The forest contributes major natural resource for maintenance of ecological balance. In India, and many other counties, natural forests are shrinking at a faster rate than expected. The density of forest cover is also diminishing. The natural forest ecosystems are the results of growth, development, interaction and stabilization of different species, both of plants and animals, over centuries. The ecological balance has been upset due to rapid growth of human population and subsequent increase in the demands for utilization of natural resources. As per FAO, 42 million acres (16.8 million ha) of forest area disappear every year globally, which in the tropics frequently lead to soil erosion and contributes to the loss of 15 million acres (6 m ha) arable land.

In Indian forests, different types of plants like timber plants, fuel wood plants, fruit plants, medicinal plants etc. are observed in large number. About 80% of the raw drugs being used by the Ayurvedic drug manufacturing units of India are obtained from wild. The supply from forest area account for 45% of the drug supply (Kurian and Sankar, 2007).

About 80% of the world depends on herbal-based alternative system of medicine. As estimated, 70,000 plants (including lower plants) are used as medicine. Indian Ayurveda utilizes about 2000 plants to cure different ailments (Daniel, 2005).

Medicinal plants collected by the tribal from wild or in cultivation, reach local market where the local trading centers purchase them. The traders in turn sell the raw material to wholesalers. At all these levels the raw materials are
stored in different containers. Right from the collection of different medicinal plant parts till their transportation to the manufacturing units, they are exposed to different environmental conditions, and they carry the mycolflora, Leaves, rhizome, fruits, seeds etc. of different plants, which are used as medicine carry mycoflora on them. Roy et al., (1988) reported association of different fungi viz. *Aspergillus flavus*, *A. niger*, *A. candidus*, *A. buchensis*, *A. ochraceus*, *A. nidulans*, *Alternaria alternata*, *Chaetomium sp.* *Fusarium moniliforme*, *Penicillium citrinum*, *Penicillium sp.* *Rhizopus*, *stolonifer* and *Trichoderma* sp. on seeds of *Emblica officinalis*, *Azadirachta indica*, *Datura metal*, *Abrus precatorius*, *Holarhena*, *antidysentrica*, *Strychnos nuxvomica*.

Seeds are used as medicine. These seeds are found to be frequently contaminated by fungi (Roy et al. 1988, Mamatha et al., 2000). Chaurasia (1990) investigated that almost all medicinal seed samples were associated with a large number of fungi. Some of these had heavy contamination of toxigenic *Aspergillus flavus* strains. The drug manufacturers without examining the raw drug samples from microbial association manufacture the finished herbal drugs. Therefore, it is essential to pay adequate attention to the medicinal seed mycoflora.

**Seed Mycoflora:**

Seeds of medicinal plants, like those of agricultural and horticultural crops, carry a wide variety of micro-organisms like fungi, bacteria and even some viruses. Seeds may be attacked by the microbes while still borne on the
trees in the field, during storage and subsequent handling before use. Therefore, the study of mycoflora of medicinal seeds is essential.

Mycoflora of 9 medicinal seeds, 8 from the forest of Aurangabad district and 1 from Gujrat was studied by both, blotter and agar plate methods. In all 8 fungi were recorded by blotter method (Table 5.1) and 32 were recorded by agar plate method (Table 5.2). By agar plate method, 24 additional fungi than blotter method were detected, 8 fungi were common in both the methods. Therefore, it can be concluded that agar plate method is superior to blotter method for isolation of seed mycoflora of medicinal seeds. Similiar observations have been made by various workers (Sahu and Agrawal, 2001; Ghyare, 2002)

Afzal et al., (2010) report that for isolation mycoflora associated with the seeds of sunflower, by both the agar plate and blotter paper methods, are nearly equally effective, as total of 12 fungi were isolated by agar plate method and 11 fungi by blotter paper method. Similar observation has been made by Shrivastava et al., (2002) with Soyabean seeds. Joshi and Gupta (1980) studied seed mycoflora of Echinochloa tomentosa, an important millet crop of Kumaun and Garhwal regions. They reported that some fungi viz. Chaetomium globosum, Nigrospora sp., Acremoniella atra, Cochliobolus spicefer and Fusarium semitectum did not appear on agar plate but were detected on moist blotter. Probably the advantage with blotter method is due to the slow growth of fungi on seed surface, in comparison to the agar plate technique, where fast growing fungi do not allow the detection of slow growing ones.
Maximum numbers of fungi were recorded on *Tectona grandis* and *Butea monosperma* followed by *Pongamia pinnata, Madhuca longifolia* and *Thespesia populnea* (Table 5.2). It is interesting to note that the fungus *Aspergillus niger* and *Rhizopus oryzae* are present on the seeds of all 9 medicinal seeds and *A. flavus* on 8 seeds. Association of species of *Aspergillus* has been observed in seeds of crop plants by various researchers (Joshi and Gupta, 1980, Basak and Mridha, 1985, Afzal *et al.*, 2010).

It appears that the fungi which could not be detected by blotter method, require certain additional nutrients for their growth, which are made available to them, on the agar media used.

Sinniah *et al.*, (1983), Roy *et al.*, (1988) have also found these and some other species of Aspergilli consistently on all the samples of Neem seeds during their studies. Roy and Kumari (1991) observed *Aspergillus* and *Penicillium* species with seeds of some medicinal plants, Mamatha *et al.*, (2000) observed seeds of some forest trees associated with *Aspergillus flavus, A. niger* and *Rhizopus* sp. Roy (2003), while discussing about mycological problems of crude herbal drugs, stated that the basic mycoflora of medicinal plants include *Aspergillus flavus, A. niger, Fusarium sp., Rhizopus sp.*etc.

When different agar media *viz.*, Czapek Dox Agar(CZA), Glucose nitrate Agar(GNA), Malt Extract Agar(MEA), Potato Dextose Agar( PDA), Rose Bengal Agar(RBA) and Seed Extract Agar (SEA) were used for the isolation of mycoflora of medicinal seeds. It was observed that PDA was the best medium
for the isolation (Table: 5.3 to 5.11). Similar observation has been made by Ghyare (2002) for the isolation of mycoflora of 5 timber tree species.

It is known that different fungi require different pH for their growth. The experiments conducted to study the effect of percent incidence of seed borne fungi of medicinal plants under investigation; it was observed that, at pH 5.6 maximum number of fungi developed (Table: 5.12-5.20). It was also observed that highly acidic (2.5) or alkaline (8.5) pH were inhibitory for the growth of seed mycoflora. Interestingly it was also noted that *Brooksia tropicalis* has grown only at pH 5.6. Certain fungi like *Cephalophora irregularis*, *Chaetomium cochliodes*, *C. globosum*, *Cunninghamella echinulata* and *Syncephalis cornu* have developed at pH 5.6 on *Tectona grandis* seeds. Occurrence of maximum number of fungi at pH 5.6 has also been observed by Bhikane (1988), and Ghyare (2002).

The data on effect of incubation period on the percent incidence of seed borne fungi of 9 medicinal plants revealed that, 7 days of incubation period caused maximum incidence (Table : 5.21 to 5.29). Ghyare (2002) also made similar observation incase of isolation of seed mycoflora of 5 timber plants. It was also noted that with increase in incubation period, there was increase in percent incidence of mycoflora. It has been observed during the course of present investigation, that incubation temperature at 25ºC was favorable for the seed mycoflora development of the medicinal seeds. (Table: 5.30 to 5.38). Similar observation has been reported by Sagar *et al.*, (2007) in case of fungi
isolated from various soil samples. They have observed 20 fungi at 5°C and 7 fungi at 55°C.

However, Tripathi (1974); Datta (1998) reported 30°C as optimum incubation temperature for mycoflora of Jowar and *Strychnos potatorum* and *S. nux-vomica* respectively.

Development of mycoflora on the seeds is certainly harmful causing loss in viability of seed and, pre and post emergence mortality of seedlings. (Asmol *et al.*, 2001). Inhibition of seed germination due to heavy infection has been observed by Khati and Pandey (2004), Dwivedi *et al.*, (2006). Variation in seed mycoflora related to the seed surface has been found. The occurrence of more mycoflora on vegetable types of beans may be due to soft seeds and tender nature of the plants. More carbohydrate content in vegetable type variety may be ascribed for more mycoflora in these varieties (Sud *et al.*, 2005). Minimum number of fungi was found adhering to the seeds of *Semecarpus anacardium*, *Madhuca longifolia* and *Plantago ovata*, which were having smooth surface.

**Storage mycoflora:**

Spoilage of seed in storage and mortality of seedlings in the nursery by seed-borne pathogens are the serious problems. These problems can be tackled in an effective manner if sufficient information of storage fungi of respective plant species is available. An attempt has been made in the present investigation to study the seed borne storage fungi. The seeds were stored in gunny bags at room temperature and the mycoflora was isolated using PDA
medium. The data obtained (Table 6.1 to 6.9) reveal some interesting results. In the seed samples of 9 medicinal plants, the mycoflora varied with respect to fungal species as well as their percent incidence.

In case of *Azadirachta indica* seeds, initially there were 7 fungi (Table 5.2) which increased to 9, during storage period of six months in gunny bags (Table 6.1). The two new fungi observed after storage were *Fusarium solani* and *Phytophthora indigoferae*. The number of storage fungi was increased to one during storage in case of *Butea monosperma* seeds (Table 6.2). *Trichoderma aureoviride* which was the only fungus eliminated during storage; while 3 fungi *viz.* *Pythium echinulatum*, *P. indigoferae* and *P. salpingophorum*, developed after storage. On the seeds of *Holarrhena pubescens*, 7 new fungi developed during storage (Table 6.3). These were *Aspergillus carbonarius*, *Cladosporium cladosporioides*, *Fusarium solani*, *Penicillium corylophilum*, *Pythium intermedium* and *P. salpingophorum*. In case of *Madhuca longifolia* seeds, two fungi *viz.* *A. terreus* and *Alternaria (Trichoconis) padwickii* (Table 6.4) were eliminated. However, 4 new fungi were recorded after the storage period of 6 months. These were *C. cladosporioides*, *Penicillium corylophilum*, *Pythium salpingophorum*, and *Syncephalis cornu*. During storage, the fungus *Pythium echinulatum* was eliminated from the seeds of *Plantago ovata* (Table 6.5). However, 5 fungi, *Aspergillus carbonarius*, *Penicillium corylophilum*, *Pythium indigoferae*, *P. intermedium* and *P. salpingophorum* were observed on the seeds after storage for 6 months. However, not a single fungus was eliminated on the seeds from *Pongamia pinnata* (Table 6.6). *Cladosporium*
cladosporioides, Pythium indigoferae and Rhizoctonia oryzae were the 3 new fungi that were observed on the seeds after storage. None of the fungus was eliminated from the seeds of Semecarpus anacardium during storage period of six months in gunny bags (Table 6.7). However, six new fungi viz. Aspergillus carbonarius, Cunnighamella echinulata, F. solani, Pythium intermedium, Syncephalis cornu and Trichoderma aureoviridae, were observed on the seeds during storage. In case of Tectona grandis seeds although three species of Chaetomimum- C. brasilience, C. cochliodes and C. globosum were eliminated during storage (Table 6.8), while 4 new species of fungi were observed. They were A. carbonarius, Penicillium corylophilum, Pythium, echinulatum and P. salpingophorum. After storage, 5 new fungi were observed on the seeds of Thespesia populnea (Table 6.9), these were A. kanagawaensis, F. oxysporum, F. solani, Monilia implicata and P. corylophilum. 

The fungi which were eliminated during storage may be because they are weak fungi. These fungi were Trichoderma aureoviridae, A. terreus, Alternaria (Trichonis) padwickii, Pythium echinulatum, Chaetomium braslence, C. cochliodes and C. globosum. It is also observed from the data presented in Table (6.1 to 6.9) that certain new fungi were observed on the seeds after storage. These were F. oxysporum, F. solani, Pythium echinulatum, P. indigoferae, P. salpingophorum, P. intermedium, A. carbonarius, A. kanagawaensis, Penicillium corylophilum, Cladosporium, cladosporioides, Syncephalis cornu, Rhizopus oryzae-sativae, Cunnighamella echinulata, Trichoderma aureoviridae, Monilia implicata. On 9 medicinal seeds tested,
*Rhizopus oryzae* was the fungus which had occurred on all the medicinal seed samples during storage. Observation of new fungi after storage was also observed by Bhikne (1988). It appears that the newly appearing fungi might be present on the seeds initially, but they might be requiring certain incubation period for their development. It was also noted that species of *Aspergillus* and *Pythium* were dominant during the storage period. Chomchalow (2003) stated that *Aspergillus and Penicillium* are the important fungi that are generally associated with stored products. *Aspergillus flavus, A. terreus, A. candidus, A. niger, F. moniliformae, Penicillium corylophilum*, were observed as common storage fungi with Chilli fruit (Prasad *et al.*, 2000).

As the number of fungi increased during storage for 6 months and, as some of the storage fungi caused heavy incidence on the seed (up to 80%); it was felt that, this may be because of the gunny bags which were used as storage containers. Vijayalaxmi and Rao (1985) also observed maximum mycoflora on the seeds of sunflower, when stored in gunny bags.

The experiment conducted to study the effect of storage containers on the incidence of seed mycoflora of 9 medicinal plants, showed promising results. When the seeds were stored in cotton bags, polypropylene bags polythene bags, tin boxes and plastic bottles; reduced the mycoflora in descending order. Storing seeds in plastic bottle eliminated maximum storage fungi during the present study.

Singh *et al.*, (1986) used different containers for storing wheat grains. They observed that the grain stored in gunny bags had significantly less
incidence of storage microbes, which may be said to be due to better ventilation. Proper ventilation condition results in lowering the temperature due to exchange of moisture between the grain and atmosphere; and thus help in reducing the level of mould attack.

Different workers have used different containers like tin boxes, cloth bags, earthen pods, gunny bags, paper bags, polythene bags, glass bottles and plastic containers etc. for storage of seeds (Dwivedi and Shukla, 1990; Verma et al., 1993; Purohit and Jamaluddin 1993). Based on their research, Dwivedi and Shukla (1990) suggested that polythene bags be preferred to other containers to preserve the seed viability and lesser seed invasion by the fungal flora during storage.

Purohit and Jamaluddin (1993) observed that glass bottles, plastic boxes and tin boxes were better for storage purpose of *Butea monosperma* seeds. In these containers, less incidence of mycoflora was observed. Nema et al., (2006) drawn an inference from their work, that the Niger seeds may be stored safely in permeable/ semi-permeable storage containers for 6 months.

Conclusion can be drawn that for reducing seed mycoflora, a proper container should be used. Storage of crude herbal seed samples under hygienic conditions with low moisture content (about 8%) and low temperature protect them from mould, insect infestation; should avoid moulding of these samples, and consequently reduce the risk of aflatoxin contamination (Roy and Kumari, 1994).
During present investigation *Aspergillus niger* and *Rhizopus oryzae* were found to persist on all the medicinal seeds tested, even after storage for 6 months. Roy *et al.*, (1988) found that none of the plant drug samples were free from storage fungi. The growth of *Aspergillus flavus* under storage not only deteriorate the quantity of drug plants but may also contaminate by elaborating aflatoxin. They observed this from the experiments with 6 medicinal seeds from different plants including *Azadirachta indica* and *Holarrhena antidysentrica*.

**Fugal metabolites and seed mycoflora:**

The culture filtrates of different fungi are known to produce inhibitory effect on seed germination and seedling growth (Parthasarthy and Hiremath, 1983; Pandey *et al.*, 1982). Probably non enzymatic phytotoxic substances play an important role in these processes (Parthasarthy and Hiremath, 1983).

Data presented in Tables 7.1 -7.8 and plate 7.1 to 7.8 reveal that the culture filtrates of 8 different seed borne fungi of 9 plants *viz.* *Azadirachta indica*, *Butea monosperma*, *Holarrhena pubescence*, *Madhuca longifolia*, *Plantago ovata*, *Pongamia pinnata*, *Semecarpus anacardium*, *Tectona grandis* and *Thespesia populnea* reduce their seed germination percentage substantially. In addition, they also reduce radicle length of the germinated seeds. The percentage inhibition of seed germination and radical length varied with different fungal species. In all the seed samples, maximum inhibition of seed germination was observed in the culture filtrate of *Aspergillus flavus* and *Penicillium corylophilum*. 
Abraham (1978) reported that the culture filtrate of *Alternaria solani*, *Fusarium moniliformae*, *F. oxysporum* f sp. *melonis*, showed pronounced inhibitory effect on seed germination. Parthsarthy and Hiremath (1983) reported 100% inhibition of germination of the seeds of Fenugreek by the culture filtrate of *Rhizoctonia solani*. Reduction in seed germination and root and shoot length has also been reported by Pandey *et al.*, (1982) in case of *Setaria italicca* seeds. However, Singh (1984) reported a different phenomenon. Of the 31 fungi isolated from wheat seeds, the metabolites of only 11 fungi exhibited appreciable inhibitory or stimulatory effect on wheat seed germination and seedling vigour. They observed that the metabolites of only 3 fungi reduced the seed germination and seedling growth, and inhibition of seedling growth by variable number of fungi. Ali and Singh (1992) reported that the culture filtrates of 15 isolates of *Sclerotinia oryzae* showed retardation of radical and plumule elongation of rice seeds. They observed more adverse effect of culture filtrates on plumule than on the radicle. Gurjar and Singh (2003) studied the effect of cultures filtrates of *Fusarium oxysporum*, *F. moniliformae* and found that it caused significant reduction in seed germination. They also observed reduction in length of radicle. Samota and Singh (2006) isolated 8 fungi from the seeds of coriander, which were *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. alternata*, *Curvularia lunata*, *F. solani*, *F. clamydiosporium* and *Rhizopus stolonifer*. They observed that culture filtrate of all the fungi caused reduction in seed germination, plumule and radicle length. Kavitha and Vijayalaxshmi (2007) observed that the culture
filtrate of *Curvularia maculans*, *A. terreus* and *Fusarium* sp. severely affected seed germination, adversely affected root elongation and hampered shoot elongation.

When the toxicity of the culture filtrates of 8 common seed borne fungi of 9 medicinal plant species under investigation *viz. Aspergillus carbonarius*, *A. flavus*, *A. niger*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Penicillium corylophilum*, *Pythium indigoferae* and *Rhizopus oryzae* was assayed, by leaf necrosis method on the leaves of these plants; it showed toxic effect. Developments of necrotic spots were observed on all the leaves by these culture filtrates. The size of the necrotic spots varied with the test leaves as well as the culture filtrates of the respective fungus (Table 7.9). The spots developed by the culture filtrate of the 8 fungi on *Plantago ovata*, *Pongamia pinnata*, *Semecarpus anacardium*, and *Thespesia populnea* were more in diameter than on other leaves. This suggests that the leaves of these plants are more susceptible for the metabolites of fungi. Sherwood and Lindbert (1963) identified the phytotoxic substance as O-nitrophenyl-beta-D-glucoside in the culture filtrate of *Rhizoctonia solani*. Presence of nonenzymatic phytotoxic substances in culture filtrates of *R. solani* has been indicated by several workers (Newton and Meyers, 1935; Vasudeva and Sikka, 1941; Nishimura and Sabaki, 1963). Shetty *et al.*, (1994) observed contamination of developing seeds of rice, sorghum and groundnut; and isolated species of *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. They found that these fungi were most toxigenic and detected Aflatoxin B1, T-2 toxin and Ochra toxin in the seeds.
Nahdi (1996) observed that in the population of *Aspergillus flavus* only some of the strains produced Aflatoxin. Singh *et al.*, (2001) also found that out of 75 isolates of *A. flavus*, 27 were toxigenic and produced Aflatoxin in culture medium. Elshafie *et al.*, (2002) while working with spices found that none of the 15 samples, contaminated by *A. flavus*, were found to contain Aflatoxin. Ahmad and Sinha (2002) reported mycotoxin producing potential of the fungi isolated from stored mustard seed. They reported that out of 66 isolates of *A. flavus* only 48 were toxigenic; of 34 isolates of *Fusarium moniliformae*, 13 were toxigenic; and of 12 isolates of *Penicillium citrinum* 4 were toxigenic. Roy and Kumari (1991), while working with seeds of some medicinal plants found that strains of *A. flavus* and *P. citrinum* produced Aflatoxin and citrinin. Koul and Sumbali (2008) detected Aflatoxin B$_1$ and B$_2$ from some medicinal important dried rhizomes of *Curcuma longa* and *Zinziber officinale*. Gupta *et al.*, (2009) isolated 389 fungal strains belonging to 18 species from Chilli. They reported that 37- 67 % isolates of *Aspergillus* spp., 14 -40% *Fusarium* sp. and 20- 42% of *Alternaria* spp. were toxin producers. All the isolate of *Aspergillus* produce Aflatoxin, while *Fusarium* spp. produced zearalenone.

**Pectolytic Enzymes:**

It is now well established fact in literature that cell wall degrading enzymes are involved during the pathogenesis by microbes. Of these, pectolytic and cellulolytic enzymes are studied in detail, in various laboratories. (Vidhyasekharan, 1978; Papdiwal 1982; Upreti and Joshi 1984; Sandhoo and
Arora, 1985; Mehta et al., 2007). Pectolytic enzymes are classified by various authors. However, broadly 4 enzymes are recognized viz., macerating enzyme, pectinesterase, polygalacturonase and transeliminase. Of these only production of macerating enzyme by the 8 storage fungi was studied during the present investigation and it was found that all the storage fungi screened, produced the macerating enzyme. It was observed that the enzyme production was adaptive in nature. Adaptive production of pectic enzymes has been reported by several workers (Gupta, 1956; Papdiwal and Deshpande 1979; Papdiwal and Korekar 2001; Pawar and Papdiwal 2009). However, constitutive production of macerating enzyme has also been reported in literature. (Wood 1955; Heale and Gupta, 1972).

Cellulolytic Enzymes:

The production of cellulolytic enzymes has been reported by several workers (Ghewande, 1976; Mehta et al., 2007; Pawar and Papdiwal, 2009). The cellulolytic enzymes have also been classified by various workers. They are classified as endoglucanase, exoglucanase and β-glucosidase. In another classification, it is classified as C₁, Cₓ, and cellobiase. During present research, the cellulase enzyme activity was tested by viscometric method. All the 8 storage fungi of the medicinal seeds produce the enzymes adaptively. Similar observation has been made by Ghewande (1976), Pawar and Papdiwal (2009). Various workers found a positive co-relation between cellulase production and virulence. This has been demonstrated in case of certain bacterial pathogens.
viz. Xanthomonos campestris pv. campestris (Gough et al., 1998) Pseudomonas solanacearum (Roberts et al., 1988) etc.

During present investigation no correlation was found between mycelial growth and enzyme production. Similar observation has been made by Sujatha et al., (2006). Very high cellulolytic and pectolytic enzyme activities of 10 seed borne fungi have reported by Ranjan et al., (2009).

**Amylase:**

Amylase produced by fungi play very important role in seed deterioration. The seeds which mainly contain starch, are degraded by fungi having amylase producing ability. During present study, all the 8 storage fungi were found to possess the ability for the production of enzyme amylase. The enzyme production was found to be adaptive. Similar observation has been made by Pawar and Papdiwal (2009a). During present investigation, it was observed that there is correlation between growth and amylase production; which has also been reported by Pawar and Papdiwal (2009).

Extracellular synthesis of two mould fungi- Aspergillus flavus and Penicillium purpurescense has been observed by Olama and Sabry (1989). Storage fungi of Rice seed viz. Aspergillus flavus, A. glaucas, A. niger, A. versicolor and Penicillium sp. were studied for α-amylase activity. It was found by Purushotham et al., (1996) that all these fungi showed the enzyme activity. Among different fungi A. flavus was the most deteriorative of rice quality followed by A. glaucas and A. versicolor. The differential activity of this
fungus may be due to production of different types of secondary metabolites. Sager et al., (2006) observed amylase production by 27 fungi. 6 highly pathogenic fungi (Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Phytophthora, sp. and Rhizoctonia solani) were isolated from different medicinal plants by Jadhav and Shinde (2008). All these fungi produced amylase, and sucrose was found stimulatory for amylase production.

**Lipase:**

The involvement of enzyme Lipase in the deterioration of oil seeds is reported in literature. Several fungi viz., A. niger, A. fumigatus, A. terreus, Penicillium chrysogenum, P. fumiculosum, Fusarium solani, F. moniliformae, F. vasinfecta, Alternaria alternata have been reported to produced enzyme Lipase (Abdel – Fattah, 2002). During present investigation also the 8 storage fungi of medicinal seeds were found to exhibit Lipase enzyme activity. Its production was found adaptive (Table 7.13). Constitutive production of enzyme Lipase has also been observed with different species of Fusarium (Waghamare et al., 2009); Saraswat and Mathur (1985) investigated 17 storage fungi from Lin seed for Lipase production. They observed that all the 17 fungi, majority of which were species of Aspergillus, produced the enzyme.
Antibacterial activity of plant extracts:

In order to manage plant disease, various chemicals are used since last several years, the world over. Prior to 1930 very little plant protection was practiced and it was in the form of calcium arsenate, copper, mercury, barium salts and crude botanicals like nicotine. However, after world war II, the scene changed with the advent of organic pesticides with discovery of BHC and DDT, followed by a large number of chlorinated insecticides like cyclodiienes and herbicides like 2, 4-D and 2,4,5-D. All these have high persistence in environment. They tend to accumulate in animal tissues posing threat to human health. ‘India Today’ (1989) narrated the effect of these chemicals on human health, viz. the diseases of heart, brain, kidney, liver, breast etc., which are caused by the consumption of food articles having the residual effect of the pesticides, to a greater extent. Moreover, the use of chemicals as plant protectant in agriculture is having several other hazardous effects like pollution, ecological imbalance, development of resistance among the pathogen etc.

Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Balandrin et al., 1985; Hostettmann and Wolfender, 1997). Reports are available on the active agent from higher plant, in place of chemical fungicide, that are non phytotoxic, more systemic and easily biodegradable (Fawcett and Spencer, 1970). Reports are also available on the use of several plant by-products, which possess
antimicrobial properties, on several pathogenic bacteria and fungi (Deans and Svoboda, 1990; Heisey and Gorham 1992; Hili et al., 1997).

In recent years, various types of plants like bryophytes, gymnosperms and angiosperms are being tried to manage diseases. Antimicrobial activity of mosses has been studied by Sanjeev et al., (1999), who have reported the effectivity of two bryophytes against 04 bacterial pathogens. Charjan (1995) reported the antifungal activity of Cycas revoluta and Thuja orientalis. There are various reports of application of plant extracts in the management of fungal pathogens (Deshpande and Rathod, 1995; Gohil and Vala, 1996; Khilare and Gangawane, 1997). There are also few reports of leaf extracts as plant virus inhibitors (Verma and Kumar, 1980; Verma and Dwivedi, 1984). There are several reports of use of plant extracts against phytopathogenic bacteria (Ansari, 1995; Singh et al., 1995; Sobti et al., 1995)

In the present investigation experiments were conducted to assess the efficacy of plant extracts against the storage fungi of 9 medicinal seeds. This study yielded positive results. In the present investigation, in a planned series of experiments the extracts of various plant parts viz. leaves, fruits, roots, seeds and barks were screened against the 8 storage fungi of 9 medicinal seeds.

The leaf extract of Acacia arabica was found fungicidal during present investigation. Shrivastava and Yadav (2008) observed mycelium inhibition of Fusarium oxysporum f sp. lycopersici with the leaf extract of A. nilotica (Table- 8.1).
Leaf extract of *Azadirachta indica* found effective against several fungi by different workers (Yadav and Mujumdar, 2004; Lakpale *et al*., 2008; Shrivastava and Yadav 2008; Gupta and Singh 2008; Jadhav and Shinde, 2009; Kandelwal and Singh 2009; Khadir and Khan 2010). During present investigation also the leaf extract was found effective against the storage fungi of medicinal seeds.

In present investigation, the leaf extract of *Lantana camera* showed antifungal activity against the 8 storage fungi. Praveen Gehlot (2002) and Lokaple *et al*., (2008); Gupta and Singh, (2008); Jadhav and Shinde (2009) found that the leaf extract of *L. camera* was more effective than other plant parts, against 5 fungal species.

The leaf extract of *Vitex negundo* exhibited good antifungal activity against the 8 storage fungi. Singh *et al*., (2004); Jadhav and Shinde (2009) observed the hydrolytic extract of *V. negundo* effective against 19 fungal species on rice seeds during storage.

However, it has been observed during present investigation that the leaf extract of *Terminalia thorelii* and *Lawsonia inermis* showed maximum inhibitory activity against all the eight storage fungi (Table 8.1). The leaf extract of *L. inermis* showed maximum inhibitory activity against all the 8 storage fungi. The leaf extract of *L. inermis* has also been found most effective against 5 phytopathogenic bacteria. (Pawar, 1999). Pawar, (1999) has also found the leaf extract of *Lawsonia inermis*, having good antibacterial activity against the 5 Phytopathogenic tested by him. Similar observation with respect
to leaf extract of *L. inermis* has been reported by Srivastava and Yadav (2008) Gupta and Singh (2008), Kandalwal and Singh (2009).

Among the roots extracts, the extract of *Withania somnifera* was found more effective against the 8 storage fungi (Table 8.2). Effectivity of root extracts of 12 plants has been reported by Ushiki *et al.*, (1996). Pawar (1999) observed that, the root extract of *Rauwolfia serpentina* more effective against the 05 bacterial pathogens he tested.

Present studies of effectivity of fruit extracts against the 8 storage fungi showed that fruit extract of *T. thorelii* was more effective amongst the fruit extract tested (Table 8.3). However, Pawar (1999) has observed better antibacterial activity with fruit extract of *Brassica juncea*.

Amongst 06 bark extracts tested, only the extract of *A. indica* showed inhibition against the 8 storage fungi (Table 8.4). Similar observation has been made by Pawar (2007) in case of *Xanthomonas campestris pv. mangiferae indicae*.

Amongst the various extracts used, seed extract of *A. indica* exhibited maximum activity, followed by the seed extract of *Semecarpus anacardium*. As the leaf extract of *Terminalia thorelii* and *Lawsonia inermis* showed maximum antifungal activity, these extracts were used to test its effect on mycoflora of 9 medicinal seeds tested (Tables : 8.2.1 and 8.2.2). It was observed that there was gradual reduction in number of fungi from 6 hrs to 12 hrs to 18 hrs. treatments with the leaf extract. However, even after 18 hrs treatment none of the extracts completely eliminated the mycoflora from the medicinal seeds. The
fungi which were found persisting were *Rhizopus oryzae* and *Aspergillus niger* on all the medicinal seeds. However, on some seeds one more fungus in addition to above two fungi was observed. It was in some cases *Trichoderma aureoviridae* or *A. flavus* or *A. carbonarius* or *F. oxysporum* or *Penicillium corylophilum*. When these observations were compared with 3 fungicides viz. Bavistin, Benlate and Captan; similar observation (i.e. either 2 or 3 fungi surviving on the seeds after treatment (table 8.3) was made. As the seeds of *Azadirachta indica*, *Butea monosperma* *Holarrhena pubescence*, *Madhuca longifolia*, *Plantago ovata*, *Pongamia pinnata*, *Semecarpus anacardium*, *Tectona grandis* and *Thespesia populnea* are used for medicinal purpose they are not to be treated with fungicide for the elimination of mycoflora. If these fungicides are used as seed dressers for the medicinal seeds, they will be unfit to be used for medicinal purpose.

Therefore, based on the present investigation, it can be suggested that the medicinal seeds should be treated either with the leaf extract of *Terminalia thorelii* or of *Lawsonia inermis*; and stored in plastic bottles preferably, or in tin boxes, alternatively. The seed treatment with the above mentioned plant extracts will be a chapter method and can be practiced in rural and tribal areas; where majority of the seeds are collected from the nearby forests.
Scope for future research:

1) The deterioration of medicinal seeds by mycoflora is reported in literature (Dutta and Roy, 1987). In some cases, it is reported to adversely affect the active principles like phenols, proteins and alkaloids. Therefore, it is felt that in case of the 9 medicinal seeds also, studies should be performed to study the loss in their active principles due to mycoflora.

2) Reduction in germination percentage of seeds, due to mycoflora, is well documented in literature. Therefore, studies on the effect on germination of the 9 medicinal seeds should be performed.

3) Several moulds are detected on the seeds during storage. In literature, it is reported that different isolates behave differently for the production of metabolites. Therefore, it is felt that application of nucleic–acid based detection methods for seed mycoflora will facilitate the strain differentiation.

4) Some other biological seed treatment methods should be devised, so that the mycoflora can be managed, without affecting the medicinal properties of the seeds.

For this, some microorganism or non-pesticidal chemical, that may enhance the defense mechanisms in the seed, against the pathogens, be investigated.