India is the largest oil economics in the world. It occupies a distinct position in terms of diversity in annual oil seed crops. The prevailing agro-ecological condition have been favourable for growing several important annual oil seeds, including edible (groundnut, rapeseed, mustard, soybean, sunflower, safflower and sesame) and non edible oil seeds (castor and linseed). India contributes a large share to the global castor production (76.9%) and also a substantial one to production of sesame (31.2%) and groundnut (25.1%). The country is the largest producer of sesame and second largest producer of groundnut.

The total oil seed production in Rabi and Kharif season is increased considerably. A record production of about 25 million tonnes of oil seeds from an area of nearly 27 million hectares and productivity of 931 kg/ha during 1996-97. The oilseeds area, production and productivity in India have increased nearly 0.5 times in 1960-61, 1.5 times in 1970-71 and 2 times 1996-97 respectively since 1950-51 (Mangala, 1999).

According to food and agricultural Organisation (FAO) about 5% of all harvested food grains are lost before consumption. In some countries it may be up to 30% of the total agricultural produce/annual harvest. Any programme leading to reduce storage losses may results in 10-20% increase in available food, in some developing countries.
The damage to agricultural product that occurs during storage is mainly due to rodents, insects and microorganisms. Rodents and insects consume grains and contaminate them with feces, webbings, body parts, foul odours and microorganisms. Beetles and moths are most ruinous of grains insects and are capable of destroying the stored products completely.

The microorganisms associated with the stored cereal grains and oil seeds are most undesirable because of their danger to public health. The actinomycetes when present may grow under certain conditions and sporulate heavily this making the atmosphere full of their spores. These spores when inhaled cause allergic disorders on sensitized subjects. Fungi are the major cause of deterioration during storage. They may cause total deterioration of grain mass because they elaborate secondary metabolites such as mycotoxins that render the product unsafe for human and animal consumptions.

In the literature it is observed that, several mycologist and plant pathologist observed and recorded association of storage fungi, especially *Aspergillus* which causes deterioration to oil seeds.

A) **Groundnut (Arachis hypogaea L.)**

This is the most important oil seed crop of India, popularly know as ‘Peanut’ make up a highly nutritious food. It is rich in number of nutrients, like protein, fat, antioxidant, copper, vitamin E, niacin etc. Groundnut belong to the family Fabaceae and are extensively grown throughout the world. They are available throughout the year, in several forms, like raw, roasted, shelled and unshelled.
Association of fungal mycoflora with Groundnut:

Association of A. *flavus* with the seeds of groundnut have been reported by number of workers. (Asplin and Cornaphan, 1961; Brock and White, 1966 and Mc Donald and Markness, 1968). Gupta and Chauhan (1970) isolated *A. niger* along with *A. flavus* from groundnut seeds. Similarly, Lalithakumari *et al.* (1971) observed presence of *A. fumigatus* along with *A. flavus* as dominant fungi on groundnut seeds. Cherry *et al.* (1975) isolated only *A. parasiticus* from groundnut seeds. Singh and Ghewande (1980) isolated *A. niger* from groundnut kernels.

Storage seed mycoflora in Groundnut:

Welty and Goper (1967) recorded species of *Fusarium* and *Aspergillus* from stored groundnut seeds. These species reduced the germination of seeds and damage the seeds during storage (Christensen, 1973). Many workers have detected different mould fungi and their toxin production ability in stored grain, which deteriorate the stored products (Rodrieks, 1976, Afzal *et al.* 1979, and Vedahayagam *et al.* 1989). Species of *Aspergillus, Penicillium* and *Rhizopus* have also been reported on groundnut seed (Lumpungu *et al.* 1989). Mukherjee *et al.* (1992) found that *Aspergillus flavus* and *A. niger* were the predominant storage fungi of groundnut seeds. Bhattcharya and Raha (2002) reported that in groundnut seeds *Aspergillus niger, A. rubber* and *A. flavus* were initially very abundantly found in storage condition. Incidence of *Aspergillus flavus* increased to 78% but *A. niger* gradually declined to 40% and *A. rubber* disappeared after the tenth months.
Abnormal seed mycoflora in Groundnut:

Chauhan and Gupta (1968) reported that seeds of groundnut when get infected with *Aspergillus flavus*, develops yellowish colouration. Lalitakumari *et al.* (1970), recorded 27 fungal species from abnormal groundnut seeds. Chauhan and Mall (1981) also claimed that discoloration in the seeds of groundnut showed maximum incidence of *Aspergillus flavus* and *A. niger*. Where as, Chavan and Mukadam (2001), isolated *Alternaria alternata* from discoloured oil seeds.

Chauhan and Mall (1981) isolated seed mycoflora from the discoloured seeds, and they stated that the major role in seed discoloration was found to be due to the association of *Aspergillus flavus*. Abnormal seed mycoflora was studied by Sobti and Sharma (1988) and they isolated *Aspergillus flavus*, *A. sydowii*, *A. niger*, *A. wentii*, Ramkrishna and Kolte (1988) showed the association of fungi like *Aspergillus niger*, *Trichoderma pseudokoningi* and *Fusarium semitectum* from abnormal seeds. Chavan and Danai (1993) reported that the presence of species of *Aspergillus*, *Macrophomina*, *Trichoderma* and *Spicaria* from abnormal oil seeds from Maharashtra state.

B) Soybean (*Glycine max* L.)

Soybean is an important pulse and oil seed crop of the hilly regions in India. It has recently been introduced on a large scale for cultivation in India. Soybeans are very rich in nutritive components. Besides the very high protein content, Soybeans contains a lot of fibre and are rich in calcium. The soya
proteins has a high biological value and contains all the essential amino acids, they are rich in unsaturated fatty acid and low in saturated fatty acids.

**Association of fungal mycoflora with Soybean:**


**Storage seed mycoflora in Soybean:**

Dorworth and Christensen (1968) suggested that the decrease in population of field fungi is due to their interaction with storage fungi. *Aspergillus flavus* and *A. niger* were adapted to the groundnut, soybean, sesame and sunflower seeds since they were predominant during storage. *Aspergillus candidus* appeared after some months of storage possible when nutrient and water availability become favourable as a result of fungal activity.

Bhattachary and Raha, (2002) reported that, *Aspergillus candidus* appeared after 5 months and increased to 44% at the end of 1 year. Singh (2009), isolated seventeen fungi namely *A. flavus, A. niger, A. ochraceus,*
Alternaria alternate, Chaetomium globosum, Fusarium solani, F. pallidoroseum, F. verticillioides, Penicillum citrinum, Phoma sp. etc. from stored oil seeds. Aspergillus flavus, A. niger, A. repens and A. versicolor were predominant at the start of the storage period. Only A. flavus gradually increased to 90%. Aspergillus versicolor was found to be disappeared after five months.

**Abnormal seed mycoflora in soybean:**


**C) Sunflower (Helianthus annus L.)**

Sunflower is an important oil seed crop, which is a rich source of vegetable oil of high quality with anticholesterol properties. Seeds, which are consumed as raw, roasted or salted, contains 32 to 45% edible oil, which is a rich source of polyunsaturated fatty acids. Seeds of sunflower are subjected to attack by several fungi. Mukewar and Sen (1979) observed the incidence of Aspergillus niger, Penicillium spp. Alternaria alternate, A. Zinnae, Phoma exique.
Storage seed mycoflora in Sunflower:

Seed infection and biodeteriorations during storage and reduction in germination is reported to be caused by *Alternaria alternata* (Prasad and Singh, 1983). Straser (1985) reported *Fusarium oxysporum* as seed borne pathogen of sunflower even from the endosperm of chemically treated seeds. Vijayalakshmi and Rao (1985) isolated *A. flavus*, *A. niger*, *A. luchuensis*, *A. nidulans*, *A. fumigatus* and *A. glaucus*. Moraghy et al., (1986) reported that different species of *Aspergillus* were associated with the seeds. Similarly, Roberts et al. (1987) isolated *A. awamori*, *A. condidus*, *A. flavipes*, *A. flavus*, *A. fumigatus*, *A. montevidensis*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. penicilliodes*, *A. tamari*, *A. terreus*, *A. termicola*, *A. versicolor* and *A. wentii* from sunflower seeds.

Association of *Fusarium* species with seeds, results in spread of several diseases in fields such as wilting (Vijayalakshmi and Rao, 1986) food rot, seedling blight, stunting, wilting and hypertrophy in sunflower (Shanaz and Ghaffar, 1990, 1991a). Neera and Mehrotra (1990) found presence of *A. nidulans*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. tamari* and *A. terreus* among the total of 28 seed borne fungi associated with sunflower seeds.

Abnormal seed mycoflora in Sunflower:

Low quality with reduced and discoloured oil contents of sunflower seeds are reported to be caused by species of *Rhizopus* (Zod, 1979, Singh and Prasad, 1977).
Shahanz and Gaffer (1991a) reported the incidence of fungi such as *Aspergillus flavus*, *A. niger*, *Alternaria alternta*, *Fusarium moniliforme*, *F. solani* and *F. semitectum* on abnormal sunflower seeds from Pakistan. Umatale (1995) studied the seed mycoflora from different varieties of oil seeds (LS 11, 21, M71-9, MSFH-8, KBSH-1, E-48414, SS-56, LDMRSH-1, Morden) which are cultivated in Marathwada region.

**D) Safflower (Carthamus tinctorius L.)**

This is one of the common oil seed of India, China, Egypt and several other African and far eastern countries. In India it is grown during rainy season in the Madhya Pradesh, Maharashtra state, Rajasthan, Uttar Pradesh, west Bengal and Gujarat.

**Storage seed mycoflora in Safflower:**

During harvest infection was mostly included by the field fungi, including *Alternaria* sp., *Curvularia* sp. etc. Their number decreased gradually during storage, because they were replaced by storage fungi, mainly by different species of *Aspergillus* as found by earlier workers (Clarke *et al.* 1967, Mukherjee *et al.* 1988). Bose and Nandi (1985), reported association of *A. flavus*, *A. fumigatus*, *A. sydoco* on safflower seeds. Tawar and Kore (1985) isolated *A. niger* and *A. flavus* from the seeds of safflower while, Singh *et al.* (1987) could get only *A. niger*, where as Prasad (1988) observed association of only *A. flavus* from the safflower seeds. Sandikar (1990) also reported dominant fungi on safflower and sesame. Chavan and Kakde (2009) isolated
nine different fungal species from safflower oil seed among these fungi *Aspergillus* sp. showed their dominance as compare to other fungi.

**E) Sesame (Sesamum indicum L.):**

Sesame seed is an important oil seed widely grown and used in some African, Asiatic countries including India (Madhya Pradesh, Rajasthan, Uttar Pradesh). About seventy two fungi, seven bacteria, one Phytoplasma (Mycoplasma) and one virus disease have been reported in India. (Vyas *et al.* 1983)

**Storage seed mycoflora in Sesame:**


**Abnormal seed mycoflora in Sesame:**

Khushi and Khare (1979) reported that discoloured seeds of sesame showed presence of *Aspergillus* species along with other fungi. Tani and Siddiqui (1981) recorded 17 fungal species from sesame seeds. Later on Yu (1981) found the pathogenic moulds from sesame seeds from Korea. Singh and Ghewande *et al.* (1984) isolated the dominant fungi like *Macrophomina phaseolina*, *Aspergillus flavus*, *A. niger*, *Alternaria* species, from the abnormal sesame seeds. Where as Vaidehi and Lalitha (1985) detected 27 fungi from
abnormal sesame seeds. Where as, Wu (1988) reported 10 seed moulds, from sesame seeds from Taiwan.

F) Mustard (*Brassica compestris*) Prain

Mustard is cultivated as one of the major oil seed crop in different parts of India viz. Bihar, Assam, Haryana, Punjab, Uttar Pradesh, West Bengal.

Mishra and Kanaujia (1973) reported that *Aspergillus fumigatus*, *A. niger*, *A. terreus* and *A. flavus* were associated with mustard. On the other hand Jain *et al.* (1982), Sing and Neigi (1984) reported only *A. flavus* was found to be associated with mustard. In 1990, Shivapuri *et al.* isolated *Aspergillus flavus*, *A. caesiellus*, *A. nidulance*, *A. sejunctus* and *A. versicolor* from *Brassica comesperis*.

**Storage seed mycoflora in Mustard:**

Gupta and Basuchaudhary (1994) observed that, infection of seed-borne fungi from different Mustard seeds samples which was mainly caused by *Alternaria alternata* during storage period. *Alternaria alternata* reduces the germinability during storage, it was also observed that as the storage period increases, there was decreased in oil percentage of seeds (Ghugal and Thanker, 2000). *Alternaria* blight is caused by *Alternaria brassicae* and *A. brassicicola* which was reported by Patni *et al.* (2006). Similarly, Kumar and Kolte (2006) studied *Alternaria* blight of mustard. Singh and Singh (2007) reported *Alternaria* blight caused by *Alternaria brassicae* and *A. brassicicola*. 
Abnormal seed mycoflora in Mustard:

The seed-borne pathogens from the abnormal Mustard seeds were isolated by Singh and Ghewande (1980), they reported *Aspergillus flavus*, *A. caesiellus*, *A. nidulance*, and *Alternaria brassicae* as the dominant fungus in abnormal seeds, while Jain *et al.* (1982) isolated *Alternaria brassicae* from the abnormal mustard seeds. Vishnuvant *et al.* (1985) found that the grey discolouration and shrivelled appearance of seeds are due to the presence of *Alternaria brassicae*.

G) Linseed (*Linum usitalissimum* L.)

Linseed is an important oil seed, grown in different parts of India such as Madhya Pradesh, Uttar Pradesh, Rajasthan, Bihar, Gujarat and Maharashtra particularly in rabbi session. Different workers have studied the seed mycoflora and seeds born disease of linseed. Kadian and Suryanaraya (1971), detected the association of *Aspergillus niger*. In 1973, Mishra and Kanaujia reported association of *Aspergillus flavus*, *A. niger* and *A. aculeatus* with the Linseed.

Thakur and Williams (1980) found that leaf spot in linseed due to presence of *Alternaria lini*. Singh and Srivastava (2001) reported *Alternaria* blight as one of the important diseases of linseed in eastern Uttar Pradesh. Similarly Singh and Singh (2002) found *Alternaria* blight of linseed caused by *Alternaria lini* and *A. linicola*. Recently Singh *et al.* (2006), reported *Alternaria* sp. on linseeds.
Abnormal seed mycoflora in Linseed:

Prasad and Prasad (1980), reported the association of *Aspergillus flavus, A. niger* and *A. lini* with the abnormal linseed. Saraswat and Mathur (1985), isolated *A. candidus, A. flavus, A. fumigatus, A. japonicas, A. tamari, A. terreus, A. nidulance, A. niger* and *A. ustus* from abnormal seeds. Agarwal and Singh (1975) reported *Alternaria lini* and *A. linicola* from abnormal linseeds.

H) Castor (*Ricus communis* L.)

Castor is one of the important oil seed crops of India, mainly used for medicine and lubrication and cultivated throughout the country. Singh (1948), studied seed mycoflora of castor seed and he found *Aspergillus niger* as a dominant fungi. Similarly Mishra and Kanaujia (1973) observed only *Aspergillus flavus* associated with Castor seeds.

Abnormal seed mycoflora in Castor:

Abnormal seed mycoflora of castor, causing seedling blight disease was studied by Culp *et al.* (1966) which was mainly caused due to *Alternaria ricini*. A detail account on seed-borne fungi of castor was published by Jain and Patel (1969). They found species of *Alternaria* to be associated with the seeds of castor. Similarly, *Aspergillus niger, A. flavus, A. ustus, Alternaria alternata, A. solani* and *A. tenuissima* were found to be associated on the castor seeds. (Nagaraja and Krishnappa, 2006)
DNA Fingerprinting

The genus *Aspergillus* has been classified based on morphological and growth criteria. Members of the *Aspergillus* section are economically important and methods of differentiating them are thus very important. Several molecular methods are developed to distinguish these strains. Also a number of biochemical and genetic studies have been used in order to provide a better means of classification (Lee, *et al.* 2004).

The most commonly used marker systems are restriction fragment length polymorphism (RFLP) (Soller and Beckmann, 1983), random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990), amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995), inter simple sequence repeats (ISSRs) (Zietkiewicz *et al.*, 1994) and microsatellites or simple sequence repeats (SSRs). Among them to characterize DNA variation patterns within species and among closely related taxa in Vigna species have been studied by ISSR (Ajibade *et al.*, 2000).

Similarly Cooke *et al.* (1998) studied interaspecific and interspecific variation. Waals (2004), also studied genetic diversity among *Alternaria solani* isolated from potato in South Africa. He further reported that isolates collect from different geographical region show relative high level of diversity amongst the isolates.

Ten dominant species of *Alternaria* were studied by Chavan (2008) for their molecular characterization by AFLP technique. All these species showed great diversity at species level which where isolated from different oil
seeds and cereals. Similarly, Mukadam et al. (2009) studied molecular characterization of *Aspergillus niger* isolates.

Dendograms which assess the likeness between different isolates has also been used (Martinez et al. 2001). Restriction fragment length polymorphism (RFLP) analysis has been applied to number of studies to detect difference between fungi and to establish relationship between them.

Aflatoxins the most frequently studied mycotoxins, are produced by certain *Aspergillus* species isolates of fungi. The aflatoxin biosynthetic pathway studies have led to a number of discoveries. Several structural and regulatory genes involved in the biosynthesis of aflatoxins have been discovered and purified (Trail et al. 1995). Aflatoxin production and contamination of agricultural crops are major causes of economic losses in agriculture. By using study of genetic diversity, characterization for both aflatoxigenic and non-aflatoxigenic isolates can be studied.

**Change in lipase enzyme activity**

Seeds contain two major types of lipids: storage and functional. Storage lipids are primarily neutral triglycerides. Functional lipids can be grouped in several classes: Phospholipids, glycolipids, sterols, sterolessters, sterolesster glucosides, etc. and are present in subcellular organelles and other compartmentalized structures. Storage lipids especially triglycerides, serve as reservations of energy to be mobilized by specific enzymes upon damage to the seed by browsing disease other stress factor or by germination. When seeds are damaged by improper storage conditions or are exposed to certain
microorganisms. Lipid degradation reactions can occur. These reactions can be catalyzed by their own endogenous enzyme systems or by enzymes from the microorganisms, depending upon the environment and/or the extent of the damage. Lipase and lipoxygenase are the two principal enzymes involved in degradation of lipids in seeds. (Allen et al. 1983).

Oil seeds are rich sources of triacylglycerides. In the germinating seed these lipids are important sources of energy. Lipases catalyze the breakdown and metabolism of these triglycerides to glycerol and fatty acids which can then be oxidized to provide energy for the newly emerging plant. Despite economic importance of the oil from soybeans, groundnut, safflower, sesame etc. most research in oil seeds has been done on the acid lipase of the castor bean, an industrial oil seed that contains on active lipase in the ungerminated seed. There are few reports on lipases in other industrial oil seeds (eg. Soybean, safflower, sesame, groundnut, cottonseed, corn and coconut). (Brockerhoff and Jensen, 1970).

Ramakrishnan and Banerjee (1951) found that fungal lipases grown on oilseeds were more active than the endogenous seed lipases and they concluded that activity of the fungal lipase increase with age of the seedling to a certain point be for decreasing.

Many researchers working on biodeterioration of oil seeds reported that, loss in seed weight as well as oil contents may have been relationship with that of the lipolytic nature of seed mycoflora. This has been reported in case of different species of Aspergillus associated with stored maize seeds (Goodman
and Christensen, 1952), *A. niger*, *A. tamari* and *A. flavus* were isolated from seeds of sesame, castor and cotton by Sharma and Chouhan (1976), *Aspergillus* sp. from gram seeds by Sinha *et al.* (1979), *A. flavus* and *A. fumigatus* from the seeds of sesame and safflower by Bose and Nandi (1985), *A. flavus* isolated from sesame and safflower by Sandikar (1990) showed significant production of lipase. While during the studies on lipase production in case of *A. flavus*, Sandikar (1990) observed that serine, tryptophan, cystine and seed powders (sesame and groundnut) supplemented in basal media supported lipase production significantly. Similarly, he also reported that *A. flavus* could produce lipase on a broad range of pH 3.5 to 7.5 but had a optimum temperature 25°C.

Saraswat and Mathur (1985) studied lipase production in *Aspergillus japonicus*, *A. terreus*, *A. nidulans*, *A. niger* and *A. ustus* isolated from the seeds of linseed, they reported that among the species *A. niger* was maximum lipolytic and which was followed by *A. flavus* and *A. candidus*, while, lipase production was very poor in *A. japonicus* and *A. nidulans*.

Similarly Roberts *et al.* (1987) studied lipase production in *A. candidus*, *A. fumigatus*, *A. tamarii*, *A. terreus*, *A. niger*, *A. versicolor*, *A. wentii* and *A. flavus* isolated from seeds of sunflower. Among the species only *A. niger*, *A. versicolor*, *A. flavus* and *A. wentii* produced lipase while, *A. candidus*, *A. flavus*, *A. tamari* and *A. terreus* could not produce lipase.
Regarding the effect of different substrates (oil sources) on lipase production, Krish (1935) reported that soybean meal is the best medium for lipase production in *A. flavus*. While Sharma and Chouhan (1976) found castor oil as an unfavourable oil source for lipase production in different species of *Aspergillus*. Recently, Kakde *et al.* (2009) and Gadgile and Chavan (2009) reported that polysaccharide induces lipase activity of *Aspergillus* species.

Bhosale (1989); and Rathod (2007) reported that nitrogen sources like casein and peptone induced lipase activity of *Aspergillus flavus, A. niger, Curvularia lunata, Fusarium oxysporum* and *Alternaria* species. Khairnar (1987), found that potassium nitrate and sodium nitrate increase amylase activity in *Alternaria alternata*. Similarly, Patil and Shastri (1982), found that potassium nitrate induced protease activity in *A. alternata*.

Rathod (2007) and Kesare (2008), reported impact of phosphorus sources on hydrolytic enzyme. They observed that, Lipase action of *Aspergillus flavus, A. fumigatus, nidulance, A. oryzae, A. terreus* and *A. ustus* were stimulated by disodium hydrogen ortho-phosphate. Similar results were observed by Kakde *et al.* (2009), in case of lipase production by *Aspergillus niger, Fusarium oxysporum, Macrophomina phaseolina* and *Penicillium notatum*, in different oil seeds.

Rathod (2007) and Kesare (2008) reported effect of antibiotic at 100 ppm concentration on different seed borne fungi. Antibiotic like Norflaxacium, Ampicilin, Trioflan, Tetracycline and Almox DT inhibited lipase action in species of *Aspergillus*. Recently Gadgile and Chavan (2009b) found that,
Almox DT significantly inhibited pectinase activity of *Penicillium* species and *Aspergillus flavus*. Gadgile and Chavan (2009b) reported that hostacetyl significantly inhibited pectinase activity of *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Colletotrichum gloeosporioides*, *Rhizopus stolonifer* and *Penicillium* species, while antibiotic induced pectinase activity of some post harvest fungi of mango fruits.

**Biodeterioration of seeds**

Many developing countries including India have been trying to increase seed production in recent years. Unfortunately, due to lack of improved post harvest preservation technique, a large portion of annual yield gets lost in storage, and these losses have been attributed partly to the microbial action in storehouses. Fungi growing on stored grains, can reduce the germination rate along with loss in the quantum of carbohydrate, protein and total oil content, induces increased moisture content, free fatty acid content enhancing other biochemical changes (Bhattacharya and Raha., 2002). Mondal *et al.* (1981) and Nandi *et al.* (1982), reported post harvest deterioration of oil yielding seeds.

Seeds of different crops are known to carry variety of fungi mainly the species of *Curvularia*, *Alternaria*, *Helminthosporium*, *Cladosporium*, *Fusarium*, *Macrophomina*, *Rhizoctonia*, *Rhizopus*, *Mucor*, *Penicillium*, *Chaetomium*, *Aspergillus* and etc. These fungi during their association with the seeds in field as well as during storage cause various types of harmful effects to the seeds. ‘Utilization and change in seed contents, affected by these type of
association is known as biodeterioration’. Biodeterioration results into different types of abnormalities, discolouration losses in weight, viability and food nutrients of the seeds. It is estimated by Clark (1966) that about 4% of the world’s grains are lost due to biodeteriorations caused by the microorganisms. While, specifically Christensen (1972) stated that the losses in food grains have been found to be more severe due to the species of *Aspergillus* than other fungi.

Among various seed contents starch (Vidyasekeran and Govindswamy, 1968), Lipid (Godman and Christensen 1952, Lalitakumari *et al.* 1971) and proteins (Sinha and Prasad 1977, Panchal 1984) were found to be reduced significantly by seed mycoflora, Vaidehi and Lalita (1973) found that proteins, starch and phenol in sesame seeds were reduced by the action of storage fungi and there was increase of amino acids and fatty acids.

There was gradual depletion of nutrients with prolonged storage period. Protein showed 24% decrease in its value when stored in teen container, whereas in Iron-bin, Pucca Kothi and Metal-bin the decline was 23%, 17% and 15% respectively within 8 month of storage. Depletion in starch was maximum in kothi (18%) minimum in metal bin (3%) like wise total sugar, reducing sugar and non reducing sugars declined in their value in different storage system.

Maximum incidence of fungi which found from Kothi storage system were *Aspergillus candidus*, *A. flavus*, *A. niger*, *Fusarium semitectum* and *Penicillium citrinum*. Mathur and Sinha (1978) studied certain biochemical changes in bajra seed during storage. They had found that reducing sugar decreased in the earlier period of storage and then increased. Change in protein,
nitrogen and total oil where only slight. Fatty acid value steadily increased on storage.

Association of different species of *Aspergillus* with the seeds has been reported to cause variety of harmful effect like

i) Loss in seed weight

ii) Decrease in percent germination of seed

iii) Seed discolouration

iv) Seed rotting

v) Biochemical changes and

vi) Production of mycotoxins

**i) Loss in seed weight**

Due to continue association of fungi with seeds during storage period results into the depletion of seed contents, (i.e. gradual or sudden loss in seed weight). This has been reported in seeds of black gram and green gram, loss in seed weight were detected by the association of *Aspergillus flavus* and *A. niger* (Bilgrami *et al.* (1976) and Bhikane, 1988). The loss in seed weight have been reported in case of other crops like jowar due to *Aspergillus flavus* and *A. niger* (Panchal, 1984), in bajra due to *Aspergillus flavus* (Girishan and Reddy, 1987), in sunflower due to *A. flavus* (Sandikar, 1990), in mustard due to *A. flavus* (Kumar and Prasad, 1993).

In case of jowar seed borne fungi, like *Curvularia lunata* (Bhatnagar, 1971), *Fusarium* spp. (Castor 1977), *Macrophomina phaseolina* (Anahosur and Patil, 1983) has been reported to cause loss in seed weight. Pedgaonkar (1973)
estimated the reduction in grain weight due to mould and reported weight loss ranged from 11.9 to 16.7% in different jowar cultivars, while Godbole (1982) recorded that weight loss was maximum i.e. 15.2% and minimum i.e. 12.1%. Similar type of reduction in Pea (Sawhney and Aulakh, 1980), Wheat (Ahmed et al. 1981) and Mustard (Randhawa and Aulakh, 1981). Sonawne (2002) reported loss in seeds weight of *Pisum sativum* due to *Alternaria alternata*. Rathod (2007) showed that *Alternaria tenuissima* in case of wheat, *A. citri* in black gram reduced the seed weight considerably.

**ii) Loss in germinability**

The associated fungi with storage fungi results in toxins production which cause inhibition of seed germination and also various types of abnormalities in seedlings. Reduction in seed germinability due to association of species of *Aspergillus* have been reported in lentil due to *A. flavus* (Gupta et al. 1982) and in pea due to *A. rubber* (Harman and Gienda, 1972). Similarly browning of stem and roots in the seedlings due to *A. terreus* and lack of secondary roots due to association of *A. niger* in case of cowpea has been studied by Singh and Chohan (1974).

In addition to the inhibition of seed germination toxins cause various types of abnormalities in seedlings. In bajra, Mathur and Sinha (1978) studied that, *Aspergillus flavus*, *A. terreus*, *A. chevalieri* and *Penicillium* sp. produces toxins which are of highly inhibitory for seed germination. The highly inhibitory nature of culture filtrates produced by respective seed borne fungi in different crops has been recorded as *Aspergillus flavus* for maize (Prasad and
Daradhiyar, 1978), *A. flavus, Trichoderma viride, Helminthosporium sativum* and *Fusarium moniliforme* for wheat (Thakur and Prasad, 1983) and *Aspergillus niger, Drechslera haloder, Fusarium moniliforme, Trichoderma viridae* and *Trichothecium roseum* for alfalfa (Singh and Gupta 1984). Mallikarjun and Bhide (1985) observed that culture filtrate of *Alternaria tenuis*, *Curvularia* spp. and *Helminthosporium* sp. interestingly inhibited seed germination in a group of cereals namely jowar, maize wheat and paddy.

Rate of seed germinability is a well known indicator of deterioration. Loss in germinability gradually increase with prolonged storage in all seeds. (Bhattacharya and Raha, 2002). The decrease in germinability during storage of seeds was perhaps due to fungal infection and thus damaging the embryo (Christensen and Kaufmann, 1969), Depletion of nutrient reserve (Christensen, 1973), or the production of toxic metabolites (Harman and Nash, 1972 and Lacey 1975). Harrington (1967) suggested that depletion of available oxidisable materials in meristematic cells might cause deterioration. Loss of viability of stored seeds may be associated with increased respiratory rate (Bass et al. 1963).

### iii) Seed discolouration

When various species of *Aspergillus* associated with seeds of different crops, they results into seed discolouration, which supposed to be undesirable condition of seeds used both for the purpose of agriculture and consumption. Species of *Aspergillus* are found to be responsible for seed discolouration and pigment production. Qasem and Christensen (1958) found
association of *A. candidus*, *A. flavus*, *A. glaucus* and *A. ruber*, with discoloured seeds of maize. Among all these species *A. ruber* caused more damages i.e. seed discolouration.

Most of the species belong to *Aspergillus* group have been reported to produce red coloured pigments. It has been observed by Chavan (1993) in case of *Aspergillus ruber*, Gould and Raistrick (1934) in case of *Aspergillus glaucus*, Smith (1954) in case of *A. nidulans*, Zeijic (1962) in case of *A. niger* and Maetoyashi *et al.* (1978) in case of *A. ochraceus*. Similarly Orange yellow coloured pigments have been reported by Gould and Raistrick (1934), Johnson and Gould (1953) in case of *Aspergillus glaucus*. Hatsuda and Kuyama (1954) reported orange yellow coloured pigment produced by *Aspergillus versicolor* and in case of *Aspergillus fumigatus* maroon coloured pigment production has been reported by Anslow (1938), Reddy *et al.* (1980) found association of *Aspergillus* along with other fungi with discoloured seed of black gram. In soybean, (Kilpartick, 1957) purple colour pigmentation was observed due to *Cercospora kikuchii* and *Alternaria tenuis* found to produce blue staining in cotton seed (Neergard, 1977).

**iv) Seed rot**

Rotting of seeds due to association of different seed borne fungi is very common (Singh and Gupta, 1984). While studying comparative effects of different species of *Aspergillus*, Singh and Gupta (1984) found that among *A. flavipes*, *A. fumigatus*, *A. flavus* and *A. niger*, only *A. flavus* caused seed rotting.
Sandikar (1990) reported rotting of sunflower was caused due to *Aspergillus flavus*, and Rotting of groundnut seed were caused due to *Aspergillus niger* reported by Dange and Sharma (1993), and Sobti and Sharma (1993). Seed rot due to *Aspergillus flavus* in case of jowar was reported by Rati and Ramlingam (1974) and Panchal (1984). While in case of maize, seed rot was caused due to *Aspergillus flavus* and *A. niger* (Aulakh *et al.* 1976 and Kulkarni 2009).

Seed rotting is attributed mainly to the microbial destruction particularly with the help of their hydrolytic enzymes. This has been reported in various crops like cereals (Grewal and Mahedrapal, 1965 and Mishra and Mishra, 1971). Pulses (Sawhney and Aulakh, 1980, Bhikane and Mukadam, 1982), and oil seeds (Shukla and Bhargava 1977). Seed rotting in bajra has been reported due to *Alternaria alternate, Curvularia lunata, C. pallescens, Penicillium spp. Dreschlera hawiiensis, D. longirosstrats, D. maydis* and *Phoma*. While according to Randhawa and Aulakh (1980) and Shetty *et al.* (1982) the maximum seed rot in bajra has been reported only due to the species of *Fusarium*.

v) Biochemical Changes

Fungal activity can cause changes during storage of seeds and seed products that are detrimental to nutritive value (Zeleny 1954). Specifically, nutrients are lost because of changes in carbohydrates, protein, lipids, and vitamins (Semenick 1954).
**Change in dry weight**

Continuous association of fungi with seeds, results into the gradual or sudden loss in weight. Loss in weight of Moong bean due to *Aspergillus flavus* was observed by Bilgrami *et. al.* (1976). Randhava and Aulakh (1980) observed the reduction in seed weight due to the mycoflora of Mustard. Similarly, Prasad (1980) reported loss in dry weight of coriander seed due to association of fungi.

Chalal (1981) reported loss in seed weight inMustard due to *Aspergillus flavus* and *A. niger*. Sandikar and Mukadam (1990) also observed the loss in seed weight due to the association of fungi in safflower.

**Change in reducing sugar content**

Conditions that favour fungal activity lead to carbohydrate decomposition. Sugars are consumed and converted into CO$_2$ and H$_2$O. At moisture levels of approximately 15%, seed loses both starch and sugar and the dry weight decrease.

Ramstad and Geddes (1942) found a marked increase in reducing sugars in soybeans stored at more than 15% moisture. The increase was followed by an equally significant decrease in non reducing sugars. Milner and Geddes (1946) demonstrated that sugars in stored soybean disappear during the biological phase of heating.

It has been correlated by various workers that seeds infested with moulds showed significant increase in reducing sugars. Mathur and Sinha (1978) reported that in case of bajra seeds associated with *Aspergillus*...
candidus, A. nidulans, A. flavus, A. fumigatus, A. niger and A. terreus. The fungi caused initial increase in reducing sugar but latter on it was found to be decreased gradually. Girisham and Reddy (1989) stated that increase in reducing sugar content of bajra seeds associated with *Aspergillus flavus* was found to be maximum at high RH. Seeds of jowar infested with *A. flavus, A. niger, A. terreus* (Bhadarih and Ramaro, 1987) and seeds of cowpea with *Aspergillus fumigatus, A. nidulans* and *A. terreus* showed considerable increase in reducing sugar. Similarly, Maheshwari and Mathur (1984), Prasad and Pathak (1987) reported that seeds of wheat, maize, and barley associated with *Aspergillus niger, A. flavus, A. terreus, A. candidus, A. sulphurous* and *A. sydowi* showed increase in reducing sugar. Prasad *et al.* (1990) also observed increase in reducing sugar in the seeds of radish infested with *Aspergillus flavus*.

**Change in Protein content**

The total protein content of grain is calculated from its nitrogen content is generally assumed to be constant during storage. However, as fungal deterioration advances and carbohydrate is used in the respiratory processes, protein increases mathematically. Daftary *et al.* (1970) demonstrated this by finding that the protein content (determined by the Kjeldahl method) was slightly, but consistently, higher in flours from mould damaged samples than in corresponding flours from sound wheat.

Bilgrami *et al.* (1976) studied that, infesting seeds with *A. flavus* results in significant decrease in the seed protein. Similarly, Sinha *et al.* (1978)
observed loss in protein contents of Arhar seeds due to *Aspergillus flavus* and *A. niger*. They also noted that among the two species, *A. niger* was found to be more active than *A. flavus*, while on the other hand Vijayakumari and Karan (1981) observed loss in protein content of cowpea seeds and which was caused more actively by *A. flavus* than the other species. This was also seen in case of lobia seeds as reported by Maheshwari (1987).

Any abnormally change in seed protein due to the association of mycoflora may affect adversely the nutritional value of the seeds. Bhattacharya and Raha (2002) reported that in groundnut seeds, the protein content reduced from 26% to 21.8%. The protein content of soybean seeds also decreased slowly from 40% to 37.6% but gradually further increased to 38.6% and finally become slightly reduced (38.4%).

Reports on degradation of protein from seeds of cereals have been made by Steward (1965), Vidyasekaran *et al.* (1973), and Cherry (1982). Bilgrami *et al.* (1981) noted a significant loss in protein content of maize seeds infested with *A. parasiticus* which had resulted into increase in free amino acids. On the contrary, in case of bajra it has been reported that *A. flavus* caused increase in seed protein content and this increase was significant during early period of storage (Mathur and Sinha, 1978). Nandi *et al.* (1989) also observed loss of protein in wheat seeds infested with *Aspergillus flavus, A. niger* and *A. candidus*. 
Change in protein content of oil seeds caused by different species of *Aspergillus* have been reported by different workers. Cherry *et al.* (1975) found in case of groundnut that seeds infested with *Aspergillus flavus* showed great loss in protein resulting it into increase in free amino acids. Similarly, Singh and Prasad (1988) noted loss in protein of sesame seeds due to *Aspergillus flavus*.

Neeti and Karan (1991) noted degradation of protein in sunflower and sesame seeds caused by *Aspergillus flavus* and *A. niger*. Among the two species of *Aspergillus*, *A. flavus* was found to be more active than *A. niger*. On the other hand, Neera and Mehrotra (1990) observed increase in soluble proteins of sunflower seeds infested with *Aspergillus flavus*, *A. tamari* and *A. niger*. Sandikar (1990) found loss in protein in sunflower and sesame seeds due to *Aspergillus flavus*.


**Change in Fat content**

Most of the moulds have a high lipolytic activity, fats and oil in seeds are readily broken down into free fatty acids and partial glycerides during the fungal deterioration of seeds. These changes are greatly accelerated when moisture and temperature are favourable for fungal growth (Goodman and
Christensen 1952; Loeb and Mayne 1952). Similarly, Singh et al. (1972) reported biodeterioration of fat in sesame and safflower.

**Change in ash, nitrogen, calcium and phosphorus content**

There are reports evidencing infection of fungi with seeds and fruits in the field and storage (Roy et al. 1887; and Datta and Roy 1987), resulting in the changes in ash, nitrogen, calcium and protein content due to *Aspergillus parasiticus* and *A. niger*. Effect of *Aspergillus flavus* and *Fusarium oxysporum* on biochemical content of three legume crops were studied by Embaby and Mona (2006), they reported changes in protein, carbohydrate, fat, fiber, ash and moisture content of legume crops.

Maheshwari and Mathur (1987) reported that during biochemical changes, infection by *Aspergillus nidulans* was more deleterious than by *A. terreus*. Ushamitin et al. (1998), reported 10.2% loss of protein, 27.02% of carbohydrates, 0.5% of fat, 5.99% of fiber and 1.08 of ash caused due to *Aspergillus flavus* in bean seeds. Similar results of biochemical changes were reported in ash, nitrogen, protein and fat by Kritzinger et al. (2003); Aziz and Mohrous (2004) and Markunas et al. (2005)

**vi) Production of Mycotoxins:**

Mycotoxins are classified by chemists as ‘natural products’ and by biologists as ‘secondary metabolites’ produced by filamentous fungi. Mycotoxins are produced under special conditions of moisture and temperature. These mycotoxins producing fungi are aerobic (use oxygen), microscopic and may colonise many kinds of food in the field. (Yu et al. 1995).
Not all fungi can produce mycotoxins. In addition, some fungi are able to produce mycotoxins only under special conditions. Even those with the ability to produce mycotoxins may not produce them all the time. The absence of mycotoxins doesn’t ensure the absence of fungal spores. Mycotoxins are also very resistant to temperature treatments and conventional food process such as freezing (Reddy and Waliyar, 2005).

There are more than 300 species of fungi with the ability to produce mycotoxins. Only about 20 mycotoxins produced by the five genera of fungi (i.e. Aspergillus, Penicillium, Fusarium, Alternaria and Claviceps) are found periodically in food at levels posing threats to humans (Benbrook, 2005). Mycotoxins have also become part of the global debate over the benefits of genetically engineered (G.E.) crops. Studies have shown that G.E. insects protected field maize is less prone to mycotoxin contamination than conventional maize (Benbrook, 2005).

Some of the most frequently studied mycotoxins belong to the genus Aspergillus. These toxins are structurally diverse and sometimes they are produced in combination by a single species. Toxicity of these toxins is also different for each species (Cotty et al. 1994).

**Aflatoxins:**

The known mycotoxins the most important which have been studied mostly because of possible hazards to human health are the aflatoxins. Tripathi (1974) observed presence of B$_1$, B$_2$ and G$_2$ in the seeds of jowar infected with *A. flavus*. Deo et al., (1981) found the twenty strains of *A. flavus* on the seeds
of lentil to be aflatoxigenic. Aflatoxins B₁, B₂ and G₁ were detected in seeds of jowar contaminated with *A. flavus* to the field (Reddy and Nusrath, 1986).

Aflatoxins (*A* for *Aspergillus*, *fla* for *flavus* and toxin the appended noun) are naturally occurring mycotoxins that are produced by many species of *Aspergillus*, but most notably *A. flavus* and *A. parasiticus* (Trail *et al.* 1995). Aflatoxins are toxic and carcinogenic to animals, including humans. Aflatoxins often occur in crops in the field prior to harvest. Post harvest contamination can occur if crop drying is delayed and during storage if water exceeds the critical value for mould growth (Trail *et al.* 1995).

At least 18 different types of aflatoxin are produced in nature (Reddy and Waliyar, 2005). Aflatoxin B₁ (Fig. 1) is considered the most toxic and is produced by both *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B₂ (Fig. 1.2) is less toxic than that of B₁ and is also produced by both *Aspergillus flavus* and *A. parasiticus*. Aflatoxin G₁ (Fig. 2.1) and aflatoxin G₂ (Fig. 2.2.) are produced exclusively by *Aspergillus parasiticus*. (Reddy and Waliyar, 2005). While the presence of *Aspergillus* in food products does not always indicate harmful levels of aflatoxins, it does imply a significant risk in consumption of that product. After entering the body, aflatoxins are metabolised by the liver to an intermediate reactive aflatoxin M₁ (Reddy and Waliyar, 2005).

Aflatoxin M₁ (Fig. 3.1) and aflatoxin M₂ (Fig. 3.2.) were first isolated from milk of lactating animals fed aflatoxin preparations, hence the M designation is given. (Eaton and Groopman, 1994).
The B designation of B\textsubscript{1} and B\textsubscript{2} aflatoxin resulted from the exhibition of blue fluorescence under UV light, while the G designation refers to the yellow green fluorescence of the relevant structures under UV light (Eaton and Groopman, 1994).

**Fig. 1.1**

Aflatoxin B\textsubscript{1}

![Aflatoxin B1](image)

**Fig. 1.2**

Aflatoxin B\textsubscript{2}

![Aflatoxin B2](image)
Fig. 2.1
Aflatoxin G$_1$

Fig. 2.2
Aflatoxin G$_2$
These toxins have closely similar structure and from a unique group of highly oxygenated, naturally occurring compounds. Their molecular formulas as established from elementary analyses and mass spectrometric determinations are:
Aflatoxin $B_1 = C_{17}H_{12}O_6$, $B_2 = C_{12}H_{14}O_6$

Aflatoxin $G_1 = C_{17}H_{12}O_7$, $G_2 = C_{12}H_{14}O_7$

Aflatoxin $B_2$ and $G_2$ were established as the dihydroxyl derivatives of $B_1$ and $G_1$ respectively. Where as $M_1$ is 4-hydroxyl Aflatoxin $B_1$ and $M_2$ is 4-hydroxyl Aflatoxin $B_2$ (Eaton and Groopman, 1994).

Different species of *Aspergillus* capable of producing aflatoxins have been reported by different workers, as Basappa et al. (1967) in *Aspergillus oryzae*, Kulik and Holady (1967), in *A. niger*, *A. wentii* and *A. rubber*, Hesseltine et al. (1975) in *A. flavus* and *A. niger*, Ohmono et al. (1973) in *A. versicolor*, Bilgrami et al. (1981) in *A. parasiticus*, Reddy and Reddy (1990) in *A. flavus*, *A. nidulans* and *A. terreus*, Dalvi and Solunke (1990) in *A. flavus*, *A. parasiticus* and *A. ochraceus*.

Lillehoy et al. (1976) studied aflatoxin production in case of corn seeds due to association of *A. flavus* and *A. parasiticus*. They also stated that simply presence mould growth was not found to be an indicative for the presence of aflatoxin. While, Diener and Davis (1966) stated that all strains of *Aspergillus flavus* were not found to be aflatoxin producer as they had screened nearly 1400 strains which were isolated from different sources but only 58% strains were reported to be aflatoxigenic, Boller and Shcroeder (1966) found 94% among 284 isolates of *A. flavus* were found to be capable of aflatoxin production in rough rice.
Codner et al. (1968) stated that different isolates of *A. flavus* vary in their ability to produce aflatoxin even on the same natural substrates. As, Hesseltine et al. (1966) grew 3 strains of *A. flavus* on 6 agriculture commodities and found that one strain produced maximum aflatoxin on sorghum, groundnut, soybean and rice while, another strain did the same on wheat and corn while, the third strain produced less aflatoxin on all the substrates. Mayne et al. (1966) found that aflatoxin production on living cotton seed kernels was found to be lower than whole cotton seeds. While, cotton seed hults and lint were found to be poor substrates for growth and aflatoxin production in *A. flavus*. Similarly in case of wheat supported maximum production than oat (with hull) has been reported by Stubblefields et al. (1967).

Frank (1966) reported aflatoxin contamination with growth of *A. flavus* on numerous foods including egg noodles, cheese, condensed and powered milk, wall nuts, Brazil nuts, poppy seeds, coconut, apple juice, potato products, dried peas, beans, lentil, apple slices, peaches and figs. Wildman et al. (1967) reported production of aflatoxin in variety of agriculture commodities like apple, apricot, grape, vegetable juice, orange peach, pear, pineapple and tomato juice.

Schindler and Eisenberg (1968) noted aflatoxin production in crushed fresh red paper but it is poor production look place on soybeans or soyproteins. Hesseltine et al. (1966) have found poor production of aflatoxin on soybean than on wheat, corn, rice, groundnut and sorghum. Hesseltine et al. (1968a) also found that sweet clover and alfalfa did not allow production of aflatoxin. It
was interesting to note that the fungi, *A. flavus*, *A. nidulance*, *A. chevalieri*, *A. niger*, *A. stellata* and *A. versicolor* did not produce aflatoxins when associated with seed of bajra (Mathur and Sinha, 1977).

**Temperature and Aflatoxin:**

Most of the fungi grow luxuriantly at between 20 and 30°C (Detroy *et al.* 1971). *Aspergillus flavus* exhibits optimum growth between 36 and 38°C (Northolt and Bullerman, 1982). The optimum temperature for aflatoxin production by *A. flavus* ranges between 25 and 35°C (Saver, 1986). Earlier several workers have reported the effect of temperature on aflatoxin production in various food and agricultural commodities (Diener and Davis 1970, Mall and Pateria, 1983).

The highest levels, (10.67 to 1.75 µg/g) of aflatoxin B₁ were detected in the samples of *Mucunan pruriens* seeds which were incubated at 25°C in 3 week of incubation period. However, the aflatoxin levels ranged from 0.30 to 0.56, 0.37 to 1.20 and 0.26 to 0.65 µg/g in the samples stored at 20, 30 and 35°C, respectively, within the same period. The lowest concentration (0.10 to 0.29 µg/g) of aflatoxin B₁ was produced at 15°C However, no visible growth of fungus or aflatoxin production was observed at 10°C. (Roy and Chourasia, 1989).

Diener and Davis (1966), reported that 25°C is optimum temperature for aflatoxin production in *Aspergillus flavus* grown on sterile groundnut. Boller and Schroeder., (1966) noted complete inhibition of growth and aflatoxin production of *A. flavus* at 45°C in groundnuts.
In 1967, Schroeder and Mein, investigated the effect of temperature on aflatoxin production on moist cotton seeds, groundnut and rough rice, growing with *A. flavus*. In groundnuts, infested with *A. flavus*, aflatoxin production was not favoured at higher temperature (45°C) and also at lower temperature (13°C) (Diener and Davis, 1968b), whereas the aflatoxin production was high at 25 to 30°C and it was low at 20°C (Schroeder and Mein, 1967). In case of rice, Souer, (1986) found that, optimum temperature for aflatoxin production was 28°C, poor at 15°C and no production at 8°C.

The occurrence of aflatoxin is usually associated with poor storage condition, although more and more evidence indicates that these compounds are produced in the field also. Lillehoj *et al.* (1975a), determined that 2.5% of 5,000 test ears of field corn from southeast Missouri and east central Illinois contained aflatoxin B1 at levels exceeding 20 µg/kg. In a subsequent study of field corn in north-eastern south Carolina, Lillehoj *et al.* (1975b) demonstrated a 49% incidence of aflatoxin in samples collected from 184 fields. Again in 1977, lillehoj *et al.* detected extensive *Aspergillus flavus* infection at harvest in 214 Iowa corn samples but only four contained 20 µg/kg or more aflatoxin B1.

According to comprehensive studies by Shotwell *et al.* (1969 a) on samples mainly from the corn belt, only nine out of 1,311 corn samples contained small amounts of aflatoxin (up to 19 µ/kg). A second survey of U.S. corn for aflatoxin showed a similarly low incidence of aflatoxin at levels up to 25 µg/kg (Shotwell *et al.* 1970).
Interaction between *Trichoderma* and *Aspergillus*.

*Trichoderma* is a potent biocontrol agent. It has been used successfully against various pathogenic fungi belonging to various genera, *Aspergillus, Fusarium* etc., they are known to produce antibiotics and toxins, which are volatile or non-volatile in nature and have a direct effect on host organisms  (Ranasingh *et al.* 2006)

The *Trichoderma* spp. i.e. *T. viride*, *T. harzianum* were tested against *Sclerotium rolfsii* the incidence of groundnut stem rot by Pushapavati and Chandrasekharrao (1999). Kore and Chavan (2000) reported the efficacy of *Trichoderma* species in the management of safflower charcoal rot disease.

Whereas, Gupta *et al.* (2002) studied the antagonistic properties of *Penicillium* sp, against different fungi viz, *Fusarium, Curvularia, Pestalotiopsis, Aspergillus* Species. Howell (2003), reported the interaction between *Trichoderma viridae* and *Rhizoctonia solani, Macrophomina phaseolina* and *Rhizopus oryzae* by different mechanisum.

**World oil seed production**

World vegetable oil and marine oil output is forecast at a record of 60.1 million tons, with all major oil increase except sunflower and coconut oil. Reduced demand in the USSR will contributed to a slowdown in world vegetable oil consumption while higher level of oil seed production in the major vegetable-oil importing countries, such as India, is expected to slow world rate. World trade in vegetable oil is forecast to drop from 20.0 to 19.8
million tons in 2001-2002. As production exceeds consumption, global stock of oil seed remain at relative high levels.

World oil seed output is forecast to jump 2.2 percent to a record 222.4 million tons in last few years, much of this gain is from the United States, where total oil seed out put is forecast at 62.8 million tons, up 3.5 percent from last year. Substantial increase in cotton seed and groundnut production underlie the 3-percent growth, while U.S. soybean production is up less than 1 percent from last year. The 1.8 –percent growth in foreign outturn includes larger ground, soybean, cotton seed, sunflower seed crops in India, larger rapeseed crops in the EC and Canada, and a larger soybean crop in Brazil. Global oilseed crush is forecast to increase slightly to 180 million tons, while trade in oilseeds is little changed from last year in response to a slowdown in demand in the USSR and Eastern Europe.
## Major oilseeds producing country:

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### Total yield of oil seeds (2007-2009)

#### 1) India

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Oil seeds</th>
<th>Trade Estimate</th>
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<td>Rabi</td>
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<tr>
<td></td>
<td>2008-09 Season</td>
<td>2007-08 Season</td>
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<td>Soybean</td>
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<td>Sesame</td>
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<td>4.</td>
<td>Safflower</td>
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</tr>
<tr>
<td>5.</td>
<td>Sunflower</td>
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#### 2) Maharashtra:

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<th>Trade Estimate</th>
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</thead>
<tbody>
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<td>Kharif®</td>
<td>Rabi</td>
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<tr>
<td></td>
<td>2008-09 Season</td>
<td>2007-08 Season</td>
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<td>2.</td>
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Data finalised at the 30th all India seminar on oilseeds, oil trade and industry during the Rabi seminar in 2009.