

Mycotoxicology Society of Nigeria Mycotoxin Contamination of Maize and Guinea corn from Markets in Plateau State, Nigeria

<sup>1\*</sup>Okeke, O.F.I, <sup>2</sup>Fapohunda, S., <sup>3</sup>Soares, C., <sup>3</sup>Lima, N and <sup>1</sup>Ayanbimpe, G. M.

<sup>1</sup> Department of Medical Microbiology, Faculty of Medical Sciences, University of Jos, Plateau State, Nigeria, West Africa
 <sup>2</sup> Department of Biosciences and Biotechnology, Babcock University, Ilishan-remo, Ogun State, Nigeria, West Africa
 <sup>3</sup> Biological Engineering Centre, Applied Mycology Group, University of Minho, Campus of Gualtar, Braga, Portugal.
 \*Corresponding Author: Okeke, O.F.I., e-mail: liciasly@yahoo.com, Tel: +2348036358489

# ABSTRACT

Maize (*Zea mays*) and guinea corn (*Sorghum bicolor*) are major food items in Plateau state, Nigeria. A multistage sampling technique was used to select the markets and store/warehouses used for this study; sample collection employed a simple random sampling method from different sampling points within designated areas. A total of 18 representative samples were collected and analyzed for the following mycotoxins: aflatoxins (Aflatoxin B<sub>1</sub> - AFB<sub>1</sub>, Aflatoxin B<sub>2</sub> - AFB<sub>2</sub>, Aflatoxin G<sub>1</sub> - AFG<sub>1</sub> and Aflatoxin G<sub>2</sub> - AFG<sub>2</sub>), fumonisins (Fumonisin B<sub>1</sub> - FB<sub>1</sub> and Fumonisin B<sub>2</sub> - FB<sub>2</sub>) and cyclopiazonic acid (CPA). Out of 12 samples analyzed for Aflatoxins, AFB<sub>1</sub> was detected in 5, AFB<sub>2</sub> in 1, AFG<sub>1</sub> in 1 and AFG<sub>2</sub> in 6 samples respectively. The highest concentration of AFB<sub>1</sub> and AFG<sub>2</sub> were found in maize samples from Pankshin market. Only maize samples from Mangu market were contaminated with AFB<sub>2</sub> and also harboured the lowest concentration of AFG<sub>2</sub> AFG<sub>1</sub> contamination occurred in only guinea corn from Shendam market. and FB<sub>1</sub> was detected in all 18 samples analyzed. The mycotoxin CPA was not detected in any of the samples. Aflatoxins levels in analyzed samples were regarded as safe based on Nigerian and European Union maximum permissible levels of 4µg/kg. With the exception of two samples, FB<sub>1</sub> levels in analyzed maize samples were within European Union maximum permissible levels of 1,000 to 3000µg/kg. The health and food safety implications of these results for the human and animal population are further discussed.

# Keywords: aflatoxins, fumonisins, cyclopiazonic acid, food safety, Nigeria

# **1.0 Introduction**

Contamination of commodities, feeds and feed ingredients by mycotoxins from different parts of the world is well documented (Bewaji et al, 2000; Chu and Li, 1994; Dongo et al, 2008; Goharty, 1995; Makun et al, 2011; Okoli et al, 2007; Rashedi et al, 2012; Rodrigues et al, 2011). The human aspects of the untoward (toxic) effects of mycotoxicoses are also well documented (Bhat et al, 2010; Bhat et al, 1997; Peraica et al, 1999; Sodeinde et al, 1995). It is estimated that about 25 to 50% of the world's food crops are affected by mycotoxins (Miller, 1995). Aflatoxin  $B_1$  has been placed on the list of type 1 human carcinogens by the International Agency for Research on Cancer (IARC), an arm of the World Health Organization (WHO). There is little doubt that high levels of exposure of people to food-borne mycotoxins pose a significant food safety and public health risk (Cardwell et al., 2001 and FRI, 2012). According to Dohlman, (2002) among grains (and other field crops), perhaps the most prevalent - if publicly unrecognized - source of food related-health risks are mycotoxins. In many low-income countries mycotoxins affect staple foods, including

groundnuts (peanuts), maize (corn), other cereals and nuts, such that exposure is continuous and often at high levels; the highest exposures occur in communities that produce and consume their own food and thus regulatory measures to control exposure are largely ineffective (Wild and Gong, 2010). Furthermore, warmer (tropical) countries, such as Nigeria, Kenya and Ghana, have a higher occurrence of mycotoxins (aflatoxins) (Rodrigues et al, 2011). Maize and guinea corn are well cultivated in the study area and both are major staples of the local diet. This study therefore focused on the mycotoxin analysis of these two important grains in Plateau state.

# 2.0 Materials and Methods

# 2.1 Description of the Study Area

This study was conducted in Plateau state, North-central Nigeria which derives its from the Jos Plateau. Plateau state has an area of about 26,899sq. km and an estimated population of about three million. It is within latitude  $8^{0}$  22' North and  $10^{0}$ 24' North and longitude  $8^{0}$  32' East and  $10^{0}$ 38' East and has a total land area of 26, 899 square kilometers.

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S/no	Senatorial District	LGA	Name of Market	Market Day(s)
1.	Northern	Barkin Ladi	Barkin Ladi	Saturday
		Jos North	Katako	Daily
		Jos South	Vom	Monday
2.	Central	Kanam	Dengi	Friday
		Mangu	Mangu	Thursday/Friday
		Pankshin	Pankshin	Monday
3.	Southern	Langtang North	Langtang	Saturday
		Mikang	Garkawa	Tuesday
		Shendam	Shendam	Saturday

#### 2.2 Sample Collection

Samples of maize and guinea corn were collected between May and June, 2014 from designated markets and store/warehouses within the state according to the three senatorial districts. Multistage sampling technique was employed in selecting the markets used for the study. Each market was sub divided into two segments for the purpose of sample collection: grains in storage in the warehouse and grains for sale directly to consumers in the open market. Using simple random sampling, 1kg each of sample was collected from ten (10) different sampling points from each market. All samples were then pooled together to obtain one composite/representative sample of each grain for each sampling location (i.e. market) in the study area. A total of 18 (9 for maize and 9 for guinea corn) composite/representative samples (comprising of 180 individual samples: 18 samples x 10 sampling points = 180) were collected for this study. Each composite sample was then blended using an electric blender to obtain 50g finely ground powder which was collected into airtight zip locked bags. All ground samples were properly packaged according to IATA (International Air Transporters Association) standards and subsequently sent via courier to the host laboratory (Micoteca da Universidade do Minho, IBB/Centre of Biological Engineering, Braga, Portugal) where they were stored at - $70^{\circ}$ C until analysis.

#### 2.3 Mycotoxin Analysis of Ground Grain Samples

Ground samples were prioritized accordingly and subsequently analyzed for aflatoxins, fumonisins and cyclopiazonic acid using the High Performance Liquid Chromatography (HPLC) technique with different extraction methodology for each mycotoxin.

# 2.3.1 Aflatoxin HPLC Procedure

## Sample Extraction

A 50g sample of ground maize/guinea corn was weighed with 5g salt (NaCl) and placed in a blender jar (Moulinex TURBO blender). 100ml of methanol:water (80:20 by volume) was added to the jar. Blender jar was covered with its lid and blending was carried out at high speed for 1minute. Blender lid was removed from the jar and extract filtered through a filter paper and collected in a clean conical vessel.

## Extract Dilution

10ml of filtered extract was poured into a clean conical flask. 40ml of Phosphate Buffered Saline (PBS) was added to dilute the extract. Extract was filtered through a microfiber filter and collected in a clean conical flask.

## Column Chromatography

10ml (10ml = 1.0g sample equivalent) of diluted extract was completely passed through AflaTest<sub>WB</sub> Mycotoxin Testing System immunoaffinity column at a rate of about 1 - 2 drops per second until air came through column. 10ml of purified water was passed through the column at a rate of 1 - 2 drops per second until air came through column. Affinity column was eluted by passing 3ml HPLC grade methanol through column at a rate of ~1 drop per second. All of the sample eluate (1.5ml) was collected in a glass cuvette. 1.5ml purified water was added to elute and the sample subsequently vortexed for 2minutes. 50µL portion of the eluate was injected into HPLC for aflatoxin.

Limit of Detection: Less than 0.25ppb

Recovery: Greater that 70% over the range of 0 - 100 ppb for aflatoxin.

## 2.3.2 Cyclopiazonic Acid HPLC Procedure

### Sample Extraction

50g of ground sample was weighed out into a blender jar and an extraction solvent (methanol:2% NaHCO3 in water, 70+30) added. The mixture was blended at high speed for 2 minutes. The blender contents were allowed to settle before opening it. Mixture was filtered through Whatman No. 4 filter paper. 25ml of the filtered extract was pipetted into a 250ml separation funnel. 100ml of nhexane (to de-fat the extract) was added and the mixture mixed gently to avoid the formation of an emulsion. After separation to two layers, the lower aqueous layer was carefully transferred into another separation funnel. 50ml of 10% KCL in water was added to the aqueous solution from the step above and the *n*-hexane discarded. The solution was acidified with 2ml of 6N HCL. 50ml of chloroform was added and the solution mixed gently. The lower organic layer was collected in an Erlenmeyer flask. The extraction process was repeated with additional 50ml chloroform and the two extracts were combined in the same Erlenmeyer flask. 50g of anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) was added and let stand for 1 hour. Extract was filtered and collected into a 200ml rotary evaporator flask. Extract was evaporated to dryness at  $40^{\circ}$ C in a rotary evaporator.

## Column Chromatography

Dried evaporated sample was resuspended in mobile phase (2mls of acetonitrile: 50mM ammonium acetate; 3:1 v/v). Extract solution was filtered through  $0.2\mu\text{m}$ cellulose filter into a fresh tube.  $20\mu\text{L}$  of the final extract sample was injected into HPLC for cyclopiazonic acid.

# 2.3.3 Fumonisin HPLC Procedure Using the SAX Extraction Protocol (Modified from Ndube *et al*, 2011)

#### Sample Extraction

20g of ground sample was placed in a blending jar and methanol:water (3;1, 100ml) was added. Solution was homogenized by blending at high speed for 3mins and subsequently centrifuged at 5,000rpm at 4<sup>o</sup>C for 10mins. Supernatant was decanted off into fresh centrifuge tubes.

# Extraction with SAX

The supernatant from above was filtered into fresh tubes using  $0.2\mu m$  cellulose filter paper. 10ml of the filtrate was taken into a fresh tube and 30ml of methanol added (i.e methanol:sample was 3:1; v/v). Another 5mls of methanol was added. SAX column was pre-conditioned with 5mls of methanol:water mixture (3:1). Filtrate was passed through the column. Extraction process was continued by washing the SAX column with 8ml of methanol:water (3:1). 3mls of methanol was used to wash the column again. The tube for the column was changed (new one put in place). Using a 10ml solution of methanol:acetic acid (99:1) fumonisin trapped in the SAX column was extracted. Final extract was eluted to dryness.

#### Column Chromatography

Evaporated sample was resuspended in 0.5ml of methanol.  $150\mu$ L was injected into HPLC for fumonisin. Further derivatization with OPA (0-phthaldialdehyde) was carried out on the sample before HPLC analysis.

# 2.4 High Performance Liquid Chromatography Technique

Analysis of aflatoxin samples was carried out by employing a HPLC equipment with the following specifications: a JascoFP-920 fluorescence detector (365 nm excitation wavelength; 435 nm emission wavelength), using a photochemical post-column derivatization reactor (PHRED unit Aura Industries, USA). Chromatographic separations were performed on a reverse phase C18 column (Waters SpherisorbODS2, 4.6 mm×250mm, 5 µm), fitted with a pre-column with the same stationary phase. The mobile phase was a mixture of water:acetonitrile: methanol (3:1:1, v/v) pumped at 1.0mL/min. The injection volume was 50µL. Aflatoxins standard (a mix containing 2  $\mu$ g/mL each of AFB<sub>1</sub> and AFG<sub>1</sub>, and 0.5 µg/mL of AFB<sub>2</sub> and AFG<sub>1</sub>) was supplied by Biopure (Austria). Cyclopiazonic acid samples were analyzed using a HPLC system equipped with a Varian 2050 UV detector (285 nm). Chromatographic separations were performed on a EuroSpher 100 NH2 column (Knauer, 4.6 mm×250 mm, 5 µm), fitted with a precolumn with the same stationary phase. The mobile phase used was pumped at 1.0 mL/min and consisted of a mixture of acetonitrile:50mM ammoniumacetate (3:1, v/v), pH5. The injection volume was 50 µL. CPA standard was supplied by Sigma (St. Louis, MO, USA). Fumonisin samples were analyzed by a HPLC method using a Jasco FP-920 fluoroscence detector (420nm excitation wavelength; 500nm emission wavelength). Chromatographic seperations were performed on a reverse phase C18 column phase. The mobile phase was acetonitrile:water:acetic acid (60:40:1, v/v) pumped at 1.0mL/min. the injection volume was 50µL. Fumonisin standard was supplied by Sigma (USA).  $\mathbf{B}_2$ 

					Aflatoxin			
No	Sample	Market	LGA	Senatorial	B1	B2	G1	G2
				District				
1.	Maize	Langtang	Langtang	Southern	0.14	nd	nd	0.19
2.	Maize	Katako	Jos North	Northern	0.16	nd	nd	0.12
3.	Maize	Pankshin	Pankshin	Central	0.22	nd	nd	0.24
4.	Maize	Mangu	Mangu	Central	nd	0.13	nd	0.09
5.	Maize	Dengi	Kanam	Central	nd	nd	nd	nd
6.	Guinea corn	Langtang	Langtang	Southern	0.17	nd	nd	0.17
7.	Guinea corn	Shendam	Shendam	Southern	nd	nd	0.67	0.21
8.	Guinea corn	Katako	Jos North	North	nd	nd	nd	nd

Table 2. Concentration (ug/kg) of Anatoxins in Samples	Table 2:	Concentration	(ug/kg) of Aflatoxins in Sampl	es
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nd - not detected

Table 3: Concentration (ug/kg) of Cyclopiazonic acid (CPA) in Samples

S/no	Sample	Market	L.G.A.	Senatorial District	СРА
1.	Maize	Katako	Jos North	Northern	nd
2.	Guinea corn	Katako	Jos North	Northern	nd
3.	Guinea corn	Mangu	Mangu	Central	nd
4.	Guinea corn	Garkawa	Mikang	Southern	nd
not detected	d	C. C.	A State of the sta		

nd –

#### **Results**

3.0

А total of 18 composite/representative samples (comprising of one hundred and eighty individual samples) were collected for this study. Out of 12 samples analyzed for aflatoxins, AFB1 was detected in 5, AFB2 in 1, AFG1 in 1 and AFG2 in 6 samples respectively. The highest concentration of AFB1 and AFG2 were found in maize samples from Pankshin market. Only maize

samples from Mangu market were contaminated with AFB2; the lowest concentration of AFG2 was also found in maize samples from Mangu market; AFG1 contamination occurred in only guinea corn from Shendam market. FB1 was detected in all 18 samples for which it was analyzed. The mycotoxin CPA was not detected in any of the samples. Results are as shown in Tables 2, 3 and 4.

					Fumonisin	
No	Sample	Market	LGA	Senatorial District	FB1	FB2
1.	Maize	Langtang	Langtang	Southern	4300	nd
2.	Maize	Garkawa	Mikang	Southern	400	nd
3.	Maize	Shendam	Shendam	Southern	3910	nd
4.	Maize	Barkin Ladi	Barkin Ladi	Northern	2830	nd
5.	Maize	Vom	Jos South	Northern	1750	nd
6.	Maize	Pankshin	Pankshin	Central	550	nd
7.	Maize	Dengi	Kanam	Central	1050	nd
8.	Maize	Mangu	Mangu	Central	5220	nd
9.	Guinea corn	Garkawa	Mikang	Southern	70	nd
10.	Guinea corn	Shendam	Shendam	Southern	4090	nd
11.	Guinea corn	Barkin Ladi	Barkin Ladi	Northern	1590	nd
12.	Guinea corn	Vom	Jos South	Northern	260	nd
13.	Guinea corn	Pankshin	Pankshin	Central	190	nd
14.	Guinea corn	Dengi	Kanam	Central	1100	nd
15.	Guinea corn	Mangu	Mangu	Central	160	nd
detected						

# Table 4: Concentration (ug/kg) of Fumonisins (FB) in Samples

nd - not detected

## 4.0 Discussion

Several studies in Nigeria have reported toxin levels far above the limits allowed by International regulatory agencies in food and agricultural products (Atanda et al, 2013) and according to Fapohunda (2010), there is a correlation between poverty and aflatoxin consumption. The results from this present study reports aflatoxins levels below Nigerian and European Union maximum permissible levels of 4µg/kg (FAO, 2003) in the analyzed maize and guinea corn. The lowest aflatoxin levels were recorded from Mangu market. Mangu market is the largest market in Plateau state (Source: Oral interview with officials of the Plateau State Traders and Marketing Association (PSTMA), Mangu District, maize section office). Observation from field visits also point to a very organized market with well-defined storage systems practicing basic sanitation measures. Application of insecticides for grains in storage is also practiced (the local insecticide - Sniper (a DDVP, 2, 2-Dichlorovinyl dimethyl phosphate compound) - in powdered or liquid form is most commonly used). Atanda et al (2013) have also reported that basic sanitation measures such as sorting out physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in aflatoxins levels. These are all plausible reasons which may have been responsible for the low levels of aflatoxins reported in this study.

FB1, the most abundant of the numerous fumonisin analogues, was classified by the IARC as a group 2B carcinogen (possibly carcinogenic in humans) (Shephard, 2008). According to Darwish et al (2014), fumonisin toxicity in humans and animals is widespread in Africa. In Nigeria, there have been a number of documented reports on fumonisin contamination in various categories of foods (Adejumo and Adejoro, 2014; Amina et al, 2012; Bankole and Mabekoje, 2014; Egbuta et al, 2013; Makun et al, 2011; Somorin et al, 2012). However, documented reports on the toxicity of fumonisins are still somewhat limited when compared to that of aflotoxins. With the exception of two samples, this study reports FB<sub>1</sub> levels in analyzed maize samples within Nigerian and European Union maximum permissible levels of 1,000 to 3000µg/kg (FAO, 2003). Only one of the guinea corn samples was found to be contaminated with FB1 levels exceeding this limit.

The presence of aflatoxins and fumonisins at concentrations as reported in this study is a welcome development which underscores the fact that successful mitigation of the mycotoxin scourge in Nigeria is indeed achievable.

#### **Conflict of Interest**

Authors declare no conflict of interest.

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