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Mycobiota and Aflatoxin Contaminations of Some Spices and Condiments Sold in Katsina Central Market, Nigeria

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Abstract

Spices and condiments get contaminated with fungi and aflatoxin due to poor agricultural and storage practices. A total of 42 dried, raw, powdered and processed samples representing fourteen different types of spices and condiments were randomly collected in new polythene bags from Katsina central market (Katsina town) and were screened for fungi and aflatoxin contaminations. These were Clove, African nutmeg, Ashanti pepper, Candlewood, Ethiopian pepper, Pineapple of the bush, Ginger, Garlic, Chillies, Kajiji, Thyme, Chilli powder (yaji), Curry and Locust bean (Dadawa). Fungi was isolated on Potato Dextros Agar by Agar plate method for dried and raw samples and Standard Dilution Plate method for powdered samples. Enzyme-linked Immunosorbent Assay (ELISA) was used for aflatoxin determination. Ten fungal spp were isolated and identified as *Aspergillus flavus, A. parasiticus, A. fumigatus, A. versicolor, A. niger, Mucor hiemalis, Rhizopus stolonifer, Phoma glomerata, Penicillium citrinum* and *Nigrospora sphaerica.* Percentage occurrence of fungal species ranged from 1.8 % for *Nigrospora sphaerica* to 35.7 % for *A. parasiticus*.. Eight out of 14 spices and condiments (57.1%) contained total aflatoxin ranging from 0.7- >20µg/kg. Two of these (14.2%) namely clove (>20 µg/kg), and ginger (11.6 µg/kg) had aflatoxin levels above maximum acceptable limit of 10µg/kg set by European Union and National Agency for Food and Drug Administration and Control and are therefore, not safe for human consumption.

Key words: Condiments, ELISA, Fungi, Spices, Total Aflatoxin

INTRODUCTION

Food and Feeds can be contaminated with toxigenic fungi either pre-harvest or post-harvest periods (Elshafie *et al*., 2002), resulting in the production of highly toxic secondary metabolites called mycotoxins. Among all known mycotoxins, aflatoxins are the most frequent contaminants of spices and condiments (Ozbey and Kabak, 2012). Aflatoxins are a group of highly toxic secondary metabolites produced by some *Aspergillus* species namely *A. flavus, A. parasiticus* and rarely *A. nomius* (Kumar *et al*., 2008). *A. flavus* produces only B aflatoxin while the other two species produce both B and G aflatoxins (Creppy, 2002). These toxins are named as aflatoxin BI (AFBI), aflatoxin B2 (AFB2), aflatoxin GI (AFGI) and aflatoxin G2 (AFG2) (Tarin *et al*., 2004). Aflatoxin BI has been described as most toxic and classified as group 1 carcinogen by International Agency for Research on Cancer (IARC, 1993). They have carcinogenic, immunosuppressive, teratogenic and mutagenic effects on both human and livestock (Paterson and Lima, 2010). Spices are exposed to contamination by fungi and aflatoxin right from the field (during growth and development), during drying, storage, transport and processing (Elshafie *et al*., 2002) or in the market due to poor hygienic conditions.

Spices are extensively used in Nigeria to flavoring food as well as for medication due to their antioxidant property. But poor handling practices by farmers such as drying of spices on bare ground exposes them to fungal spore and subsequent aflatoxin contamination. Spices are also usually processed in poorly hygienic form which favours growth of moulds and aflatoxin production. In Nigeria little work has been carried out on mycobiota and aflatoxin contamination and most from the southern part of the country (Ezekiel *et al*., 2013; Oranusi *et al*., 2013). There is no available documented report on fungi and aflatoxin contamination in spices and condiments in Katsina State. Therefore, this study is aimed at studying the fungi and aflatoxin contaminations of spices and condiments obtained from Katsina central market.

Materials and Methods

Sample Collection

A total of 42 dried, raw, powdered and processed samples representing fourteen different types of spices and condiments were randomly collected in new polythene bags from Katsina central market, Katsina, Katsina state, Nigeria. For each spice and condiment, three (3) replicates were obtained and mixed to prepare one composite sample (Farid *et al*., 2013). Spices and condiments were chosen based on their availability in the market and popularity of usage. Table 1 shows list of spices and condiments with their Scientific, English, Hausa names as well as parts of plant used.

Mycological Studies

Isolation of fungi by Agar plate method was used for dried and raw spices which are the most commonly used by the populace (Jha, 1995). Each sample was surface sterilized by putting it into 1% sodium hypochloride for two minutes. Each sample was rinsed three times with sterile distilled water. Disinfected samples were transferred with sterile forceps into petri dishes containing sterilized 15ml PDA (supplemented with chloronphenicol) at the rate of four pieces

Table 1: Spices and Condiments with their Respective Scientific, English, Hausa names as well as Part of Plant Used

Scientific Name	English Name	Hausa Name	Part of Plant Used
Eugenia aromatica (L.)	Clove	Karamfani	Bud
Monodora myristica (Gaertan)	African nutmeg	Gyadar miya	Seeds
Piper guineense (Schumach)	Ashanti pepper	Masoro	Seeds
Fagara zanthoxyloides (Lam.)	Candlewood	Fasakwari	Stem
Xylopia aethiopica (Dunal)	Ethiopian pepper	Kimba	Fruit
Thonningia sanguinea (Vahl.)	Pineapple of the bush	Kulla	Root
Zingiber officinale (Roscoe)	Ginger	Citta mai yatsu	Rhizome
Allium sativum (L.)	Garlic	Tafarnuwa	Bulb
Capsicum frutescence (L.)	Chillies	Barkono	Fruit
Cyperus tonkinensis (Hooper)		Kajiji	Corm
Thyme vulgaris (L)	Thyme	Thyme	-
Capsicum frutescence (L.)	Chilli powder	Yaji	-
-	Curry	Kori	-
Parkia biglobosa (Jacq.)	Locust bean cake	Dadawa	-

per plate, larger samples were cut into smaller pieces before being sterilized. These were made in triplicates and plates were incubated at room temperature for 7 days.

Isolation of fungi by Standard Dilution Plate was used for powdered samples (Aziz *et al*., 1998). One gram (1g) of each composite samples was transferred into McCartney bottle containing 9ml of sterile distilled water. Solution was mechanically homogenized using a mechanical shaker at constant speed for 15 minutes. The sample water suspension was allowed to stand for 10 minutes. Ten fold serial dilution was prepared and 1ml portions of suitable dilution (10^{-2} and 10^{-4}) was used to inoculate petri dishes. Plates were incubated at room temperature for 7 days. Pure cultures of isolates were obtained by repeated subculture on PDA.

Percentage of fungal occurrence were calculated using the formula:

$$\% = \frac{\text{Total number of individual fungal occurrence}}{\text{Total number of fungal occurrence}} \times 100$$

Fungi colonies and isolates were identified according to morphological and microscopic characteristics using identification keys (Robert *et al*., 2004; Davise, 2002; Klich, 2002).

Aflatoxin Assay

Spices and condiments were individually and finely ground using laboratory mill. ELISA (AqraQuant Total Aflatoxin Assay 1/20) test kit was used for aflatoxin determination in the samples. This process occurred in three stages namely:

Sample Extraction

Sample extraction was performed according to the manufacturer's instruction (Aqra Quant Total Aflatoxin Assay 1/20) test kit. 25ml of acetonitrile /water (84/16) was added to 5g of each ground spice and condiment sample and the solution was extracted by shaking for 30 minutes using orbital shaker. The sample was allowed to settle and the top layer of extract was filtered through a Whatman no 1 filter paper and the filtrate was collected for clean up.

2.5 Sample Cleanup

Sample Cleanup was performed with MycoSep 226 aflazon according to the manufacturer's instruction, so as to remove interfering substances such as colour and oil. 4ml of extract was transferred into a glass tube, MycoSep column was placed firmly into the top portion of the glass tube and pushed it through until 0.5ml of purified extract was removed. The purified extract (0.5ml) was transfered into vial and evaporated to dryness. The residue in the vial was reconstituted with 0.5ml of 70/30 methanol/water, which was used for ELISA analysis.

ELISA Test

According to AqraQuant Total aflatoxin Assay 1/20 test kit manual. Two hundred µL of conjugate was dispensed into each green-bordered Dilution well. One hundred µL of each standard and sample was added into the appropriate Dilution well containing 200µL of conjugate. Each well was carefully mixed by pipetting it up and down three times and 100µL of the contents from each Dilution well was immediately transferred into a corresponding Antibody Coated Microwell, it was then incubated at room temperature for 15 minutes. The contents of the microwell strips were discarded followed by washing each microwell by filling it with distilled water, and then the water from the microwell strips was discarded. 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 µL of the substrate was added into each microwell and incubated at room temperature for five minutes and blue color developed. One hundred µL of stop solution was added into each microwell strip, the color changed from blue to yellow. The strips were read with microwell reader using an absorbance filter of 450 nm.

RESULTS

Spices and Condiments Mycobiota

Fifty six fungal isolates representing 10 species of 6 genera were isolated from samples obtained from Katsina central market. *Aspergillus* was the most common genus, represented by 5 species and 43 isolates. Highest occurring fungi were *A. parasiticus* and *A flavus* and were found to contaminate (8 and 9) samples respectively. Moderate level of occurrence was recorded in *Rhizopus stolonifer*, *A. niger*, *A. versicolor* and *A. fumigatus*. The least occurring fungi were *Phoma glomerata*, *Penicillium citrinum*, *Mucor hiemalis* and *Nigrospora sphaerica* (Table 2).

Spices that had highest contamination with fungi from Katsina central market were clove (16.07%), thyme and kajiji (12.50%), Ethiopian pepper (10.71%), African nutmeg and chilli powder (8.93%). Moderately contaminated samples were garlic, ginger and locust bean cake (5.36%). Lowest contaminated samples were Chillies, Pineapple of the bush and Candlewood (3.57%) followed by curry and Ashanti pepper (1.79%) (Fig. 1).

Aflatoxin Contents of Spices and Condiments

Eight out of 14 spices and condiments samples (57.1%) contained total aflatoxins in the range of 0.7->20µg/kg. Highest level of aflatoxin contamination was recorded in clove (>20 µg/kg), followed by ginger (11.6 µg/kg) and African nutmeg (9.9 µg/kg). Moderate level of contamination was recorded in yaji (8.1 µg/kg) and Ethiopian pepper (7.3 µg/kg). The lowest aflatoxin contamination was recorded in thyme (2.6 µg/kg) followed by kajiji (0.8 µg/kg) and candlewood (0.7 µg/kg). Aflatoxin was not detected in garlic, pineapple of the bush, dadawa, chillies, curry and Ashanti pepper (Table 3).

Table 2: Percentage Occurrence of Fungal Isolates in Spices and Condiments from Katsina Central Market

Samples	*A. flavus*	*A. parasiticus*	*A. niger*	*A. versicolor*	*A. fumigatus*	*Mucor hiemalis*	*Phoma glomerata*	*Rhizopus stolonifer*	*Penicillium citrinum*	*Nigrospora sphaerica*
Chillies	1	-	1	-	-	-	-	-	-	-
Garlic	-	1	2	-	-	-	-	-	-	-
Ginger	1	-	-	2	-	-	-	-	-	-
Dadawa	1	2	-	-	-	-	-	-	-	-
Curry	-	-	-	-	-	-	-	1	-	-
Thyme	1	3	-	-	2	-	-	1	-	-
African Nutmeg	3	1	-	-	-	-	-	-	-	1
Ashanti Pepper	-	-	-	-	-	-	-	1	-	-
Clove	5	3	1	-	-	-	-	-	-	-
Kajiji	1	4	-	-	-	1	-	1	-	-
Candle wood	1	-	-	-	-		-	-	1	-
Chilli powder	1	1	-	1	1	-	-	1	-	-
Pineapple of the bush	-	-	-	-	-	-	2	-	-	-
Ethiopian pepper	-	5	-	-	-	-	-	-	1	-
Total	15	20	4	3	3	1	2	5	2	1
% Occurrence	26.8	35.7	7.1	5.4	5.4	1.8	3.6	8.9	3.6	1.8

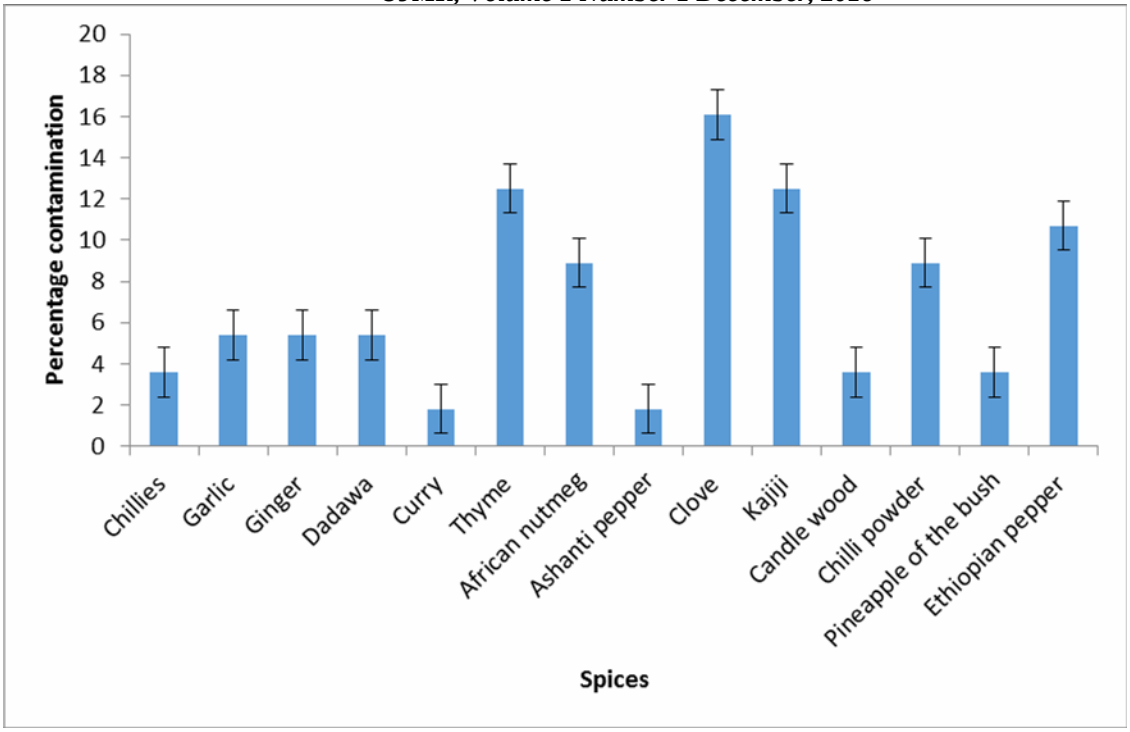

Figure 1: Fungi Contamination of Spices and Condiments from Katsina Central Market

DISCUSSION

Aspergillus had the highest frequency of occurrence in all spices and condiments studied. The reason for their high incidence might be due to their cosmopolitan nature and are known to be obligate saprophyte that can survive in the environment with a wide range of temperature varying from 18ºC-32ºC. This result is in agreement with the result obtained by Bokhari in Saudi Arabia (Bokhari, 2007) and Sumanth in India (Sumanth *et al*., 2010) who revealed that *Aspergillus* was the most common genus isolated from tested spices. Several researches reported that spices were greatly contaminated by different fungi including *Aspergillus*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Trichoderma*, *Mucor* and *Stachybotrys* (Freire *et al*., 2000; Elshafie *et al*., 2002; Bokhari, 2007; Hashem and Alamri, 2010). Among the *Aspergillus* species encountered, *A. flavus* and *A. parasiticus* were most prevalent. This result is in agreement with the result obtained by Hashem and Alamri (Hashem and Alamri, 2010) who reported that among the species of *Aspergillus* previously reported to contaminate spices globally, *A. flavus*, *A. parasiticus* and *A. tamarii* were prevalent with *A. flavus* occurring more frequently. Moderate level of incidence was found in *Rhizopus stolonifer*. In a similar work conducted by Hashem and Alamri in Saudi Arabia also reported moderate occurrence of *Aspergillus*, *Rhizopus* and *Penicillium*. The least level of incidence was recorded for *Nigrospora sphaerica*, *A. fumigatus*, *A. niger*, *A. versicolor*, *A. terreus*, *Rhizoctonia sp*, *Muccor hiemalis*, *Penicillium citrinum* and *Phoma glomerata*. Several researchers reported moderate or low frequency of occurrence of *A. fumigatus*, *A. terreus* and *A. versicolor* in different spices (Moharram *et al*., 1989; El-kady *et al*., 1992; Abdulkadir *et al*., 2003).

Table 3: Aflatoxin Contamination of Spices and Condiments from Katsina Central Market

Sample	Absorbance (A_{450nm})	Total Aflatoxin concentration (pbb)
Garlic	2.997	N.D
Pineapple of the bush	3.00	N.D
Thyme	2.139	2.6
Locust bean cake	2.875	N.D
Ginger	1.052	11.6
African nutmeg	1.179	9.9
Chilli Powder	1.345	8.1
Kajiji	2.521	0.8
Candlewood	2.553	0.7
Chillies	3.00	N.D
Curry	2.698	N.D
Clove	0.495	> 20
Ashanti pepper	3.00	N.D
Ethiopian pepper	1.429	7.3

N.D = Not Detected

Most of these fungi were previously isolated from various kinds of spices (El-kady *et al*., 1995; Freire *et al*., 2000; Ekhuemelo and Ebenezer, 2013; Farid *et al*., 2013; Gnonlonfin *et al*., 2013). The fungi contamination of spices and condiments could be attributed to the contamination from the environment (field, storage facility and market), as well as handling practices by farmers and people involve in marketing these products. In this study aflatoxin assay of spices and condiments revealed that 8 out of 14 spices and condiments samples (57.1%) were contaminated with total aflatoxins in the range of 0.7->20µg/kg. Two of these (14.3 %) namely, clove and ginger had aflatoxin levels above maximum acceptable limit of 10µg/kg set by EU and NAFDAC. Clove had aflatoxin level of >20 µg/kg which is higher than the one obtained by William et al. (2014) who reported mean aflatoxin contamination in Clove obtained from Nyahururu retail market in Kenya at the level of 7µg/kg. This might be associated to the poor handling practice of clove during transport or at the market. High contamination of ginger by aflatoxin (11.6 µg/kg) might be associated to long period of ginger storage in the shops and nature of

handling the product during packaging. Farmers mostly sprinkle water on ginger during packaging so as to reduce the peppery irritation in the eye. After packaging, the little moisture present on the ginger will be enough to support mould growth. In a similar report by Patel et al. (1996) who reported aflatoxin levels in ginger between 4.2-13.5µg/kg. African nutmeg had aflatoxin concentration of 9.9 µg/kg and this might be due to the nature of it's matrice which is highly rich with nutrient content that promotes fungal growth and subsequent aflatoxin production. In a similar work in Lagos Nigeria (Ezekiel *et al*., 2013) reported aflatoxin B1 concentration of African nutmeg at 20µg/kg. Aflatoxin concentration of 8.1 µg/kg was recorded in chilli powder. In a similar work conducted by Klieber (2000) who reported aflatoxin contamination in chilli powder in Australia at the range of 5-10µg/kg. Ethiopian pepper had aflatoxin concentration of 7.3 µg/kg which is within aflatoxin acceptable limit. Contamination of Ethiopian pepper with aflatoxin might have occurred during transportation and poor handling practice in the markets.

Low aflatoxin concentration of 2.6 µg/kg was recorded in thyme which might be due to the presence of thyme essential oil (thymol) that inhibits mould growth as well as industrial processing. Aflatoxin concentrations of 0.8 and 0.7 µg/kg were recorded in Kajiji and Candlewood respectively and this migh be attributed to unsuitable substrate for aflatoxin production.

Conclusion

It is concluded from the results that some spices and condiments sold commercially in Katsina central market were contaminated with different fungi species including aflatoxigenic ones. However, of the different fungi isolated *Aspergillus* was the most dominant genus encountered. Aflatoxin analysis revealed that eight samples of spices and condiments were contaminated by aflatoxin out of which two of them had levels above maximum acceptable limit set by EU and NAFDAC of 10 µg/kg.

Recommendations

Spices are usually spread on bare ground or by the roadside for drying, therefore, drying should be done in a hygienic place so as to prevent mould contamination during drying. Market hygienic conditions need to be improved.

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References

Abdulkadir, E., Tahiya, A., Saif, A. and Charles, B. (2003). Fungi and aflatoxins associated with spices in the sultanate of Oman. *Mycopathologia*,**155**: 155-160.

Aziz, N. H., Youssef, Y. A. and El-fouly, M. Z. (1998).Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Botanical Bulletin of Academia Sinica*, **39**:279-285.

Bokhari, F. M. (2007). Spices mycobiota and mycotoxins available in Saudi Arabia and their ability to inhibit the growth of some toxigenic fungi. *Mycobiology*, **35**(2): 47-53.

Creppy, E. E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*, **127**:19-28.

Davise, H. (2002). *Important Fungi. A guide to identification*. American society for microbiology Press,Washington D.C., USA, pp 1-111.

Ekhuemelo, C. and Ebenezer, J. E. (2013). Seed borne fungi of chilli pepper (*Capsicum frutescens*) from pepper producing areas of Benue State, Nigeria. *Agriculture and Biology Journal of North America*, **4**(4):370-374.

El-Kady, S., El-Maraghy, S. M. and Mostafa, E. M. (1992).Contribution of the mesophilic fungi of different spices in Egypt. *Mycopathologia*, **120**: 93-101.

El-Kady, I. A., El-Maraghy, S. S. and Eman, M. M. (1995). Natural occurrence of mycotoxins in different spices in Egypt. *Folia Microbiology*, **40**: 297- 300.

Elshafie, E. A., Al-Rashdi, T. A., Al-Bahry, S. N. and Bakheit, C. S. (2002). Fungi and aflatoxins associated with spices in the Sultanate of Oman. *Mycopathologia*, **155**(3):155–160.

Ezekiel, C. N., Fapohundra, S. O. and Olarunfemi, M. F. (2013). Mycobiota and aflatoxin B1 contamination of *Piper guineense*, *Piper nigrum* . and *Monodora myristica* from Lagos Nigeria. *International Food Research Journal*, **20** (1):111-116.

Farid, M. T., Nareen, Q. and Fagi, A. (2013). Isolation and identification of fungi from spices and medicinal plants. *Reserch Journal of Environmental and Earth Science*, **5** (3):131-138.

Freire, F. D., Kozakiewicz, Z. and Paterson, R. M. (2000). Mycoflora and mycotoxins in Brazilian black peppe r, white pepper and Brazilnuts.*Mycopath ologia*, **149**:13–19.

Gnonlonfin, G. J., Adjovi, Y. C., Tokfo, A. F., Agbekponon, E. D., Ameyapoh, Y., de Souza, C., Brimer, C. and Sanni, A. (2013). Mycobiota and identification of aflatoxin gene cluster in marketed spices in West Africa. *Food Control*, **34** (1):115-120.

Hashem, M. and Alamri, S. (2010). Contamination of common spices in Saudi Arabia markets with potential mycototoxin- producing fungi. *Saudi Journal of Biological Science*, **17**:167-175.

IARC, (1993). IARC Monographs on the evaluation of carcinogenic risk to humans. international agency for research on cancer, Lyon, France **56**: 245-395.

Jha, D. (1995). *Laboratory manual on seed pathology*.Vikas Publishing House, New Delhi, India, pp 7-9.

Klich, M. A. (2002). Identification of common *Aspergillus species*. Urecht, the Netherlands Central Bureau Voor Schimmer Cultures, 116pp.

Klieber, A. (2000). Aflatoxin contamination and it's management in chilli and paprika products in Australia. *Food Australia*, **53** (3):90-92.

Kumar, V., Basu, M. S. and Rajendran, T. P. (2008). Mycotoxin research and mycoflora in somecommercially important agricultural commodities. *Crop Protection*, **27**:891-905.

Moharram, A. M., Abdel-Mallek, A. Y. and Abdel-Hafez, A. I. (1989). Mycoflora of anise and fennel seeds in Egypt. *Journal of Basic Microbiology*, **29**:427-435.

Oranusi, S., Braide, W., Nwodo, C. F. and Nwosu, U. P. (2013). Assay for aflatoxins in some local food condiments. *International Journal of Biology Pharmacy and Allied Sciences*, **2**(3): 529-537.

Ozbey, F and Kabak, B. (2012). Natural co-occurrence of aflatoxins and ochratoxin A in spices. *Food Control*, **28**(2):354-361.

Patel, S., Hazel, C. M., Winterton, A. G. and Mortby, E. (1996). Survey of ethnic foods for mycotoxins. *Food Additive and Contaminant*, **13**: 833-841.

Paterson, R. M. and Lima, N. (2010). Molecular, clinical and environmental toxicology. *Toxicology of Mycotoxins*, 31-63.

Robert, A. S., Ellen, S., Hoekstra, J. and Frisvad, C. (2004). Introduction to food and airborne fungi. Centraal Bureau Voor Schimnal Cultures, Utrecht, the Netherlands, pp 1-111.

Sumanth, G. T., Bhagawan, M. W. and Surendra, R. S. (2010). Incidence of mycoflora from seeds of Indian main spices. *African Journal of Agriculture Research*, **5** (22): 3122-3125.

Tarin, A., Rosell, M. G. and Guardino, X. (2004). Use of high performance liquid chromatography to assess airborne mycotoxins, aflatoxins and ochratoxin A. *Journal of Chromatography A*, **1047**: 235-240.

William, W., Mwangi, C. M., Nguta, B. and Muriuki, G. (2014). Aflatoxin contamination in selected spices and preparations in the Nyahururu retail market, Kenya. *Journal of Scientific Reserach and Reports*, **3**(7): 919-920.