibitory for *Curvularia lunata and Alternaria alternata* respectively. While bliton showed stimulatory effect on growth of *Penicillium notatum*. Such type of work have been supported by different workers such as Rao and Ramkrishna (1999) studied comparative efficacy of leaf extract of piper betle and carbendazim on *Colletotrichum fulcatum*.

Kamble *et al.*, (2006) studied twelve plant leaf extracts along with Agrochemicals like endosulphan, Monocrotolos, Streptomycin, Mycostatin, Aureofungin, Sodium chloride and Boron gave the promising results in the management of *Alternaria alternata, Fusarium oxysporum, Rhizoctonia bataticola*. Among the twelve leaf extracts *Salvia aegyptiaca* at 10 %
concentration with 10 ppm, monocrotofos completely inhibited the growth of *Fusarium oxysporum f. Spinaciae*.

**Biological control:**

During the past three decades crop production has been greatly influenced by the dramatic increase in the use of pesticides. Indeed, these compounds have played an externally important role in obtaining the maximal yield potential of almost every commercial crop by reducing yield losses caused by plant pathogens and other pests. However, due to indiscriminate and excessive use of these potentially hazardous, chemicals, several environmental threat and health problems to mankind and livestock’s etc. have arisen. Intensive use of chemical fertilizers and pesticides combined with poor management of water shades has resulted in severe water stress, pesticides contamination of water and agricultural products in addition to unacceptable degradation of soil, resulting in disruption of ecosystem over large areas. Pesticide contamination of food, water reservoirs and soil, has become a fact of life.

Marathwada possess a very rich flora with a great biodiversity. Naik (1998) reported 155 families with 746 genera and 1645 species from this region. Most of the higher plants contribute their uses in medicines and the uses of these plants are in consequently. Reports regarding the uses of different botanicals related to Indian medicine clearly indicates that the families like Fabaceae, Poaceae, Euphorbiaceae, Asteraceae, Rubiaceae,
Cucurbitaceae, Solanaceae, Malvaceae and Convolvulaceae play an important role in antifungal property (Fig. 12).

Considering the importance of eco-friendly management of the pathogens experiments were carried out by using aqueous extracts of separate part against the five test fungi.

It is understood from the results summarized in the table 11 that all the tested fungi inhibited the growth and sporulation at more or less degree. However, NLE was proved highly inhibitory for the growth and sporulation followed by *Datura stramonium* and *Ocimum sanctum*. Sporulation of *Alternaria alternata* was totally inhibited by *Azadirachta indica*, *Datura Stramonium* and *Ocimum sanctum*, *Polyalthia longifolia* and *Vitex negundo*. Whereas, *Fusarium roseum* inhibited the sporulation in *Aegle marmelos*, *Azadirachta indica* and *Ocimum sanctum*. However, it is interesting to note that sporulation of *Aspergillus flavus* and *Penicillium notatum* do not inhibited completely in any leaf extracts of tested botanicals. Bhowmick and Vardhan (1981) studied the ten medicinal plants tested for antifungal activity against *Curvularia lunata*. Whereas *Cinnamomum camphora*, *Catharanthus roseus* were inhibited the growth, sporulation and spore germination. Similarly, Elkaffash *et al.* (1998) screened 48 plants for their antifungal activity against *Fusarium oxysporum* f. sp. nivenum, *Rhizoctonia solani*, *Botrytis cinera* etc. and they showed that eight plants having high level of antifungal activity.
The effect of stem extracts at 10% aqueous concentration was studied against the five tested pathogens (table 26). It was also found that *Penicillium notatum* did not inhibited with the toxicity of botanicals. But *Fusarium roseum*, *Alternaria alternata* inhibited with *Aegle marmelos* and *Curvularia lunata* with *Polyalthia longifolia* and *Tridex procumbens*. Gourinath and Manoharachary (1991) observed that the different concentration of stem extracts of *Eucalyptus lanceolatus* exhibited 50 to 70% inhibition in the conidial germination of four pathogenic fungi. However, Gahangaonkar and Mukadam (2001) tested the bark extracts of the neem tree against the *Alternaria pori*, *Fusarium oxysporum*, *Aspergillus niger*, *A. flavus*, *Penicillium notatum*, *Macrophomina phaseolina* from the onion bulbs for their antifungal activity and they found the extracts of stem was to be highly inhibitory for the most of the fungi. While, Gawai (2004) showed the effect of extracts of stem of *Lantana camera* and *Curcuma longa* on the growth of two fungal pathogens of cabbage and tomato. The stem extract of *Curcuma longa* were found to be inhibitory for the growth of *Alternaria solani* than the *A. brassicae*.

Root extracts are very commonly used against several diseases to the plants and human beings. Fungitoxicity of root extracts of ten botanicals were studied (Table 27). It was observed that all the tested fungi inhibited in the extract of *Azadirachta indica* followed by *Datura stramonium*, *Ocimum sanctum* and *Polyalthia longifolia*. Similar types of observations were reported earlier by several workers. Charya et al., (1979) used the root
extracts of *Lawsonia inermis* and *Prosopis juliflora* against the *Drechslera rostrata* and *Curvularia lunata*. However, Gourinath and Manoharachary (1991) stated that the root extracts of *Eucalyptus lanceolatus* were found to be least toxic and inhibited to some extent the spore germination of phytopathogens.

Toxicity of flower extracts was also studied (Table 28) and it was interesting to note that flower extract of *A. indica* inhibited in all tested fungi. However, *Alternaria alternata*, *Curvularia lunata* and *Fusarium roseum* also inhibited the mycelium growth in other flower extracts. Selvamani and Latha (2005) reported antimicrobial capacity of the flowers of *Cassia alata*. The effectivity of seed extracts against the tested fungi was studied (Table 29) and it was found that the seed extract of *Aegle marmelos* and *Azadirachta indica* proved highly inhibitory than the seed extracts of other botanicals.

In order to know the toxicity of plant products like essential oils latex and gum were supplemented in the nutrient media (Tables 30 to 32). Clove oil, camphor oil, Eucalyptus oil and Tulsi oil are proved highly inhibitory. However, black piper and castor oil inhibited the growth of *Aspergillus flavus* and *Curvularia lunata*. Camphor oil and blackpiper oil retarded the growth of *Fusarium roseum*. Castor oil inhibited the growth of *Alternaria alternata*. Considering the results application of oil is strongly recommended for the stored seeds for longer duration. Similar types of
inhibitory activities were recorded with plant gum and latex against five test fungi.

In order to know the fungal toxicity in presence of NLE mixed with different solvents (Table 33) against the five test fungi. It was also observed that the NLE mixed with solvents like ether, acetone inhibited *Alternaria alternata*. Acetone, diethyl ether and methanol extracts inhibited the growth of *Aspergillus flavus*. Such types of work have been reported by earlier workers such as Ramesh *et al.*, (1991); Mishra and Tiwari (1992); Madhavi Adhav (1999); Kamble and Bhale (2001); Ganesan *et al.*, (2004).

To observe the role of *Trichoderma* sps. in the management of seed-borne fungi (Table 34) it was observed that species of *Trichoderma* play ecofriendly role in the management of five tested fungi. Considering this fact the application of *Trichoderma* is strongly recommended against the seed-borne pathogens. And such type of work have been supported by many workers such as Weindling (1932); Elad *et al.*, (1981); Sivan and Chet (1986); Baker (1989); Adams (1990); Devaki *et al.*, (1992); Mathivanan *et al.*, (2005) and recently Patale (2005).

To know the antifungal activity of algal biomass fresh water algae were tested (Table 35). And it was found that *Chara grovesii, Cladophora callicoma* and *Syctonema coactile* extracts showed inhibitory to the fungi like *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Penicillium notatum* and *Fusarium roseum* respectively. This clearly indicates that the
algal extracts have some antimicrobial properties and hence this work should be carried out.

Fungi produces secondary metabolites which includes toxic substances (toxins) which may kill the host tissues and results into severiority of the disease studies were carried out in order to understood the effect of leaf extracts of certain plants on toxin production in fungi. And it is clearly understood from the results summerised in the tables 37 to 40 that the leaf extracts of *Datura stramonium*, *Ocimum sanctum*, *Polyalthia longifolia*, *Vitex negundo* and neem significantly reduce the toxin production in the fungi. This clearly suggests that use of botanicals could be a beneficial aspect for the control of toxin production in fungi.

The seed-borne fungi specially during storage deteriorate seeds and this is mostly harmful if the fungi are efficient in production of amylase, protease and lipase enzymes. These enzymes degredate starch, protein and lipids respectively present in the seeds. Therefore the studies were carried out to see the effect of plant extracts on production of these enzymes. It is clear from the results summerised in the tables 41 to 43 that the use of plant extracts definitely would be helpful in the control of seed biodeterioration by inhibiting growth and enzyme production of the pathogens. Control of protease, lipase and amylase by using various chemicals have been reported by several workers such as Bhowmick and Choudhary (1982); Abraham and Prakashan (2001).

Seeds are loaded with microbes, which causes biodeterioration of seeds. However, several chemicals are used to control the associated mycoflora but in order to avoid exclusive reliance of chemicals, Henceforth
role of effective microorganisms and botanicals are seem to be effective and eco-friendly to manage the activity of seed borne pathogens.

**SUMMARY**

A) Effect of seed treatments on seed mycoflora:

1) Qualitative and quantitative composition of seed mycoflora was isolated from two crops of each group like cereals, pulses, oil seeds, vegetables, spices and condiments.

2) Among cereals jowar yielded twentyone fungi and bajra twenty fungi. Among pulses pea yielded fifteen fungi and gram thirteen fungi and among oil seeds groundnut yielded eighteen fungi. Similarly, safflower seventeen fungi. Among vegetables, tomato yielded nineteen fungi and brinjal seventeen fungi. Whereas, among spices and condiments dhania yielded fifteen fungi and jeera thirteen fungi.

3) Seed treated with aqueous neem leaf extract reduced qualitative as well as quantitative seed mycoflora.

4) Five pathogenic fungi namely *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium roseum* and *Penicillium notatum* are found in dominance.
5) Screening of 151 commonly occurring wild and cultivated plants are studied for their antifungal activities and toxicity.

6) Aqueous leaf extract of *Azadirachta indica* is found significantly suitable against the test fungi followed by leaf extracts of *Datura stramonium* L. and *Ocimum sanctum* L.


8) Aqueous leaf extract at 10% concentration was found effective for the management of growth and sporulation of seed-borne pathogens.

**B) Botanicals and Nutritional factors:**

9) The neem leaf extract delayed incubation period for growth and sporulation in fungi. However, other physical factors did not affect the toxicity of neem leaf extract against seed-borne pathogens.

10) In case of all the test fungi growth was found to be inhibited in the presence of neem leaf extract mixed with CMC as a source, of carbohydrates.

11) *Aspergillus flavus* did not respond to the toxicity of neem leaf extract in the presence of different nitrogen sources. Whereas, among phosphorus sources sodium phosphate and calcium phosphate
stimulated growth of the fungi in presence of neem leaf extract. Whereas, sulphur sources did not affect the toxicity of neem.

12) Supplement of vitamins in the presence of neem leaf extract proved stimulatory for the growth of the fungi.

13) Amongs fungicide at 100 ppm concentration bavistin and neem leaf extract is together proved highly inhibitory for the fungi.

14) *Alternaria alternata* and *Fusarium roseum* showed inhibition of growth in presence of neem leaf extract and antibiotics like Streptomycin and Hostacyclin.

C) **Biological control:**

15) Among aqueous leaf extracts at 10% concentration of different plants, *Azadirachta indica* proved more highly inhibitory to the growth and sporulation of test pathogens followed by *Datura stramonium* and *Ocimum sanctum*.

16) The leaf extract did not inhibit growth of *Penicillium notatum*. However *Alternaria alternata* was inhibited by *Aegle marmelos*, *Lantana camera*; *Curvularia lunata* by *Polyalthia longifolia*; *Fusarium roseum* by *Aegle marmelos* and *Tridex procumbens*.

17) Aqueous root extracts of *Azadirachta indica* and *Datura stramonium*, *Ocimum sanctum* and *Polyalthia longifolia* inhibited growth of the seed-borne fungi.
18) Flower extract of *Azadirachta indica* was suitable to control all the tested fungi. Whereas, *Alternaria alternata*, *Curvularia lunata*, *Fusarium roseum* also were inhibited for growth.

19) Among seed extract *Azadirachta indica* and *Aegle marmelos* proved highly toxic against growth of fungi.

20) Among essential oils, clove oil, camphor oil, Eucalyptus oil, neem oil and tulsi oil were highly inhibitory to the growth of seed-borne pathogens.

21) Neem leaf extract in ether and acetone inhibited the growth of *Alternaria alternata*, neem leaf extract in ether, diethylether and methanol inhibited to growth of *Aspergillus flavus*.

22) Fresh water algal extract also proved inhibitory to the fungi. *Chara grovesii* extract inhibited the growth of *Alternaria alternata* and *Aspergillus flavus*. Similarly, *Cladophora callicoma* inhibited *Curvularia lunata* and *Penicillium notatum* whereas *Syctonema coactile* inhibited *Curvularia lunata*, *Penicillium notatum* and *Alternaria alternata*. Which indicates that fresh water algae also have antifungal properties.

23) *Trichoderma viride* inhibited the growth of *Aspergillus flavus*, *Fusarium roseum* and *Penicillium notatum*. Whereas, *Trichoderma harzianum* inhibited the growth of *Curvularia lunata*.

24) Glucose nitrate medium proved favourable for the growth of tested seed-borne fungi.
D) **Toxin:**

25) Aqueous leaf extract proved effective against the toxin production of *Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium roseum* and *Penicillium notatum*. Whereas, *Datura stramonium, Ocimum sanctum, Polyalthia longifolia* and *Vitex negundo* also check the toxicity of tested seed-borne fungi. Due to which the percentage inhibition of seed, root length and shoot length also showed great variation.

E) **Enzymes:**

26) Leaf extract of *Azadirachta indica, Datura stramonium* and *Ocimum sanctum* inhibited amylase production of the seed-borne pathogens.

27) Protease production of *Alternaria alternata* was checked by leaf extracts of *Lantana camera* and protease activity of *Curvularia lunata* was controlled by *Azadirachta indica* and *Datura stramonium, Fusarium roseum* was by *Azadirachta indica* and *Penicillium notatum* inhibited in the presence of *Azadirachta indica, Datura stramonium* and *Ocimum sanctum* respectively.

28) Lipase production of tested seed-borne pathogens was controlled in the presence of leaf extract of *Azadirachta indica, Datura stramonium, Ocimum sanctum, Polyalthia longifolia* and *Vitex negundo*.

29) Aqueous leaf extract of *Azadirachta indica* followed by *Datura stramonium* and *Ocimum sanctum* inhibited growth of tested fungi.
Hence it proved eco-friendly biological control against the seed-borne pathogens.
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Screening of plant (leaf) extracts as a biopesticide

One hundred fifty one different plants (leaf) extracts were screened in order to know their antifungal nature against the five tested fungi at 10% concentration and the results are summarised in table 11.

It is observed that all the leaf extracts showed their toxicity against fungal growth. However, leaf extracts of Azadirachta indica proved highly inhibitory against all five test fungi followed by leaf extracts of Ocimum sanctum and Datura stramonium. However, Polyalthia longifolia, Lantana camera, Aegle marmelos, Jatropha curcas, Catharanthus roseus, Tridex procumbens and Vitex negundo are also proved their antifungal nature.

Effect of different concentrations of leaf extract on fungal growth

In order to know the fungitoxicity of Neem, Ocimum and Datura aqueous leaf extract of fresh leaves at different concentrations were studied and the results are given in table 12.

It is clear from results that the leaf extracts of Azadirachta indica showed highly inhibitory against test fungi at 10% concentration as compared with the Ocimum sanctum and Datura stramonium. However, the leaf extracts of Neem, Ocimum and Datura at 20% concentration showed moderately inhibition and proved completely inhibitory against the growth of test fungi at 25% concentrations.