Prevalence of aflatoxin in dried okra (*Abelmoschus esculentus*) and tomatoes (*Lycoperisicon esculentum*) commercialized in Ibadan metropolis

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**Introduction**

Aflatoxin as defined by Busby and Wogan [1] are group of carcinogenic, tetragenic and mutagenic mycotoxins commonly associated with fruits, vegetables and other products whilemycotoxins generally areadsed to be poisonous secondary metabolites produced by moulds when growing on different food products [2].

In West African sub-regions, aflatoxin B1, B2, G1 and G2 are the major ones because they are thermo-stable [3] and due to their high prevalence in nature and toxicity are said to be the most important mycotoxins in food and feeds [4].

Historically, scientific research on Aflatoxin started after the incidence that took place the year 1960 in England where a large number of turkey poults died after eating contaminated groundnut meal that was imported from Brazil, the atoxigenic fungus was identified as *Aspergillus flavus* and the toxic principle named Aflatoxin meaning Aspergillus toxin [5].

The significance of this study is to give insight on the causes of aflatoxicosis outbreak in Ibadan city. It also provides adequate information on the level of aflatoxins in the vegetables as they form a vital nutritious component of the daily diet of the citizens. It will also be vital in setting up prevention, control and management programs on aflatoxin contamination in Ibadan as a public health issues.

Aflatoxin contamination of food is said by Bhat and Miller [6] to be of serious problem as they bind to DNA and consequently prevent transcription of genetic information which eventually has an adverse effect in humans and other animals. More so, they have been reported by Stoloff [7] to be acutely and chronically toxic causing acute Liver damage, Liver cirrhosis, induction of tumors and teratogenic effects.

Report on the various infestations of Okra and tomatoes by *Aspergillus* species [4] and the ability of some *Aspergillus* strains to produce aflatoxin [8] justifies the need to determine the possible contamination of these vegetables with aflatoxin. The aim of this research work is to provide information on the natural occurrence of aflatoxin in the two vegetables sold in Ibadan metropolis in lieu of the very scarce data on it.

The objective of the study is to determine:

- The incidence and concentration of aflatoxins in these commodities and
- The occurrence of the fungi on the them

**Materials and methods**

**Sterilization of materials used for the research**

All materials used for the research work were sterilized and the media was prepared according to manufacturer’s instruction and autoclave at 121°C for 10 minutes [9-11].

**Samples collection**

Hundred grams of each sample were collected from the four markets namely Oje, Bodiga, Shasha and Orita-merin in five replicate of 20gram in separate airtight sterile polythene bag to prevent further contamination until aflatoxin analysis was done and subsequent isolation and identification of fungi.

**Aflatoxins Extraction**

Aflatoxins was extracted from the samples as described by Hell, et al. [12] with modifications employed due to different weight and dryness of the samples. Dichloromethan was used to extract the toxin and allowed to evaporate to dryness in laminar air flow-hood chamber for 48 hours until analysed.

**Qualitative analysis**

Four micro litres of the dissolved extract were spotted on the thin layer chromatography (TLC) plates 20*10cm with aflatoxin standards G and I and then allowed to develop in a tank containing diethyl ether, methanol and water at the ratio of 96: 3: 1 respectively. The spots intensities were visually compared with those of standards under ultraviolet light 366 nm wavelengths [13].

**Quantitative analysis**

Aflatoxin quantification was done by scanning with CAMAG TLC Scanner3 (densitometer), which measured the absorbance and fluorescence of the toxin extracted [14].

**Isolation of fungi**

Direct isolation method was employed for fungi isolation. The samples were surface sterilized in 70% ethanol for 10minutes and
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Results

Aflatoxins (ppb) content of dried okra and tomato sampled from four markets in Ibadan

The aflatoxin content (ppb) in dried okra sampled from four markets in Ibadan is shown in Figure 1. The figure revealed that aflatoxin B1 was highest in samples from Oje market (33.490ppb) and lowest in samples from Bodija market (26.690ppb). Aflatoxin B2 was highest in samples from Bodija market (3.736) and lowest in samples from Ojota-merin market (0.932ppb). In all the markets, the percentage occurrence of aflatoxin B2 was highest in samples from Ojota-merin market (60%) and Shashs market (60%) while reported the highest occurrence in Bodija market (100 %) and Oje market (100%). A. niger recorded the highest occurrence in Oritamerin market (95%) while that of A. flavus was the least (50%). The aflatoxinproducing fungi showed a significant difference in their percentage occurrence between the various markets (p<0.05).

Percentage occurrence of aflatoxin producing fungi in okra and tomato sampled from various markets in Ibadan

The percentage occurrence of aflatoxin producing fungi in dried Okra sampled from four markets in Ibadan is shown in Table 1. A total of three aflatoxin producing fungi include A. flavus, A. niger and A. parasiticus were isolated from dried okra seed samples from the four markets. A. flavus reported the highest occurrence in Bodija market (100 %) and Oje market (100%). A. niger recorded the highest occurrence in Oritamerin market (60%) and Shashs market (60%) while A. parasiticus showed the highest occurrence in Oje market (80%) and Shashs market (80%). In all the markets, the percentage occurrence of A. flavus was the highest (95%) while that of A. Niger was the least (50%). The aflatoxinproducing fungi showed a significant difference in their percentage occurrence between the various markets (p<0.05).

The percentage occurrence of aflatoxin producing fungi in dried tomatoes sampled from four markets in Ibadan is shown in Table 2.

Identification of fungi

Isolates were identified based on colony characteristics, strain morphology, macroscopic feature and microscopic feature [16]. The pure cultures were characterized and subsequently identified with the aid of a compound microscope as the representatives of the different colonies/fungi [16].

Calculation of the percentage occurrence of the different fungi isolates were done to determine their frequencies from the 4 different markets. Five plates from each market were used, the number of occurrence of each of the isolates was recorded, the mean taken and calculated as a ratio of the total number of occurrence and then expressed as a percentage using the formula:

\[
\text{Percentage occurrence} = \frac{X}{N} \times 100
\]

Where:

- \(X\) = Total number of each isolate in all the market samples
- \(N\) = Total number of all isolates in all the market samples

Statistical analysis

The experimental design was a complete randomized one. The levels of aflatoxin contamination on the samples were illustrated with an error bar chart at 95% cl. The chart was obtained by plotting the aflatoxin concentrations against the different markets. The incidences of the fungi were determined by calculating their percentage frequency.

IBM SPSS Statistical data editor version 21.00 was used to perform the ANOVA and chi-square analysis at P<0.05 level of significance.

Table 1. Aflatoxin content (ppb) in dried okra sampled from four markets in Ibadan

| Market       | Aflatoxin B1 (ppb) | Aflatoxin G1 (ppb) | Aflatoxin G2 (ppb) | Aflatoxin B2 (ppb) |
|--------------|-------------------|--------------------|--------------------|-------------------|
| Oje Market   | 33.490            | 0.853              | 0.878              | 0.700             |
| Oritamerin Market | 26.690            | 9.011              | 9.583              | 0.878             |
| Shasha Market | 20.010            | 6.878              | 7.878              | 0.878             |
| Bodija Market | 16.980            | 5.878              | 6.878              | 0.878             |

Table 2. Aflatoxin content (ppb) in dried tomato sampled from four markets in Ibadan

| Market       | Aflatoxin B1 (ppb) | Aflatoxin G1 (ppb) | Aflatoxin G2 (ppb) | Aflatoxin B2 (ppb) |
|--------------|-------------------|--------------------|--------------------|-------------------|
| Oje Market   | 33.490            | 0.853              | 0.878              | 0.700             |
| Oritamerin Market | 26.690            | 9.011              | 9.583              | 0.878             |
| Shasha Market | 20.010            | 6.878              | 7.878              | 0.878             |
| Bodija Market | 16.980            | 5.878              | 6.878              | 0.878             |
Aflatoxin producing fungi were associated with production of aflatoxins in the dry commodities. The detection of aflatoxin in these samples might be due to the market sanitation and handling by the sellers. Okigbo, et al. [21] has also emphasized that factors such as harvesting method, handling, processing, storage and even climate can influence the presence and abundance of aflatoxins producing fungi in food products.

Conclusions and recommendation

Hazard analysis critical control point (HACCP) should be adopted at every point during food processing chain to help reduce fungal infection and subsequent aflatoxin contamination of food commodities.

Government should also enforce enlightenment programs to educate the citizens about food safety. Also, Mycotoxin regulations should be adopted in our country to help regulate the level of mycotoxin in locally consumed food to ensure food security which is basic for good health and better economy. There is also a need for further research on how best to prevent and control these toxins.

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