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Level of Mycotoxins Consumption and Burden of Aflatoxin-Induced Hepatocellular Carcinoma in People Subsisting on Sorghum Based Products in Nigeria: A Risk Assessment

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ABSTRACT

Background: The level of risk associated with the preponderance of hepatocellular carcinoma due to consumption of Sorghum based product is evaluated in this study. Sorghum is a local grain that is widely consumed in Northern Nigeria and other parts of the world.

Research Methods: A quantitative food frequency questionnaire (QFFQ) was used to sample sorghum based products while delineation method was employed in the sampling of the sorghum samples. High performance liquid chromatography (HPLC) was employed to determine mycotoxin concentration in both the raw and processed samples. The burden of aflatoxin induced Hepato cellular carcinoma (HCC) in communities that subsist on sorghum and sorghum-based products was determined from the amount of mycotoxins consumed by respondents from different age groups.

Research Result: With the exemption of zearalenone from the Southern guinea savannah (SGS) that was observed to increase by 272.3% in pap (kamu/ogi) sample and aflatoxin and ochratoxin in porridge sample from the Sudan savannah that was observed to increase by 70.0 and 66.7% respectively, there was a significant difference (P = 0.05) between the concentration of the mycotoxins in the raw and the processed sorghum samples in all the agro-ecological zones.

Conclusion: The processing methods employed does not appear to have effect in reducing the toxin level below the PTDI and TDI levels set by the regulatory agencies. Average daily consumption of sorghum based products based on age range was found to be increasing in ascending order for the infants, children, adults and elderly respectively. The incidence and burden of aflatoxin induced HCC in the HbeAg and the HbsAg populations alarmingly high and appeared to be dependent on increasing humidity. Despite the nutritional value of sorghum as a source of food and feed material, its contamination by fungi and fungal metabolites calls for urgent mitigation strategies to avoid health emergencies particularly in the poverty stricken countries of the sub Saharan Africa.

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1. INTRODUCTION

In the world agricultural economy, sorghum is the fifth largest most important cereal after wheat, maize, rice and barley, and ranked second after maize in sub-Saharan Africa. A Global area of 42.3 million hectares was cropped with sorghum in 2013 giving an output of 61.5 million metric tons. According to the USDA data, sorghum production was 63.08 million metric tonnes in 2016, while a total land of 5.35 million hectares was cropped in Nigeria with an output of 6.55m metric tons in the 2017/18 cropping season. Nigeria's sorghum consumption for the year 2017/18 stood at 6.45and 150,000 million metric tonnes for food and feed respectively (Yahaya, A. et al., 2022). Sorghum is a local grain that grows predominantly in the semi-arid, savannah and grassland regions of Northern Nigeria and other parts of the world (Ilaria et al., 2015).

The USA, Nigeria, Mexico, India and Ethiopia are the main producers. As of 2022, the production of sorghum in Nigeria was estimated at seven million metric tons. Between 2010 and 2022, sorghum production in the country generally increased. Sorghum represents one of the main crops of the country. Nigeria is among the main producers of sorghum worldwide (Yahaya, A. et al., 2022).

Unlike in the developed economies, sorghum together with millet, represents a main source of energy and protein for about one billion people in the semi-arid region of the tropics (Sub Saharan Africa) and it is part of the staple diet of more than 300 million people in developing countries (of which Nigeria forms an integral part), representing their major source of energy and nutrients (Ilaria et al., 2015). Sorghum is a basic staple food for many rural communities, in Africa, and Nigeria in particular especially in drought prone areas, which makes it a subsistence food crop for many food insecure people (De Cardoso et al., 2014).

Sorghum is said to be nutritionally rich and serves as a staple food in most parts of Northern Nigeria. The grain has assumed commercial relevance in recent years, especially in the food and beverage sector. It is said to be a valuable ingredient next to malted barley used in the brewery industry. A wide variety of traditional food products and recipes are based on sorghum. The cereal is boiled like rice, brewed for beer production, baked into flatbreads or cracked for porridge preparation. Besides providing calories, sorghum has actual nutritional value in principle, because of its content of protein, vitamins, both fat-soluble (D, E and K) and of B group (except for B12), as well as minerals, such as iron, phosphorus and zinc.

Sorghum has many and varied outlets. Sorghum fodder is used for silage production (dairy farms, beef cattle, etc.) and bioenergies such as biogas for methanisation and electricity production. One hectare of biomass sorghum can produce around 7,000m3 of biogas, and 1 tonne of grain sorghum can produce 290 to 410 litres of ethanol, so sorghum has a place in many of today's energy projects to reduce our dependence on oil. Fibre-rich biomass sorghum is also used in the manufacture of biomaterials.

Sorghum is highly adaptable to climate change, with low input and water requirements (over 80% of agricultural land has no irrigation). It therefore tolerates drought and high temperatures well in medium to deep soil without irrigation.

Fungi are ubiquitous plant pathogens that are major spoilage agents of foods and feedstuffs (Pawlowska et al., 2012). They are saprophytes that acquire their food by absorbing dissolved molecules, typically by extracellular digestion of the organic molecules around them (Blackwell, 2011). The infection of plants by various fungi not only results in reduction in crop yield and quality with significant economic losses, but also contamination of grains with poisonous fungal secondary metabolites called mycotoxins (Reyes-García, 2010).

The proven presence of toxigenic fungi and toxic and carcinogenic mycotoxins in sorghum a highly consumed grain makes it a major source of mycotoxins exposure to animals and human beings and therefore of great public health concern. With regards to economic impact of mycotoxins on human health, the loss of 40% labour productivity in Africa due to diseases and deaths exacerbated by Aflatoxins (Felicia et al., 2011; Miller, 1995) is quite worrisome. Despite its anticipated high contribution to mycotoxin exposure particularly in Asia, sub Saharan Africa and Nigeria in particular, there are no known methods of control of fungi and mycotoxins specific for sorghum. Meanwhile there are physical, chemical and biological intervention strategies against mycotoxins in wheat, groundnut, rice, maize etc. (Anthony, 2016).

Studies so far carried out ranked aflatoxins as the most potent carcinogens in animal and human populations (Thomas et al., 2011) and were confirmed to be potent immune-suppressors (Björn and Ijaz, 2014). In 1993, the International Agency for Research on Cancer (IARC) assessed and classified naturally occurring mixtures of aflatoxins as a class1 human carcinogen (IARC, 2016). Deoxynivalenol (DON) is one of the most common mycotoxins found in grains. It is known to cause nausea, vomiting and diarrhoea when ingested in high doses by agricultural animals, while at lower doses, pigs and other farm animals exhibit weight loss and food refusal. For this reason, deoxynivalenol is sometimes called vomitoxin or food refusal factor (Matejova et al., 2015). Ochratoxin A is a mycotoxin that has been known to play a major role in the aetiology of endemic Balkan nephropathy (BEN) that often is accompanied by upper urinary tract urothelial cancer (Lalini and Kanti, 2010), and is recognized as a potent nephrotoxin. Additionally, animal studies have also indicated that, ochratoxinA has effect on hepatocytes, an immune suppressant, a potent teratogen, and a carcinogen (Li et al., 2014). Fumonisins on the other hand were found to affect animals in different ways by interfering with sphingolipid metabolism. They are the main cause of equine leukoencephalomalacia (ELEM) (hole in the head syndrome), a fatal disease associated with equines (Gunther et al., 2014), they were also observed by Gelderblom et al. (2002) to have hepatotoxic and carcinogenic effects in experimental rats. The occurrence of fumonisin B1 has been correlated with the occurrence of a higher incidence of oesophageal cancer in regions of Transkei (South Africa), China and northeast Italy (Candida et al., 2013). The IARC has evaluated the cancer risk of fumonisins to humans and classified them as group 2B (probably carcinogenic) (Muhammed, 2011). Zearalenone has been implicated as an important factor in the etiology of # numerous mycotoxicoses in farm animals, causing infertility, abortion or other breeding problems especially in pigs. It has been observed by Kowalska et al. (2016) that, zearalenone and its metabolite in blood, zearalanol bind to human sex hormone binding globulin to some extent is associated with outbreaks of precocious pubertal changes in children in Puerto Rico, and has been suggested to have a possible involvement in human cervical cancer (Wild and Gong 2010).

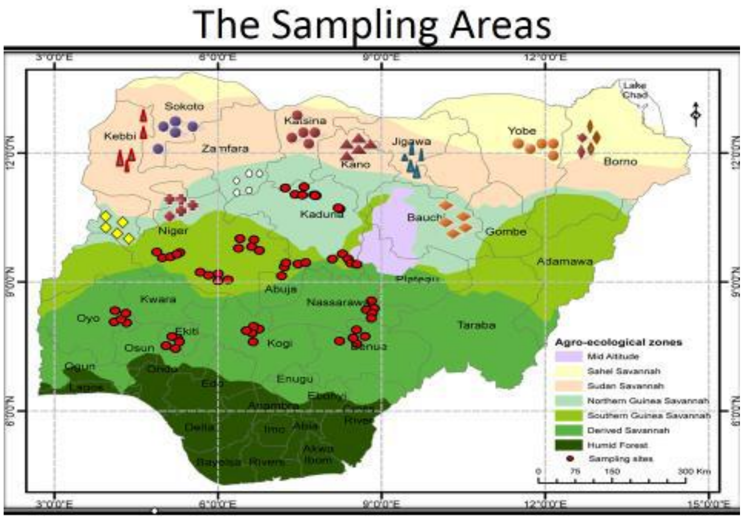
This research work therefore set to establish the effect of processing and the daily dietary intake of the five major mycotoxins from sorghum food products in Nigeria and at the same time determine the risk burden of HCC associated with such consumption.

2. METHODS

2.1. Sampling of *Sorghum bicolor* (SB) from The Six (6) Agro-Ecological Zones

Five districts where sorghum is produced were selected for the study, that is: Derived Savannah(DS) (Ado-Ekiti, Lafia, Lokoja, Makurdi, and Ogbomosho), the Southern Guinea Savannah (SGS) (Abuja, Akwanga, Bida, Minna, and Mokwa), the Northern Guinea Savannah (NGS) (Zaria, Kontagora, Kaduna, Bauchi and Rijau),the Sudan Savannah,(SS) (Sokoto, Daura, Kebbi, Dutse and Dawanau), and five districts (Goronyo, SabonBirni,Baure, Kirikasamma and

Guri/Nguru) from the Sahel savannah SHS while Riyom, Toro, Langtang and Wase constitute districts from the Mid- Altitude. (Atenkengh et al., (2008). In each district, Sorghum grains in stores and bunches in the field and in the markets were sampled from five locations, each approximately 20 km from the previous sampling location. At each location, a single farmer who grew sorghum in the previous season was identified and 1kg of sorghum with or without visible signs of fungal growth was arbitrarily selected from the farmer's store. Only sorghum that had been in storage for up to 2 months were sampled from each farmer during the survey. This duration is long enough for mycotoxin to accumulate in fungi infected sorghum grains (Olakojo and Akinlosotu 2004). All the samples were placed in bags, properly sealed and transported to our laboratory in Federal University of Technology, Minna. A total of 435samples was collected from the field, store, and market (each measuring 100g). A total of 100g was taken from each kg in each eco-niche of the locations and these 100g were composited and then sub slotted to the final 100g which was used for fungal isolation. To prevent further postharvest accumulation of moulds prior to analysis, all the samples were properly sealed and stored at 4^oC.



Figures 1. The Sampling Area

2.2. Sorghum - based products consumption survey

Briefly, purposive sampling was carried out in 8 communities from the six agro-ecological zones identified to subsist on sorghum almost on daily basis. In these communities, One hundred and sixty three (163) individuals of various age groups were also purposively targeted to assess the risk of burden of HCC associated with consumption of sorghum in the study area . Their body weights, age and the weight of the sorghum products consumed per day were recorded. Individuals were categorised as infants, children, adults and elderly based on the following age range thus: 0 -3, 4 – 17, 18 – 49 and 50 and above years. The main food items consumed which include Gruel (tuwo), Pap/kunu/kamu/ogi, Porridge (Fura), Guinea corn cake (Masa/waina), Chincoins (Dambu) and the local alcoholic beverage, burkutu/fito were the sorghum derived product purposively targeted.

2.3. Extraction and clean-up procedures

A multi-mycotoxin extraction method (multi-mycotoxin screen) devised by Patterson and Roberts (1979) as modified by Makun et al. (2011) with modifications was used for extraction of the selected mycotoxins (AFs, ZEA, OTA, DON). Twentyfive (25) grams of the pulverised sorghum based food product was placed in a volumetric flask followed by addition of o100 mL of Acetonitrile/KCl (9:1 v/m). The mixture was thoroughly shaken and filtered using No 1 filter paper. The filtrate was then placed in a separating funnel held on a tripod stand. Fat impurities were removed from the filtered sample by washing three times with 25 mL of 2,3,4, Trimethylpentane (Iso-octane). The lower layer (Pellet) was carefully separeted into another flask while the supernatant (Iso-octane+fat) was discarded. The defatted extract was returned back into the separating funnel followed by addition of 30 mL freshly prepared Sodium bicarbonate (NaHCO3) and 20 mL distilled water. After shaking the contents and later allowed to settles, the bottom layer (Neutral fraction) was passed through a bed of anhydrous Sodium Sulphate (NaSO4) overlaid on a filter paper into a receiving flask. This filtered portion was then placed into a rotary flask and dried using vacuum rotatory evaporator (Yamato Scientific RE-211, Analog220V. The dried extract was reconstituted using 1 mL Acetonitrile (ACN) and placed into a prepared 10 mm dialysis tube, which was knotted from the two ends and placed into a 50 mL boiling tube to which 50 mL of 30% acetone was added. The test tube was then sealed firmly with a parafilm to avoid leakage. The sealed boiling tube was placed into a conical flask then shaken overnight (Vortex-2 GENIE, Scientific industries). After shaking, the dialysis tube was removed from the test tube and discarded while the 30% acetone solution was again placed back into the separating funnel and held on a tripod stand and washed/back extracted three times with 25 mL dichloromethane (DCM) into a round bottom flask and dried using rotatory evaporator. This was reconstituted with 1 mL DCM and dried using nitrogen gas stream and stored at 4oC. To the remaining upper layer (from which the neutral fraction forms lower layer), 50 mL of 1 molar (M) sulphuric acid (H2SO4) was added to form acid fraction. This was moderately shaken with a slight opening of the valve of the separation funnel. The bubbling was allowed to subside and washed three times with 25 mL of DCM. The extract was collected into a round bottom flask and dried using rotatory evaporator. It was then reconstituted with 1 mL DCM and dried using nitrogen gas stream and stored at 4oC.

Because fumonisins (FB) are only soluble in lower alcohols especially methanol and water, a different extraction method to that for the other mycotoxins was employed. The extraction of FBs and clean-up was done according to the method of Sydenham et al. (1992) without modification. A Sub-sample (25 g) was mixed with 50 mL of methanol/water (3:1 v/v) in a volumetric flask and shaken for one hour and filtered through Whatman No1 filter paper. The pH of the extract was adjusted to 6-6.5 using acetic acid (to enable the binding of the fumonisin on the SAX column). The SAX cartridge was first conditioned by washing with 5 mL methanol (MeOH) followed by 5 mL MeOH/H2O (3:1, v/v). The flow rate was maintained at 2 mL/min as described by Sydenham et al. (1992). The column was then washed with 3 mL MeOH. The fumonisin (FB1) was finally eluted at the flow rate of 1 mL/min with 10 mL of 1% acetic acid in MeOH. The eluate was dried under the stream of nitrogen gas at 60oC and stored at 4 – 8OC until further analysis.

2.4. Mycotoxin Analysis

Quantification of aflatoxins, ochratoxin A, zearalenone and deoxynivalenol was performed using high performance liquid chromatography (HPLC, Agilent 1260 Infinity-HPLC-Series, Agilent, Santa Clara, CA, USA) as described by Makun et al. (2011) In briefAFs (AFB1, AFB2, AFG1 and AFG2) were individually determined using HPLC with fluorescence detection after post column electrochemical derivatization with bromine using KOBRA cell (Reif and Metzger, 1995). The eluent (Mobile phase) was water/methanol/Acetonitrile (60:20:20, v/v/v) with addition of 25 µL of Triflouroacetic acid (TFA) per litre at a flow-rate of 1.00 mL/min (isocratic). The AFs were detected using a scanning Photo diode array (PDA) detector

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(λex.= 365 nm, λem.=500 nm) while ZEA was analysed by fluorescent detector at excitation and emission wavelengths of 274 nm and 455 nm as described by Abdulkadir et al. (2004). The injection volume was set at 20 µl, while the mobile phase used was acetonitrile/water/Methanol, (46: 46: 8, v/ v/v) was pumped at the rate of 1 mL/min. OTA analysis was performed accordingly, by fluorescence detection. The mobile phase (acetonitrile/water/acetic acid, 50:48:2 v/v/v) was pumped at a rate of 0.8 mL/min. Respective fluorescence excitation and emission wavelengths of 333 nm and 443 nm was set and used. Residues for FB analysis were reconstituted in methanol and aliquots derivatized with o-phthaldialdehyde (OPA) prior to separation on a reversed-phase HPLC system using fluorescence detection at excitation and emission wavelengths of 335 and 440 nm respectively. The isocratic mobile phase made up of 0.1mol/L sodium dihydrogen phosphate/methanol (80:20, v/v) that had its pH adjusted to 3.5 using Acetic acid, and was pumped at a rate of 1 mL/min. DON was also analysed on a photodiode array detector (PDA) at 220 nm. The mobile phase was Water/Methanol (85:15, v/v) and at a flow rate of 0.4 mL/min with an injection volume of 20 µl. Mycotoxins were quantified using peak area and external calibration curves.

2.5. Validation of mycotoxins analytical methods

In order to be sure of the reliability of the results, the typical parameters for validation methods such as: specificity, accuracy, linearity and detection limits were used in addition to the ensuring that, validated methods were employed in the course of the determination process. Both internal and external quality control experiments were conducted. Visual determination was used to the asses the limit of detection. This was as thus: Known concentrations of mycotoxin standards were prepared and successively diluted and subjected to TLC and HPLC until the minimum concentration at which the analyte can be detected was established. This was taken as the limit of detection. Sorghum samples that were known not to contain mycotoxins or those with known concentrations were spiked with 100 µg/kg of AFs, OTA, ZEA, FM and DON for determination of recoveries. for DON and FUM, a spiking level of 500 µg/kg was used. Correlation coefficients of the calibration curves of the known concentrations of the standards, was used to check the linearity of the HPLC method. The RF values and retention time of the mycotoxin standards are indicative of the specificity of the methods. The low detection limit, recoveries of 71.2 – 96.4% for the various mycotoxins and the chromatographic separation indicated that the sensitivity and reliability of the methods employed was sufficient for the purpose of food analysis.

2.6. Determination of Dietary Intake of Mycotoxins in Sorghum

During this process, the levels (i.e mean values of aflatoxins in sorghum based products in Nigerian communities that subsist on this grains that was determined. The average amount of food(s) made from the grain consumed by the populace in different district of the sampling areas was the second information utilised. The concentration of toxins in the Sorhum- based product was taken into account and was calculated thus:

$$\left[100\% - \left[\frac{\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{UPS} - \overline{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}}{\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{UPS}}\right] x\ 100\right]$$

Where: $\overline{\mathrm{X}}_{\mathrm{m}}UPS$ = Mean of mycotoxin concentration in unprocessed sample
$\overline{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}$ = Mean of mycotoxin concentration in processed sample

Based on the values (data) for the mycotoxins concentrations obtained in figure 1, the average daily mycotoxin exposure per person from sorghum based products in 30 districts that forms the sampling sites and invariably Nigeria was estimated in accordance to the methods of

Kimanya *et al.* (2008) and Bandyopadhyay *et al.* (2007) using the modelled formula thus:

$$\frac{\sum \left[\frac{\bar{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}}{1000}\ X\ \bar{\mathrm{X}}_{\mathrm{m}}\right]^{T,F,K}}{\bar{\mathrm{X}}bw}$$

where: $\frac{\bar{\mathrm{X}}_{\mathrm{m}}\mathrm{PS}}{1000}$ = average mycotoxin concentration in processed sample in µg/kg

$\bar{\mathrm{X}}_{\mathrm{m}}{}^{\mathrm{TFK}}$ = average of the amount(weight) of the three food items consumed daily

T,F,K = tuwo, fura and kunu

$\bar{\mathrm{X}}bw$ = Mean body weight of the studied group/age range

2.7. Determination of Burden of Aflatoxin-Induced Hepatocellular Carcinoma

As reported by Liu and Wu (2010), for cancer risk assessment, it is tradition¬ally assumed that there is no threshold of exposure to a carcinogen below which there is no observable adverse effect (National Research Council, 2008). Cancer potency fac¬tors are estimated from the slope of the dose–response relationship, which is assumed to be linear, between doses of the carcinogen and cancer incidence in a population. The IPSC/WHO aflatoxin risk assessment selected two different cancer potency factors for aflatoxin: 0.01 cases/100,000/year/nanogram/kilogram body weight per day aflatoxin exposure for individuals without chronic HBV infection, and 0.30 corresponding cases for individuals with chronic HBV infection. This was based on one cohort study that estimated can¬cer potency in individuals positive for the HBV surface antigen (HBsAg; a biomarker of chronic HBV infection) and in HBsAg-negative individuals (Yeah et al. 1989), as well as other human studies that assessed cancer potency among either HBsAg-positive or HBsAg-negative individuals. These values were used in the course of our estimation throughout and was multiplied by the aflatoxin exposure in ng/kgbw/day to determine the annual HCC cases per 100,000 individuals, while the values for the annual HCC cases/100,000 in both the HBsAg negative and positive indiduals were multiplied by their respective population (determined) to arrive at the burden of the disease within a given agro-ecological region and Nigeria at large. The formulae below were modelled and employed as thus:

Population of HBsAg positive $= \frac{13.2}{100}\,\mathrm{x\ N(given\ population)}$

While population of the HBsAg negative $\left| = \mathrm{N} - \left(\frac{13.2}{100}\ \mathrm{x\ N\ (given\ population)}\right)\right|$

where is N the total population of the individuals

13.2% is the determined percentage of Nigerian population that are HBsAg positive

Estimated annual HCC cases per 100,000 for HBsAg negative individual is given by:

$$\mathrm{Aflatoxin\ exposure}\left(\frac{\frac{\mathrm{ng}}{\mathrm{kgbw}}}{\mathrm{day}}\right)\ \mathrm{x}\ \ 0.01$$

where 0.01 is the cancer potecy factor for HBsAg negative subjects

While estimated annual HCC cases per 100,000 for HBsAg positive subjects is given by:

$$\mathrm{Aflatoxin\ exposure}\left(\frac{\frac{\mathrm{ng}}{\mathrm{kgbw}}}{\mathrm{day}}\right)\ \mathrm{x}\ \ 0.3$$

where 0.3 is the AFB_1 cancer potecy factor for HBsAg negative subjects

The annual HCC cases was calculated thus:

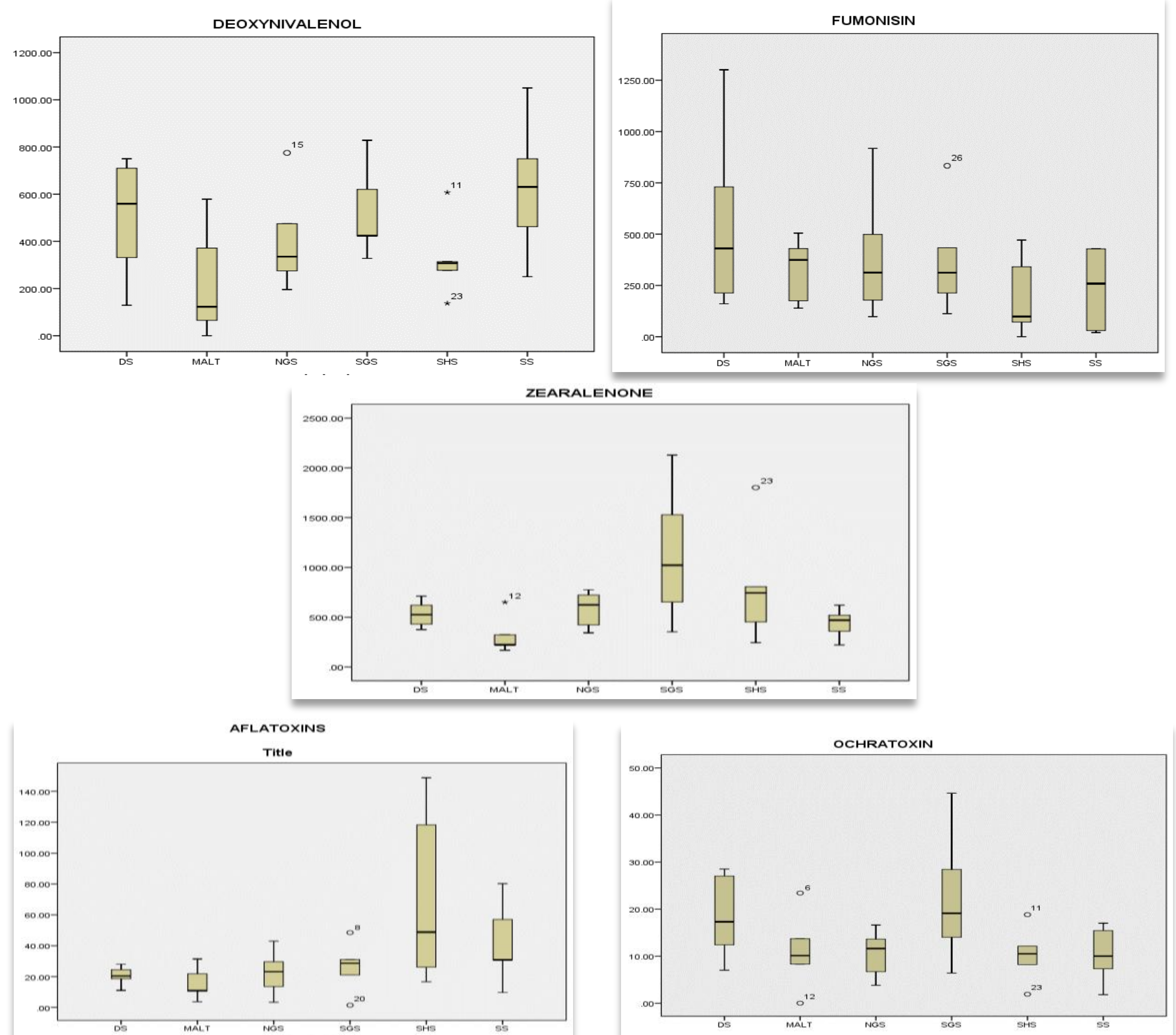
$$\frac{\text{Aflatoxin exposure x AFB1potency factor}(0.01 \text{ or } 0.3)}{100{,}000} \text{ x } \mathrm{N}(\mathrm{HBsAg} - \text{ve or} + \text{ve})$$

where is N the total population of the individuals

3. RESULTS AND DISCUSSION

3.1. Summary on The Effect of Processing Methods on The Mycotoxin Concentration (µg/Kg) In Sorghum Across The Six Agro-Ecological Zones

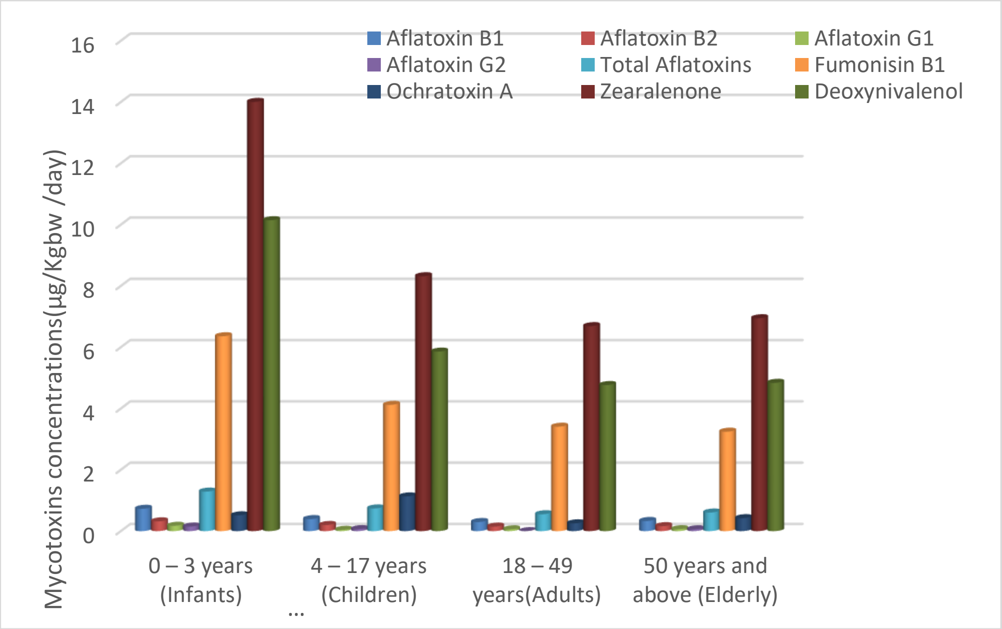
The effect of processing methods seems to have a profound effect on the mycotoxin concentration in sorghum and the daily mycotoxin intake from sorghum-derived products in the six Agro-ecological zones. There appeared to be a significant reduction in the concentration of OTA in Masa/ Waina sample from the SS. In all the remaining samples, there was a marked decrease in the mycotoxin concentration as observed in - of mycotoxin content when compared with the raw sample. In all the samples, only a Gruel sample from SGS showed 272.3% increase in ZEA concentration while there was



Figures 2. Distribution of levels of total afatoxins, total ochratoxins, total zearlenone, total deoxynivalenol and total fumonisins, (in µg/kg) in Sorghum bicolor samples across the six agroecological zones. The dots, error bars, and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively

3.2. Average mycotoxins consumption from sorghum based products (µg/Kgbw /day) by people from different age groups in the six agro-ecological zones

The purposive sampling carried out in 8 communities identified to subsist on sorghum almost on daily basis and the subsequent categorisation of individuals as infants, children, adults and elderly based on the following age range thus:
0 -3, 4 – 17, 18 – 49 and 50 and above years forms the preliminary data. Based on these data the average daily mycotoxin exposure per person from sorghum in 29 districts that forms the sampling sites and invariably Nigeria was estimated as shown in figure 4.1 – 4.7 below:



Figures 3. Average mycotoxins consumption from sorghum based products (µg/Kgbw/day) by people from different age groups in Nigeria

Key:
Average daily consumption of the three common sorghum derived products

Sorghum product	Age range			
	0 – 3 years	4 – 17 years	18 – 49 years	50 year and ≥
Gruel (Tuwo)	55.3 g	294.8 g	432.8 g	371.0 g
Porridge (Fura)	22.6 g	68.2 g	112.1 g	239.1 g
Pap (Kunu)	114.6 g	254.0 g	265.3 g	136.0 g

3.3. Hepatocellular Carcinoma (HCC) Incidence Attributable To AflatoxinB1 Consumption From Sorghum Based Products (ng/Kgbw/day) In Nigeria

Burden of Aflatoxin-Induced Hepatocellular Carcinoma was assessed in people that subsist on sorghum and sorghum based products in Nigeria and also within the six agro-ecological regions of the country. Tables 3.7 – 3.10 clearly revealed the estimated aflatoxin exposure level and the annual Hepato cellular carcinoma (HCC) per 100,000 persons in both HBsAg negative and HBsAg positive in Nigeria and also in the respective six agro-ecological regions of the country.

Table 1. Estimated HCC incidence attributable to aflatoxin B_1 from sorghum based products consumption (ng/kgbw/day) in Nigeria

Age range (years)	Aflatoxin exposure (ng/kgbw/day)	Estimated annual HCC (per 100,000)	
		HBsAg negative	HBsAg positive
0 - 3	728.0	7.28	218.4
4 - 17	392.0	3.92	117.6
18 - 49	297.0	2.97	89.1
50 and above	330.0	3.30	99.0

Table 2. Estimated annual burden of HCC cases attributable to aflatoxin B_1 exposure due to sorghum based foods consumption (ng/kgbw/day) in HBsAg-positive and HBsAg-negative populations in Nigeria

Age range (years)	Population (millions)	Annual HCC cases	
		HBsAg negative	HBsAg positive
0 - 3	18,003,405	1137.6	5190.2
4 - 17	68,642,593	2335.6	10655.5
18 - 49	78,729,014	2029.6	9259.5
50 and above	17,859,779	511.6	2333.9

Table 3. Estimated HCC incidence attributable to aflatoxin B_1 from sorghum based products consumption (ng/kgbw/day) in the six agro-ecological regions in Nigeria

Age range (years)	Aflatoxin exposure (ng/kgbw/day)	Estimated annual HCC (per 100,000)	
		HBsAg negative	HBsAg positive
DERIVED SAVANNAH			
0 - 3	392.0	3.92	117.6
4 - 17	191.0	1.91	57.3
18 - 49	157.0	1.57	47.1
50 and above	152.0	1.52	45.6
SOUTHERN GUINEA SAVANNAH			
0 - 3	389.0	3.89	116.7
4 - 17	236.0	2.36	70.8
18 - 49	199.0	1.99	59.7
50 and above	184.0	1.84	55.2
NORTHERN GUINEA SAVANNAH			
0 - 3	497.0	4.97	149.1
4 - 17	279.0	2.79	83.7
18 - 49	256.0	2.56	76.8
50 and above	270.0	2.70	81.0
SUDAN SAVANNAH			
0 - 3	516.0	5.16	154.8
4 - 17	293.0	2.93	87.9
18 - 49	258.0	2.58	77.4
50 and above	306.0	3.06	91.8
SAHEL SAVANNAH			
0 - 3	1763.0	17.63	528.9
4 - 17	1095.0	10.95	238.5
18 - 49	698.0	6.98	209.4
50 and above	878.0	8.78	236.4
MID - ALTITUDE			
0 - 3	436.0	4.36	130.8
4 - 17	256.0	2.56	76.8

Age range (years)	Aflatoxin exposure (ng/kgbw/day)	Estimated annual HCC (per 100,000)	
		HBsAg negative	HBsAg positive
18 - 49	215.0	2.15	64.5
50 and above	192.0	1.92	57.6

Table 4. Estimated annual burden of HCC cases attributable to aflatoxin B_1 exposure due to sorghum based foods consumption in HBsAg-positive and HBsAg-negative populations in the six agro-ecological regions of Nigeria

Age range (years)	Population (millions)	Annual HCC cases	
		HBsAg negative	HBsAg positive
DERIVED SAVANNAH			
0 - 3	2,530,405	86.1	392.8
4 - 17	9,440,432	156.5	714
18 - 49	13,090,058	178.4	809.3
50 and above	2,828,124	37.3	170.2
SOUTHERN GUINEA SAVANNAH			
0 - 3	2,037,364	68.5	313.8
4 - 17	11,432,440	234.0	1068.4
18 - 49	13,055,909	225.5	1034.2
50 and above	4,159,539	66.4	303.3
NORTHERN GUINEA SAVANNAH			
0 - 3	4,300,567	185.5	846.4
4 - 17	13,036,009	315.7	1440.3
18 - 49	13,709,110	304.6	1389.8
50 and above	3,635,934	85.2	388.8
SUDAN SAVANNAH			
0 - 3	3,400,675	152.3	694.9
4 - 17	12, 356,786	314.3	1433.2
18 - 49	12,709,110	284.6	3512.9
50 and above	2,451,567	65.1	297.1
SAHEL SAVANNAH			
0 - 3	2,298,439	351.7	1604.7
4 - 17	10,312, 100	980.1	3246.5
18 - 49	12,565,842	761.3	3473.3
50 and above	1,861,110	141.8	580.8
MID - ALTITUDE			
0 - 3	3,156,224	119.5	545.0
4 - 17	11,064,789	245.9	1121.7
18 - 49	13,011,673	242.8	1107.8
50 and above	2,923,500	48.7	222.3

Note : Cancer potency for HBsAg –ve is 0.01*
Cancer potency for HBsAg +ve is 0.3
Population of HBsAg -ve = Total population – population of HBsAg +ve
Population of HBsAg +ve = 13.2% of total population of Nigeria or AEZ.

3.4. Discussion

Based on the findings made by Micha et al. (2015) Several Sub-Saharan African nations had high intakes of whole grains, likely representing amaranth grain, millet, teff, sorghum and fonio, but very low intakes of nuts/seeds. The case of high sorghum consumption can therefore be expected to be quite high in Nigeria which is the second largest world producer of this grain crop and at the same time, the highest consumer of this grain in the form of human foods. The high consumption rate is attributable to the widespread cultivation of the crop and the extreme poverty level in the country which the relatively low cost of the grain attracted the poor masses' attention towards it.

Findings made from this study clearly revealed that there is co-occurrence of virtually all the five major mycotoxins in the sorghum samples from the six agro-ecological zones. Co-occurrence of AF/OTA/FB/ZEA/DON was observed in the samples from Derived savannah (DS), Southern guinea savannah (SGS) and Northern guinea savannah (NGS). While, co-occurrence of AF/OTA/ZEA/DON and AF/FB/ZEA/DON was observed in samples from Sudan savannah, Sahel savannah and Mid-altitude respectively. The co-occurrence of mycotoxins has been reported to worsen the already complicated situation of toxicity exerted by these mycotoxins, as a result of their synergistic and in some cases, additive effects. Therefore, the occurrence of some unusual (strange) ailments with some unfamiliar symptoms which are often attributed to witchcraft or spiritual attacks in the study area, may be attributed (based on our opinion) to the consumption of these matrices of mycotoxins, which either synergistically or additively interact to cause such anusual events.

Various processing methods employed in the preparation of the sorghum based product seems to be ineffective in reducing the OTA (46.5%), ZEA (34.8%) and DON (18.7%) in the Derived savannah (DS) and 10.2 and 18.7% in DON in the SGS and NGS respectively.

It is worth mentioning that an observation made in the Sudan savannah agro-ecological zone, where there was an increase by 70% and 66.7% in the aflatoxin B1 and zearalenone concentration repectively in porridge (Fura) sample compared to the raw sample from which it was made. This observation may not be unconnected to the fact that in the study area, it is the tradition of the people there to constantly refill the container in which food product is kept without allowing it to dry as this according to their tradition is a sign of poverty and hunger in the family. Therefore, based on our opinion, the continuous fungal growth in the reservoir container allow for the continuous accumulation of their secondary metabolite (mycotoxin) therein. The additional source of concern is the observed rise in the aflatoxin concentration which is a known potent carcinogen and has been known to be reduced by so many processing methods. The case of an increase in the concentration of DON may emanate from the fact that, it is less degraded by so many processing methods in addition to the tradition that encourage more of its production by the undisturbed fungal species in the family reservoir (container).

According to FAOSTAT (2010), maize, cassava, sorghum and millet are amongst the six most cultivated and consumed crops in Nigeria. According to the United States Department of Agriculture (USDA), five million and three hundred and fifty hectares of land was cultivated with sorghum in 2017 which gave an output of Six million and five hundred and fifty tonnes of sorghum grain within the same year. Also within the same year (2017), about Six million and four hundred and fifty thousand metric tonnes were directly consumed by humans while the balance was utilised in animal feed production. Our finding revealed that, probably, due to the gloomy economy and extreme poverty experienced from 2015 to 2017, the consumption of sorghum based products has surpassed the values in previous findings (Makun et al., 2013; Odoemelam and Osu (2009). We found an average daily consumption of sorghum based products based on age range as 192.5g/day, 617.0g/day, 810.2g/day and 746.1g/day for infants, children, adults and elderly respectively. The implication of these high levels of consumption of sorghum-based products may also have to be correlated to the findings again thus made in this study in which it was found that despite the seeming efficiency of the processing methods observed, the mycotoxins concentration still remain much higher than the Health based guidance value (HBGV) such as: as low as possible for aflatoxins (IARC, 2012; EFSA, 2007), 120ng/kgBW/PTWI (EFSA, 2006), 2000ng/kgBW PTDI/TDI (JECFA, 2012; SCF, 2003), 1000ng/kgBW TDI/PTDI (SCF, 2002; EFSA, 2004), 500ng/kgBW/day or 250ng/kgBW/day PTDI/TDI (JECFA, 2000; EFSA, 2011b) for ochratoxin, fumonisinB1, deoxynivalenol and zearalenone set by the regulatory agencies respectively.

A point of major concern is the fact that the highest consumption of virtually all the mycotoxins occurred within the age range of 0-3 (infants) followed by those within the age range of 4-17 (children). This is due to the fact that exposure to aflatoxins has been speculated to be a causative factor for child stunting and underweight, neurological impairment, immunosuppression and child mortality, according to WHO Expert Group Meeting in July 2005 (Henry et al., 2001). A possible association between aflatoxin exposure and growth faltering, particularly stunting in young children and in children with kwashiorkor has been established by series of studies (Gong et al., 2008; Turner et al., 2007). Immune suppression resulting in increased susceptibility to infectious disease, inhibition of protein synthesis caused by aflatoxin-induced disruption to RNA, or intestinal malabsorption has been attributed to aflatoxin exposure which eventually manifests into negative tendencies in child growth (Gong et al., 2008; Williams et al., 2004). Reduction in adult size, reduced work capacity and adverse reproductive outcomes are all attributed to stunting growth during childhood caused by aflatoxin consumption at that stage (Wild and Gong, 2010). It is interesting to note that, the observation made of high sorghum and by extrapolation mycotoxin consumption in our studies, is corroborated by Martani (2014) where he reported 41.0 and 26.7% stunting and underweight prevalence in Nigeria children which is quite worrisome. With regards the effect of over-exposure to fumonisin (FB) beyond PTDI level, it was found in one study by Kimanya et al. (2008) that children with FB intakes greater than the PMTDI were significantly shorter (1.3 cm) and lighter (328 g) than children with FB intakes less than the PMTDI. Despite the fact that the effects of DON exposure on growth in children have not yet been studied it is expected that (based on animal studies) DON has a negative effect on growth because of decreased food intake and reduced weight gain (Martani, 2014). It was observed in a study by Goyarts et al. (2007) that DON crosses the placenta which makes it more likely that utero exposure to DON will occur in humans as well. However, concerning ZEA, very little is known on the relationship between infant and maternal exposure levels in Africa, mostly due to the lack of valid biomarkers. However, the primary symptoms of ZEA toxicosis include nausea, vomiting and diarrhoea (Goyarts et al. 2007). All of which could influence growth negatively especially in terms of food refusal and weight loss. Interestingly, zearalenone or its metabolites have been suspected to cause precocious pubertal changes in young children in Puerto Rico. The occurrence of this phenomenon in other countries needs to be confirmed On the other hand, exposure to OTA beyond the PTDI level has long been associated with Balkan endemic nephropathy (BEN) and the incidence of epithelial tumours of the upper urinary tract (Castegnaro et al. 2006). OTA was classified by the IARC as possibly carcinogenic to humans (group 2B carcinogen) (IARC 1993b).

Due to high sorghum consumption across all the age groups observed in this study and the observed inefficiency of the processing methods to significantly reduce the mycotoxins concentration to levels lower than the PTDI, coupled with the high value of daily consumption determined. It became apparent that, the burden of Aflatoxin-induced hepatocellular carcinoma may probably be a source for serious concern, particularly with Nigeria ranking third on the Estimates of HBV prevalence in select countries based on HBsAg seroprevalence with 13.2% (Fasola et al.,2008) after the Gambia with 15-20% (Kirk et al. 2004) and Sudan with 6-26% (Elsheikh et al. 2007) respectively. Despite the fact that it is unclear the extent to which aflatoxin exposure alone causes HCC in exposed populations, findings by Qian et al. (1994) and Ross et al. (1992) has shown it (aflatoxin) to modify the risk associated with persistent

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hepatitis B infection by increasing it about tenfold.

Findings in this study as revealed by table 3.8 clearly showed a direct relation between the amount of sorghum product consumed per day and the burden aflatoxin-induced HCC in Nigeria. As clearly indicated in figures 3.1 – 3.7, the subjects within the age ranges of 4-17 and 18-49 consumed higher amounts of sorghum product per day and hence by extrapolation higher amounts of mycotoxins (aflatoxin inclusive). The higher HCC incidence within the age range of 0-3 (infants) observed in Table 3.7 is clearly a function of the body weight of the individuals that was used in the calculation of the exposure per day. Therefore, it can be said with a degree of certainty that, intake of high concentrations of aflatoxin by a lower age group may translate into high incidence of HCC per 100,000. This incidence cannot be avoided in poverty-stricken countries like Nigeria, Niger, Sudan, Uganda et cetra where staples are still used as weaning foods particularly in the rural areas.

Findings made by Joseph et al. (2012) revealed that, there is a marked geographical difference in the prevalence of HBeAg among HBsAg-positive patients in Nigeria, with the savanna zone of the broadly northern Nigeria showing a higher prevalence when compared with the southern forest zone. Results obtained in this studies follows similar trend as indicated in table 3.9 in which the incidence was observed to increase from the derived savannah (DS) down into the hinterland to the Sahel savannah. However, the mid-altitude with its characteristic cool temperature and temperate like climate showed low incidence and HCC aflatoxin induced burden. With regards to the burden in the seropositive and negative populations in the agro ecological zones, the annual HCC cases followed similar pattern observed by Joseph et al. (2012) with the mid-altitude also following aforementioned trend. However, worthy of mention is the contrary findings made by Ojo et al. (1995) in Ile-Ife and Ola et al. (2009) in Ibadan, in which in a dramatic twist, reported 48.4% and 19% HBeAg seropositivity among HBsAg-positive patients—all in the forest zone of Nigeria. Taken collectively and following documented evidence in Nigeria, it is clear that high infectivity of the virus is widespread among Nigerians with HBV infection and whenever HBeAg was present in Nigeria and most developing countries, it was associated with active liver disease.

4. CONCLUSION

From the findings thus made from this work, it suffice to assert that, three fundamental issues come to the fore, which include, high concentration of mycotoxins in the grain sorghum from all the agro-ecological zones and invariably Nigeria, insufficiency of the processing methods to lower the mycotoxins to tolerable limits, high consumption of sorghum-based products across all the age groups. These taken together will certainly translate into a precarious emergency situation with regards to mycotoxicosis and aflatoxin-induced burden of Hepatocellular carcinoma (HCC) in all the agricultural zones studied and invariably Nigeria. Therefore, a necessary mitigation strategy by all the stakeholders becomes imperative.

5. REFERENCES

Anthony, P. K. (2016). Utility of a cucumber plant bioassay to assess fungicide efficacy against *Pseudoperonospora cubensis. Plant Disease,* 1002: 490-499

Bandyopadhyay, R., Kumar, M. & Leslie, J. F. (2007). Relative severity of aflatoxin contamination of cereal crops in West Africa. *Food Additives and Contaminants,* 24**:** 1109–1114.

Björn, L. D. M., Brücher, I. & Jamall, S. (2014). Epistemology of the origin of cancer: A new paradigm. *BMC Cancer*, 14: 331. Doi: 10.1186/1471-2407-14-331

Blackwell, M. (2011). "The fungi: 1, 2, 3 ... 5.1 million species?". *American Journal of Botany,* 98 (3): 426–38. Doi:10.3732/ajb.1000298.

Candida, P., Shirima, M. Kimanya, E.., Kinabo, J. L., Michael N. R., Chou, S., Christopher, Wild, P. & Gong, Y. Y. (2013). Dietary exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. *Molecular and Nutritional Food Research*, 57(10): 1874–1881. Doi: 10.1002/mnfr.201300116

Castegnaro M, Canadas D, Vrabcheva T, Petkova-Bocharova T, Chernozemsky IN, Pfohl-Leszkowicz A. 2006. Balkan endemic nephropathy: Role of ochratoxin A through biomarkers. *Molecular Nutrition and Food Research,* 50:519–529.

De Cardoso L.M., Pinheiro S.S., da Silva L.L., de Menezes C.B., de Carvalho C.W., Tardin F.D., Queiroz V.A., Martino H.S., Pinheiro-Sant'Ana H.M. Tocochromanols and carotenoids in sorghum (*Sorghum bicolor L.*): Diversity and stability to the heat treatment. *Food Chem,* 172: 900–908. Doi: 10.1016/j.foodchem.2014.09.117.

EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2007) European food safety authority (EFSA) scientific opinion 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 J,* 446: 1–127

EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2006) EFSA scientific opinion on ochratoxin a in food. *EFSA J,* 365: 1–56

EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2004) EFSA scientific opinion on deoxynivalenol (DON) as undesirable substance in animal feed. *EFSA J,* 73: 1–42

EFSA, European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM) (2011b) EFSA scientific opinion on the risks for public health related to the presence of zearalenone in food. *EFSA J*, 9: 2197

Elsheikh R, Daak A, Elsheikh M, Karsany M, Adam I. 2007. Hepatitis B virus and hepatitis C virus in pregnant Sudanese women. *Virol J,* 4: 104. Doi:10.1186/1743-422X-4-104.

FAOSTAT. (2010). *Crops Primary Equivalent*. Retrieved from: www. faostat.fao.org.

Fasola F, Kotila T, Akinyemi J. (2008). Trends in transfusion-transmitted viral infections from 2001 to 2006 in Ibadan, Nigeria. *Intervirology*, 51(6): 427–431.

Felicia, W. & Pornsri, K. (2011). Evaluating the technical feasibility of aflatoxin risk reduction strategies in Africa. *Food Additives and Contaminants,* 27(5): 658–676. Doi: 10.1080/19440041003639582

Gelderblom, W.C.A., Marasas, W.F.O., Lebepe-Mazur, S., Swanevelder, S., Vessey, C.J., Hall, P. & de La, M. (2002). Interaction of FBs B_1 and aflatoxin B_1 in a short term carcinogenesis model in rat liver. *Toxicology,* 171: 161–173.

Gong YY, Turner C, Hall AJ and CP Wild Aflatoxin exposure and impaired child growth: An unexplored International Public Health Burden: In Leslie *et al* (2008). Mycotoxins: Detection methods, management, public health and agricultural trade. *CAB International*, 6: 53-54.

Goyarts T, Danicke S, Brussow KP, ValentaH, Ueberschar KH, Tiemann U: On the transfer of the *Fusarium* toxins deoxynivalenol (DON) and zearalenone (ZON) from sows to their foetuses during days 35–70 of gestation. *Toxicol Lett*, 171: 38–49.

Gunther, A., Siska, C., Frank, P., Richard, D., Venessa, E., Mathias, D., Marc V., Freddy, H., Mia, E., Sarah, D-S., Birgit, A., Barbara, N., An M. & Van Immerseel, F. (2014). Fumonisins affect the intestinal microbial homeostasis in broiler chickens, predisposing to necrotic enteritis. *Veterenary Research,* 46(1), 98.

Henry SH, Whitaker T, Rabbani I, Bowers J, Park D, Price W, Bosch FX, Pennington J, Verger P, Yoshizawa T, van Egmond H, Jonker MA and R Coker Aflatoxin M1. (2001). *Food and Drug Administration.* Washington DC: JECFA 47.

Ilaria Proietti, Chiara Frazzoli, and Alberto Mantovani (2015). Exploiting Nutritional Value of Staple Foods in the World's Semi-Arid Areas: Risks, Benefits, Challenges and Opportunities of Sorghum. Healthcare (Basel) 3(2): 172 193 doi: 10.3390/healthcare3020172

International Agency for Research on Cancer (IARC). (1993b). Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. Ochratoxin A. WHO IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, *yon: IARC*, 56: 489–521.

International Agency for Research on Cancer (IARC). (2012). Monographs on the evaluation of carcinogenic risks to humans volume 100F: chemical agents and related occupations. *PRESS RELEASE N° 374*

International Agency for Research on Cancer (IARC). (2016). Mycotoxin Control in Low- and Middle-Income Countries*:* A Working Group of world-leading experts convened by the International Agency for Research on Cancer (IARC) with support from the Bill & Melinda Gates Foundation (BMGF. Lyon, France, 17 February 2016. *PRESS RELEASE N° 242*

JECFA, Joint FAO/WHO Expert Committee on Food Additives. (2000). Safety evaluation of certain food additives and contaminants, Prepared by the 51st JECFA meeting. *WHO Technical Report Series*, No. 891

JECFA, Joint FAO/WHO Expert Committee on Food Additives. (2012). Safety evaluation of certain food additives and contaminants, Prepared by the 76th JECFA meeting, *WHO Technical Report Series*, No. 974

Joseph C. Forbi, Odunayo H Iperepolu, Timothy Zungwe, and Simon M. Agwale. (2012). Prevalence of Hepatitis B e Antigen in Chronic HBV Carriers in North-central Nigeria. *J Health Popul Nutr*, 30(4): 377–382.

Kimanya ME, De Meulenaer B, Tiisekwa B, Ndomondo-Sigonda M, Devlieghere F, Van Camp J, Kolsteren P. (2008). Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2008*, 25: 1353–1364.

Kimanya, M D- M., BTiisekwa, B. N-S.M. & Kolsteren, P. (2008). Human exposure to fumonisins from home grown maize in Tanzania**.** *World Mycotoxin Journal,* 1(3): 307-313

Kirk GD, Lesi OA, Mendy M, Akano AO, Sam O, Goedert JJ, *et al*. (2004). The Gambia Liver Cancer Study: infection with hepatitis B and C and the risk of hepatocellular carcinoma in West Africa. *Hepatology*, 39(1):211–219.

Kowalska, K, Habrowska-Górczyńska, D.E. & Piastowska-Ciesielska, A.W. (2016). Zearalenone as an endocrine disruptor in humans (A Review). *Environmental Toxicology and Pharmacology*, 48: 141-149. Doi: 10.1016/j.etap.2016.10.015

Lalini, R. & Kanti, B. (2010). Ochratoxins—food contaminants: impact on human health. *Toxins (Basel)*, 2(4):771–779. Doi: 10.3390/toxins2040771

Li, Y., Zhang, B., He, X., Cheng, W.H., Xu, W., Luo, Y., Liang, R., Luo, H. & Huang, K. (2014). Analysis of individual and combined effects of ochratoxin A and zearalenone on HepG2 and KK-1 Cells with mathematical models. *Toxins* (*Basel),* 6(4),1177–1192. doi: 10.3390/toxins6041177

Liu, Y & Wu, F. (2010*).* Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental Health Perspectives,* 118(6): 818 – 824.

Makun, H. A., Michael F. D., Patrick, B. N., Mulunda, M. & Kabiru, A.Y. (2011). Natural multi-mycotoxin occurrence in rice from Niger State, Nigeria. DOI: 10.1007/s12550-010-0080-

5. *Mycotoxin Research*, 27 (2), 97-104.

Makun ,H. A., Adeniran A. L., Mailafiya, S. C., Ayanda, I. S., Mudashiru, A. T., Ojukwu , U. J., Jagaba, A. S., Usman , Z., & Salihu, D. A. (2013). Natural occurrence of ochratoxin A in some marketed Nigerian foods. *Food Control,* 31 (2013): 566e571

Martani J. L. (2014). Mycotoxin exposure and infant and young child growth in Africa: What do we know? *Ann Nutr Metab*, 64(2):42–52

Matejova, M., Vicenova, L., Vojtek, H., Kudlackova, K., Nedbalcova, M., Faldyna, E., Sisperova, H. & Modra, Z. S. (2015). Effect of the mycotoxin deoxynivalenol on the immune responses of rainbow trout (*Oncorhynchus mykiss*). *Veterinarni Medicina, 60* (*9): 515–521* Doi: 10.17221/8443

Micha, R., Khatibzadeh, S., Shi, P., Andrews, K. G., Engell, R. E., and Mozaffarian, D. (2015). Global, regional and national consumption of major food groups in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys worldwide. *BMJ Open*. 5(9): e008705. Doi: 10.1136/bmjopen-2015-008705

Miller, J. D. (1995). Fungi and mycotoxins in grains: implications for stored product research. *Journal of Stored Products Research,* 31(1): 1–16

Mohammed, E. Z. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society ,*15(2): 129–144

National Research Council (2008). *Science and Decisions: Advancing Risk Assessment*. Washington, DC: National Academy Press.

Odoemelam, S. A. and Osu, C. I. (2009). Aflatoxin B1 contamination of some edible grains marketed in Nigeria. *E-Journal of Chemistry*, 6(2): 308-314

Ojo OS, Thursz M, Thomas HC, Ndububa DA, Adeodu OO, Rotimi O, et al. Hepatitis B virus markers, hepatitis D virus antigen and hepatitis C virus antibodies in Nigerian patients with chronic liver disease. *East Afr Med J*, 72:719–21.

Ola SO, Otegbayo JA, Yakubu A, Aje AO, Odaibo GN, Shokunbi W. (2009). Pitfalls in diagnosis of hepatitis B virus infection among adults Nigerians. *Niger J Clin Pract*. 12:350–4.

Pawlowska, A.M., Zannini, E., Coffey, A. & Arendt, E. K. (2012). Green preservatives": combating fungi in the food and feed industry by applying antifungal lactic acid bacteria. *Advances in Food and Nutrition Research*, 66: 217-38. Doi: 10.1016/B978-0-12-394597-6.00005-7.

Qian GS, Ross RK, Yu MC *et al.* (1994) A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, Peoples Republic of China. *Cancer Epidemiology, Biomarkers and Prevention,* 3: 3–10.

Reyes-García, V. J. (2010). The relevance of traditional knowledge systems for ethnopharmacological research: theoretical and methodological contributions. *Journal of Ethnobiology and Ethnomedicine*, 6,32. doi:10.1186/1746-4269-6-32

Ross RK, Yuan JM, Yu MC *et al.* (1992) Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. *Lancet*. 339: 943–946.

SCF, Scientific Committee for Food. (2002). *Opinion Of The Scientific Committee On Food On Fusarium Toxins. Part 6: Group Evaluation Of T-2 Toxin, HT-2 Toxin, Nivalenol And Deoxynivalenol.* Retrievied from: http://ec.europa.eu/food/fs/sc/scf/out123_en.

SCF, Scientific Committee for Food. (2003). *Updated Opinion Of The Scientific Committee On Food On Fumonisin B1, B2 And B3.* Retrievied from: http://europa.eu.int/comm/food/fs/sc/scf/index_en.html

Sydenham, E. W., Marasas, W. F. O., Shephard, G. S., Thiel, P. G. & Hirooka, E. Y. (1992). Fumonisin concentrations in Brazilian feeds associated with field outbreaks of confirmed and suspected animal mycotoxicoses. *Journal of Agric. and Food Chemistry*, 40: 994-997.

Thomas, W., Kensler, B., Roebuck, S. Gerald, N., Wogan, J. & Groopman, D. (2011). Aflatoxin: A 50-Year odyssey of mechanistic and translational toxicology. *Toxicological* Science. 120(1): 28–S48. Doi: 10.1093/toxsci/kfq283

Turner PC, Collinson AC, Cheung YB, Gong YY, Hall AJ and AM Prentice Aflatoxin exposure in utero causes growth faltering in Gambian infants. *Int J Epidemiol, 36*:1119-1125.

Wild, C. P. & Gong, Y. Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis*, 31:71–82. DOI: 10.1159/000365126

Williams JH, Phillips TD, Jolly PE, Stiles JK, Curtis M and CM Jolly. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition,* 80:1106-1122.

Yahaya, M. A., Shimelis, H., Nebie, B., Ojiewo, C. O & Danso-Abbeam, G. (2022). Sorghum production in Nigeria: opportunities, constraints, and recommendations, *Acta Agriculturae Scandinavica,* Section B. *Soil & Plant Science*, 72(1): 660-672, Doi: 10.1080/09064710.2022.2047771

Yeah, F. S., Yu, M.C., Mo, C. C., Luo, S., Tong, M. J. & Henderson, B. E. (1989). Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Research,* 49(9): 2506–2509