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EVALUATION OF MOULDS CONTAMINATION OF CEREALS AND LEGUMES SOLD IN DUTSINMA METROPOLIS AND THEIR AFLATOXIN PRODUCTION POTENTIAL

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ABSTRACT
Contamination of food materials by aflatoxigenic moulds put the health and well-being of consumers of such food materials at risk of hepatocellular carcinoma. The aim of this study was to isolate and identify moulds capable of producing mycotoxins from maize millet, guinea corn, groundnut and groundnut cake sold to consumers within Dutsinma metropolis, Katsina State of Nigeria. Fungal isolation was done using the direct plating of serially diluted samples of the cereals and legumes, and direct plating technique of surface-sterilized samples of same on potato dextrose agar. Fungi colonies were purified by sub-culturing on fresh potato dextrose agar. The Isolates were identified by comparison of colony morphology and microscopic characteristics with standard reference guide for identification. Determination of aflatoxin producing potential of fungal isolates was carried out using fluorescence under UV-light at 365nm. A total of 124 fungi were isolated and identified. Fifty five (55) of the isolates were identified as *Aspergillus Spp.*, giving a prevalence of 44.4%, and 69 as non-aflatoxigenic moulds (55.6%). Among the *Aspergillus*group, *A.niger* had the highest prevalence (35.0%), followed by *A.flavus* (31.7%), *A.fumigatus* (15.0%). *A.lentulus* (10.0%) were less prevalent. Determination of aflatoxin production potential using UV-light, showed that 15 isolates fluoresced blue, 21 fluoresced green and 15 fluoresced blue/yellow (reverse plate), indicating potential production of various toxins including aflatoxins B1, B2, G1, and G2. The results showed that cereals and legumes sold in Dutsinma metropolis were highly contaminated with *Aspergillus Spp.*, which showed potential for aflatoxin production.

Keywords*: Evaluation, Aflatoxin, Potential, Moulds, cereals.*

INTRODUCTION

When food safety issues are raised, more attention is paid to perceived problems of pesticides or other man-made chemicals (heavy metals), bacterial and viral food contamination, antibiotic resistance and environmental degradation (Byloos, 2011). However, natural toxins such as mycotoxins produced by a range of fungi (moulds), are equally of great importance to food safety as they are potent toxins and carcinogens and therefore a great threat to food safety as manmade chemicals (WHO, 2018).

Mycotoxins are natural secondary metabolites produced by fungi on agricultural commodities in field during production and storage and processing, under certain environmental conditions (Williams *et al*., 2004; Michael, 2013). They are common contaminants of a number of staple foods, including maize, ground nuts, rice and sorghum. The toxins are widely distributed in nature and pose serious public health hazards to humans as a result of their toxic, teratogenic, mutagenic, and highly carcinogenic properties (IARC, 2002; Williams *et al*., 2004). In USA the Food and Drug Administration (FDA) agency has implemented regulations that required special attention to the management of the fungi contamination. It therefore behooves on the producers and other stake holders to avoid mould contamination of their produce as this might constitute huge losses and health hazard (Mady, 2001).

The Food and Agriculture Organization (1997) estimated that mycotoxins contaminate 25% of agricultural crops worldwide, however, only a few mycotoxins particularly those affecting cereals are considered to be significant to humans. The food borne mycotoxins that are of great significance to human health in tropical developing countries are the fumonisins and aflatoxins (COT, 1997). Consumption of mycotoxin contaminated grains kills hundreds of people every year in developing countries (Kung'u, 2005). The most important mycotoxins in terms of economic importance, incidence and toxicity in agricultural products are aflatoxins, deoxynivalenol, fumonisins, ochratoxins and zearalenones produced by *A. flavus, A. parasiticus, F. moniliforme, A. ochraceus* and *F. graminearum*, respectively (FAO, 1999).

Two closely related species of fungi are mainly responsible for producing the aflatoxins of public health significance: *Aspergillusflavus* and *A. parasiticus*. (WHO, 2018). Under favourable conditions typically found in tropical and subtropical regions, including high temperatures and high humidity, these moulds, normally found on dead and decaying vegetation, can invade food crops. Drought stress, insect damage and poor storage can also contribute to higher occurrence of the mould seven in temperate regions (WHO, 2018).

Several types of aflatoxin (14 or more) occur in nature, but four – aflatoxins B1, B2, G1 and G2 are particularly dangerous to humans and animals as they are more potent toxin and have been found in all major food crops; but most human exposure comes from contaminated nuts, grains and their derived products(WHO, 2018).At the farm level, the real problem is that mycotoxin contaminated cereals and groundnuts may appear just like the normal grains without any outward physical signs of fungal infection. Ingestion of high doses of such mycotoxin contaminated cereals and groundnuts can result in acute aflatoxicosis which manifest in hepatotoxicity (Fung and Clark, 2004). Aflatoxins have been implicated in human health disorder including hepatocellular carcinoma, Rey syndrome and chronic hepatitis (IARC, 2002; Williams *et al*., 2004; WHO, 2018). This research was aimed at detection of aflatoxigenic moulds in some cereals and legumes and their products, sold in Dutsinma metropolis.

MATERIALS AND METHODS

Sample collection

A total of sixty (60) samples comprising millet, maize, guinea corn, groundnut and groundnut cake, twelve (12) each were collected from different shop outlets within Dutsinma metropolis, Katsina State in clean, freshly opened polythene bags and transported immediately to the laboratory and stored at ambient temperature until ready to be analyzed.

Isolation and identification of fungi

Ninemillilitres (9ml) of distilled water was dispensed into 20ml test-tubes, covered with aluminum foil sheet and sterilized at 121^{o}C for 15 minute in the autoclave.

Each of the maize, millet, guinea corn, ground nuts and ground nut cake were aseptically pounded using mortar and pestle, and 1gram of each was weighed and dispensed into a separate test-tubes containing 9ml of sterile distilled water (Abubakar, 2017). Serial dilution was done and 1ml of each dilution was inoculated on Potato dextrose agar (PDA) plates prepared according to manufacturer's instruction to which streptomycin (0.5g/ml) was incorporated (i.e. 1ml of Streptomycin-Sulphate to each 99ml of PDA). The inoculated plates were incubated upright in a locker at ambient temperature for 7-14 days for possible colony growth.

All observed colonies were sub-cultured on PDA to obtain pure colonies. The growth rate (time taken for appearance of fungal colonies on culture plates) and colony pigmentation of each sample was examined macroscopically.

Microscopy

Tease mount using lacto-phenol cotton blue was adopted (Cumitech II, 1980). One drop of Lacto-phenol cotton blue was placed on a clean glass slide and a portion of the fungal colony was collected aseptically using a sterile straight wire and was mixed with the lacto-phenol blue on the slide and a cover slip was used to cover the preparation. The prepared slide was placed on the stage of the Microscope and viewed using X10 and X40 objective lens of the microscope.

Morphological characterization was done based on colony pigmentation and morphology of the conidia head. Viewed moulds were compared to standard reference keys (ATLAS) for possible identification.

Detection of aflatoxigenic moulds using ultraviolet (UV) light

The black light or bright greenish-yellow fluorescence (BGYF) test based on the BGYF observed under ultraviolet (UV) light at 365nm is a presumptive test used to identify pathogenic fungi, bacterial and moulds (Yabe *et al.,* 1987). The plates containing the fungi colonies were placed in dark room and the UV-light was switched on and held 4 to 5inches away from the plates. The plates were gently opened and exposed to the UV-light for about five or less minute. The samples were inspected under the black-ray lamp in the dark by shining a long wave black light for a characteristic bright greenish-yellow (BGY) fluorescence or bluish-green fluorescent band presence of fluorescence is presumptive of Aflatoxin.

RESULTS

The observable features of mould isolates of the genus Aspergillus based on colonial morphology and microscopy and the identified moulds are presented in Table 1 below. Members of the genus identified in this were *Aspergillus .flavus, A. niger, A. fumigatus* and *A. lentulus.*

Table 1: Identification of moulds by Colony and microscopic appearance

S/N	Colony Description	Microscopy	Probable identity
1	Light green or olive green with whitish margin.	Septate and aerial hyphae bearing long conidioopore.	*Aspergillusflavus*
2	Brown to black colonies terminate in vessels	Long conidiophores arising from septate hyphae.	*Aspergillusniger*
	Light green and powdery colonies	Having central vesicles completely covered with conidia	
3	Smokey grey-green and powdery growth with a reverse white, pale-yellow colony	Short conidiophores and septate hyphae	*Aspergillusfumigatus*
4	white with interspersed grey-green patches of conidia	conidial heads are short, columnar and uniseriate	*Aspergilluslentulus*

The prevalence of *Aspergillus* isolates in the various samples is shown in Table 2.*Aspergillusniger* was shown to prevalent in all types of samples (i.e. maize, millet, guinea corn, groundnut and groundnut cake). *A. flavus* also occurred in all sample types except groundnut cake. A greater prevalence of *A. niger* and *A. flavus* was observed in millet samples (50% each). Two other genera of moulds were also prevalent in the study including Mucor spp. and Rhizopus spp.

Table 2: prevalence of Aspergillus species in cereals and legumes sampled from Dutsin-Ma Wednesday market

S/N	***Aspergillus***	**No. and percentage of samples positive (Prevalence)**					**Total**
	Spp	**Maize**	**Millet**	**Groundnut**	**G/nut cake**	**G/Corn**	**Prevalence**
		(n=12)	**(n=12)**	**(n=12)**	**(n=12)**	**(n=12)**	**(n=60)**
1	*A.lentulus*	3(25.0)	3(25.0)	-	-	-	6(10.0)
2	*A.niger*	3(25.0)	6(50.0)	6(50.0)	3(25.0)	3(25.0)	21(35.0)
3	*A.flavus*	3(25.0)	6(50.0)	3(25.0)	-	3(25.0)	19(31.7)
4	*A.fumigatus*	3(25.0)	3(25.0)	-	-	3(25.0)	9(15.0)
5	*Mucorspp*	12(100)	6(50.0)	6(50.0)	6(50.0)	12(100)	42(70.0)
6	*Rhizopusspp*	6(50.0)	3(25.0)	9(75.0)	3(25.0)	6(50.0)	27(45.0)

G/nut - Groundnut; G/Corn - Guinea corn

The study shows the various colours of fluorescence observed for *Aspergillus Spp.* isolated from the various samples analyzed and the type of Aflatoxin associated with such fluorescence under UV-light at 365nm. Fifteen isolates each showed blue and blue/yellow fluorescence, while 21 isolates were observed to fluoresce green under UV. These colours are affiliated with production of various mycotoxins, including aflatoxin B1, B2, G1, and G2 etc. as shown in Table 3.

Table 4: Aflatoxin detection using Fluorescence of *Aspergillus Spp.* under UV-light and the prevalence of associated toxin.

Type of fluorescence	No. of isolates showing fluorescence by sample type					Total(%) prevalence	Presumptive Aflatoxin
	Millet	Maize	Groundnut cake	Guinea corn	Groundnut		
Blue	6	6	-	3	-	15(25.0)	Gliotoxin, terrain, auranthine & Cyclopiazonic acid.
Green	6	3	3	3	6	21(35.0)	OTA, ZON and AFB1
Blue/yellow (reverse plate)	6	3	-	3	3	15(25.0)	AFB1, AFB2, AFG1 and AFG2

Key: AFB1 and AFB2 (Aflatoxin Blue1 and Blue2), AFG1 (Aflatoxin Green1), OTA (Ochratoxin), ZON (zearalenone).

DISCUSSION

The occurrence of *Aspergillus spp*. in the various samples could be attributed to factors such as temperature, humidity and storage conditions which might have permitted improper drying of the samples thus predisposing them to the moulds at pre-harvest stage in the field and post-harvest stage in storage (Okoth *et al.,* 2012). Growth requirements of the Genera of *Aspergillus* (i.e. ability to grow on minimal medium or nutrients) suggest greatly of their presence in the analyzed samples. Bankole *et al.,* (2003), asserted that the ability of moulds to grow on minimal media is responsible for their successful contamination of stored grains. The results in this study agrees with the findings of Muthomi *et al*. (2012) where higher *Aspergillus* spp. isolation frequencies were recorded in grain/cereal samples from the semi-arid region than those from the humid. This can be attributed to the poor storage and preservation condition or the unhygienic handling of grains.

Michael, (2013) asserted that, contamination of crops and toxin production are particularly likely to occur in subsistence farming communities in tropical and sub-tropical regions with high temperatures and humidity. These environmental conditions, in addition to the moisture content of plants, are important factors in determining growth of, and toxin production by, these moulds.

Contamination of crops with aflatoxins is also likely to occur in communities and regions where food drying and storage facilities are suboptimal, the resources, technology, and infrastructure necessary for routine food monitoring are absent or inadequate, and regulations to control exposure to the moulds are either non-existent or unenforceable in practice (Strosnider *et al*., 2006; Liu and Wu, 2010; Liu *et al*., 2012).In addition, storage, particularly prolonged storage, of crops in hot and humid conditions promotes growth of the aflatoxin-producing fungi and accumulation of the toxin (Strosnider *et al*., 2006: Liu *et al.,* 2012). These conditions as explained above apply to this study area.

A variety of factors influencing invasion of fungi include stress conditions such as drought (Sanders and Fitter, 1992), high temperature (Charles, 2014), insect damage and plant diseases predispose maize to contamination by mycotoxigenic fungi (Laura and Allen, 2009). Consumption of the mouldy grains, exposes the consumers to the mycotoxins and their associated health complications (WHO, 2018).

The detection of aflatoxin production potential of isolates using UV-light revealed various fluorescence by the isolates, including blue, green or blue/yellow suggesting positive potential for mycotoxin production. Blue fluorescence is associated with Aflatoxin B1, Gliotoxin, terrain, auranthine and Cyclopiazonic acid production (Abdel Hameed, *et al.,* 2012). Green fluorescence suggest production of OTA (Ochratoxin), ZON (Zearalenone) and Aflatoxin-B1(Abdel Hameed, *et al.,* 2012).The blue/yellow (reverse plate) fluorescence on the other hand suggest a potential for production of AflatoxinsB1, B2, G1 and AflatoxinG2 whereas the non-aflatoxin producing isolates did not produce any fluorescence. It should be noted, however, that, although the methods used in the current study could identify the Aflatoxin-producing potential of the isolates, they are not sensitive enough for the detail identification and quantification of the toxins. Advanced molecular techniques are needed to characterize the various *Aspergillus spp.* conclusively

CONCLUSION

The study revealed a heavy contamination of grains with moulds of the genera *Aspergillus, Mucor and Rhizopus.*

Detection of aflatoxin production potential revealed that majority of the *Aspergillus* isolates have aflatoxin producing potential, making the consumption of these food items a public health risk.

Mycotoxins, especially aflatoxin are silent killers and there is, an urgent need to make Nigerian food grains safe from the deadly mycotoxins.

Possible intervention strategies including good agricultural practices like early harvesting, proper drying, sanitation, proper storage and insect management, breeding for resistance, use of antimicrobial agents to protect the grains, surveillance and

awareness creation should be looked into to curtail the menace of mycotoxins.

Policy makers should establish and enforce quality standards and regulations related to moulds and mycotoxin contamination across the country to minimize health hazards related to consumption of contaminated grains and their products.

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