Front. Agric. China 2011, 5(4): 497–503
DOI 10.1007/s11703-011-1115-1

RESEARCH ARTICLE

Occurrence of major mycotoxins in maize from Hebei Province, China

Yizhi FENG, Bu TAO, Minhao PANG, Yingchao LIU (✉), Jingao DONG (✉)

The National Maize Industry Technology RD Center, MOA, Mycotoxin Laboratory, Agricultural University of Hebei, Baoding 071001, China

Abstract **To investigate the frequency of occurrence and the concentrations of aflatoxins (AFs), deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FBs) in naturally infected maize, 25 samples of maize collected from fields in Hebei Province, China, were analyzed by high-performance liquid chromatography (HPLC). The maize samples were found to be frequently contaminated with DON (68%), ZEN (60%) and FBs (32%) in the range from 28 to 2533 μg/kg, 60 to 1239 μg/kg and 150 to 4480 μg/kg, respectively. The average concentration found for DON, ZEN and FB1 + FB2 were 605, 238 and 418 μg/kg, respectively. The average concentration of DON (605 μg/kg) in our samples was below the maximum tolerable limit of 1000 μg/kg set as the Chinese standard for maize, while ZEN (238 μg/kg) was almost four times as high as the maximum tolerable limit of 60 μg/kg. The overall level of FB (FB1 + FB2) contamination was relatively low, with an average concentration of 418 μg/kg in 32% (8 of 25) of maize samples from Hebei. AFs were not detected in any of the tested samples. This is the first report on the natural occurrence of multimycotoxin in maize in China.**

Keywords occurrence, mycotoxins, maize, China

Introduction

Food crops and feed materials can be easily infected by fungal species which may produce mycotoxins during growth, harvest, and storage (Monbaliu et al., 2010). When considering the heat stability of mycotoxins, these substances constitute a potential risk for human and animal health (Zinedine and Mañes, 2009).

Currently, more than 400 mycotoxins have been identified in the world (Salem and Ahmad, 2010). Among the many known toxins, aflatoxins (AFs) are highly toxic and carcinogenic compounds that can cause diseases in livestock and humans (Groopman and Donahue, 1988; Simon et al., 1998). AFs are a group of mycotoxins produced as secondary metabolites by the spoilage of fungi *Aspergillus*, particularly *A. flavus* and *A. parasiticus* (Kurtzman et al., 1987). The most important members of AFs are AFB1, AFB2, AFG1 and AFG2. The International Agency for Research on Cancer (IARC) has classified AFB1, AFB2, AFG1 and AFG2 as Group I human carcinogens (IARC, 2002).

Received January 11, 2010; accepted May 6, 2010
Correspondence: [a]Yingchao LIU; [b]Jingao DONG
E-mail: [a]liuyingchao@hebau.edu.cn; [b]dongjingao@126.com

Deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FBs) are mycotoxins produced by several species of the grain pathogen ascomycete genus *Fusarium* spp. (Gelderblom et al., 1988; Schaafsma et al., 1998; Bennett and Klich, 2003). The toxicity of DON and ZEN is relatively lower compared to that of AFs. However, acute exposure to high levels of DON doses causes vomiting, diarrhea and even gastrointestinal hemorrhage; chronic exposure may lead to reduced weight gain and anorexia in animals (Pestka, 2007). ZEN has been associated with early puberty, hyperplasic and neoplastic endometrium, and cervical cancer in humans (Thongrussamee et al., 2008). Additionally, ingestion of foods highly contaminated with FBs results in increased risk of esophageal cancer and neural tube defects in humans (Chu and Li, 1994; Missmer et al., 2006). In pigs, high levels of FB1 in feeds can be hepatotoxic, inducing pulmonary edema and cardiovascular changes (Haschek et al., 2001; Marin et al., 2007). Mycotoxins can cause serious problems on human and animal health. The maximum tolerating levels to mycotoxin in foodstuffs and feedstuffs have been established in many countries (Wang et al., 2008).

Mycotoxins can occur both in temperate and tropical regions of the world, depending on the species of fungi (Salem and Ahmad, 2010). Maize is a very good substrate for fungal growth and toxinogenesis (Trung et al., 2008). The

natural occurrence of mycotoxins in maize, maize-based food and feeds has been steadily reported from all over the world (Doko and Visconti, 1994; Fandohan et al., 2005; Lino et al., 2007; Martins et al., 2007; Ok et al., 2007; Silva et al., 2007; Jajić et al., 2008; Marinamartins et al., 2008; Gong et al., 2009; Kimanya et al., 2009). However, there is no information available on the natural occurrence of some mycotoxins in maize throughout China. The purpose of this study was to investigate AFs (mostly including AFB1, AFB2, AFG1 and AFG2), DON, ZEN, FB1 and FB2 contamination incidence and their levels in maize collected in Hebei Province of northern China.

Materials and methods

Chemicals and apparatus

HPLC-grade acetonitrile, water and methanol were purchased from Dikma (Lake Forest, USA), AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1 and FB2 standards were obtained from Sigma Chemicals Co. (St. Louis, USA). The multifunctional columns, PuriToxSR column TC-M160 and PuriToxSR column TC-T200, were obtained from Trilogy Analytical Laboratory (Washington, USA). Strong anion-exchange columns (SAX) (500 mg, 3 mL) were obtained from Thermo Electron Corporation (Boston, USA). O-phthaldialdehyde (OPA), 2-mercaptoethanol and potassium borate buffer (o-phtalaldehyde diluent, OD104) were supplied by Pickering Laboratories (Mountain View, USA).

A 20 mg/L stock solution of DON was prepared in methanol and stored at 4°C. A 20 mg/L stock solution of ZEN was prepared in acetonitrile and stored at −20°C. A 20 mg/L stock solutions of AFB1, AFB2, AFG1 and AFG2 were prepared in benzene:acetonitrile (98∶2, v:v). Solutions for AFB1, AFG1 and AFG2 were stored at 4°C and at −20°C for AFB2. Stock solutions of FB1 and FB2 (20 mg/L) were prepared in acetonitrile:water (50∶50, v:v) and stored at 4°C. The HPLC (Agilent Technologies 1200 Series) apparatus used in this study consisted of a quaternary pump (model G1311A), a vacuum degasser (model G1322A), a thermostated autosampler (model G1329A), a column compartment (model G1316A), a DAD detector (model G1315D) and a fluorescence detector (model G1321A). Online post-column chemical derivatization was performed with a Pinnacle PCX reactor supplied by Pickering Laboratories (Mountain View, USA). The ZORBAX SB-C18 column (250 mm × 4.6 mm i.d., particle size 5 μm), was supplied by Agilent Technologies.

Chromatographic conditions

For AFs analysis

The injection volume was 10 μL and the column temperature was set at 42°C. The mobile phase was methanol-acetonitrile-water (22∶22∶56, v:v:v) and pumped at a flow rate of 1 mL/min. Fluorescence detection was performed at the optimized excitation and emission wavelengths of 365 and 430 nm, respectively. The post-column derivatization conditions were set as the following: runtime of 20 min; equilibration time for 1 min; reactor temperature at 95°C; reactor volume of 1.4 mL; and pump1 (100 mg/L, iodine solution) flow rate of 0.3 mL/min.

For DON analysis

The injection volume was 10 μL and the column temperature was set at 30°C. The mobile phase was methanol-water (20∶80, v:v) pumped at a flow rate of 1 mL/min. DON was detected at 220 nm with a DAD detection.

For ZEN analysis

The injection volume was 10 L and the column temperature was set at 30°C. The mobile phase was methanol:water (80∶20, v:v) and pumped at a rate of 1 mL/min. Fluorescence detection excitation and emission wavelengths were 274 and 440 nm, respectively.

For FBs analysis

The injection volume was 100 μL and the column temperature was set at 40°C. The mobile phase consisted of methanol (A) and 0.05 M citric acid buffer (pH 4) (B) at a flow rate of 1.0 mL/min. The optimized elution gradient was 7 min isocratic step at 62% A and 38% B; 3 min linear gradient to 66% (A) and 34% (B) and held constantly for 5 min, followed by the initial mobile phase composition, whose system was re-equilibrated for 10 min. Fluorescence detection was performed at the optimized excitation and emission wavelengths of 335 and 440 nm, respectively. The OPA derivatization reagent was prepared fresh with 2.0 g OPA dissolved in 10 mL methanol and diluted with OD104, then added with 6 mL 2-mercaptoethanol, and finally diluted with OD104 to 500 mL. The post-column derivatization solution was under a 25 min runtime; 1 min equilibration time; reactor at 40°C; 1.4 mL reactor volume; at pump1 (2% sodium hydroxide) flow rate of 0.4 mL/min and pump2 (OPA) flow rate of 0.8 mL/min.

Sampling and sample preparation

A total of 25 samples of corn harvested in 2009 were collected from the main corn growing areas of Hebei Province in the northern part of China. The location selected for sampling was divided into plots of 80 m^2 each. Ten ears of corn were sampled from each plot, taken as one sample, from which a 1 kg subsample was taken. The samples were kept at 4°C until analysis showed they came from the same maize breed, i.e., Zhengdan 958. All the samples were finely milled in the switching apparatus. A ground sample (10 g) was placed into a blender jar and mixed with 40 mL acetonitrile:

water at 84∶16 (v:v). The mixture was blended at a high speed for 3 min, filtered through Neutral filter paper, and collected in a 50 mL centrifuge tube.

A 4 mL aliquot of the filtrate was loaded onto a multifunctional cleanup column TC-M160 without pre-conditioning and was pushed completely through the column using a plunger while being collected directly in a 15 mm × 125 mm test tube. A 2 mL sample of the filtrate was evaporated in a 60°C water bath under a stream of nitrogen. The residue was reconstituted in 0.5 mL of acetonitrile. After filtration (0.45 μm pore size), the samples were subjected to HPLC analysis for their AFs and ZEN contents.

A 2 mL sample of the filtrate from the initial processing was loaded to a multifunctional cleanup column TC-T200, and pushed through with a plunger while the filtrate was being collected in a 15 mm × 125 mm test tube. The plunger was removed, 4 mL acetonitrile:water (84∶16, v:v) was added into the top of the column, pushed all through the column and collected in the same test tube. The rinsing process was repeated. All the filtrate was evaporated in a 60°C water bath under a stream of nitrogen. The residue was redissolved in 0.5 mL acetonitrile. After filtration (0.45 μm pore size), the sample was subjected to HPLC analysis for DON content.

Eight mL of the filtrate was loaded onto a SAX cartridge preconditioned with 5 mL of methanol followed by 5 mL of acetonitrile/water (3∶1, v:v) at a flow rate of approximately 1 mL/min. The cartridge was washed with 5 mL of acetonitrile/water (3∶1, v:v), followed by 3 mL of methanol. Finally, fumonisins were eluted with 10 mL of 1% methanolic acetic acid (v:v). The eluate was evaporated in a 60°C water bath under a stream of nitrogen, and redissolved in 1 mL of acetonitrile/water (50∶50, v:v). After filtration (0.45 μm pore size), the sample was subjected to HPLC analysis for FB content.

Results

Method validation

The analytical method was within the laboratory validated with respect to linearity, recovery, precision, limits of detection (LODs) and limits of quantification (LOQs).

The standard curves for AFs, DON, ZEN and FBs at two determinations of eight concentration levels, were linear, with correlation coefficients (R^2) from 0.9990 to 0.9999 (Table 1). Precision was evaluated in terms of intraday repeatability and inter-day reproducibility as RSD%. The intraday variability of the assay was tested by six consecutive injections on the same day. The interday reproducibility of the assay was determined by six injections on five different days. The results are shown in Table 1. The accuracy of the method was confirmed by determining the recovery. Average recovery rates of AFs, DON, ZEN and FBs, which varied from 74.9% to 104.8% with a relative standard deviation from 2.4% to 8.9%, by adding different spiking levels to analyte-free corn samples are presented in Table 2. The limits of detection (LODs) for AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1 and FB2 were 0.05, 0.1, 0.1, 0.2, 11, 12, 10 and 9 μg/kg, respectively at a signal-to-noise ratio of 3∶1, and the limits of quantitation (LOQs) for AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1 and FB2 were 0.2, 0.4, 0.4, 0.7, 35, 40, 65 and 62 μg/kg, respectively at 10∶1 (Table 2).

Determination of mycotoxins in maize

In this investigation, a total of 25 samples were analyzed for mycotoxins. The occurrences of AFs, DON, ZEN and FBs in the analyzed samples are presented in Table 3. 68% of the 25 samples analyzed were contaminated with DON at levels ranging from 28 to 2533 μg/kg. The average level of DON in all samples analyzed in the present study was 605 μg/kg.

For ZEN analysis, 15 samples (60%) were found to be contaminated with ZEN at levels ranging from 60 to 1239 μg/kg, and the average level of ZEN in all samples was 238 μg/kg.

The presence of FB1 and FB2 was verified in 8 samples (32%) and 3 (12%) samples, respectively. In positive samples, the concentration of FB1 ranged from 150 to 3480 μg/kg and the concentration of FB2 ranged from 170 to 1000 μg/kg. The average concentration in this study for the totality of maize samples was 360 μg/kg for FB1 and 58 μg/kg for FB2.

AFs were not detected in maize in our study.

Table 1 Results of the intraday and interday precision study (both expressed as RSD%) obtained and calibration data for AFs, DON, ZEN and FBs

Mycotoxins	Correlation coefficient (R^2)	Calibration curve	Precision (RSD%, n = 6)	
			Intraday	Interday
AFB1	0.9996	$y = 0.4951x + 0.3049$	2.7	3.5
AFB2	0.9997	$y = 0.4185x + 0.1707$	3.1	6.2
AFG1	0.9995	$y = 0.1909x + 0.0841$	3.4	4.7
AFG2	0.9995	$y = 0.2298x + 0.0951$	4.1	5.2
DON	0.9996	$y = 9.7035x + 1.727$	5.1	6.8
ZEN	0.9999	$y = 4.2504x - 0.1183$	3.2	4.9
FB1	0.9994	$y = 84.443x - 7.3308$	3.7	7.7
FB2	0.9990	$y = 91.342x - 8.3462$	4.8	9.0

RSD stands for relative standard deviation.

Table 2 Recovery, limits of quantification (LOQs) and limits of detection (LODs) obtained for AFs, DON, ZEN and FBs by HPLC (n = 5)

Mycotoxins	Spiking level (μg/kg)	RSD (%)		Average recovery ±RSD (%)	LODs (μg/kg)	LOQs (μg/kg)
		Intraday (n = 6)	Interday (n = 6)			
AFB1	4	3.2	6.3	94.2±6.7	0.05	0.2
	8	2.7	5.8	96.4±5.2		
	16	3.1	5.2	92.8±3.2		
AFB2	2	2.4	4.8	99.2±4.7	0.1	0.4
	4	1.9	3.9	94.7±3.9		
	8	2.6	4.3	92.3±2.4		
AFG1	4	3.5	6.0	95.5±4.3	0.1	0.4
	8	3.4	5.6	84.6±7.1		
	16	2.5	5.3	90.6±6.8		
AFG2	2	2.6	4.6	94.2±5.7	0.2	0.7
	4	3.6	7.1	104.8±8.9		
	8	4.0	6.0	92.4±7.2		
DON	400	1.8	3.9	80.2±5.4	11	35
	800	3.2	4.5	82.4±6.9		
	1600	2.9	4.5	90.6±5.1		
ZEN	250	3.4	5.1	85.2±8.1	12	40
	500	2.9	5.0	87.4±5.6		
	1000	2.1	4.3	83.8±4.2		
FB1	200	3.7	7.7	95.1±6.7	10	65
	400	1.7	3.5	89.4±6.9		
	800	2.7	5.7	88.9±3.2		
FB2	200	4.8	9.0	77.1±7.0	9	62
	400	2.3	4.9	75.0±5.6		
	800	2.4	4.7	74.9±3.4		

Discussion

The study was undertaken to evaluate the natural occurrence of some mycotoxins (AFB1, AFB2, AFG1, AFG2, DON, ZEN, FB1 and FB2) in maize from Hebei Province. To the best of our knowledge, there have been no previous reports on the occurrence of multiple mycotoxins in corn throughout China.

As reported in the available literature, the incidence and levels of DON in maize varied widely. The average contamination level of DON was found to be 605 μg/kg, much higher than the 0–338 μg/kg reported in Argentina, France and Serbia (González et al., 1999; Jajić et al., 2008; Scudamore and Patel, 2009). However, higher values were also reported in maize samples from Austria, with an average contamination level of 753 μg/kg (Berthiller et al., 2009). Among all the samples, 7 samples were found to have a higher level of DON than the maximum tolerable limit of 1000 μg/kg set as the Chinese standard in maize, although the average of DON did not exceed the Standard (GB, 2005).

Low levels of ZEN were reported in Argentina (7 μg/kg), France (26 μg/kg) and Italy (42 μg/kg) according to some reports (Visconti and Pascale, 1998; González et al., 1999; Scudamore and Patel, 2009), whereas higher levels of ZEN were found in previous papers (Silva and Vargas, 2001; Shim et al., 2009). Thongrussamee et al. (2008) reported that ZEN was found at a maximum level of 686 μg/kg by HPLC in maize. The similar level was found by Silva and Vargas (2001) in Brazilian corn. In our work, 60% samples contaminated with ZEN were found and all the positive samples were above the maximum level (60 μg/kg in maize) adopted by China. The average concentration of ZEN (238 μg/kg) in our samples was almost four times as high as the maximum tolerable limit of 60 μg/kg set as the Chinese standard in maize (GB, 2005).

In this study, we found that the mean concentration of FB2 (58 μg/kg) was lower than that of FB1 (360 μg/kg), which was usual in other studies (Scudamore and Patel, 2000; Domijan et al., 2005; Lino et al., 2006). The mean concentration in our study for the totality of maize samples was 360 μg/kg for FB1 and 58 μg/kg for FB2, which was similar to that reported in Croatia (459.8 μg/kg for FB1) and Portugal (329 μg/kg for FB1 and 131 μg/kg for FB2) (Domijan et al., 2005; Lino et al., 2006), but lower than that reported in Spain (4800 μg/kg for FB1 and 1900 μg/kg for FB2), Morocco (1930 μg/kg for FB1) and China (6662 μg/kg for FB1) (Castellá et al., 1999; Zinedine et al., 2006; Gong et al., 2009). The maximum permitted level of FBs in feed and groceries has not yet been set in China. No samples, except one (Maize-01), had levels of FBs exceeding

Table 3 Occurrence of AFs, DON, ZEN and FBs in maize from Hebei Province

Samples	AFs mean (μg/kg) ±RSD (%)	DON mean (μg/kg) ±RSD (%)	ZEN mean (μg/kg) ±RSD (%)	FB1 mean (μg/kg) ±RSD (%)	FB2 mean (μg/kg) ±RSD (%)	Moldy
Maize-01	nd	2533±6.7	1239±8.3	3480±5.4	1000±6.0	+++
Maize-02	nd	28±4.4	nd	nd	nd	–
Maize-03	nd	nd	nd	nd	nd	–
Maize-04	nd	nd	nd	nd	nd	–
Maize-05	nd	114±5.2	60±2.4	nd	nd	–
Maize-06	nd	1905±6.2	780±4.3	1660±6.7	280±7.3	+++
Maize-07	nd	140±4.2	110±3.4	180±6.5	nd	+
Maize-08	nd	1505±6.9	568±3.8	1660±5.3	nd	+++
Maize-09	nd	1850±7.7	782±4.9	1040±4.0	170±3.1	+++
Maize-10	nd	1484±6.8	370±5.1	640±3.9	nd	++
Maize-11	nd	nd	nd	nd	nd	–
Maize-12	nd	nd	nd	nd	nd	–
Maize-13	nd	nd	nd	nd	nd	–
Maize-14	nd	nd	nd	nd	nd	–
Maize-15	nd	nd	nd	nd	nd	–
Maize-16	nd	1280±4.5	386±3.1	150±4.1	nd	++
Maize-17	nd	580±5.8	285±3.9	nd	nd	–
Maize-18	nd	62±5.3	nd	nd	nd	–
Maize-19	nd	780±3.9	276±5.1	nd	nd	+
Maize-20	nd	1358±4.0	402±3.8	180±3.3	nd	++
Maize-21	nd	nd	nd	nd	nd	–
Maize-22	nd	138±4.7	125±4.8	nd	nd	–
Maize-23	nd	688±4.3	230±2.8	nd	nd	+
Maize-24	nd	160±2.9	158±3.6	nd	nd	–
Maize-25	nd	520±5.7	179±4.4	nd	nd	+
Maize-average		605±7.9	238±8.9	360±8.7	58±7.9	

nd means not detected. RSD means relative standard deviation. The grade of moldy was defined according to Yang et al. (2010). – means normal samples. + means slightly moldy samples with no remarkably moldy spots; + + means moderately moldy samples with small moldy spots with visible mycelia. + + + means heavily moldy samples with remarkably moldy spots with profuse mycelia.

the maximum level of 4000 μg/kg in unprocessed corn for direct human consumption established by the European Union (EU) (European Commission, 2007).

AFs were not detected in maize in our study, although *A. flavus*, *A. niger* and *Fusarium* were found in maize samples (further study is on going). Aflatoxin contamination may be influenced by many factors including the nature of the substrate and the presence of other competing fungi (Betina, 1989). Although fungi, particularly *A. flavus*, was detected at very high levels in the market melon seeds, the incidence and level of aflatoxin were low (Bankole et al., 2004). This agrees with an earlier report in which *A. flavus* constituted 60% of total fungi isolated from melon ball snacks, but the aflatoxin contamination was actually detected in only 11% of samples (Bankole, 1995). Similar results were reported in spices from Egypt by Oman. Abou-Arab et al. (1999) and Elshafie et al. (2002) did not detect aflatoxins, but isolated aflatoxigenic strains of *A. flavus*. ElShafie et al. (2002) concluded that spices and other condiments are unsuitable substrates for aflatoxin production because of their essential oils, which may inhibit toxin production. Horn and Wicklow (1983) have reported that each fungus carries a factor that determines its growth and ability to compete with other fungal species. Thus, it has been pointed out that fungal interactions from competition could lead to a decrease in mycotoxin contamination.

Although the number of samples analyzed was very limited, the investigation showed that the maize in Hebei Province frequently contained DON, ZEN and FBs. As AFs were not detected in maize in this study, continuing detection of AFs is still an essential part of monitoring. Further monitoring of mycotoxins in China is necessary. Furthermore, the producing fungi and their competition need to be thoroughly investigated.

Acknowledgements

This research was supported by the earmarked fund for China Agriculture Research System (No. nycytx-02).

References

Abou-Arab A A K, Soliman Kawther M, EI Tantawy M E, Badeaa R I,

Khayria N (1999). Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. Food Chem, 67(4): 357–363

Bankole S A (1995). Mycoflora, mycotoxin producing fungi and mycotoxins from melon ball snacks in Nigeria. Chemie, Mikrobiologie, Technologie der Lebensmittel, 17: 164–168

Bankole S A, Ogunsanwo B M, Mabekoje O O (2004). Natural occurrence of moulds and aflatoxin B1 in melon seeds from markets in Nigeria. Food Chem Toxicol, 42(8): 1309–1314

Bennett J W, Klich M (2003). Mycotoxins. Clin Microbiol Rev, 16(3): 497–516

Berthiller F, Dall'asta C, Corradini R, Marchelli R, Sulyok M, Krska R, Adam G, Schuhmacher R (2009). Occurrence of deoxynivalenol and its 3-beta-D-glucoside in wheat and maize. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 26(4): 507–511

Betina V (1989). Biological effects of mycotoxins. In: Betina V, ed. Mycotoxins: Chemical, Biological and Environmental Aspects. Amsterdam: Elsevier Science Publishers, 42–58

Castellá G, Bragulat M R, Cabañes F J (1999). Surveillance of fumonisins in maize-based feeds and cereals from Spain. J Agric Food Chem, 47(11): 4707–4710

Chu F S, Li G Y (1994). Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer. Appl Environ Microbiol, 60(3): 847–852

Doko M B, Visconti A (1994). Occurrence of fumonisins B1 and B2 in corn and corn-based human foodstuffs in Italy. Food Addit Contam, 11(4): 433–439

Domijan A M, Peraica M, Jurjević Ž, Ivić D, Cvjetković B (2005). Fumonisin B1, fumonisin B2, zearalenone and ochratoxin A contamination of maize in Croatia. Food Addit Contam, 22(7): 677–680

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

European Commission (EC) (2007). Commission Regulation (EC) No 1126/2007, of 28 September 2007 amending Regulation (EC) No1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. Off J Eur Union, L255: 14–17

Fandohan P, Gnonlonfin B, Hell K, Marasas W F O, Wingfield M J (2005). Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. Int J Food Microbiol, 99(2): 173–183

GB (2005). Hygienic standardard for gains (GB2715–2005). In: Mycotoxin limits, Beijing, Standardization Administration of the People's Republic of China, 2 (in Chinese)

Gelderblom W C, Jaskiewicz K, Marasas W F, Thiel P G, Horak R M, Vleggaar R, Kriek N P (1988). Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Appl Environ Microbiol, 54(7): 1806–1811

Gong H Z, Ji R, Li Y X, Zhang H Y, Li B, Zhao Y, Sun L, Yu F, Yang J (2009). Occurrence of fumonisin B(1) in corn from the main corn-producing areas of China. Mycopathologia, 167(1): 31–36

González H H L, Martínez E J, Pacin A M, Resnik S L, Sydenham E W (1999). Natural co-occurrence of fumonisins, deoxynivalenol, zearalenone and aflatoxins in field trial corn in Argentina. Food Addit Contam, 16(12): 565–569

Groopman J D, Donahue K F (1988). Aflatoxin, a human carcinogen: determination in foods and biological samples by monoclonal antibody affinity chromatography. J Assoc Off Anal Chem, 71(5): 861–867

Haschek W M, Gumprecht L A, Smith G, Tumbleson M E, Constable P D (2001). Fumonisin toxicosis in swine: an overview of porcine pulmonary edema and current perspectives. Environ Health Perspect, 109(Suppl 2): 251–257

Horn B W, Wicklow D T (1983). Factors influencing the inhibition of aflatoxin production in corn by *Aspergillus niger.* Can J Microbiol, 29 (9): 1087–1091

IARC (2002). Monograph on the Evaluation of Carcinogenic Risk to Humans. In: Some Traditional Herbal Medicines, Some Mycotoxins, Naphtalene and Styrene, vol. 82. Lyon: IARC, 171–300

Jajić I, Jurić V, Glamočić D, Abramović B (2008). Occurrence of deoxynivalenol in maize and wheat in Serbia. Int J Mol Sci, 9(11): 2114–2126

Kimanya M E, De Meulenaer B, Tiisekwa B, Ugullum C, Devlieghere F, Van Camp J, Samapundo S, Kolsteren P (2009). Fumonisins exposure from freshly harvested and stored maize and its relationship with traditional agronomic practices in Rombo district, Tanzania. Food Addit Contam, 26(8): 1199–1208

Kurtzman C P, Horn B W, Hesseltine C W (1987). *Aspergillus nomius*, a new aflatoxin-producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. Antonie van Leeuwenhoek, 53(3): 147–158

Lino C M, Silva L J G, Pena A L S, Silveira M I (2006). Determination of fumonisins B1 and B2 in Portuguese maize and maize-based samples by HPLC with fluorescence detection. Anal Bioanal Chem, 384(5): 1214–1220

Lino C M, Silva L J G, Pena A, Fernández M, Mañes J (2007). Occurrence of fumonisins B1 and B2 in broa, typical Portuguese maize bread. Int J Food Microbiol, 118(1): 79–82

Marin D E, Gouze M E, Taranu I, Oswald I P (2007). Fumonisin B1 alters cell cycle progression and interleukin-2 synthesis in swine peripheral blood mononuclear cells. Mol Nutr Food Res, 51(11): 1406–1412

Marinamartins H, Almeida I, Marques M F, Guerra M M (2008). Fumonisins and deoxynivalenol in corn-based food products in Portugal. Food Chem Toxicol, 46(7): 2585–2587

Martins H M, Guerra M M M, Bernardo F M D (2007). Presencia de aflatoxina B1 en piensos para ganado lechero en Portugal durante el periodo 1995–2004. Rev Iberoam Micol, 24(1): 69–71

Missmer S A, Suarez L, Felkner M, Wang E, Merrill A H Jr, Rothman K J, Hendricks K A (2006). Exposure to fumonisins and the occurrence of neural tube defects along the Texas-Mexico border. Environ Health Perspect, 114(2): 237–241

Monbaliu S, Van Poucke C, Detavernier C, Dumoulin F, Van De Velde M, Schoeters E, Van Dyck S, Averkieva O, Van Peteghem C, De Saeger S (2010). Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS/MS method. J Agric Food Chem, 58(1): 66–71

Ok H E, Kim H J, Shim W B, Lee H, Bae D H, Chung D H, Chun H S (2007). Natural occurrence of aflatoxin B1 in marketed foods and risk estimates of dietary exposure in Koreans. J Food Prot, 70(12): 2824–2828

Pestka J J (2007). Deoxynivalenol: Toxicity, mechanisms and animal

health risks. Anim Feed Sci Technol, 137(3–4): 283–298

Salem N M, Ahmad R (2010). Mycotoxins in food from Jordan: preliminary survey. Food Contr, 21(8): 1099–1103

Schaafsma A W, Nicol R W, Savard M E, Sinha R C, Reid L M, Rottinghaus G (1998). Analysis of *Fusarium* toxins in maize and wheat using thin layer chromatography. Mycopathologia, 142(2): 107–113

Scudamore K A, Patel S (2000). Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom. Food Addit Contam, 17(5): 407–416

Scudamore K A, Patel S (2009). Occurrence of *Fusarium* mycotoxins in maize imported into the UK, 2004–2007. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 26(3): 363–371

Shim W B, Kim K Y, Chung D H (2009). Development and validation of a gold nanoparticle immunochromatographic assay (ICG) for the detection of zearalenone. J Agric Food Chem, 57(10): 4035–4041

Silva C M G, Vargas E A (2001). A survey of zearalenone in corn using Romer Mycosep^{TM} 224 column and high performance liquid chromatography. Food Addit Contam, 18(1): 39–45

Silva L J G, Lino C M, Pena A, Moltó J C (2007). Occurrence of fumonisins B1 and B2 in Portuguese maize and maize-based foods intended for human consumption. Food Addit Contam, 24(4): 381–390

Simon P, Delsaut P, Lafontaine M, Morele Y, Nicot T (1998). Automated column-switching high-performance liquid chromatography for the determination of aflatoxin M1. J Chromatogr B Biomed Sci Appl, 712(1–2): 95–104

Thongrussamee T, Kuzmina N S, Shim W B, Jiratpong T, Eremin S A, Intrasook J, Chung D H (2008). Monoclonal-based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals. Food Addit Contam, 25(8): 997–1006

Trung T S, Tabuc C, Bailly S, Querin A, Guerre P, Bailly J D (2008). Fungal mycoflora and contamination of maize from Vietnam with aflatoxin B_1 and fumonisin B_1. World Mycotoxin Journal, 1(1): 87–94

Visconti A, Pascale M (1998). Determination of zearalenone in corn by means of immunoaffinity clean-up and high-performance liquid chromatography with fluorescence detection. J Chromatogr A, 815 (1): 133–140

Wang Y, Chai T, Lu G, Quan C, Duan H, Yao M, Zucker B A, Schlenker G (2008). Simultaneous detection of airborne aflatoxin, ochratoxin and zearalenone in a poultry house by immunoaffinity clean-up and high-performance liquid chromatography. Environ Res, 107(2): 139–144

Yang L, Wang L, Pan J Y, Xiang L, Yang M H, Logrieco A F (2010). Determination of ochratoxin A in traditional Chinese medicinal plants by HPLC-FLD. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 27(7): 989–997

Zinedine A, Brera C, Elakhdari S, Catano C, Debegnach F, Angelini S, Desantis B, Faid M, Benlemlih M, Minardi V, Miraglia M (2006). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. Food Contr, 17(11): 868–874

Zinedine A, Mañes J (2009). Occurrence and legislation of mycotoxins in food and feed from Morocco. Food Contr, 20(4): 334–344