

INCIDENCE OF FUNGAL FLORA AND AFLATOXIN CONTENT OF MILLET AND MAIZE CEREAL GRAINS SOLD IN GUINEA SAVANNA ZONES OF KEBBI STATE

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ABSTRACT

Cereal grains get contaminated with fungi and aflatoxin due to poor agricultural, displayed in market and storage practices. A total of three local governments in Guinea savanna zone of Kebbi State were selected for samples collection namely; Zuru, Yauri and Danko/Wasgu. Five heads of millet and five heads of maize were collected in each site, marked total of Thirty (30) head of both millet and maize in all. Fungi were isolated on Potato Dextrose Agar (PDA) using Agar plate method. Eight (8) fungal species were isolated and identified as *Aspergillus flavus*, *Aspergillus fumigates*, *Fusarium* spp., *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor hiemalis*, *Eurotium harvarium* and *Penicillium* spp. The highest incidence of fungi were obtained in *Aspergillus flavus* with (30.9%) and lowest were obtained in *Eurotium harvarium* (4.4%). Enzyme-linked immunosorbent Assay (ELISA) were used for the determination of Aflatoxin. From the results obtained Zuru had the highest level of aflatoxin 58.00 ± 3.65^a $\mu\text{g}/\text{kg}$ in millet while Danko/Wsagu with less 12.72 ± 2.99^b $\mu\text{g}/\text{kg}$ in maize. Implication of this findings showed that both millet and maize had aflatoxin levels above maximum acceptable limit of $10 \mu\text{g}/\text{kg}$ set EU and NAFDAC. Therefore, adequate and rapid drying of grains to water content level may be employed as this could reduce the growth of aflatoxingenic fungi to the minimum possible level.

Keywords: Aflatoxin contents, fungi, mille grains, maize, Market and Guinea Savanna

INTRODUCTION

Over 16 million metric tons of cereal grains are produced annually in Nigeria and 4.8 million metric tons are produced in Kebbi State alone (Sharma, 1992; Bankole and Adebajo, 2003; Uzundu and Udo, 2007). Approximately, thirty percent (30 %) of these grains lost in Nigeria have been estimated to run into billions of Naira annually which is attributed largely to fungal contaminations (Aworch, 2004). It was also reported by Hill *et al.* (1985) that 2.3 million bags of corn harvested in Kenya were found to be contaminated with aflatoxins. (Njoroe, 2010) reported that, mycotoxins get into human food as a result of growth of specific fungi on food crops either in the field or at the storage level. Aflatoxin B₁, B₂ and M₁ has been detected in other animal tissues used as meat and therefore presents a potential health hazard for the humans and live stocks. Mycotoxins cause cancer, liver

disorder and weaken the human immune system (Bennett and Klich, 2003).

Aflatoxins are a group of mycotoxins produced by some *Aspergillus* species, notably *Aspergillus flavus* and *Aspergillus parasiticus* (Creepy, 2002). Although the presence of *Aspergillus* molds does not necessarily indicate aflatoxin contamination, there is certainly an increased risk (Robertson, 2005).

The presence of aflatoxin in grains constitutes a serious health hazard to both human beings and animals due to their toxic and carcinogenic property which could lead to diseases such as hemorrhage, edema, acute liver damage, anemia, jaundice, poor growth, embryonic toxicity, recurrent infections as well as death. Among the known aflatoxins, aflatoxin B₁ (AFB₁) remains the most prevalent in foods (Lee *et al.*, 2004). AFB₁ is also the most potent toxic metabolite capable of inducing hepato-carcinogenicity (Sweeney and Dobson, 1998), genotoxicity in reproductive and blood cells (Fapohunda *et al.*, 2008; Ezekiel *et al.*, 2011), as well as some other toxic conditions. They are also considered to cause liver cancer in humans, particularly in a number of developing countries, where high levels of aflatoxins are found in some staple foods (Mathew, 2005). Aflatoxin contamination in foods and agricultural commodities has significant economic implications especially in developing nations where the skills and the financial ability to effectively control this problem are lacking (Bankole and Adebajo, 2003). As agriculture forms the main source of income in the study area, it therefore becomes necessary to a certain the level of aflatoxin and aflatoxin contaminations in food and agricultural products in the study area. Prior to this study, such information were not documented in Kebbi State the little available information in Nigeria were the reports of (Bankole and Adebajo, 2003) in Ogun and Oyo States, (Bankole and Mabekoje, 2004) in Ogun and Oyo states, (Makun *et al.*, 2006) in Niger State and (Odoemelam and Osu, 2008), in Southwestern Nigeria and (Obida *et al.*, 2012) in Borno State respectively. Millet and Maize are routinely used in Kebbi State and Nigeria homes at large for different purposes. Regardless of the daily use of these cereal grains there are very few documents reports on mycobiota and aflatoxin contaminations in theses cereal grains more especially in Guinea savannah of Kebbi State. The aim of this study was to identify the fungi species and aflatoxin content contaminations in two different cereal grains sold in Guinea savannah of Kebbi State.

MATERIALS AND METHODS

Study Area

The study was conducted in Kebbi State Nigeria located in Northwestern Nigeria with its capital at Birnin Kebbi. Kebbi State lays between latitudes 10 – 13 °N and longitudes 3.5 – 6 °E. The state is characterized by Sudan and Guinea Savanna vegetation and a minimum temperature of 21 - 24 °C with relative humidity range of 17 – 80 % (Njorope, 2010).

Sample Collection

Sampling of cereal grains millet and maize were made using stratified sampling design. Samples were collected based on geographical and vegetation distribution of Kebbi State. Zuru, Danko/Wsagu and Yauri local government areas Guinea Savanna Zone are marked as the collection centers. Four villages were selected for samples collection. Four heads of cereal grains were bought in each markets place and each were collected in a sterilized polythene bags and labelled.

Isolation of fungi from Sample

Using the method of Harrigan and McCance (2005), one gram of the sample was poured into 9ml of sterile distilled water and the tube was shaken gently to ensure thorough mixing. After mixing, a serial dilution was then carried out by transferring 1ml from the first test-tube into the second test-tube that contains 9ml of distilled water. The second tube was mixed gently and 1ml was taken from the second tube into the third test-tube, and so on till the fifth test-tube. Isolation of fungi were done using the pour plate method (Harrigan and McCance, 2005). One milliliter (1ml) each from the third test-tube was pipetted using a new sterile pipette into one empty sterile Petri-dishes, for potato dextrose agar. Also, 1ml was pipetted from the third test-tube and poured empty sterile Petri-dishes. After the samples have been poured into the Petri-dishes, the media (PDA) were poured into their respective plates (about 15ml was poured into the plates). After the plates had set, they were then incubated at 25°C for 5-7 days. Percentage frequencies of isolation (PFI) of all the fungi were calculated by the formular:

$$PFI = \frac{\text{No. of times a fungus is encountered}}{\text{Total no. of times all fungi were encountered}} \times 100$$

Preparation of the Grain Samples for determination of Aflatoxins

The grains collected in markets were threshed from the heads of the samples and separated by hand. The dirt was removed by winnowing and picking. One kg of each cereal grains collected in market were measured in the Laboratory using weighing balance and the samples from each village were made into composite samples. Cleaned grains were surfaced sterilized by immersing in 70 % alcohol for 20 seconds and in a solution of sodium hypo chloride containing 1 % chloride for 10 minutes (Cooke, 1977). The grains were rinsed with sterile distilled water blotted dry with filter paper. A portion of each grain sample was weighed (500 g) ground to fine powder using electrical blender (Philips, HR2815, Japan) and packaged in a sterilized bottle and kept at 4°C and later taken to National Agency For Food and Drug Administration and Control (NAFDAC) Kaduna, Nigeria for aflatoxin analysis.

Determination of Aflatoxin Content in Grain Samples

Samples of millet and maize obtained from Guinea savanna Zone were ground and 25 ml of 70 % methanol was added to 5 g of each ground grain sample, and allowed to stand for 10 minutes for extraction of aflatoxin. The samples were filtered using No. 1 Whatman filter paper and 50 µL filtrate was mixed with 100 µL of conjugate in a separate dilution wells (Green border well), 100 µL was taken out from the filtrate/standard conjugate mixture and dispensed into the antibody coated wells and incubated at room temperature for 15 minutes. The content of the wells were discarded and the wells were washed 3-4 times with deionizer water, 3-4 times. 100 µL of substrate was added to each well, and incubated for 5 minutes and allowed for color changes (different shades of blue to colourless) and added 100 µL of stock solution was added to the wells which convert the blue end point to yellow. Using an ELISA reader, the optical density of standards (0, 4, 10, 20, and 40 µg/kg) and those of the samples were read at 450 nm. Filter results for sample analysis were obtained after a colour change from blue to yellow. Dilution factors were taken into consideration using a micro plate reader with an absorbance filter of 450 nm and a differential filter of 630 nm, which is optically measured from which the results were calculated to determine the level of total aflatoxin in the grain samples. Data obtained were analyzed using SPSS.

Results

The fungal species identified from the study area were presented in (Table 1). However, the highest prevalent of occurrence were obtained in *Aspergillus flavus* and lowest were recorded in *Eurotium harvarium* as shown in Table 1. Total Aflatoxin Concentrations (µg/kg) in two cereal grains sold in Markets Guinea Savanna Zones of Kebbi State were seen in Table 2). From the results showed that Zuru L.G.A had highest aflatoxin content of (58.00±3.65^a) in millet while lowest were obtained in Danko/Wasagu with level of (12.72±2.99^b) in maize samples as presented in (Table 2) respectively.

Table 1: Incidence of fungi isolated from two cereal grains in Guinea savanna, Kebbi State.

Fungal isolated	Frequency of occurrence (%)
<i>Aspergillus flavus</i>	30.9
<i>Aspergillus fumigates</i>	20.1
<i>Fusarium spp</i>	6.2
<i>Aspergillus niger</i>	16.6
<i>Rhizopus stolonifer</i>	7.1
<i>Mucor hiemalis</i>	9.7
<i>Eurotium harvarium</i>	4.4
<i>Penicillium/rubrum</i>	5
Total	100

Table 2: Total Aflatoxin Concentrations ($\mu\text{g}/\text{kg}$) in Cereal Grains sold in Markets Guinea Savanna Zones of Kebbi State

L.G.A.	Millet	Maize
Zuru	58.00 \pm 3.65 ^a	57.60 \pm 8.11 ^a
Yauri	48.40 \pm 3.11 ^a	42.40 \pm 4.17 ^b
Danko/Wasagu	41.80 \pm 4.03 ^a	12.72 \pm 2.99 ^b

Values are means of triplicate determinations \pm S.D (Standard Deviation) Means in the same row followed by same letter(s) are not significantly different ($P > 0.05$)

Discussion

Fungi species isolated in this study indicate the extent of genera contamination of cereal grains in Kebbi State. Molds species reported in this study were found reported on same cereal grains and other cereals in other parts of the country (Gbodi *et al.*, 1986; Okoye, 1992). The market fungi identified in this study have been showed in other studies (Obada *et al.*, 2012; Vasenthi and Bhat, 1998; Hill *et al.*, 1985). The species of fungi isolated in this study are said to be undesirable in foods. The presence of *Aspergillus* species in the cereal samples is an indication of possible health hazards as some species of *Aspergillus* are known to cause food intoxication and food poison (Hill *et al.*, 1985). It was however observed that market had incidence of fungi this could be due to the fact that, grains are mostly transported to markets after harvest for sales in locally made woven baskets and sacks under conditions that encourage the incubation of these contaminants and left exposed in open bowls in the market (Bankole and Adebanyo, 2003). The observed variation in incidence of fungi could be climate dependent (Viguez *et al.*, 2004) and the fungi may differ from those of other studied area.

In Guinea Savanna markets, the highest aflatoxin concentrations (58.00 \pm 3.65 $\mu\text{g}/\text{kg}$) were obtained in maize grains from Zuru markets and lowest (12.72 \pm 2.99^b $\mu\text{g}/\text{kg}$) from Danko/wasagu markets in Maize grains respectively. This finding was collaborated with research conducted by Williams *et al.* (2004) reported aflatoxin levels ranging between 17- 2110 $\mu\text{g}/\text{kg}$. Although, the levels of aflatoxin reported in Nigeria and other African countries are within the range reported in this present study the differences between these results and that of the present study may be due to differences in harvest and storage conditions as well as agricultural practices in each country. A difference in geographical and environmental conditions may influence the production of aflatoxins in the field and during storage (Bankole and Mabekiye, 2004). This was supported by Uzundu and Udo (1992) who reported that 33% of maize samples from different ecological zones of Nigeria were contaminated with aflatoxin. This finding was agree by the report of Sharma, (1992) who observed that the differences in harvest and storage conditions as well as the agricultural and storage practices may influence the level of aflatoxin contamination of each grain and also environmental factors influence the production of aflatoxin in the field and during storage. Similarly, Uzundu and Udo (1992) reported that the difference in the mean annual rainfall between Sudan and Guinea Savanna regions is over 4000 mm. Cereals stored without adequate drying as well as weather conditions,

moisture/humidity (14%) and warm temperature (20 $^{\circ}\text{C}$) can potentially become contaminated and damaged. Studies done on the effect of environmental conditions on aflatoxin contamination on corn showed that, when the conditions were favorable, the occurrence of aflatoxin was highly related to these factors (Viguez *et al.*, 1994).

The occurrence of high aflatoxin content in Zuru market in Guinea Savanna may be due to variation in environmental factors which are favourable for the growth of aflatoxigenic moulds which may be responsible for the detection of high aflatoxin level in this area which is more to the tropic when compared with Guinea Savanna. This statement was supported by Ellis *et al.* (1991) who observed that temperature is an important factor for the growth of some toxigenic fungi especially *Aspergillus* spp. Viguez *et al.* (1994) supported this findings as he amplified that aflatoxin levels was significantly ($P < 0.05$) affected by temperature Mishra and Daradhiyar (1991) reported that mycotoxigenic fungi are notable to inhabit a good number of food and grains to build up mycotoxins when conditions of the environment are complimentary for their development in the field and at storage locations. Furthermore, Giray *et al.* (2009) in Turkey reported on the effects of Ochratoxin A and total aflatoxin levels in corn, and reported corn grain to be highly contaminated above permitted limit 10 $\mu\text{g}/\text{kg}$, this reported agreed with the present study in which corn was found to have higher level of contaminant. The demonstrated presence of mycotoxigenic fungi in cereal crops millet and maize in this study has public health implications as low grade, cheap, mouldy grains are consumed by animals and humans in the study area and other parts of the country resulting in high risk of human and animals mycotoxicoses with adverse effects on crop and livestock production, and therefore national economy and trade. This makes regulation of mycotoxins in our foods and feedstuffs, an imperative.

Conclusion

Different species of fungi were isolated from this two cereal grains. The grains are shown to be contaminated with aflatoxins at levels that cannot be said to be completely not safe. Therefore, prolonged consumption of large quantities of these grains ingested for long time may lead to aflatoxin and aflatoxin related intoxications in livestock and human beings. Therefore, adequate and rapid drying of grains to water content level may be employed as this could reduce the growth of aflatoxigenic fungi to the minimum possible level in the studied area. These cereal grains points to potential serious hazards to human and animals in Kebbi State and indeed Nigeria as a whole.

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