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**INVESTIGATION OF FUMONISIN LEVELS IN MAIZE GRAIN COLLECTED  
FROM AGRO-ECOLOGICAL ZONES OF NIGERIA**

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**ABSTRACT**

Fungi cause kernel rots of maize grain in the field and in storage, and *Fusarium* is the most important genus of the field fungi. Strains of *F. moniliforme* and *F. proliferatum* produce toxic metabolites known as fumonisins. *F. moniliforme* is a frequent contaminant of maize grain in Nigeria, but information on fumonisin in maize grain in West Africa is only available from Benin. This study was undertaken to identify *Fusarium* spp. and determine the levels of fumonisin in maize grain from farmers' stores in Nigeria. Maize grain samples from farmers' stores in three ecological zones of Nigeria were analysed for mould contents using the direct agar plating technique and fumonisin contamination using analytical High-Performance Liquid Chromatography (HPLC) methods with detection limits of 20ng/g. The first sampling involved taking maize grain that was between one and four months in stores, while samples for the second survey were taken when the maize grain had been in stores for not less than six months. The predominant genera of fungi isolated in all the agro ecological zones were *Fusarium*, *Aspergillus* and *Penicillium*. In the maize grains stored for one to four months, the mean levels of fumonisins were 1.25, 0.91 and 0.78 ppm in the rain forest, Guinea savannah and Sudan savannah respectively. In maize grains that were stored above six months, the mean fumonisin levels were 0.71, 0.73 and 0.65 ppm respectively. These results revealed that maize grain consumers across all the ecological zones of Nigeria are exposed to the risk of fumonisin toxicoses. Furthermore since aflatoxins have been found to be unavoidable contaminants of Nigerian maize grain, this study could show that consumers are likely exposed to multiple mycotoxins, the interactive effect of which is still poorly understood, the synergistic or additive effect of mycotoxin combinations could be applied.

**Keywords:** Agro-ecological zones, Fumonisin, Maize, Nigeria, Storage,



## INTRODUCTION

Maize (*Zea mays L.*) is the world's largest cultivated cereal crop with over 872 million metric tons (MT) produced in 2012 (FAO, 2014), and one of the most important cereals used for food and feed production (IITA, 2014). African adults consume an average of 400g of maize-based diets/person/day which is about 40 times higher than maize grain intake in the developed world (Pitt *et al.*, 2013); this emphasizes the importance of maize in the African diet. In Nigeria, maize grain is an important cereal crop with an estimated production of 4,779,000 valued at USD555, 220,000 in 2005 (FAO, 2005), making the country the second largest producer after South Africa in Africa. The International Institute for Tropical Agriculture (IITA) nutritional survey shows that it is the most consumed staple food in all the ecological zones of Nigeria (Maziya-Dixon, 2004). Several fungi attack maize at pre-harvest and postharvest stages, with some of them producing mycotoxins under suitable conditions. The aflatoxin produced by *Aspergillus flavus* and *A. parasiticus* and fumonisins produced by *Fusarium verticillioides* and *F. proliferatum* are the most important and of high priority in sub-Saharan Africa because of their frequent distribution in food products (Marin *et al.*, 2001, Kpodo and Bankole 2008; Asiki *et al.*, 2014). The occurrence of aflatoxins in food crops of African origin has been a subject of intense study since its discovery in 1960 (Shephard, 2003), and the FAO (2004) listed Nigeria as one of the 15 African countries regulating aflatoxin in foods.

Fumonisin are polypeptide mycotoxins associated mostly with maize grain. The *Fusarium* species attacking maize in Africa include *Fusarium verticillioides* (Bankole, 1994; Kedera *et al.*, 1999; Afolabi *et al.*, 2006). Though, fumonisin was discovered in maize in Africa, however its distribution has only been studied in details in South Africa (Dutton, 2003) while there are relatively few data on corn from other parts of the continent. A preliminary survey has shown that fumonisin B1 occur in up to 78% of preharvest maize from south western Nigeria located in the rain forest of Nigeria (Bankole and Mabekoje, 2004). Existing reports



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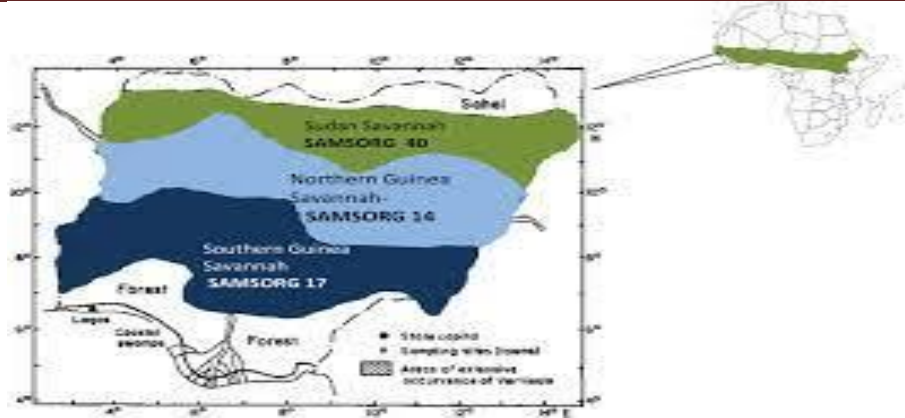
show that fumonisins cause cancer and neural tube defects in laboratory rodents (Abbas *et al.*, 2013). The fumonisins also cause fatal toxicoses (equine encephalomalacia) in horses and porcine pulmonary oedema in swine (Gnonlonfin *et al.*, 2013). Though, the impact of fumonisin on human health is not yet fully understood, but ingestion of corn with high fumonisin concentrations has been correlated with high oesophageal cancer rates in the Transkei region of South Africa and in the Linxian region of China (Thiel *et al.*, 1992; Darwish *et al.*, 2014). It has also been suggested that fumonisins are connected to the high incidence of infant neural tube defects in Texas Mexico border, where consumption of corn tortillas is very high (Gelineau-van Waes *et al.*, 2005). Recent findings suggest that fumonisin exposure increases the risk of neural tube defects, proportionate to dose, up to a threshold level, at which point foetal death may be more likely to occur (Wild *et al.*, 2016; Akbari *et al.*, 2017). International Agency for Research on Cancer (IARC) classified FBs in group 2B i.e. that they are possibly carcinogenic to humans (IARC Working Group, 2012).

Farmers in Nigeria store maize grain for varied period of time but usually less than one year depending on market price. This study was conducted to determine the fumonisin contamination of maize grain from farmers' stores in three ecological zones of Nigeria. The results of this study will provide data on the occurrence of fumonisin in maize from different parts of Nigeria.

### MATERIALS AND METHODS

#### **Agroecological zones covered by the survey**

The surveys were carried out in three (rain forest, guinea savannah and Sudan savannah) agro-ecological zones of Nigeria. The agroecological zones as identified by Iloeje (1981) and described by Udoh *et al.* (2000) and Sowunmi and Akintola (2010).



### Sampling

Five local governments in each ecological zone were used for the study. Five villages were selected in each local government area based on the following criteria: easy accessibility to the village by road transportation, populations higher than 1000, previous visitation by scientist and maize being a significant component of their daily diet. The first batch of maize samples selected were less than four months in stores, while the second sampling was carried out on maize that were six months and above in stores. The same farmers' stores were used for the first and second surveys. However, some of the farmers had completely sold off the maize in their stores thus, the number of samples in the second surveys were less than the first.

### Moisture Content Determination

The samples were analysed for moisture content by oven drying 5 g of milled grains at 105<sup>0</sup>C for 4 hrs. The percentage of the difference between initial weight and dry weight divided by the initial weight represented the moisture content.



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### **Determination of Visually Infected Grains**

The number of visibly mould, rotted or discoloured grains in a random sample of 200 grains from each sample was counted and expressed as a percentage.

### **Isolation and Identification of Fungi**

One hundred gram from each sample were surface disinfected in 1% sodium hypochlorite solution for two minutes, rinsed three times with changes of sterile distilled water, and blotted dry between folds of sterilised filter paper. From each sample, 50 grains randomly selected were plated directly in 90mm diameter Petri dishes (at the rate of five kernels) per dish containing dichloran rose bengal agar. The plates were incubated in the dark in upright positions at room temperature  $27\pm 2^{\circ}\text{C}$  for 5-7 days. The emerging fungal colonies were counted, grouped and transferred into identification media. The species of *Aspergillus*, *Penicillium* and other genera except *Fusarium* were identified by culture characteristics in Potato Dextrose Agar (PDA), malt extract agar and Czapez agar (Gough *et al.*, 2017) and by microscopic examinations (Barnett and Hunter, 1982), *Fusarium* isolates were identified by observing their micro-morphological features on synthetic nutrient agar and carnation leaf agar (Nelson *et al.*, 1980; Gough *et al.*, 2017).

### **Fumonisin Analysis**

The extraction, clean-up and assay of fumonisin were based mainly on the method of Shephard *et al.* (1990). Twenty grams (20g) of powdered maize mixed with 100mL methanol/water (3:1 v/v) was blended at high speed for approximately one minute, followed by filtration through fluted filter papers. The pH of the filtered extract was adjusted to 5.8 to 6.5 with 1M NaOH solution. The clean-up, the Bond-Elut strong anion exchange (SAX) cartridge was conditioned with 5 mL of methanol and then with 5 ml methanol/water (3:1 v/v) and 3 mL of methanol. Then 10 ml of supernatant of extracted maize was applied to the cartridge at a flow rate of one or two drops per min. Then, the cartridge was washed with methanol/water (3:1



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v/v) and methanol. Fumonisin were eluted by passing 10 ml of an acetic: methanol (1:99) solution through the cartridge at one drop per sec. The eluate was evaporated to dryness at 40°C under a gentle stream of nitrogen, and kept at 4°C in a refrigerator until use.

The dried residue was reconstituted in 200µl of acetonitrile/water (1/1) by vortexing and filtered with a membrane filter. 50µL of the solution was derivatised with 50µL of *o*-phthalaldehyde reagent (40mg of *o*-phthalaldehyde in 1 ml methanol, mixed with 5 ml of 0.1M sodium tetra borate and 50µl of 2-ercaptoethanol), mixed very well with a vortex mixer and 20µL of the derivatised solution was injected into the HPLC system with an R1:55 fluorescent detector (excitation wavelength 355 nm), 25:2 ratio methanol and injected into the isocratic HPLC system ( reverse phase C18 column; flow rate, 1.0 ml/min; loop, 100µl, analysis). The fumonisin derivatives were separated on reverse-phase isocratic high-performance liquid chromatography (HPLC), (emission wavelength 440 nm and the mobile phase was methanol/sodium acetate buffer (7:3 v/v, pH 3.6) as described by Bankole and Mabekoje (2004).

### Recovery Experiments

Ground fumonisin free maize was spiked with FB1:FB2 in the ratio 2:1 at total concentrations levels of 100, 500 and 1000 ng/g adding 10, 50 and 100µg of spiking solution (containing 250µg total fumonisins per ml), respectively. The average calculated recoveries were 88±3.5% for FB1 and 83±5.6% for FB2. The detection limit of the method was 20ng for the two fumonisin.

## RESULTS AND DISCUSSION

The results presented in Table 1 shows that the moisture content of maize from farmers stores for one to four months was highest in the rain forest with a mean of 14.8%, followed by that



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of the Guinea savanna(12.7%), and lowest in Sudan savanna(12.5%). However, no significant differences were found in the moisture content values within the four months of storage.

For the maize samples that were more than six months in stores (Table 2), the moisture content of Rainforest samples (12.5%) was significantly higher than the values from the Guinea Savannah (10.3%) and Sudan Savannah (9.7%), but no statistical difference was found between the moisture content of Guinea savanna and Sudan savannah samples.

**Table 1:** Moisture content of maize in stores for one to four months from three ecological zones of Nigeria

Ecological zone	No of samples	Moisture content (%)		Visually infected grain %	
		Range	Mean	Range	Mean
Rainforest	25	11.3-18.8	14.8	4-15	7.6
Guinea Savannah	25	10.5-15.7	12.7	3-9	5.3
Sudan savanna	25	10.7-14.8	12.5	4-7	4.7

**Table 2:** Moisture content of maize in stores for more than six months from three ecological zones of Nigeria

Ecological zone	No of samples	Moisture content (%)		Visually infected grain %	
		Range	Mean	Range	Mean
Rainforest	18	9.5-13.8	12.5	7-25	16.3
Guinea Savannah	16	9.3-11.7	10.3	5-16	11.2
Sudan savanna	22	8.9-10.6	9.7	4-14	9.5



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The moisture content represents one of the most critical factors that influence mould growth and toxin elaboration in stored grains, and Olafur-Reykdal (2018) put the safe moisture content of maize at 15%. For maize grains that were in stores for one to four months, seven samples in the rain forest and three samples in the Guinea savanna and only one in the Sudan Savanna had moisture content above 15%. However, all the maize samples that were above six months in stores had moisture content less than 15%. Majority of the maize samples of one to four months, and all the samples of above six months, had less than 15% safe moisture level. In the survey of maize from Ugandan traders, Mugabi and Driscoll (2016) reported moisture content ranged of 8.22 to 15.1%. That the maize grain continue to dry in stores as evidenced from the moisture content of maize grains stored for over six months, which was less than that of one to four months in stores agrees with the report of Mugabi and Driscoll (2016).

The proportion of visually infected grains was significantly higher in rain forest for both samples of one to four months and those more than six months in stores (Tables 1 and 2). There was no significant difference in the proportion of damaged seeds in Guinea and Sudan savanna samples for samples of one to four months in stores. However, the proportion of damaged seeds was significantly higher in Guinea savanna than that of Sudan savanna samples for samples stored for more than six months.





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**Table 3:** Distribution of Fungi on Maize in Stores for One to Four months from Three Ecological Zones of Nigeria

Fungi	Forest (n=25)		Guinea savannah (n=25)		Sudan savannah (n=25)	
	NSI	Mean	NSI	Mean	NSI	Mean
<i>Aspergillus clavatus</i>	2(8)	2.5	4(16)	1.5	-	-
<i>Aspergillus flavus</i>	8(32)	6.7	6(24)	4.5	9(36)	4.9
<i>A. nidulans</i>	-	-	3(12)	1.1	-	-
<i>A. niger</i>	8(32)	8.8	10(40)	5.2	6(24)	3.4***
<i>A. fumigatus</i>	5(24)	2.4			2(8)	1.3
<i>A. ochraceus</i>	2(8)	2.0	1(4)	2.0	-	-
<i>Curvularia lunata</i>	2(8)	1.57	1(8)	3.0	-	-
<i>Penicillium chrysogenum</i>	3(12)	2.3	2(8)	2.5	1(4)	3.0
<i>P. citrinum</i>	2(8)	3.5	-	-	2(8)	2.5
<i>P. pinophilum</i>	-	-	-	-	3(12)	2.3
<i>Fusarium graminearum</i>	6(24)	4.7	3(12)	3.3	4(16)	2.7
<i>F. pallidoroseum</i>	2(8)		5(20)		3(15)	
<i>F. verticillioides</i>	20(80)	28.3	15(60)	16.7	18(72)	21.5
<i>F. solani</i>	3(12)	3.7	-	-	-	-
<i>Rhizopus spp</i>	5(20)	2.7	3(12)	2.5	7(28)	3.5
<i>Mucor spp</i>	5(20)	3.3	4(16)	2.7	2(8)	1.5

Fungi belonging to five genera were identified on the 75 maize samples in stores for one to four months (Table 3). *Fusarium* spp was the most frequent genera followed by *Aspergillus* and *Penicillium* in all the three agroecological zones surveyed. Four species of *Fusarium* were identified with *F. verticillioides* distinctly being the most frequent as it occurred in 80%, 60% and 72 % of samples with a mean grain infection of 28.3%, 16.7% and 21.5% of Rainforest, Guinea savanna and Sudan savanna



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respectively. *F. graminearum* was also found in the three zones with mean grain infection ranging from 2.7 to 4.7%. *Aspegillus niger* and *Aspergillus flavus* were found in maize samples from the three zones with 24-40%, and 24 to 36% infected respectively, while the percentage grain infection ranged from 4% to 80%. *Fusarium verticillioides*, *Aspergillus flavus*, *Aspergillus niger*, *F. graminearum* in decreasing order were the most frequent fungi identified on maize stored for one to four months.

**Table 4:** Distribution of Fungi on Maize in Stores for More than Six months from Three Ecological Zones of Nigeria

Fungi	Forest (n=18)		Guinea savannah(n=14)		Sudan savannah (n=17)	
	NSI	Mean	NSI	Mean	NSI	Mean
<i>Aspergillus clavatus</i>	2(8)	3.5	4(16)	2.3	-	-
<i>Aspergillus flavus</i>	15(32)	23.5	11(24)	17.6	14(36)	19.7
<i>A. nidulans</i>	4	2.3	7(12)	3.3	-	-
<i>A. niger</i>	16(32)	32.3	10(40)	15.2	15(24)	24.4
<i>A. fumigatus</i>	7(24)	5.5	4	2.7	7(8)	4.3
<i>A. ochraceus</i>	5(8)	5.7	5(4)	7.0	-	-
<i>Curvularia lunata</i>	2(8)	1.0	1(8)	2.0	-	-
<i>Penicillium chrysogenum</i>	6(12)	3.5	6(8)	4.6	3(4)	5.3
<i>P. citrinum</i>	2(8)	3.5	-	-	2(8)	2.5
<i>P. pinophilum</i>	-	-	-	-	3(12)	2.3
<i>Fusarium graminearum</i>	6(24)	4.7	3(12)	3.3	4(16)	2.7
<i>F. pallidoroseum</i>	2(8)		5(20)	-	3(15)	-
<i>F. verticillioides</i>	20(80)	28.3	15(60)	16.7	18(72)	21.5
<i>F.solani</i>	3(12)	3.7	-	-	-	-
<i>Rhizopus spp</i>	5(20)	2.7	3(12)	2.5	7(28)	3.5
<i>Mucor spp</i>	5(20)	3.3	4(16)	2.7	2(8)	1.5



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In table 4, the predominant genera of fungi identified on maize in stores for more than six months were *Aspergillus*, *Fusarium* and *Penicillium*. Seven species of *Aspergillus* were identified, with *A. niger* predominating, and was recovered in 83.7% of the samples with a mean grain infection ranging from 15.2% to 32.3%. *A. flavus* which was the second most frequent fungi occurred in 81.6% of samples with mean grain infection ranging from 17.6% to 23.5%.

That *F. verticillioides* predominated above other fungi is in accordance with the report of other workers that *F. verticillioides* is the most common seed borne fungi in Africa (Kpodo *et al.*, 2000, Leslie and Bandyopadhyay 2005, Afolabi, *et al.*, 2006, Kaaya and Kyamuhangire 2007; Yli-Mttila and Sundheim, 2022). The field fungi require moisture content of 20-25% for their activity, thus the decrease in the incidence and abundance of *Fusarium* spp within store is to be expected since the moisture content in the storage systems was far below the levels required for their activity.

**Table 5:** Fumonisin Contamination of Maize Stored for One to Four months obtained from Farmers Stores in Three Agro-ecological Zones of Nigeria

Zone	No of Samples	Fumonisin B1 (ppm)			Fumonisin B2 (ppm)			Total fumonisins (ppm)		
		%+ves	Range	Mean	%+ves	Range	Mean	%+ves	Range	Mean
Rainforest	25	100	0.25-2.89	1.25A	88	0.20-0.47	0.46A	100	0.25-3.03	1.62A
Guinea savanna	25	100	0.37-1.93	0.91B	80	0.22-0.57	0.36AB	100	0.37-2.33	1.20B
Sudan savanna	25	100	0.20-2.15	0.78B	64	0.15-0.28	0.29B	100	0.20-2.53	0.96B
<b>Total</b>	75	100	0.20-2.89	0.87	77	0.15-0.57	0.30	100	0.20-3.03	1.28

Total fumonisins= (FB1+ FB2) within a column, means with different letters are significantly different

The results in Table 5 shows that fumonisins were detected in all the maize stored for one to four months had detectable levels of fumonisins, irrespective of the ecological zone. Fumonisin B2 was found in 77% of all samples, 88%, 80% and 64% from the forest, guinea savanna and from Sudan savanna samples respectively. Maize samples from the forest had significantly higher levels of fumonisin B1, followed by guinea savanna, while the samples from Sudan savanna had the least, though no significant differences was found between the last two. Four samples from the forest had



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fumonisin levels above the 2ppm (levels), while only one samples from Sudan savanna had fumonisin levels (2.15ppm) exceeding 2ppm.

**Table 6:** Fumonisin Contamination of Maize Stored for more than Six months obtained from Farmers Stores in Three Agro-ecological Zones of Nigeria

Zone	No of Samples	Fumonisin B1 (ppm)			Fumonisin B2 (ppm)			Total fumonisins (ppm)		
		%+ves	Range	Mean	%+ves	Range	Mean	%+ves	Range	Mean
Rainforest	18	100	0.20-1.83	0.71A	83	0.16-0.35	0.24A	100	0.20-2.13	0.91A
Guinea savanna	14	86	0.17-1.75	0.73	80	0.14-0.39	0.20	86	0.17-1.87	0.93
Sudan savanna	17	79	0.13-1.56	0.65	57	0.15-0.41	0.29	79	0.13-1.73	0.90
<b>Total</b>	49	88	0.13-1.83	0.87	75	0.14-0.41	0.24	88	0.13-2.13	1.28

Total fumonisins= (FB1+ FB2) within a column, means with different letters are significantly different

The results presented in Table 6 shows that maize samples stored for more than six months had 88% fumonisin contamination. Maize from the forest had the highest rate of contamination (100%) followed by Guinea savanna samples (86%), while maize from Sudan Savanna had 79% contamination. The levels of fumonisin B1 followed the same trend, where maize from the forest still had the highest fumonisin levels, though no significant differences were found.

The presence of fumonisins in maize grains which is the staple mostly consumed by Nigerians is a serious public health threat. It is important to carry out regular survey for the occurrence of toxins that have been found to be prominent in sub Saharan Africa such as fumonisin so that human and animal exposure to these toxins could be accurately assessed. Bryla *et al.* (2017) have proposed that fumonisin levels in raw maize are influenced by environmental factors such as rainfall, temperature and humidity during the pre harvest and harvest period exert tremendous impact on fumonisin contamination of raw maize. The fumonisin levels found in corn samples from the three ecological zones follow the same trend as *F. verticillioides* i.e. comparatively higher levels of fumonisins in rain forest than in the other two zones. This is in consonance with the findings of *Ghiasian et al.* (2006) in which highest levels of



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fumonisin in maize were obtained from the area with the highest amount of rainfall and humidity in Iran.

### CONCLUSION

A wide range of mould species contaminate stored maize in Nigeria, the degree varying with different climatic conditions and storage time.

The high incidence of fungi in maize and the significant levels of fumonisins mycotoxins recorded in present study warrants that there is urgent need to undertake mycotoxin awareness creation programs among different foods and feedstuffs. The control manipulation of environmental factors particularly the moisture content of cereals during various steps of production before reach to consumer assist reduction of the risk of harmful effects of toxigenic fungi and their toxins to human and other farm animals health.

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