

Occurrence of Mycotoxins in Maize and Status of their Management by the Farmers and Marketers in Abuja, Nigeria

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Abstract
The occurrence and extent of usage of control strategies against mycotoxins in maize (*Zea mays* L.) by the farmers and marketers in the Federal Capital Territory (FCT) Abuja, Nigeria was investigated using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS-MS) and structured questionnaires involving 263 respondents respectively. Four out of the nine major mycotoxins was detected in $\geq$59.00% of the 30 investigated composite samples. About 50.00% of the samples were contaminated with Aflatoxin B_1 ($AFB_{1)}$ above the EU regulatory limit of 5 µg kg^{-1} in the maize raw grain samples. About 26.67% of the grains were contaminated with Aflatoxin B_2 (AFB_2) above the regulatory limit of 10 µg kg^{-1}. Up to 80.00% of the raw grains were contaminated with Fumonisin B_1 ($FB_{1)}$ above the regulatory limit of 1000 µg kg^{-1}. It was indicated that only 19.87% of the farmers regularly use the management strategies against seed-borne fungi infection and mycotoxins build-up before and during maize harvesting while up to 48.80% of marketers regularly do so after harvesting. There was an indication that maize grains in the territory were contaminated with toxigenic fungi in view of mycotoxins load and due to inadequate usage of management practices. Increasing awareness on the management strategies to mitigate mycotoxin load in maize in the FCT, Abuja, Nigeria is imperative.
Keywords: Farmers; Liquid Chromatography-Tandem Mass Spectrometry, Maize grains, Management strategies, Marketers, Regulated mycotoxins

1. Introduction

Nigerians have maize (*Zea mays* L.*)* as part of their dietary staples and maize is increasingly demanded in the feed milling industry (Idem and Sowemimo eds, 2005). In developing countries like Nigeria, most staples obtained from local markets are often consumed irrespective of quality due to ignorance and conservatism, food scarcity problems and shortage of mycotoxin analysis facilities (Milićević et al, 2010). Food safety results only when microbial contaminants and chemical toxicants are present below tolerance levels in foods and feeds.

Food and Agriculture Organisation estimated that 25% of the world's food crops are lost due to mycotoxins each year (Grenier and Oswald, 2011). Mycotoxins can have adverse impacts on the health of humans and other animals as well as negative economic impacts on agriculture and associated industries (CAST, 2003). According to International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, 2011) the volume of cereal grains wasted by mycotoxins each year worldwide, especially in developing Asian and African countries was 378,000 tons. There are reports of multiple mycotoxins contamination in raw maize grains and in the animal feed formulated from the grains in Northern Nigeria (Gwary, 2012; Kayode *et al*, 2014) The occurrence cuts across sub-Saharan Africa (Mafurah et al, 2012).

Mycotoxins such as aflatoxins or fumonisins (FUMs) are the major potentially toxic microbial metabolites in grains (Atanda et al, 2013; Makun *et al,* 2010). In developing countries like Nigeria, many individuals are ignorant of mycotoxin as a chemical hazard in their diet and how to control them from the field and after crop harvest (Hell and Mutegi, 2011). Thus these vulnerable people are not only food insecure, but also are chronically exposed to high levels of mycotoxins (Rasmussen et al, 2010; FAO, 2012).

There are however, limited data on the co-occurrence of multi-mycotoxins on maize and their products in the Federal Capital Territory, Abuja, Nigeria due to the novelty and shortage of LC-MS/MS facility for such analysis. This study therefore investigated the mycotoxin profile of regulated mycotoxins in maize across the FCT Abuja, Nigeria and assessed the level of usage of management measures against the toxins. This is with the view of determining the potential risk of the toxicants and facilitating awareness on the management of mycotoxins in the staple.

2. Materials and Methods

***2.1** Chemicals and reagents*

Methanol (LC gradient grade) and glacial acetic acid (pa) were purchased from Merck (Darmstadt, Germany); acetonitrile (LC gradient grade) from VWR (Leuven, Belgium); and ammonium acetate (MS grade) was obtained from Sigma-Aldrich (Vienna, Austria). Water was purified successively by reverse osmosis, and an Elga Purelab ultra analytic system was used from Veolia Water (Bucks, UK). Standards of fungal metabolites were obtained as gifts either from various research groups or from various commercial sources such as Romer Labs (Tulln, Austria), Sigma-Aldrich (Vienna, Austria), Iris Biotech GmbH (Marktredwitz, Germany), Axxora Europe (Lausen, Switzerland) and LGC Promochem GmbH (Wesel, Germany). The purity of the solid substances was ≥ 95%.

2.2 *Sampling and Samples*

Surveys were conducted in the FCT, Abuja Nigeria (between Lat. 9^o 40' N, Long. 7^o 29' E and Lat. 8^o 83'N, Long. 7^o 17' E, 388 - 566m asl.) between January and February, 2015. The farmers' stores were located in the six Area Councils (Table 1).

A total of 30 maize grains samples were collected from farmers store in the six area councils of the FCT namely Abuja Municipal Area Council (AMAC), Abaji, Bwari, Gwagwalada (GWA), Kuje and Kwali. The sampled locations and number of sample types collected from each district were uneven as the number of stores for each type of grain in the respective zone varied. Only shelled samples stored for less than 30 days after harvest were collected. Each sample was collected as a bulk sample (1.8 - 2.0 kg) and comprised of four subsamples of 0.5 ±0.05kg each. The subsamples were obtained from random points in farmer's basins or other storage containers and mixed to form an aggregate sample. The samples were comminuted and quartered such that 100 -150g of representative samples was obtained from each bulk as described by Ezekiel et al (2012). Representative samples were stored at 4^oC until they were transported to Centre for Analytical Chemistry Laboratory, Department of Agrobiotechnology (IFA-Tulln), Vienna, Tulln, Austria, for multimycotoxin analysis.

2.3 *Sampling area*

The sampling areas were 30 locations (Table 1) in the six Area Councils of the Federal Capital Territory, Abuja (Table 1).

Table 1. Samples of maize grains collected and number of respondents in the FCT Abuja, Nigeria

Area council	Total no. of maize samples/ Area Council	No of Questionnaires distributed (retrieved)/Area council	
		Farmers	Marketers
Abaji	5	27(26)	18(17)
AMAC	6	29(27)	17(16)
Bwari	4	30(28)	17(15)
Gwagwalada	6	28(27)	19(17)
Kuje	6	27(26)	20(19)
Kwali	3	28(27)	19(18)
Total	30	169(161)	110(102)

2.4 *LC-MS/MS Determination*

To 5 g of milled sample, 20 mL of extraction solvent (acetonitrile/water/acetic acid 79: 20:1, v/v/v) was added. Extraction, dilution, and analysis were performed as described by (Malachova et al, 2014). Briefly, LC-MS/MS screening of target fungal toxins was performed with a QTrap 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA) equipped with a TurboIon Spray electrospray ionization (ESI) source and an 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 ºC on a Gemini C_{18}-column, 150 x 4.6 mm i.d, 5 µm particle size, equipped with a C_{18} 4 x 3 mm i.d. security guard cartridge (all from Phenomenex, Torrance, CA). The chromatographic method as well as chromatographic and mass spectrometric parameters for the investigated analytes was also as described by (Malachova et al, 2014). Quantification was based on external, 1/x weighed calibration in connection with correction for apparent recoveries that were obtained during method validation for maize. The accuracy of the method is verified on a routine basis by participation in proficiency tests.

2.5 *Questionnaire administration*

Questionnaires were designed to measure the level of awareness of maize seed-borne fungi and mycotoxins control strategies. 279 questionnaires were distributed and 263 were retrieved from the respondents (Table 1). Sampling technique was purposive, with a focus on the maize farmers and marketers.

2.6 *Data analysis*

Median, mean, maximum and standard deviation (SD) of the concentration for each of the toxins was calculated using Excel package 2007 (SPSS Inc, Chicago, IL, USA). To determine the level of usage of management strategies by the farmers and the marketers, a four point Likert scale was used. The responses were ranked into 'regularly', 'occasionally', 'rarely' and 'not use'. Nominal values of 4,3,2 and 1 were assigned to the ranks respectively.

3. Results

3.1 *Contamination levels of regulated toxins in maize grains in the FCT Abuja*

The percentage of contaminated samples, the mean, median and maximum contamination level of the regulated mycotoxins, in maize samples from the FCT, Abuja are shown in Table 2. The mean toxin concentrations for AFB_1 =1749µg kg^{-1}, AFB_2 =273µg kg^{-1} and FB_1 = 4142 µg kg^{-1}. Usually, contaminated maize samples exhibited high levels of AFB_2, AFG_1, FB_1, FB_2, and FB_3. In this study, up to 96% of the maize samples were contaminated with FB_1. Also, 50% of the samples were contaminated with AFB_1 above the EU regulatory limit. This gave an indication that maize was not safe for consumption in the territory in view of mycotoxins contamination. It was found that FB_1 occurred in 96% of the maize samples, and FB_1, FB_2 and FB_3 contaminated all maize grains at higher concentrations (mean = 4142, 1881, 460µg kg^{-1} respectively).

Table 2. Occurrence and concentrations of regulated toxins in maize grains from the FCT, Abuja, Nigeria, by LC-MS/MS

Mycotoxins	%. positive in maize (n=30)	Maize contamination level (µg kg^{-1})			
		Median	Maximum	Mean	SD[a]
Aflatoxin B_1 (AFB_1)	46.7	221	5818	1,749	2,260
Aflatoxin B_2 (AFB_2)	43.3	72.7	1,102	273	368
Aflatoxin G_1 (AFG_1)	10	109	381	165	195
Fumonisin B1 (FB_1)	96	2349	18,245	4,142	4,585
Fumonisin B_2 (FB_2)	96	817	7,493	1,881	2,193
Fumonisin B_3 (FB_3)	93.3	355	2,349	460	501
Deoxynivalenol (DON)	60	1.3	17.2	2.7	4
Zearalenol (ZEN)	16.7	1.0	1.4	0.9	0.5
Citrinin (CIT)	40	605	4,260	1,147	1,435

[a]Standard deviation

3.2 *Multi-metabolites occurrence in maize grain samples in Abuja Nigeria*

Apart from AFM1, OTA and patulin, all the other mycotoxins addressed by regulatory limits in the EU were detected in the 46 samples. Among the Area Councils, the highest occurrence of the detected metabolites in maize grain was from Gwagwalada and those from Abaji and Kuje had the least of 44 each (Table 2).

3.3 *Level of usage of management strategies against mycotoxins on maize grains by the Abuja farmers and marketers*

Only 19.30% of the farmers regularly sow improved seeds but in order to improve yield (Table 3). Most of the respondents (47.80%) practice mixed cropping to produce varieties of crops per season and also to reduce the spread of diseases. As high as 44.09% rarely used fertilizer on their maize farm while only 11.80% used fertilizer regularly. In order to control mycotoxins, none of the farmers occasionally or regularly used atoxigenic fungi strains to competitively displace toxigenic fungi as they are not aware of it. It was only 1.24%

of the respondents that rarely used atoxigenic fungi and was on a trial level. As high as 43.50% of the respondents occasionally applied seed dressing fungicides while 31.05% did not used it at all. Up to 54.04% of the farmers regularly harvest maize at maturity, however only 6.20% regularly used to bend down maize husk on the stalk and allowed drying on the field. According to the farmers, this practice of bending maize husks involves additional labour. Among the farmers, most of them rarely use pre-harvest management practice against mycotoxins (27.81%) and the least regularly (19.87%) used it

On post-harvest practices, 61.50% of the farmers regularly remove the damaged cobs immediately after harvest (Table 4). About 50.90% of the farmers and 41.20% of the marketers regularly store their maize in aerated stores. Most of the farmers (42.20%) and the marketers (46.72%) do not use smoking to preserve their harvested maize as they claimed that it is archaic. Only 0.62% that regularly use smoking on maize with husk reserved for next season planting. Both the farmers (37.90%) and the marketers (69.60%) used to dry their maize grains but mostly on bare open flat grounds under the sun. The management practice with highest Index among the farmers was the harvesting of maize at maturity while the least was the use of atoxigenic bio-control agents.

Generally, higher mean number of the marketers (48.80%) regularly sort, transport, dry, use pesticides and triple bag storage for their maize grains (Table 4). The management practice with highest Index among the marketers was the sorting and cleaning while the least was the use of Purdue Improved Crop Storage (PICS) hermetic triple bag.

Table 3.Level of usage of management practices against mycotoxin in maize by the farmers in the FCT, Abuja

S/No	Management Practice (161 respondents) **(Preharvest)**	Regularly (freq./%)	Occasionally (freq./%)	Rarely (freq./%)	Not use (freq./%)	Management Practice index (Rank)
1	Sowing of improved variety	32(19.3)	40(24.8)	48(29.8)	41 (25.5)	2.39(3rd)
2	Maize in mixed cropping	77(47.8)	35(21.7)	27 (16.8	22 (13.7)	3.03(2nd)
3	Diammonium phosphate fertilizer	19(11.8)	41(25.5)	71 (44.09)	30(18.6	2.30(4th)
4	Use of atoxigenic fungi strains	0	0	2(1.24)	159(98.8)	1.01(7th)
5	Apply fungicide on seeds/field	0	70(43.5)	41 (25.5)	50 (31.05	2.12(5th)
	Good Harvest Practice					
6	Harvest at crop maturity	87(54.04)	42(26.1)	2(1.24)	30 (18.6	3.15(1st)
7	Bend down husk on the stalk and allow to dry	10(6.2)	19(11.8)	61 (37.8)	71 (44.1)	1.80(6th)
	Mean %	19.87	21.91	22.35	27.81	

Table 4.Level of usage of management practices against mycotoxins on maize by the marketers in the FCT, Abuja

S/No	Management Practice (110 respondents)	Regularly (freq./%)	Occasionally (freq./%)	Rarely (freq./%)	Not use(freq./%)	Management Practice index (Rank)
1	Immediate removal of damaged cobs	39(38.2)	36 (35.3)	23(22.5)	4(3.9)	2.85(5th)
2	Maize stored in aerated stores	42(41.2)	31 (30.4)	16(15.7)	12(11.8)	2.77(6th)
3	Use smoking	0	18 (17.6)	35(34.3)	19(18.6)	1.30(8th)
4	Rapid and proper drying of maize without the husk	71(69.6)	21 (20.6)	6(5.8)	4(3.9)	3.30(2nd)
5	Sun drying on platform	59(57.8)	22(21.6)	5(4.9)	16(15.7)	2.97(4th)
6	Proper transportation and packaging	76(74.5)	10(9.8)	9(8.8)	7(6.86)	2.57(7th)
7	Sorting and cleaning	77(75.5)	15(14.7)	8(7.8)	2(1.96)	3.37(1st)
8	Use of botanicals/synthetic pesticides in the store	75(73.5)	14(13.7)	4(3.9)	9(8.8	3.26(3rd)
9	Use of Purdue Improved Crop Storage (PIC) hermetic triple bag	5(4.9)	7(6.8)	5(4.9	89(87.3)	1.27(9th)
	Mean %	(48.80)	(18.94)	(12.07)	(17.64)	

4. Discussion

Maize grains are known for harbouring several fungal strains many of which produce mycotoxins (Raghavender *et al*, 2007). Maize produced, stored or sold in most North central States of Nigeria have been shown over many seasons to be contaminated with agriculturally important toxins (Atehnkeng et al, 2008; Makun et al 2010). This is possibly due to favourable climatic and crop storage conditions that promote fungal growth and mycotoxin production, resulting from over reliance on subsistence farming and unregulated local markets. The occurrence of many of these mycotoxins in same matrices is a source of concern as co-occurrence may result in synergistic deleterious effect on the human and animal consumers (Tang, 2013; Spanjer, 2008).

In this study, there were relatively higher concentration levels of regulated toxins with an exception of DON and ZEN in the maize. Maize grains contained relatively higher levels of AFs above the EU maximum tolerable levels, with highest concentrations of 5817.6µg kg^{-1}. Ezekiel et al (2014) observed that mycotoxin levels were higher in the Nigerian maize-based kunu-zaki (<LOQ [limit of quantitation] - 123 µg kg-1) than in the sorghum- based *pito* (<LOQ - 5 µg kg-1). FB_1 mean concentration was as high as 4142 µg kg^{-1}while FB_2 concentration level was about 1881µg kg^{-1}. Adetunji et al (2014) and Adejumo and Adejoro (2014) had similar reports from their study of fungal toxins of stored maize grains in Nigeria that AFB_1 and FB_1 were quantified in 67.10% and 92.90% of the grains, and that 64.10% and 57.10% exceeded the EU maximum acceptable limit (MAL) for AFB_1 and FUMs, respectively.

From the review of Soriano and Dragacci (2004) FUM contamination of maize powder was not only more frequent but also accompanied by higher toxin concentrations. Kpodo and Bankole (2008) in their review similarly reported that among the regulated mycotoxins, maize was more susceptible to fumonisin contamination in West Africa. Also, Silva et al (2007) reported that FB_1 was always in higher concentrations than FB_2 and FB_3; following the general pattern of FUM contamination in maize and maize-based foods in Portugal

In Brazil, Souza (2013) reported the detection of maximum contamination level of 30µg kg^{-1} DON in maize samples which were below the maximum tolerable limit of 1000 µg kg^{-1} set as the US standard for maize. Biselli and Hummert (2005) analyzed DON toxin in maize and found an average of 140 µg kg^{-1} and maximum level of 1950 µg kg^{-1} respectively. It was also reported that Fusarium toxin, zearalenone (ZEN) concentration reached 1.4µg kg^{-1} for maize; thus, it did not exceed the maximum acceptable level (MAL) by EU for the mycotoxin at 400 µg kg^{-1} in 2012 but now propose a MAL of 150 µg $kg^{-1.}$

There was no OTA toxin detected in the maize grains. Kayode *et al* (2014) and Adetunji et al (2014) had a similar report from their analysis of mycotoxins and fungal metabolites in stored maize and maize-based snacks from Nigeria. Shephard et al (2013) reported that no AFs, OTA, or T-2 or HT-2 toxins were detected on their maize grains produced in the rural subsistence farmers in Transkei, South Africa. They associated this to the type of varieties planted and good agricultural practices.

The regular use of management strategies against mycotoxins contamination was low among the maize farmers and marketers in Abuja Nigeria. Past studies have posited that agricultural extension service is very germane to awareness, usage and crop protection information-seeking behaviour of farmers (Babalola et al, 2010). Thus there is need for urgent extension strategies and public awareness campaigns focused at farmers and marketers in order to enlighten them. Hell et al, (2008) reported that maize mono cropping, sowing of non-healthy seeds, intercropping with cowpea, peanut or cassava, non-application of fertilizer and delayed harvest, to be capable of enhancing mycotoxins build up in maize farms and stores. Other unwholesome practices are late shelling of cobs, delayed drying and no sorting at harvest, storage of maize in poorly aerated and dirty stores and inadequate insect pest control.

This indicated that more awareness and assistance should be directed toward the farmers in order to mitigate the problem of mycotoxin in cereals. However, the marketers need to know that once the maize have been harvested, dried and sorted, it should be packaged in clean sacks such as triple bags to prevent further contamination by moulds and toxin liberation in the maize seeds.

5. Conclusion

The Abuja maize samples analysed were contaminated with AFB_1 and AFB_2, FB_1 and FB_2 with their contamination levels exceeded the maximum levels established by the EU. There is need for a comprehensive and effective mycotoxin management and monitoring programmes with cost-effective sampling and analytical methods on grains and other food commodities, in order to reduce the risk of mycotoxins in them.

The authors declare no conflict of interest

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Biography

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