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Mycodeterioration of Lipids Contents in Sesame Seeds during Storage in Koshi Region of Bihar State

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ABSTRACT

Sesamum indicum L. (Til) is an important source of oil and protein. The quality and quantity of oil and protein is adversely affected by biological agents, which consequently influence the product manufactured by sesamum seed or its derivatives. The health of *sesamum* plant is affected by fungi by causing infection of root, foliage and seeds. Sesame is grown in marginal and submarginal lands. The products and byproducts of sesamum have tremendous value. The sesame seeds yield oil content of 46-52%. Fungi associated with diseased seeds of sesamum indicum L. from markets of kosi region were isolated to the study the deteriorative changes in lipid content. In terms of number abundance *Aspergillus flavus* had the highest percentage occurrence in the market. Visually healthy seeds of *Sesamum indicum* were inoculated with spores of each of the ten fungi isolated from diseased seeds and incubated at 26 $\pm$ 2^0c 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. *A. niger* was responsible for the maximum depletion of the lipid content of the seeds. The result clearly indicate that these fungal species are capable of deterioration in the lipid content in storage.

INTRODUCTION

Sesanum indicum ((Til)), 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 others. It is commonly called Beni seed in English, Ridi (Hausa), Ishwa (Tiv), Eeku (Yoruba), Igorigo (Ebira). The seeds are small, about 3 to 4mm long and 1-2 mm thick. They are ovate in shape, slightly flattered and usually range in colour from white, brown to black). Sesame seeds contain 40-60% oil 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 under storage reduce germination rate and also deteriorate the available carbohydrate, protein, oil content of the seed. During storage condition moisture content is also increased and other biochemical changes takes place (Chavan, 2011). Considering above facts in view emphasis is given on to study the mycoflora of the seeds in this environment and the effect on the lipid content of the seeds.

MATERIALS AND METHODS

Seeds of *Sesamum indicum* were obtained from different sites (A, B,C, D and E) from kosi region Markets. The samples were preserved in plastic bags and stored in refrigerator for further investigation. The standard blotter and agar methods were used for the detection and isolation of fungi (ISTA, 1996). The seeds were surface sterilized with 1% Sodium hypochlorite (NaOCl) solution and rinsed 3 times with sterile distilled water to remove surface contaminants. One hundred seeds were used for each location for the blotter method. The sterilized seeds were plated out on moist Whatman No.1 filter paper in Petri dishes at the rate of 20 seeds per plate. The plates were incubated at 26 $\pm$ 2^0c for 7 days. During the incubation period the seeds were examined regularly (daily) for the evidence of fungal growth. At the end of the incubation period (7 days), the number of infested seeds were recorded as percentage incidence. The following formula was used to record the percentage incidence.

Incidence (%) = No. of infected seeds/Total no. of seeds×100

Results were calculated on the basis of the mean of 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) medium to obtain pure cultures. The cultures were incubated for 7 days at 26 $\pm$ 2^0C, after which they were examined for fungal growth and pure cultures obtained for identification. Based on the morphological characteristics of vegetative mycelium and spores, the fungal flora was identified after the reference of (Ellis and Ellis, 1987).

50 milliliters of sterile distilled water were aseptically poured into each of the pure culture plates of the fungi. A sterile glass road 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 $2x10^6$ spores were used to inoculate potato dextrose agar medium) in Petri dishes. Five apparently healthy surface disinfected seeds were introduced into the potato dextrose agar plates containing the spores of fungi. The plates were incubated at 26 $\pm$ 2^0C 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 of new experiment. This proved the pathogenicity of the fungi isolated.

LIPID CONTENT DETERMINATION:

The lipid content of healthy and infected sesame seeds were 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 heathy seeds) were placed separately in whatman no. 1 filter paper in a thimble, the mouth of the thimble was plugged with free absorbent cotton wool. Solvent was added in a dry 250 ml 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 60^0C. After extraction 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 95^0C, it was then cooled in a dessicator and weighed. The amount of lipid was measured form 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 ten fungi and non-infested seeds which had previously been surface sterilized served as the control. The results obtained were the mean of 3 replicates. The lipid content of the infested and sterilized seeds was recorded in terms of mean value with Standard deviation.

RESULTS AND DISCUSSION

The results showed that 10 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 (Christensen and Kaufmann, 1974). Species of *Aspergillus*, *Penicillium*, *Alternaria*, *Rhizopus*, *Cladosporium and Mucor* were reported to reduce the germination of seeds and damage the seeds in storage.

Table 1. Frequency of occurrence of fungi from each location

Fungi Isolated	**Locations**					**Mean**
	A	**B**	**C**	**D**	**E**	
Alternaria alternata	1.5	1.4	1.6	1.3	1.7	1.5
Aspergillus chevalieri	2.2	1.5	1. 2	1.4	1.7	1.6
Aspergillus niger	2.2	2.5	2.1	2.3	1.9	**2.2**
Aspergillus oryzae	1.5	1.9	1.3	1.2	1.1	1.4
Aspergillus flavus	2.1	1.5	2.4	1.7	1.8	1.8
Aspergillus terreus	2.3	2.1	1.5	2.2	1.8	1.9
Penicillium Spp.	1.1	1.2	1.4	1.5	1.3	1.3
Rhizopus stolonifer	1.1	1.9	2.0	1.2	2.1	1.7
Cladosporium spp.	1.4	1.3	1.9	2.0	2.1	1.7
Mucor mucedo	1.2	1.7	1.3	1.5	1.4	1.4

Each value is a mean of three samples.

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

Fungi Incidence	Locations					
	A	**B**	**C**	**D**	**E**	**% Incidence**
Alternaria alternata	6.6	3.6	3.2	2.0	0.0	15.4
Aspergillus chevalieri	10.3	4.2	5.0	0.0	0.0	19.5
Aspergillus niger	0.0	6.1	7.4	2.6	5.2	**21.3**
Aspergillus oryzae	3.3	4.0	0.0	0.0	0.0	7.3
Aspergillus flavus	4.8	11.2	2.4	13.0	0.0	**31.4**
Aspergillus terreus	3.9	3.1	2.7	0.0	0.0	9.7
Penicillium Spp.	9.5	0.0	0.0	0.0	0.0	9.5
Rhizopus stolonifer	1.0	1.0	1.5	0.0	3.5	7.0
Cladosporium spp.	5.9	2.0	0.0	0.0	0.0	7.9
Mucor mucedo	3.7	4.2	0.0	5.9	0.0	13.8

Each value is a mean of three samples.

From the above table it apparent that *Aspergillus flavus* had the highest percentage occurrence of 31.4% from the market samples which was followed by *A. niger* (21.3), *Aspergillus chevalieri* (19.5), *Alternaria alternata* (15.4) and *Mucor mucedo*(13.8) respectively. Amongst the fungi isolated fungi *A. terreus* (9.7), *Penicillium spp.* (9.5), *Cladosporium spp.* (7.9) and *A. oryzae* (7.3) were found to have less incidence, however *Rhizopus stolonifer* had minimum incidence(7.0). These fungi were similarly isolated from other seeds like groundnut, soybean, 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. From the result all of the 10 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). Kakde and Chavan, (2011) 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.

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

Fungi	**Lipid content (%)**
Aspergillus niger	**49.40**
Rhizopus stolonifer	49.15
Aspergillus flavus	48.70
Aspergillus terreus	48.10
Penicillium Spp.	47.80
Aspergillus oryzae	45.45
Cladosporium spp.	45.00
Aspergillus chevalieri	43.50
Alternaria alternata	42.40
Mucor mucedo	**41.5**
Control	**50.0**
Sum	511
Mean	51.1
Variance	5.97
S/deviation	+2.46

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

The values obtained from changes in lipid content of sesame seeds was found to be maximum by, *Aspergillus niger* (**49.40**) which is followed by *Rhizopus stolonifer* (49.15), *Aspergillus flavus* (48.70), *Aspergillus terreus* (48.10), *Penicillium Spp.* (47.80), *Aspergillus oryzae* (45.45), *Cladosporium spp.* (45.00), *Aspergillus chevalieri* (43.50) and *Alternaria alternate* (42.40)respectively. However, it was observed minimum in case of Mucor mucedo (41.5). The mean was calculate as 51.1, Variance was 5.97 and Standard deviation was +2.46. The value for the control was 50.0%).

CONCLUSION

The statistical analysis of the overall mean gave the upper and lower boundaries of 49.40 and 41.5 (95% confidence limit) as acceptable limit. A comparison of individual means shows that the lipid content of samples infested with *A. chevalieri, A. terreus, A. flavus, A. alternata* and

Cladosporium 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. niger* 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.

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