**DISCUSSION**

India is known for its agriculture products. The environmental conditions throughout the country are favorable to cultivate the crop throughout the year. This is a favorable situation where maximum utilization of available land is converted into agriculture. Depending upon climatic condition farmers selected crop for cultivation and ultimately on which Indian economy is based.

Around 70 percent of average land is used for agriculture in both Rabi and Kharif. But it observed that there is a heavy loss takes place in production as proper care is not taken by farmers. Hence the modern tools and technique in agricultural engineering is strongly recommended to protect and preserve the production for maximum utilization of mankind. It was observed in literature that a heavy loss of some crops in some state created a history. This loss is mainly takes place in field in an average more than 30 percent, this loss is reported till it reaches to consumers.

Microbes play a vital role in the loss of food crop both in field as well as in storage conditions. Among microbes more than 70 percent loss is reported due to interaction of moulds in the field and group of fungi in different storage conditions. Oil seed crop are cultivated as commercial crop in India. It is also plays an important role in Indian economy. Hence this research work is carried out...
DISCUSSION

to know the present scenario of storage fungi with oil seeds and its role in its biodeterioration.

Fungi, those involved in the deterioration of cereal grains and oil seeds and other agricultural products have been classified as field fungi, storage fungi, and advanced decay fungi depending on the time of their invasion of colorization of grain and whether they occur before or after the harvest.

I) Field fungi:

The invade grain kernel before harvest either while the crop is growing in the field or after it has been cut and swathed but before it is threshed. They require a minimum water activity (a_w) of 0.85 for their growth. The field fungi have been further divides into two groups.

1) **Specialized parasite** – Such as smuts, bunts, and ergot.

2) **Facultative parasite** – Such as *Alternaria*, *Bipolaris*, *Curvularia*, *Cladosporium*, *Epicoccum*, *Helminthosporium*, *Fusarium*, *Nigrospora*, etc.

Some of the well known toxicogenic moulds like *Aspergillus flavus*, and *A. parasiticus* are also known to elaborate aflatoxins in the maize cobs and groundnut pods even in the fields. In field, seeds are known to be colonized by varied types of microorganisms among which many are plant pathogens. About
1500 types of micro-organism are known that are associated with seed borne disease. The organisms can be grouped into:

i) Obligate parasite

ii) Facultative saprophytes

iii) Facultative parasites

In case of obligate parasites like *Sclerospora graminicola*, the causal agent of downy mildew of pearl millet and *Peronoslerospora sorghii*, the parasitism is a part of their life cycles. These fungi transform the floral primordial into vegetative leafy structure, stimulate or induce sterility in seeds. In facultative parasitic group, many fungi are known to cause considerable damage to seeds of food crops.

Seed infection also occur during seed development through various ways. In many oil seed, infection by *Aspergillus flavus* results in boll penetration by Pink Boll Worms (*Pectiophora gossypiella*). The fungus enters the boll through boll worm exit holes and invades the seeds during boll rotting, finally causing seed deterioration and elaboration of aflatoxin in seeds.
II) Storage fungi:

Storage fungi are those, which do not invade grain before harvest. Most of these fungi belong to genus *Aspergillus* and *Penicillium*. These are capable to grow at water activity in a range of 0.65-0.90 $a_w$ and can grow at a very wide range of temperature. On the basis of temperature and $a_w$ relationship of storage microorganisms they have been classified into following six physiological groups as follows:

1) Psychrotolerant (10°-35°C):  
2) Mesophilic (2°-50°C):  
3) Lower mesophilic (2°-37°):  
4) Upper mesophilic (5°-50°):  
5) Thermophilic (100-57°C):  
6) Extremely thermophilic (25°-70°C):

III) Advance decay fungi and other organisms:

In stored products microbial succession leads to the development of heat and moisture which favor the growth of most thermophilic fungi and bacteria including actinomycetes. However, the stage at which these organisms start developing the product is so deteriorated that it can not be consumed by man or even by animals. Hence, it can be said that the main cause of cereal grains and oil seeds spoilage occurs in field by the field fungi and other parasitic microorganisms and in storage mainly by the storage fungi.
Aspergillus is one of the commonly occurring fungi in the storage condition. During the storage the seeds contaminated by large number of fungal species. Among them the species of Aspergillus play major role in the seed spoilage or seed biodeterioration. Along with Aspergillus species other fungi like Penicillium, Fusarium, Curvullaria etc also occur and contaminated the storage seeds which are rejected by the seed industry.

**Incidence of Aspergillus species on different oil seeds**

Oil seeds are known to carry pathogenic and non-pathogenic fungi. These fungi associates with oil seeds at two different place-1) in the field, commonly called as field fungi; 2) in the storage, commonly called as storage fungi. This association of fungi with the seeds is known as seed-borne fungi.

In different storage ambiance, physical factor and chemical composition are responsible for interaction of pathogen. In storage condition among group of fungi different Aspergillus species were predominantly occurring. While studying the incidence of Aspergillus species on groundnut, soybean, sesame, sunflower and safflower, ten different Aspergillus species were commonly available on the seeds. The qualitative and quantitative incidence varies within the seeds, its varieties and type of abnormality.
Several scientists also reported the qualitative and quantitative variation in the incidence of *Aspergillus* species. Gupta and Chouhan (1970) and Cherry *et al.*, (1975) isolated qualitative seed mycoflora in ground. Where as similar observation were reported by Gowda and Sullia (1987) and Sinha *et al.* (1991) in soybean, Neera and Mehrotra (1990) in sunflower and Rathod (2007) in oil seed.

It was also found that maximum *Aspergillus* species were isolated by agar plate method as compare to blotter paper method. It is clearly indicated that blotter agar plate method proved to be favorable as compare to blotter paper method for isolation of more number of *Aspergillus* species. On PDA medium, maximum *Aspergillus* species isolated as compare to RBA and GNA medium. This clearly indicates that, PDA medium isolate maximum *Aspergillus* species than RBA and GNA. It indicates that, the chemical composition of PDA favors the growth of *Aspergillus* species as compare to GNA and RBA.

Similar results has been reported by Neergaard 1973. During isolation study it has been found that percentage incidence of *Aspergillus* species was found to be differ with the oil seeds and different varieties of oil seeds. Hence it can be concluded that physical and chemical characteristic of seeds might be playing vital role for the association of *Aspergillus* species. This variation in mycoflora in different varieties may occur due to recently develop new varieties by using modern technique are disease resistance. Sandikar (1990), observed variation in
seed mycoflora in sunflower and sesame varieties collected from Parbhani of Marathwada region.

Pasri, tag45 and ghungru varieties of groundnut, Js335, S1B and eagle varieties of soybean, C1L, C1P and C1B of safflower, Lsu, Lsf-8 and suraj of sunflower showed maximum percentage incidence of *Aspergillus* species. Kesare (2008) isolated seed mycoflora of soybean varieties Js335, Js80, Musa-2, Musa-8, Musa-38, Pooja, Pk-1029, Pk-1059 and MACS-13, which were commonly cultivated in the agro-climatic condition, but not favorable for storage of soybean for more than 15 months.

**Growth pattern and cultural characteristic of *Aspergillus* species.**

All the selected *Aspergillus* species showed great variation in diameter color and growth pattern of colony on solid medium and sporulation and mycelium weight on liquid medium. It was also found that, there is maximum morphology difference among *Aspergillus flavus* in the form of colony diameter, growth pattern, colony color, Sporulation and mycelia weight on solid and liquid medium.

This diversity in the morphology may be due to, isolates of *Aspergillus* species from different oil seeds have their own choice of food were it grows. However it may adjust contents of other media. Danai (1994) reported that, the growth pattern of *Aspergillus* species varies in different growth media, however
the physical and nutritional factor also shows variation in Sporulation count. Similarly, Umatale (1995) reported that *Aspergillus flavus* and *A. niger* isolates from different groundnut growing on PDA media shows variation in its growth pattern and colony character.

**DNA fingerprinting of *Aspergillus flavus* isolates.**

*Aspergillus flavus* was reported on all types of seeds in all varieties and in every abnormalities of seeds. It indicates that, *Aspergillus flavus* easily adjust the change in chemical composition in seeds as well as agro-chemical conditions. This may be because of the genetic pattern of *Aspergillus flavus*. Therefore twelve isolates of *A. flavus* showing different morphological behavior isolated from different oil seeds were selected and the genetic diversity was study by ISSR technique.

The dendogram clearly represent five distinct clusters of genetic similarity observed, among which isolate Asf 5 isolated from groundnut (GP.F1) do not shows any closer relationship with other isolates, where as Asf 3(GT45.F3) and Asf 12 (So80.F1), Asf 1 (GT11.F1), Asf 2 (GT45.F1), Asf 6 (SaKa.F2), Asf 7 (GT11.F3), Asf 8 (Gt45.F2), Asf 9 (SaSu.F1), Asf 10 (So335.F2), shows genetic similarity. It is interested to note that Asf 9, Asf 10, Asf 11 and Asf 4 are proved to be more toxic and carcinogenic. This type of study in different species of genera
is helpful to point out the group of genetic material showing pathogenic and non-pathogenic nature.

Similarly different cluster isolation from different oil seed interpret the relationship in its growth pattern and chemical composition. Such type of work would be helpful to remove the carcinogenic gene from *Aspergillus flavus*, as it is harmful for humans, hence further mycotoxic and aflatoxic work in relation to genetic diversity should be carried out.

Earlier the work on DNA fingerprinting was carried out and also support by several scientist (Cook *et al.* 1998; Morris *et al.*, 2000; Walls, 2004; Patale, 2005; Phalak, 2007; Chavan, 2008 and Hatti *et al.*, 2010).

**Lipase production**

Lipase production by fungi is responsible for degradation of oil seeds (Goodman and Christensen, 1952). As the oil seeds are rich in oil, studies were carried out to observe lipase production by *Aspergillus* species. Substrate medium induce lipase production as compare to non-substrate medium. It can be concluded that substrate like Tween-20 may induce lipase activity of *Aspergillus* species in substrate medium.

Among the carbohydrates lactose, fructose and sucrose induced lipase action in some *Aspergillus species* whereas polysaccharides like CMC and starch inhibits lipase activity of *Aspergillus* species. Result clearly indicates that
polysaccharide induces lipase activity of *Aspergillus* species. Recently Kakde *et al.* (2009) and Gadgile and Chavan (2009) found the same result in case of lipase and cellulase activity respectively.

Nitrogen source like casein and peptone induced lipase activity of all *Aspergillus* species. Similar results were reported by Khairnar (1987); Bhosale (1989); and Rathod (2007) in case of *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium oxysporum* and *Alternaria* species. It was also observed that, sodium nitrate and sodium nitrite induced lipase activity of all *Aspergillus* species expect *A. oryzae* and *A. ustus*. 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*.

Sandikar (1990), observed same results about impact of nitrogen source on lipase production in seed borne fungi of sesame. Recently, Kakde *et al.* (2009) recorded same results in case of lipase activity in *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium notatum*, where as Gadgile and Chavan (2009a), reported that calcium nitrite, sodium nitrite, urea and peptone induce cellulase activity of *Colletotrichum gloesporioides*, *Penicillium* species, *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *Rhizopus stolonifer* of mango fruits.
Regarding the phosphorus source, potassium di-hydrogen ortho-phosphate induces lipase action in *Aspergillus fumigatus, A. oryzae* and *A. versicolor* while sodium di-hydrogen ortho-phosphate stimulated lipase production of *A. glaucus, A. niger, A. parasiticus* and *A. versicolor*. Lipase action of *A. flavus, A. fumigatus, nidulance, A. oryzae, A. terreus* and *A. ustus* were stimulated by disodium hydrogen ortho-phosphate. Rathod (2007) and Kesare (2008), reported more or less similar results about impact of phosphorus sources on hydrolytic enzyme. 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.

Among the sulphur source, ferrus sulphate and disodium sulphate increase lipase production of all *Aspergillus* species. Where as zinc sulphate retarded the lipase production of some *Aspergillus* species. Recently, Kakde *et al.* (2009) found that sulphur sources, zinc sulphate, ferrous sulphate, calcium sulphate and disodium sulphate inhibited the lipase action of *Aspergillus niger, Fusarium oxysporum, F. equiste, Macrophomina phaseolina* and *Penicillium notatum*.

Gadgile and Chavan (2009a), reported that ferrus sulphate, zinc sulphate and copper sulphate significantly reduced the cellulase action of *Aspergillus niger, A. fumigatus, A. flavus, Colletotrichum gloesporioides, Penicillium* species and *Rhizopus stolonifer* from mango fruits.
Antibiotic like Norflaxacium, Ampicilin, Trioflan, Tetracycline and Almox DT at 100 ppm inhibited lipase action in some of the Aspergillus species. and Rathod (2007) and Kesare (2008) reported more or less similar finding about impact of antibiotic at 100 ppm concentration on different seed borne fungi. 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 hostacyelin 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.

Among vitamins, vitamins C activated lipase activity of all selected Aspergillus species. While Thiamin HCl retard the lipase activity of Aspergillus niger, A. parasiticus, A. terreus and A. ustus. lipase production of A. fumigatus was totally retarded with folic acid. Folic acid induce lipase action of A. flavus, A. oryzae and A. terreus. Riboflavin inhibited lipase production of A. glaucus, A. oryzae, A. ustus and A. versicolor. Bhikane (1988), reported that Thiamin and nicotinic acid were proved to stimulatory for protease production in A. flavus while pyridoxin was found to be inhibitory in case of Curvularia lunata, Fusarium oxysporum and Rhizoctonia soloni for protease production. Recently Gadgile and Chavan (2009b), reported that Vitamin C, Vitamin A, Thiamin HCl and Riboflavin induce pectinase activity of A. niger, while pyridoxine retarded the
same for *Aspergillus niger*. Bharaswadkar (2003) reported that *Aspergillus flavus* inhibited protease action with pyridoxine.

Impact of physical factor like incubation period, temperature, pH and light on lipase was studied. Maximum lipase activity of all *Aspergillus* species was found in between 15 to 20 days of incubation period. Lipase activity of all *Aspergillus* species was stimulated at pH 6.5 to 7.5 and at 20 to 30°C. Continuous light stimulated lipase action in all *Aspergillus* species as compare to dark and alternate light and dark.

Rathod (2007) and Kesare (2008) reported same results for effect of incubation, temperature, pH and light on hydrolytic enzyme of seed-borne fungi.

**Biodeterioration of oil seeds**

Biodeterioration of oil seed content by *Aspergillus* species was studied by artificial inoculating at laboratory condition. All the selected *Aspergillus* species deteriorate dry weight, cured fiber, reducing sugar, protein, nitrogen, ash, calcium, phosphorus and fat content of groundnut, soybean, sesame, sunflower and safflower.

Maximum loss of dry weight in groundnut, soybean and safflower was due to *Aspergillus flavus* while *A. niger* deteriorate maximum dry weight of sunflower and sesame. This clearly indicates that *Aspergillus* species are found to be capable in resulting the dry weight of oil seeds. Loss in dry weight of seed in

*Aspergillus flavus* reduces the fiber content of in groundnut, soybean, sesame, sunflower and safflower. It was interested to note that *Aspergillus niger*, *A. parasiticus* and *A. ustus* increased cured fiber content of groundnut, soybean and safflower. Rathod (2007) found that crude fiber content was considerably deteriorated due to species of *Alternaria*.

Similarly maximum fat content of soybean, safflower was reduced by *Aspergillus flavus*, where as *A. fumigatus* was responsible for maximum loss of fat content in sesame. Rathod (2007), reported that *Alternaria alternata* was mainly responsible for change in cured fat in wheat, black gram and safflower seeds. Recently Gadgile and Chavan (2009c) observed that *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Rhizoctonia soloni*, *Colletotrichum gloeosporioides*, *Rhizopus stolonifer* and *Penicillium* species were responsible for fat content in post mango fruit.
Maximum reduction of nitrogen in groundnut, soybean, sesame, sunflower and safflower seeds were caused due to *Aspergillus terreus* while *A. flavus* deteriorate maximum nitrogen content of sesame and safflower. Kesare (2008), found that *Alternaria alternata* and *Fusarium oxysporum* were mainly responsible for deteriorating nitrogen content in soybean.

*Aspergillus versicolor* deteriorate maximum protein content in groundnut, soybean and sesame, while *A. ustus* was found to be responsible for maximum reduction of protein content in sunflower. Rathod (2007), reported that *Alternaria dianthicola, A. citri, A. macrospore* and *A. crassa* were mainly responsible for change in protein content of safflower, wheat and black gram. Ivanonv *et al.* (1989) reported loss in protein content in sunflower due to associated fungi. Similarly change in protein content was observed by Sing and Prasad (1988) in mustard and kumar and Prasad (1993) in oil seeds.

In soybean maximum ash content was reduced due to *Aspergillus fumigatus, A. nidulance, A. oryzae* and *A. terreus*. *Aspergillus glaucus* was most responsible for maximum loss of ash content of groundnut. *Aspergillus nidulance* and *A. oryzae* were reduced maximum ash content in sunflower, while *A. fumigatus, A. glaucus* and *A. versicolor* were reduced maximum loss of ash content of safflower.
Kesare (2008), reported that *Aspergillus flavus* and *A. ustus* were mainly responsible for change in ash content of soybean. Where as Rathod (2007), found that *Alternaria dianthicola* and *Aspergillus terreus* were mainly responsible in reducing the ash content of safflower, wheat and black gram seeds. Currently, Gadgile and Chavan (2009d) found that *Aspergillus niger, A. flavus, A. fumigatus, Colletotrichum gloeosporioids, Rhizopus stolonifer* and *Penicillium* species were responsible for loss in ash content of post-harvest mango fruits.

Maximum loss of calcium in groundnut and safflower seeds was caused by *Aspergillus flavus*. Rathod (2007), observed that calcium and phosphorus content of wheat, black gram and safflower was due to *Alternaria* species. While Kulkarni (2009), reported that maximum loss in calcium of maize seed varieties supper 900 was due to *Penicillium notatum, Helminthosporium tetramera, Alternaria alternata, Aspergillus terreus* and *Fusarium oxysporum*. Gadgile and Chavan (2009e) reported that loss of calcium content of mango fruit were due to *Aspergillus niger*.

*Aspergillus flavus, A. ustus* and *A. versicolor* were found to be responsible for loss in phosphorus content of sesame, where as *A. niger, A. parasiticus* and *A. flavus* were responsible for loss in phosphorus content in groundnut, safflower and sunflower respectively. Kesare (2008) concluded that, *Aspergillus flavus* is mainly responsible for loss in phosphorus content of soybean. Kulkarni (2009) reported that, *Alternaria alternata, Aspergillus niger, Curvularia lunata, Helminthosporium tetramera, Penicillium notatum* and *Fusarium oxysporum* were responsible for loss in phosphorus content of soybean.
*Helminthosporium tetramera, Penicillium notatum, and Trichoderma viridae* were responsible for loss of phosphorus content in maize seeds.

**Effect of mycotoxins on oil seed germination**

Effect of culture filtrate of different *Aspergillus* species on oil seed germination was studied. Culture filtrate of all selected *Aspergillus* species was found to be inhibit the seed germination in all oil seeds. Among all *Aspergillus* species, *A. flavus, A. niger* and *A. ustus* showed more toxic to seed germination of groundnut, soybean, sesame, sunflower and safflower. Also the effect of toxic and non toxic culture filtrate of *Aspergillus flavus* isolates on oil seeds germination was studied. The culture filtrate of toxic strain affected the germination of all the oil seeds as compare to non toxic culture filtrate and control. Similarly, the root length and shoot length were also affected.

Ibraheem (1987) reported, soybean seeds soaked in culture filtrate of *Fusarium solani, F. oxysporium, Aspergillus flavus, A. niger, Alternaria tenuis* and *A. alternata* for 14 hours showed reduction in percent seed germination. Hilty and Lee (1988) also reported the role of toxic metabolites of stored fungi on soybean seed germination. Similarly, Haikal (2008) reported that toxic metabolites secreted by culture filtrate of *Aspergillus niger, Fusarium culmorium* and *Rhizoctonia solani* reduces the percentage of seeds germination of soybean.
Qualitative estimation of aflatoxin

Totally forty eight isolates of *Aspergillus flavus* were isolated from different category of oil seeds which were screened for aflatoxin production by ammonia vapor test and HPLC. Isolates Gp11.F3, Gp45.F3 and So80.F3 were isolated from groundnut and soybean and all these isolates were highly toxic according to ammonia vapor test. In case of sunflower seeds, 5 isolates were isolated, out of which three isolates were moderate toxic and one was non toxic. In case of safflower and sesame none of isolates were found to be highly toxic. In safflower, two isolates were found to be mildly toxic.

Reliable, fast, and simple method for the detection of aflatoxigenic and non- aflatoxigenic *Aspergillus* flavus isolates was used by Fente *et al.* (2001). Tseng *et al.* (1995) reported that, the infected beans from Taiwan were contaminated with aflatoxin B1, B2, G1 and G2, by HPLC method. Similar type of work was also carried out by Christensen and Kaufmann (1969); Shephard *et al.*, (1990) and Tseng (1985);

The effect of temperature and incubation period was also studied. Most of the *Aspergillus* isolates did not produces aflatoxin on 10\textsuperscript{th} day of incubation. Expect few isolates there was no production of aflatoxin at 15 and 45\textsuperscript{0}C, where as maximum production of aflatoxin was at 25 and 30\textsuperscript{0}C.
Similar, several workers have reported the effect of temperature and incubation period on aflatoxin production. Sauer (1986), reported that the optimum temperature for aflatoxin production by *Aspergillus flavus* ranges between 25 to 35 °C. Roy and Chourasia (1989), reported that the highest level of aflatoxin was detected in *Mucuna* seeds at 25 °C in week 3 of incubation period. Aflatoxin production declines with decreasing temperature and has been reported to cause between 10 and 13 °C (Schindler, *et al.* 1967; Northolt and Sonentoro, 1988; Hesseltine, *et al.* 1966; Kozakiewicz and Smith, 1994)

**Interaction between *Trichoderma viridae* and *Aspergillus* species**

The interaction between *Aspergillus* species and *Trichoderma viridae* was studied. In this interaction the conidial mass of *Trichoderma viridae* is get attracted towards *Aspergillus* species in different way. It was observed that *T. viride* inhibits the growth of *Aspergillus* species by two ways i.e. either by coiling the *Aspergillus* species or by penetrating haustoria in mycelium of *Aspergillus* species.

The *Trichoderma* *spp.* i.e. *T. viridae, 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.
Similar type of work is carried out by Howell (2003), he studied the interaction between *Trichoderma viridae* and *Rhizoctonia solani, Macrophomina phaseolina* and *Rhizopus oryzae.*