Biogeological studies have revealed that the land plants emerge from the paleozoic eras more than 3300 million years ago. Mythological stories tell us, that in the beginning man had no knowledge of agriculture. The primitive man use to hunt animals and gather fruits, nuts, seed and roots from the naturally growing plants for food. Most of the natural vegetation used for food use to dry at the end of the season forcing him to shift to another region, where different type of vegetation was growing, and could be used as food during that season. He had, therefore, to lead to nomadic life till he discovered that the seeds produced by some plants could be collected and used for sowing and producing the same plants again, after the end of season, or store these till the next season for sowing. It is believed that this art was revealed to some saintly men through Gods or Goddess appearing in the dreams. Whatever way it might have happened, it was a great achievement because after this he gave up the nomadic habit of shifting from place to place at the end of the season. He built houses where suitable land for sowing the seeds, for growing the crops and water sources were available. Growing plants from seed is a superior way of providing food. Therefore, he must have settled down to a family way leading a civilized life. Farming naturally must have become an indicator of civilization (Siddiqui, 1992).

Since the dawn of civilization, the plant or plant parts have been used to cure the human sufferings. Despite manifold development in synthetic medicines, the herbal drugs are still maintaining its popularity among majority of population in almost all Asian countries including India. The earlier
literature from Atharveda, Rigveda to Materia medica unveils the hidden importance of medicinal plants. As plant science developed, many more plants got entry in Indian pharmacopoeia. One of the ancient Ayurveda treatises “Charak” (600 B.C.) has mentioned the entry of only 341 plants, further more in the beginning of first century Shushruta Samhita, listed 760 plants of medicinal value. With the advent of modern scientific technique, many of these medicinal plants were put under chemical scrutiny leading to the isolation and characterization of active principles and establishment of their pharmaceutical activities. During last few decades many of the synthetic drugs were found to have side effects, when used for longer periods. Due to such cumulative derogatory effect, now a day the western population is looking for natural remedies which are safe and effective.

India sitting on a goldmine of well recorded and traditionally well practiced knowledge of herbal medicine. India officially recognizes over 300 plants for medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine representing about 75% of medicinal needs, of the 3rd world countries. All India ethnobiology survey carried out by the Ministry of Environment and Forests estimates that over 7500 species of plants are used for human and veterinary health care across the country (Roy, 2010).

India among the Asian countries occupies the top most position in the production and usage of plant drugs. However, two main drawbacks viz. lack of proper identification and unscientific storage methods exists still in this system.
The traditional storage practices provide conducive condition for the association and growth of several fungi. Some of them are toxigenic and during storage elaborate adequate amount of aflatoxin. Aflatoxin are well studied for health hazards in man. (Roy and Kumari, 1994).

Roy and Kumari (1994) observed during their investigation clearly suggest that almost all medicinal seed samples were associated with a large number of fungi. Some of them had heavy contamination of toxigenic Aspergillus flavus strains. The drugs manufactures without examining the raw drugs samples for microbial association, manufacture the finished herbal drugs. This may be account for high incidence of aflatoxin content in powdered herbal drugs (Chaurasia, 1990). This is a matter of concern, since these medicines may un-intentionally cause another hazard to human health. Therefore, care must be taken for quality control of raw drugs samples during storage and also that of finished the herbal drugs (Roy and Kumari, 1992).

Seeds of forest trees, like those of agriculture and horticulture crops carry a wild variety of microorganism like bacteria, fungi and even some viruses, either inside the seeds, or between the seed coat and embryo, or as external contaminant. Seeds may be attacked by the pathogen while still borne on the trees in the field, during storage and subsequent handling prior to sowing (Khullar et al., 1991).

Screening of literature on seed pathology reveal that voluminous work has been done in agricultural and horticultural crops, on the role of seed borne fungi, in deteriorating seed quality and causing diseases, both in the storage and
in the field (Doyer, 1938; Cristenson and Kaufmann, 1965; Neergard, 1977; Afzal et al., 2010). The earliest record of well established seed-borne diseases relates to stinking smut of wheat (caused by *Tilletia caries* and *T. foetida*) now termed as bunt. (Remnant, 1637)

Seeds are living organelles and they suffer from invasion of insects moulds, bacteria, viruses and mites. Consequently the seed vigour and viability or the nutritional qualities are adversely affected. Paul Neergaard (1977) concentrated on these aspects. He believed that the healthy seed is the basic ingredient for the green revolution. Because of his voluminous work a new branch of plant pathology—“Seed pathology” has developed and recent years it has occupied a prominent position in the field of plant research.

Seed pathology involved the study and management of diseases affecting seed production and utilization as well as disease management practices applied to seeds. Presently, the world over, the seed pathology research revolves around 1) Research innovation in detection of seed borne pathogens and elucidation of their epidemiology; 2) Advances in development and use of seed treatments and 3) Seed health testing methods (Munkvold, 2009). For detection of seed borne pathogens several methods are used, like examination of dry seeds, examination of seed washing, Blotter method, Agar Plate method, rolled paper towel method, 2,4-D method, Deep freezing method etc. However, in recent years the invention of polymerase chain reaction (PCR) in 1980 revolutionized biological diagnostics, opening a new era in pathogen detection. Since that time, many PCR-based detection methods have been
developed and applied to seed borne pathogens (Munkvold, 2009). Agrawal (2006) reviewed traditional health testing methods along with recent developments in immunoassays and non destructive seed health tests such as ultrasound, optical and infrared analysis and biopsis.

**Seed Mycoflora:**

Seed is exceedingly dynamic in an ecosystem. It changes its habitat owing to its migration from one place to another i.e. from plant in the field to distributing or disseminating agencies or to storage and ultimately to the field soil. Once the seed is drilled in the soil, a new environment is set up and is controlled by seed activity and soil composition. Fungi in spermosphere, in or around the seed, respond to various microclimatic pressures, such as temperature, moisture and also the structure and composition of seed and its physiological activity. The fungi play an important role in germination and development of the seedling and also in the deterioration of the seed in storage (Manoharachary and Kunwar, 2006).

Seed mycoflora of different plants have been studied by several researchers. Mycoflora associated with seeds of cereals have been studied by many workers (Joshi and Gupta, 1980; Basak and Mridha, 1985; Satyanarayana et al., 1993; Singh, 2006; Rizvi, 2006; Reddy et al., 2008; Islam et al., 2009). These workers observed saprophytic fungi on the seed. These include *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Fusarium oxysporum* and *Chaetomium* species etc. They were isolated by blotter and Agar plate method.
Seed mycoflora of oil seed crops has also been studied in different laboratories. (Afzal et al., 2010) isolated 13 fungal species associated with seed of sunflower. The fungi include *Alternaria altenata*, *A. helianthi*, *Asperillus flavus*, *a fumigatus*, *A. Niger*, *Curvularia lunata*, *Deshlera tetramera*, *Fusarium solani*, *F. moniliformae*, *Macrophomina phaeosolina*, *Mucor mucedo*, *Penicillium* and *Rhizopus* species. Khati and Panday (2004; 2008) studied seed mycoflora of sesame and their role in plant health. Association of *A. flavus* with groundnut seeds has been prominently reported in literature (Nahadi, 1996). Vishvanathan (1996) reported 13 fungi, while Singh et al., (1987) reported 11 fungal species associated with sunflower and safflower respectively. Investigation in seed mycoflora of *Bhuanania lanzen*, an oil seed of forest origin, revealed that four fungi were present with fresh seeds kernels, 8 were recorded on stored seeds. *Aspegillus flavus*, was the most common fungus appearing on all seed samples (Singh, 2009) Reddy et al.,(2008) observed species of *Aspergillus, Fusarium, Curvularia, Helminthosporium, Trichoderma, Alternaria, Penicillium, Rhizopus, Bioplars*, on seeds of rice. They found that *Alternaria padwickii* was the predominant species on the seeds.) Seed mycoflora of vegetables has also been studied (Teggi and Hiremath, 1990; Kumar and Singh, 2004). Species of *Aspergillus, Alternaria* and some other moulds have been found associated with the seeds of vegetables (Samota and Singh, 2006; Rathod and Paul, 2004; Asamol et al., 2001, Sud et al., 2005 stated that 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 varieties may be ascribed for more mycoflora in these varieties. Dwivedi et al., (2006) while studying the seed mycoflora of coriander observed that seed germination was hampered due to heavy inflection of fungi. Elshafie et al., (2002) stated that very little information is available on the mycoflora and mycotoxins of spices worldwide. Seed mycoflora of forest trees have been studied by few workers (Watanabe and Sato, 1988; Mamtha et al. 2000; Gupta and Sharma, 2007; Bhanumathi and Rai, 2008). Singh et al. (2001) detected aflatoxins in almost all samples of different forest seeds. They have observed different aflatoxin viz. Afl. B₁, Afl.B₂, Afl.G₁, Alf. G₂, Ochratoxin, Zearalenone, Critinin in the seeds of *Aegle marmelos*, *Buchanania lanzen* and *Pinus gerardiana*, inflected with *Aspergillus flavus*, *A. ochraceus*, *Fusarium verticilloides*, and *Penicillium citrinum*.

Medicinal seeds are used in our country as important constituent of many herbal drugs, or the seeds are given raw in the treatment of some diseases. They have antimicrobial properties. The seeds become contaminated by fungi, in the field during storage, transportation, processing and handling. The mycoflora and mycotoxins of many agricultural products have been investigated by many researchers (Neergaard, 1977; Jha, 1995). However, worldwide very little information is available on the mycoflora of medicinal seeds. Roy et al., (1988) have studied the association of mycoflora with drug plants and aflatoxin producing potentiality of *Aspergillus flavus*. They have studied the roots of Sarpagandha (*Rauwolfia serpentina*. Benth. ex Kurz),
rhizomes of saunth (Zingiber officinalis Rasc), fruits of amla (Emplica officinalis Gaerth), seeds of neem (Azadirachta indica A Jass), datura (Datura metel Linn), gunja (Abras precatorius Linn), Indrajav (Holarrhena antidysenterica A. D. C. and Kuchal (Strychnos nux-vomica Linn). They observed 13 fungal species - Aspergillus flavus, A niger, A candidus, A. luchensis, A. nidulans, Fusarium moniliformae and Penicillium citrinum occurring frequently, while Alternaria alternata, Chaetomium species, Penicillium sp. and Rhizopus stolonifer were of rare occurrence. Of the 50 isolate of A. flavus, 21 were found by them to be toxigenic; among these 21 isolate, 12 had potentiality to produce aflatoxin B₁, only 7 had both B₁ and B₂ and only 2 produced B₁, B₂, and G₁. They found none of the samples free from storage fungi. The growth of A. flavus under storage not only deteriorates the quantity of drug plants but may also contaminate by elaborating aflatoxins.

Purohit and Jamaluddin (1993) studied the seed mycoflora of palas (Butea monosperma, (Lam) Taub). They isolated 11 fungi belonging to four genera from the seeds. Out of them, species of Aspergillus (viz. A. flavus, A fumigatus, A. niger, A nidulans, A. sulphurius etc.) were dominant over Penicillium sp., Chaetomium sp. and Rhizopus stolonifer. They used Blotter and Agar plate method for the isolation of seed mycoflora and found that the range of infestation did not differ much in the two test methods.

Purohit et al., (1998) studied seed mycoflora of Butea monosperma, Dendrocalamus, strictus, Lagarstromia parviflora and Pongamia pinnata. They isolated 12 fungi, of which Aspergillus niger and A. flavus were dominant
on seeds of all the four tree species. The maximum species of fungi were recorded on *P. pinnata* (8 spp.) followed by *B. monosperma* (5 spp.)

Singh *et al.*, (2001) studied seed mycoflora of tree species of *Aegle marmelos, Azadirachta indica, Emblica officinalis, Plantago ovata, Terminalia chebula, T. bellerica and Vitex negundo*. A total of 115 fungi viz., *Aspergillus candidus, A. flavus, A. niger, A. ochraceus, Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata, Fusarium oxysporum, F. semitectum, Gloethecium sp., Penicillium citrinum, Penicillium sp., Trichoderma roseum* and *Rhizopus stolonifer* were found associated with the tested seeds.

Roy (2003) reviewed the mycological problems of crude herbal drugs. He has reviewed mycological problems that include association of mycoflora with crude herbals under storage, their role on biodeterioration and mycotoxic contamination. These aspects have drawn worldwide attention for quality maintenance and therapeutic potentials of plants drugs. He has stated that during storage the fungal organisms thrive on drug plants by utilizing various components including the active ingredients. Biodeterioration in alkaloid, phenol and protein contents of seed, fruit and root samples of 21 plants by *A. flavus* and *P. citrinum* was studied at optimum temperature (28º± 2ºC) and RH (90 ± 6 %). A general trend of decrease (10-14%) in the concentration of all the important constituents (except phenols) was recorded under single infestation within 60 days of storage. Degradation of alkaloids in *Strychnos potatorum* and *S. nux-vomica* seeds within 90 days of infestation by *A. flavus, A. niger* and *P.*
citrinum was studied. Strychnine and brucine were identified in the seeds as major alkaloids, their concentration was significantly reduced under investigation.

**Isolation of seed mycoflora by blotter and agar plate method:**

While studying the seed mycoflora of *Lagarstromia floas reginae*, Sahu and Agrawal, (2001) observed that a total of 13 fungi viz. *Alternaria tenuissima*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Curvularia clavata*, *C. lunata*, *Fusarium moniliformae var. subglutinum*, *F. pallidoroseum*, *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. purpurogenum*, *Phomopsis sp.* and *Schizophyllum commune* associated with the seeds. Amongst these, the fungi detected by blotter method were *Aspergillus flavus*, *A. niger*, *Fusarium*, *palliolorosrum*, *Penicillium purpurogenum* and *Schizophyllum commune*. The seed mycoflora detected by agar plate method were *Alternaria tenuissima*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Curvularia clavata*, *C. lunata*, *Fusarium moniliformae var. subglutinus*, *F. pallidoroseum*, *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. purpurogennum*, *Phomopsis sp.* and *Schizophyllum commune*. However, some fungi viz. *Aspergillus flavus*, *A. niger*, *Fusarium pallidorosrum*, *Penicillium purpurogenum*, and *Schizophyllum commune* were isolated by both, blotter and agar plate method. The percent incidence of *Aspergillus flavus* was maximum (50 %) in comparison with other fungi. They observed that agar plate was the most successful means for detecting seed borne fungi than the blotter method.
Sharma and Chaudhary (1985) while working with the seed mycoflora of Paddy, observed 34 fungi. Of these, maximum number of fungi (31) was observed on blotter method, followed by deep freeze (20) and agar plate (18) methods. They have also observed maximum infection percentage of *Drechslera oryzae*, *Trichoconis podwickii* and *Rhizoctoni solani*. This method reveals the presence of *R. solani*, *Curvularia verruculosa*, *Fusarium moniliforme var. subglutinans* and *Sarocladium attenuatum*.

Muley and Baig (2007), while working with mycoflora of oil seeds found that in agar plate method higher incidence of seed mycoflora was observed; while Sable et al., (2008) found lowest number of fungi(4) on agar plate method and highest number of fungi (39) by standard blotter method. They studied the seed mycoflora of groundnut. However, Shrivastava et al. (2009) found blotter and agar plate method equally suitable for the isolation of mycoflora of soybean seeds. Nagaraja and Krishnappa (2009) observed standard blotter method superior over other methods for detection of seed borne mycoflora of Niger. They used standard blotter method, PDA method, water agar method and 2-4D method. They also stated that the seed borne fungi reduce the seed germination method.

**Physical Factors and Seed Mycoflora:**

Environmental conditions like relative humidity, temperature, seed moisture, storage conditions etc. have been known to affect establishment of seed mycoflora.
**Relative humidity:**

In most of the cases, seed invasion by moulds is severe under high humidity and rainfall during the seed development (Tarr, 1992; Rao and Williams, 1977). The invasion of different storage moulds at different levels of relative humidity was studied by (Christensen, 1974) and found that it is different with different fungi.

The relative humidity 96% was found favourable for maximum incidence of fungi on *Strychnos potatorum* and *S. nux-vomica* seeds by Dutta and Rao (1987). Chourasia and Roy (1991) found 75% relative humidity as lowest and 96% as highest limit for the association of seed mycoflora of Neem and Datura.

**Temperature:**

Literature regarding the effect of temperature on establishment of seed mycoflora is meager. Galliogeos and Castor (1977) observe that temperatures between 24º to 30º C with high humidity as favourable for the attack of *Curvularia lunata* on jawar seeds.

**Effect of Storage Temperature:**

Bahuguna *et al.*, (1987) studied storage of seeds of *Terminalia myriocarpa* at different temperatures, and found that there was total loss of viability at 30ºC, within 3 months. At 5ºC and 15ºC, the viability could be maintained up to 6 and 4 months respectively. In case of *Syzygium cumini* the seeds lost their viability within 15 days, when stored at room temperature,
while the seed retained viability up to 60 days when stored at low temperature (i.e. 5º C) Bahuguna and Rawat, 1989).

Gupta and Sood (1978) noted a very significant effect of storage temperature in seeds of *Dendrocalamus strictus*. Seeds stored at room temperature lost the germination capacity within a year; even when the moisture content of the seed was reduced to 5 percent. The seed stored at 5ºC, on the other hand, remained viable beyond 2-3 years, depending on moisture content. Moubeshwar et al. (1979) found that groundnut seeds at 28ºC showed maximum incidence of *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. fumigatus*, *A. ochraceous* and *Penicillium* sp., but at 40ºC temperature only *A. fumigatus* and *A. terreus* could grow.

Datta (1988) reported maximum incidence of seed mould at 30ºC in *Strychnos potatorum* and *S. nux-vomica*. Roy (1989) observed maximum incidence of seed borne fungi on number of medicinal plant parts and seeds stored at 39.2ºC.

**Seed Moisture and Storage Mycoflora:**

Most seeds loose their viability rapidly at relative humidity approaching 80% and temperature of 25º to 30ºC, but can be maintained with high viability for ten years or longer at relative humidity of 50% or less, and a temperature of 5ºC or lower (Toole, 1950).

Harrington (1973) suggested that the sum of the percentage or relative humidity and the temperature of storage environment should not exceed 100ºF for safer storage. He had earlier proposed two rules of thumb regarding
optimum seed storage (Harrington, 1972): (i) each 1 percent reduction in seed moisture doubles the life of seeds (applicable between temperature range 5-14ºC) and (ii) each 5ºC reduction in seed temperature doubles the life of the seed. Reduction in moisture content cause a reduction in respiration and thus slows down ageing of the seeds and prolongs viability. There can be two ways in which seed can be stored with respect to moisture content. In the first case, manipulation of relative humidity can effectively change the moisture content of seed to the optimum for storage. Using this approach, moisture content of Bombusa tulda seeds was maintained at different moisture levels. i.e. 26 percent (initial), 19 percent, 14 percent, 10 percent and 4.5 percent and they were stored in sealed polythene bags at room temperature. Seeds stored at initial moisture content of 26% deteriorate completely, while those stored with 10% and 4.5% moisture content retained 65% and 76% germination capacity respectively, even after 13 months (Thapliyal et al., 1991). Gupta and Sood (1978) observed that in Dendrocalamus strictus, seed with moisture content reduced to 8% and stored at 3-5ºC in refrigerator, in sealed bottle, retained good germination (59%) even after 30 months.

Seed moisture is important parameter in deciding the quality and quantity of seed mycoflora. Castor and Fredrisen (1981) found that 20 to 22% seed moisture was favourable to the growth of field fungi, and 13 to 17% moisture for the growth of storage fungi. Siddiqui (1977) categorised three types of seed mycoflora, on the basis of seed moisture requirement, as field, post harvest, and storage mycoflora. Girisham and Reedy (1986) noticed that
increase in mycoflora was proportional with increase in seed moisture in case of bajra.

Chourasia and Roy (1991) reported that high moisture content on Neem and Datura seeds as favourable for growth of maximum moulds along with *A. flavus*.

**Storage of Seeds:**

Storage products include materials, which may be dried, rendering them, storable for future use of food, industrial raw materials, medicines or as planting materials. Insects, fungi, bacteria, Actinomycetes etc. cause damage to stored products (Chomchalow, 2003). Insect and fungal infestation is major contributor to quality deterioration of seeds. Growth of both mould and insect is affected by moisture content of the seed and prevailing environmental conditions (Sharma, 2007).

Storage fungi are generally present as mycelia below the pericarp, or as dormant spores on the surface of seeds. They cause spoilage of stored seeds through discoloration, loss of viability, biochemical changes leading to quality loss and production of toxins. Among the most serious is *Aspergillus flavus*, which produces toxin on many grains and oil seeds, and causes quality deterioration. Aflatoxin and other mycotoxins are highly poisonous and carcinogenic compounds *Aspergillus* and *Penicillium* are important fungi that are generally associated with stored products (Chomchalow, 2003).

The storage fungi differ in their ability to deteriorate seeds under conditions which favor fungal growth. This is an important fact which attracts
special attention since seed mycoflora varies depending upon the storage environment, especially seed moisture content and temperature (Purushotham et al. 1996).

The fungal flora observed on the seeds of *Vigna sinensis* soil, varied qualitatively as well as quantitatively with respect to the period of storage. Six fungi viz. *Aspergillus flavus, A. fumigatus, A. nidulaus, A. niger, A. terreus* and *Fusarium moniliforme* persisted over other associated fungi (Maheshwari et al. 1985), Sahu and Behera (1996) observed *Aspergillus* groups as the dominant one amongst the storage fungi, followed by *Penicillium* and *Fusarium* on the seeds of Jute.

**Effect of Storage Container:**

Storage containers used for seed storage for various periods, influence seed mycoflora. Mathur and Sinha (1978) stored the bajra seeds for eight months at farmers place, godown and laboratory, and found that maximum mycoflora was on the seeds stored at godown. Girisham and Reddy (1985) studied influence of various storage structures (tin pot, cloths and basket lined with dung) on seed mycoflora of bajra and concluded that fungi varied not only with storage structure but also with the period and moisture during the storage. They have recorded maximum fungi (28 spp.) on seeds in basket lined with dung.

Singh and Singh (1986) used gunny bags, polythene lined gunny bags, tin boxes and earthen pots for storage of wheat samples. Dwivedi and
Shukla (1990) used cloth bag, in addition to above cited containers for the mycoflora studies of gram.

Maithani et al (1987) observed that storage of seeds of *Holoptelia integrifolia* at 30° exhibited wide variation in deterioration in germination capacity. Seeds stored in perforated poly bags rapidly lost germination capacity within 4 months, while seeds in sealed containers remained viable up to 16 months, exhibiting 16.3% germination. Singh and Singh (1986) used 4 different storage containers *viz.* gunny bag, polythene lined gunny bag, tin boxes and earthen pots for storing wheat grain and found that grain stored in gunny bag had significantly less incidence of storage microbes. This may be due to better ventilation.

Bhikane (1988) while studying the effect of storage containers on incidence of mycoflora on the seeds of green gram, observed that seeds of all the three varieties *viz.* Pusa baisakhi, PS-16 and S-8, had maximum mycoflora when stored in gunny bags, followed by seeds stored in polythene bags; whereas, seeds in paper bags showed less number of fungi. Dwivedi and Shukla (1990) used 5 different types of containers found that polythene bags provided much protection to grain seeds in preventing the development of fungal colonies.

Verma and Singh (1993) have used 700 gauge polyethylene, polylined jute, cloth and gunny bags for storing the mustard seeds. They found that 700-gauge polythene bags maintained more than 85% germination up to 30 months.
storage. It was probably because 700-gauge polyethylene, did not allow atmospheric moisture vapors to penetrate.

After proper sun-drying medicinal seeds of *Butea monosperma* were stored in glass bottles, plastic boxes, tin boxes, polythene bags, jute bags and cloth bags for 2 years by Purohit and Jamaluddin (1993). They reported that comparatively glass bottles, plastic boxes and tin boxes were better for storage purpose than the bags of polythene jut and cloth.

Sahai (1999) while studying the effect of storage on seed viability of 26 leguminous taxa, observed that seeds stored in air tight bottle and paper bags at the ambient temperature, the seeds of 4 taxa *viz.* *Bauhinia purpurea*, *Leucaena glauca*, *Prosopis juliflora* and *Pithecellobium dulce* remained highly viable; whereas seeds of *Cassia glauca*, *Caesalpinia coriraria*, *Mimosa pudica* and *Peltoforum ferrugineum* showed poor viability. Rest of the eighteen leguminous taxa lost their viability after seven years of storage.

Chauhan *et al.*, (2002) stored the seeds of Neem under 7 storage conditions separately *viz.* clay coating, gum coating, plastic container, poly bags, earthen container and gunny bags,. They stated that plastic container and poly bags were detrimental for storing the seeds.

Nema *et al.*, (2006) used 3 types of storage containers *viz.* permeable (gunny bags), semi-permeable (baked earthen pitcher mud plastered outside pot) and impermeable (plastic container with screw lid) for storing Niger seeds. They inferred that Niger seeds may be stored safely in permeable/semi-
permeable storage containers with 6-8% moisture content for period of 6 months.

**Fungal Metabolites:**

Singh (1981) worked on effect of the metabolites of seed borne fungi on wheat seed germination and seedling vigour. The metabolites of *Aspergillus terreus*, *A. niger* and *A. flavus* reduced and seed germination appreciably. The maximum inhibition of both, shoot and root growth, was recorded with the culture filtrated of *A. terreus*, followed by those of *A. flavus*, *A niger* and *Helminthosporium sativum*. He further reported that the culture filtrates of *Memnoniella echinata* and *Chaetominum globossum* retarded root growth only. Reduction in seed germination and seedling vigour due to treatment with the metabolites of seed borne fungi have also been reported for barley(Armolik *et al.*, 1956), for sorghum(Bhale *et al.*, 1982; Mathur and Sehagal,1964), for rice (Vidysekharna *et al.*, 1970) etc.

Seed deterioration by seed borne fungi has been reported by various workers. They found that these fungi produce certain enzymes which breakdown the complex ingredients of the seeds into simpler compounds, thereby affecting the viability and nutritive value of the seeds. In such type of seed deterioration, the enzymes amylase, cellulose, pectinase and lipase play an important role (Charya and Reddy, 1980, 1983; Jha, 1995; Muley and Baig, 2007).

For assay of the fungal metabolities different workers have proposed different techniques. The techniques adapted in present investigation have been
described under Materials and Methods. These methods are based on use of culture filtrates for the assay. Hankin and Anagnostakis (1975) suggested use of solid media that permit rapid screening of large population of fungi for the presence or absences of specific enzymes. This also allowed a search for genetic varients to be made more easily. They have used solid media for the detection of some extracellular enzymes produced by fungi; viz., Amylase, Lipase, Pectinase, Protease, Urease, Kitinase, DNA- and RAN ase.

Use of solid media for the detection of amylase, cellulase, lipase, pectinase, protease, produced by fungi, has been reported by Das et al., (2009).

For detection of macerating enzyme in the culture filtrates, potato discs are used. Sharma and Chauhan (1980) clearly showed that if potato discs from green potato tubers are used, maceration was delayed. Therefore, they have stated that for the assay of macerating enzyme potato discs should be from healthy potato tubers. They stated that artificial exposure to sunlight create greening in potato tubers and such tubers were reported to be poisonous for human beings due to high glycoalkoloid contents. A simple low cost solid media containing wheat flour as natural source of starch was developed by Upadhyay et al., (2001) to detect amylase production by fungi. They stated that hydrolysis of starch was very rapid, as evidenced by appearance of clear zone around the colonies within 3 days of incubation.

Toxin Study:

Since De Bary(1986) postulated that plant pathogenic organisms might be producing pathogenic toxins, a large number of investigators have studied
plant pathogenic fungi and bacteria producing toxins in culture media and which are toxic to host plants. Gauman (1954) gave a general conclusion that micro-organisms are pathogenic only if they are toxigenic. The toxin produced by *Helminthosporium victoriae*, the causel organism of Victoria blight of oats was termed a ‘Victorin’ by Wheeler and Luke (1954). *Pseudomonas tabaci*, the causal agent of wild fire disease of tobacco, produces a toxin called as wild fire toxin. (Wheeler and Luke, 1963). Suraiya Begum and Deshpande (1977) have studied toxic metabolites produced by *Phoma herbarum* in culture and its phytotoxic effects. Patil et al., (1993) studied toxic effect of culture filtrate of *Phyllosticta arachidis hypogaeae* and found that the pathogen produces a thermostable toxin, which on inoculation develops necrotic brown spots on leaves. Marimuthu(1983) observed that cysteine sulphur containing amino acid inhibited toxin production by *Xanthomonas campestris pv. phaseoli*. Jahani et al., (2006) state that *Bipolaris sorokiniana* causing spot blotch of wheat produces toxin, which play role in pathogenicity. Saikia et al., (2004) purified toxin produced by *Colletorichium capsici*, causing ripe rot in chilli, by column chromatography and high performance liquid chromatography (HPCL); and have done partial characterization by U.V. / Vis. Spectroscopy and Infrared (IR) spectroscopy.

Kishun and Shukla (2000) evaluated 25 strains of *Xanthomonas campestris pv. mangiferae indicae* for toxins and their role in disease development. Fourteen strains were found positive for toxins, while 02 showed variable reactions. The culture filtrate showed complete killing of seedlings,
where as diluted culture filtrate (1:10) had inhibitory effects on roots of germinating wheat seeds. They observed that toxin is heat stable. This is the first report on production of toxin by Xc mpi and its role in MBCD development.

It is known that varying degree of pathogenesity of a fungus results with variation in production of phytotoxic metabolites. Nandi and Santra (1974) studied the differential phytotoxicity of metabolic by products of *Nigrospora oryzae*. They recorded the inhibition of root and shoot growth of *Oryza sativa* by the culture filtrates. Abraham (1978) studied the inhibitory effect of culture filtrate of *Alternaria solani, Colletotrichum* sp., *Helminthosporium* sp. *Fusarium oxysporum, F. melonis, f. moniliformae* and *F. vas infecta* on brinjal seed germination. Gurjar and Singh (2003) studied the effect of toxic metabolites of Okra seed borne fungi *viz.* *Curvularia lunata, Macrophomina phaseolin, Fusarium oxysporum, F. moniliformae* and *F. pallidoroseum*, on seed germination and *seedling* vigour. Samota and Singh (2006) observed that the culture filtrates of fungi caused reduction in germination, plumule and radicle length. They observed maximum inhibition in germination with the culture filtrate of *A. flavus*. Kavita and Vijaya Laxmi (2007) observed that the culture filtrate of seed borne fungi of Foxtail Millet *viz.*, *Alternaria terreus, P. citrinum, C. lunata and Alternaria alternata*; hampered root and shoot elongation. Ahamad (2009) observed that seed germination and seedling growth of mustard reduced significantly by the aflatoxin produced by *A. flavus*. Detailed study of aflatoxin has been done by several workers. (Kehari and Basu, 2003, Kumar *et al.*, 2008)
Major Classes of Mycotoxins:

A review of mycotoxins has been presented by Chauhan (2004). He has described the major classes of mycotoxins. Mycotoxins which are commonly considered to pose high risk to human and animal health, as food and feed contaminant are described below.

Aflatoxins: The aflatoxins are produced by some strains of *Aspergillus flavus* and most if not all strains of *A. parasiticus* (Chauhan, *et al.*, 2000). There are four major types of aflatoxins, B$_1$, B$_2$, G$_1$ and G$_2$ plus two additional metabolic products, M$_1$ and M$_2$.

Sterigmatocystin: This mycotoxin is produced by several species of *Aspergillus, Penicillium luteum* and a *Bipolaries* species; chemically sterigmatocystin resembles aflatoxins and is a precursor in biosynthesis of aflatoxin.

Trichothecenes: Trichothecenes are a family of over 48 structurally relate4d compounds produced by several genera (*Fusarium, Cephalosporium, Myrothecium, Stachybotrys and Trichoderma.*) There are several naturally occurring trichothecene mycotoxins produced in foods and feeds by *Fusarium* species including deoxynivalenoll, T-2 toxin, nivalenol and diacetoxyscirpeniol.

Zearalenone: An estrogenic mycotoxin causes vulvo vaginitis and estrogenic response in swine. This mycotoxin in produced by *F. graminiearum* occurring in high moisture corn, moldy hay and palleted seeds.
**Ochratoxins:** They are a group of structurally related metabolites that are produced by *Aspergillus ochraceous* and related species and also by *Penicillium viridicatum*. The most important in this group is Ochratoxin A. This mycotoxin has been reported to cause Balkan endemic nephropathy in human beings.

**Citrinin:** It is a yellow coloured mycotoxin which is produced by many species of *Penicillium* and *Aspergillus*. Like Ochratoxin A, citrinin damages kidney in laboratory experiments of animal and at times acts synergistically with ochratoxins A in case of swine nephropathy.

There are few less studied mycotoxins found under certain unusual circumstances e.g. Citreoviridin was isolated form mouldy rice causing cardiac beriberi in Japan. Several species of *Penicillium* and a single species of *Aspergillus* have been reported to produce this mycotoxin. This mycotoxin causes paralysis, dyspnea, cardiovascular disturbances and loss of eyesight.

**Cyclopiazonic Acid (CPA):** Occurs as natural contaminant of foods and feeds infested with several moulds. The mould species are *A. flavus, A. tamari, A. versicolor, P.camemberti* etc.

**Fumonisins** are comparatively newly described mycotoxins isolated from *F. moniliforme*. This mycotoxin produces leukoencephalomalacia in horses in United Sates. Fumonisin B₁ showed cancer promoting activity in experimental rats. The fungus *F. moniliforme* is universal inhabitant of corn There are many fungi which produce tremorgenic mycotoxins e.g. *Penicillium, Aspergillus, Claviceps and Acremonium.*
Mycotoxicoses and Animal Health

The toxic metabolites produced by fungi cause wide range of injurious effects on animals, both naturally and in laboratory. This is in addition to serving as food borne hazards to humans. The economic impact of reduced productivity increased disease incidence because of immune suppression subtle but chronic damage to vital organs and tissues and interferences with reproductive capacity is many times greater than that of acute livestock death.

The aflatoxins cause liver damage, decreased milk and egg production and suppression of immunity in animals. The young ones of all animals are most susceptible and the clinical symptoms include gastrointestinal dysfunction, reduced reproductivity, decreased food utilization, anemia and jaundice. Nursing animals may be affected by aflatoxins produced in milk.

The trichothecene mycotoxins are a large group of mycotoxins that cause necrosis and hemorrhage in the digestive tract, depress blood regenerative processes in the bone marrow and spleen and cause changes in the reproductive organs. The affected animals show loss in weight, poor feed utilization, vomiting, bloody diarrhea, abortion and death.

Ochratoxin: A damages the kidneys of wide variety of domestic and wild animals that consume contaminated feed. High concentration of dietary ochratoxin A can also cause liver damage, intestinal necrosis and haemorrhage. It is also found to be carcinogenic and to suppress immunity.

The Zearalenone: mimics the effects of female hormone estrogen and induced feminization at low dietary concentrations(less than 1 ppm), while higher
concentrations may interfere with conception, ovulation, implantation, fetal development and viability of new born animals.

The mycotoxins produce variety of other effects like embryonic death, inhibitions of foetal development and abortions. These symptoms have been observed due to the presence of ergot, aflatoxin, rubratoxin and zearalenone toxins in the animal feed.

Teratogenicity has been documentaed due to consumption of contaminated feed by aflatoxin, ochratoxin, rubratoxin T-2 mycotoxin, zearaleanone and sterigomatomocystin.

Pectolytic enzymes:

The establishment of the pathogen in the host and the subsequent development of disease involve a chemical interaction between host and the pathogen. The pathogen employs offensive chemical weapons to breach host barriers. Enzymes, toxins, growth regulators and polysaccharides are important chemical weapons of the pathogen (Bilgrami and Dube, 1982).

As a result of the work by several researchers, it is now accepted that production of pectic enzymes is of great importance in the development of disease by different plant pathogens (Brown, 1915, 1936; Wood, 1955, 1959, 1960; Diamond, 1955; Sadasivan and Subramanian, 1963; Hancook et al., 1964; Keen and Erwin, 1971; Heale and Gupta, 1972; Vidyasekaran et al., 1973; Papdiwal, 1982; Pawar and Papdiwal, 2009). The conditions affecting the in vitro production of pectolytic enzymes, their properties and characterization, their relation with the virulence of the pathogen
and with host specificity; form the major lines of investigation. These aspects were studied in case of several pathogens. Review of such work by Brown (1936, 1955); Wood (1955, 1960); Demanin and Phaff (1957) and Sadasivan and Subramaninan (1963) are particularly relevant.

Pathogens like Botrytis cinerea, Xanthomonas axonopodis pv. malvacearum, Pythium debaryanum, Fusarium moniliformae, Rhizopus stolonifer, Verticillium dahlia, Rhizoctonia solani, Erwinia carotovora and other differ very much in their nutritional requirements for the production of pectolytic enzymes. Some of them produce pectic enzymes in a very simple synthetic media devoid of pectic substances (Brown, 1915; Wood, 1955), and is termed as constitutive production.

Another group of pathogens is adaptive in the production of pectic enzymes; unless pectic substances are present in the media, the pathogens do not produce them. Some of them are Fusarium moniliformae (Singh, and Wood, 1956), Xanthomonas spp. (Bilimoria, 1966), Verticillium albo-atrum (Keen and Erwin, 1971), Cercospora herpotrichoides (Henseller, 1973) Xanthomonas campestris pv. malvacearum (Papdiwal and Deshpande, 1993).

In the third group are included Penicillium chrysogenum (Phaff, 1974), Fusarium lycopersici (Waggoner and Dimond, 1955), Fusarium vasinfectum (Laxminarayan, 1957), Verticillium spp. (Talboys and Busch, 1970). They are characterized by being partly adaptive in secretion i.e. they give greater secretion in the presence of pectic substances in the medium.
The ability to degrade pectic polymers found in plant cell walls is a feature of several plant pathogenic bacteria. Diseases caused by xanthomonads do not involve extensive maceration of host tissues, but pectolytic activity has been observed in these pathogens (Dye, 1960; Starr and Nasuno, 1967).

It is now well established that the break down of plant tissues by certain facultative parasites is caused by the activity of pectolytic enzymes which they secrete. Pectolytic enzyme production of *Pythium debaryanum* has been studied by Gupta (1958). He has observed no relation of growth with the protopectinase production. Gupta and Pandey (1959) studied the factors affecting the production of protopectinase by *Rhizopus solani*. Deshpande (1959) studied the properties of protopectinase secreted by *Rhizotonia solani* and reported that it is thermolabile. Constitutive production of pectic enzymes of *Macrophomina phaseolina* has been reported by Dube and Gour (1975). Adaptive production of pectolytic enzymes by *Phoma herbarum* has been reported by Suraiya Begum and Deshpande (1979) studies on polygalacturonase produced by *Scelerotinia sclerotiorum* were carried out by Hancock (1966). Hancock (1966) worked on degradative enzymes of *Colletotrichum trifolii* and reported that it produces polygalacturonase tans-eliminase. Pectin esterase and transelimianse production of *Fusarium solani* has been reported by Hancock (1968). Trans-eliminases of *Phytophthora rubra* has been reported by Mantri and Deshpande (1970).
In the degradation of green gram seeds by *Fusarium oxysporum*, involvement of cellulolytic and pectolytic enzymes have been reported by Chaube and Usha (1978).

Enzymes produced by seed borne fungi are involved in seed deterioration. Researchers have found that the seed borne fungi produce certain enzymes which break down the complex ingredients of the seeds into other compounds, thereby affecting the viability and nutritive value of the seeds. In such type of seed deterioration the enzyme pectinases, cellulases and amylases play an important role (Charya and Reddy, 1980, 1983; Jha, 1995).

Mehta *et al.*, (2007) studied the pectolytic and cellulolytic enzymes produced by *Fusarium solani* and *Trichothecium roseum*, which are associated with fruit rot of apple. They observed that these enzymes play an important role in disease development.

**Cellulolytic Enzymes:**

*Erwinia carotovora* subsp. *carotovora*, the pathogen causing soft rot of fruits, vegetables and other plant parts produces number of extracellular degradative enzymes including pectinases, cellulases, amylases and proteases (Willis *et al.*, 1987)

As a result of work by several investigators, it is now well understood that production of cellulolytic enzymes is of great importance in the development of diseases cause by different plant pathoges (Wood, 1960; Husain, 1958; Bateman, 1969; Kelman and Cowling, 1965; Ghewande and Deshpande, 1976)
*In vitro* production of cellulases by various microorganisms is affected by different conditions. This type of work has been carried out in various research laboratories and which has been reviewed by Rees (1956), Basu and Ghosh (1960), Norkrans(1963), Mandels and Reese (1965), Walker *et al.*, (1994) observed that cellulase secreted by *Erwinia carotovora* subsp. *carotovora* as soft rot virulence factor.

Bose and Nandi (1985) studied the role of enzymes of storage fungi in deterioration of stored sunflower and sesame seeds. They observed that the storage fungi *Aspergillus fumigatus*, *A. candidus* and *Rhizoctonia solani* as good producers of Cx enzymes. They also noted a correlation of production of Cx to mycelial growth. Sujatha *et al.*, (2006) studied production of cellulases by 3 thermophilic fungi, *viz.* *Chaetomium thermophile* var. *dissitum*, *Torula thermophila* and *Malbranchea pulchalla* var. *sulfurea*. They found that the production of the enzyme increased with the increase in incubation temperature up to 45°C. They have noted that incubation period of 9 days as optimum for enzyme production. The final pH of the culture medium shifted towards alkaline side (8.0-9.0), which was initially 5.5.
Nine isolates Chaetominum globosum, which is identified as potential antagonist of several seed borne and soil borne fungi have been found to produce cellulolytic enzyme (Ahmad et al., 2008). The enzyme production was maximum on 9th day of incubation. Pawar and Papdiwal (2009) reported production of cellulase by Xanthomonas campestris pv. mangiferae indicae as adaptive in nature; while Waghmare et al., (2009) report cellulose production in Fusarium spp. as constitutive in nature.

While working with occurrence of pre-harvest seed borne fungi of crops and their cellulolytic and pectolytic enzyme activity, Ranjan et al.(2009) observed that activities of these enzymes was very high in 10 pre harvest fungi, isolated by them.

**Amylase Assay:**

Starch is the principal storage carbohydrate in plant cells. It contains long chains of glucose linked together by oxygen bridges. Starch is made up of amyllose and amylopectin. Amylose possesses unbranched chains and is readily soluble in water, while amylopectin consists of unbranched chains and is less soluble. Amylase hydrolyse starch. Amylase cleaves amylose molecule to the disaccharide maltose, while amylase yields dextrins, short chains of glucose molecules with 6-12 glucose residues.

Plant pathogens produce these enzymes and their role is of interest in studies dealing with carbohydrate metabolism of diseased tissues (Mahadevan and Sridhar,1996) Amylolytic enzymes of mesophilic fungi, especially Aspergillus terreus, A. corneus, A. oryzae, Penicillium expansum, Fusarium
and Phoma sorghina are well described (Olama and Sabry, 1989). Extracellular amylase synthesis by Aspergillus flavus and Penicillium purpurescens was investigated by Olama and Sabry (1998) and they observed that maximum amylase production reached on 7th days of incubation. While studying in vitro α amylase and protease produced by Aspergillus flavus, Brown et al., (2001) observed that the hydrolytic enzyme excreted extracellularly by this fungus, play an important role in colonization of host plant tissue. Amylase activity of 27 psychrophilic fungi and 7 thermophilic fungi isolated from cold dessert of Himachal Pradesh were observed by Sagar et al. (2006). Jadhav and Shinde (2008) found sucrose as stimulatory for amylase production by Alternaria alternata, Aspergillus flavus, Curvularia lunata, Fusarium oxysporum, Phytophthora sp. and Rhizoctonia solani.

Adaptive production of Amylase has been reported by Pawar and Papdiwal (2009) in case of Xanthomonas campestris pv. mangiferae indicae; while it’s constitutive production has been reported by Waghmare et al., (2009).

Lipsae:

The Lipase production by microorganisms is important from economic and industrial view point (Arnold et al., 1975; Mutua and Akoh, 1993). The Lipase production by fungi is known to be affected by several factors such as pH (Abd-Alla, 1999; Ferreira Costa and Peralta, 1999), temperature (Ferreira costa and Peralta 1999; Venkateshwarlu and Reddy, 1993), Carbon source
Oil seeds are reported to carry many moulds, both in field and during storage (Chauhan and Kaur, 1975). The association of moulds adversely affects health of seeds. The seeds carry number of fungi which include species of Aspergillus, Fusarium, Rhizopus, Penicillium, Alternaria, Cladosporium, Curvularia and Cephalosporium (Singh et al., 1987). The fungi associated with seeds at the stage of harvest and under storage bring about several undesirable changes making them unfit for consumption and sowing. (Vaidehi and Lalitha, 1985) Abdel-Fattah and Hammad (2002) identified Aspergillus niger and A. terreus as highest Lipase producer. They obtain maximum lipase production in 5 days cultures having 4% corn oil as carbon source. Optimum pH for crude Lipase production was 6.0. Lipase production by A. flavus and A. alternata has been studied by Muley and Baig, (2002). Constitutive production of amylase has been reported by Waghmare et. al., (2009) in Fusarium species.

Antibacterial Activity of Plant Extracts:

Indian literature is wealthy with regard to the scientific information and knowledge about plants and their uses. Many ancient texts provide vital information about plants. Brahatsamhits of Varahmihira (6th century A.C.) describes the remedies for plant diseases, in the chapter No.55 (Sadhale, 1996). ‘Vrukshayurveda’ of Surapala, also describes about various plant diseases. Classifies them, and also suggests the possible measures to correct those
diseases. ‘Sarngadharapaddhati’ of Sarngadhara also provides the remedies for plant disease in 20 versus (Sadhale, 1996).

Work on the medicinal properties of the plants did not remain a monopoly of one region. The civilization in Egypt, Greece and China also acknowledged the utility of plants as medicine (Patwardhan and Hpooe, 1991). British people also explored the plants for their medicinal values. Around 15th and 16th century A.D. many books were published, which gave information about ‘Herbes’. The above period is also called as ‘Age of Herbals’. As time passed, the scientific community began to explore the knowledge about the possible medicinal properties of plants; this led to the screen the available plants for their antipathogenic action. Osborn (1943) screened 2300 plant species, so as to know their antibacterial activity against the bacteria like Escherichia coli and Staphylococcus aureus. In the year 1946, Atkinson tried to search the antibacterial property of 1100 species of higher plants against S. aureus and Bacterium typhosum.

In India, the screening plants in search of the their antipathogenic activity was pioneered by George and Pandalia (1949). They used Gram negative as well as some gram positive bacteria for their survey. Joshi and Magar soon followed in 1952, with their testing of 63 Indian plant species against E. coli and S. aureus. Martinez et al., (1996) screened 12 plant species, that were known to the citizens of Cuba as traditional medicinal plants against gram +ve and garm –ve bacteria;; While Taylor et al., (1996) took interest in the traditional Nepalese medicinal plants. They reported that Bauhinia,
Carissa, Mittetia, Mallotus, Tumex, Stoblus and Terminalia plant species were antiviral in action. Papdiwal and Korekar (1996) studied the effect of Cassia spp. against some phytopathogenic bacteria, Khilare and Gangawan (1997) studied the inhibitory activity of the plant species like Azadirachta indica, Calotropis procera, Terminalia chebula, Curcuma longa, Oscimum sanctum and Zingiber officinale in the management of thiophanate methyl resistant Penicillium digitatum causing green mould of mosambi.

Perumalswamy and Raja (1996) reported many weeds as antibacterial. Hulloi et al., (1998) observed that neem based formulations were much better than the chemical pesticides.

Gohil and Vala (1996) studied the effect of extracts of some medicinal plants like Allamanda cathartica, Adhatoda vasica, Acacia guriculiformis, Vinca rosea, Ricinus communis, Datura stramonium, Allium sativum, Zingiber officinalis, Lawsonia inermis, Terminalia chebula, Azadirachta indica, Allium cepa, Curcuma linga on the growth of Fusicrurium moniliforme. Zafar et al. (1989) reported the antimicrobial activity of the methanolic extract of Butea monosperma against E. coli and S. aureus.

Efficacy of different plant extracts against plant pathogens is reported recently from various laboratories in India. Shinde and Patel (2004) evaluated 12 phytoextracts against Rhizoctnia solani, the incident of black scurf disease in potato. They observed that garlic extract at 10% concentration showed complete inhibition of growth. The leaf extract from Datura officinalis, Eucalyptus globules, Oscimum sanctum and rhizome extracts of Zingiber
*Emblica officinalis* inhibited mycelial growth of *R. solani*, but their efficacy varied. However, the leaf extract of *Emblica officinalis, Adhatoda vasica, Zizypus mauritiana, Azadirachta indica* and *Ricinus communis* exhibited no inhibition on the growth of the fungus. Mamtha and Rai (2004) observed that leaf extracts of *Lantana camera, Azadirachta indica, Acalypha indica* and *Bacopa mommirri* effective in inhibiting the growth of *Fusarium solani in vitro*. Bhatnagar *et al.*, (2004) screened plant extracts of 17 species against *Fusarium oxysporum, f. sp. cumini* and found that plant extracts of *Datura alba and Plantago ovata* effective *in vitro*. Tiwari *et al.*, (2004) reported efficacy of 213 medicinal plant extracts and found that 90 extracts significantly effective in checking mycelial growth of *Sclerotium rolfsii*.

Pawar (1999) has screened 110 leaf extracts, 09 root extracts, 36 fruits extracts, 05 stem extracts, 10 seeds extracts, 04 bark extracts, 08 gums and 06 latex against 05 bacterial phytopathogens. Of the extracts used, he found that leaf extracts of *Terminalia thorelii* and *Lawsonia inermis* effective against all the five phytopathogenic bacteria. Leaf extract of *Lawsonia inermis* showed promising antifungal activity with cent percent inhibition *A. flavus and M. phaseolina* (Beebi and Ahmad, 2007). Different plant parts like seed, leaf, root, flower, tuber; fruit plup has been used against *Sclerotinia, rolfsii* by Tiwari *et al.*, (2004).

Leaf extract of neem has been found inhibiting the mycelial growth and spore germination of *Fusarium oxysporum*, (Bansal and Gupta, 2000), and its methanol extract toxic to *Sclerotinia sclerotiorum* (Zewani, 2004)
The antifungal activity of 43 plant extracts against *Aspergillus niger* was studied by Bobbarala *et. al.*, (2009), who used extract of *Holarrhena antidysenterica, Lantana camera, Pongamia pinnata, Vitex negundo* etc. Efficacy some plant extracts like *Lawsonia inermis, Plantago ovata, Lantana sp. Azadirachta indica* etc. has been studied by Bhatnager *et al.*, (2004) Ethanol extract of some plants like *Butea monosperma* has been found strong antibacterial (Tambekar and Khante, 2010).

While studying antifungal activity of some medicinal plants against *Fusarium oxysporum f. sp. Lycopersici*, Srivastava and Yadav, (2008) observed that leaf extract of *Azadirachta indica, Lawsonia inermis* and few other extracts caused mycelial inhibition. In addition, several workers (Sharma and Kumar, 2009); Wabale (2009); Khadir and Khan (2009) have reported antifungal activity of different plant extracts.

**Effect of Fungicides:**

Fungicides are used as seed dressers for the crop plants; the world over. Several workers observed the efficacy of different fungicides in checking the seed mycoflora (Karivaratharagu *et al.*, 1987) used different fungicides *viz.* captan, Bavistin for seed treatment of brinjal. For seed treatment of lentil Vishunavat and Shukla (1982) used 11fungicides *viz.*, Bavistin, Benlate, Vitavax, Plantaivx, Thiram, Dithane M-45.Aretan, Agrosan GN, Ceresan Dry, Captan and Brassicol. Of these, Captan eliminated all the fungi associated with Lentil seeds. Thiram and Bavistin also give complete control of all the fungi except *Alternaria alternata* in case of former and *Rhizopus arrhizus* in the
latter case. Savita et al., (1994) found that the fungicide Thiram control seed borne fungi of sorghum effectively. Thiram was also effective in controlling seed borne fungi of groundnut effectively by Sativitri et al., (1998). Seed treatment with fungicides viz. Thiram, Rovral, Dithane M-45, Dithane Z 78, Ridomil MZ and Bavistin considerably reduced seed borne pathogens viz. *Bipolaris maydis, Botrydiploidia theobromae, Curvularia lunata, C. pallescens and Fusarium moniliformae* (Kumar and Agarwal, 1998).

Pathonia et al., (2000) studied the effect of different fungicides and antibiotics on seed germination and storage fungi of *Acacia catechu*. They observed that the combination of Bavistin (0.025%) + Streptocycline (50 ppm.) was best in controlling fungal as well as bacterial infection. Kareappa and Kalburge (2002) studied the effect of fungicides on *Aspergillus flavus* which was a dominant seed borne pathogen of groundnut. They observed the sensitivity of this fungus against carbendazim and Thiram.

Evaluation of 4 fungicides i.e. Topsin, Benlate, Dithane M-45, Captan, against seed borne mycoflora of egg plant, was done by Habib et al., (2007). They observed that these fungicides proved effective in inhibiting the most pathogenic fungi - *F. solani* and *Alternatia alternata*. Afzal et al., (2010) found that two systemic fungicides viz. Topsin and Bayleton were effective in elimination of seed borne fungi of sunflower cultivars.