Aflatoxin Contamination In Some Fresh Vegetables And A Spice Sold At Samaru Market Zaria, Nigeria

[1]Haruna, M, [2]Saleh, A., [3]Dangora, D. B., [3]Zongoma, A. M. and [4]Ali, M.
[1]Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria,
[2]National Root Crop Research Institute Umudike, Maro Sub-station, Kaduna, Nigeria,
[3]Department of Botany, Ahmadu Bello University Zaria, Kaduna, Nigeria and
[4]National Population Commission, Dutse Jigawa State
Correspondence: mustaphaharuna65@yahoo.com (08069587120/+27719637814)

Abstract: Aflatoxin is a common contaminant of agricultural produce resulting from the growth of *Aspergillus* species under conditions of favourable temperature and moisture. This study was conducted to assess the level of aflatoxin contamination in fresh vegetables and tamarind used on daily basis by the populace. A total of 9 different fresh vegetables and tamarind samples were randomly collected in new polythene bags from Samaru market, Zaria, Nigeria, and were screened for aflatoxin contamination. The vegetables were onion, cabbage, bell pepper, pepper, pumpkin, tomato, peas, cucumber and amaranthus, and tamarind. Aflatoxin contamination was determined by using Enzyme-linked Immunosorbent Assay (ELISA). All the samples tested positive to total aflatoxin contamination ranging from 2 – 100 µg/kg. Six out of the 10 samples namely onion, cabbage, amaranthus, tomato, cucumber and tamarind contained total aflatoxin above the acceptable limit of 10 µg/kg set by National Agency for Food and Drugs Administration and Control (NAFDAC), therefore, are not safe for human consumption.
Key words: ELISA, Vegetables, Spice, Total Aflatoxin, NAFDAC

INTRODUCTION

Vegetables are a rich source of vitamins, minerals and fibres required for the growth, development, and normal functioning of the human body (Said, 2012; Silva *et al*., 2017). However, vegetables are suitable substrates for the growth of a wide range of microorganisms which contaminate and undermine their quality, nutritional and health benefits leading to increasing outbreaks of human infections associated with the consumption of fresh or minimally processed fruits and vegetables (Suleiman *et al*., 2017). Fungi are cosmopolitan and may form a part of the vegetable or fruit micro-flora as incidental contaminants from the soil, dust and surroundings, or because of poor agricultural and unsanitary handling practices (Ofor *et al*., 2009). This fungal contamination will subsequently lead to aflatoxin contamination under favourable environmental conditions (Haruna *et al.,* 2016). Furthermore, fungal contamination and subsequent aflatoxin production may occur at various stages, including pre-harvest and post-harvest, post-harvest contamination may lead to changes in the quality and nutritional value of the fruits and vegetables (Barkai-Golan and Paster, 2008).

Aflato**x**ins are a group of toxic, mutagenic, teratogenic and carcinogenic secondary metabolites of fungal origin produced by different *Aspergillus* species such as *A. flavus*, *A. parasiticus* and in rare cases, *A. nomius* and *A. pseudotamari* (Jackson and Al-Taher, 2008; Mazaheri, 2009). A lot of factors influence the production of aflatoxins such as pH, water activity (a_w of 0.95), redox potential of the substrate, relative humidity, temperature (13–40▪ C) and oxygen availability of the environment where the commodities are produced, stored or processed (Whitlow and Hagler, 2004; Fernández-Cruz *et al.*, 2010; Nagwa *et al*., 2013). Aflatoxicosis is the disease associated with the ingestion of aflatoxins (Adejumo and Adejoro, 2014). Samaru market is a popular local market with poor or no drainage systems and untarred ground situated amidst Samaru residential area and many government institutions where it serves as a source of vegetables and other food commodities. Unhygienic handling of vegetables and spices is a common norm among the sellers in the market which may increase the risk of aflatoxin contamination. Therefore, this research is aimed at assessing the level of aflatoxin contaminations in fresh vegetables and tamarind sold at Samaru market Zaria, Nigeria.

MATERIALS AND METHODS

Sample Collection

Fresh- and healthy-looking samples representing 9 different types of vegetables, and tamarind were randomly collected in new polythene bags from Samaru open market located in Zaria, Kaduna state, Nigeria. Three (3) replicates of each vegetable, and tamarind were obtained and mixed to prepare one composite sample as described by Haruna *et al.* (2016). Samples were oven dried at 60ºC for 48 hours (Aziz, 1987), afterwards, the dried samples were individually and finely ground using a laboratory mill (Romer). Table 1 shows list of samples with their scientific, common and local names.

Aflatoxin Assay

Enzyme linked immunosorbent assay ELISA (Beacon) test kit was used to determine the content of total aflatoxins in the samples. This process occurred in three stages namely;

Sample Extraction

Sample extraction was performed according to the manufacturer's instruction (Beacon) test kit. Two and half gram (2.5 g) of sodium chloride was added to 25 g of each ground sample, and 50 ml of 80% (v/v) methanol was added in the mixture. The resulting mixture was blended for one minute in a high-speed blender, and this was followed by filtration using a coffee filter paper. Afterwards, 5 ml of the extract was diluted with 20 ml distilled water and mixed thoroughly, and then filtered through a glass fiber filter.

Table 1: Vegetables with their Respective Scientific, Common and Local Names

Serial No.	Scientific Name	Common Name	Local Name
	Vegetables		
1	*Allium cepa* L	Onion	Albasa
2	*Brassica oleracea* L	Cabbage	Kabeji
3	*Capsicum annuum* L	Bell pepper	Tattasai
4	*Capsicum frutescence* L	Pepper	Attarugu
5	*Cucurbita pepo* L	Pumpkin	Kabewa
6	*Solanum lycopersicum* L	Tomato	Tumatir
7	*Pisum sativum* L	Peas	Wake
8	*Cucumis sativus* L	Cucumber	Kokwamba
9	*Amaranthus caudatus* L	Amaranthus	Alayyahu
	Spice		
10	*Tamarindus indica* L	Tamarind	Tsamiya

ELISA Test

ELISA test was carried out according to Beacon test kit manual (2018 instruction booklet). Fifty microlitre (50 µL) of enzyme conjugate was dispensed into each test well. Fifty microlitre (50 µL) of each standards and samples were added into each test well containing 50 µL of enzyme conjugate, afterwards, 50 µL of antibody solution was added into the plate and shaken gently to mix the contents, and then incubated at room temperature for 10 minutes. After 10 minutes, the contents of the microwell strips were discarded, followed by washing each microwell by filling it with distilled water, and then dumping the water from the microwell strips. This was repeated for a total of five washes. Microwell strips were tapped using absorbent paper towels to expel as much residual water as possible after the fifth wash. The bottom of the microwells were dried with a dry towel. One hundred microlitre (100 µL) of the substrate was added into each microwell and incubated at the room temperature for 10 minutes, afterwards, blue colour developed.

One hundred microlitre (100 µL) of stop solution was added into each microwell strip, the colour changed from blue to yellow. The strips were read with microwell reader using an absorbance filter of 450 nm.

RESULTS

All the tested samples (100 %) were contaminated with total aflatoxins in the range of 2-100 µg/kg. Highest contamination was recorded in tamarind (100 µg/kg) followed by onion, cabbage, amaranthus, tomato and cucumber (25 µg/kg). Pepper and Pumpkin had moderate levels of contamination (7.5 µg/kg), while least contaminated samples were bell pepper and peas (2.0 µg/kg) (Table 2).

Table 2: Total Aflatoxin Contamination of Vegetables Obtained from Samaru Market Zaria, Kaduna, Nigeria

Sample	Absorbance (OD)450nm	Total Aflatoxin Concentration (µg/kg)
Vegetables		
Onion	0.929	25
Cabbage	0.771	25
Bell pepper	1.421	2
Pepper	1.123	7.5
Pumpkin rem	1.142	7.5
Tomato	0.958	25
Peas	1.288	2
Cucumber	0.918	25
Amaranthus	0.753	25
Spice		
Tamarind	0.217	100

DISCUSSION

In this study, all vegetable and spice samples tested positive to total aflatoxins contamination. However, tamarind has the highest contamination with 100 µg/kg which is above the maximum acceptable limit of 10 µg/kg set by National Agency for Food and Drugs Administration and Control (NAFDAC) for raw food (Haruna *et al*., 2016). This might be due to storage in unhygienic condition. Tamarind is commonly utilized by rural dwellers to treat certain ailments or used in drinks to add flavour, because of this, it is a common belief among the locals that tamarind that has been stored for a long period has more healing power than the newly harvested one. There is a high risk in consuming tamarind that has been stored for a long period because the fruits are sticky by nature and can easily attract dust particles particularly if it is not stored in a hygienic condition. These dust particles may contain spores of aflatoxin producing fungi which may cause fungal contamination and subsequent aflatoxin production. Vegetable samples that contained high concentrations of total aflatoxins above the maximum acceptable limit of 10 µg/kg set by NAFDAC were onion, cabbage, amaranthus, tomato and cucumber (25 µg/kg). These were followed by pepper and pumpkin (7.5 µg/kg), and bell pepper as well as peas (2 µg/kg). Aflatoxin contamination of vegetables were previously reported in onion at the concentration of 1.2-5.2 µg/kg (Jonathan *et al*., 2012), 0.4-26 µg/kg in cabbage (Hariprasad *et al*., 2013), 0.2-9.6 µg/kg in amaranthus sp (Hariprasad *et al*., 2013), 0.5-7.1 µg/kg in tomato (Suleiman *et al*., 2017), 1.7 µg/kg in cucumber (Hacibekiroghi and Kolak, 2013), 97.5 µg/kg in pepper (Heperkan, 2006), 10.66 µg/kg AFB1 and 12.74 µg/kg AFG1 in pumpkin leaf, 4.1 µg/kg in split peas (Lutfullah and Hussain, 2012).

Aflatoxin contamination of vegetables in this study might have occurred right from the farm due to the proximity of the growing vegetables to the soil, during harvesting or in the market due to poor hygienic conditions. During harvesting, farmers mostly place the harvested produce in either dirty wooden baskets, or old metal containers which may contain fungal spores that contaminate the produce. In the market, vegetables were put in containers placed on mats that were spread on bare ground. The vegetables could easily get contaminated due to their proximity to the bare ground or from the dust particles in the raised air because of movement by the people.

CONCLUSION

In this study, aflatoxin assay showed that all the samples contained aflatoxins. Six out of 10 samples were contaminated with total aflatoxins above acceptable limit of 10 µg/kg set by NAFDAC and EU with highest concentration in tamarind (100 µg/kg), followed by onion, cabbage, amaranthus, tomato and cucumber (25 µg/kg). These vegetables were healthy and did not show any visible sign of deterioration during sample purchase. This implies that the populace is at high risk of aflatoxin exposure from routine vegetables consumption which may lead to aflatoxicosis.

RECOMMENDATIONS

1) Farmers should employ good and sanitary practices especially during harvest by placing their harvested produce in clean containers. Additionally, sanitary practices should also be employed at selling points.
2) Since vegetables are perishable products, good storage conditions should be considered to decrease the chances of rapid contamination by fungi which is the cause of aflatoxin contamination.
3) Regulatory agencies such as NAFDAC should ensure regular monitoring of aflatoxin contamination in vegetables.

REFERENCES

Adejumo, T. O. and Adejoro, D. O. (2014). Incidence of Aflatoxins, Fumonisins, Trichothecenes and Ochratoxins in Nigerian Foods and Possible Intervention Strategies. *Food Science and Quality Management*, 31: 127.

Apeh, D.O., Ochai, D.O., Adejumo, A., Muhammad, H.L. and Saidu, A.N. (2016) Mycotoxicological Concerns with Sorghum, Millet and Sesame in Northern Nigeria. *Journal of Analytical and Bioanalytical Techniques*, 7: 336.

Aziz, N.H. (1987). Etiology of Toxin-Producing Fungi from the Class of Deuteromycetes Occurring in Various Feed Products, PhD. Thesis, Agricultural University, Cracow, Poland.

Barkai-Golan, R. and Paster, N. (2008). *Mycotoxins in Fruits and Vegetables*. Academic Press, San Diego, USA. p3-11.

Fernández-Cruz, M.L., Mansilla, M.L. and Tadeo, J.L. (2010). Mycotoxins in Fruits and Their Processed Products: Analysis, Occurrence and Health Implications. *Journal of Advanced Research*, 1: 113–122.

Hacıbekiroğlu, I. and Kolak, U. (2013). Aflatoxins in Various Food from Istanbul, Turkey. *Food Additives and Contaminants*: Part B, 6 (4): 260-264.

Hariprasad, P., Durivadivel, P., Snigdha, M. and Venkateswaran, G. (2013). Natural Occurrence of Aflatoxin in Green Leafy Vegetables. *Food Chemistry*, 138 (2-3): 1908–1913.

Haruna, M., Dangora, D.B. and Khan, A.U. (2016). Fungal and Aflatoxin Contaminations of Spices Sold in Tsohuwar Kasuwa Market, Katsina, Nigeria. *Nigerian Journal of Scientific Research*, 15(1): 64-68.

Heperkan, D. (2006). The Importance of Mycotoxins and a Brief History of Mycotoxin Studies in Turkey. The

Bulletin of the Istanbul Technical University, 54(4) :24-25.

Jackson, L. S. and Al-Taher, F. (2008). Factors Affecting Mycotoxin Production in Fruits. In: Barkai-Golan R, Paster N. (Ed), *Mycotoxins in Fruits and Vegetables*, Elsevier. p75-104.

Jonathan, S.G., Olawuyi, O.J., Aina, D.A., Dada, H.G. and Oyelakin, A.O. (2012). Influence of Storage Time on Biodeterioration, Aflatoxin Contamination and Food Values of Onion (*Allium cepa*). *Nature and Science*, 10(11):26-32.

Lutfullah, G., and Hussain, A. (2012). Studies on Contamination Level of Aflatoxins in Some Cereals and Beans of Pakistan. *Food Control*, 2: 32-36.

Mazaheri, M. (2009). Determination of Aflatoxins in Imported Rice to Iran. *Food and Chemical Toxicology*, 47: 2064-2066.

Nagwa, J. A., Alarousy, R.M. and Zaki, M.S. (2013). Mycotoxicosis in Animals and Fish. *Life Science Journal*, 10(1).1133-1135. 11

Ofor, M.O., Okorie, V.C., Ibeawuchi, I.I., Ihejirika, G.O., Obilo, O.P. and Dialoke, S.A. (2009). Microbial Contaminants in Fresh Tomato Wash Water and Food Safety Considerations in South-Eastern Nigeria. *Life Science Journal*, 6(3): 80– 82.

Said, D.E. (2012). Detection Parasites in Commonly Consumed Raw Vegetables. *Alexandria Journal of Medicine*, 48(4):345–352.

Silva, O.O., Ayankogbe, O.O. and Odugbemi, T.O. (2017). Knowledge and Consumption of Fruits and Vegetables Among Secondary School Students of Obele Community Junior High School, Surulere, Lagos State, Nigeria. *Journal of Clinical Science*, 14: 68-73.

Suleiman, M.S., Nuntah, L.C., Muhammad, H.L., Mai lafiya, S.C., Makun, H.A., Saidu, A.N., Apeh, D.O. and Iheanacho, H.E. (2017). Fungi and Aflatoxin Occurrence in Fresh and Dried Vegetables Marketed in Minna, Niger State, Nigeria. *Journal of Plant Biochemistry and Physiology*, 5(1): 2-3.

Whitlow, L.W. and Hagler, W.M. (2004). Mycotoxins in Feeds. *Feedstuffs*, 76 (38): 66-76.