LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS USED

(Source : Plant Pathologists Pocket Book 3rd Edition)

CD	-	Critical difference
Cm	-	Centimeter
et al.	-	And other co workers
Fig.	-	Figure
g	-	gram
hr	-	hour
i.e.,	-	that is
kg/ha	-	kilogram per hectare
l	-	Litre
lbs	-	Pounds
ml	-	milliliter
mm	-	millimeter
PDA	-	potato dextrose agar
SE	-	Standard error of mean
sp. & spp	-	Species singular and plural
Viz.,	-	namely
%	-	per cent
^{0}C	-	Degree Celsius
µm	-	Micro metre
inch^{-2}	-	Per square inch
m t		million tones
m ha		million hectare
M		Molarity
N		Normality

LIST OF PLATES

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Place: Bapatla

Date: **(K.MURALI KRISHNA)**

ABSTRACT

Name of the Author	:	**K. MURALI KRISHNA**
Title of the Thesis	:	**STUDIES ON SEED MYCOFLORA OF SESAMUM (*Sesamum indicum* L.)**
Degree to which it is submitted	:	Master of Science in Agriculture
Faculty	:	Agriculture
Department	:	**Plant Pathology**
Major Advisor	:	**Dr.K.V.M.KRISHNA MURTHY**
University	:	Acharya N.G. Ranga Agricultural University
Year of submission	:	**2006**

Seeds of sesamum varieties, YLM-17 followed by Tanuku brown and Swetha til yielded the higher per cent incidence of the fungal species.

Significant increase in the incidence of *Aspergillus* spp., *Penicillium* sp. and *Rhizopus* sp. and decrease in that of *Alternaria, Curvularia, Sclerotium* and *Rhizoctonia* species were observed. *Fusarium* sp. completely disappeared at the end of storage.

Maximum reduction in seed germination was caused by the culture of *Aspergillus niger* and the culture filtrates of *A. terreus*. Culture of *Curvularia* sp. and culture filtrates of *A. terreus* resulted in maximum reduction in shoot length. The culture of *A. niger* and culture filtrate of *Rhizoctonia* sp. caused the maximum reduction in root length under laboratory conditions. In general, culture filtrates were more effective than the cultures of fungal species.

It is observed that there was significant reduction in the protein content but a slight decrease in the oil content of the seed treated with different fungal species with increase in the incubation period. Regarding physico-chemical properties of oil a change in colour and odour of oil was observed. Significant increase in the free fatty acid content, saponification values and peroxide values was recorded in the oil from seed inoculated with different fungal species separately, irrespectively of incubation period. In general, iodine value decreased in respect of all fungal species except *Rhizoctonia* sp., but the decrease was not significant.

CHAPTER - I

INTRODUCTION

Sesamum (*Sesamum indicum* L.) known variously as sesame, til, gingelly, simsim, gergelin *etc.,* is an important oil-yielding crop cultivated in India, Myanmar, Indo-China, China and Japan. Sesamum is regarded as the oldest oil yielding plant known to man.

It is grown in the tropics as well as temperate zones mostly between altitudes of 40^{o} N and 40^{0}S. In World, it occupies an area of 6.58 m ha with an annual production of 2.35 million tonnes and productivity of 357 kg/ha. In India, it occupies an area of 2.20 m ha with an annual production of 0.60 million tonnes and productivity of 273 kg/ha. In Andhra Pradesh it occupies an area of 2 lakh ha. with an annual production of 55,000 tonnes and with an average productivity of 204 kg/ha (*Vyavasaya panchangam*, 2005-'06).

Sesamum is called as" Queen of edible oils" in view of the rich oil content (40-50%), seed protein (20%), carbohydrates and minerals such as calcium (1%) and phosphorus (0.7%). It is also a rich source of vitamine E. Among cultivated crops of India, sesamum is unique in that the plant and its produce, find wide range of uses not only in daily life of people but also in various industries.

The importance of clean and healthy seed in crop production needs no special emphasis. In many crops including sesamum, the seed is colonized by several fungi when the seed is developing or before harvest, and also when the

seed is in the storage. The fungi associated with the seed cause a variety of adverse effects, posing a constraint on the production of crops. Seed serves not only as better medium for dissemination of fungi but also as a substratum for survival and growth.

The important pathogenic fungi found to be transmitted through sesamum seeds are *Fusarium moniliforme, Aspergillus flavus, Aspergillus ochraceous, Aspergillus niger. Alternaria sesami, Curvularia lunata, Penicillium spp. Macrophomina phaseolina, Cladosporium oxysporum, Fusarium semitectum* and *Acremonium strictum* (Tini Pillai *et al.,* 2003). Besides these, *Rhizopus stolonifer, Mucor haemalis, Aspergillus flavus* and *Alternaria sesami* were also found associated with sesamum seed (Sulochana and Bala Krishnan, 1997).

Seed-borne fungi may cause reduction of volume of storage tissue and the amount of reserve food available to the developing seedling, which accounts for poor germination and seedling growth. Poor seed germination often results in poor crop stand in the field. Some of these fungi also produce toxic metabolites which make the seed unfit for consumption besides adversely affecting seedling vigour. The fungi associated with the seed may also cause adverse changes in the constituents of the seed like proteins and also adversely affect the quality of the oil content of sesamum seeds.

Several workers reported earlier on the effect of seed mycoflora on seed health, nutritional status of the seed and quantity and quality of the oil in the case of groundnut, soybean, and sunflower. However, no precise information is

available on the effect of seed mycoflora on sesamum seeds and oil, particularly in Andhra Pradesh. Hence, the present work has been taken up with the following objectives.

1. To isolate and identify seed mycoflora from different varieties of sesamum.
2. To study the effect of storage period on dynamics of seed mycoflora.
3. To study the effect of seed mycoflora on seed germination and seedling growth
4. To study the effect of seed mycoflora on protein and oil content of seed.

CHAPTER - II

REVIEW OF LITERATURE

Literature on various aspects of the present study has been reviewed hereunder

1. ISOLATION OF SEED MYCOFLORA FROM DIFFERENT SESAMUM VARIETIES

Different fungal species were reported to be isolated from different varieties of sesamum earlier.

Name of the author & year	Name of sesamum varieties	Fungal species isolated from sesamum
Vidyasekharan *et al.* (1972)	Not mentioned	*Rhizopus nigricans*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus versicolor*, *Aspergillus fumigatus*, *Aspergillus tamarii*, *Aspergillus terreus*, *Emericella vercicolor*, *Cladosporium herbarum*, *Sporotrichum roseolum*, *Fusarium moniliforme*, *Alternaria brassicola*, *Curvularia lunata*, *Helminthosporium tetramera*, *Syncephalastrum racemosum*, *Chaetomium globosum*, *Memnoniella echinata*.
Sing Tribhuvan and Singh Dalbir (1981)	Not mentioned	*Alternaria sesami*, *Cephalosporium acremonium*, *Fusarium oxysporum f.sp. sesami*, *Fusarium solani* and *Macrophomina Phaseolina.*

Vaidehi and Lalitha (1985)	Not mentioned	*Alternaria, Curvularia*, *Drechslera, Fusarium, Cladosporium, Aspergillus niger* and *Aspergillus flavus.*
Wu-Ws (1988)	Not mentioned	*Alternaria longissima, Alternaria sesami, Alternaria sesamicola, Alternaria tenuis* and *Corynespora cassiicola.*
Khamees and Schlosser (1990)	Not mentioned	*Alternaria sesamicola, Macrophomina phaseolina, Phoma sorghina, Aschochyta gossypii, Fusarium* sp. and *Aspergillus flavus.*
Bahkali and Moslem (1996)	Not mentioned	*Aspergillus* sp., *Alternaria* sp., *Chaetomium* sp., *Curvularia* sp., *Drechslera* sp., *Fusarium* sp., *Helminthosporium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp. *Setosphaeria* sp., *Stemphylium* sp., *Syncephalastrum* sp., *Trichoderma* sp. and *Ulocladium.*
Neeti-saxena *et al.,* (1991)	Cultivar T – 85	*Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Fusarium moniliforme.*

Sulochana and Balakrishnan (1997)	Kayamkulum 1, TC 30, No.42, Assam local, B 64, Si 866, Kayamkulum 2, T 13, Timbi 9 and Trivandrum local	*Rhizopus stolonifer, Aspergillus flavus, Mucor haemalis, Aspergillus niger, Penicillium chrysogenum and Alternaria sesami.*
Gooya *et al.* (2000)	Not mentioned	*Acremonium, Alternaria, Aspergillus, Chaetomium, Cladosporium, Fusarium, Paecilomyces, penicillium, Rhizopus, Stemphylium, Trichoderma* and *Ulocladium.*
Wagan *et al.* (2002)	PR-125, S-17, PR-19-9, and PR-14-2.	*Alternaria sesami, Curvularia lunata, Alternaria sesamicola* and *Fusarium oxysporum.*
Mashooda-Begum *et al.* (2003)	Not mentioned	*Aspergillus flavus, Aspergillus niger, Rhizopus* spp. and *Fusarium* spp.
Tini-Pilli *et al.* (2003)	TC-25, Phule Til-7, JLT-7 and AKT-64.	*Acremonium strictum, Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Cladosporium oxysporum, Curvularia lunata, Fusarium moniliforme, Fusarium semitectum, Macrophomina phaseolina* and *Penicillium sp*.

Nasira-Altaf *et al.* (2004)	Til-93, Ti-89, Lateefi, Tabrazi, Nagari, Johi-1, Johi-2, Sehwani-1, Quallandari and P-37-40.	*Alternaria brassicola*, *Alternaria redicina*, *Aspergillus alba*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus viridis*, *Cephalosporium* sp., *Curvularia* sp., *Drechslera* sp., *Fusarium* sp. and *Penicillium* sp.

2.2 EFFECT OF STORAGE PERIOD ON THE INCIDENCE OF SEED MYCOFLORA IN SESAMUM

Nandi *et al.* (1982) reported that with the increase in storage period, seed moisture content decreased. A gradual decrease in field fungi with simultaneous increase in storage fungi was recorded as storage proceeded.

Vaidehi and Lalitha (1985) studied the incidence of seed mycoflora of sesamum variety N-62-30 during pre and post harvest stages in *Kharif* and *Rabi* seasons and in storage. In all 54 fungal species comprising mostly field fungi were isolated during pre and post harvest stages. Species of *Alternaria, Curvularia, Drechslera, Fusarium* and *Cladosporium* including some pathogens, *Alternaria sesami, Cercospora sesami, Cylindrosporium sesami* and *Fusarium oxysporum* which were present in large numbers in pre-harvest stages considerably declined in post-harvest stage and some were totally eliminated during storage of six months giving way to storage fungi. *Aspergillus flavus* and *Aspergillus niger* which were recorded in very low

numbers in pre and post-harvest stages increased significantly during storage. The number of fungal species reached its peak in storage, which consisted of mostly different species of *Aspergillus* and *Penicillium.*

Singh *et al.* (2003) isolated some 29 to 35 species of fungi at varying frequencies from stored seeds of sesamum, groundnut, sunflower and castor. *Aspergillus* spp. outnumbered the other species, with *Aspergillus flavus* recording the highest frequency (81-100%). *Alternaria, Curvularia,* and *Cladosporium* spp. were found in all seeds.

Similar changes were observed in other oil seed crops by several workers in soybean (Kabeere and Taligola, 1983; Dwivedi and Shukla,1990 ; Tripathi and Singh, 1991), sunflower (Charjan and Tarar, 1992), mustard (Ahmad *et al.,* 1996) and groundnut (Krishnappa *et al.,* 2003).

2.4 EFFECT OF SEED MYCOFLORA ON SEED GERMINATION AND SEEDLING GROWTH OF SESAMUM

Vidhyasekharan *et al.* (1972) reported that seed germination and root and shoot elongation in sesamum was severely reduced by seed-borne fungi. *Aspergillus niger* was highly effective in reducing germination percentage, root and shoot elongation.

Nandi *et al.* (1982) reported that in sesamum, mustard and linseed, seed germination was reduced due to the invasion of storage fungi.

Bose and Nandi (1985) found in sesamum and safflower seeds inoculated with *Aspergillus flavus* and *Rhizoctonia solani* complete loss of germinability. But *Aspergillus sydowi* in safflower and *Aspergillus candidus* in sesamum decreased germinability to a lesser extent.

Nasira-Altaf *et al.* (2004) using standard rolled paper towel method studied sesamum seed borne mycoflora on germination and seedling health and reported a decrease in germination potential due to the effect of seed mycoflora. Eleven fungi were isolated from abnormal sesamum seedlings and ungerminated seeds.

Similar adverse effects were observed in other oil seed crops *viz.*, sunflower (Kushal *et al.*, 1994) and groundnut (Abid-Riaz *et al.*, 2002)

2.5 EFFECT OF CULTURE FILTRATES OF SEED MYCOFLORA ON SEED GERMINATION AND SEEDLING GROWTH OF SESAMUM

Mishra and Kanaujia (1973) studied the effect of culture filtrate of *Aspergillus flavus* on seven different samples of oil seeds. They reported that the germination of oil seeds was affected with the increase in the concentration of the culture filtrate. The effect of 15 days old culture filtrate of *Aspergillus flavus* was also studied on seedling growth and it was observed that in all the cases there was a considerable effect on growth of seedlings.

The effect of culture filtrates of *Aspergillus flavus, Aspergillus niger, Penicillium* sp. and *Rhizopus* sp.on seed germination and seedling growth of sesamum, soybean, mustard, linseed, sunflower, niger, safflower and groundnut was studied by Chandra *et al.* (1985). A reduction in seedling growth expressed in terms of root length, compared to control was reported in respective of all oil seeds. With respect to sesamum, highest reduction was caused by *Penicillium* followed by *Rhizopus* sp**.**, *Aspergillus niger* and *Aspergillus flavus.*

Wasnikar *et al.* (1991) studied toxicity of fungal metabolites on germination and growth of sesamum. The culture filtrates of the four most important seed-borne pathogens of sesamum (*Alternaria sesami, Fusarium oxysporum* f. sp. *sesami, Helminthosporium sesami* and *Myrothecium roridum*) were found to show pronounced effects on germination, root and shoot elongation. *Alternaria sesami* and *Myrothecium roridum* were the most toxic to germination while *Alternaria sesami* was the most inhibitory to shoot growth and *Fusarium oxysporum* f. sp. *sesami* to root growth.

Pinkey khati and Pandey (2005) studied the effect of fungal metabolites on germination, sprouting and growth of sesame. They reported that the maximum inhibition in seed germination was caused by *Fusarium semitectum* (59%) followed by *Stachybotrys atra* (51%), *Aspergillus niger* (47%) and *Aspergillus flavus, Curvularia lunata* and *Penicillium chrysogenum*, when seeds were treated with 30 days old culture filtrates.The effect of culture filtrates on sprouting was more pronounced than on germination. The maximum inhibition in sprouting was recorded in seed treated with culture filtrates of *Aspergillus niger* (67%) followed by *Curvularia lunata, Fusarium oxysporum* (47% each), *Penicillium pupurogenum* (43% each), *Alternaria alternata* and *Cladosporium oxosporum* (39% each). The sesamum seeds treated with culture filtrates showed a considerable decrease in root and shoot length. The maximum inhibition in root length was recorded in the seeds treated with culture filtrates of *Fusarium oxysporum* (87%) followed by *Stachybotrys atra* (60%), *Alternaria brassicae* (52%), *Penicillium chrysogenum* (49%) and *Curvularia lunata* (45%). Corresponding figures for shoot length were in seed treated with culture filtrates of *Aspergillus niger* (86%) followed by *Stachybotrys atra* (57%), *Alternaria brassicae* (43%) and *Alternaria tenuissima* (36%).

2.6 EFFECT OF SEED MYCOFLORA ON PROTEIN CONTENT OF SESAMUM SEED

Sharma (1977) made a comparative study between healthy and stored oil seeds (sesamum, castor and cotton) collected from local warehouses of Agra region, in order to know the extent of bio-deterioration of oil seeds in storage and reported a marked reduction in the seed germination and oil and protein contents of storage seeds.

Neeti Saxena *et al.,* (1991) isolated predominantly *Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Fusarium moniliforme* from seeds of sesamum cultivar T-85. Seed protein and carbohydrate contents were analysed before and 10, 20 and 30 days after inoculation with each of the predominant fungi identified. *Aspergillus flavus, Aspergillus niger* and *Alternaria alternata* caused decreases in protein and carbohydrate contents of seed.

Similar changes were observed by several workers in other oil seed crops like soybean and pea seeds artificially infested with *Cephalosporium* sp., (Rehim *et al.,*1977). However, Marcinkowska *et al.* (1986) observed an increase in the protein content of soybean seeds encrusted with *Peronospora manshurica* and *Pseudomonas syringae* pv *glycinea*.

2.7 EFFECT OF SEED MYCOFLORA ON OIL CONTENT OF SESAMUM SEED

Vidyasekahran *et al.* (1972) studied the role of seed-borne fungi on the deterioration of quality of gingelly seeds. They observed that gingelly seeds infected by 17 fungal species in storage, showed reduction in the oil content of the seeds appreciably.

Mondal and Nandi (1984) studied deteriorative efficacy of storage fungi of sesame, rape seed and linseed. Maximum loss of oil in both rape and lin seed was recorded with *Aspergillus fumigatus* and in black cultivar of sesame with *Aspergillus niger*.

Bose and Nandi (1985) studied role of enzymes of storage fungi in deterioration of sesamum and safflower seeds. They observed reduction in germinability and oil content and increase in fat acidity value more pronouncedly in seeds inoculated with *Aspergillus flavus, Aspergillus fumigatus* and *Rhizoctonia solani.*

A decrease in oil content of seeds of sesame due to mycoflora, particularly *Macrophomina phaseolila, Aspergillus flavus, Drechslera hawaiiensis* and *Fusarium moniliforme* was reported (Singh, 1972, 1987).

Bhargava and Shukla (1980) observed reduction in the oil content of sesamum seed due to *Fusarium equiseti* and *Fusarium oxysporum.*

Sharma (1981) found a decline in the oil content in the sesamum seeds infested with *Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Aspergillus tamarii, Penicillium funiculosum, Penicillium citrinum, Penicillium multicolor* and *Cladosporium herbarum*.

Singh *et al.* (2003) isolated some 29-35 species of fungi from stored seeds of sesamum, groundnut, sunflower and castor. They observed that the maximum loss in seed oil content was caused by *Aspergillus flavus* followed by *Aspergillus niger, Fusarium moniliforme* and *Alternaria tenuissima*.

Similar changes were observed by several workers in other oil seed crops like sunflower (Prasad and Singh 1983; Neera and Mehrotra, 1990) and safflower (Singh and Sinha, 1979).

2.8 EFFECT OF SEED MYCOFLORA ON PHYSICO – CHEMICAL PROPERTIES OF SESAMUM OIL

2.8.1 Effect of Seed mycoflora on colour and odour of oil

Mondal and Nandi (1984) studied the role of fungi on stored seeds of sesamum, rape and linseed and reported an increase of red and yellow units in the oil color during storage. *Aspergillus fumigatus* produced blue units.

Mashooda-Begum *et al.* (2003) studied physico-chemical characteristics of oil obtained from the infested sesamum, sunflower and toria seeds and reported a change in the colour intensity by *Aspergillus niger* and *Aspergillus flavus.*

2.8.2 Effect of seed mycoflora on free fatty acid content of sesamum oil

Sharma (1981) reported an increase in the free fatty acid content of sesamum seed infested with eight fungi.

Free fatty acid content of sesamum, safflower and sunflower seeds infested with *Aspergillus ochraceous, Rhizoctonia solani*, *Aspergillus flavus* and *Alternaria alternata* showed an increase (Bose and Nandi, 1982; Prasad and Singh, 1983).

Mondal and Nandi (1984) reported an increase in the free fatty acid content of sesamum and linseed infested with *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum.*

Mashooda-Begum *et al.* (2003) reported a great increase in free fatty acid content in sesamum, toria and sunflower. *Aspergillus flavus* caused the highest increase of free fatty acid content in sesamum.

Similar changes were observed by several workers in other oil seed crops like groundnut (Ward and Diener, 1961; Lalithakumari *et al.*, 1971), soybean (Lisker *et al.*,1985) and sunflower (Lakhsmidevi *et al.*, 1992).

2.8.3 Effect of seed mycoflora on saponification values of sesamum oil

Increase in the saponification value of sesamum seed infested with *Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Aspergillus tamarii, Penicillium funiculosum, Penicillium citrinum, Penicillium multicolor* and *Cladosporium herbarum* was reported (Sharma, 1981).

Saponification values increased in sesamum, safflower and sunflower seeds infested with *Aspergillus ochraceous, Rhizoctonia solani, Aspergillus flavus and Alternaria alternata* (Bose and Nandi, 1982; Prasad and Singh, 1983).

Sesamum and linseed seeds infested with *Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum* showed an increase in the saponification value (Mondal and Nandi, 1984).

2.8.4 Effect of seed mycoflora on iodine values of sesamum oil

Sharma (1981) reported that sesamum infested with *Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Aspergillus tamarii, Penicillium funiculosum, Penicillium citrinum, Penicillium multicolor, Cladosporium herbarum* and *Botryodiplodia* sp. showed reduction in the iodine number.

Iodine value decreased in sesamum, safflower and sunflower seeds infested with *Aspergillus ochraceous, Rhizoctonia solani, Aspergillus flavus* and *Alternaria alternata* (Bose and Nandi, 1982; Prasad and Singh, 1983).

Singh (1987) reported that the iodine values decreased in sesamum infested with *Aspergillus flavus, Drechslera hawaiiensis* and *Fusarium moniliforme.*

Mashooda-Begum *et al.* (2003) reported that the iodine values decreased in sesamum, sunflower and toria seeds when treated with *Aspergillus niger* and *Aspergillus flavus.* The reduction in iodine value was more in respect of *Aspergillus flavus* than *Aspergillus niger.*

2.8.5 Effect of seed mycoflora on peroxide values of sesamum oil

Sharma (1981) observed increased peroxide values in sesamum seeds infested with *Aspergillus flavus, Aspergillus niger, Aspergillus versicolor, Aspergillus tamarii, Pencillium funiculosum, Pencillium citrinum, Pencillium multicolor,*and *Cladosporium herbarum.*

Mashooda-Begum *et al.* (2003) reported slight increase in peroxide values in sesamum, sunflower and toria seeds when these were treated with *Aspergillus niger* and *Aspergillus flavus.*

In soybean and cotton also an increase in peroxide values was reported as a result of infestation by *Cercospora kikuchii* and *Rhizoctonia solani* seedlings respectively (Park *et al.*, 1982; Veech, 1976).

CHAPTER – III

MATERIALS AND METHODS

The conventional techniques employed in the present investigation such as preparation of media, isolation of fungi, maintenance of cultures are standard methods (Riker and Riker, 1936; Tuite, 1969). Mention was made wherever special methods or procedures were adopted.

3.1 SEED MATERIAL

The seed samples of five popular sesamum varieties, of both white seeded and black seeded types were collected from Agricultural Research Station, Yellamanchili, in Visakhapatanam district of Andhra Pradesh. The varieties *viz.*, Swetha til (white seeded), YLM-17, Gowri, Madhavi and Tanuku brown were used to isolate seed mycoflora. The effect of the isolated seed mycoflora and their cultured filtrates on seed germination, seedling growth, and protein and oil content of seed were studied with sesamum variety, YLM-17.

3.2 GLASSWARE

Borosil and Corning brand glassware was used. The glassware was cleaned with detergent solution and rinsed with water. Later they were kept overnight in cleaning solution (potassium dichromate 75g, concentrated sulphuric acid 500 ml and water 1000 ml) and washed with tap water followed by rinsing with distilled water.

3.3 CHEMICALS

The analar grade chemicals of B.D.H and S. Merek make were used.

3.4 NUTRIENT MEDIA

The nutrient media used in the present investigation were prepared as given below.

3.4.1 Preparation of potato Dextrose Agar (PDA) Medium

Peeled potato : 200 g

Agar agar : 20g

Dextrose : 20g

Distilled water : 1000 ml

Potato dextrose agar medium used for isolation and culture of fungi was prepared by boiling the small pieces of peeled potato tubers in 500ml of distilled water for about 30 minutes. Agar-agar powder was melted in another 500ml of distilled water. Both the solutions were filtered through a muslin cloth into a third container. Dextrose was added to the filtrate. The final volume of the medium was made up to one liter by adding distilled water. The pH of the medium was adjusted to 6.0 by using 1.0 N sodium hydroxide or 1.0 N hydrochloric acid before sterilization. About 100ml of nutrient medium was distributed to 250ml conical flasks.

3.4.2 Preparation of Czapek – Dox liquid medium

Sodium nitrate ($Na\ NO_3$)	: 2.0 g
Magnesium sulphate ($Mg\ SO_4$)	: 0.50g
Potassium chloride (KCl)	: 0.50g
Ferrous sulphate ($FeSO_4$)	: 0.01g
Sucrose ($C_{12}H_2O_{11}$)	: 30.0g
Water (H_2O)	: 1.0 litre

The chemicals in the order listed were mixed in water and the mixture was autoclaved.

3.5 STERILIZATION

All the glassware used in the present investigation was sterilized in a hot air oven at 160^0C for two hours. All the nutrient media were sterilized in an autoclave at 15 lb $inch^{-2}$ for 15 minutes at 121^0C.

Seed material, wherever necessary, was surface sterilized with one per cent sodium hypochlorite and washed in three changes of sterile distilled water.

The blotting paper discs used in laboratory experiments were sterilized in an autoclave at 15 lb in ch^{-2} for 30 minutes.

3.6 ISOLATION OF SEED MYCOFLORA FROM DIFFERENT SESAMUM VARIETIES

Methods employed to isolate seed mycoflora were standard blotter method with unsterilised and surface sterilized seed and agar plate method with unsterilised and with surface sterilized seed (ISTA, 1976). Seeds of sesamum varieties Swetha til, YLM-17, Gowri, Madhavi and Tanuku brown were used.

3.6.1 Standard blotter method with unsterilised seed

The discs of sterilized blotting papers were placed in sterile perti dishes (9.0 cm diameter) and moistened with sterile distilled water, such that a little surplus water was left on the surface the paper discs. Unsterilised sesamum seeds were then placed on the moist blotter at equal distance from each other at the rate of 20 seeds per plate, and each petri dish constituted one replication. Four replications were maintained. The petri dishes were incubated at 25 ± 1^0C with alternating cycles of 12 h light and 12 h darkness. White fluorescent light was provided by placing two 40 W day light tubes in a horizontal position, 20 cm apart at a height of approximately 41 cm above the seed (*i.e.*, 40 cm above the lids of the petri dishes, Plate 1). The seeds were examined daily under stereoscopic binocular microscope and observations on the incidence of different fungal species were recorded in all replications and the per cent incidence of each fungal species was calculated. The fungal species, as soon as they appeared, were transferred to agar slants for maintenance and further investigation.

3.6.2 Standard Blotter method with surface sterilized seed

The method is the same as the standard blotter method with unsterilised seed except that the seeds were used after surface sterilization with 1 per cent sodium hypochlorite. Twenty surface sterilized seeds were placed in each petri dish, which constituted one replication. Four replications were maintained. All petri dishes were incubated and observations on the incidence of different fungal species were recorded and per cent incidence of each fungal species was calculated as described earlier. The fungal species, as soon as they appeared, were transferred to agar slants for maintenance and further investigation.

3.6.3 Agar plate method with unsterilised seed

Unsterilised seeds of sesamum were spaced out at equal distance aseptically in each petri dish containing sterile potato dextrose agar medium at the rate of 20 seeds per plate, and this constituted one replication and four replications were maintained. Petri dishes were incubated (Plate 2) as described in the case of standard blotter method with unsterilised seed. The seeds were examined under stereoscopic binocular microscope. Observations the incidence of different fungal species were recorded in all the replications and the per cent incidence of each fungal species was calculated. The fungal species, as soon as they appeared, were transferred to agar slants for maintenance and further investigation.

3.6.4 Agar plate method with surface sterilized seed

The method is same as the agar plate method with unsterilised seed except that seeds were used after surface sterilization with 1 per cent sodium

hypochlorite. Twenty surface sterilized seeds were placed in each petri dish containing sterile potato dextrose agar medium. A petri dish with twenty seeds constituted one replication and four such replications were maintained. All petri dishes of different fungal species were recorded and per cent incidence of each fungal species was calculated as described elsewhere. The fungal species as soon as they appeared were transferred to agar slants for maintenance and further investigation.

3.7 IDENTIFICATION OF THE FUNGI

Fungal species obtained during the present study were transferred to fresh agar petri plates and agar slants, and purified by single spore isolation. To purify the fungal culture by single spore isolation, the spore suspension was prepared as described elsewhere. After confirming the presence of spores, the suspension was poured into petri dishes containing 2 per cent sterilized plain agar medium aseptically. The petri dishes were gently rotated for the even distribution of the spores on the medium.

After incubation of petri dishes for 12 hr, the spores well-spaced out were located using a microscope and marked on the bottom of the dishes. Each marked single spore along with a bit of agar medium was scooped with a sterilized cork borer and transferred to sterilised petri dishes containing potato dextrose agar medium. When the growth of the fungus was seen in the petri dish, the culture was transferred aseptically to potato dextrose agar slants. The culture was maintained by sub-culturing regularly at 15-20 days interval. Identification of fungal species

was done based on their colony and cultural characters, and on the basis of characters of conidiophores and conidia which were recorded and compared with the descriptions given in the "C.M.I descriptions of pathogenic fungi and bacteria (1952)" and "Illustrated genera of imperfect fungi" (Barnet and Hunter 1972). For identification of Aspergillus cultures "The Genus *Aspergillus*" by Raper and Fennel (1965) was consulted. The following formula was employed for comparison of measurements of fungal species with standard measurements.

$$\mu = x \pm t_{0.05} \times S.E$$

Where

μ = size (length/width) of the conidia/Conidiophore

X = mean of 100 observations.

$t_{0.05}$ = test significance at 5 percent level= 1.96

S.E = Standard error of the mean

The fungal species were identified based on the 'μ' values falling between confidence levels.

3.8 PERCENT INCIDENCE OF FUNGAL SPECIES

As suggested by Jha (1995) the incidence of fungal species was recorded by counting the number of seeds colonized by a particular fungal species in each of the replications, and the percent incidence of the species was calculated as follows.

$$\text{Percent incidence} = \frac{\text{No. of seeds colonized in each plate by a particular species}}{\text{Total no. of seeds in each plate}} \times 100$$

3.9 EFFECT OF STORAGE PERIOD ON THE INCIDENCE OF SEED MYCOFLORA IN SESAMUM

To study the effect of storage period on seed mycoflora, seeds of sesamum variety YLM-17 were collected and stored in a cloth bag at room temperature for different periods *viz.*, 15,30,45,60 and 75 days respectively.

The fungi associated with the seed samples stored for different periods were isolated using agar plate method as described earlier. For each storage period four replications were maintained. Each replication consisted of twenty seeds distributed in one petri dish. At the end of each storage period, observations on the incidence of different fungal species were recorded and percent incidence was calculated.

3.10 EFFECT OF SEED MYCOFLORA AND THEIR CULTURE FILTRATES ON SESAMUM SEED GERMINATION AND SHOOT AND ROOT LENGTH OF SESAMUM SEEDLINGS UNDER LABORATORY CONDITIONS

The effect of seed mycoflora and their culture filtrates on seed germination, shoot and root lengths of seedlings was studied under laboratory conditions.

3.10.1 Effect of seed mycoflora on seed germination

Pure cultures of different fungal species isolated from sesamum seed were multiplied on potato dextrose agar (PDA) medium and spore suspension of each fungal species was prepared as described below.

Preparation of spore suspension

The surface of the 10-day old fungal growth of each fungal species on the agar plate was scraped, and the scrapings were made into a concentrated suspension by adding them to 1ml sterile water which was further diluted so as to have, a spore suspension of 10^4 spores ml^{-1}.

Surface sterilized sesamum seeds were inoculated with each fungal species by soaking the seed for 24 h in the spore suspensions of respective fungal species and removing the excess moisture with a blotting paper. The inoculated seeds were placed on the wet sterile blotters contained in a sterile petri dish at the rate of twenty seeds per dish. Twenty seeds in one dish constituted one replication and four replications were maintained for each fungal species. Surface sterilized seeds soaked in sterile distilled water and placed on wet blotters served as control. The number of seeds germinated in each replication was count and expressed as per cent seed germination.

3.10.2 Effect of seed mycoflora on shoot and root length of sesamum seedlings

Surface sterilized sesamum seeds were inoculated with each fungal species by soaking the seed for 24 hours in the spore suspensions of respective fungal species and removing the excess moisture with a blotting paper. The inoculated seeds were placed on sterile wet blotters in a petri dishes. Twenty seeds in one petri dish constituted one replication and four such replications were maintained. A suitable control was maintained by soaking the surface sterilized seeds in sterile distilled water. After 7 days of incubation, observations were recorded on the shoot and root length of seedlings in each replication.

3.10.3 Effect of culture filtrates of seed mycoflora on seed germination and shoot and root length of sesamum seedlings

In order to study the effect of culture filtrates of different fungal species on seed germination and seedling growth, cultural filtrates were prepared as follows.

A small disc of 5 mm diameter was cut from the margin of actively growing 3-day old fungal colony with the help of a sterile cork borer and the fungal disc was transferred aseptically into a 100ml Erlenmeyer flask containing 20ml of sterile Czapek-Dox liquid medium. The flasks were incubated at room temperature ($28^0 \pm 1^0$c) for 15 days (Plate 6&7) . Uninoculated sterile Czapek-Dox liquid medium served as a common control. At the end of incubation, when full growth of all isolates was seen, the fungal mats were filtered repeatedly through a double layer of what man's No. 41 filter papers until the filtrate was free of fungal spores.

Surface sterilized seeds were soaked in the culture filtrate of each fungal isolate separately and also in the control for 24 hr.

The treated seeds were placed on sterilized wet blotters in petri dishes and observations on seed germination, shoot and root length were recorded explained earlier.

3.11 EFFECT OF SEED MYCOFLORA ON PROTEIN AND OIL CONTENT OF SESAMUM SEEDS AND PHYSICO-CHEMICAL PROPERTIES OF OIL

The spores suspension of each fungal species was made from 10-day old culture on PDA as described earlier and spore concentration was adjusted to 10^4 spores in 1ml. About 100g of sesamum seeds (YLM-17) were autoclaved in an auto clave at 15 lb $inch^{-2}$ for 20 min, and were inoculated with spore suspension of fungal species separately following the methods of Gupta *et al* (1993). The seeds were arranged into a layer of one seed thickness. Spore suspension was sprayed evenly on the seeds at the rate of one ml suspension per 200 seeds (50 spores per seed). The inoculated seeds were incubated separately for 15 and 30 days in the laboratory at room temperature ($28^0 \pm 1^0$ c). At the end of incubation, protein and oil content of the seed and the physico-chemical properties of the oil extracted from the inoculated seed were studied. Four replications were maintained for each fungal species and for each incubation period. Seeds treated with sterile water and incubated for 15 days and 30 days separately constituted the control.

3.11.1 Estimation of proteins.

For estimation of protein content the method developed by Lowry *et al.* (1951) was followed.

3.11.1.1 Extraction of protein from sample.

Extraction was carried out by grinding 500 mg of the seed sample in five to ten ml of 0.1M acetate buffer of p^H 4.8. After centrifugation of the suspension, 0.1 and 0.2 ml of the supernatant was taken in separate test tubes and volume was made up to one ml. A test tube with one ml of distilled water served as the blank.

To these test tubes 5ml of alkaline copper solution prepared by adding one ml of 0.5% copper sulphate in 1.0% potassium sodium tartarate to 50 ml of 2% sodium carbonate in 0.1N sodium hydroxide, was added and allowed to stand for 10 minutes and then 0.5 ml of Folin-ciocalteau reagent was added. The contents were mixed well and incubated at room temperature in darkness for 30 minutes. After the development of blue colour, the light absorbance by the sample at 660nm was recorded with the help of Spectronic 20.

The same procedure was followed for obtaining a standard curve using serial dilutions of the working standard prepared by taking 10 mg of bovine serum albumin in 50 ml of distilled water.

The standard graph was obtained by plotting the protein concentration of the standard on the X- axis and the corresponding absorbance on the Y-axis. The protein content of the samples was read from the graph and expressed as percentage on dry weight basis.

3.11.2 Estimation of oil content

The oil content of the seeds was estimated by Nuclear Magnetic Resonance method (Kashalkar *et al.,* 1988) at National seed project, Hyderabad.

3.11.2.1 Extraction of oil

For estimation of free fatty acids, saponification, iodine and peroxide values, the oil was extracted from the inoculated sesamum seed. Extraction was carried out by grinding 100g of the dried seed sample in a micro-sample mill with minimum exposure to air. The seed meal was placed in a folded filter paper which was wrapped with a second filter paper in such a way that it was left open at the top like a thimble. A piece of cotton wool was placed at the top for even distribution of the solvent during extraction. The sample packet was placed in the butt tubes of the soxhlet extraction apparatus. Extraction was carried out with petroleum ether by gentle heating for 6 hours with out interruption. After complete extraction of the oil the extraction flask was allowed to cool and later it was dismantled. The ether present in the oil was evaporated on a steam or water bath until no odour of ether remained. The remaining oil after evaporation of ether was collected.

3.11.2.2 Determination of colour and odour

The colour of the oil extracted from the seed inoculated with each fungal species was recorded.

3.11.3 Estimation of free fatty acids.

The free fatty acid content of the oil was estimated by titrating it against potassium hydroxide (KOH) in the presence of phenolphthalein indicator (AOAC, 1960).

Five grams of oil was dissolved in 50ml of the neutral solvent in a 250ml conical flask to which a few drops of phenolphthalein indicator were added. The contents were titrated against 0.1N potassium hydroxide (KOH) and constantly shaken until pink colour was produced. Acid value was calculated using the following formula.

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre value} \times \text{Normality of KOH}}{\text{Weight of the sample(g)}} \times 56.1$$

Free fatty acid was calculated as % oleic acid using the equation 1 ml N/10KOH = 0.028g oleic acid.

3.11.4 Estimation of saponification value.

Saponification value of the oil was estimated by the method (AOAC, 1960). One gram of oil sample was taken into a clean conical flask to which 25ml of 0.5N Alcoholic KOH was added. A long condenser tube was fixed to the conical flask. The contents were reflexed by placing the flask in boiling water bath for 30 minutes after which the flask was removed and 2 drops of phenolphthalein indicator was added to the flask, when pink colour was observed. The contents were titrated against 0.5N Hydrochloric acid (HCl) till the pink colour just

disappeared. A blank experiment was done in a similar way but without oil sample. The difference between blank and experimental titration value being equivalent to the KOH was used for the saponification. Saponification value was calculated as follows and expressed as mg KOH per gram of oil.

$$\text{Saponification value} = \frac{(\text{Titre value of blank} - \text{Titre value of sample})}{\text{Weight of the sample (g)}} \times 28.05$$

3.11.5 Estimation of Iodine value

Iodine value of the oil was estimated by the method of (AOAC, 1960). An oil sample of 0.25g was transferred into a clean dry iodine flask and 10ml of chloroform was added to this with the help of a pipette. Twenty five ml of Hanus iodine solution was then added. The contents were mixed well and allowed to stand in darkness for 30 minutes with occasional shaking. After 30minutes, 10ml of 15 per cent potassium iodide solution was added to the flask and the contents were titrated against 0.1N sodium thio-sulphate until yellow solution turned colour less. A few drops of starch was added as indicator and titration was continued until the blue colour just disappeared. A blank experiment was done in a similar manner but without oil sample.

The difference between the blank and experimental titration value gave the exact volume of 0.1N Sodium thio-sulphate needed to react with equivalent volume of Iodine. Iodine value of oil was calculated as follows and expressed as mg Iodine per gram of oil.

$$\text{Iodine value} = \frac{(\text{Blank} - \text{Experimental value}) \times N \times 12.69}{\text{Weight of the sample (g)}}$$

3.11.6 Estimation of peroxide value

The peroxide value of the oil was estimated by the following method (AOAC, 1960).

One gram of the oil sample was taken into a clean dry boiling tube and 1g of powdered potassium iodide and 20ml of solvent mixture consisting glacial acetic acid and chloroform (2:1) was added to it. The tube was placed in boiling water and the contents were boiled for 60 seconds. The contents were transferred quickly to a conical flask containing 20ml of 5% potassium iodide solution. The tube was washed twice with 25 ml of water each time and the washings were collected into a conical flask. The content of the flask were titrated against 1/500 N sodium thio-sulphate solution until yellow colour disappeared. Then the starch solution (0.5ml) was added to the contents which were thoroughly shaken and titration was continued till the blue colour just disappeared. A black was set similarly without the oil sample. Peroxide value was calculated as follows and expressed as milliequivalents peroxide per g of oil sample.

$$\text{Peroxide value} = \frac{(\text{Test} - \text{Blank}) \times N}{\text{Weight of the sample}}$$

3.12 STATISTICAL ANALYSIS

In the present investigation the experiment on the effect of storage period on the incidence of seed mycoflora was conducted in complete randomized design. Factorial complete randomized design was adopted for the experiments conducted to study the effect of seed mycoflora on seed constituents and oil. The data obtained from all the experiments were statistically analysed following the standard methods (Gomez and Gomez, 1984). The data showing percentages were transformed into arc sine or square root transformation, as it is required, before statistical analysis.

Plate 1 : Plate showing the Standard blotter paper method adopted for the isolation of seed mycoflora of sesamum

Plate 2 : Plate showing the Agar plate method adopted for the isolation of seed mycoflora of sesamum

Plate 6 : Plate showing incubation of culture filtrates of seed mycoflora

1. *Aspergillus niger* 2. *A. terreus* 3. *A. flavus*
4. *Rhizopus sp.* 5. *Alternaria sp.*

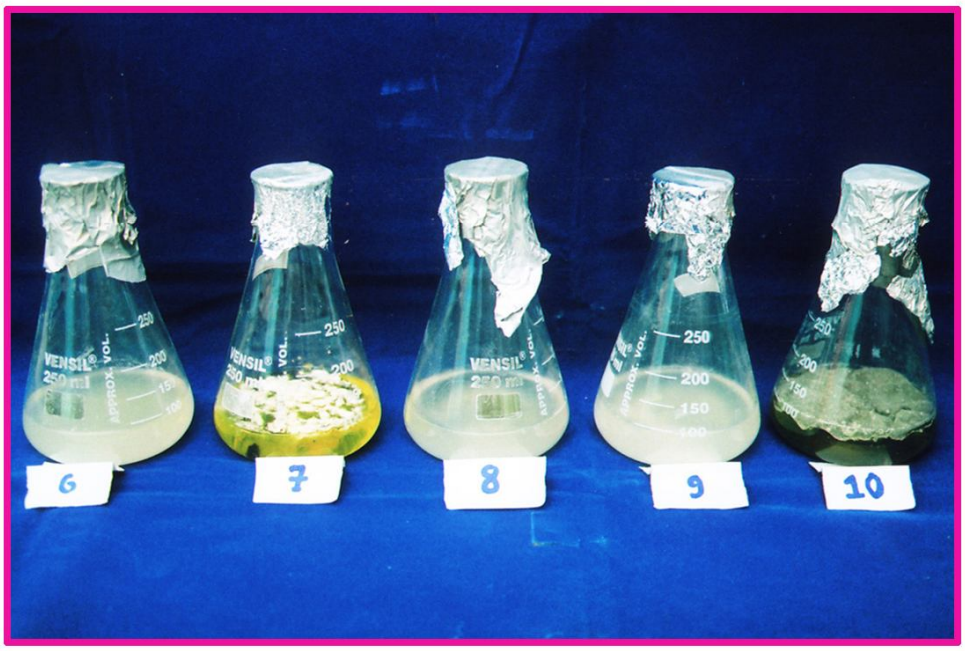

Plate 7 : Plate showing incubation of culture filtrates of seed mycoflora

6. *Fusarium* sp. 7. *Curvularia* sp. 8. *Sclerotium* sp.
9. *Rhizoctonia* sp. 10. *Penicillium* sp.

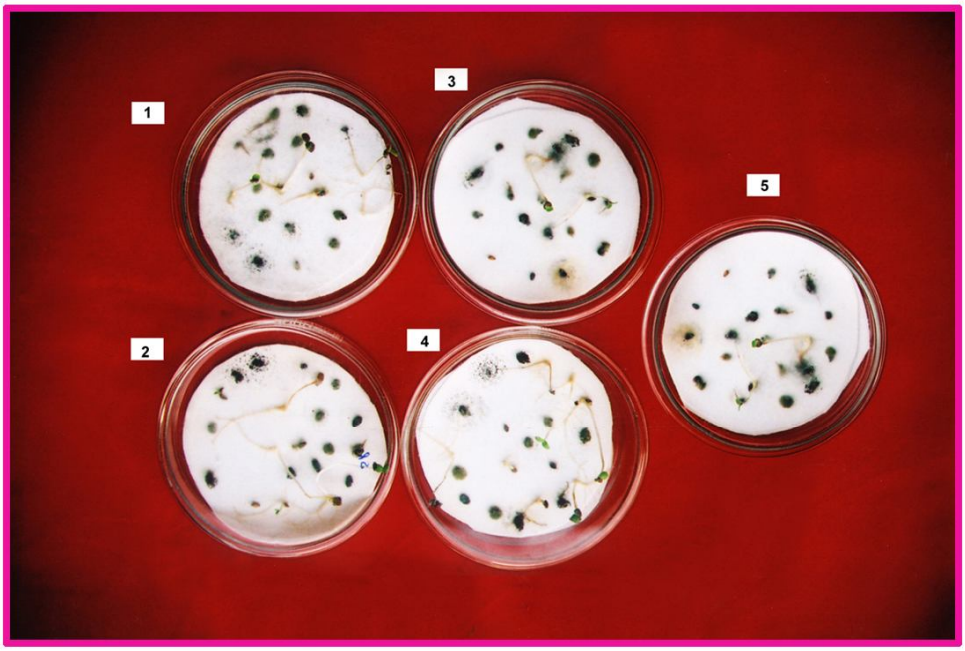

Plate 3 : Incidence of seed mycoflora in sesamum varieties (Standard blotter paper method)

1. Swetha til 2. YLM -17 3. Madhavi
4. Tanuku brown 5. Gowri

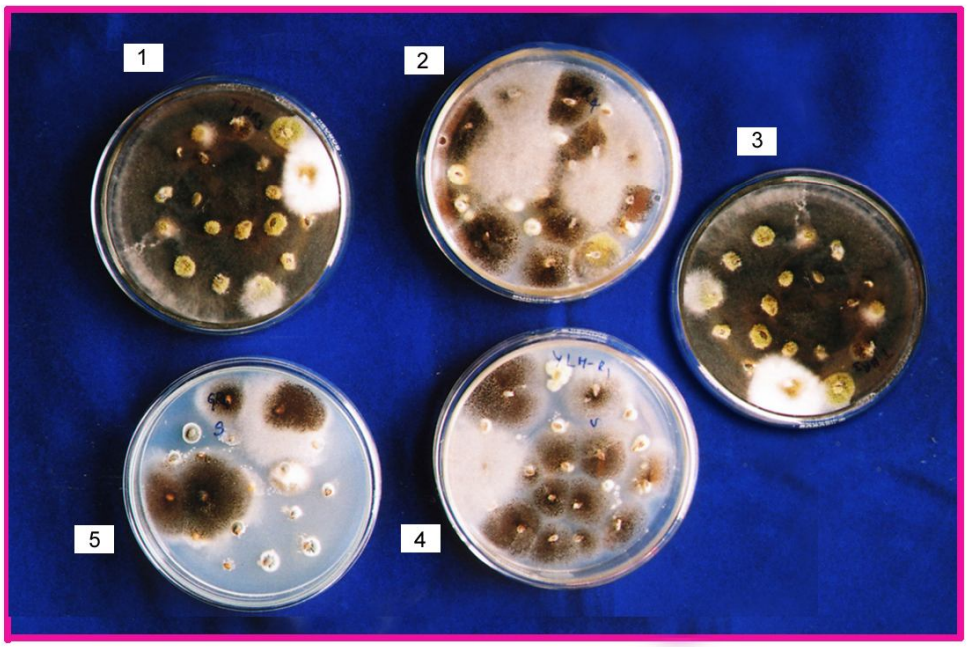

Plate 4 : Incidence of seed mycoflora in sesamum varieties (Agar plate method)

1. Swetha til 2. YLM -17 3. Madhavi
4. Tanuku brown 5. Gowri

CHAPTER - IV

RESULTS

4.1 ISOLATION OF SEED MYCOFLORA FROM DIFFERENT SESAMUM VARIETIES

4.1.1 Standard blotter paper method

The incidence of different fungal species in both surface sterilized and unsterilised seeds of five sesamum varieties *viz.,* Swetha til, YLM-17, Madhavi, Tanuku brown and Gowri collected from Agricultural Research Station-Yellamanchili, Andhra Pradesh, was studied. The results were presented in the Table 1

Three fungal species *viz., Aspergillus flavus, Alternaria* sp. and *Rhizopus* sp. were isolated from each of the 5 varieties. While *Aspergillus niger, Curvularia* sp. and *Penicillium* sp. were isolated from four varieties, *Aspergillus terreus* and *Sclerotium* sp. were isolated from three varieties. *Fusarium* sp. and *Rhizoctonia* sp. were isolated from two varieties only.

Fungal species showed significant differences in their mean per cent incidence. *Rhizopus* sp. was found to have the highest mean per cent incidence (28.00%) followed by *Aspergillus flavus* (22.25%), *Aspergillus niger* (11.00%), *Aspergillus terreus* (6.13%), *Penicillium* sp. (5.63%), *Curvularia* sp. (3.88%), *Rhizoctonia* sp. (3.64%), *Sclerotium* sp. (1.89%) and *Fusarium* sp. (1.51%).(Table 1.a)

The varieties showed significant differences in respect of incidence of mycoflora. The variety Swetha til showed the highest mean per cent incidence

(12.13%) of fungal species followed by YLM-17 (9.94%), Madhavi (8.69%) and Gowri (8.07%). The least mean per cent incidence was observed in case of Tanuku brown (7.44%).

Methods of sterilization showed significant differences in respect of incidence of fungal species. The mean per cent incidence of fungal species in respect of unsterilised seeds was 11.70 per cent where as with sterilized seeds it was 6.80 per cent.

The interaction between fungal species and varieties was found to be significant. The individual fungal species differed from one another in respect of colonization of varieties. *Rhizopus* sp. was observed to be the most frequent in Madhavi (52.50%) and Swetha til (40.00%). *Aspergillus flavus* was found to be of the highest incidence (33.75%) followed by *Aspergillus niger* (15.62%) in Swetha til compared to other varieties. *Curvularia* sp. was found to of be the highest incidence (14.37%) in YLM-17. *Alternaria* sp. (13.75%) in Tanuku brown and *Penicillium* sp. (19.37%) in Gowri were dominant.

The interaction between fungal species and methods of sterilization was found to be significant. *Rhizopus* sp. was found to be of the highest incidence in both unsterilised (36.00%)and sterilized seed (20.00%) irrespective of the variety. followed by *Aspergillus flavus* (27.50% and 17.00%), *Aspergillus niger* (13.75% and 8.26%), *Alternaria* sp. (10.50% and 6.75%), *Aspergillus terreus* (8.01% and 4.26%), *Penicillium* sp. (7.25% and 4.01%) *Rhizoctonia* sp. (5.01% and 2.26%) *Curvularia* sp. (4.50% and 3.25%), *Sclerotium* sp. (2.51% and 1.27%) and *Fusarium* sp. (2.01% and 1.02%). The lowest mean per cent incidence was recorded by *Fusarium* sp. in both the methods of sterilization.

The interaction between varieties and methods of sterilization found to be non-significant. However, among the varieties Swetha til harboured the greatest mean per cent incidence of fungal species in both unsterilised (14.63%) and sterilized seed (9.63%) followed by YLM-17 (12.37% and 7.50%), Madhavi (11.88% and 5.51%) Tanuku brown (10.13% and 4.75%) and Gowri (9.31% and 6.63%) .

The interaction between fungal species, varieties and methods of sterilization was found to be non-significant.

4.1.2 Agar plate method

The incidence of different fungal species in both surface sterilized and unsterilised seeds of five varieties *viz.,* Swetha til, YLM-17, Madhavi, Tanuku brown and Gowri collected from Agricultural Research Station-Yellamanchili, Andhra Pradesh were studied. The results were presented in the Table 2

Four fungal species viz., *Alternaria* sp., *Aspergillus flavus, Aspergillus terreus* and *Rhizopus* sp. were isolated from each of the five varieties. While *Aspergillus niger, Curvularia* sp., *Fusarium* sp. and *Penicillium* sp. were isolated from four varieties, *Rhizoctonia* sp. *Sclerotium* sp.were isolated from two varieties only.

Fungal species showed significant differences in mean per cent incidence. *Rhizopus* sp. was found to have the highest mean per cent incidence (31.37%) followed by *Aspergillus flavus* (27.73%), *Aspergillus niger* (18.88%), *Alternaria* sp. (13.62%) *Aspergillus terreus* (10.62%), *Penicillium* sp. (8.38%), *Rhizoctonia* sp. (5.64%) and *Sclerotium* sp. (3.52%).

Varieties showed significant differences in respect of incidence of mycoflora. YLM-17 (17.81%) showed the highest mean per cent incidence followed by Tanuku brown (15.75%), Swetha til (15.08%), Madhavi (9.19%) and Gowri (5.02%). The variety, Gowri had shown the lowest mean per cent incidence.

Methods of sterilization showed significant differences in the mean per cent incidence of fungal species. The total mean per cent incidence of fungal species with unsterilised seeds was 14.78% where as with sterilized seeds it was 10.40%.

The interaction between fungal species and varieties was found to be significant. Individual fungal species differed from one another in respect of their preference of the varieties. *Rhizopus* sp. was observed most frequently (57.50% and 50.00%) in the varieties Madhavi and Swetha til respectively. *Aspergillus flavus* was found to be of the highest incidence (46.87%) in Swetha til. *Aspergillus niger* (37.50%), *Alternaria* sp. (23.12%) and *Curvularia* sp. (21.25%) were found to be of the highest incidence in YLM-17. *Penicillium* sp. was found to be the highest in the variety Gowri and *Rhizoctonia* sp. (13.75%) and *Sclerotium* sp. (15.00%) in Tanuku brown which were on par with each other.

The interaction between fungal isolates and methods of sterilization was found to be significant. *Rhizopus* sp. was found to be of the highest mean per cent incidence in unsterilised (35.25%) and sterilized seed (27.50%) followed by *Aspergillus flavus* (29.25% and 21.00%), *Aspergillus niger* (20.26% and 17.51%), *Alternaria* sp. (16.75% and 9.50%), *Aspergillus terreus* (14.25% and

7.00%), *Penicillium* sp. (9.01% and 7.76%), *Fusarium* sp. (6.67% and 1.02%), *Curvularia* sp. (6.51% and 4.52%), *Rhizoctonia* sp. (6.26% and 5.02%) and *Sclerotium* sp. (3.52% and 3.53%).

The interaction between varieties and methods of sterilization was found to be non-significant. Among the varieties YLM-17 (22.62% and 13.00%) harboured greatest per cent of fungal species in both the methods of sterilization, followed by Tanuku brown (18.50% and 13.00%), Swetha til (16.41% and 14.02%), Madhavi (10.26% and 8.13 per cent) and Gowri (6.39% and 4.01%).

The interaction between fungal isolates, varieties and methods of sterilization was found to be no- significant.

4.2 EFFECT OF STORAGE PERIOD ON THE INCIDENCE OF SEED MYCOFLORA IN SESAMUM

In order to find out the effect of storage period on sesamum seed mycoflora, sesamum seeds of the variety YLM-17 (Fig.1) were stored in a small cloth bags for 15, 30, 45, 60 and 75 days separately and seed mycoflora was isolated (Plate 5) after each storage period. The data were presented in Table 3.

Significant differences were observed in the mean per cent incidence of fungal species irrespective of storage period. Among the different fungal species, *Aspergillus flavus* was of the highest incidence (22.25%) followed by *Rhizopus* sp. (16.00%), *Penicillium* sp. (15.25%), *Curvularia* sp.(14.00%), *Aspergillus niger* (12.76%), *Rhizoctonia* sp. (10.00%), *Alternaria* sp. (9.25%),

Fusarium sp. (9.25%), *Aspergillus terreus* (8.00%) was found to be of low incidence. The lowest incidence was recorded in the case of the *Sclerotium* sp. (7.25%).

Among the storage periods significant differences were found. There was increase in the population of fungal species with increase in the storage period. After 15 days of storage, irrespective of fungal species, the incidence of mycoflora was 5.87% which increased to 10.25% after 30 days of storage and to 13.37, 16.37 and 16.12 per cent after 45, 60 and 75 days of storage respectively.

The interaction between the fungal species and storage period was found to be significant. The fungal species *Aspergillus flavus, Rhizopus* sp., *Penicillium* sp., *Aspergillus niger* and *Aspergillus terreus* showed increase with increase in storage period from 15 to 75 days. In contrast, *Fusarium* sp. increased initially upto 30 days, and later it decreased when storage period was extended to 75 days. *Curvularia* sp. and *Alternaria* sp. increased initially up to 45 days and later decreased when the storage period was extended to 75 days. *Sclerotium* sp., and *Rhizoctonia* sp. increased initially upto 60 days of storage,but later decreased when the storage period was extended to 75 days. *Rhizopus* sp. recorded the highest incidence of 31.45% after 75 days of storage, followed by *Aspergillus flavus* (30.00%).

4.3.1 Effect of seed mycoflora on sesamum seed germination under laboratory conditions.

In order to study the effect of seed mycoflora on seed germination, sesamum seeds were inoculated with spore suspensions of different fungal

species separately and placed on wet blotters contained in Petri dishes for germination as explained under Materials and Methods. The data on seed germination were recorded and presented in the Table 5.

The data indicate that there was significant reduction in the seed germination in respect of all the fungal species, compared to control (78.57%). However, the fungal species *Aspergillus niger, Rhizoctonia* sp. *Aspergillus flavus, Sclerotium* sp. and *Fusarium* sp. brought about significant reduction in seed germination. Among the species *Aspergillus niger* resulted in 42.50 per cent seed germination with the maximum reduction of 45.90% over control followed by *Rhizoctonia* sp., *Sclerotium* sp., *Aspergillus flavus* and *Fusarium* sp. reduction over control. *Penicillium* sp., *Curvularia* sp., *Alternaria* sp. and *Aspergillus terreus* reduced the germination to 62.50, 66.25, 67.50 and 72.50 from 78.57 per cent in control respectively. *Rhizopus* sp. was found to be the least effective, reducing the seed germination to 76.25 per cent.

4.3.2 Effect of seed mycoflora on shoot length of sesamum seedlings under laboratory conditions

In the above experiment observations were recorded on shoot length of sesamum seedlings seven days after seed germination(Plate 16) and the data were presented in the Table 6.

It is clear that shoot length was significantly reduced by all the fungal species. *Curvularia* sp. showed the maximum adverse effect on shoot length, reducing it to 3.10 cm from 5.30 cm in control followed by *Aspergillus niger, Aspergillus flavus* and *Fusarium* sp. which reduced the shoot length to 3.37 cm, 3.65 cm and 3.82 cm from 5.30 cm in control were found to be on par with

each other. Among the other fungal species *Alternaria* sp., *Sclerotium* sp., *Aspergillus terreus* , *Rhizoctonia* sp. *Penicillium* sp. and *Rhizopus* sp. reduced shoot length to 4.15, 4.25, 4.40, 4.42, 4.60 and 4.75 cm respectively from 5.30 cm which were on par with each other.

4.3.3 Effect of seed mycoflora on root length of sesamum seedlings under laboratory conditions

The data on root length of sesamum seedlings recorded at seven days after seed germination (Plate 16) reveal that there was significant reduction in the root lengths due to seed mycoflora compared with control. The maximum (3.70 cm from 6.60 cm in control) reduction in root length was observed in respect of *Aspergillus niger*. *Penicillium* sp., *Curvularia* sp., *Sclerotium* sp. *Rhizoctonia* sp., *Aspergillus terreus*, *Aspergillus flavus*, and *Fusarium* sp. reduced root length to 3.87 cm, 4.72 cm, 5.07 cm, 5.10 cm, 5.10 cm , 5.15 cm, and 5.22 cm respectively and were found to be on par with each other in reducing root length. *Alternaria* sp. (5.80 cm) and *Rhizopus* sp. (6.17 cm) were found to have least effect on root length.

4.4.1 Effect of culture filtrates of seed mycoflora on sesamum seed germination under laboratory conditions.

With a view to finding out the effect of culture filtrates of the fungal species on seed germination under laboratory conditions, the seed were treated with culture filtrates of different fungal species separately and placed on wet blotters. The percentage of seed germination was recorded and the data were presented in Table 8.

The culture filtrates of all fungal species significantly reduced the seed germination over the control. The culture filtrates of *Aspergillus terreus* significantly reduced the seed germination from 83.75% in control to 42.50% with the maximum per cent reduction of 49.25 over control followed by culture filtrates of *Fusarium* sp. (35.82%) *Aspergillus flavus* (34.32%), *Rhizoctonia* sp. (26.86%) and *Curvularia* sp. (20.89%) reduction over control. Culture filtrates of Alternaria sp. and Rhizopus sp. each reduced the germination to 68.75% and 67.50% respectively. Culture filtrates of *Aspergillus niger*, *Penicillium* sp. and *Sclerotium* sp. were found to be the least effective, reducing the germination.

4.4.2 Effect of culture filtrates of seed mycoflora on shoot length of sesamum seedlings under laboratory conditions

The results presented in the (Table 9) reveal that culture filtrate *Aspergillus terreus* was highly effective in reducing the shoot length to 2.90 cm from 5.22 cm in control, the reduction being 44.44%. This was followed by *Aspergillus niger* (2.97 cm), *Aspergillus flavus* (3.05 cm), *Sclerotium* sp. (3.72 cm) and *Alternaria* sp. (3.87 cm). The reduction in shoot length in respect of the above species was significant(Plate 17) and they were on par with each other. The culture filtrates of *Penicillium* sp., *Rhizoctonia* sp. and *Rhizopus* sp. were also found effective in reducing the shoot length to 4.35, 4.50 and 4.85 cm respectively, and they were on par with each other. *Fusarium* sp. and *Curvularia* sp. were found to be the less effective in reducing the shoot length.

4.4.3 Effect of culture filtrates of seed mycoflora on root length on sesamum seedlings under laboratory conditions.

The effect of culture filtrates on root length of sesamum seedlings was also studied in the above experiment. The data indicated that culture filtrates of *Sclerotium* sp., *Rhizoctonia* sp. *Aspergillus flavus and Curvularia* sp. were significantly effective in reducing the root length to 3.92, 4.05, 4.22 and 4.37 cm respectively over control (Plate 17). Culture filtrates of *Penicillium* sp. (4.45 cm), *Fusarium* sp. (5.20 cm), *Aspergillus terreus* (4.87 cm), *Aspergillus niger* (5.30 cm), *Rhizopus* sp. (5.47 cm) and *Alternaria* sp. (6.17 cm) showed considerable reduction in root length over the control and were on par with each other. Of all the fungal species, culture filtrates of *Alternaria* sp. recorded the minimum reduction in root length reducing it to 6.17 cm from 7.25 cm in control.

4.5. EFFECT OF SEED MYCOFLORA ON PROTEIN CONTENT OF SESAMUM SEED

With a view to finding out the effect of seed mycoflora on protein content of sesamum seed, the seed was inoculated with the spore suspension of different fungal species individually and incubated for 15 and 30 days separately and protein content at the end of each incubation period was estimated. The data were presented in the Table 11.

Significant differences in the protein content of sesamum seeds inoculated with different fungal species were recorded irrespective of incubation period. All the fungal species significantly reduced protein content. *Rhizoctonia* sp. caused the highest reduction of protein content from 12.60 in

control to 8.87 per cent followed by *Sclerotium* sp. (9.13), *Curvularia* sp. (9.17%) and *Alternaria* sp. (9.40%) which were superior to the rest. Among the other fungi *Fusarium* sp., *Penicillium* sp., *Aspergillus terreus, Rhizopus* sp., *Aspergillus flavus* and *Aspergillus niger* reduced the protein content to 9.77, 10.11, 10.50, 10.92, 11.12 and 11.27 per cent respectively.

In general the protein content of the seed inoculated with fungal species significantly declined with increase in the incubation period irrespective of fungal species (Fig.5). While the protein content of the inoculated seed was 10.65% after 15 days of incubation, it was 9.87% after 30 days of incubation.

The interaction between fungal species and incubation period was found to be significant. After 15 days of incubation protein content of the seed was significantly reduced to the extent of 9.42% in respect of *Rhizoctonia* sp. which was significantly superior to the rest of the fungal species. This was followed by *Curvularia* sp. (9.72%), *Alternaria* sp. (9.75%) and *Fusarium* sp. (10.07%) which were on par with each other and significantly superior to the control.

After 30 days of incubation, reduction in the protein content of seed was observed to be the highest with *Rhizoctonia* sp. and *Curvularia* sp. followed by *Sclerotium* sp., *Alternaria* sp., *Fusarium* sp. and *Penicillium* sp. However, the reduction in the protein content by *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus* over control was not significant even after 30 days of incubation. *Aspergillus niger* and *Aspergillus flavus* brought about the less reduction in the protein content of the seed after 15 and 30 days of incubation of inoculated seed.

The isolates *Rhizopus sp., Aspergillus niger*, *Curvularia* sp. and *Rhizoctonia* sp. in the order mentioned caused a significant reduction in protein content of the seed with increase in the incubation period of inoculated seed from 15 days to 30 days. Though there was a perceptible reduction in the protein content with increase in the incubation period, in respect of other fungal isolates, the reduction was non-significant

4.6 EFFECT OF SEED MYCOFLORA ON OIL CONTENT OF SESAMUM SEED

In order to find out the effect of seed mycoflora on oil content of sesamum, the seed was inoculated with the spore suspensions of different fungal species individually and incubated for 15 days and 30 days separately and after each incubation period oil content of estimated and expressed as per cent oil.

From the results it is evident that all the fungal species irrespective of incubation period reduced the oil content compared to control (Fig. 6). Among the fungal species *Aspergillus niger* caused maximum reduction in the oil content of the inoculated seed from 49.78% to 43.98 per cent followed by *Penicillium* sp. (47.32 per cent) *Fusarium* sp. (47.55 per cent), *Rhizoctonia* sp. (47.70%) and *Rhizopus* sp. (47.96%) which was found to be significantly superior to the rest in reducing the oil content. These were followed by *Sclerotium* sp., *Alternaria* sp., *Aspergillus terreus, Aspergillus flavus* and *Curvularia* sp. which were effective in reducing the oil content to 48.01, 48.18, 48.22, 48.46 and 48.76 per cent respectively and on par with each other.

There was a slight but significant decrease in the oil content with increase in the incubation period irrespective of fungal species with 48.05 per cent oil content recorded after 15 days of incubation and 47.55% oil content after 30 days of incubation.

The interaction between fungal species and incubation period was found significant. After 15 days of incubation period the oil content was found to be reduced to the maximum of 47. 37 per cent in respect of seed inoculated with *Fusarium* sp. followed by *Penicillium* sp. (47.55%) and *Rhizopus* sp. (47.55%) and these were superior to the rest of the fungi. *Rhizoctonia* sp., *Rhizopus sp*., *Penicillium* sp. and *Alternaria* sp. were effective in reducing the oil content to 47.52, 47.77, 47.82, 47.85 and 47.87 per cent from 50.55 per cent in control. *Fusarium* sp. reduced oil content from 49.02 in control to 47.37% both after 15 days and 30 days incubation period. The reduction of oil control in respect of *Alternaria* sp., *Aspergillus flavus, Aspergillus terreus* and *Curvularia* sp. was not superior to the control after both 15 days and 30 days incubation period. Oil content showed the least decline as compared to control in seed treated with *Curvularia* sp. (48.69 and 48.85%) after both 15 and 30 days of incubation.

Notwithstanding the general decline in the oil content of the inoculated seed with increase in incubation period. The change in the oil content in respect of the individual fungal species with increase in incubation period was variable. While the oil content of the seed inoculated with *Aspergillus flavus, Aspergillus terreus, Curvularia* sp., *Sclerotium* sp., *Penicillium* sp. and *Rhizopus* sp. showed increase when incubation of the seed was continued to 30 days. *Aspergillus niger, Alternaria* sp. and *Rhizoctonia* sp. brought about a decrease in the oil content. However, the change was found non significant except in case of *Aspergillus niger*.

4.7 EFFECT OF SEED MYCOFLORA ON PHYSICO-CHEMICAL PROPERTIES OF SESAMUM OIL

4.7.1 Physical properties

4.7.1.1 Colour

Visual examination revealed colour variation in oil extracted from the seed inoculated with *Aspergillus niger, Aspergillus flavus, Alternaria* sp. and *Fusarium* sp. in both 15 and 30 days of incubation.

The colour of the oil extracted from uninoculated seeds was golden yellow. *Aspergillus niger* produced red coloured oil, *Aspergillus flavus* produced light red coloured oil, *Alternaria* sp. and *Fusarium* sp. produced yellow coloured oil with slight colour difference that of the oil produced from the uninoculated seeds after 15 days of incubation period.

After 30 days of incubation the colour of the oil changed to blue in the case of the seeds which were inoculated with *Aspergillus niger.* Deep red coloured oil was produced by *Aspergillus flavus*. In the case of *Alternaria* sp. and *Fusarium* sp. the colour changed from yellow to reddish yellow. In the case of other fungi, *Aspergillus terreus, Curvularia* sp., *Penicillium* sp., *Rhizopus sp., Rhizoctonia* sp. and *Sclerotium* sp. the colour variation was not noticed after both the incubation periods.

4.7.1.2 Odour

The odour of the oil extracted from the seed inoculated with different fungal species was assessed by smelling.

In respect of 15 days of incubation the rancid order was observed in the case of *Aspergillus niger, Fusarium* sp., *Alternaria* sp. and *Rhizoctonia* sp. But in the case of the other fungi no rancid order was produced..

In respect of 30 days of incubation the rancid order was observed in the case of *Aspergillus niger, A. terreus, Fusarium* sp. *Rhizoctonia* sp. *Alternaria* sp. and *Sclerotium* sp. Rancid odour was not observed in the case of *A.flavus, Curvularia* sp., *Rhizopus* sp. and *Penicillum* sp.

4.7.2 Chemical properties

4.7.2.1 Effect of seed mycoflora on free fatty acid content of sesamum oil

In order to study the effect of seed mycoflora on free fatty acid content of sesamum oil, the seeds was inoculated with spore suspensions of different fungal species and incubated separately for 15 and 30 days separately and free fatty acid content after each incubation period was estimated. The results were presented in the Table 12. From the results it is discernible there was a significant increase in free fatty acid per cent of the oil extracted from the seed treated with fungal species irrespective of incubation period, when the incubation period was increased from 15 to 30 days there was a marked increase in the free fatty acid content overall of the fungal species(Fig. 7).

Among the fungal species, maximum increase was observed in the seed treated with *Fusarium* sp., (39.25%) followed by *Aspergillus flavus* (38.70%) and *Curvularia* sp. (36.37%). These were followed by *Rhizopus* sp. (30.62%), *Aspergillus terreus* (30.07%) and *Sclerotium* sp. (25.01%), *Aspergillus niger* (12.50%), and *Alternaria* sp. (9.00 %) and *Rhizoctonia* sp. (8.72%).

The interaction between fungal species and incubation period was found to be significant. After 15 days of incubation, highly increased free fatty acid content was observed in seed treated with *Fusarium* sp. (37.62%) followed by *Curvularia* sp.(34.12%), *Rhizopus* sp. (28.62%), *Aspergillus terreus* (27.87%), *Aspergillus flavus* (25.52%), *Sclerotium* sp. (22.10%) and *Aspergillus niger* (9.35). However, free fatty acid content recorded a less pronounced increase in seed treated with *Alternaria* sp. (6.67%), *Rhizoctonia* sp. (6.37%) and *Penicillium* sp. (4.55%) but the increase was significant compared to control.

After 30 days of incubation free fatty acid content showed a maximum of 51.87% in the case of *Aspergillus flavus*, 40.87% with *Fusarium* sp., 38.62% with *Curvularia* sp., 32.62% with *Rhizopus* sp., 32.27% with *Aspergillus terreus* and 27.92% with *Sclerotium* sp. But in respect of *Aspergillus niger, Penicillium* sp., *Alternaria* sp. and *Rhizoctonia* sp. the increase was markedly small. A slight increase in the free fatty acid content was observed with increase in incubation period from 15 to 30 days in respect of control.

4.7.2.2 Effect of seed mycoflora on saponification values of sesamum oil

In order to study the changes that may occur in the chemical properties of sesamum oil due to seed mycoflora, saponification, iodine and peroxide values of the oil extracted from sesamum seed inoculated with spore suspensions of different fungal species and incubated for 15 and 30 days separately were studied as explained under Materials and Methods. The results were presented in the Table 14.

The results presented in the Table 14 reveal that there was a significant increase in saponification values of the oil extracted from the seed treated with the fungal species as compared to control, irrespective of the incubation period. It is also evident that the saponification of oil showed a significant increase with increase in incubation period in the case of fungal species (Fig. 8). The saponification value of the oil after 15 days of incubation was 220.50 where as it was 236.09 after 30 days of incubation. Among the species *Rhizoctonia* sp. caused the highest increase in the saponification value from 187.87 to 308.50 followed by *Penicillium* sp. which were significantly superior to the rest. Of the rest *Alternaria* sp., *Sclerotium* sp., *Curvularia* sp., *Aspergillus niger* and *Fusarium* sp. resulted in a significant increase in saponification values to 232.37, 218.25, 213.62, 204.62 and 204.62 .But only a slight increase in the saponification values was observed due to *Aspergillus terreus, Aspergillus flavus* and *Rhizopus* sp.

The interaction between fungal species and incubation period was significant. The increase of saponification values due to *Rhizoctonia* sp. *and Penicillium* sp. was found to be significantly higher after 15 days of incubation while after 30 days of incubation, *Penicillium* sp. followed by *Rhizoctonia* sp. *and Alternaria* sp. resulted in increased saponification values.

Saponification values showed least increase in respect of *Aspergillus flavus* (187.00 and 209.25) followed by *Aspergillus terreus* (178.25 and 220.75) after 15 and 30 days of incubation period. When the incubation period of inoculated seed increased from 15 to 30 days the increase in saponification value in respect of different fungal species showed significant differences. The increase was maximum in case of *Penicillium* sp. followed by *Rhizoctonia*

sp., *Alternaria* sp. *Sclerotium* sp. *Aspergillus terreus, Curvularia* sp. and *Aspergillus niger.* Compared to above fungal isolates *Fusarium* sp., *Rhizopus flavus* resulted in small increase of saponification values. Even in the control the increase of saponification values was detected.

4.7.2.3 Effect of seed mycoflora on iodine values of sesamum oil

All the fungal species reduced iodine value irrespective of incubation period. The iodine values were found to have decreased with increase in incubation period (Fig. 9) in general over all the fungal species but the decrease was not statistically significant.

The highest reduction in iodine value to 65.87 from 117.50 in control was recorded in the seeds treated with *Aspergillus flavus* (65.87) followed by *Aspergillus terreus* (72.62) which were significantly superior to the other species. Ranked next to them were *Fusarium* sp. (92.00), *Aspergillus niger* (91.75), *Rhizopus* sp. (102.12) *Sclerotium* sp. (104.62), *Curvularia* sp. (108.12), *Alternaria* sp. (111.87) and *Penicillium* sp. (113.00) effectively reducing the iodine value over control. *Rhizoctonia* sp. was found to be the least effective and also on par with control.

The interaction between fungal species and incubation period was found to be significant. The maximum reduction in the iodine value was observed in seed treated with *Aspergillus flavus* and *Aspergillus terreus* after 15 and 30 days of incubation. Maximum reduction was observed in the case of seed treated with *Rhizoctonia* sp. and *Curvularia* sp. after 15 days of incubation. *Rhizopus* sp. and *Penicillium* sp. were observed to be the least effective in reducing the iodine values after 30 days of incubation. *Aspergillus niger*,

Rhizopus sp. and *Penicillium* sp. which decreased the iodine value after 15 days of incubation resulted in an increase in the iodine value after 30 days of incubation.

When the incubation period was increased from 15 to 30 days the change observed in iodine values of inoculated seed was found to be variable. Iodine values showed increase with increase in incubation period in respect of *Aspergillus niger*, *Penicillium* sp., *Rhizopus* sp., and *Rhizoctonia* sp.. Other fungal species caused a decrease in iodine values. In control also a decrease in iodine values was found when the incubation period of the seed was extended.

4.7.2.4 Effect of seed mycoflora on peroxide values of sesamum oil

The data presented in the Table 16, reveal significant increase in peroxide values of the oil extracted from the seed treated with different fungal species irrespective of incubation period. When the incubation period was prolonged to 30 days significant increase in peroxide values was recorded over control (Fig.10).

Seeds inoculated with *Sclerotium* sp. and *Rhizoctonia* sp. showed increased peroxide values to 5.66 and 5.33 respectively from 4.13 in control. *Curvularia* sp. (4.05), *Rhizopus* sp. (4.18), *Alternaria* sp. (4.25), *Aspergillus flavus*, (4.34), *Penicillium* sp. (4.36), *Aspergillus niger*, (4.42), *Aspergillus terreus* (4.55) and *Fusarium* sp. (4.83) showed a small increase over control but on par with each other. *Curvularia* sp. was found to have reduced the peroxide value from 4.13 in control to 4.05.

The interaction between fungal species and incubation period was found to be significant. Among the species maximum increase in peroxide value was observed in the seed treated with *Sclerotium* sp., after both 15 and 30 days incubation. Next to it *Rhizoctonia* sp., *Fusarium* sp. and *Aspergillus terreus* were effective in increasing the peroxide values after 15 and 30 days incubation. Minimum reduction in peroxide value was observed in the seed treated with *Curvularia* sp. (2.61) after 15 days of incubation.

Maximum increase in peroxide value was recorded in respect of *Sclerotium* sp. followed by *Rhizoctonia* sp., *Aspergillus terreus* and *Aspergillus niger* when incubation period was increased from 15 to 30 days.

Table 1 : Per cent incidence of fungi isolated with unsterilised and sterilised seeds of five sesamum varieties by standard blotter paper method

Fungal species	Varieties											
	Sweta til		YLM-17		Madhavi		Tanuku brown		Gowri		Mean	
	US	S	US	S	US	S	US	S	US	S	US	S
Alternaria sp.	12.50 (18.03)	6.25 (10.30)	13.75 (21.69)	6.25 (10.30)	8.75 (12.33)	8.75 (14.91)	15.00 (19.83)	12.50 (20.61)	2.50 (6.46)	0.00 (0.45)	10.50 (15.67)	6.75 (11.31)
Aspergillus flavus	40.00 (39.21)	27.50 (35.51)	16.25 (20.69)	13.75 (18.97)	37.50 (37.71)	16.25 (20.69)	25.00 (29.94)	13.75 (21.25)	18.75 (25.62)	13.75 (21.69)	27.50 (30.63)	17.00 (22.88)
Aspergillus niger	22.50 (24.87)	8.75 (14.91)	16.25 (20.69)	8.75 (14.91)	7.05 (13.10)	6.27 (10.32)	0.00 (0.45)	0.00 (0.45)	22.50 (28.14)	17.50 (24.67)	13.75 (17.44)	8.26 (13.05)
Aspergillus terreus	11.25 (14.14)	6.25 (10.30)	15.00 (22.64)	11.25 (19.52)	0.00 (0.45)	0.00 (0.45)	13.75 (18.97)	3.75 (7.83)	0.00 (0.45)	0.00 (0.45)	8.01 (11.33)	4.26 (7.71)
Curvularia sp.	0.00 (0.45)	0.00 (0.45)	17.50 (24.40)	11.25 (19.52)	1.25 (3.23)	1.25 (3.23)	2.50 (6.46)	2.50 (6.46)	1.25 (3.23)	1.25 (3.25)	4.50 (6.49)	3.25 (6.57)
Fusarium sp.	0.00 (0.45)	0.00 (0.45)	7.50 (11.39)	5.00 (11.06)	0.00 (0.45)	0.00 (0.45)	2.50 (6.46)	0.00 (0.45)	0.00 (0.45)	0.00 (0.45)	2.00 (3.84)	1.25 (2.57)
Penicillium sp.	1.25 (3.33)	0.00 (0.45)	8.75 (14.91)	2.50 (4.60)	3.25 (7.83)	1.25 (3.23)	0.00 (0.45)	0.00 (0.45)	22.50 (28.04)	16.25 (23.73)	7.25 (10.89)	4.10 (6.49)
Rhizoctonia sp.	0.00 (0.45)	0.00 (0.45)	13.75 (18.75)	6.25 (10.30)	0.00 (0.45)	0.00 (0.45)	11.25 (6.94)	5.00 (11.06)	0.00 (0.45)	0.00 (0.45)	2.42 (5..01)	2.26 (4.54)
Rhizopus sp.	57.50 (49.34)	47.50 (43.56)	15.00 (22.64)	10.00 (18.14)	58.75 (50.10)	21.25 (27.28)	21.25 (24.01)	3.75 (5.69)	27.50 (23.93)	17.50 (24.67)	36.00 (34.00)	20.00 (23.87)
Sclerolium sp.	1.25 (3.23)	0.00 (0.45)	0.00 (0.45)	0.00 (0.45)	1.25 (3.23)	0.00 (0.45)	10.00 (17.71)	6.25 (10.30)	0.00 (0.45)	0.00 (0.45)	2.51 (5.01)	1.27 (2.42)
Mean	14.63 (15.34)	9.63 (11.28)	12.37 (17.82)	7.50 (12.78)	11.88 (12.89)	5.51 (8.14)	10.13 (14.12)	4.75 (8.48)	9.51 (11.72)	6.63 (10.02)	11.70 (14.38)	6.80 (10.14)

	SE	CD (0.05)
Fungal species	1.76	3.45
Varieties	3.45	2.44
Methods	0.78	1.54
Fungal species × varieties	3.94	7.72
Fungal species × methods	2.49	4.88
Varieties × method	1.76	3.45
Fungal species × varieties × method	5.57	10.92

* Mean of 4 replications

Values in the parenthesis are arc sine transformed values

US – Un sterilised

S- sterilised

Table 2 : Per cent incidence of fungi isolated with unsterilised and sterilised seeds of five sesamum varieties by agar plate method

Fungal species	Varieties											
	Sweta til		YLM-17		Madhavi		Tanuku brown		Gowri		Mean	
	US	S	US	S	US	S	US	S	US	S	US	S
Alternaria sp.	18.75 (25.62)	13.75 (21.62)	27.50 (31.60)	18.75 (10.30)	6.25 (10.30)	1.25 (3.23)	27.50 (31.51)	12.50 (20.61)	3.75 (9.69)	1.25 (3.23)	16.25 (21.72)	9.50 (4.86)
Aspergillus flavus	51.25 (45.71)	42.50 (40.68)	31.25 (33.97)	16.25 (20.78)	8.75 (17.05)	3.75 (9.69)	45.00 (42.11)	36.25 (37.01)	10.00 (16.00)	6.25 (14.29)	29.25 (30.97)	21.00 (24.49)
Aspergillus niger	0.00 (0.64)	0.00 (0.64)	41.25 (39.95)	33.75 (35.50)	22.50 (28.80)	20.00 (26.56)	35.00 (36.27)	31.25 (33.97)	2.50 (6.46)	2.50 (6.46)	20.26 (22.32)	17.51 (20.62)
Aspergillus terreus	27.50 (31.60)	16.25 (23.73)	23.73 (29.14)	12.50 (20.61)	5.00 (9.21)	2.50 (6.46)	6.25 (14.29)	2.50 (6.46)	8.75 (16.76)	1.25 (3.23)	14.25 (20.20)	7.00 (12.09)
Curvularia sp.	1.25 (3.23)	0.00 (0.64)	25.00 (30.00)	17.50 (24.67)	1.25 (3.23)	1.25 (3.23)	5.00 (12.92)	3.75 (7.83)	0.00 (0.64)	0.00 (0.64)	6.51 (10.00)	4.52 (7.40)
Fusarium sp.	3.75 (9.69)	0.00 (0.64)	15.00 (19.92)	2.50 (4.60)	0.00 (0.64)	0.00 (0.64)	8.75 (14.91)	1.25 (3.23)	6.25 (14.29)	1.25 (3.25)	6.76 (11.89)	1.02 (2.47)
Penicillium sp.	0.00 (0.64)	0.00 (0.64)	12.50 (18.03)	6.25 (12.15)	5.00 (11.06)	2.50 (6.46)	12.50 (20.32)	12.50 (20.61)	15.00 (22.20)	17.50 (24.58)	9.01 (14.45)	7.76 (12.89)
Rhizoctonia sp.	7.50 (15.67)	3.75 (9.69)	10.00 (16.00)	7.50 (12.15)	0.00 (0.64)	0.00 (0.64)	13.75 (18.79)	13.75 (21.55)	0.00 (0.64)	0.00 (0.64)	6.27 (10.38)	5.00 (9.27)
Rhizopus sp.	63.75 (53.01)	51.25 (41.82)	38.75 (38.04)	15.00 (22.64)	53.75 (46.43)	46.25 (42.84)	15.00 (16.44)	2.50 (4.60)	17.50 (18.13)	10.00 (13.28)	35.25 (32.18)	27.27 (27.50)
Sclerolium sp.	0.00 (0.64)	0.00 (0.64)	1.25 (3.23	0.00 (0.64)	3.75 (7.83)	0.00 (0.64)	16.25 (20.78)	13.75 (21.55)	0.00 (0.64)	0.00 (0.64)	3.52 (5.05)	3.50 (6.26)
Mean	16.14 (17.50)	14.02 (15.20)	22.62 (25.99)	13.00 (18.09)	10.26 (12.68)	8.13 (10.75)	18.50 (22.85)	13.00 (17.74)	6.39 (10.54)	4.01 (7.02)	14.78 (17.92)	10.43 (13.76)

	SE	CD (0.05)
Fungal species	1.65	3.24
Varieties	1.16	2.29
Methods	0.73	1.24
Fungal species× varieties	3.69	7.24
Fungal species× method	2.33	4.58
Varieties × method	1.65	3.24
Fungal species ×varieties × method	5.22	10.24

* Mean of 4 replications
Values in the parenthesis are arc sine transformed values
US – Un sterlised
S- sterilised

Table 3 : Effect of storage period on the incidence of seed mycoflora in sesamum

Fungal species	Per cent incidence*					Mean
	15 days of storage	30 days of storage	45 days of storage	60 days of storage	75 days of storage	
Alternaria sp.	7.50 (15.67)	8.75 (17.05)	13.75 (21.69)	10.00 (18.43)	6.25 (14.29)	9.25 (17.43)
Aspergillus flavus	16.25 (23.73	17.50 (24.67)	21.25 (27.42)	26.25 (30.80)	30.00 (33.21)	22.25 (27.96)
Aspergillus niger	3.75 (9.69)	8.75 (17.05)	10.00 (18.43)	18.75 (25.62)	22.50 (28.20)	12.75 (19.81)
Aspergillus terreus	0.00 (0.45)	3.75 (9.69)	7.50 (15.67)	10.00 (18.43)	18.75 (25.62)	8.00 (13.88)
Curvularia sp.	5.00 (12.92)	12.50 (20.61)	22.50 (28.28)	21.25 (27.42)	8.75 (17.05)	14.00 (21.25)
Fusarium sp.	12.50 (20.61)	16.25 (23.73)	13.75 (21.69)	3.75 (9.69)	0.00 (0.45)	9.25 (15.14)
Penicillium sp.	7.50 (15.67)	10.00 (18.43)	12.50 (20.61)	20.00 (26.56)	26.45 (30.80)	15.25 (22.41)
Rhizoctonia sp.	3.75 (9.69)	7.50 (15.67)	11.25 (19.52)	15.00 (22.78)	12.50 (20.61)	10.00 (17.65)
Rhizopus sp.	0.00 (0.45)	11.25 (19.52)	12.50 (20.61)	25.00 (30.00)	31.45 (33.97)	16.00 (23.14)
Sclerotium sp.	2.50 (6.46)	6.25 (14.29)	8.75 (17.05)	13.75 (21.69)	5.00 (12.92)	7.25 (14.48)
Mean	5.87 (11.44)	10.25 (18.07)	13.37 (21.10)	16.37 (23.14)	16.12 (21.67	

	S.E.	CD (0.05)
Fungal species	0.87	1.71
Storage period	0.61	1.21
Fungal species x storage period	1.96	3.85

* Mean of four replications
Figures in the parenthesis are arcsine transformed values.

Table 4 : Effect of seed mycoflora on sesamum seed germination under laboratory conditions

Fungal species	Per cent seed germination	Per cent reduction over control
Alternaria sp.	67.50 (55.25)	14.08
Aspergillus flavus	58.75 (50.04)	25.22
Aspergillus niger	42.50 (40.68)	45.90
Aspergillus terreus	72.50 (58.39)	7.72
Curvularia sp.	66.25 (54.49)	15.68
Fusarium sp.	61.25 (51.50)	22.04
Penicillium sp.	62.50 (52.24)	20.45
Rhizoctonia sp.	43.75 (41.40)	44.31
Rhizopus sp.	76.25 (60.85)	2.95
Sclerotium sp.	57.50 (49.50)	26.81
Control	78.51 (62.57)	0.00
S.E.	0. 56	
CD (0.05)	1.63	

* Mean of four replications
Figures in the parenthesis are arcsine transformed values.

Table 5 : Effect of seed mycoflora on shoot length of sesamum seedlings under laboratory conditions

Fungal species	Mean shoot length (cm)	Per cent reduction over control
Alternaria sp.	4.15	21.69
Aspergillus flavus	3.65	31.13
Aspergillus niger	3.37	36.41
Aspergillus terreus	4.40	16.98
Curvularia sp.	3.10	41.50
Fusarium sp.	3.82	27.92
Penicillium sp.	4.60	13.20
Rhizoctonia sp.	4.42	16.60
Rhizopus sp.	4.75	10.37
Sclerotium sp.	4.25	19.81
Control	5.30	0.00
S.E.	0.11	
CD (0.05)	0.32	

* Mean of four replications

Table 6: Effect of seed mycoflora on root length of sesamum seedlings under laboratory conditions

Fungal species	Mean root length (cm)	Per cent reduction over control
Alternaria sp.	5.80	12.12
Aspergillus flavus	5.15	21.96
Aspergillus niger	3.70	43.93
Aspergillus terreus	5.10	22.72
Curvularia sp.	4.72	28.48
Fusarium sp.	5.22	20.90
Penicillium sp.	3.87	41.36
Rhizoctonia sp.	5.10	22.72
Rhizopus sp.	6.17	6.51
Sclerotium sp.	5.07	23.18
Control	6.60	0.00
S.E.	0.42	
CD (0.05)	1.20	

* Mean of four replications

Table 7 : Effect of culture filtrates on germination of sesamum seed under laboratory conditions

Fungal species	Per cent seed germination	Per cent reduction over control
Alternaria sp.	68.75 (56.02)	17.92
Aspergillus flavus	55.00 (47.87)	34.32
Aspergillus niger	71.25 (57.59)	14.92
Aspergillus terreus	42.50 (40.68)	49.25
Curvularia sp.	66.25 (54.49)	20.89
Fusarium sp.	53.75 (47.15)	35.82
Penicillium sp.	73.75 (59.19)	11.94
Rhizoctonia sp.	61.25 (51.52)	26.86
Rhizopus sp.	67.50 (55.25)	19.40
Sclerotium sp.	76.25 (60.85)	8.95
Control	83.75 (66.26)	0.00

S.E.	0. 92
CD (0.05)	2.26

* Mean of four replications
Figures in the parenthesis are arcsine transformed values.

Table 8 : Effect of culture filtrates on shoot length of sesamum seedlings under laboratory conditions

Fungal species	Mean shoot length (cm)	Per cent reduction over control
Alternaria sp.	3.87	25.86
Aspergillus flavus	3.05	41.25
Aspergillus niger	2.97	43.10
Aspergillus terreus	2.90	44.14
Curvularia sp.	4.95	5.17
Fusarium sp.	4.95	5.17
Penicillium sp.	4.35	16.66
Rhizoctonia sp.	4.50	13.79
Rhizopus sp.	4.85	7.08
Sclerotium sp.	3.72	28.73
Control	5.22	0.00
S.E.	0.18	
CD (0.05)	0.53	

* Mean of four replications

Table 9 : Effect of culture filtrates on root length of sesamum seedlings under laboratory conditions

Fungal species	Mean root length (cm)	Per cent reduction over control
Alternaria sp.	6.17	14.89
Aspergillus flavus	4.22	41.79
Aspergillus niger	5.30	26.89
Aspergillus terreus	4.87	32.82
Curvularia sp.	4.37	39.72
Fusarium sp.	5.20	28.27
Penicillium sp.	4.45	38.62
Rhizoctonia sp.	4.05	44.13
Rhizopus sp.	5.47	24.55
Sclerotium sp.	3.92	45.93
Control	7.25	0.00

S.E.	0. 13
CD (0.05)	0.40

* Mean of four replications

Table 10: Effect of seed mycoflora on protein content of sesamum seed

Fungal species	Per cent protein*		Mean
	15 days of incubation	30 days of incubation	
Alternaria sp.	9.75 (18.19)	9.05 (17.50)	9.40 (17.85)
Aspergillus flavus	11.37 (19.70)	10.87 (19.25)	11.12 (19.48)
Aspergillus niger	11.80 (20.09)	10.75 (19.13)	11.27 (19.61)
Aspergillus terreus	10.62 (19.02)	10.37 (18.79)	10.5 (18.90)
Curvularia sp.	9.72 (18.17)	8.62 (17.07)	9.17 (17.62)
Fusarium sp.	10.07 (18.50)	9.47 (17.92)	9.77 (18.21)
Penicillium sp.	10.30 (18.71)	9.92 (18.36)	10.11 (18.54)
Rhizoctonia sp.	9.42 (17.87)	8.32 (16.77)	8.87 (17.32)
Rhizopus sp.	11.77 (20.06)	10.07 (18.50)	10.92 (19.28)
Sclerotium sp.	9.55 (18.00)	8.72 (17.18)	9.13 (17.59)
Control	12.75 (20.92)	12.45 (20.66)	12.60 (20.79)
Mean	10.65 (19.02)	9.87 (18.28)	

	S.E.	CD (0.05)
Fungal species	0.054	0.110
Days of incubation	0.023	0.047
Fungal species x Days of incubation	0.076	0.156

* Mean of four replications
Figures in the parenthesis are arcsine transformed values.

Table 11 : Effect of seed mycoflora on oil content of sesamum seed

Fungal species	Per cent oil *		Mean
	15 days of incubation	30 days of incubation	
Alternaria sp.	48.50	47.87	48.18
Aspergillus flavus	48.25	48.65	48.46
Aspergillus niger	47.85	40.12	43.98
Aspergillus terreus	48.02	48.42	48.22
Curvularia sp.	48.67	48.35	48.76
Fusarium sp.	47.37	47.75	47.56
Penicillium sp.	47.55	47.85	47.32
Rhizoctonia sp.	48.40	47.52	47.70
Rhizopus sp.	47.55	47.82	47.96
Sclerotium sp.	47.95	48.07	48.01
Control	49.02	50.55	49.8
Mean	48.07	47.55	

	S.E.	CD (0.05)
Fungal species	0.046	0.095
Days of incubation	0.020	0.040
Fungal species x Days of incubation	0.066	0.135

* Mean of four replications

The data were not transformed before statistical analysis as the percentage lie with in the range of 30 to 70 per cent. .

Table 12 : Effect of seed mycoflora on free fatty acid content of sesamum oil

Fungal species	Per cent fatty acid *		Mean
	15 days of incubation	30 days of incubation	
Alternaria sp.	6.67 (14.97)	11.3 (19.66)	9.00 (17.31)
Aspergillus flavus	25.52 (30.34)	51.87 (46.07)	38.70 (38.21)
Aspergillus niger	9.35 (17.80)	15.65 (23.30)	12.50 (20.55)
Aspergillus terreus	27.87 (31.86)	32.27 (34.61)	30.07 (33.24)
Curvularia sp.	34.12 (35.74)	38.62 (38.42)	36.37 (37.08)
Fusarium sp.	37.62 (37.83)	40.87 (39.74)	39.25 (38.78)
Penicillium sp.	4.55 (12.31)	5.20 (13.18)	4.87 (12.74)
Rhizoctonia sp.	6.37 (14.62)	11.07 (19.43)	8.72 (17.03)
Rhizopus sp.	28.62 (32.34)	32.62 (34.82)	30.62 (33.58)
Sclerotium sp.	22.10 (28.03)	27.92 (31.90)	25.01 (29.96)
Control	3.17 (10.26)	3.32 (10.50)	3.25 (10.38)

	S.E.	CD (0.05)
Fungal species	0.19	0.39
Days of incubation	0.08	0.16
Fungal species x Days of incubation	0.27	0.55

* Mean of 4 replications
Figures in the parenthesis are Arcsine Transformed values

Table 13 : Effect of seed mycoflora on saponification values of sesamum oil

Fungal species	Saponification value*		Mean
	15 days of incubation	30 days of incubation	
Alternaria sp.	228.25	236.50	232.37
Aspergillus flavus	187.50	209.25	198.37
Aspergillus niger	195.00	214.25	204.62
Aspergillus terreus	178.25	220.75	199.50
Curvularia sp.	209.00	218.25	213.62
Fusarium sp.	200.25	209.00	204.62
Penicillium sp.	263.50	348.75	306.12
Rhizoctonia sp.	287.50	329.25	308.50
Rhizopus sp.	196.00	210.75	203.37
Sclerotium sp.	210.75	225.75	218.25
Control	186.50	189.25	187.87
Mean	212.50	236.09	

	S.E.	CD (0.05)
Fungal species	4.32	8.80
Days of incubation	1.84	8.75
Fungal species x Days of incubation	6.11	12.44

* Mean of 4 replications

Table 14 : Effect of seed mycoflora on iodine values of sesamum oil

Fungal species	Iodine value *		Mean
	15 days of incubation	30 days of incubation	
Alternaria sp.	114.50	109.25	111.87
Aspergillus flavus	68.25	63.50	65.87
Aspergillus niger	90.50	93.00	91.75
Aspergillus terreus	74.25	71.00	72.62
Curvularia sp.	117.00	101.25	108.12
Fusarium sp.	95.50	88.50	92.00
Penicillium sp.	110.75	115.25	113.00
Rhizoctonia sp.	117.25	115.75	116.50
Rhizopus sp.	96.50	107.75	102.12
Sclerotium sp.	106.25	103.00	104.62
Control	118.25	116.75	117.50
Mean	100.81	98.63	

	S.E.	CD (0.05)
Fungal species	0.67	1.36
Days of incubation	0.28	0.58
Fungal species x Days of incubation	0.94	1.93

* Mean of 4 replications

Table 15: Effect of seed mycoflora on Peroxide values of sesamum oil

Fungal species	Peroxide value *		Mean
	15 days of incubation	30 days of incubation	
Alternaria sp.	2.73	5.77	4.25
Aspergillus flavus	2.83	5.85	4.34
Aspergillus niger	2.91	5.93	4.42
Aspergillus terreus	3.02	6.07	4.55
Curvularia sp.	2.61	5.50	4.05
Fusarium sp.	3.20	6.47	4.83
Penicillium sp.	2.90	5.82	4.36
Rhizoctonia sp.	3.77	6.88	5.33
Rhizopus sp.	2.75	5.62	4.18
Sclerotium sp.	4.07	7.25	5.66
Control	2.71	5.50	4.13
Mean	3.04	6.06	

	S.E.	CD (0.05)
Fungal species	0.02	0.04
Days of incubation	0.008	0.017
Fungal species x Days of incubation	0.028	0.058

* Mean of 4 replications

Table 1a: Interaction between fungal species and varieties

Fungal species	Varieties					
	Swetha til	**YLM-17**	**Madhavi**	**Tanuku brown**	**Gowri**	**Mean**
Alternaria sp.	9.37 (14.16)	10.00 (16.00)	8.75 (13.62)	13.75 (20.22)	1.26 (3.45)	8.62 (13.49)
Aspergillus flavus	33.75 (35.36)	15.00 (19.83)	26.87 (29.20)	19.37 (25.74)	16.25 (23.65)	22.42 (26.76)
Aspergillus niger	15.62 (19.89)	12.50 (17.77)	6.88 (11.71)	0.00 (0.45)	20.00 (26.40)	11.00 (15.24)
Aspergillus terreus	8.57 (12.22)	13.12 (21.08)	0.00 (0.45)	8.75 (13.40)	0.00 (0.45)	6.13 (9.52)
Curvularia sp.	0.00 (0.45)	14.37 (21.98)	1.25 (3.23)	2.50 (6.46)	1.25 (3.23)	3.88 (7.07)
Fusarium sp.	0.00 (0.45)	6.25 (11.23)	0.00 (0.45)	1.26 (3.45)	0.00 (0.45)	1.51 (3.20)
Penicillium sp.	0.00 (0.45)	5.26 (9.76)	2.50 (5.53)	0.00 (0.45)	19.37 (25.88)	5.63 (8.69)
Rhizoctonia sp.	0.00 (0.45)	10.00 (14.52)	0.00 (0.45)	8.12 (14.00)	0.00 (0.45)	3.64 (5.97)
Rhizopus sp.	52.50 (46.45)	12.50 (20.39)	40.00 (38.69)	12.50 (14.85)	22.50 (24.30)	28.00 (29.94)
Sclerotium sp.	0.00 (0.45)	0.00 (0.45)	0.00 (0.45)	8.12 (14.00)	0.00 (0.45)	1.89 (3.71)
Mean	12.13 (13.31)	9.93 (15.30)	8.62 (10.52)	7.44 (11.30)	8.07 (10.87)	

Table 2a: Interaction between fungal species and varieties

Fungal species	Varieties					
	Swetha til	**YLM-17**	**Madhavi**	**Tanuku brown**	**Gowri**	**Mean**
Alternaria sp.	16.25 (23.65)	23.12 (28.57)	3.75 (6.76)	20.00 (26.06)	2.50 (6.46)	13.12 (18.30)
Aspergillus flavus	46.87 (43.19)	23.75 (27.37)	6.25 (13.37)	40.62 (39.56)	8.12 (15.15)	25.12 (27.73)
Aspergillus niger	0.00 (0.64)	37.50 (37.73)	21.25 (27.42)	33.12 (35.12)	2.50 (6.46)	18.88 (21.47)
Aspergillus terreus	21.87 (27.66)	18.12 (24.87)	3.75 (7.83)	4.37 (10.38)	5.00 (9.99)	10.62 (16.15)
Curvularia sp.	0.00 (0.64)	21.25 (27.33)	1.25 (3.23)	4.37 (10.38)	0.00 (0.64)	5.51 (8.70)
Fusarium sp.	1.90 (5.16)	8.75 (12.26)	0.00 (0.64)	5.00 (9.02)	3.75 (8.76)	3.89 (7.18)
Penicillium sp.	0.00 (0.64)	9.37 (15.09)	3.75 (8.76)	12.50 (20.46)	16.25 (23.39)	8.38 (13.67)
Rhizoctonia sp.	5.62 (12.68)	8.75 (14.91)	0.00 (0.64)	13.75 (20.26)	0.00 (0.64)	5.64 (9.82)
Rhizopus sp.	57.50 (47.42)	26.87 (30.36)	50.00 (44.63)	8.75 (10.52)	13.75 (15.70)	31.35 (29.73)
Sclerotium sp.	0.00 (0.64)	0.00 (0.64)	1.87 (3.91)	15.00 (21.16)	0.00 (0.64)	3.52 (5.66)
Mean	15.08 (16.36)	17.81 (22.04)	9.19 (11.72)	15.75 (20.30)	5.20 (8.78)	

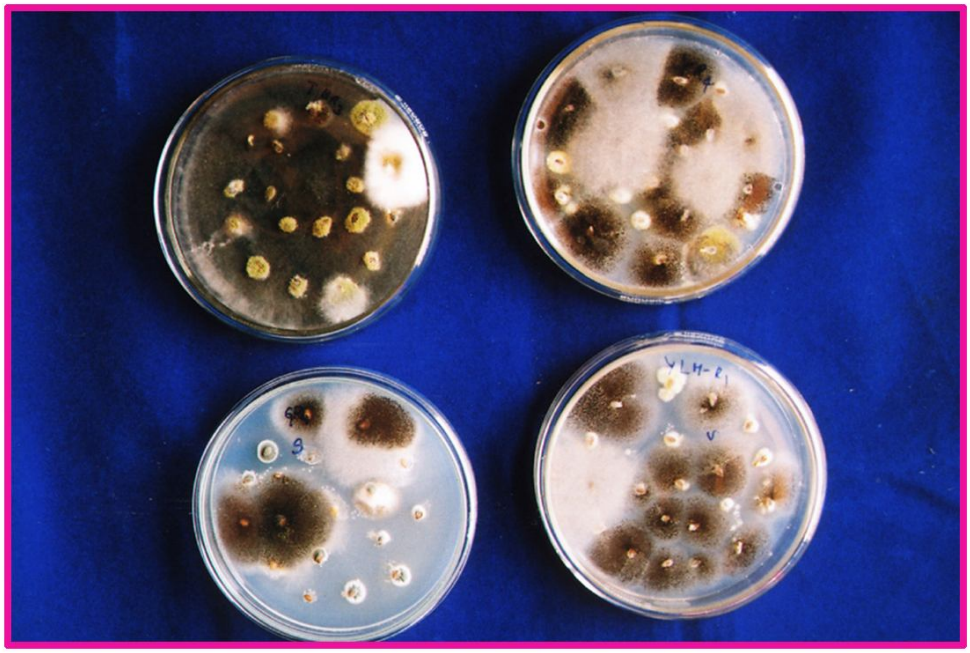

Plate 5 : Effect of storage period on the incidence of seed mycoflora in sesamum

Plate 8 : Conidia of *Alternaria* sp. (400x)

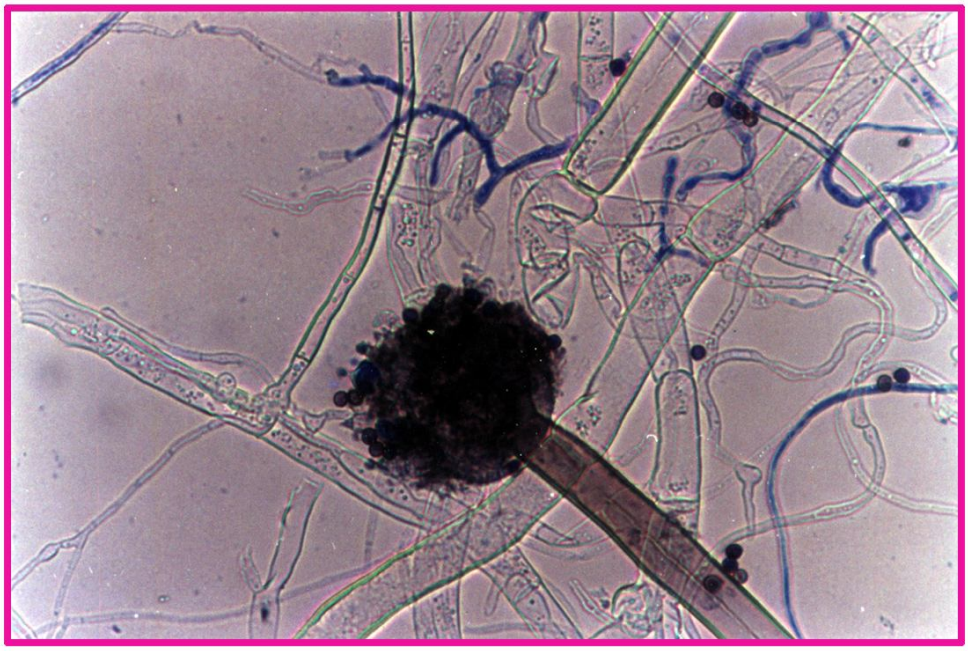

Plate 9 : Conidiophore and conidial head of *Aspergillus flavus* (400 x)

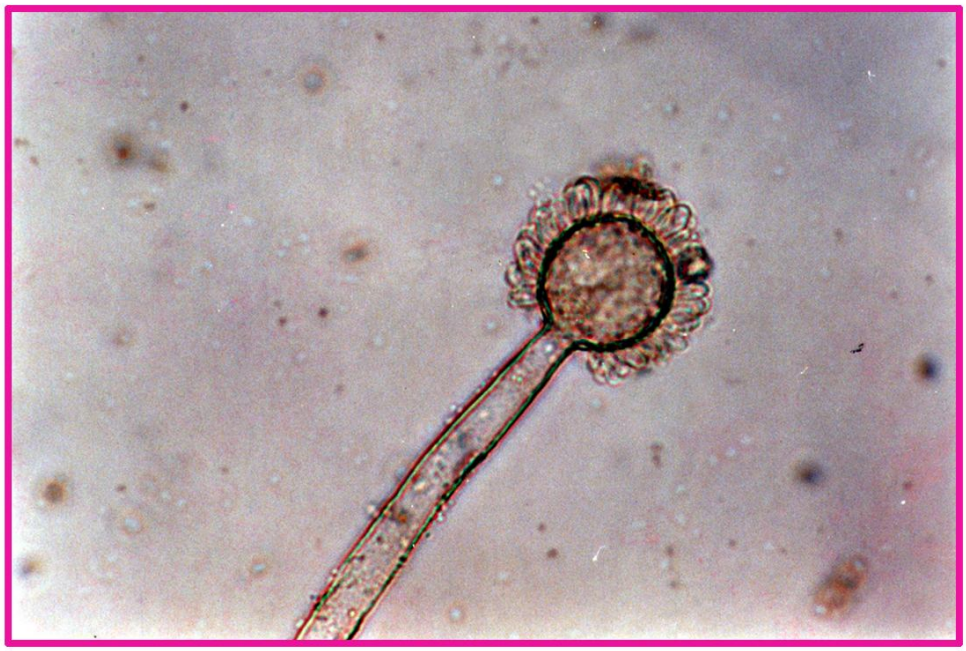

Plate 10 : Conidiophore and conidial head of *A.niger* (400 x)

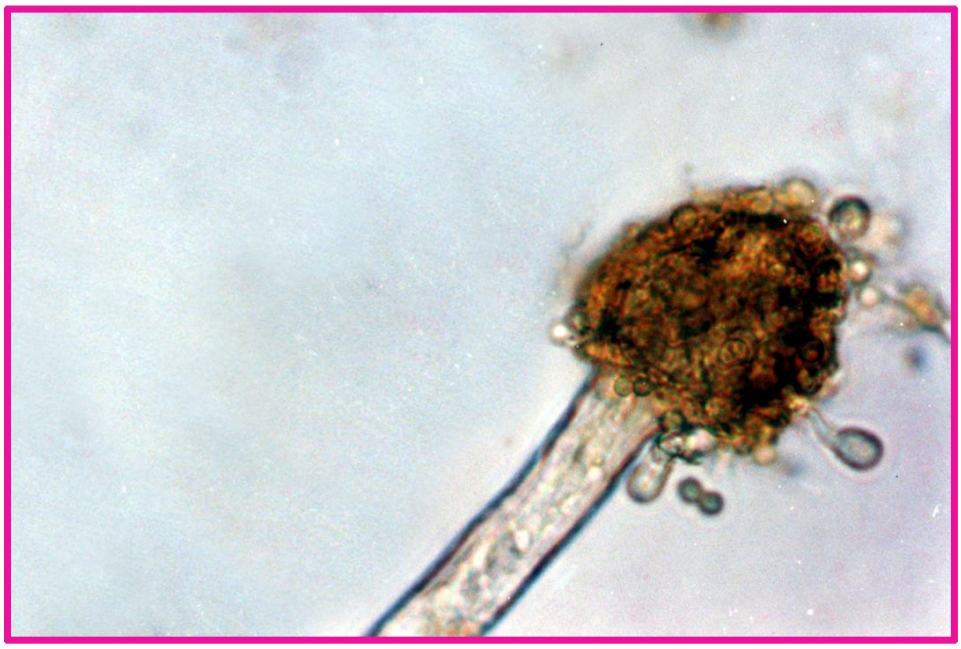

Plate 11 : Conidiophore and conidial head of *A. terreus* (400 x)

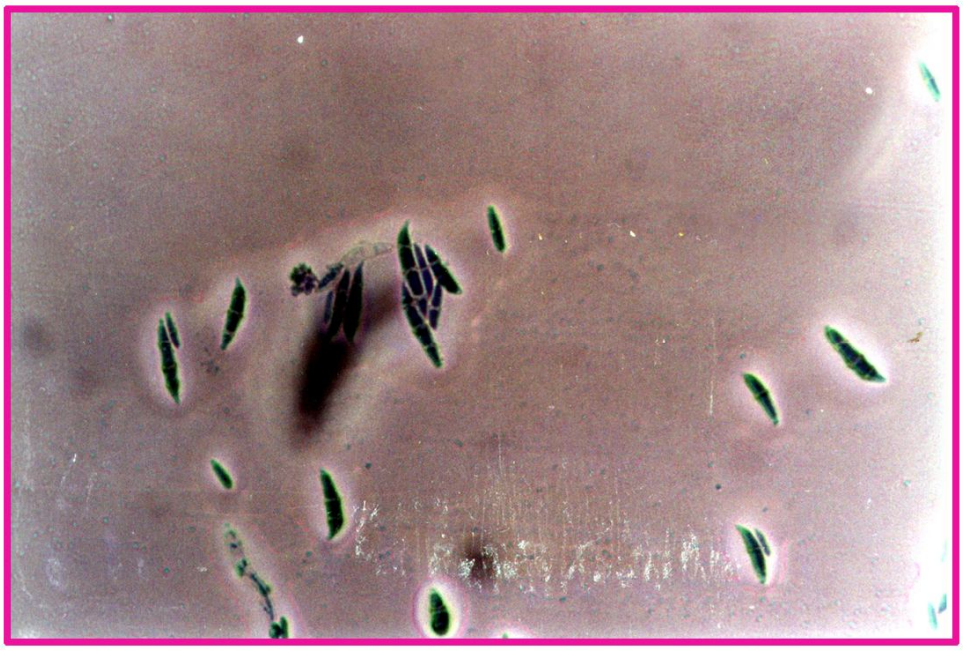

Plate 12 : Conidia of *Fusarium* sp.

Plate 13 : Conidia of *Curvularia* sp.

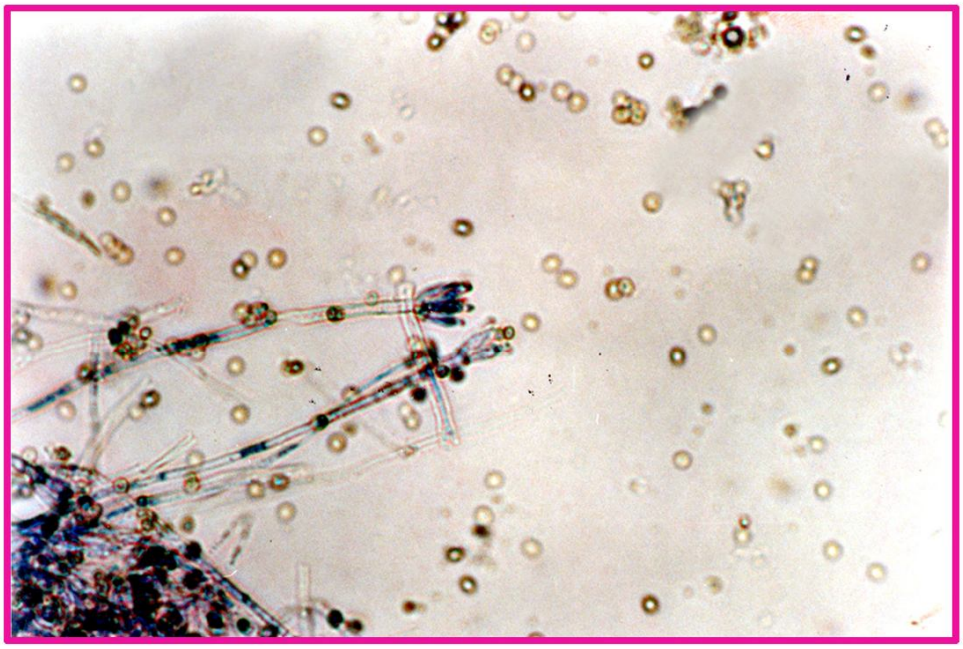

Plate 14 : Phialides and phialospores of *Penicillium* sp. (400 x)

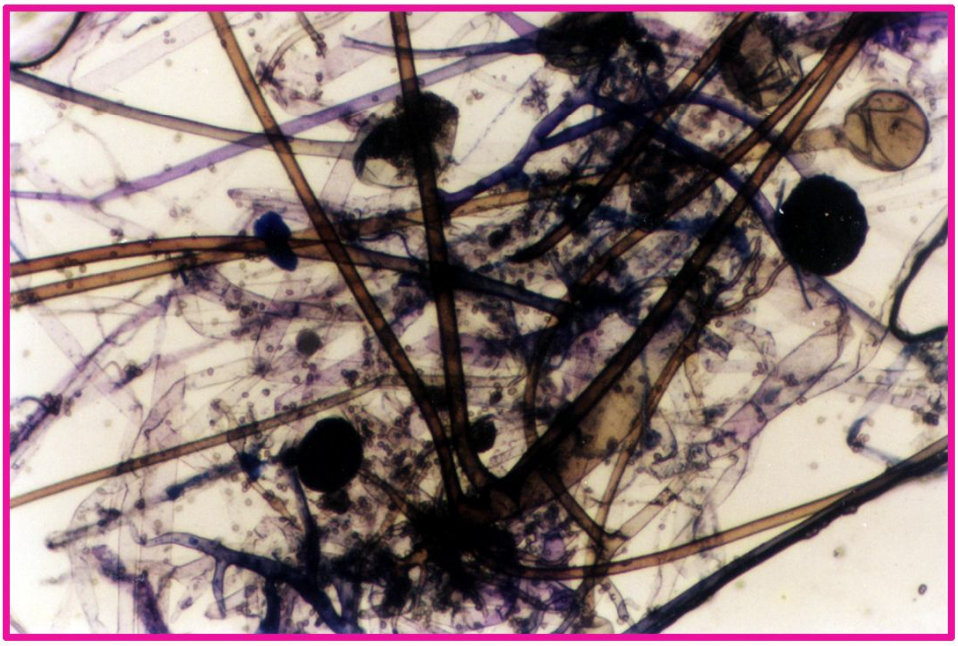

Plate 15 : Sporangiophore and sporangial head of *Rhizopus* sp. (400 x)

CHAPTER - V

DISCUSSION

5.1 ISOLATION OF SEED MYCOFLORA FROM DIFFERENT SESAMUM VARIETIES

In order to explore the possible differences in the composition of seed mycoflora due to varietal reaction, the quantitative and qualitative composition of mycoflora associated with the seeds of 5 sesamum varieties collected from Agricultural Research Station, Yellamanchili, Andhra Pradesh were studied.

The results showed that four fungal species *viz.*, *Alternaria* sp., *Aspergillus flavus*, *Aspergillus terreus* and *Rhizopus* sp. were commonly isolated from each of five varieties. *Aspergillus niger*, *Curvularia* sp. *Fusarium* sp. and *Penicillium* sp. were isolated from four varieties *Rhizoctonia* sp. and *Sclerotium* sp. were isolated from two varieties only. This revealed the influence of variety on the composition of seed mycoflora. The varieties also showed differences in the relative abundance of seed mycoflora, with YLM-17 recording the highest mean per cent incidence of fungal species and Gowri the lowest mean per cent incidence of seed mycoflora. Among the fungal species, *Rhizopus* sp. was found to be of maximum incidence followed by *Aspergillus flavus* and *Aspergillus niger* over all the varieties. These results suggested that the fungal species associated with seed tend to change in their frequency and type with the varieties.

The earlier work revealed the differences in type of fungal species associated with the seeds of different sesamum varieties corroborating the

present results. Vaidehi and Lalitha (1985) found *Alternaria* sp., *Curvularia* sp., *Drechslera* sp., *Fusarium* sp., *Cladosporium* sp. *Aspergillus niger* and *Aspergillus flavus* to be dominant fungi in sesamum seeds.

Alternaria alternata, Aspergillus flavus, Aspergillus niger and Fusrium moniliforme were found to be dominant fungi from the sesamum cultivar T-85 (Neeti saxena *et al.*, 1991). Sulochana and Balakrishnan (1997) isolated *Rhizopus stolonifer, Aspergillus flavus, Mucor haemalis, Aspergillus niger, Penicillium chrysogenum* and *Alternaria sesami* from 10 sesamum varieties. Wagon *et al.* (2002) found *Alternaria sesami, Curvularia lunata, Alternaria sesamicola* and *Fusarium oxyporium* from 4 sesamum varieties *viz.*, PR-125, S-17, PR-19-9 and PR-14-2.

From the above reports, it is obvious, that the seed mycoflora isolated from different varieties of sesamum seeds showed variation. The results of the present study are in agreement with the above reports.

The variation in the qualitative and quantitative composition of seed mycoflora in different sesamum cultivars may suggest that the varietal differences, might play a role in determining the nature of seed mycoflora.

5.2 EFFECT OF STORAGE PERIOD ON THE INCIDENCE OF SEED MYCOFLORA IN SESAMUM

It is well known that seed mycoflora undergoes both quantitative and qualitative changes when seeds are stored. In the present investigation when sesamum seeds were stored for 15, 30, 45, 60 and 75 days, five fungal species viz., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium* sp.

and *Rhizopus* sp. appeared to be less frequent in the initial periods of storage, but occurred more frequently and abundantly with increase in storage period. Contrastingly four fungal species *viz., Alternaria* sp., *Curvularia* sp. *Rhizoctonia* sp. and *Sclerotium* sp. occurred relatively in large numbers in the initial periods of storage but declined in the later period of storage. *Fusarium* sp. showed increase initially up to 30 days of storage but then slightly declined after 60 days of storage and later disappeared completely in the seed stored for 75 days. Variation was observed among different fungal species in the extent of changes in number as storage period was increased.

Following the terminology of Christen and Kaufmann (1965) species of *Aspergillus, Penicillium* and *Rhizopus* were storage fungi while the rest, *Fusarium* sp., *Alternaria* sp., *Curvularia* sp. *Rhizoctonia* sp. and *Sclerotium* sp. were field fungi. Storage fungi were observed to increase with increase in storage period (Christensen and Sauer, 1962; Dwivedi and Shukla, 1990; Kabeere and Taligola, 1983; Komaraiah and Reddy, 1985; Tripathi and Singh, 1991) while the population of field fungi decreased with increased storage period (Fallstad and Christensen, 1964; Nandi *et al.*, 1982; Mian and Fakir, 1989).

Vaidehi and Lalitha (1985) studied the incidence of seed mycoflora of sesamum variety N-62-30 during pre and post harvest stages in *kharif* and *rabi* seasons and in storage. Species of *Alternaria*, *Curvularia*, *Drechslera*, *Fusarium* and *Cladosporium*, *Alternaria sesami*, *Cercospora sesami*, *Cylindrosporium sesami and Fusarium oxysporum* which were of high percentages in pre-harvest stages considerably declined in post harvest stage and some were totally eliminated during storage of six months giving way to

storage fungi. The storage fungi were mostly *Aspergillus* and *Penicillium* sp. Similar observations were made by others (Charjan and Tarar, 1992; Ahmad *et al.*, 1996 and Krishnappa *et al.*, 2003).

The results of the present study which indicate the increase of storage fungi during the later stages of storage and increased occurrence of field fungi initially in the storage are corroborated by the above reports.

The change in moisture content of seed during storage might be responsible for the shifts observed in storage and field fungi. Christensen and Kaufmann (1965) observed that the amount of moisture was responsible for differential colonization of seed by storage and field fungi.

5.3 EFFECT OF SEED MYCOFLORA ON SESAMUM SEED GERMINATION UNDER LABORATORY CONDITIONS

It is generally known that the fungi colonizing seed may adversely affect seed germination and seed health. These fungi may cause deterioration of seed in storage when the optimal conditions are present in the storage (Kennedy, 1964, 1978; Christensen and Kaufmann, 1965 and Williams and Mc Donald, 1983). The adverse effects may range from discoloration of the seed, inhibition of seed germination and seedling growth to total decay of the seed (Christensen, 1978).

The results of present study revealed that in general all the fungal species tested significantly inhibited seed germination ranging from 2.95 to 45.90 per cent *Aspergillus niger, Rhizoctonia* sp. *Sclerotium* sp. and *Aspergillus flavus*, showed more adverse effects than others on seed germination. Minimum inhibitory effect on seed germination was noticed with *Rhizopus* sp.

The adverse effect of seed mycoflora on seed germination was well documented. Vidyasekharan *et al*. (1972) reported that the seed germination was severely reduced by seed-borne fungi, *Aspergillus niger* was found to be highly effective in reducing germination percentage in sesamum. Bose and Nandi (1980) reported that when sesamum seeds were inoculated with *Aspergillus flavus* and *Rhizoctonia solani* the germinability was completely lost. Sesamum seed germination was reduced due to the invasion of storage fungi (Nandi *et al.,* 1980). Similar decrease in the germinability was reported due to seed-borne fungi in other oil seed crops *viz.,* sunflower (Kushal and Saharan, 1994), Groundnut (Abid-Riaz *et al*., 2002)

The inhibition of seed germination due to seed-borne fungi may be attributed to toxins produced by the fungi which would kill the embryonic tissue and also to the production of the enzymes (Singh *et al.,* 1981). However, Vidyasekharan *et al.* (1970) expressed that the toxins produced by seed-borne fungi did not inhibit seed germination but affected seedling vigour. Gupta *et al.* (1993) observed that the storage fungi could invade the layers of seed coat and kill the seed by producing toxins and the first effect of the invasion was the weakening of embryo followed by death and discolouration.

5.3.1 Effect of seed mycoflora on shoot length of sesamum seedlings under laboratory conditions

With regard to the effect of seed mycoflora on shoot length under laboratory conditions *Curvularia* sp. caused a maximum reduction of 41.50 per cent over control. The other fungal species reduced the shoot length ranging from 10.37 per cent to 36.41 per cent. The results of the present study are in

agreement with the observations of earlier workers. Lalithakumari *et al.* (1972) found *Rhizoctonia bataticola* highly effective in reducing shoot length in groundnut. Vidhyasekharan *et al.* (1972) found *Aspergillus niger* highly effective in reducing root and shoot elongation in sesamum. Haider *et al.* (1986) reported reduction in the shoot length by *Aspergillus flavus, Aspergillus niger, Alternaria alternata* and *Fusarium equiseti*. Similar inhibitory effect of *Aspergillus flavus, Aspergillus niger, Rhizoctonia* sp. and *Fusarium moniliforme* on the shoot length was reported by Reddy *et al.* (1991).

5.3.2 Effect of seed mycoflora on root length of sesamum seedlings under laboratory conditions

It was observed in the present study that there was reduction in the root length, over control due to fungal species. *Aspergillus niger* followed by *Penicillium* sp. showed more adverse effect on root while *Rhizopus* sp. and *Alternaria* sp. were least effective. Some of the fungi associated with the seed were reported to adversely affect the seeding vigour as a result of the invasion of embryonic tissues and weakening them (Singh *et al.*, 1981). Vidhyasekharan *et al.*(1970) reported that toxins produced by seed-borne fungi affected the seedling vigour. Inhibition in root growth by fungi associated with the seed was reported by Lalithakumari *et al.* (1972) in groundnut.

5.4 EFFECT OF CULTURE FILTRATES OF SEED MYCOFLORA ON SESAMUM SEED GERMINATION UNDER LABORATORY CONDITIONS

In order to explore the possibility of the production of toxic metabolites by seed mycoflora and their effect on seed germination and seedling health, the

experiment was conducted. Seed germination was significantly inhibited by the culture filtrates of all the fungal species. The culture filtrates of *A.terreus, Fusarium* sp. and *Aspergillus flavus* were more effective than the culture filtrates of the other fungi on seed germination. The adverse effect of *Rhizopus* sp. was found to be the least. The inhibition of seed germination ranged from 8.95 to 49.25 per cent.

The results of the present study are corroborated by similar reports of earlier workers in the case of sesamum (Chandra *et al.,* 1985; Wasnikar *et al.,* 1991; Pinkey khali and Pandey, 2005).

In contrast, Tervet (1944) reported that culture filtrates of *Aspergillus fumigatus* increased seed germinability in soybean. The increase in seed germination due to certain seed-borne fungi might be due to the production of auxin like substances by the fungi (Sinha and Prasad, 1981). However the metabolites of *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Fusarium oxysporum* and *Penicillium oxalicum* were found inhibitory to germination of soybean seed (Tripathi and Singh, 1991), corroborating the findings of the present study.

When a comparison is made between the cultures and culture filtrates of the fungi the inhibition of germination appear to be more pronounced with culture filtrates in the case of *Aspergillus terreus, Fusarium sp. and Aspergillus flavus,* while cultures of *A. niger* and *Rhizoctonia* sp. appeared to be more effective than their culture filtrates (Fig. 1). Tervet (1944) observed culture filtrates of *A.niger* was more inhibitory on germination of soybean seed than the culture of the fungus.

5.4.1 Effect of culture filtrates of seed mycoflora on shoot length of sesamum seedlings under laboratory conditions.

The results presented in the (Table 8) showed that the culture filtrates of all the fungal species reduced shoot length over control. Significant reduction was recorded with culture filtrates of *Aspergillus terreus, Aspergillus niger* and *Aspergillus flavus.* The culture filtrates were found more effective than the fungal cultures in reducing the shoot length. The present results are corroborated by the report of Wasnikar *et al.* (1991) that culture filtrates of *Alternaria sesami* caused reduction in the shoot length (Fig. 2). The culture filtrates of *Aspergillus niger, Stachybotrys atra, Alternaria brassicae* and *Alternaria tenuissima* were reported to cause reduction in shoot length in sesamum (Pinkeykhati and Pandey, 2005). Contrasting results were obtained by Vishunavat and Shukla (1981) with certain seed-borne fungi on shoot length of lentil. An increase in shoot length was observed due to the culture filtrates of *Aspergillus fumigatus, Alternaria alternata, Fusarium equiseti* and *F. oxysporum.*

5.4.2 Effect of culture filtrates of seed mycoflora on root length of sesamum seedlings under laboratory conditions

The results revealed significant reduction of root length with culture filtrates of *Sclerotium* sp. followed by *Rhizoctonia* sp. *Aspergillus flavus* and *Curvularia* sp. However, culture filtrates of all fungal species reduced root length over control ranging from 14.89 to 45.93 per cent. Between the cultures and culture filtrates (Fig. 3), the latter were found to be more effective in the case of *Aspergillus flavus, Sclerotium* sp., *Rhizoctonia* sp., *Penicillium* sp. and *Curvularia* sp. in their adverse effect on root length.

Similar adverse effects of culture filtrates of seed-borne fungi were reported earlier. Mishra and Kanaujia (1973) reported that culture filtrates of *Aspergillus flavus* caused maximum reduction of root length in soybean.

Chandra *et al.*(1985) reported that culture filtrates of *Penicillium* sp., *Rhizopus* sp. *Aspergillus niger* and *Aspergillus flavus* caused highest reduction of root length in sesamum. The culture filtrates of *Fusarium oxysporum* f.sp *sesami* caused reduction of root growth in sesamum (Wasnikar *et al.*, 1991).

The present results also showed that between root length and shoot length the effect of both cultures and culture filtrates was more on root length than on shoot length. From these observations it can be assumed that root was more prone to be attacked by the fungi or their toxic metabolites than the shoot. This assumption is confirmed by Lalithakumari *et al.* (1972) who reported that root development was more reduced than shoot development by fungi associated with groundnut seed. Vidhyasekharan *et al.* (1970) pointed out that the root appeared to be more susceptible part of the plant to toxins contained in the culture filtrates. Kanta (1982) found that root of blackgram was more susceptible to culture filtrates of seed mycoflora than shoot.

5.5 EFFECT OF SEED MYCOFLORA ON PROTEIN CONTENT OF SESAMUM SEED

In the present work *Rhizoctonia* sp., *Sclerotium* sp. and *Curvularia* sp. were found to be highly effective in reducing the protein content after both 15 and 30 days of incubation. *Aspergillus niger*, *Rhizopus sp.* and *Aspergillus flavus* were found to be the least effective in reducing the protein content after 15 days of incubation. *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus*

terreus were found to be the least effective after 30 days of incubation. Significant reduction in protein content was observed with increase in incubation period from 15 to 30 days in respect of *Rhizopus* sp., *Aspergillus niger, Curvularia* sp. and *Rhizoctonia* sp.

Neeti Saxena *et al.* (1991) observed decrease in protein content of sesamum seeds when the seeds were inoculated with *Aspergillus flavus, Aspergillus niger* and *Alternaria alternata.* The ability of seed-borne fungi like *Aspergillus flavus*, *Aspergillus niger* and *Alternaria alternata* was also observed in sesamum and sunflower (Singh and Prasad, 1981; Saxena and Karan, 1991) and in other crops like moong (Gupta and Gupta, 1984), arhar (Shukla *et al.*, 1988), cow pea (Maheswari and Mathur, 1984) and horse gram (Alka Pandey, 1988). The findings of the present study on decrease of protein content due to seed mycoflora agree with the above reports.

The reduction in the protein content of seeds due to seed-borne fungi may be attributed to the breakdown of proteins to amino acids and their subsequent utilisation by fungi (Bilgrami *et al.*, 1979). Sharma and Wahab (1975) reported the use of proteins by fungal organisms after successive deamination in soybean.

The differences in reduction of proteins produced by different fungal species observed in the present investigation may be due to the differences in the intrinsic individual abilities to produce necessary enzymes and utilize the seed protein.

5.6 EFFECT OF SEED MYCOFLORA ON OIL CONTENT OF SESAMUM SEED

In the present study all the fungal species reduced the oil content of inoculated sesamum seed but the significant decrease was found in respect of *Aspergillus niger, Penicillium* sp., *Fusarium* sp. and *Rhizoctonia* sp. *Curvularia* sp. *Aspergillus flavus* and *Aspergillus terreus* showed the minimum reduction of oil content of inoculated seeds after 15 and 30 days of incubation respectively.

The earlier reports on the efficacy of seed-borne fungi in reducing the oil content were variable. Appreciable reduction in oil content of gingelly, after the seeds were infected by seed-borne fungi was reported by Vidhyasekharan *et al.* (1972). A decrease in oil content of sesame due to mycoflora, particularly *Macrophomina phaseolina, Aspergillus flavus, Drechslera hawaiiensis* and *Fusarium moniliforme* was reported (Singh, 1972, 1980). Bhargava and Shukla (1980) observed the reduction in the oil content of sesame seed due to *Fusarium equiseti* and *Fusarium oxysporum*. Sharma (1981) observed maximum reduction in the oil content of sesamum seeds inoculated with *Aspergillus niger* and *Aspergillus tamarii*. However, Shivapuri *et al.* (1990) found an increase in the oil content of mustard seed due to six fungal species but, a significant reduction in the oil content with *Fusarium* spp. and *Phoma* spp.

In the present study increase in the incubation period of inoculated seed caused a slight but not significant decrease of the oil content in general. Inrespect of certain individual fungal species a negligible increase of oil content was recorded with increase of incubation period.

The cause for the reduction in oil content due to fungal invasion is not well understood (Ward and Diener, 1961). The reduction in the oil content may be attributed to lipolytic activity of the seed-borne fungi. Vidhyasekharan and Govindaswamy (1968) observed accumulation of reducing sugars in paddy seed due to fungal invasion, which directly or indirectly influenced the lipolytic activity of seed-borne fungi. Saraswat and Mathur (1985) showed the production of lipase by storage fungi in linseed *in vitro*. The increase in the oil content of infected seed was attributed to selective utilization of proteins in preference to oil by invading fungi (Pattinson and Thornton, 1965). Sharma and Chauhan (1976) suggested production of oil in the mycelium of fungi. He also observed that the enzymes like lipases reduced by the seed-borne fungi might have activated the formation of oil from the seed tissue. From the foregoing discussion it may be concluded that the ability of seed-borne fungi to affect the oil content depends on the lipolytic activity of the fungi.

5.7 EFFECT OF SEED MYCOFLORA ON PHYSICO-CHEMICAL PROPERTIES OF SESAMUM OIL

5.7.1 Physical properties

5.7.1.1 Colour and odour

1. Colour

In the present investigation, colour change was observed in the seeds treated with *Aspergillus niger, A. flavus, Alternaria* sp. and *Fusarium* sp. and incubated for 15 and 30 days separately.

2. Odour

Rancid odour was noticed in the seed inoculated with *A. niger, Fusarium* sp., *Sclerotium* sp. and *Rhizoctonia* sp. after 15 days of incubation.

After 30 days of incubation, the rancid odour was observed in respect of *A. niger, A. flavus, Curvularia* sp., *Fusarium*sp., *Rhizoctonia* sp., *Alternaria* sp., and *Sclerotium* sp. Rancid odour was not observed in the case of *A. terreus, Rhizopus* sp. and *Penicillium* sp.

Ward and Diener (1961) reported that of the fungi associated with groundnut seed, *Penicillium citrinum* caused a marked change in colour of the oil, but none of the fungi caused rancid odour. He suggested that the change in the colour of the oil might be due to pigments synthesized by invading fungi and the absence of rancid odour was explained by the fact that the fungi were unable to increase peroxides which cause oxidative rancidity. Rai and Saxena (1980) observed that *A. flavus* was responsible for changing the mustard oil colour to green and all the fungi induced unpleasant odour in the mustard oil. The role of seed-borne fungi in rancid odour development in oil was reported in peanuts (Wilson, 1947).

Similar changes in the colour of oil in sesamum and other oil seeds was reported (Mondal and Nandi, 1984; Mashooda-Begum *et al.,* 2003). The results of the present study are in accordance with the above reports.

5.7.2 Chemical properties

5.7.2.1 Effect of seed mycoflora on free fatty acid content of sesamum oil

Fungi associated with the seed in storage are generally known to bring about the deterioration of the seed. The adverse effects produced by storage fungi include poor germinability of seed, weakening of seedling vigour and impairing the biochemical constituents of the seed and products of seed such as oil. In several oil seed crops the deteriorative changes due to storage fungi like loss in organic matter, decrease in the oil content, loss of sucrose and increase in unsaturated fatty acids were recorded (Ward and Diener, 1961; Mondal and Nandi, 1981).

In the present study, it is evident that all the fungal species except *Penicillium* sp. significantly increased the free fatty acid content of inoculated sesamum seeds. Of these fungal species, *Aspergillus flavus* and *Fusarium* sp. caused the maximum increase in free fatty acid content. Similar observations on the ability of seed-borne fungi to increase the free fatty acid content of oil were made in various oil seed crops (Ward and Diener, 1961; Lalithakumari *et al.*, 1971 and Oso, 1978).

In the present study *A. flavus*, among other fungi caused the maximum increase of free fatty acid content of oil. Similar reports on the ability of *A.flavus* to maximize free fatty acid content were made by Sharma (1981), Prasad and Singh (1983), Mondal and Nandi (1985) and Mashooda – Begum *et al.* (2003) in sesamum.

Increase in the incubation period from 15 to 30 days resulted in increase in the free fatty acid content. This observation is in accordance with report of Rai and Saxena (1980) who reported an increase in the free fatty acid content with increase in the incubation period of infested mustard seed upto 30 days.

It is known fact that naturally occurring glyceride mixtures in oils contain only a small amount of free fatty acids produced by hydrolysis (Lalithakumari *et al.,* 1971). It observed that the fungi infesting seed in storage spoiled the quality of oil from such seed by increasing the free fatty acid content in contrast to the minimum level of fatty acids in oil from healthy seeds (Sharma, 1981). The present results are corroborated by the above reports. The increase in free fatty acid content in the present study may be attributed to the high lipase activity of storage fungi. McGee and Christensen (1970) stated that fats in grains were rapidly broken down by lipases into freefatty acids and glycerol.

5.7.2.2 Effect of seed myhcoflora on saponification values of sesamum oil

Saponification value is one of the important criteria for the assessment of quality of oils. Saponification value is an index of mean molecular weight of the fatty acid glycerides comprising fat or oil. Higher saponification values are indicative of large quantities of short chain fatty acid glycerides (Sankaran, 1966). Earlier literature on storage fungi revealed higher saponification values of oil from oilseeds infested with fungi and incubated for varying lengths of time (Sahasrabudhe and Kale, 1933; Sankaram, 1966 and Lalithakumari *et al.* 1972).

Sankaram (1966) postulated that the increase in Saponification value is due to the formation of large quantities of short chain fattyacid glycerides

during the lipolysis of the oil by the enzyme. Increase in the saponificaiton value due to fungal infestation was reported by several workers in different crops *viz.*, Singh and Prasad (1977, 1983) in Sunflower, Sharma (1981), Mondal and Nandi (1984) and Singh (1987) in sesamum, Rai and Saxena (1980) in mustard.

The significant increase in saponification value with increase in incubation period, observed in present study, was supported by the reports of Rai and Saxena (1980), Sharma (1981) and Bose and Nandi (1982) who detected similar increase in saponification values in crops like mustard, sesamum and safflower.

5.7.2.3 Effect of seed mycoflora on iodine values of sesamum oil

The quality of the oil is determined by a number of parameters of which the iodine value is one. Iodine number is a measure the degree of unsaturation in oil and the decrease in iodine number indicates absence or low presence of unsaturated free fatty acids (Sadasivam and Manickam, 1966).

In the present study all the fungal species significantly reduced the iodine number except *Rhizoctonia* sp. and *Penicillium* sp. These two were found to be least effective in reducing the iodine number. The increase in the incubation period of inoculated seed also did not produce any significant effect on the iodine values in general.

The results of the present study are in accordance with study of Lalithakumari *et al.* (1971) in groundnut and Sharma (1981) in sesamum.

5.7.2.4 Effect of seed mycoflora on peroxide values of sesamum oil

Peroxide value is a measure of the peroxides contained in the oil. Peroxides are formed as a result of auto-oxidation of unsatuarated oil. The present study revealed that the all fungal species increased the peroxide values. *Sclerotium* sp. and *Rhizoctonia* sp. resulted in maximum increase in peroxide value of inoculated seeds. From the above results it may assumed that the infestation of sesamum seed by fungal species resulted in the increased production of unsaturated fatty acid, linoleic acid. The increased levels of linoleic acid might have become auto-oxidized which increased peroxide values. Holman (1954) and Morris (1954) observed auto-oxidation of oil usually occurs when the oil has a higher linoleic acid.

Sharma (1981) reported that infestation of sesamum seed with fungal species caused an increase in peroxide content. Mashooda-Begum *et al.* (2003) showed slight increase in peroxide values when the seeds are treated with *A. flavus* in sesamum. The findings of the present study are in accordance with the above reports.

CHAPTER – VI

SUMMARY

In the present investigation isolation of seed mycoflora from different varieties of sesamum, effect of storage period on incidence of seed mycoflora of sesamum, effect of seed mycoflora and their culture filtrates on seed germination and seedling health and the effect of seed mycoflora on protein and oil content of seed and physico-chemical properties of oil were studied.

Differences were observed among the five varieties of sesamum regarding per cent incidence and composition of the mycoflora. Higher per cent incidence of fungi was observed in the case of YLM-17 followed by Tanuku brown and Swetha til. While four fungal species *viz.*, *Alternaria* sp., *Aspergillus flavus, Aspergillus terreus*, and *Rhizopus* sp. were common to all the varieties, *Aspergillus niger, Curvularia* sp., *Fusarium* sp. and *Penicillium* sp. were isolated from four varieties. *Rhizoctonia* sp. and *Slerotium* sp. were isolated from two varieties only.

The duration of storage period was also found to influence the composition of seed mycoflora, though significant variation was not seen among the different durations of storage in the mean per cent incidence of mycoflora. The increase in the duration of storage caused the increase in the incidence of *Aspergillus, Rhizopus* and *Penicilium* species and decrease in that of *Alternaria, Curvularia, Rhizoctonia* and *Sclerotium* species. *Fusarium* sp. was completely eliminated after 75 days of storage period.

Significant inhibition in seed germination and reduction in root length of seedlings of sesamum were observed with the culture and culture filtrates of the seed mycoflora. Maximum reduction in seed germination was produced by the

culture of *Aspergillus niger and* the culture filtrates of *Aspergillus terreus* under laboratory conditions. Minimum reduction was observed by the culture of *Rhizopus* sp.

Cultures of *Curvularia* sp. and culture filtrates of *Aspergillus terreus* resulted in maximum reduction in shoot length under laboratory conditions while minimum reduction was recorded with the culture of *Rhizopus* sp. and the culture filtrates of *Curvularia* sp. and *Fusarium* sp. In the case of root length the culture of *Aspergillus niger* and the culture filtrates of *Sclerotium* sp. resulted in maximum reduction. The minimum reduction in root length was recorded by the culture of *Rhizopus* sp. and the culture filtrates of *Alternaria* sp. under laboratory conditions.

In general, the culture filtrates of the fungal species were found to be more effective than their cultures in reducing seed germination, shoot length and root length under laboratory condition. Between root and shoot the adverse effect of both cultures and culture filtrates was more on root than on shoot under laboratory condition.

Inoculation of the seed with different fungal species followed by incubation for 15 and 30 days separately resulted in significant reduction of protein content. The percentage of reduction of protein content increased with increase in the incubation period with all the fungal species. *Rhizoctonia* sp. was found to be the most effective in reducing the protein content from 12.75 per cent in control to 9.42 per cent after 15 days and from 12.45 in control to 8.32 per cent after 30 days of incubating, while *Aspergillus niger* caused the minimum reduction after 15 days of incubation and *Aspergillus flavus* after 30 days of incubation.

Irrespective of the incubation periods, *A. niger* caused the maximum reduction in the oil content of inoculated sesamum seed while *Curvularia* sp. was least effective. With increase in the incubation period, all the fungal species except *A. niger, Rhizoctonia* sp. and *Alternaria* sp. caused increase in the oil content.

The colour of the oil was changed in the seeds inoculated with *A. niger, A. flavus, Alternaria* sp. and *Fusarium* sp. in both 15 and 30 days of incubation. Rest of the other fungi did not change the colour of the oil.

Rancid odour was of the oil was changed in the oil extracted from the sesamum seed inoculated with *A. niger*, *Fusarium* sp., *Sclerotium* and *Rhizoctonia* sp. in 15 days of incubation. After of 30 days of incubation the rancid odour was observed in respect of all the fungal species except *A. flavus, Curvularia* sp. *Rhizopus* sp. and *Penicillium* sp.

All the fungal species produced significant increase in the free fatty acid content of seed as compared to control. Significant increase of free fatty acid content of inoculated seed was observed with increase in incubation period. Maximum increase in free fatty acid content was recorded with *Fusarium* sp.. Minimum increase was observed with *Penicillium* sp. both after 15 and 30 days of incubation.

Significant increase in the saponification values of oil from the seed treated with all the fungal species was observed. *Rhizoctonia* sp. increased saponification value to 287.75 from 186.50 in control after 15 days of incubation and in 30 days of incubation *Penicillium* sp. was resulted in increase in saponification value from 189.25 in control to 348.75. Minimum increase was observed in the case of *A. flavus* in 15 days and *Fusarium* sp. after 30 days of incubation.

Reduction in iodine values was observed in the oil from the seed inoculated with all fungal species. Irrespective of incubation period the maximum reduction was observed in the case of *A. flavus* (117.50 to 65.87) and the minimum in the case of *Rhizoctonia* sp.

Significant increase in peroxide values was observed with all the fungal species irrespective of the incubation period. *Sclerotium* sp. was found to be most effective in increasing the peroxide values from 4.13 to 5.66, while minimum increase was observed with *Curvularia* sp.

LITERATURE CITED

*Abd-EL-Rehim M A Michail S H Abu Elgascin E A 1977 Changes in the Aminoacid patterns of soybean and pea seeds due to infestation by *Cephalosporium* sp., Acta phyto pathogloica academicae sceintiarum Hungarical 12: 273-276.

*Abid-Riaz, Khan A S and Mohammad-Irfan-Ul-Haq 2002 Frequency of seed borne fungi of groundnut and their impact on seed germination. Pakistan Journal of Phytopathology 14(1); 36-39.

Ahmad M A Khan B A Masood A Shamsuddin Z A 1996 Mycoflora of sotred and freshly harvested mustard and rapeseed grown in Pakistan. Pakistan Journal of Scientific and Industrial Research 39(5-8): 139-143.

Alka Pandey 1988 Aminoacid changes in horse gram seeds infested with *Rhizoctonia bataticola*. Indian Journal of Mycology and Plant Pathology 18: 306.

AOAC 1960 Official methods of analysis of the association of official Agricultural Chemists pp: 832.

Bahkali A K and Moslem M A 1996 Seed borne fungal contaminants of sesame (*Sesamum indicum* L.) seed in Saudi Arabia. Arab. Gulf. Journal of Scientific Research 14(1): 155-167.

Barnettt and Hunter 1972 Illustrated genera of imperfect fungi

.Bhargava S N and Shukla D N 1980 Interrelationship studies on some pulse and oil crop seeds wit seed borne fungi. Proceedings of the National Academy of Sciences India 49: 81-84.

Bilgrami K S Prasad T and Sinha R K 1979 In changes in nutritional components of stored seed due to fungal association. Today and Tomorrows Printers and Publishers, New Delhi.

Bose A and Nandi B 1982 Effect of some predominant storage fungi on physico-chemical properties of safflower and sesame oil. Phytopathogiche Zeitschrift 109: 357-363.

Bose and Nandi B 1985 Role of Enzymes of storage fungi in deterioration of stored safflower and sesame seeds. Seed Research 13(2); 19-28.

Chandra S Narang M and Srivastabva R K 1985 Studies on mycoflora of oil seeds in India. Inter relationship between mycoflora and seed. Seed Science and Technology.

Charjan S K V and Tarar J L 1992 Influence of storage container on germinability and mycoflora of sunflower seed (*Helianthus annulus* L.) Seeds and Farms 18(4): 3-5.

Christen sen C M and Sauer D B 1962 Mycoflora in storage of cereal grains and their products (Ed.Christensen C M) American Association of cereal chemistry,St paul Mlnn.219

Christensen C M and Kaufmann M M 1965 Deterioration of stored grains by fungi. Annual Review of Phytopathology 3: 69-84.

*Christensen C M 1978 Storage fungi in : Food and Beverage Mycology (Ed Berry R) AVL Publishing Co. Inc. Westport Connecticut. pp : 173 – 190.

CMI 1952 Description of pathogenic fungi and bacteria. Common Wealth Mycological Institute, Kew.

Dwivedi S N and Shukle T N 1990 Effect of methods of storage on germinability and mycoflora of gram (*Cicer arietinum*) seed Research 18: 82-85.

Fallstad M N and Christensen C M 1964 Effect of storage for three months at 14.1% moisture content and 21°C upon barely kernels. Phytopathology 54 : 399-400.

FAO Food and Agricultural Organization.

Gomez K A Gomez A A 1984 Statistical procedure for agricultural research. 2nd edition John Wiley and Sons Singapore.

*Gooya M Ershad D and Riahi H 2000 An investigation on mycoflora of sesame seeds in Iran. Rostaniha 1(1-4): 27-31.

Gupta P K and Gupta J S 1984 Storage deterioration of moong been seeds by fungi. Seed Science and Technology 21: 281-591.

Gupta I J Schmittener A E and Mc Donald M B 1993 Effect of storage fungi on seed vigour of soybean. Seed Science and Technology 21:581-591

Haider M M Sulaiman E D and Dawwood R K 1986 Effect of culture filtrate of species of fungi and their mixture on seed germination and seedling development of safflower, Journal of Biological Sciences Research 17: 140-150

Holman R T 1954 Auto-oxidation of fats and related substances. In progress in the chemistry of fats and other lipids. Vol. 2 Academic Press, New York.

International rules for seed testing annexes 1976 Seed Science and Technology 4:51-77

Jha D K 1995 Laboratory manual on seed pathology Vikas publishing house Pvt. Ltd., New Delhi.

Kabeere F and Taligola H K 1983 Mycoflora and deterioration of soybean seeds in Uganda. Seed Science and Technology 11: 381-392.

Kennedy B W 1964 Moisture content, mold invasion and seed viability of stored soybean. Phytopathology 54:771-773

Krishnappa N Narayanaswamy S Balakrishna P Lokesh K and Prashant Shankhinamath 2003 Influence of storage mycoflora on seed quality of groundnut varieties stored in different package materials. Research on Crops 4(3): 409-417.

Kanta S 1982 In effect of culture filtrate of some fungi on seed germination and on seedling of *Phaseolus mungo.* Biological Bulletin India 4 : 78 – 84.

Kashalkar P D Weginwar D G and Dani R G 1988 Variability of oil and lint traits and effects at harvest in upland cotton (*Gossypium hirsutum*). Indian Journal of Agricultural Science 58: 554-555.

*Khamees MAF and Sehlosser E 1990 Seed borne fungi on sesame in the Sudan. Mededelingen van de facultiet land bouwwetenschappen-Rijkeeniversiteit Gent 55(3a): 877-887.

Komaraiah M and Reddy S M 1985 Seasonal variation in seed mycoflora of two varieties of methi (*Trigonella foenum-graceum*). Seed Research 13(1): 45 -49.

Kushal Raj Saharan G S and Raj K 1994 Seed mycoflora of sunflower and its effect on seed and plant health. Indian Journal of Mycology and Plant Pathology 24(1): 15-19.

Lakshmidevi N Prakash H S and Shettly H S 1992 Effect of seed borne *Macrophomina phaseolina* (Jassi) Gold on physico-chemical properties of sunflower oil. International Journal of Tropical Plant Diseases 10(1): 79-84.

Lalithakumari D Govindaswamy C V Vidhyasekharan P 1971 Effect of seed borne fungi on the physico chemical properties of groundnut oil. Indian Phytopathology 24: 283-289.

Lalithakumari D Govindaswamy C V Vidhya Sekharan P 1972 Isolation of seed borne fungi form stored groundnut seed and their role on seed spoilage. Madras Agricultural Journal 59: 1-6.

*Lisker N Ben-Efrai M A and Henis Y 1985 Involvement of fungi in the increase of free fatty acids in stored soybean. Canadan Journal of Microbiology 37: 799-803.

Lowry O H Rosebrough N J Farr A L and Randall R J 1951 Journal of Biological Chemistry 193-265.

Maheswari R K and Mathur S K 1984 Deterioration of various seed constituents of lobia (*Vigna sinensis*) due to some Aspergilli. Seed Research 12 : 116 – 118.

*Marcinkowska J Schollen berger M and Boros L 1986 Influence of infection of soybean seeds with *Perenospora manschuria* and *Pseudomonas syringae* Pv. *Glycinea* on protein, oil and fattyacids content. Acta Agrobotanica 37: 157-167.

*Marium – Tariq Shahnaz – Dawar Muhammad Abid and Shaukat S 2005 Seeds borne mycoflora of soybean. International Journal of Biology and Biotechnology 2(3) : 711 – 713.

Mashooda-Begun Ravishankar Rai V and Lokesh S 2003 Effect of mycoflora on the physico-chemical characteristics of oil obtained from the infected sunflower, foria and sesame seeds. Journal of Food Science and Technology 40(6): 626-628.

Mc Gee D C and Chrishtensen C M 1970 Storage fungi and fatty acids in seeds held thirty days at moisture contents of fourteen and sixteen per cent . Phytopathology 60: 1775-1777.

*Milner M 1950 Biological processes in stored soybeans. IN soybeans and soybean production. (Markely, K. S. Ed), Inter Science, New York Vol. Pp: 483-501.

Mishra R R and Kanaujia R S 1973 Studies on certain aspects of seed-borne fungi. Indian Phytopathology 26: 284-294.

Mondal G C Nandi D and Nandi B 1981 Studies on deterioration of some oilseeds in storage. Variation in seed moisture infection and germinability. Mycologia 73: 157-167.

Mondal G C and Nandi B 1984 Role of fungi on oil quality of stored seeds of sesame, rape and linseed. Journal of Food Science 49: 1394-1395, 1400.

Morris S G 1954 Fat rancidity recent studies on mechanisms of fat oxidation in its relation to rancidity. J. Agri. Food Chem. 2 : 126 – 132.

Nandi D Mondal G S and Nandi B 1982 Studies on deterioration of some oil seeds in storage III. Effects of different storage temperatures and relative humidities on seeds moisture, germinability and infection. Seed Science and Technology 10: 141-150.

*Nasira – Altaf Khan S A Mustaq Ahmad Rehana – Asgar Ahmad R A Shabnum Shabeen Zafar M and Saquib 2004 Seed borne mycoflora of sesame and their effect on germination and seedling. Pakistan Journal of Biological Sciences 7(2): 243-245.

Neergard P 1979 Seed pathology vol.1 Mc Millan Press, Ltd, London Basinstore.

Neeti-Saxena Karan D, Saxena N 1991 Effect of seed borne fungi on protein and carbohydrate contents of sesame and sunflower seeds. Indian Phytopathology 44(1): 134-136.

Oso B A 1978 The lipase activity of *Talaromyces emersonii*. Can. J. Bot 56(16): 1840-3.

Pandey K N Gupta R C 1986 Inhibitory effect of fungal metabolites on germination and sprouting of cucubit seeds. Indian Journal of Mycology and Plant Pathology 16(3): 253-256.

Park W M Ko Y H Yoo Y J and Lee J Y 1982 The change of peroxide activity in soybean seed followed by infection with *Cercospora kikuchii.* Korean Journal of Plant Protection 21: 23-26.

Pattinson I and Thornton I 1965 The quality of unshelled groundnuts in the Gambia with special reference to insect and fungal attack. Tropical Science 7: 67-74.

Pinkey Khati and Pandey K N 2005 Effect of fungal metabolites on germiantion, sprouting and growth of sesame. Journal of Mycology and Plant Pathology 35(2): 385-387.

Prasad A and Prasad B K 1987 Retarding effect of the extract of *Aspergillus niger* inoculated seeds on the enzyme activity of lab lab bean F D seeds. Indian Journal of Mycology and Plant Pathology 17: 64.

Prasad T and Singh B K 1983 Effect of R H on oil properties of fungal infested sunflower seeds. Biological Bulletin of Indian 5: 85-88.

Prasad R B Thakur M K and Afsar M R 1983 Changes in energy content of linseed due to seed borne fungi. National Academic Science Letter.

Rai J N and Saxena A 1980 Effect of some seed-borne fungi on the physico-chemical properties of the oil of Indian mustard. Indian Journal of Agricultural Science 50(10): 769-772.

Raper and Fennel 1965 The Genus Aspergillus

Reddy G R Reddy A G R and Rao K C 1991 Effect of certain seed – borne fungi on seed germination and seedling vigor of groundnut. Journal of Research APAU 19 : 77 – 78.

Riker and Riker R S 1936 Introduction to research on plant disease. John Swift and Company USA.

Sadasivan S and Manickam A 1996 2nd. Edition New Age International (P) Limited Publishers.

Sahasrabuddhe D L and Kale N P 1933 A biochemical study of the formation of oil in niger seed (*Guizotia abessinica*). Indian Journal of Agricultural Science 3(7) : 57 – 88.

Sankaram A 1966 A laboratory manual for agricultural chemistry. Asia Pub. House Bombay.

Saraswat R P and Mathur S K 1995 Lipase production by seed borne fungi of linseed (*Linum usitatissimum* L.). Seed Research 13(2) : 123 – 124.

Saxena N and Karan D 1991 Effect of seed borne fungi on protein and carbohydrate contents of sesame and sunflower seeds. Indian Phytopathology 4 : 636 – 638.

*Shahid Ahmad and Mukesh Srivastava 2002 Fungi associated with Ethiopian mustard seeds Pathogenicity and Chemical Control. Annals of Agricultural Research 23(4): 748-751.

*Shahnaz Dawar Ghaffar A and Dawar S 1991 Detection of seed borne mycoflora of sunflower. Pakistan Journal of Botany 23(2): 173-178.

*Sharfun Nhar Muhammad Mustaq and Hashmi M H 2005 Seed borne mycoflora of sunflower (*Helianthus annuus* L.). Pakistan Journal of Botany 37(2): 451-457.

Sharma K D 1977 Biochemical changes in stored oil seeds. Indian Journal of Agricultural Research 11(3): 137-141.

Sharma K D 1981 Biodeterioration of sesamum oil *in situ* by fungi. Indian Phytopathology 34(1): 50-53.

Shivaprui A Siradhana B S and Bansal R K 1990 Effect of seed borne mycoflora on quality and quality of mustard oil. Indian Phytopathology 43(3): 404-407.

Shukla H S Dube K S and Tripathi 1988 Change in protein content of arhar due to fungal association. Legume Research 11: 85-88.

Singh A K Sinha R N Hussain M A and Prasad B K 2003 Effect of storage fungi on oil yielding seeds on germination and seedling diseases. Journal of Phytopathological Research 16(2): 163-168.

Singh B K and Prasad T 1983 Effect of seed-borne fungi on the physico-chemical properties of sesamum seed oil. Journal of Indian Botanical Society 62: 48-53.

Singh B K and Prasad T 1981 Biol. Bull. Of India 3: 125.

Singh B K and Prasad T 1985 Changes in cholesterol content in sunflower seeds due to fungal infestation. Indian Phytopathology 38(4): 666-667.

Singh H G and Sinha S 1979 Prevalence of fungi and their role on the activities of the seeds of three oil yielding crops. Seeds and Farms 5: 27-29.

Singh Tribhuvan and Singh Dalbir 1981 Seed borne mycoflora of sesame with special reference to Rajastan. Indian Journal of Mycology and Plant Pathology 13(1): 32-41.

Singh B P Sharma Y K and Shukla B N 1972 Role of seed-borne pathogens on reducing nutritive value of *Sesamum indicum* seeds proceedings of National Academic Sciences India 42: 440-441.

Singh B K 1987 Oil properties of sesamum sees at different R H under infestation. Indian Phytopathology 40: 356-359.

Singh B K and Prasad T 1977 Effect of seed borne fungi on the physico-chemical properties of sunflower oil. Phytopathology 90: 337-341.

Singh V Sinha R K P and Sinha K 1986 Effect of cultural filtrates of seed mycoflora of soybean upon seed germinability and seedling length. Indian Journal of Mycology and Plant Pathology.

Sinha R K and Prasad T 1981 Effect of fungal metabolites on seed germination, mycobial association and seedling growth of mung. Phytopathological Notes 34: 515-517.

Sulochana K and Balakrishnan S 1997 Seed borne mycoflora of sesame (*Sesamum indicum* L.). Journal of Tropical Agricultural 35(1-2): 64-66.

Tervet I W 1944 The influence of fungi on storage on seed viability and seedling vigour. Phytopathology 35 : 3 – 15.

Tinipilli Rout B T Paulkar P K Agarkar G D and Thakare A R 2003 Assessment of different methods for detection of mycoflora of sesamum. Annals of Plant Physiology 17(2): 153-156.

Tripathi D P and Singh B R 1991 Metabolites of soybean seed mycoflora affecting seed viability and seedling vigour. Madras Agricultural Journal 78: 133-135.

*Tuiet J 1969 Plant Pathological methods. Burgers Publishing Company, Minneapolis, Minnesota.

Vaidehi B K and Lalitha P 1985 Fungal succession in sesamum seeds. Indian Journal of Botany 8(1): 39-48.

Veech J A 1976 Localization of peroxidase in *Rhizoctonia solani* infested cotton seedlings. Phyto Pathology 66: 1072-1076.

Vidhyasekharan P and Govinda Swamy C V 1968 Role of seed borne fungi in paddy seed spoilage III. Production of Carbon dioxide, Fatty acids and reducing sugars. Indian Phytopathological Society Bulletin 4: 71-78.

Vidhyasekharan P Lalithakumari D and Govindaswamy C V 1972 Role of seed-borne fungi on the determination of quality of gingelly. Indian Journal of Microbiology 12: 104-107.

Vishunavath K and Shukla P 1981 Screening of culture filtrates of seed mycoflora of lentil upon seed germinability and seedling length. Indian Phytpathology 34 : 510 – 512..

Vyavasayapanchangam 2005-2006 pp: 76-77.

Wagan K H Pathan M A and Jiskani M M 2002 Effect of stored grain fungi on seed germination of sesame and their control. Sarhad Journal of Agricultural 18(1): 53-57.

Wasnikar A R Sharma S M Prasad K V V 1987 Seed borne Mycoflora of sesamum and their significance. Journal of Oil Seeds Research 4: 141-144.

Wasnikar A R Deshmukh M R and Sharma S M 1991 Toxicity of fungal metabolites on germination and growth of sesamum. Current Research University of Agricultural Sciences of Bangalore 23(9): 185-186.

Williams R J and Mc Donald D 1983 Grain molds in tropics: problems and importance . Annual Review of Phytopathology 21: 153-178.

Wu W S 1988 Studies on seed-borne diseases of sesame in Taiwan. Plant Protection Bulletin, Taiwan 30(3): 314-319.

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