

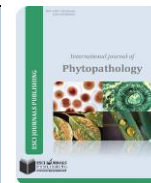


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## EFFECTS OF FUNGAL DETERIORATION ON LIPID CONTENT OF SESAME SEEDS (*SESANUM INDICUM* L.)

<sup>a</sup>Charity A. Amienyo, <sup>b</sup>Filibus M. Gugu, <sup>a</sup>Mary Oshatuyi

<sup>a</sup>Department of Plant Science and Technology, Faculty of Natural Sciences, University of Jos, Nigeria.

<sup>b</sup>Department of Microbiology, Faculty of Science, Plateau State University, Bokkos, Plateau State, Nigeria.

### ABSTRACT

Fungi associated with diseased seeds of *Sesamum indicum* L. from four markets in Jos and Okene central market were isolated to study the deteriorative changes in lipid content. In terms of number and fungal species abundance, Faringada market had the highest fungal occurrence, while *Aspergillus niger* showed the highest percentage occurrence in that market. Visually healthy seeds of *Sesamum indicum* were inoculated with spores of each of the nine fungi isolated from diseased seeds and incubated at 25+20c for 7days. The healthy and fungal infected seeds were analysed for their lipid content. Significant decreases in lipid content were observed in the seeds inoculated with all the fungi except in seeds inoculated with *G. candidum*, which showed increase in lipid content. *A. chevalieri* was responsible for the maximum depletion of the lipid content of the seeds. The results clearly indicate that these fungal species are capable of depleting the lipid content in storage.

**Keywords:** *Sesamum indicum* L., lipids, sesame seeds.

### INTRODUCTION

*Sesamum indicum* (family *Pedaliaceae*) is a high value ancient oil seed which is considered to be the oldest oil seed crop known to man for over 5000 years (Bedigian, 2012). Presently China, India and Myanmar are the leading producers of Sesame followed by Sudan, Nigeria, Pakistan, Bangladesh, Ethiopia, Thailand, Turkey and Mexico (FAO, 2004). Major producing areas in Nigeria are Benue, Gombe, Jigawa, Kano, Katsina, Kogi, Nassarawa, Plateau and Gombe states.

It is commonly called Beniseed in English, Ridi (Hausa), Ishwa (Tiv), Eeku (Yoruba), Igorigo (Ebira). The seeds are small, about 3 to 4mm long by 2mm inch and 1mm thick. They are ovate in shape, slightly flattered and usually range in colour from white, brown to black). Sesame seeds contain 40-60% oil content with a good stability due to the presence of antioxidants (FAO, 2010). The seeds are used extensively in manufacturing sesame oil used for cooking, perfumed oils and medicine purposes (Bedigian, 2000). They can be used to produce

flour for baking and preparation of sweets and confectionaries such as cakes (Frederick, 2004). They are a rich source of protein, carbohydrate and nutrients like calcium and phosphorus and forms a valuable and nutritious feeds for mulch cattle (Tjai, 2006).

Although sesame is extensively used for numerous purposes, the crop has very low yielding capacity as compared to other plants due to various factors especially disease susceptibility (Ashri, 1998). Numerous deteriorative microorganisms (fungi) have constituted a problem to the production and storage of the seeds (Mbah and Akueshi, 2009). Previous works on sesame seeds have indicated the presence of *A. flavus* among other fungi (Mbah and Akueshi, 2001). These organisms on the seeds affects their palatability and germinability, thereby predisposing the seeds to other pathogens (McDonald, 1999). Fungi growing on stored grains reduce not only germination rate but also carbohydrate, protein total oil content, increase moisture content and enhancing other biochemical changes (Chavan, 2011). Considering the above facts, emphasis is given on to study the mycoflora of the seeds in this environment and the effect of growth of fungi on the lipid content of the seeds.

\* Corresponding Author:

Email: [camienyo@gmail.com](mailto:camienyo@gmail.com)

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## MATERIALS AND METHODS

Seeds of *Sesamum indicum* used in this study were obtained from Faringada, Terminus, Chobe, Rukuba Road Markets Jos and Okene central markets, Nigeria. The samples were preserved in plastic bags and stored in refrigerator until required.

The standard blotter and agar methods were used for the detection and isolation of fungi (Association, 1996). The seeds were surface sterilized with 1% Sodium hypochlorite solution and rinsed in 3 changes of sterile distilled water to remove surface contaminants. One hundred and fifty seeds were used for each location for the blotter method, the sterilized seeds were plated out on moistened filter paper (Whatman No.1) in Petri dishes at the rate of 10 seeds per plate. The plates were incubated at  $25 \pm 2^\circ\text{C}$  for 7 days. During the period, the incubated seeds were examined daily for evidence of fungal growth. At the end of the incubation period, the number of infested seeds were recorded as percentage incidence.

The following formula was used to record the percentage incidence.

$$\text{Incidence (\%)} = \frac{\text{No. of infested seeds}}{\text{Total no. of seeds}} \times 100$$

Results obtained were the mean for five replicates. Sampling from each location was done three times. The fungal colonies observed on the filter paper in the Petri dishes were transferred into sterilized Petri dishes containing freshly prepared Potato Dextrose Agar (PDA) to obtain pure cultures. The cultures were incubated for 7 days at  $25 \pm 2^\circ\text{C}$ , after which they were examined for fungal growth and pure cultures obtained for identification. Based on the morphological characteristics of vegetative hyphae and spores, the fungal flora was identified after the reference of (Ellis and Ellis, 1987).

Fifty milliliters of sterile distilled water were aseptically poured into each of the pure culture plates of the fungi. A sterile glass rod was used to dislodge the spores from the mycelia in the culture plates. The spore load for inoculation of the seeds was calculated with the aid of a haemocytometer. One milliliter of each inoculum (containing  $2 \times 10^6$  spores were used to inoculate potato dextrose agar) in Petri dishes. Five (5) apparently healthy surface disinfected seeds were introduced into the potato dextrose agar plates containing the spores of fungi. The plates were incubated at  $25 \pm 2^\circ\text{C}$  for 7 days. A re-isolation of the fungi was made from the sesame

seeds. The similarities observed in the previous infested seeds and the fungi isolated were compared to that produced in the new experiment. This proved the pathogenicity of the fungi isolated.

**Lipid Content Determination:** This was estimated by the Standard Soxhlet method given by (Chemists and Horwitz, 1980). The Lipid present in the seed was extracted in petroleum ether in Soxhlet extraction apparatus. Two (2) g of each sample (infested seeds and uninfested seeds) were placed separately in whatman filter paper (No.1) in a thimble, the mouth of the thimble was plugged with free absorbent cotton wool. Solvent was added in a dry 250ml receiver flask from the Soxhlet assembly, just to reach the level of the neck. The thimble with sample was introduced into the Soxhlet. The apparatus was placed in a heating mantle with temperature controlling device. The extraction was carried out for eight (8) hours at  $60^\circ\text{C}$ . When the extraction was over, thimble was removed from Soxhlet from the receiver flask. About 250ml solvent along with the extracted lipid was left in the receiver flask, the receiver flask was disconnected. The solvent was then transferred in a clean, previously weighed beaker. After drying in a hot air oven at  $95^\circ\text{C}$ , it was then cooled in a dessicator and weighed. The amount of lipid was measured from extracted per 2 of the sample and amount of lipid as percent of dry matter (DM) was calculated. The above procedures were repeated for sesame seeds infested with each of the nine fungi and non-infested seeds which had previously been surface sterilized served as the control. The results obtained were the mean for 3 replicates. The lipid content of the infested and sterilized seeds were recorded in terms of mean value with Standard deviation.

## RESULTS AND DISCUSSION

The results showed that nine (9) fungal species were associated with the diseased sesame seeds (Table 1). The results of this study showed that fungi were isolated from sesame seeds, indicating that the seed samples were highly infected with pathogens and could cause diseases in seeds. The presence of fungi on sesame seeds is in agreement with (Christensen and Kaufmann, 1974), who stated that fungi were the major cause of spoilage in stored grains and seeds. Species of *Aspergillus*, *Penicillium* and *Rhizopus* are reported to reduce the germination of seeds and damage the seeds in storage. On a general note, Faringada area market had the highest percentage fungal occurrence of 44.3% (Table 2).

Table 1. Frequency of occurrence of fungi from each location.

Fungi Isolated	Location				
	A	B	C	D	E
<i>Alternaria alternate</i>	1.5	1.5	1.5	1.5	1.0
<i>Aspergillus chevalieri</i>	2.0	1.5	1.5	1.0	1.0
<i>Aspergillus niger</i>	1.0	2.0	1.5	1.5	1.5
<i>Aspergillus oryzae</i>	1.5	2.0	1.0	1.0	1.0
<i>Aspergillus flavus</i>	2.0	1.5	2.0	1.0	1.0
<i>Aspergillus terreus</i>	2.0	2.0	1.5	2.0	1.0
<i>Cochliobolus Spp.</i>	2.0	1.0	1.0	1.0	1.0
<i>Geotrichum candidum</i>	1.0	1.0	1.0	1.0	2.0
<i>Phoma Spp.</i>	2.0	1.5	1.0	1.0	1.0

Each value is a mean of three samples. A=Samples from Faringada; B=Samples from Terminus; C=Samples from Chobe; D=Samples from Rukuba Rd; E=Samples from Okene.

Scores based on a scale in which 1= absence of fungus and 2= presence of fungus; therefore any means score above 1 indicates presence of fungi (Ataga and Akueshi, 1986).

Table 2. Percentage incidence of fungi isolated from *Sesamum indicum*.

Fungi incidence	Location					Incidence (%)
	A	B	C	D	E	
<i>Alternaria alternate</i>	6.6	3.6	3.2	2.0	0.0	15.4
<i>Aspergillus chevalieri</i>	10.3	4.2	5.0	0.0	0.0	19.5
<i>Aspergillus niger</i>	0.0	6.1	7.4	2.6	5.2	21.3
<i>Aspergillus oryzae</i>	3.3	4.0	0.0	0.0	0.0	7.3
<i>Aspergillus flavus</i>	3.9	3.1	2.7	0.0	0.0	9.7
<i>Aspergillus terreus</i>	4.8	11.2	2.4	13.0	0.0	31.4
<i>Cochliobolus Spp.</i>	9.5	0.0	0.0	0.0	0.0	9.5
<i>Geotrichum candidum</i>	0.0	0.0	0.0	0.0	3.5	3.5
<i>Phoma Spp.</i>	5.9	2.0	0.0	0.0	0.0	7.9
Incidence/Location (%)	44.3	34.2	20.7	17.6	8.7	-

Each value is a mean of three samples. A=Samples from Faringada;B=Samples from Terminus;C=Samples from Chobe; D=Samples from Rukuba Rd; E=Samples from Okene.

From the result, *Aspergillus niger* had the highest percentage occurrence of 10.3% from the Faringada samples (Table 2). *A. niger* and *G. candidum* were isolated from samples from Okene market, but absent from Faringada samples. All other isolated fungi were present in Faringada. Amongst the fungi isolated were *A. terreus*, *A. niger* and *A. oryzae*, which spoil seeds. These fungi were similarly isolated from groundnut, soybean, sesame and sunflower seeds (Chavan, 2011).

On the other hand, these fungi are known to produce mycotoxins which are harmful for human health. Fungi belonging to the genus *Aspergillus* commonly invade oil-rich seeds and grains, such as peanuts, corn and cottonseed, in which they produce the carcinogenic aflatoxins (Ghafoor and Khan, 1976). From the result, 8 of the 9 fungi caused a decrease in the lipid content of sesame seeds which

showed that these fungi were associated with the deterioration of sesame seeds and cause changes in the lipid content of the seeds. (Table 3).This is in agreement with (Kakde and Chavan, 2011), who found that storage fungi were responsible for the decrease in fat content of oil seeds, as the fungi secrete enzymes necessary to degrade the lipid content of seeds.

The values obtained from changes in lipid content of sesame seeds in this work by *A. niger* (45.0%); *A. flavus* (48.70%); *A. oryzae* (43.50%) and *A. terreus* (45.45%) were within the range of other findings on the changes in oilseeds by *Aspergillus* Spp. (Chavan, 2011) reported 47.0%, 42.3%, 43.3% and 44.0% lipid content in sesame seeds infested with *A. niger*, *A. flavus*, *A. oryzae* and *A. terreus* respectively. However, the value for the control (50.0%) was higher than the 49.35% obtained for this work.

Table 3. Changes (%) in lipid content of *Sesamum indicum* due to fungi.

Fungi	Lipid content (%)
<i>Aspergillus chevalieri</i>	42.40
<i>Aspergillus oryzae</i>	43.50
<i>Aspergillus niger</i>	45.00
<i>Aspergillus terreus</i>	45.45
<i>Alternaria alternata</i>	47.80
<i>Cochliobolus Spp</i>	48.10
<i>Aspergillus flavus</i>	48.70
<i>Phoma Spp.</i>	49.15
<i>Geotrichum candidum</i>	49.40
Sum	419.5
Mean	46.62
Variance	5.95
S/deviation	+2.44

Values are expressed as mean+standard deviation (% mean  $\pm$ SD)

The statistical analysis of the overall mean gave the upper and lower boundaries of 49.05 and 44.17 (95% confidence limit) as acceptable limit. A comparison of individual means shows that the lipid content of samples infested with *A. niger*, *A. terreus*, *A. flavus*, *A. alternata* and *Cochliobolus* with means of 45.0, 45.45, 47.80, 48.10, 48.70% respectively fall within confidence limit). The effect of infestation of the seeds by these fungi was not significantly different in terms of the lipid content. *A. chevalieri* was responsible for maximum depletion of the lipid content (6.95%) lower than the values obtained from the control.

The differences in lipid content of the fungal infected seeds could be mainly due to the influence of their pathways to use the lipid as energy source. This is due to fact that fungi utilize basic compounds of the seeds for their metabolism and growth. Thus there is a need to prevent fungal growth by employing various management techniques to ensure improvement of seed health which ultimately increase crop quality and human health.

#### REFERENCES

Ashri, A. 1998. Sesame breeding. Plant breeding reviews. 16: 179-228.

Association, I. S. T. International rules for seed testing: rules 1996, 1996: International Seed Testing Association.

Ataga, A. and C. Akueshi. 1986. Changes in oil and free fatty acid contents of Sunflower seeds

inoculated with *Alternaria tenuis* Auct., *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme* Sheld. and *Macrophomina phaseolina* (Tassi) Goid. *Phytopathologia Mediterranea*. 44-6.

- Bedigian, D. 2000. Sesame. The Cambridge world history of food. 1: 411-21.
- Bedigian, D. 2012. *Sesame: the genus Sesamum*. CRC Press.
- Chavan, A. M. 2011. Nutritional changes in oilseeds due to *Aspergillus* spp. *Journal of Experimental Sciences*. 2.
- Chemists, A. O. O. A. and W. Horwitz. 1980. *Official methods of analysis*. AOAC Washington, DC.
- Christensen, C. and H. Kaufmann. 1974. Microflora. CHRISTENSEN, CM Storage of cereal grain and their products. St. Paul: American Association of Cereal Chemists. 158-92.
- Ellis, M. and P. Ellis. 1987. Microfungi on Land Plants. Croom Helm Austraria. New South Wales. 289pp. 2-3.
- FAO. 2004. Agricultural Data. In. *In Agricultural Statistics database*. Rome. Italy: Food and Agriculture Organisation of the United Nations.
- FAO. 2010. Production Crops. Sesame seeds. In.: Food and Agricultural Organisation of the United Nations, 150.
- Ghafoor, A. and S. a. J. Khan. 1976. List of diseases of economic plants in Pakistan.
- Kakde, R. B. and A. M. Chavan. 2011. Deteriorative changes in oilseeds due to storage fungi and efficacy of botanicals. *Curr. Bot.* 2: 17-22.
- Mbah, M. and C. Akueshi. 2001. Some Physico-chemical changes induced by *Aspergillus flavus* and *Aspergillus niger* on *Sesamum indicum* and *Sesamum radiatum*. *J. Sci. Agric. Food Technol. Environ.* 1: 65-9.
- Mbah, M. and C. Akueshi. 2009. Aflatoxin in mould infested sesame seeds. *African Journal of Biotechnology*. 8.
- Mcdonald, M. 1999. Seed deterioration: physiology, repair and assessment. *Seed Science and technology*. 27: 177-237.
- Tjai, P. 2006. *Sesame: Higher value oilseed.*: Dover publications.