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ASSESSMENT OF AFLATOXIN CONTAMINATION OF SOME FOODS IN SOUTH EASTERN NIGERIA

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ABSTRACT

Mycotoxins contamination of foods is a major threat associated with harvested food products sold in open markets. This appalling situation is made worse in the tropics where the warm and humid climate is stable for microbial proliferation. This research work is aimed at assessing the mycotoxin contamination of *Citrullus colocynthis*, *Irvingia wombolu* and cassava chips sold in open markets in South-Eastern Nigeria. Simple random sampling technique was used to collect samples of *Citrullus colocynthis*, *Irvingia wombolu* and cassava chips from storage structures in three waves (Wet season, Harmattan and Dry season) between February, 2015 and March, 2016 from three senatorial zones in each of the states in south eastern Nigeria. The food samples were analyzed for aflatoxins (AFB1, AFB2, AFG1 and AFG2). Thin Layer Chromatographic technique was used for the analysis of Aflatoxins. Aflatoxin was detected in all the food samples, highest in *Citrullus colocynthis* and lowest in cassava chips; relatively higher concentrations of Aflatoxins were detected in Anambra and Enugu state during Wet season and Harmattan. There was no significant ($P<0.05$) difference in the concentration of Aflatoxins in *Irvingia wombolu* across the wave of collection, in cassava chips aflatoxins was detected more during the Harmattan while in *Citrullus colocynthis* it was detected more during Wet season. AFB1 and AFB2 occur in all the food material across the waves of collection, while only insignificant quantity of AFG1 (0.033±0.100ppb) occur in cassava chips across the wave of collection. The level of aflatoxins in these food materials across the states were lower than the NAFDAC maximum permissible level (4ppb) but frequent contamination of these food materials and constant consuming of these contaminated foodstuffs may result in bio-acumulation of these toxins in the human system, the presence of AFB1 and AFB2 in their varying concentrations may portend serious health risks for the human population and also has implications for food safety. Inference of this study showed that these foodstuff are partially safe for consumption because of the presence of aflatoxins.

Keywords: Aflatoxins, Egusi, ogbono, cassava chips, South Eastern Nigeria,

INTRODUCTION

World Health Organization (2002) estimates that about a quarter of the diseases facing human being today occur due to bio-accumulation of toxins in human system as a result of consumption of food materials that are improperly handled and processed. The notion that consumption of foods that are improperly handled is sometimes hazardous to human health cannot be overemphasized. Improper handling of food materials from the field, store to the market place is a common phenomenon in Nigeria. These food materials sold in the open market are often contaminated with fungi which produce mycotoxins, when these foods are consumed by human beings and animals, the toxins accumulates in the body with serious health effects (Rotich *et al.,* 2006; UNDP, 2006; USEPA, 2002).

Risk of mycotoxin contamination of food, feed and raw material in Africa is increased due to environmental, agronomic and socio-economic factors. The socio-economic and food security status of the majority of inhabitants of sub-Saharan Africa leaves them with little option in choosing good quality products (Hell *et al.,* 2010). Many individuals in developing countries are not only food insecure but chronically exposed to high levels of mycotoxins through their diets (Adetunji *et al.,* 2014). In many developing countries such as Nigeria, mycotoxins affect staple foods such as maize, groundnut, rice etc such that exposure is continuous and often at high levels. The exposures occur in towns, villages and communities that produce and consume their own food; hence regulatory measures to control exposure are largely ineffective (Wild and Gong, 2010).

Approximately 25% of the world's crops are affected by fungal growth, and commodities may be, both pre- and post-harvest contaminated with mycotoxins (Anukwuorji *et al.* 2012, 2013; Francesca and Chiara, 2012, Okigbo *et al.,* 2013). Occurrence and concentrations of mycotoxins were variable by year, which is expected because of the annual variation in weather

conditions and plant stresses known to affect mycotoxin formation (Coulumbe, 1993). Also mycotoxins that can be expected in food differ from location to location in relation to the different crops, agronomic practices and climatic conditions (Bryden, 2007). Since climate changes affect the growth of mycotoxigenic fungi, mycotoxin production is also influenced (Makun *et al.*,2010).

Previous studies have shown an increase in the aflatoxin contamination of food materials during periods of high temperature and humidity with subsequent increase in cases of health issues (Okigbo *et al.*, 2015). South eastern Nigeria, the area of this research is characterized by hot, moist and wet humid environment. Hence the reports of so many scientists showed that such environments encourage the proliferation of fungal producing mycotoxins and contamination of foods by heavy metals especially in inadequate dried, processed and stored food commodities. The World Bank estimates that currently nine African countries including Nigeria will have 64% of their annual export of nuts, fruits and cereals valued at about $674million in trade annually reduced as a result of rejections in overseas' markets caused by mycotoxin contamination and other Sanitatry and Phyto-Sanitary (SPS) related issues (Awolowo, 2014). The Food and Agricultural Organization (FAO, 2005) has equally estimated that 25% of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins. It has also been estimated by the Centre for Disease Control (CDC) that more than 4.5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxin through contaminated food (Awolowo, 2014).

'Egusi' (*Citrullus colocynthis* (L.) Schrader), 'Ogbono'(*Irvingia wombolu* Vermoesen) and cassava chips are important staple food in south eastern Nigeria (Akusu and Kiin-kabari, 2016). These food materials are susceptible to fungal infection both in the stores and in the

growing field (Pereira, *et. al.,* 2014). Hence the possibility of mycotoxins occurrence in these food materials (Devi, *et al.,* 2009).

Myriads of works have been done globally to assess quality of common foods such as rice, maize etc (FAO, 2005). But none of these studies looked into the problems of mycotoxins contamination of local food materials such as egusi, ogbono and cassava chips in south eastern Nigeria, with the aim of supplying stakeholders (farmers, traders, government ministries/agencies etc) with information on how to improve the quality of these products sold in the market and consumed by the masses. Therefore, the inference of this study will elucidate some information on the quality of these food materials with the aim of alerting the public on the potential dangers of consuming foods that are improperly handled and to ensure the safety of consumers.

MATERIALS AND METHODS

Sample collection

The method of Adetunji *et al.,* (2014) was adopted in sampling food materials; the food materials sampled were *I. wombolu*, *C. colocynthis* and cassava chips. Food samples that were locally sourced, sun dried, not sorted and had been stored for at least four months in their respective state of sampling were collected in dry season (February- April), wet season (May-August) and Harmattan (November- January) from designated markets and stores in the three senatorial districts of each state. Using simple random sampling technique a total of three (3) markets from each senatorial zone were sampled, 10g each of sample was collected from three (3) different sampling points from each market. Each of the plant materials purchased from all senatorial zones within a state were pooled together to form a single bulk, from where the secondary sample was made. Two hundred grams (200g) working sample was drawn at random from the mixed samples. The 20g working sample was ground to a powdered form. These

working samples represented plant materials from the states in which they were collected. Hence a total of five samples per plant materials at each analysis time (dry season, wet season and harmattan) were used for the experiment.

Analysis of Aflatoxin

The samples were screened and analyzed for aflatoxin B1, B2, G1 and G2. The Thin Layer Chromatographic method of the Association of Analytical Chemistry (AOAC) (1996) was adopted for the analysis.

Extraction of Aflatoxin from cassava chips

Cassava chips samples were carefully ground with commercial waring blender (Waring blade series L HP blender, made in USA) and thoroughly mixed. Twenty gram (20g) of the ground sample was randomly picked and weighed out (in 3 replications) for extraction purpose. Twenty gram (20g) ground working sample was thoroughly mixed with 100 ml of 80% methanol for three minutes using waring blender. Blended mixture was poured into a 250 ml Pyrex conical flask, and the flask was sealed with parafilm. Mixture from the step above was vigorously shaken using orbit shaker at 4 x 100 rpm for 30 minutes. Thereafter, it was filtered into a clean conical flask (that had been previously rinsed with absolute methanol) using No 1 quantitative Whatman filter paper, 185 mm. The filtrate (40 ml) was poured into a separating flask. Twenty milliliters (20 ml) of distilled water was added. Twenty five milliliters **(**25 ml) of dichloromethane was also added. The separating flask containing the mixture was hand-shook gently and left for few minutes to separate (i.e. allow mixture to separate into top and bottom phases). The extract or bottom phase was drained through a bed of 20g *anhydrous sodium sulphate* into a 150 ml white plastic beaker. To the remaining mixture in the separating flask, 10 ml of dichloromethane was added. This was gently shaken and allowed to separate. The extract or bottom phase was drained through a bed of 20g *anhydrous sodium sulphate* into a 150 ml white plastic beaker (that contains the first extract). The extract was allowed to stay in the fume

hood to dry overnight. Dried extract was reconstituted with 1 ml of dichloromethane and transferred into a 1.5 ml Eppendorf tube and left in the hood to dry overnight.

Extraction of aflatoxin from *I. wombolu* and *C. colocynthis*

Ogbono (*I. wombolu*) or melon (*C.colocynthis*) samples were carefully ground with commercial waring blender and thoroughly mixed. Twenty gram (20g) of ground Ogbono or melon was randomly picked and weighed out (in 2 replications) for extraction purpose. Twenty gram (20g) working sample of ogbono or melon was thoroughly mixed with 100 ml of 80% methanol for three minutes using waring blender. Blended mixture was poured into a 250 ml Pyrex conical flask, and the flask was sealed with parafilm. Mixture from step 4 above was shaking vigorously using orbit shaker at 4 x 100 rpm for 30 minutes. Thereafter, it was filtered into a clean conical flask (that had been previously rinsed with absolute methanol) using No 1 quantitative Whatman filter paper, 185 mm. The filtrate (40 ml) was poured into a separating flask. Forty milliliters (40 ml) of 10% Sodium chloride was added. Twenty-five milliliters (25 ml) of hexane was also added. The mixture was shaking vigorously by hand for 1 minute and allowed to separate. The extract or the bottom phase was drained into a 250 ml conical flask and what remained in the separating flask was discarded. At this point, the filtrate was poured back into the cleaned separating flask. Twenty five milliliters (25 ml) of dichloromethane was added. The separating flask containing the mixture was hand-shook gently and left for few minutes to separate (i.e. allow mixture to separate into top and bottom phases). The extract or bottom phase was drained through a bed of 20g *anhydrous sodium sulphate* into a 150 ml white plastic beaker. To the remaining mixture in the separating flask, 10 ml of dichloromethane was added. This was hand-shook gently and allowed to separate. The extract or bottom phase was drained through a bed of 20g *anhydrous sodium sulphate* into a 150 ml white plastic beaker (that contains the first extract). The extract was allowed to stay in the fume hood to dry overnight. Dried extract was

reconstituted with 1 ml of dichloromethane and transferred into a 1.5 ml eppendorf tube and left in the hood to dry overnight.

Quantitative assay of extracts

Reconstituted dried extract was dissolved in 1 ml dichloromethane and vortex to homogenize the mixture. High Performance Thin Layer Chromatography (HPTLC) plate to be used for spotting was calibrated according to the standard format. A cleaned micro-capillary tube was inserted into a bulb assembly through silicon tip and made sure it was firm. Carefully and gently the capillary tube was cleaned with acetone solution in three changes. Carefully, 4 ul of aflatoxin **G** and **I** standards was spotted on 6^{th} and 8^{th} marked spots respectively on calibrated HPTLC. Carefully, 4ul of each sample extract was spotted on remaining 1cm interval marked spots on the HPTLC plate. Spotted and air-dried plate was developed in a solution of diethyl ether, methanol and distilled water in ratio 96:3:1. The developed plates was viewed under the ultraviolet light- box (wavelength = 365 nm) to see whether each extract fluoresces or not. Those with fluorescence and those without are compared with the standards. Quantitatively, extracts were subjected to quantitative analysis to ascertain total amount aflatoxins (B_1, B_2, G_1 and G_2) in each of the samples. This was done with the aid of *CAMAG* Thin Layer Chromatography scanner 3; which enables quantitative evaluation of densitometric data to be generated.

RESULTS

Concentration of Aflatoxins in food samples

For the comparison of aflatoxins in food samples, there was significant difference in the concentrations of AFB1 across all the food samples, the highest value of AFB1 (0.154±0.012 (ppb) was observed from *I.wombolu*, next to this was 0.090±0.038 ppb recorded from *C. colocynthis* and the least concentration (0.018±0.063 ppb) was observed from cassava chips. For AFB2, the highest value was detected in *C. colocynthis* (0.136±0.001ppb) this was significantly higher ($P<0.05$) than 0.014±0.056ppb and 0.034±0.073ppb recorded from cassava chips and *C.*

colocynthis respectively. *I. wombolu* has the highest concentration of AFG1 (0.073±0.018 ppb), the least in AFG1 was cassava chips (0.001±0.003 ppb). AFG2 was not detected *I. wombolu*, while very small quantities of 0.0067±0.045 ppb and 0.0004±0.000 ppb were observed in cassava chips and *C. colocynthis* respectively. For total aflatoxins, there was no significant difference between the values recorded from *C. colocynthis* (0.234±0.278 ppb) and *I. wombolu* (0.262±0.274 ppb), the least concentration of total Aflatoxin was recorded from cassava chips (Table 1).

Table 1: Comparison of Aflatoxins in the Food Samples

Food Samples	Aflatoxin Concentration (ppb) B1	B2	G1	G2	Total
cassava chips	0.018±0.063^{c}	0.014±0.056^{b}	0.001±0.003^{b}	0.0067±0.045^{a}	0.040±0.091^{b}
C.colocynthis	0.090±0.038^{b}	0.136±0.001^{a}	0.008±0.019^{b}	0.0004±0.000^{b}	0.234±0.278^{a}
I. wombolu	0.154±0.012^{a}	0.034±0.073^{b}	0.073±0.018^{a}	0.0000±0.000^{c}	0.262±0.274^{a}
p-value	0.000	0.000	0.002	0.395	0.000

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)

Concentrations of Aflatoxins in cassava chips

There was no significant difference in the concentration of AFB1 in cassava chips across the states except in Imo State where AFB1 was not detected, for concentrations of AFB2 in cassava chips, the values of 0.040±0.105ppb and 0.022±0.067ppb were detected in samples collected from Enugu state and Abia state respectively, these were significantly higher than 0.009±0.012ppb recorded from samples collected from Ebonyi state, AFB2 was not detected in Anambra and Imo state. AFG1 not detected in all the samples across the states except in Ebonyi state with a value of 0.003±0.008ppb, AFG2 was also not detected in all the states except in Anambra State (0.033±0.100ppb). For total aflatoxin, the highest values was recorded from Anambra state (0.086±0.142ppb), this was significantly (P>0.05) higher than 0.047±0.140ppb and 0.044±0.021ppb recorded from samples collected from Abia state and Enugu state respectively, the least concentration of 0.023±0.014ppb was obtained from Ebonyi state, while

no aflatoxin was detected from Imo state. With respect to the wave of collection, there was no significant difference in the values of AFB1 and AFB2 recorded across the wave of collection, although the highest value of AFB1 (0.026±0.082ppb) and AFB2 (0.01±0.0240ppb) were recorded during the dry season but these values were not significantly (P>0.05) higher than values recorded from samples collected during harmattan and Wet season. AFG1 and AFG2 were not detected from samples collected across the waves of collection except during harmattan with values of 0.002±0.006ppb and 0.020±0.077ppb for AFG1 and AFG2 respectively. For total aflatoxin there was significant difference in the values obtained, the highest values of 0.056±0.136ppb was recorded during the wet season, this was significantly higher than 0.036±0.028ppb recorded during harmattan at P>0.05 level of significance while the least value of 0.017±0.028 was observed during dry season. There was significant difference in total aflatoxins across the wave of collection, 0.056±0.136ppb was recorded during wet season this was significantly (P>0.05) higher than 0.036±0.028ppb observed during dry season, the least value of 0.027±0.077ppb was recorded during harmattan (Table 2).

Table 2: Concentrations of Aflatoxins in Cassava chips

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	(Total)	0.024±0.073[a]	0.022±0.067[a]	0.000±0.000[b]	0.000±0.000[b]	0.047±0.140[b]
Anambra		0.050±0.120[a]	0.000±0.000[c]	0.000±0.000[b]	0.033±0.100[a]	0.086±0.142[a]
Ebonyi		0.010±0.011[a]	0.009±0.012[b]	0.003±0.008[a]	0.000±0.000[b]	0.023±0.014[c]
Enugu		0.004±0.009[a]	0.040±0.105[a]	0.000±0.000[b]	0.000±0.000[b]	0.044±0.021[b]
Imo		0.000±0.000[b]	0.000±0.000[c]	0.000±0.000[b]	0.000±0.000[b]	0.000±0.000[d]
Total	HM	0.003±0.005[a]	0.002±0.005[a]	0.002±0.006[a]	0.020±0.077[a]	0.027±0.077[c]
	DS	0.026±0.082[a]	0.01±0.0240[a]	0.000±0.000[b]	0.000±0.000[b]	0.036±0.028[b]
	WS	0.014±0.052[a]	0.041±0.105[a]	0.000±0.000[b]	0.000±0.000[b]	0.056±0.136[a]

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)
HM is Harmattan
DS is Dry season
WS is Wet season

Concentrations of Aflatoxins in *Citrullus colocynthis*

For the concentrations of AFB1 in *C.colocynthis* sampled across the states, samples collected from Anambra State and Enugu State with values of 0.184±0.153ppb and 0.180±0.208ppb were significantly (P>0.05) higher than values recorded from other states, the least value of 0.015±0.018ppb was observed in sample collected from Abia state, for AFB2, the highest concentration was detected from sample collected from Anambra state with value of 0.447±0.147ppb this was significantly (P>0.05) higher than 0.129±0.198ppb obtained from samples collected from Enugu State, lower values of 0.024±0.024ppb, 0.053±0.116ppb and 0.024±0.055ppb were recorded from Abia, Ebonyi and Imo state respectively, these values were not significantly different from each other. For AFG1, the values recorded across the states ranged between 0.002±0.004ppb and 0.017±0.033ppb although there was no significant difference in the concentrations across the state but the highest value was recorded from Abia state while the least value was recorded from Anambra state. AFG2 was not detected in all the samples across the state of collection except in Anambra state (0.002±0.004ppb). For total aflatoxin, there was significant difference in values obtained from samples across the states, the highest value of 0.636±0.078ppb was recorded from Anambra state, next to this was 0.323±0.352ppb obtained from sample collected from Enugu state, then 0.086±0.038ppb from Ebonyi state while a significantly lower values of 0.056±0.047ppb and 0.068±0.073ppb observed from Imo and Abia state respectively, all the values were significantly (P>0.05) different from each other except the values from Ebonyi and Enugu that were not significantly different from eachother, total aflatoxin was not detected in Imo state. With respect to the wave of collection, there was no significant difference in the concentration of AFB1 across the wave of collection, although the highest value of 0.026±0.082ppb was obtained during dry season while the least value of 0.003±0.005ppb was obtained during harmattan, on the same hand, there was no significant difference in the concentration of AFB2 across the wave of collection but the

highest value was detected during the wet season (0.041±0.105ppb) while the least during harmattan, AFG1and AFG2 were not detected in samples collected across the waves except during harmattan with values of 0.002±0.006ppb and 0.020±0.077 respectively. For total aflatoxin there was significant difference in *C. colocynthis* across the wave of collection, the highest value of bnn0.346±0.354ppb was obtained during the harmattan season this was significantly (P>0.05) higher than 0.0179±0.260ppb and 0.0176±0.210ppb recorded from samples collected during dry season and wet season respectively (Table 3).

Table 3: Concentrations of Aflatoxins in *Citrullus colocynthis*

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	(Total)	0.015±0.018^{b}	0.024±0.024^{d}	0.017±0.033^{a}	0.000±0.000^{b}	0.056±0.047^{c}
Anambra		0.184±0.153^{a}	0.447±0.147^{a}	0.002±0.004^{a}	0.002±0.004^{a}	0.636±0.078^{a}
Ebonyi		0.029±0.017^{b}	0.053±0.116^{c}	0.004±0.013^{a}	0.000±0.000^{b}	0.086±0.038^{c}
Enugu		0.180±0.208^{a}	0.129±0.198^{b}	0.014±0.021^{a}	0.000±0.000^{b}	0.323±0.352^{b}
Imo		0.040±0.069^{b}	0.024±0.055^{d}	0.004±0.013^{a}	0.000±0.000^{b}	0.068±0.073^{c}
Total	HM	0.142±0.190^{a}	0.196±0.222^{a}	0.008±0.022^{b}	0.000±0.000^{b}	0.346±0.354^{a}
	DS	0.041±0.070^{c}	0.122±0.220^{b}	0.017±0.023^{a}	0.000±0.000^{b}	0.179±0.260^{b}
	WS	0.086±0.114^{b}	0.089±0.152^{c}	0.000±0.000^{c}	0.001±0.003^{a}	0.176±0.210^{b}

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)
HM is Harmattan
DS is Dry season
WS is Wet season

Concentrations of Aflatoxins in *Irvingia wombolu*

For the concentration of AFB1 in *Irvingia wombolu*, there was a significant difference in the values obtained across the states, values of 0.221±0.140ppb and 0.367±0.150ppb recorded from Abia and Anambra were not significantly different from eachother but significantly higher than values recorded from other states at P>0.05 level of significance, next to this was 0.118±0.110ppb obtained from sample collected from Enugu state while the least in AFB1 was 0.017±0.032ppb obtained from Imo state. There was significant difference in the values of AFB2

in *Irvingia wombolu* across the states, relatively higher values of 0.093±0.036ppb and 0.046±0.057ppb were recorded from samples collected from Anambra state and Enugu state respectively these were not significantly different from eachother but significantly higher that values obtained from other states at P>0.05 level of significance, next to these were 0.021±0.013ppb and 0.011±0.033ppb recorded from Abia and Ebonyi respectively, AFB2 was not detected in *I. wombolu* collected from Imo state. For AFG1, values of 0.180±0.047ppb and 0.152±0.084ppb were not significantly different from eachother but significantly higher than values recorded from other states, next were 0.029±0.048ppb obtained in samples collected from Imo state, lower concentrations of 0.001±0.002ppb and 0.005±0.007ppb were recorded from Enugu and Ebonyi respectively. AFG2 was not detected in *I. wombolu* across all the states. For total aflatoxin, Anambra with value of 0.612±0.019ppb and Abia with value of 0.412±0.058ppb were significantly higher than values obtained from other states at P>0.05 level of significance, Ebonyi with a value of 0.074±0.011ppb was next, relatively lower values of total aflatoxin were obtained from Enugu (0.165±0.032ppb) and Imo state (0.047±0.063ppb). With respect to wave of collection, there was no significant difference in the concentration of AFB1 across the wave of collection, although the highest value of AFB1 was detected from sample collected during wet season with value of 0.222±0.055ppb while the least in AFB1 was from sample collected during dry season (0.107±0.017ppb), on the same hand, there was no significant difference in the concentration of AFB2 recorded from the samples across the waves of collection, the values ranged between 0.030±0.031ppb and 0.037±0.004ppb, the highest concentration of AFG1 was recorded from sample collected during harmattan (0.097±0.026ppb) this was not significantly higher than 0.081±0.015ppb obtained during dry season while least value of AFG1 (0.041±0.047ppb) was obtained during wet season, AFG2 was not detected from *I. wombolu* in all the samples collected across the waves. For total aflatoxin values of 0.300±0.005ppb and 0.268±0.044ppb recorded during wet season and harmattan respectively were not significantly

different from eachother but significantly higher than 0.218±0.083ppb obtained during dry season at P>0.05 level of significance (Table 4).

Table 4: Concentrations of Aflatoxins in *Irvingia wombolu*

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		BI	B2	GI	G2	Total
Abia	(Total)	0.221±0.140[a]	0.011±0.033[b]	0.180±0.047[a]	0.000±0.000[a]	0.412±0.058[a]
Anambra		0.367±0.150[a]	0.093±0.036[a]	0.152±0.084[a]	0.000±0.000[a]	0.612±0.019[a]
Ebonyi		0.049±0.016[c]	0.021±0.013[b]	0.005±0.007[c]	0.000±0.000[a]	0.074±0.011[b]
Enugu		0.118±0.110[b]	0.046±0.057[a]	0.001±0.002[c]	0.000±0.000[a]	0.165±0.032[c]
Imo		0.017±0.032[d]	0.001±0.000[c]	0.029±0.048[b]	0.000±0.000[a]	0.047±0.063[c]
Total	HM	0.134±0.145[a]	0.036±0.070[a]	0.097±0.026[a]	0.000±0.000[a]	0.268±0.044[a]
	DS	0.107±0.017[a]	0.030±0.031[a]	0.081±0.015[a]	0.000±0.000[a]	0.218±0.083[b]
	WS	0.222±0.055[a]	0.037±0.004[a]	0.041±0.047[b]	0.000±0.000[a]	0.300±0.005[a]

Results are in Mean ± Standard Deviation
Means with the same letter in a column are not significantly different (p>0.05)
HM is Harmattan
DS is Dry season
WS is Wet season

DISCUSSION

Aflatoxin Contamination of Food Samples

Aflatoxin was detected in all samples, highest in *C. colocynthis* and lowest in *M. esculenta* (cassava chips), although the levels of contamination in this study were lower than the NAFDAC and Codex Alimantarius Commission (CAC) maximum permissible levels of aflatoxins of 10 µg/kg and 4ug/kg or 4ppb respectively in the food samples (Atanda *et al.,* 2011; Marco *et al.,* 2008) (Table 2). This agrees with the reports of Muzanila *et al.*, (2000) and Chiona *et al.* (2014) who recorded a very low aflatoxin contamination in cassava chips, but differs with the study of Salau *et al.,* (2017) who reported that the aflatoxins level contained in food materials

were above the Nigerian (10 µg/kg) limits and the European Union tolerance level of 2µg/kg for AFB1 and 4µg/kg for Total Aflatoxin. The result of this research work also differs with the documentations of Okigbo *et al.,* (2015) who reported that there were more concentrations of aflatoxins in *I. wombolu* than *C. colocynthis*. Even though the levels of Aflatoxins detected in this work is below the maximum permissible level, the frequent contamination of these food materials at a reasonable concentration by these potent carcinogen especially AFB1 and AFB2 that were detected in relatively higher quantities call for serious concern. Moreso, these toxins however small when consumed in food materials bio-accumulates in the body with serious health effects. Human Exposure to multiple chemical combinations in food samples has led to series of human health disorder (Rotich *et al.,* 2006; UNDP, 2006; USEPA, 2002). The occurrence of all the different types of aflatoxins in the food materials points to the diversity of fungal species that colonized the food materials from the field to the market (Adetunji *et al.,*2014). The significant differences in the concentrations of aflatoxins detected in the food materials observed in this study can be linked to the method of drying these food materials across the states of sampling (Atanda *et al.,*2011, Turner *et al.,*2005), hence cassava chips has the least concentration because of its dry nature.

The concentration of aflatoxins in the food materials with respect to the states of collection increases with increase in temperature and rainfall of the states except for Abia State (temperature) and Imo State (Rainfall). Among all the states in south eastern states of Nigeria, Anambra State (37°C) and Enugu State (32.5°C) have a relatively higher mean annual temperature than the other three states (Annual Abstract of Statistics, 2012). From this research, the highest concentration of aflatoxin was detected in Anambra and Enugu except in *I. wombolu*, this can be attributed to the slight difference in the climatic conditions of these states from other states in south eastern Nigeria, this does not agree with the report of Atanda *et al.,* (2013) who stated that more toxins accumulates and are produced between the temperature of 4 to 10°C.

The concentrations of aflatoxins in the food materials with respect to wave of collection was more during wet season in all the samples (except in *C. colocynthis*), then in harmattan and the least was in dry Season, this agrees with the result of Makun *et al.,* (2007) who reported that the quantity of aflatoxin detected from samples collected during the wet season were significantly higher than those detected during harmattan and Dry Season in all the food materials. The growth and proliferation of mycotoxigenic fungi is influenced by water (Atanda *et al.,* 2013), hence the higher concentration of aflatoxins detected during wet season. This vital condition is adequately fulfilled more during rainy seasons and in wetter areas than in drier seasons and places with resultant higher fungal contamination of food materials in wet conditions than dry ones. This explains why fewer fungi incidences and less mycotoxin were detected and recorded in dry seasons than in wet season. On the other hand, in *C.colocynthis*, more mycotoxins were detected during harmattan; this can be linked to the fact that more fungi were isolated from *C. colocynthis* during harmattan.

CONCLUSION

The occurrence of aflatoxins in food samples from south eastern Nigeria even though at a low concentration indicates that the region's population can be at risk of cancer and aflatoxicoses because these food materials are staple foods in the region. Although values obtained for aflatoxins were not above the maximum permissible limit, but long term exposure to low levels of these toxins in the food supply system may bio-accumulate in the human system and consequently cause various health challenges (USDA, 2005). The implication of the findings of this research is that most of the food materials presently on sale in our markets are partially acceptable for human consumption. Since these food materials are also distributed from south eastern Nigeria to other parts of Nigeria, it is possible that even more people are consuming contaminated foodstuffs in the country. Hence there is need for consistent sensitization of

consumers in South Eastern Nigeria and awareness campaign on dangers/possibility of aflatoxin contamination of various food materials exposed in open for sale in the market.

Though the amount of aflatoxins obtained in the foodstuff analyzed fall below the 10µg/kg limit set in Nigeria for unprocessed food products by NAFDAC. However, it is crucial to devise natural means of preventing the survival of aflatoxin-producing species in food products prior to consumption in south eastern Nigeria, especially during storage where they are prone to be colonized by these toxin-producing pathogenic organisms due to the prevalent environmental conditions in many of the storage facilities used by commercial retailers.

This research has elucidated so many facts about the quality of foodstuffs sold in the open markets in south eastern Nigeria and by reason of these glaring facts; there is need for proper handling of food materials sold in the market to avoid aflatoxin and other mycotoxins contamination.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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