Health of seed is affected due to improper handling and ill storage conditions. During this, fungi affect the quality of seeds. In this regards experiments were carried out to study the impact of association of fungi with oilseeds.

PART I

A) ISOLATION OF STORAGE FUNGI OF OILSEEDS

a) Collection of oilseed samples

Oilseeds samples of groundnut, soybean, sesame, safflower and sunflower of different varieties were collected from market places, store houses and fields from different parts of Marathwada region of Maharashtra state. These seeds were then packed in pre-sterilized polythene bags and kept in laboratory conditions until use (Plate 1).

b) Isolation of oilseed mycoflora

For detection of seed mycoflora associated with seed samples, the method recommended by ISTA (1966), Neergaard (1973) and Agarwal (1976) were adopted. Seeds were further categorized according to their abnormalities like shrunkened seeds, discoloured seeds, cracked seeds and rottened seed to know the fungi responsible for their abnormal nature. 10 seeds per pre-sterilized petriplates were equispaced aseptically on autoclaved Potato Dextrose Agar (PDA), Czapek Dox Agar (CZA), Glucose Nitrate Agar (GNA) and Rose Bengal Agar (RBA) media. Plates were then allowed to incubate at room temperature for seven days.
Composition of media used in isolation

1) **Potato Dextrose Agar (PDA)**

Peeled potato-200gm, Dextrose- 20g, Agar- 20 gm, Distilled water-1000ml, pH - 5.6.

Peeled potatoes were boiled until soft and pass through muslin cloth. Then dextrose was added in it and final volume of solution was made up to 1000ml. In this solution agar was added, pH was adjusted to 5.6.

2) **Czapek Dox Agar (CZA)**

Sucrose – 30g, NaNO₃ – 2.0g, KH₂PO₄ – 1.0gm, MgSO₄\(\cdot\)\(\text{H}_2\text{O}\) – 0.5g, KCl – 0.5 gm, FeSO₄\(\cdot\)\(\text{H}_2\text{O}\) – 0.01gm, Agar – 15 gm, Distilled water - 1000 ml, pH – 5.6.

3) **Glucose Nitrate Agar (GNA)**

Glucose – 10gm, KNO₃ – 2.5gm, KH₂OPO₄ – 1.0gm, MgSO₄\(\cdot\)\(\text{H}_2\text{O}\) – 0.5g, Agar – 20 gm and distilled water 1000 ml, pH – 5.6.

4) **Martin’s Rose Bengal Agar (RBA)**

Glucose – 10gm, Peptone – 5.0gm, KH₂PO₄ – 1.0gm, MgSO₄\(\cdot\)\(\text{H}_2\text{O}\) – 0.5g, Rose Bengal – 0.0001 gm, Agar – 20 gm, Distilled water - 1000 ml, pH – 5.6.

B) **CHARACTERIZATION OF STORAGE FUNGI**

**Characterization of storage fungi**

On seventh day of incubation the seeds were examined under stereoscopic microscope for the preliminary determination of fungal growth on them. Detail observations of fungal characters were done under the binocular microscope and their identification was confirmed with standard literature (Ellies, 1971 and Mukadam et al., 2006). Identification was done on the basis of size, shape, septation, colour of conidia and presence or absence of chlamydospore. Pure culture of these fungi were made and maintained separately on Potato Dextrose Agar (PDA) slants.
C) DNA FINGERPRINTING OF *Fusarium* SPP. ISOLATES BY USING RAPD TECHNIQUE

a) RAPD (Random Amplified Polymorphic DNA)
Ten isolates of *Fusarium* used in the present study were isolated from abnormal oilseeds collected from different market places of Marathwada region of Maharashtra. The isolate were isolated and purified on GNA agar medium.

**DNA Extraction method**-
The total genomic DNA of *Fusarium* was isolated from dry mycelia. Isolates were incubated at 28 °C for eight days on GNA media. Mycelia were harvested by scrapping with the help of sterile spatula and used for isolation of the DNA.

DNA isolation was done by CTAB method. Small quantity of dry mycelium mat was taken in a sterile eppendorf tube and crushed to very fine powder. Then prewarm CTAB buffer (600µl) was added. Further it was kept for lysis more than 2-3 hrs in water bath at 50°C. Tubes were removed and centrifuged @10,000 rpm for 20 min. at room temperature. The supernatant was transfered to other sterile eppendorf tube. Further, 24:1 chloroform-isoamyl alcohol was added to it (600µl) and vortexed and centrifuged at 10,000 rpm for 20 min at room temperature. After that 0.5 µl of 3 M sodium acetate (pH-5.2) to each tube was added, mixed and chilled on ice for 15 min. and centrifuged for 30 min at 10,000 rpm at 4°C. The supernatant was removed and transferred to new sterile eppendorf tube. Then ice cool ed isopropanol (600µl) was added and kept in -20°C for 15 min and centrifuged @ 10,000 rpm for 10 min at 4°C. Further the isopropanol was removed and drained out. Then 70% ethanol (600µl) was added and kept for 15 min followed by centrifugation @ 10,000 rpm for 5 min. The pellets were dried overnight and 100 µl ultrapure water was added for better dissolve of DNA and further used for electrophoresis.
Materials and Methods

RAPD Primers

Three decamer RAPD primers were used for initial screening. Following primers gave the amplification. The primer sequences are as follows.

Primer OPAD-4 - GTAGGCCTCA
Primer OPAD-7 - CCCTACTGGT
Primer OPAD-18 – ACGAGAGGCA

PCR amplification of DNA with RAPD primers:

PCR technique has promoted the development of a range of molecular assay systems which detect polymorphism at molecular level. In this study we used the most widely adopted PCR based RAPD marker technology for characterizing the natural variation amongst the *Fusarium* spp. isolates. PCR reactions were carried out in a thermal cycler.

Master mixture for PCR:

The master mix for PCR was prepared by mixing the following components in the given proportion as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq buffer (10X)</td>
<td>2.5µl</td>
</tr>
<tr>
<td>MgCl₂ (25mM)</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>dNTP’s (2.5mM)</td>
<td>2µl</td>
</tr>
<tr>
<td>Taq polyerase (5Unit/µl)</td>
<td>0.2µl</td>
</tr>
<tr>
<td>MQ water</td>
<td>14.8µl</td>
</tr>
<tr>
<td>Primer (10pm/µL)</td>
<td>2.0µl</td>
</tr>
<tr>
<td>Template DNA</td>
<td>1.0µl(100ng/µL)</td>
</tr>
</tbody>
</table>

Total Reaction Volume - 25 µl

PCR thermal cycler –

The polymerase chain reaction, usually called PCR, is an extremely powerful procedure that allows one to amplify a selected DNA sequence. The PCR procedure involves using synthetic oligonucleotides complementary to the known sequences spanning the region of interest to
prime enzymatic amplification of this segment of DNA in the test tubes. DNA was amplified by Applied biosystem, Veriti 96 well Thermal Cycler programme. The PCR procedure involves three steps, each repeated many times to produce cycles of amplification.

First denaturation step is for 3 min at 95°C followed by 35 cycles of 30 sec at 95°C. Annealing temperature 35°C for 30 sec followed by renaturing temperature 68°C for 1min and final extension of 68°C for 1min. After completing 35 cycles, the samples were loaded on 1X TBE gel.

The products of first cycle of replication were then denatured, annealed to oligonucleotide primers and replicated again with DNA polymerase. The cycle is repeated many times until the desired level of amplification was achieved.

**Post PCR processes**

PCR products were resolved by horizontal electrophoresis using agarose gel (1.5%) with TBE buffer (1%) containing ethidium bromide

**Electrophoresis:**

**Principle:** Electrophoresis is the movement of charged molecules under the electric field. Electrophoresis was carried out by using agarose gels. It is used to separate large sized macromolecules like DNA or RNA. DNA is negatively charged and move towards anode when an electric field is applied.

**Procedure:**

To prepare 1.5 % of agarose gel, 1.5 gm of agarose was dissolve in 100ml of 1X TBE buffer. It is then kept for cooling. Meanwhile, the gel tray was sealed at both the sides with tape. The comb was inserted in such a way that 1mm gap was created between the teeth and surface of tray could be made. Agarose solution was then poured into the tray to get the thickness of gel of about 4-5 mm and kept for about 30-40 min without disturbing. After solidifying the gel, the comb was removed gently. The gel tray was transferred into electrophoresis tank and TBE buffer was poured into the tank. DNA samples were loaded carefully into slots of submerged gel. The
electric lead was connected in such a way that a negative terminal should be at the end where sample had been loaded. Electrophoresis was ran at 75 V until loading dye bromophenol blue migrate to the other end of the gel. Turn the button off and disconnect the electric leads after 1 hr. Agarose gel take out from the electrophoresis tank.

**Band scoring and data analysis**

The banding patterns generated through RAPD assay in the present study were used to differentiate between three *Fusarium* spp. isolates from different locations to deduce genetic diversity among them. A total of 10 samples were analysed using three arbitrarily selected decamer primers revealed varying degree of polymorphism. For each fragment that was amplified using RAPD primers was treated as a unit rearrangement in genome. The gel pictures were taken by BioRad molecular imager GelDoc™ XR and documented to computer.

**Phylogeny analysis**

The bands obtained were scored and absent/present matrix was prepared for all the RAPD primers. This matrix was used as a input file for PAST Ver. 1.83 software for further Phylogeny analysis (Hammer et al., 2001).
PART II

1) STUDY OF LIPASE ENZYME

Production of Lipase

Lipase activity was studied by growing the fungi in liquid medium at pH 5.6 containing oil-10ml, KNO3 -2.5g, KH$_2$PO$_4$-1.0g, MgSO$_4$-0.5g and distilled water 1000ml. To study the impact of physical factors on lipase enzyme production, treatments of different physical factors such as temperature, pH, light and incubation period were given to above basal medium. In order to study the effect of different nutritional sources; carbon sources, nitrogen sources, phosphorus sources, sulphur sources, antibiotics and vitamins were added to above basal medium. 50ml of the medium was poured in 100ml conical flasks and autoclaved at 15 lbs pressure for 30 minutes and then on cooling, the flasks were inoculated separately with 1.0ml spore suspension of the fungi. On 7th day, the flasks were harvested by filtering the contents through Whatman filter paper no.1. The filtrates were collected in pre-sterilized culture filtrate bottles and termed as crude lipase.

Assay Method (Cup-plate method)

Determination of lipase activity was done with the help of cup-plate method (Sierra, 1957). The medium contains Difco peptone-10g, NaCl-5g, CaCl$_2$.2H$_2$O-1.0g, Agar 20g and 10ml lipid substrate Serbitan monolaurate (Tween-20) (Pre-sterilized), Distilled water- 1000ml was added to it. The pH of the medium was adjusted to 6.00. The medium was poured in each petri plate. On solidifying the medium, with the help of a cork borer (No.4) of 8mm diameter well was made in the centre of the each plate and was filled with 0.1ml culture filtrate. The plates were incubated at 28°C. After 24 hours, a clear circular zone was measured (mm) as lipase activity.
2) **BIODETERIORATION**

a) **Biodeterioration of oilseeds**

Healthy oilseeds i.e. groundnut, soybean, sesame, sunflower and safflower were surfaced sterilized with 0.1% mercuric chloride solution and subsequently washed and soaked in sterile distilled water for two hours. Excess of water was decanted from the seeds. The seeds of each variety were distributed into eleven flasks (100g per flask) and were inoculated separately with 2ml spore suspension of the ten test fungi and one flask without spore suspension was kept as a control. The flasks were incubated at room temperature for 14 days. After this incubation period mycelium mat was removed with the help of blotter paper (Plate 10). Powder of each sample was prepared in Electrical grinder. These powders were further used for the estimation of physical and chemical parameters.

**Estimation of dry matter (DM)**

Dry weight was estimated by the method recommended by Mungikar (1999). Dry matter (DM) was calculated by weighing the sample after drying to a constant weight in an oven at 95±5°C. For this purpose, 100g of sample was taken in a clean dry pre-weighed tray and is kept in oven for 48 hours or more, till constant weight. Weight of the dried sample recorded as percent dry matter (DM).

**Estimation of crude fat**

The crude fat in the seed sample was estimated by the standard Soxhlet method (A.O.A.C., 1970). The fat present in the seed material was extracted in the solvent mixture of chloroform (CHCl₃) and methanol (CH₃OH) in Soxhlet extraction assembly. 2g dry seed powder was placed in a thimble of Whatman filter paper No.1. The mouth of thimble was plugged with fat free absorbent cotton. Solvent was added in dry 250ml receiver flask from the soxhlet assembly just to reach the level of the neck. The thimble with sample was introduced into the soxhlet. The apparatus was
assembled and placed on heating mental with temperature controlling device. The fat was extracted for 8 hours at 60°C. When the extraction was over, thimble was removed from soxhlet. Apparatus was again assembled and heated to recover most of the solvent from the receiver flask. About 25ml solvent along with the extracted fat was left in the receiver flask, the receiver flask was disconnected. The solvent was then transferred in a clean, previously weighed beaker. After drying in a hot air oven at 95°C, it was then cooled in desiccators and weighed. The amount of fat was measured from extracted per 2g of the sample and amount of crude fat as percent of dry matter (DM) was calculated.

**Estimation of crude fibre**

Crude fibre (CF) was determined as that fraction remaining after digestion with dilute solutions of sulphuric acid (H$_2$SO$_4$) and sodium hydroxide (NaOH) under carefully controlled conditions. The major part of it contains carbohydrates and it is valuable parameter in deciding the nutritive quality of animal feed (A.O.A.C 1970).

2g seed powder was taken in a 500ml spotless beaker and 200ml 1.25% H$_2$SO$_4$ was added to it. The lumps were broken with the help of glass rod having a rubber policeman. The beaker was covered with a conical flask, half filled with cold water, which severed as water condenser, boiled for 30 minutes. Any loss in volume during the boiling with hot distilled water was made up. It was then filter through Whatman filter paper No. 54, by washing the residue several times with hot distilled water. The residue was then taken in the beaker with 100ml water and to it 100ml of 2.5% NaOH was added. Boiled for 30minutes as earlier, filtered through previously weighed Whatman filter paper No. 54. The residue was washed several times with hot water and lastly with 70% alcohol. It was then dried over night at 100°C to a constant weight, cooled and weighed. The residue was then incinerated along with filter paper in a crucible at 600±20°C for 2hrs in a muffle furnace until all the carbonaceous matter was burnt. The crucible was
cooled in a desiccator and weighed. The loss in weight as crude fibre recorded.

**Estimation of reducing sugar**

The sugar content in the plant material was estimated by the procedure recommended by Oser (1979) as follows.

500mg of seed powder was taken in 50ml distilled water and boiled, then filtered. Further filtrate was diluted up to 100ml. Three Folin-wu tubes were taken and to it following content were added

(1) Blank tube - D. W. 2ml (2) 2ml glucose 'C’ solution. (3) 2ml filtrate. In each tube 3ml alkaline solution of copper was added. Then tube was boiled in boiling water bath for 8 minutes. The tubes were cooled under tap water and 2ml of phosphomolybdic acid solution was added which gave blue colour. Then this solution was diluted up to 25ml distilled water and optical density was determined at 420nm and the amount of reducing sugar present in seed powder was calculated.

**Estimation of crude protein:**

(Reagents: A. 2% \( \text{Na}_2\text{CO}_3 \) in 0.1 N NaOH; B. 1% NaK Tartrate in \( \text{H}_2\text{O} \); C. 0.5% \( \text{CuSO}_4\cdot\text{5 H}_2\text{O} \) in \( \text{H}_2\text{O} \); D. 48 mL of A, 1 mL of B, 1 mL C; E. Phenol Reagent - 1 part Folin-Phenol [2 N] : 1 part water; BSA Standard – 50mg BSA in 50ml D.W.).

0.2, 0.4, 0.6, 0.8 and 1 ml of working standard BSA was pipetted out in a series of test tubes. 0.1 ml of sample extract was pipetted out in another test tube. In all test tubes volume of 1 ml was made and tube with 1 ml of water served as a control. Then 5 ml of reagent C was added in all the test tubes including blank. It was then mixed well and incubate for 10 minutes at room temperature. 0.5 mL of dilute Folin-phenol solution was added to each tube. Each tube was vortexed immediately and incubated at room temperature for 30 minutes. Blue colour was appered and at 660 nm
readings were taken. Absorbance vs mg protein graph was plotted to obtain standard curve.

**Estimation of ash**

The residue after incineration of sample at 550-600°C is known as ash. For this purpose the sample was subjected to a high temperature up to 600°C and then the ash content was determined. During ignition to such a high temperature all organic compounds decompose and pass off in the form of gases, while the material elements remain in the form of ash (A.O.A.C., 1970 and Mungikar 1999).

2g of oven dry seed powder was placed in a previously weighed crucible and it was subjected for heating on hot plate till the sample was sufficiently turned black about 30 minutes. Then it was placed in muffle furnace, pre-heated to 600°C for 2 hours with automatic control. Crucible were transferred directly to dessicator, cooled and weighed immediately. Weight of ash was obtained per 2g of sample and the ash content was further calculated as percent of dry matter (DM).

**Estimation of calcium**

Estimation of calcium was done by the method recommended by A.O.A.C. (1970). An aliquat (25ml) of the acid solution ash portion was diluted to about 150ml with distilled water. Few drops of methyl red were added and the mixture was neutralized with ammonia (NH₃) solution till the pink color changed to yellow. The solution was heated to boiling and the 10ml ammonium oxalate solution was added. The mixture was allowed to boil for a few minutes. Glacial acetic acid was then added till distinctly pink color reappeared. The mixture was then kept aside for 12 to 24 hours at room temperature.

Calcium oxalate was settled down when the precipitation appeared. It was filtered through Whatman filter paper No.42. The precipitate was washed several times with water, to make it free from acid. It was then
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transferred in a small beaker by piercing a hole in the filter paper and by pouring over it about 15ml 2N H$_2$SO$_4$. This is heated to above 40°C and titrated against 0.01N KMnO$_4$ solution until the first drop which gave the solution a pink coloration persisting for at least 30 second. The amount of calcium was calculated using an equation. 1ml of KMnO$_4$=0.2004mg of Ca. The percent Ca on DM basis was then calculated on the basis of the amount of sample used for ashing, the volume to which acid solution of ash is diluted and the volume of the aliquat taken for the precipitation of calcium.

**Estimation of phosphorus**

The estimation of phosphorus was carried out by the method given by Fiske and Subba, (1925) and recommended by Oser (1979).

0.5ml of acid soluble portion of ash was taken in a test tube. It was diluted to a volume of 10ml with distilled water. Simultaneously, blank containing only 10ml distilled water was taken. 1ml molybdate solution to each test tube was added and mixed. Then 0.4ml ANSA reagent was added and again mixed. Further, it was allowed to stand for 5 minutes and the optical density (O.D.) at 660mn was taken using colorimeter by setting it to a zero with the blank.

The O.D. of standard phosphorus solution was established by preparing a standard graph containing 0 to 1ml standard phosphorus solutions in series of test tubes. The amount of phosphorus in an aliquot was determined with the help of standard graph and the phosphorus content in the seed powder was calculated by considering its amount taken for ashing, volume of the acid soluble ash and amount of aliquot used for the reaction.

b) Biodeaterioration of oil

1) Physical parameters

**Estimation of moisture from oil:**

Moisture content was estimated by A.O.C.S. (1976). About 1 gm of oil was taken into a moisture dish made of aluminum sheet provided with
Materials and Methods

 tight filling slip-over cover. Dish was dried previously, cooled in the desiccator (containing an efficient desiccant such as phosphorous pentaoxide) and weighed. The dish was placed in the air oven for approximately two hours at 105°C. The dish was removed from the oven, cooled in the desiccator at room temperature and weighed. This procedure was repeated but the dish kept in the oven only for half an hour each time until the difference between the two successive weighing does not exceed one milligram.

Moisture content was calculated by following formula

$$\text{Moisture } \% = \frac{100 \times (M_1-M_2)}{M_1-M}$$

Where, $M_1$= mass in gm of the dish with the material before drying

$M_2$= mass in gm of the dish with the material after drying

$M$= mass in gm of the empty dish.

**Estimation of colour of oil:**

Colour of biodeteriorated oil was determined by observing the grade of the yellow colour as yellow, bright yellow, dark yellow, amber yellow and pale yellow.

**Odor of biodeteriorated oil:**

Odor of biodeteriorated oil was determined by smelling the sample.

**Absorbance of biodeteriorated oil:**

By taking the O.D. at 420nm, absorbance of biodeteriorated oil was recorded.
**Estimation of specific gravity**

Specific gravity was estimated by A.O.C.S. (1976). The weight of dry specific gravity bottle was taken (B). The dry specific gravity bottle filled with the 5ml of sample. After fixing the stopper, weight was taken (A). Weight of the specific gravity bottle containing 5ml of distilled water was taken (C). Specific gravity was calculated by the formula:

\[
\text{Specific gravity at } 30^\circ C = \frac{A-B}{C}
\]

Where, 
- \(A\) = Weight in gm of specific gravity bottle with oil at \(30^\circ C\).
- \(B\) = Weight in gm of specific gravity bottle at \(30^\circ C\).
- \(C\) = Weight in gm of specific gravity bottle with distilled water at \(30^\circ C\).

2) **Chemical parameters**

**Estimation of peroxide value**

Peroxide value of biodeteriorated oil was calculated according to Cox and Pearson (1962). 1 gm of sample of oil was taken in test tube. 20 ml acetic acid- chloroform solution (2:3 volume) and 1g powdered potassium iodide was added. Tube was placed in boiling water bath until liquid boil vigorously. Contents were quickly transferred to the flask containing 20ml of 5% KI solution. Tube was washed quickly with 25ml Distilled water each time and collected in conical flask. Yellow colour was appeared. This was then titrated with 0.1 N sodium thiosulphate solution with constant and vigorous shaking. The titration was continued till the yellow colour almost disappeared. 0.5 ml of starch solution was added and continued titration till the blue colour just disappeared. A blank determination of reagent was conducted. Peroxide value was calculated by formula

\[
\text{Peroxide value} = \frac{(S-B) \times N \times 100}{\text{Wt. of the sample}}
\]
Materials and Methods

Where,

- \( B \) = Titration of blank test ml.
- \( S \) = Titration of sample ml.
- \( N \) = Normality of sodium thiosulphate solution.

**Estimation of iodine value**

Iodine value was determined according to the titrometric method of Pearson (1970). 2g of oil sample was weighed into a dry glass stopper bottle of 250ml capacity and 10ml of carbon tetrachloride was added to the oil. About 20ml of Wij’s solution (Mix 1.5 % of Iodine monochloride and 98% of Glacial acetic acid) was then added and allowed to stand in the dark for 30 min. 15ml of (10%) potassium iodide and 100ml of water was added and then titrated with 0.1M sodium thiosulphate solution using starch as indicator just before the end point. A blank was also prepared alongside the oil samples. Iodine value was calculated from the formula:

\[
\text{Iodine value (Wij’s)} = \frac{(V_2-V_1) \times 1.269}{\text{Weight of sample (g)}}
\]

Where: \( V_2 \) = titer value for blank, \( V_1 \) = titer value for sample (s)

**Estimation of free fatty acid content**

Free fatty acid content was estimated by the method recommended by Cox and Pearson (1962). 2ml of oil was dissolved in 50ml of neutral solvent in 250ml conical flask. Few drops of phenolphthelein indicator were added and titrated against 0.1N potassium hydroxide. Constant shaking was done until pink colour was persisted for fifteen seconds. Acid value was calculated by formula

\[
\text{Acid value} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of sample (g)}}
\]
Saponification value:

The Saponification value was determined according to the titre metric method of Pearson (1981). 2g of oil sample was weighed into a conical flask and 25ml of alcoholic potassium hydroxide was added. Solution was heated in boiling water for 1h. 1ml of 1% phenolphthalein was added and titrated with 0.5N HCl. A blank was prepared alongside the oil samples. The value was calculated by the formula:

\[
\text{Saponification no} = \frac{56.1 \times (B-S) \times N}{W}
\]

Where,

- \( B \) = Volume in ml of 0.5 N Hydrochloric acid required for blank test
- \( S \) = Volume in ml of 0.5 Hydrochloric acid required for the sample
- \( N \) = Normality of Hydrochloric Acid
- \( W \) = Weight of oil in gm.
A) STUDY OF MYCOTOXINS

Culture filtrate preparation

A disc (0.5cm diameter) of mycelia and spores was taken from the periphery of 7-day-old cultures of fungus grown on PDA medium was inoculated into 250ml conical flasks, each containing 100 ml of Glucose nitrate broth. The broth contains (g/l): glucose 1g, potassium nitrate 0.25g, potassium dihydrogen ortho-phosphate 0.1g, magnesium phosphate 0.5g. The flasks were allowed to incubate at room temperature for 15 days. Three flasks were used for each fungus per incubation period. The fungal filtrates were obtained by passing the culture through sterile Whatman No. 1 filter paper to obtain a cell-free filtrate.

i) Effect of fungal filtrate on seed germination

Seeds of groundnut, soybean, sunflower, safflower and mustard were surface sterilized with 1% Mercuric chloride solution for 1 min and rinsed several times in sterile distilled water. All these five oilseeds were then allowed to presoak in fungal culture filtrate for 3h. At the end of presoaking period, the seeds were removed from the filtrates and washed in sterile distilled water. It was then transferred into the petriplates containing two layered blotter papers soaked with sterile distilled water. About 10 seeds were kept per dish and it was then allowed to incubate for two days for room temperature. Germination counts were made after incubation period of 48h and 72h.

ii) Effect of fungal filtrate on chlorophyll content of spinach

Leaves of spinach were washed with sterile distilled water and later on treated with fungal filtrate of all the storage fungi with 30min, 60min,
90min, 120min and 150min time period. Later on O.D. (Chl-a at 645nm; Chl-b at 663nm) of each sample was taken and chlorophyll content was estimated.

iii) Effect of fungal filtrate on betalain pigment content of beet root

3cm cylinders were made with the help of 1cm diameter borer. Cylinders were continuously washed with distilled water until water becomes colourless. These cylinders were then treated with fungal filtrates of storage fungi for specific time. Treated cylinders were then soaked in distilled water for 5min. Finally, O.D. at 525nm of these coloured samples was taken.

iv) Effect of fungal filtrate on seedlings of oilseeds

Seedlings of sesame, safflower, soybean, groundnut and sunflower were dipped in the culture filtrate of storage fungi. The fates of all these seedlings were recorded after 24 hrs.

B) ECO-FRIENDLY MANAGEMENT OF STORAGE FUNGI

i. Antagonistic activity of Trichoderma viride and Trichoderma harzianum against storage fungi.

Trichoderma harzianum and Trichoderma viride were isolated from the sesame on PDA. A mycelial disc (1.2 cm diam), obtained from the peripheral region of 5-7-day-old cultures of Alternaria dianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum and Penicillium chrysogenum was placed on a fresh PDA plate (3 cm from the center) and incubated at 28°C for 48 h to initiate growth. Then a 1cm diameter mycelial disc, obtained from the periphery of a 5-7day old culture of Trichoderma harziaum and Trichoderma viride was placed 3 cm away from the inoculum of the pathogen, the plates were incubated at 28°C and measurements were taken after 7 days. In the control experiment a
sterile agar disc (1.2 cm diam) was placed in the dish. At the end of the incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control (Edington et al., 1971) as follows:

\[
\frac{C-T}{C} \times 100 = \% \text{ Inhibition of radial mycelial growth}
\]

Where, \( C \) = radial growth measurement of the pathogen in control
\( T \) = radial growth of the pathogen in the presence of \( \text{Trichoderma} \)

ii. Antifungal activity of botanicals

Fungitoxic properties of ten selected medicinal plants (10% aqueous leaf extract) screened against test fungi (Nene and Thapliyal, 1993). Glucose nitrate medium was prepared in flasks and sterilized. To this medium, the requisite quantity of the plant extract was added. The plant extract was prepared by collecting fresh plant parts, washed thoroughly in distilled water and grinded in distilled water. The plant extract was thoroughly mixed by stirring. The medium was then autoclaved at 15 lbs pressure for 20 minutes. After cooling the medium, fungi were inoculated in aseptic condition and incubated for seven days at room temperature. Suitable checks were kept where the fungi were grown under the same condition in glucose nitrate without plant extract. Mycelial growth of the test fungi was measured after harvesting. The mycelial weight of the fungi compared with check, was taken as a measure of the fungal toxicity. Similar procedure was followed to study the antifungal activity of essential oils, medicinal plant gums and latex of some medicinal plant.