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## Aflatoxin contamination of maize vended in Ondo state, Nigeria, and health risk assessment

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### ABSTRACT

Aflatoxin contamination of maize is a serious food safety problem worldwide. Despite the widespread consumption of maize in Nigeria, there is limited data on aflatoxin contents of maize vended in open markets in Ondo state, Nigeria. A total of 140 maize samples randomly purchased from major markets in four locations in Ondo state, were screened for total aflatoxins using an ELISA method. Exposure and health risk assessments were performed for the maize consumers by the deterministic and Margin of exposure (MOE) approaches, respectively. About 99% of the maize were contaminated with total aflatoxins (range: 0.65–265 µg/kg; mean: 125.9 µg/kg). Aflatoxin levels exceeding the 4 µg/kg set by the European Union for total aflatoxins were found in 88% of the maize whilst more than one half contained at least 100 µg/kg aflatoxins. The average probable daily intake values were 830, 332 and 138 ng/kg bw/day for the average children, adolescent and adult populations, respectively. Consequently, MOEs for the respective populations were 0.20, 0.51 and 1.23, suggesting a high level of health risk for consumers of maize vended in open markets in Ondo state due to high aflatoxin levels. Maize farmers and households in Ondo state need urgent aflatoxin mitigation interventions.

### Introduction

Maize (*Zea mays* L.) is a cereal produced in most parts of the world (Nuss and Tanumihardjo, 2010; Ranum et al., 2014). It is the most widely cultivated food crop in sub-Saharan Africa, where it contributes significantly to human calorie intake (ten Berge et al., 2019; Tesfaye et al., 2015). In Nigeria, maize production was estimated at 11 million tons in 2019 (FAO, 2019), which makes it one of the most important cereals produced in the country. About 10–15 % of maize produced in Nigeria is directly utilized for household consumption (USDA, 2019). Nutritionally, maize contains carbohydrate, protein, fat, fibre, vitamins and minerals (Ranum et al., 2014; Shah et al., 2016). Apart from being a major staple consumed directly by boiling or roasting, maize serves as the prime raw material for a range of traditionally processed Nigerian meals such as *aadun*, *ogi*, *kokoro*,

*tuwo masara* and beverage (e.g. *kunu*) (Abdulrahman and Kolawole, 2006). In addition, maize is a major ingredient in the formulation of animal feeds (Amudalat, 2015).

Despite the importance of maize in Nigeria, its safety is often compromised by the presence of toxic chemicals of fungal origin, e.g. aflatoxins (Adetunji et al., 2014a, 2014b; Ogara et al., 2017; Onyedum et al., 2020). This could be attributed to poor farming and storage practices that encourage frequent contamination by aflatoxigenic fungi (Bankole et al., 2006). Aflatoxins are toxic secondary metabolites produced by toxigenic strains within the *Aspergillus* section *Flavi* (Frisvad et al., 2019). Factors that could trigger aflatoxin production by this group of fungi include climatic changes (Battilani et al., 2016; Medina et al., 2017), oxidative stress (Reverberi et al., 2010) and light (Kovac et al., 2018). Depending on the concentration ingested via contaminated foods, human

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exposure to aflatoxins could result to acute aflatoxicosis or chronic effects such as cancers, immune system modulation, and impaired child growth (Gong et al., 2016; IARC, 2015). In worst cases, consumption of foods contaminated with high levels of aflatoxin can lead to human death (Kamala et al., 2018).

Analytical methods such as Enzyme-linked immunosorbent assay (ELISA) (Kos et al., 2013), high performance thin-layer chromatography (Atehnkeng et al., 2008; Bandyopadhyay et al., 2019), high performance liquid chromatography (Kamika et al., 2016), and liquid chromatography tandem mass spectrometry (LC-MS/MS) (Adetunji et al., 2014a,b) have been applied for the detection of aflatoxins in maize. However, for surveillance studies, ELISA remains a low-cost and rapid technique available to researchers in developing countries such as Nigeria. The application of ELISA in aflatoxin surveillance research in developing countries are mainly due to limited funds available to support mycotoxin analysis on high-end equipment (Makinde et al., 2020).

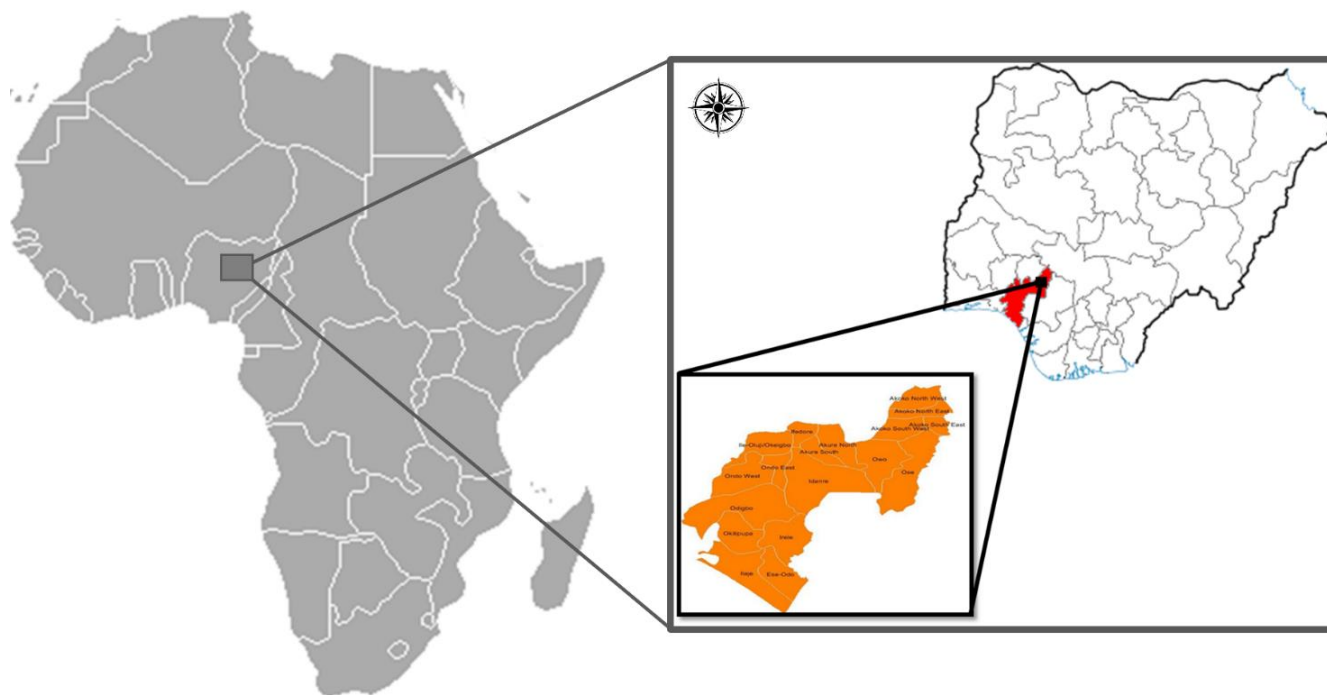
A study by Adetunji et al. (2014b) recently revealed Ondo state as a likely hotspot for aflatoxin exposure. Precisely, aflatoxin levels up to 1,548.96  $\mu\text{g}/\text{kg}$  were quantified in maize stored in different structures in Ondo state. However, data on the aflatoxin contents of maize vended in the open market in this state is sparse. Maize available at markets is often stored in different storage structures prior to sale. In view of the need to

continuously provide data on aflatoxin contamination of food crops and dietary exposure among consumers and considering that maize is a major food crop for households in Nigeria, this study was designed. This study aimed to screen maize from major markets in Ondo state for the presence of aflatoxins and to estimate exposures resulting from maize consumption in households, with a view to providing recent data that could trigger more mitigation efforts and safeguard consumer health.

## Materials and methods

### Study area

This study was carried out in four locations in Ondo state, Nigeria (Figure 1). Ondo state is located within the Derived savannah agro-ecological zone (AEZ) with a bimodal rainfall distribution averaging between 1,000 and 1,300 mm per year and maximum temperature varying from 26 to 38°C (Adetunji et al., 2014b; Atehnkeng et al., 2008). Maize consumed is mostly cultivated by subsistence farmers within the state (Ayodele and Akindele, 2018). The four locations were Akure, Ondo, Ore and Owo. These locations were purposely selected based on their large population, status as major economic hubs of Ondo state, and the presence of major markets.



**Fig. 1** Study area (Ondo state, Nigeria)

### Sampling and sample preparation

In total, 140 maize samples were randomly purchased from major markets in the four locations in December 2019. Two major markets were visited in each of the four locations. In each location, 35 samples (17 or 18 samples per market) were randomly purchased from maize vendors in open markets. At most two samples were purchased from a vendor. Each sample (0.8–1 kg) was collected in clean polyethylene bags and transported to the laboratory for further processing. All samples were pulverized to fine powder in an electric blender (MX-AC400, Panasonic, India) within 3 days of collection, quartered repetitively to obtain representative samples (50 g each) and stored at -20°C for not more than 5 days when aflatoxin analysis was performed.

### Determination of total aflatoxin by ELISA

Total aflatoxin (sum of B and G aflatoxins) in the maize samples was assayed by a quantitative ELISA kit assay (AFL01LM-96; Helica Biosystems, Inc., USA) according to manufacturer's instructions. All reagents and ground maize samples were allowed to reach ambient temperature. Methanol (HPLC grade, Merck, Germany) and distilled water was used for extraction of total aflatoxin from the maize samples. In all cases, 20g sub-sample was weighed into a 250 mL conical flask and 100 mL of the extraction solution was added to give a 1:5 w/v ratio of sample to extraction solvent. The mixture was vortexed laterally in a shaker (UNISCOPE SM101, England) for 3 mins and allowed to stand for 5 mins. The supernatant was carefully decanted through Whatmann No. 1 filter paper into a 25 mL screw cap vial.

Assay buffer (200 µL) was dispensed into each mixing well, and 100 µL of the sample extract and aflatoxin standards (0, 0.02, 0.05, 0.1, 0.2 and 0.4 ppb) was added to the appropriate mixing well containing the assay buffer. A 100 µL portion of this mixture was added to the antibody coated microtiter wells and incubated in the dark at ambient temperature for 30 mins. After incubation, the content of each micro well was discarded and the micro wells were washed three times with PBS-Tween wash buffer and dried. Aliquots (100 µL) of a conjugate and a substrate were added consecutively with a step of wash in-between and two steps of incubation in the dark at ambient temperature for 30 and 10 mins, respectively after each solution was added. A stop solution (100 µL) was then added and the optical densities (OD) of the reaction solution in the microtiter plates were read at 450 nm using Microplate reader (LABTRON LMPR-A30, United Kingdom). The corresponding aflatoxin concentration in each well was estimated from standard curve plotted using the percentage binding against the total aflatoxin standards.

The recovery (%) of the assay was tested by spiking blank samples at five concentration levels (0.02, 0.05, 0.1, 0.2 and 0.4 ppb). Recovery of the method ranged 80.7–104.2% (mean±SD: 96.6±9.41).

### Exposure assessment and risk characterization

The exposure assessment of maize consumers in Ondo state via consumption of aflatoxin contaminated maize was determined by the deterministic approach; this involved estimating the average probable daily intake (APDI) (EFSA, 2007). As shown in the formula below, to obtain the APDI (ng/kg bw/day) for the average population, the mean concentration of aflatoxins in the maize samples was multiplied by the average maize consumption in Nigeria (65.9 g/person/day; NBS/World Bank, 2016) and then divided by the assumed body weights depending on the target population. Assumed body weights were 10, 25 and 60 kg for children, adolescents and adults, respectively (Rodríguez-Carrasco et al., 2013).

$$\text{APDI} = [\text{aflatoxin concentration (ng/g)} \times \text{maize consumption (g/person/day)}] / \text{body weight (kg)}$$

In order to assess the health risk due to aflatoxin consumption of maize in the populations, the Margin of exposure (MOEs) approach was adopted (EFSA, 2007). Thus, the MOE for each population group was calculated, as described in the formula below, by dividing the benchmark dose limit (BMDL<sub>10</sub>) of 170 ng/kgbw/day for aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) by the APDI estimated for the group. It is important to mention that total aflatoxin comprises AFB<sub>1</sub> + AFB<sub>2</sub> + AFG<sub>1</sub> + AFG<sub>2</sub>, and that AFB<sub>1</sub> usually constitutes a higher proportion (more than 75%) in foods (IARC, 2015); thus the application of the BMDL<sub>10</sub> for AFB<sub>1</sub> in the MOE calculation.

$$\text{MOE} = \text{benchmark dose limit (BMDL}_{10}) / \text{APDI}$$

### Statistical analysis

Data obtained for total aflatoxins in the maize samples were subjected to descriptive statistics. Overall mean value was computed using Microsoft Excel 2013.

## Results and discussion

### Concentration of aflatoxins in maize

Results from this study indicated that 98.6% (n=138) of the 140 maize samples were contaminated with aflatoxins (µg/kg). The total aflatoxin levels in the samples ranged between 0.65 µg/kg and 265 µg/kg (mean±SD: 126±109). The observed incidence of aflatoxin contamination in this study is similar to the 100% incidence previously reported in maize from

Nigeria (Onyedum et al., 2020; Williams et al., 2015) and Kenya (Nduti et al., 2017) analyzed by ELISA. The result obtained in the present study is, however, higher than the aflatoxin incidence of 68.5% (137/200) and 78% (25/32) documented for Serbian and Indian maize, respectively, screened by an ELISA method (Chandra et al., 2013; Kos et al., 2013).

The percentage contaminated samples for various categories of aflatoxin levels in the maize samples are given in Table 1. In Nigeria, maize available in the local market is not regulated; however, the maize may find their way to the international market if local vendors are linked to maize aggregators who purchase for export. Only 12% of the maize samples were below 4 µg/kg, the European Union threshold for total aflatoxins in foods. A higher proportion (36%) was, however, found to be below the 20 µg/kg limit set by the United States Food and Drug Administration (FAO, 2004). In addition, about 53% of the maize samples contained aflatoxins above 100 µg/kg. Similar aflatoxin levels (>100 µg/kg) were reported in maize in India screened by an ELISA method (Chandra et al., 2013) and also in stored maize from Nigeria (Adetunji et al., 2014a). Our results, however, contradict the recent findings of (Oyeka et al., 2019) who did not detect aflatoxins in 36 maize samples from local markets in Anambra state, Nigeria, using LC-MS/MS. Aflatoxin levels reported in the present study compared to the study by Oyeka et al. (2019) may have been influenced by several factors such as geographical variation, pre- and post-harvest practices including length and condition of grain storage and even analytical method applied. Anambra state belongs to the Humid forest AEZ, where lower levels of aflatoxins

in maize have been reported when compared with Derived savannah AEZ (Adetunji et al., 2014a, b). Precisely, aflatoxin levels in maize from Derived savannah AEZ to which Ondo state belong were two times higher than maize from Humid forest AEZ (Adetunji et al., 2014b). Perhaps, differences in climatic conditions between the two AEZ could be a contributing factor to the observed disparity in aflatoxin levels (Paterson and Lima, 2010). In addition, maize grown in Anambra state is mostly at small-scale level and for household consumption, as such, there may not have been prolonged storage, which could have influenced aflatoxin levels. A more important factor that could have led to the disparity in results between our study and that of Oyeka et al. (2019), since the latter applied a highly sensitive analytical technique, may be sampling period and sampling technique. ELISA is good for rapid screening, however, it is limited by cross-reactivity and possible underestimation of total aflatoxin contents (Oplatowska-Stachowiak et al., 2016). Consequently, there is a possibility that the aflatoxin levels reported in maize in this study could be relatively higher or lower. This assertion may require verification by applying a more sensitive analytical technique such as LC-MS/MS.

#### *Estimated exposure and health risk for maize consumers*

Exposure to compounds that are both carcinogenic and genotoxic such as aflatoxins should be as low as reasonably achievable (ALARA) (IPCS, 2009). In addition, MOE <10,000 for a carcinogenic and genotoxic substance is considered a public health risk (EFSA, 2005).

**Table 1.** Incidence of aflatoxins in 140 maize samples from Ondo state, Nigeria.

Location	N	N <sup>c</sup>	% contaminated			
			≤ 4µg/kg *	≤ 20µg/kg **	≤ 100µg/kg	>100µg/kg
Akure	35	34	17.7	29.4	55.9	44.1
Ondo	35	35	8.57	25.7	25.7	74.3
Ore	35	35	14.3	57.1	65.7	34.3
Owo	35	34	5.88	32.4	41.2	58.8
Total	140	138	11.6	36.2	47.1	52.9

N = number of samples; N<sup>c</sup> = number contaminated; % = percentage; \*European Union regulatory limit for total aflatoxins in foods; \*\*United States Food and Drug Administration regulatory limit for total aflatoxins in foods

**Table 2.** Aflatoxin exposure and risk characterization of maize consumers in Ondo state, Nigeria

Population	Average Probable Daily Intake (ng/kg bw/day)	Margin of exposure
Children	830	0.21
Adolescents	332	0.51
Adults	138	1.23

In this study, the estimated APDIs were 830 ng/kg bw/day, 332 ng/kg bw/day and 138 ng/kg bw/day for the average children, adolescent and adult populations, respectively, who consume maize vended in major markets in Ondo state (Table 2). Consequently, the calculated average MOEs for the children, adolescent and adult populations were 0.20, 0.51 and 1.23, respectively (Table 2). The MOEs were substantially lower than the 10,000 threshold indicating that dietary exposure to aflatoxins would constitute public health risk in the consumers. As such, our results add to existing literatures that suggests that maize consumers especially children in Ondo state may be at high risk of chronic aflatoxin exposure (Adetunji et al., 2014b; Adetunji et al., 2017).

## Conclusion

This surveillance study suggests that maize vended in open markets in Ondo state contain high levels of aflatoxins that may constitute a risk to public health especially children who consume the maize. We therefore recommend that appropriate pre- and post-harvest measures should be adopted to reduce aflatoxin contamination of maize in this state. This includes early harvesting of maize by farmers, use of bio-pesticides for aflatoxin control, proper drying and storage by farmers and vendors (Bandyopadhyay et al., 2016; Ezekiel et al., 2018; Misihairabgwi et al., 2017). In addition, households are advised to properly sort out bad maize kernels before being applied to make foods.

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