

Full Length Research Paper

Evaluation of aflatoxins levels and molecular identification of toxigenic molds in cereals and cereal-derived breakfast foods in Nigeria

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In the last decade, there have been an increase in safety concerns on the aflatoxins contents of cereals and cereal-derived food products in Nigeria. In the current study, evaluation of aflatoxins levels and molecular identification of toxigenic molds recovered from cereals and cereal-derived foods products in Nigeria were studied. Enzyme Link Immunosorbent Assay (ELISA) and high performance liquid chromatography (HPLC) methods were used for qualitative and quantitative aflatoxins evaluation, respectively. The result showed that aflatoxins were detected in 53 out of the 120 samples of the cereals and their products studied. The detected levels of aflatoxins in some of the samples were over the permissible (4 µg/kg) as recommended by relevant food regulatory authorities. Aflatoxins B₁, B₂, G₁, and G₂ were all detected in the sample, aflatoxin B₁ was the most predominant in the samples. *Aspergillus flavus* (AZ19), was isolated and identified as the major contaminating mold. Thus, findings of this research provide strong evidence that incidence of aflatoxins contamination of food crops still remain a major problem in Nigeria agricultural sector.

Key words: Aflatoxins, toxigenic molds, cereals, cereal-derived foods, *Aspergillus flavus*.

INTRODUCTION

Aflatoxins are toxic chemical compounds produced in foods and food products by *Aspergillus flavus* and *Aspergillus parasiticus*. These mycotoxins have been shown to induce both genotoxic and carcinogenic effects in humans (EFSA, 2007). Cereals and its products have been widely reported to be prone to contamination by potentially toxigenic fungi (Achaglinkame et al., 2017). However, incidences of aflatoxins contamination of cereals and associated food products are more prevalent in developing countries such as Africa compared to Europe (EFSA, 2013; Wagacha and Muthomi, 2008). The

prevalence of aflatoxins contamination of cereals and cereals derived food products in Africa is due to improper post-harvest handling and/or storage in addition to the inability to control environmental factors (high temperature, high relative humidity and moisture content) that promotes the growth of the toxigenic mold and mycotoxins production (Gong et al., 2003; Beuchat, 2002; Burger et al., 2013). Studies have shown that high aflatoxins exposure in young children in Africa is due to feeding in contaminated cereals and cereal-food products either as complementary or breakfast food (Gong et al.,

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2003; Magoha et al., 2016; Achaglinkame et al., 2017). The maximum permissible levels of aflatoxins B1 and aflatoxins B2, G1 and G2 in all cereals and cereal-based human foods are 2 and 4 µg/kg, respectively as recommended by EFSA (2013). There are significant adverse carcinogenic potentials of exposure to aflatoxins over the permissible limits for long period. The most dangerous effects of AFs in human especially in children are the immune suppression effect, low birth weight, and growth impairment (Lombard, 2014; Wagacha et al., 2008). Whereas chronic exposure to aflatoxins have been shown to cause different kinds of cancer (Gong et al., 2016).

On the other, hand maize (*Zea mays*), millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) are common foods in use in Nigeria. These cereals are very popular for their use in making “*pap*”, quick breakfast beverages in Nigeria. *Pap* is a naturally fermented porridge that serves as both complementary weaning food for infant and breakfast food for adults in Nigeria and other African countries. Several studies have shown that due to improper post-harvest handling and bad storage conditions in Africa, these cereals are widely contaminated by potentially toxigenic fungi together with their toxins and these cereals must be subjected to examination before being used in making *pap* and breakfast beverages (Gong et al., 2003; Enyisi et al., 2015). Hence, the major setbacks to the utilization of these cereals in Africa are their susceptibility to aflatoxins contamination.

Furthermore, according to the European Commission all aflatoxins must be absent in agricultural products to be fit for human consumption (European Commission, 2006a). However, aflatoxins, in foods are stable in foods under different processing conditions including under cooking temperatures, hence difficult to eliminate (Magoha et al., 2016). Thus, the study by the Joint Expert Committee on Food Additives (JECFA) recommended that aflatoxins levels in foods and food products should be under permissible limit (Strosnider et al., 2006).

Moreover, EFSA (2011) reported that developing are the major countries exposed to aflatoxins in food commodities and hence appropriate preventive measures must be applied to reduce incidences of aflatoxins contamination.

Therefore, the purpose of this study is to evaluate aflatoxins contamination and molecular identification of toxigenic molds in cereal and cereal-derived breakfast food in Nigeria.

MATERIALS AND METHODS

Sample collection

One hundred and twenty (120) samples were collected and analyzed; twenty samples each of maize, millet and sorghum and the respective *pap* from sellers covering different markets in Abakaliki, Nigeria.

Isolation and identification of molds

The samples were first screened for the presence of viable mold spores using potato dextrose agar (PDA). The cereals and their products were cultured on PDA and incubated at room temperature of 25 to 28°C for 3 to 5 days. The recovered fungal cells were identified microscopically using morphological and microscopic features and were further identified by molecular biology to accurate species level using internally transcribed spacer (ITS) and partial calmodulin and transcriptional elongation factor (TEF) rDNA sequencing according to the method described by Azi et al., (2017).

Molecular identification of fungal isolate

The recovered mold isolates identified as *Aspergillus* species were further identified to species level at Centre for Agriculture and Biotechnology International (CABI), Microbial Identification Services (United Kingdom). ITS, partial calmodulin and TEF rDNA sequencing analyses were used for the identification of the isolates. All procedures were validated and processed in accordance with CABI's in-house method as documented in TPs 61-68 and TP70. Molecular assays were carried out using nucleic acid as a template. A proprietary formulation [microLYSISR-PLUS (MLP) Microzone, UK] was subjected to the rapid heating and cooling of a thermal cycler, to lyse cells and release deoxyribonucleic acid (DNA). Following DNA extraction, polymerase chain reaction (PCR) was employed to amplify copies of the rDNA *in vitro*. The quality of the PCR product was assessed by undertaking gel electrophoresis. PCR purification step was carried out to remove unutilized dNTPs, primers, polymerase and other PCR mixture compounds and obtained a highly purified DNA template for sequencing. Sequencing reactions were undertaken using BigDyeR Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which utilizes fluorescent labeling of the chain terminator ddNTPs, to permit sequencing.

Removal of excess unincorporated dye terminators was carried out to ensure problem-free electrophoresis of fluorescently labeled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx™ 2.0 (Qiagen, UK). Modules containing prehydrated gel-filtration resin were optimized for clean-up of sequencing reactions containing BigDyeR terminators. Dye removal was followed by suspension of the purified products in highly deionized formamide Hi-Di™ (Life Technologies, UK) to prevent rapid sample evaporation and secondary structure formation. Samples were loaded onto the AB 3130 Genetic Analyzer and sequencing were undertaken to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide. Following sequencing, identifications were undertaken by comparing the sequence obtained with those available in the European Molecular Biology Laboratory (EMBL) via the European Bioinformatics Institute (EBI).

Qualitative ELISA aflatoxins detection in the cereals and cereal-derived products

Detection of total aflatoxins in cereals and their *pap* samples was done by Enzyme Link Immunosorbent Assay (ELISA) method as described by Azi et al., (2017). Briefly, the samples were first ground into fine powder and aflatoxins content was extracted by addition of tween-ethanol (25 ml) to 5 g of each sample and mixed properly. Then the sample solution was centrifuged for 3 min at 250 rpm and filtered with Whatman No. 1 filter paper. Aflatoxin conjugate (200 µl) was dropped in a clean mixing wall and 100 µl of the sample analyte were added. The mixture of the aflatoxin conjugate and the sample was then transferred into antibody incubated micro-walls and incubated under dark condition at room

Table 1. Concentrations of total aflatoxins in the cereals and their *pap*.

Cereals/Cereal products	Number sample/positive	Aflatoxins concentrations ($\mu\text{g}/\text{kg}$)		Average value ($\mu\text{g}/\text{kg}$)
		Below limit	Above limit	
Maize	20/13	9 (4.3-5.7)	4 (11.3 -14.7)	9.52
MP	20/8	4 (4.6-6.4)	4 (10.2-12.3)	8.64
Millet	20/11	3 (1.8-3.8)	8 (5.7-7.8)	6.51
MiP	20/9	5 (2.1-3.3)	4 (4.1-5.6)	3.73
Sorghum	20/9	7 (1.9-3.1)	2 (4.9-5.2)	2.85
SP	20/3	2 (2.5-3.7)	1 (4.0)	2.31

MP= Maize *pap*, MiP=millet *pap*, SP=sorghum *pap*.

temperature for 15 min which allowed antibody/antigen reaction to take place. At the end of the incubation period, the solution was then washed off 5 times using deionized water and then 100 μl of the substrate were added and allowed to stand for 5 min. Finally, the solution was subjected for reading the aflatoxins content using ELISA machine.

Quantitative HPLC analysis of aflatoxins B₁, B₂, G₁ and G₂

The analyses of samples for aflatoxins B₁, B₂, G₁, and G₂ for all samples were also performed using Waters Alliance 2695 HPLC System, Column (C18, 4.6 \times 150 mm) equipped with a 2475 Scanning Fluorescence detector. In this method, the mobile phase was water: methanol: acetonitrile (60:25:15 v/v/v) with a flow rate of 1.0 mL/min and the column temperature was 28°C and injection volume 50 μl . The fluorescence detector was operated at an excitation wavelength of 365 nm and an emission wavelength of 455 nm. The fluorescence of G₁ and B₁ was enhanced using electrochemically generated bromine. The analog out was sample energy while the scaling factor was 10 with the filter as digital (hamming) at 20 s. Post-column electrochemical derivatization was performed in order to enable detection of B₁ and G₁ aflatoxins. Detection was carried out at 220 nm. The resolution peaks were recorded on the HPLC chart according to the retention time of each aflatoxin. The concentrations of the aflatoxins were quantified from standard curves.

RESULTS AND DISCUSSION

Molecular identification of isolate

The natural adverse environmental conditions together with improper long-term storage are the major factors responsible for the presence of *Aspergillus* in food products (Campos et al., 2017; Burger et al., 2013). The *Aspergillus* contamination of cereals at different stages of the production chain such as pre-harvest, harvest, and post-harvest handling in Africa have been widely reported (Smith et al., 2016; Atanda et al., 2011). The findings of the current study revealed that the cereals and cereal-based foods studied were contaminated by *Aspergillus* spp. (Figure 1). While molecular identification showed that the cereals and their *pap* were mostly contaminated by toxigenic *A. flavus* (AZ19) strains which produce AfB and AfG (Lutfullah and Hussain, 2011). These cereals

and their products are widely and frequently consumed in Nigeria as well as other African countries as basic staple foods. The presence of these potent toxigenic molds in these cereals and its product (*pap*) results in great health risk for a large number of human populations (children and adult) in Nigeria. This contamination could be as a result of bad storage and unhygienic processing facilities which might have provided optimum good conditions for the growth and proliferation of these fungi. The growth and toxin production by *Aspergillus* require about 13% moisture, relative humidity of 65% (water activity, a_w , of 0.65) (Medina et al., 2014; Achaglinkame et al., 2017). These factors together with improper drying might have predisposed the contamination of the cereals with *Aspergillus* and progressive proliferation.

Total aflatoxins in the cereals and their *pap*

The result of total aflatoxins (sum of B₁, B₂, G₁, G₂) in the cereals and their *pap* is shown in Table 1. Total aflatoxins levels in some of the cereals and their *pap* were above the maximum permissible limit as stipulated by relevant international regulatory authorities (4 $\mu\text{g}/\text{kg}$ for cereals and cereal-derived products except maize for which maximum levels are 10 $\mu\text{g}/\text{kg}$) (European Commission, 2006b; EFSA, 2013). Currently, the maize sample was highly contaminated by fungi (13 out of 20 samples) followed by the millet sample while the least contamination was recovered from sorghum samples. This is in line with the findings of EFSA (2013) in which maize was the most contaminated cereals studied. On the other hand, fungal contamination of the *pap* produced from millet samples was more than those produced from sorghum. Among the contaminated maize samples, 4 samples were beyond safe limit of 10 $\mu\text{g}/\text{kg}$ stipulated by European Union (EU) regulations for total aflatoxins whereas nine samples (2.3 to 5.7 $\mu\text{g}/\text{kg}$) had aflatoxins below the safe limit. Four samples of the maize *pap* also had unacceptable levels of total aflatoxins contaminations. The millet had eleven (11) contaminated samples out of the samples evaluated. While three of the samples had below maximum acceptable limit of aflatoxins

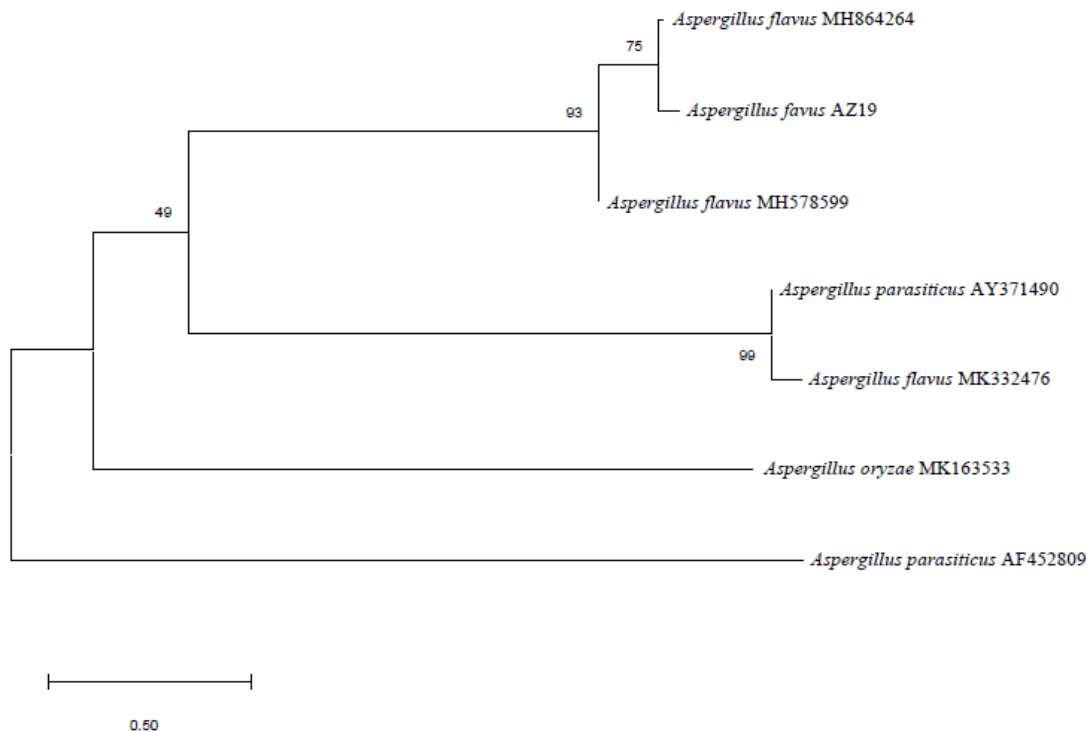


Figure 1. Phylogenetic tree of the isolated *Aspergillus flavus* (AZ19).

contamination, eight samples had aflatoxins levels beyond the acceptable limit of 4 $\mu\text{g}/\text{kg}$ according to European regulations. The millet *pap* also had nine samples contaminated above the limit of detection (LOD) of 1 $\mu\text{g}/\text{kg}$ and four out of the nine samples were above the acceptable limit. Sorghum and its *pap* were the least contaminated of the cereals evaluated. It was only two out of the nine aflatoxins contaminated sorghum samples that had above limit contamination while one out of the three contaminated sorghum *pap* had above the acceptable limit of aflatoxins contamination. Mean occurrence values for maize and its *pap* were 9.54 $\mu\text{g}/\text{kg}$ and 8.64, respectively. The millets and its *pap* were 6.51 and 3.73 $\mu\text{g}/\text{kg}$ while the sorghum and its *pap* had the least average values of 2.85 and 2.31 $\mu\text{g}/\text{kg}$, respectively.

In general, 53 (44.16%) out of the 120 samples of cereals and their *pap* evaluated had values above 1 $\mu\text{g}/\text{kg}$ LOD for the sum of aflatoxins B₁, B₂, G₁, and G₂. Fourteen percent (43.39%) samples had total aflatoxins contaminations above the permissible limit according to the regulations of the European Commission.

Thus, cereals and their *pap* with aflatoxins contamination above the permissible limit portend significant health risk for human exposure to the toxicants (European Commission, 2006a; EFSA, 2013). The high level of aflatoxins contamination of these cereals and their products might have been as a result of poor post-harvest handling/storage facilities. Most of the storage and processing structures commonly used by farmers/

local cereal processors in Nigeria as well as other Africa countries are traditional hence predisposes the cereals to *Aspergillus* contamination and subsequent aflatoxins production (Atanda et al., 2011). Environmental factors such as temperature and high relative humidity might have also played a significant role in increasing the levels of aflatoxins contaminations of these cereals. Temperature above 20°C has been reported to enhance the growth of *Aspergillus* while aflatoxins production is optimum at the temperature between 25 and 37°C (Smith et al., 2016; Strosnider et al., 2006). These temperatures are the ambient temperature in Nigeria and might have high relative humidity to be responsible for the rapid growth and multiplication of the toxigenic molds.

The variation in the prevalence and concentrations of aflatoxins in the different cereals and their product could be attributed to the differences in environmental factors during storage and processing as well as nutrient composition of the cereals. The level and rate of production of aflatoxins in food substrates have been reported to be influenced by available nutrients (Williams et al., 2003; Achaglinkame et al., 2017). Foods that have high concentrations of sucrose, maltose, and glucose have been noted to be more vulnerable to aflatoxins contamination (Magoha et al., 2016; Smith et al., 2016; Achaglinkame et al., 2017). Thus, the levels of aflatoxins and prevalence in the different cereals and their *pap* could also be partly attributed to the varying concentrations of the sugars present in the cereals.

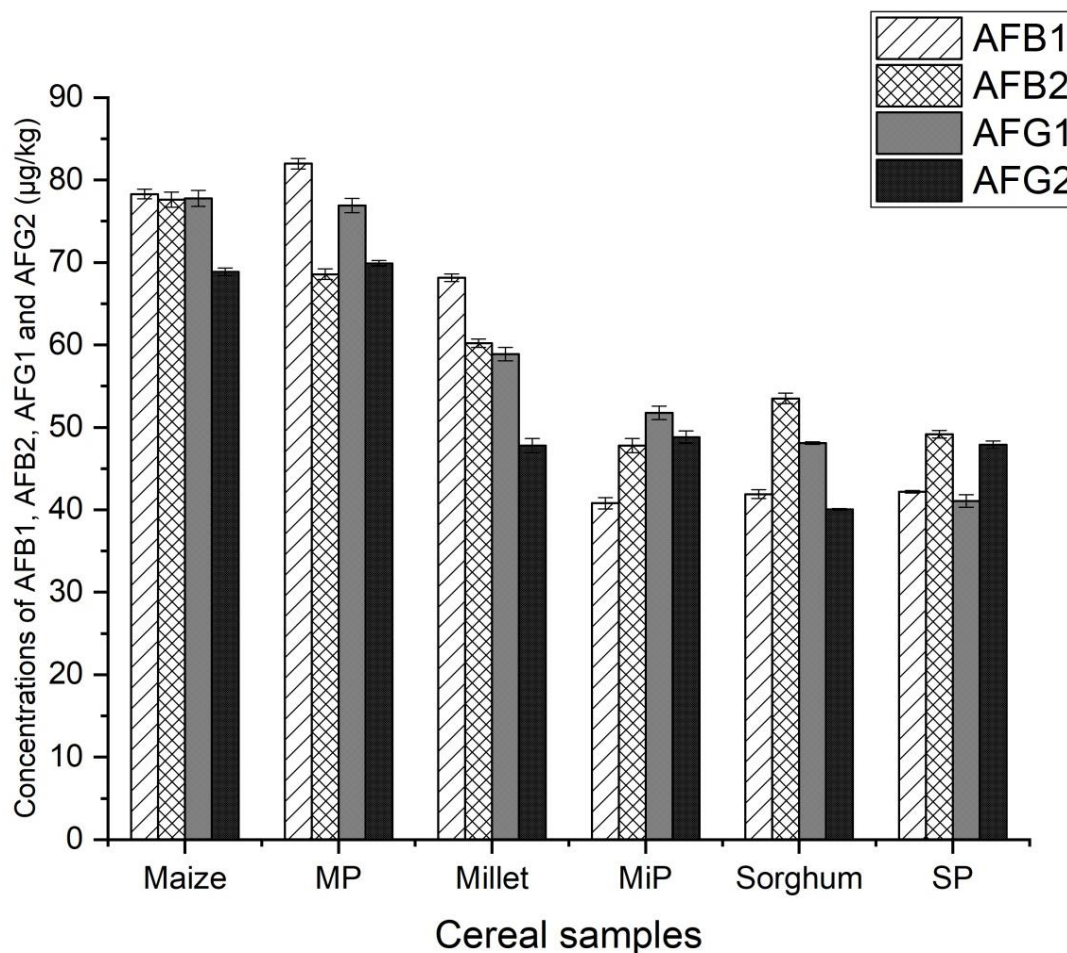


Figure 2. Average concentrations of aflatoxins B₁, B₂, G₁ and G₂ in the cereals and their *pap*.

Aflatoxins B₁, B₂, G₁ and G₂ contaminations in the cereals and their *pap*

Results of 120 cereal samples and their *pap* samples are summarized (Figure 2). The result showed that aflatoxins B₁, B₂, G₁, and G₂ were significantly detected in all the cereals analyzed with reference aflatoxins B₁ being the most detected form in all the cereals and their products. Aflatoxin B₁ is produced by *A. flavus* hence Figure 1 shows the evidence for its abundance in the cereals and their *pap* studied in this research.

Aflatoxin B₁ is the most toxic and carcinogenic type than other aflatoxins (B₁ > G₁ > B₂ > G₂) and has been implicated in the etiology of hepatocarcinoma as well as tumors of the lungs and kidneys (Shephard, 2008). Chronic exposure increases the risk of developing liver and gallbladder cancer (Gong et al., 2016). Aflatoxins interact with the basic metabolic pathways of the human cells and disrupt key enzymatic processes including those involved in carbohydrate and lipid metabolism as well as protein synthesis (Shephard, 2008; Quist et al.,

2000). It also induces changes in insulin-like growth protein factor, hence resulting in inhibition of mineral bioavailability especially in children and young adult (Sowley, 2016).

The impact of aflatoxins exposure is often most severe among children and pregnant mothers. Based on the exposure level, the effect can range from low birth weight to growth impairment, immunosuppression and mental retardation (Gong et al., 2004). In addition, Gong et al. (2016) reported that infants and young children who had the toxin's exposure above tolerable daily limit were shorter and under-weight. It was also recorded that in some cases it caused various kinds of cancer and resultant deaths depending on the type, period and amount of exposure (Lombard, 2014). From the finding of this study, cereals and their *pap* represent significant health risk considering that they are widely used as breakfast and weaning food in Nigeria. Similar studies carried out on cereals cultivated and consumed in other Africa countries such as Ghana, Kenya, Tanzania, Mali, and Benin show similar results of high aflatoxins

contamination with its attendant health risk (Magoha et al., 2016; Wagacha and Muthomi, 2008; Gong et al., 2004; Achaglinkame et al., 2017).

Conclusion

The findings of this study detected aflatoxins contamination of food crops especially cereals in Africa particularly in Nigeria. Unfortunately, daily consumption of these contaminated cereals and their products as either breakfast food or weaning food by infants and children still remains a reality in Nigeria as well as other African countries. Therefore, the present study warrants that urgent step be taken to raise more awareness on the incidences of mycotoxins contamination of our food crops. Furthermore, there is need for regular examination of cereals and their products for aflatoxins contamination.

It is therefore imperative that aflatoxins surveillance research findings such as the one presented in this study should form the basis for taking different prevention approach especially in the formulation/vigorous implementation of already existing national food policy that will help to reduce, if not eradicate, aflatoxins contamination of Nigeria agricultural produce. Critical among the strategies to be adopted should be a public campaign on the impact of aflatoxins on our health and the best control strategies to be adopted by all relevant stakeholders in the Nigerian agricultural sector.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Achaglinkame MA, Opoku N, Amagloh FK (2017). Aflatoxin contamination in cereals and legumes to reconsider usage as complementary food ingredients for Ghanaian infants: a review. *Journal of Nutrition & Intermediary Metabolism* 10:1-7
- Atanda SA, Pessu PO, Agoda S, Isong IU, Adekalu OA, Echendu MA (2011). Fungi and mycotoxins in stored foods. *African Journal Microbiological Resource* 5(25):4373-4382.
- Azi F, Odo MO, Okorie PA, Njoku HA, Nwobasi VN, Nwankwegu AS (2017). Fungi and aflatoxin analysis of processed ogiri-egusi and ogiri-ugba consumed in Abakaliki metropolis. *African Journal of Biotechnology* 16(42):2024-2030.
- Beuchat LR (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes Infection* 4(4):413-423
- Burger HM, Shephard GS, Louw W, Rheeder JP, Gelderblom WCA (2013). The mycotoxin distribution in maize milling fractions under experimental conditions. *International Journal Food Microbiology* 165(1):57-64.
- Campos WEO, Rosas LB, Neto AP, Mello RA, Vasconcelos AA (2017). Extended validation of a sensitive and robust method for simultaneous quantification of aflatoxins B1, B2, g1 and g2 in Brazil nuts by HPLC-FLD. *Journal of Food Composition and Analysis* 60:90-96.
- Enyisi SI, Orukotan AA, Adewumi AAJ (2015). Total aflatoxin level and fungi contamination of maize and maize products. *African Journal of Food Science and Technology* 6(8):229-233.
- European Food Safety Authority (EFSA) (2007). Opinion of the scientific panel on contaminants in the food chain [CONTAM] related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. *EFSA Journal* 446:1-127. <https://www.efsa.europa.eu/en/efsajournal/pub/446>
- European Food Safety Authority (EFSA) (2011). Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. *EFSA Journal* 9(3):1970. DOI: 10.2903/j.efsa.2011.1970
- European Food Safety Authority (EFSA) (2013). Aflatoxins (sum of B1, B2, g1, g2) in cereals and cereal-derived food products. *EFSA Supporting Publications* 10(3) DOI: 10.2903/sp.efsa.2013.EN-406
- European Commission (2006a). Commission Regulation No. 1881/2006, setting maximum levels of certain contaminants in foodstuffs. *Official Journal of the European Union* L364/5.
- European Commission (2006b). Commission Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, L364/5-L364/24
- Gong Y, Egal S, Hounsa A, Turner P, Hall A, Cardwell K (2003). Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning. *International Journal of Epidemiology* 32(4):556-562.
- Gong Y, Hounsa A, Egal S, Turner PC, Sutcliffe AE, Hall AJ (2004). Post weaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. *Environmental Health Perspectives* 112(13):1334-1338.
- Gong YY, Watson S, Routledge MN (2016). Aflatoxin exposure and associated human health effects, a review of epidemiological studies. *Food Safety* 4(1):14-27.
- Lombard MJ (2014). Mycotoxin exposure and infant and young child growth in Africa: what do we know. *Annals of Nutrition Metabolism* 64:42-52
- Lutfullah G, Hussain A (2011). Studies on contamination level of aflatoxins in some dried fruits and nuts of Pakistan. *Food Control*, 22(3-4):426-429.
- Magoha H, Kimanya M, Meulenaer B, Roberfroid D, Lachat C, Kolsteren P (2016). Risk of dietary exposure to aflatoxins and fumonisins in infants less than 6 months of age in Rombo, Northern Tanzania. *Maternal Child Nutrition* 12:234-247
- Medina A, Rodriguez A, Magan N (2014). Effect of climate change on *Aspergillus flavus* and aflatoxin B1 production. *Frontiers in Microbiology* 5:348.
- Quist CF, Bounous DI, Kilburn JV, Nettles VF, Wyatt RD (2000). The Effect of dietary aflatoxin on wild turkey poults. *Journal of Wildlife Diseases* 36(3):436-444.
- Shephard GS (2008). Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminant* 25(2):146-151.
- Smith LE, Stasiewicz M, Hestrin R, Morales L, Mutiga S, Nelson RJ (2016). Examining environmental drivers of spatial variability in aflatoxin accumulation in Kenyan maize: potential utility in risk prediction models. *African Journal Food, Agriculture Nutrition and Development* 16(3):11086-111105.
- Sowley ENK (2016). Aflatoxins: a silent threat in developing countries. *African Journal Biotechnology* 15(35):1864-1870.
- Strosnider H, Azziz-Baumgartner E, Banziger M, Bhat RV, Breiman R, Brune MN, DeCock K, Dilley A, Groopman J, Hell K, Henry SH (2006). Workgroup Report: Public Health Strategies for Reducing Aflatoxin exposure in Developing Countries. *Environmental Health* 47:567-575.
- Wagacha JM, Muthomi JW (2008). Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal Food Microbiology* 124(1):1-12.
- Williams J, Philips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal J (2003). Human Aflatoxicosis in Developing Countries. A review of toxicology, exposure, potential health consequences and interventions. *American Journal of Clinical Nutrition* 80:1106-1122.