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EXTRAPOLATING HEPATOCELLULAR CARCINOMA (HCC) RISK FROM AFLATOXIN EXPOSURE IN FOOD GRAINS AND LEGUMES USING DIETARY EXPOSURE RISK ASSESSMENT (DERA) CALCULATIONS

Baha'uddeen Salisu[a,b], Siti Marwanis Anua[a]*, Wan Ishak Wan Rosli[a], Nurzafirah Mazlan[c]

[a]School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[b]Department of Microbiology, Umaru Musa Yar'adua University, PMB 2218, Katsina, Nigeria
[c]Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

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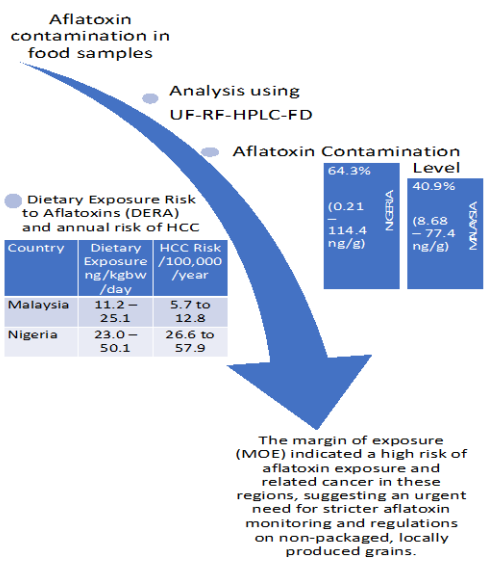
*Corresponding author
smarwanis@usm.my

Graphical abstract



Abstract

This study quantifies aflatoxins, which are linked to about 20% of cancer-related deaths globally, in food samples from open markets in Malaysia and Nigeria. Using optimized ultra-fast high-performance liquid chromatography (HPLC) aflatoxin levels were measured in 44 Malaysian and 84 Nigerian samples of peanuts, maize, wheat, and rice by matrix-matched calibration approach. Contamination was found in 40.9% of Malaysian samples, in the range of 8.68 – 77.4 ng/g, and in 64.3% of Nigerian samples, with levels between 0.21 and 114.4 ng/g. The Dietary Exposure Risk to Aflatoxins (DERA) was calculated, yielding a mean exposure of 11.2 – 25.1 ng/kgbw/day in Malaysia and 23.0 – 50.1 ng/kgbw/day in Nigeria. These exposure levels correspond to an estimated annual risk of HCC of 5.7 to 12.8 cases per 100,000 people in Malaysia and 26.6 to 57.9 cases per 100,000 in Nigeria. The margin of exposure (MOE) indicated a high risk of aflatoxin exposure and related cancer in these regions, suggesting an urgent need for stricter aflatoxin monitoring and regulations on non-packaged, locally produced grains.

Keywords: Aflatoxins, exposure risk, food grains, liver cancer

Abstrak

Kajian ini mengukur tahap aflatoksin, yang dikaitkan dengan kira-kira 20% kematian akibat kanser di seluruh dunia, dalam sampel makanan dari pasar terbuka di Malaysia dan Nigeria. Menggunakan HPLC ultra-pantas yang dioptimumkan, tahap aflatoksin dianalisis dalam 44 sampel dari Malaysia dan 84 sampel dari Nigeria yang melibatkan kacang tanah, jagung, gandum, dan beras melalui pendekatan penentukuran matriks. Pencemaran dikesan dalam 40.9% sampel dari Malaysia (8.68–77.4 ng/g) dan 64.3% sampel dari Nigeria (0.21–114.4 ng/g). Risiko Pendedahan Pemakanan kepada Aflatoksin (DERA) menunjukkan purata pendedahan 11.2–25.1 ng/kgbw/hari bagi Malaysia dan 23.0–50.1 ng/kgbw/hari bagi Nigeria. Risiko tahunan kanser hepatoselular (HCC) dianggarkan sebanyak

5.7–12.8 kes bagi setiap 100,000 orang di Malaysia dan 26.6–57.9 kes di Nigeria. Margin pendedahan (MOE) menunjukkan risiko tinggi pendedahan aflatoksin, menekankan keperluan pemantauan dan peraturan yang lebih ketat terhadap bijirin tempatan yang tidak dibungkus.

Kata kunci: Aflatoksin, risiko pendedahan, bijirin makanan, kanser hati

1.0 INTRODUCTION

Essential legumes and grains such as peanuts, beans, peas, rice, wheat, and maize are widely cultivated as primary food and animal feed sources globally. These staples, with a high consumption value of over 2.6 to 2.9 billion tonnes worldwide [1], are highly susceptible to invasion by aflatoxigenic fungi, notably *Aspergillus* spp., which produce aflatoxins that are extremely carcinogenic, genotoxic, nephrotoxic, neurotoxic, and enterotoxic mycotoxins [2–4]. Studies have found a strong association between aflatoxin exposure and several health effects, including stunted growth in children [5,6], immunosuppression [7, 8], and various types of cancer [9], necessitating awareness and management throughout the value chain. Carcinogens currently pose a significant risk to public health [10, 11], making their continuous detection, monitoring, characterization, and control very critical. Generally, four primary kinds of aflatoxins (AFB_1, AFB_2, AFG_1, and AFG_2) have been categorized, by the International Agency for Research on Cancer (IARC), as a group I hepatocarcinogenic fungal metabolites, requiring continuous monitoring and regulations [12].

In tropical and subtropical regions, the incidence and proliferation of toxigenic fungi, especially *Aspergillus* spp., and their mycotoxin production is amplified due to the conducive warm and humid conditions. This correlation between environmental conditions and aflatoxin contamination is exacerbated by factors such as food moisture, temperature, and storage duration [13,14], leading to significant health risks from contaminated grains. Despite extensive research and regulatory measures, the prevalence of aflatoxin contamination in grains and legumes persists, presenting an ongoing risk to public health. Markets serve as crucial nodes in the distribution of these commodities [15], making them focal points for assessing aflatoxin levels and understanding the associated risks to communities reliant on these food sources.

Despite significant advancements in the detection and regulation of aflatoxin contamination, there is a marked gap in the comprehensive assessment of these toxins at the consumer level, particularly in open markets within tropical and subtropical regions such as Malaysia and Nigeria. Existing studies predominantly focus on contamination during the production and importation stages [2,4,16–31], neglecting the final points of sale where consumers are directly exposed to contaminated food products. This oversight is critical, as it undermines efforts to mitigate the public health risks associated with aflatoxin exposure. Moreover, poor storage practices by the staple sellers in marketplaces (locked up stores without sufficient ventilation outlets or air conditioning) along with the dietary habits in these regions, which heavily rely on staple grains and legumes, exacerbate the potential for chronic exposure to aflatoxins, increasing the risk of severe health outcomes, including hepatocellular carcinoma (HCC).

In fact, a risk assessment of aflatoxin in 2017 reported that the estimated aflatoxin-related liver cancer cases could go as high as 13 per 100,000 Malaysians [32]. However, these studies were carried out in developed states only, such as Penang and Selangor, which have high population density. But few or no studies were conducted in rural states, where local farm products are produced in abundance and not screened or quarantined by the authorities. Similarly, high aflatoxin exposure levels of up to 1.7×10^{-4} to 9.9×10^{3} ng/kgbw/day, potentially contributing to an estimated incidence of hepatocellular carcinoma (HCC) cases within a range of 84.03 to 1,052.50% per 100,000 population annually between 2009 and 2018 was reported in Nigeria [33]. In another report [34], aflatoxin-related HCC cases in Nigeria have claimed around 5,000 lives and incurred USD155 million in economic losses each year. These reports emphasize the critical need for effective aflatoxin control measures in Nigeria and Malaysia.

It has been reported that the HCC burden resulting from exposure to aflatoxins in developing nations, such as Malaysia and Nigeria, is underestimated [12], necessitating further research to safeguard people's lives. Given the limited data on the prevalence, concentration, and health implications of aflatoxins in locally marketed food items, this study addresses a significant gap in the literature by providing a detailed analysis of aflatoxin contamination in staple foods sold in Malaysian and Nigerian open markets and the associated cancer risk. We recently reported a bioburden and distribution of mycotoxigenic fungi in non-packaged, locally-produced grains (rice, maize, wheat and peanut) distributed across Kelantan State open markets in Malaysia [25], and Katsina State in Nigeria [20], and characterize their aflatoxigenic potential [35,36]. These grain types are considered

"neglected" because they are not screened for aflatoxins, to ensure their safety for consumption, as done to the imported food items. Therefore, this study (1) quantifies total aflatoxins in the same food categories sourced from the same open markets in Malaysia and Nigeria using optimized ultra-fast reverse-phase high-performance liquid chromatography with a fluorescent detection (UF-RP-HPLC-FD); and (2) calculates the dietary exposure risks to aflatoxins (DERA) and associable liver cancers in the proximal communities of both countries through elucidating the correlation between the contamination and the associated exposure levels based on the probable daily intake (PDI), as well as the assessment and characterization of liver cancer risks using the margin of exposure (MOE) within the impacted communities. By addressing these intertwined aspects (contamination levels and associated public health risks), this research aims to lay a foundation for understanding complex challenges aflatoxins bring to the locally produced food supply chain, especially for communities that are directly impacted, thereby informing the development of relevant targeted interventions and regulatory policies to improve food safety in these regions.

2.0 METHODOLOGY

2.1 Study Location and Samples

The samples in this study comprised three grains and a legume (rice, maize, wheat and peanut). These same samples had been assessed in our previous studies that reported the bioburden and distribution of mycotoxigenic fungi in Malaysia [25] and Nigeria [20]. Both study areas, Kelantan State in Malaysia and Katsina State in Nigeria, are located in the tropics and sub-tropic regions with geographical coordinates of 5.1151° N and 101.8892° E, and 12.3797° N and 7.6306° E, respectively. As reported, a stratified research design was adopted to select one state from each country (Kelantan in Malaysia and Katsina in Nigeria). A total of 84 non-imported, non-packaged locally produced food samples were purchased from Nigeria, consisting of 21 triplicated samples each of rice, maize, wheat, and peanut [20]. In Malaysia, 44 samples were collected, consisting of 11 triplicate samples each of the same non-imported, non-packaged locally produced food types [25].

2.2 Aflatoxin Extraction and Quantification

The aflatoxins were extracted from the samples and quantified using UF-RP-HPLC-FD (Nexera-XR, LC-20ADXR, Shimadzu, Kyoto, Japan), following a validated protocol [37,38]. Briefly, an electric grinder (Bosch Teknologi, Penang, Malaysia) was used to grind each sample into crystalline powder. After that, a small portion (20 g) was blended for three min in 100 mL of solvent mixture consisting of 5 g mixture of magnesium sulphate (AR grade, Thermo Fisher Scientific, Kuala Lumpur, Malaysia) and sodium chloride (Thermo Fisher Scientific, Kuala Lumpur, Malaysia) and (4:1 w/w) plus methanol and water (8:2 v/v). Then the organic layer underwent decantation and filtration using grade 1 Whatman's qualitative filter paper (Whatman, Maidstone, England). The leftover residual material was re-suspended again in 50 mL of fresh solvent mixture with same composition mentioned above. This mixture was blended for one minute in high-powered blender, left to settle in 10 min, and then filtered as previously described. This second step was repeated twice more. Subsequently, the entire filtrate was evaporated to dryness at 30°C in vacuum evaporation oven (Memmert, Schwabach, Germany). The processed extract for each sample was dissolved again in 2 mL of the extraction solvent (omitting salts), filtered anew using a 0.2 µm filter (Thermo Fisher Scientific, Kuala Lumpur, Malaysia) before loading into the UF-RV-HPLC-FD system for aflatoxin quantifications.

As mentioned above, the ultra-fast Nexera-XR LC-20AD$_{XR}$ HPLC system (Shimadzu, Kyoto, Japan), was used to quantify the aflatoxins in each sample. A HC-C18(2) reverse-phase Agilent Zorbex column with a dimension of 150 × 4.6 mm and 5 µm particle size (Agilent Technologies, Amsterdam, Netherlands) was used with a mobile phase containing analytical grade acetonitrile (Thermo Fisher Scientific, Kuala Lumpur, Malaysia) + methanol (Thermo Fisher Scientific, Kuala Lumpur, Malaysia) + water (3:5:30 v/v) mixture at a column oven temperature of 40°C. The system was operated using a 10 µL injection volume at a 0.50 mL/minute flow rate for 25 min. Aflatoxin signals were detected between 360 nm and 450 nm wavelengths using the of the UF-RV-HPLC-FD system's prominence fluorescence detector. A serial dilution of the aflatoxin standard (STD#1048) [39] (0.1, 0.2, 0.4, 0.6, and 0.8 ng/mL) was prepared to generate a calibration curve for each aflatoxin based on the matrix-matched calibration approach, which was used to extrapolate the aflatoxin levels in samples using the LC Solutions post-run software (Shimadzu, Kyoto, Japan).

As a further optimization step, negative samples [40] of rice, maize, wheat and peanut were spiked with aflatoxin standards' mixture (2.0, 5.0 and 10.0 ng/g) in triplicates and reextracted as described, before being analyzed using HPLC to assess the recovery of aflatoxins.

2.3 Risk Assessment of Exposure to Aflatoxins

Following the recommendations of European Food Safety Authority (EFSA) [3] for evaluating DERA in foods, the mean contamination level and 95th percentile (lower and upper bound limits) of AFB_1 and total aflatoxins (AFB_1 + AFB_2 + AFG_1 + AFG_2) for each food type were used to calculate the DERA among consumers using average daily food intake (DFI) per person in each country (Equation 1) [41].

$$\text{Estimated DERA}\left(\frac{\frac{\text{ng}}{\text{kgBw}}}{\text{day}}\right) = \frac{\text{Aflatoxin level in foodstuff (ng/g)} \times \text{per person DFI (g/day)}}{\text{Average body weight (kgBw)}}$$

Eq. 1

The following data were used in estimating DERA:

- The consumption of rice in Malaysia per person per day is 202.47 g [42], followed by 143.04 g for wheat [43], 33.0 g for maize [43] and 4.47 g for peanuts [44].
- The corresponding consumption in Nigeria is 60 g for maize [45], 36.85 g for peanuts [46], 101.37 g for rice [47] and 54.79 g for wheat [48].
- The average adult person's body-weight (Bw) in Malaysia and Nigeria is 62.65 kg [17] and 60.0 kg [49], respectively.

2.4 Aflatoxin Exposure Risk Characterization

For carcinogenic and genotoxic substances like aflatoxins, risk characterization typically involves utilizing the Margin of Exposure (MOE) method [3,50]. This calculation involves dividing the toxin's Benchmark Dose Lower Limit (BDML) by the exposure observed in individual consumers [49]. Based on MOE values, the exposure risk for each food grain was either high, requiring public health risk management action, or low and not a public health threat. In this study, the MOE of each food type was determined using Equation 2 [49] based on the mean and 95% (lower to upper bound limits) of the estimated DERA [3].

$$\text{MOE to Aflatoxin} = \frac{BMDL\ for\ Aflatoxins}{Dietary\ exposure\ to\ aflatoxins\ for\ the\ food\ grain}$$

Eq. 2

Where:

- Reference BDML for aflatoxins is given as 400 ng/kgBw/day [3], which is the lowest permitted defined as $_{BDML10}$ (10% extra cancer risk).

2.5 Estimated HCC Risks Due to Consumption of Each Food Grain

The estimated HCC risks due to the consumption of each food grain per 100,000 population of consumers per year were evaluated according to Equation 3 [41] based on the mean and 95th percentile (lower to upper bound limits) of the estimated DERA [3].

$$HCC_{aflatoxin}\ \text{per 100,000 consumers/year} = \text{Estimated DERA} \times \text{Average population's HCC potency}$$

Eq. 3

Where:

- The average liver cancer potency (cancers/year/100,000 per ng of aflatoxin per kgbw of person per day) is given as 0.025 in Malaysia [17] and 0.0825 in Nigeria [49].

Finally, the attributable HCC values were used to calculate the percentage incidence of HCC attributable to dietary aflatoxins for each food type using Equation 4 [49].

$$\%\ HCC_{aflatoxin}\ \text{Incidence per}\ \frac{100{,}000\ \text{people}}{\text{year}} = \frac{HCC_{aflatoxin}\ \text{per 100,000/year}}{\text{Mean incidence of liver cancer in the population}}\ \text{X 100\%}$$

Eq. 4

Where:

- The average liver cancer incidence is 4.9/100,000 population/year in Malaysia [51]
- The average liver cancer incidence is 7.13/100,000 population/year in Nigeria [52].

3.0 RESULTS AND DISCUSSION

3.1 Optimization of the UF-RP-HPLC-FD

Figure 1 compares chromatograms of the serial dilutions of the aflatoxin standard solution at various concentrations (0.1 to 0.8 ng/mL) and the chromatogram of aflatoxins in the food samples fortified with 2 ng/g of the standard. The results indicated the remarkable specificity of the HPLC quantification method since there was no interference between the sample matrix and aflatoxin peaks [53].

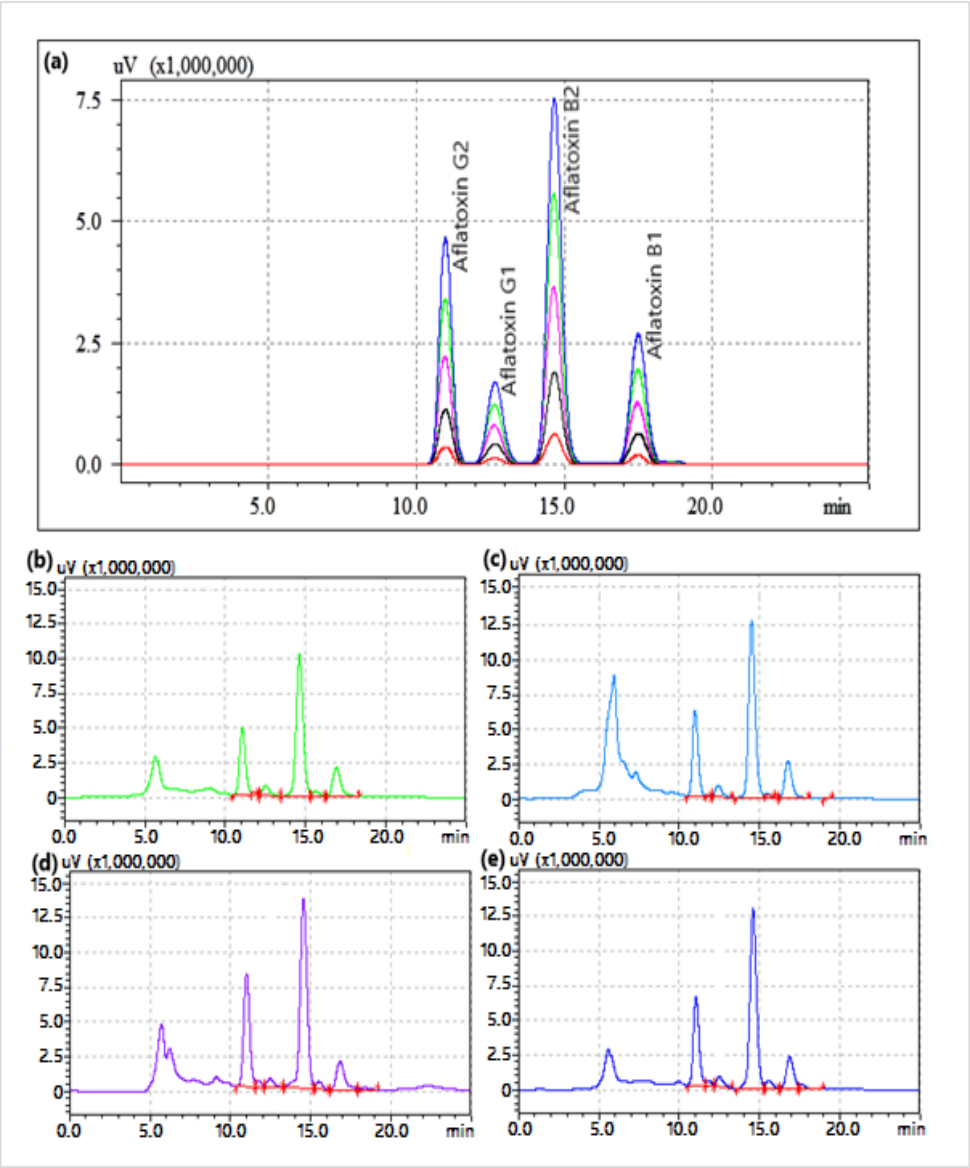


Figure 1 HPLC chromatograms: (a) data comparison of five concentrations (0.1, 0.2, 0.4, 0.6, and 0.8 ng/mL) of aflatoxin standards showing the linearity of the method; (b) rice sample fortified with 2 ng/g of each aflatoxin; (c) peanut sample fortified with 2 ng/g of each aflatoxin; (d) maize sample fortified with 2 ng/g of each aflatoxin; (e) wheat sample fortified with 2 ng/g of each aflatoxin

Similarly, the calibration curves produced from the serial dilutions of the four aflatoxin's standard solution (0.1 to 0.8 ng/mL) displayed exceptional linearity and sensitivity of the HPLC technique, as indicated by higher coefficients of determination (R^2 > 99.9%). Using the calibration of the pure aflatoxin standard solution, the recovery of the aflatoxins in the spiked samples were extrapolated. The average recovery of each aflatoxin in samples spiked with 2 ng/g, 5 ng/g, and 10 ng/g of the standard varied from 91% to 103%, which was within acceptable range defined by the European Commission rule [54] for aflatoxins assessed at values between 1 and 10 g/kg. Hence, the method was adopted to determine the total aflatoxin in the market samples using matrix-based calibration curves for each food type to improve the accuracy of quantification of the aflatoxins. An example of the chromatograms of aflatoxin standards prepared using aflatoxin-negative peanut sample matrix and the resulting calibration curves of the aflatoxins when the calibration line was forced to pass through the zero is shown in Figure 2.

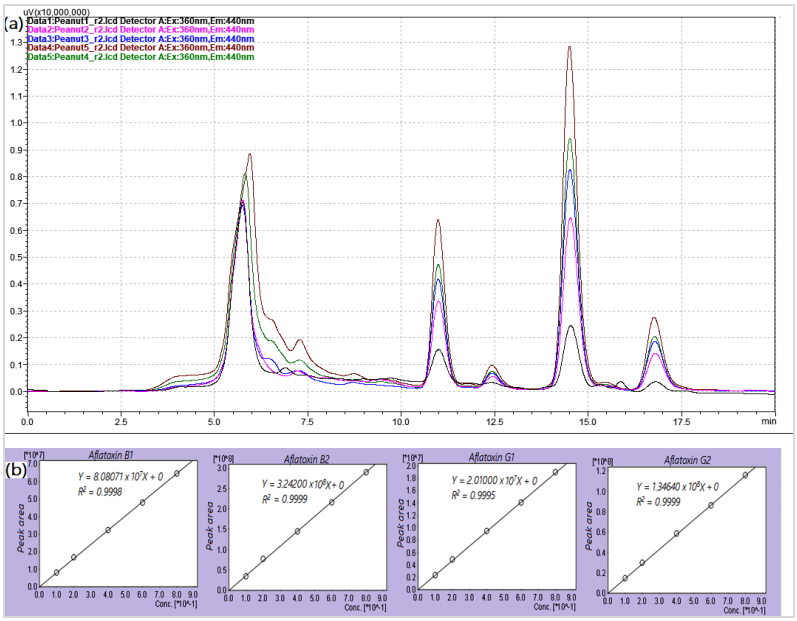


Figure 2 (a) Compared chromatograms of aflatoxin standards' dilutions (0.1, 0.2, 0.4, 0.6, and 0.8 ng/mL per milligram of peanut), prepared with blank peanut sample showing the specificity and precision of the UF-RP-HPLC-FD method. (b) Resulting calibration curves from data in (a). The curves showed the linear correlation between the chromatogram's peak areas (Y) against the respective concentrations (ng/g) for each aflatoxin. R^2 stands for coefficient of determination and X in each calibration equation represent the slope of the curve

3.2 Quantification of Aflatoxins in Malaysian and Nigerian Food Samples

Table 1 presents a summary of the distribution, frequency, and average concentration of individual aflatoxins, and that of total aflatoxin content in each food category. The levels of aflatoxins in the analyzed staples from Nigeria were significantly higher than those found in the samples from Malaysia ($p<0.05$). This discrepancy is anticipated as over 80% of food grains and legumes distributed in Nigerian markets were not screened for aflatoxin as they come directly from fields and farmers [33]. Conversely, in Malaysia, a significant portion of the consumed food grains and legumes are imported [42], subjected to quarantine and screening by regulatory authorities before reaching the market.

3.2.1 Aflatoxin Levels in Malaysian Food Samples

Eighteen (40.9%) of the 44 composite Malaysian food samples exhibited contamination with at least one of the four aflatoxins. The concentrations ranged from 8.68 to 77.40 ng/g, with mean total aflatoxin levels varying between 11.4 ± 5.1 and 43.8 ± 22.5 ng/g (Table 1). The wheat samples exhibited the highest levels of aflatoxins, followed by those of maize, peanuts, and rice. However, despite differences in the number of aflatoxin-positive samples, only rice had significantly lower levels of total aflatoxin ($p<0.05$) than wheat, peanut and maize samples.

In terms of aflatoxin relative distribution in the positive samples, AFB_1 was found in 14 (77.8%) of the total 18 positive food samples, whereas AFB_2, AFG_1, and AFG_2 were found in five (27.8%), eight (44.4%), and six (33.3%) samples, respectively. Across all contaminated food samples, AFB_1 and AFB_2 had the highest overall mean levels of 29.9 ± 7.9 ng/g and 35.7 ± 3.9 ng/g, respectively, which were significantly different ($p<0.001$) from AFG_1 (17.6 ± 9.9 ng/g) and AFG_2 (19.6 ± 9.5 ng/g).

Overall, aflatoxin levels in 11 (61%) out of the 18 positive samples were higher than the 35 ng/g allowed limit in Malaysia and Europe [55]. However, the percentage of positive samples obtained in the present study was less than those documented previously for Malaysian spices [30], red rice [29], chilies [28, 56, 57], nuts and their products [58], food products [59], raw peanut kernels [60], peanuts samples [61, 62], and corn [62]. Similarly, the aflatoxin concentrations in grains and legume samples in the present study were also less than those published in previously [61, 63], but higher than those from some studies on cereals [29, 63–65] and peanuts [61, 66, 67].

3.2.2 Aflatoxin Levels in Nigerian Food Samples

In total, 64.3% (54 samples) out of the 84 Nigerian food samples analyzed had detectable levels of at least one or more of the aflatoxins in the range of 0.2 to 114.4 ng/g, with a total aflatoxin mean range of 24.2 ± 8.5 to 32.9 ± 20.4 ng/g. Among the tested staples, the peanut samples were the most contaminated and exhibited the highest levels of aflatoxins, followed by maize, wheat and rice. Notably, despite these differences, there was no statistically significant variance ($p>0.05$) observed in the mean aflatoxin levels across the four types of food samples. Aflatoxin levels in 46% of the

positive samples were higher than the 20 µg/kg permitted limit that set in Nigeria and many other nations, including the United States, Kenya, Nepal, Brazil, Philippines, Austria etc. [55].

On the frequency of occurrence, AFB_1 was the most detected. It contaminated about 81% (44 samples) out the 54 total aflatoxin-positive samples. Of these, 18 samples contain only AFB_1, eight samples contain all four aflatoxins, while the remaining 18 samples contain AFB_1 together with one or two of the three other aflatoxins. On the other hand, AFB_2, AFG_1 and AFG_2, contaminated only 26 (48%), 25 (46%), and 15 (28%) of the 54 aflatoxin-positive samples, respectively. But, in terms of the overall mean concentration, AFB_2 was the highest, then AFB_1, AFG_2, and AFG_1.

However, the concentrations of aflatoxins in the analyzed Nigerian food samples were less than those published in previous studies from some local food stuffs in other parts of Nigeria such as peanut cake (mean range = 151 to 1,428 µg/kg) [68], snacks (14 to 1,041 µg/kg) [69], stored maize (26.5 to 15, 489.6 µg/kg) [70], dried tomatoes (853 to 1, 430 µg/kg) [71], peanuts (0.0 to 2,076 µg/kg) [72], and cereals (0.88 to 589.8 µg/kg) [73]. However, the aflatoxin levels in our study were also higher than those published by other studies from Nigeria [74–80]. Furthermore, a study in Mali, Burkina Faso, and Niger found mean aflatoxin concentrations in maize, sorghum, and groundnut were high, with exposure values ranging from 6 to 69, 29 to 432, and 310 to 2100 ng/kg bw/day, respectively [81]. Some studies in Uganda reported that 45% to 60% of maize and groundnut foods in Uganda were contaminated with aflatoxins with levels higher than 20 ppb [82, 83].

Overall, the high levels of Aflatoxins found in this study, particularly in Nigerian samples, can be attributed to poor storage conditions and limited regulatory enforcement. This highlights the urgent need for enhanced market surveillance and stricter aflatoxin regulations in Nigeria and Malaysia to mitigate the health risks in these communities. Essential preventative measures, including better storage practices, routine screening of food products, and public awareness campaigns should be enhanced to reduce aflatoxin exposure and protect public health.

3.3 Dietary Exposure Risk Assessment and Characterization of Consumers

Research had shown that in the absence of AFB_1, detection of other aflatoxins is not observed [84]. Hence, estimations of dietary exposure for total aflatoxin in food stuffs or feeds are calculated based on the AFB_1 toxigenic potency. For exposure assessments using urine or milk, the excretory form of AFB_1 (AFM1) is used [78]. Therefore, in this study, the DERA and attributable HCC risk in exposed populations were determined using the level of AFB_1 and TAFs in the 128 Nigerian and Malaysian composite grains and legume samples analyzed (Table 2).

The level of aflatoxins obtained in Malaysian samples indicated a very low dietary exposure risk in the mean range of 11.2 to 25.1 ng/kgBw/day with statistical boundary at 95% confidence interval (CI) in the range of 3.8 to 16.1 ng/kgBw/day (lower bound, LB) and 18.8 to 34.1 ng/kgBw/day (upper bound, UB) (Table 2). Consequently, the dietary level of aflatoxins could lead to an estimated 5.7 to 12.8% mean HCC incidence /100,000 people/year in the range of 1.8 to 8.2% LB and 9.6 to 17.4% UB at the 95th percentile.

Among the four staples examined, consumption of rice could pose the highest mean DERA of 25.1 ng/kgbw/day in the 95th percentile LB – UB range of 16.1 – 34.1 ng/kgbw/day in Malaysia, which might result in an estimated 12.8% yearly mean incidence of HCC/100,000 people with a 95% CI range of 8.2 – 17.4% (LB – UB) HCC/100,000 people/year. This was followed by an estimated mean incidence of 8.8%, 7.8%, and 7.0% of HCC/100,000 people in the proximal consumers in Malaysia per year with a 95% CI ranges between 4.7 – 12.9%, 5.9 – 7.9% and 3.2 – 10.8% due to consumption of peanut, maize and wheat and maize, respectively.

The calculated percentage HCC incidence rates attributable to the DERA via consumption of grains and legume in Malaysia were similar to previously documented Malaysian incidence of 5.5 % in 2010 [60], 0.6 to 14.9% in 2011 [58], 13.5% in 2012 [85], and 12.4 to 17.3% in 2012 [17]. However, there were also relatively higher percentage of incidence reported in 2010 (91 to 857 %) [41] and 2013 (14.7 to 29.6%) [86]. A recent literature survey revealed that the dietary intake of aflatoxins among Malaysians has significantly elevated compared to other Asian nations, varying from 0.002 to 34.00 ng/kg body weight/day. The increased dietary exposure was linked to Malaysia's extreme weather conditions, global climate change, and less stringent regulatory standards for aflatoxins compared to developed countries [4].

Generally, based on the current $BDML_{10}$ (10% extra cancer risk) of 400 ng/kgbw/day for aflatoxins (EFSA, 2020), the Scientific Committee of EFSA had suggested that a MOE value of 10,000 and above would be of minimal risk to public health. Thus, aflatoxin exposure could be considered a public health issue in situations where MOE values were less than 10,000 [49]. Hence, exposure of more than 0.04 ng/kgbw/day (calculated by dividing 400 ng/kgbw/day by 10,000) would pose a danger to public health. In the present study, the MOE values for all the food grains and legume were less than the reference value (10,000). Hence, the exposure data for both AFB_1 and TAF could be characterized as a public health issue, and taking measures to alleviate the risk should become a priority. The findings of this study have been corroborated by recent studies in Malaysia that reported a high prevalence of aflatoxin contamination in Malaysian food products and linked it with a significant risk of HCC. According to a study by Schrenk *et al*. [87], there is a strong correlation between aflatoxin exposure and the incidence of liver cancer in Malaysia. The study found that individuals with high levels of aflatoxin biomarkers had a significantly increased risk of developing HCC [87].

Table 1 Aflatoxin levels in the Malaysian and Nigerian food grains (ng/g)

Country	Sample type	N	AFB_1		AFB_2		AFG_1		AFG_2		TAFs	
			n	Concentration	N	Concentration	n	Concentration	n	Concentration	N_t	Concentration
Malaysia	Rice	11	6	14.9 ± 5.1	1	10.3 ± 2.9*	1	8.9 ± 4.0*	nd	nd	7	11.4 ± 5.1
	Maize	11	1	40.3 ± 4.0*	1	39.5 ± 2.1*	2	14.3 ± 8.1	1	23.9 ± 3.6*	3	29.5 ± 8.1
	Wheat	11	4	36.2 ± 12.2	1	69.8 ± 8.9*	2	30.5 ± 22.2	3	39.2 ± 33.1	5	43.8 ± 22.5
	Peanut	11	3	28.0 ± 10.5	2	23.0 ± 1.7	3	16.9 ± 5.4	2	15.2 ± 1.3	4	20.8 ± 4.7
Nigeria	Rice	21	9	18.3 ± 5.5	10	41.3 ± 19.4	3	17.7± 3.6	6	19.5 ± 6.2	16	24.2 ± 8.5
	Maize	21	11	28.7 ± 10.4	2	32.9 ± 9.4	7	23.6 ± 12.4	2	25.5 ± 0.8	13	27.7 ± 8.2
	Wheat	21	10	32.7 ± 26.3	7	46.4 ± 31.2	5	12.3 ± 3.7	4	20.9 ± 9.0	11	28.1 ± 17.6
	Peanut	21	14	45.7 ± 33.7	7	42.6 ± 21.0	5	19.5 ± 8.5	3	23.8 ± 17.9	14	32.9 ± 20.4

Note: Values for the concentration are mean ± standard deviation (SD), N refers to the total number of composite samples analyzed, n refers to the number of samples positive for individual aflatoxin in the N samples analyzed, TAFs stands for total aflatoxins, N_t is the number of samples positive for all the aflatoxins (TAFs), * indicates that only one sample is positive, and the mean ± SD value is for the triplicate measurement of the respective aflatoxin level in that positive samples.

Table 2 Assessed dietary exposure to aflatoxins and corresponding incidences of primary liver cancer (HCC) in Malaysia and Nigeria

Country	Sample categories	Aflatoxins	Mean level of aflatoxins (ng/g)			Estimated Level of dietary Exposure (ng/kgBw/day)			Margins of Exposure (MOEs) based on $BDML_{10}$ of 400 ng/kgBw/day			Attributable HCC per 100,000/year			% Incidence of the HCC per 100,000/year		
				95% CI			95% CI			95% CI			95% CI			95% CI	
			$\bar{x}$	LB	UB	$\bar{x}$	LB	UB	$\bar{x}$	LB	UB	$\bar{x}$	LB	UB	$\bar{x}$	LB	UB
Malaysia	Rice	AFB_1	14.9	9.5	20.3	25.1	16.1	34.1	26.4	16.9	35.8	0.6	0.4	0.8	12.8	8.2	17.4
		TAFs	11.4	6.7	16.1	19.2	11.3	27.1	20.9	12.3	29.6	0.5	0.3	0.7	9.8	5.7	13.9
	Maize	AFB_1	40.3	30.4	50.2	15.3	11.5	19.1	26.1	19.7	32.6	0.4	0.3	0.5	7.8	5.9	9.7
		TAFs	29.5	9.4	49.6	11.2	3.6	18.8	35.8	11.4	60.2	0.3	0.1	0.5	5.7	1.8	9.6
	Wheat	AFB_1	36.2	16.8	55.6	13.7	6.4	21.0	29.2	13.5	44.8	0.3	0.1	0.5	7.0	3.2	10.8
		TAFs	43.8	15.9	71.7	16.7	6.0	27.4	24.0	8.7	39.3	0.4	0.1	0.7	8.5	3.1	13.9
	Peanut	AFB_1	28.0	15.0	41.0	17.2	9.2	25.2	23.3	12.4	34.1	0.4	0.2	0.6	8.8	4.7	12.9
		TAFs	20.8	15.0	26.6	12.8	9.2	16.4	31.3	22.5	40.1	0.3	0.2	0.4	6.5	4.7	8.3
Nigeria	Rice	AFB_1	18.3	14.1	22.5	50.1	38.5	61.7	8.0	3.8	6.1	4.1	3.2	5.0	57.9	44.5	71.3
		TAFs	24.2	19.7	28.7	40.9	33.2	48.6	9.9	8.0	11.7	3.4	2.8	4.0	47.3	38.4	56.2
	Maize	AFB_1	28.7	21.7	35.7	28.4	21.5	35.3	26.1	20.0	32.8	2.3	1.7	2.9	32.8	24.8	40.8
		TAFs	27.7	22.7	32.7	27.7	22.7	32.7	35.5	29.2	41.9	2.3	1.9	2.7	32.0	26.3	37.7
	Wheat	AFB_1	32.7	13.9	51.5	42.3	18.0	66.6	9.4	2.8	10.4	3.5	1.5	5.5	48.9	20.8	77.0
		TAFs	28.1	16.3	39.9	25.7	14.9	36.5	15.6	9.0	22.2	2.1	1.2	3.0	29.7	17.2	42.2
	Peanut	AFB_1	45.7	26.2	65.2	23	13.2	32.8	17.4	12.2	30.3	1.9	1.1	2.7	26.7	15.3	38.1
		TAFs	32.9	21.1	44.7	20.2	13.0	27.4	19.8	12.7	26.8	1.7	1.1	2.3	23.4	15.0	31.8

Note: TAFs stands for total aflatoxins, $\bar{x}$ stands for mean, LB refers to lower bound, UB refers to upper bound, CI refers to confidence interval.

Conversely, the level of aflatoxins in Nigerian staples analyzed in this study indicated a very high DERA with a mean range of 23.0 to 50.1 ng/kgBw/day and 95% CI mean range (LB to UB) between 13.2 – 38.5 ng/kgBw/day to 32.8 – 61.7 ng/kgBw/day which could lead to an estimated 26.6 to 57.9% mean yearly incidence of HCC/100,000 people with a 95% CI mean range (LB to UB) between 15.3 – 44.5% HCC/100,000 people/year and 38.1 – 71.3% HCC/100,000 people/year (Table 2). Similar to Malaysia, consumption of rice in Nigeria was associated with the highest DERA of 50.1 ng/kgBw/day that could lead to an estimated mean HCC/100,000 people of 57.9% incidence per year with a 95% CI range of 44.5 – 71.3% (LB – UB) HCC/100,000 people/year. This was followed by an estimated mean incidence of 48.9%, 32.8%, and 26.7% of HCC/100,000 people in the proximal consumers in Nigeria per year with a 95% CI ranges (LB – UB) between 20.8 – 77.0%, 24.8 – 40.8%, and 15.3 – 38.1% due to consumption of wheat, maize and peanut, respectively.

Compared to Malaysia, Nigeria faces even higher health risks due to higher contamination levels of aflatoxins and less stringent food safety measures. Chronic aflatoxin exposure contributes to the high incidence of HCC in the country. A study by Ayeni *et al*. [88] highlights the severe impact of aflatoxin exposure on liver health in Nigeria, noting that the prevalence of HCC is alarmingly high in regions with high aflatoxin contamination in staple foods [88]. Another recent report from Nigeria highlighted that maize, rice, peanut and wheat consumptions could lead to aflatoxin exposures in the range of 0.000017 ng/kgbw/day to 980.56 ng/kgbw/day, 1.23 to 628.2 ng/kgBw/day, 0.60 to 397 ng/kgbw/day, and 1.50 to 18.80 ng/kgbw/day, respectively. These might lead to an estimated percentage incidence of HCC from 0.002 to 708% /100,000 people/year in the mean range of 161 to 176% between 1998 and 2008. Between 2009 and 2018, the percentage was 0.0046 to 45,602% /100,000 people/year, in the mean range of 84.0 to 1,052.5% [33].

Furthermore, the findings of this study corroborated a previous report [89], which found a connection to aflatoxin poisoning in the post-mortem of people who died of liver cancer in Nigeria. Accordingly, doctors have been observing increasing cases of aflatoxicosis and other mycotoxicoses in Nigeria, but most of the data were not published [89]. Furthermore, the MOE values for all Nigerian food types in this study were less than the reference value of 10,000 set by EFSA [3]. Hence, the exposure risks signified by the aflatoxin levels in the samples were also of public health concern to the exposed population and urgently required risk management action.

Other health hazards on the proximal communities in the study that are implied by aflatoxin levels in the analysed staples in this study could be:

Stunted Growth in Children: Aflatoxin exposure has been linked to stunted growth and developmental delays in children. Chronic intake of aflatoxin-contaminated food can impair nutrient absorption and immune function, leading to malnutrition. Given the obtained levels of aflatoxins exposure in Malaysia (11.2 to 25.1 ng/kgBw/day) and Nigeria (23.0 to 50.1 ng/kgBw/day) in this study, the children in the affected communities may be at risk of growth impairments. A study by Ahlberg and colleagues [90] found that an aflatoxin exposure of 3.5 ng/kgBw/day in Kenyan children has resulted in −0.340 height for age z-score reduction in growth. Research by Nazhand *et al*. [91] indicates that children in regions of Malaysia with high aflatoxin levels in food have higher rates of stunted growth and developmental issues compared to regions with lower contamination levels. The impact of aflatoxin on child growth is even more pronounced in Nigeria, where food insecurity and poor nutrition exacerbate the effects of aflatoxin exposure. A previous study had found that children in aflatoxin-endemic areas of Nigeria exhibited significantly higher rates of stunting and underweight compared to children in areas with lower aflatoxin exposure [84].

Immune System Suppression: Aflatoxins can suppress the immune system, increasing susceptibility to infectious diseases. This is particularly concerning in areas with high aflatoxin contamination. Numerous studies in Africa have demonstrated a strong correlation between aflatoxin exposure and an elevated risk of hepatocellular carcinoma (HCC), immunodeficiency, and the development of infectious diseases, as well as vaccine failure [92, 93]. In Nigeria, immune suppression due to aflatoxin exposure contributes to the high prevalence of infectious diseases, compounding public health challenges. According to Mupunga *et al*. [94], aflatoxin exposure in Nigerian communities is associated with increased incidence and severity of infectious diseases, including malaria and respiratory infections. A study by Turna *et al*. [95] demonstrated that aflatoxin exposure in Malaysian populations leads to a decrease in immune function, making individuals more vulnerable to infections such as hepatitis and tuberculosis. Research has also indicated a significant link between unsafe sexual practices and the development of aflatoxicosis in HIV-endemic areas [96]. This implies that aflatoxins may play a role in AIDS-related immunosuppression and fatalities, potentially contributing to the higher incidence of aflatoxicosis. For example, in Nigeria, the HIV prevalence rate is 1.4%, with approximately 1.9 million people living with HIV and around 160,000 AIDS-related deaths reported in 2016 alone [97]. These statistics suggest a potential correlation between aflatoxin exposure and AIDS.

Therefore, the health implications of aflatoxin contamination in Malaysia and Nigeria are profound, contributing to liver cancer, stunted growth in children, and immune system suppression. These findings highlight the urgent need for strengthened regulatory frameworks, public health interventions, and technological innovations to mitigate aflatoxin exposure and protect public health.

4.0 CONCLUSION

This pioneering study highlights a critical public health concern regarding aflatoxin exposure and HCC risk in local markets across Kelantan State, Malaysia, and Katsina State in Nigeria. The results showed that aflatoxins contamination was above the acceptable limit in more than 50% of the samples analyzed in both countries. These findings highlight the need for targeted interventions, including improved storage practices, regular market surveillance, and stricter regulatory enforcement to protect public health. According to the calculated MOE values, proximal populations within the study areas were at high risk of aflatoxin exposure and consequent HCC. Therefore, it was justifiable to infer that the elevated risk of aflatoxin exposure in the regions examined stemmed from the substantial quantity of positive samples. Therefore, extensive screening should be prioritized to ensure that aflatoxin-contaminated food grains and legumes in the open markets are withdrawn to prevent the risk of aflatoxicosis in local communities. Intervention studies were also recommended to assess the knowledge and practice of farmers, traders and the general public regarding fungal and aflatoxin contamination in grain and legume products, as well as to develop an effective awareness campaign on the dangers of aflatoxicosis.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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