EXPERIMENTAL RESULTS

Part I

A) Isolation of seed-born fungi

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

To study the incidence of different *Aspergillus* species associated with commonly grown varieties of different oil seeds like groundnut (Pasari, Tag-11, Tag-24, and Ghungru), soybean (Js-335, S1B, S1P, Eagle), sesame (Se1N, Se1P, Se2N, Se1N, Se2B and Se2B), sunflower (Lsu, LsF-8, Surajj, Kargill) and safflower (C1L, C1P, C1B and Bhima), were collected from different regions of Marathwada of Maharashtra state. These oil seed were then surface sterilized and inoculated on different media and incubated at 30°C for 7 days.

As the results observed in the table 1 total ten *Aspergillus* species were isolated. The fungi like *A. flavus, A. fumigatus, A. glaucus, A. niger, A. terreus, A. ustus* and *A. versicolor* showed their incidence on all selected oil seeds. *A. oryzae* and *A. parasiticus* were found to be totally absent on soybean seeds, similarly *A. nidulans* on sesame and *A. oryzae* on sunflower were found to be absent. It is clear from the table 1 that the percent incidence of *A. flavus* and *A. niger* were more dominated on all selected five oil seeds as compare to other isolated fungi.
2. Percent incidence of *Aspergillus* on different media

It is clear from the result observed in table 2 that, blotter paper method yield less *Aspergillus* species from oil seeds than that of agar plate method. Totally six *Aspergillus* species viz. *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus* and *A. ustus* were isolated on blotter paper. The *Aspergillus* species like *A. flavus*, *A. fumigatus*, *A. niger* and *A. ustus* were found to be dominant on blotter paper as compare to other *Aspergillus* species.

It was interesting to note that the fungi like *A. glaucus*, *A. oryzae*, *A. parasiticus* and *A. versicolor* were totally absent on blotter paper but they were recovered from agar plate method. PDA was found to more favorable for isolation of fungi as compared to other media.

On PDA and RBA medium, ten *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated from groundnut. On the other hand, seven *Aspergillus* species were isolated viz. *A. flavus*, *A. glaucus*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. ustus* and *A. versicolor* from groundnut on GNA media.

In case of soybean, eight *Aspergillus* species i.e. *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus*, *A. ustus*, *A. versicolor* were isolated on PDA and seven species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* were isolated on RBA. On GNA only five
Aspergillus species were isolated namely A. flavus, A. niger, A. ustus and A. versicolor.

Eight species of Aspergillus were found to be isolated on sesame on PDA namely Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. parasiticus, A. terreus, A. ustus and A. versicolor where as on RBA number of species isolated to six viz. A. flavus, A. fumigatus, A. glaucus, A. niger, A. terreus and A. versicolor. Number of Aspergillus species isolated on sesame on GNA found to be reduced to five namely A. flavus, A. fumigatus, A. niger, A. parasiticus and A. ustus.

In case of Sunflower, eight Aspergillus species were i.e. Aspergillus flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. terreus, A. ustus and A. versicolor were isolated on PDA, on RBA medium seven species viz. A. flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. terreus and A. ustus where isolated and on GNA, only six Aspergillus species namely A. flavus, A. fumigatus, A. niger, A. parasiticus, A. ustus and A. versicolor were isolated.

3. **Percent incidence of *Aspergillus* species on abnormal oil seeds:**

To study the relationship between seed abnormality and associated fungi, all the collected oil seeds were categorized in four types of abnormalities viz. Shrunken (Sh), Undersized (Us), Discolored (Dc) and Cracked (Cr). These seeds were plated separately on agar media and results were recorded in table 3.

It is clear from the result in the table no.4, that the undersized and discolored seeds yield maximum number of *Aspergillus* species. The percent incidence of *A. flavus*, *A. fumigatus*, *A. oryzae* and *A. terreus* was found to be maximum on discolored seeds i.e. 41.90%, 4.47%, 5.11 and 5.81% respectively. Similarly, *A. glaucus* (4.37%), *A. nidulans* (4.56%) and *A. versicolor* (7.75%) shows maximum incidence on undersized seeds. On the other hand *A. niger* (49.5), *A. parasiticus* (6.25) and *A. ustus* (3.81) showed maximum incidence on cracked seeds.

It was interesting to observe that *A. oryzae* on cracked seeds and *Aspergillus parasiticus* on shrunken seeds were totally absent in all the varieties of oil seeds.

4. **Incidence of *Aspergillus* species on different variety of oil seeds:**

To study the variety wise variation, percentage incidence of *Aspergillus* species in the oil seeds, different varieties of groundnut, soybean, sesame, sunflower and safflower were plated on agar media and the results are given in table 4.
It is clear from the table 5.I, that among four varieties, Pasari, Tag-45 and ghungru showed maximum percent incidence of *Aspergillus* species, while Tag-11 showed presence of only eight *Aspergillus* species, *A. glaucus* and *A. nidulans* were totally absent in Tag-11 varieties.

Incidence of *Aspergillus* species on four variety of soybean (Js-335, S1B, S1P and Eagle) showed (table %.II) considerable qualitative and quantitative variations. In all total eight *Aspergillus* species were recorded on all the four different varieties of soybean seeds, from which maximum incidence of total seven *Aspergillus* species were recorded on Js-335, S1B and Eagle, while six *Aspergillus* species were recorded on S1P variety. *A. terreus* (26.5%) showed highest incidence on Js-335 variety followed by *A. niger* (23.0%) and *A. flavus* (6.0%). In variety S1B *A. niger* (12.5%) showed maximum incidence followed by *A. flavus* (8.8%) and *A. terreus* (6.5%), similarly *A. niger* (25.0%) showed maximum incidence followed by *A. flavus* (7.0%) and *A. terreus* (4.5%) in S1P variety, were as *A. flavus* (62.5) showed maximum incidence among all the fungi on eagle variety followed by *A. niger* (10.0%) and *A. terreus* (5.0%). It was interesting to note that *A. versicolor* was present only on Eagle variety and totally absent on rest of three varieties, similarly *Aspergillus fumigatus* and *A. nidulans* were absent on S1P and Eagle variety respectively.

In case of Sesame (table no. 5.III), the seeds of six different variety (Se1N, Se1P, Se2N, Se1B, Se2B and Se3B) were employed for the isolation of *Aspergillus* species. Totally nine number of *Aspergillus* species were recorded
from these varieties. Se2N variety yield highest number (ten) of *Aspergillus* species, from which *A. flavus* (48.75) and *A. niger* (46.5) were more dominant as compare to other *Aspergillus* species. It was interesting to not that *A. parasiticus* was totally absent in variety Se1N, but present in rest of all varieties, on the other hand *A. glaucus* (1%) was present only on Se2N variety and absent on rest of all varieties. Similarly *A. ustus* and *A. versicolor* were found to be present on all varieties except Se1B and Se2B respectively. It was also observed that *A. oryzae* was totally absent on Se1P and Se3B variety.

Four different variety (C1L, C1P, C1B and Bhima) of safflower were selected for isolation of different *Aspergillus* species. C1L and C1P showed maximum (ten) incidence of *Aspergillus* species, were as C1P and Bhima variety showed nine and eight species. In all the variety *A. niger* was found to the most dominant fungi followed by *A. flavus* as compare to other fungi. It was interesting to observe that *A. glaucus* was totally absent in C1P variety and in variety Bhima *A. nidulans* *A. parasiticus* were totally absent.

The incidence of *Aspergillus* species on four different variety (Ls u, LsF-8, Suraj and Kargill) of sunflower showed (table 5.V) considerable variation. Three variety, i.e. Ls u, Ls F-8 and Suraj showed maximum association of *Aspergillus* species (ten) as compared to kargill, *A. terreus* and *A. ustus* were found to be totally absent on Kargill variety.
5. Growth pattern and culture characteristics of different *Aspergillus* species.

The growth pattern of different *Aspergillus* species were studied on solid and liquid CZA media. On solid media the results were recorded after 7 days and results of liquid media were recorded after 15 days and the results of liquid media were recorded after 15 days (Table 5).

It is clear from the table (Table 5), that the different *Aspergillus* species showed great variation in diameter, color and growth pattern of colony on solid media and sporulation and mycelia weight on liquid media. The maximum diameter was observed in *Aspergillus flavus* (5.5cm) followed by *A. versicolor* (5.2cm) and the minimum diameter observed by *A. terreus* (2.3cm) and *A. ustus* (3.4cm). *Aspergillus flavus* and *A. oryzae* showed zonate colony pattern on CZA media were as rest of all *Aspergillus* species showed smooth colony pattern on the same media.

On CZA broth, *A. flavus*, *A. fumigatus* and *A. niger* showed maximum Sporulation, where as *A. glaucus*, *A. nidulance*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* showed moderate sporulation on CZA broth. It was interested to observe that only *A. oryzae* showed poor Sporulation. Maximum mycelia weight was observed in *Aspergillus flavus* (1.075gm) and *A. niger* (1.120gm) while *A. oryzae* (0.444gm) showed minimum mycelia weight.
6. **Growth pattern and culture characteristic of *Aspergillus flavus* isolates.**

Total 48 different isolates of *Aspergillus flavus* were isolated from different category of different oil seed varieties. Total 14 different strains of *A. flavus* were isolated from four different groundnut varieties. Similarly 5 different strain from 4 variety of sunflower, 7 strains from 4 variety of safflower, 14 strains from 4 different variety of soybean and 8 strains from 4 variety of sesame were isolated. All these isolates showed morphological difference such as colony diameter, growth pattern, colony color (reverse and front), sporulation and mycelia weight in solid and liquid media and the results are shown in the table 6.

7. **DNA Finger printing of *Aspergillus flavus* isolates by ISSR technique.**

In order to know the genetic diversity of *Aspergillus flavus* for molecular characterization, 12 toxic and non toxic *A. flavus* isolates were isolated from oil seeds. Polymerase Chain Reaction (PCR)-based, single sequence repeats (SSR) micro satellites analyzed DNA relatedness of these isolates was carried out. These Inter simple sequence repeats (ISSR) represent genome region between micro satellite loci. Dendograms which evaluate the likeness between different isolates has also been used.

PCR reactions were carried out in a Finnzyme make thermal cycler using ISSR primers. Three primes were used for this study. These three ISSR primer sets were ordered from University of British Columbia (UBC). The primer sequences are as follows.
Primer 809 - AGA GAG AGA GAG AGA GG
Primer 810 - GAG AGA GAG AGA GAG AT
Primer 811 - GAG AGA GAG AGA GAG AC

UBC Primer No. 809 produced 10 polymorphic bands (table 7), UBC primer No. 810 produced 9 polymorphic bands (table 8) and UBC primer no. 811 produced 9 polymorphic bands (table 9). An average 9.33 polymorphic bands per primer was produced.

The dendrogram analysis (photo plate) divided the total 12 samples into five different clusters. Cluster I comprises of Asf 4 (GP.F4), Asf 11 (So80.F2), Asf 10 (So335.F2) and Asf 9 (SaSu.F1); while cluster II contains Asf 7 (GT11.F3) and Asf 8 (Gt45.F2) only. Further cluster III possesses Asf 6 (SaKa.F2), Asf 1 (GT11.F1) and Asf 2 (GT45.F1). The IV$^{th}$ cluster have Asf 5 (GP.F1), Asf 12 (So80.F1) and Asf 3 (GT45.F3). Asf 5 i.e. isolate GP.F1 grouped into cluster V showed its separate identity in comparison with other samples.

Similarly genetic similarity matrix of Aspergillus flavus based on PCR marker was generated by using software NTSYS – PC with Jaccard’s coefficient of similarity. The similarity matrix of these 12 genotypes is presented in table 8.

Phylogeny analysis was carried out using Neighbor Joining tree construction method. The Distance Similarity matrix was calculated and shown maximum value of 1.0000 in Sample 9 and Sample 10 while minimum value 0.23077 was observed in Sample 2. Other Samples ranges their Matrix values in between 1.0000 and 0.23077.
After all bioinformatics analysis of the produced bands, it is analyzed that the Samples 1 to Sample 12 shows the genetic divergence with regards to its banding pattern produced by the primers, distance similarity matrix and dendrogram analysis. Also all above samples produced the polymorphic bands which also confirms about its genetic divergence from each other.

The dendrogram analysis divided the total 12 samples into five different clusters (Table 10). Cluster I comprises of Sample 4, Sample 11, Sample 10 and Sample 9; while cluster II contains Sample 7 and Sample 8 only. Further cluster III possesses Sample 6, Sample 1 and Sample 2. The IVth cluster have Sample 6, Sample 1 and Sample 2. Sample 5 grouped into cluster V showed its separate identity in comparison with other samples.
PART – II

Lipase enzyme activity

Many of hydrolytic enzymes are known to play a vital role in biodeterioration of oil seeds. Lipase is one of the enzymes produced by fungi associated with oil seeds during storage period. In present investigation ten dominant *Aspergillus* species were screened out to test their ability to produce enzymes and role in biodeterioration.

These ten fungi were grown on GN (Glucose nitrate) media, serve as a control. Similarly fungi were also grown separately grown on media containing oil. After eight days the culture filtrate were used as cured enzyme. Lipase activity of *Aspergillus* species showed interesting results (table 11 and graph 1) in all the ten fungi, maximum activity was observed in oil based medium as compared to GN media.

a) Nutritional factor

1. Effect of carbon source on lipase enzyme production

Six different carbohydrates sources were selected at 1% concentration to study the activity of lipase enzymes against ten dominant *Aspergillus* species and the results are summarized in table 12 and graph 2.

It is clear from data summarized in the table 12, that the lactose induced the lipase production of *Aspergillus versicolor*, *A. flavus* and *A. niger*.

Starch increased lipase activity of *A. parasiticus*, *A. oryzae* and *A. terreus*. were as it reduces the lipase activity of *A. ustus*, *A. flavus*, *A. glaucus* and *A.
versicolor. It was interesting to observer that fructose as monosaccharide induced lipase action of all most all the Aspergillus species expect A. fumigatus, A. parasiticus and A. versicolor.

It was interested to observed that most of the Aspergillus species were found to reduced the lipase action in Polysaccharide. The species like A. glaucus, A. oryzae, A. parasiticus, A. ustus and A. versicolor reduced the lipase action in Carboxyl Methyl Cellulose (CMC), where as starch inhibits the lipase action of A. flavus, A. glaucus, A. ustus and A. versicolor.

2. Effect of nitrogen source on lipase enzyme production

Seven different nitrogen sources including sodium nitrate as control were tested against lipase production of ten dominant Aspergillus species and the results are given in table 13 and graph 3.

It was observed that casein and peptone induced Lipase action of all Aspergillus species viz. A. flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. oryzae, A. parasiticus, A. terreus, A. ustus and A. versicolor.

It was also observed that Calcium nitrate, sodium nitrate and sodium nitrite increased the production of Lipase activity of all Aspergillus species except A. oryzae and A. ustus. It was interested to observe that A. ustus produced maximum lipase in urea and sodium nitrite as compare to other Aspergillus species.
3. Effect of phosphorus source on lipase enzyme production

Five different sources of phosphorous at 0.1% concentration were tested for lipase production in the selected ten fungi and the results are mentioned in Table 14 and Graph 4. It was observed from the results that in Aspergillus species, A. fumigatus, A. oryzae and A. versicolor produced minimum lipase in presence of potassium dihydrogen orthophosphate. On the other hand, lipase activity A. flavus, A. fumigatus, A. nidulans, A. oryzae, A. terreus and A. ustus stimulated in presence of disodium hydrogen orthophosphate. Sodium hydrogen orthophosphate induced lipase action of A. glaucus, A. niger, A. parasiticus and A. versicolor. Disodium hydrogen orthophosphate did not show any impact on Lipase production of A. versicolor.

4. Effect of Sulphur source on lipase enzyme production

Six different sources of sulphur were tested against the lipase production of ten dominant Aspergillus species and the results are given in Table 15 and Graph 5.

It was found that ferrus sulphate and disodium sulphate increased lipase production of all the Aspergillus species viz. A. flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. oryzae, A. parasiticus, A. terreus, A. ustus and A. versicolor.

It was also observed that Calcium sulphate inhibits lipase activity of Aspergillus flavus, A. fumigatus, A. glaucus and A. ustus as compared to other Aspergillus species. Lipase action of A. flavus, A. oryzae and A. parasiticus were inhibited by zinc sulphate. A. glaucus, A. ustus and A. versicolor produced maximum lipase in presence of copper sulphate.
5. Effect of Antibiotics on lipase enzyme production

In order to study the impact of antibiotics on the production of lipase enzyme five different antibiotics at 100 pm concentration were tested against ten dominant Aspergillus species and the results are given in table 16 and graph 6.

It was interesting to observe that the most of the antibiotics were decreased the lipase action of Aspergillus species. The Aspergillus species like A. fumigatus, A. glaucus, A. niger and A. ustus were produced less lipase in presence of Norflaxacin. Similarly, Ampicilin inhibited lipase action of A. flavus and A. glaucus. A. glaucus, A. niger, A. terreus and A. ustus produces minimum lipase in presence of Trioflan. It was also observed that, the lipase action of A. parasiticus and A. nidulance were inhibited by Tetracycline and Almox DT.

Effect of Vitamins on lipase enzyme production

Effects of different vitamin sources on lipase production of Aspergillus species were studied and the results are given in the table 17 graph 7.

It was observed that vitamin C induced lipase action of all Aspergillus species viz. A. flavus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. oryzae, A. parasiticus, A. terreus, A. ustus and A. versicolor.

The lipase production of Aspergillus fumigatus was totally inhibited by folic acid. Folic acid increased lipase production of A. flavus, A. glaucus, A. oryzae and A. terreus.
Aspergillus glaucus, A. oryzae, A. ustus and A. versicolor produced less lipase in presence of riboflavin. Nicotinic acid inhibited lipase activity of A. parasiticus. Lipase production of A. niger, A. parasiticus, A. terreus and A. ustus were inhibited in presence of Thymine HCl and A. parasiticus produced minimum lipase with Pyridoxin.

b. Physical factor:

1. Effect of incubation period on lipase enzyme production

In order find out the optimum period for lipase enzyme activity, 5th day to 25th day of incubation period were studied and results are shown in table 18 and graph 8.

It is observed that lipase action of Aspergillus species were inhibited on 5th day whereas it was increased on 15th day and gradually minimized from 20th to 25th day.

Lipase production of Aspergillus flavus, A. nidulance, A. niger and A. ustus was totally inhibited on 5th day. On 10th day, lipase production was less as compared to 20th and 25th day. On 15th and 20th day, A. oryzae showed the maximum lipase production as compared to other Aspergillus species.

2. Effect of pH on lipase enzyme production

Lipase production of Aspergillus species at different pH was studied and the results are mention in the table 19 and graph 9.
It was observed that lipase action of *Aspergillus flavus*, *A. glaucus*, *A. niger*, *A. oryzae*, *A. terreus*, *A. ustus* and *A. versicolor* were totally inhibited at 3.5 pH. The lipase activity *Aspergillus* species was stimulated at pH 4.5, were at pH 5.5 to 7.5 lipase action of all *Aspergillus* species were maximum. It was also observed that *A. glaucus*, *A. oryzae* and *A. terreus* ceased their activity at pH 7.5.

It was observed that at pH 3.5 species like *Aspergillus fumigatus*, *A. nidulans* and *A. parasiticus* failed to produce lipase enzyme. Maximum lipase action of *Aspergillus* species was observed at pH 6.5. At pH 8.5 there was very less production of lipase enzyme by all *Aspergillus* species. In between pH 5.5 to 7.5 there was moderate lipase activity in all *Aspergillus* species.

3. Effect of temperature on lipase enzyme production

To study the role of temperature in the enzyme production, the ten selected dominant *Aspergillus* species were incubated at six different temperature and the results are given in the table 20 and graph 10.

It was found that at 10°C and 50°C temperature all *Aspergillus* species produced very less lipase enzyme. Lipase production of *A. flavus*, *A. fumigatus*, *A. niger* and *A. ustus* was totally stopped at 10°C temperature. Lipase action of *A. fumigatus*, *A. nidulance*, *A. niger*, *A. terreus*, *A. ustus* and *A. versicolor* was totally inhibited at 50°C temperature.
30\(^{\circ}\)C to 40\(^{\circ}\)C temperature was found to be optimum temperature for lipase production in all *Aspergillus* species. The maximum lipase production was observed at 30\(^{\circ}\)C by *A. parasiticus* and *A. versicolor* followed by *A. niger* and *A. ustus*.

4. Effect of light on enzyme production

In order to know the impact of light at different conditions i.e. continuous dark, continuous light and alternate dark and light on lipase production of *Aspergillus* species was studied and results are given in table 21 and table 11.

It was found that continuous light induced lipase action in all the *Aspergillus* species where as, continuous dark inhibited the lipase activity of *A. flavus* and *A. fumigatus*. It was interested to note that alternate dark and light condition induced the lipase activity of *A. fumigatus*.

Continuous light induced lipase action in all *Aspergillus* species where as continuous dark inhibited the same.

II) Biodeterioration of oil seed

Biodeterioration of oil seeds by *Aspergillus* species was studied by inoculating the spore suspension of these species in 100 gm of oil seeds, separately. These seeds were incubated for 25 days and biochemical parameters were estimated by standard method.
EXPERIMENTAL RESULTS

1) Change in dry weight:

Change in dry weight of oil seeds due to utilization of their contents by fungi was studied in five oil seeds and the results are given in the table 22 and graph 12.

It was observed that ten *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. nidulans*, *A. niger*, *A. oryzae*, *A. parasiticus*, *A. terreus*, *A. ustus* and *A. versicolor* decreased dry weight in all the five oil seeds i.e. groundnut, soybean, sesame, sunflower, and safflower. *A. flavus*, deteriorated maximum dry weight in groundnut, soybean and safflower.

In groundnut the maximum loss in dry weight was observed due to *Aspergillus flavus* and *A. oryzae* followed by *A. ustus* and *A. terreus*. *A. flavus* and *A. ustus* deteriorate maximum dry weight in soybean.

Maximum loss of sesame and sunflower dry weight was caused due to *A. niger* followed by *A. versicolor* and *A. nidulance*.

2) Change in crude fat

Biodeterioration of crude fat was estimated by soxhlet method and results are given in table 23 and graph 13.

It is clear from the results given in table 31, that all *Aspergillus* species were capable of reducing the fat content. *Aspergillus flavus* was responsible for change in fat content in all oil seeds. In case of soybean and sesame seed, *A. fumigatus* reduced the fat content. In sunflower maximum fat content was reduced due to *A. niger*. 
3) Change in crude fiber:

To study the change in fiber seeds were artificially inoculated with ten dominant *Aspergillus* species, and the results are given in the table 24 and graph 14.

It is clear from the results that, most of the *Aspergillus* species are found to be responsible for change in crude fiber content. In groundnut, *A. flavus*, *A. fumigatus*, *A. nidulans* and *A. versicolor* were found to reduce the fiber content. Similar types of results were obtained in rest of oil seeds.

In soybean, the maximum loss in crude fiber was observed due to *Aspergillus nidulans*, *A. flavus*, followed by *A. oryzae*. On the other hand *A. fumigatus, A. terreus, A. ustus* reduced minimum crude fiber soybean as compare to other *Aspergillus* species.

It was interested to note that in sesame and sunflower all the *Aspergillus* species were found to reduced the crude fiber content. The species like *A. oryzae*, *A. glaucus, A. versicolor, A. fumigatus* and *A. parasiticus* were found to reduce the fiber content effectively in sesame and in sunflower *A. versicolor, A. ustus, A. oryzae* and *A. terreus* were found to reduced the maximum crude fiber.

*Aspergillus flavus* and *A. versicolor* were found to reduced the crude fiber in safflower where as *A. nidulans, A. terreus, A. niger* and *A. oryzae* were found to increased the fiber content of soybean.
4) **Change in Nitrogen content**

Table 25 and graph 15 showed that nitrogen content of all oil seeds was reduced due to all *Aspergillus* species. Maximum loss of nitrogen content in groundnut, soybean and sunflower seeds were caused due to *A. terreus*. Maximum deterioration of nitrogen in sesame and safflower were caused by *A. flavus*.

5) **Change in protein content:**

Protein is one of the important content of legume it was estimated with the help of microKjeldahl technique and results are summarized in table 26 and graph 16.

From results it was observed that all *Aspergillus* species reduced protein content in sunflower, sesame, soybean, safflower and groundnut. In groundnut the maximum loss in protein content was due to *A. terreus* followed by *A. niger*, *A. ustus* and *A. versicolor*. The fungi like *A. niger*, *A. terreus*, *A. parasiticus* and *A. fumigatus* were found to reduced the maximum protein content very effectively in soybean. Where as, *A. fumigatus* reduced the protein content in sesame followed by *A. niger*, *A. flavus*, *A. versicolor* and *A. parasiticus*.

In case of sunflower the maximum loss in protein content was observed due to *Aspergillus ustus*, *A. versicolor*, *A. flavus* and *A. terreus*. *Aspergillus* species like *A. flavus* followed by *A. nidulans*, *A. glaucus* and *A. terreus* reduced the protein content in safflower seeds.
6) **Change in reducing sugar content**

Change in reducing sugar was estimated by Follin-u tube method and results are given in table 27 and graph 17.

From the results it is clear that all *Aspergillus* species reduced sugar in all oil seeds. It was interested to note that *A. versicolor* was found to the most effective fungi among all the ten *Aspergillus* species, it reduced maximum amount of sugar content in groundnut, soybean and sesame. *A. terreus* and *A. fumigatus* were responsible for maximum deterioration of sugar content in sunflower and safflower respectively.

7) **Change in ash content:**

The seeds were when artificially inoculated by ten dominant *Aspergillus* species at room temperature for twenty five days, the seeds showed remarkable loss in crude ash content and the results are mention in table 28 and graph 18.

In groundnut the maximum loss in ash content was due to *Aspergillus glaucus* followed by *A. fumigatus, A. ustus, A. oryzae, A. nidulans, A. niger, A. parasiticus* and *A. versicolor*. In case of soybean, the maximum ash loss was observed due to *A. fumigatus, A. nidulans, A. oryzae* and *A. terreus*. Similarly in case of sunflower maximum loss was due to *A. nidulans* and *A. oryzae*. The species like *A. fumigatus, A. glaucus* and *A. versicolor* were found to caused the maximum loss in ash content of safflower.
8) **Change in calcium content**

Estimation of calcium content of the biodeteriorated oil seed due to *Aspergillus* species were studied and results are summarized in table 29 and graph 19.

It was observed from the results that the all *Aspergillus* species where or less responsible for reducing in calcium content in all the selected oil seeds. In groundnut, *A. flavus* was found to be more effective in reducing the calcium content.

In case of soybean, the maximum loss in calcium content was observed due to *A. parasiticus* followed by *A. glaucus* and *A. ustus*, Similarly *A. parasiticus*, *A. niger*, followed *A. glaucus* were found to be effective in reducing the calcium content in sesame. The maximum loss in calcium content of sunflower and safflower were caused due to *A. glaucus* and *A. flavus* respectively.

9) **Change in phosphorus content**

Phosphorus content in five oil seeds were estimated by the method given by A.O.A.C. (1970). and results are given in table 30 and graph 20.

From the results (table 30), it was observed that all *Aspergillus* species were decreased the phosphorus content in all oil seeds. It was observed that in sesame seeds *A. flavus, A. ustus* and *A. versicolor* reduced maximum phosphorous content.
In groundnut seeds, \textit{A. niger} reduced maximum phosphorous content, followed by \textit{A. flavus} and \textit{A. ustus}. The \textit{Aspergillus} species like \textit{A. parasiticus}, was responsible for maximum deterioration of phosphorous content in sunflower seeds. While in case of safflower, \textit{A. flavus} was most responsible for reducing the phosphorous content.

III. Study of mycotoxins

1. Aflatoxin study by Rapid detection test:

Qualitative detection of toxic and non toxic isolates of \textit{Aspergillus flavus} by Ammonia vapor test:

Forty eight isolates of \textit{Aspergillus flavus} were isolated from five oil seeds and these isolates were alternatively screened for aflatoxin by Ammonia vapor test (Fente \textit{et al.}, 2001) and the results are given in table 31.

Fourteen isolate were isolated from different unhealthy seeds of groundnut, out of which two isolates i.e. GT11.F3 and Gt45.F2 were highly toxic, three isolates i.e. GP.F2, GP. F3 and GP.F4 were moderate toxic and five isolates i.e. GP.F1, GT11.F2, GT11.F4, GT45 and GG.F1 were also moderate toxic in nature.

Fourteen isolates were isolated from soybean seeds, among these isolates only one isolate i.e. So80. F3 was highly toxic, nine isolates were moderate toxic and remaining isolates were non toxic.
In case of sunflower seeds, 5 isolates were isolated, out of which three isolates were moderate toxic (Su Lsu.F1, Sa Ls.8. F1 and Sa Ka.F1), one isolate was mildly toxic (Sa Su.F1) and one was non toxic (Sa Ka.F2). It was interested to note 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 (Sa PB.F2 and Sa A1.F1) and two were moderate (Sa PB.F1 and Sa Bh.F1), where as in sesame, only one isolate was found to be mildly toxic (Se-2B.F1) and three were moderate toxic (Se-1N.F1, Se-1N.F2 and Se-2N.F2).

2. **Qualitative detection of Aflatoxin by HPLC (High performance liquid chromatography) method:**

Aflatoxin qualitative estimation was done by High performance liquid Chromatography (HPLC). Forty eight isolates of *Aspergillus flavus* were isolated from different abnormal oil seed varieties for Aflatoxin production potential and results are summarized in Table 32.

It was note that from all the forty eight isolates, thirty two isolates were found to be toxic and sixteen isolates were found to be non toxic and it was also observed that all the isolates strains consist of aflatoxin B1, but none of the isolates produces aflatoxin G2.

In groundnut variety, out of ten toxic isolates, two isolates (GT11.F3 and GT45.F2) were found to produce aflatoxin B1, B2, and G1, only one isolates (GP. F4) produce aflatoxin B1 and B2, Where as seven isolates (GP.F1, GP.F2, GP.F3,
GT11.F2, GT11.F4, GT45.F4 and GG.F1) produce only aflatoxin B1. Out of five isolates of *A. flavus* from sunflower, only one produced B1 and B2 aflatoxin, three isolates produced only B1 and one isolate did not produce aflatoxin.

Out of fourteen isolates isolated from soybean abnormal seed category, ten isolates were found to be toxic. Two isolates (So80.F3 and So80.F4) produces aflatoxin B1, B2 and G1, three isolates produces (So335.F2, So80.F2 and SoMu.F1) aflatoxin B1 and B2, were as five isolates produces (So335.F1, So335.F3, SoEg.F4, SoMu.F1 and SoMu.F2) B1 aflatoxin.

Out of seven isolates of safflower, four isolates were toxic and three were non toxic. One isolates (SaPB.F2) produced aflatoxin B1 and B2, three isolates (SaPB.F1, SaBh.F1 and SaA1.F1) produced aflatoxin B1 only. Similarly in sesame isolates, out of eight isolates, four isolates are toxic and four were non toxic. Out of toxic isolates, three isolates (Se-1N.F1, Se-2N.F2 and Se-2B.F1) produced aflatoxin B1 and B2, where as only one isolate (Se-1N. F2) produced aflatoxin B1.

**3. Effect of incubation period on aflatoxin production.**

In order to study the effect of incubation period for aflatoxin production, ten different toxic *Aspergillus flavus* isolates were selected from ground nut and soybean oil seeds and the aflatoxin detection was carried out on 10th, 15th, 20th, 25th and 30th day. The results are given in table 33.
It was interested to observe that most of the isolates does not produces aflatoxin up to 10\textsuperscript{th} day of incubation. Out of ten isolates only three isolates, viz. GT11.F3, So335.F2 and So80.F3 produces aflatoxin B1. The isolates incubated for 15\textsuperscript{th} days showed significant production of aflatoxin B1 and B2 and only one isolate i.e. GT11.F3 showed production of aflatoxin G1. Up to 20\textsuperscript{th} and 25\textsuperscript{th} all the isolates showed the production of aflatoxin. It was interested to observe that many of the isolates failed to produce aflatoxin after 30\textsuperscript{th} day of incubation. Isolate GP.F2, GP.f3, GT45.F2 and So335.F2 were failed to produce aflatoxin.

4. Effect of temperature on aflatoxin production

To study the effect of temperature for aflatoxin production, ten different toxic *Aspergillus flavus* isolates were selected from ground nut and soybean seeds and the aflatoxin detection was carried out at different incubation temperature. The results are given in table 34.

From results it was observed that the isolates GP.F1, GP.F4, GT11.F3 from ground and So335.F2, So80.F3, So80.F4 from soybean could produce aflatoxin even at low temperature (15\textdegree C), but rest of isolates were found to be incapable for aflatoxin production. Maximum amount of aflatoxin was found in all the isolates at 25 \textdegree C to 35 \textdegree C. It was interested to observe that as the temperature increase the aflatoxin production ability decrease. At 45 \textdegree C, the isolates from groundnut viz. GP.F2, GP.F3, GP.F4, GT11.F3 and isolates of soybean viz. So335.F2, So80.F2, So 80. F3 and So80.F4 were failed to produce aflatoxin were fail to produce aflatoxin at 45 \textdegree C.
5. Effect of toxin on percent inhibition of germination and Root length:

In order to study the toxic effect of *Aspergillus* species culture filtrate of oil seeds. Oil seeds were soaked in culture filtrate of *Aspergillus* species for 12 hr and then incubated in petriplates for 7 days at room temperature. The results are summarized in table 35.

It is clear from the results that, the culture filtrates of all the selected *Aspergillus* species inhibit the seed germination. Among all the *Aspergillus* species, *A. flavus*, *A. niger* and *A. ustus* showed more toxicity on seed physiology of all the oil seeds. Similarly the retardation of root length of oil seed was observed in case of all the oil seeds with more or less degree of inhibition of germination. It was also observed that *A. niger*, and *A. nidulance* showed maximum inhibition of germination.

Similar experiment was carried out to study the effect of toxic and non toxic culture filtrate of *Aspergillus flavus* isolates on oil seeds germination (Table 36).

It is clear from the results in table 36 that, 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. In case of groundnut and sesame the shoot length was totally aborted, were as root length was aborted in sesame due to toxic culture filtrate.
Experimental Results

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

The interesting part of our research is to study the mycoparasitism and antibiosis in *Trichoderma* and *Aspergillus* species. For this, *Trichoderma viridae* strain is selected to test against the different *Aspergillus* species. The mycelium of *A. flavus* and the spores of *T. viridae* where co cultured in 1% sucrose solution in cavity slide and observations were recorded at regular intervals. The results where found to be very interesting and are shown in photographs. It was observed that *T. viride* inhibits the growth of *Aspergillus* species by two ways i.e. either by coiling the host species or by penetrating haustoria in mycelium of host species.

In photo plate 16, *Aspergillus flavus* and *Tricoderma viridae* were allowed to grow together with 1percent sucrose solution. At early stage of growth, the spores of *Tricoderma viridae* grow faster and cluster around the *Aspergillus flavus*. The mycelia developed around the spores, vesicle and conidoiphore. The spores and mycelia mass of *Tricoderma viridae* do not allowed *Aspergillus flavus* to grow further. It clearly indicates that the extra cellular toxin secreted during antibiosis mechanism by *Tricoderma viridae* inhibits the growth of *A. flavus* which ultimately results the inhibition of *A. flavus* by *Tricoderma viridae*.

*Aspergillus glaucus* and *Tricoderma viridae* were also grown together for studying their antagonistic activity where spores of *Tricoderma viridae* grows much faster than spores of *Aspergillus glaucus*. Spores of *Tricoderma viridae* clusters towards *Aspergillus glaucus* it penetrates the wall and inhibits the growth of *Aspergillus flavus*. 
In order to study the antibiosis of *Aspergillus niger* and *Tricoderma viridae*, both the fungi were allowed to grow in 1 percent sucrose solution. In this case it is observed that spores of both fungi grow separately initially, later on the mycelia of *Tricoderma viridae* when comes close to mycelia of *Aspergillus niger* it releases its housteria, in mycelia of *Aspergillus niger* (photo plate 18), it starts coiling in clockwise direction which results into restricting the growth of *Aspergillus niger*.

Similar types of experiments were carried out with other species of *Tricoderma viridae, Aspergillus nidulance* and *Tricoderma viridae*, it shows the formation of cluster of spores of *Tricoderma viridae* around the mycelia and spores of *Aspergillus oryzae* and *A. nidulance* (Photo plate 19 a, b), where as the spiral coiling of mycelia of *Tricoderma viridae* and *A. fumigatus* (Photo plate 19 c), *T. viridae* and *A. terreus* (Photo plate d), *Tricoderma viridae* and *A. versicolor* (Photo plate 19 e) clearly reported, which ultimately results in to inhibition of *Aspergillus species*. 