

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 6th and 8th 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 (B₁, B₂, G₁ and G₂) 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
<i>C.colocynthis</i>	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>I. wombolu</i>	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 ± 0.082 ppb) and AFB2 (0.01 ± 0.0240 ppb) 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 ± 0.006 ppb and 0.020 ± 0.077 ppb for AFG1 and AFG2 respectively. For total aflatoxin there was significant difference in the values obtained, the highest values of 0.056 ± 0.136 ppb was recorded during the wet season, this was significantly higher than 0.036 ± 0.028 ppb 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 ± 0.136 ppb was recorded during wet season this was significantly ($P > 0.05$) higher than 0.036 ± 0.028 ppb observed during dry season, the least value of 0.027 ± 0.077 ppb was recorded during harmattan (Table 2).

Table 2: Concentrations of Aflatoxins in Cassava chips

States	Wave of Collection	Average Aflatoxin Concentrations (ppb)				
		B1	B2	G1	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 \pm 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 ± 0.153 ppb and 0.180 ± 0.208 ppb were significantly ($P>0.05$) higher than values recorded from other states, the least value of 0.015 ± 0.018 ppb 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 ± 0.147 ppb this was significantly ($P>0.05$) higher than 0.129 ± 0.198 ppb obtained from samples collected from Enugu State, lower values of 0.024 ± 0.024 ppb, 0.053 ± 0.116 ppb and 0.024 ± 0.055 ppb 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 ± 0.004 ppb and 0.017 ± 0.033 ppb 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 ± 0.004 ppb). For total aflatoxin, there was significant difference in values obtained from samples across the states, the highest value of 0.636 ± 0.078 ppb was recorded from Anambra state, next to this was 0.323 ± 0.352 ppb obtained from sample collected from Enugu state, then 0.086 ± 0.038 ppb from Ebonyi state while a significantly lower values of 0.056 ± 0.047 ppb and 0.068 ± 0.073 ppb 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 each other, 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 ± 0.082 ppb was obtained during dry season while the least value of 0.003 ± 0.005 ppb 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 ± 0.105 ppb) while the least during harmattan, AFG1 and AFG2 were not detected in samples collected across the waves except during harmattan with values of 0.002 ± 0.006 ppb 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 0.346 ± 0.354 ppb was obtained during the harmattan season this was significantly ($P > 0.05$) higher than 0.0179 ± 0.260 ppb and 0.0176 ± 0.210 ppb 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)				
		B1	B2	G1	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 \pm 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 ± 0.140 ppb and 0.367 ± 0.150 ppb recorded from Abia and Anambra were not significantly different from each other but significantly higher than values recorded from other states at $P > 0.05$ level of significance, next to this was 0.118 ± 0.110 ppb obtained from sample collected from Enugu state while the least in AFB1 was 0.017 ± 0.032 ppb obtained from Imo state. There was significant difference in the values of AFB2

in *Irvingia wimbolu* across the states, relatively higher values of 0.093 ± 0.036 ppb and 0.046 ± 0.057 ppb were recorded from samples collected from Anambra state and Enugu state respectively these were not significantly different from each other but significantly higher than values obtained from other states at $P>0.05$ level of significance, next to these were 0.021 ± 0.013 ppb and 0.011 ± 0.033 ppb recorded from Abia and Ebonyi respectively, AFB2 was not detected in *I. wimbolu* collected from Imo state. For AFG1, values of 0.180 ± 0.047 ppb and 0.152 ± 0.084 ppb were not significantly different from each other but significantly higher than values recorded from other states, next were 0.029 ± 0.048 ppb obtained in samples collected from Imo state, lower concentrations of 0.001 ± 0.002 ppb and 0.005 ± 0.007 ppb were recorded from Enugu and Ebonyi respectively. AFG2 was not detected in *I. wimbolu* across all the states. For total aflatoxin, Anambra with value of 0.612 ± 0.019 ppb and Abia with value of 0.412 ± 0.058 ppb were significantly higher than values obtained from other states at $P>0.05$ level of significance, Ebonyi with a value of 0.074 ± 0.011 ppb was next, relatively lower values of total aflatoxin were obtained from Enugu (0.165 ± 0.032 ppb) and Imo state (0.047 ± 0.063 ppb). 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 ± 0.055 ppb while the least in AFB1 was from sample collected during dry season (0.107 ± 0.017 ppb), 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 ± 0.031 ppb and 0.037 ± 0.004 ppb, the highest concentration of AFG1 was recorded from sample collected during harmattan (0.097 ± 0.026 ppb) this was not significantly higher than 0.081 ± 0.015 ppb obtained during dry season while least value of AFG1 (0.041 ± 0.047 ppb) was obtained during wet season, AFG2 was not detected from *I. wimbolu* in all the samples collected across the waves. For total aflatoxin values of 0.300 ± 0.005 ppb and 0.268 ± 0.044 ppb recorded during wet season and harmattan respectively were not significantly

different from each other but significantly higher than 0.218 ± 0.083 ppb 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 \pm 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 Alimentarius Commission (CAC) maximum permissible levels of aflatoxins of $10 \mu\text{g}/\text{kg}$ and $4 \mu\text{g}/\text{kg}$ 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.



The authors declared that they have no conflict of interest.

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