Food Additives & Contaminants: Part A

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tfac20

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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 (18 Dec 2023): Assessment of dietary exposure and levels of mycotoxins in sorghum from Niger State of Nigeria, Food Additives & Contaminants: Part A, DOI: 10.1080/19440049.2023.2293998

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

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https://doi.org/10.1080/19440049.2023.2293998

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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 dominant fungal species followed by *F. verticilloides*, *A. oryzae* and *P. verrucosum*. Aflatoxins (mean: 29.97 μg/kg) were detected in all samples, whereas OTA (mean: 37.5 μg/kg) and FUMs (mean: 3269.8 μg/kg) were detected in 72% and 50% of the samples, respectively. Mycotoxins frequently co-occurred in binary mixtures of AFs + OTA and AFs + FUMs. 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 exposure to AFs and OTA. The Risk of hepatocellular carcinoma cases (HCC/year) attributable to AFs and OTA exposure from sorghum was estimated to be 5.99×10^5 and 0.24×10^5 cases for HBsAg + individuals based on 13.6% HBV incidence. Similarly, the HCC/year for AFs and OTA were assessed to be 3.59×10^5 and 0.14×10^5 at an 8.1% prevalence rate. Therefore, the results of this study demonstrate the high prevalence and dietary exposure to mycotoxins 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 metabolites, 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 mycotoxins worldwide (Eskola et al. 2020). There are many known mycotoxins but the ones that are significant, with regards to public health and international trade include aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), cyclopiazonic acid (CPA) and fumonisins (FUMs) (Dumitru et al. 2019; Alam et al. 2022).

Aflatoxin B1 (AFB_1), 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 associated 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.

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, abortions, vomiting, haemorrhaging of internal organs, immunosuppression, and blood disorder in humans and animals (Singh and Kumari 2022). The economically important trichothecenes 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 leukoencephalomalacia 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 (FB_1) 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 apoptosis in cultured human cells and in rat kidneys and are now recognised as possible human carcinogens (Group 2B) as classified by IARC (IARC 2002; Malir et al. 2023). ZEN, being a non-steroidal 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 possible 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, dehydration, 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 disease, along with aflatoxins and Koduo millet poisoning syndrome in Northern India (Kumar et al. 2020).

Sorghum is a drought resistant crop indigenous 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 sorghum 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 substrate for the growth of various species of fungi and therefore it is disposed to mycotoxin contamination (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 presence of AFB_1, 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 sorghum 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 dietary 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 contamination 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,

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 associated with sorghum consumption.

Materials and methods

Sample collection

Niger State is in Central Nigeria and the largest State in the country with 76,363 km^2 (29, 484 sq 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 1400 mm, wet and dry zone having an annual rainfall between 1200–1400 mm and 1000–1200 mm respectively, and the driest zone having annual rainfall of less than 1000 mm (Muhammad et al. 2019).

A total of 240 samples of sorghum were collected 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): 1 kg of each sample was collected, labelled, packaged in polythene bags, and taken to the laboratory. The samples were pooled together to form thirty-two (32) composite samples, 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 (driest 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 0.5–1 mm 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 sample (1 g) was weighed into a sterile tube and suspended in ringer solution (9 mL) and shaken for 2 min on a vortex mixer. The suspension was serially diluted 10 fold. Aliquots (1 mL) of each tube from the 10^6 diluents were used for inoculation in triplicate using potato dextrose agar (PDA). The first set of the media was incubated at 28 ± 2 °C for 3–5 d, intended for the enumeration of *Aspergillus species*, while the second set was incubated for 7 d at 25 °C for the enumeration 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).

$$\text{CFU/g} = \frac{\text{Number of colonies}}{\text{Dilution factor} \times \text{plating volumes (ml)}} \quad (1)$$

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 12.5 g of previously milled sorghum sample was placed in a 100 ml conical flask and extracted with 50 ml of phosphate buffered saline (PBS) using an orbit shaker operated at 200 rpm for 30 min. The supernatant (first extract) was collected and centrifuged at an RCF value of 1693 g for 15 min using a Thermo fisher scientific centrifuge (Fiberlite F9-4x1000y, rotor radius; 16.8 cm) and then filtered through a glass microfiber filter. Afterwards, 35 ml 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 15 min and subsequently centrifuged at 3810 g (RCF) at 4 °C. Then, 20 ml of the resultant extract was diluted with 90 ml PBS and filtered through a glass microfiber filter (second extract). Firstly, 50 ml of the second extract was passed through a

VICAM Myco6in1™ column at 0.2 ml drops/s, and the column was washed with 25 ml PBS to eliminate methanol residues before adding 5 ml of the 'first' extract. A subsequent washing step was followed with 30 ml distilled de-ionized water to completely remove PBS residue and matrix interfering compounds. The toxins were eluted with 3 ml methanol added in two steps (0.1 ml each at one drop/s) with 5 min 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: 0.05 ml of acetonitrile/0.01 ml 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 applicability of the chosen method allows a broad-spectrum detection for a wide range of compounds, making it suitable for analysing multiple mycotoxins 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 420 Pa × 1 ml/ml (AFs), 420 Pa × 0.8 ml/ml (DON/NIV) and 420 Pa × 0.6 ml/ml (CPA), for separation and quantification of selected mycotoxins and internal standards. An injection volume of 20 µl was used for the prepared samples 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 0.4 mM aqueous $ZnSO_4.7H_2O$: methanol (70:30 v/v) for CPA. AFs were detected at a wavelength of 365 nm, DON and NIV at 220 nm, and CPA was detected at 248 nm. 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 sorghum samples following method described herein. The method was validated in terms of linearity (quantification), accuracy (recovery) and sensitivity (limit of detection, LOD and limit of quantification, LOQ) using blank matrices of sorghum, 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 dilutions, as presented in Supplementary Table S1, into the HPLC column. Linear calibration curves generated for the mycotoxin standards were considered satisfactory when correlation coefficients (r^2) were greater than 0.90.

Apparent recovery experiments were carried out in duplicates by spiking five least contaminated samples (5 g of each) with 100 µL of the analyte standard of known concentration. The spiked samples were left overnight in a fume cupboard 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 mycotoxin extraction method described above. From each spiked sample, 20 µL 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 mycotoxin standard. Recovery was calculated by taking the percentage of the concentration (peak area) measured from the spiked sample per toxin concentration used for spiking the sample. The sensitivity parameters (i.e. LOD and LOQ) for mycotoxins in the sorghum samples were calculated 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: AgraQuant® Ochratoxin A (COKAQ2000), AgraQuant® Fumonisin (COKAQ3000) and AgraQuant® Zearalenone (COKAQ5000). Extraction of fumonisins, ochratoxin A and zearalenone from sorghum samples was carried out following the procedure recommended by the manufacturer. A known mass (10 g each) of the milled samples was weighed into labelled conical flasks. About 20 mL of the mixture of methanol and water

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

Approximately 100 µL of a standard solution or prepared sample (as above) was added into appropriate wells of microtiter plate, and 200 µL 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 incubated for 15 min at room temperature. The content 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 striking the plate against an absorbent paper towel. Subsequently, 100 µL of substrate solutions was pipetted into each well. This was mixed gently by shaking the plate manually and incubated for 5 min at 25 °C. The reaction was allowed to develop in the dark and the plate was not subjected to temperature fluctuations. Lastly, 100 μL of stop solution (0.18 M of H_2SO_4) 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 microplate reader set to 450 nm and 630 nm differential filter. All samples, controls, and standards were assayed in duplicate.

The range of quantification and detection limits (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 deviations of uncontaminated samples spiked at 20 µg/kg for OTA, 250 µg/kg for FUM and 300 µg/kg for ZEN (Table S2). 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 20–60 years. 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 Rodríguez-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).

$$EDI_m = \frac{c_m \times k}{b_w} \tag{2}$$

where EDI_m is the estimated daily intake (ng/kg bw/day) for mycotoxin m; c_m is the average level of a mycotoxin present in a sample (ng/kg); k is the daily consumption of sorghum (kg/day); b_w 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 328.79 g/person/day (0.32879 kg/person/day) as estimated from the questionnaire and then divided by mean body weight of 63.03, 64.29 and 61.58 kg for adult male, adult female, and total population groups, respectively.

Risk characterisation

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

$$\%TDI_m = \frac{EDI_m}{TDI} \times 100 \tag{3}$$

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 (400 ng/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 130 ng/kg bw/week (JECFA/WHO 2001) and 120 ng/kg bw/week (EFSA 2006), resulting in an average of 125 ng/kg bw/week (equivalent to 17.86 ng/kg bw/day)

$$\text{MOE} = \frac{\text{Benchmark dose lower limit}}{\text{Estimated daily intake}} \tag{4}$$

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 determining the annual burden and HCC incidence attributable 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 (HBsAg+), 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 HBsAg + prevalence 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 $HBsAg^+$ and $HBsAg^-$ individuals were estimated using Equation (5), whereas the annual HCC cases based on populations that are $HBsAg^+$ and $HBsAg^-$ were estimated using Equation (6).

$$\text{Annual HCC cases}/100,000 = \text{AFs/OTA EDI} \times \text{Potency factor} \tag{5}$$

$$\text{Annual HCC cases} = \frac{\text{AFs/OTA EDI} \times \text{Potency factor}}{100,000} \times \text{N}\left(HBsAg^+ \text{ or } HBsAg^-\right) \tag{6}$$

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

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

$$\text{Cancer potency} = 0.3 \times \text{Annual HCC cases}\left(HBsAg^+\right) + 0.01 \times \text{Annual HCC cases}\left(HBsAg^-\right) \tag{7}$$

$$\text{HCC Population Risk} = EDI \times \text{Cancer potency} \tag{8}$$

Results

Performance of HPLC and ELISA methods

The LOD, LOQ, and mean recoveries of mycotoxins in spiked samples for the HPLC and ELISA methods are provided in Supplementary Tables S1 and S2, respectively. The recovery rates for the selected mycotoxins are within the allowable limits of the recovery and RSD recommended 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

2008). Correlation co-efficient (r^2) for the calibration 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 S2). 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 ($p > .05$) difference in colony forming units (CFU) between white and red sorghum varieties and between 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 ($13.00 \pm 5.98^{b} \times 10^{6}$ CFU/g) > Zone 4 ($5.00 \pm 1.73^{a} \times 10^{6}$ CFU/g) ≈ Zone 2 ($4.75 \pm 1.25^{a} \times 10^{6}$ CFU/g) = Zone 3 ($4.75 \pm 1.23^{a} \times 10^{6}$ CFU/g), meaning the wettest and driest zones had the most contamination (Figure 1). Based on the categorization of Gimeno (2002), in which samples can be categorized as good (CFU $< 3 \times 10^{4}$ cfu/g), regular (count range between 3×10^{4} and 7×10^{4} cfu/g) and bad ($> 7 \times 10^{4}$ cfu/g), the sorghum samples were found to fall in the bad category.

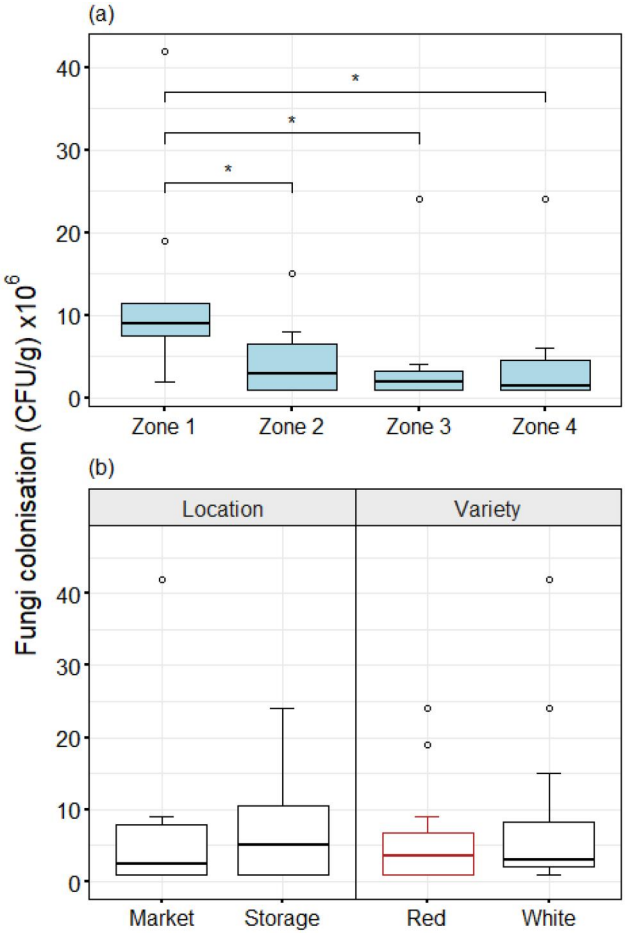

Figure 1. Levels of fungal colonisation (CFU/g) of sorghum samples (a) across the four micro-climatic zones (see text) and (b) categorised by sample location and sorghum type. * Indicates zones that have significant differences ($p = .05$). The dots, error bars and upper and lower ends of the box represent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

In the present study, a total of 159 fungi isolates 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* + *F.nygamai* + *F.semitectum* + *F.solani* + *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. oryzae*, *P. verrucosum*, *A. parasiticus* 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 species) 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). The commonest forms of such multi-occurrence were

the simultaneous occurrence of *A. flavus, A. fumigatus, 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 multiple mycotoxins (Figure 2; Supplementary Table S4), 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 sorghum 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

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	Market (*n = 16*)	Store (*n = 16*)	White (n = 16)	Red (*n = 16*)	Zone 1 (*n = 8*)	Zone 2 (*n = 8*)	Zone 3 (*n = 8*)	Zone 4 (*n = 8*)	Total (*n = 32*)
A.flavus	9 (56.3)	14 (87.5)	13 (81.3)	10 (62.5)	5 (62.5)	7 (87.5)	5 (62.5)	6 (75.0)	23 (71.9)
A.fumigatus	10 (62.5)	8 (50.0)	9 (56.3)	9 (56.3)	4 (50.0)	6 (75.0)	3 (37.5)	5 (62.5)	18 (56.3)
A.oryzae	8 (50.0)	8 (50.0)	9 (56.3)	7 (43.8)	5 (62.5)	3 (37.5)	2 (25.0)	6 (75.0)	16 (50.0)
A.ustus	2 (12.5)	4 (25.0)	4 (25.0)	2 (12.5)	2 (25.0)	1 (12.5)	3 (37.5)	0 (0.00)	6 (18.8)
A.parasiticus	5 (31.3)	7 (43.8)	7 (43.8)	5 (31.3)	4 (50.0)	1 (12.5)	3 (37.5)	4 (50.0)	12 (37.5)
A.niger	4 (25.0)	2 (12.5)	1 (6.3)	5 (31.3)	2 (25.0)	2 (25.0)	1 (12.5)	1 (12.5)	6 (18.8)
F. verticilloides	8 (50.0)	9 (56.3)	11 (68.8)	6 (37.5)	4 (50.0)	6 (75.0)	4 (50.0)	3 (37.5)	17 (53.1)
F. nygamai	6 (37.5)	3 (18.8)	4 (25.0)	5 (31.3)	3 (37.5)	1 (12.5)	3 (37.5)	2 (25.0)	9 (28.1)
F.semitectum	8 (50.0)	2 (12.5)	7 (43.8)	3 (18.8)	2 (25.0)	2 (25.0)	2 (25.0)	4 (50.0)	10 (31.3)
F. solani	4 (25.0)	2 (12.5)	3 (18.8)	3 (18.8)	3 (37.5)	0 (0.00)	1 (12.5)	2 (25.0)	6 (18.8)
F. oxysporum	2 (12.5)	3 (18.8)	1 (6.3)	4 (25.0)	2 (25.0)	1 (12.5)	1 (12.5)	1 (12.5)	5 (15.6)
P.expansum	2 (12.5)	4 (25.0)	2 (12.5)	4 (25.0)	3 (37.5)	1 (12.5)	1 (12.5)	1 (12.5)	6 (18.8)
P.griseofulvum	1 (6.3)	3 (18.8)	2 (12.5)	2 (12.5)	1 (12.5)	2 (25.0)	0 (0.00)	1 (12.5)	4 (12.5)
P.verrucosum	8 (50.0)	6 (37.5)	6 (37.5)	8 (50.0)	3 (37.5)	2 (25.0)	6 (75.0)	3 (37.5)	14 43.8)
Rhizopus spp.	3 (18.8)	4 (25.0)	2 (12.5)	5 (31.3)	3 (37.5)	1 (12.5)	2 (25.0)	1 (12.5)	7 (21.9)

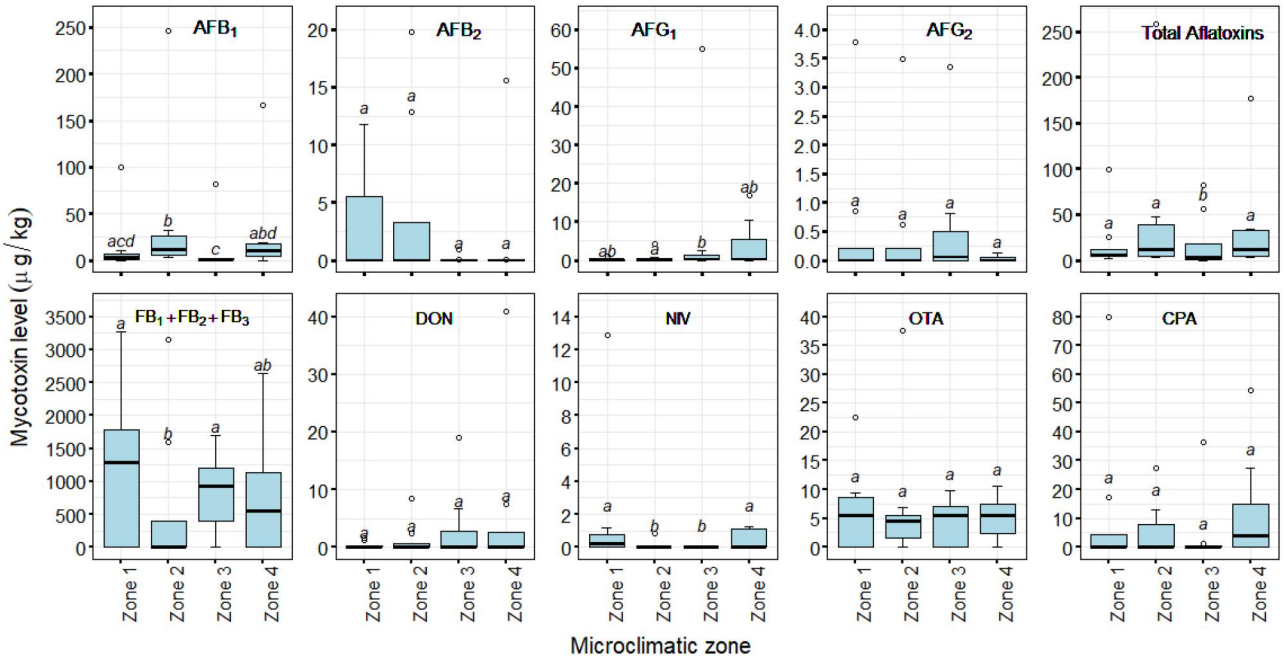

Figure 2. Distribution of levels of mycotoxins in sorghum samples across the four microclimatic zones of Niger State (see text). Zones with different letters have significant differences ($p = .05$). The dots, error bars and upper and lower ends of the box represent outliers, spread, and first and third 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.

Table 2. Estimated annual burden of HCC cases and risk of HCC/year attributable to aflatoxin exposure from sorghum consumers in $HBsAg^+$ and $HBsAg^-$ Populations in Niger State, Nigeria.

			AFB_1	AFB_2	AFG_1	AFG_2	AFs	OTA
Average concentration ($\times 10^3$ ng/kg)			24.26	2.27	3.03	0.43	29.97	5.95
Estimated daily Intake (EDI) (ng/kg bw/day)		Male	126.55	11.84	15.81	2.24	156.34	31.04
		Total Population	129.53	12.12	16.18	2.3	160.02	31.77
		Female	124.07	11.61	15.5	2.2	153.27	30.43
Margin of Exposure (MOE)		Male	3.16	33.78	25.30	178.57	2.56	0.58
		Total Population	3.09	33.00	24.72	173.91	2.50	0.56
		Female	3.22	34.45	25.81	181.82	2.61	0.59
Estimated Annual HCC (per 100,000)	$HBsAg^+$	Male	37.97	3.55	4.74	0.67	46.9	9.31
		Total Population	38.86	3.64	4.85	0.69	48.01	9.53
		Female	37.22	3.48	4.65	0.66	45.98	9.13
	$HBsAg^-$	Male	1.27	0.12	0.16	0.02	1.56	0.31
		Total Population	1.3	0.12	0.16	0.02	1.6	0.32
		Female	1.24	0.12	0.16	0.02	1.53	0.30
Annual HCC cases (HBsAg Prevalence = 13.6%) ($\times 10^3$)	$HBsAg^+$	Male	9.81	0.92	1.23	0.17	12.12	2.41
		Total Population	10.04	0.94	1.25	0.18	12.4	2.46
		Female	9.62	0.9	1.20	0.17	11.88	2.36
	$HBsAg^-$	Male	2.08	0.19	0.26	0.04	2.57	0.51
		Total Population	2.13	0.2	0.27	0.04	2.63	0.52
		Female	2.04	0.19	0.25	0.04	2.52	0.50
HCC Risk/year (13.6%) ($\times 10^5$)		Male	3.75	0.03	0.06	0.001	5.72	0.23
		Total Population	3.93	0.03	0.06	0.001	5.99	0.24
		Female	3.61	0.03	0.06	0.001	5.5	0.22
Annual HCC cases (HBsAg Prevalence = 8.1%) ($\times 10^3$)	$HBsAg^+$	Male	5.84	0.55	0.73	0.1	7.22	1.43
		Total Population	5.98	0.56	0.75	0.11	7.39	1.47
		Female	5.73	0.54	0.72	0.1	7.08	1.40
	$HBsAg^-$	Male	2.21	0.21	0.28	0.04	2.73	0.54
		Total Population	2.26	0.21	0.28	0.04	2.79	0.55
		Female	2.17	0.2	0.27	0.04	2.68	0.53
HCC Risk/year (8.1%) ($\times 10^5$)		Male	2.25	0.02	0.04	0.001	3.43	0.13
		Total Population	2.35	0.02	0.04	0.001	3.59	0.14
		Female	2.16	0.02	0.03	0.001	3.30	0.13

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

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

The aflatoxin profile of the studied sorghum followed the natural occurrence pattern with AFB_1 (84%, 24 ± 9 µg/kg) and AFG_1 (69%, 3 ± 1 µg/kg) occurring in higher frequency and levels than AFB_2 (31%, 2 ± 1 µg/kg) and AFG_2 (37%, 0.4 ± 0.1 µg/kg) and the concentration of AFB_1 is less than that of total aflatoxins (Table S4). Additionally, 66% and 69% of all positive samples exceeded the 2 µg/kg and 4 µg/kg EU maximum regulatory limit for AFB_1 and total aflatoxins, respectively, in all cereals (including sorghum) intended for direct human consumption (EC 2010). Further, 44% of FUM positive samples were contaminated at levels above the EU maximum regulatory limit (1000 µg/kg) and 47% of the samples analysed were found to be contaminated with OTA at levels above the EU maximum regulatory limit (5 µg/kg) for cereal based food intended for direct human consumption (Table S4). 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 1250 µg/kg and only 6% of the analysed samples were contaminated with ZEN above the EU maximum limit of 100 µg/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 AFB_2 was detected at significantly higher ($p < .05$) concentrations in market samples than stored samples (supplementary Figure S1). Levels of FUMs and NIV were higher in Zone 1 (wettest) and Zone 4 (driest) than other zones, whereas levels of total aflatoxins 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

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

Mycotoxins	Average concentration ($\times 10^3$ ng/kg)	Estimated daily Intake (EDI) (ng/kg bw/day)			TDI ($\times 10^3$ ng/kg)	Risk characterization (% TDI)		
		Male	Total Population	Female		Male	Total Population	Female
DON	2.82	14.71	15.06	14.42	1	1.47	1.51	1.44
FUM	862.44	4498.84	4604.77	4410.66	2	224.94	230.24	220.53
NIV	0.6	3.13	3.2	3.07	0.7	0.45	0.46	0.44
ZEN	16.43	85.71	87.72	84.03	0.5	17.14	17.54	16.81
CPA	8.84	46.11	47.2	45.21	NA	NA	NA	NA
OTA	5.95	31.04	31.77	30.43	0.014	221.71	226.93	217.36

one sample each from other zones (Figure 2; Table S4). 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 combination of AFs + OTA and AFs + FUM. The other most frequently found mycotoxin co-occurrences were AFs + OTA + NIV, AFs + CPA + FUM + OTA and AFs + CPA + DON + FUM + OTA (Supplementary Table S5).

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 329 g/d. While the mean weight of the sampled population was 61.6 kg, with an average weight of 64.3 kg and 63.0 kg for women and men, respectively. Sorghum is consumed in the study area as pap, *tuwo* (dough), *masa-waina* (slightly fermented and fried sorghum 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 estimated based on two prevalence rates as presented in Table 2. Liver cancer risk for AFB_1 (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 AFB_1, 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 AFB_1, 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 aflatoxins and ochratoxin were higher in males than in females and obviously also higher in $HBsAg^+$ 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, concentrations, 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 mycotoxin contaminants of sorghum from Nigeria and elsewhere around the world (Hussaini et al. 2009;

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 respective mycotoxins in the sorghum samples is attributed to (i) their predominance (particularly *A. flavus*) in soil which serves as a reservoir of inoculum 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 conditions 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 881 mm, high relative humidity (average 73.1%) offers favourable conditions for fungal proliferation and mycotoxin production (Benkerroum 2020). In addition, the nutrient composition of sorghum; 73.8 wt% starch and a substantial amount of protein (12.3%) with rich deposit of B-complex vitamins (niacin, riboflavin and pyridoxin), oil (3.6%) and moisture (11–12%) contents (FAO 1994) makes it an excellent 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 samples 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 concentrations of up to 259 µg/kg, with 69% having levels above EU legislated limits, whereas 47% and 44% of positive samples exceeded the EU regulatory levels for OTA (5 µg/kg) and FUMs (1000 µg/kg), respectively (Table S4). 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 regulated 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 100 µg/kg for unprocessed grain other than maize. CPA was detected in 37% occurrence, with an average concentration of 8.8 µg/kg in the 32 composite samples analysed (Table S4).

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 AFB_1 levels of up to 1164 µg/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 (141 µg/kg) in the market and store samples while Onyedum et al. (2020) observed a 90% incidence and lower concentration range of between 1.4 µg/kg and 5.6 µg/kg from Niger State. For FUMs, Onyedum et al. (2020) reported comparable levels, but at a higher incidence rate (100%, $n = 20$) of the toxin in sorghum 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 (1170–1890 µg/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 3092 µg/kg from Nigerian grown sorghum that were kept for over a year before analysis. A prolonged storage period exacerbates mycotoxin contamination (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 producers 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

on the occurrence of CPA in Nigeria are in peanut at concentration between 9.6 and 114 µg/kg (Onyeke 2020) and maize at levels between 0 and 417 µg/kg (Ogara et al. 2017). Therefore, the current study seems to report for the first-time prevalence of CPA in sorghum in Nigeria at 37.5% occurrence and an average concentration of 8.84 µg/kg in the 32 composite samples. The differences in the reported levels of contamination and co-occurrences of aflatoxins, ochratoxin A, and fumonisins reflects growing season to season 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 several fungi species producing different mycotoxins. Several species of fungi isolated in our study are multi-mycotoxin producers; *A. flavus* (AFB_1, AFB_2 and CPA), *A. fumigatus* (gliotoxins and fumitremorgin), *A. niger* (OTA and FUM), *A.parasiticus* (AFB_1, AFB_2, AFG_1 and AFG_2), *A. oryzae* (melinolin), *A. ustus* (austacystins, versicolous/sterigmatocystin), *F. verticillioides* (FUMs and moniliformin), *F. semitectum* (ZEN), *F. nygamai* (FUM), *F. solani* (fusarins and fusaric acid), *F. oxysporum* (T-2 and moniliformin), *P. verrucosum* (OTA, citrinin, patulin and CPA), *P. expansum* (citrinin and patulin), *P. griseofulvum* (patulin and CPA) and *Rhizopus* (rhizonins and rhizoxins) (Ismaiel and Papenbrock 2015; Kostić 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 31.8 ng/kg/bw/day as against a tolerable level of 14 ng/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 established safety standards (Table 3) suggests that the population are exposed to over twice the tolerable daily intake limit (2000 ng/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 consumption. 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 0.1 µg/kg bw/day and 10 µg/kg bw/day, were proposed by De Waal (2002) and Burdock and Flamm (2000), respectively. Our calculated EDI for CPA from sorghum (47 ng/kg/bw/day which is equivalent to 0.047 µg/kg bw/day) is below the more stringent TDI of 0.1 µg/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 AFB_1 are highly carcinogenic (IARC 2002; McMillan 2018; FAO/WHO 2019) and their association with increased HCC rates have been reported in China (Wu et al. 2013). Even EDI levels as low as 0.001 µg/kg bw/day may induce liver cancer (American Cancer Society 2011). The result reported in this study lead to the conclusion 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 susceptibility to infectious diseases such as pneumonia, stunted growth, and HIV/AIDS (Smith et al. 2017; McMillan et al. 2018). Exposure to unsafe levels of OTA can lead to nephropathy and urinary 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

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 immunosuppressive 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 kidney damage in laboratory rats, human oesophageal and liver cancers, and neural tube defects in human babies (Ezekiel et al. 2021). CPA is a calcium 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 infertility in both human beings and animals (Ropejko and Twarużek, 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 monkey kidney (vero) cell, increased DNA fragmentation 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 AFB_1 + DON had a synergistic cytotoxicity effect on HepG2 and RAW cell lines, whilst Sun et al. (2017) showed that combinations of AFB_1 + ZEN, AFB_1 + DON, and AFB_1 + ZEN + DON had synergetic toxic effects on BRL 3 A rat liver cells. A significant synergistic cytotoxic effect, with about 26% loss in cell viability, was exerted by a ternary combination of AFB_1+FB_1+OTA 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 contamination of sorghum found in this work and considering 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 enforcement of regulatory limits on both local and imported products to reduce the hazards of mycotoxins are necessary. Research to elucidate the toxicological 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 understand their public health risks. The outcome of such an investigation will surely influence establishing 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 dietary mycotoxin exposure and health/disease outcomes 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).

Authors contributions

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, validation, writing – review & editing; Maurice Monjerezi, Limbikani Matumba: Validation, visualization, writing – review & editing.

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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References

Abbas HK, Yoshizawa T, Shier WT. 2013. Cytotoxicity and phytotoxicity of trichothecenemycotoxins produced by *Fusarium* spp. Toxicon. 74:68–75. doi: 10.1016/j.toxicon.2013.07.026.

Abia WA, Warth B, Sulyok M, Krska R, Tchana AN, Njobeh PB, Dutton MF, Moundipa PF. 2013. Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). Food Control. 31(2):438–453. doi: 10.1016/j.foodcont.2012.10.006.

ACS (American Cancer Society). 2011. Cancer facts and figures 2011. Atlanta. ACS. http://www.cancer.org.>damPDF.

Adetunji MC, Alika OP, Awa NP, Atanda OO, Mwanza M. 2018. Microbiological quality and risk assessment for aflatoxins in groundnuts and roasted cashew nuts meant for human consumption. J Toxicol. 2018:1308748–1308811. doi: 10.1155/2018/1308748.

Adeyinka DA, Olakunde BO, Oladimeji O, Ezeanolue EE. 2019. HIV indicator and impact survey, consideration for Nigeria. Lancet Hiv. 6(6):e348–e350. doi: 10.1016/S2352-3018(19)30153-5.

Alam S, Nisa S, Daud S. 2022. Mycotoxins in environment and its health implications. In: Ahmed T, Hashmi MZ, editors. Hazardous environmental micro-pollutants, health impacts and allied treatment technologies. Cham: Springer International Publishing. p. 289–318.

Amir M, Shahzad A, Faraz A, Sajid M, Afzal K, Naeem I, IA, Mumtaz Z. 2022. Health effects of aflatoxins in fetus, infants, and children. In: Aflatoxins in Food: a Recent Perspective. Cham: springer International Publishing. p. 99–122.

AOAC (Association of Official Analytical Chemist). 1995. Sub-committee on feed additives and contaminants. Rockville (MD): AOAC. p. 41–42.

Arumugam T, Ghazi T, Abdul NS, Chuturgoon AA. 2021. A review on the oxidative effects of the fusariotoxins: fumonisin B1 and fusaric acid. Toxicology. 19:181–190.

Astoreca AL, Emateguy LG, Alconada TM. 2019. Fungal contamination and mycotoxins associated with sorghum crop: its relevance today. Eur J Plant Pathol. 155(2):381–392. doi: 10.1007/s10658-019-01797-w.

Atanda O, Makun HA, Ogara IM, Edema M, Idahor KO, Eshiett ME, Oluwabamiwo BF. 2013. Fungal and mycotoxin contamination of Nigerian foods and feeds. In: Makun HA, edtior. Mycotoxin and food safety in developing countries. London (UK): IntechOpen Limited; p. 3–38. doi: 10.5772/3414.

Battilani P, Barbano C, Piva G. 2008. Aflatoxin B1 contamination in maize related to the aridity index in North Italy. World Mycotoxin J. 1: 449–456. doi: 10.3920/wmj2008.x043.

Battilani P, Leggieri MC, Rossi V, Giorni P. 2013. AFLA-maize, a mechanistic model for Aspergillus flavus infection and aflatoxin B1 contamination in maize. Comput Electron Agric. 94:38–46. doi: 10.1016/j.compag.2013.03.005.

Benkerroum N. 2020. Aflatoxins: producing-molds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African Countries. IJERPH. 17(4):1215. doi: 10.3390/ijerph17041215.

Braun MS, Wink M. 2018. Exposure, occurrence, and chemistry of fumonisins and their cryptic derivatives. Compr Rev Food Sci Food Saf. 17(3):769–791. doi: 10.1111/1541-4337.12334.

Burdock GA, Flamm WG. 2000. Safety assessment of the mycotoxincyclopiazonic acid. Int J Toxicol. 19(3):195–218. doi: 10.1080/10915810050074964.

CAC (Codex Committee on Contaminants in Food). 2012. Discussion paper on fungi and mycotoxins in sorghum. Rome (Italy): FAO, WHO.

Casquete R, Benito MJ, Aranda E, Martín A, Hernández A, de Guía Córdoba M. 2021. Cyclopiazonic acid gene expression as strategy to minimizing mycotoxin contamination in cheese. Fungal Biol. 125(2):160–165. doi: 10.1016/j.funbio.2019.06.011.

CAST (Council for Agriculture Science and Technology). 2003. Mycotoxins: risks in plant, animal, and human systems. Task Force Report Number 139. Ames, Iowa.

Chang CH, Yu FY, Wang LT, Lin YS, Liu BH. 2009. Activation of ERK and JNK signaling pathways by mycotoxincitrinin in human cells. Toxicol Appl Pharmacol. 237(3):281–287. doi: 10.1016/j.taap.2009.03.021.

Chauhan NM, Washe AP, Minota T. 2016. Fungal infection and aflatoxin contamination in maize collected from Gedeo zone, Ethiopia. Springerplus. 5(1):753. doi: 10.1186/s40064-016-2485-x.

Chen J, Wen J, Tang Y, Shi J, Mu G, Yan R, Cai J, Long M. 2021. Research progress on fumonisin b1 contamination and toxicity: a review. Molecules. 26(17):5238. doi: 10.3390/molecules26175238.

Chilaka C, De Boevre M, Atanda O, De Saeger S. 2016. Occurrence of *Fusarium*mycotoxins in cereal crops and processed products (ogi) from Nigeria. Toxins (Basel). 8(11):342. doi: 10.3390/toxins8110342.

Chilaka CA, De Boevre M, Atanda OO, De Saeger S. 2017. The status of *Fusarium*mycotoxins in sub-Saharan Africa: a review of emerging trends and post-harvest mitigation strategies towards food control. Toxins. 9(1):19. doi: 10.3390/toxins9010019.

Clarke R, Connolly L, Frizzell C, Elliott CT. 2014. Cytotoxic assessment of the regulated, co-existing mycotoxinsaflatoxin B_1, fumonisin B_1 and ochratoxin, in single, binary and tertiary mixtures. Toxicon. 90:70–81. doi: 10.1016/j.toxicon.2014.07.019.

Codex (Codex Allimentarius Commission). 2008. Proposed draft code of practice for the prevention (reduction) of mycotoxin contamination in cereals, including annexes on ochratoxin A, Zearalenone, Fumonisins and Tricothecenes. Codex Committee on Food Additives and Contaminants, Thirty-fourth Session. Codex Alimentarius Commission/RCP 51–2003.

Daneil AO, Daneil Ochi O, Adejumo A, Hadiza LM, Abubakar NS, Atehnkeng J, Rinde HA, Mailafiya C S, Hussaini AM. 2016. Mycotoxicological concerns with sorghum, millet and sesame in Northern Nigeria. J Anal Bioanal Tech. 7(5):5. doi: 10.4172/2155-9872.1000336.

De Waal EJ. 2002. Safety assessment of cyclopiazonic acid. Int J Toxicol. 21(5):425–427; discussion 429, 431. doi: 10.1080/10915810290096658.

Del Palacio A, Pan D. 2020. Occurrence and toxigenic potential of Aspergillus section Flavi on wheat and sorghum silages in Uruguay. Mycology. 11(2):147–157. doi: 10.1080/21501203.2020.1752321.

Di Paola D, Iaria C, Capparucci F, Arangia A, Crupi R, Cuzzocrea S, Spanò N, Gugliandolo E, Peritore AF. 2022. Impact of mycotoxin contaminations on aquatic organisms: toxic effect of aflatoxin B_1 and fumonisin B_1 mixture. Toxins. 14(8):518. doi: 10.3390/toxins14080518.

Dumitru G, Ciornea ET, Sandu I. 2019. Effect of glyphosate herbicide on some hematological and biochemical parameters in Carassiusauratus L. Rev Chim. 70(2):518–521. doi: 10.37358/RC.19.2.6947.

EC (European Commission Regulation). 2010. EC.NO./65/2010 of 26 February 2010 amending regulation (EC). NO.1881/2016.Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Off J Eur Union. 150:8–12.

EC (European Union Commission). 2006. Commission regulation (EC) No 401/2006 of 23 February, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Off J Eur Union. 70:12–34.

Edia H. 2018. Sorghum production, cultivation and economic importance in Nigeria. www.blog.farmcrowdy.com

Ediage EN, Van Poucke C, De Saeger S. 2015. A multi-analyte LC–MS/MS method for the analysis of 23 mycotoxins in different sorghum varieties: the forgotten sample matrix. Food Chem. 177:397–404. doi: 10.1016/j.foodchem.2015.01.060.

EFSA (European Food Safety Authority). 2006. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ochratoxin A in food. EFSA J. 4(6):365.

EFSA (European Food Safety Authority). 2007. Opinion of the scientific panel on contaminants in the food chain on a request from the commission 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). 2020. Outcome of a public consultation on the risk assessment of ochratoxin A in food. EFSA J. 17(5):1845E.

EFSA Panel on Contaminants in the Food Chain (CONTAM), Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, Hogstrand C, Leblanc JC, Nielsen E. Hoogenboom L. 2022. Assessment of information as regards the toxicity of fumonisins for pigs, poultry and horses. EFSA J. 20(8):e07534.

Ekwomadu TI, Akinola SA, Mwanza M. 2021. Fusarium mycotoxins, their metabolites (free, emerging, and masked), food safety concerns, and health impacts. IJERPH. 18(22):11741. doi: 10.3390/ijerph182211741.

Eskola M, Kos G, Elliott CT, Hajšlová J, Mayar S, Krska R. 2020. Worldwide contamination of food-crops with mycotoxins: validity of the widely cited 'FAO estimate' of 25. Crit Rev Food Sci Nutr. 60(16):2773–2789. doi: 10.1080/10408398.2019.1658570.

Ezekiel CN, Ayeni KI, Akinyemi MO, Sulyok M, Oyedele OA, Babalola DA, Ogara IM, Krska R. 2021. Dietary risk assessment and consumer awareness of mycotoxins among household consumers of cereals, nuts and legumes in north-central Nigeria. Toxins. 13 (9):635. doi: 10.3390/toxins13090635.

FAO (Food and Agriculture Organization). 1994. Components of sweet sorghum stem juice. In: Integrated energy systems in China - the cold north eastern region experience. FAO corporate document repository. [accessed 2012 April 06]. http://www.fao.org/docrep/T4470E/t4470e07.htm#4.1.%20components%20of%20sweet%20sorghum%20stem%20juice

FAO/WHO (Joint FAO/WHO Food Standards Programme, CAC). 2019. Report of the 13th session of the Codex Committee on Contaminants in Foods.

FAOSTAT (Food and Agriculture Organization of the United Nations Statistics). 2018. Statistical Databases, Fisheries Data, 2001. Rome, Italy: Food and Agriculture Organization of the United Nations.

Frangiamone M, Cimbalo A, Alonso-Garrido M, Vila-Donat P, Manyes L. 2022. In vitro and in vivo evaluation of AFB1 and OTA-toxicity through immunofluorescence and flow cytometry techniques: a systematic review. Food Chem Toxicol. 160:112798. doi: 10.1016/j.fct.2021.112798.

Garba MH, Makun HA, Jigam AA, Muhammad HL, Patrick BN. 2017. Incidence and toxigenicity of fungi contaminating sorghum from Nigeria. World J. Microbiol. 4:105–114.

Garcia-Cela E, Kiaitsi E, Sulyok M, Krska R, Medina A, Petit Damico I, Magan N. 2019. Influence of storage environment on maize grain: CO_2 production, dry matter losses and aflatoxins contamination. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 36(1):175–185. doi: 10.1080/19440049.2018.1556403.

Gbashi S, Njobeh PB, Madala NE, De Boevre M, Kagot V, De Saeger S. 2020. Parallel validation of a green-solvent extraction method and quantitative estimation of multi-mycotoxins in staple cereals using LC-MS/MS. Sci Rep. 10(1):10334. doi: 10.1038/s41598-020-66787-z.

Gimeno A. 2002. Los Hongos y lasMicotoxinas en la Alimentación Animal; Conceptos, Problemas, Control Y Recomendaciones. http://www.engormix.com/MA-micotoxinas/ articulos/criterios-calidad-micologica-t353./p0.htm.

Gupta RC, Doss RB, Lall R, Srivastava A, Sinha A. 2022. Aflatoxins, ochratoxins, and citrinin. In: Gupta RC, editor. Reproductive and developmental toxicology. New York: Academic press. p. 983–1002.

Herman D, Mantle P. 2017. Immunohistochemical analysis of rat renal tumours caused by ochratoxin A. Toxins. 9(12):384. doi: 10.3390/toxins9120384.

Hertveldt K. 2016. Mycotoxins occurrence in Nigerian cereal crops (sorghum and millet).

Hussaini AM, Timothy AG, Olufunmilayo HA, Ezekiel AS, Ho G. 2009. Fungi and some mycotoxins found in mouldy sorghum in Niger State, Nigeria. World J Agric Sci. 5(1):5–17.

IARC (International Agency for Research on Cancer). 1993. Ochratoxin A. Some naturally occurring substances: some food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monogr Eval Carcinog Risk Chem Hum. 56:489–521.

IARC (International Agency for Research on Cancer). 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monogr Eval Carcinog Risks Hum. 82:1–556.

Ismaiel AA, Papenbrock J. 2015. Review mycotoxins: producing fungi and mechanisms of phytotoxicity. Agriculture. 5(3):492–537. doi: 10.3390/agriculture5030492.

Janik E, Niemcewicz M, Ceremuga M, Stela M, Saluk-Bijak J, Siadkowski A, Bijak M. 2020. Molecular aspects of mycotoxins – A serious problem for human health. Int J Mol Sci. 21(21):8187. doi: 10.3390/ijms21218187.

JECFA/WHO (Joint FAO/WHO Expert Committee on Food Additives. Meeting, & World Health Organization). 2001. Safety evaluation of certain mycotoxins in food. Vol. 74. Geneva: Food & Agriculture Org.

Kaufman DD, Williams LE, Sumner CB. 1963. Effect of plating medium and incubation temperature on growth of fungi in soil-dilution plates. Can J Microbiol. 9(6): 741–751. doi: 10.1139/m63-100.

Kos J, Anić M, Radić B, Zadravec M, Janić Hajnal E, Pleadin J. 2023. Climate change – A global threat resulting in increasing mycotoxin occurrence. Foods. 12(14): 2704. doi: 10.3390/foods12142704.

Kostić AŽ, Milinčić DD, Petrović TS, Krnjaja VS, Stanojević SP, Barać MB, Tešić ŽL, Pešić MB. 2019. Mycotoxins and mycotoxin producing fungi in pollen. Toxins. 11(2):64. doi: 10.3390/toxins11020064.

Kumar S, Sinha A, Kumar R, Singh V, Hooda KS, Nath K. 2020. Storage fungi and mycotoxins. In: Kumar R, Gupta A, editors. Seed-borne diseases of agricultural crops: detection, diagnosis & management. Singapore: Springer. p. 821–861.

Liu J, Applegate T. 2020. Zearalenone (ZEN) in livestock and poultry: dose, toxicokinetics, toxicity and estrogenicity. Toxins. 12(6):377. doi: 10.3390/toxins12060377.

Longobardi C, Ferrara G, Andretta E, Montagnaro S, Damiano S, Ciarcia R. 2022. Ochratoxin A and kidney oxidative stress: the role of nutraceuticals in veterinary medicine – A review. Toxins. 14(6):398. doi: 10.3390/toxins14060398.

Makun HA, Dutton MF, Njobeh PB, Mwanza M, Kabiru AY. 2011. Natural multi-mycotoxin occurrence in rice from Niger State, Nigeria. Mycotoxin Res. 27(2):97–104. doi: 10.1007/s12550-010-0080-5.

Malir F, Ostry V, Pfohl-Leszkowicz A, Novotna E. 2013. Ochratoxin A: developmental and reproductive toxicity—An overview. Birth Defects Res B Dev Reprod Toxicol. 98(6):493–502. doi: 10.1002/bdrb.21091.

Malir F, Pickova D, Toman J, Grosse Y, Ostry V. 2023. Hazard characterisation for significant mycotoxins in food. Mycotoxin Res. 39(2):81–93. doi: 10.1007/s12550-023-00478-2.

Mateo EM, Gómez JV, Romera D, Tarazona A, Gimeno-Adelantado JV, Mateo-Castro R, Jiménez M. 2018. Comparative study of different cereals as substrates for T-2 and HT-2 production by *Fusarium langsethiae*. Int J Food Eng. 4(1):40–45.

Matumba L, Namaumbo S, Ngoma T, Meleke N, De Boevre M, Logrieco AF, De Saeger S. 2021. Five keys to prevention and control of mycotoxins in grains: a proposal. Glob Food Sec. 30:100562. doi: 10.1016/j.gfs.2021.100562.

McMillan A, Renaud JB, Burgess KM, Orimadegun AE, Akinyinka OO, Allen SJ, Miller JD, Reid G, Sumarah MW. 2018. Aflatoxin exposure in Nigerian children with severe acute malnutrition. Food Chem Toxicol. 111:356–362. doi: 10.1016/j.fct.2017.11.030.

Merem EC, Twumasi Y, Wesley J, Isokpehi P, Shenge M, Fageir S, Crisler M, Romorno C, Hines A, Hirse G, et al. 2017. Analyzing rice production issues in the Niger State area of Nigeria's middle belt. Food Public Health. 7(1):7–22. doi: 10.5923/j.fph.20170701.02.

Monbaliu S, Van Poucke C, Detavernier CL, Dumoulin F, Van De Velde M, Schoeters E, Van Dyck S, Averkieva O, Van Peteghem C, De Saeger S. 2010. Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS/MS method. J Agric Food Chem. 58(1):66–71. doi: 10.1021/jf903859z.

Muhammad HK, Apeh DO, Muhammad HL, Olorunmowaju YB, Ifeji E, Makun HA. 2019. Mycoflora

of maize in Niger state, Nigeria. Adv Res Life Sci. 3(1): 40–45. doi: 10.2478/arls-2019-0009.

Musa BM, Bussell S, Borodo MM, Samaila AA, Femi OL. 2015. Prevalence of hepatitis B virus infection in Nigeria, 2000–2013: a systematic review and meta-analysis. Niger J Clin Pract. 18(2):163–172.

Nayak S, Mukherjee A, Sengupta C, Samanta S. 2019. Association of microbial diversity with post-harvest crops and bioprospecting of endophytic microorganisms for management. In: Mitra S, Banik AK, Mani A, Kuchi VS, and Meena NK, editor. Trends & prospectsin post harvest management of horticultural crops. Cuttack, India: ICAR-NRRI. p. 263.

Ndoro J, Manduna IT, Nyoni M, de Smidt O. 2022. Multiple mycotoxin contamination in medicinal plants frequently sold in the free state province, South Africa detected using UPLC-ESI-MS/MS. Toxins. 14(10):690. doi: 10.3390/toxins14100690.

Nishimwe K, Mandap JAL, Munkvold GP. 2020. Advances in understanding fungal contamination in cereals. In: Advances in postharvest management of cereals and grains. Taylor and Francis; p. 31–66.

Ogara IM, Zarafi AB, Alabi O, Banwo O, Ezekiel CN, Warth B, Sulyok M, Krska R. 2017. Mycotoxin patterns in ear rot infected maize: a comprehensive case study in Nigeria. Food Control. 73:1159–1168. doi: 10.1016/j.foodcont.2016.10.034.

Onyedum SC, Adefolalu FS, Muhammad HL, Apeh DO, Agada MS, Imienwanrin MR, Makun HA. 2020. Occurrence of major mycotoxins and their dietary exposure in North-Central Nigeria staples. Sci Afr. 7:e00188. doi: 10.1016/j.sciaf.2019.e00188.

Onyeke CC. 2020. Review of mycotoxins in foods in Nigeria. Food Control. 118:107376. doi: 10.1016/j.foodcont.2020.107376.

Patterson DS, Roberts BA. 1979. Mycotoxins in animal feedstuffs: sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone, and T-2 toxin. J Assoc Off Anal Chem. 62(6):1265–1267.

Peter Mshelia L, Selamat J, Iskandar Putra Samsudin N, Rafii MY, Abdul Mutalib NA, Nordin N, Berthiller F. 2020. Effect of temperature, water activity and carbon dioxide on fungal growth and mycotoxin production of acclimatised isolates of Fusarium verticillioides and F. graminearum. Toxins. 12(8):478. doi: 10.3390/toxins12080478.

Reddy KRN, Salleh B, Saad B, Abbas HK, Abel CA, Shier WT. 2010. An overview of mycotoxin contamination in foods and its implications for human health. Toxin Rev. 29(1):3–26. doi: 10.3109/15569541003598553.

Rodríguez-Carrasco Y, Ruiz MJ, Font G, Berrada H. 2013. Exposure estimates to *Fusarium* mycotoxins through cereals intake. Chemosphere. 93(10):2297–2303. doi: 10.1016/j.chemosphere.2013.07.086.

Ropejko K, Twarużek M. 2021. Zearalenone and its metabolites – General overview, occurrence, and toxicity. Toxins. 13(1):35. doi: 10.3390/toxins13010035.

Singh K, Kumari A. 2022. Traditional mycotoxins and their health implications. In: Mycotoxins and Mycotoxicoses. New York: Springer. p. 27–64.

Smith LE, Prendergast AJ, Turner PC, Humphrey JH, Stoltzfus RJ. 2017. Aflatoxin exposure during pregnancy, maternal anemia, and adverse birth outcomes. Am J Trop Med Hyg. 96(4):770–776. doi: 10.4269/ajtmh.16-0730.

Ssepuuya G, Van Poucke C, Ediage EN, Mulholland C, Tritscher A, Verger P, Kenny M, Bessy C, De Saeger S. 2018. Mycotoxin contamination of sorghum and its contribution to human dietary exposure in four sub-Saharan countries. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 35(7):1384–1393. doi: 10.1080/19440049.2018.1461253.

Stoev SD. 2022. Studies on teratogenic effect of ochratoxin A given via mouldy diet in mice in various sensitive periods of the pregnancy and the putative protection of phenylalanine. Toxicon. 210:32–38. doi: 10.1016/j.toxicon.2022.02.012.

Sun XD, Su P, Shan H. 2017. Mycotoxin contamination of maize in China. Compr Rev Food Sci Food Saf. 16(5): 835–849. doi: 10.1111/1541-4337.12286.

Taghizadeh SF, Rezaee R, Badiebostan H, Giesy JP, Karimi G. 2020. Occurrence of mycotoxins in rice consumed by Iranians: a probabilistic assessment of risk to health. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 37(2):342–354. doi: 10.1080/19440049.2019.1684572.

Udovicki B, Tomic N, Trifunovic BS, Despotovic S, Jovanovic J, Jacxsens L, Rajkovic A. 2021. Risk assessment of dietary exposure to aflatoxin B1 in Serbia. Food Chem Toxicol. 151:112116. doi: 10.1016/j.fct.2021.112116.

Vlachou M, Pexara A, Solomakos N, Govaris A. 2022. Ochratoxin A in slaughtered pigs and pork products. Toxins. 14(2):67. doi: 10.3390/toxins14020067.

Wu F, Stacy SL, Kensler TW. 2013. Global risk assessment of aflatoxins in maize and peanuts: are regulatory standards adequately protective? Toxicol Sci. 135(1):251–259. doi: 10.1093/toxsci/kft132.

Yassin MA, El-Samawaty AR, Bahkali A, Moslem M, Abd-Elsalam KA, Hyde KD. 2010. Mycotoxin-producing fungi occurring in sorghum grains from Saudi Arabia. Fungal Divers. 44(1):45–52. doi: 10.1007/s13225-010-0058-9.

Zain ME. 2011. Impact of mycotoxins on humans and animals. J Saudi Chem Soc. 15(2):129–144. doi: 10.1016/j.jscs.2010.06.006.

Zhou H, George S, Li C, Gurusamy S, Sun X, Gong Z, Qian H. 2017. Combined toxicity of prevalent mycotoxins studied in fish cell line and zebrafish larvae revealed that type of interactions is dose-dependent. Aquat Toxicol. 193:60–71. doi: 10.1016/j.aquatox.2017.09.030.

Zinedine A, Fernández-Franzón M, Mañes J, Manyes L. 2017. Multi-mycotoxin contamination of couscous semolina commercialized in Morocco. Food Chem. 214:440–446. doi: 10.1016/j.foodchem.2016.07.098.