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Occurrence of Aflatoxin Levels in Harvest and Stored Groundnut Kernels in Kaduna State, Nigeria

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Abstract: *The occurrence of moulds and aflatoxins in groundnut kernels are of great concern to food processors and consumers because of their ability to cause spoilage resulting to economic losses and public health problem such as aflatoxicosis. This study was aimed at determining the presence of Aspergillus species and aflatoxin levels in fresh harvested and stored groundnuts kernels from non-mechanized groundnut oil processors in parts of Kaduna State. Enumeration and identification of Aspergillus spp of groundnut kernels; freshly harvested and stored samples were carried out using standard methods. Aflatoxin levels were determined using Enzyme linked immunosorbent assay (ELISA). The result showed that both harvest and stored products had significantly (P<0.05) higher number of samples containing Aspergillus flavus than Aspergillus parasiticus. The four Aspergillus species isolated in descending order were: Aspergillus flavus, Aspergillus parasiticus, Aspergillus niger and Aspergillus terreus. Though only about 9.02% of the total 260 samples screened had aflatoxin levels above the 20ppb recommended standard limit by Nigeria National Agency for Food and Drug Administration and Control, it was obvious that the persistent detection of moulds and total aflatoxin in this research could be a health threat to both human and animal groundnut products (cake and oil) consumers.*

Keywords: *Arachis hypogea, Aspergillus spp, aflatoxin.*

I. Introduction

Groundnut (Arachis hypogea Link (L.) belong to the family Leguminosae. It is an herbaceous plant grown as an annual crop chiefly because of its kernel. The kernel contain essential oil between 45-55% , high energy, fat-soluble vitamins (A, D, E, and K) and essential fatty acids [1] . It has been estimated that about 1.4million hectares are used for groundnut cultivation in Nigeria [1,2] .

In Northern Nigeria Arachis hypogea is used in the form of groundnut paste for preparation of liquid beverage, vegetable delicacies, groundnut cake and for oil**.** Groundnut kernels have also been processed into ready to eat roasted groundnuts, corn-groundnut snacks and often eaten raw or boiled. Although Arachis hypogea is widely consumed in a variety of food products, however groundnuts have been reported to be associated with growth of certain moulds species which are aflatoxigenic [3]. This aflatoxigenic species are capable of producing secondary metabolites, called aflatoxins. These moulds are of worldwide importance causing problems in agriculture and public health. In agriculture, due to unstandardized methods of processing and storage of agricultural produce, most Nigerian farmers spread their harvest to dry under the sun, which require longer duration for the crop to attain most probable safe moisture level [4,3]. Consequently, these moulds being ubiquitous [5,6,7] in nature, they grow on these groundnut kernels as suitable substrates and subsequently produce secondary metabolites such as aflatoxins [8] when environmental conditions (moisture, temperature and relative humidity) are optimum [9,10] . Aflatoxin contamination of Arachis hypogea is not only a major hazard to both human and animal health but has also been a constraint to groundnuts international trade to Nigeria.

Aflatoxins have been considered to be an important cause of hepatocellular carcinoma **[11]** , a common cancer in developing tropical countries. Studies on aflatoxin revealed that there are four major classes of aflatoxins; B1, B2, G1 and G2 [12]. Fundamentally, aflatoxins are produced by the moulds; Aspergillus flavus which typically produces aflatoxins B1 and aflatoxin B2, and Aspergillus parasiticus which produces aflatoxins G1 and G2 as well as aflatoxins B1and B2. Four other aflatoxins M1, M2, B2A, and G2A are produced in minor quantities [13, 14].

In Nigeria, contamination of groundnut with aflatoxin levels up to 2000ug/kg had been reported [12] with an ample evidence that the inhabitants of sub-Saharan Africa are experiencing heavy dietary exposure to food-borne mycotoxins particularly aflatoxins [13]. A situation that could be responsible for the current public health hazard associated with the prevalence of cancer in humans [15]. This study was prompted by the wide application of groundnut and groundnuts products among the inhabitants of the study area, and thus the need for continuous evaluation of moulds and prevalence of total aflatoxin levels in stored groundnuts in the area.

II. Materials And Method

2.1 Study area / Sample collection site

The study was carried out in Kaduna State located in North West of Nigeria. The study area largely cultivates groundnuts and is a commercial city in the North.

Many non-mechanized groundnut oil processors were identified and registered for the purpose of this study. A dip basket was acquired and each processor`s name was written on a piece of paper and placed in the basket and mixed. Ten processors were then picked from the lot by simple random sampling and were given a numerical code (redesignated number codes 001-010).

2.2 Sample collection

A total of 260 samples of shelled groundnut kernels were collected for both stored and harvest samples using sterilized low density polyvinyl chloride (PVC) bags. The cellophane bags were sterilized by swapping with methylated spirit and were kept under ultraviolet rays before wrapping in a sterilized aluminium foil. A surface point on groundnut sack was disinfected with methylated spirit and allowed to dry. The sack was then punched at the point sterilized with a sterile sampler and aliquots 200g of samples was taken in duplicate into a sterile cellophane bags from the local processors once every week for 6 months for each category. The samples were then taken to the laboratory for analysis.

2.3 Isolation and identification of Aspergillus spp

Fungal isolation was carried out following the Standard Methods of the American Public Health Association [16] APHA & adopted method of [4] with slight modifications. Aliquot ten grams (10g) of the sample were aseptically weighed into 90 ml of sterile physiological saline solution in 250ml sterile conical flask. The mixture was stirred for 5minutes using magnetic stirrer and serial dilutions were prepared. Aliquot of 0.1ml of the 10^{-2} dilution was surface spread on sterile chloronphenicol supplemented potato dextrose agar with sterile bent glass rod and incubated at room temperature for 3-5days.The developed colonies were counted using GallenKamp colony counter and the counts expressed as colony forming unit per gram (CFU/g).

The mould colonies were each teased out a bit with sterile shape pointed needle and placed in a drop of lacto phenol cotton blue already mounted on clean microscope slide. The slide was covered with clean cover slip and examined under microscope for identification according to the methods of [17, 18]. Features observed included spore sizes, colour, arrangement, shape and morphology of the conidia. The mould isolates were further confirmed at Crop Protection Department, Institute of Agricultural Research, Zaria.

2.4 Detection and quantification of total aflatoxin content in groundnut kernels.

2.4.1 Aflatoxin extraction

Representative samples were ground to a particle size of about 250µm using clean Nakai blender 446. Aliquot (20g) ground portion of the sample was weighed out and added to 100mls of extraction solvent (70% methanol; prepared by adding 30mls of distilled water to 70mls of absolute methanol) in a clean bottle with cover. The mixture was shaken for five minutes and allowed to settle. The suspension was filtered through whatman number one filter paper. The filtrate was kept in a tight container for detection and quantification of the total aflatoxins.

2.4.2 Aflatoxin detection and quantification

Total aflatoxin was detected and quantified using Enzyme Linked Immunosorbent Assay (ELISA) kits obtained from Helica Biosystems Inc,U.S.A. The manufacturer's instruction was strictly followed. One dilution well was placed in a micro well holder each for standard and samples to be tested. An equal number of antibody coated micro titer wells were placed in another micro well holder. Then 200µl of the conjugate was dispensed into each dilution well followed by 100µl of each standard and the sample extracts was added to appropriate dilution well containing conjugate. This was then mixed by priming at least three times and the corresponding antibody coated micro titer well was also added to 100µl of each content of the dilution well and was incubated at room temperature for 15minutes.The contents of the micro wells were decanted and washed with distilled water. The washing was repeated five times. The micro wells were then taped face down on a layer of absorbent towel to remove residual water. One hundred micro litres (100µl) of the substrate was added to each micro well and incubated at room temperature for five minutes. In addition 100µl of stop solution was added in the same sequence and at the same pace as that of the substrate. Finally, the optical density (OD) of each micro well was read with a micro titer plate reader using a 450nm filter.

III. Result And Discussion

Table 1 presents the percentage occurrence of Aspergillus spp isolated from groundnut kernel from ten non-mechanized processors. The dominant Aspergillus spp in decreasing order were Aspergillus flavus,

Aspergillus parasiticus, Aspergillus niger and Aspergillus terreus. However, mean count of stored samples showed significant higher count than harvest samples. Highest mould growth from the non-mechanized processors was observed in samples collected from harvest samples of manufacturer 004. The high moulds contamination especially from manufacturer 004, may be related to unavailability of proper storage structures as some bags were seen stacked on bare floor of the store house during the course of sample collection. The presence of Aspergillus spp on these products might possibly be due to contamination of the groundnuts kernels right from the farm due to broken pods, uncontrolled condition of sun drying before sacking and storage as reported by [19] hence the high occurrence of the genera and total aflatoxins as was seen from these research.

Although, control of kernel moisture plays great roll in controlling Aspergillus species [20] , the high 14.8 and 13.5% (data not shown) detected from fresh and stored sample respectively could be responsible for the high moulds count. In addition, the production of spores by these molds on dried food products makes it possible for the microorganism to survive, since their spores are to some extent more resistant to dry conditions than the vegetative mycelia as reported by other researchers [21,4] consequently, the contamination on groundnut as substrate.

Aspergillus flavus had significantly ($p<0.05$) higher occurrence on both harvest and stored kernels from the different non-mechanized processors than Aspergillus parasiticus. This report is similar to the findings of [22] on pistachio nuts and the report of [4] . However, contrary to the reports of [23] who registered Aspergillus parasiticus to have more occurrence than Aspergillus flavus. Though factors such as moulds strains, or probably growth climatic stress factors were not investigated during this research, they could be responsible for these differences. More so, these differences could be probably due to the variety of the different samples, since there are resistant forms of groundnut kernels, or due to population advantage, since both organisms are not good competitive mycoflora as reported by [4]. All the same, the implication of the two principal organisms and some strains of Aspergillus niger in aflatoxin production in Arachis hypogea have been reported by many researchers; [24] (Akano and Atanda, 1990; [25] Ndiaye et al.,1999; [26] Koirara et al.,2005; [27] Groopman et al.,2008, [14] Carlos et al.,2009).

This study has demonstrated that the presence of the main aflatoxin producing organisms, Aspergillus flavus and Aspergillus parasiticus was consistent on the groundnut samples. In line with this, since 90-95% of these natural strains of Aspergillus parasiticus produce aflatoxins [7] , the Aspergillus species contaminants obtained from this research therefore could be useful in assessing the wholesomeness of nuts and prevention of incidence of aflatoxicosis.

Table 1 The percentage occurrence of Aspergillus spp isolates of groundnut kernel from ten local processors.

Aspergillus spp	Harvest(%)	Storage(%)	n(%)
Aspergillus flavus	20.3	44.7	65.0
Aspergillus parasiticus	10.1	10.2	20.3
Aspergillus niger	1.5	5.5	7.0
Aspergillus terreus	0.2	1.0	1.2
Values are means for eight determinations based on eight weeks sampling. n(%), % positive			

Fig.1 presents incidence of Aspergillus spp and aflatoxin on groundnut kernels surveyed in Kaduna state. The highest aflatoxin value was seen in harvest sample (28.75%) collected from processor 004. However, the mean total aflatoxin level of stored samples showed significantly ($p<0.05$) higher level than harvest samples. The mean total aflatoxin levels of groundnut kernels from the processors were in the range of 6.00ppb to 28.75ppb. Though the mean total aflatoxin levels detected from mechanized processors (control, data not shown) and on most samples from all the non-mechanized manufacturers were within the limits recommended by the Nigerian National Agency for Food and Drug Administration and control (NAFDAC) (20ppb), only 9.20% of the later total samples were above the (NAFDAC) limit.

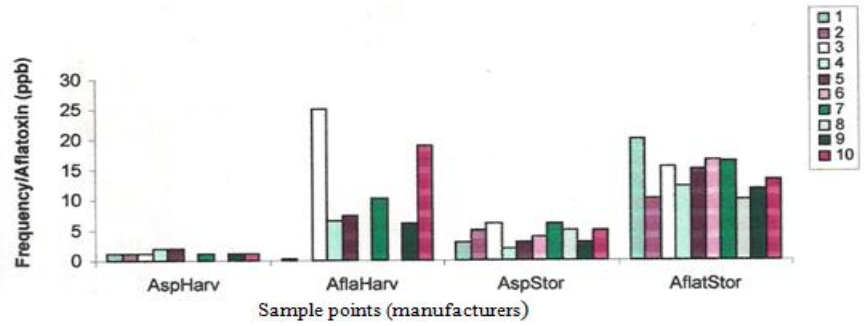

Fig.1 Incidence of Aspergillus spp and aflatoxin levels of groundnut kernels for ten different manufacturers

Key
AspHarvest- Aspergillus spp on harvest groundnut kernels
AspStorage- Aspergillus spp in stored groundnut kernels
AflHarvest- aflatoxin in harvest groundnut kernels
AflaHarvest- Aflatoxin in stored groundnut kernels
1-10 -non-mechanized processors

However, [27] reported the deleterious impact of low levels of aflatoxin in a diet can be a threat to human and animal health. The inability to detect aflatoxin from mechanized processors samples was largely due to standardized procedures; moisture control, good storage system, crushing the kernels before use and probably due to the absence of aflatoxigenic strains. On the same hand, it might be that the conditions were not adequate for aflatoxin production as reported by [28]. The total aflatoxin positive contamination by the processors out of 260 samples showed a prevalence of 28.4%.

Therefore, the detection of total aflatoxins from the nuts investigated by this research means that such mycotoxins could be inoculated into groundnut products. This of course constitutes health hazard to the consumers of groundnut and its products such as groundnut cake and oil when the processing method is not mechanized.

IV. Conclusion

Sufficient evidence from this survey showed that some samples were within aflatoxin regulatory standard ranges especially samples from mechanized processors. However, the 28.4% prevalence as was recorded in this research could suggest that human population in the study area subsisting on groundnut products (cake and oil) could be at risk of consuming aflatoxin contaminated kernels, and thus the need for continuous evaluation and government intervention through the supply of improved resistant seeds and good storage facilities to farmers.

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