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Evaluation of Aflatoxin in *Gossypium hirsutum* (Cottonseeds) and *Arachis hypogaea* (Peanuts)

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Abtstract
A total of ten (10) samples, five (5) samples each of peanuts and cotton seeds were obtained for analysis from different locations/markets in Funtua, Katsina state, Nigeria. The analyses include mould isolation and aflatoxin extraction was carried out to isolate the major aflatoxigenic fungi and identify the type of aflatoxin present in the samples. *Aspergillus flavus* was present in only 1(20%) cottonseed and 4(80%) peanut samples. Other fungal species isolated were *Aspergillus niger* in 3(60%) peanut samples and *Rhizopus* spp. in all the 5(100%) cottonseeds and 3(60%) peanut samples. The samples were defatted and aflatoxin extraction was carried out using chloroform and water (100:10) as the extraction solvent. The extracts were concentrated using rotary evaporating machine and characterized using Thin Layer Chromatography (TLC) technique. Aflatoxin was detected in eight (8) samples (five samples of cotton seed and three samples of pea nut).
Keywords: Aflatoxins; Moulds; Thin Layer Chromatography

INTRODUCTION

Arachis hypogaea, Peanut (groundnut), is an important and commonly grown legume crop in Nigeria. While *Gossypium hirsutum* (cottonseed) is a popular and excellent feed for dairy animals due to its high level of fat, protein (20%), crude fiber (22%) and TDN (87%) in a compact package (Lane *et. al.* 2012; Ramon *et al.,* 2013). Whole cottonseed and Peanut are used for variety of purposes; they are very importants for use as feed or to be crushed for oil. Agricultural commodities implicated with aflatoxin are peanuts (groundnuts), barley, beans, cottonseeds, rice, wheat, copra, cassava and peas (Flor *et al.,* 2002; Tijjani *et al.,* 2013).

Aflatoxin is the most important type of mycotoxins usually produced by the aflatoxigenic Aspergilli. Studies have revealed that there are four major aflatoxins: B1, B2, G1, G2, plus M1 and M2 (Cornell, 2015). The Aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2) are produced by *Aspergillus flavus*, while all four isoforms (B1, B2, G1, and G2) are produced by *Aspergillus parasiticus* (Bennett and Klich, 2003).

Aflatoxin can be a problem in feeding animals with whole cottonseed and peanut. The worldwide contamination of foods and feeds with mycotoxins is a significant problem. Often more than one mycotoxin is found on a contaminated substrate (Ashiq *et al.*, 2014). Plants under stress (drought, insects, improper handling) can develop high levels of aflatoxin due to fungal infestation. The dairy cow is very efficient in converting aflatoxin B in the plant to aflatoxin (M1 and M2) in the milk, so every load of milk and diet must be tested for aflatoxin to ensure safety (Lane, 2012).

Aflatoxins are known to be the causes of acute aflatoxicosis in humans, more chronic disease such as hepatocellular carcinomas and other human maladies (CAST, 2013). It is estimated that aflatoxins cause between 5% and 30% of all liver cancer in the world, with the highest incidence of 40% occurring in Africa (CAST, 2013). An estimated 4.5 billion people in developing countries may be exposed chronically to aflatoxins through their diets (Willey *et al.*, 2011). The aim of this work is therefore to evaluate groundnut and cotton seeds for the presence of moulds and aflatoxins in Funtua markets, Katsina State, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of ten (10) samples Five (5) each of the peanuts and cottonseeds were obtained for analysis from different locations/markets within Funtua metropolis (part of Katsina State known to produce groundnuts and cotton). These samples were transported in polythene bags for analysis (Tijjani *et al.,* 2013; Sule *et al.,* 2014) to the Department of Microbiology Laboratory at Umaru Musa Yar'adua University, Katsina for analysis.

Mould Isolation and Identification

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instruction. The fungal isolation was carried out by placing one seed at the centre of each media plate and one seed on each quadrant of the plate. This was done for all the ten (10) samples i.e. each plate was inoculated with five (5) cottonseeds/peanuts. The plates were labeled accordingly and incubated at room temperature for 5 days. Moulds were isolated and sub-cultured to obtain a pure culture and identified according to (Mukhtar *et al.*, 2010).

Defatting of Samples

Samples of the cottonseeds and peanuts were ground using a clean mortar and pestle. The samples were defatted by suspending 30 g of the ground samples in petroleum ether for four (4) hours and then dried-off by heating in hot air oven for 30 minutes (Jones, 1972; Wilson, 2015).

Aflatoxin Extraction

Ten grams (10g) of the defatted sample was measured into conical flasks. A volume, 100:10 milliliter (v/v) chloroform: water was added to each sample and mixed for 30 minutes using an electric Stuart flask shaker. The samples were allowed to settle and filtered while the residues were discarded. The filtrate was then concentrated using a rotary evaporator and the concentrate was finally poured into clean bijou bottles and kept in refrigerator for further analysis (PACA, 2016; Sule *et al.*, 2014).

Determination of Aflatoxin content

Aflatoxin was determined using Thin-layer Chromatography (TLC) technique as described by Shamsuddeen and Kabir (2015) and Jones (1972). The chromatogram was ran with 97ml of chloroform and 3ml of methanol (i.e. chloroform : methanol mixture at 97:3ml) as the chromatographic solvent for the TLC. Following the TLC the plates were illuminated using ultraviolet (UV) light after evaporation of he solvent. Blue and green fluorescent spots were observed under the UV lamp to detect the aflatoxin present. The aflatoxin Retention Factor (R_f) values were estimated using the mathematical relation formulae below (Srisit, 2016).

$$R_f = \frac{\text{Distance moved by substance(spot)}}{\text{Distance moved by solvent (solvent front)}} = \frac{DMS}{SF}$$

RESULTS

Isolation of Mould from Cottonseed and Peanut samples

The result of the study (Table 1) reveals that, only one cottonseed sample (C2) was found to be infested by *Aspergillus flavus* and no other *Aspergillus* specie was isolated in the cottonseed samples. All cottonseed samples were found to be contaminated with *Rhizopus* spp. Out of the total five samples of the peanuts, *Aspergillus flavus* was found to be present in four (4) samples, *Aspergillus niger* in three (3) samples and *Rhizopus* spp. in three (3) samples as well. Only one sample (P1) was found to be infested with all the three fungal species. *Rhizopus* spp. was predominantly found present in the samples.

Thin Layer Chromatography Result and Type of Aflatoxin Detected

From Table 2, it can be inferred that aflatoxin was found to be present in five (5) samples cotton seeds and three (3) samples of peanuts

Number and Percentage Occurrence of Fungi and Aflatoxin

Table 4 shows that, *Aspergillus flavus* was present in only 1(20%) cottonseed and 4(80%) peanut samples, *Aspergillus niger* in 3(60%) peanut samples and *Rhizopus* spp. in all 5(100%) cottonseeds and 3(60%) peanut samples.

Aflatoxin B was detected in all 5(100%) of the cottonseed samples and 3(60%) of the peanuts. Among the peanut samples, aflatoxin G was detected in 2(40) of the samples, in addition to the Aflatoxin B.

Table 1: Isolation of Mould from cottonseed and peanut samples

Sample	*Aspergillus flavus*	*Aspergillus niger*	*Rhizopus* species
C1	-	-	+
C2	+	-	+
C3	-	-	+
C4	-	-	+
C5	-	-	+
Sub total	01(20%)	00(0%)	05(100%)
P1	+	+	+
P2	-	-	+
P3	+	+	-
P4	+	-	+
P5	+	+	-
Sub total	04(80%)	03(60%)	03(60%)
Total	**5(50%)**	**03(30%)**	**08(80%)**

KEY: C= Cottonseed P= Peanut += Present - = Absent

Table 2: Thin Layer Chromatography Result and Aflatoxin Type Detected in the samples

Sample	DMS (cm)	Solvent front (cm)	Retention factor (R_f)	Aflatoxin detection	
				Fluorescence	Aflatoxin type
C1	5.4	10	0.54	Blue	Aflatoxin B
C2	5.1	10	0.51	Blue	Aflatoxin B
C3	5.1	10	0.51	Blue	Aflatoxin B
C4	5.1	10	0.51	Blue	Aflatoxin B
C5	4.6	10	0.46	Blue	Aflatoxin B
P1	ND	ND	ND	ND	ND
P2	ND	ND	ND	ND	ND
P3	3.7; 6.3	10	0.37; 0.63	Green and Blue	Aflatoxin G; Aflatoxin B
P4	1.0; 4.3	10	0.1; 0.43	Green and Blue	Aflatoxin G; Aflatoxin B
P5	4.0	10	0.40	Blue	Aflatoxin B

KEY: C= Cottonseed P= Peanut DMS= Distance moved by substance (spot) ND= Not detected

Table 3: Percentage Occurrence of Fungi and Aflatoxin Detected in Cottonseed and Peanut

Samples	Mould isolates			Aflatoxin	
	A. flavus	*A. niger*	*Rhizopus*	Aflatoxin B	Aflatoxin G
Cottonseeds	1(20%)	0(0%)	5(100%)	5(100%)	0(0%)
Peanuts	4(80%)	3(60%)	3(60%)	3(60%)	2(40%)
Total	5(50%)	3(30%)	8(80%)	8(80%)	2(20%)

DISCUSSION

From mould isolation, one cotton seed and three peanuts samples yielded *Aspergillus flavus*, the producer of aflatoxins. This is not unexpected as it is common for a substrate to be contaminated with more than one fungus at the same time or fungi to colonize a substrate in succession since the conditions may favour one species over another as reported in (CAST, 2013).

However, aflatoxin was still detected in all samples of the cottonseeds. Mazen *et al.*, (1991) reported *A. flavus* to be the most common species encountered in cottonseeds. Conversely, the presence of *Aspergillus flavus, Aspergillus niger* and *Rhizopus* spp. in high proportions of the peanut samples shows the extent of contamination and food safety hazard that maybe posed on the consumers when ingested. This result is in conformity with the work of Patricia *et al.*, (2013) who also reported the presence of these fungi (*Aspergillus flavus, Aspergillus niger* and *Rhizopus* spp.) in peanut samples.

Presence of *A. flavus* and aflatoxins in peanuts is in line with the findings of Embaby and Mona (2014) who reported the presence of *Aspergillus flavus*, *A. niger and Rhizopus* spp. and aflatoxin in peanut samples. In one sample (P2), neither aflatoxin nor any aflatoxigenic fungus was isolated. Thereby indicating that; aflatoxin was not produced due to the absence of the aflatoxigenic fungi in the sample.

Conversely, four (4) samples of the cottonseeds (C1, C3, C4 and C5) do not habour *A. flavus* but aflatoxin was still detected in them. This could be due to the fact that spores of the aflatoxigenic *Aspergilli* (notably, *A. flavus and A. parasiticus*) might be present but not evenly distributed as reported by Wilson, (2015) or the spores might have being cleared away during handling. It could also be because *Aspergilli* present in the sample were no more viable at the time of culture/isolation, or, other species (which may have not being isolated/identified) might have produced the aflatoxin present as reported by (Zain, 2011; Fonseca, 2012; Patricia *et al.*, 2013).

With regards to the Retention factor (R_f), the R_f values of aflatoxin G tend to be lower than those of aflatoxin B obtained from a single extract. Similar pattern was reported in Jones, (1972).

Few literatures also reported the production of aflatoxins by some other rare aflatoxigenic species such as the *Rhizopus* spp. and *A. niger* (Frank, 1970) (Claude and Muarice, 1979) (Peterson *et al.*, 2001). It is therefore possible that this aflatoxin might have been produced by these non-aspergillus fungi (*Rhizopus* spp. and *A. niger*). The *Aspergillus niger* present in the samples may indicate the possible presence of other toxins such as ochratoxin (Zain, 2011; Ashiq *et al.*, 2014) or even the production of aflatoxin itself, though this is rare (Zain, 2011).

CONCLUSION

Gossypium hirsutum (cottonseed) and *Arachis hypogaea* (peanuts) were found to be infested by *Aspergillus flavus* along with other contaminating fungi (*Rhizopus* spp. and *A. niger*) with the presence of aflatoxin B in eight samples 8(80%) and Aflatoxin G in few samples 2(20%).

RECOMMENDATIONS

From the result of this research, the following are highly recommendations are made:

i. Emphasis should be laid on proper harvesting, handling and storage of cottonseeds and peanuts by maintenance and monitoring of food storage facilities and ensuring strict and good hygiene practice. Other environmental factors such as temperature and humidity should be monitored.
ii. Aflatoxin content of food products such as peanuts, cottonseeds and animal feeds should be adequately assessed before being released to markets for sale or supplied for consumption.
iii. Public awareness on the risks associated with aflatoxins, its control and preventive measures should be intensified most especially to those people dealing with the usage, harvesting and consumption of these cottonseeds and peanuts.

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