Fungi Associated with Stored Sorghum Grains and Occurrence of Aflatoxin Contamination in Southwest Nigeria

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Abstract

This study evaluated the incidence and aflatoxin-producing potential of *Aspergillus flavus* in sorghum kernels in Oyo state. Samples were randomly collected from wholesales traders' stores in eight major towns: Ibadan, Ogbomoso, Oyo, Saki, Eruwa, Iseyin, Igbo-Ora and Oke-Ogun. Five samples were obtained from each of the eight towns that were surveyed and a total of 40 samples were evaluated. Data were collected on incidences of seed-borne fungi, *Aspergillus* species and level of aflatoxin contamination. Proximate analysis of sorghum kernels artificially infected with six seed-borne fungal isolates: *Aspergillus niger*, *A. flavus*, *Fusarium* sp., *Colletotrichum* sp., *Penicillium* spp. and *Rhizopus* sp. was carried out to determine their effect on nutrient composition. Data were analyzed using analysis of variance (ANOVA) and mean separation was done using LSD at $P<0.05$. *Aspergillus* had the highest incidence which varied from 32.3-67.8% among the fungal genera that were associated with the sorghum kernels. Among the *Aspergillus* species, *A. flavus* was the most predominant with an incidence of 31.1-62.8%. The atoxigenic strains of *A. flavus* were more prevalent than the toxigenic strains in all the eight surveyed locations with incidences of 54.4-81.2%. The highest level of the carcinogenic B_1-aflatoxins was detected in Eruwa and Oke-Ogun with concentrations of 48.1 and 43.6 µg/kg respectively. However, Iseyin location had the highest Aflatoxins G_1 and G_2 contaminations with concentrations of 14.3 and 12.8 µg/kg respectively. All the seed-borne fungi significantly ($p<0.05$) reduced the protein, carbohydrate and fat content of infected sorghum kernels. This study has shown that *A. flavus* strains with the ability to produce aflatoxins are present in sorghum kernels across major towns of Oyo state posing a health risk.

Key words: Aflatoxins, *Aspergillus flavus,* Atoxigenic strains,Carcinogenic, Proximate analysis.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moech) is the fifth most important world cereal and an important native cereal in Africa. It is the second most important staple cereal in Nigeria with an annual production of 8 million tons from 6.1 million hectares and is used for food, fodder and the production of alcoholic beverages (Danazumi *et al.,* 2015). Sorghum is a principal source of energy, and significant quantity of protein, vitamins and minerals to the poorest people of the semi-arid tropics. The grains are an important source of carbohydrate in meals while the seeds serve as input for arable cultivation that determines the potential production and ultimate quality and quantity of yield. It is commonly processed into paste and used in making pap for children in southwestern Nigeria.

Sorghum contamination by fungi not only renders the grains unfit for human consumption by discoloration and reduction of nutritional value, but can lead to production of harmful mycotoxin (Sreenivasa *et al.,* 2010; Sule, 2014).

Aflatoxins are naturally occurring toxins produced by certain fungi: mainly *Aspergillus flavus* and *Aspergillus parasiticus*. Several types of aflatoxins (B_1, B_2, G_1, and G_2) are produced by these fungi (Heather *et al.,* 2006). The B_1 form is recognized by the International Agency for Research on Cancer as one of the most naturally occurring toxic and carcinogenic substances found in nature (IARC, 2002). All *A. flavus* isolates have been reported to produce only B-aflatoxins due to a deletion in the aflatoxin biosynthesis gene required for G-aflatoxin production (Ehrlich *et al.,* 2004). Aflatoxin-producing fungi can contaminate several dozen food commodities, including many of Africa's important staple crops: maize, sorghum, millet, rice, oil seeds, spices, groundnuts, tree nuts, and cassava (Bandyopadhyay *et al.,* 2007)

Countries that are located between 40ºN and 40ºS latitude, such as Nigeria, offer suitable growing conditions for the fungi (Bankole and Adebanjo, 2003; Bankole and Mabekoje, 2003). *Aspergillus* spp. originates in the soil, therefore, the biochemical risk of aflatoxin contamination begins with planting, and can be worsened later through inappropriate harvesting, handling, storage, processing, and transport practices. Contamination during crop development and after harvest depends on environmental conditions that are optimal for the growth of fungi. Damage by pests (birds, mammals, and insects) or the stress of hot, dry conditions can contribute to significant *Aspergillus* infection. Drought stressors such as elevated temperature and low relative humidity increase the number of *Aspergillus* spores in the air, increasing the chance of contamination. Heavy rain can cause spores to splash onto fruit and grain. After harvest, high crop moisture coupled with warm temperatures, inadequate drying and poor storage can further increase the risk of contamination (AAI, 2012).

Toxigenic fungi can attack sorghum prior to harvest and cause further decay during storage. As a result mycotoxins may form during crop development and storage. The species of *Aspergillus*, *Fusarium*, *Penicilium* and *Cladosporium* are the predominant fungal genera associated with grains in storage (CAST, 2003; Atehnkeng *et al.,* 2008). Due to the carcinogenic and immune-suppressing effects on both man and animals and the huge yield losses and export reduction, aflatoxins are probably the most important mycotoxins worldwide (Turner *et al.,* 2003; Wu, 2006). Thus, aflatoxin contamination can adversely affect both individual livelihoods and agricultural sector output. Despite aflatoxin standards, unpackaged foods bound for domestic consumption are not strictly regulated in Nigeria. This means that aflatoxin-contaminated grain can easily enter the consumption stream (AAI, 2012).

Although cereal crops are most vulnerable to *Aspergillus* infection and subsequent contamination with aflatoxins, greater emphasis has been placed on aflatoxin contamination in maize and groundnuts to the detriment of sorghum in Nigeria. Mould associated with sorghum have been reported (Abdulsalaam and Shenge, 2011; Danazumi *et al.,* 2015), but there is limited information on the occurrence of aflatoxin contamination of the produce. A knowledge of the aflatoxin producing ability of these fungi will be invaluable in the formulation of suitable management strategies of pre and postharvest aflaoxin contamination. Therefore, this study sought to evaluate seed-borne fungi and the distribution of aflatoxin-producing *Aspergillusflavus* in sorghum grains used for consumption in Oyo state.

Materials and Methods

Sampling sites

Sorghum grains were collected from wholesale traders' stores in eight major towns in Oyo state: Ogbomoso, Oyo, Saki, Ibadan, Eruwa, Iseyin, Igbo-Ora and Oke-Ogun. Samples were collected according to the methods described by Hurburgh (2005) and Makun *et al.* (2010) at two to three months of storage in order to provide sufficient time after harvest for fungal colonization and possible aflatoxin accumulation in infected grains (Atehnkeng *et al.,* 2008). Five samples were randomly collected from each of the eight towns that were surveyed, thus a total of 40 samples were evaluated. Sorghum grains weighing 200 g were purchased from each of five traders' store; thus 1 kg of sample was collected from each town. The samples from each town were thoroughly mixed to form a composite sample per location. One hundred and twenty kernels were removed from each composite sample for isolation of fungi while the remaining seed lot was stored in clearly labelled envelops at 100 g per sample for aflatoxin analysis. All the samples were stored in a refrigerator at 4°C preparatory to analysis in order to inhibit fungal growth.

Fungal isolation and identification

The 120 sorghum kernels earlier separated from the composite sample were surface-sterilized using 10% sodium hypochlorite for one minute and rinsed with three changes of sterile distilled water. Potato dextrose agar (PDA) was prepared following standard procedure with the addition of 1 ml lactic acid to 1 litre of PDA in order to inhibit bacterial contaminants. Twelve small kernels were uniformly spread in each Petri dish containing PDA at equidistant positions with a total of ten plates per composite sample and incubated at 28±2ºC for three days. Purification of isolates was done through subculturing of individual fungus in order to obtain pure cultures before the identification process. A selective medium consisting of 5% V8 medium, 2% agar and adjusted to pH 5.2 was prepared to enhance distinctive sporulation for the identification of *Aspergillus* species (Cotty, 1994). While malachite green agar 2.5 medium was used for *Fusarium* spp. (Bragulat *et al.,*2004). Colony morphology, mycelium structure and fruiting body production were carefully observed under a compound microscope following standard procedures (Singh *et al.,* 1991: Barnett and Hunter, 1998: Samson *et al.,* 2010). The incidence of fungal isolates was calculated as:

$$\frac{\text{Number of times each fungus was isolated}}{\text{Total number of fungal isolates}} \times 100$$

Aflatoxin extraction and analysis

Samples of sorghum kernels were ground using high speed blender (Warring Commercial, Springfield, M.O. USA) and aflatoxin extraction process was carried out following modified standard procedures (Hell *et al.,* 2000; Giorni *et al.,* 2007; Odoemelam and Osu, 2009). Twenty five grammes of the ground sample was dispensed into a 500 mL Erlenmeyer flask containing 100 mL of *n*-hexane-petroleum ether (1:10) and maintained at a temperature of 28-30ºC for 24 h then filtered using cheese cloth. An aliquot of 100 ml of 50% acetone was thereafter added to the filterate and shaken for 1 h using an orbital shaker at 250 rpm before a second filtration. Aflatoxins were extracted into 20 ml chloroform in 250 ml round bottomed flasks, filtered through dehydrated sodium sulphate and evaporated to dryness using a rotary evaporator. The extracted aflatoxins were separated by thin-layer chromatography (TLC) plates coated

with silica gel (60,250 µm) and developed using 3% methanolic chloroform solution. Aflatoxin extract was spotted on a silica-gel pre-coated plates and developed unidimensionally in a TLC tank in a chloroform/acetone (96:4v/v) solvent system. The plates were visualized using ultraviolet light and scored for presence or absence of aflatoxin with a 2ng limit of detection. Aflatoxins spots were observed under long-wave ultraviolet light (λ=363 nm, γ=22,000 nm) and identified by comparison to the fluorescence standard for each aflatoxin type. Samples that were positive for presence of aflatoxins were classified as toxigenic while negative samples were atoxigenic. Aflatoxins were quantified using the method of Opadokun (1979):

$A = \gamma c$

Where A = Absorbance

γ = Molar extinction coefficient

C = Concentration

Determination of proximate composition of infected sorghum kernels

Clean viable sorghum kernels were inoculated with six predominant fungal species that were previously isolated in the experiment: *Aspergillus flavus, A. niger, Fusarium solani*, *Colletotrichum* sp., *Penicillium* sp. and *Rhizpopus* sp. The samples were incubated at 28±2°C for 14 days. Non inoculated samples served as control. Crude fibre, ash and moisture content of infected and healthy sorghum grains were determined using standard chemical methods described by AOAC (2005). Moisture content was determined by drying 5 g of sample at 75°C for 24 h. Ash content was determined using 2 g of each sample; the samples were incinerated in muffle furnance at 500°C for 2 h. Petroleum ether at 45°C was used to determine fat content of sorghum samples by soxhlet extraction method (Pearson *et al.,* 1981), while Kjeldahl method was used in determining crude protein content (AOAC, 2005). Carbohydrate content was determined by differential method (Sule, 2014): where % carbohydrate = 100% - (% moisture + % fat + % protein + % fibre).Calcium, magnesium and iron mineral content were determined using flame absorption spectrophotometer using the method described by Khan and Zeb (2007).

Data analysis

Data obtained were analyzed by one way analysis of variance and means were compared according to Least Significant Difference (LSD) test (SPSS 11.5 version). Differences were considered significant at $P<0.05$.

Results

Six fungal genera, *Aspergillus, Fusarium, Colletotrichum, Penicillium, Macrophomina* and *Rhizopus* were associated with samples of sorghum grains that were collected during the field survey. *Aspergillus* species were the most predominant fungi with the highest incidence across all the locations. This was followed by *Rhizopus, Colletotrichum* and *Fusarium*, while *Macrophomina* and *Penicillium* were the least prevalent (Figure 1). The incidence of *Aspergillus* was significantly ($p<0.05$) higher than other fungal genera at various locations except at Eruwa and Oyo locations. *Aspergillus flavus* was most prevalent within *Aspergillus* section *Flavi* in six of the eight surveyed locations and had the highest incidence of 64.2% at Iseyin location (Figure 2). The incidence of atoxigenic strains of *Aspergillus flavus* varied across all the surveyed locations and was significantly ($p<0.05$) higher than the toxigenic strains (Figure 3).

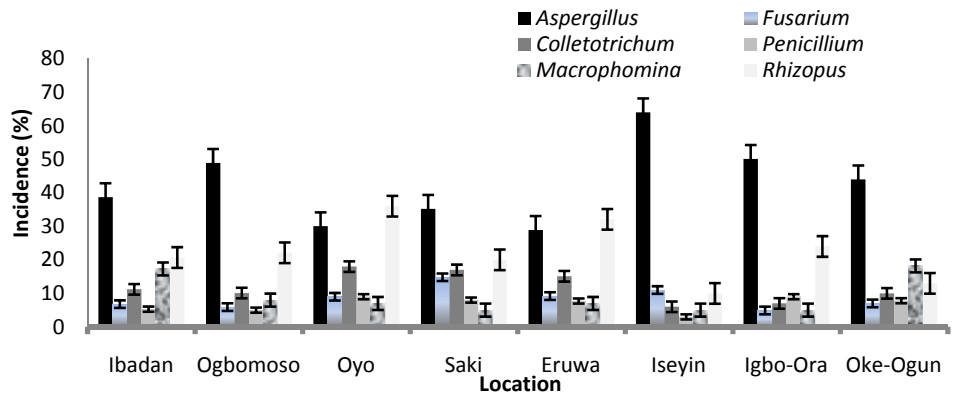

Figure 1: Incidence of fungi isolated from sorghum kernel samples collected from eight locations in Oyo state

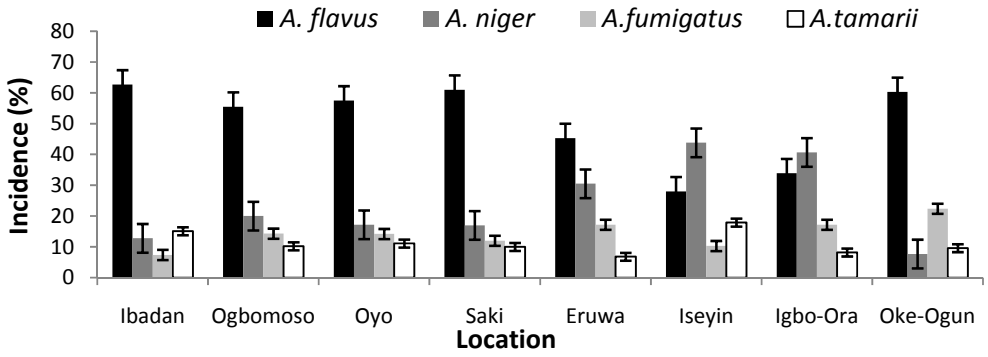

Figure 2: Incidence of *Aspergillus* species isolated from sorghum kernel samples collected from eight locations in Oyo state

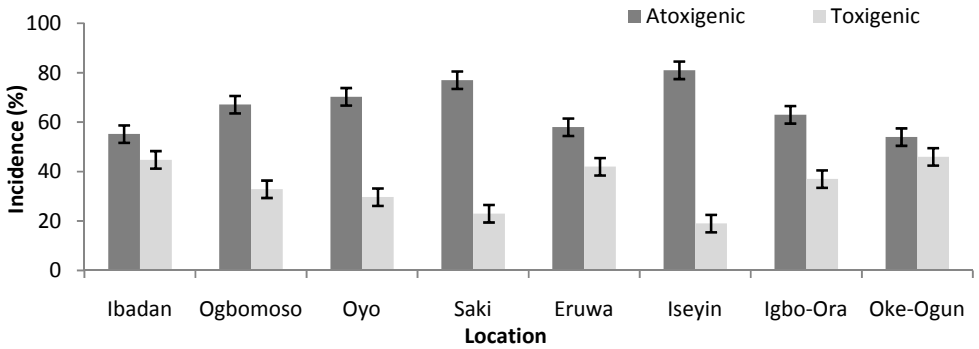

Figure 3: Incidence of toxigenic and atoxigenic *Aspergillus flavus* in sorghum kernels collected from different locations in Oyo state

The highest incidence of atoxigenic strains (78.1%) of *A. flavus* was recorded at Iseyin location, while Ibadan and Oke-Ogun had the least incidence of 57.6%. Comparatively, atoxigenic strains had higher levels of prevalence than the toxigenic strains in all the surveyed locations. There was significant difference ($p<0.05$) in the incidence of toxigenic strains across the various locations. The level of aflatoxin contamination recorded in the sorghum kernels that were collected during the survey showed the presence of four types of aflatoxins B_1, B_2, G_1 and G_2 with significant variation along the locations. Aflatoxin B_1 had the highest concentration of 48.2 µg/kg in sorghum samples collected from Eruwa (Table 1).This was followed by samples obtained from Oke-Ogun with a concentration of 43.6 µg/kg. The lowest concentration of 21.0 µg/kg was detected in samples collected from Ibadan.

Table 1: Levels of aflatoxin contamination(µg/kg) in sorghum kernel samples collected from various locations in Oyo state

	Afllatoxin concentration (µg/kg)							
Location	B_1	Range	B_2	Range	G_1	Range	G_2	Range
Ibadan	20.0^{cd}	0-77.1	12.82^{bc}	0-44.2	8.8^{b}	0-11.1	1.7^{bc}	0-10.5
Ogbomoso	16.77^{c}	0-100	18.17^{b}	0-58.3	ND	ND	ND	ND
Oyo	39.12^{b}	11.3-98	22.16^{ab}	0-35.5	3.1^{c}	0.7.2	10.4^{ab}	2.1-44.4
Saki	15.15^{d}	0-51.3	10.09^{c}	0.1-22.3	ND	ND	ND	ND
Eruwa	48.15^{a}	0-108.3	31.11^{a}	0-77.4	ND	ND	ND	ND
Iseyin	17.66^{d}	0-67.2	20.17^{b}	0.4-18.6	14.3^{a}	2.6-30.5	12.8^{a}	2.8-25.4
Igbo-Ora	31.51^{bc}	0.3-101	22.99^{ab}	0-29.3	ND		ND	ND
Oke-Ogun	43.64^{ab}	0.2-9.4	29.88^{a}	0-51.3	7.1^{ab}	1.2-28.6	5.3^{b}	0-9.7
WHO	20.0	-	20.0	-	20.0	-	20.0	-

Means followed by the same superscripts letter along a column are not significantly different using Least Significant Difference ($P<0.05$). ND = Not determined. WHO = World Health Organization standard (20µg/kg body weight per day)

AflatoxinB_2 contamination was highest in sorghum kernels collected from Eruwa (31.1 µg/kg), which was followed by samples at Oke-Ogun location with a concentration of 29.9 µg/kg. The least concentration of 10.2 µg/kg of aflatoxin B_2 was detected in samples obtained from Saki. Samples collected from Iseyin had the highest concentration of aflatoxin G_1 and G_2 with values of 14.3 and 12.8 µg/kg respectively. There was significant difference ($P<0.05$) in mean aflatoxin concentration across the various surveyed locations. However, aflatoxins G_1 and G_2 were not detected in sorghum kernels that were obtained from Ogbomoso and Igbo-ora locations. Samples that were collected from four of the locations: Oyo, Eruwa, Igbo-Ora and Oke-Ogun all had aflatoxin B_1 and B_2 contamination levels that were

above World Health Organization (WHO) limit of 20 µg/kg.

Sorghum kernels that were infected with *Colletotrichum* species had the highest moisture content of 16.0% relative to healthy uninfected kernels that served as control. This was followed by samples that were inoculated with *Aspergillus flavus* and *Rhizopus* species with 14.4 and 13.3% respectively (Table 2). Similarly, samples infected with *Colletotrichum* had the highest amount of protein (13.3%), while *Rhizopus* infected kernels had the lowest amount of protein (5.2%). However, *Penicillium*-infected samples had the highest amount of carbohydrate (52.1%) relative to control. There was significant ($P<0.05$) increase in the amount of moisture in sorghum kernels inoculated with the test pathogens. Kernels inoculated with *Fusarium* species had the highest calcium content (30.8%), while those inoculated with *Aspergillus* species had the highest amount of magnesium (32.8%). The highest amount of iron (30.1%) was recorded in sorghum kernels inoculated with *A. niger*.

Table 2: Effect of Fungal isolates on nutrient composition of infected sorghum kernels

Fungus	Nutrient composition (%)								
	Moisture	Protein	Crude fibre	Carbohydrate	Ash	Fat	Ca	Mg	Fe
Aspergillus flavus	14.1^{ab}	7.4^{b}	7.8^{a}	44.2^{bc}	5.4^{a}	4.7^{bc}	16.1^{c}	32.8^{a}	21.24^{b}
Aspergilus niger	12.8^{b}	5.1^{bc}	5.5^{ab}	35.7^{cd}	3.1^{ab}	5.1^{b}	28.4^{ab}	22.9^{bc}	30.1^{a}
Fusarium solani	11.3^{b}	7.9^{b}	8.1^{a}	41.4^{c}	5.8^{a}	6.1^{b}	20.8^{bc}	27.5^{ab}	27.2^{ab}
Colletotrichum sp.	16.0^{a}	9.3^{ab}	2.7^{bc}	48.7^{b}	3.5^{ab}	8.8^{ab}	24.4^{b}	24.3^{b}	15.08^{c}
Penicillium spp.	11.8^{b}	10.7^{ab}	7.2^{a}	52.1^{ab}	2.8^{ab}	8.4^{ab}	15.9^{c}	20.3^{c}	18.7^{bc}
Rhizopus spp.	12.3^{b}	9.7^{ab}	2.4^{bc}	39.2^{d}	6.8^{a}	3.7^{bc}	30.8^{a}	22.8^{ba}	13.3^{cd}
Control	9.7^{bc}	13.1^{a}	4.2^{b}	55.5^{a}	5.1^{a}	12.1^{a}	21.1^{bc}	32.1^{a}	25.1^{b}

Means followed by the same superscripts letter along a column are not significantly different using Least Significant Difference ($P<0.05$).

Discussion

Fungal infection and biodeterioration of sorghum kernels is frequently enhanced by poor condition of the produce at harvest, improper handling, unhygienic storage methods and favourable environmental conditions such as high moisture content and temperature. This often leads to significant quality and market value reduction with a concomitant decrease in famers' income. However, produce contamination becomes a very serious problem when mycotoxin-producing fungi such as *Aspergillus* section *Flavi* that are characterized by the production of aflatoxins is involved. *Aspergillus flavus* was the most prevalent among the six fungal genera associated with sorghum kernels across the various surveyed locations in this study. This agrees with the reports of previous authors who had implicated the pathogen in postharvest spoilage of stored produce, especially cereals (Probst *et al.,* 2007; Giorni *et al.,* 2007; Atehnkeng *et al.,* 2008). The high incidence of *A. flavus* relative to other fungal species could probably be attributed to its bioecology as a soil-borne pathogen. This implies that the soil and plant debris act as suitable source of inoculum for infection of sorghum seeds planted in the

field which is eventually transferred from harvested grains in the field to storage.

The presence of *Aspergillus flavus* in sorghum products does not necessarily imply an obvious occurrence of aflatoxin in the products. Factors that favour mould growth may also enhance aflatoxin production but mould growth may occur with little or no mycotoxin production (Sheriff, 2004). Although other species of *Aspergillus* such as *A. niger, A. tamarii* and *A. fimigatus* were also isolated from sorghum kernels in this study, they have not been implicated in the production of aflatoxins, which are secondary metabolites mainly attributed to *A. flavus* and *A. parasiticus* (Frisvad *et al.,* 2007). However, *A. tamarii* has the ability to produce other secondary metabolites such as cyclopiazonic acid which is an indole tetramic acid mycotoxin and some strains of*A. niger*have been reported to produce ochratoxin, a potent mycotoxin (Groopman *et al.,* 2008). In addition to the production of these toxins, *Aspergillus* species can cause allergic reaction and Aspergillosis in humans and animals (Stack and Carlson, 2006*).*

Atoxigenic strains of *A. flavus* were most predominant and had significantly higher incidence than the toxigenic strains across all the surveyed locations. Vermonde *et al.* (2003) had reported significant variability in aflatoxin production by *Aspergillus* section *Flavi* from different substrates. Bioaccumulation of toxigenic strains may be influenced by time of harvest, storage conditions and modern agricultural practices that create unique ecological niches which select toxigenic fungi and the extent of these selective forces affect the relative proportion of toxigenic and atoxigenic strains in a given area (Atehnkeng *et al.,* 2008). The levels of aflatoxin B_1 and B_2 obtained from sorghum kernels did not vary significantly probably because the samples were collected from the same Derived savanna agroecology with similar environmental factors.

Environmental factors such as temperature, relative humidity and amount of rainfall influence the production of aflatoxin in the field and during storage. Bioaccumulation of aflatoxin in maize has been reported to be highly dependent on the suitability of these factors (Odoemelam and Osu, 2009). *Aspergillus* species are generally xerophilic being tolerant to high temperatures varying between 30-40ºC and are capable of producing mycotoxins under these unfavourable conditions (Vermonde *et al.,* 2003). Aflatoxin contamination of food and feed are highly dependent on environmental conditions that lead to mould growth and toxin production. If commodities are stored in an improper dried state or under high humidity with inadequate protection; fungi will inevitably grow and possibly produce toxins. Duration of storage is an important factor when considering aflatoxin formation. Longer retention in storage, results in build-up of environmental condition conducive for *Aspergillus flavus* proliferation and production of aflatoxin (Kumar *et al*., 2008). All these conditions could probably have influenced variability in the occurrence of aflatoxin in sorghum kernels across the surveyed locations.

Aflatoxin B_1 and B_2 concentrations in the sorghum samples that were collected from four of the surveyed locations: Oyo, Eruwa, Igbo-ora and Oke-Ogun exceeded the recommended FDA limit of 20 $\mu g/kg^{-1}$. This result agrees with previous findings (Odoelami and Osu 2009) that reported high levels of aflatoxin contamination in sorghum purchased from some open markets in Nigeria. However, considering the FDA tolerance level for total

aflatoxin in food for human consumption, the high level of aflatoxin reported in this study signals a note of caution against prolonged consumption of the commodity in view of the health hazard associated with aflatoxins. All *A. flavus* isolates produce only B-aflatoxins due to deletion in the aflatoxin biosynthesis gene *cypA* required for G-aflatoxin production (Ehrlich *et al.,* 2004).An unnamed taxon, called S_{BG}, which is phylogenetically divergent from but morphologically similar to the S-type *A. flavus* produces small sclerotia and large amounts of both the B and G aflatoxins (Ehrlich *et al.,* 2003). Aflatoxins G_1 and G_2 were detected in half of the surveyed locations. This corroborates earlier reports of Ehrlich *et al.* (2003, 2004) and Jaime-Garcia and Cotty (2006) that all *Aspergillus* isolates produce only B aflatoxins. Therefore, the detection of G-aflatoxins in four of the locations probably implies the presence of the unnamed taxon S_{BG} that is characterized by the production of this type of mycotoxins.

There was a significant decrease in the protein, carbohydrate and fat content of sorghum kernels that were inoculated with the seed-borne pathogens relative to the control, suggesting that the presence of these food substances is of primary importance for the survival and successful establishment of these pathogens within the sorghum kernels. These findings agree with Embaby *et al.* (2006) that reported a reduction in carbohydrate, reducing sugar and crude fat due to *Fusarium oxysporum* in legume seeds. Similarly, Kakde and Chavan (2011) submitted that *Aspergillusflavus* and *Fusariumequisetti* were responsible for maximum depletion of fat content and reducing sugar in soybean and groundnut. Decrease in carbohydrate content may be due to the hydrolytic breakdown into simple sugars or due to exudation by loss of cell membrane integrity. Moisture content increased significantly in sorghum kernels that were inoculated with the seed-borne pathogens and was higher than values contained in the healthy kernels that served as control. The higher moisture content in the infected kernels could be attributed to metabolic activities of the pathogen such as respiration and enzymatic activities. Panchal *et al.* (2009) and Nweke (2015) had reported increase in moisture content of sweet potato and yam tubers infected by rot-causing fungi respectively. Net changes in nutritional value and the risk of mycotoxin contamination are difficult to predict because they depend on a complex interaction of factors such as temperature, moisture, storage time, fungal species composition, nature of enzymatic activity kind of grain, and previous storage history (Vikas and Mishra, 2010). Also, disparity in research reports in this regard may be due to differences in geographical origin, seasonal variation of the samples tested and weather conditions.

This study showed the presence of aflatoxin B_1 and B_2 contaminations in all the analyzed samples with some concentration levels above the permissible WHO limit of 20 µg/kg. This implies that prolonged consumption of large quantities of the sorghum grains investigated may lead to aflatoxin related intoxications when used in feed formulation for livestock and as food by humans. Therefore, there is need to prevent aflatoxin contamination in the grains by use of good agricultural practices for planting, pre harvest, and post-harvest handling and adequate drying of grains to reduce aflatoxin contamination to the barest minimum. The relevant national agencies should enforce commodity standards and

conduct laboratory tests to detect aflatoxins in Nigeria. However, this is only done for packaged foods and produce bound for the formal export market. The vast majority of foods consumed by the Nigerian population are not regulated for aflatoxins. Consequently, aflatoxin-contaminated grain can enter the domestic markets and the informal international markets due to low awareness about aflatoxins and their health impact among consumers and sellers. There is, therefore, the urgent need for improvement in the awareness of the presence and risks of aflatoxins in the food and feed system by all stakeholders and create market-based incentives for safer food.

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