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SURVEY OF AFLATOXINS CONTAMINATION IN SELECTED AGRICULTURAL COMMODITIES OBTAINED FROM MAJOR MARKETS IN SIX GEOGRAPHICAL ZONES IN NIGERIA

***Pessu, P. O., Peters, O. A., Aina, J. A. and Akande, S. A.**

Nigerian Stored Products Research Institute Headquarters, Km 3, Asa-Dam Road, Ilorin, Nigeria.
*Correspondence address; pattypessu@yahoo.com;Phone; +2348033151997

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ABSTRACT

This study evaluated total aflatoxin contents of six agricultural commodities obtained from major markets in six geographical zones of Nigeria. Results showed that total aflatoxin levels in cowpea, yam chips and dried fish from six zones were less than European Union (EU) recommended maximum safe level (4 ppb) while 16.67% of groundnut samples and 83.33% each of melon and maize had total aflatoxin above 4 ppb. Moisture content of groundnut, melon, dried fish, yam chips, cowpea and maize significantly ranged from 3.92–7.74%, 3.15–5.73%, 7.76–14.39%, 9.66–17.04%, 7.84–10.94% and 10.13–13.31% respectively. Insect infestations of food commodities were light and most of the few insects found were dead. Thus cowpea, dried fish and yam chips from six zones with maize and melon from N. West zone of Nigeria could be regarded as being free from aflatoxins contaminations. It was also demonstrated that proper postharvest handling and adherence to simple good agricultural practice (GAP) were enough to improve the quality of produce internationally. Food commodities from Southern zones had significant (p<0.05) higher moisture than those from the Northern zones.

***Keywords**: Nigeria, mycotoxins, hazards, safety, food, feeds*

INTRODUCTION

Animal feedstuffs and agricultural commodities may contribute to the transfer of certain health hazards such as dioxins, mycotoxins, heavy metals, drug and pesticides residues, and microbiological hazards into food chain, raising global concerns about food safety (FAO/WHO, 2014). Mycotoxins are prioritized as unavoidable natural contaminants in food and feedstuffs that if consumed might cause serious consequences related to several acute and chronic diseases in human as well as animals (Hussein and Brasel, 2001; Adeyeye, 2016).

Aflatoxins are probably the most common and widely known mycotoxin contaminants and they have been implicated in human diseases including liver cancer, Reye's syndrome, Indian childhood cirrhosis, chronic gastritis, kwashiorkor and certain occupational respiratory diseases in various parts of the world and particularly in Africa (FAO, 1986; Adeyeye, 2016). Aflatoxins, a group of about 20 chemically related toxic substances are mainly produced by the *Aspergillius flavus* and *Aspergillius* *parasiticus* that are commonly encountered in foodstuffs and animal feeds worldwide (Cotty, 1994; Bhathagar *et al*., 2003; Wu, 2013). Beside, Makun *et al*. (2014) stated that there are not less than 14 naturally occurring aflatoxins known to exist. However, only six have public health and agricultural significance which comprises of aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2) while the other two, aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) are the hydroxylated forms of AFB1 and AFB2 respectively that are secreted in animals (including humans) tissues and fluids. Aflatoxin exposure is particularly problematic in low-income populations in the tropics that consume relatively large quantities of staples, particularly maize and groundnuts (Unnevehr and Delia, 2013).

The current administration in Nigeria is encouraging non-oil income generation especially in Agricultural sector in order to attract more foreign exchange. However, several cases of rejections of Nigerian Agricultural commodities overseas would hamper this development; hence a pragmatic approach must be developed and the need for investigation of the causes of low quality of exportable crops is therefore imperative. The Nigerian Stored Products Research Institute (NSPRI) has been saddled with the responsibility of securing the quality of agricultural produce after harvest. In addition, moisture content is a determining factor for mould growth and subsequently toxins production. This study therefore was designed to evaluate the aflatoxins contaminations and other quality parameters including moisture contents of six exportable food commodities obtained from six geographical locations in Nigeria with a view to evaluate the level of their compliance to international standards especially the European Union (EU).

MATERIALS AND METHODS

Materials

Methanol was obtained from BDH Chemicals Limited Poole England. RIDA®QUICK SCAN Aflatoxin (RQS-R5205) with accessories, AFLACARD TOTAL (RBRP38) and AFLACARD B1 (RBRP27) with other accessories were purchased from R-Biopharm Rhone Ltd. AG Darmstadt. Germany.

Sampling and sample treatment

Two major markets each were selected in North-Central (Ilorin), North-East (Maiduguri), North-West (Kano), South-East (Enugu), South-South (Port-Harcourt) and South-West (Lagos), Nigeria for sampling. Using a simple random sampling technique, 30 samples each of *Arachis hypogaea* (groundnut), *Citrullus colocynthis* (melon), *Dioscorea spp*. (dried yam chips), *Vigna unguiculata* (cowpea), *Zeamays* (maize)and *Clarias gariepinus* (smoked African catfish) were purchased from 30 traders in each zone making a total of 1080 samples. They were brought to the Quality Control Laboratory of Nigerian Stored Products Research Institute (NSPRI) headquarters, Ilorin. Groundnut, melon seeds, cowpea and maize were bulked individually and divided by quartering technique according to method of Hammonds (1982) by using Grain Divider (Seeburo Equipment Company, Chicago, Illinois) while yam chips and dried fish were each mixed manually. This operation

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was carried out in order to obtain a representative sample (1 kg) for further analyses.

Aflatoxin analysis

The aflatoxin analysis was carried out in two stages; RIDA®QUICK Lateral Flow tests which are immuno-chromatographic tests for the quantitative evaluation (RIDA®QUICK SCAN), using 'Aflaquant's method for the determination of total aflatoxins. It is applicable to maize, cowpea and yam chips with detection limit of 4-100 ppb. The second stage involved the use of Test cards; AFLACARD TOTAL and AFLACARD B1 for qualitative screening of total aflatoxins and aflatoxin B1 at various levels applicable to oil seeds, cereals and spices with detection limit of 2-30ppb.Both aflaquant and aflacard were done for aflatoxin total.

Aflaquant method

To 10 g of grounded raw sample was added 20 mL of 70% methanol (% v/v) in a screw tube and shaken vigorously for 5 minutes. The sample was filtered through Whatman No. 4 filter paper to get a clear filtrate. Fifty micro litres (50 µL) of clear filtrate were added to 100 micro litres of mobile solvent provided and thoroughly mixed together. One hundred micro litres (100 µL) of the mixture were placed on the application area of the test strip with the aid of micro pipette; the test strip was then incubated for exactly 5 minutes after which it was inserted into the RIDA®QUICK SCAN for reading.

Aflacard method

To 50 g of grounded raw sample was added 100 mL of 80% methanol (% v/v) in 1 litre capacity solvent resistant blender jar and blended at high speed for 2 minutes. The sample was filtered through Whatman filter paper No. 4. The filtrate was passed through the solid phase clean-up column provided with the kit by applying pressure with the plunger until it became clear. The clean-up filtrate was then collected into collection tube ready for analysis. Depending on the screening level required, the filtrate was further diluted with 80% methanol into dilution tube provided; at 4 ppb screening level, 1 mL of filtrate was diluted with 1 mL of 80% methanol (% v/v), and 1 mL of diluted filtrate was added to the vials containing 3 mL of sample diluents buffer mixed very well and 500 µL was applied into the AFLACARD membrane for reading.

Physical control method

This was carried out on maize and melon samples. They were subjected to some physical control measures after being found with total aflatoxins contents above recommended maximum EU level (4 ppb). The physical process includes: for maize; sorting, washing with distilled water, soaking (24 hours) and drying. For melon seeds; sorting, washing with distilled water and drying.

Determination of moisture content

The moisture content was determined (as soon as the samples were received) using hot air oven method in accordance with AOAC (2000) methods.

Insects' infestation analysis

Food samples were incubated for some weeks in glass jars under laboratory conditions (27–29^{0}C, 70–80% RH) following the methods described by Eze *et* *al*. (2006). After the incubation period, yam chips were broken into pieces of less than 5 mm using mortar and pestle before sieving, smoked dried fish was as well broken manually with hands before sieving while other grains were sieved directly to separate insect pests. Recovered insects were counted and conserved in a flask containing 70% alcohol for safeguarding and identification (Loko *et al*., 2013; Uneke, 2015).

Statistical analysis

Data collected were subjected to statistical test by one-way Analysis of Variance (ANOVA) using SPSS Statistics for Windows software package version 20.0.0 (IBM SPSS Statistics, IBM Corporation 2011. Armonk NY. USA). Means were separated using New Duncan's Multiple Range F-Test (DMRT) According to Duncan (1955). Significance was accepted at 5% confidence limit ($p<0.05$).

RESULTS

Total Aflatoxins

The total aflatoxin contents (ppb) of six food commodities sampled in October, 2017 from different geographical zones in Nigeria was as shown (Figure 1). The results obtained could be discussed in two categories; firstly, total aflatoxins levels recorded for cowpea (*V. ungiculata*), yam chips (*Dioscorea spp.*) and smoked dried African catfish (*C. gariepinus*) were lower than EU recommended maximum acceptable level (4 ppb) and therefore they may be regarded as being safe for human consumption.

The second category consisted of groundnut (*A. hypogaea*), melon (*C. colocynthis*) and maize (*Z. mays*) as shown (Figure 1); levels of total aflatoxin recorded for groundnut from S. South (Port-Harcourt) was above the acceptable limit (4 ppb) while groundnut samples from other zones were normal. On the other hand, melon and maize from all zones with exception of Northwest (Kano) had levels of total aflatoxins higher than the acceptable limit of 4 ppb, hence only maize and melon from Northwest (Kano) of the country can be regarded as being within the safe limit for human consumption in terms of aflatoxins contaminations.

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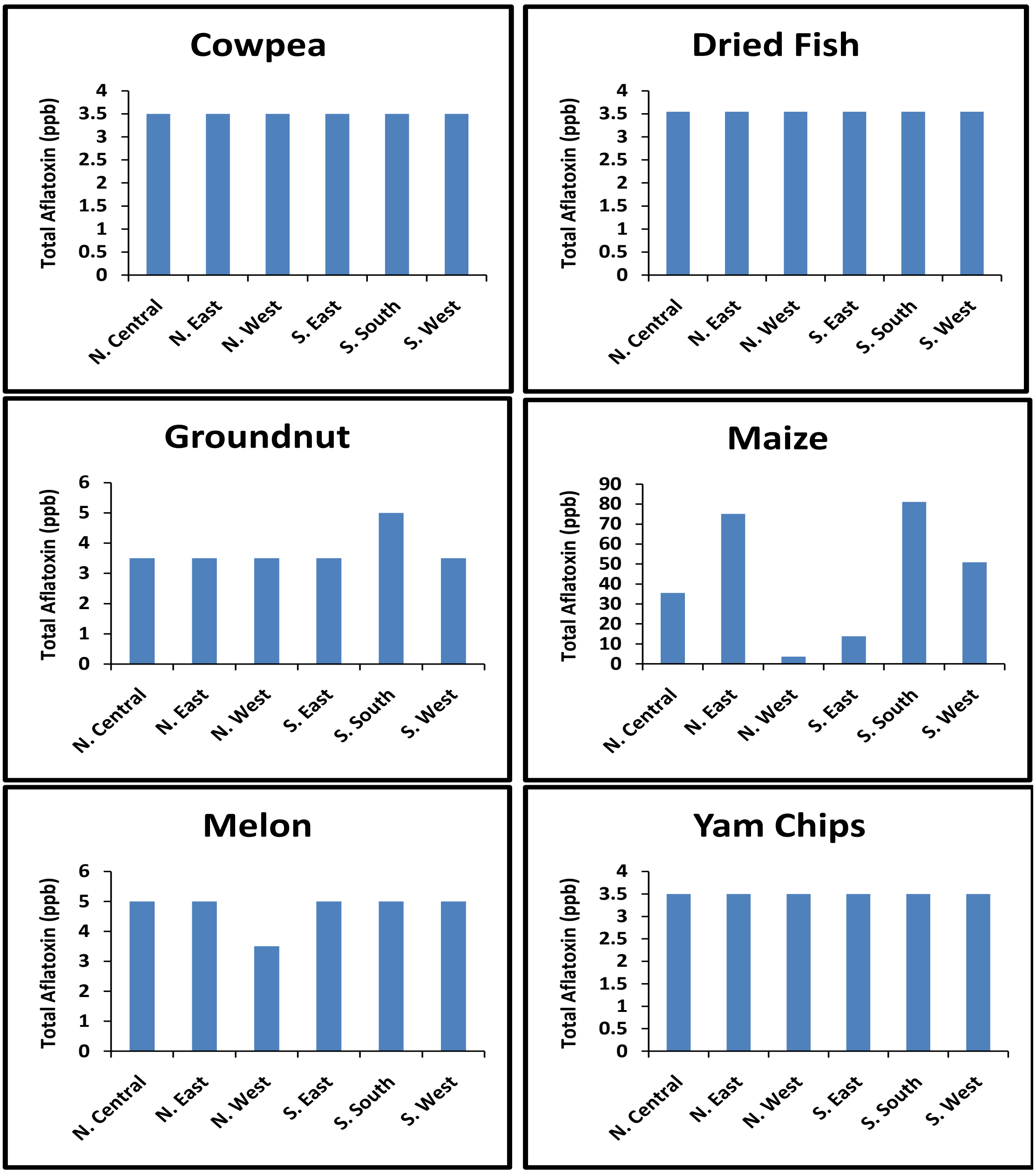

Figure 1: Effect of geographical locations on total aflatoxins contents (ppb) of agricultural produce from six geographical zones in Nigeria. Each bar represents mean of triplicate readings (n=3). *Total Aflatoxins above 4 ppb exceeds EU recommendation

Mean value (%) of food commodity with total aflatoxin above 4 ppb

The percentage mean value of aflatoxins contaminations in groundnut, melon seeds, smoked dried fish, yam chips, cowpea and maize sampled from Nigerian markets in October, 2017 was as shown (Table 1). Mean values for cowpea, smoked dried fish and yam chips with total aflatoxin above 4 ppb were 0%, this indicates that total aflatoxin levels were all less than 4 ppb which is the current EU recommended maximum safe level. The mean value for groundnut showed that 16.67% of samples collected were having total aflatoxin above 4 ppb while 83.33% of melon and maize samples each contained total aflatoxin above 4 ppb.

Table 1: Mean value (%) of groundnut, melon, smoked dried fish, yam chips, cowpea and maize sold in Nigerian markets in October 2017 with Total Aflatoxin level above 4 ppb

Commodity	Total aflatoxin level (ppb)	% Mean of Commodity
Groundnut (*A.hypogaea*)	>4	16.67*
Melon (*C. colocynthis*)	>4	83.33*
Smoked dried fish (*C. gariepinus*)	>4	0
Yam chips (Dioscorea spp.)	>4	0
Cowpea (V. ungiculata)	>4	0
Maize (Z. mays)	>4	83.33*

*The Aflatoxin value greater than 4ppb is above the EU recommendation

Effect of physical control system on total aflatoxins content

The effect of physical control system on the total aflatoxins contents of melon and maize was as shown (Figure 2).

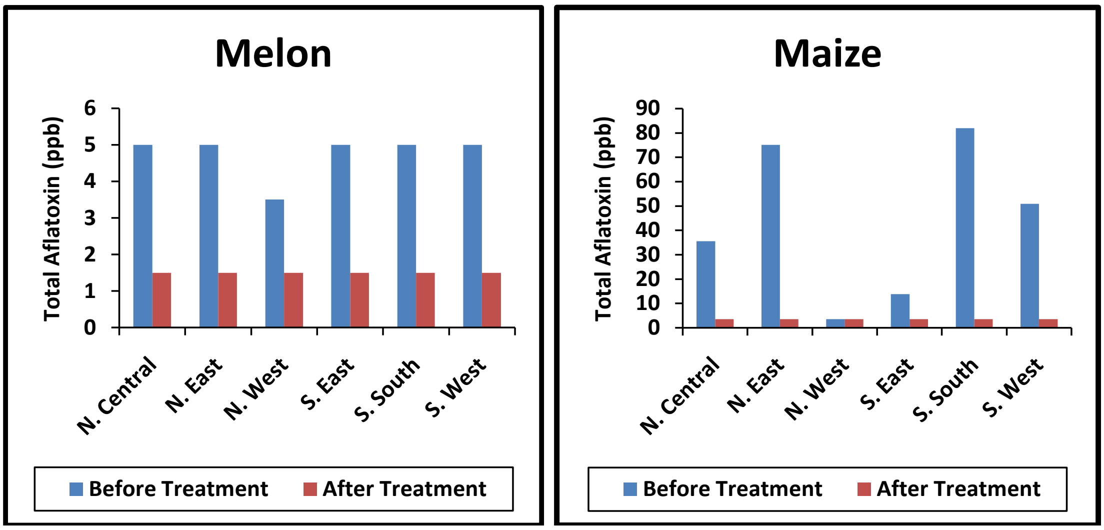

Figure 2: Effect of physical control systems on the Total Aflatoxins contents (ppb) of melon and maize from six geographical zones of Nigeria. Each bar represent mean of three readings (n=3). *Total Aflatoxins values above 4 ppb exceeds EU recommendation

Total Aflatoxins in melon and maize reduced significantly ($p<0.05$) when the commodities were subjected to some physical control processes such as sorting, washing and drying. For maize samples; sorting, washing, soaking and drying were carried out on the contaminated sample. Thereafter, the total aflatoxin level were found to be lowered significantly below the EU recommended safe level of 4 ppb and hence could be regarded as safe for human consumption after such treatments.

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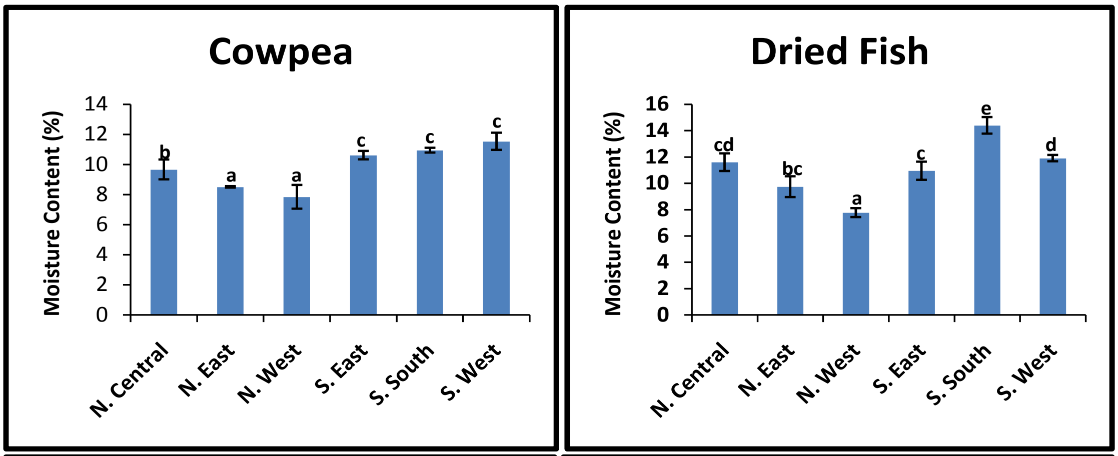
Moisture contents

The moisture content (MC) of food commodities from the six geographical locations in Nigeria sampled in October, 2017 was as shown in Figure 3. The results showed that MC ranged from 7.84–10.94%, 7.76–14.39%, 3.92–7.74%, 10.13–13.30%, 3.15–5.78%, and 9.66–17.04% for cowpea, dried fish, groundnut, maize, melon and yam chips respectively. The results indicated that groundnut and yam chips samples from N. East (3.92% and 9.66% moisture contents respectively), cowpea, dried fish maize and melon from N. West (7.84%, 7.76%, 10.13% and 3.15% moisture contents respectively) were the lowest significant ($p<0.05$) recorded values of moisture contents for those produce. Conversely, dried fish, groundnut, maize and yam chips, from S. South (14.39%, 7.74%, 13.31% and 17.04% moisture contents respectively) with cowpea and melon from S. West (11.53% and 5.78% moisture contents respectively) had the highest recorded values.

Insect infestation

The insect infestation level in all food commodities was generally low (Table 2). Most of the few insects were found dead, this could be an indication of high pesticide usage among traders as recorded for yam chips, white cowpea and yellow maize where 54.20%, 89.02% and 58.57% of the insects found respectively were dead. According to Okunade (2006), among the common insect pests are; maize weevil-*Sitophilus zeamais L.* from maize, Khapara beetle-*Trogodermagranarium* (Even) from groundnut, skin or hide beetle-*Dermestes maculates* (Deg) from dried fish, meat or skin, cowpea beetle-*Callosobruchus maculatus F.* from cowpea and rust red flour beetle-*Tribolium castaneum* (Hearbst).



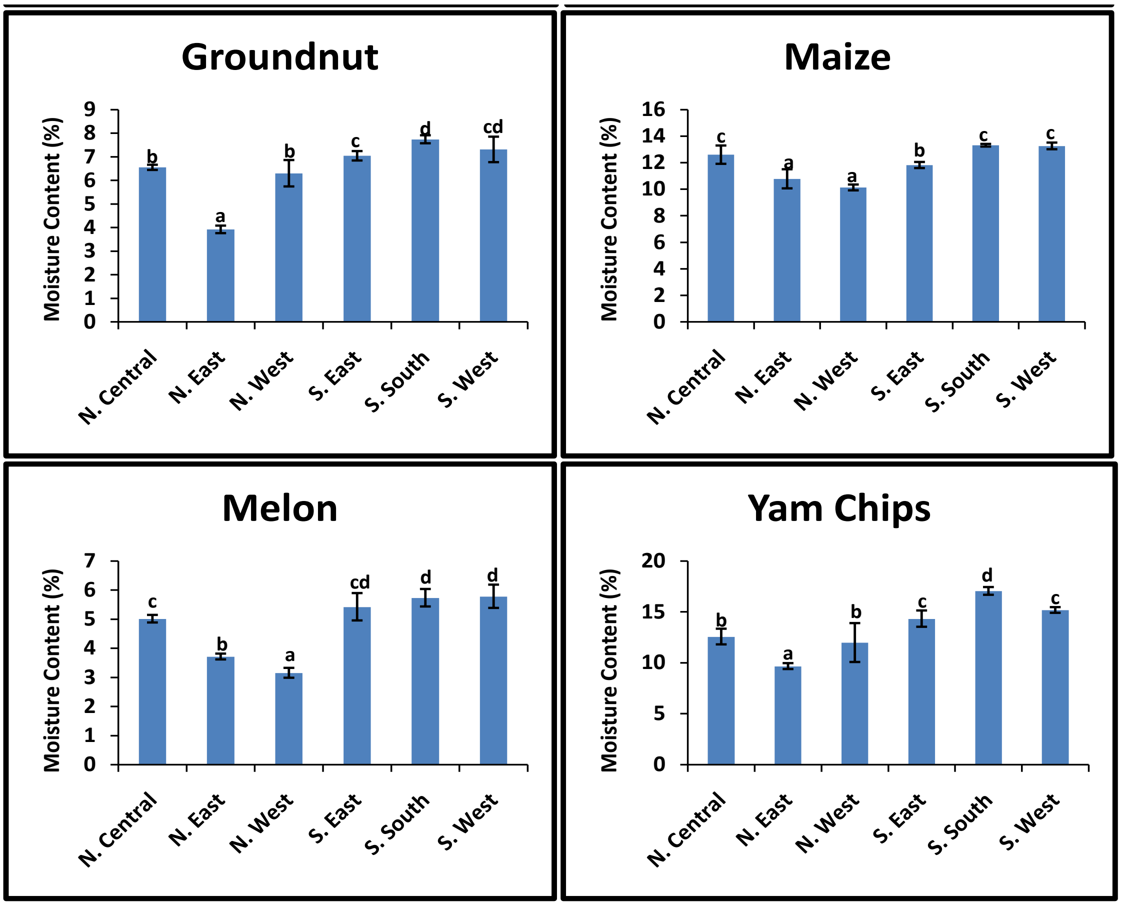


Figure 3: Effect of locations on the moisture contents of agricultural produce from six geographical zones of Nigeria. Bars represent mean of triplicate readings (n=3) while error bars represent standard deviation of the means. Bars with unshared alphabets are significantly different ($p<0.05$).

Table 2: Insect counts of food commodities from six geographical zones of Nigeria

Commodity	Geographical Location	Total insects found	Percentage (%) dead insects	Insects found
Cowpea (*V. unguiculata*)	All zones	164	89.02	*Callosobruchus maculates*
Dried Fish (*C. gariepinus*)	N. East and S. East	6	0	*Dermestes maculates.*
Groundnut (*A. hypogea*)	N. East and S. South	7	14.30	*Tribolium castaneum* and *Trogoderma granarium.*
Maize (*Z. mays*)	All zones	70	58.57	*Sitophilus zeamais*
Melon (*C. colocynthis*)	N. East, S. East and S. South	3	0	*Tribolium castaneum*
Yam chips (*D. rotundata*)	N. Central, N. West, S. East, S. South and S. West	24	54.20	*Tribolium castaneum*

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DISCUSSION

Total Aflatoxins

The lower levels of aflatoxins recovered from these agricultural commodities (cowpea, dried fish and yam chips) were in tandem with available data in the literatures. Low aflatoxin contaminations of cowpea, soybean and their products were recorded in many parts of the world according to Makun *et al.* (2014). For instance, only 3 positive samples were demonstrated in 268 cowpea samples analysed between 1975 and 1983 from Nigeria (Opadokun, 1992). High seed coat integrity, ensuring limited access and low moisture contents might be some of the factors responsible for low susceptibilities of these nuts to aflatoxigenic fungi with consequent rare occurrence of toxins in them (Makun *et al*., 2014). Similarly, all the eleven samples of smoked dried fish collected in Nigeria by another researchers contained mean level of AFB1 and AFG1 (3.46 and 2.94 ppb respectively), a level below the EU recommended value (Adebayo-Tayo *et al*., 2008).

On the other hand, high levels of contaminations in cereals (especially maize), nuts, oilseeds and their products especially groundnuts have been reported in literatures. According to a report by Makun *et al*. (2014); nuts, oilseeds and their products are the most investigated of all foods with regards to aflatoxins contaminations because they are the most susceptible especially due to their high protein content particularly in the case of groundnut which has made them priceless components of many animal and human diets. Serious contamination of Nigerian groundnut is a recurring safety threat since 1960 when up to 8000 ppb were reported from the Northern region of Nigeria (Opadokun, 1992). Out of 106 samples of roasted groundnut samples collected in Nigeria, 68 had aflatoxin B1 (AFB1) in the range of 5–165 ppb, 28 out 106 had aflatoxin B2 (AFB2) in the range of 6–26 ppb, 12 out 106 samples had AFG1 in the range of 5–20 ppb while only 3 contained AFG2 in range of 7–10 ppb (Bankole *et al*., 2003). Similarly, the level of aflatoxin in groundnut cake from Nigeria was as high as between 20–455 ppb which was considered to be toxicologically unsafe according to a report by Bankole and Adebajo (2003). Melon seed which is another important oil seed in West Africa has been shown to be prone to fungal attack and aflatoxin contamination at largely unsafe levels (Bankole *et al*., 2006). Earlier report by Opadokun (1992) had demonstrated high incidence level of 73% aflatoxin in Nigerian melon seeds at mean level of 19 ppb. Also, monitoring of aflatoxin in maize samples from different region of Africa showed a disturbingly high levels of contaminations above 100 ppb with many of the samples containing aflatoxins which exceeded the CODEX regulatory limit of 20 ppb (five times more lenient than EU guideline of 4 ppb) as stated by Makun *et al*. (2014). Atehnkeng *et al*. (2008) had also published a report on analysis of 55 maize samples from 11 districts across three agro-ecological zones of Nigeria and found out that the mean value of aflatoxins ranged from 30.9 – 507.9 ppb in 10 districts. This level of aflatoxins was far beyond any known acceptable limits. In addition, reports showed that incidence levels of aflatoxins vary with seasons (Mutegi *et al*., 2009; Makun *et al.*, 2014). Highly contaminated samples were obtained during the rainy season (90% frequency at 12–939 ppb) than in the dry season (53.2% frequency at 15–390 ppb) according to a report by Kamike and Takoy (2011).

The production of mycotoxins is stimulated by certain environmental factors; therefore, the extent of contamination will differ with geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods (Adeyeye, 2016). This reason might explain why some food commodities from the southern regions were highly contaminated with aflatoxins in the present study.

The reduction in aflatoxins contents after some physical treatments on maize and melon in this study corroborates the assumptions put forward by Adeyeye (2016) who stated that some methods employed in removal of aflatoxin include physical separations (sorting), washing, milling, nixtamalization, heat-treatment, radiation, extraction with solvents, and the use of chemical or biological agents.

Moisture contents (%)

It was generally observed in this study that the MC of food commodities from the southern geographical zones of Nigeria were significantly ($p<0.05$) higher than those from the northern geographical zones. These results might be a reflection of the atmospheric condition of different zones in Nigeria. The southern part is humid and moist especially during the rainy season which normally takes about nine (9) months of the year (February–October) while the Northern part is always hot and dry especially during dry season spanning about six (6) months of the year (November–April). This observation is in line with the report of Okunade (2006) who stated that moisture contents of stored grains have direct bearing with the humidity of the surrounding air. Similarly, 70% relative humidity (RH) is regarded as a safe limit for most commodities; hence, dry produce tends to gain moisture if kept in an environment of high humidity (Amadi and Adegbola, 2008).

One of the factors responsible for deterioration or spoilage of food commodities is the moisture content (Okunade, 2006). The author stated further that; the presence of moisture above the safe level in food grains could aggravate the activities of insects and microorganisms including moulds that produce mycotoxins and consequently reducing its quality. Thus, it was observed in the study that those food commodities with high total aflatoxins contents are also those with high moisture contents especially the products from the southern parts of the country.

From literature, data of moisture contents on smoked dried fish from S. Eastern Nigeria and maize from N. Western Nigeria in the present study are in agreement. For instance, Uneke (2015) reported the moisture contents of smoked dried catfish from two markets in Abakaliki, Ebonyi State (S. East) Nigeria ranging from 10.67–11.67% as compared with 10.94% (S. East) in the present study. Also, Oluwabamiwo *et al.* (2015) in their work on four Agro-Ecological Zones (Sudan Savanna-Sokoto, Nothern Guinea Savanna-Kaduna, Derived Savanna-Enugu and Oyo States, Humid Forest-Anambra and Lagos States)

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recorded the moisture contents of melon seeds from Kaduna and Sokoto States (N. West) Nigeria as 2.67 and 3.51 respectively compared to 3.15% reported for the same geographical zone (N. West) in the present study. On the other hand, moisture contents of groundnut (S. West), maize (S. East), melon seeds (S. East and S. West) were found to be higher than the available data in the literatures for the same zones. For instance, recently, Adetunji *et al*. (2018) reported the moisture contents of groundnut from five locations in Ogun State (S. West) Nigeria as ranging from 5.10–7.20% which was slightly lower than 7.31% recorded for S. West zone in the present study. Also, Ape *et al*. (2016) reported the moisture content of maize sample from Ogbete main market in Enugu State (S. East) Nigeria as 7.16% as against 11.82% recorded for maize from the same zone in this present report. Oluwabamiwo *et al*. (2015) as well reported the moisture contents of melon seeds from Lagos and Ibadan (S. West) Nigeria as 3.68 and 5.04% respectively as against 5.78% recorded in the current study. In addition, 4.46 and 4.03% were reported for melon seeds from Enugu and Anambra States (S. East) Nigeria respectively by the same authors as against 5.42% which was recorded in the present study. One of the reasons for the differences in the data reported in the current study and the available in literature data may be due the variation in the period of the year in which a particular sample was taken as some of these authors did not mention their sampling time.

Insect infestations

The pests isolated from the food commodities in the present study were; *Callosobruchus maculatus, Dermestes maculates, Tribolium castaneum, Trigoderma granrium*, and *Sitophilus zeamais*. Similar results have been published in literatures; for instance, Uneke (2015) isolated 20–25 insects from dried catfish obtained from two markets in Ebonyi State (S. East) Nigeria which also included some mentioned above.

CONCLUSION

The study has shown that cowpea, dried fish and yam chips sampled in October, 2017 from six geographical zones of Nigeria with maize and melon sampled from N. Western Nigeria were free from aflatoxins contaminations. Therefore, they could be considered as being safe for human consumption. On the contrary, groundnut from S. South, maize and melon from all zones except North Western Nigeria were contaminated with aflatoxins above safe limit which could render them unsafe for human consumption. However, this study has established the fact that proper postharvest handling and strict adherence to good agricultural practices (GAP) could improve the quality of Nigerian agricultural commodities internationally thereby adding value. Furthermore, the moisture contents of food commodities sampled from the Southern geographical zones were found to be higher than those from the Northern geographical zones of Nigeria in October, 2017. Insects found in the food commodities were generally few and majorities were dead indicating that the commodities were probably heavily treated with synthetic chemicals (pesticides) by handlers. There is need for proper and periodic surveillance on aflatoxins and pesticide residue contaminations in Nigeria so as to ensure reduced risks of exposure of human and livestock to these chemicals which constitute serious health hazards.

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Conflict of interest

Authors declared no conflict of interest in this study.

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