Food Additives & Contaminants: Part A

ISSN: (Print) (Online) Journal homepage: [www.tandfonline.com/journals/tfac20](https://www.tandfonline.com/journals/tfac20?src=pdf)

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To cite this article: Fatimah Omolola Badmos, Hadiza Lami Muhammad, Achi Dabara, Funmilola Adefolalu, Susan Salubuyi, Abdullahi Abdulkadir, Victor Tope Oyetunji, Daniel Ojochenemi Apeh, Hadiza Kudu Muhammad, Mulunda Mwanza, Maurice Monjerezi, Limbikani Matumba & Hussaini Anthony Makun (2024) Assessment of dietary exposure and levels of mycotoxins in sorghum from Niger State of Nigeria, Food Additives & Contaminants: Part A, 41:1, 74-90, DOI: [10.1080/19440049.2023.2293998](https://www.tandfonline.com/action/showCitFormats?doi=10.1080/19440049.2023.2293998)

To link to this article: <https://doi.org/10.1080/19440049.2023.2293998>

[View supplementary material](https://www.tandfonline.com/doi/suppl/10.1080/19440049.2023.2293998)

Published online: 18 Dec 2023.

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FOOD ADDITIVES & CONTAMINANTS: PART A 2024, VOL. 41, NO. 1, 74–90 <https://doi.org/10.1080/19440049.2023.2293998>

RESEARCH ARTICLE

**Assessment of dietary exposure and levels of mycotoxins in sorghum from Niger State of Nigeria**

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ABSTRACT

This study reports levels of mycotoxins in sorghum from Niger State, Nigeria, and provides a comprehensive assessment of their potential health risks by combining mycotoxin levels and dietary exposure assessment. A total of 240 samples of red and white sorghum were collected from both stores and markets across four microclimatic zones. Fungal species were identified using a dilution plate method. Aflatoxins (AFs), deoxynivalenol, nivalenol, and ochratoxin (OTA) were quantified using HPLC, whereas cyclopiazonic acid, fumonisins (FUMs) and zearalenone were quantified using ELISA. *A. flavus* and *A. fumigatus* were domin-ant fungal species followed by *F. verticilloides*, *A. oryzae* and *P. verrucosum*. Aflatoxins (mean: 29.97mg/kg) were detected in all samples, whereas OTA (mean: 37.5 mg/kg) and FUMs (mean: 3269.8mg/kg) were detected in 72% and 50% of the samples, respectively. Mycotoxins frequently co-occurred in binary mixtures of AFsþOTA and AFsþFUMs. Dietary exposure estimates were highest for FUMs at 230% of TDI and margin of exposures (MOEs) for both AFs and OTA (*<*10,000) indicating a potential risk associated with combined expos-

ure to AFs and OTA. The Risk of hepatocellular carcinoma cases (HCC/year) attributable to

AFs and OTA exposure from sorghum was estimated to be 5.99�105 and 0.24�105 cases

for HBsAgþindividuals based on 13.6% HBV incidence. Similarly, the HCC/year for AFs and

OTA were assessed to be 3.59�105 and 0.14�105 at an 8.1% prevalence rate. Therefore, the results of this study demonstrate the high prevalence and dietary exposure to mycotox-ins through sorghum consumption, raising public health and trade concerns.

ARTICLE HISTORY Received 11 September 2023 Accepted 6 December 2023

KEYWORDS

Aflatoxins; cyclopiazonic acid; deoxynivalenol; nivalenol; ochratoxin A; Niger State; sorghum

**Introduction**

The presence of fungi and their secondary metabo-lites, known as mycotoxins, in agricultural produce has profound adverse effect on food and feed, impacting human health, animal productivity and trade (Ekwomadu et al. 2021). Global estimates of mycotoxin occurrence in food crops show that 60-80% of agricultural produce is lost to mycotox-ins worldwide (Eskola et al. 2020). There are many known mycotoxins but the ones that are signifi-cant, with regards to public health and

international trade include aflatoxins (AFs), ochra-toxin A (OTA), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), cyclopiazonic acid (CPA) and fumonisins (FUMs) (Dumitru et al. 2019; Alam et al. 2022).

Aflatoxin B1 (AFB1), the most potent among the AFs, is classified as a Group 1 human liver carcinogen by the International Agency for Research on Cancer (IARC 2002). AFs are associ-ated with increased hepatocellular carcinoma (HCC), child stunting, pregnancy loss, premature

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Supplemental data for this article can be accessed online at [https://doi.org/10.1080/19440049.2023.2293998.](https://doi.org/10.1080/19440049.2023.2293998) � 2023 Taylor & Francis Group, LLC

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birth, and death in human beings (Smith et al. 2017; McMillan et al. 2018; Udovicki et al. 2021; Amir et al. 2022). Trichothecenes comprise a large group (about 150 related compounds) of structurally related fungal metabolites that inhibit protein synthesis, leading to weight loss, abor-tions, vomiting, haemorrhaging of internal organs, immunosuppression, and blood disorder in humans and animals (Singh and Kumari 2022). The economically important trichothe-cenes include T-2 toxin, HT-2 toxin, DON, and NIV because they occur frequently in foods and feed and can severely affect human health (Abbas et al. 2013). FUMs are associated with equine leu-koencephalomalacia and porcine pulmonary oedema (EFSA Panel on Contaminants in the Food Chain (CONTAM) et al. 2022). Of major concern is the extent of association of Fumonisin B1 (FB1) with human oesophageal cancer, liver cancers, and neural tube defects in human babies (Arumugam et al. 2021; Chen et al. 2021). It has also been demonstrated that FUMs induce apop-tosis in cultured human cells and in rat kidneys and are now recognised as possible human carci-nogens (Group 2B) as classified by IARC (IARC 2002; Malir et al. 2023). ZEN, being a non-ster-oidal oestrogenic mycotoxin, adversely affects humans and animals and its metabolite has been found to bind competitively to oestrogen receptor in various species resulting in hypoestrogenism and infertility in livestock (Liu and Applegate 2020). It has also been suggested to have a pos-sible involvement in human cervical cancer (Zinedine et al. 2017). OTA is responsible for nephrotoxic, immunosuppressive and teratogen complications and it was classified by the IARC as possible human carcinogen because of its well-known carcinogenicity effect on both male and female rats (IARC 1993; Janik et al. 2020; Gupta et al. 2022). The most significant adverse effect of OTA is its connection with human kidney disease known as Balkan endemic nephropathy (Longobardi et al. 2022). OTA has also been reported to cause kidney and liver impairment in human and animals, especially in pigs (Vlachou et al. 2022). CPA causes diarrhoea, fever, dehy-dration, anorexia, and weight loss in chickens, rats, dogs, and pigs (CAST 2003; Casquete et al. 2021). The role of CPA in naturally occurring

toxicoses is not clear, but it occurs together with aflatoxin and may be involved in Turkey X dis-ease, along with aflatoxins and Koduo millet poi-soning syndrome in Northern India (Kumar et al. 2020).

Sorghum is a drought resistant crop indigen-ous to Africa and it serves as a staple for almost a billion people in Africa, Asia and Latin America. It is the fourth most consumed cereal in Africa after maize, rice, and wheat (FAOSTAT 2018). In Nigeria, sorghum is the 5th source of energy after cassava, yam, maize, and rice. Nigeria is the second largest producer of sor-ghum in the world after the USA, with almost 7 million tons (Edia 2018; Ssepuuya et al. 2018). Niger State, the study area of this work, is the second largest producer of sorghum throughout the country for commercial purpose (Merem et al. 2017). However, sorghum is a suitable sub-strate for the growth of various species of fungi and therefore it is disposed to mycotoxin con-tamination (Mateo et al. 2018). There are several reports on fungi and mycotoxin contamination of sorghum in Nigeria and elsewhere (Hussaini et al. 2009; Yassin et al. 2010; Atanda et al. 2013; Hertveldt 2016; Garba et al. 2017; Onyedum et al. 2020). These publications have reported the pres-ence of AFB1, FUMs, DON and OTA in sorghum from Niger State and three agro-ecological zones of Nigeria. However, there is still a paucity of information on other mycotoxins, particularly CPA and NIV contamination, and co-occurrence with other mycotoxins, across varieties of sor-ghum and micro-climatic zones from Niger state and Nigeria at large. The previous survey on the occurrence of fungi and mycotoxins in sorghum in the state used a limited sample size (Onyedum et al. 2020). In addition, except for Onyedum et al. (2020), there is no information on the diet-ary intake and risk characterization of the studied toxin in sorghum from Niger State, Nigeria. Further, surveillance studies need to be updated regularly to capture changes in mycotoxin con-tamination resulting from changes in climate which exacerbates mycotoxin contamination in agricultural produce (Kos et al. 2023). In this context, the present study was conducted to ascertain the presence and distribution of fungi and the level of contamination of AFs, DON,

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NIV, OTA, FUMs, ZEN and CPA in sorghum from Niger State using HPLC and Enzyme Linked Immunosorbent Assay (ELISA). This study was also designed to elucidate the possible risk associated with the ingestion of the toxins with a view to assessing the health impact associ-ated with sorghum consumption.

**Materials and methods**

***Sample collection***

Niger State is in Central Nigeria and the largest State in the country with 76,363km2 (29, 484sq mi). The State experiences a hot (annual average of 29.5�C) and humid climate (average 73%) for the most part of the year, especially between the fifth and tenth month of every year, which is favourable for fungal growth and mycotoxin production. Niger State is categorised into four microclimatic zones namely: the wettest zone with an annual rainfall greater than 1400mm, wet and dry zone having an annual rainfall between 1200–1400mm and 1000–1200mm respectively, and the driest zone having annual rainfall of less than 1000mm (Muhammad et al. 2019).

A total of 240 samples of sorghum were col-lected from both stores and markets in July 2019 through purchases and donations from the four microclimatic zones of Niger state. Samples were collected following the European Commission Regulation (2010): 1kg of each sample was col-lected, labelled, packaged in polythene bags, and taken to the laboratory. The samples were pooled together to form thirty-two (32) composite sam-ples, consisting of 16 stored sorghum (white: 8, red: 8) and 16 market sorghum (white: 8, red: 8). A total of eight (8) composite samples each were formed from the samples collected from the four micro-climatic zones; Zone 1 (wettest zone), Zone 2 (wet zone), Zone 3 (dry zone) and Zone 4 (dri-est zone). Prior to analysis, the samples were blended into fine flour using a milling machine (Greiffenberger Antriebstenchnic, Marktredwitz, Germany), and sieved to pass sieve size of 0.5– 1mm and then stored at −20�C.

***Fungal isolation and identification***

Fungi isolation was carried out using the plate dilution method as described by Kaufman et al. (1963) with slight modification. The milled sam-ple (1g) was weighed into a sterile tube and sus-pended in ringer solution (9mL) and shaken for 2min on a vortex mixer. The suspension was serially diluted 10 fold. Aliquots (1mL) of each tube from the 106 diluents were used for inocula-tion in triplicate using potato dextrose agar (PDA). The first set of the media was incubated at 28±2�C for 3–5d, intended for the enumer-ation of *Aspergillus species*, while the second set was incubated for 7d at 25�C for the enumer-ation of *Fusarium* and *Penicillium species*. Fungi growth and sporulation was observed using a compound microscope and the plate count was noted. After incubation, the fungal colonies were counted using a colony counter and the number of colonies/g of sample were counted and expressed in colony forming unit/g as represented in Equation (1).

Number of colonies

Dilution factor � plating volumes ðmlÞ (1)

***Determination of mycotoxins using HPLC method***

A multi-mycotoxin extraction method devised by Patterson and Roberts (1979) was used for the extraction of AFs, CPA, DON, and NIV. Approximately 12.5g of previously milled sorghum sample was placed in a 100ml conical flask and extracted with 50ml of phosphate buffered saline (PBS) using an orbit shaker operated at 200rpm for 30min. The supernatant (first extract) was col-lected and centrifuged at an RCF value of 1693g for 15min using a Thermo fisher scientific centri-fuge (Fiberlite F9-4x1000y, rotor radius; 16.8cm) and then filtered through a glass microfiber filter. Afterwards, 35ml of methanol/PBS mixture (80:20 and 70:30, v/v) was added to the obtained product and the mycotoxins were extracted by shaking for 15min and subsequently centrifuged at 3810g (RCF) at 4�C. Then, 20ml of the resultant extract was diluted with 90ml PBS and filtered through a glass microfiber filter (second extract). Firstly, 50ml of the second extract was passed through a

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VICAM Myco6in1TM column at 0.2ml drops/s, and the column was washed with 25ml PBS to eliminate methanol residues before adding 5ml of the ‘first’ extract. A subsequent washing step was followed with 30ml distilled de-ionized water to completely remove PBS residue and matrix inter-fering compounds. The toxins were eluted with 3ml methanol added in two steps (0.1ml each at one drop/s) with 5min interval between steps. The eluate was evaporated to dryness at 50�C under a nitrogen gas stream and the obtained residue was reconstituted in mobile phase: 0.05ml of aceto-nitrile/0.01ml of dichloromethane (70:30, v/v).

AFs, DON, NIV, and CPA were quantified using an HPLC modular system (model LC98II, Searchtech) with UV detection. The broad applic-ability of the chosen method allows a broad-spectrum detection for a wide range of compounds, making it suitable for analysing multiple myco-toxins simultaneously and thus it is cost-effective to operate and eliminates the requirement of a fluorescent reagent. Chromatography separation was performed at column temperature of 40�C, 30�C, 30�C and 37�C for AFs, DON, NIV, and CPA, respectively. The system was accomplished with column types C-18, pressure max per flow rate of 420Pa � 1ml/ml (AFs), 420Pa � 0.8ml/ ml (DON/NIV) and 420Pa � 0.6ml/ml (CPA), for separation and quantification of selected mycotoxins and internal standards. An injection volume of 20ml was used for the prepared sam-ples with different mobile phase for individual toxin: that is, water: methanol: acetonitrile (60:20:20) for AFs, water: acetonitrile (90:10) for DON and NIV, and 0.4mM aqueous ZnSO4.7H2O: methanol (70:30 v/v) for CPA. AFs were detected at a wavelength of 365nm, DON and NIV at 220nm, and CPA was detected at 248nm. Different run time was used for HPLC analysis of each mycotoxin.

***Validation of the HPLC method***

Method validation was carried out to ascertain the accuracy of results of mycotoxin levels in sor-ghum samples following method described herein. The method was validated in terms of lin-earity (quantification), accuracy (recovery) and sensitivity (limit of detection, LOD and limit of

quantification, LOQ) using blank matrices of sor-ghum, according to the method of Monbaliu et al. (2010), European Union Commission (2006) and Abia et al. (2013). For quantification, external calibration curves were established by injecting mycotoxin standards at five serial dilu-tions, as presented in [Supplementary Table S1](https://doi.org/10.1080/19440049.2023.2293998), into the HPLC column. Linear calibration curves generated for the mycotoxin standards were con-sidered satisfactory when correlation coefficients (*r*2) were greater than 0.90.

Apparent recovery experiments were carried out in duplicates by spiking five least contami-nated samples (5g of each) with 100mL of the analyte standard of known concentration. The spiked samples were left overnight in a fume cup-board at room temperature to allow for solvent evaporation to establish equilibrium between the sample matrix and the toxins. Mycotoxin from the spiked samples was extracted using the myco-toxin extraction method described above. From each spiked sample, 20mL of the extract was injected into the HPLC. Each analyte detected was quantified by comparing its peak area on the calibration plot to that of the equivalent myco-toxin standard. Recovery was calculated by taking the percentage of the concentration (peak area) measured from the spiked sample per toxin con-centration used for spiking the sample. The sensi-tivity parameters (i.e. LOD and LOQ) for mycotoxins in the sorghum samples were calcu-lated from the signal to noise ratios (S/N) of the respective UV absorbance chromatogram derived from the analysis of the spiked samples.

***Determination of fumonisins, ochratoxin A and zearalenone using ELISA***

Commercial ELISA kits were obtained from Romer Labs Singapore Pte Ltd: AgraQuantR Ochratoxin A (COKAQ2000), AgraQuantR Fumonisin (COKAQ3000) and AgraQuantR Zearalenone (COKAQ5000). Extraction of fumo-nisins, ochratoxin A and zearalenone from sor-ghum samples was carried out following the procedure recommended by the manufacturer. A known mass (10g each) of the milled samples was weighed into labelled conical flasks. About 20mL of the mixture of methanol and water

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(70:30 v/v) was added to the samples under con-tinuous stirring and shaken in a mechanical shaker for 2h. The reaction mixture was filtered through a Whatman No. 2 filter paper. A portion of the filtrate (about 5mL) was cleaned in a microfilter (5mm pore size: Maidstone, UK), pre-conditioned with methanol and water in ratio 3:1 v/v. Afterwards, the filtrate was stored at 4�C until further analyses.

Approximately 100mL of a standard solution or prepared sample (as above) was added into appropriate wells of microtiter plate, and 200mL of Horseradish Peroxidase (HRP) conjugated antibody working solution were added into each well separately. Thereafter, the microtiter plate was sealed with a cover membrane, and incu-bated for 15min at room temperature. The con-tent was dispensed (by aspiration or dumping) from each dilution well into a corresponding antibody coated microwell and washed repeatedly five times. Washing was done by filling each microwell with distilled de-ionized water using a multi-channel pipette. Complete removal of the residual liquid at each step was ensured by strik-ing the plate against an absorbent paper towel. Subsequently, 100mL of substrate solutions was pipetted into each well. This was mixed gently by shaking the plate manually and incubated for 5min at 25�C. The reaction was allowed to develop in the dark and the plate was not sub-jected to temperature fluctuations. Lastly, 100lL of stop solution (0.18M of H2SO4) was added to each well and mixed gently by shaking the plate. After thorough mixing, the optical density of each well was measured using an ELISA micro-plate reader set to 450nm and 630nm differential filter. All samples, controls, and standards were assayed in duplicate.

The range of quantification and detection lim-its (LODs and LOQs values) was determined according to the manufacturer’s description. Validation of ELISA was conducted by evaluating the apparent recoveries and the standard devia-tions of uncontaminated samples spiked at 20mg/ kg for OTA, 250mg/kg for FUM and 300mg/kg for ZEN [(Table S2](https://doi.org/10.1080/19440049.2023.2293998)). In addition, repeatability and reproducibility were examined at spiking levels. Concerning repeatability, the parameters were evaluated under repetitive conditions at the same

day whereas, reproducibility were evaluated at a different time interval.

***Data collection for exposure assessment***

A total of 120 respondents were enrolled to fill a questionnaire to collect data to determine the exposure rate. Based on the selected criteria, the survey was age- and gender- weighted and they represent adult population groups between the ages of 20–60years. All the subjects from each population group returned and completed the questionnaire within a few min. A portable scale was used to determine the weight of each respondent from the studied region. Also, the questionnaire assessed whether the respondent consumed sorghum, and explored the extent to which they consumed sorghum products (if they were consumed on a weekly basis or not at all).

***Calculation of dietary intake of mycotoxins***

Human exposure to mycotoxins was determined by calculating estimated daily intake (EDI) and percentage tolerable daily intake (%TDI) using the method of Rodrıguez-Carrasco et al. (2013) and confirmed by Joint FAO/WHO Expert Committee on Food Additives (JECFA). The EDI of individual mycotoxin was calculated using the formula presented in Equation (2).

*EDIm* ¼ *cm* � *k* (2) *w*

where *EDIm* is the estimated daily intake (ng/kg bw/day) for mycotoxin *m*; *cm* is the average level of a mycotoxin present in a sample (ng/kg); *k* is the daily consumption of sorghum (kg/day); *bw* is the body weight of individuals in kg

In this study, the values of *EDI* were calculated using Equation (2) by multiplying the average level of each mycotoxin present in the sorghum samples with the average sorghum consumption of 328.79g/person/day (0.32879kg/person/day) as estimated from the questionnaire and then div-ided by mean body weight of 63.03, 64.29 and 61.58kg for adult male, adult female, and total population groups, respectively.

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***Risk characterisation***

The calculated *EDIm* was used to evaluate the risk of each mycotoxin (except aflatoxins) by dividing the *EDIm* value with the tolerable daily intake (relevant TDI) (ng/kg bw/day), as desig-nated in Equation (3).

%*TDIm* ¼ *ETDI* � 100 (3)

***Margin of exposure (MOE) characterization for aflatoxins and ochratoxin A***

MOE, designated by the European Food Safety Authority (EFSA), was used to estimate the degree of concern required for managing health related risk associated with consumption of AFs and OTA in contaminated sorghum. MOE was estimated by dividing the Benchmark dose limit (BDML) for aflatoxins (400ng/kg bw/day) (EFSA 2020; Adetunji et al. 2018) by the corresponding toxins exposure as represented in Equation (4). Likewise, the BMDL for OTA was reported to be 130ng/kg bw/week (JECFA/WHO 2001) and 120ng/kg bw/week (EFSA 2006), resulting in an average of 125ng/kg bw/week (equivalent to 17.86ng/kg bw/day)

Benchmark dose lower limit Estimated daily intake

A public health alert is triggered when the margin of Exposure (MOE) are less than 10,000 for both AFs and OTA.

***Estimated hepatocellular carcinoma risk due to consumption of sorghum***

The JECFA estimated cancer potency values for aflatoxins/ochratoxin A were adopted in determin-ing the annual burden and HCC incidence attrib-utable to AFs and OTA exposure from sorghum. The values which corresponded to 0.3 cases of cancer per 100,000 population annually, for each ng/kg bw/day, among populations infected with hepatitis B virus (HBsAgþ), and 30 times lesser (0.01 cases of cancer per 100,000 population per ng/kg bw/day) among people not infected were employed for this estimation (EFSA 2007). The HBsAgþprevalence rate used was 13.6% in Nigeria based on previous studies (Musa et al.

2015). Similarly, a recent report by the National HIV/AIDs Indicator and Impact Survey (NAIIS) highlighted that HBV prevalence in Nigeria was 8.1% and affirmed Nigeria populations to be approximately 190 million (Adeyinka et al. 2019). Hence, the annual HCC cases per 100,000 for HBsAgþ and HBsAg- individuals were estimated using Equation (5), whereas the annual HCC cases based on populations that are HBsAgþ and HBsAg- were estimated using Equation (6).

Annual HCC cases*=*100,000

¼ AFs*=*OTA EDI � Potency factor (5)

Annual HCC cases ¼ AFs*=*OTA EDI � Potency factor � N HBsAgþ or HBsAg−�

(6)

where *N*, represents prevalence rate multiplied by the total population of the individual (190 million).

Based on the prevalence of *HBsAg*þ (13.6% or 8.1%) individuals in Nigerian total population, the risk for liver cancer was estimated for differ-ent population groups consuming sorghum in Niger State using the relation presented in Equations (7) and (8), respectively.

Cancer potency ¼ 0*:*3 � Annual HCC cases HBsAgþ þ0*:*01 � Annual HCC cases ðHBsAg−Þ

(7)

HCC Population Risk ¼*EDI* � Cancer potency

(8)

**Results**

***Performance of HPLC and ELISA methods***

The LOD, LOQ, and mean recoveries of myco-toxins in spiked samples for the HPLC and ELISA methods are provided in [Supplementary](https://doi.org/10.1080/19440049.2023.2293998) [Tables S1 and S2,](https://doi.org/10.1080/19440049.2023.2293998) respectively. The recovery rates for the selected mycotoxins are within the allow-able limits of the recovery and RSD recom-mended by Codex and the AOAC International (AOAC 1995). The Codex recommends 60–120% recovery rates of mycotoxins and the guideline for the recoveries by AOAC is 70–125% and RSDr below 15% of mycotoxins (AOAC 1995; Codex Committee on Contaminants in Food

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2008). Correlation co-efficient (*r*2) for the calibra-tion curves indicated good linearity with *r*2 values ranging from 0.9018 to 0.9998 for the various mycotoxin standards. For the ELISA methods, repeatability and reproducibility results varied between 0.98–3.5% and 2.4–12%, respectively, for the concerned mycotoxins [(Supplementary Table](https://doi.org/10.1080/19440049.2023.2293998) [S2)](https://doi.org/10.1080/19440049.2023.2293998). The performance characteristics of the ELISA method also fell within the acceptable range stated in Commission Regulation No. 401/ 2006, for a method of sampling and analysis of official control of mycotoxins (EC 2006).

***Fungal colonisation of sorghum samples***

The results show no significant (*p>*.05) differ-ence in colony forming units (CFU) between white and red sorghum varieties and between

**Figure 1.** Levels of fungal colonisation (CFU/g) of sorghum samples (a) across the four micro-climatic zones (see text) and (b) categorised by sample location and sorghum type. Indicates zones that have significant differences (*p* ¼ .05). The dots, error bars and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively.

stored and market sorghum (Figure 1). The CFU in sorghum samples across the four micro-climatic zones is presented in decreasing order as follows: Zone 1 (13.00±5.98b � 106 CFU/g) *>* Zone 4 (5.00±1.73a � 106 CFU/g) � Zone 2 (4.75±1.25a � 106 CFU/g) ¼ Zone 3 (4.75±1.23a �106 CFU/g), meaning the wettest and driest zones had the most contamination (Figure 1). Based on the categoriza-tion of Gimeno (2002), in which samples can be categorized as good (CFU *<* 3�104 cfu/g), regular (count range between 3�104 and 7�104 cfu/g) and bad (*>* 7�104 cfu/g), the sorghum samples were found to fall in the bad category.

In the present study, a total of 159 fungi iso-lates belonging to four fungal genera namely *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* were isolated from sorghum samples (Table 1). In total, *Aspergillus* spp. was the most frequently isolated genera regardless of sample type, variety, or zone; with corresponding frequency values (out of 32 composite samples) of 23 (*A.flavus*), 18 (*A.fumigatus*), 16 (*A.oryzae*), 6 (*A.ustus*), 12 (*A.parasiticus*) and 6 (*A.niger*) respectively (Table 1). *Aspergillus* was followed by *Fusarium* spp with a total of 47/159 isolated fungi (*F.verticilloides* þ *F.nygamai* þ *F.semitectum* þ *F.solani* þ *F.oxysporum*), *Penicllium* spp at 24/ 159 (15.09%), and less frequent members of *Rhizopus* spp (7/159). In total, 15 species were found and of these, the dominant species in decreasing order were *A. flavus*, *A. fumigatus*, *F. verticillioides*, *A. oryazae*, *P. verrucosum*, *A. para-siticus* and *F. semitectum*. The other less common fungal contaminants were *F. nygamai*, *Rhizopus* spp, *A. ustus*, *A. niger*, *F. solani*, *P. expansum*, *F. oxysporum* with *P. griseofulvum* as the least occurring fungi species. In addition, *F. solani*, *P. griseofulvum* and *A. ustus* were not reported in the wet (Zone 2), dry (Zone 3) and driest (Zone 4) zones of the state. It is noteworthy that the extreme microclimatic conditions, wettest (46 spe-cies) and driest zones (40 species) registered higher fungal species than the moderate climatic conditions. Additionally, the mycological survey showed that none of the samples were free from fungal infestation with some samples having a high frequency of co-occurrence of between 3 and 12 fungal species [(Supplementary Table S3)](https://doi.org/10.1080/19440049.2023.2293998). The commonest forms of such multi-occurrence were

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the simultaneous occurrence of *A. flavus, A. fumi-gatus, F. verticillioides, A. oryzae*, *A. parasiticus* and *P.verrucosum.*

***Levels of mycotoxins in sorghum samples***

The findings in the present study show that sorghum samples were contaminated with mul-tiple mycotoxins (Figure 2; [Supplementary Table](https://doi.org/10.1080/19440049.2023.2293998)

[S4](https://doi.org/10.1080/19440049.2023.2293998)), in accordance with the fungal profile across the microclimatic zones. Overall, across all microclimatic zones, total aflatoxins (AFs) and OTA were the most found mycotoxins in the sor-ghum samples at the incidence of 100% and 72%, respectively, followed by FUM (56%), CPA (37%), DON (31%), NIV (25%) and ZEN (9%). With regards to levels of mycotoxin contamin-

**Table 1.** Distribution of isolated fungal species in sorghum samples from Niger State, arranged by sample location, varieties and zones, with occurrence rates in parenthesis.

Sample Places Sample Varieties Sample Location/Zones

Fungi Species

*A.flavus A.fumigatus A.oryzae A.ustus A.parasiticus A.niger*

*F. verticilloides F. nygamai F.semitectum F. solani*

*F. oxysporum P.expansum P.griseofulvum P.verrucosum Rhizopus spp.*

Market (*n* ¼ *16*)

9 (56.3) 10 (62.5) 8 (50.0) 2 (12.5) 5 (31.3) 4 (25.0) 8 (50.0) 6 (37.5) 8 (50.0) 4 (25.0) 2 (12.5) 2 (12.5) 1 (6.3)

8 (50.0) 3 (18.8)

Store (*n* ¼ *16*)

14 (87.5) 8 (50.0) 8 (50.0) 4 (25.0) 7 (43.8) 2 (12.5) 9 (56.3) 3 (18.8) 2 (12.5) 2 (12.5) 3 (18.8) 4 (25.0) 3 (18.8) 6 (37.5) 4 (25.0)

White (n ¼ 16)

13 (81.3) 9 (56.3) 9 (56.3) 4 (25.0) 7 (43.8) 1 (6.3) 11 (68.8) 4 (25.0) 7 (43.8) 3 (18.8) 1 (6.3)

2 (12.5) 2 (12.5) 6 (37.5) 2 (12.5)

Red (*n* ¼ *16*)

10 (62.5) 9 (56.3) 7 (43.8) 2 (12.5) 5 (31.3) 5 (31.3) 6 (37.5) 5 (31.3) 3 (18.8) 3 (18.8) 4 (25.0) 4 (25.0) 2 (12.5) 8 (50.0) 5 (31.3)

Zone 1 (*n* ¼ *8*)

5 (62.5) 4 (50.0) 5 (62.5) 2 (25.0) 4 (50.0) 2 (25.0) 4 (50.0) 3 (37.5) 2 (25.0) 3 (37.5) 2 (25.0) 3 (37.5) 1 (12.5) 3 (37.5) 3 (37.5)

Zone 2 (*n* ¼ *8*)

7 (87.5) 6 (75.0) 3 (37.5) 1 (12.5) 1 (12.5) 2 (25.0) 6 (75.0) 1 (12.5) 2 (25.0)

0 (0.00) 1 (12.5) 1 (12.5) 2 (25.0) 2 (25.0) 1 (12.5)

Zone 3 (*n* ¼ *8*)

5 (62.5) 3 (37.5) 2 (25.0) 3 (37.5) 3 (37.5) 1 (12.5) 4 (50.0) 3 (37.5) 2 (25.0) 1 (12.5) 1 (12.5) 1 (12.5)

0 (0.00) 6 (75.0) 2 (25.0)

Zone 4 (*n* ¼ *8*)

6 (75.0) 5 (62.5) 6 (75.0)

0 (0.00) 4 (50.0) 1 (12.5) 3 (37.5) 2 (25.0) 4 (50.0) 2 (25.0) 1 (12.5) 1 (12.5) 1 (12.5) 3 (37.5) 1 (12.5)

Total (*n* ¼*32*)

23 (71.9) 18 (56.3) 16 (50.0) 6 (18.8) 12 (37.5) 6 (18.8) 17 (53.1) 9 (28.1) 10 (31.3) 6 (18.8) 5 (15.6) 6 (18.8) 4 (12.5) 14 43.8) 7 (21.9)

**Figure 2.** Distribution of levels of mycotoxins in sorghum samples across the four microclimatic zones of Niger State (see text). Zones with different letters have significant differences (*p* ¼ .05). The dots, error bars and upper and lower ends of the box repre-sent outliers, spread, and first and third quartiles, respectively. ZEN is not included in the boxplots because ZEN was not detected in Zone 1 and only detected in one sample in the other zones.

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**Table 2.** Estimated annual burden of HCC cases and risk of HCC/year attributable to aflatoxin exposure from sorghum consumers

in HBsAgþ and HBsAg− Populations in Niger State, Nigeria.

Average concentration (� 103 ng/kg) Estimated daily Intake (EDI) (ng/kg bw/day)

Margin of Exposure (MOE)

Estimated Annual HCC (per 100,000) HBsAgþ

HBsAg−

Annual HCC cases (HBsAg Prevalence ¼ 13.6%) (�103) HBsAgþ

HBsAg−

HCC Risk/year (13.6%) (�105)

Annual HCC cases (HBsAg Prevalence ¼ 8.1%) (�103) HBsAgþ

HBsAg−

HCC Risk/year (8.1%) (�105)

AFB1

24.26 Male 126.55 Total Population 129.53 Female 124.07 Male 3.16 Total Population 3.09 Female 3.22 Male 37.97 Total Population 38.86 Female 37.22 Male 1.27 Total Population 1.3 Female 1.24 Male 9.81 Total Population 10.04 Female 9.62 Male 2.08 Total Population 2.13 Female 2.04 Male 3.75 Total Population 3.93 Female 3.61 Male 5.84 Total Population 5.98 Female 5.73 Male 2.21 Total Population 2.26 Female 2.17 Male 2.25 Total Population 2.35 Female 2.16

AFB2 AFG1

2.27 3.03 11.84 15.81 12.12 16.18 11.61 15.5 33.78 25.30 33.00 24.72 34.45 25.81

3.55 4.74 3.64 4.85 3.48 4.65 0.12 0.16 0.12 0.16 0.12 0.16 0.92 1.23 0.94 1.25 0.9 1.20 0.19 0.26 0.2 0.27 0.19 0.25 0.03 0.06 0.03 0.06 0.03 0.06 0.55 0.73 0.56 0.75 0.54 0.72 0.21 0.28 0.21 0.28 0.2 0.27 0.02 0.04 0.02 0.04 0.02 0.03

AFG2 AFs OTA

0.43 29.97 5.95 2.24 156.34 31.04 2.3 160.02 31.77 2.2 153.27 30.43 178.57 2.56 0.58 173.91 2.50 0.56 181.82 2.61 0.59 0.67 46.9 9.31 0.69 48.01 9.53 0.66 45.98 9.13 0.02 1.56 0.31 0.02 1.6 0.32 0.02 1.53 0.30 0.17 12.12 2.41 0.18 12.4 2.46 0.17 11.88 2.36 0.04 2.57 0.51 0.04 2.63 0.52 0.04 2.52 0.50 0.001 5.72 0.23 0.001 5.99 0.24 0.001 5.5 0.22 0.1 7.22 1.43 0.11 7.39 1.47 0.1 7.08 1.40 0.04 2.73 0.54 0.04 2.79 0.55 0.04 2.68 0.53 0.001 3.43 0.13 0.001 3.59 0.14 0.001 3.30 0.13

EDI: Estimated daily intake; TDI: Tolerable daily intake; %TDI: Percentage tolerable daily intake; HCC: Hepatocellular carcinoma.

ation in sorghum, the *Fusarium* mycotoxins, FUM (862.4±502.2mg/kg) were detected at the highest level, followed by the *Aspergillus* myco-toxins, AFs (30±15mg/kg). The others, ZEN (16±8mg/kg), CPA (9±3mg/kg), OTA (6±2mg/ kg), DON (3±1mg/kg) and NIV (0.6±0.1mg/kg) occurred in relatively low concentrations.

The aflatoxin profile of the studied sorghum followed the natural occurrence pattern with AFB1 (84%, 24±9mg/kg) and AFG1 (69%, 3±1mg/kg) occurring in higher frequency and levels than AFB2 (31%, 2±1mg/kg) and AFG2 (37%, 0.4±0.1mg/kg) and the concentration of AFB1 is less than that of total aflatoxins ([Table](https://doi.org/10.1080/19440049.2023.2293998) [S4)](https://doi.org/10.1080/19440049.2023.2293998). Additionally, 66% and 69% of all positive samples exceeded the 2mg/kg and 4mg/kg EU maximum regulatory limit for AFB1 and total aflatoxins, respectively, in all cereals (including sorghum) intended for direct human consump-tion (EC 2010). Further, 44% of FUM positive samples were contaminated at levels above the EU maximum regulatory limit (1000mg/kg) and 47% of the samples analysed were found to be contaminated with OTA at levels above the EU

maximum regulatory limit (5mg/kg) for cereal based food intended for direct human consump-tion [(Table S4)](https://doi.org/10.1080/19440049.2023.2293998). However, none of the sorghum samples, regardless of the sample type, variety, or location (zones) were found contaminated with DON above the EU maximum limit of 1250mg/kg and only 6% of the analysed samples were contaminated with ZEN above the EU max-imum limit of 100mg/kg for unprocessed cereals other than maize. Presently, no maximum limit has been fixed for NIV and CPA in foods and feeds.

Levels of CPA were significantly higher (*p<*.05) in the red sorghum variety than white sorghum variety, whereas AFB2 was detected at significantly higher (*p<*.05) concentrations in market samples than stored samples ([supplemen-tary Figure S1)](https://doi.org/10.1080/19440049.2023.2293998). Levels of FUMs and NIV were higher in Zone 1 (wettest) and Zone 4 (driest) than other zones, whereas levels of total aflatox-ins were lowest in Zones 3 (dry). There were no significant differences in levels of OTA, DON and CPA across the microclimatic zones. ZEN was not detected in zone 1 and was detected in

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**Table 3.** Risk characterisation from dietary exposure of DON, FUMs, NIV, ZEN, CPA and OTA from sorghum in Niger State, Nigeria.

Average concentration

Mycotoxins (� 103 ng/kg)

Estimated daily Intake (EDI) (ng/kg bw/day)

Male Total Population Female

TDI

(� 103 ng/kg)

Risk characterization (% TDI)

Male Total Population Female

DON 2.82 FUM 862.44 NIV 0.6 ZEN 16.43 CPA 8.84 OTA 5.95

14.71 4498.84 3.13 85.71 46.11 31.04

15.06 4604.77

3.2 87.72 47.2 31.77

14.42 4410.66 3.07 84.03 45.21 30.43

1 1.47 1.51 1.44 2 224.94 230.24 220.53 0.7 0.45 0.46 0.44 0.5 17.14 17.54 16.81 NA NA NA NA 0.014 221.71 226.93 217.36

one sample each from other zones (Figure 2; [Table S4](https://doi.org/10.1080/19440049.2023.2293998)). The mycotoxins analysed were found to co-occur in the samples. There were 18 different mycotoxin combinations observed and the commonest of them were the binary combin-ation of AFsþOTA and AFsþFUM. The other most frequently found mycotoxin co-occurrences were AFsþOTAþNIV, AFsþCPAþFUMþ OTA and AFsþCPAþDONþFUMþOTA ([Supplementary Table S5)](https://doi.org/10.1080/19440049.2023.2293998).

***Exposure estimates and risk characterization of multi-mycotoxins from sorghum***

The information derived from the questionnaire shows that sorghum is mostly consumed in the driest zone (Zone 4) of the State, at an average daily consumption of 329g/d. While the mean weight of the sampled population was 61.6kg, with an average weight of 64.3kg and 63.0kg for women and men, respectively. Sorghum is con-sumed in the study area as pap, *tuwo* (dough), *masa-waina* (slightly fermented and fried sor-ghum meal) and *burukutu* (local alcoholic drink). FUMs and AFs had the highest Estimated Daily Intake (EDI) levels (Tables 2 and 3). Generally, dietary intake of FUM and OTA were above the EU tolerable daily intake levels and therefore, these toxins pose the highest risk in the studied population as their %TDI is above the hundredth value. Presently, no tolerable daily intake (TDI) level has been set for aflatoxins in food and feeds. The others, ZEN, DON, and NIV had EDI and %TDI values below the EU regulated levels (Table 3).

The MOE values for aflatoxins range between 2.5 and 181.8, whereas MOE for OTA was calculated to be 0.56, 0.58 and 0.59 for the total population, male and female, respectively (Table

2). The risk of HCC for AFs and OTA were esti-mated based on two prevalence rates as presented in Table 2. Liver cancer risk for AFB1 (being the most potent of aflatoxins) was observed to be the highest among the overall population groups. Based on the results, annual HCC cases of 38.9, 48.0 and 9.5 per 100,000 persons is anticipated due to the intake of AFB1, AFs and OTA, respectively. At 13.6% HBsAg prevalence rate, an estimated 393,000, 599,000 and 24,000 new HCC cases are likely to occur annually in over 190 million population of Nigeria due to AFB1, AFs and OTA in the grain but if the HBsAg prevalence is assumed to be 8.1%, the figures will be 235,000, 359,000 and 14,000 cases respectively (Table 2). The estimated HCC cases due to afla-toxins and ochratoxin were higher in males than in females and obviously also higher in HBsAgþ than in HBsAg− populations (Table 2).

**Discussion**

The study provides evidence of the presence of fifteen fungal species belonging to four genera namely *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* in stored and marketed grains of the white and red sorghum varieties from the four microclimatic zones of Niger State, Nigeria. It also records the natural occurrence, concentra-tions, and associated risks of dietary exposure to AFs, CPA, DON, FUMs, NIV, OTA, and ZEN in the studied grain.

***Occurrence of fungal species and mycotoxins in sorghum samples***

All the fungal species and mycotoxins recorded in this investigation are common fungal and myco-toxin contaminants of sorghum from Nigeria and elsewhere around the world (Hussaini et al. 2009;

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Reddy et al. 2010; CAC 2012; Garba et al. 2017; Chilaka et al. 2017; Astoreca et al. 2019; Onyedum et al. 2020; Benkerroum 2020). The presence of these filamentous storage and field fungi (*Aspergillus*, *Fusarium* and *Penicillum*) and advanced decay fungi (*Rhizopus*) and their respect-ive mycotoxins in the sorghum samples is attrib-uted to (i) their predominance (particularly *A. flavus*) in soil which serves as a reservoir of inocu-lum for infestation of grains in the field (Nayak et al. 2019; Nishimwe et al. 2020; Peter Mshelia et al. 2020), and (ii) the favourable climatic condi-tions for proliferation and mycotoxin production. For this study, the tropical savannah climate of Niger State, with a mean annual temperature of 29.5�C, mean annual rainfall of 881mm, high relative humidity (average 73.1%) offers favourable conditions for fungal proliferation and mycotoxin production (Benkerroum 2020). In addition, the nutrient composition of sorghum; 73.8wt% starch and a substantial amount of protein (12.3%) with rich deposit of B-complex vitamins (niacin, ribo-flavin and pyridoxin), oil (3.6%) and moisture (11–12%) contents (FAO 1994) makes it an excel-lent substrate for fungal growth and mycotoxin production. Generally, high relative humidity and temperature are the most critical climatic risk factors for fungal colonisation of grains in the field, and during drying and storage (Battilani et al. 2008, 2013; Chauhan et al. 2016; Matumba et al. 2021). In this study, fungal load in the sam-ples also varied across the microclimatic zones, with the wettest (Zone 1) showing the highest contamination.

In this investigation, all the samples analysed were contaminated by aflatoxins at concentra-tions of up to 259mg/kg, with 69% having levels above EU legislated limits, whereas 47% and 44% of positive samples exceeded the EU regulatory levels for OTA (5mg/kg) and FUMs (1000mg/kg), respectively [(Table S4)](https://doi.org/10.1080/19440049.2023.2293998). The two trichothecenes extracted from the studied samples (DON and NIV) and ZEN occurred at low incidence rates and concentrations. DON and NIV were detected in 31% and 25% of the samples, respectively, with an EDI and %TDI much below EU regu-lated levels, whereas ZEN was detected in only 3 (of the 32) composite samples, with two of the samples contaminated with the toxin at levels

exceeding the EU legislated limit of 100mg/kg for unprocessed grain other than maize. CPA was detected in 37% occurrence, with an average con-centration of 8.8mg/kg in the 32 composite sam-ples analysed ([Table S4](https://doi.org/10.1080/19440049.2023.2293998)).

Our results on incidence rates and levels of aflatoxins agree with those reported earlier for sorghum from Niger Sate (Daneil et al. 2016; Onyedum et al. 2020), across Nigeria (Garba et al. 2017) and the globe (Astoreca et al. 2019). However, Hussaini et al. (2009) reported higher AFB1 levels of up to 1164mg/kg in 93 of 168 mouldy sorghum samples collected from Niger State. Similar incidence rates and contamination levels of sorghum with OTA were also reported by Gbashi et al. (2020) in samples from Nigeria. Garba et al. (2017) reported higher frequency (80%) and mean concentration (141mg/kg) in the market and store samples while Onyedum et al. (2020) observed a 90% incidence and lower con-centration range of between 1.4mg/kg and 5.6mg/ kg from Niger State. For FUMs, Onyedum et al. (2020) reported comparable levels, but at a higher incidence rate (100%, *n*¼20) of the toxin in sor-ghum samples from Niger State. However, lower incidence rates and concentrations of the toxins were reported in sorghum samples from Northern and Southern Nigeria (Chilaka et al. 2016; Gbashi et al. 2020), but higher levels (1170–1890mg/kg) were recorded from samples across the six agro-ecological zones of Nigeria (Garba et al. 2017). In addition, there are quite a few studies in agreement with our results of low incidence and levels of ZEN, DON, and NIV (Ediage et al. 2015; Chilaka et al. 2016; Gbashi et al. 2020). Garba et al. (2017) found ZEN at high frequency and concentration of up to 3092mg/kg from Nigerian grown sorghum that were kept for over a year before analysis. A pro-longed storage period exacerbates mycotoxin con-tamination (Garcia-Cela et al. 2019). The trichothecenes are associated with a temperate climate and hence their low incidence in unfavourable tropical climates such as in Nigeria. It is noteworthy that no DON and NIV pro-ducers were isolated in this work which might also account for their low prevalence in grain samples. There is a paucity of reports on the prevalence of CPA in sorghum. The two reports

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on the occurrence of CPA in Nigeria are in pea-nut at concentration between 9.6 and 114mg/kg (Onyeke 2020) and maize at levels between 0 and 417mg/kg (Ogara et al. 2017). Therefore, the cur-rent study seems to report for the first-time prevalence of CPA in sorghum in Nigeria at 37.5% occurrence and an average concentration of 8.84mg/kg in the 32 composite samples. The differences in the reported levels of contamin-ation and co-occurrences of aflatoxins, ochratoxin A, and fumonisins reflects growing season to sea-son variation and shows that the mycotoxin problem is persistent over time.

The co-occurrence of mycotoxins in sorghum, and other cereals, is in line with previous studies (Makun et al. 2011; Chilaka et al. 2016; Ogara et al. 2017; Onyedum et al. 2020), and results from colonization by multi-mycotoxin producing fungi and/or co-colonisation of a sample by sev-eral fungi species producing different mycotoxins. Several species of fungi isolated in our study are multi-mycotoxin producers; *A. flavus* (AFB1, AFB2 and CPA), *A. fumigatus* (gliotoxins and fumitremorgin), *A.* *niger* (OTA and FUM), *A.parasiticus* (AFB1, AFB2, AFG1 and AFG2), *A. oryzae* (melinolin), *A. ustus* (austacystins, versico-lous/sterigmatocystin), *F. verticillioides* (FUMs and moniliformin), *F. semitectum* (ZEN), *F. nyga-mai* (FUM), *F. solani* (fusarins and fusaric acid), *F. oxysporum* (T-2 and moniliformin), *P. verruco-sum* (OTA, citrinin, patulin and CPA), *P. expan-sum* (citrinin and patulin), *P.* *griseofulvum* (patulin and CPA) and *Rhizopus* (rhizonins and rhizoxins) (Ismaiel and Papenbrock 2015; Kostic et al. 2019).

***Public health risk from mycotoxins in sorghum***

The assessment of mycotoxin levels in sorghum from Niger State, Nigeria, has revealed critical insights into the potential public health risks associated with the consumption of this staple cereal. With observed EDI value of 31.8ng/kg/ bw/day as against a tolerable level of 14ng/kg and %TDI of 227 in the samples, the studied population are at risk of OTA poisoning. A %TDI of FUM (230) at levels exceeding estab-lished safety standards (Table 3) suggests that the population are exposed to over twice the tolerable

daily intake limit (2000ng/kg) *via* consumption of sorghum. For aflatoxins and ochratoxin A, all MOE values were below 10,000 indicating a potential health concern for neoplastic effect (EFSA 2020). Further, the EDI value for AFs and OTA (Table 2) were used to estimate the annual burden and HCC/year attributable to aflatoxins and ochratoxin A exposure from sorghum con-sumption. Overall, higher EDIs than reported in this study were reported for AFs and OTA in sorghum samples from Niger State (Onyedum et al. 2020) and across Nigeria’s six agroecological zones (Garba et al. 2017). Currently, there are no legislated maximum levels and TDI for CPA, however TDI values of 0.1lg/kg bw/day and 10lg/kg bw/day, were proposed by De Waal (2002) and Burdock and Flamm (2000), respect-ively. Our calculated EDI for CPA from sorghum (47ng/kg/bw/day which is equivalent to 0.047lg/ kg bw/day) is below the more stringent TDI of 0.1lg/kg bw/day suggesting that Nigerians are not at risk of CPA poisoning from consumption of sorghum.

As a staple, chronic contamination of sorghum with multiple mycotoxins poses a public health risk, which may be particularly serious for rural subsistence farming communities and children (Braun and Wink 2018). Aflatoxins especially AFB1 are highly carcinogenic (IARC 2002; McMillan 2018; FAO/WHO 2019) and their asso-ciation with increased HCC rates have been reported in China (Wu et al. 2013). Even EDI lev-els as low as 0.001lg/kg bw/day may induce liver cancer (American Cancer Society 2011). The result reported in this study lead to the conclu-sion that about 599,000 (13.6% prevalence) and 359, 000 (8.1% prevalence) cases of HCC annually are anticipated from the intake of aflatoxins in sorghum in Nigeria. Intake of such doses of aflatoxin could increase still-births and neonatal mortality, immunosuppression with increased sus-ceptibility to infectious diseases such as pneumo-nia, stunted growth, and HIV/AIDS (Smith et al. 2017; McMillan et al. 2018). Exposure to unsafe levels of OTA can lead to nephropathy and urin-ary tract tumours as the toxin has been classified as a group 2B possible human carcinogen by IARC (1993). Several studies have suggested that OTA poses teratogenic effects, implying that it

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could interfere with fatal development and increase the risk of congenital abnormalities if exposure occurs during pregnancy (Malir et al. 2013; Stoev 2022). There is evidence that OTA may be linked to the development of kidney tumours in animals, although the evidence in humans is less clear (Herman and Mantle 2017). OTA has also been reported to cause immuno-suppressive and hepatotoxic effect in humans and animals (Frangiamone et al. 2022). FUM elicit neurotoxicity, hepatotoxicity and nephrotoxicity in animals causing equine leukoencephalomalacia porcine pulmonary oedema in pigs, liver and kid-ney damage in laboratory rats, human oesopha-geal and liver cancers, and neural tube defects in human babies (Ezekiel et al. 2021). CPA is a cal-cium uptake disrupters which results in increased muscle contraction and the ‘kodua poisoning’ toxic syndrome in cattle and humans (Chang et al. 2009; Del Palacio and Pan 2020). Lastly, ZEN has been associated with gynecomastia with testicular atrophy in rural males in Southern Africa (Ndoro et al. 2022), precocious pubertal changes in children (Zain 2011) and general infer-tility in both human beings and animals (Ropejko and Twaruzek, 2021).

The mycotoxins frequently co-occurred in the sorghum samples considered in this study, which exacerbates the public health risk. AFs and FUMS act synergistically in cancer initiation and promotion (Di Paola et al. 2022). The combined additive effect of AFs and OTA have also been reported to have decreased cell viability in mon-key kidney (vero) cell, increased DNA fragmenta-tion and expression of p53 activation of the antiapoptotic factors bcl-2 protein (Taghizadeh et al. 2020). Zhou et al. (2017) demonstrated that the combination of AFB1 þ DON had a synergis-tic cytotoxicity effect on HepG2 and RAW cell lines, whilst Sun et al. (2017) showed that combi-nations of AFB1 þ ZEN, AFB1 þ DON, and AFB1 þ ZENþDON had synergetic toxic effects on BRL 3A rat liver cells. A significant synergis-tic cytotoxic effect, with about 26% loss in cell viability, was exerted by a ternary combination of AFB1þFB1þOTA at concentrations above EU limits in Madin-Darby Bovine kidney (MDBK) cells (Clarke et al. 2014).

In summary, our comprehensive study on mycotoxin levels in sorghum from Niger State, Nigeria, has highlighted the high presence of these toxins and their potential health impact *via* dietary exposure. Considering the high rate of contamin-ation of sorghum found in this work and consider-ing the public health and economic implications of mycotoxins, there is a need to deploy efficient and cost-effective mycotoxin mitigation strategies along the food value chain. Public enlightenment of farmers and traders on mycotoxins, and enforce-ment of regulatory limits on both local and imported products to reduce the hazards of myco-toxins are necessary. Research to elucidate the toxi-cological effects of the various mycotoxin combinations observed in this study particularly those with between three and six toxins on animals and human beings need to be conducted to under-stand their public health risks. The outcome of such an investigation will surely influence estab-lishing maximum limits that will take cognizance of the co-occurrence of mycotoxins. It is also appropriate to conduct longitudinal follow up studies to determine the association between diet-ary mycotoxin exposure and health/disease out-comes in African communities.

**Acknowledgements**

We appreciate the technical assistance from the Department of Animal Health, Northwest University, Mafikeng, South Africa. This work was partly funded by the Tertiary Education Fund (TETFUND), Nigeria (TETFUND/FUTMINNA/2019/ B7/16).

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Fatimah Omolola Badmos: Data collection and curation, investigation, methodology, validation, writing – original draft, writing – review & editing; Hadiza Lami Muhammad, Achi Dabara, Funmilola Adefolalu, Susan Salubuyi, Abdullahi Abdulkadir, Victor Tope Oyetunji, Daniel O Apeh, Hadiza Kudu Muhammad, Mulunda Mwanza: Conceptualization, methodology, resources, supervision, val-idation, writing – review & editing; Maurice Monjerezi, Limbikani Matumba: Validation, visualization, writing – review & editing.

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**Ethical approval**

All the experiments carried out in this work comply with the current laws of the federal republic of Nigeria and South Africa where the work was performed.

**Disclosure statement**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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