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Full Length Research Paper

Aflatoxin in mould infested sesame seeds

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Experiments were carried out with two species of seeds of sesame (Benniseed) (*Sesamum indicum* Link, and *Sesamum radiatum* Schumach) inoculated with a storage fungus (*Aspergillus flavus*) previously isolated from seeds of sesame The inoculated seeds were incubated for 10, 15 and 20 day intervals at 30°C. Results showed that *S. indicum* inoculated with the test fungus *A. flavus* and incubated for a period of 20 days showed the presence of aflatoxin B_1 estimated to be 25 ppb. While seeds of *S. rediatum* inoculated with the same test fungus and inculcated for the same length of time did not show any presence of aflatoxin. All the seeds of the two species of sesamum inoculated with the test fungus and incubated for 10 and 15 day intervals showed no presence of aflatoxin. The results portray the danger of consuming infested seeds of sesame which usually appear uninfested to a casual observer when *A. flavus* grows on them and the inherent danger of using such seeds for livestock feed.

Key words: Sesame seed, aflatoxin, Aspergillus flavus, storage fungus.

INTRODUCTION

Sesame seed (S*esamum* sp. Benniseed) is an oil seed grown in Nigeria. The most common and popular species are *Sesamum indicum* Link and *Sesamum radiatum* Schumach. The seed apart from its utilization for the production of vegetable oil is also used in the preparation of a variety of local delicacies and livestock feed. It is also important in pharmaceutical industries.

However, deteriorative micro-organisms (fungi) have constituted a problem to the production and storage of the seeds. Previous works in sesame seeds have indicated the presence of *A. flavus* among other fungi (Mbah and Akueshi, 2000, 2001). These organisms apart from their deteriorative effect on the seeds may produce toxic substances (mycotoxins), the most important of which is aflatoxin, (Alexopoules and Mims, 1979). Sesame seeds infested with *Aspergillus flavus* in most cases especially on *S. indicum* appear normal. *A. flavus* Link ex. Fries has long been shown to produce aflatoxins (Blount, 1961; Sargeant et al., 1961; Bennett and Christensen, 1983). Hartley et al. (1963) showed that four parent aflatoxins, aflatoxins B_1, B_2, G_1, and G_2, are deposited in the substrate food-stuff during fungal deterioration. Experimental data from the various biological actions of aflatoxin in several animal systems consistently indicate that aflatoxin B_1 is the most potent of the four. Aflatoxin B_1 is also found as food contaminant and it is for these reasons that aflatoxin B_1 is in the most studied of the four (Okoye, 1985). Cole (1976) reported that although *A. flavus* is best known for its ability to produce aflatoxins, the fungus is capable of producing other toxins. Hesseltine (1974) cited research suggesting that aflatoxin B_1 may cause liver cancer in humans and animals.

Aletor (1990), reported that aflatoxin B_1 caused liver damage in animals while Asuzu and Shetty (1986) reported that feed contaminated with aflatoxin B_1 caused the death of over 1000 6-weeks old birds in University of Nigeria. Okoye (1984) showed that there is evidence that

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Table 1. Incidence of *Aspergillus flavus* on sesame seeds.

Year	Number of *samples	% of samples with *Aspergillus flavus* above 25%
1995	25	75
1998	20	65
2000	20	60
2002	25	75

**A. flavus* was isolated from every sample. Samples were collected from farmers and markets. Each sample was 100 g.

traces of aflatoxin B_1 circulating in blood may introduce errors in diagnostic blood test. Likewise, Onyemelukwe et al. (1982) reported that aflatoxins have been detected in the blood of Nigerian liver cancer patients.

The proliferation and ubiquity of the toxin producing mould, *A. flavus* on sesame seeds under the warm humid tropical condition in Nigeria led to the design of this investigation which was aimed at finding out if this mould (*A. flavus)* produce aflatoxins in the sesame seeds of which it is usually associated with .

MATERIALS AND METHODS

Seeds of sesame (S. *indicum and S. radiatum*) used in this study were obtained from farmers in Plateau state Nigeria. The study was carried out in the Department of Botany University of Jos, Nigeria.

Inoculum preparation

Pure cultures of *A. flavus* Link ex. Fries previously isolated from sesame seeds were used (Table 1). Fifty millilitres of sterile distilled water containing two drops of Tween 20 (polyoxethylene sorbitan monolaurate) were aseptically poured into each of the plates containing pure cultures of the fungus. A sterile glass rod was used to dislodge conidia from the mycelia in the culture plates. The plates were then rocked carefully to collect all the dislodged conidia. The spore load for inoculation of the seeds was calculated with the aid of a heamocymeter.

Inoculation of the seeds

One millilitre of the inoculum (containing 2 x 10^6 spores) was used to inoculate 10 g of surface disinfected seeds (A.O.A.C 1980) contained in sterilized 100 ml flasks plugged with cotton wool (60 flasks containing either of the seeds were inoculated with the inoculum giving a total of 120 flasks). The inoculated samples were incubated at 30°C for 10, 15 and 20 days respectively.

Aflatoxin extraction

Each set of the sample was milled defatted (A.O.A.C. 1980 method) weighed into 100 ml flasks into which some quantities of chloroform were added. The ratio of the weight of sample and volume of chloroform used in each extraction was 3:1 (6 g of sample was used in each case). The samples were then shaken mechanically at a fixed speed of 100 rpm for 2 h on an orbital shaker. After this the chloroform layer of each sample was collected. The procedure was repeated and extracts from each sample were pooled together and evaporated *in vacuo* using a rotary film evaporator. The resulting residue in each case was redissolved in 0.1 – 1 ml chloroform depending on the quantity of the residue (Howell and Taylor, 1981).

Thin layer chromatography of the extracts

Thin layer chromatographic (TLC) plates of size 20 x 20 x 0.15 cm were used. The plates were coated with silica gel and activated by heating in oven for one hour at 100°C and finally allowed to cool at room temperature in desiccators. The silica gel coated plates were spotted with aflatoxin standards and the already prepared samples at the same level on either side of the plates. After development, the plates were allowed to dry and viewed under long wave lengths (365 nm) ultraviolet (UV) light. Aflatoxin B_1 and B_2 fluorescence bright blue and G_1 greenish blue (Howell and Taylor, 1981).

The aflatoxin standards 5 µg aflatoxin B_1/ml of benzene : acetonitrile (98:2, v/v) prepared from crystaline standard purchased from Makor chemicals, Jerusalem. 10% H_2SO_4 in methanol was used to spray on developed plates. Aflatoxin gives yellow fluorescence under UV light (Romer and Campbell, 1976).

Purification

The detected bands of aflatoxin on the silica gel coated plates were marked under UV light. The required band containing the toxin was scraped and placed into a column of 1 cm diameter containing from bottom to top; glass wool, anhydrous Na_2S0_4(5 g), silica gel for column chromatography (5 g) and anhydrous Na_2S0_4. The silica gel containing the toxin on top of the column was washed down with 10 ml toluene followed by 5 ml (ether : n-hexane 3:1, v/v) and then 5 ml $CHCl_3$ (Howels and Taylor, 1981). This was repeated until the eluate appeared colourless, the clear eluate obtained was allowed to evaporate in a dark cupboard and was redissolved in 3 ml $CHCl_3$. The absorbance of the sample was used to estimate concentration of the toxin by comparing with the absorbance of the known standard and read at 363 nm (Howell and Taylor, 1981).

RESULTS OF DISCUSSION

The presence of aflatoxin B_1 was detected in some of the inoculated seeds of *S. indicum* after 20 days of incubation (Table 2). No aflatoxin was detected in all the seeds of *S. rediatum*. None of the two species of seeds showed the presence of aflatoxin G_1. Four samples out of

Table 2. Incidence of aflatoxin in seeds of *Sesamum* sp. after incubation with *Aspergillus flavus*.

Type of seed	No of samples	Incubation period (days)	No of samples showing presence of aflatoxin B_1	No of samples showing presence of aflatoxin G_1	% of Samples showing presence of aflatoxin	Estimated aflatoxin (ppb)	
						B_1	G_1
Sesamum indicum	20	10	ND	ND	ND	ND	ND
	20	15	ND	ND	ND	ND	ND
	20	20	4	ND	20	25	ND
Sesamum rediatum	20	10	ND	ND	ND	ND	ND
	20	15	ND	ND	ND	ND	ND
	20	20	ND	ND	ND	ND	ND

the 20 samples incubated for 20 days had aflatoxin B_1 contents in excess of 20 ppb, the level adopted by many countries as maximum level of aflatoxin in food stuffs meant for human consumption (Salunkhe and Desai, 1986). The estimated level of aflatoxin detected in this study was 25 ppb.

The absence of aflatoxin in all the seeds of *S. radiatum* in this study may not suggest that the fungus *A. flavus* did not produce aflatoxin in the seeds. On the other hand it may be possible that the level of aflatoxin produced was too small to be detected (Pepeljnjak and Cvetnic, 1984). This may also be a possible reason why aflatoxin G_1 was not detected in all the seeds. Unlike *S. rediatum*, luxuriant growth of *A. flavus* was observed on *S. indicum* which appeared normal to a casual observer. This may be the reason why *S. radiatum* showed no presence of the mycotoxin. Payne et al. (1988) showed that aflatoxin appeared one week after inoculation in their study and generally peaked 7 to 9 weeks after inoculation. It may be possible that after a longer period of incubation mycotoxins may be detected in *S. radiatum*.

The 25 ppb level of aflatoxin detected from seeds of *S. indicum* incubated for 20 days after inoculation with the test fungus indicates the danger inherent in consuming mouldy sesame seeds. Salunkhe and Desai (1986) reported that the hazard is not only confined to direct consumption of foods or foods processed from produce containing aflatoxin. They pointed out that sensitive and reliable tests have shown that aflatoxin was present in the edible tissues and milk of animals fed large amounts of aflatoxin-infested food. Thus consumption of such animal tissues and milk are equally dangerous. Diener et al. (1982) observed that of the mycotoxins that played important roles in human and animal health in the last century, aflatoxin appears to be the most significant threat to modern agriculture. The level of aflatoxin detected in this study calls for serious concern in order to enhance the much talked food security. The dangers are even more here in Nigeria where the warm humid tropical conditions provide conducive environment for luxuriant fungal growth. It is important that farmers and consumers alike would be more educated on possible ways of storage that would not encourage the growth of storage fungi especially *A. flavus* that has always been found associated with sesame seeds and other agricultural produce in order to ensure food security. This would enhance the nutritional qualities of the food and feed consumed by humans and animals respectively and eliminate poisoning by mycotoxins.

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