

Zonal Evaluation of Aflatoxin Contamination of Local Maize Products in Nigeria

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Abstract

Aflatoxin contamination of food and feeds continue to be a worldwide food safety concern due to its health and economic hazards. There is need to maintain a close monitoring of the level of contamination so as to assess the effectiveness of various control strategies and interventions. This study seeks to provide recent data on aflatoxin contamination of local maize products in Nigerian open markets. Maize grains, Ogi/Akamu (a fermented maize condiment), local and industrial maize flour were obtained from the six geo-political zones of Nigeria and assessed for mycological profile and aflatoxin content using standard fungal isolation/identification methods and Enzyme-Linked Immunosorbent Assay respectively. Results showed mean fungal count ranging from $1.10 \times 10^3 \pm 0.00$ cfu/g to $2.60 \times 10^7 \pm 0.75$ cfu/g with no significant difference ($p < 0.05$) in counts across the zones. Fungal genera identified include; *Aspergillus*, *Penicillium*, *Mucor*, *Neospora*, *Choanophora*, *Cladosporium*, *Rhizopus*, *Rhodotorula*, *Sacharomyces*, *Fusarium*, *Botrydoplodia*, *Helminthosporium*, *Trichoderma*, *Cuninghamella* and *Geotricum*. *Aspergillus* genera was most frequently isolated from the maize samples across the zones with an incidence of 28.5% while the least isolated were *Cuninghamella* and *Geotricum* spp. with 1.00 % incidence. Aflatoxin levels ranged from -11.24 ± 0.40 ppb to 66.80 ± 2.36 ppb with the southern zones having higher level of aflatoxin contamination. Fifty-six percent (56.00 %) of maize products had greater than 20 ppb maximum allowable limit across the zones. There is need for timely and a more target oriented intervention to prevent the serious health implications of this moderate dose exposures to this carcinogenic toxin.

Key words: Aflatoxin, Contamination, Maize products, Nigeria

Introduction

Aflatoxins are highly potent toxic fungal metabolites produced by some strains of *Aspergillus flavus*, *A. parasiticus* as well as related species like *A. nomius*, *A. niger*, *A. terreus* and *A. oryzae* (1,2). The toxicity of the six most potent aflatoxins in decreasing order are AFB1 > AFM1 > AFG1 > AFB2 > AFM2 > AFG2. The B aflatoxins exhibit a blue fluorescence under UV light while the G series fluoresce green. The M series are toxic metabolic derivatives of the B series found in milk hence their designation M. *Aspergillus flavus* produces only the B aflatoxins while *A. parasiticus* produces both the B and G series. Of these, AFB1 has been classified as the most toxic and most potent chemical carcinogen that occurs in nature (3). The occurrence of aflatoxin contamination of food products is a global problem due to its ability to cause severe health hazards such as carcinogenic, mutagenic, teratogenic and immune-suppressive disease conditions (4). In addition, Strosnider *et al.* (5) reported an increased susceptibility to infectious diseases such as Malaria and HIV/AIDS. A recent health hazard reported is the implication of aflatoxin in chronic hepatomegaly in school children (6). Expectedly, foods containing high levels of aflatoxins are often consumed in less-developed countries than in developed countries, with obviously more threats to the health of humans and animals.

Maize and peanuts are the main source of human exposure to aflatoxin largely because they are the most susceptible crops to its contamination and they unfortunately happen to be the agricultural crops that are highly consumed worldwide (7). Nigerian population depends on maize, millet and sorghum as the principal source of food. In many parts, maize has become the preferred cereal for food, feed and industrial use displacing millet and sorghum. However, it has been found to be significantly more colonized by aflatoxin-producing *Aspergillus* spp. than either sorghum or millet (8). Contamination of maize by these fungi renders the grain unfit for human consumption due to discolouration, a reduction in nutritional value and most importantly, it leads to the production of mycotoxins (9).

Various control strategies and interventions by governments of nations and non-governmental international bodies to eliminate aflatoxin contamination worldwide, have achieved varying levels of success and these include; stopping the infection process (host plant resistance, biocontrol); control of environmental factors (temp, rainfall, relative humidity, evapotranspiration, soil type) including efforts to build predictive models; pre-harvest crop management practices; post-harvest management strategies (timely harvesting, proper drying, sorting, proper storage, proper transportation, use of plant extracts and preservatives, good manufacturing practice and finding alternative uses for contaminated grain (10).

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The ultimate goal of these control strategies is to reduce / eliminate the deadly toxin from all points of the food chain, so as to protect consumers and the nation at large from the numerous health and economic hazards of aflatoxin. Constant monitoring of the effectiveness of any chosen strategy of control is however fundamental to its success. To this end, regular evaluation of total aflatoxin content of food products especially at the point of consumption will greatly validate the success and effectiveness of any control or intervention, as it is a crucial strategy to estimate indirect exposure of consumers to the deadly toxin.. Several researches have targeted evaluation of susceptible agricultural products in the farm, at harvest and during storage, but little work has been done to evaluate the susceptible crops at the point of consumption, hence this study. In this work, Maize grains, the number one susceptible crop to aflatoxin contamination, as well as its local by-products were assessed for their fungal load and aflatoxin contamination.

Materials and Methods

Study Areas

Random sampling was carried out in selected states from the six (6) geo-political zones of Nigeria. Samples of maize products were obtained from major local markets of the representative states. These were obtained from three main markets to form a sample pool and required aliquots were taken as representative test samples. The maize products of interest were collected from Enugu state representing the South-East zone, Edo state representing the South-South zone, Ondo state representing the South-West while Gombe, Katsina and Benue represented the North-East, North-West and North-Central zones respectively.

Experimental Design

This study was done as a six by four by three randomized setup where four kinds of indigenous maize products were sampled from three major markets in the six geo-political zones of Nigeria, with climatic variations in the zones of study expected to influence fungal and aflatoxin contamination.

Sample Collection

Local maize products analyzed were raw grains, Ogi(Akamu), local maize flour and industrial maize flour. Representative samples were randomly collected during rainy and dry seasons in order to evaluate possible effect of seasonal variations in aflatoxin content in the food samples. These were obtained with polyethylene containers and taken to the laboratory for analysis. When immediate analysis was not possible, samples were preserved by reducing the moisture content to below 13.00 % (to hinder the growth of the fungi and stop further production of aflatoxins) by drying at 60°C for 4 hours and then, storing at 4°C until needed for analysis.

Media Preparation

The media used in this study to isolate fungi from test samples were; Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Sabouraud Dextrose Agar (SDA), Yeast Extract Sucrose Agar (YES Agar) and Rose Bengal Agar (RBA). All media preparations were carried out in accordance with manufacturer's instructions. Sterilization of media was by autoclaving for 15 minutes at 121°C and 15 pounds pressure. Antibiotics (Streptomycin and Chloramphenicol – 50 mg/L each) were introduced into the dissolved media after sterilization was carried out.

Mycological Evaluations

Isolation of fungi / *Aspergillus* species from the various food samples was carried out using ten-fold serial dilution method and direct plating on SDA, PDA and RBA then incubating at 27±2°C for 72 hours. Colonies were counted and expressed in Colony Forming Units per gram (cfu/g).

Purification of Cultures

Distinct colonies were sub-cultured on Yeast Extract Sucrose (YES) agar and incubated at 27±2°C for 72hours. Then the various fungal isolates were passed through preliminary identification using their morphological and microscopic characteristics on viewing fungal mycelium stained with lactophenol cotton blue (11).

Identification of fungal isolates

Cultural and morphological characteristics like shapes, size, pigmentation, ornamentation and/or mode of attachment, asexual or sexual structures were determined with the aid of a microscope from observation of fungal mycelium stained with lactophenol cotton blue. Fungal keys and manuals were used for further preliminary identification of the isolates (12,13).

Determination of Incidence / Prevalence of Aspergillus Isolates.

The incidence of *Aspergillus* species in the food samples analyzed were assessed using the presence index and relative frequency criteria. These values were obtained using the formulae postulated by (14):

$$Pif = Nsc / Tnse \text{ and } Rif = Ncpc / Tnil$$

Where Pif=Presence index of the fungus; Nsc=Number of samples contaminated; Tnse=Total number of samples examined; Rif=Relative index of the fungus; Ncpc=Number of food materials contaminated by each fungus; Tnil=number of isolates obtained per zone.

Determination of Aflatoxin Content in the Various Food Samples

Aflatoxin content of the food samples were determined using Max Signal Total Aflatoxin Enzyme Linked Immunosorbent Assay (ELISA) test kits and these were purchased from BioOscientific Corporation, USA. The

tests (food sample preparation, ELISA test protocols) were performed according to the manufacturer's instructions.

Sample Preparation for ELISA

Solid Samples

- 1g of the sample was weighed out aseptically into a sterile plain container.
- 5ml of 70% methanol was added to the weighed sample
- The sample-methanol mixture was thoroughly shaken to make a homogenous mixture
- The homogenous mixture was centrifuged at 4000 revolutions per minute (rpm) for 10 minutes
- 2.0 ml of the supernatant (fluid) was measured aseptically into sterile pilot bottles.

Liquid Sample

- 0.5ml of the sample was measured aseptically into a plain sterile container
- 4.5ml of 70% methanol was added to the measured sample
- The mixture shaken thoroughly to homogenize
- 2.0ml of the mixture was transferred aseptically into a pilot bottle

ELISA Test Protocol

A 50 μ L of each of the aflatoxin B₁ standards was added into different wells in duplicate in the order from low concentration to high concentration. Then 50 μ L of each of the samples were added into different sample wells in duplicate followed by the addition of 100 μ L of antibody #1 which were then well mixed by gently rocking the plate manually for 1 minute. The plate was then incubated for 30 minutes at a temperature of 20-25°C. The plate was washed 3 times with 250 μ L of 1X wash solution. After the last wash, the plate was inverted and gently tapped dry on paper towels. Then 150 μ L of 1X antibody #2 was added and the plate incubated at room temperature for 30 minutes with the microtitre plate covered. The plate was washed 3 times with 250 μ L of 1X wash solution. After the last wash, the plate was inverted and tapped dry on paper towels. 100 μ L of TMB substrate and then incubated for 15 minutes. After incubation, 100 μ L of stop buffer was added to stop the enzyme reaction. The plate was then read as soon as possible following the addition of the stop buffer on a plate reader 450 nm wavelength.

Total Aflatoxin Concentration Calculations

A standard curve was constructed by plotting the mean relative absorbance (%) obtained from each of reference standard against its concentration in ng/ml on a logarithmic curve.

$$\text{Relative Absorbance (\%)} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100$$

Absorbance zero standard

The mean relative absorbance values for each sample were then used to determine the corresponding concentration of the tested sample in ng/g from the standard curve.

Statistical Analysis

Statistical analysis of all data generated in this research was performed using SPSS 15.0. Analysis of Variance (ANOVA) and Duncan's multiple comparison tests were used to compare the means of cfu/g of all fungal counts. Student's t-test was used to evaluate fungal counts and incidence in the zones (15).

Results and Discussion

Aflatoxins are highly toxic carcinogens that contaminate crops worldwide (16). Hence, governments of various countries, have established or proposed regulations for controlling aflatoxin levels in foods and feeds (17). Continuous evaluation and the establishment of maximum allowable limit of aflatoxins in foods and feeds constitute part of the regulations.

This work aims to achieve this purpose by providing recent data on level of aflatoxin contamination in maize products from six geo-political zones of Nigeria, with a view of facilitating a more target oriented intervention. Thus, maize grains and some of its local products (Akamu, local maize flour, industrial maize flour) were randomly purchased from representative states of the six geo-political zones of Nigeria and analyzed for mycological and aflatoxin contamination with a view of assessing indirect exposure levels to aflatoxins at final point of distribution prior to consumption.

Results indicate a highly diverse pattern of fungal distribution on the local maize products analyzed. Mean fungal count in all maize products across the zones ranged from $1.10 \times 10^3 \pm 0.00$ cfu/g to $2.60 \times 10^7 \pm 0.75$ cfu/g. No significant difference ($p < 0.05$), was observed in most fungal counts across the zones. Fungal genera identified include; *Aspergillus*, *Penicillium*, *Mucor*, *Neospora*, *Choanophora*, *Cladosporium*, *Rhizopus*, *Rhodotorula*, *Sacharomyces*, *Fusarium*, *Botrydoplodia*, *Helminthosporium*, *Trichoderma*, *Cuninghamella* and *Geotricum* as depicted in Figure 1. *Aspergillus* genera was most frequently isolated from the maize samples across the zones with an incidence of 28.5% while the least isolated were *Cuninghamella* and *Geotricum* spp with 1.0% incidence. The diversity and distribution of fungi in maize products was observed not to differ much across the zones.

Table1: Mean Fungal Counts (cfu/g) of Maize Products in the Geo-political zones of Nigeria

	Edo	Benue	Enugu	Gombe	Katsina	Ondo	p-Value
	$\bar{x}\pm SD$						
Raw grains	2.296±1.787	0.665±0.746	1.078±0.036	0.903±0.777	1.041±0.000	0.732±0.631	p>0.05
Local Maize flour	0.588±0.541	0.794±0.685	0.423±0.599	0.025±0.028	0.829±1.074	0.669±0.597	p>0.05
Industrial Corn flour	0.722±0.617	0.005±0.000	0.222±0.294	0.162±0.208	0.005±0.001	0.382±0.605	p>0.05
Akamu	1.673±1.383	0.592±0.541	0.972±0.794	0.601±0.502	0.060±0.043	0.511±0.510	p>0.05

All figures gotten from the laboratory analysis were unified by dividing by 10^5 and transformed using $\text{Log}_{10}(1+X)$

p>0.05 – No Significant Difference

Key: Zones and their representative states

North-West zone: Katsina State

North-East zone: Gombe State

North-Central zones: Benue State

South-West zone: Ondo State

South-East zone: Enugu State

South-South zone: Edo State

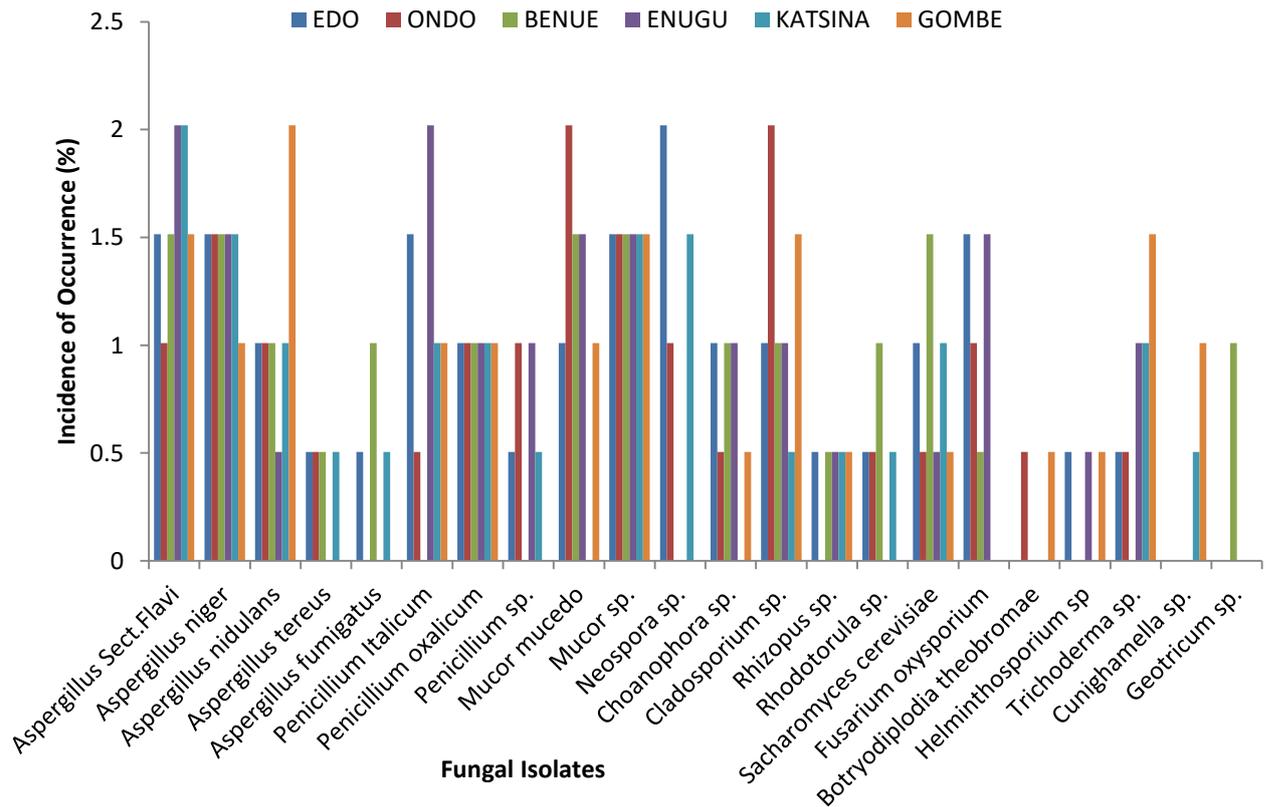


Figure 1 : Incidence of fungal isolates from maize products in the geo-political zones of Nigeria

Key: Zones and their representative states

North-West zone: Katsina State

North-East zone: Gombe State

North-Central zones: Benue State

South-West zone: Ondo State

South-East zone: Enugu State

South-South zone: Edo State

Fungal contamination showed a uniform pattern in maize products with the grains slightly having higher fungal counts. *Aspergillus* spp., *Penicillium* spp. and *Mucor* sp. isolated from maize grains in this study, have been previously reported by (18) in Ghana who isolated *Aspergilli* as the predominant species in maize followed by *Penicillium*, *Fusarium* and other fungi. Atehnkeng *et al.* (19) reported the presence of *Aspergillus*, *Penicillium*, *Fusarium* and other fungi in maize grains while (20) reported the predominance of *Aspergillus* and *Fusarium* species in maize obtained from Kogi and Niger states in Nigeria. In contrast to this study, (21) from Togo and Benin reported *Fusarium* spp. as the predominant fungi in maize. These differences might be as a result of sampling strategy because these workers collected freshly harvested maize samples while in our study, samples were collected from previously stored market maize. Under storage conditions, *Aspergillus* and *Penicillium* being storage fungi are most active (2). Muthomi *et al.* (9) also isolated similar *Aspergillus* species from processed and unprocessed maize products, to the ones isolated in this work. The number of fungal genera was higher in maize grains than the processed maize products such as the flour. Local maize flour is however expected to be more contaminated with fungi because of local milling of the grains to flour, during which contamination usually occurs from un-sanitized equipments, inadequate personal hygiene of handlers and lack of Good Manufacturing Practices (GMP). Also, seed coat integrity is usually destroyed when grains are milled to flour which could further encourage fungal invasion and contamination (12). Industrial maize flour consistently had low fungal contamination across the zones probably due to strident processing and standardization. Similarly, the presence of *Trichoderma* sp., *Curvalaria* sp., *Mucor* sp. and other fungi was reported as seed borne mycoflora of maize in Pakistan (22). Most of the fungi isolated in this study have been isolated in maize grains in Karnataka, India (23). Mycological profile is however not a direct reflection of aflatoxin contamination in a food product, though the type and distribution of the *Aspergillus* sect. Flavi group as well as incidence of the S or L morphotypes of *A. flavus* may greatly determine aflatoxin levels. Maize has been known to be highly susceptible to aflatoxin contamination and this was also observed in this work. Aflatoxin levels of the maize products in this study are shown in Figure2.

The maize products analyzed had aflatoxin levels ranging from -11.24±0.40ppb to 66.80±2.36ppb. Maize grain was most contaminated with aflatoxin in Benue (North Central Zone) while Gombe and Katsina had no detectable contamination. Fermented maize grains (Ogi or Akamu), is a staple food of the Yorubas in Nigeria and is the first local food given to babies at weaning. It is produced by soaking maize grains in water for 2-3days followed by milling and sieving through a screen mesh. Fungal contamination of Ogi (Akamu) has previously been reported (24) but aflatoxin contamination is expected to be low due to the long fermentation of the maize grains. Akamu being a fermented food product is expected to have low levels of aflatoxin as fermentation has been reported to significantly reduce aflatoxins in food (25). This was contrary to some results obtained in this work probably due to high fungal contamination and aflatoxin content prior to fermentation. It may also be due to short fermentation period as well as processing methods used (26).

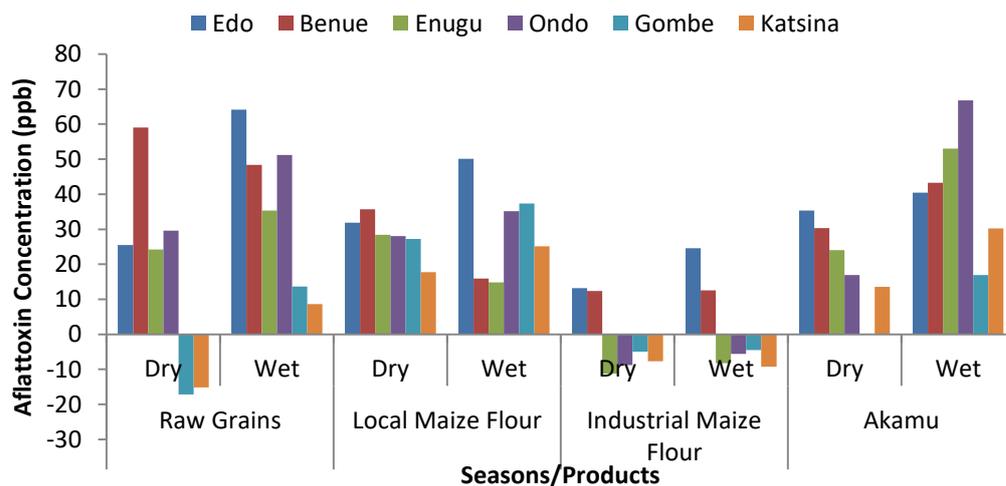


Figure 2: Total aflatoxin content (ppb) of maize products from the geo-political zones of Nigeria

Key: Zones and their representative states

North-West zone: Katsina State

North-East zone: Gombe State

North-Central zones: Benue State

South-West zone: Ondo State

South-East zone: Enugu State

South-South zone: Edo State

The results in this study shows that the local condiment Akamu from Ondo, unexpectedly had the highest aflatoxin contamination of $66.80\text{ppb} \pm 2.36$ (Figure 2) while the least contamination occurred in industrial maize flour from Ondo, Benue, Gombe and Katsina, with no detectable aflatoxin. North-West (Katsina State) and North-East (Gombe State) zones consistently had low levels of aflatoxin contamination in all their maize products analysed in this study while South-West (Ondo State), South-East (Enugu State) and South-South (Edo State) zones had high levels of contamination. This is unexpected considering the drought situations and extreme temperatures in the northern zones. A possible explanation is that the northern zones represents the mainstay of commercial agriculture in Nigeria, hence intervention strategies are concentrated there. Also, the southern zones engage in long term storage of grains produced in the north and aflatoxin contamination of food has been shown to increase with storage period (27). This may be attributed to variations in climatic conditions of the zones. The more humid Southern zones may encourage the growth/proliferation of aflatoxigenic fungi and subsequent production of the toxins than the drier Northern Nigeria. These reasons may have led to the high significant difference ($p < 0.01$) in the various maize samples analyzed across the six zones. Bankole and Mabekoje (28) have reported the occurrence of aflatoxin in maize from southwestern Nigeria with levels ranging from 3-138ng/g. Matumba *et al.* (29) detected levels of aflatoxin B1 in maize from Malawi exceeding the median aflatoxin maximum tolerable limit of 5ng/g. Surveys from other countries have reported the occurrence of aflatoxins in maize and by products from China (30) Tanzania (31), Kenya (9). Contrary to this study, results of (32) showed that aflatoxin B1 content of maize samples collected in Lagos, Nigeria ranged from 2.51-3.94ng/g which is below the set aflatoxin B1 level.

Kilonzo *et al.* (33) reported a higher level of aflatoxin contamination in maize products in Kenya, than the level observed in this work. They stated aflatoxin content between 18-480 $\mu\text{g}/\text{kg}$ while the highest level of aflatoxins for maize products in this study was 64ppb ($\mu\text{g}/\text{kg}$) in maize grains from Edo State (South-South zone) during the wet season. The high variation may be due to the sampling location (Kenya) being a high risk zone of aflatoxin outbreaks and acute aflatoxicosis. Karthikeyan *et al.* (1) observed a range of aflatoxin level of 0-149.32 $\mu\text{g}/\text{kg}$ in pre and post-harvest maize sampled in Tamil Nadu, India.

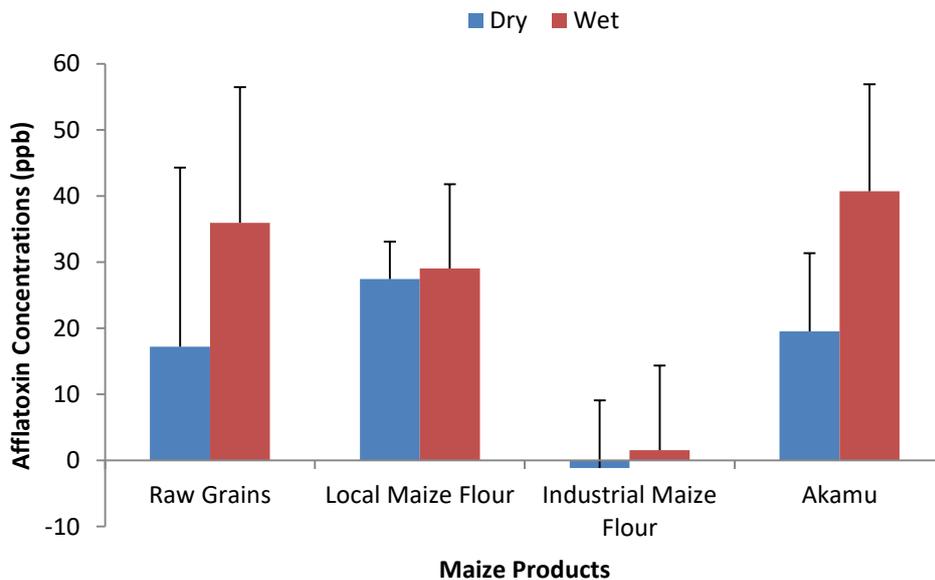


Figure 3: Effect of Season on Aflatoxin Content of Maize Products in the Geo-political Zones of Nigeria
 $P > 0.05$: No significant difference in Aflatoxin content except in Akamu where there is a highly significant difference at $P < 0.01$

Though higher levels of aflatoxin contamination were observed in many maize products during the rainy season as compared to dry seasons, their difference were not statistically significant ($p > 0.05$) except for Akamu that had a highly significant increase in aflatoxin content ($p < 0.01$) during the rainy season.

Several researchers have however reported that climatic factors greatly affect aflatoxin contamination of food (34). Astronomically high aflatoxin levels of 2072 $\mu\text{g}/\text{kg}$ have been reported in maize from Croatian farms and feed factories and this was attributed to a high weather temperature and drought conditions during the period of cultivation of the maize (35).

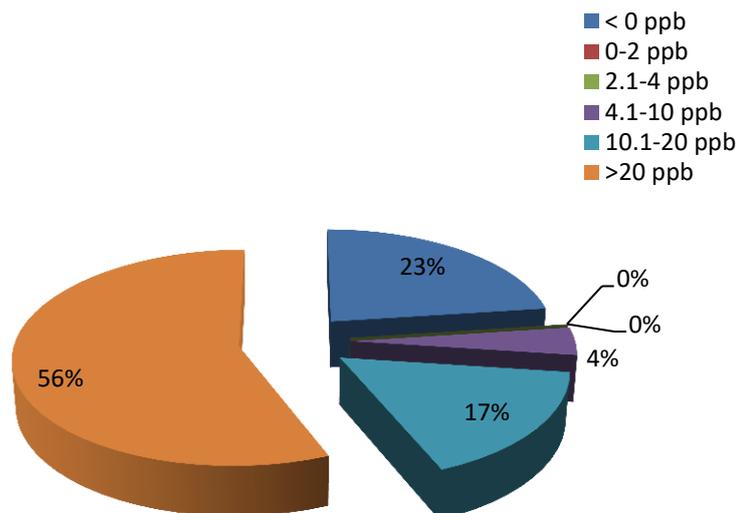


Figure 4: Aflatoxin Contamination of Maize Products in Relation to National and International Standards

Results from this study indicated that 56% of maize products exceeded the Nigerian standard of 20ppb. In related investigations, 81% of 364 maize samples analyzed by (36) had detectable levels of aflatoxins and 47% of the samples had levels exceeding the 20 μ g/kg US limit for total aflatoxins. This is a big economic threat to Nigeria as half of the maize produced locally cannot be exported to the US or EU countries.

Conclusion:

This study provides recent data on the mycological profile and aflatoxin contamination of local maize products in the six geo-political zones of Nigeria. The level of aflatoxin contamination suggests a moderate level of exposure to the deadly toxin with its associated health hazards. It also revealed a possible economic crisis due to low export potential of the staple food. There is need to extend various interventions to the southern zones to achieve a more effective control of aflatoxins in Nigeria.

Conflict of Interest

There is no conflict of interest associated with this work.

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