International Food Research Journal 20(1): 111-116 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Mycobiota and aflatoxin B1 contamination of *Piper guineense* (Ashanti pepper), *P. nigrum* L. (black pepper) and *Monodora myristica* (calabash nutmeg) from Lagos, Nigeria

[1,*]Ezekiel, C. N., [1]Fapohunda, S. O., [2]Olorunfemi, M. F., [2]Oyebanji, A. O. and [1]Obi, I.

[1]*Department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Ogun State. Nigeria*
[2]*Department of Microbiology, Nigerian Stored Product Research Institute, Ibadan, Nigeria*

Article history

Received: 2 December 2011
Received in revised form: 27 May 2012
Accepted:29 May 2012

Keywords

Aflatoxin
Aspergillus
food safety
fungi
spices

Abstract

The incidence of moulds including toxigenic *Aspergillus* section *Flavi*, and aflatoxin B1 (AFB1) were determined in 36 samples of three spices. Moulds were isolated and characterized by conventional mycological techniques while AFB1 was analyzed by Thin-layer chromatography with fluorescent detection coupled with an immunoaffinity clean up step. About 67% (24 out of 36) of the spices were contaminated by moulds belonging to four genera: *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus*. *Aspergillus* was the most predominant (78.9%) genera and a total of 220 *Aspergillus* section *Flavi* isolates were obtained. The incidence of *A. flavus* (63.2%) was higher than that of *A. tamarii* (36.8%). Approximately, 68% of *A. flavus* isolates from the spices produced aflatoxins in neutral red desiccated coconut agar (NRDCA). Only 19.4% of the spices were contaminated with AFB1 and the concentrations in 8.3% of calabash nutmeg exceeded the NAFDAC permissible limit of 20 µg/kg aflatoxin in foods in Nigeria. Of the three spices, calabash nutmeg showed the highest significant ($p<0.05$) mould count (3.45 Log_{10}CFU), incidence of toxigenic *Aspergillus* section *Flavi* (50%) and AFB1 (50%). Spices especially calabash nutmeg are prone to contamination by moulds including toxigenic *Aspergillus*. Consequently, the risk of aflatoxicosis may be high and as such may threaten public health safety due to regular consumption of the spices though aflatoxin levels were low.

Introduction

Food contamination by toxigenic fungi and consequent mycotoxins remain an issue requiring continuous attention by food mycology and safety specialists. The notable moulds that have been reported to contaminate spices include *Aspergillus*, *Penicillium*, *Rhizopus*, *Eurotium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Stachybotrys* (Freire *et al*., 2000; Elshafie *et al*., 2002; Bokhari, 2007; Hashem and Alamri, 2010). Among these, *Aspergillus* remains the major threat due to the ability of majority of its members to liberate toxic metabolites. The *Aspergillus* section *Flavi* group is the remarkable producer of aflatoxins in food matrices that they invade and colonize. The toxigenic species affecting food in the tropical regions include *A. flavus*, *A. parasiticus*, *A. nomius* and S_{BG} unnamed taxon (Egel *et al*., 1994; Atehnkeng *et al*., 2008). *A. tamarii* is a known atoxigenic member of the *Aspergillus* section *Flavi*. Toxigenic moulds have the capacity to invade and colonize a variety of food matrices and liberate aflatoxins depending on the prevailing conditions of temperature, relative humidity and moisture levels of the food (Rosi *et al*., 2007).

Aflatoxins are the best known dietary human carcinogens of fungal origin thus making their occurrence in food commodities a significant threat to public health. Of the known aflatoxins, aflatoxin B1 (AFB1) remains the most prevalent in foods (Lee *et al*., 2004). AFB1 is also the most potent toxic metabolite capable of inducing hepato-carcinogenicity (Sweeney and Dobson, 1998), genotoxicity in reproductive and blood cells (Fapohunda *et al*., 2008; Ezekiel *et al*., 2011), as well as some other toxic conditions. This toxin has been reported to contaminate a wide

*Corresponding author.
Email: *chaugez@gmail.com*

variety of spices such as chilies, red pepper, curry, ginger and black pepper (Freire *et al*., 2000; Bokhari, 2007; Ardic *et al*., 2008; Cho *et al*., 2008; Iqbal *et al*., 2011). However, there is paucity of data for the occurrence of toxigenic *Aspergillus* section *Flavi* and or aflatoxins in spices from Nigeria.

Spices are vegetables grown mostly in the tropics but are used to cook in all homes around the world due to their aromatic or pungent fragrances. Spices in minute quantities are capable of masking flavors of foods. In Nigeria, several spices are routinely used in homes for cooking and they include: curry, thyme, calabash nutmeg, red pepper, leaves of Ashanti pepper, ginger, and many others. The fruits of black pepper (*Piper nigrum* L.; local name: *uda*) and seeds of Ashanti pepper (also called the West African pepper; *Piper guineense*; local name: *uziza*), which we investigated in this study, are boiled daily as soup for women who were delivered of their babies within 1.5 months. This is used to stabilize their womb and bowels. The fruit of the calabash nutmeg (*Monodora myristica*) is usually sold dry and used in stews, soups, cakes and desserts.

Regardless of the daily use of these spices in Nigerian homes, very few documented investigations have been carried out on the distribution, density and frequency of the occurrence of mycobiota in these spices. The paucity of data in respect to AFB1 contamination of Nigerian spices may have been due to the difficulty in the clean up step of aflatoxin extraction from spices. Spices are known to contain colored compounds, thus making aflatoxin analysis difficult due to matrix interference (Cho *et al*., 2008). Therefore we applied an immunoaffinity column (IAC) clean up step coupled with thin-layer chromatographic extraction steps in this study as we investigated the contamination level of AFB1 in Ashanti pepper, black pepper and calabash nutmeg sold in three markets in Lagos, Nigeria. The mycobiota including toxigenic species of *Aspergillus* section *Flavi* present in the spices were also enumerated.

Materials and Methods

Sample collection

A total of 36 spices samples comprising of 12 samples each of Ashanti pepper, black pepper, and calabash nutmeg were purchased from three markets in Lagos, Nigeria. The markets are situated at Oyingbo, Mushin and Ikorodu. Each bulk sample (400g) was obtained by adding five parts each of about 80g representative samples collected from various parts of a trader's storage bag or tray. The bulk samples were collected into transparent *Zip-lock* bags and transported to the Microbiology Laboratory of Babcock University, Nigeria for further analysis. The samples were communited immediately in order to reduce particle size and stored at 4^{o}C prior to further analysis.

Mycological analysis of spices

The moulds in the spices were isolated by the dilution plating technique. One gram of each sample was appropriately diluted in 10ml sterile distilled water and inoculated on a set of triplicate plates containing Potato dextrose agar (PDA) supplemented with 5g/L chloramphenicol and 2.5g/L streptomycin. Similar dilutions were inoculated on a set of triplicate modified Rose Bengal Agar (mRBA) plates for the recovery of *Aspergillus* section *Flavi* from the spices. The colonies on PDA were counted and purified by repeated transfers to freshly prepared PDA in order to morphologically characterize the isolates down to the genus level. On the other hand, five colonies on each mRBA plate per sample were transferred to freshly prepared 5/2 agar (5% V8 juice and 2% agar, pH 5.2) for proper identification of members of the *Aspergillus* section *Flavi*. The 5/2 plates were incubated in the dark at 31^{o}C for 5 days while PDA and mRBA plates were incubated at 31^{o}C for 3 days. Identification of all moulds was based on assessment of macro- and micro-characteristics specific for each genus or species as in the case of members of the section *Flavi* (Samson *et al*., 1995; Cotty and Cardwell, 1999; Ehrlich *et al*., 2003).

Aflatoxigenicity testing of isolates

Two hundred and twenty isolates of *Aspergillus* section *Flavi* obtained from the spices were tested for their capacity to produce aflatoxins on neutral red desiccated coconut agar (NRDCA). The agar medium was prepared as described by Atanda *et al*. (2011). Each of the isolates were inoculated at the center of triplicate NRDCA Petri dishes and incubated at 31^{o}C for 5days in the dark. On the third day of incubation the plates were checked under UV at 365nm for blue fluorescence characterizing aflatoxin production while on the fifth day, plates that showed fluorescence were subjected to quantitative analysis for AFB1 according to a modification of the method of AOAC International (2000).

For the quantitative determination of AFB1 produced in culture, approximately 25g of the entire content of each NRDCA plate was weighed into a Waring blender (Marlex Emerald UNIT III, Daman). This was extracted with 125 ml of 55% aqueous methanol (v/v) and 1g of NaCl for 3 minutes. The mixture was emptied into a 250 ml conical flask and

20 ml n-hexane was added. The mixture was shaken for 30 minutes on an orbital shaker and filtered through Whatman No.1 filter paper. The filtrates were partitioned in 250 ml separatory funnels by adding 15 ml n-hexane and shaken vigorously for 30 seconds. The funnel was allowed to stand for separation of the mixture into two layers. The lower layer that contained the sample was drained into another 250 ml separating funnel and 20 ml of chloroform was added and shaken vigorously. The resulting lower layer was passed through a bed of anhydrous sodium sulphate into a polypropylene cup to remove residual water and the extract was concentrated on a hot plate.

The extract was re-constituted in 500 µl chloroform, and 40 µl extract and 50 µl aliquots of 0.50 µg/ml total aflatoxin standards were separated on pre-coated TLC plates (silica gel 60 F_{254}; 20 × 10cm; Merck, Germany) in chloroform-acetone-water (88/12/1.5). The developed plates were dried in a fume cupboard and visualized under long wavelength UV light (366nm). The AFB1 band in each sample spot was identified on the basis of co-migration and characteristic blue fluorescence with aflatoxin standard. The suspected sample and standard spots on dried TLC plates were sprayed with trifluoroacetic acid and tetra oxo sulphate (VI) acid according to Bankole *et al*. (2006). AFB1 concentration (µg/kg) in the samples was estimated using the formula described in Atanda *et al*. (2011).

Estimation of AFB1 in the spices

The concentration levels of AFB1 in the 36 spices samples were determined by Thin-layer chromatographic technique with fluorescent detection at 366nm. Twenty-five grams of a spice sample was weighed and extracted with 125 ml of 55% aqueous methanol (v/v) and 1g of NaCl for 3 minutes in a Waring blender. The mixture was emptied into a 250 ml conical flask and 10 ml n-hexane was added before the flask was vigorously shaken for 10 minutes on an orbital shaker. The mixture was filtered through Whatman No.1 filter paper and the filtrate was collected in a polypropylene cup. For the separation and clean up of the extract, the RIDA® Aflatoxin columns (Art. No.: R5001 / R5002) was used according to manufacturer's instruction. The IACs were purchased from R-Biopharm AG, Germany. Equilibration of the column was carried out by rinsing in 2 ml distilled water and the flow rate of extract through the IAC was approximately 1drop/second. The subsequent aflatoxin detection steps involving spotting and development of spotted plates were carried out as stated above. The developed plates were dried, visualized under long wavelength UV light (366nm) and scored for the presence or absence of AFB1 band as described above. AFB1 concentration (µg/kg) in the spice samples was estimated using the formula described in Atanda *et al*. (2011).

Analysis of data

The data obtained were subjected to statistical analysis using SPSS® 15.0 package (SPSS Inc., Chicago, IL). Means and standard error of each set of data were determined and compared for significance at 95% confidence level by One-way Analysis of Variance (ANOVA).

Results and Discussion

Occurrence of fungi in spices

Spices have been reported to be heavily contaminated by a wide array of storage fungi including *Aspergillus*, *Penicillium*, *Rhizopus*, *Eurotium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Stachybotrys* (Freire *et al*., 2000; Elshafie *et al*., 2002; Bokhari, 2007; Hashem and Alamri, 2010). Mycological investigation of the three spices analyzed in this study showed that 25%, 75% and 100% of black pepper, Ashanti pepper and calabash nutmeg, respectively, were contaminated with various fungi (Table 1). The isolated fungi belonged to four genera: *Aspergillus*, *Penicillium*, *Fusarium* and *Rhizopus*. *Aspergillus* was the most predominant (78.9%) genera in the spices followed by *Rhizopus*, *Penicillium* and *Fusarium*. The trend of fungal predominance which we observed agreed with the reports of Bokhari (2007) and Hashem and Alamri (2010) who worked on diverse spices including black pepper.

On the overall, about 67% of the spices were contaminated by fungi. There were significant ($p<0.05$) differences in the fungal load of the spices as calabash nutmeg had the highest (3.45 Log_{10}CFU) count and black pepper, the least (0.80 Log_{10}CFU). The highest fungal load observed in the case of calabash nutmeg in contrast to black pepper and Ashanti pepper may have been due to the structure of the fruit of the calabash nutmeg. We observed that this spice has two thick woody pericaps surrounding the mesh-like fragrant pulp. The pericaps are joined by an extension of the pulp. This dried pulp has perforations which we suggest may be an avenue for entry of fungal spores as well as a store for these spores during drying or storage practices. Contamination of spices by fungi and aflatoxins has been reported to take place in the field, and during drying and storage (Martins *et al*., 2001; Elshafie *et al*., 2002; Fazekas *et al*., 2005).

Table 1. Total mould count and occurrence of fungal genera in three spices

Spices	N+	N*	Log_{10}CFU/g#	Incidence of isolated fungal genera^			
				Aspergillus	*Penicillium*	*Fusarium*	*Rhizopus*
Black pepper	12	3	0.80 ± 0.37c	97.22	0.00	0.00	2.18
Ashantipepper	12	9	2.47 ± 0.36b	89.70	4.21	2.02	4.49
Nutmeg	12	12	3.45 ± 0.20a	93.94	2.66	1.49	1.91
Total	36	24	---	---	---	---	---
Mean	---	---	2.25 ± 0.26	92.82	2.79	1.47	3.03

+Number of analyzed samples.
*Number of positive samples for fungal contamination.
#Mean logarithmic value and standard error of colony forming units (CFU) in analyzed samples.
^Incidence was calculated based on N*.
CFU values with different superscripts in a column are significantly different (p<0.05).

Table 2. Aflatoxin B1-producing capacity of *A. flavus* isolated from three spices on neutral red desiccated coconut agar.

Spices	Incidence (%) of toxigenic strains and respective AFBl concentrations (µg/kg)			
	<10.0	10.0 – 100.0	100.1 – 200.0	>200
Black pepper (*N*+= 10)	50.0	50.0	0.0	0.0
Ashanti pepper (*N*= 24)	16.7	37.5	37.5	8.3
Nutmeg (*N*= 60)	61.7	28.3	10.0	0.0

+Number of toxigenic *Aspergillus* section *Flavi* isolated from each spice

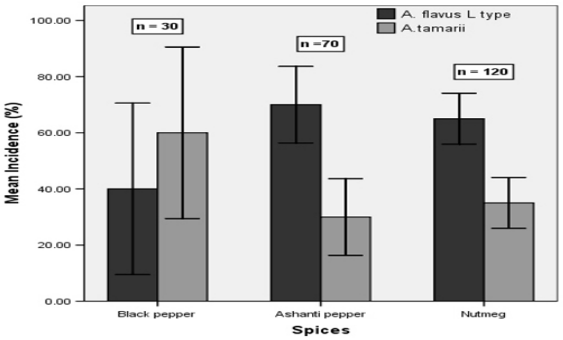

Figure 1. Incidence of *Aspergillus* section *Flavi* in three spices
n = number of isolated *Aspergillus* section *Flavi*

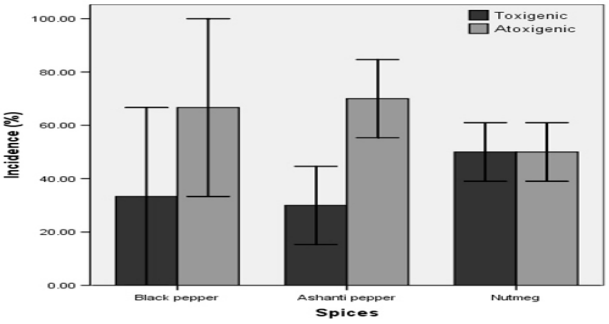

Figure 2. Incidence of toxigenic and atoxigenic *Aspergillus flavus* in three spices

Incidence of Aspergillus section Flavi in spices

A total of 220 isolates belonging to only two members of the *Aspergillus* section *Flavi* were isolated from the spices (Figure 1). *A. flavus* was more predominant (63.2%) than *A. tamarii* (36.8%) but there was no significant (p>0.05) difference in the incidence of *A. flavus* or *A. tamarii* among spices. However, when the incidence of *A. flavus* and *A. tamarii* was compared in each spice, we observed significant (p<0.05) differences between these species in only calabash nutmeg. Among the species of *Aspergillus* previously reported to contaminate spices globally, *A. flavus* and *A. tamarii* were prevalent with *A. flavus* occurring more frequently (Bokhari, 2007; Hashem and Alamri, 2010). This is similar to the observations from our study. Although these two species of *Aspergillus* and some others like *A. parasiticus* have been reported to contaminate spices and some drug plants (El-Kady *et al*., 1992; Abdulkadir *et al*., 2007), their occurrence as a group (*Aspergillus* section *Flavi*) in the spices have not been considered. This makes our report the first on the incidence of *Aspergillus* section *Flavi* in spices.

Incidence and aflatoxin B1 production by A. flavus isolated from spices

Only 94 of the 220 *Aspergillus* section *Flavi* isolates had the ability to produce aflatoxins in neutral red desiccated coconut agar (NRDCA). Specifically, the toxigenic isolates belonged to *A. flavus*. The incidence of atoxigenic strains in black pepper and Ashanti pepper were higher but insignificant (p>0.05) than that of toxigenic strains (Figure 2). In calabash nutmeg, the ratio of occurring toxigenic strains equaled that of atoxigenic strains. When the capacity of each toxigenic strain was tested for AFB1 production on NRDCA (Table 2), we observed that all isolates from black pepper produced AFB1 at concentrations below 100 µg/kg. About 46% of toxigenic isolates from Ashanti pepper and 10% from calabash nutmeg produced greater than 100µg/kg AFB1 in culture.

Black pepper alongside other spices which we did not investigate in this study has been reported to inhibit aflatoxin biosynthesis but not the growth of the toxigenic fungi (Madhyastha and Bhat, 1984; Bokhari, 2007). Specifically we noted that *A. flavus* flourished at varying proportions on the three spices examined in this study albeit with lower aflatoxin production in the spices than in culture. This is in agreement with the reports of Elshafie *et al*. (2002) who did not detect any aflatoxins in 15 selected samples out of 105 spices samples including cumin, black pepper, cinnamon, cloves, cardamom, ginger and coriander, but found 45% of their 20 *A. flavus* isolates to be aflatoxigenic. It is also important to note that it has been established that spices may support fungal growth (e.g. *Aspergillus flavus*) but inhibit the production of aflatoxins than in cereals (MacDonald and Castle, 1996). Therefore, we may suggest that not all *A. flavus* strains are aflatoxigenic on this kind of matrix (Elshafie *et al*., 2002). Not much has been

Table 3. Occurrence of aflatoxin B1 in three spices

Spices	N+	N*	Samples contaminated with AFB1 (µg/kg) <10	10 – 20	>20
Black pepper	12	0	0.0%	0.0%	0.0%
Ashanti pepper	12	1	0.0%	8.3%	0.0%
Nutmeg	12	6	25.0%	16.7%	8.3%

+Number of samples analyzed for aflatoxin B1
*Number of positive samples for AFB1 contamination

reported on the inhibitory activity of Ashanti pepper and calabash nutmeg against aflatoxin biosynthesis and growth of toxigenic fungi.

Aflatoxin B1 contamination in spices

Only 7/36 of the spices were contaminated with AFB1 (Table 3). No sample of black pepper had AFB1 contamination while 8.3% (1/12) of calabash nutmeg were contaminated with at concentrations above the NAFDAC permissible limit of 20 µg/kg aflatoxin in foods in Nigeria. Aflatoxin contamination of spices has been reported to be low to moderate depending on the kind of spice. In Korea, Cho *et al*. (2008) investigated 88 spice samples (including black pepper) and found aflatoxin contamination in only 13.6% (exclusive of black pepper) at concentrations below 5 µg/kg. Santos *et al*. (2010), in a study carried out in Spain, found aflatoxins in 40% of 35 chilli samples, all at concentrations below the maximum allowable limits. The very low incidence of AFB1 in Ashanti pepper and exclusion of AFB1 in black pepper reported in our present study follow the previous reports above. This may have been due to the presence of piperine, a potent alkaloid responsible for the pungency of the *Piper* species. Piperine has been reported by Madhyastha and Bhat (1984) to inhibit aflatoxin biosynthesis in *A. parasiticus* (IC_{50}<35 µM). The fact that the toxin concentrations in the spices were low does not guarantee safety of this product since these spices are frequently used to prepare dishes especially the calabash nutmeg which had 50% AFB1 contamination.

Conclusion

This study has shown that spices especially calabash nutmeg are prone to contamination by moulds including toxigenic *Aspergillus* and although aflatoxin levels may be low, the risk of aflatoxicosis resulting from the continuous ingestion of these foods may be high. Since nutmeg had the highest fungal load, aflatoxigenic strains and AFB1 concentrations; we suggest that its cultivation, post-harvest including drying and storage practices be improved so as to reduce the invasion and colonization by storage and toxigenic moulds. This will enhance food and public health safety and in turn reduce the risk of mycotoxicosis. This is because the calabash nutmeg is the most frequently used of the three spices.

Acknowledgement

The authors are grateful to Dr. Atanda O.O. for supplying useful literature.

References

AOAC International, 2000. Official methods of analysis, 17th edition. Association of Official Analytical Chemists International, Gaitersburg, MD, USA.

Ardic, M., Karakaya, Y., Atasever, M. and Durmaz, H. 2008. Determination of aflatoxin B1 levels in deep-red ground pepper (isot) using immunoaffinity column combined with ELISA. Food and Chemical Toxicology 46: 1596–1599

Atanda, O.O., Ogunrinu, M.C. and Olorunfemi, F.M. 2011. A neutral red desiccated coconut agar for rapid detection of aflatoxigenic fungi and visual determination of aflatoxins. World Mycotoxin Journal 4(2): 147–155.

Atehnkeng, J., Ojiambo, P.S., Donner, M., Ikotun, T., Sikora, R.A., Cotty, P.J. and Bandyopadhyay, R. 2008. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. International Journal of Food Microbiology 122 (1-2): 74–84.

Bankole, S.A., Ogunsanwo, B.M., Osho, A. and Adewuyi, G.O. 2006. Fungal contamination and aflatoxin B1 of 'egusi' melon seeds in Nigeria. Food Control 17: 814–818.

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.

Cho, S.-H., Lee, C.-H., Jang, M.-R., Son, Y.-W., Lee, S.-M., Choi, I.-S. Kim, S.-H. and Kim, D.-B. 2008. Aflatoxins contamination in spices and processed spice products commercialized in Korea. Food Chemistry 107: 1283-1288.

Cotty, P.J. and Cardwell, K.F. 1999. Divergence of West African and North American communities of *Aspergillus* section *Flavi*. Applied and Environmental Microbiology 65: 2264–2266.

Egel, D.S., Cotty, P.J. and Elias, K.S. 1994. Relationships among isolates of *Aspergillus* section *Flavi* that vary in aflatoxin production. Phytopathology 84: 906–912.

Ehrlich, K.C., Montalbano, B.G. and Cotty, P.J. 2003. Sequence comparison of aflR from different *Aspergillus* species provides evidence for variability in regulation of aflatoxin production. Fungal Genetics and Biology 38: 63–74.

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., Alabi, O.A., Anokwuru, C.P. and Oginni

O. 2011. Studies on dietary aflatoxin-induced genotoxicity using two *in vivo* bioassays. Archives of Applied Science Research 3(2): 97–106.

Fapohunda, S.O., Ezekiel, C.N., Alabi, O.A., Omole, A. and Chioma, S.O. 2008. Aflatoxin mediated sperm and blood cell abnormalities in mice fed with contaminated corn. Mycobiology 36(4): 255–259.

Fazekas, B., Tar, A. and Kovács, M. 2005. Aflatoxin and ochratoxin A content of spices in Hungary. Food Additives and Contamination 22(9): 856–863.

Freire, F.D.O., Kozakiewicz, Z. and Paterson, R.R.M. 2000. Mycoflora and mycotoxins in Brazilian black pepper, white pepper and Brazil nuts. Mycopathologia 149: 13–19.

Hashem, M. and Alamri, S. 2010. Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. Saudi Journal of Biological Sciences 17:167-175.

Iqbal, S.Z., Paterson R.R.M., Bhatti I.A. and Asi M.R. 2011. Aflatoxin concentrations in chilies vary depending on variety. Mycoscience 52: 296-299.

Lee, N. A., Wang, S., Allan, R. D. and Kennedy, I. R. 2004. A rapid aflatoxin B1 ELISA: Development and validation with reduced matrix effects for peanuts, corn, pistachio, and soybeans. Journal of Agricultural and Food Chemistry 52: 2746–2755.

Macdonald, S. and Castle, L. 1996. A UK retail survey of aflatoxin in herbs and spices and their fate during cooking. Food Additives & Contaminants 13: 121–128.

Madhyastha, M.S. and Bhat, R.V. 1984. A*spergillus parasiticus* growth and aflatoxin production on black and white pepper and the inhibitory action of their chemical constituents. Applied and Environmental Microbiology 48: 376-379.

Martins, M.L., Martins H.M. and Bernardo, F. 2001. Aflatoxins in spices marketed in Portugal. Food Additives & Contaminants 18: 315–319.

Rosi, P., Borsari, A., Lasi, G., Lodi, S., Galanti, A., Fava, A., Girotti, S. and Ferri, E. 2007. Aflatoxin M1 in milk: Reliability of the immunoenzymatic assay. International Dairy Journal 17: 429–435.

Samson, R.A., Hoekstra, E.S., Frisvad, J.S. and Filtenborg, O. 1995. Introduction to Food-borne Fungi. 4th Ed. CentraalBureau voor Schimmelcultures, The Netherlands. pp. 3–165.

Santos, L. Marín, S., Sanchis, V. and Ramos, A.J. 2010. Co-occurrence of aflatoxins, ochratoxin A and zearalenone in Capsicum powder samples available on the Spanish market. Food chemistry. 122: 826-830.

Sweeney, M.J. and Dobson, A.D.W. 1998. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* species. International Journal of Food Microbiology 43: 141–158.