REVIEW OF LITERATURE

Fungal diseases on medicinal plants in field:

It is clear from the literature that there is a significant information on the incidence of fungal diseases on crop plants. But such information is very insignificant in case of medicinal plants under cultivation as well as naturally growing.

*Chlorophytum borivilianum* is commonly known as safed musli. It’s an important medicinal plant for it’s energetic roots which contain saponins. Therefore farmers have started cultivation of this crop on large scale in Maharashtra. The crop has been found to be attacked by number of fungal pathogens in the field. Ramakrishnan and Ramakrishnan (1948) observed incidence of leaf rust caused by *Uromyces loculiformis*. Similarly, Chandra and Tandon (1965) recorded leaf spot due to *Colletotrichum chlorophytum*. Narsimhan et. al., (1966) rust due to *Uromyces cligniyi* and Rao and Narendra (1974) reported anthracnose due to *Colletotrichum dematium*. Mukherji et. al., (1986) recorded leaf spot due to two species of *Colletotrichum* viz. *Colletotrichum chlorophyti* and *C. dematium* and rust due to two species of *Uromyces* viz. *Uromyces loculiformis* and *U. cligniyi* on the crop of *Chlorophytum*. Recently Kunal Mandal et. al., (2004) recorded *Macrophomina phseolina* for the first time on the crop, causing leaf disease.
Rauwolfia serpentina is another important drug plant in Gujrat state, but in Maharashtra it’s cultivation has been found in some private gardens. It’s root contain different alkoloids and it is used in ayurvedic medicines. The plant has been reported to be infected by number of fungi in the field. Ramakrishnan et. al., (1950) reported leaf spot disease caused by Mycosphaerella rauwolfiae. Mohanty and Addy (1957) leaf spot due to Cercospora rauwalfiae, Chandra (1957) leaf blotch disease due to Cercospora rauwalfiae and Mohanty (1958) target spot disease due to Corynespora cassicola. Similarly, Ganguly and Pandotra (1962) reported leaf spot due to Alternaria alternata, wilting due to Fusarium oxysporum and powdery mildew due to Leveillula taurica. Janardhanan et. al., (1964) found wilting due to Fusarium sp., Varadrajan (1964) observed leaf spot due to Pellicularia filamentosa and anthracnose due to Colletotrichum gloeosporioides. Lal and Tandon (1966) reported leaf spot disease caused by Colletotrichum sp. Lele and Ram (1968) reported die-back due to Collectotrichum dematium. Mehrotra and Das (1976) reported disease on leaves due to Cercospora rauwalfiae and wilting due to Fusarium sp. Varadarajan (1996) recorded leaf spot due to Curvularia lunata.

Aloe barbadensis a member of family Liliaceae. The plant can be cultivated in almost all parts of Maharashtra, even under constant drought conditions. Aloe have long been in use in traditional medicine for treatment of digestive system, wounds, burns and skin troubles. Aloe gel can be obtained from the leaves. It contains mixture of glycosides called aloin. The
leaves of Aloe plants found to be infected by various pathogens in field. Ajrekar and Tonapy (1923) and Parndekar (1964) reported rust due to \textit{Uromyces aloes}. Roy (1976) observed \textit{Colletotrichum gloeosporioides}, \textit{Fusarium solani}, \textit{Pestolotiopsis vesicular}, \textit{Phoma sorghina}, \textit{Phomopsis aloes percrassae} and \textit{Alternaria alternata} on leaves. Kate \textit{et. al.}, (1997) and Gupta and Masood (2004) found leaf blight and leaf spot due to \textit{Alternaria alternata}.

\textit{Adathoda vasica} is another important drug plant. It’s cultivation on large scale around farm house in konkan region. The leaves contain important alkaloid vasicine, used in various cough syrups. Some fungal pathogens infected on leaves have been recorded as, Sydow \textit{et. al.}, (1966) reported rust disease due to \textit{Aecidium adhathode}, Chowdhary (1948) observed \textit{Cercospora adhathode} on leaves, Pandotra and Ganguly (1964) leaf spot due to \textit{Phyllosticta vasicae}. Similarly, Shreemali (1972) found new disease on stem due to \textit{Phoma vasicae}. Roy (1976) and Roy \textit{et. al.}, (1988) observed leaf spot due to \textit{Colletotrichum capsici}, \textit{Alternaria alternata}, \textit{Colletotrichum gloeosporioides} and \textit{Curvularia lunata}, anthracnose disease due to \textit{Drechslera speciferum}.

\textit{Centella asiatica} is commonly called as brahmi. It is also creeping plant. Now farmers have started cultivation of this crop under polyhouse condition in Maharashtra. Brahmi mainly contains saponins in the form of asiaticoside, madecassic acid. It is used for nervic tonic and improving memory power. This crop plant has been attacked by number of fungi on

*Emblica officinalis* belonging to family *Euphorbiaceae*. It’s fresh and dried fruits are use in various ayurvedic preparations like ‘Triphala churn’ and ‘Chyawanprash’. Amla fruit is a rich natural source of vitamin ‘C’. it also contains tannins. Amla fruits are largely used in Indian medicine. Fruits and leaves of plant has been attacked by number of fungi in field. Joshi (1958) recorded leaf, stem, fruit rust due to *Ravenelia emblicae*. Similarly Jain *et. al.*, (1960) observed same pathogen in Gwalior and Indore region. Tyagi and Prasad (1972) leaf rust due to *Ravenelia emblicae*. Nagaraja (1990) found same pathogen in Kolhapur district in Maharashtra.

*Zingiber officinale* is also called as ginger. It is medicinal as well as cash crop of Maharashtra. It’s cultivation almost in all the areas. The fresh and dry rhizomes of ginger used in medicines and condiments, ginger consist of volatile oil i.e. ginger oil. Aroma and flavour are the main character of ginger. The crop is particularly susceptible to the disease and often heavy losses occur in different localities. Mix (1949) reported infection of leaves due to *Taphrina maculans*. Leaves of ginger infected by different pathogens *Phyllosticta zingiberi* (Ramakrishnan, 1952), *Colletotrichum zingiberis* (Neema and Agarwal, 1960), *Colletotrichum*
Introduction


*Hemidesmus indicus* is also called as ‘Anantmul’. It’s cultivation largely in Gujrat, Rajasthan, U.P. etc. But in Maharashtra plants has been found in Western ghats in wild stage. The leaves of plants attacked by number of pathogens. Agarwal and Hasija (1961) recorded leaf spot due to *Pestalotiopsis carbonacea*. Kar and Mandal (1969) observed new cercospora sp. in West Bengal, Khanna and Chandra (1977) leaf spot due to *Fusarium equiseti*. Recently Pawar and Deotare (2001) reported white powdery mildew caused by *Oidium hemidesmi* from Gautala forest in Maharashtra.

*Azadirachta indica* belonging to member of family Meliaceae. The plant is found throughout India. In India it is very common in Maharashtra, Rajasthan, M.P., U.P., and Tamil Nadu. The neem seed contains non-edible fixed oil. It also contains Nimbin, Nimbidin possess anti-viral activity. It also contains glycerides of saturated and unsaturated fatty acids. The leaf extracts is useful for blood purification. The leaves and fruits infected by different fungi. Mitra (1935) recorded leaf spot due to *Cercospora*
*subsessilis*, same pathogen observed by Mundkar (1938) and Rao (1962). Uppal *et. al.*, (1935) found powdery mildew and leaf spot due to *Oidium* sp. and *Cercospora leucosticta*. Chawdhary (1957) and Jain *et. al.*, (1960) observed rust due to *Cercospora leucostica*, Narayanaswamy *et. al.*, (1968) powdery mildew due to *Oidium azadirachtae*, same pathogen observed by Sharma and Jain (1974). Recently Pawar and Deotare (2001) found powdery mildew and rust due to *Oidium azadirachtae* and *Cercospora* sp.

*Glycyrrhiza glabra* is commonly called as liquorice. It’s cultivation largely in Spain, England, Russia. But in India it is cultivated small scale in field. Nowadays in Maharashtra it is also cultivated in agricultural sector. It’s root contain triterpenoid saponin known as glycyrrhizin. The plant has been attacked by fungi. Chona *et. al.*, (1959) recorded leaf spot due to *Cercospora cavarae*, similarly same pathogen observed by Banerjee *et. al.*, (1966) on leaf.

*Curcuma longa* (Turmeric) is multipurpose ayurvedic as well as spice plant in India. It is main species of commerce and is cultivated for it’s rhizomes. The extraction of powder is carried out from rhizomes. Turmeric contains chief yellow colouring substances curcuminoids is known as curcumin. It’s official use in various pharmacopoeias. Turmeric plant is largely cultivated in Sangli district in Maharashtra. The leaves and rhizomes of plant has been attacked by number of fungi in field. Ramakrishnana (1954) recorded *Colletotrichum capsici* on leaves. Summanwar and Bhide (1962) leaf spot disease due to *Phyllosticta zingiberis*, Chowdhary (1966)

Andrographis paniculata belonging to family Acanthaceae. It’s common name is Kalmegh or Kirayat. It’s cultivation throughout India, in Maharashtra it is cultivated in small scale. The dried leaves and tender shoots are used in medicines. It contains active principles andrographolide. The plant affected by number of fungi in field. Thirumalachur and Govinda (1953) recorded leaf spot due to *Cercospora andrographidis*. Recently Roy (1989) found incidence of *Alternaria alternata* and *Botrydiploidia theobromae* on stems of Kalmegh.

*Datura metel* plant belonging to family *Solanaceae*. The plants are cultivated in agricultural field as well as in wild condition. The plant parts attacked by different pathogens recorded as, Rao (1962) recorded leaf spot of *Datura* caused by *Alternaria crassa*, same pathogen recorded by Siddiqui (1963). Ganguly and Pandotra (1962) observed leaf spot due to *Alternaria tenuissima*, Rao (1967) found *Phyllosticta solani* on infected leaves. Narayanswamy and Ramakrishanan (1968) observed powdery mildew due to *Oidium cyparissiac*. Recently Roy (1989) found
Sepegazzinia sundera and Cochliobolus specifer on seeds, tubers and stems of Datura.

Solanum viarum is commonly known as ringini. The plants actually grow in wild condition, but nowadays plants grow on agricultural sector on large scale. The fruits contain high amount of alkaloids. Kapoor and Hingorani (1958) recorded leaf spot and fruit rot of Solanum due to Alternaria alternata. Rao (1965) leaf blight due to Phytophthora parasitica. Singh and Seth (1970) observed Alternaria tenuis on leaves.

Asparagus officinalis and A. racemosus is commonly known as Shataveri. Asparagus has two species, A. officinalis is used as vegetables. It’s tubers are used in preparation of Shataveri soup. Another species A. racemosus is used in preparation of readymade energetic food. Both the species attacked by number of fungi in field. Kheswala (1936) reported Phoma disease due to Phoma asparagi on stem, Thirumalachur (1947) rust on cladodes due to Puccinia phyllocladiae. Shreemali (1973) observed fungus on stem due to Phomopsis armericae. Falloon and Grogan (1988) observed Phytophthora sp. on stem. Recently Roy (1989) observed fungi Pestalopsis laprogena and Nigrospora sphaerica on stems of Asparagus racemosus.

Abrus precatorius is another important drug plant, it is commonly called Gunj. It’s leaves has medicinal properties. Plants are generally grow in climbing habit. It’s leaves infected by airborne pathogens. Ramakrishnan and Ramakrishnan (1948) reported leaf rust due to Ravenelja ornata,
Sanwal (1951) found leaf rust due to *Ravenelia ornata*, Siddiqui (1957) leaf rust due to *Ravenelia ornata* and Tyagi and Prasad (1972) leaf rust due to same pathogen. Patwardhan (1966) reported powdery mildew due to *Acrosporium* sp.

*Withania somnifera*, it’s member of family *Solanaceae*. It is commonly called Ashwagandha. It’s roots are used in tonic. The roots contain major amount of alkoloids viz. Withanine. The different pathogens infected on plant parts in field. Sydow *et al.*, (1912) reported leaf rust caused due to *Aecidium withaniae*. Munjal recorded leaf spot due to *Cercospora withaniae*, Pavgi *et al.*, (1970) observed leaf spot due to *Cercospora withaniae*.

*Mentha arvensis* is one of the important medicinal plant. It’s leaves aromatic and scented due to volatile oil in their leaves. Ahmed (1990) recorded rust disease due to *Puccinia menthae*. Shukla *et al.*, (2001) observed stem blackening and stem rot due to *Botrydiploia theobromae*.

*Tinospora cardifolia* is a member of family *Menispermasae*. It’s commonly called as ‘Gulvel’. It’s stem has medicinal value and used in Jaundice, stem is generally climber. Ajrekar and Oza (1932) reported *Glomaerella cingulata* on leaves. Mundkar (1938) and Thirumalachur and Chupp (1948) observed leaf spot due to *Cercospora tinosporae*, Thirumalachur and Lacy (1951) leaf spot caused by *Phyllachora dolichospora*. Tilak and Kale (1970) found parasitic stem fungus due to


Piper longum is commonly called as ‘Pimpli’. It’s member of family Piperaceae. The fruit contains high amount of phenolic compounds. The quality of fruits is depend on infectious nature of the plant. Mundkar (1938) recorded leaf rot due to Phytophthora parasitica. Asthana (1946) observed leaf spot caused by pathogens Cercospora piperata. Rao (1962) leaf spot due to Cercospora piperata.

Ocimum sanctum is a sacred plant of Hindu religion. It’s cultivation is in Tulsi vrndavan and in field. Ocimum is a medicinal as well as aromatic plant, it contains volatile oil in their leaves. The leaves infected by different fungal pathogens. Munjal et. al., (1959) reported leaf spot due to Cercospora guatemalensis, Munjal (1960) leaf spot caused by Cercospora ocimicola, Pandotra and Ganguly (1964) leaf spot due to Cercospora canescans. Ahmed (1990) observed powdery mildew due to Oidium sp. in Arunachal Pradesh.
**Incidence of fungi on medicinal plant parts during storage:**

Medicinal plants undergo drastic chemical changes from field to factory due to microbial action. The plant samples collected from field or forests are stored in traditional warehouses where they are usually packed in gunny bags or spread as such as ground and have to face fluctuating environment and diverse range of microbes. During transport of medicinal plant parts to the market may involve various types of damages which may result into infections. Roy (1973) studied white rot disease on roots of *Chlorophytum borivilianum* caused by pathogen *Sclerotium rolfsii*. Cooke (1978) found that *Aspergillus phaeocephalus* was found on the roots of *Asparagus racemosus*. Malvia and Jain (1981) isolated root rot fungus *Macrophomina phaseolina* from roots of *Rauwolfia serpentina*. Roy et al (1988) isolated about seven fungi viz. *Aspergillus flavus*, *A. niger*, *A. candidus*, *A. luchuensis*, *A. ocharaceus*, *Fusarium moniliforme* and *Penicillium* sp., from infected roots of *Rauwolfia serpentina*. Chourasia (1990) reported that *Aspergillus flavus*, *A. niger*, *A. ocharaceus*, *Penicillium citrinum*, *Penicillium* sp. *Fusarium moniliforme* and *Fusarium* sp. were found to be associated on the surface of roots and seeds of *Withania somnifera*. Bordia *et. al.*, (1995) observed that *Chlorophytum borivilianum* tubers are infected by fungus *Aspergillus* sp. and *Fusarium* sp. during the storage of fleshy tubers. Chourasia (1990) isolated fungal pathogens *Aspergillus flavus*, *A. niger*, *A. ocharaceus*, *Penicillium citrinum*,
Penicillium sp., Fusarium moniliforme, Fusarium sp., were present on roots of Asparagus racemosus.

Rhizome rot of Zingiber officinale caused and reported by Pythium myriotylum (Bhagwat 1960), Curvularia lunata (Sahni 1966), Fusarium oxysporum (Rao 1966), Diplodia notalensis (Wilson and Balagopal, 1971), Sclerotium rolfsii (Haware and Joshi, 1973), Macrophomina phaseolina (Sarma and Nambiar, 1974). Roy et. al., (1988) isolated fungi Aspergillus flavus, A. niger, A. ocharaceus, Chaetomium sp., Penicillium citrinum, Rhizopus stolonifer from the rhizomes of Zingiber officinale. Chourasia (1990) reported rhizomes of Acorus calamus infected by saprophytic fungi Aspergillus flavus, A. niger, A. ocharaceus, Penicillium citrinum, Penicillium sp. Fusarium moniliforme, Fusarium sp. Dohroo (2001) reported fungal pathogens Pythium ultimum, Fusarium oxysporum, Verticillium sp. and Chlamydosporium sp. associated with storage rot of ginger. This disease was noticed in storage pits from January, which reached it's maximum intensity in April at 18.5°C temperature and 67.5% relative humidity.

Setty (1959) reported Penicillium islandicum a fruit rot disease of Emblica officinalis. Fruit rot disease of Emblica officinalis was caused and reported by Cladosporium herbarum, Pestalotia cruenta, Phoma sp.(Tandon and Verma 1964), Pestalotia cruenta (Tandon and Srivastava 1964), Pestalotia cruenta, Aspergillus niger (Srivastava et. al., 1964), Aspergillus niger, Fusarium sp, Penicillium oxalicum (Rao 1966),
Aspergillus niger (Srivastava and Tandon 1968), Penicillium oxalicum (Kulkarni and Sharma (1971), Herdersonula toruloides (Khanna and Chandra, 1975), Cladosporium cladosporioides (Jamaluddin, 1978), Phoma putaminum (Pandey et. al., 1980). Manoharachary (1975) isolated fungus Cladosporium oxysporum from fruit rot of Strychnos nux-vomica. Rajiv Kumar et. al., (1979) made an extensive screening of fungi associated with stored samples of Triphala using blotter test and two culture media. They found Asperglli were most frequent contaminant and Aspergillus niger was recorded in every sample and in all the tests. Chourasia et. al., (1987) reported Aspergillus flavus, A. niger, A. ochraceus, A. candidus, A. luchuensis, Chaetomium sp., Botrytis sp. on fruits of Piper longum.

Janardhanan and Ganguly (1963) was studied fungal flora of seeds of Belladona, Digitalis and Pyrethrum and their data indicated that most of fungi associated with seeds of medicinal plants were apparently externally borne and could be substantially eliminated by surface sterilization. Narayan and Prasad (1981) reported that minimum number of fungi were recorded on fresh seeds of Foeniculum vulgare (Fennel) and their number was found to increase gradually up to second year of storage and it remained constant in the third year. They further observed that Asperglli invaded the seeds during the first year of storage and their frequency increased during successive years. Dutta and Roy (1987) showed association of Aspergillus flavus, A. niger and Penicillium citrinum on seeds of Strychnos nux-vomica and S. potatorum. Roy et. al., (1988) isolated fungi from seeds of different


The above literature review shows that the micro-organisms containing different kinds of diseases on medicinal plants such as leaf spot, twig blight, root rots, rhizosphere mycoflora etc. However, the mycoflora of
stored raw medicines or crude drugs which cure the human diseases have not been investigated so far.

**Physical factors and storage mycoflora:**

A number of drugs absorb moisture during their storage and become susceptible to the microbial growth. The environmental conditions like relative humidity, temperature, moisture and storage conditions have been reported to affect establishment of drug mycoflora, their role on biodeterioration and mycotoxin contamination.

1. **Relative humidity:**

   It has been observed in most of the cases that a large number of fungi have been associated with stored medicinal plants/plant parts (Roy *et al.*, 1987). The effect of different levels of relative humidity on fungal association and aflatoxin production in *Piper longum* fruits was reported by Chourasia and Roy (1989). Christensen (1974) recorded different levels of relative humidity affecting invasion of different storage moulds.

   Dutta *et al.*, (1987) reported 96% relative humidity as favourable for maximum incidence of fungi on *Strychnos potatorum* and *S. nux-vomica* seeds. Roy (1989) noted that relative humidity above 90% was highly favourable for the maximum incidence of *Aspergillus niger, A. flavus, Fusarium sp. and Penicillium sp.* on twenty one crude herbal plant samples. Chourasia and Roy (1991) found 75% relative humidity as lowest limit and 96% relative humidity as highest limit for association of mycoflora on the seeds of *Neem* and *Datura*. Dohroo (2001) reported
storage rot of ginger disease was reached it’s maximum intensity in 67.5% relative humidity. Earlier investigations reported on association of various fungi with herbal drugs under storage, the highest incidence of fungi was recorded during monsoon season when the relative humidity ranged between 79-91% (Roy, 2003).

2. Temperature:

Temperature is also an important factor in preservation of the drugs. Bhikane and Mukadam(1982) reported the seed mycoflora of urid bean showed variations in types of seed-born fungi and intensity of fungal attack due to influence of temperature ranged between 10°C - 35°C. The variation in the occurrence of fungi as basic mycoflora and periodical mycoflora showed their appearance throughout the year due to wide range of temperature, which has been reported by Armolic et al.,(1956), Kennedy(1964), Sinha (1979) and Dutta (1988).

Dutta (1988) reported maximum incidence of seed mould at 30°C in the seed of *Strychnos potatorum* and *Strychnos nux-vomica*. Roy (1989) observed maximum incidence of seed born fungi on number of medicinal plant parts and seeds stored at 28.1°C to 33°C temperature. Dahroo (2001) reported storage rot of ginger disease reached it’s maximum intensity at 18.5°C.

3. Moisture:

Moisture is one of the important prime factors in colonization of moulds. A number of drugs absorb moisture during their storage and
become susceptible to the microbial growth. Some drugs absorb moisture to the extent of 25% of their weight. The moisture, not only increases the bulk of the drugs, but also causes impairment in the quality of crude drug. The excessive moisture facilitates enzymatic reactions resulting in decomposition of active constituents. Drug plant materials dried such as that moisture levels remain below to the limits might salvage from mould infestation reported by Bewly and Black (1985). Similarly, Chourasia and Roy (1991) reported that high moisture content of Neem seeds and Datura seeds allowed growth of maximum moulds along with *Aspergillus flavus*. Bagwan and Meshram (2004) reported that moisture content is 15-22% was found to be more suitable for mycoflora association and aflatoxin production.

**Fungal enzymes of mycoflora:**

Role of extracellular enzymes produced by stored drug plant parts fungi, during the process of deterioration of plants parts has been considered to be important ability to the fungi. The role of such enzymes specially amylases, lipases, proteases etc. in case of seed pathogens have been studied by few workers, as Vidhyasekaran *et. al.*, (1966) claimed that the production of extracellular hydrolytic enzymes by *Fusarium moniliforme* and *Aspergillus flavus* was found to be responsible in the spoilage of paddy seeds.

During the process of biodeterioration extracellular hydrolytic enzymes plays very important role in the invasion and establishment of
plant pathogen (Bateson and Miller, 1966, Wood, 1967). Regarding medicinal plant parts fungi very scanty information is available about their role in biodeterioration of medicinal plant parts.

**Carbohydrates and amylase production:-**

Preliminary studies on amylase production by various types of micro-organism in-vitro have been done by several workers as the species of *Penicillium and Aspergillus* (Le-Mense *et. al.*, 1947), *Alternaria tenuis, Fusarium coeruleum and Curvularia lunata* (Tondon *et. al.*, 1949). Das *et. al.*, (1961) reported amylase production in some pathogenic fungi. Production of amylase by fungi was found to be increased with the increase in the concentration of starch in the medium (Chapman *et. al.*, 1975). Effect of different carbohydrates on amylase production have been studied by Adams and Deploey (1976) and found that glucose, fructose and lactose when supplemented in the basal medium to be stimulatory. Wadje and Deshpande (1977) studied amylase production in seed mycoflora of jawar and found that the jawar meal medium superior than the starch medium.

Fashim *et. al.*, (1985) noted that *Aspergillus flavus* as more efficient amylase producer than *A. niger*. Stimulatory effect of different carbohydrates on amylase production have been reported in case of *A. niger* due to maltose (Barton *et. al.*, 1972), due to dextrin in case of *A. awamori* (Musaeva, 1967) and glucose along with starch in case of *A. flavus* (Khairnar, 1987). Charya and Reddy (1980) found in case of *Phoma exigua* and *Graphium penicillioides* from the seeds of Vigna radiata
that glucose, maltose, dextrin and starch were best carbon sources for amylase production. On the other hand, glucose alone proved inhibitory for amylase production in *Aspergillus oryzae* (Fenikrova *et. al.*, 1965), *A. fumigatus* and *A. terreus* (Venkateswarlu and Reddy, 1987).

**Nitrogen sources and amylase production:**

Among various inorganic nitrogen sources potassium nitrate and sodium nitrate stimulatory for amylase production in case of *Aspergillus flavus, A. fumigatus, P. italicum* (Singh and Agarwal, 1981). Regarding the effect of organic nitrogen sources peptone was found to be stimulatory in some seed-borne fungi of bajra (Khairnar, 1987). *Aspergillus flavus* also showed similar result in sorghum (Bhosale, 1989).

Effect of ammonium sulphate in *Aspergillus flavus* was stimulatory (Yang and Kong, 1976), while some of the ammonium forms of nitrogen proved to inhibitory for amylase production in seed mycoflora of bajra (Khairnar, 1987). On the other hand Bhosale (1989) reported that gelatin and urea proved inhibitory for amylase production in *Curvularia lunata* and *Fusarium oxysporum*.

**Phosphorus sources and amylase production:**

Yurkevich *et. al.*, (1967) reported *Aspergillus oryzae* produced maximum amylase in the absence of phosphorus. While Mahmoud *et. al.*, (1978) reported stimulatory effect of amylase in the presence of dipotassium hydrogen phosphate and potassium dihydrogen phosphate in case of *Aspergillus niger*. Similarly, El-Zalaki *et. al.*, (1980) found that low
concentration of potassium dihydrogen phosphate and dipotassium hydrogen phosphate stimulatory for amylase production. Whereas dipotassium hydrogen phosphate used in the basal medium at very low stimulatory for amylase production in *Aspergillus awamori* (Chung Won Hwi *et. al.*, 1987).

**Sulphur sources and amylase production:**

It is clear from the literature that among sulphur sources used in the based medium, effect of ammonium sulphate in *Aspergillus niger* was stimulatory for amylase production (Yang and Kong, 1976). Panchal (1984) noted that sulphur at 500 ppm concentration were tested against common seed borne fungi of jawar for the production of amylase where magnesium sulphate was found to be the best sulphur source for amylase production. Bhosale (1989) noted the same sulphur concentration against *Aspergillus flavus*, *Fusarium oxysporum*, *Curvularia lunata*, *Helminthosporium* sp. He found that magnesium sulphate was to be the best sulphur source. Whereas sodium bisulphate and ferrous sulphate are highly, *Curvularia lunata* and *Helminthosporium* sp. inhibitory for amylase production in *Fusarium oxysporum* as compared to *Aspergillus flavus*

**Vitamins and amylase production:**

Among the vitamins riboflavin was found to be stimulatory for amylase production by *Aspergillus terreus* (Jayaraman and Prasad, 1971) while in case of bajra vitamins at 50 ppm concentration proved inhibitory for enzyme production by seed borne fungi (Khairnar, 1987) whereas Yang
and Kong (1976) reported that vitamins like nicotinamide and biotin stimulatory in case of *Rhizopus niveus*.

**Antibiotics and Fungicides and amylase production:**

It is clear from the literature that, terramycin among the antibiotics was found to be stimulatory for amylase production when tested against rhizosphere mycoflora of cauliflower (Rao and Sharma, 1987). Similarly Khairnar (1987) found stimulatory effect of Streptomycin and Streptopenicillin for amylase production while Hostacyclin show inhibitory effect. Bhosale (1989) reported stimulatory effect of Penicillin, Streptomycin and Aureofungin in sorghum.

Among the fungicides like Benomyll and Bavistin proved highly inhibitory for amylase production in *Fusarium oxysporum*. On the contrary, Hexathir, Difoltan, Cuman, Dithane Z-78, Mancozeb with more or less degree proved inhibitory for amylase production in *Fusarium oxysporum* and *Helminthosporium langirostrata* (Bhosale, 1989).

**Incubation period and amylase production:**

**pH and amylase production:**

H\(^+\) ion concentration also affects the in-vitro secretion of enzymes. On the basis of pH, amylases produced by the microorganisms are of three types viz. acid amylases, alkaline amylases and neutral amylases. The optimum pH for the production of these amylases required is variable and having broad range.

Micro-organisms so far studied by various workers showed acidic pH required for amylase production. Charya and Reddy (1980) found pH 4.5 as optimum for amylase production in *Phoma exigua* and *Graphium penicillioides*. Otutiola (1982) has shown that the Maize mould *Aspergillus chevalieria* produced maximum amylase at a pH 5.5. Whereas Mangallam *et al.*, (1977) recorded pH 6.7 as optimum for *Sporotrichum thermophile* and it was pH 5.6 in case of *Talaromyces emersonii* (Oso, 1979).

**Temperature and amylase production:**

Temperature plays an important role in enzyme production by different fungi. Regarding the effect of temperature on amylase production, Khadilkar and Choudhari (1977) reported that in case of plant pathogenic fungi, *Alternaria solani*, *A. fumigatus*, *Curvularia lunata*, *Helminthosporium sativa* and *Rhizoctonia solani* temperature range 22°C to 30°C was found to be favourable for amylase production. In thermophilic fungi *Sporotrichum thermophile* optimum temperature for amylase production was 50°C (Mangallam *et al.*, 1977) and it was 40°C to 45°C for *Talaromyces emersonii* (Oso, 1979). Charya and Reddy (1980) found 40°C
temperature as optimum for amylase production in *Phoma exigua* and *Graphium Penicillioides*.

Micro-organisms having temperature range between 35°C and 40°C are reported as *Aspergillus flavus* and *A. fumigatus* (Adisa, 1985), While Bhosale (1989) reported that 25°C–30°C temperature range was optimum for amylase production in *Aspergillus flavus, Fusarium oxysporum, Helminthosporium* sp. and *Curvularia lunata*.

**Carbohydrates and lipase production:**

It is clear from literature that feeding of carbohydrates as a source of carbon affects the rate of lipase production. Glucose was found to be stimulatory in many cases such as *Candida regosa* (Valero et al., 1991) and *Penicillium requefostii* (Petrovic et al., 1990), *Rhizopus nigricans* (Chander et al., 1981), *Mucor racemosus* (Chopra et al., 1981), *Aspergillus wentii* (Chander et al., 1980). Maltose was also reported to be stimulatory in case of *Rhizopus rhizophodiformis* (Samad et al., 1990). *Penicillium verrrucosum* (Glenza & Jaballah, 1985). Dextrin was noticed in case of *Rhizopus delemar* (Martinez et al., 1993).

Sandikar and Mukadam (1992) found that, fructose, xylose and sucrose stimulated lipase production in *Alternaria carthami*, but glucose was found to have no effect on lipase synthesis in case of *Aspergillus ustus* (Reddy and Reddy, 1988). Chopra & Chander (1983) reported that in *Syncephalastrum racemosum* lipase production was found to be better in
presence of fructose than due to other carbohydrates. Glucose was proved to be inhibitory for lipase production in *Rhizopus delemar* (Haas *et al*., 1993).

**Nitrogen sources and lipase Production:**


**Amino acids and lipase production:**

Regarding the effect of amino acids, some work has been reported & it is clear that during the studies on lipase production in case of *Aspergillus flavus* (Sandikar & Mukadam, 1990) observed that serine, tryptophan cystine and seed powder of sesamum & groundnut supplemented in basal media supported lipase production significantly.
Incubation period and lipase production:

It is clear from the literature that, micro-organisms show varieties in their optimum period of incubation for lipase production. Incubation period is recorded two days in case of *Syncephalastrum racemosum* (Chopra and Chander, 1983), three days in *Penicillium simplicissimum* (Sztajer et al., 1992), *Penicillium expansum* (Stocklein et al., 1993), *Trichosporon fermentans* (Chen et al., 1992), *Aspergillus fumigatus* (Mohawed et al., 1990), *Rhizopus oligosporus* (Usami et al., 1989), *Rhizopus nigricans* (Chander et al., 1981) required incubation period of 4-5 days (Patil and Shastri, 1982). Micro-organisms which required optimum period more than a week for lipase production are *Thermomyces lanugenosus* (Venkateshwarlu et al., 1993), *Acromonium strictum* (Okeke and Okolo, 1990).

pH and lipase production:

On the basis of pH, lipases produced by the micro-organisms are of three viz. acid lipases, alkaline lipases and neutral lipases. The optimum pH for the production of these three lipases required is variable and having broad range. Micro-organisms so far studied by various workers showed acidic pH required for lipase production on *Rhizopus oryzae* (Salbh et al., 1993), *Aspergillus flavipes* (Savith and Ratledge, 1992), *Aspergillus fumigatus* (Mohawed et al, 1990), *Rhizopus oligosporus* (Nahar Ely et al., 1988), *Aspergillus sydowii* (Elwan et al., 1986), *Sacchromycopsis lypolytica* (Kralova et al., 1988), *Macrophomina phaseolina* (Reddy and

**Temperature and lipase production:**

Temperature is very important factor, which control various activities of micro-organisms. Optimum temperature can be classified into two groups, where the micro-organisms having optimum temperature below 30°C and another group of micro-organisms having optimum temperature range between 30°C and 50°C. In case of *Rhizopus oryzae* (Salbh et. al., 1993), *Aspergillus flaviceps* (Savith and Ratledge, 1992), *Candida regusa* (Wu et. al., 1990), *Aspergillus fumigatus* (El Sahed et. al., 1990), *Humicola lanuginosa* (Omar et. al., 1987). Micro-organisms having optimum temperature range between 20-30° are *Mucor racemosus* (Chopra et. al., 1981). However, some thermophilic micro-organisms have optimum temperature up to 60°C in case of *Thermomyces lanuginosus* (Venkateshwarlu et. al., 1993).
Mycoflora and Biodeterioration:

Medicinal plant parts are known to carry variety of fungi which during their association in field as well as during storage cause various types of harmful effects to the plant parts. The whole process is termed as biodeterioration.

During storage, the fungal organisms thrive in drug plant parts such as root, rhizome, fruit, and seeds by utilizing various components including degradation of protein, carbohydrates, lipids etc, where as loss in medicinally active ingredients from the drug plant parts have been reported by few workers.

a) Protein:

Proteins are the important constituents of the drug plant parts. During storage, change in seed content of medicinal plants are influenced both by fungi as well as physical factor (Wallace et. al., 1976 and Mondal et. al., 1981). The decrease in protein might be due to their enzymatic degradation into simpler components which are subsequently utilized by fungi (Cherry et. al., 1975). Loss in protein content of seed due to associated mycoflora has also been reported in case of different oil seeds as in groundnut (Nager and Chauhan, 1977), Sunflower (Ivanov, 1989) and safflower (Sandikar and Mukadam,1990), Mustard (Kumar and Prasad, 1993). On the other hand increase in protein content in the infested seeds have been reported by Conte et. al., 1989) in case of sunflower seeds.
Loss in protein content in the medicinal plants have also been reported by some workers. Dutta et. al., (1987) found in case of *Strychnos potatorum* and *Strychnos nux-vomica* seeds stored for 60 days showed maximum loss in protein content due to high incidence of mycoflora. Roy (1989) observed that *Aspergillus flavus* caused maximum loss in protein content of *Strychnos nux-vomica, Strychnos potatorum, Datura metal* and *Piper longum* seeds. While *Penicillium citrinum* was found to be more destructive of protein in case of *Syzygium cumini* and *Azadirachta indica* seeds. Bavaji and Sreeramula (2002) observed sesame leaves, where as decrease in protein content in infected leaves is due to the utilization of protein by the pathogen.

**b) Oil content:**

Seeds of some medicinal plants are mainly used for the extraction of oil for it's further uses in ayurvedic medicines. Therefore moulds associated with the seeds of some medicinal plants may cause degradation of oil both qualitatively and quantitatively. Unfortunately no work on these lines is available in the literature. In case of neem seeds but degradation of oil content of the seeds due to mycoflora have been reported by different workers in case of different crop seeds. Diener (1959) found in case of groundnut, Singh et. al., (1972) in sesame reported that association of mycoflora caused significant reduction in oil content resulting into increase in free fatty acid content. Loss in oil content of seeds has also been reported in case of mustard (Chahal and Kang, 1979), Sunflower (Singh and Prasad,
1985). On the other hand Lalithakumari et. al., (1971) showed increase in fat content with the infection of *Rhizoctonia bataticola*.

Sharma and Bhawmik (1987) found that groundnut seeds infected with *Macrophomina phaseolina* showed loss in oil content and change in the oil colour. Among the total mycoflora *Aspergillus niger* and *A. flavus* were found to be highly destructive to oil in the seeds of sesame (Singh and Prasad, 1979). Similarly Sharma (1981) found in case of sesame deterioration of oil due to *Aspergillus niger*, *A. flavus*, *A tamari*, *Penicillium citrinum*, *P. lilicenum*, *P. petulum* and *Cladosporium herbarum*. Prasad (1988) studied in case of safflower and reported that *Alternaria carthami*, *A. alternara* and *Aspergillus flavus* caused loss in oil content, change in colour and other physiochemical properties of oil. Rashmi and Mehrotra (1990) found that sunflower deteriorated seeds caused loss in oil content.

c) Degradation of active ingredients:

In case of medicinal plant parts, root, stem, fruit, and seeds undergo drastic chemical changes due to active principles like alkaloids, glycosides, phenolic compounds, essential oils are found to be degraded by number of fungi. Dutta and Roy (1987) in case of *Strychnos nux-vomica* and *S. potatorum* seeds found that degradation of alkaloids is due to *Aspergillus candidas*, *A.clavatus*. *A. flavus*, *A.luchuensis*, *A.niger*, *A. nidulans*, *A. ochraceus* and *A. sydowi*. Similarly Dutta (1988) also reported degradation of maximum alkaloids from *Strychnos* seeds due to *A. flavus*. He also showed that *Aspergillus niger* effectively reduces strychnine. Roy
changes in alkaloid, phenol and protein content of seed, fruit, and root samples of different drug plants by *A. flavus*, *P. citrinum*, *A. niger*, *Fusarium moniliforme*. The decrease in the concentration of all the important constituents (10-40%) was recorded under fungal infestation within 60 days of storage. The decrease in phenolics might be due to their enzymatic degradation of fungi.

Girisham and Reddy (1989) found in case of bajra seeds that there was increase in phenolic content due to *A. flavus*, while on other hand Roy and Chourasia (1988) reported decrease in phenolic & alkaloid content of *Mucuna pruriens* seeds associated with *A.flavus*. Roy (1989) also reported reduction in alkaloid content of *Strychnos potatorum & Strychnos nux-vomica* seed due to attack of storage fungi *A. niger, A. flavus and Penicillium citrinum*. Kumar & Roy (1996) reported that the inhibitory growth of *A. flavus* as well as toxin production due to presence of active ingrediants like alkaloids, phenolics & essential oils. *Plumbago zeylanica* contained highest amount of alkaliiods i.e 6.3%, *Ocimum sanctum* didnot contain alkaliiods, but highest amount of essential oil ie. 7%. Phenolics were detected maximal in the fruits of *Piper nigrum*. Roy (2003) reported degradation of alkaloids in *Strychnos potatorum* and *S. nux-vomica* seeds with in 90 days of infestation by *A.flavus A. niger, & Penicillium citrinum* was studied. Strychnine & brucine were identified in the seeds as the major alkaliiods, their concentration was significantly reduced under infestation.
Mycoflora and Aflatoxin:

Aflatoxins are known to produce very commonly by two species of *Aspergillus* viz, *Aspergillus flavus* and *Aspergillus parasiticus*. The former is more common then latter. Aflatoxins have been identified as $B_1$ and $B_2$ from isolates of *Aspergillus flavus*, while $B_1$, $B_2$, $G_1$ and $G_2$ from *Aspergillus parasiticus* (Nagarajan and Bhat, 1973). Hasseltine *et. al.*, (1966) reported Aflatoxigenic contamination in seeds of groundnut, maize, rice, wheat, rye, jawar, soyabean etc. due to attack of *Aspergillus flavus*.

Initial reports from India shows that less than 10% isolates of *Aspergillus flavus* are toxigenic affecting groundnut crop (Rao *et. al.*, 1965). Most of the *Aspergillus flavus* strains are able to produce only aflatoxin $B_1$ and $B_2$. Indulkar *et. al.*, (1971) reported in cotton seeds that 90% of strains out of 1800 strains of *Aspergillus flavus* were found toxigenic. Mehan and Chohan (1973) screened 21 isolates of *Aspergillus flavus* from cotton, maize, and wheat out of which 16 were toxigenic producing aflatoxins. Bilgrami *et. al.*, (1980) reported that out of 404 strains of *Aspergillus flavus* screened, 269 were positive for aflatoxin from the standing maize crop. Nagarajan and Bhat (1973) demonstrated more production of aflatoxin by the strains of *A. parasiticus* than those of *A. flavus*. Deo *et. al.*, (1981) reported twenty strains of *Aspergillus flavus* on lentil to be aflatoxigenic. Gupta *et. al.*, (1982) isolated nineteen strains of *Aspergillus flavus* from moth bean, out of which sixteen strains were aflatoxigenic. Reddy and Subbayya (1985) studied aflatoxin production in
blackgram seeds and stated that moisture is the limiting factor. Roy et. al., (1988) isolated fifty A. flavus strains from drug plant seeds out of which 21 were aflatoxigenic.

Roy et. al., (1988) reported 50 isolates of Aspergillus flavus obtained from different drug plants /plant parts, 21 isolates were found to be toxigenic, 12 isolates had potentiality to produce aflatoxin B₁, only seven had both B₁ and B₂ and only two produced B₁, B₂ and G₁. The growth of A. flavus under storage not only deteriorate the quality of drug plants but may also contaminate by elaborating aflatoxins. Roy (1989) screened 33 plant part samples for aflatoxin and found that all are aflatoxin contaminated. Bagwan and Meshram (2004) reported that ninety seven samples of dried fig fruits were collected from different places. The aflatoxin contamination in 97 sample varied from 0.6 to 39.0 µg/Ka. The moisture content in 15-22% was found to be suitable for aflatoxin production.

Chourasia and Roy (1989) reported aflatoxin contamination in fruits of Piper longum (Pippali) and stated that the level of aflatoxin B₁ production was high when the relative humidity was between 75 and 96%. Chourasia. (1990) reported five drug plant samples used for the preparation of churna, four were aflatoxin B₁ positive where it’s concentration ranged from 1.27 to 0.47 µg/g. of the 49 strains of Aspergillus flavus isolated from different drug plant samples, 22 were toxigenic and their potentiality to produce aflatoxin B₁ was in the range of 0.09 to 0.88 µg/ml of culture
fillrate, each drug contained aflatoxin B₁. Shivendra Kumar and A.K. Roy (1996) recorded an aqueous extracts of twenty four medicinal plants (parts) which have medicinal properties in curing different human diseases were screened for aflatoxin prevention. The root extracts of *Plumbago zeylanica* showed maximum aflatoxin B₁ prevention (81.54%), followed by *Ocimum sanctum* (80.03%), *Moringa oleifera* (78.81%), *Piper longum* (75.95%), *Lawsonia inermis* (73.88%), *Curcuma aromatica* (71.45%) and *Azadirachta indica* (70.46%). The growth of *Aspergillus flavus* was also significantly reduced by medicinal plant extracts.
MATERIAL AND METHODS

PART – I

A) Isolation of fungi from medicinal plant parts:

1) Collection of fungal diseases of medicinal plant parts from field:

An extensive survey on medicinal plant diseases in field was undertaken for two years (2003-2004). The repeated visits were made to observe disease incidence at different age of the crops during rainy, winter and summer seasons. The diseased plant parts such as roots, rhizomes, leaves and fruits of different varieties were collected from different localities of Maharashtra comprising four regions viz. Marathwada, West Maharashtra, Konkan and North Maharashtra. The diseased plant parts samples were kept separately in pre-sterilized polythene bags and brought into the laboratory, for the identification of the fungal diseases based on the necessary documents of literature.

2) Detection of storage mycoflora from different medicinal plant parts:

The storage mycoflora of different parts of medicinal plants such as roots, rhizomes, fruits and seeds were collected from store houses, godowns and ayurvedic shops of various localities of Maharashtra. The stored plant materials were kept seperately in pre-sterilized polythene bags and brought into the laboratory, for the further identification of storage mycoflora on the different plant materials such as roots, rhizomes, fruits and seeds were isolated by using Standard Blotter Method (SBM) and Agar Plate Method.
(APM) as recommended by International Seed Testing Association (ISTA, 1966) and Neergard (1973).

**a) Standard Blotter Method (SBM):**

A pair of white blotter papers of 8.5 cm diameter was jointly soaked in sterile distilled water and were placed in pre-sterilized petriplates of 10 cm diameter. The diseased plant parts such as root, rhizome were cut aseptically into pieces using sterile blades. These pieces were surface sterilized by using 0.1 percent solution of mercuric chloride for 2 minutes and subsequently thoroughly washed thrice with sterilized distilled water and root, rhizome and fruit samples were then placed at equal distance on the moist blotters. These plates were incubated at 25 ± 2°C under diurnal conditions for seven days.

**b) Agar plate method (APM):**

In this method, presterilized corning glass petriplates of 10 cm diameter were poured with 15 ml of autoclaved Potato Dextrose Agar (PDA) medium, on cooling the medium 10 seeds, root and rhizome pieces were placed in separate petriplate at equal distance aseptically. Incubation conditions and other details were same as described for the blotter test method.

In order to isolate only internal root, rhizome, fruit and seed borne mycoflora, these plant parts were pretreated with 0.1 percent solution of mercuric chloride for 2 minute and subsequently thoroughly washed thrice.
with sterilized distilled water and placed on solid agar plates. These plates were incubated at 25 ± 2 °C for 7 days.

c) Identification of fungi:

The fungi occurring on each and every diseased tissue portion in the plates were identified preliminary on the basis of sporulation characters like asexual or sexual spores and fruiting structures with the help of stereoscopic binocular microscope. The identification and further confirmation of the fungi was made by preparing slides of the fungal growth and observing them under compound microscope. Pure cultures of these fungi prepared and maintained on potato dextrose agar slants.

3) Composition of media used in isolation:

a) Potato Dextrose Agar (PDA):

Peeled potato–200 g, Dextrose–20 g, Agar-15 g, and distilled water 1000 ml, pH 5.6. Peeled potatoes were boiled until soft and passed through muslin cloth. Then dextrose was added in it and final volume of solution was made up to 1000 ml. In this solution agar was added, pH was adjusted.

b) Czapek Dox Agar (CDA):

Sucrose – 30 g, NaNO₃ – 2.0 g, K₂HPO₄ – 1.0 g., MgSO₄.7H₂O-0.5 g, Rose Bengal – 0.0001 g,

Agar – 20 g and distilled water – 1000 ml,

pH - 5.6
c) Martin’s Rose Bengal Agar:

Glucose –10 g, Peptone – 5.0 g, KH$_2$PO$_4$ – 1.0 g, MgSO$_4$. 7H$_2$O – 0.5 g, Rose Bengal – 0.001 g, Agar – 20 g, and distilled water – 1000 ml, pH–5.6.

B) Pathogenicity test:

Pathogenicity was tested using Koch’s postulates. The healthy and disease free plants were collected from field and planted in earthen pots filled with soil. The whole plant were surface sterilized with 0.1 percent mercuric chloride and washed with sterile distilled water for 3 times.

The fruits were injured by pin prick method and some leaves were injured by sterile blade at certain places. The inoculum was prepared from 7 days old culture of fungus grown on PDA slants. The spore suspension was inoculated or sprayed on the injured leaf and fruit portions under aseptically. The whole potted plant was then kept separately in disease free conditions for 7 to 15 days. The plant treated with sterile distilled water served as control. Both inoculated and control plants were kept in aseptic condition. The similar symptoms were observed on inoculated plants and pathogenicity were tested.

C) Enzymes of Fungal Pathogens:

1) Amylases:

a) Production:

Production of amylase was studied by growing the fungi on liquid medium containing starch 1%, KNO$_3$ 0.25%, KH$_2$PO$_4$ 0.1% and MgSO$_4$. 7H$_2$O  0.05%, pH of the medium was adjusted to 5.5 . Twenty five ml of the
medium was poured in 100 ml Erlenmeyer flasks and autoclaved at 15 lbs pressure for 20 minutes. The flasks on cooling were inoculated separately with 1 ml 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 7th day, the flasks were harvested by filtering the contents through Whatman No.1 filter paper. The filtrates were collected in pre-sterilized bottles and termed as crude enzyme preparations.

b) Enzyme assay (cup – plate method):

Determination of amylase activity was done with the help of cup-plate method which was adopted by Singh and Saksena (1982), Where 25 ml of starch agar assay medium (soluble starch – 10 gm, Na₂PO₄- 2.84 gm, Nacl – 0.35 gm, agar 20 gm, D.W. – 1000 ml and at pH 6.9). 15 ml of the medium were poured in each petriplate. On solidifying the medium, a cavity (8 mm diameter) was made in the center with the help of a cork borer (No.4) and was filled with 1 ml culture filtrate (Crude enzyme preparation). The plate 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 was obtained surrounding the central cavity. The diameter, which was measured (mm) as the amylase activity zone. Similar procedure was followed for the control except pouring of autoclaved culture filtrate in the central cavity instead of the active enzyme.
**Composition of media used for amylase production:**

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

1) **Starch nitrate medium :**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble starch</td>
<td>10 gm</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>2.5 gm</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>MgSO$_4$.7H$_2$O</td>
<td>0.5 gm</td>
</tr>
</tbody>
</table>

Dissolved in 1000 ml of D.W.

2) **Glucose nitrate medium :**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10 gm</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>2.5 gm</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>MgSO$_4$.7H$_2$O</td>
<td>1.0 gm</td>
</tr>
</tbody>
</table>

Dissolved in 1000 ml of D.W.

2) **Lipase:**

a) **Production:**

Lipase production was studied by using liquid medium containing crude groundnut oil 1%, KNO$_3$–0.25%, KH$_2$PO$_4$-0.1% and MgSO$_4$.7H$_2$O-0.05%, pH of medium was adjusted to 5.0. Twenty five ml of the medium was prepared in 100 ml Erlenmeyer flask and autoclaved at 15 lbs pressure for 30 minutes. The flasks on cooling were inoculated separately with 1 ml 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 7th day the flasks were harvested by filtering the contents through Whatman No.1 filter paper. The filtrates were collected in presterilized bottles and termed as crude enzyme preparations.

b) Enzyme assay (Cup plate method)

Determination of lipase activity was done with the help of cup plate method, where 20 ml of oil agar assay medium (oil 1% and agar 2%) were poured in each petriplate. On solidifying the medium, a cavity was made in the center with the help of a cork borer (No.4) and was filled with 1 ml culture filtrate (Crude enzyme). The plates were incubated at 28°C for 24 hours then they were flooded with 1% phenolphthalein as an indicator kept it 20-40 minutes activity zones are clearly seen. The diameter zone was measured (mm) as lipase activity.

**Composition of media used for lipase production:**

The synthetic media were employed for the production of lipase (s) in the preliminary experiment. Composition of media is given below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>1%</td>
</tr>
<tr>
<td>KN03</td>
<td>0.25%</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>0.1%</td>
</tr>
<tr>
<td>MgSO4.7H2O</td>
<td>0.05%</td>
</tr>
</tbody>
</table>

Dissolved in 100 ml of D. W.
ii) **Glucose nitrate medium:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10 gm</td>
</tr>
<tr>
<td>KNO₃</td>
<td>2.5 gm</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.0 gm</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.5 gm</td>
</tr>
</tbody>
</table>

Dissolved in 1000 ml of D. W.
PART – II

A) Biodeterioration of medicinal plant parts:

The roots, rhizomes, stems, fruits and seeds of different drug plant were surface sterilized separately with 0.1% mercuric chloride solution and washed twice with sterile distilled water. Excess water was discarded, the plant parts were distributed into sterilized conical flasks (25 g/flasks) and were inoculated separately with 2 ml spore suspension of different fungi of drug plants. The flasks were incubate at room temperature 1, 3, 6 and 12 months respectively and were harvested for recording chemical changes in the drug plant parts due to fungi. For which the plant parts were thoroughly washed under running tap water in order to remove mycelial growth from their surface. Subsequently the drug plant parts were dried at 60°C for 48 hours and crushed into fine powder for the estimation of different phytochemicals. For the control, plant parts were incubated in a similar manner but without inoculating the spore suspension.

Estimation of Dry weight:

Surface sterilized dry root, stem, and seeds of different drug plants were distributed into presterilized conical flasks (10 gm/flask). These flasks were inoculated separately with 2ml spore suspension of test fungi and incubated at room temperature for 1, 3, 6 and 12 months respectively. The roots, stems, and seeds were then washed under running tap water to remove mycelial growth from their surface. Subsequently the plant parts
were dried in oven at 60°C for 48 hours and loss in dry weight was calculated.

**Estimation of alkaloid:**

20 gms of finely powdered roots sample was soaked with 28% ammonium hydroxide (NH₄OH) solution for a few hours and then little dried up. This was latter soxhleted with the mixture of chloroform and ethanol (3:1) for eight hours. After that, 100 ml of solvent extract shaken with 25 ml N/2 sulphuric acid (H₂SO₄) and collected acidic extract. This process was repeated thrice for the total extraction of alkaloids. Collected acidic extract was then made alkaline with ammonia hydroxide solution, followed by chloroform extraction. Chloroform extraction was made twice with 20 ml and 15 ml chloroform respectively. Extract obtained was distilled on water both up to dryness and weighted on monopan balance.

**Estimation of glycoside:**

10 gm of coarsely powdered rhizome and macerate with 50 ml of 70% alcohol for 1 hour. Filter and retain the filtrate, then slowly add strong solution of lead subacetate until precipitation is complete, centrifuge and retain the supernatant. Then slowly add 6.3% sodium sulphate solution to precipitate excess lead, and centrifuge or filter as before. The aqueous supernatant contains the glycosides. Extract with successive 25 ml portions of chloroform and reduce the volume of chloroform to 5 ml by distillation. The glycosides are in the chloroform layer and can be crystallized out with evaporate the chloroform layer and weighed on monopan balance.
**Estimation of protein:**

Estimation of crude protein was made by Microkjeldahl method (A.O.A.C., 1965). 300 mg root powder was placed in 50 ml Microkjeldahl flask to which 60 mg catalyst and 7.5 ml of H$_2$SO$_4$ were added. The flasks were heated for 6-8 hours (digestion) till colourless digest is obtained. On cooling, the digest was diluted to 50 ml in a volumetric flask. 5 ml of the aliquot was introduced in a Markham’s distillation unit through the side tube funnel to which glass stopper was fitted. 10 ml of 10% NaOH solution was added into the funnel of side tube and was allowed it into the digest. The quantity of ammonia liberated was collected in 50 ml conical flask containing 5 ml of 2 % boric acid as an indicator. The distillate was titrated against 0.035 N Hcl till the end point (faint pink) was achieved.

1 ml of 0.035 N Hcl = 0.5 mg nitrogen. The amount of nitrogen obtained in aliquot and subsequently in total volume of digest per 300 mg root powder was calculated and the value was expressed as percent nitrogen. The crude protein was calculated by using the formula.

Percent crude protein = 6.25 × percent nitrogen.

**Estimation of phenolics:**

200 gms finely powdered sample was homogenized with 20 ml 80% warm ethanol and centrifuged. Supernatant was collected and mixed with equal volume of ferric chloride (0.3% ferric chloride in 0.4 N Hcl) and potassium ferricyanide (0.3%). Presence of blue green precipitation
confirms the presence of phenolics. This was further quantitated by spectrophometrically at 530 nm.

**Estimation of fat:**

Fat estimation was done by ether extraction method. 1 gm of seed powder was placed in a thimble, placed in an extractor at 40-45°C for one hour. Then the solvent ether along with extracted seed fat was poured in a preweighed disc. Ether was evaporated under fan and residue was dried overnight in an oven at 60°C. The dish was immediately transferred in a desiccator. On cooling, it was adjusted and the amount of fat extracted was reported as percent crude fat.

**B) Studies on Aflatoxins:**

**a) Screening of *Aspergillus flavus* isolates for aflatoxigenic nature:**

The strains of *Aspergillus flavus* were isolated from seeds, fruits, rhizomes, roots and stem samples of different medicinal plants and maintained on Czapek dox agar medium. These isolates were first screened for aflatoxigenic nature by organic yellow or reverse pigmentation method using *Aspergillus* differential medium (Bothast and Fennel, 1974) containing 1.5% tryptone, 1.0% yeast extract, 0.05% Ferric citrate and 1.5% agar. Intensity of organic yellow pigmentation on coconut agar medium (Lin and Dianese, 1976) was used as index for determining the aflatoxigenic nature of *Aspergillus flavus* isolates.
b) Production and Extraction of Aflatoxins:

The aflatoxin producing potential of *A. flavus* isolates was done on semisynthetic SMKY liquid medium (Diener and Devis, 1966) containing sucrose –200g, MgSO₄ 7H₂O-0.5 g, KN₀₃- 3.0 g and yeast extract 7.0 g, dissolved in 1000 ml water. Isolates of *A. flavus* were grown on SMKY medium contained in 100 ml Erlenmeyer flasks and incubated for 8 days at 25°C ± 1°C. After incubation period, contents of each flasks were filtered through Whatman No.1 filter paper. The filtrates of each isolates were collected in pre-sterilized bottles and termed as crude toxin preparations. The crude toxin preparation obtained was mixed with equal volume of chloroform in a separating flask and shaken well. After the preparation of two phases, the chloroform phase taken into a flask containing 10 g sodium sulphate to absorb any remaining water. The clear chloroform solution was concentrated to a known volume by evaporating chloroform and stored in a amber coloured vials under refrigeration until analysis.

c) Assay of Aflatoxins (TLC method):

The analysis of aflatoxins was done by using the method of Jones (1972). 30g.of silica gel ‘G’ (with CaSO₄ as binder) was taken in a stopper flask and mixed with 60 ml distilled water. The slurry was uniformly spread using applicator on clean plate keeping the thickness of layer 0.25 mm. The dry plate was activated at 110°C for 30 min. in hot air oven. The gel on plate was divided into number of lines by drawing lines with sharp needle.
The chloroform extract of toxins were then spotted in known volume in various lines carefully with microcapillary tube on an imaginary line 2.5 cm away from one end of the plate. The plate was developed in solvent system of toluene : ethyl acetate : formic acid (6:3:1) in a chromatographic tank for 50 min. till solvent reached up to 20 mm below the top end of the plate. Plate was dried at room temperature and the flourescing spots of toxins were visualized with UV lamp in a UV cabinet (360 nm). The blue flourescent spot were identified as aflatoxin B\textsubscript{1} and B\textsubscript{2} and on the basis of the intensity of the flourescent spots.

C) Biocontrol studies:

Use of plant extracts:

Fungitoxicity of plant extracts was studied by the poisoned food technique. Rose Bengal Agar medium was prepared in flasks and sterilized. To this medium, was added the requisite quantity of the plant extract to get 1:1 final concentration. The plant extract was thoroughly mixed by stirring. The medium was then poured into petriplates. Small disc (0.7 cm) of the fungus culture grown on Potato Dextrose Agar for 7 days was cut with a sterile cork borer and transferred aseptically in the center of the petridish containing the plant extracts. Suitable checks were kept where the culture discs were grown under the same conditions on Rose Bengal Agar without plant extract. The fungus colony diameter compared with check, was taken as a measure of the fungitoxicity.

Percent inhibition was calculated by using the formula.

\[
\text{Percent inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100
\]
EXPERIMENTAL RESULTS

PART - I

A) Fungal diseases of medicinal plants in field:

1) Leaf spot disease of *Aloe barbadensis*: (Plate-I/A-1)

During the course of survey of *Aloe* plant under field condition. The leaves of *Aloe* were found to be infected with leaf spot disease. The symptoms appeared in the form of small black spots, 1-2 mm size and which become ashy in the centre with blackish ring.

The mycelium of the pathogen was brown, septate and irregularly branched. The conidiophores were short, straight to slightly curved with varying number of septa with yellow brown colour, conidia were ovoid, beakless or with short beak, in chain with transverse and longitudinal septa. The pathogen was identified as *Alternaria alternata*.

2) Leaf spot disease of *Adathoda vasica*: (Plate-I/B-1)

The *Adathoda vasica* plants were cultivated around farm house in Konkan region. During the survey of *Adathoda* plants under field condition. The infected leaves showed large, irregular, dark black spots scattered over the leaf lamina. In severe cases the spots coalesced to involve larger surface area resulting into complete drying of infected leaves.

Conidiophores were dark, broader than hypha, straight, erect conidia in chains at the apex. The conidia were dark brown coloured, septate, muriform having rounded bases, tapering gradually towards the apices, hyaline beak. The fungus was identified as *Alternaria tenuis*. 

52
3) Leaf spot disease of *Centella asiatica*: (Plate-I/C-1)

The *Centella asiatica* plants were cultivated by Mr. Govande R. S. from Sangli district. He cultivated brahmi plants in polyhouse condition in 10 gunta. The leaf spot disease appeared in large numbers on upper surface of the leaf i.e. epiphyllous. Spots showed small, circular, semicircular brown coloured. The diameter of spot is 1-3 mm with thin dark brown margin and light brown in the centre. Attacked plants are not killed but due to excessive spotting and destruction of the green tissue of the leaf, there is stunted growth of plants and leaves are reduced in size.

The pathogen was identified as *Cercospora centellae*. Stroma poorly developed, substomatal, dull brown, pseudoparenchymatus, conidiophores caespitose, geniculate, uniformly thick. Conidia are straight to slightly curved, acicular, smooth, hyaline to pale brown, septa 6-14 apices acute to subacute, base truncate.

4) Leaf spot disease of *Chlorophytum borivilianum*: (Plate-II/A-1)

These types of medicinal plants were cultivated on large scale in field condition. Mr. Rajesh Sharma, Chopada, Jalgaon district developed 2 hectar farm under safed musli. The drug plant is severely affected by leaf spot disease. The symptoms appeared near the midrib of lamina. Generally eye shaped spot with thin dark brown margin and ash whitish centre. The centre often got detached to produce hole symptoms. While in the older plants large elongated spot with dark brown margin and light brown or ash coloured center appeared on infected leaves.
The mycelium was hyaline, septate and irregularly branched in the host cells. It produces single celled, club shaped conidiophores arise from the hymenial layer below the epidermis, and emerge directly through the epidermis. The conidiophores bear single conidia which are cylindrical to falcate, single celled, hyaline and mostly have blunt ends. The pathogen was identified as *Colletotrichum dematium*.

5) **Leaf spot disease of *Curcuma longa***: (Plate-II/B-1)

The *Curcuma longa* plants were cultivated by Agriculture Research Institute, Kasbe Degraj, Dist. Sangli. This institute cultivated various varieties of *Curcuma longa* plants on large scale. The spots appear in large number on both surfaces of the leaf, and covering a great portion of the leaf blade. Individual spots are 1-2 mm. in diameter. Infected leaves appear reddish brown, in contrast to yellowish green of healthy leaves.

The fungal hyphae present in the cuticle and epidermal layers of the host, when the spot are fully developed the hyphae collect at the subcuticular region and produce basal cells. From which short cylindrical to club shaped structures arise to push out from the host tissue and become transformed into asci and it contain ‘8’ ovoid, hyaline, unicellular ascospores. The fungus was identified as *Taphrina maculans*.

6) **Fruit rot disease of *Solanum viarum***: (Plate-II/C)

*Solanum viarum* is a very important medicinal plant in ayurvedic medicine. These plants were cultivated by Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar. The fruits of *Solanum* were found