



**Full Length Article**

# Occurrence of Aflatoxins in Maize Grains from Central Areas of Punjab, Pakistan

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## ABSTARCT

Mycotoxins (MTs) are natural contaminants of food and feed, chiefly produced by moulds of genera *Penicillium*, *Aspergillus*, and *Fusarium* and are toxic to humans and animals. Aflatoxins are toxic metabolites produced mainly by *Aspergillus flavus*, *parasiticus* and *nomius*. Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a potent carcinogen, teratogen and mutagen. Forty samples of maize grains were collected randomly from urban, semi-urban and rural areas. These were ground to 20 mesh and extracted using 100 mL mixture of acetonitrile and water (84:16). The filtered extract was cleaned up with MycoSep # 226 column. The presence of aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>) was determined by using reverse-phase high performance liquid chromatography (RP-HPLC) using fluorescent detection system. Out of 40 maize samples, 34 were found contaminated with aflatoxins. The percentage of aflatoxins contamination in maize samples was 80%, 87% and 90% with their respective mean values of 45 µg kg<sup>-1</sup>, 54 µg kg<sup>-1</sup> and 62 µg kg<sup>-1</sup> in urban, semi-urban and rural areas, respectively. The method proposed in this study is convenient either for routine analysis or for research purposes. © 2010 Friends Science Publishers

**Key Words:** Mycotoxins; Aflatoxins, Maize grains, RP-HPLC

## INTRODUCTION

Huge quantities of food are wasted every year, because they are invaded by toxic fungi and get contaminated by fungal metabolic products. Such spoilage is prominent in hotter countries, where problems of food shortages already exist. One reliable estimate (CAST, 2003) is that mycotoxins affect a quarter of the world's food crops, including many basic foodstuffs such as animal feed, crops like maize, rice and wheat. The aflatoxins are hepatotoxic in animals; aflatoxin B<sub>1</sub> is the most potent mutagenic and carcinogenic metabolite known and ranked as Class 1 human carcinogen (IARC, 2002). Mycotoxins are associated with many chronic health risks, including the induction of cancer, immune suppression and blood and nerve defects (Shephard, 2006).

Maize (*Zea mays* L.) is one of the most widely distributed food plants in the world and its infection by fungi can result mycotoxin contamination during the growing, harvesting, storage, transporting and processing stages (Bradburn *et al.*, 1993). The main fungal species, which infect the maize are *Aspergillus flavus*, *parasiticus* and *nomius*. These fungi species grow well in the range of 19-35°C and produce maximum aflatoxins at 28°C (Sanchis & Magan, 2004). Inefficient drying or water ingress can cause pockets of wetter grain resulting in a higher MC

(Magan & Aldred, 2007) and substantially higher production of aflatoxins.

In Pakistan agriculture is a primary driver for the economic development, almost 21.8% share of GDP is obtained from this sector that play a vital role in the economy of the country. During 2007-08 maize was grown on an area of 1052 thousand hectares and produced 3605 thousand tonnes with average yield of 343 kg ha<sup>-1</sup> (Anonymous, 2008). It is rich in vitamins, fibre, carbohydrates and oil.

The economic consequences of mycotoxin contamination are profound, as the crops contaminated with high levels of mycotoxin often destroyed. The effected crops are sometimes diverted to animal feed, which may reduce growth rates, lead to illness of animal consuming contaminated feeds and result in meat and milk containing toxic residues or biotransformation products. In many parts of the world, dietary staples are highly susceptible to contamination by aflatoxin e.g., peanuts and maize are consumed daily and may constitute more than 50% of the diet in West Africa (Wild & Turner, 2002).

The presence of mycotoxins other than aflatoxins (OTA included) has been demonstrated in breakfast cereals and infant cereals (Candlish *et al.*, 2000; Biffi *et al.*, 2004; Araguas *et al.*, 2005; Molini *et al.*, 2005). Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) had been detected in muesli baby food (0.05 ng g<sup>-1</sup>)

sold in UK supermarkets and traces of total aflatoxins in corn flakes (Candlish *et al.*, 2000; Food Standard Agency, 2004) and highly contaminated corn flakes with aflatoxins consumed by the people of Egypt (El-Sayed *et al.*, 2003).

The metrological conditions of the country, specially, the province of Punjab, are very good for the cultivation of maize. During maize growth to harvest, the temperature and humidity and other plant stresses are conducive for the invasion of *Aspergillus* species, which produce aflatoxins as a secondary metabolites. Extensive review has been made but not a single study was conducted on the presence of aflatoxins in maize grown in Pakistan. The objective of the present study is to explore the levels of aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> & G<sub>2</sub>) in maize grains collected from different localities of urban, semi-urban and rural in Faisalabad Division, Pakistan.

## MATERIALS AND METHODS

**Maize samples:** Samples of maize grains were procured directly from whole sale market (grain market), vendors (grocery shops) and super markets during January 2008 to December 2008 covering the two harvesting seasons of maize production. It was pre-planned to get random sample from urban, semi-urban and rural areas and stored at 4°C in polythene bags with proper identification codes until these were analyzed for aflatoxins.

**Chemicals and standards:** Acetonitrile, methanol (HPLC grade), sodium chloride (analytical grade) of Merck (Darmstadt, Germany) were used. Trifluoroacetic acid (TFA) of Riedel-de Haen used as derivatizing agent to enhance fluorescence. The MycoSep<sup>®</sup> columns-226 (Romer Lab., USA) and standards of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> (50 µg mL<sup>-1</sup> in acetonitrile), Supelco, USA were purchased locally. A series of working standard solutions (0.1 to 0.5 µg mL<sup>-1</sup>) were prepared from stock solution and were stored in a borosilicate glass vials below 4°C.

**Aflatoxins extraction:** The analysis method used for aflatoxins in maize grain samples was made following the procedure of Fu *et al.* (2008) with little modifications. The samples of maize were thawed for two hours at room temperature (22°C±5) and ground 250 g samples of each collected from different localities Faisalabad Division, Pakistan using Centrifugal Grinding Mill (Retsch, ZM 200, Germany). A portion of 25 g of ground maize sample was taken in an Erlenmeyer flask (250 mL) and added 20% sodium chloride. Aflatoxins were extracted in 100 mL of a mixture of acetonitrile–water (84:16) by blending at high speed with Braun Multimix blender (MX-32), Germany for 3 min. The extract was filtered through Whatman (Maidstone, UK) filter paper (N0. 1). The filtrate (9 mL) was transferred to a glass tube, acidified with acetic acid (70 µL) and then passed through a Mycosep - 226 AflaZon<sup>+</sup> columns (Romer Labs.) with a flow rate of 2 mL/min. A portion of 2 mL of solution was evaporated to dryness with gentle stream of N<sub>2</sub> at 60°C in a centrifuge glass tube and

the residue was derivatized with trifluoroacetic acid (TFA).

**Liquid chromatographic determination with UV-Vis and fluorescence detections:** The LC system used for aflatoxins analysis was of Shimadzu, LC-10A Series (Shimadzu, Japan), equipped with manual injection system with loop size (20 µL), column oven (CTO-10A), dual liquid pumps (LC-10AS), system controller (SCL-10A), UV-Vis detector (SPD 10A) and fluorescence detector (RF-530) with excitation and emission wavelength of 360 nm and 440 nm, respectively. Discovery<sup>®</sup> HS C<sub>18</sub> (Octadecyl silane chemically bonded to porous silica) column (Supelco, Bellefonte, PA, USA), 250 x 4.6 mm with particle size 5 µm in diameter, was used. Acetonitrile, methanol and double distilled water in the ratio of 22.5:22.5:55 were used as mobile phase at a flow rate of 1.5 mL min<sup>-1</sup>. These chromatographic conditions gave good resolution of peaks. Calibration curve was drawn using a series of calibration solutions of aflatoxin in acetonitrile with concentration of 0.05, 0.1, 0.5, 1.0, 5.0, and 10 µg L<sup>-1</sup>. All analyses were performed in triplicate in isocratic mode at 30°C.

**Statistical analysis:** The results of aflatoxins concentration were statistically analyzed by applying one-way analysis of variance using (ANOVA) MSTAT-C software.

## RESULTS AND DISCUSSION

Linearity of HPLC system was checked to inject different concentrations of reference standards. The system was calibrated using the working solutions of aflatoxin in the range of 1-10 µg mL<sup>-1</sup> in acetonitrile. Parameters of linear regression measured for AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub> using Discovery column, Supelco are reported in Table II. The calibration curve was also evaluated by its correlation coefficient, slope and intercept. Recovery percentage of aflatoxins spiked at different levels was studied and found in the range of 88–91% (Table I).

The analyzed working solution gave excellent values of regression coefficient, slope and intercept for aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> & AFG<sub>2</sub>). Regression coefficient (R<sup>2</sup>) values are ≥0.9959, whereas slope and intercept are in the range of 6218–19043 and 432–4541, respectively.

Maize is usually grown during hot weather and harvested during humid summer in Pakistan. These conditions promote the attack of fungi with maximum production of aflatoxins. Samples of maize (corn) were collected randomly, extracted with organic solvent, cleaned up and analyzed using Shimadzu HPLC (LC-10A). The data after analyses and computation are given in Tables III-V.

A total of 40 samples collected from Faisalabad Division were analyzed with fluorescent detector in reverse-phase mode for aflatoxins. Data presented in Table III show that the contamination intensity with aflatoxins is > 80% with mean residues greater than 45 µg kg<sup>-1</sup> in the samples of maize collected from Faisalabad Division. Most of maize samples were found contaminated with fungi producing metabolites of aflatoxins. Aflatoxin-producing fungi had the

highest frequency of occurrence in rural areas as compared to urban and semi-urban. Samples of maize grain and processed maize (flour) were collected in *kharif* (rainy season) and *rabi* (winter season) and overall frequency of aflatoxins was very high among samples (85%). A few samples (15%) were found free from aflatoxins contamination. The maize samples analyzed representing different cultivars, collected from diverse agro-climatic areas of Faisalabad Division revealed that mean residues in rural samples is high (62  $\mu\text{g kg}^{-1}$ ) and lower mean residues of aflatoxin was found in urban i.e. 45  $\mu\text{g kg}^{-1}$ .

It is clear that maize consumed by residents and animals belonging to the Faisalabad Division may suffer with certain diseases due to the toxicity of aflatoxins. Peak area under each compound was computed to find out the concentration of aflatoxins. The figures given in Table IV were compared with the established limits of aflatoxins for various foodstuffs in different countries. The data of the present study indicate that out of 34 contaminated samples, 6 contained residue of aflatoxins  $\leq 20 \mu\text{g kg}^{-1}$  and most of the samples with  $> 30 \mu\text{g kg}^{-1}$  (40%) of the collected samples. Among analyzed samples, 10% contained aflatoxins with concentration range 26-30  $\mu\text{g kg}^{-1}$ , whereas few samples (20%) had residues in the range of 21-25  $\mu\text{g kg}^{-1}$ .

The samples contaminated with aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> & AFG<sub>2</sub>) were categorized on the basis of locality; percent incidence and concentration range (Table IV). The data illustrate that AFB<sub>1</sub> is present in 8 samples in the range of 8-46  $\mu\text{g kg}^{-1}$  and sample was in the shape of mixture. Out of 34 contaminated samples, 6 contained two types of aflatoxins (AFB<sub>1</sub>+AFB<sub>2</sub>) with incidence percentage of 15. Total aflatoxins was more frequent in maize and maize flour samples (40%) with a concentration range 12-75  $\mu\text{g kg}^{-1}$  and least samples contained metabolites (AFG<sub>1</sub> & AFG<sub>2</sub>) in the concentration range 0.2-12  $\mu\text{g kg}^{-1}$ . The aflatoxins concentration among samples of the same area (urban or rural) was not consistent. All the data computed for maize aflatoxins show substantial variation ( $> 2.40$  standard deviation) among concentration of aflatoxins. The data variation may be due to differences in moisture content, period of storage or nature of storage (steel bin, clay bin etc.). From the data, it is indicated that most of the samples of maize were contaminated with total aflatoxins (AFB<sub>1</sub>+AFB<sub>2</sub>+AFG<sub>1</sub> & AFG<sub>2</sub>) and least samples were found contaminated with AFG<sub>1</sub> and AFG<sub>2</sub> as compared to other aflatoxins.

The contamination of corn seed and flour were evaluated in the undertaking study and found high levels of aflatoxins in it (Tables III-V). The samples of maize analyzed showed the presence of aflatoxins ( $>80\%$ ) of which rural maize contamination is high (90%). The aflatoxin content of the maize under investigation was found to be lower than those reported by Abbas *et al.* (2006). According to their findings aflatoxins content in conventional hybrid corn ranged from 21 to 699  $\mu\text{g kg}^{-1}$  with a mean of 215 $\pm$ 49  $\mu\text{g kg}^{-1}$ , while those of Bt hybrids

**Table I: Recovery % of aflatoxins in cereals samples**

| Aflatoxin | Spiking level ( $\mu\text{g kg}^{-1}$ ) | Maize         |
|-----------|---|---------------|
| AFB1      | 10                                      | 88 $\pm$ 0.18 |
| AFB2      | 5                                       | 91 $\pm$ 0.14 |
| AFG1      | 10                                      | 88 $\pm$ 0.12 |
| AFG2      | 5                                       | 89 $\pm$ 0.16 |

Data is mean of 5 replicate  $\pm$  standard deviation

**Table II: Parameters of linear regression\* measured for aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> & AFG<sub>2</sub>) in HPLC**

| Aflatoxin        | Concentration                 | Slope (a) | Intercept (b) | R <sup>2</sup> |
|------------------|-------------------------------|-----------|---------------|----------------|
| AFB <sub>1</sub> | 1 – 10 $\mu\text{g mL}^{-1}$  | 16654     | 432.23        | 0.9989         |
| AFB <sub>2</sub> | 0.1 – 5 $\mu\text{g mL}^{-1}$ | 19043     | 1608          | 0.9981         |
| AFG <sub>1</sub> | 1 – 10 $\mu\text{g mL}^{-1}$  | 6851.7    | 4541.8        | 0.9959         |
| AFG <sub>2</sub> | 0.1 – 5 $\mu\text{g mL}^{-1}$ | 6218.6    | 597.39        | 0.9987         |

\*y = ax + b; y = peak area, x = ng injected, R<sup>2</sup> = regression coefficient

**Table III: Aflatoxins\* level ( $\mu\text{g kg}^{-1}$ ) in Maize samples collected from urban, semi-urban and rural area of Faisalabad Division**

| Commodity                       | Area       | Total samples | Contaminated samples (%) | *Mean $\pm$ SD |
|---------------------------------|------------|---------------|--------------------------|----------------|
| Maize grain                     | Urban      | 15            | 13 (87%)                 | 45 $\pm$ 2.40  |
| Maize grain and Semi-corn floor | Semi-urban | 15            | 12 (80%)                 | 54 $\pm$ 4.05  |
| Maize grain                     | Rural      | 10            | 9 (90%)                  | 62 $\pm$ 5.64  |

\*Aflatoxins = AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>

**Table IV: Distribution of aflatoxin levels in Maize collected from Faisalabad Division**

| Area of sample | Total sample | Maize Aflatoxin   |           |           |                |
|----------------|--------------|-------------------|-----------|-----------|----------------|
|                |              | $\leq 20$ ppb (%) | 21-25 ppb | 26-30 ppb | $> 30$ ppb (%) |
| Urban          | 15           | 2 (13)            | 4 (27)    | 2 (13)    | 5 (33)         |
| Semi-urban     | 15           | 3 (20)            | 2 (13)    | Nil       | 7 (47)         |
| Rural          | 10           | 1(10)             | 2 (20)    | 2 (20)    | 4 (40)         |

Acceptable upper limit for aflatoxin in grains is 20 ppb (FDA, 1987); Values in parenthesis are in %

**Table V: Occurrence of the four major types of aflatoxins in \*Maize from Faisalabad Division**

| Aflatoxin                           | No. of sample | of (%) | Conc. Range $\mu\text{g kg}^{-1}$ | Condition of sample |
|-------------------------------------|---------------|--------|-----------------------------------|---------------------|
| AFB <sub>1</sub>                    | 8             | 20     | 8-46                              | Maize grain + flour |
| AFB <sub>1</sub> + AFB <sub>2</sub> | 6             | 15     | 10-58                             | Maize grain + flour |
| AFG <sub>1</sub> + AFG <sub>2</sub> | 4             | 10     | 0.2-12                            | Maize grain + flour |

\*samples were analyzed with two detectors (UV-Vis and Fluorescence detectors) and compared the concentration with working standard

fell in the range of 66 to 428  $\mu\text{g kg}^{-1}$  with a mean value of 197 $\pm$ 85  $\mu\text{g kg}^{-1}$ . However, recent reports of aflatoxins in food showed the presence of aflatoxins in maize analyzed in Argentina and India ranged (5-560  $\mu\text{g kg}^{-1}$ ) and (5-666  $\mu\text{g kg}^{-1}$ ), respectively reported by Moss (2002).

Of the 40 maize samples that were tested, 34 (85%) were found contaminated with aflatoxins. Some of these samples contain aflatoxins that exceed the legal limits of 20  $\mu\text{g kg}^{-1}$  as imposed in many countries (Moss, 2002). Aflatoxin distribution on the basis of legal limits was evaluated for maize grains. The computed data indicate that

most of the samples (>84%) contained high aflatoxins level than the permitted limits; however, some samples (15%) were found aflatoxin below than the recommended limits. Surveys from other countries have reported the occurrence of aflatoxins in corn and related products consumed in Turkey and China (Li *et al.*, 2001; Castells *et al.*, 2008).

It also has been reported that increased aflatoxin formation was registered by heavy rains during the storage, by delayed storage and high moisture contents (Kumar *et al.*, 2008). Furthermore the storage conditions and their effects on aflatoxin production was studied by Saleemullah *et al.* (2006) Paddy rice samples were analysed after 2-3 and 12-18 month storage, where an increase of the aflatoxin content from 17.7 to 23.3  $\mu\text{g kg}^{-1}$  was registered during the storage (Saleemullah *et al.*, 2006). Additionally, Liu *et al.* (2006) evaluated the relationship between aflatoxin contamination and storage in whole grain rice and analyzed samples (37) showed an average aflatoxin content of 3.87  $\mu\text{g kg}^{-1}$ . Moreover, the aflatoxin content was determined in rice, which has been stored for 1 to 10 years (2.79–2.93  $\mu\text{g kg}^{-1}$ ). The highest concentration (6.23  $\mu\text{g kg}^{-1}$ ) was registered after 7-8 year storage (Liu *et al.*, 2006). The mould producing aflatoxins is only a small number of species that are widespread in the tropics and sub-tropics and are associated with important food commodities consumed in all parts of the world. However, if the plant is at all stressed (drought), then significant level of aflatoxin may be produced in plant tissue during growth in the field. Under these circumstances food commodities may already be contaminated at harvest (Hill *et al.*, 1985). The presence of high level in the present study may be attributed due to storage period or due to other factors (temperature, humidity, handling during harvesting & conditions during storage) as mentioned by authors worked on mycotoxins (Smith & Moss, 1985). The data (Table IV & V) show that the concentration of aflatoxin B<sub>1</sub> overpassed the authorized limits of EU. Similar findings were reported in conventional breakfast cereals of aflatoxins in the range of 2.29-4.30  $\mu\text{g kg}^{-1}$  (Entwisle *et al.*, 2000).

## CONCLUSION

The method validated for the assay of aflatoxins is simple, cost-effective and precise. The maize samples collected from Faisalabad Division were contaminated with mycotoxins. The samples of maize taken from rural localities were most toxigenic for inhabitants and animals. The long use of such cereal crop may cause diseases in humans and animals. There is a dire need to create awareness among the masses especially in the rural areas about health risks involved regarding this type of contaminations. The samples from the storage outlets must be taken after regular intervals during storage for the detection and measurements of aflatoxins. The authorities of the area must take this issue of contamination on urgent basis to solve the problem.

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## REFERENCES

- Anonymous, 2008. *Agricultural Statistics of Pakistan (2007-2008)*, Government of Pakistan, Ministry of Food, Agriculture and Livestock (Economic, Trade & Investment Wing), Islamabad, Pakistan
- Abbas, H.K., R.D. Cartwright, W. Xie and W.T. Shier, 2006. Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. *Crop Prot.*, 25: 1–9
- Araguas, C., E. Gonzalez-Penas and A. Lopez De Cerain, 2005. Study on ochratoxin A in cereal-derived products from Spain. *Food Chem.*, 92: 459–464
- Battilani, P., A. Scandolara, S. Formenti, V. Rossi, A. Pietri, A. Marocco and C. Ramponi, 2007. *L'acqua Nelle Cariossidi Facilita L'accumulo Di Fumonisine*, Vol. 58, pp: 49–52. L'informatore Agrario
- Biffi, R., M. Munari, L. Dioguardi, C. Ballabio, A. Cattaneo and C.L. Galli, 2004. Ochratoxin A in conventional and organic cereal derivatives: A survey of the Italian market, 2001-2002. *Food Additives Contaminants*, 21: 586–591
- Bradburn, N., G. Blunden, R.D. Coker and Jewers, K., 1993. Aflatoxin contamination of maize. *Trop. Sci.*, 33: 418–428
- Candlish, A.A.G., K.E. Aidoo, J.E. Smith and S.M. Pearson, 2000. A limited survey of aflatoxins and fumonisins in retail maize-based products in the UK using immunoassay detection. *Mycotoxin Res.*, 16: 2–8
- CAST, 2003. *Mycotoxins: Risks in Plant, Animal and Human Systems*. Council for Agricultural Science and Technology, Ames, Iowa
- El-Sayed, A.M.A.A., E.A. Soher and A.F. Sahab, 2003. Occurrence of certain mycotoxins in corn and corn-based foods and thermostability of fumonisin B<sub>1</sub> during processing. *Nahrung*, 47: 224–225
- Entwisle, A.C., A.C. Williams, P.J. Man, P.T. Slack and J. Gilbert, 2000. Liquid chromatographic method with immunoaffinity column cleanup for determination of ochratoxin A in barley: Collaborative study. *J. Association Official Analytical Chemists*, 83: 1377–1383
- Food Standards Agency, 2004. *Survey of Baby Foods for Mycotoxins*. Food Survey Information Sheet No. 68/04 (available at: <http://www.food.gov.uk/multimedia/pdfs/fsis6804.pdf>).
- Fu, Z., X. Huang and S. Min, 2008. Rapid determination of aflatoxins in corn and peanuts. *J. Chromatogr. A.*, 1209: 271–274
- Hill, R.A., D.M. Wilson, W.W. McMillan, N.W. Widstrom, R.J. Cole, T.H. Sanders and P.D. Blankenship, 1985. Ecology of the *Aspergillus flavus* group and aflatoxin formation in maize and groundnuts. In: Lacey, J. (ed.), *Trichothecenes and Other Mycotoxins*, pp: 79–95. Wiley, New York
- IARC, 2002. Working Group on the Evaluation of Carcinogenic Risks to Humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monog. Eval. Carcinogenic Risks Humans*, 82: 1–556
- Kumar, V., M.S. Basu and T.P. Rajendran, 2008. Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop Prot.*, 27: 891–905
- Liu, Z., J. Gao and J. Yu, 2006. Aflatoxins in stored maize and rice grains in Liaoning Province, China. *J. Stored Prod. Res.*, 42: 468–479
- Magan, N. and D. Aldred, 2007. Post-harvest control strategies: minimizing mycotoxins in the food chain. *Int. J. Food Microbiol.*, 119: 131–139
- Molini, A., V. Faucet, M. Castegnaro and A. Pfohl-Leschkiewicz, 2005. Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B<sub>1</sub>: Development of a method for simultaneous extraction of ochratoxin A and citrinin. *Food Chem.*, 92: 391–400
- Moss, M.O., 2002. Risk assessment for aflatoxins in foodstuffs. *Int. Biodeter. Biodegrad.*, 50: 137–142

- Saleemullah, A. Iqbal, I.A. Khalil and H. Shah, 2006. Aflatoxin contents of stored and artificially inoculated cereals and nuts. *Food Chem.*, 98: 699–703
- Sanchis, V. and N. Magan, 2004. Environmental profiles for growth and mycotoxin production. In: Magan, N., M. Olsen, (eds.), *Mycotoxins in Food: Detection and Control*. Woodhead Publishing Ltd., Cambridge, UK
- Shephard, G.S., 2006. Mycotoxins in the context of food risks and nutrition issues. In: Barug, D., D. Bhatnagar, H.P. Van Egmond, J.W. Van Der Kamp, W.A. Van Osenbruggen and A. Visconti (eds.), *The Mycotoxin Factbook: Food and Feed Topics*, pp: 21–36. Wageningen Academic Publishers, Wageningen, The Netherlands
- Smith, J.E. and M.O. Moss, 1985. *Mycotoxins: Formation, Analysis and Significance*. Wiley, Chichester, UK
- Wild, C.P. and P.C. Turner, 2002. The toxicology of aflatoxins as a basis for public health decisions. *Mutagenesis*, 17: 471–481

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